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(54) Titre : IMMUNOTHERAPIE A BASE DE CELLULES POUVANT ETRE GREFFEES POUR L'ADMINISTRATION SUR
UNE LONGUE PERIODE DE PROTEINES THERAPEUTIQUES

(54) Title: ENGRAFTABLE CELL-BASED IMMUNOTHERAPY FOR LONG-TERM DELIVERY OF THERAPEUTIC
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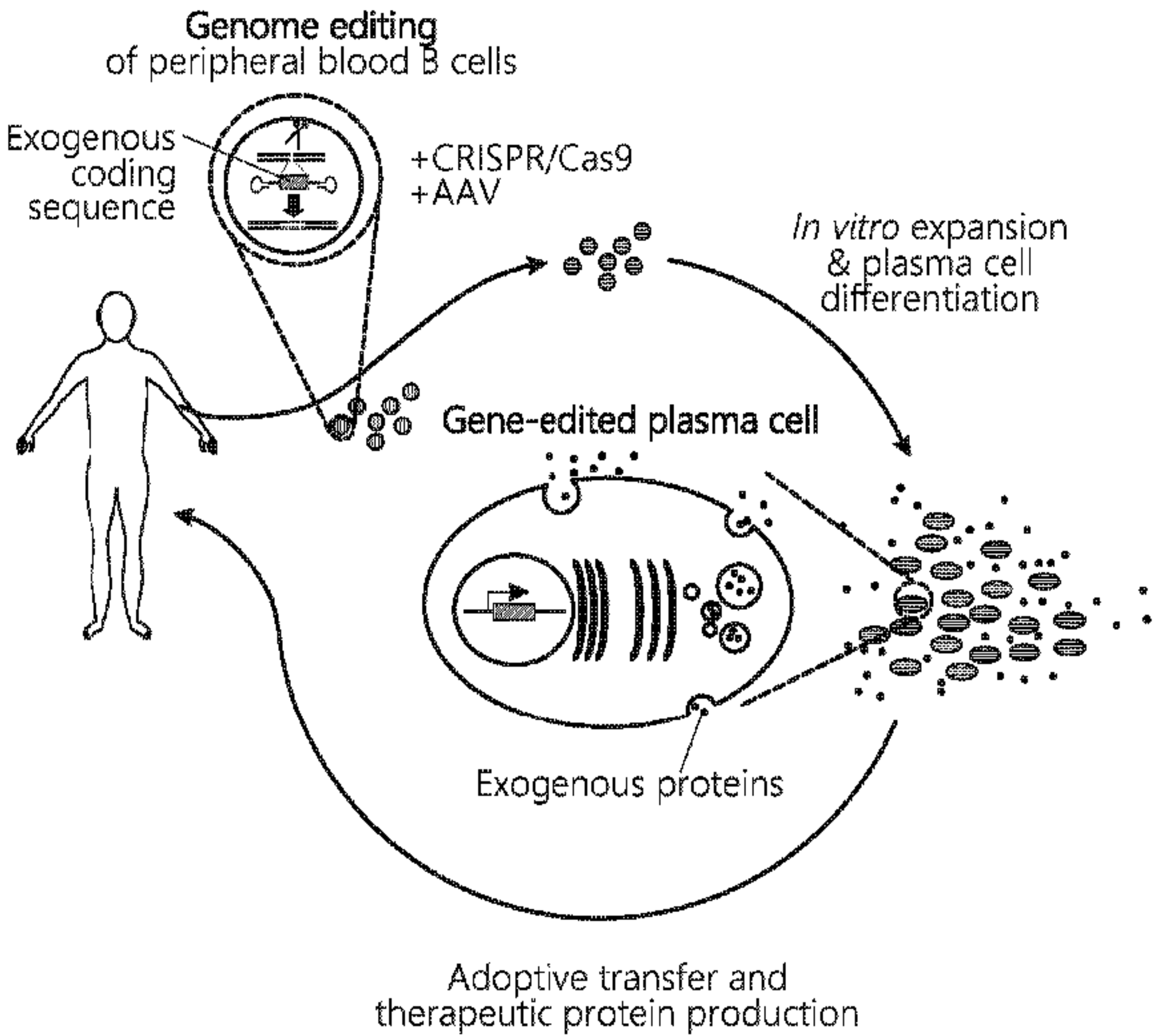


FIG. 1

(57) **Abrégé/Abstract:**
The present application relates to plasma cells and plasma cell precursors that express a macromolecule, such as a protein, protein mimetic or a peptide and compositions comprising these plasma cells or plasma cell precursors. The application further

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relates to methods of using and making the plasma cells and plasma cell precursors that express the macromolecule. Methods of treatment comprising administering the plasma cells or plasma cell precursors are also contemplated.

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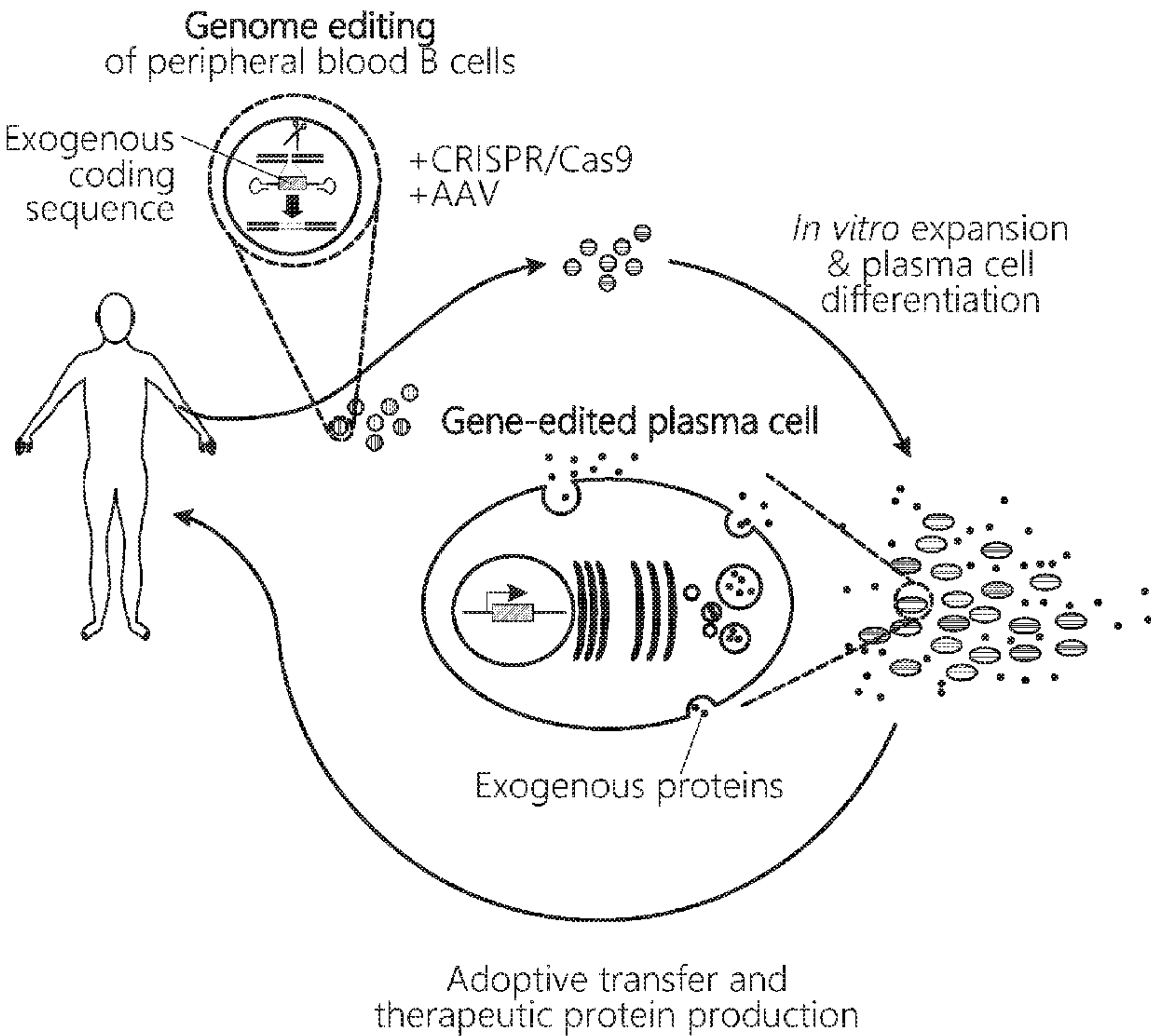
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(54) Title: ENGRAFTABLE CELL-BASED IMMUNOTHERAPY FOR LONG-TERM DELIVERY OF THERAPEUTIC PROTEINS



(57) Abstract: The present application relates to plasma cells and plasma cell precursors that express a macromolecule, such as a protein, protein mimetic or a peptide and compositions comprising these plasma cells or plasma cell precursors. The application further relates to methods of using and making the plasma cells and plasma cell precursors that express the macromolecule. Methods of treatment comprising administering the plasma cells or plasma cell precursors are also contemplated.

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JUMBO APPLICATIONS/PATENTS

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ENGRAFTABLE CELL-BASED IMMUNOTHERAPY FOR LONG-TERM DELIVERY OF THERAPEUTIC PROTEINS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority to U.S. Provisional Patent Application No. 62/472,493, filed March 16, 2017, U.S. Provisional Patent Application No. 62/549,385, filed August 23, 2017, and U.S. Provisional Patent Application No. 62/571,918, filed October 13, 2017. The entire disclosures of the aforementioned applications are expressly incorporated by reference in their entireties.

REFERENCE TO SEQUENCE LISTING, TABLE, OR COMPUTER PROGRAM LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled, was created March 14, 2018 and is 171kB in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] A new approach for engineering human plasma cells or plasma cell precursors to express macromolecules such as proteins for therapeutic purposes, has been discovered. The alternatives described herein include methods requiring isolation and activation of B cells from subject-derived (autologous) or allogeneic peripheral blood mononuclear cells; genome engineering of these B cells or B cell precursors so that they express molecules, such as macromolecules, protein mimetics, proteins or peptides of interest in the absence of viral integration (with or without additional genetic modifications that modulate their eventual function and survival); expansion and differentiation of these cells into $>1e^{-6}$ long-lived plasma cells *in vitro*; and administration of these autologous or allogeneic engineered protein-producing plasma cells into human recipients for therapeutic application.

BACKGROUND OF THE INVENTION

[0004] To date there are limited reports of genome editing in primary B cells. These limited reports can be found, for example, in Cheong *et al.* 2016 and Chu *et al.* 2016 (“Editing of mouse and human immunoglobulin genes by CRISPR-Cas9 system.” Nat Commun. 2016 Mar 9; 7:10934. and “Efficient CRISPR-mediated mutagenesis in primary immune cells using CrispRGold and a C57BL/6 Cas9 transgenic mouse line.” Proc Natl Acad Sci U S A. 2016 Nov 1; 113(44):12514-12519.; both incorporated by reference in their entireties herein). These reports have used either transient transfection of plasmid DNA or lentiviral vector delivery to facilitate gene disruption. However, there are no reports available in the medical literature of using nucleases to achieve homologous recombination in primary human B cells.

[0005] One of the main problems with the use of plasmids is their low efficiency. For example, plasmid-based methods of DNA delivery to primary B cells are extremely toxic, which is likely due to innate DNA sensing and they also exhibit low efficiency (<1%). Lentiviral-based gene delivery also has low efficiency in these cells, for example, in most cases less than 5% of the cells can be transduced, and gene targeting in primary B cells has historically required use of cells from animals with transgenic expression of CAS9. In contrast, the genome engineering approach used in the alternatives described herein enables one to selectively edit the genes with introduction of gene expression cassettes by homologous recombination in more than 30% of primary human B cells and to selectively expand these cells to generate an enriched cell product expressing transgenes of interest.

[0006] Selectivity is also an issue with lentiviral and plasmid DNA. For example, lentiviral vectors integrate randomly and primarily in sites of active transcription and have the potential to be oncogenic. In contrast, nuclease targeting and homology directed repair (HDR) dependent integration of payload allow for selectivity with minimal off-target effects. The need for new approaches that allow one to achieve homologous recombination in primary human B cells is manifest.

SUMMARY OF THE INVENTION

[0007] Aspects of the alternatives described herein include, but are not limited to: (1) the use of blood-derived human B cells and/or B cell precursors as a starting material for a plasma cell molecule producing immunotherapy; (2) RNA- and protein-based transfection

to facilitate delivery of candidate designer nucleases targeting a broad range of genetic loci in primary B cells that include, but are not limited to zinc finger nucleases, transcription activator-like effector nucleases (TALEN), homing endonucleases (HEs), combined TALEN-HE proteins (megaTALs) and CRISPR/Cas systems; (3) transfection of long single-stranded DNA oligonucleotides or transduction with recombinant adeno-associated virus to facilitate efficient delivery of donor DNA templates carrying therapeutic expression cassettes into primary human B cells and/or B cell precursors in order to facilitate efficient homologous recombination into a range of candidate genetic loci; (4) integrity measures that include, but are not limited to, methods to prevent somatic hypermutation of the B cell antibody locus during the engineering process including, but not limited, to disruption of the AID gene; (5) production enhancers that include, but are not limited to methods to introduce dimerizable drug-inducible activating proteins to enable selectable expansion of engineered human B cells and/or B cell precursors *in vitro* or *in vivo*; (6) safety measures that include, but are not limited to, introduction of sequences from cell surface proteins including, but not limited to, the CD20 protein into B cells and/or B cell precursors to enable targeted removal from recipients using rituxan or alternative therapeutic approaches; and (7) the use of a proprietary multi-step cytokine and co-culture based systems to facilitate differentiation of blood-derived B cells and/or B cell precursors into long-lived plasma cells and their survival and expansion *in vitro*. The human B cells as described herein, can include B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cell, plasmablast, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. Without being limiting, the molecule comprises macromolecules, proteins, protein mimetics and peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, of the CRISPR/Cas system described herein, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9.

[0008] Multiple categories of use of a plasma cell producing a molecule, such as a macromolecule, protein or a peptide in immunotherapy are also envisioned. Exemplary alternatives are listed below:

[0009] (1) Prophylactic or therapeutic protection from infection (viral, bacterial, or parasitic) in healthy subjects or following stem cell administration or solid-organ transplantation in pediatric and adult subjects including, but not limited to neutralizing antibodies that block influenza, parainfluenza, rhinovirus, Respiratory Syncytial Virus (RSV), HIV, pathogenic bacteria, and/or parasites;

[0010] (2) Protein replacement, enzyme replacement and rescue of enzyme deficiencies including, but not limited to Factor VIII (Hemophilia A), Factor IX (Hemophilia B), ADAMTS13 (Hereditary TTP), LIPA (lysosomal acid deficiency), SERPING1 (hereditary angioedema), SERPINA1 (alpha1 anti-trypsin deficiency), GLA (Fabry disease), and/or ALPL (Hypophosphatasia);

[0011] (3) Immune modulation via expressed cytokines, cytokine receptors, complement proteins or other inhibitory proteins including, but not limited to: IL1 receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes; complement inhibitory proteins (including Factor H, Factor I) for treatment or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis; and/or C1 inhibitor for hereditary angioedema;

[0012] (4) Anti-fibrotic molecules including, but not limited to SCGB1A1 for the treatment of pulmonary fibrosis;

[0013] (5) Therapeutic antibodies or at least one binding portion thereof for treating or ameliorating autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer including but not limited to: anti-IL1 monoclonal antibodies or a binding portion thereof for the treatment or amelioration of periodic fever/autoinflammatory syndromes; anti-TNF antibodies or a binding portion thereof for the treatment or inhibition of inflammatory arthritis/inflammatory bowel disease, anti-IL33 antibodies or a binding portion thereof for the treatment or inhibition of asthma and/or anti-C5 antibodies or a binding portion thereof for the treatment or inhibition of paroxysmal nocturnal hemoglobinuria/atypical HUS;

[0014] (6) Anti-thrombotic molecules including, but not limited to APLN to block platelet function. Antithrombotic molecules are further described by Adam *et al.* (“Apelin: an antithrombotic factor that inhibits platelet function.” Blood. 2016 Feb 18; 127(7):908-20.; incorporated by reference in its entirety herein);

[0015] (7) Glucose response elements upstream of insulin for the treatment or inhibition of diabetic conditions; and

[0016] (8) Therapeutic monoclonal antibodies for hyper-cholesterolemia, including anti-PCSK9 inhibitory antibodies or a binding portion thereof. Preferred alternatives include the following alternatives. In some alternatives herein, the protein is a neutralizing antibody that block influenza, parainfluenza, rhinovirus, Respiratory Syncytial Virus (RSV), HIV, pathogenic bacteria, and/or parasites. In some alternatives herein, the protein is an enzyme. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the plasma cell expresses cytokines, cytokine receptors, complement proteins or other inhibitory proteins.

[0017] In a first aspect, a method of making plasma cells or plasma cell precursors that expresses a molecule, such as a macromolecule, protein, or peptide is provided, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for expression of a molecule in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells or plasma cell precursors that express the molecule. In some alternatives, the B cells in step (a) comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the molecule is a macromolecule, such as a protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the protein comprises an enzyme, monoclonal antibody or a binding portion thereof, neutralizing

antibodies or a binding portions thereof, therapeutic antibodies or binding portions thereof, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment of periodic fever/autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs), or a binding portion thereof. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed by a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed on a viral, fungal, parasitic or bacterial infection. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein is an enzyme. In some alternatives, the enzyme comprises a cofactor for enhanced enzymatic activity. In some alternatives, the B cells in step (a) comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B

cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are patient derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMK, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a CAS nuclease. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a CAS nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cell with a

recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cell. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cell comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing the proportion of gene edited B cells comprises the steps of : (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cell short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding of the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfecting into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method

further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or any other length defined by a range that is set forth by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the cells were washed with 1X PBS and seeded in medium with IL-6 (50 ng/ml), IL-15 (10 ng/ml) and human interferon-α 2B (100 U/ml, Sigma-Aldrich) for three days to stimulate plasma cell differentiation. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFNα. In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element and/or a synthetic

engineered protein. In some alternatives, the protein is IFN-alpha, IL-6, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist that is used for the treatment or amelioration of periodic fever/autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, a therapeutic antibody or binding portion thereof is used e.g., an antibody or binding portion thereof that is specific for a protein or other molecule expressed in an autoimmune disorder, autoinflammatory disorder, immune dysregulation and/or cancer. In some alternatives, the antibody or binding portion thereof is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion thereof. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic and/or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cell, plasmablast, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the molecule is a macromolecule, protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic

group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the method further comprises purifying the plasma cells or plasma cell precursors after step e) by positive selection against CD138. In some alternatives, purifying comprises selecting the plasma cells by an anti-CD138 antibody for targeted removal of the plasma cells. In some alternatives, the purifying comprises using anti-CD138 beads for plasma cell selection.

[0018] In a second aspect, a method of making a long lived plasma cell is provided, wherein the method comprises: isolating B cells; activating the B cells; a first round of genome editing of the B cells for molecule expression, such as a protein, protein mimetic or a peptide in the absence of viral integration; expanding the B cells; and differentiating the B cells, thereby producing the long lived plasma cell. In some alternatives, the method further comprises selecting the long lived plasma cell by CD138 selection of the plasma cells. In some alternatives, the macromolecule is a protein, protein mimetic or peptide. In some alternatives, the protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the macromolecule comprises a carbohydrate or lipid moiety. In some alternatives, the macromolecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for the treatment or inhibition of periodic fever or autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for the treatment or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angioedema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or a binding portion thereof are specific for a protein expressed in autoimmune disorders,

autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody or a binding portion thereof is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the molecule is a macromolecule, such as a protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein is an enzyme. In some alternatives, the enzyme comprises a cofactor for enhanced enzymatic activity. In some alternatives, the B cells in step (a) comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the B cells from the isolating step comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cell. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or

Cas9. In some alternatives, the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the first round of genome editing comprises transducing the B cell with a recombinant adeno-associated virus vector for homologous recombination into a candidate genetic loci. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cell. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cell comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cell or disruption of candidate loci within the B cell to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L, 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2,

IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angioedema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody or binding portion thereof is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNABs). In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cell, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the molecule is selected from a group selected from macromolecules, proteins, protein mimetics and peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a

carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the isolating is performed by negative selection isolation of hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the method further comprises purifying long lived plasma cells are purified by positive selection against CD138. In some alternatives, purifying comprises selecting the plasma cells by an anti-CD138 antibody for targeted removal of the plasma cells. In some alternatives, the purifying comprises using anti-CD138 beads for plasma cell selection.

[0019] In a third aspect, a plasma cell that expresses a molecule, such as a heterologous protein, protein mimetic or a peptide is provided. In some alternatives, the cell is a B cell. In some alternatives, the heterologous protein comprises an enzyme, neutralizing antibody, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody, anti-thrombotic molecule, glucose response element and/or monoclonal antibody or binding portion thereof. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and cancer. In some alternatives, the antibody or binding portion thereof is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9

inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNABs). In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the plasma cell is a long lived plasma cell. In some alternatives, the plasma cell is manufactured by any one of the alternative methods provided herein. In some alternatives, the plasma cell is manufactured by a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMK, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the

nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing the proportion of gene edited B cells comprises: (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative

selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies,

therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the plasma cell is manufactured by a method of making a long lived plasma cell, wherein the method comprises: isolating B cells; activating the B cells; a first round of genome editing of the B cells for protein expression in the absence of viral integration; expanding the B cells; and differentiating the B cells. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cells. In some alternatives, the

nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector for homologous recombination into a candidate genetic loci. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cells or disruption of candidate loci within the B cells to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in

the presence any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cell, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the

molecule is selected from a group of macromolecules, proteins, protein mimetics and/or peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the plasma cell further comprises at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20.

[0020] In a fourth aspect, a composition comprising the plasma cell manufactured by any one of the alternatives provided herein or comprising the cell of any one of the alternatives herein, is provided. In some alternatives, the plasma cell is derived from a B cell. In some alternatives, the plasma cell expresses a molecule, such as a heterologous protein, protein mimetic or a peptide. In some alternatives, the cell is a B cell precursor. In some alternatives, the B cell precursor comprises hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the protein comprises an enzyme, neutralizing antibody, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody, anti-thrombotic molecule, glucose response element or a monoclonal antibody. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angioedema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the

antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the plasma cell is a long lived plasma cell. In some alternatives, the plasma cell is manufactured by any one of the alternative methods provided herein. In some alternatives, the plasma cell is manufactured by a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMK, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-

like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing the proportion of gene edited B cells comprises: (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further

comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6,

IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the plasma cell is manufactured by a method of making a long lived plasma cell, wherein the method comprises: isolating B cells; activating the B cells; a first round of genome editing of the B cells for protein expression in the absence of viral integration; expanding the B cells; and differentiating the B cells. In some

alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cell. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transducing the B cell with a recombinant adeno-associated virus vector for homologous recombination into a candidate genetic loci. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cells or disruption of candidate loci within the B cells to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by

negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFNα. In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever or autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angioedema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In

some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cells, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the molecule is selected from macromolecules, proteins, protein mimetics and/or peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity.

[0021] In a fifth aspect, a method of expressing a molecule, such as a macromolecule in a subject in need is provided, wherein the method comprises: providing or administering a plasma cell manufactured by any one of the alternative methods provided herein, the plasma cell that expresses a molecule of any one of any one of the alternative plasma cells provided herein, or the composition of any one of the alternatives described herein, into the subject in need. In some alternatives, the method further comprises purifying the plasma cells of step d), wherein the purifying is performed after the expanding step. In some alternatives, the purifying comprises positive selecting of cells against CD138. In some alternatives, the selecting the plasma cells is performed by an anti-CD138 antibody for targeted removal of the plasma cells. In some alternatives, the purifying comprises using anti-CD138 beads for plasma cell selection. In some alternatives, the molecule is a macromolecule, such as a protein, protein mimetic or a peptide. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein is an enzyme. In some alternatives, the enzyme comprises a cofactor for enhanced enzymatic activity. In some alternatives, the composition comprises the plasma cell manufactured by any one of the alternatives provided

herein or comprises the cell of any one of the alternatives provide herein. In some alternatives, the cell is a B cell. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the protein comprises an enzyme, neutralizing antibody, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody, anti-thrombotic molecule, glucose response element or monoclonal antibody. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, the protein is the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the plasma cell is a long lived plasma cell. In some alternatives, the plasma cell is manufactured by any one of the alternative methods provided herein. In some alternatives, the plasma cell is manufactured by a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule, wherein the method comprises (a) isolating B cells; (b) developing the B

cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. In some alternatives, the B cell is an early pro-B cell, late pro-B cell, large pre-B cell, small pre-B cell, immature B cell, T1 B cell, T2 B cell, marginal zone B cell, mature B cell, naïve B cell, plasmablast (short lived) cell, GC B cell, memory B cell, plasmablast cell and/or long lived plasma cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMK, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a CAS nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified.

In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing the proportion of gene edited B cells comprises the steps of : (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfecting into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-

transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever or autoinflammatory syndromes or

complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or portion thereof, wherein the antibody or portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the plasma cell is manufactured by a method of making a long lived plasma cell, wherein the method comprises: isolating B cells; activating the B cells; a first round of genome editing of the B cells for protein expression in the absence of viral integration; expanding the B cells; and differentiating the B cells. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cells. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of

genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector for homologous recombination into a candidate genetic loci. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cells or disruption of candidate loci within the B cells to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L, 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2,

IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives of the method of expressing a molecule, such as a macromolecule in a subject in need, the administering is performed by adoptive transfer. In some alternatives of the method of expressing a molecule, such as a macromolecule in a subject in need, the subject has received a stem cell administration or solid organ transplantation. In some alternatives of the method of expressing protein in a subject in need, the subject suffers from influenza, parainfluenza, rhinovirus, respiratory syncytial virus, HIV, or other pathogenic bacteria, virus, fungus or parasite infection. In some alternatives of the method of expressing protein in a subject in

need, the subject suffers from an enzyme deficiency. In some alternatives of the method of expressing a molecule, such as a macromolecule in a subject in need, the subject suffers from pulmonary fibrosis. In some alternatives of the method of expressing a molecule, such as a macromolecule in a subject in need, the subject suffers from an autoimmune disorder, immune dysregulation or cancer. In some alternatives, the subject suffers from HIV. In some alternatives of the method of expressing a molecule, such as a macromolecule in a subject in need, the subject suffers from diabetes. In some alternatives of the method of expressing a molecule, such as a macromolecule in a subject in need, the subject suffers from hypercholesterolemia. In some alternatives of the method of expressing a molecule, such as a macromolecule in a subject in need, the subject is also receiving CAR T-cell therapy. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cells, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the molecule is selected from macromolecules, proteins, protein mimetics and/or peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the method comprises monitoring of the subject following plasma cell transplantation. In some alternatives, the subject is determined to no longer need treatment or therapy and the method further comprises administering rituxan or an anti-CD20 antibody or binding portion thereof for targeted removal of the plasma cells.

[0022] In a sixth aspect, a composition comprising the plasma cell manufactured by any one of the alternatives herein and a second B cell, wherein the second B cell secretes a macromolecule for inducing tolerance of a peptide for immunotherapeutic application or for inducing engraftment of the plasma cell manufactured by any one of anyone of the alternatives herein is provided. In some alternatives, the plasma cell is derived from a B cell. In some alternatives, the plasma cell expresses a molecule, such as a heterologous protein,

protein mimetic or a peptide. In some alternatives, the cell is a B cell precursor. In some alternatives, the B cell precursor comprises hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the protein comprises an enzyme, neutralizing antibody, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody, anti-thrombotic molecule, glucose response element or a monoclonal antibody. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angioedema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the plasma cell is a long lived plasma cell. In some alternatives, the plasma cell is manufactured by any one of the alternative methods provided herein. In some alternatives, the plasma cell is manufactured by a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In

some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMK, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous

recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing the proportion of gene edited B cells comprises: (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one

cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders,

autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the plasma cell is manufactured by a method of making a long lived plasma cell, wherein the method comprises: isolating B cells; activating the B cells; a first round of genome editing of the B cells for protein expression in the absence of viral integration; expanding the B cells; and differentiating the B cells. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cell. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transducing the B cell with a recombinant adeno-associated virus vector for homologous recombination into a

candidate genetic loci. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cells or disruption of candidate loci within the B cells to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some

alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever or autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cells, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the molecule is selected from macromolecules, proteins, protein mimetics and/or peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the method further comprises purifying the plasma cells or plasma cell

precursors by positive selection against CD138. In some alternatives, purifying comprises selecting the plasma cells by an anti-CD138 antibody for targeted removal of the plasma cells. In some alternatives, the purifying comprises using anti-CD138 beads for plasma cell selection.

[0023] In a seventh aspect a method of treating, ameliorating or inhibiting a disease, such as a cancer, in a subject in need, is provided, the method comprises administering a plasma cell manufactured by any one of the alternative methods herein or the plasma cell that expresses a molecule, such as a macromolecule manufactured by any one of the alternative methods herein, or the composition of any one of the alternative compositions herein into the subject in need. In some alternatives, the composition comprises the plasma cell manufactured by any one of the alternatives provided herein or comprises the cell of any one of the alternatives provided herein. In some alternatives, the cell is a B cell. In some alternatives, the protein comprises an enzyme, neutralizing antibody, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody, anti-thrombotic molecule, glucose response element or a monoclonal antibody. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the

plasma cell is a long lived plasma cell. In some alternatives, the plasma cell is manufactured by any one of the alternative methods provided herein. In some alternatives, the plasma cell is manufactured by a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMK, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a CAS nuclease are delivered via

translatable RNA or recombinant protein. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing the proportion of gene edited B cells comprises: (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome

editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha,

Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever or autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody or a binding portion thereof that is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the plasma cell is manufactured by a method of making a long lived plasma cell, wherein the method comprises: isolating B cells; activating the B cells; a first round of genome editing of the B cells for protein expression in the absence of viral integration; expanding the B cells; and differentiating the B cells. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cell. In some alternatives, the nuclease is a zinc finger

nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector for homologous recombination into a candidate genetic loci. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cells or disruption of candidate loci within the B cell to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of

MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives of the method of treating, inhibiting or ameliorating a disease, such as cancer, in a subject in need, the administering is performed by adoptive transfer. In some alternatives of the method of treating, inhibiting, or ameliorating a disease, such as cancer, in a subject in need, the subject has received a stem cell administration or solid organ transplantation. In some

alternatives of the method of ameliorating, treating, or inhibiting a disease in a subject in need, the subject suffers from influenza, parainfluenza, rhinovirus, respiratory syncytial virus, HIV, or other pathogenic bacteria, virus, fungus or parasite infection. In some alternatives of the method of ameliorating, treating, or inhibiting a disease in a subject in need, the subject suffers from an enzyme deficiency. In some alternatives of the method of ameliorating, treating, or inhibiting a disease in a subject in need, the subject suffers from pulmonary fibrosis. In some alternatives of the method of ameliorating, treating, or inhibiting a disease in a subject in need, the subject suffers from an autoimmune disorder, immune dysregulation and/or cancer. In some alternatives of the method of ameliorating, treating, or inhibiting a disease in a subject in need, the subject suffers from diabetes. In some alternatives of the method of ameliorating, treating, or inhibiting a disease in a subject in need, the subject suffers from hypercholesterolemia. In some alternatives of the method of ameliorating, treating, or inhibiting a disease in a subject in need, the subject is receiving therapy. In some alternatives of the method of ameliorating, treating, or inhibiting a disease in a subject in need, the subject is selected to receive cancer therapy, diabetes therapy, HIV treatment, or viral therapy. In some alternatives of the method of ameliorating, treating, or inhibiting a disease in a subject in need, the subject is also receiving CAR T-cell therapy. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cells, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the molecule is selected from macromolecules, proteins, protein mimetics and/or peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the method comprises monitoring of subject following plasma cell transplantation. In some alternatives, the subject is determined to no longer need treatment or

therapy and the method further comprises administering rituxan or an anti-CD20 antibody or binding portion thereof for targeted removal of the plasma cells.

[0024] In an eighth aspect, a method of expressing a molecule, such as a macromolecule in a subject of need is provided, wherein the method comprises: (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for molecule expression in absence of viral integration; (d) expanding the B cells; differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein; and (e) administering the B cells into the subject in need. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the method further comprises purifying the plasma cells of step d), wherein the purifying is performed after the expanding step. In some alternatives, the purifying comprises positive selecting of cells against CD138. In some alternatives, the selecting the plasma cells is performed by an anti-CD138 antibody for targeted removal of the plasma cells. In some alternatives, the purifying comprises using anti-CD138 beads for plasma cell selection. In some alternatives, the molecule is a macromolecule, such as a protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein is an enzyme. In some alternatives, the enzyme comprises a cofactor for enhanced enzymatic activity. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear

cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMG, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing the proportion of gene edited B cells comprises the steps of : (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth

enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, wherein step (a) performing a second round of genome editing on the B cells to excise a region, further comprises removing IgM positive cells. In some alternatives, the method further comprises, activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation

step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L, 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/auto-inflammatory syndromes or complement inhibitory proteins. In some alternatives, the compliment inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is

specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the administering is performed by adoptive transfer. In some alternatives, the subject has received a stem cell by administration or solid organ transplantation. In some alternatives, the subject suffers from influenza, parainfluenza, rhinovirus, respiratory syncytial virus, HIV, or other pathogenic bacteria, virus, fungus or parasite infection. In some alternatives, the subject suffers from an enzyme deficiency. In some alternatives, the subject suffers from pulmonary fibrosis. In some alternatives, the subject suffers from an autoimmune disorder, immune dysregulation and/or cancer. In some alternatives, the subject suffers from diabetes. In some alternatives, the subject suffers from hypercholesterolemia. In some alternatives, the subject is receiving a conventional therapy for the disease, malady or condition. In some alternatives, the subject is selected to receive cancer therapy, diabetes therapy, HIV treatment, or viral therapy. In some alternatives, the subject is also receiving CAR T-cell therapy. In some alternatives, the method comprises monitoring of subject following plasma cell transplantation. In some alternatives, the subject is determined to no longer need treatment or therapy and the method further comprises administering rituxan or an anti-CD20 antibody or binding portion thereof for targeted removal of the plasma cells.

[0025] In a ninth aspect, a method of ameliorating a disease in a subject in need is provided, the method comprising: administering a plasma cell manufactured by any one of the alternatives herein or the plasma cell that expresses a molecule, such as a macromolecule manufactured by any one of the alternatives herein, or the composition of the alternatives herein into the subject in need. In some alternatives, the administering is performed by adoptive transfer. In some alternatives, the subject has received a stem cell administration or solid organ transplantation. In some alternatives, the subject suffers from influenza, parainfluenza, rhinovirus, respiratory syncytial virus, HIV, or other pathogenic bacteria, virus, fungus or parasite infection. In some alternatives, the composition comprises the plasma cell manufactured by any one of the alternatives provided herein or comprises the cell of any one of the alternatives provided herein. In some alternatives, the plasma cell is derived from a B cell. In some alternatives, the plasma cell expresses a molecule, such as a

heterologous protein, protein mimetic or a peptide. In some alternatives, the cell is a B cell precursor. In some alternatives, the B cell precursor comprises hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the protein comprises an enzyme, neutralizing antibody, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody, anti-thrombotic molecule, glucose response element or a monoclonal antibody. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the plasma cell is a long lived plasma cell. In some alternatives, the plasma cell is manufactured by any one of the alternative methods provided herein. In some alternatives, the plasma cell is manufactured by a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In

some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMK, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous

recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing the proportion of gene edited B cells comprises: (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfecting into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one

cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever or autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders,

autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the plasma cell is manufactured by a method of making a long lived plasma cell, wherein the method comprises: isolating B cells; activating the B cells; a first round of genome editing of the B cells for protein expression in the absence of viral integration; expanding the B cells; and differentiating the B cells. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cell. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector for homologous recombination into a

candidate genetic loci. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cells or disruption of candidate loci within the B cells to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some

alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cells, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the molecule is selected from macromolecules, proteins, protein mimetics and/or peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the method comprises monitoring of subject following plasma cell

transplantation. In some alternatives, the subject is determined to no longer need treatment or therapy and the method further comprises administering rituxan or an anti-CD20 antibody or binding portion thereof for targeted removal of the plasma cells.

[0026] In a tenth aspect method of enhancing immune tolerance to protein therapeutics in a subject in need is provided, the method comprising: administering a plasma cell manufactured by any one of the alternatives herein or the plasma cell that a macromolecule manufactured by any one of the alternatives herein, or the composition of the alternatives herein into the subject in need. In some alternatives, the macromolecule is a protein for inducing tolerance of a protein or peptide for therapy or induces or promotes engraftment and/or survival. In some alternatives, the macromolecule is a protein. The protein may be BAFF, APRIL, IFN-alpha, IL-10 and/or IL-6. In some alternatives the protein is a viral vector capsid protein. In some alternatives, the method further comprises purifying the plasma cells of step D), wherein the purifying is performed after the differentiating step. In some alternatives, the purifying comprises positive selecting of cells against CD138. In some alternatives, the selecting the plasma cells is performed by an anti-CD138 antibody for targeted removal of the plasma cells. In some alternatives, the purifying comprises using anti-CD138 beads for plasma cell selection. In some alternatives, the viral vector capsid protein comprises one or more of the following serotypes: AAV1 (VP1, VP2 and VP3), AAV2 (VP1, VP2 and VP3), AAV4 (VP1, VP2 and VP3), AAV5 (VP1, VP2 and VP3), AAV6 (VP1, VP2 and VP3), AAV1 (VP1, VP2 and VP3), AAV8 (VP1, VP2 and VP3), AAV1 (VP1, VP2 and VP3) or AAV9 (VP1, VP2 and VP3). In some alternatives, the composition comprises the plasma cell manufactured by any one of the alternatives provided herein or comprises the cell of any one of the alternatives provided herein. In some alternatives, the plasma cell is derived from a B cell. In some alternatives, the plasma cell expresses a molecule, such as a heterologous protein, protein mimetic or a peptide. In some alternatives, the cell is a B cell precursor. In some alternatives, the B cell precursor comprises hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the protein comprises an enzyme, neutralizing antibody, cytokine, cytokine receptor, complement protein, inhibitory protein,

anti-fibrotic molecule, therapeutic antibody, anti-thrombotic molecule, glucose response element or a monoclonal antibody. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the plasma cell is a long lived plasma cell. In some alternatives, the plasma cell is manufactured by any one of the alternative methods provided herein. In some alternatives, the plasma cell is manufactured by a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing

the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cells. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMG, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing

the proportion of gene edited B cells comprises: (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the

isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion

thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the plasma cell is manufactured by a method of making a long lived plasma cell, wherein the method comprises: isolating B cells; activating the B cells; a first round of genome editing of the B cells for protein expression in the absence of viral integration; expanding the B cells; and differentiating the B cells. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cells. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector for homologous recombination into a candidate genetic loci. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cells or disruption of candidate loci within the B cells to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing a

nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement

inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cells, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the molecule is selected from macromolecules, proteins, protein mimetics and/or peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the method comprises monitoring of subject following plasma cell transplantation. In some alternatives, the subject is determined to no longer need treatment or therapy and the method further comprises administering rituxan or an anti-CD20 antibody or binding portion thereof for targeted removal of the plasma cells.

[0027] In an eleventh aspect, a method of enhancing immune tolerance to a protein therapeutic in a subject in need is provided. The method comprises administering a B cell manufactured by any one of the alternatives provided herein or the B cell that expresses a molecule, such as a macromolecule manufactured by any one of the alternatives provided

herein, or the composition of any one of the alternatives provided herein into the subject in need. In some alternatives, the subject suffers from Hemophilia A, Hemophilia B, Hereditary TPP, lysosomal acid deficiency, hereditary angioedema, alpha1 anti-trypsin deficiency, Fabry disease or hypophosphatasia. B cells can include B cell precursors, stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, activated B cells derived from any starting B cell population, plasmablasts (short-lived) cells, GC B cells, memory B cells, and/or long- or short-lived plasma cells and/or any mixture or combination thereof. In some alternatives, the composition comprises the plasma cell manufactured by any one of the alternatives provided herein or comprises the cell of any one of the alternatives provided herein. In some alternatives, the plasma cell is derived from a B cell. In some alternatives, the plasma cell expresses a molecule, such as a heterologous protein, protein mimetic or a peptide. In some alternatives, the cell is a B cell precursor. In some alternatives, the B cell precursor comprises hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the protein comprises an enzyme, neutralizing antibody, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody, anti-thrombotic molecule, glucose response element or a monoclonal antibody. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever or autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-

C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the plasma cell is a long lived plasma cell. In some alternatives, the plasma cell is manufactured by any one of the alternative methods provided herein. In some alternatives, the plasma cell is manufactured by a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMK, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein

(megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing the proportion of gene edited B cells comprises: (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein

the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides

or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the plasma cell is manufactured by a method of making a long lived plasma cell, wherein the method comprises: isolating B cells; activating the B cells; a first round of genome editing of the B cells for protein expression in the absence of viral integration; expanding the B cells; and differentiating the B cells. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear

cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cell. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector for homologous recombination into a candidate genetic loci. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cells or disruption of candidate loci within the B cells to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or

memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the

B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cells, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the molecule is selected from macromolecules, proteins, protein mimetics and/or peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the method comprises monitoring of subject following plasma cell transplantation. In some alternatives, the subject is determined to no longer need treatment or therapy and the method further comprises administering rituxan or an anti-CD20 antibody or binding portion thereof for targeted removal of the plasma cells.

[0028] In a twelfth aspect, a method of inducing tolerance of a macromolecule secreted by a B cell in a subject in need *in vivo* is provided, the method comprising: A) isolating B cells; B) performing a first round of genome editing of the B cells for protein expression in absence of viral integration, wherein the performing is under conditions of rapid B cell expansion; C) expanding the B cells; D) differentiating the B cells, optionally, after step (c) or (d), thereby producing activated B cells or naïve activated B cells that express the molecule; and E) providing or administering the B cells that expresses the macromolecule into a subject in need. In some alternatives, the macromolecule, is a protein, protein mimetic or peptide. In some alternatives, the macromolecule comprises natural amino acids and/or unnatural amino acids. In some alternatives, the protein is an enzyme. In some alternatives, the method further comprises activating the B cells prior to gene editing, where inactivating is performed using at least one stimulant. In some alternatives, the at least one stimulant is a CD40 ligand, CpG, IL2, IL10 and/or IL15. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 µg/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days. In some alternatives, the CD40 ligand is artificially oligermized. In some alternatives,

the CD40 ligand is oligomerized with two linked CD40L trimers. In some alternatives, the cells are cultured for at least 13 days with the at least one stimulant. In some alternatives, the cells are a naïve B cell phenotype, activated B cell or activated edited B cell after differentiation. In some alternatives, the cells are CD27-CD138-CD38^{low}/⁻CD19^{high}IgM⁺IgD⁺ after differentiation. In some alternatives, the providing or administering is performed by adoptive transfer. In some alternatives, the subject has received a stem cell administration or solid organ transplantation. In some alternatives, the subject suffers from influenza, parainfluenza, rhinovirus, respiratory syncytial virus, HIV, or other pathogenic bacteria, virus, fungus or a parasite infection. In some alternatives, the subject suffers from an enzyme deficiency. In some alternatives, the subject suffers from pulmonary fibrosis. In some alternatives, the subject suffers from an autoimmune disorder, immune dysregulation or cancer. In some alternatives, the subject suffers from diabetes. In some alternatives, the subject suffers from hypercholesterolemia. In some alternatives, the subject is also receiving CAR T-cell therapy. In some alternatives, the subject suffers from HIV. In some alternatives, the subject suffers from Hemophilia A, Hemophilia B, Hereditary TPP, lysosomal acid deficiency, hereditary angioedema, alpha1 anti-trypsin deficiency, Fabry disease or hypophosphatasia. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cells and co-delivering the nuclease with a single stranded nucleic acid donor template. In some alternatives, the nuclease is a synthetic guide RNA targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. B cells can include B cell precursors, stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, activated B cells derived from any starting B cell population, plasmablasts (short-lived) cells, GC B cells,

memory B cells, and/or long- or short-lived plasma cells and/or any mixtures or combinations thereof. In some alternatives, the method comprises monitoring of subject following plasma cell transplantation. In some alternatives, the subject is determined to no longer need treatment or therapy and the method further comprises administering rituxan or an anti-CD20 antibody or binding portion thereof for targeted removal of the plasma cells.

[0029] In a thirteenth aspect, a method of inducing tolerance of a peptide useful for immunotherapeutic application, secreted by a B cell in a subject in need *in vivo* and/or increasing engraftment of the B cell in a subject in need, the method comprising: A) isolating a first population of B cells; B) performing a first round of genome editing of the first population of B cells for protein or peptide expression in absence of viral integration, wherein the performing is under conditions of rapid B cell expansion; C) expanding the first population of B cells; D) differentiating the first population of B cells, optionally, after step (c) or (d), thereby producing an activated first population of B cells or a naïve first population of activated B cells that express the molecule; E) providing or administering the first population of B cells that expresses the peptide into a subject in need; and F) administering a second population of B cells that expresses a macromolecule, such as BAFF, APRIL, IFN-alpha, IL-10 or IL-6. In some alternatives, the immunotherapeutic application is treatment or amelioration of cancer. In some alternatives, the cancer comprises a solid tumor. In some alternatives, the peptide is an anti-PD1 antibody or a PDL-1 ligand. In some alternatives, the second population of B cells express BAFF, APRIL, IFN-alpha, IL-10 or IL-6. In some alternatives, the second population of B cells express a second peptide that is expressed and presented as a MHC class II molecule on a surface of the B cells. B cells can include B cell precursors, stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, activated B cells derived from any starting B cell population, plasmablasts (short-lived) cells, GC B cells, memory B cells, and/or long- or short-lived plasma cells and/or any mixtures or combinations thereof. In some alternatives, the method comprises monitoring of subject following plasma cell transplantation. In some alternatives, the subject is determined to no longer need treatment or therapy and the method further comprises administering rituxan or an anti-CD20 antibody or binding portion thereof for targeted removal of the plasma cells.

[0030] In a fourteenth aspect, a method of inducing tolerance of a peptide for immunotherapeutic application, secreted by a B cell in a subject in need *in vivo* and/or increasing engraftment or survival of the B cells in a subject in need, the method comprising: A) isolating a first population of B cells; B) performing a first round of genome editing of the first population of B cells for protein or peptide expression in absence of viral integration, wherein the performing is under conditions of rapid B cell expansion; C) expanding the first population of B cells; D) differentiating the first population of B cells, optionally, after step (c) or (d), thereby producing activated B cells or naïve activated B cells that express the protein or peptide; E) providing or administering the first population of B cells that express the protein or peptide into a subject in need F) providing or administering a second population of B cells that express a macromolecule, wherein the second population of B cells allows tolerance of the protein or peptide and/or promotes engraftment. In some alternatives, the first population of B cells and the second population of B cells are co-administered. In some alternatives, the macromolecule comprises BAFF, APRIL, IL-10, IFN-alpha or IL-6. In some alternatives, the protein comprises an enzyme, neutralizing antibody or a binding portion thereof, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody or a binding portion thereof, anti-thrombotic molecule, glucose response element or monoclonal antibody or a binding portion thereof. In some alternatives the protein is a viral vector capsid protein. In some alternatives, the viral vector capsid protein comprises one or more of one of the following serotypes: AAV1 (VP1, VP2 and VP3), AAV2 (VP1, VP2 and VP3), AAV4 (VP1, VP2 and VP3), AAV5 (VP1, VP2 and VP3), AAV6 (VP1, VP2 and VP3), AAV1 (VP1, VP2 and VP3), AAV8 (VP1, VP2 and VP3), AAV1 (VP1, VP2 and VP3) or AAV9 (VP1, VP2 and VP3). In some alternatives, the protein is a receptor antagonist for the treatment or inhibition of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for the treatment or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angioedema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the protein comprises therapeutic antibodies or a binding portion thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some

alternatives, the antibody or a binding portion thereof is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. B cells can include B cell precursors, stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, activated B cells derived from any starting B cell population, plasmablasts (short-lived) cells, GC B cells, memory B cells, and/or long- or short-lived plasma cells and/or any mixtures or combinations thereof. In some alternatives, the method comprises monitoring of subject following plasma cell transplantation. In some alternatives, the subject is determined to no longer need treatment or therapy and the method further comprises administering rituxan or an anti-CD20 antibody or binding portion thereof for targeted removal of the plasma cells.

[0031] In a fifteenth aspect a composition is provided, wherein the composition comprises a first population of B cells manufactured by any one of the alternatives herein and a second population of B cells, wherein the second population of B cells secretes a macromolecule for inducing tolerance of a peptide for immunotherapeutic application or for inducing engraftment of the plasma cell manufactured by any one of the alternatives herein. In some alternatives, the macromolecule expressed by the second population of B cells comprises BAFF, IFN-alpha, APRIL, IL-10 or IL-6. In some alternatives the macromolecule is a viral vector capsid protein. In some alternatives, the viral vector capsid protein comprises one or more of one of the following serotypes: AAV1 (VP1, VP2 and VP3), AAV2 (VP1, VP2 and VP3), AAV4 (VP1, VP2 and VP3), AAV5 (VP1, VP2 and VP3), AAV6 (VP1, VP2 and VP3), AAV1 (VP1, VP2 and VP3), AAV8 (VP1, VP2 and VP3), AAV1 (VP1, VP2 and VP3) or AAV9 (VP1, VP2 and VP3). B cells can include B cell precursors, stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, activated B cells derived from any starting B cell population, plasmablasts (short-lived) cells, GC B cells, memory B cells, and/or long- or short-lived plasma cells and/or any mixtures or

combinations thereof. In some alternatives, the method of making plasma cells or plasma cell precursors that expresses a molecule, such as a macromolecule, protein, or peptide comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells or plasma cell precursors that express the molecule. In some alternatives, the B cells in step (a) comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the molecule is a macromolecule, such as a protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein is an enzyme. In some alternatives, the enzyme comprises a cofactor for enhanced enzymatic activity. In some alternatives, the B cells in step (a) comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some

alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cells. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMG, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a CAS nuclease. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a CAS nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the increasing the proportion of gene edited B cells comprises the steps of : (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth

enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding of the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or any other length defined by a range that is set forth by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells,

memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element and/or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, IL-6, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist that is used for the treatment or amelioration of periodic fever/autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, a therapeutic antibody or binding portion thereof is used e.g., an antibody or binding portion thereof that is specific for a protein or other molecule expressed in an autoimmune disorder, autoinflammatory disorder, immune dysregulation and/or cancer. In some alternatives, the antibody or binding portion thereof is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion thereof. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies. In some alternatives, the protein is an engineered protein, wherein the engineered

protein is protective in viral, fungal, parasitic and/or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cell, plasmablast, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and memory B cells. In some alternatives, the molecule is a macromolecule, protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the method of making a long lived plasma cell comprises isolating B cells; activating the B cells; a first round of genome editing of the B cells for molecule expression, such as a protein, protein mimetic or a peptide in the absence of viral integration; expanding the B cells; and differentiating the B cells. In some alternatives, the molecule is a macromolecule, such as a protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein is an enzyme. In some alternatives, the enzyme comprises a cofactor for enhanced enzymatic activity. In some alternatives, the B cells in step (a) comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B

cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the B cells from the isolating step comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cells. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector for homologous recombination into a candidate genetic loci. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cells. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cells or disruption of candidate loci within the B cells to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing a nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or

recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, the plasmablast differentiation step is performed in the presence of any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence of any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment or amelioration of periodic fever or autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody or binding portion thereof is an anti-IL1 monoclonal antibody, anti-TNF antibody,

anti-IL33 antibody or anti-C5 antibody or a binding portion of any of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNABs). In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cell, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the molecule is selected from macromolecules, proteins, protein mimetics and/or peptides. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acetylated. In some alternatives, the protein comprises a cofactor for enhanced enzymatic activity. In some alternatives, the isolating is performed by negative selection isolation of hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] **Figure 1** shows a schematic of the potential clinical application of plasma cell therapy.

[0033] **Figure 2** highlights the advantages of using engineered plasma cells for a protein delivery platform, such as longevity, protein production and its potential for *ex vivo* manipulation and generation.

[0034] **Figure 3** shows candidate nuclease platforms that can be used in the alternatives described herein, such as, for example, zinc finger nucleases, CRISPR/Cas Nuclease systems, TAL effector nucleases and homing endonucleases.

[0035] **Figure 4** provides a table that shows different methods of CRISPR editing of the primary B cells and the benefits of the methods.

[0036] **Figure 5** shows the ribonucleoprotein strategy for the editing of B cells.

[0037] **Figure 6** shows the indel in a Sanger sequencing gel. As shown, increasing the concentration of ribonuclease increased the T7 cleavage during a 5 day DNA cleavage protocol. For the experiment, B cells were isolated from peripheral blood mononuclear cells at day zero and transfected with ribonucleoproteins and a PR domain zinc finger protein at day 1. The cells were then activated with mCD40L, CpG and IL2, IL10, and IL15. At day 5, the DNA was extracted from the cell to test for DNA cleavage.

[0038] **Figure 7** shows a Western blot, which illustrates results from a T7 cleavage. The antibodies were directed to PRDM1 and TUBB1.

[0039] **Figure 8** shows that PRDM1 disruption limits plasmablast generation.

[0040] **Figure 9** shows homologous-directed repair (HDR) using single-stranded DNA templates.

[0041] **Figure 10** shows homologous-directed repair (HDR) using single-stranded DNA templates.

[0042] **Figure 11** shows dose response of short oligonucleotide HDR.

[0043] **Figure 12** shows low toxicity in setting of high-efficiency HDR.

[0044] **Figure 13** shows long oligonucleotide templates for HDR.

[0045] **Figure 14** shows that long template HDR is effective.

[0046] **Figure 15** shows results from the testing of primary B cell transduction with AAV vectors. Provided in the right panel is the percent GFP expression with different AAV serotypes. From left to right are AAV serotypes by culture volume from 2%, 5% and 10%. The bottom panel shows the percent viability of the cells after 48 hours post transduction. From left to right consecutively in the graph are the AAV amount by culture volume of 2%, 5% and 10%.

[0047] **Figure 16** shows results from the testing of primary B cell transduction with AAV vectors.

[0048] **Figure 17** shows results from transduction of B cells using AAV6.

[0049] **Figure 18** shows the strategy of homologous-directed repair (HDR) using AAV repair templates.

[0050] **Figure 19** shows that CRISPR and AAV repair template co-delivery mediates HDR in primary B cells.

[0051] **Figure 20** shows the strategy of Homologous-directed repair (HDR) using AAV repair templates.

[0052] **Figure 21** shows CRISPR and AAV repair template co-delivery mediates HDR in primary B cells.

[0053] **Figure 22** shows the culturing and genome editing strategy using negative selection isolation of B cells from healthy donor PBMCs.

[0054] **Figure 23** shows B cell phenotype at end of expansion phase.

[0055] **Figure 24** shows B cell expansion results. The left panel shows the cell expansion curve and the right panel shows the viability by DAPI staining.

[0056] **Figure 25** shows Plasma cell differentiation using the 3-step culture system.

[0057] **Figure 26** shows results of plasma cell differentiation at pre-differentiation and at post-differentiation (day 12).

[0058] **Figure 27** shows the demonstration of quantifiable secretion of an exogenous protein in an animal model.

[0059] **Figure 28** shows the demonstration of the ability of B cells to produce functionally active Factor IX.

[0060] **Figure 29A and Figure 29B** shows sustained expression of human proteins in murine serum between 10 and 21 days.

[0061] **Figure 30** shows the experimental design for gene disruption and protein expression.

[0062] **Figure 31** shows the long lived human antibody production in mice.

[0063] **Figure 32** shows expression of human IL6 in mice confers a growth advantage for transplanted *ex vivo* differentiated human plasma cells.

[0064] **Figure 33** shows high IL6 expression correlates with high plasma cell secretion.

[0065] **Figure 34** shows *in vitro* human APRIL treatment correlates with increased plasma cell antibody secretion *in vivo*.

[0066] **Figure 35** shows the results of the gene disruption and protein expression.

[0067] **Figure 36** shows targeting of abundant plasma cell genes using CRISPR.

[0068] **Figure 37** shows multiplexed genome editing to introduce FIX and increase protein secretion in human plasma cells.

[0069] **Figure 38** shows knock-out of BANK1 promotes plasma cell differentiation and antibody production.

[0070] **Figure 39** shows long-lived plasma cells secrete more immunoglobulin on a per cell basis.

[0071] **Figure 40A** shows B cell activation cocktail leads to expansion of naïve B cells *in vitro*. (40A-40C) Primary human CD19⁺ B cells were isolated from PBMCs and cultured with MEGACD40L, CpG, IL2, IL10 and IL15 for 13 days. Fresh medium with the soluble factors was replenished every one or two days to maintain a cell density of $\sim 1 \times 10^6$ cells/ml. Cells were counted every day (a) and viabilities were measured using 4',6-diamidino-2-phenylindole (DAPI) staining (b) (n=2). Line graphs show means $\pm$ SEM. (c) Cell samples were stained using anti-CD27, CD138, CD19, CD38, IgD and IgM antibodies after 0, 2, 8, and 13 days of culture. Immunophenotypes were analyzed by flow cytometry.

[0072] **Figure 41** shows primary B cells are effectively electroporated with mRNA encoding blue fluorescent protein (BFP). B cells were activated for two days in culture and transfected with BFP mRNA using the optimal electroporation settings. Percentages of BFP⁺ cells were measured 24 hours after transfection by flow cytometry. Left: representative flow plots showing BFP expression of mock and BFP mRNA electroporated B cells. Right: bar graph showing percentages of BFP⁺ cells (n=5). All values are means $\pm$ SEM.

[0073] **Figure 42** shows Cas9 RNP induces site-specific indels or a precise single-nucleotide change in the presence of an ssODN in primary human B cells. (42A) CD19⁺ B cells were isolated and activated *in vitro* for two days, mock treated or transfected with Cas9 RNPs targeting CCR5 or PRDM1 (CCR5g, PRDM1g-1 or PRDM1g-2) and cultured for five additional days. Total genomic DNA was isolated on day 5, target regions were PCR amplified and analyzed using the T7 endonuclease 1 (T7E1) assay (one representative experiment shown) or sequencing for percentages of on-target indels (112,000 reads per experimental condition). (42B) Diagram of wild type PRDM1 locus, PRDM1g-2 target location and the ssODN donor template containing a single-nucleotide change. (42C,

D) B cells were activated for two days and mock treated, electroporated with Cas9 RNP-PRDM1g-2 alone, or with the ssODN donor template at serially increasing doses from 7.5 pmol to 120 pmol. **(42C)** Viabilities of B cells 2 days and 5 days after genome editing (n=3, three donors). No significant difference in viability was observed between mock and up to 30 pmol ssODN plus RNP either on day 2 or day 5. The bar graph shows means $\pm$ SEM. **(42D)** Percentage of HDR, indel and wildtype (WT) alleles in total genomic DNA extracted 5 days after genome editing as assessed by sequencing (greater than 1 million reads per experimental condition). N represents the number of independent experiments. One-way ANOVA with the Sidak correction for multiple comparisons is used; ***p < 0.001; ns, not significant. WT, wildtype. As shown in **42D** of the bar graphs, the top black portions of the bar are wild type, light grey is HDR and the bottom grey is indel.

[0074] **Figure 43A and 43B** shows Cas9-mediated gene disruption is associated with protein reduction and high cell viabilities. **(43A)** TMD8 cells were mock treated or transfected with either of the two PRDM1-targeting RNPs and protein levels were analyzed five days later by western blot. **(43B)** Viabilities of primary human B cells two days or five days after genome editing.

[0075] **Figure 44A-44E** shows Cas9-mediated disruption of PRDM1, IRF4, PAX5, or BACH2 alters B cell phenotype. **(44A)** Experimental workflow of the plasma cell differentiation assay. This workflow includes a three-step differentiation culture that is divided by a B cell activation phase (phase 1), a plasmablast differentiation phase (phase 2) and a plasma cell differentiation phase (phase 3) using the different cocktails of soluble factors and cytokines specified. Cells were transfected with Cas9 RNP after two days of initial activation, indels were assessed in genomic DNA on day 5, and phenotypes and IgM/IgG secretion were quantified on day 11. **(44B)** Schematic diagram of the roles of transcription factors PRDM1, IRF4, PAX5, and BACH2 as established by murine studies. **(44C-44E)** Primary B cells were activated and either mock treated, or transfected with Cas9 RNP targeting CCR5, PRDM1, IRF4, PAX5, or BACH2 and were subsequently differentiated *in vitro*. **(44C)** Top: representative flow plots showing CD19 and CD38 expression in live singlets and bar graph showing mean percentages of CD19^{low}CD38^{high} plasmablasts 11 days post transfection. Bottom: representative flow plots showing CD27 and CD138 expression and bar graph showing percentage of CD27⁺CD138⁺ plasma cells 11

days post transfection (n=5, three donors). **(44D)** Genomic DNA was isolated five days after RNP transfection for allelic indel analysis by sequencing (n=5). **(44E)** Amounts of IgM (left) and IgG (right) in each culture as measured by ELISA (n=4, three donors). All bar graphs show means $\pm$ SEM. n represents the number of independent experiments. Dotted lines mark mock levels. One -way ANOVA with the Sidak correction for multiple comparisons is used; *p < 0.05; **p < 0.01; ***p < 0.001; ns, not significant. Statistical comparisons were made compared to mock. IFN- α , interferon α ; PB, plasmablast; PC, plasma cell.

[0076] **Figure 45A-45C** shows Cas9 RNPs targeting CCR5, PRDM1, IRF4, PAX5, and BACH2 lead to high levels of gene disruption in primary human B cells. **(Figure 45A-45C)** Primary B cells were activated for two days and either mock treated, or transfected with Cas9 RNP targeting CCR5, PRDM1, IRF4, PAX5, or BACH2 and were subsequently differentiated *in vitro*. **(45A)** IgG and IgM levels were quantified on day 11 by ELISA and normalized to cell numbers. IgG/IgM mass ratios were calculated and shown in bar graph (n=4). **(45B)** Cells were immunophenotyped on day 11 and gated on live, singlet, CD19^{low}CD38^{high} plasmablasts. Left: representative CD19 histograms. Right: mean fluorescence intensities of CD19 (n=5). One-way ANOVA with the Sidak correction for multiple comparisons; *p < 0.05; ***p < 0.001; ns, not significant. Statistical comparisons were made compared to mock. As shown in Figure 45 B from top.

[0077] **Figure 46A-46B** shows AAV-transduced B cells are marked by rapid recovery and comparable viabilities to mock at experimental endpoint. **(46A)** Primary B cells were activated for two days in culture and transduced with scAAV GFP of various serotypes at 25,000 MOI. Bar graph shows viabilities two days after transduction (n=3). **(46B)** Primary B cells were activated for two days and gene-edited using PRDM1-targeting RNP and AAV PRDM1-GFP at different MOIs. Bar graph shows cell viabilities on day 2 and 11 in mock, BFP-no- HA control or PRDM1-GFP edited B cell cultures (n=4). All values are means $\pm$ SEM. As shown in 46B in the bar graphs are Day 2 and Day 11 consecutively.

[0078] **Figure 47A-47D** shows HDR-mediated GFP addition at the PRDM1 locus using Cas9 RNP and an AAV6 donor template leads to progressively increased GFP expression in a dose-dependent manner. **(47A)** B cells were activated for two days and were either mock treated or transduced with the GFP-expressing scAAV packaged using a comprehensive panel of AAV serotypes (1, 2, 2.5, 5, 6, 8, 9 or D-J) at a multiplicity of

infection (MOI) of 25,000. Shown are the percentages of GFP⁺ cells (left) and mean fluorescence intensities (right) (n=3, three donors). **(47B)** Schematic of wild type PRDM1 locus, PRDM1g-2 target location and an AAV GFP expression cassette with 400 bp flanking PRDM1 homology arms (AAV PRDM1-GFP). The 3-base PAM sequence is deleted from the AAV template homology sequence. **(47C, 47D)** B cells were activated for two days and were either mock treated or transfected with Cas9 RNP-PRDM1g-2 with or without AAV transduction. Cells were subsequently cultured under the same activating condition for 11 days. **(47C)** Top: representative flow plots showing BFP and GFP expressions on day 2 and day 11 after genome editing. The boxed area of the bottom row second panel highlights the GFP⁺ population in the gene-edited B cells. Bottom: bar graph showing percentages of GFP⁺ cells on day 2 and day 11 after gene-editing (n=4, four donors). All bar graphs show means $\pm$ SEM. n represents the number of independent experiments. One-way ANOVA with the Sidak correction for multiple comparisons is used; *p < 0.05; ***p < 0.001; ns, not significant. MFI, mean fluorescence intensity. PAM, protospacer adjacent motif; WT, wildtype; HA, homology arm; pA, SV40 poly-adenylation signal.

[0079] **Figure 48A-48B** shows targeted addition of GFP at the PRDM1 locus using RNP and AAV donor templates containing either 400 bp or 1.0 kb homology arms leads to stable GFP expression. **(48A)** Schematic of AAV PRDM1-GFP cassettes flanked by either 400 bp (top) or 1.0 kb (bottom) homology arms. The template on the top is identical to the one shown in Figure 3. Both templates leave out the 3 bp PAM sequence. **(48B)** Primary B cells were activated for two days and gene-edited. Bar graph shows percentages of GFP⁺ cells on day 2 and day 11 after genome editing (n=3). All values are means $\pm$ SEM. Unpaired two-tailed t-test; ns, not significant. HA, homology arm; ns, not significant.

[0080] **Figure 49A to 49E** shows HDR-mediated integration of BAFF coding sequence at the CCR5 locus results in persistent BAFF secretion by gene-edited plasma cells and increases plasma cell differentiation and viability. **(49A)** Schematic of wild type CCR5 locus, CCR5g target location, an AAV construct that co-expresses GFP and BAFF via T2A linkage (AAV CCR5-GFP-BAFF) and a BAFF-expressing AAV construct (AAV CCR5-BAFF) with identical 800 bp flanking CCR5 homology arms. **(49B)** B cells were gene-edited after two days of *in vitro* activation, and were subsequently differentiated into plasma cells using the three-step culture system. Left: representative flow plots showing GFP expression

on day 2 and day 11 post gene-editing in mock, CCR5-GFP-BAFF transduced cells with or without Cas9 RNP. Right: bar graph summarizing percentages of GFP⁺ cells on day 2 and day 11 post gene-editing (n=4, three donors). **(49C)** B cells were mock electroporated or transduced with AAV CCR5-GFP-BAFF or AAV CCR5-BAFF, with or without Cas9 RNP. Bar graph shows BAFF production as measured by ELISA at day 11 (n=4, three donors). **(49D)** Left: cells were counted at day 5 and day 11 post gene-editing. Bar graph shows fold changes in cell numbers. Right: viabilities at day 11 by flow cytometry. (n=5, four donors) **(49E)** Left: representative flow plots showing CD19 and CD38 expression at day 11 after genome editing using the AAV CCR5-BAFF vector. Right: bar graph summarizing percentages of CD19^{low}CD38^{high} plasmablasts/plasma cells at day 11 (n=5, four donors). Both AAV CCR5-GFP-BAFF and CCR5-BAFF were added at 20,000 MOI. All bar graphs show means $\pm$ SEM. N represents the number of independent experiments. One-way ANOVA was used with the Sidak correction for multiple comparisons; **p < 0.01; ***p < 0.001. PAM, protospacer adjacent motif; IL2ss, IL2 signal sequence; WT, wildtype; HA, homology arm; pA, SV40 poly-adenylation signal.

[0081] **Figure 50** shows that using AAV without CCR5 homology leads to minimal fluorochrome expression. B cells were either mock electroporated or co-treated with CCR5-targeting RNP and an AAV BFP-noHA control after two days of *in vitro* activation, and were subsequently differentiated into plasma cells using the three-step culture system. Representative flow plots on day 2 and day 11 (left) and bar graph showing mean percentages of BFP⁺ cells $\pm$ SEM (n=4).

[0082] **Figure 51A-51D** shows HDR-mediated integration of FIX coding sequence at the CCR5 locus leads to high levels of FIX secretion by gene-edited plasma cells *ex vivo*. **(51A)** Schematic of wild type CCR5 locus, CCR5g target location, and a FIX-expressing AAV construct with 800 bp flanking CCR5 homology arms (AAV CCR5-FIX). **(51B-51D)** Primary B cells were gene-edited using the AAV CCR5-FIX donor template and CCR5-targeting RNP (CCR5g RNP) with or without PAX5-targeting RNP (PAX5g RNP). B cells were subsequently differentiated *in vitro*. **(51B)** Frequency of on-target FIX integration in total alleles on day 11 after genome editing as assessed by digital droplet PCR. **(51C)** CD19 and CD38 expression on day 11. Left: representative flow plots; right: mean percentages of CD19^{low}CD38^{high} plasmablasts (n=2). **(51D)** FIX production at day 11 after

genome editing as measured by ELISA (n=3, two donors). AAV was added at 20,000 MOI. Bar graphs show means $\pm$ SEM. n represents the number of independent experiments. One-way ANOVA with the Sidak correction for multiple comparisons were used; **p < 0.01; ***p < 0.001. PAM, protospacer adjacent motif; WPRE3, a shortened woodchuck hepatitis virus posttranscriptional regulatory element 55; WT, wildtype; HA, homology arm; pA, SV40 poly-adenylation signal.

[0083] **Figure 52A to 52C** shows Outline of flow cytometry gating strategy. **(52A-52B)** Activated B cells were analyzed by flow cytometry. **(52A)** Preliminary gates for live cells (left) and singlets (middle) were used to define a viable cell population in unstained B cells before measuring fluorescence (GFP shown on right). **(52B)** Cells were stained with DAPI to mark dead cells. Viability levels were confirmed by quantifying percentages of DAPI- cells. **(52C)** Demonstration of general flow cytometry gating strategy for analyzing B cell immunophenotypes. B cells were stained with fluorochrome-conjugated anti-CD27, CD138, CD19, CD38 and live/dead stain-Alexa Fluor 350. Live singlets were defined using FSC/SSC, FSC-W/FSC-H and AF350- gates, and the resulting cell population was analyzed for expression of CD markers. Positive and negative populations were separated based on unstained controls. DAPI, 4,6-diamidino-2-phenylindole.

[0084] **Figure 53A-53D** shows a description of library preparation and molecular analysis of gene disruption and ssODN editing studies. **(53A)** Genomic DNA flanking the cut site was initially amplified using sequence specific primers to introduce molecular adapters (orange bars). After bead-based purification to remove primers and enzyme, these sequences were amplified with primers containing molecular indices unique to each experiment (red bar) and cluster-generating sequences (purple bars). The samples were purified, pooled and analyzed using a NextSeq 500 Mid-kit. Finally, the data were de-multiplexed, indel and HDR frequencies were quantified using the CRISPResso software package. **(53B)** An example from one replicate showing indel frequency and read-depth for the IRF4 guide. **(53C)** A histogram showing the percent insertion, deletion and substitutions plotted relative to the guide cut site (dotted line) in the 150 bp read sequence. **(53D)** The number of sequences corresponding to each indel size quantified in this experiment (negative and positive numbers represent deletions and insertions, respectively).

[0085] **Figure 54** shows Gene-edited, BAFF-expressing plasma cells stably secrete BAFF and human immunoglobulins in NSG mice. **(54A)** Experimental layout of NSG mouse transplant. Gene-edited B cells were generated as before after two days of *in vitro* B cell activation and were subsequently differentiated into plasma cells using the three-step culture system. 11 days post genome editing, cells were delivered intravenously into NSG mice (conditioned with Busulfan a day before transplant) at 10 million cells/animal. Blood samples were collected at day 10 and, finally, at day 21 when mice were sacrificed. **(54B-54C)** Blood serum proteins were quantified by ELISA at day 10 and day 21 (n=3). Shown are serum BAFF levels **(54B)** and serum human IgM and IgG levels **(54C)**. All graphs represent means $\pm$ SEM. n represents the number of mice per group. One-way ANOVA with the Sidak correction for multiple comparisons between groups was used, and paired two-tailed t-test for comparisons between two time points (day 10 vs 21); *p<0.05; **p < 0.01; ***p < 0.001. NSG, NOD/SCID/gamma-c null; PCs, plasma cells; IV, intravenous.

[0086] **Figure 55** shows data that demonstrates that in vivo delivery of human APRIL or IL6 enables increased long-term secretion by edited long-lived plasma cells. Primary human B cells were expanded in culture and edited as described in the alternatives herein. Following differentiation into plasma cells, 10 million were injected into NSG mice. Human BAFF was quantified using ELISA. Each dot represents data from an individual recipient mouse at the indicated time point.

Definitions

[0087] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

[0088] As used herein, “a” or “an” may mean one or more than one.

[0089] “About” as used herein when referring to a measurable value is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value.

[0090] “Nucleic acid” or “nucleic acid molecule” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for

example, polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term “nucleic acid molecule” also includes so-called “peptide nucleic acids,” which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded. In some alternatives, a nucleic acid sequence encoding a protein is provided. In some alternatives, the nucleic acid is RNA or DNA.

[0091] “Macromolecule” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a large molecule, such as a protein. The most common macromolecules in biochemistry can include nucleic acids, proteins, peptides, carbohydrates and macrocycles, for example. In the alternatives herein, plasma cells or plasma cell precursors are provided, wherein the plasma cells or plasma cell precursors express a macromolecule, such as a protein, protein mimetic, or a peptide. In some alternatives, the macromolecule is a prodrug.

[0092] “Prodrug” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, an inactive molecule, or macromolecule that is metabolized into a pharmacologically active drug. After

administration, the prodrug is metabolized into a pharmacologically active drug. Prodrugs can be classified into two types, Type I prodrugs may be bioactivated inside the cells (intracellularly). Examples of these are anti-viral nucleoside analogs that must be phosphorylated and the lipid-lowering statins. Type II prodrugs are bioactivated outside cells (extracellularly), especially in digestive fluids or in the body's circulatory system, particularly in the blood. Examples of Type II prodrugs are salicin (described above) and certain antibody-, gene- or virus-directed enzyme prodrugs used in chemotherapy or immunotherapy.

[0093] “Proenzyme” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a precursor of an enzyme. In some alternatives, the macromolecule is a protein, such as a proenzyme. In some alternatives, the proenzyme is Factor XI. In some alternatives, the proenzyme is from the family of the coagulation system. In some alternatives the proenzyme comprises fibrinogen.

[0094] “Protein” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, large macromolecules that comprise long chains of amino acid residues. Protein, polypeptide, and peptide can be ambiguous terms and can overlap in meaning. The term “protein” can refer to the complete biological molecule in a stable conformation, whereas “peptide” can be reserved for short amino acid oligomers that can lack a stable three-dimensional structure. The boundary between the two terms is not well defined and the number of amino acids can be close to about 20–30 residues for a peptide. The term “polypeptide” refers to a single linear chain of amino acids, usually regardless of length. Without being limiting proteins can be a serum protein, glycoprotein, lipoprotein, enzyme, nucleoprotein, structural protein, antibody, or prodrug, for example.

[0095] “B-cell activating factor” (BAFF), can also be referred to as “tumor necrosis factor ligand superfamily member 13B.” BAFF has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a protein that is encoded by the *TNFSF 13B* gene. BAFF is a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. In some alternatives herein, a B cell or plasma cell is provided, wherein the B cell or plasma cell expresses BAFF. BAFF has been shown to interact with and activate noncanonical NF- κ B signaling pathways. This interaction triggers

signals essential for the formation and maintenance of B cell, thus it is important for a B-cell survival. In some alternatives, the cells are manufactured to secrete BAFF to improve or enhance B-cell survival.

[0096] MHC class II molecules, have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a class of major histocompatibility complex (MHC) molecules normally found only on antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells, and B cells. These cells are important in initiating immune responses.

[0097] A protein, polypeptide or peptide can also be found with a functional group or an additional moiety for functional purposes such as an enzyme co-factor, for example. Without being limiting a protein, polypeptide or peptide can comprise a hydrophobic group for membrane localization. Hydrophobic groups can be added by myristoylation, palmitoylation, isoprenylation, prenylation, farnesylation, geranylgeranylation or glypiation, for example.

[0098] Cofactors are another functional group, which are used for enhanced enzymatic activity. Without being limiting, cofactors can include lipoylation, Flavin moiety, heme C attachment, phosphopantetheinylation, retinylidene formations.

[0099] Proteins and peptides can also comprise modified amino acids or non-natural amino acids. In some alternatives, the macromolecule comprises a protein mimetic. In some alternatives, the macromolecule is a prodrug.

[0100] In some alternatives herein, a method for making plasma cells or plasma cell precursors that expresses a molecule, such as a protein, peptide or macromolecule is provided, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells or plasma cell precursors that express the molecule. In some alternatives, the molecule is a prophylactic or therapeutic protection from infection (viral, bacterial, or parasitic) following stem cell administration or solid-organ transplantation in pediatric and adult subjects including, but not limited to neutralizing antibodies that block influenza, parainfluenza, rhinovirus, Respiratory Syncytial Virus (RSV), HIV, pathogenic bacteria, and/or parasites. In

some alternatives, the molecule is a macromolecule, such as a protein or an enzyme. In some alternatives, the protein or enzyme is for protein replacement, enzyme replacement and rescue of enzyme or protein deficiencies. In some alternatives, the macromolecule comprises Factor VIII, Factor IX, ADAMTS13, LIPA, SERPING1, SERPINA1, GLA, and/or ALPL. In some alternatives, the macromolecule is a prodrug.

[0101] “Cytokines” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a group of proteins that function in cell signaling. For example cytokines can be involved in autocrine signaling, paracrine signaling and endocrine signaling as immunomodulating agents. Cytokines are important in health and disease, for example, playing a role in host responses to infection, immune responses, inflammation, trauma, sepsis, cancer, and reproduction. In some alternatives herein, a method for making plasma cells or plasma cell precursors that expresses a molecule, such as a protein, peptide or macromolecule is provided, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells or plasma cell precursors that express the molecule. In some alternatives, the molecule is a macromolecule, such as a cytokine, cytokine receptor, complement protein or other inhibitory protein including, but not limited to: IL1 receptor antagonist for treatment or amelioration of periodic fever/autoinflammatory syndromes; complement inhibitory proteins (including Factor H, Factor I) for treatment or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis; and/or C1 inhibitor for hereditary angioedema.

[0102] “Anti-fibrotic molecule” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a molecule that is used in the treatment of fibrosis, or excess fibrous connective tissue in an organ or tissue in a reparative or reactive process. Fibrosis can include pulmonary fibrosis, cystic fibrosis, Idiopathic pulmonary fibrosis, liver cirrhosis, atrial fibrosis, endomyocardial fibrosis, myocardial infarction, glial scars of the brain, arterial stiffness, Arthrofibrosis (knee, shoulder, other joints), Crohn's Disease (intestine), Dupuytren's contracture (hands, fingers), Keloid (skin), Mediastinal fibrosis (soft tissue of the mediastinum), Myelofibrosis (bone

marrow), Peyronie's disease, Nephrogenic systemic fibrosis (skin), Progressive massive fibrosis (lungs), Retroperitoneal fibrosis (soft tissue of the retroperitoneum), Scleroderma/systemic sclerosis (skin, lungs) and/or some forms of adhesive capsulitis (shoulder). Without being limiting, anti-fibrotic molecules can include but is not limited to SCGB1A1 for the treatment of pulmonary fibrosis.

[0103] “Antibodies” also known as “immunoglobulins” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, large Y-shaped proteins that are secreted by plasma cells to neutralize pathogens. In some alternatives herein, a method for of making plasma cells or plasma cell precursors that expresses a molecule, such as a protein, peptide or macromolecule is provided, wherein the method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells or plasma cell precursors that express the molecule. In some alternatives, the molecule is an antibody, or a portion thereof. In some alternatives herein, the plasma cell or plasma cell precursor expresses an antibody.

[0104] “Coding for” or “encoding” are used herein, has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, the property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other macromolecules such as a defined sequence of amino acids. Thus, a gene codes for a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system.

[0105] “Vector,” “Expression vector” or “construct” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a nucleic acid used to introduce heterologous nucleic acids into a cell that has regulatory elements to provide expression of the heterologous nucleic acids in the cell.

[0106] “B cells” or “B lymphocytes” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a white blood cell type of the lymphocyte subtype. B cells are unlike lymphocytes such as T cells and natural killer cells, as B cells express B cell receptors on their cell membrane. The

B cell receptors allow the B cell to bind a specific antigen, which will initiate an antibody response. B cells develop from hematopoietic stem cells. As described herein, B cells can include B cell precursors, stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, activated B cells derived from any starting B cell population, plasmablasts (short-lived) cells, GC B cells, memory B cells, and/or long- or short-lived plasma cells and/or any mixtures or combinations thereof depending on the context.

[0107] B cell precursors include the cells from which the B cells are derived. Like T cells, B cells are lymphatic cells that are originated from the bone marrow, where they can reside until they are mature. The B cells, as described in the alternatives herein, include stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablasts (short-lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives of the plasma cell for expressing a molecule such as a macromolecule, protein or a peptide, the plasma cell is derived from a B cell. In some alternatives, the B cell is a memory B cell. In some alternatives, the B cell is a stem cell, early pro-B cell, late pro-B cell, large pre-B cell, small pre-B cell, immature B cell, T1 B cell, T2 B cell, marginal zone B cell, mature B cell, naïve B cell, plasmablasts (short-lived) cell, GC B cell, memory B cell, plasmablast cell or a long lived plasma cell. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells (HSCs), multipotent progenitor (MPP) cells, lymphoid progenitor (CLP) cells, naïve B cells, GC B cell, plasmablasts, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells and/or memory B cells. In some alternatives, the macromolecule is a prodrug.

[0108] “Memory B cells” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, the B cell subtypes that are formed within germinal centers following primary infection and are important in generating an accelerated and more robust antibody-mediated immune response in the case of re-infection. The B lymphocytes form the memory cells that can remember the same pathogen for future antibody production during future infections. In some alternatives of the plasma cell for expressing a molecule such as a macromolecule, protein or a peptide, the

plasma cell is derived from a B cell. In some alternatives, the B cell is a memory B cell. In some alternatives, the macromolecule is a prodrug.

[0109] “Naïve B cell” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a B cell that has not yet been exposed to an antigen. Once exposed to an antigen, the naïve B cell becomes a memory B cell. In some alternatives of the plasma cell for expressing a molecule such as a macromolecule, protein or a peptide, the plasma cell is derived from a B cell. In some alternatives, the B cell is a memory B cell. In some alternatives, the macromolecule is a prodrug. “Peripheral blood mononuclear cells” (PBNC) as described herein are peripheral blood cells having a round nucleus. These cells consist of lymphocytes (T cells, B cells, NK cells) and monocytes, whereas erythrocytes and platelets have no nuclei, and neutrophils, basophils, and eosinophils have multi-lobed nuclei. In the alternatives described herein, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells.

[0110] “Plasma cells” as described herein, are also called plasma B cells, plasmocytes, plasmacytes, or effector B cells. Plasma cells have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, white blood cells that secrete antibodies and are transported by the blood plasma and the lymphatic system.

[0111] “Plasma cell precursor” can begin as an immature plasma cell. The most immature blood cell of the plasma cell lineage is called the plasmablast which can differentiate into a mature fully differentiated plasma cells. Plasmablasts can secrete more antibodies than a B cell, but less than a plasma cell. In some alternatives, a method of making a plasma cell that expresses a molecule is provided. In some alternatives, the plasma cell is a plasma cell precursor. In some alternatives, the plasma cell precursor is a plasmablast.

[0112] “Cell isolation,” also referred to as “isolating of cells,” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a cell separation technique. Such techniques are important in immunology and the techniques can be appreciated by those of skill in the art. Isolation or the separation of the cells can be performed, for example, by the use of antibodies against differentially expressed cell-surface molecules of targets. Without being limiting, B cells can be isolated

by using a commercially available kit. Without being limiting, there are commercially available kits for B cell isolation by Miltenyi Biotec, EasySep™ Human B Cell Isolation Kit by StemCell™ Technologies, and many others. In some alternatives herein, a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule is provided, wherein the method comprises a step for isolating B cells. In some alternatives, the macromolecule is a protein, a protein mimetic or a peptide. In some alternatives, a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule is provided, the method comprising isolating B cells, developing the B cells, performing a first round of genome editing of the B cells for protein expression in absence of viral integration, expanding the B cells; and differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express the molecule.

[0113] “Development” of a cell describes a cell's differentiation to a cell type. For example, development into a B cell can occur in several stages, wherein each stage is marked by various gene expression patterns and immunoglobulin H chain and L chain gene loci arrangement. For example the B cells undergo VJ recombination as they develop. B cells develop from hematopoietic stem cells that originate from bone marrow. In some alternatives herein, a method of making plasma cells or plasma cell precursors that express a molecule such as a macromolecule is provided, wherein the method comprises a step for developing the B cells. In some alternatives, the cells are memory B cells or naïve B cells. In some alternatives, the macromolecule is a prodrug.

[0114] B cells undergo two types of selection while developing in the bone marrow to ensure proper development. For example, positive selection occurs through antigen independent signaling. Negative selection occurs through the binding of self-antigen with the B cell receptor.

[0115] “Genome editing” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a process that includes methods for genetic engineering in which DNA is inserted, deleted or replaced in the genome of a living organism. Editing a gene is also known as gene editing. In some alternatives described herein, a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule is provided, in which B cells or B cell precursors are

subjected to at least one round of genome editing. Methods of genome editing can include, but is not limited to nucleic acid being inserted, deleted or replaced in the genome of a cell. In some alternatives, a nuclease is used to achieve this process. In some alternatives, the nuclease is engineered. In some alternatives, the methods include inducing double strand breaks that are repaired through nonhomologous end-joining (NHEJ) or homologous recombination (HR). In some alternatives, the step of genome editing is performed by introduction of a single stranded nucleic acid. In some alternatives, the at least one round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, a second round of genome editing is performed to excise a region. In some alternatives, a third round of genome editing is performed to result in expression of a drug activatable growth enhancer. In some alternatives herein, the genome editing is performed by nonpathogenic AAV mediated editing by direct homologous recombination.

[0116] Genome editing can also employ the use of RNA and protein based transfection. For example the CRISPR/Cas system can be modified to edit genomes. This technique requires the delivery of the Cas nuclease complexed with a synthetic guide RNA (gRNA) into a cell, thus the cell's genome can be cut at a specific location and allow existing genes to be removed and/or add new ones. Thus, CRISPR/Cas and related programmable endonuclease systems are rapidly becoming significant genome editing tools of the biomedical research laboratory, with their application for gene disruption and/or gene targeting as demonstrated in a variety of cultured cell and model organism systems. In some alternatives, of the CRISPR/Cas system described herein, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9.

[0117] The basic components of CRISPR/Cas system comprise a target gene, a protospacer adjacent motif (PAM), a guide RNA, Cas endonuclease. An important aspect of applying CRISPR/Cas for genome editing is the need for a system to deliver the guide RNAs efficiently to a wide variety of cell types. This could, for example, involve delivery of an in vitro generated guide RNA as a nucleic acid (the guide RNA generated by in vitro

transcription or chemical synthesis). In some alternatives, the nucleic acid could be rendered nuclease resistant by incorporation of modified bases.

[0118] The CRISPR-Cas system falls into two classes. The Class 1 system has a complex of multiple Cas proteins for the degradation of foreign nucleic acids. The Class 2 system has a single large Cas protein for a same purpose for the degradation of foreign nucleic acids. There are a 93 cas genes that are grouped into 35 families. 11 of the 35 families form a cas core which includes the protein families CAS1 to CAS9. As described herein, Cas comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9.

[0119] Gene editing may also be performed by a novel non-nuclease based gene editing platform. A novel family of AAVs were previously isolated from human hematopoietic stem cells. These nonpathogenic AAVs are naturally present in healthy individuals and may possess unique gene editing and gene transfer properties. This technique is also described as AAV mediated editing by direct homologous recombination (AmENDR™). This process is homologous recombination by a natural biological mechanism that is used by cells to ensure highly precise DNA repair.

[0120] AAV mediated editing by direct homologous recombination is initiated by design of homology sequence “arms” that are specific to a region of the genome and results in a permanent correction in the DNA when administered to cells. In some alternatives herein, the gene editing is performed by nonpathogenic AAV mediated editing by direct homologous recombination. The identification of novel AAV genomes are described in Smith *et al.* (Mol Ther. 2014 Sep; 22(9): 1625–1634; incorporated by reference in its entirety herein). The novel AAVs described by Smith *et al.*, represents a new class of genetic vector for the manipulation of HSC genomes. Furthermore, these vectors may greatly expand the ability to deliver genes to targeted tissues and cells including cells that are refractory to gene transfer which circumventing prevalent preexisting immunity to AAV2. In some alternatives, the gene editing is performed by nonpathogenic AAVs naturally present in hematopoietic cells, wherein the editing is performed by AAV mediated editing by direct homologous recombination using the nonpathogenic AAVs as described in Smith *et al.*

[0121] “Engineered nucleases” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, enzymes that are engineered to be hybrid enzymes which can be used to specifically recognize a DNA

sequence and efficiently edit the genome by the introduction of double-strand breaks. Without being limiting, there are four families of engineered nucleases are meganucleases, zinc finger nucleases (ZFN), transcription activator like effector-based nucleases (TALEN), and the CRISPR-Cas system.

[0122] “Meganucleases” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, endodeoxyribonucleases characterized by a large recognition site (double-stranded DNA sequences of 12 to 40 base pairs). In some alternative methods for making a plasma cell or plasma cell precursor that expresses a molecule such as a macromolecule, the method comprises: (a) isolating B cells, (b) developing the B cells, (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration, (d) expanding the B cells, and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the nuclease is a meganuclease.

[0123] “Zinc finger nucleases (ZFN)” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, engineered restriction enzymes that are generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target specific desired DNA sequences and this enables zinc-finger nucleases to target unique sequences within complex genomes. In some alternative methods for making a plasma cell that expresses a molecule, such as a macromolecule, the method comprises: (a) isolating B cells, (b) developing the B cells, (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration, (d) expanding the B cells, and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the nuclease is a zinc finger nuclease.

[0124] “Transcription activator-like effector nucleases,” (TALEN), have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, restriction enzymes that can be engineered to cut specific

sequences or sites in DNA. They are made by fusing a TAL effector DNA-binding domain to a DNA cleavage domain (a nuclease which cuts DNA strands). Transcription activator-like effectors (TALEs) can be engineered to bind to a desired DNA sequence, so when combined with a nuclease, the DNA can be cut at specific locations. Thus, the restriction enzymes can be introduced into cells, for use in genome editing or for genome editing in situ, a technique known as genome editing with engineered nucleases. The use of TALEN is known to those of skill in the art. In some alternatives described herein, a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule is provided, in which B cells or B cell precursors are subjected to at least one round of genome editing. Methods of genome editing can include, but is not limited to nucleic acid being inserted, deleted or replaced in the genome of a cell. In some alternatives, a nuclease is used to achieve this process. In some alternatives, the nuclease is engineered. In some alternatives, the methods include inducing double strand breaks that are repaired through nonhomologous end-joining (NHEJ) or homologous recombination (HR). In some alternatives, the method includes a first round of genome editing or genome editing. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMG, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transducing the B cell with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary.

[0125] “Expansion” as described herein, refers to steps to increase the yield of the cells. In order for a group of cells to be useful or beneficial, the expansion of cells following isolation or extraction is necessary. The ability to expand B cell populations ex

vivo is valuable for downstream applications including high throughput B cell assays and *ex vivo* differentiation of the cells. For example steps for increasing the number of B cells or gene edited B cells can include but are not limited to methods for introducing genetic modifications that artificially induce non-transformative expansion of gene edited B cells. Furthermore, there are also commercially available kits that comprise reagents for the growth and expansion of B cells. These methods and commercially available kits for B cell expansion can be appreciated by those of skill in the art. In some alternatives herein, the gene editing is performed by nonpathogenic AAV mediated editing by direct homologous recombination.

[0126] For “rapid B cell expansion,” as described herein, edits to a B cell are introduced two days after activating and expanding for five additional days. It is expected that the cells will be expanded 50-fold.

[0127] “Differentiation” as described herein, refers to a cell changing from one cell type into another. Without being limiting, B cells can be differentiated based on their exposure to T cell-derived cytokines bound by B cell cytokine receptors. For example, CD40L can serve as a necessary stimulatory factor for B cell activation by binding the B cell surface receptor CD40, which can also affect differentiation. In the alternative methods described herein, the B cell is differentiated in a three step culture system comprising activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, the plasmablast differentiation step is performed in the presence any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence any combination of IL-6, IL-15, APRIL and/or IFN α .

[0128] “Somatic hypermutation” (or SHM) has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a cellular mechanism by which the immune system adapts to the new foreign elements that confront it (e.g. microbes), as seen during class switching. SHM diversifies B cell receptors used to recognize foreign elements (antigens) and allows the immune system to adapt its response to new threats during the lifetime of an organism. Somatic hypermutation involves

a programmed process of mutation affecting the variable regions of immunoglobulin genes. Without being limiting, a cell may be stimulated to divide or proliferate after antigen recognition. During proliferation, the B cell receptor can undergo a high rate of somatic mutation that is at least 10^5 to 10^6 greater than the normal rate of mutation across the cellular genome. “Activation-induced cytidine deaminase,” (AICDA and also known as AID), as described herein as AID, is a 24 kDa enzyme which in humans is encoded by the AICDA gene. AID creates mutations in DNA by deamination of cytosine base, which turns it into uracil (which is recognized as a thymine). Through an unknown mechanism, AID changes a C:G base pair into a U:G mismatch. The cell's DNA replication machinery recognizes the U as a T, and hence C:G is converted to a T:A base pair. During germinal center development of B lymphocytes, AID also generates other types of mutations, such as C:G to A:T. In the alternative methods described herein, the method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule, further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cell or B cell precursor. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cell comprises disruption of an AID gene.

[0129] “Synthetically engineered protein” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a protein that is made by protein or expressed from a synthetic gene that is manufactured through the method of genetic engineering. The synthetically engineered protein is usually made through rational protein design and may include unnatural amino acids as well. Furthermore the engineered protein can be transcribed and translated from a gene that is codon optimized for expression in a mammal, such as a human. In some alternatives, a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule is provided. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. In some alternatives, the protein is a genetically engineered protein. In some alternatives, the genetically engineered protein is encoded by a codon optimized gene. Genetically engineered proteins can also be a fusion or a chimeric protein, which are proteins created through the joining of two or more genes that originally coded for separate proteins or portions of proteins. The fusion proteins can also be made up of specific protein domains from two or more separate proteins. Translation of this fusion gene can result in a single or

multiple polypeptides with functional properties derived from each of the original proteins. Recombinant fusion proteins can be created artificially by recombinant DNA technology for use in biological research or therapeutics. Such methods for creating fusion proteins are known to those skilled in the art. Some fusion proteins combine whole peptides and therefore can contain all domains, especially functional domains, of the original proteins. However, other fusion proteins, especially those that are non-naturally occurring, combine only portions of coding sequences and therefore do not maintain the original functions of the parental genes that formed them. In some alternatives, the protein is for prophylactic or therapeutic protection from infection (viral, bacterial, or parasitic) in healthy subject or following stem cell administration or solid-organ transplantation in pediatric and adult subjects including, but not limited to neutralizing antibodies that block influenza, parainfluenza, rhinovirus, Respiratory Syncytial Virus (RSV), HIV, pathogenic bacteria, and parasites. In some alternatives, the protein is for protein replacement, enzyme replacement or rescue of enzyme deficiencies including, but not limited to Factor VIII (Hemophilia A), Factor IX (Hemophilia B), ADAMTS13 (Hereditary TTP), LIPA (lysosomal acid deficiency), SERPING1 (hereditary angioedema), SERPINA1 (alpha1 anti-trypsin deficiency), GLA (Fabry disease), and/or ALPL (Hypophosphatasia). In some alternatives, the protein is for immune modulation via expressed cytokines, cytokine receptors, complement proteins or other inhibitory proteins including, but not limited to: IL1 receptor antagonist for treatment of periodic fever/autoinflammatory syndromes; complement inhibitory proteins (including Factor H, Factor I) for treatment or amelioration of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis; and C1 inhibitor for hereditary angioedema. In some alternatives, the protein expressed is an anti-fibrotic molecule, including, but not limited to SCGB1A1 for the treatment or amelioration of pulmonary fibrosis. In some alternatives, the plasma cell or plasma cell precursor expresses a macromolecule, such as a protein, wherein the protein comprises therapeutic antibodies for autoimmune disorders, autoinflammatory disorders, immune dysregulation and cancer including but not limited to: anti-IL1 monoclonal antibodies for treatment of periodic fever/autoinflammatory syndromes; anti-TNF antibodies for inflammatory arthritis/inflammatory bowel disease, anti-IL33 antibodies for the treatment of asthma and anti-C5 antibodies for treatment of paroxysmal nocturnal hemoglobinuria/atypical HUS. In

some alternatives, the plasma cell or plasma cell precursor expresses a macromolecule, such as a protein, wherein the protein is an Anti-thrombotic molecules including, but not limited to APLN to block platelet function. Antithrombotic molecules are further described by Adam *et al.* (“Apelin: an antithrombotic factor that inhibits platelet function.” Blood. 2016 Feb 18;127(7):908-20.; incorporated by reference in its entirety herein). In some alternatives, the plasma cell or plasma cell precursor expresses a macromolecule, such as a protein, wherein the protein is a glucose responses element for treatment of diabetic conditions. Synthetically engineered proteins or antibodies that are protective in viral, fungal, parasitic or bacterial infection are also contemplated. Without being limiting the synthetically engineered proteins or antibodies can be specific for HIV, viral pneumonia, or fungal infections.

[0130] “Engraftment” as described herein, refers to the expansion of cells and their cell progeny so that they can re-initiate the immune system or become incorporated into the body of the host. As such the cells may be able to grow and reproduce within the recipient. In some alternatives herein, a method of inducing engraftment of a cell that expresses a peptide for immunotherapeutic application, secreted by a B cell in a subject in need in vivo, is provided. In some alternatives, the cell expresses BAFF, APRIL, IL-10, IFN-alpha or IL-6.

[0131] A proliferation-inducing ligand (APRIL), as described herein, is also known as tumor necrosis factor ligand superfamily member 13 (TNFSF13), is a protein of the TNF superfamily recognized by the cell surface receptor TACI. In some alternatives herein, a B cell or plasma cell is provided, wherein the B cell or plasma cell expresses APRIL. In some alternatives, the plasma cell secretes IL-10 and/or IL-6. In some alternatives, the cell expresses BAFF, APRIL, IL-10, IFN-alpha or IL-6.

[0132] Those skilled in the art will appreciate that gene expression levels are dependent on many factors, such as promoter sequences and regulatory elements. Another factor for maximal protein selection is adaptation of codons of the transcript gene to the typical codon usage of a host. Many synthetic genes can be designed to increase their protein expression level. The design process of codon optimization can be to alter rare codons to codons known to increase maximum protein expression efficiency. In some alternatives, codon selection is described, wherein codon selection is performed by using algorithms that are known to those skilled in the art to create synthetic genetic transcripts optimized for

higher levels of transcription and protein yield. Programs containing algorithms for codon optimization are known to those skilled in the art. Programs can include, for example, OptimumGene™, GeneGPS® algorithms, etc. Additionally synthetic codon optimized sequences can be obtained commercially for example from Integrated DNA Technologies and other commercially available DNA sequencing services. In some alternatives herein, the engineered protein is encoded by a gene, wherein the gene is codon optimized for expression in a human cell. In some alternatives, the genetically engineered protein is encoded by a codon optimized gene.

[0133] “Proprotein convertase subtilisin/kexin type 9 (PCSK9)” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, an enzyme that is expressed in many tissues and cell types. PCSK9 binds to the receptor for low-density lipoprotein particles (LDL), which typically transport 3,000 to 6,000 fat molecules (including cholesterol) per particle, within extracellular water. If PCSK9 is blocked, more LDLRs are recycled and are present on the surface of cells to remove LDL-particles from the extracellular water. Therefore, blocking PCSK9 can lower blood LDL-particle concentrations. PCSK9 orthologs are found in many species. Agents which block PCSK9 can lower LDL particle concentrations. In some alternatives, a method of making plasma cells or plasma cell precursors that express a molecule, such as a macromolecule is provided. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. In some alternatives, the protein is a genetically engineered protein. In some alternatives, the genetically engineered protein is encoded by a gene that has been codon optimized for expression in humans. In some alternatives, the genetically engineered protein is specific for PCSK9. In some alternatives, the plasma cell expressing the protein is administered to a subjects suffering from high cholesterol. In some alternatives, subject has at least 200 mg/dL of total cholesterol, wherein total cholesterol includes the levels HDL and LDL cholesterol.

[0134] In some alternatives, the plasma cell expressing the protein is administered to a subjects suffering from HIV, CMV or an autoimmune disorder. In some alternatives, the subject is suffering from multiple sclerosis. In some alternatives, the subject is suffering from Crohn’s disease. In some alternatives, the protein is an antibody. In some alternatives, the protein comprises Tysabri (natalizumab) antibody or a portion thereof.

[0135] “Rituxan” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a medication sold under the brand name Rituxan among others, which is a monoclonal antibody specific for CD20 and is used to treat or inhibit certain autoimmune diseases and types of cancer. It is used for non-Hodgkin's lymphoma, chronic lymphocytic leukemia, rheumatoid arthritis, idiopathic thrombocytopenic purpura, and/or pemphigus vulgaris. Rituximab is another name for this anti-CD20 monoclonal antibody. This anti-CD20 antibody is also used to treat rheumatoid arthritis. In RA, this medicine slows the inflammatory process and help reduce joint pain and swelling. This medicine is often used with other cancer or arthritis medications. The success of rituximab likely relates to a number of factors, one of which is that it is a chimeric rather than a murine antibody (Grillo-Lopez, 2000). Rituximab retains the murine CD20-binding Fab regions, but uses a human Fc portion.

[0136] “Negative selection of cells” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, selecting out cells, for example, in which B-cells and T-cells that recognize MHC molecules bound to peptides of self-origin, or just MHC molecules with high affinity are deleted from the repertoire of immune cells. Negative selection can be performed with a commercial kit, such as a human B cell isolation kit (Miltenyi Biotec, Auburn, CA).

[0137] “Rapamycin” also known as “sirolimus” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, a macrolide compound that inhibits activation of T cells and B cells by reducing the production of interleukin-2 (IL-2). Rapamycin-inducible FKBP11 dimers has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, FKBP (FKBP1A) which will form a dimer in the presence of rapamycin.

[0138] The HPRT1 gene has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, a gene that provides instructions for producing an enzyme called hypoxanthine phosphoribosyltransferase 1. This enzyme allows cells to recycle purines, a type of building block of DNA and its chemical cousin RNA. Manufacturing purines uses more energy and takes more time than recycling purines, which makes recycling these molecules more efficient. Recycling purines ensures that cells have a plentiful supply of building blocks for the production of DNA and RNA. The process of

recycling purines is also known as the purine salvage pathway. More than 200 mutations in the HPRT1 gene have been identified. These mutations include changes in single DNA building blocks (nucleotides) or insertions or deletions of small amounts of DNA within the gene. These changes result in either nonfunctional or very low-function hypoxanthine phosphoribosyltransferase 1. Under these conditions, uric acid, a waste product of purine breakdown, accumulates in the body and can cause gouty arthritis (arthritis caused by uric acid in the joints), kidney stones, and bladder stones. It is unclear how this enzyme deficiency causes the neurological and certain mutations in the HPRT1 gene can also cause a condition featuring gouty arthritis called HPRT-related gout, previously known as Kelley-Seegmiller syndrome. Individuals with this condition have lower than normal levels of hypoxanthine phosphoribosyltransferase 1.

[0139] “CD138” or “syndecan-1” can be used interchangeably and has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, a transmembrane (type I) heparan sulfate proteoglycan and is a member of the syndecan proteoglycan family. CD138 is a surface antigen that is expressed at high levels in plasma cells. The CD138 antigen is expressed on normal and malignant plasma cells but not mature B cells. As such, plasma cells may be purified, for example by use of CD138 positive selection and through use of CD138 antibodies attached to beads for cell purification. There are commercial kits that may be used to purify cells that are CD138 positive (e.g. The EasySep™ Human CD138 Positive Selection Kit by STEMCELL™, The CD138+ Plasma Cell Isolation Kit by Miltenyl Biotech). CD138 may be used to purify long lived plasma cells, as this population should engraft better and express more exogenous protein.

DETAILED DESCRIPTION

[0140] As described herein are the methods of making protein producing B cells, and the use of the protein producing B cells for immunotherapies. Additional alternatives can also include the protein producing B cells as well as compositions comprising the protein producing B cells. In some alternatives, the B cells express a macromolecule, such as a protein mimetic or a peptide.

[0141] Compositions and methods for inducing tolerance for a peptide secreted by a B cell or inducing engraftment is also provided herein. In some alternatives, these cells express BAFF, APRIL, IL-10, IFN-alpha or IL-6.

[0142] Methods for a reproducible system for enrichment and long-term *in vitro* culture of human B cell progenitors have previously been described by Rawlings *et al.* 1995, Rawlings *et al.* 1997, and Fluckinger *et al.* 1998 (“Long-term culture system for selective growth of human B-cell progenitors.” Proc. Natl. Acad. Sci. USA Vol. 92, pp. 1570-1574, February 1995, “Differentiation of human CD34+CD38- cord blood stem cells into B cell progenitors *in vitro*.” Exp Hematol. 1997 Jan; 25 (1):66-72; and “In vitro reconstitution of human B-cell ontogeny: from CD34 (+) multipotent progenitors to Ig-secreting cells.” Blood. 1998 Dec 15; 92(12):4509-20; all references incorporated by reference in their entireties). These types of systems, as previously reported, are important in the production of normal human B-lineage development and includes the production of mature Ig-secreting B cells and were used in studies of normal and abnormal early human B-lymphopoieses. In some systems, the long term *in vitro* culture system can be initiated with CD34+ or CD34+CD38- umbilical cord blood hematopoietic progenitors that can support normal human B-lineage development and can include the production of mature Ig-secreting B cells (Fluckiger *et al.* 1998). Although there are methods for isolating and developing B cells for Ig secretion, the development of plasma cells from differentiated B cells that have undergone genome engineering to express a protein has previously not been reported.

[0143] As previously reported, there has been genome editing of primary human hematopoietic cells. Co-delivery of designer nuclease mRNA and AAV donor provides a reliable approach for targeted gene modification in primary human hematopoietic cells. Such methods average in about 60% homology-directed repair (HDR) in primary T cells, multiple loci, with multiple experiments and independent donors and averages about 30-50% HDR in adult-mobilized CD34+ cells as shown in Sather *et al.* (“Efficient modification of CCR5 in primary human hematopoietic cells using a megaTAL nuclease and AAV donor template.” Sci Transl Med. 2015 Sep 30; 7(307):307ra156; incorporated by reference in its entirety herein). This has also led to efficient delivery of therapeutic gene cassettes into candidate loci, such as HIV therapeutics into CCR5 locus: C46, CD19 CAR, & HIV CAR (Sather *et al.*, Sci Transl Med. 2015 Sep 30;7(307); Roman-Ibarra *et al.*, Mol Ther Nucleic Acids.

2016;5:e352; Hale *et al.*, Mol Ther Nucleic Acids. 2016, Hale Molec Ther Methods 2016; all incorporated by reference in their entireties herein), safe Harbor or TCRA locus- CARs and other therapeutic cassettes (Hale *et al.* Molec Ther Methods 2016; incorporated by reference in its entirety herein), CD40L gene in hyper-IgM syndrome to restores CD40L deficient T cell function (Hubbard *et al.* Blood 2016; incorporated by reference in its entirety herein), and methods to further enhance genome editing using viral helper proteins (Gwiazda *et al.* Mol Ther. 2016 Sep 29;24(9):1570-80; incorporated by reference in its entirety herein). Thus, modification of T cells (or CD34+ HSC) using this co-delivery approach is translatable to a wide range of clinical applications. However this approach was not contemplated for use in B cells.

[0144] In T cells, for example, gene targeting was used to disrupt the *CCR5* gene. This was done by stable integration of gene cassette at target locus. Homology-directed repair mechanism was used using a megaTAL nuclease and rAAV donor template (Sather and Romano Ibarra *et al.*, Sci Trans Med 2015; incorporated by reference in its entirety herein). Disruption of genes are also used to manufacture HIV protected anti-CD19CAR T cells (Milone et al, 2009 Mol Ther; Porter et al, 2011 NEJM; incorporated by reference in its entirety herein). Thus, successful techniques for genetic editing of B cells, which would also result in cells that have a long life, are much needed. As described herein, are some alternatives for B cell genetic editing, wherein the genome editing is performed by nonpathogenic AAV mediated editing by direct homologous recombination.

[0145] As described herein, are engineered plasma cells as a macromolecule delivery platform. The macromolecule comprises proteins, protein mimetics, or peptides. The potential for the *ex vivo* manipulation and generation of cells include the increased longevity of the cells and protein production. For example, human influenza antibody titers can persist for over 90 years. These cells can persist in non-dividing state within bone marrow survival niche without need for ongoing antigen exposure and are relatively resistant to immunosuppression/chemotherapy. In regards to protein production, plasma cells can produce about 2000-10,000 immunoglobulin molecules per second, which can translate to about ~50-340 pg protein/cell/day. Industrial cell-line protein production can lead to about 20-90 pg protein/cell/day. The alternatives herein have the benefits of using the plasma cells, which are highlighted in Figure 2.

[0146] There are several types of plasma cell-based therapies that are contemplated in the alternative methods described herein. This can include plasma cells that express antibodies that are specific to proteins that are expressed due to infection with a pathogen such as influenza virus, EBV, CMV, HIV, or malaria, for example. Therapeutic proteins are also contemplated. Without being limiting, therapeutic proteins can include proteins to supplement for enzyme deficiencies such as Factor VIII and Factor IX in those suffering from hemophilia, for example. In some alternatives, the therapeutic proteins can bind proteins of the lipid envelope of the influenza virus. In some alternatives, the lipid envelop proteins comprises glycoproteins hemagglutinin and neuraminidase. In some alternatives, the therapeutic proteins can bind proteins of the CMV virus. In some alternatives, the proteins of the CMV virus comprises glycoprotein B.

[0147] Requirements for edited plasma cell regeneration can include, B cell activation and genome editing, B cell expansion B cell differentiation into long-lived plasma cells (LLPC). LLPC make up about 0.1–1% of all bone-marrow cells in healthy individuals. In mice there are about $\sim 10^6$ plasma cells, and in humans there are about $\sim 10^9$ plasma cells. There are also antigen specific LLPCs as described in Radbruch *et al.* (“Competence and competition: the challenge of becoming a long-lived plasma cell.” Nat Rev Immunol. 2006 Oct; 6(10):741-50; incorporated by reference in its entirety herein). Exposure to tetanus toxoid led to tetanus-specific IgG concentrations of 10-20 μ g/mL, which also implies a number of $0.8-1.6 \times 10^6$ tetanus-specific LLPCs. However there is a competition for the LLPC to reside in the bone marrow or spleen survival niche. Plasma cell longevity in the bone marrow was shown to be more than 90 days of survival without turnover. It was shown in Radbruch *et al.*, and Slifka *et al.*, adoptive transfer of 1.5×10^4 virus-specific plasma cells in absence of conditioning resulted in readily detectable anti-viral antibody (“Competence and competition: the challenge of becoming a long-lived plasma cell.” Nat Rev Immunol. 2006 Oct; 6(10):741-50; and “Humoral Immunity Due to Long-Lived Plasma Cells.” Immunity, Vol. 8, 363–372, March, 1998; incorporated by reference in their entireties herein). Thus, for clinical efficacy, the number of engrafted engineered plasma cells should be at least $1-10 \times 10^6$ of edited long-lived plasma cells, and may be sufficient for many applications described herein. In some alternatives of the methods of treating, inhibiting or ameliorating a disease, such as cancer, in a subject in need or expressing a protein in a

subject in need, the subject is administered at least $1-10 \times 10^6$ of edited long-lived plasma cells that express a protein, or a composition which is used to deliver at least $1-10 \times 10^6$ of edited long-lived plasma cells that express a protein. In some alternatives, the subject receives 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 10×10^6 , 1×10^7 , 2×10^7 , 3×10^7 , 4×10^7 , 5×10^7 , 6×10^7 , 7×10^7 , 8×10^7 , 9×10^7 , 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 or 9×10^9 edited long-lived plasma cells that express a protein or any number of cells in between a range defined by any two aforementioned values. In some alternatives herein, the genome editing is performed by nonpathogenic AAV mediated editing by direct homologous recombination.

[0148] The generation of gene-edited long-lived plasma cells also requires specific steps in the editing of the primary B cells as well as optimization of B cell differentiation. To date, there is existing literature in murine as well as human B cell editing. In murine studies, sgRNA was delivered into CAS9 transgenic murine B cells which led to gene disruption of candidate genetic loci including application to sgRNA screens. It was also shown that retroviral delivery of sgRNA (plus antibiotic drug selection) in primary B cells leads to up 80% gene disruption. However, was HR not tested in the B cells, furthermore, it is not clinically feasible to used transgenic CAS9 and integrating viral vector for sgRNA delivery into B cells (“Efficient CRISPR-mediated mutagenesis in primary immune cells using CrispRGold and a C57BL/6 Cas9 transgenic mouse line.” Proc Natl Acad Sci U S A. 2016 Nov 1; 113(44):12514-12519; incorporated by reference in its entirety herein). Retroviral and LV-based, CRISPR/CAS9 delivery in murine B cells and hybridomas was also performed by Cheong *et al.* (“Editing of mouse and human immunoglobulin genes by CRISPR-Cas9 system.” Nat Commun. 2016 Mar 9; 7:10934; incorporated by reference in its entirety herein). Editing was performed of the Ig locus to mediate Ig class-switch. However, the technique had very low efficiency and again, HR was not tested by Cheong *et al.* Additionally, the techniques provided were not clinically feasible for use in B cells because of the long-term CAS9 expression from an integrated viral vector (Cheong *et al.*).

[0149] For the preferred alternatives described herein, the candidate nuclease platform used for the methods of making the plasma cell from the B cell includes genetically engineered nucleases, zinc finger nucleases, CRISPR/Cas Nucleases, TAL Effector

Nucleases, and Homing Endonucleases (Figure 3). Options for genetic modification of B cells in the alternatives described herein are RNA (or RNP) plus AAV co-delivery as well as RNP/ODN (oligodeoxynucleotides) delivery. As shown in the tables of Figure 4, this table shows qualitative comparisons of the approaches described herein, where 4 plus indicates a positive result and the 1 plus sign describes negative outcome. These tables show that RNP + AAV or RNP+ODN are both specific, cost-effective and efficient.

[0150] Also contemplated are ribonucleoprotein strategies for editing B cells. As shown in Figure 5, complex CAS nuclease and RNA guides are prepared *in vitro*. The CAS nuclease and guides are then used to transfect the isolated primary B cells to cleave the desired target in the B cell genome. In some alternatives, the guide sequence comprises a sequence set forth in any one of SEQ ID NO: 63-112.

Genome modification rates, NHEJ and site-specific homology directed repair (HDR) genome editing in primary human B cells.

[0151] Genome modification rates of human B cell lines as described in the alternatives herein, are 10-fold greater than previous methods for genome editing reported; and provide the first demonstration of NHEJ and, most importantly, of site-specific homology directed repair (HDR) genome editing in primary human B cells. In fact, the literature show methods that do not provide a clinically translatable methodology for use in primary human B cells. In one study, CAS9 transgenic mice were generated and utilized. In this setting, CAS9 expression was achieved using a ubiquitous transgenic expression vector and guide RNAs were subsequently introduced via lentiviral vectors into CAS9 transgenic murine B cells, as described by Chu *et al.* ("Efficient CRISPR-mediated mutagenesis in primary immune cells using CrispRGold and a C57BL/6 Cas9 transgenic mouse line," Proc. Natl. Sci., 2016, Nov 1; 113(44); 12514-12519; incorporated by reference in its entirety herein). Chu *et al.* did not show evidence of homology directed repair (HDR) and did not perform any work using primary human B cells. In a second report, retroviral delivery of CAS9 and guide RNAs to initiate gene disruption (NHEJ) in murine primary B cells and in human B cell lines was used by Cheong *et al.* ("Editing of mouse and human immunoglobulin genes by CRISPR-Cas9 system," Nature Commun, 2016 Mar 9;7:10934; incorporated by reference in its entirety herein). This disruption strategy was used to initiate

class switch recombination and drive switching to specific antibody constant regions. In this case, disruption percentage (NHEJ) rates were low (5-10%) and there was no demonstration of HDR. In addition, no work was performed in primary human B cells. More recently, homology directed repair (HDR) was used by Pogson *et al.* in B cell lines to alter the antibody specificity of a hybridoma cell line (“Immunogenomic engineering of a plug-and-(dis)-play hybridoma platform; Nat. Commun. 2016 Aug 17; 7: 12535; incorporated by reference in its entirety herein). In this case, the repair rates were modest (5%) and the authors utilized plasmid-based DNA delivery of the repair template and the CRISPR nuclease reagents. Again, no work was performed using primary human B cells. As described herein, none of these delivery systems are likely to result in the high efficiency genome editing that have been observed in the primary B cells (rates of 80% NHEJ or rates of ~40% HDR) of the alternatives herein. In fact, the editing rates of the alternatives described herein are surprising, because they significantly outpace the efforts of others in the literature regarding sustained gene expression following either transgene integration with lentiviral vectors or any existing genome editing approach utilized in B cells.

[0152] In some alternatives herein, the genome editing is performed by nonpathogenic AAV mediated editing by direct homologous recombination.

The yield of DNA delivery is greater than the published rates of DNA delivery to primary human B cells and has a sustained gene expression.

[0153] The alternatives as described herein, have increased the yield of DNA delivery >1 log over published rates to primary human B cells. This advance is achieved in greatly facilitating HDR rates by either transfecting with single-stranded DNA oligonucleotides or transduction with capsid-specific AAV. Previous studies by Kim *et al.* using recombinant adenovirus have shown that vectors utilizing the serotype Ad-K35 can transduce primary human B cells at a rate of ~75% (“Enhanced antitumor immunotherapeutic effect of B-cell-based vaccine transduced with modified adenoviral vector containing type 35 fiber structures.” Gene Ther. 2014, Jan; 21(1): 106-114). This vector serotype was designed for delivery of B cell vaccines, but in contrast to the sustained gene expression that is observed in the alternatives described herein, the HDR gene-edited loci in primary B cells, adenovirus delivery does not lead to long-term gene expression in B cells. In addition,

adenoviral infected B cells are likely to be subject to immune responses *in vivo* that would make delivery of *ex vivo* adenoviral infected B cells highly problematic. In previous studies, recombinant Epstein-Barr virus (EBV) vector systems have also been developed for episomal-based gene delivery. Modified EBV vectors are capable of transducing of >80% of primary human B cells or CLL tumor cells as shown in Hellebrand *et al.* (“Epstein-Barr virus vector-mediated gene transfer into human B cells: potential for antitumor vaccination.” *Gene Ther.* 2006, Jan; 13(2): 150-162). However, this vector class is associated with loss in episomal DNA over time leading to absence of long-term expression; further, recombinant EBV infected B cells express EBV viral proteins and therefore remain capable of triggering T cell mediated immune responses in EBV exposed individuals (the vast majority of the human subjects). In contrast to the relative efficiency of transient gene delivery using adenovirus or EBV, integrating gamma-retroviral or lentiviral (LV) vectors are extremely inefficient for transducing human B cells as shown by Serafini *et al.*, Bovia *et al.* and Janssens *et al.* (“Molecular evidence of inefficient transduction of proliferating human B lymphocytes by VSV-pseudotyped HIV-1-derived lentivectors.” *Virology.* 2004 Aug 1; 325(2); 413-424, “Efficient transduction of primary human B lymphocytes and nondividing myeloma B cells with HIV-1-derived lentiviral vectors.” *Blood.* 2003 Mar 1; 101(5): 1727-1733, “Efficient and stable transduction of resting B lymphocytes and primary chronic lymphocyte leukemia cells using measles virus gp displaying lentiviral vectors.” *Blood.* 2009 Oct 8; 114(15): 3173-3180, “Efficiency of onco-retroviral and lentiviral gene transfer into primary mouse and human B-lymphocytes is pseudotype dependent.” *Hum Gene Ther.* 2003 Feb 10; 14(3):263-76; all incorporated by reference in their entireties herein). Several studies by Amirache *et al.* have shown that the VSV-G envelope typically used for LV vectors is ineffective in B cells (<5% transduction) because primary B cells lack the LDL receptor used by the VSV-G coat protein (“Mystery solved: VSV-G-LVs do not allow efficient gene transfer into unstimulated T cells, B cells, and HSCs because they lack the LDL receptor.” *Blood.* 2014 Feb 27; 123(9):1422-4; incorporated by reference in its entirety herein). In addition to lacking LV receptors, B cells likely express uncharacterized factors downstream of the receptor that limit LV transduction as described by Serafini *et al.* (“Molecular evidence of inefficient transduction of proliferating human B lymphocytes by VSV-pseudotyped HIV-1-derived lentivectors.” *Virology.* 2004 Aug 1; 325(2):413-24;

incorporated by reference in its entirety herein). As reported by Bovia *et al.*, higher levels of transduction with VSV-G LV in primary human B cells (~25%) in the setting of sustained 4-5 day co-cultures with a mouse T cell tumor line (EL-4) or following co-infection with Epstein Barr virus. However, transduction using a clinically translatable system (e.g., without tumor cell lines and using only cytokine and CD40L-based stimulation) remained very low at 2% as shown by Bovia *et al.* (“Efficient transduction of primary human B lymphocytes and nondividing myeloma B cells with HIV-1-derived lentiviral vectors.” *Blood*. 2003 Mar 1; 101(5):1727-33, incorporated by reference in its entirety herein). Newer LV vectors employing alternative envelopes including either baboon retrovirus envelope protein (BaEV), measles virus envelope (MV) or gibbon-ape leukemia virus envelope (GALV) have demonstrated increased transduction efficiency of up to 50% with baboon endogenous retrovirus (BaEV), measles virus (MV) and Gibbon ape leukemia virus (GALV) as shown by Levy *et al.*, (“Baboon envelope pseudotyped lentiviral vectors efficiently transduce human B cells and allow active factor IX B cell secretion in vivo in NOD/SCID γ c^{-/-} mice.” *J Thromb Haemost*. 2016 Dec;14(12):2478-2492; “Baboon envelope pseudotyped lentiviral vectors efficiently transduce human B cells and allow active factor IX B cell secretion in vivo in NOD/SCID γ c^{-/-} mice.” *J Thromb Haemost*. 2016 Dec;14(12):2478-2492; “Lentiviral vectors displaying modified measles virus gp overcome pre-existing immunity in in vivo-like transduction of human T and B cells,” *Mol Ther*. 2012 Sep; 20(9):1699-712; “Efficient transduction of healthy and malignant plasma cells by lentiviral vectors pseudotyped with measles virus glycoproteins.” *Leukemia*. 2012 Jul; 26(7):1663-70; “Measles virus glycoprotein-pseudotyped lentiviral vector-mediated gene transfer into quiescent lymphocytes requires binding to both SLAM and CD46 entry receptors.” *J Virol*. 2011 Jun; 85(12):5975-85; “Efficient and stable transduction of resting B lymphocytes and primary chronic lymphocyte leukemia cells using measles virus gp displaying lentiviral vectors.” *Blood*. 2009 Oct 8; 114(15):3173-80; and “Efficient lentiviral transduction and transgene expression in primary human B cells.” *Hum Gene Ther Methods*. 2012 Dec; 23(6):408-15; all references incorporated in their entireties herein), but at the cost of drastically lower viral titers. Because the titers of BaEV, GALV and MV LV vectors are much lower (>10-20-fold lower for BaEV, GALV and 100-fold lower for MV) than LV vectors using VSV-G coats, the practicality of transducing large numbers of primary B cells is limited, because high

efficiency integration requires viral quantities that result in unacceptable toxicity. In summary, the demonstration of 40% stable expression of reporter genes following HDR into target loci in primary B cells with concomitant high-levels of cell viability greatly exceeds the frequency of sustained gene expression reported for either integrating or non-integrating viral vectors, which is shown by the results of the alternative methods described herein.

Several steps are used to limit the deleterious impact of innate immune signaling that is triggered by foreign DNA in primary B cells.

[0154] As described herein, there are several steps to limit the deleterious impact of innate immune signaling triggered by foreign DNA in primary B cells. In essentially all cell types, cytosolic self- or foreign-DNA (viral, bacterial, plasmid or oligonucleotide) binds to and activates the DNA sensor cGAS, leading to synthesis of the second messenger 2'3'-cGAMP which, in turn, triggers STING-dependent downstream signaling leading to type-I interferon production and additional transcriptional changes which is described in Crowl *et al.* ("Intracellular Nucleic Acid Detection in Autoimmunity." *Annu Rev Immunol.* 2017 Jan 30; "cGAS-cGAMP-STING: The three musketeers of cytosolic DNA sensing and signaling." *IUBMB Life.* 2016 Nov; 68(11):858-870). In addition to cytosolic DNA sensing, many cell types (including human B cells) express endosomal toll-like receptors (TLRs) that recognize and respond to endosomal-delivered double-stranded DNA (dsDNA) ligands, triggering MyD88 dependent signals that promote type-I interferon and pro-inflammatory gene expression. The outcome of exogenous DNA-triggered innate signals is dependent upon the route of DNA delivery, the quantity of DNA delivered, DNA structure and cell lineage. In primary B, and T lymphocytes, transfection of dsDNA oligonucleotides promotes rapid cell death via apoptosis as described by Seiffert *et al.*, Bell *et al.*, Van Tendeloo *et al.*, Cotten *et al.*, and Ebert *et al.* ("Efficient nucleofection of primary human B cells and B-CLL cells induces apoptosis, which depends on the microenvironment and on the structure of transfected nucleic acids." *Leukemia.* 2007 Sep; 21(9):1977-83); "The analysis of costimulatory receptor signaling cascades in normal T lymphocytes using *in vitro* gene transfer and reporter gene analysis." *Nat Med.* 2001 Oct; 7(10):1155-8; "High-level transgene expression in primary human T lymphocytes and adult bone marrow CD34+ cells via electroporation-mediated gene delivery." *Gene Ther.* 2000 Aug; 7(16):1431-7;

“Intracellular delivery of lipopolysaccharide during DNA transfection activates a lipid A-dependent cell death response that can be prevented by polymyxin B.” *Hum Gene Ther.* 1997 Mar 20; 8(5):555-61.; and “Lymphocyte apoptosis: induction by gene transfer techniques.” *Gene Ther.* 1997 Apr; 4(4):296-302; all incorporated by reference in their entireties herein). Introduction of plasmid DNA by transfection in primary T lymphocytes has been tested by multiple investigators as a means to achieve transgene expression. The effectiveness of this approach has been limited by low efficiency and high toxicity. Previous studies demonstrate expression ranging from 15-75% with progressively poorer viability (<30%) as expression increases (“The analysis of costimulatory receptor signaling cascades in normal T lymphocytes using in vitro gene transfer and reporter gene analysis.” *Nat Med.* 2001 Oct; 7(10):1155-8.; “High-level transgene expression in primary human T lymphocytes and adult bone marrow CD34+ cells via electroporation-mediated gene delivery.” *Gene Ther.* 2000 Aug; 7(16):1431-7; “Gene transfection and expression in resting and activated murine CD4 T cell subsets.” *J Immunol Methods.* 2003 Nov; 282(1-2):93-102; all incorporated by reference in their entireties herein). Low cell viability correlates with transfection triggered cell apoptosis (“Lymphocyte apoptosis: induction by gene transfer techniques.” *Gene Ther.* 1997 Apr; 4(4):296-302; incorporated by reference in its entirety herein). While higher viability is observed using plasmid “Nucleofection” (perhaps due to reduced cytosolic DNA content), expression using this approach was also shown to be low (“High-efficiency transfection of primary human and mouse T lymphocytes using RNA electroporation.” *Mol Ther.* 2006 Jan; 13(1):151-9.; incorporated by reference in its entirety herein). While limited published data exist for B cells, the results from the experiments of the alternatives described herein suggest even poorer DNA transfection efficiencies with higher toxicity in primary B cells. These observations have lead most groups to abandoning plasmid DNA transfection for gene delivery. While not directly tested, cytosolic or endosomal DNA sensing likely explains this observed toxicity and strongly imply that this approach would not work for DNA template delivery for B cell genome editing. Consistent with this idea, in primary B cells plasmid DNA transfection also triggers TLR9-dependent type-I interferon and pro-inflammatory gene expression (“Oligonucleotide delivery by nucleofection does not rescue the reduced proliferation phenotype of gene-edited cells.” *Nucleic Acid Ther.* 2012 Dec; 22(6):405-13; incorporated by reference in its entirety herein); and this plasmid driven type-I interferon

production is independent of CpG motifs. The alternatives described herein, avoids the toxicity problem associated with transducing primary B cells, which have not been previously reported.

[0155] Thus, triggering of these pathways is likely highly problematic for efficient B cell editing and HDR. Taking these observations into consideration, the alternative methods described herein utilize single-stranded DNA (ssDNA) delivery to limit these deleterious responses to foreign DNA. The rationale for choosing ssDNA donor templates is that the binding constant of cGAS for ssDNA (1.5 μ M) is dramatically higher than that for dsDNA (87 nM), leading to the hypothesis that ssDNA is likely to elicit lower innate immune signaling in response to transfection than dsDNA (“Structure of human cGAS reveals a conserved family of second-messenger enzymes in innate immunity.” Cell Rep. 2013 May 30; 3(5):1362-8; incorporated by reference in its entirety herein). Further, ssDNA are poorly recognized by TLR9 and hence not expected to trigger TLR9 innate signaling- an additional challenge not encountered in T cell editing. Therefore two exemplary alternatives for delivery ssDNA are described: a) modified ssDNA donor oligonucleotides via transfection (“Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells.” Nat Biotechnol. 2015 Sep; 33(9):985-9; and “Enhancing homology-directed genome editing by catalytically active and inactive CRISPR-Cas9 using asymmetric donor DNA.” Nat Biotechnol. 2016 Mar; 34(3):339-44; both references incorporated by reference in their entireties herein); or b) ssDNA as recombinant AAV genomes.

B cells have a high capacity for site-specific HDR editing

[0156] The alternatives described herein have unexpectedly demonstrated that B cells have a high capacity for site-specific HDR editing. Site-specific genome editing (HDR) in ~100% of primary B cells have been observed in which one can detect transduction or transfection with templates for homology-directed-repair by using the alternative methods of editing as described herein. While others have described methods for high rates of HDR-genome editing in primary T cells, even in the most optimized settings, HDR rates (~20-50%) remain below the levels of AAV or oligonucleotide gene delivery (80-100%) (“Highly efficient homology-driven genome editing in human T cells by combining zinc-finger nuclease mRNA and AAV6 donor delivery.” Nucleic Acids Res. 2016 Feb 18; 44(3):e30;

Sather et al., Sci Transl Med. 2015 Sep 30; 7(307); and “Generation of knock-in primary human T cells using Cas9 ribonucleoproteins.” Proc Natl Acad Sci U S A. 2015 Aug 18; 112(33):10437-42; both references incorporated by reference in their entireties herein). Importantly, despite advances in modulating AAV turnover using co-delivery of other viral proteins leading to marked increase in AAV genomes and/or reduced AAV turnover in T cells, HDR rates are only modestly increased (“High Efficiency CRISPR/Cas9-mediated Gene Editing in Primary Human T-cells Using Mutant Adenoviral E4orf6/E1b55k "Helper" Proteins.” Mol Ther. 2016 Sep 29; 24(9):1570-80; incorporated by reference in its entirety herein). Thus, relative to other primary cell types commonly used for site-specific genome editing applications, a remarkable efficiency for HDR in primary human B cells have uncovered, which has not been demonstrated or previously reported. It is hypothesized that these unexpected findings showing surprisingly efficient HDR in human B cells reflects an unusual aspect of primary B cell biology and is perhaps related to an increased permissiveness to DNA breaks and resolution by HDR that has evolved to facilitate safe genomic modification during germinal center B cell activation and class-switch recombination.

Optimal B cell culturing conditions to enable high rates of site-specific genome editing and subsequent expansion and differentiation of gene-edited cells.

[0157] As described herein, the alternative methods have determined optimal B cell culturing conditions to enable high rates of site-specific genome editing and subsequent expansion and differentiation of gene edited cells. In several other primary cell types, several researchers have found that persistence in the G2 phase of cell cycle or high degrees of cell cycling promotes HDR during site-specific genome editing (“High Efficiency CRISPR/Cas9-mediated Gene Editing in Primary Human T-cells Using Mutant Adenoviral E4orf6/E1b55k "Helper" Proteins.” Mol Ther. 2016 Sep 29; 24(9):1570-80; incorporated by reference in its entirety herein). In this exemplary alternative, the cell culture conditions were optimized to enable rapid cycling of undifferentiated B cells, and thus increased the time the cells spend in G2 (“Regulation of homologous recombination in eukaryotes.” Annu Rev Genet. 2010; 44:113-39. “Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery.” Elife. 2014 Dec 15; 3:e04766; “High Efficiency CRISPR/Cas9-

mediated Gene Editing in Primary Human T-cells Using Mutant Adenoviral E4orf6/E1b55k "Helper" Proteins.” *Mol Ther.* 2016 Sep 29; 24(9):1570-80; incorporated by reference in its entirety herein). In some alternatives, reagents are delivered for site-specific genome editing during this cycling phase, leading to efficient HDR-gene editing. In some alternatives, the culture conditions were further optimized, wherein the edited cell populations are expanded to high numbers and to subsequently enable the terminal differentiation of these edited and expanded B cells into long-lived plasma B cells during secondary and tertiary culture phases, respectively. This experimental design has not been previously considered for genome engineering of primary B human cells. In some alternatives herein, the gene editing is performed by nonpathogenic AAV mediated editing by direct homologous recombination.

[0158] Several alternatives are described herein, included among these are: (a) designing editing strategies for target loci that were found in several alternatives to be highly expressed in antibody secreting B cells (JCHAIN, IGKC, IGMC, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1, and others) and that are not subject to aberrant somatic hypermutation or required for differentiation into antibody secreting cells; (b) inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells including, but not limited to, disruption of HPRT locus and selection of gene edited cells using 6-TG; (c) development of a targeted sequencing method to ensure the integrity of the B cell product at loci that are known to confer susceptibility to non-Hodgkin's lymphoma or multiple myeloma; (d) designing protein producing products for clinical situations where stable delivery of the protein by a B cell provides clinical benefit by avoiding organ toxicity or immune responses observed in some AAV delivery studies (for example, Factor IX), by enabling delivery of multiple different protein simultaneously (delivery of multiple neutralizing antibodies for HIV or other pathogens), or promoting stable, steady-state levels of protein delivery over time. These would not be subject to aberrant somatic hypermutation, as previously described (“Two levels of protection for the B cell genome during somatic hypermutation.” *Nature.* 2008 Feb 14; 451(7180):841-5; “DNA damage defines sites of recurrent chromosomal translocations in B lymphocytes.” *Nature.* 2012 Feb 7; 484(7392):69-74; “Translocation-capture sequencing reveals the extent and nature of chromosomal

rearrangements in B lymphocytes.” Cell. 2011 Sep 30; 147(1):95-106.; all incorporated by reference in their entireties herein).

[0159] In some alternatives herein, the gene editing is performed by nonpathogenic AAV mediated editing by direct homologous recombination.

Development of *in vivo* pre-clinical models

[0160] Additional alternatives contemplated herein are *in vivo* pre-clinical models that can be used to assess the efficacy of the B cells. Murine models exist that could be used for this purpose.

CRISPR/Cas9, crRNA, tracrRNA

[0161] In the alternatives herein, CRISPR guide targets at the BLIMP1, IRF4, BCL6, PAX5, MITF, BACH2, IRF8 loci were designed for several alternative methods described herein, using the online MIT CRISPR design tool (<http://crispr.mit.edu/>) and the Broad Institute sgRNA design tool (<http://portals.broadinstitute.org/gpp/public/analysis-tools/sgRNA-design>). A tracrRNA recognition sequence (GUUUUAGAGCUAUGCU; SEQ ID NO: 1) was added to the 3' end of each selected guide target to form the complete synthetic crRNA sequence. The crRNA guides were synthesized by IDT with additional modifications – phosphorothioate linkages between the four nucleotides on the 5' end and the 3' end, as well as 2'O-methyl groups on the three nucleotides on the 5' end and the 3' end). The tracrRNA with proprietary chemical modifications, as well as the recombinant Cas9 nuclease were also purchased from IDT.

[0162] Prior to delivery of the nuclease into cells (method shown in Figure 5), the crRNA and tracrRNA were mixed at a 1:1 molar ratio. The mixture was heat-shocked at 95°C for 5 minutes and then incubated at room temperature for 30-60 minutes to allow crRNA: tracrRNA hybrids to form. Next, the crRNA:tracrRNA hybrids were mixed with the Cas9 nuclease at a 1.2:1 molar ratio and incubated at room temperature for 10-20 minutes to allow Cas9:crRNA:tracrRNA complexes to form. The Cas9 ribonucleoprotein (RNP) complexes were then delivered into cells by electroporation.

Single-stranded ODNs

[0163] All single-stranded ODNs (ssODNs) were commercially synthesized by IDT (Ultramer® DNA Oligonucleotides) with phosphorothioate linkages between the three nucleotides on the 5' end as well as the 3' end.

Production of recombinant AAV

[0164] AAV stocks were produced as previously described in Khan *et al.* (Khan IF, Hirata RK, Russell DW. AAV-mediated gene targeting methods for human cells. Nature Protocols (2011) 6, 482–501.; incorporated by reference in its entirety herein). The AAV vector, serotype 6 helper plasmid (pRepCap6) and HgT1-adenovirus helper plasmid were transfected into HEK293T cells. Cells were harvested 48 hours later, lysed by 3 freeze-thaw cycles, treated with benzonase, and the cell lysate was purified using an iodixanol density column.

Primary human CD19⁺ B cell genome editing

[0165] Peripheral blood mononuclear cells (PBMCs) collected from CD34⁺ negative selection flow-through from whole blood of healthy male donors post hematopoietic stem cell mobilization were purchased from Fred Hutchinson Cancer Research Center. Alternatively, PBMC were collected from healthy donors using an IRB approved protocol. CD19⁺ B cells were isolated from PBMCs by negative selection using a human B cell isolation kit (Miltenyi Biotec, Auburn, CA) and cultured in Iscove's modified Dulbecco's medium (IMDM, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum and 55μM beta-mercaptoethanol at 1-1.5×10⁶ cells/ml. B cells were activated with 100ng/ml of recombinant human MEGACD40L (Enzo Life Sciences), 1μg/ml of CpG oligodeoxynucleotide 2006 (Invitrogen), 50ng/ml of IL-2 (Peprotech), 50ng/ml of IL-10 (Peprotech) and 10ng/ml of IL-15 (Peprotech) for 48 hours. Cells were then electroporated with Cas9 RNP complexes using the Neon Transfection System (ThermoFisher Scientific) as follows. Cells were washed with PBS and resuspended in Neon Buffer T. 30.5pmol Cas9 RNP per 3×10⁵ cells was added to the resuspension so that the final cell density was 3×10⁷ cells/ml. Cells were then electroporated (1700V, 20ms, 1 pulse) in 10-μl Neon tips, and then transferred into pre-warmed B cell culture medium with MEGACD40L, CpG, IL-2, IL-10 and IL-15 and cultured at 1.5×10⁶ cells/ml. For samples transfected with an ssODN donor

template, ssODN was added concurrently with Cas9 RNP at 30 pmol per 3×10^5 cells, unless otherwise specified. For samples transduced with AAV, AAV was added to the culture immediately after electroporation. The added AAV volume was 20% of the cell culture volume, unless otherwise specified. Culture volume was doubled 24 hours after electroporation, and medium was replenished every two to three days thereafter to maintain a cell density of 1×10^6 cells/ml.

Flow cytometry

[0166] Flow cytometric analysis was done on an LSR II flow cytometer (BD Biosciences) and data were analyzed using FlowJo software (TreeStar). To assess B cell surface marker expressions, cells were stained with fluorophore-conjugated antibodies: CD19-PECy7 (clone HIB19, eBioscience), CD27-APC (clone O323, Life Technologies), CD20-PE (clone L27, BD Biosciences), CD38-PerCPCy5.5 (clone HIT2, BD Biosciences), CD138-Alexa Fluor 700 (clone MI15, BioLegend), HLA-DR-FITC (clone L243, BD Biosciences); CD19-PECy7 (clone HIB19, eBioscience), CD27-APC (clone O323, Life Technologies), CD20-FITC (clone L27, BD Biosciences), CD38-PerCPCy5.5 (clone HIT2, BD Biosciences), CD138-Alexa Fluor 700 (clone MI15, BioLegend), IgD-PE (clone IA6-2, BD Biosciences), IgM-Pacific Blue (clone MHM-88, BioLegend). Dead cells were excluded using Fixable Live/Dead stain-Alexa Fluor 350 (LifeTechnologies).

Plasma cell differentiation assays

[0167] In the alternatives herein, plasma cells were differentiated in vitro using a three-step culture system as previously characterized in Jourdan *et al.* (Jourdan M, Caraux A, De Vos J, Fiol G, Larroque M, Cognot C, Bret C, Duperray C, Hose D, Klein B. An in vitro model of differentiation of memory B cells into plasmablasts and plasma cells including detailed phenotypic and molecular characterization. *Blood* (2009) 114(25), 5173-5181; incorporated by reference in its entirety herein). CD19⁺ B cells isolated from PBMCs were activated for 2 days with MEGACD40L (100ng/ml), CpG (1μg/ml), IL-2 (50ng/ml), IL-10 (50ng/ml) and IL-15 (10ng/ml) and edited as outlined. Cells were then expanded for another 5 days in the same cocktail. Next, cells were washed and seeded in medium with IL-2 (50ng/ml), IL-6 (50ng/ml), IL-10 (50ng/ml) and IL-15 (10ng/ml) for 3 days. At day 10, cells

were washed and seeded in medium with IL-6 (50ng/ml), IL-15 (10ng/ml) and human interferon- α 2B (100U/ml, Sigma-Aldrich) for 3 days to stimulate plasma cell differentiation. At day 13, cell phenotypes were analyzed by immunofluorescence. To obtain a purified population of plasma cells, a BD FACSAria II cell sorter (BD Biosciences) was used to sort cells stained with CD27-APC (clone O323, Life Technologies) and CD138-Alexa Fluor 700 (clone MI15, BioLegend).

[0168] In the alternatives herein, experiments were performed to optimize the long-term maintenance of *in vitro* generated plasma cells in the culture. In order to do this, plasma cell viability were compared using the following culture systems or combinations thereof: i) plasma cell culture on M2-10B4 stromal cells as described in Cocco *et al.* (Cocco M, Stephenson S, Care MA, Newton D, Barnes NA, Davison A, Rawstron A, Westhead DR, Doody GM, Tooze RM. *In Vitro* Generation of Long-lived Human Plasma Cells. *J Immunol* (2012) 189(12), 5773-5785.; incorporated by reference in its entirety herein); ii) culture without stromal cells in media supplemented with combinations of IL-6 (10-50mg/mL), APRIL (200ng/mL), BAFF (200ng/mL), IGF1 (10ng/mL) and/or SDF-1 (200ng/mL) as described in Jourdan *et al.* (Jourdan M, Cren M, Robert N, Bolloré K, Fest T, Duperray C, Guilloton F, Hose D, Tarte K, Klein B. IL-6 supports the generation of human long-lived plasma cells in combination with either APRIL or stromal cell-soluble factors. *Leukemia* (2014) 28, 1647–1656.; incorporated by reference in its entirety herein). It is anticipated that these culture conditions will allow long-term maintenance of *in vitro* generated plasma cells for > 3 months.

Molecular analyses - PCR, T7, sequences

[0169] To assess the efficacy of guide RNA-CAS9 transfection for gene disruption applications in primary B cells, following transfection of PRDM1-targeting guide complexes, a variety of molecular analyses were used including a T7 endonuclease cleavage assay, Sanger sequencing and western blot. To sequence individual alleles, the DNA flanking the cleavage site was amplified via polymerase chain reaction (PCR) using locus-specific primers. Individual DNA molecules from this PCR reaction were cloned into bacterial plasmids, which were subsequently transformed to yield colonies, each expressing unique sequences. These were individually sequenced to assess the percentage of insertions or

deletions initiated by the PRDM1 guides (**Fig. 6**). As shown in the gel, an increase in the dose of RNP led to increased cleavage. In parallel, T7 endonuclease cleavage was used of the same locus-specific PCR products as described above. To do, so the PCR products were melted by heating to 90 degrees Celsius and allowed to reanneal by lowering the temperature. Treatment of these re-annealed products with T7 endonuclease, which cleaves mis-matched DNA, initiates the formation of higher mobility DNA that enabled visualization and quantification of insertion and deletion frequency (**Fig. 6**). Finally, western blots using antibodies that specifically bind PRDM1 demonstrate that disruption of the gene with PRDM1 guides also blocks protein expression from that locus (**Fig. 7**).

Target Guide RNA sequences

Target guide sequences used herein are as follows in the below table:

Gene	Sequence	SEQ ID NO:
PAX5	UGU GAA UGG ACG GCC ACU CC	2
PAX5	UGU AGU CCG CCA GAG GAU AG	3
IRF8	AUU GAC AGU AGC AUG UAU CC	4
IRF8	CGG AAA UGU CCA GUU GGG AC	5
BACH2	GUU CCU GCG CAU GCA CAA CC	6
BACH2	CUG UGA CGU GAC UUU GAU CG	7
CCR5	CAA UGU GUC AAC UCU UGA CA	8
CCR5	GCU GUG UUU GCG UCU CUC CC	9
CARD11	CAAUGACCUUACACUGACGC	10
PRDM1	UGAUGGCGGUACUUCGGUUC	11
PRDM1	AGGAUGCGGAUAUGACUCUG	12
PRDM1	GGGGAGCGAGUGAUGUACGU	13

Table 1: Guide RNA sequences

Methods for inducing tolerance of a peptide for immunotherapeutic application, secreted by a B cell in a subject in need in vivo and/or increasing engraftment of the B cell in a subject in need.

[0170] In some alternatives, a method of making B cells, plasma cells or plasma cell precursors that expresses a macromolecule is provided. The B cell or plasma cell can be administered in some alternative methods described herein, with a second cell that expresses a macromolecule that promotes tolerance of a protein or a peptide and/or promotes engraftment of the B cell or plasma cell. Thus, the two cells can be administered in parallel

in order to provide a subject with a therapeutic protein as well as a second protein that allows sustained protein replacement in a subject in need.

[0171] The method comprises (a) isolating B cells; (b) developing the B cells; (c) performing a first round of genome editing of the B cells for protein expression in absence of viral integration; (d) expanding the B cells; and (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells or plasma cell precursors that express the macromolecule. In some alternatives, the macromolecule is a protein, protein mimetic or peptide.

[0172] In some alternatives, the protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids.

[0173] In some alternatives, the B cells in step (a) comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. Mixtures of different types of B cells can also be utilized. As described herein, B cells can include B cell precursors, stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, activated B cells derived from any starting B cell population, plasmablasts (short-lived) cells, GC B cells, memory B cells, and/or long- or short-lived plasma cells and/or any mixtures or combinations thereof.

[0174] In some alternatives, the B cells in step (a) comprise memory B cells and/or naïve B cells.

[0175] In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation.

[0176] In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell.

[0177] In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration.

[0178] In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid.

[0179] In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells.

[0180] In some alternatives, the B cells are blood-derived human B cells.

[0181] In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection.

[0182] In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell.

[0183] In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMG, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1.

[0184] In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease.

[0185] In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9.

[0186] In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to the CAS nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the guide sequence comprises a sequence set forth in any one of SEQ ID NO: 2-13 or 63-112.

[0187] In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In other alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates.

[0188] In some alternatives, the first round of genome editing comprises transducing the B cell with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci.

[0189] In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary.

[0190] In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cell.

[0191] In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cell comprises disruption of an AID gene.

[0192] In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells.

[0193] In some alternatives, the increasing the proportion of gene edited B cells comprises the steps of : (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cell short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair.

[0194] In some alternatives, the second round of genome editing results in the excision of the IgM constant region.

[0195] In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells.

[0196] In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells.

[0197] In some alternatives, method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1.

[0198] In some alternatives, the IgM positive cells are removed by negative selection.

[0199] In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1.

[0200] In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers.

[0201] In some alternatives, the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers.

[0202] In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells.

[0203] In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine.

[0204] In some alternatives, the method further comprising introducing nucleic acid, wherein the nucleic acid encodes at least one cell surface protein.

[0205] In some alternatives, the at least one cell surface protein is CD20.

[0206] In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci.

[0207] In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned lengths.

[0208] In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells and/or any mixtures or combinations thereof..

[0209] In some alternatives, the isolating is performed by isolation of naïve or memory B cells.

[0210] In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections.

[0211] In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step.

[0212] In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15.

[0213] In some alternatives, the plasmablast differentiation step is performed in the presence any combination of IL-2, IL-6, IL-10 and/or IL-15.

[0214] In some alternatives, the plasma cell differentiation step is performed in the presence any combination of IL-6, IL-15, APRIL and/or IFN α .

[0215] In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein.

[0216] In some alternatives, the protein comprises an enzyme, monoclonal antibody or a binding portion thereof, neutralizing antibodies or a binding portions thereof, therapeutic antibodies or binding portions thereof, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein.

[0217] In some alternatives, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1, wherein the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1.

[0218] In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL.

[0219] In some alternatives, the protein is a receptor antagonist for treatment of periodic fever/autoinflammatory syndromes or complement inhibitory proteins.

[0220] In some alternatives, the compliment inhibitory protein is Factor H, Factor I or a C1 inhibitor.

[0221] In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1.

[0222] In some alternatives, the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer.

[0223] In some alternatives, the antibody is an anti-IL1 monoclonal antibody, anti-TNF antibody, anti-IL33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies.

[0224] In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN.

[0225] In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof.

[0226] In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs), or a binding portion thereof.

[0227] In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection.

[0228] In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed by a virus, fungus, parasite or bacteria.

[0229] In some alternatives, the protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection.

[0230] In some alternatives, the B cell or plasma cell manufactured by any one of the alternatives described herein is provided. In some alternatives, the B cell or plasma cell that expresses the macromolecule is provided to subject in need. In some alternatives, the subject has a disease. In some alternatives, the subject is in need of engraftment of the B cell or plasma cell. In some alternatives, a method of inducing tolerance of a peptide for immunotherapeutic application, secreted by a B cell in a subject in need *in vivo* and/or increasing engraftment or survival of the B cell in a subject in need.

[0231] In some alternatives, the method comprises administering the plasma cell or B cell manufactured by any one of the alternatives described herein, wherein the plasma cell or B cell expresses a macromolecule for therapy or a peptide for immunotherapy and administration of a second B cell. In some alternatives, the second B cell expresses a second macromolecule, wherein the second macromolecule promotes tolerance of a peptide or protein for therapeutic application and/or engraftment. In some alternatives, the second macromolecule comprises IFN-alpha, BAFF, APRIL, IL-10 or IL-6.

[0232] Gene editing can be performed to manufacture an active B cell line that also co-expresses inhibitory cytokines such as IL-10.

[0233] In some alternatives, of the methods for inducing tolerance, about 700K cells/mL for a subject in need of treatment or therapy, may be therapeutic. That equates to ~700 million cells per liter in a person. These cells will likely dissipate within a month, but could provide therapeutic benefit for much longer periods. For inflammatory disease, these

treatments would likely have to be repeated. For graft tolerization, it may be at least a one-time therapy.

Alternative 1: Editing of Primary B cells

[0234] As shown in **Figure 7**, primary B cells were edited. Peripheral blood mononuclear cells (PBMCs) collected from CD34+ negative selection flow-through from whole blood of healthy male donors post hematopoietic stem cell mobilization were purchased from Fred Hutchinson Cancer Research Center. Alternatively, PBMC were collected from healthy donors using an IRB approved protocol. CD19+ B cells were isolated from PBMCs by negative selection using a human B cell isolation kit (Miltenyi Biotec, Auburn, CA) and cultured in Iscove's modified Dulbecco's medium (IMDM, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum and 55 μ M beta-mercaptoethanol at 1-1.5 $\times 10^6$ cells/ml. B cells were activated with 100ng/ml of recombinant human MEGACD40L (Enzo Life Sciences), 1 μ g/ml of CpG oligodeoxynucleotide 2006 (Invitrogen), 50ng/ml of IL-2 (Peprotech), 50ng/ml of IL-10 (Peprotech) and 10ng/ml of IL-15 (Peprotech) for 48 hours. Cells were then electroporated with Cas9 RNP complexes using the Neon Transfection System (ThermoFisher Scientific) as follows. Cells were washed with PBS and resuspended in Neon Buffer T. 30.5pmol Cas9 RNP per 3 $\times 10^5$ cells was added to the resuspension so that the final cell density was 3 $\times 10^7$ cells/ml. Cells were then electroporated (1700V, 20ms, 1 pulse) in 10- μ l Neon tips, and then transferred into pre-warmed B cell culture medium with MEGACD40L, CpG, IL-2, IL-10 and IL-15 and cultured at 1.5 $\times 10^6$ cells/ml. For samples transfected with an ssODN donor template, ssODN was added concurrently with Cas9 RNP at 30 pmol per 3 $\times 10^5$ cells, unless otherwise specified. For samples transduced with AAV, AAV was added to the culture immediately after electroporation. The added AAV volume was 20% of the cell culture volume, unless otherwise specified. Culture volume was doubled 24 hours after electroporation, and medium was replenished every two to three days thereafter to maintain a cell density of 1 $\times 10^6$ cells/ml.

Alternative 2: PRDM1 disruption limits plasmablast generation

[0235] Plasma cell differentiation was performed as described in the section entitled “Plasma cell differentiation assays” of this paper. Sanger sequencing was used to calculate the total allelic disruption frequencies of PRDM1 in this experiment (69%). Based on these numbers, the expected biallelic disruption frequency was 49%. Because biallelic disruption of PRDM1 blocks plasmablast differentiation, it is expected that the number of plasmablasts to decrease ~49%. To quantify plasmablasts, flow cytometry was used. Flow cytometric analysis was done on an LSR II flow cytometer (BD Biosciences) and data were analyzed using FlowJo software (TreeStar). To assess B cell surface marker expressions, cells were stained with fluorophore-conjugated antibodies: CD19-PECy7 (clone HIB19, eBioscience), CD27-APC (clone O323, Life Technologies), CD20-PE (clone L27, BD Biosciences), CD38-PerCPCy5.5 (clone HIT2, BD Biosciences), CD138-Alexa Fluor 700 (clone MI15, BioLegend), HLA-DR-FITC (clone L243, BD Biosciences); CD19-PECy7 (clone HIB19, eBioscience), CD27-APC (clone O323, Life Technologies), CD20-FITC (clone L27, BD Biosciences), CD38-PerCPCy5.5 (clone HIT2, BD Biosciences), CD138-Alexa Fluor 700 (clone MI15, BioLegend), IgD-PE (clone IA6-2, BD Biosciences), IgM-Pacific Blue (clone MHM-88, BioLegend). Dead cells were excluded using Fixable Live/Dead stain-Alexa Fluor 350 (LifeTechnologies). Consistent with the hypothesis, the observed depletion of plasmablasts (CD38⁺/CD27⁺) was at 44%, which closely mimics the observed bi-allelic disruption frequency (**Figure 8**).

Alternative 3: Homologous-directed repair (HDR) using single-stranded DNA templates

[0236] Homologous directed repair was performed using single stranded ODNs. The ssODNs were commercially synthesized by IDT (Ultramer® DNA Oligonucleotides) with phosphorothioate linkages between the three nucleotides on the 5' end as well as the 3' end. For samples transfected with an ssODN donor template, ssODN was added concurrently with Cas9 RNP at 30 pmol per 3×10^5 cells, unless otherwise specified.

[0237] The expression of the Blimp-1 protein (PR domain zinc finger protein 1) in B lymphocytes can lead to an immune response through proliferation and differentiation of antibody secreting plasma cells. As schematized in **Figure 9**, the initial strategy for testing HDR in B cells was to use single-stranded oligonucleotides (ssODNs) to insert sequence into

the PRDM1 locus at Exon 5 (**Figure 9-14**). This site is shown in the light grey in Exon 5 of **Figure 9**. Varying amounts of Cas9:PRDM1-C with the single stranded ODNs were tested to see how they both can affect HDR rate. These were initially analyzed by PCR cloning combined with colony sequencing (Please see section entitled Molecular Analyses-PCT, T7, sequences). The sequencing traces showed the single base-change which were used to assess HDR, which was quantified (**Figure 10**, bold dark box under the table headed HDR rate). Next, allele-specific digital PCR was used to quantify HDR rates over a wider range of repair template concentration and found that 50pM ssODN resulted in the highest rates of HDR (**Figure 11**). In the same experiment, B cell viability was quantified by flow cytometry (See section entitled PRDM1 disruption limits plasmablast generation for methods of flow cytometry), demonstrating that although 30pM ssODN combined with 2.5uM guide RNA results in a decrease of viability at day 1, the cells recover by day 5 (**Figure 12**). As shown in **Figure 12**, the days are consecutively D1 followed by D5 on the bar graphs. Next, the ssODN approach was expanded to include larger oligonucleotides (schematized in **Figure 13**). As shown in **Figure 13**, the repair template is inserted within exon 5 at the cutsite. As shown in **Figure 14**, long template HDR is also effective in the editing of B cells, which has not been previously reported. In summary, there was surprisingly low toxicity as well as high-efficiency HDR as compared to using high titer AAV repair templates, which were shown to cause toxicity at 3×10^5 copies/cell.

[0238] The AAV repair template sequences are shown below as follows:

[0239] 1079_pscAAV-MND.GFP (SEQ ID NO: 14)

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1 AAGCTTCCCG GGGGATCTG GGCCACTCCC TCTCTGCGCG CTCGCTCGCT CACTGAGGCC
61 GGGCGACCAA AGGTCGCCCC ACGCCCGGGC TTTGCCCGGG CGGCCTCAGT GAGCGAGCGA
121 GCGCGCAGAG AGGGAGTGGC CAACTCCATC ACTAGGGGTT CCTGGAGGGG TGGAGTCGTG
181 ACCTAGGGAA CAGAGAAACA GGAGAATATG GGCCAAACAG GATATCTGTG GTAAGCAGTT
241 CCTGCCCCGG CTCAGGGCCA AGAACAGTTG GAACAGCAGA ATATGGGCCA AACAGGATAT
301 CTGTGGTAAG CAGTTCCTGC CCCGGCTCAG GGCCAAGAAC AGATGGTCCC CAGATGCGGT
361 CCCGCCCTCA GCAGTTTCTA GAGAACCATC AGATGTTTCC AGGGTGCCCC AAGGACCTGA
421 AATGACCCTG TGCCTTATTT GAACTAACCA ATCAGTTCGC TTCTCGCTTC GTTTCGCGCG
481 CTTCTGCTCC CCGAGCTCTA TATAAGCAGA GCTCGTTTAT TGAACCGTCA GATCGCCTGG
541 AGACGCCATC CACGCTGTTT TGACTTCCAT AGAAGGATCC TCGAGGCCAC CATGGTGAGC
601 AAGGGCGAGG AGCTGTTCAC CGGGGTGGTG CCCATCCTGG TCGAGCTGGA CGGCGACGTA
661 AACGGCCACA AGTTCAGCGT GTCCGGCGAG GGCGAGGGCG ATGCCACCTA CGGCAAGCTG
721 ACCCTGAAGT TCATCTGCAC CACCGGCAAG CTGCCCGTGC CCTGGCCCAC CCTCGTGACC
781 ACCCTGACCT ACGGCGTGCA GTGCTTCAGC CGCTACCCCG ACCACATGAA GCAGCACGAC
841 TTCTTCAAGT CCGCCATGCC CGAAGGCTAC GTCCAGGAGC GCACCATCTT CTTCAAGGAC
901 GACGGCAACT ACAAGACCCG CGCCGAGGTG AAGTTCGAGG GCGACACCCT GGTGAACCGC
961 ATCGAGCTGA AGGGCATCGA CTTCAAGGAG GACGGCAACA TCCTGGGGCA CAAGCTGGAG
1021 TACAACTACA ACAGCCACAA CGTCTATATC ATGGCCGACA AGCAGAAGAA CGGCATCAAG
1081 GTGAACTTCA AGATCCGCCA CAACATCGAG GACGGCAGCG TGCAGCTCGC CGACCACTAC

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1141 CAGCAGAACA CCCCCATCGG CGACGGCCCC GTGCTGCTGC CCGACAACCA CTACCTGAGC
1201 ACCCAGTCCG CCCTGAGCAA AGACCCCAAC GAGAAGCGCG ATCACATGGT CCTGCTGGAG
1261 TTCGTGACCG CCGCCGGGAT CACTCTCGGC ATGGACGAGC TGTACAAGTA AGCGGCCGCA
1321 ATTCACCCCA CCAGTGCAGG CTGCCTATCA GAAAGTGGTG GCTGGTGTGG CTAATGCCCT
1381 GGCCCAACAAG TATCACTAAG CTCGCTTTCT TGCTGTCCAA TTTCTATTAA AGGTTCCCTT
1441 GTTCCCTAAG TCCAAC TACT AAAGTGGGGG ATATTATGAA GGGCCTTGAG CATCTGGATT
1501 CTGCCTAATA AAAAACATTT ATTTTCATTG CAATGATGTA TTTAAATTAT TTCTGAATAT
1561 TTTACTAAAA AGGGAATGTG GGAGGTCAGT GCATTTAAAA CATAAAGAAA TGAAGAGCTA
1621 GTTCAAACCT TGGGAAAATA CACTATATCT TAAACTCCAT GAAAGAAGGT GAGGCTGCAA
1681 ACAGCTAATG CACATTGGCA ACAGCCCCTG ATGCCTATGC CTTATTCATC CCTCAGAAAA
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[0242] 1361_pAAV.Blimp1.1.0kb.MND.GFP.pA (SEQ ID NO: 17)

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5341	TGGGAACCGG	AGCTGAATGA	AGCCATACCA	AACGACGAGC	GTGACACCAC	GATGCCTGTA
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LOCUS #1366\CCR5.MND.B 6707 bp DNA circular SYN 10-MAR-2017

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LOCUS #1367\CCR5.MND.B 7494 bp DNA circular SYN 10-MAR-2017

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LOCUS #1378 8330 bp DNA circular UNA 09-AUG-2017

DEFINITION Gibson Assembly of mCherry T2A - coFIXpadua into 1367 XhoI+SaliI (6.2kb).

ORIGIN

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SEQ ID NO: 60

Description: pAAV CCR5.MND.IL6.Frun.T2A.GFP

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SEQ ID NO: 61

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Alternative 4: Testing Primary B cell Transduction with AAV

[0245] Collection of cells and transduction of the B cells with AAV are described in the section entitled “Primary human CD19⁺ cell gene editing” of this paper. Cells were also pretreated with neuraminidase at least two hours prior to transduction for the appropriate conditions. As shown in **Figure 15**, the AAV amount transduced into the cells varied by culture volume. As shown in the bar graphs the AAV is in order on the x axis as the AAV amount by culture volume at 2%, 5% and 10% consecutively. As shown, the percent GFP expressed with different AAV serotypes was increased using serotype AAV6. As shown in **Figure 16**, AAV6 transduction in primary B cells is more effective in activated B cells. As expected, larger AAV6 vectors (AAV6.MNDGFP with payload) exhibit lower transduction rates than smaller AAV6 vectors (**Figure 17**).

[0246] The strategy for homologous directed repair using AAV repair templates are shown in **Figure 18 and 20**. Briefly, peripheral blood mononuclear cells (PBMCs) collected from CD34⁺ negative selection flow-through from whole blood of healthy male donors post hematopoietic stem cell mobilization were purchased (**Figure 22**). CD19⁺ B cells were isolated from PBMCs by negative selection using a human B cell isolation kit (Miltenyi Biotec, Auburn, CA) and cultured in Iscove's modified Dulbecco's medium (IMDM, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum and 55μM beta-mercaptoethanol at 1-1.5×10⁶ cells/ml. B cells were activated with 100ng/ml of recombinant human MEGACD40L (Enzo Life Sciences), 1μg/ml of CpG oligodeoxynucleotide 2006 (Invitrogen), 50ng/ml of IL-2 (Peprotech), 50ng/ml of IL-10 (Peprotech) and 10ng/ml of IL-15 (Peprotech) for 48 hours. Cells were then electroporated with Cas9 RNP complexes using the Neon Transfection System (ThermoFisher Scientific) as follows. Cells were washed with PBS and resuspended in Neon Buffer T. 30.5pmol Cas9 RNP per 3×10⁵ cells was added to the resuspension so that the final cell density was 3×10⁷ cells/ml. Cells were then electroporated (1700V, 20ms, 1 pulse) in 10-μl Neon tips, and then transferred into pre-warmed B cell culture medium with MEGACD40L, CpG, IL-2, IL-10 and IL-15 and cultured at 1.5×10⁶ cells/ml. For samples transfected with an ssODN donor template, ssODN was added concurrently with Cas9 RNP at 30 pmol per 3×10⁵ cells, unless otherwise specified. For samples transduced with AAV, AAV was added to the culture

immediately after electroporation. The added AAV volume was 20% of the cell culture volume, unless otherwise specified. Culture volume was doubled 24 hours after electroporation, and medium was replenished every two to three days thereafter to maintain a cell density of 1×10^6 cells/ml.

[0247] To test HDR using the RNP-AAV approach, B cells were transduced under 3 conditions: (1) PRDM1 RNPs and AAV vector lacking PRDM1 homology arms (MND.BFP), (2) no RNP and AAV vector with PRDM1 homology arms (HDR-MND.GFP) and (3) PRDM1 RNPs and AAV vector with PRDM1 homology arms (HDR-MND.GFP). Shortly after transfection and transduction and following plasmablast differentiation, the degree of BFP and GFP were quantified in B cells using flow cytometry to determine the HDR frequency. As expected, AAV6-driven expression is evident at day 2 in all conditions, but stable GFP expression is only detectable in the condition that had matching RNPs and repair templates. As shown in **Figure 19 and 21**, this initial experiment shows that CRISPR and AAV repair template co-delivery mediates high-efficiency HDR in primary B cells.

Alternative 5: Optimization of plasma cell differentiation.

[0248] Another required step for edited plasma cell generation includes B cell expansion. Experimental set up is shown in **Figure 20**. As shown in **Figure 22**, B cells are negatively selected from healthy donor PBMCs. B cells were obtained using the B Cell Isolation Kit II from Miltenyi Biotec. The Non-B cells ($CD2^+$, $CD14^+$, $CD16^+$, $CD36^+$, $CD43^+$, and $CD235a^+$) are magnetically labeled and labeled non-B cells are retained in MACS column. The untouched B cells are then collected.

[0249] The culture and editing protocol comprises culturing isolated B cells in Mega- $CD40L^+$ CpG $^+$ IL-2 $^+$ IL-10 $^+$ IL-15 at 1.5×10^6 cells/ml for 48h. This is then followed by editing, transfecting and transducing the cells, which are then reseeded at $1.0 \times 10^6 - 1.5 \times 10^6$ cells/ml on day 2. The cells are then split on day 3, then every 2-3 days thereafter. The stimulation conditions are optimized for B cell activation, proliferation and RNA/protein delivery by electroporation with a naïve/memory B cell phenotype.

[0250] The cells were then phenotyped at the end of the expansion phase at day 9 during the culture. Shown in **Figure 23** is a flow cytometry analysis, which was gated for CD27 and CD138. Most of the cells remained CD138-CD27- after 9 days in culture, and this

population was primarily $CD19^{high}CD38^{low}CD20^{+}$, which is equivalent to naïve and activated B cells. The number of B cells were also shown to be at 8-10 fold greater at day 9 and were shown to have a viability of about 80—85% (**Figure 24**).

B cell differentiation into long-lived plasma cells

[0251] Cells were differentiated using a three step culture system as shown in **Figure 25**. Activation and proliferation steps included addition of MCD40L + CpG + IL-2 + IL-10 + IL-15 to the culture for the first 7 days. Afterwards, at phase II, IL-2 + IL-6 + IL-10 + IL-15 was added to the culture for plasmablast differentiation for the next three days. The final step, phase III, included addition of IL-6 + IL-15 + IFN α to allow for plasma cell differentiation. The cells were obtained and washed between the phases before addition of the culture additives.

[0252] The cells were tested before and after the B cell differentiation steps. Cells were subjected to FACs analysis gated for CD27 and CD138. In comparison of the pre differentiated and the post differentiated cells, at day 12, there is an increase of CD27+CD138+CD19neg CD38+ CD138+ Human PC cells (**Figure 26**). There is also an increase of viability of the cells by FSC/SSC on day 12 which was greater than 60-80%.

[0253] In summary, the alternatives provided herein established methods for efficient/non-toxic genome editing in primary human B cells using RNPs, and methods for efficient HDR (>30% using RNP and short ODN). There is also initial demonstration of HDR-mediated introduction of larger 2-4Kb expression cassettes (using RNP with long ODN or AAV) which has not previously been seen before for B cells. Furthermore, the alternatives have established sequential culture systems to generate and maintain human plasma cells.

Alternative 6: Demonstration of quantifiable secretion of an exogenous protein in an animal model

[0254] Naïve human B cells were isolated from PBMCs, and expanded in B cell activation cocktail (mCD40, CpG, IL2/10/15) for 2 days. At this point, 1 million cells were mock transfected or transfected with CCR5-targeting RNPs and transduced with homology-directed repair templates sufficient to deliver mCherry-Factor IX or eGFP-BAFF to the B cells. Following 5 additional days of activation, the cells were differentiated into plasmablasts for 3 days via incubation with IL2/6/15 and subsequently into plasma cells by

incubation with IL6/15 and IFN α . 3 million of this population of plasma cells from each condition was implanted into NSG mice (NOD, Cg-Prkcd^{SCID}, Il2rg^{tm1Wjl}/SzJ) using retroorbital injection. Following 7 days, a blood draw was taken from each mouse and the quantity of human IgG, human IgM and human BAFF was determined by ELISA. As observed in the left two panels, plasma cells expressing BAFF produce more IgM and IgG than unedited cells. Furthermore, human BAFF is quantifiable in these animals implanted with BAFF-edited plasma cells. (**Figure 27**)

Data showing sustained *in vivo* engraftment of gene-edited human plasma B cells and increased engraftment of edited B cells that express BAFF (introduced by HDR based gene editing).

[0255] Naïve human B cells from peripheral blood mononuclear cells were expanded in B cell activation cocktail (mCD40, CpG, IL2/10/15) for 2 days. At this point, 1 million cells were mock transfected or transfected with CCR5-targeting RNPs and transduced with homology-directed repair templates sufficient to deliver eGFP-BAFF to the B cells. Following 5 additional days of activation, the cells were differentiated into plasmablasts for 3 days via incubation with IL2/6/15 and subsequently into plasma cells by incubation with IL6/15 and IFN α . 10 million cells from this population from each condition was adoptively transferred into NSG mice (NOD, Cg-Prkcd^{SCID}, Il2rg^{tm1Wjl}/SzJ) via either retroorbital or intraosseous injection. At Day 10 (**Figure. 29A**) and 21 (**Fig. 29B**) following transplantation, serum abundance of human IgM, human IgG and hBAFF was measured by ELISA. Comparison of **Figure 29A** and **Figure 29B** show sustained expression of human proteins in murine serum between 10 and 21 days, indicating that edited human plasma cells are stably engraftable in mice. Further, these data show that secretion of hBAFF by gene-edited plasma cells enhances PC engraftment. (**Figure 28**).

Alternative 7: Demonstration of the ability of B cells to produce functionally active Factor IX

[0256] Naïve human B cells were isolated from PBMCs, and expanded in B cell activation cocktail (mCD40, CpG, IL2/10/15) for 2 days. At this point, 1 million cells were mock transfected, RNP transfected, AAV transduced or transfected with CCR5-targeting RNPs and transduced with homology-directed repair templates sufficient to deliver Factor IX

to the B cells. Following 5 additional days of activation, the cells were differentiated into plasmablasts for 3 days via incubation with IL2/6/15 and subsequently into plasma cells by incubation with IL6/15 and IFN α . 6 days prior to the termination of the study, 1 million cells per mL from each condition were incubated with Vitamin K1 (5ug/mL). The supernatants from all conditions were subjected to a chromogenic assay of Factor IX activity. Using this assay, it was found that plasma cells edited to integrate exogenous Factor IX produce enzymatically active protein.

Alternative 8: Assessing the impact of disruption and survival

[0257] The objective of the further experimentation was to determine the impact of gene disruption on the survival and differentiation of the cells. Also performed, was a combinatorial gene disruption with HDR at CCR5 (FIX) (**Figure 30**).

[0258] For the first phase at -2 days, B cells were isolated. At day 0, the cells were edited with a RNP/AAV vector (phase 1). In the first phase, the plasma cell differentiation culture comprised MCD40L, CpG, and a mixture comprising IL2/IL10/IL15. The first phase of plasma cell differentiation was carried out for 5 days. At phase 2 (3 days), the plasma cell differentiation culture comprised IL2/IL6/IL15. At phase 3 (3 days), the plasma cell differentiation culture comprised IFN- α , IL6 and IL15.

Alternative 9: Long lived human antibody production in mice

[0259] Primary B cells were expanded in culture and edited as described. Following differentiation into plasma cells, 10 million were injected into NSG mice using either retro-orbital (RO) or intra-osseous (IO) injection. The mice were bled at the indicated time points. Human IgG (IgM not shown) was quantified using ELISA. Each dot represents data from an individual recipient mouse at the indicated time point. These data show that gene edited human plasma cells can live for long periods in this murine model despite the absence of human cytokines. These data also show that autocrine production of human BAFF can further promote sustained survival of gene edited PCs.

[0260] Several groups of mice were under four editing conditions: group 1) mock unedited cells; group 2) cells edited with the CCR5-RPN + AAV-GFP-BAFF; group 3) Mock unedited cells and group 4) CCR5-RNP + AAV-GFP-BAFF. Groups 1 and 2 were administered the cells by retro-orbital injection (10E+06 cells/ animal) and groups 3 and 4

were administered the cells by intra-osseous injection ($10E+06$ cells/ animal). Each group had 5 mice. As shown, the sets of cells from left to right under the “no cells” area are the week one, week 3, week 5, week 5, week 7, week 9, week 11, week 13, week 15, week 17 and week 19 cells cells, this order is kept throughout the x-axis for mock IO, BAFF IO, Mock RO and BAFF RO. The mock unedited cells (Group 1) have minimal expression of the IgG. However, cells of group 2 have an increase of IgG expression that peaks at week 3 and 5. The cells of Group 3 (mock unedited) also show minimal expression of IgG, whereas the cells of Group 4 have an increase of IgG that peaks at week 3 and 5. As shown, cells administered with the CCR5-RNP with the AAV-GFP-BAFF with either retro-orbital injection or intra-osseous injection show long-lived human antibody production in mice.

[0261] Primary B cells were expanded in culture and edited as described. Following differentiation into plasma cells, 10 million were injected into NSG mice using either retro-orbital (RO) or intra-osseous (IO) injection. The mice were bled at the indicated time points. Human IgG (IgM not shown) was quantified using ELISA. Each dot represents data from an individual recipient mouse at the indicated time point. These data show that gene edited human plasma cells can live for long periods in this murine model despite the absence of human cytokines. These data also show that autocrine production of human BAFF can further promote sustained survival of gene edited PCs. (**Figure 31**).

Alternative 10: Expression of human IL6 in mice confers a growth advantage for transplanted ex vivo differentiated human plasma cells.

[0262] Neonatal NSG mice were injected with lentiviral vectors expressing human IL6 and used for adoptive cell transfer at approximately 8-12 wk of age. Primary human B cells were expanded in culture and edited as described (mock or BAFF delivered to the CCR5 locus). Following differentiation into plasma cells, 10 million were injected into NSG mice using retro-orbital (RO) injection. The mice were bled at the indicated time points. Human BAFF or IgM was quantified using ELISA. Each dot represents data from an individual recipient mouse at the indicated time point. These data show that human IL6 secretion can confer an *in vivo* survival advantage to transplanted human plasma cells and that this can synergize with autocrine expression of human BAFF in gene edited cells (**Figure 32**).

Alternative 11: High IL6 expression correlates with high plasma cell secretion.

[0263] Neonatal NSG mice were injected with lentiviral vectors expressing human IL6 and used for adoptive transfer of human cells at 8-12 wks of age. Primary human B cells were expanded in culture and edited as described (mock or BAFF delivered to the CCR5 locus). Following differentiation into plasma cells, 10 million were injected into NSG mice using either retroorbital (RO) injection. The mice were bled at the indicated time points. Human IL6, BAFF or IgM was quantified using ELISA. Each dot represents data from an individual recipient mouse at the indicated time point. These data demonstrate that the levels of human IL6 directly correlated with the levels of human protein production by the gene-edited transplanted cells, and likely the number of surviving long-lived plasma cells (**Figure 33**).

Alternative 12: *In vitro* human APRIL treatment correlates with increased plasma cell antibody secretion *in vivo*.

[0264] Primary B cells were expanded in culture and edited so that BAFF or mock is delivered to the CCR5 locus. During differentiation into plasma cells, the cells were cultured in the presence of trimeric APRIL (Mega-April) or mock. After 12 days in culture, IgM and IgG secretion was quantified by ELISA. Each dot is from an experiment from an individual donor. These data show that April promotes antibody production in in vitro derived gene-edited human plasma cells. (**Figure 34**). Shown in **Figure 35**, are the results of the gene disruption and protein expression.

Alternative 13: Targeting of abundant plasma cell genes using CRISPR.

[0265] Primary human B cells were expanded in culture and edited to disrupt the indicated loci. 5 days following transfection, genomic DNA was harvested for amplification and quantification of insertions and deletions using a T7 endonuclease I cleavage assay. As demonstrated and quantified, multiple guides successfully disrupt the target loci. (**Figure 36**).

Alternative 14: Multiplexed genome editing to introduce FIX and increase protein secretion in human plasma cells.

[0266] Primary human B cells were expanded in culture and edited to disrupt the indicated loci. Using multiplexed editing, FIX was also introduced into the CCR5 locus by homology-directed repair. After three-step differentiation into plasma cells, IgG and FIX were quantified by ELISA. These data show that knocking out a subset of highly expressed genes, including the heavy chain (IGMC), JCHAIN and CRELD2, production of IgG and FIX in gene edited plasma cells can be increased. (**Figure 37**).

Alternative 15: Knock-out of BANK1 promotes plasma cell differentiation and antibody production.

[0267] Primary human B cells were expanded in culture and edited to disrupt BANK1. After three-step differentiation into plasma cells, IgG and IgM were quantified by ELISA. These data show that knocking out the B cell developmental regulator BANK1, IgG and IgM can lead to increased production of in gene edited plasma cells. (**Figure 38**). Consistent with these data disruption of BANK1 also promotes PC differentiation.

Alternative 16: Long-lived plasma cells secrete more immunoglobulin on a per cell basis.

[0268] Primary human B cells were expanded in culture. After three-step differentiation into plasma cells, plasma cells (CD138 enriched cells) were cultured for an additional 3 weeks in the presence of IFN-beta, IL6, IL15 and trimeric APRIL. Three days following a media change, IgG was quantified by ELISA at day 11 and at 4 weeks. These data show that long-lived plasma cells produce substantially more IgG on a per cell basis (n = 2 donors). (**Figure 39**).

Alternative 17. In vivo delivery of human APRIL or IL6 enables increased long-term secretion by edited long-lived plasma cells

[0269] Neonatal NSG mice were injected with lentiviral vectors expressing human IL6 and/or APRIL and used for adoptive cell transfer at approximately 8-12 wk of age. Primary human B cells were expanded in culture and edited as described (mock or BAFF delivered to the CCR5 locus). Following differentiation into plasma cells, 10 million were injected into NSG mice using retro-orbital (RO) injection. The mice were bled at the indicated time points. Human BAFF was quantified using ELISA. Each dot represents data from an individual recipient mouse at the indicated time point. These data show that human

IL6 and APRIL secretion can confer increase the long-term production of an exogenously delivered protein (BAFF) by edited human plasma cells. (Figure

Alternative 18: Engineering protein-secreting plasma cells by homology-directed repair in primary human B cells

[0270] A method is presented for high-efficiency, homology-directed genome editing in primary human B cells for the purposes of producing therapeutic proteins.

[0271] B cells differentiate into long-lived plasma cells that provide humoral immunity by secreting large quantities of antibodies. The ability to engineer primary human B cells to secrete a *de novo* protein may allow the creation of novel plasma cell therapies for protein deficiency diseases and other clinical applications. To achieve this goal, methods for efficient genome editing of primary B cells isolated from peripheral blood were developed, followed by *ex vivo* differentiation into plasma cells. By delivering CRISPR/Cas9 ribonucleoprotein (RNP) complexes under conditions of rapid B cell expansion, site-specific gene disruption at multiple loci in primary human B cells was achieved (with editing rates up to 94%). This method was first used to modulate plasma cell differentiation by disrupting key developmental regulatory genes. Next, RNPs were co-delivered with either single-stranded DNA oligonucleotide or adeno-associated viruses containing a homologous repair template. Using either delivery method, targeted sequence integration at high efficiency (up to 40%) via homology-directed repair was achieved. This method enabled engineering of plasma cells to secrete B-cell activating factor (BAFF) or factor IX (FIX) at high levels. These results highlight the utility of genome editing in studying human B cell biology and demonstrate a novel strategy for modifying human plasma cells to secrete therapeutic proteins.

[0272] Long-lived plasma cells stably reside in the bone marrow for decades and secrete large quantities of antibodies. Consequently, plasma cells engineered to produce *de novo* proteins have the potential to be curative therapies for protein deficiency diseases, prophylaxis for infectious diseases and many other applications. However, the development of plasma cell therapeutics has been limited by technical challenges in the *in vitro* modification, culture, expansion and differentiation of primary human B cells. B cells can be transduced at high rates by recombinant adenovirus or Epstein-Barr virus (EBV) vectors, which deliver transgenes as episomes. However, episomal DNA expression is lost over time, limiting use of these vectors in applications that require long-term transgene expression.

Unlike non-integrating vectors, gamma retrovirus (γRV) and lentivirus (LV) randomly integrate into the host genome and can be used to introduce stably expressing transgenes. However, these vectors are inefficient at transducing primary human B cells. LV that employ alternative envelopes, including that of baboon retrovirus, measles virus, or gibbon-ape leukemia virus exhibit higher B cell transduction rates (up to ~50%), but have low viral titers that make large-scale production challenging. Because γRV and LV vectors do not efficiently transduce B cells while transduction by non-integrating vectors results in only transient transgene expression, neither platform is currently effective for delivering long-term expression of exogenous genes to B cells on a therapeutic scale.

[0273] An alternative method for introducing stable protein expression is genome editing via homology-directed repair (HDR). Following cleavage by an engineered site-specific nuclease, DNA double-strand breaks were resolved through non-homologous end joining (NHEJ), an error-prone DNA repair pathway that typically leads to variable insertions or deletions (indels), or HDR, which repairs DNA by copying a homologous donor template. Delivery of exogenous DNA flanked by DNA homologous to the genomic sequence around the break site can lead to incorporation of the exogenous sequence in a site-specific manner. HDR-mediated genome editing in B cells may have several advantages over viral vector transduction for therapeutic applications, including decreased risk of insertional mutagenesis and sustained transgene expression. Many have recently achieved high-efficiency HDR delivery of therapeutic transgenes to hematopoietic cells including primary human T cells and hematopoietic stem cells, but similar approaches are yet to be applied in modification of primary human B cells.

[0274] The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system is an RNA-guided nuclease platform that is easily engineered to efficiently target specific sites in the genome for cleavage, generating double-strand DNA breaks. The use of site-specific nucleases for gene disruption or HDR in B cells is currently limited to transformed or lymphoma-derived cell lines and murine models and has required plasmid- or LV-based CRISPR/Cas9 delivery. Described herein is a method of high-efficiency genome editing in human peripheral blood B cells (75-90% gene disruption or 10-40% HDR) by delivering CRISPR/Cas9 ribonucleoprotein (RNP) complexes alone or in combination with single-stranded DNA oligonucleotide (ssODN) or

adeno-associated virus (AAV) repair templates, respectively. In the alternatives herein it is shown that edited primary B cells can subsequently be differentiated in culture into plasma cells that produce physiological doses of therapeutic proteins including human Factor IX (FIX).

Conditions for expansion of primary naïve human B cells

[0275] Rapid cell cycling and/or persistence in the S/G2 phases of the cell cycle promote HDR in both cell lines and primary hematopoietic cells. Based on previous reports demonstrating rapid expansion of primary human B cells *ex vivo*, a combination of stimulants (hereafter called "B cell activation cocktail") was initially used that included artificially oligomerized CD40 ligand (MEGACD40L; two linked CD40L trimers) in association with CpG, IL2, IL10, and IL15. Primary human CD19⁺ B cells from peripheral blood mononuclear cells (PBMCs) were isolated and cultured them for 13 days with this B cell activation cocktail. This approach resulted in a ~36-fold expansion of B cells (**Figure 40A**), while preserving viability at > 60% (**Figure 40B**). Despite having proliferated over 13 days, most cells maintained a naïve B cell phenotype (CD27⁻CD138⁻CD38^{low/-}CD19^{high}IgM⁺IgD⁺; **Figure 40C**), demonstrating that the B cell activation cocktail facilitates rapid cycling and *ex vivo* maintenance of naïve primary human B cells.

Cas9-mediated disruption of CCR5 and PRDM1 in primary human B cells

[0276] To assess the efficiency of Cas9-induced indels in B cells, CRISPR guide RNAs (gRNAs) were designed that target *CCR5* (which is not expressed in human B cells and has no known significance in plasma cell development) or *PRDM1* (encoding BLIMP1, a protein required for B cell differentiation into plasma cells). After optimizing electroporation based upon mRNA (**Figure 41**), 30 pmol Cas9-guide ribonucleoprotein (RNP) complexes were transfected into B cells, cultured for five additional days under activating conditions and extracted total genomic DNA to assess nuclease- induced indels using the T7 endonuclease 1 assay (**Figure 42**). Sequencing confirmed on-target indels and revealed that the RNPs induced high indel frequencies at each guide target site (43-84%; **Figure 42A**). Western blot also verified concomitant PRDM1 protein reduction in the PRDM1-expressing TMD8 lymphoma cell line (**Figure 43A**). Finally, it was found that B

cells remained viable despite these genome modifications (**Figure 43B**). These data show that high-efficiency Cas9-mediated gene disruption is achievable in primary human B cells.

HDR-mediated single-nucleotide substitution at the PRDM1 locus using an ssODN donor template

[0277] Cas9-induced DNA lesions can be seamlessly repaired via the HDR pathway in the presence of a donor template with homology sequences flanking the lesion. An ssODN was initially tested as a donor template based on promising results in other cell types. A 120-base ssODN was designed containing asymmetric homology arms (89 bases 5' and 30 bases 3') that flank the PRDM1g-2 target site, with a single-nucleotide change at the 90th position that mutates the last nucleotide of the protospacer adjacent motif (PAM; GGG to GGT; **Figure 42B**). This single nucleotide change was designed to both prevent Cas9-mediated cleavage of the repaired sequence and to serve as a molecular marker for HDR. In this experiment, primary human B cells were activated for two days and then transfected with Cas9 RNP in conjunction with various doses of the ssODN. Two and five days following transfection, cells transfected with ≤ 30 pmol ssODN had comparable viabilities to Cas9 RNP transfected control cells (**Figure 42C**). In cells receiving 15 or 30 pmol ssODN, sequencing of the *PRDM1* target region on day 5 post-transfection revealed 20-22% of alleles had undergone HDR while another 37-41% had indels (**Figure 42D**), an overall editing rate marginally higher than that observed in the Cas9 RNP control (**Figure 42D**). Thus, ssODN donor templates can be used to achieve high rates of HDR in primary human B cells with low cytotoxicity.

Cas9-mediated disruption of genes that regulate plasma cell development

[0278] It was next investigated whether Cas9-induced gene disruption can be used to study gene roles in human plasma cell development and antibody production. To do so, an experimental workflow was developed that includes inducing NHEJ-mediated gene disruption in activated naïve human B cells using Cas9 RNPs and subsequently differentiating naïve B cells into plasma cells (CD19^{low}CD38^{high}CD27⁺CD138⁺) using a three-step plasma cell culture system (see **Figure 44A**). As a proof of concept, four genes were studied encoding transcription factors previously reported to regulate plasma cell development in murine studies: IRF4 and PRDM1/BLIMP1 are required for plasma cell

differentiation (**Figure 44B**), while PAX5 and BACH2 antagonize plasma cell differentiation in mice (**Figure 44B**). CRISPR guides were designed targeting each of these four genes, and were transfected into primary B cells with Cas9 RNP targeting each gene independently and subsequently induced *in vitro* plasma cell differentiation (**Figure 44A**). As a control, cells were also transfected with Cas9 RNPs targeting *CCR5*. Following differentiation, significantly lower percentages of CD19^{low}CD38^{high} and CD27⁺CD138⁺ plasma cells were detected in cultures transfected with *PRDM1*- or *IRF4*-targeting RNPs compared to cells transfected with *CCR5*-targeting RNP or mock transfected cells (mean indel percentages = 89%, 83% and 92% at the *PRDM1*, *IRF4* and *CCR5* target sites, respectively; **Figure 44C, 44D**). As antibody secretion is a major function of plasma cells, IgM and IgG levels were measured in cell culture supernatants by enzyme-linked immunosorbent assay (ELISA) and found IgG to be significantly decreased in the *PRDM1*- and *IRF4*-targeted B cell cultures (**Figure 44E**). Together, as predicted, these data imply that both PRDM1 and IRF4 are required for human plasma cell differentiation and antibody production. Conversely, significant increases in the percentages of plasma cells in cultures transfected with either *PAX5* or *BACH2*-targeting RNPs were observed (mean indel percentages = 80% and 86% at the *PAX5* and *BACH2* target sites, respectively; **Figure 44C, 44D**). Concomitant increases in IgM and IgG secretion in *PAX5*-targeted and an increase in IgM secretion in *BACH2*-targeted cultures were also observed (**Figure 44E**). Again, as predicted, these data indicate that Cas9-mediated disruption of *PAX5* or *BACH2* enhances human plasma cell differentiation. Further, the IgG/IgM ratio in *BACH2*-targeted B cell cultures was markedly lower than mock or *CCR5*- targeted cultures (**Figure 44E, Figure 45A**) and plasmablasts in *PAX5*-targeted cultures exhibited decreased surface CD19 expression (**Figure 45B**), both consistent with previous studies in mice. Taken together, these results demonstrate that CRISPR/Cas9-induced gene disruption in primary B cells is useful for interrogating gene products that may modulate human plasma cell development and function.

Site-specific HDR at the PRDM1 locus using co-delivery of RNP and AAV donor template

[0279] HDR using ssODN donor templates is not suitable for delivering payloads larger than ~400 bases due to current limitations of the fidelity of ssODN synthesis. In

contrast, adeno-associated virus (AAV) can package up to ~4.7 kilobases (kb) of ssDNA donor template. Many have used AAV to deliver candidate HDR templates leading to high levels of HDR in multiple cell types and at a variety of loci. Of note, there is no published data regarding the capacity of AAV to transduce primary human B cells. To investigate AAV transduction efficiency in human B cells, a self-complementary AAV (scAAV) was designed with a green fluorescent protein (GFP) coding sequence driven by MND, a robust retroviral-derived ubiquitous promoter. Activated B cells was transduced with this vector packaged using various serotypes and quantified GFP expression two days post transduction by flow cytometry. Regardless of serotype, it was observed minimal loss of cell viability following viral exposure (**Figure 46A**). It was observed the highest percentage of GFP⁺ cells (mean = 43%) and highest mean fluorescence intensity in B cells transduced with AAV serotype 6 (**Figure 47A**).

[0280] Because the packaging size of single-stranded AAV (ssAAV) is greater than scAAV, the efficacy of HDR-mediated integration of larger payloads was next assessed by delivering ssAAV into B cells. Initially, an AAV6 donor template containing an MND-driven GFP cassette flanked by 400 bp homology arms at the PRDM1g-2 target site was designed (PRDM1-GFP; **Figure 47B**). The PAM was not included in the repair template, thereby rendering the repaired sequence non-cleavable by Cas9 (**Figure 47B**). After activation and electroporation with or without Cas9 RNP, B cells were transduced with AAV6 PRDM1- GFP at various MOI and cultured for 11 additional days with the same activating factors. Although it was initially observed a 30% viability loss at the highest AAV dose, these cultures eventually recovered and exhibited comparable viabilities by day 11 (**Figure 46B**). At the highest AAV MOI, it was observed that there was persistent GFP expression in ~10% of cells that received both the *PRDM1*-targeting RNP and AAV repair template, while 2% of cells that were treated with the AAV alone had persistent GFP expression (**Figure 47C**). In addition, cells were co-treated with the *PRDM1*-targeting RNP and a non-targeting AAV BFP control (MND-BFP without homology arms; BFP-noHA) and observed ~1% BFP expression at the endpoint (**Figure 47C**), indicating that the majority of GFP expression resulting from co- delivery of *PRDM1*-targeting RNP and PRDM1-GFP was likely driven by HDR-mediated integration. It was also found that higher AAV doses correlated with increased percentages of GFP⁺ cells. Finally, it was found that increasing

homology arm lengths from 400bp to 1.0kb did not lead to higher levels of HDR (measured as persistent GFP expression; **Figure 48A-48B**). Together, these data support the conclusion that co-delivery of Cas9 RNP and AAV can lead to efficient, targeted genomic integration of transgene, likely via the HDR pathway.

Introduction of a BAFF expression cassette into CCR5 leads to secretion of functionally active BAFF

[0281] It was next assessed whether primary B cells could be engineered to secrete active proteins with predicted functionality. As a first test of this approach, HDR-mediated knock-in strategy to engineer *de novo* expression of the B-cell activating factor (BAFF) was used. Of note, the HDR studies targeting the *PRDM1* locus (described above) were anticipated to underrepresent HDR efficiencies due to the negative impact of *PRDM1* disruption on plasma B cell differentiation and the relative inefficiency of the guide used in the studies (PRDM1g-2; **Figure 42A**). Therefore, the *CCR5* locus was targeted as a “safe harbor” because: (a) it is not transcriptionally active in human B cells; (b) it is not required for plasma cell differentiation (**Figure 44A-44D**); and (c) heterozygous and homozygous null mutations in *CCR5* are innocuous to human carriers. Two AAV6 repair templates were designed: one contained MND-driven GFP and BAFF linked with a T2A self-cleaving peptide (CCR5-GFP-BAFF) and the other contained only an MND-BAFF expression cassette (CCR5-BAFF). Both donor constructs contained 800 bp homology arms around the *CCR5* guide target site (**Figure 49A**). Following RNP transfection and/or AAV transduction, cells were cultured using the three-step plasma cell culture system (**Figure 44A**). As in earlier *PRDM1*-targeting experiments, persistent GFP expression was observed (mean = 33% GFP⁺) only in the condition containing both *CCR5*-targeting RNP and AAV (**Figure 49B**, **Figure 50**). While use of both donor templates led to BAFF secretion, B cells targeted by CCR5-BAFF (repair template solely containing BAFF) exhibited much higher levels of BAFF secretion (4.1 fold increase) relative to those targeted by CCR5-GFP-BAFF (template containing BAFF downstream of the T2A linkage) (**Figure 49C**). To determine if this exogenous BAFF is functional, cell expansion and plasmablast formation was assessed following genome editing and *in vitro* differentiation. In the CCR5-BAFF edited culture that contained the highest concentration of exogenous BAFF, increases in cell number was

observed, viability and percentage of CD19^{low}CD38^{high} plasmablasts relative to controls that lacked Cas9 RNP (**Figure 49D-49E**). This is consistent with previous studies showing that BAFF promotes both human and murine B cell survival and differentiation. Taken together, these results show that Cas9 RNP- and AAV-mediated genome editing of primary human B cells can be used to generate functional, protein-secreting human plasma cells.

Generation of FIX-secreting human plasma cells via HDR-mediated integration at the CCR5 locus

[0282] Engineering exogenous protein production in plasma cells may have therapeutic applications in protein deficiency diseases such as hemophilia B, which is caused by a deficiency of FIX. To generate plasma B cells capable of secreting exogenous human FIX, an AAV vector containing an MND-driven FIX (codon-optimized FIX-R338L Padua variant) expression cassette was co-delivered, with the same flanking CCR5 homology arms as described above (CCR5-FIX), into primary human B cells with or without *CCR5*-targeting RNP (**Figure 51A**). To also boost plasma cell differentiation in these cultures, *PAX5*-targeting RNPs were also co-delivered in a subset of CCR5-targeted cells. Although similar rates of HDR at the *CCR5* locus across conditions were observed (**Figure 51B**), following plasma cell differentiation increases in CD19^{low}CD38^{high} plasmablast percentages in the cultures that received *CCR5*- and *PAX5*-targeting RNPs were observed (**Figure 51C**). With or without delivery of *PAX5*-targeting RNPs, high levels of *de novo* FIX secretion in the cultures were detected (**Figure 51D**). In addition, HDR cultures co-treated with both *CCR5*- and *PAX5*-targeting RNPs exhibited similar FIX production in dually-edited cell populations (**Figure 51D**). These data demonstrate FIX production via targeted HDR in gene-modified human B cells and the use of multi-locus modifications to simultaneously drive plasma cell differentiation and *de novo* protein secretion.

BAFF-secreting human plasma cells exhibited high secretory capabilities in NSG mice

It was then tested whether autocrine BAFF secretion can promote plasma cell survival in humanized mice. Gene-edited B cells were first generated using the *CCR5*-targeting RNP and the CCR5-GFP-BAFF AAV donor template. These cells were differentiated into plasma cells *in vitro* using the three-step culture system as before and

subsequently transplanted into immuno-deficient NOD/SCID/gamma-c null (NSG) mice via intravenous infusion (**Figure 54A**). Equal numbers of unedited plasma cells were also transplanted into parallel recipient mice as controls. Blood samples were collected at days 10 and 21 and serum human proteins were quantified by ELISA (**Figure 54A**). As expected, human BAFF was detected in mice that received BAFF-expressing plasma cells, but not in mice that received unedited plasma cells (**Figure 54B**). Also as predicted, human IgM and IgG were detected only in mice that received plasma cells but not in the untreated mice (**Figure 54C**). It was also found that serum BAFF and IgM levels remained stable from day 10 to day 21, while IgG levels significantly increased in this period (**Figures 54B, 54C**). Most strikingly, significantly higher levels of serum IgM and IgG were observed in mice that received BAFF-expressing plasma cells compared to mice that received unedited plasma cells (**Figure 54C**; difference in IgG levels at day 10 was not significant). These findings demonstrate that gene-modified plasma cells maintain stable secretory capacities in an immune-compromised murine setting for at least 3 weeks and support the hypothesis that expression of a survival factor such as BAFF via genome editing promotes plasma cell secretory function and/or survival *in vivo*.

[0283] As described, high rates of gene disruption in primary human B cells at multiple loci using CRISPR/Cas9 RNPs were accomplished. This method is applied to modeling the impact of genetic changes on human plasma B cell differentiation. It also demonstrates the use of Cas9 RNP in combination with ssODN or AAV6 repair templates to achieve high-efficiency HDR in B cells and to engineer secretion of functional and therapeutically relevant proteins. Importantly, gene-modified cells obtained a plasma cell phenotype and remained viable for several weeks in culture, providing evidence of utility for adoptive cell therapies using engineered human plasma cells.

[0284] Cas9-mediated disruption of *PRDM1*, *IRF4*, *PAX5*, or *BACH2*, which either promote (*PRDM1*, *IRF4*) or inhibit (*PAX5*, *BACH2*) plasma cell differentiation in mice, led to human B cell phenotypes consistent with these regulatory roles. The ability to easily disrupt genes or induce single-base changes using Cas9 RNP with or without ssODN-mediated HDR will facilitate further study of the impact of genetic changes on human B cell development. When combined with differentiation *in vitro*, this system is useful as a high-throughput model for studying genetic mutations that alter human B cell development,

including somatic and/or germ-line mutations associated with a range of human B cell disorders (in B cell lymphoma and in autoimmune disorders including systemic lupus erythematosus); as well as a range of mechanistic studies designed to assess candidate genes identified by genome-wide association studies.

[0285] The results of the alternative methods described herein show that Cas9 RNP and ssODN or AAV co-delivery into B cells leads to high levels of HDR with minimal toxicity. Compared to double-stranded DNA transfection, which promotes apoptosis in primary B and T cells, single-stranded DNA delivery, either as ssODN or recombinant AAV, is relatively non-toxic to primary B cells. It is hypothesized that both approaches may be protected from cyclic GMP-AMP synthase (cGAS)-dependent type I interferon responses prevalent in primary B cells. Consistent with this, compared with dsDNA, ssDNA exhibits significantly lower binding affinity for the cytosolic DNA sensor cGAS.

[0286] The development of an alternative method for the efficient, site-specific introduction of transgenes via HDR opens the possibility of engineering plasma cells to act as autologous “cell factories,” capable of delivering sustained, high doses of therapeutic proteins to subjects. As a proof of concept, plasma cells were engineered to secrete FIX. Deficiency of FIX is the cause of hemophilia B, a genetic disease characterized by blood clotting defects. Current treatment for hemophilia B is limited to protein replacement therapy, which is costly and non-curative. Recent gene therapy trials reported long-term FIX expression in patients after intravenous injection of a liver-tropic AAV8-FIX vector; however, pre-existing AAV neutralizing antibodies to all known AAV serotypes are prevalent in humans and greatly limit *in vivo* AAV transduction, making a significant group of patients with anti-AAV antibodies ineligible for treatment. In addition, systemic AAV delivery induces humoral immunity against AAV that prevents subsequent delivery, thus limiting AAV therapy to a single dose and/or necessitating use of alternative serotypes. Delivery of *ex vivo* gene-edited plasma cells producing FIX would remove the requirement for systemic AAV delivery and likely avoid limitations posed by AAV neutralizing antibodies.

[0287] In addition, immune responses to therapeutic proteins comprise a key unaddressed challenge. Based on evidence that B cell delivery can induce tolerance, an additional application of B cell editing technology is to deliver smaller numbers of short-

lived B cells producing FIX, or other therapeutic proteins, with the goal of inducing tolerance to the secreted product.

[0288] The experiments above demonstrate high rates of locus-specific genome editing in primary human B cells. These engineered B cells retain the ability to differentiate into plasma cells *ex vivo* and secrete physiological doses of therapeutic proteins such as FIX. The ability to efficiently target specific loci enables us to drive B cell differentiation and secretory programs. Thus, creative multiplexing of gene disruption and HDR will provide ever more powerful tools to coordinately optimize protein secretion, cellular phenotype and long-term survival.

CRISPR/Cas9 reagents and ssODNs

[0289] CRISPR guide targets at the *CCR5*, *PRDM1*, *IRF4*, *PAX5*, *BACH2* loci were identified using an online MIT CRISPR design tool and the Broad Institute sgRNA design tool. A tracrRNA recognition sequence (5' GUUUUAGAGCUAUGCU 3') was added to the 3' end of each selected guide target to form the complete synthetic crRNA sequence. As shown in Table 2 below are guide sequences used in the alternative herein:

Guide designation	Protospacer	tracrRNA binding sequence
CCR5g	CAAUGUGUCAACUCUUGACA	GUUUUAGAGCUAUGCU
PRDM1g-1	AGGATGCGGATATGACTCTG	
PRDM1g-2	GGGGAGCGAGTGATGTACGT	
IRF4g	CAAGCAGGACTACAACCGCG	
PAX5g	UGUGAAUGGACGGCCACUCC	
BACH2g	GUUCCUGCGCAUGCACAACC	

Table 2: Guide RNA sequences for CCR5, PRDM1, IRF4, PAX5, and BACH2. Each synthesized crRNA comprises a protospacer immediately followed by a tracrRNA binding sequence.

[0290] As shown in the Table 2 above are the sequences for CCR5G (SEQ ID NO: 20), PRDM1g-1 (SEQ ID NO: 21), PRDM1g-2 (SEQ ID NO: 22), IRF4g (SEQ ID NO: 23), PAX5G (SEQ ID NO: 24), BACH2g (SEQ ID NO: 25) and the tracrRNA binding sequence (SEQ ID NO: 26). The crRNA guides were synthesized by IDT with additional modifications: phosphorothioate linkages between the four nucleotides on each end, as well as 2'O-methyl groups on the three nucleotides on each end. The tracrRNA with proprietary

chemical modifications, as well as the recombinant Cas9 nuclease were also purchased from IDT. In some alternatives, the guide sequence comprises a sequence set forth in any one of SEQ ID NO: 2-13, 20-25 or 63-112.

[0291] Prior to delivery of the nuclease into cells, the crRNA and tracrRNA were mixed at an equimolar ratio. The mixture was heat-shocked at 95°C for 5 minutes and then incubated at room temperature for 30-60 minutes to allow crRNA: tracrRNA hybrids to form. Next, the crRNA:tracrRNA hybrids were mixed with the Cas9 nuclease at a 1.2:1 molar ratio and incubated at room temperature for 10-20 minutes to allow Cas9:crRNA:tracrRNA complexes to form. The Cas9 ribonucleoprotein (RNP) complexes were then delivered into cells by electroporation.

[0292] Single-stranded oligonucleotides (ssODNs) were commercially synthesized by IDT (Ultramer® DNA Oligonucleotides) with phosphorothioate linkages between the three nucleotides on each end.

Production of recombinant AAV vectors

[0293] The self-complementary AAV GFP (scAAV GFP) construct contains an MND promoter, enhanced green fluorescence protein (eGFP) and an α globin polyadenylation (pA) signal in an scAAV plasmid backbone. In contrast, all AAV donor templates designed for HDR experiments were cloned into single-stranded AAV plasmid backbones. PRDM1-GFP (400 bp homology arms) contains an MND promoter followed by eGFP and an SV40 pA signal. This MND-eGFP-SV40pA cassette is flanked by two 400 bp sequences homologous to *PRDM1*, with the 5' arm spanning from 106,104,721 to 106,105,120 and the 3' arm spanning from 106,105,124 to 106,105,523 on chromosome 6, with reference to the UCSC December 2013 human genome assembly (GRCh38/hg38). PRDM1-GFP (1 kb homology arms) contains an identical MND-eGFP-SV40pA cassette that is flanked by two 1 kb sequences homologous to *PRDM1*, with the 5' arm spanning from 106,104,121 to 106,105,120 and the 3' arm spanning from 106,105,124 to 106,106,123 on chromosome 6 (GRCh38/hg38). BFP-noHA contains MND promoter-driven blue fluorescent protein (mTagBFP; Evrogen) and an SV40 pA tail without genomic homology. CCR5-GFP-BAFF comprises an MND promoter-driven eGFP and T2A cleavage peptide linking a BAFF coding sequence, which is preceded by an IL2 signal sequence (IL2ss), and

an SV40 pA tail. This cassette is flanked by a 823 bp 5' *CCR5* homology arm, spanning from 46,372,387 to 46,373,209, and a 804 bp 3' *CCR5* homology arm, spanning from 46,373,221 to 46,374,024 on chromosome 3 (GRCh38/hg38). *CCR5*-BAFF comprises identical *CCR5* homology arms flanking a similar cassette, to the exclusion of eGFP and the T2A peptide (i.e. BAFF directly under the MND promoter). Finally, *CCR5*-FIX substitutes a FIX coding sequence and a succeeding modified woodchuck hepatitis virus posttranscriptional regulatory element (WPRE3) for the IL2ss and BAFF coding sequence in *CCR5*-BAFF.

[0294] AAV stocks were produced as known in the art. The AAV vector, serotype helper and HgT1-adenoviral helper plasmids were transfected into HEK293T cells. Cells were harvested 48 hours later, lysed by 3 freeze-thaw cycles, and the cell lysate was treated with benzonase. Virions with recombinant AAV genomes were purified using an iodixanol density gradient.

[0295] All multiplicity of infection (MOI) calculations were based on qPCR-based titers of AAV genomes using ITR specific primers and probe.

Primary human CD19⁺ B cell genome editing

[0296] Peripheral blood mononuclear cells (PBMCs) were collected from whole blood of consented donors and cryopreserved. After thaw, PBMCs were treated with 1X ACK buffer; CD19⁺ B cells were subsequently isolated from PBMCs by negative selection using a human B cell isolation kit (Miltenyi Biotec, Auburn, CA) and cultured in Iscove's modified Dulbecco's medium (IMDM; Thermo Fisher Scientific) supplemented with 10% fetal bovine serum and 55 μ M beta-mercaptoethanol at $1-1.5 \times 10^6$ cells/ml. B cells were activated with 100 ng/ml of recombinant human MEGACD40L (Enzo Life Sciences), 1 μ g/ml of CpG oligodeoxynucleotide 2006 (Invitrogen), 50 ng/ml of IL2 (Peprotech), 50 ng/ml of IL10 (Peprotech) and 10 ng/ml of IL15 (Peprotech) for two days. Cells were then electroporated with Cas9 RNP complexes using the Neon Transfection System (ThermoFisher Scientific) as follows. Cells were washed with PBS and resuspended in Neon Buffer T. 30 pmol Cas9 RNP per 3×10^5 cells was added to the resuspension so that the final cell density was 3×10^7 cells/ml. Cells were electroporated (1700 V, 20 ms, 1 pulse) in 10- μ l Neon tips, and then transferred into pre-warmed B cell culture medium with MEGACD40L, CpG, IL2, IL10 and IL15 and cultured at 1.5×10^6 cells/ml. For samples transfected with an ssODN donor template, ssODN was transfected concurrently with Cas9 RNP at the specified

amount. For samples transduced with AAV, AAV was added to the culture immediately after electroporation at MOIs ranging from 10,000 to 100,000. Culture volume was doubled 24 hours after electroporation, and medium was replenished every two to three days thereafter to maintain a cell density of 1×10^6 cells/ml. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L, 1 μ g/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days.

Flow cytometry

[0297] Flow cytometric analysis was done on an LSR II flow cytometer (BD Biosciences) and data were analyzed using FlowJo software (TreeStar). To assess fluorochrome expression in B cells, flow gates were drawn on FSC/SSC populations corresponding to live cell sizes and singlets were defined using FSC-W/FSC-H gates; BFP⁺ or GFP⁺ gates were then used to define fluorescent cells (**Figure 52A**). Viabilities of these cells were also confirmed by staining them separately with 4,6-Diamidino-2-phenylindole (DAPI) and subsequently quantifying percentages of DAPI- live cells by flow cytometry (**Figure 52B**). To assess B cell surface marker expressions, cells were stained with fluorophore-conjugated antibodies: CD19-PECy7 (clone HIB19, eBioscience), CD27-APC (clone O323, Life Technologies), CD38-PerCPCy5.5 (clone HIT2, BD Biosciences) or CD38-FITC (clone T16, Beckman Coulter), CD138-Alexa Fluor 700 (clone MI15, BioLegend), IgD-PE (clone IA6-2, BD Biosciences), IgM-Pacific Blue (clone MHM-88, BioLegend). Dead cells were excluded using Fixable Live/Dead stain-Alexa Fluor 350 (Life Technologies) (**Figure 52C** for the general gating strategy used for analyzing B cell immunophenotypes).

Plasma cell differentiation culture

[0298] Plasma cells were differentiated *in vitro* using a three-step culture system as known in the art. CD19⁺ B cells isolated from PBMCs were activated for 2 days with MEGACD40L (100 ng/ml), CpG (1 μ g/ml), IL2 (50 ng/ml), IL10 (50 ng/ml) and IL15 (10 ng/ml) and gene-edited as outlined above. Cells were then expanded for another 5 days in the same cocktail. Next, cells were washed with 1X PBS and seeded in medium with IL2 (50 ng/ml), IL6 (50 ng/ml), IL10 (50 ng/ml) and IL15 (10 ng/ml) for 3 days. At day 8 after gene-editing, cells were washed with 1X PBS and seeded in medium with IL6 (50 ng/ml), IL15

(10 ng/ml) and human interferon- α 2B {100 U/ml, Sigma-Aldrich) for 3 days to stimulate plasma cell differentiation. At day 11 after gene-editing, cell phenotypes were analyzed by immunofluorescence.

[0299] In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L), 1 μ g/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days.

Molecular analyses of gene disruption and single-nucleotide HDR

[0300] Total genomic DNA was isolated from $0.2-1.0 \times 10^6$ cells using a DNeasy Blood & Tissue Kit (Qiagen). To assess gene disruption using the T7 endonuclease 1 assay, guide target genomic regions were first amplified using either AccuPrime™ Pfx DNA Polymerase (ThermoFisher Scientific) or PrimeSTAR GXL DNA Polymerase (Takara Bio) with primers about 250 – 350 bp away from the guide target site (See table 3 below).

Assay	Genomic target	Forward primer	Reverse primer	
T7E1 assay	CCR5g	ATGTGAAGCAAATCGCAGCC	TCCCGAGTAGCAGATGACCA	
	PRDM1g-1	TAGCATTAAACCTTGCTTCTTTCAAG	TACAGATTCTCAGAGGTTTTCAGAGA	
	PRDM1g-2	CAGTCTAAAGCAACCGAGCAC	CTTGGGGTAGTGAGCGTTGTA	
Illumina sequencing	CCR5g	GGCAACATGCTGGTCATCCT	GGTGACCGTCTGGCTTTTA	
	PRDM1g-1	CTCTCAGAAGGAGCLACAGG	TTGGTGGLATACITGAAAAGC	
	PRDM1g-2	CCCTCACATCAGAAAAGGA	CTGGAGCTCTTGAGGCTTTG	
	IRF4g	AGATCGACAGCGGCAAGTA	GAGGCCTCCTTTCCTCCTC	
	PAX5g	CACAGCGGTGCTTCTCCTAT	GCCTCGAGCTACTGCCTTTA	
	BACH2g	TGAGGGATTCGGGACAATAG	AGTTCTCGCAGTCCTCGTGT	
In-out ddPCR	CCR5 (HDR)	GGTATGATGCTTAGAACAGTGAT	CCATATTCTGCTGTTCCAACT	Probe CCTGGGCAACATAGTGTGATC
	ActB	ACTCTGCAGGTTCTATTTGC	AATGATCTGAGGAGGGAAGG	ATCAAGGTGGGTGTCTTTCC

Table 3 Primers used for molecular analysis of gene-edited B cells.

[0301] As shown in Table 3 above, the forward primers for CCR5g, PRDM1g-1, and PRDM1g-2 for the T7E1 assay are SEQ ID NO: 27, 28 and 29, respectively. The reverse primers for CCR5g, PRDM1g-1, and PRDM1g-2 for the T7E1 assay are SEQ ID NO: 30, 31 and 32 respectively. As shown in table 3, the forward primers for CCR5g, PRDM1g-1, PRDM1g-2, IRF4g, PAX5g, and BACH2g are SEQ ID NO: 33, 34, 35, 36, 37 and 38 respectively, for the sequencing in the Illumina sequencing. The reverse primers for CCR5g, PRDM1g-1, PRDM1g-2, IRF4g, PAX5g, and BACH2g are SEQ ID NO: 39, 40, 41, 42, 43 and 44, respectively, for in the Illumina sequencing. The forward primers for CCR5 (HDR) and ActB are SEQ ID NO: 45 and 46, respectively, for the in-out ddPCR. The reverse

primers for CCR5 (HDR) and ActB are SEQ ID NO: 47 and 48, respectively, for the in-out ddPCR. The probe for CCR5 comprises SEQ ID NO: 49 and the probe for ActC comprises SEQ ID NO: 50.

[0302] PCR products were then purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel). 200 ng purified PCR product were denatured and re-annealed in 1×NEB Buffer 2 (New England Biolabs, Ipswich, MA) in 19 µl total volume, after which 10 U of T7 endonuclease I (New England Biolabs) was added to the re-annealed PCR product and the samples were incubated for 1 hour at 37°C. The reactions were immediately run on an agarose gel for imaging.

[0303] To quantify frequencies of on-target indels or nucleotide substitutions, allelic modifications by sequencing was assessed (**Figure 53A-53D**). Locus-specific primers were first designed (Table 3) within 150 bp from each guide target site, each primer also containing an adaptor sequence on the 5' end. To prepare samples for sequencing, amplification was performed each using qPCR starting with 250 ng of genomic DNA using PrimeSTAR GXL Polymerase with SYBR Green I Nucleic Acid Gel Stain until reaction reached the exponential phase. The samples were purified with Agencourt AMPure XP and amplified using qPCR for another 6 cycles with primers that contain unique index sequences for each experimental condition, as well as the p5 and p7 cluster generating sequences. The primers were removed using Agencourt Ampure XP and purity of the amplicon was confirmed using a 4-12% Novex TBE PAGE Gel. Next, amplicons from each locus and experiment were pooled at equimolar concentrations and sequenced on a NextSeq 500 in paired-end run mode with a NextSeq 500/550 Mid Output Kit v2. Reads were filtered for quality and grouped by sample index. Next, 112,000 reads per condition and experiment were aligned to the wild type sequences of each amplicon using the CRISPResso⁵⁷ aligner and analysis suite (the aligner runs a variant of the Needleman-Wunsch algorithm). The minimum average read quality score (phred33) was set to 30 and indels/substitutions were specifically quantified within a 30 bp window around each guide target.

[0304] To calculate editing rates at the CCR5 locus, “in-out” ddPCR was performed with forward oligo binding the CCR5 locus outside the homology region and reverse within the AAV insert. A similar size amplicon (1.3kb) was generated for *ActB* gene to serve as a control. Probes for both amplicons were labeled with FAM and reactions for

each were performed in different wells. The oligo and probe sequences are included in Table 3. The PCR reactions were partitioned into droplets using droplet-generating oil for probes on a QX200 Droplet Generator. Amplification was performed using ddPCR Supermix for Probes without UTP (Bio-Rad), 900 nM of primers and 250 nM of Probe. 50 ng of genomic DNA was used in a 25 μ l amplification reaction containing 1% DMSO and run according to manufacturer's directions. The droplets were scanned using the QX200 Droplet Digital PCR System (Bio-Rad) and analyzed using QuantaSoft software. The editing rates were calculated as a ratio of the copies/ μ l from *CCR5/ActB* positive droplets.

ELISA

[0305] 3 days prior to measuring culture protein levels, cells were collected, washed with PBS and then resuspended in new culture medium at 1×10^6 cells/ml. After 3 days, the culture supernatant was collected and Ig or recombinant protein secretion levels were determined by enzyme-linked immunosorbent assay (ELISA). IgG and IgM concentrations were measured using Human IgG total ELISA Ready-SET-Go and Human IgM total ELISA Ready-SET-Go kits (eBioscience, San Diego, CA). BAFF concentrations were measured using a Human BAFF/BLyS/Quantikine ELISA Kit (R&D Systems, Minneapolis, MN), and FIX concentrations were measured using a FIX Human ELISA Kit (Abcam, Cambridge, MA).

Western blot

[0306] TMD8 cells were mock treated or transfected with 30 pmol of either of the *PRDM1*-targeting RNPs. After five additional days of culture, cells were lysed in RIPA and the lysate was run on a NuPAGE 4-12% Bis-Tris protein gel. Cell Signaling Blimp-1/PRDI-BF1 Rabbit mAb #9115 was used as the primary antibody for the *PRDM1* locus and Licor IRDye[®] 800CW Goat anti-Rabbit IgG (H + L) was used as the secondary antibody.

Statistical analysis

[0307] Statistical analyses were performed using Graphpad Prism 7 (GraphPad, San Diego, CA). p-values between two groups were calculated using the unpaired two-tailed t-test, while p values in multiple comparisons were calculated using one-way ANOVA with

the Sidak correction as specified. Values from independent experiments are shown as means $\pm$ SEM.

Data availability

[0308] Accession codes: all sequencing data are accessible at the NCBI Sequence Read Archive through the following study accession number: SRP113557.

More alternatives

[0309] The ability to engineer primary human B cells to differentiate into long-lived plasma cells and secrete a *de novo* protein may allow the creation of novel plasma cell therapies for protein deficiency diseases and other clinical applications. Methods for efficient genome editing of primary B cells isolated from peripheral blood were initially developed as described in the alternatives herein. By delivering CRISPR/Cas9 ribonucleoprotein (RNP) complexes under conditions of rapid B cell expansion, site-specific gene disruption at multiple loci in primary human B cells was achieved (with editing rates up to 94%). This method was used to alter *ex vivo* plasma cell differentiation by disrupting developmental regulatory genes. Next, RNPs were co-delivered with either single-stranded DNA oligonucleotide or adeno-associated viruses containing homologous repair template. Using either delivery method, targeted sequence integration at high efficiency (up to 40%) via homology-directed repair was achieved. This method enabled engineering of plasma cells to secrete factor IX (FIX) or B-cell activating factor (BAFF) at high levels. Finally, as shown in the alternatives herein, introduction of BAFF into plasma cells promotes their engraftment into humanized mice. The results as shown in the alternatives herein, highlight the utility of genome editing in studying human B cell biology and demonstrate a novel strategy for modifying human plasma cells to secrete therapeutic proteins.

[0310] Long-lived plasma cells stably reside in the bone marrow for decades and secrete large quantities of antibodies. Consequently, plasma cells engineered to produce *de novo* proteins have the potential to be curative therapies for protein deficiency diseases, prophylaxis for infectious diseases and many other applications. However, the development of plasma cell therapeutics has been limited by technical challenges in the *in vitro* modification, culture, expansion and differentiation of primary human B cells. B cells can be

transduced at high rates by recombinant adenovirus or Epstein-Barr virus (EBV) vectors, which deliver transgenes as episomes. However, episomal DNA expression is lost over time, limiting use of these vectors in applications that require long-term transgene expression. Unlike non-integrating vectors, gamma retrovirus (γ RV) and lentivirus (LV) randomly integrate into the host genome and can be used to introduce stably expressing transgenes. However, these vectors are inefficient at transducing primary human B cells. LV that employ alternative envelopes, including that of baboon retrovirus, measles virus, or gibbon-ape leukemia virus exhibit higher B cell transduction rates (up to ~50%), but have low viral titers that make large-scale production challenging. Because γ RV and LV vectors do not efficiently transduce B cells while transduction by non-integrating vectors results in only transient transgene expression, neither platform is currently effective for delivering long-term expression of exogenous genes to B cells on a therapeutic scale.

[0311] An alternative method for introducing stable protein expression is genome editing via homology-directed repair (HDR). As described herein, in several alternatives, genome editing may be performed by HDR. Following cleavage by an engineered site-specific nuclease, DNA double-strand breaks are resolved through non-homologous end joining (NHEJ), an error-prone DNA repair pathway that typically leads to variable insertions or deletions (indels), or HDR, which repairs DNA by copying a homologous donor template. Delivery of exogenous DNA flanked by DNA homologous to the genomic sequence around the break site can lead to incorporation of the exogenous sequence in a site-specific manner. HDR-mediated genome editing in B cells may have several advantages over viral vector transduction for therapeutic applications, including decreased risk of insertional mutagenesis and sustained transgene expression. As described herein, high-efficiency HDR delivery of therapeutic transgenes to hematopoietic cells including primary human T cells and hematopoietic stem cells may be achieved, which was also a technique that is performed by others, but similar approaches are yet to be applied in modification of primary human B cells.

[0312] The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system is an RNA-guided nuclease platform that is easily engineered to efficiently target specific sites in the genome for cleavage, generating double-strand DNA breaks. The use of site-specific nucleases for gene disruption

or HDR in B cells is currently limited to transformed or lymphoma-derived cell lines and murine models and has required plasmid- or LV-based CRISPR/Cas9 delivery. Here, as described in the alternatives herein, is high-efficiency genome editing in human peripheral blood B cells (75-90% gene disruption or 10-40% HDR) by delivering CRISPR/Cas9 ribonucleoprotein (RNP) complexes alone or in combination with single-stranded DNA oligonucleotide (ssODN) or adeno-associated virus (AAV) repair templates, respectively. As shown in the alternatives herein, edited primary B cells can subsequently be differentiated in culture into plasma cells that produce physiological doses of therapeutic proteins including human factor IX (FIX).

Conditions for expansion of primary naïve human B cells

[0313] Rapid cell cycling and/or persistence in the S/G2 phases of the cell cycle promote HDR in both cell lines and primary hematopoietic cells. Based on previous reports demonstrating rapid expansion of primary human B cells *ex vivo*, a combination of stimulants were initially used (hereafter called "B cell activation cocktail") that included artificially oligomerized CD40 ligand (MEGACD40L; two linked CD40L trimers) in association with CpG, IL-2, IL-10, and IL-15. Primary human CD19⁺ B cells from peripheral blood mononuclear cells (PBMCs) were isolated and cultured them for 13 days with this B cell activation cocktail. This treatment resulted in a ~36-fold expansion of B cells (**Figure 40**), while preserving viability at > 60% (**Figure 41**). Most cells maintained a naïve B cell phenotype (CD27⁺CD138⁻CD38^{low/-}CD19^{high}IgM⁺IgD⁺; **Figure 40C**) over the 13-day culture; however, IgD expression was gradually downregulated (**Figure 40C**), demonstrating some *ex vivo* skewing due to stimulation. Together, these data demonstrate that the B cell activation cocktail facilitates rapid cycling of naïve and activated primary human B cells.

Cas9-mediated disruption of CCR5 and PRDM1 in primary human B cells

[0314] To assess the efficiency of Cas9-induced indels in B cells, CRISPR guide RNAs (gRNAs) were designed that target *CCR5* (which is not expressed in human B cells and has no known significance in plasma cell development) or *PRDM1* (encoding BLIMP1, a protein required for B cell differentiation into plasma cells). After optimizing electroporation using mRNA (**Figure 41**), 30 pmol Cas9-guide ribonucleoprotein (RNP)

complexes were transfected into B cells, cultured them for five additional days under activating conditions and extracted total genomic DNA to assess nuclease-induced indels using the T7 endonuclease 1 assay (**Figure 42A**). Sequencing confirmed on-target indels and revealed that the RNPs induced high indel frequencies at each guide target site (43-84%; **Figure 42A**). Western blot also verified concomitant PRDM1 protein reduction in the PRDM1-expressing TMD8 lymphoma cell line (**Figure 47A**; ~65% knockdown). Finally, it was discovered that B cells remained viable despite these genome modifications (**Figure 47B**). These data show that high-efficiency Cas9-mediated gene disruption is achievable in primary human B cells.

HDR-mediated single-nucleotide substitution at the PRDM1 locus using an ssODN donor template

[0315] Cas9-induced DNA lesions can be seamlessly repaired via the HDR pathway in the presence of a donor template with homology sequences flanking the lesion. An ssODN was initially tested as a donor template based on promising results in other cell types. A 120-base ssODN containing asymmetric homology arms (89 bases 5' and 30 bases 3') was designed that flank the PRDM1g-2 target site, with a single-nucleotide change at the 90th position that mutates the last nucleotide of the protospacer adjacent motif (PAM; GGG to GGT; **Figure 42B**). This single nucleotide change was designed to both prevent Cas9-mediated cleavage of the repaired sequence and to serve as a molecular marker for HDR. In this experiment, primary human B cells were activated for two days and then transfected with Cas9 RNP in conjunction with various doses of the ssODN. Two and five days following transfection, cells transfected with ≤ 30 pmol ssODN had comparable viabilities to Cas9 RNP transfected control cells (**Figure 42C**). In cells receiving 15 or 30 pmol ssODN, sequencing of the *PRDM1* target region on day 5 post-transfection revealed 20-22% of alleles had undergone HDR while another 37-41% had indels (**Figure 42D**), an overall editing rate marginally higher than that observed in the Cas9 RNP control (**Figure 42D**). Thus, ssODN donor templates can be used to achieve high rates of HDR in primary human B cells with low cytotoxicity.

Cas9-mediated disruption of genes that regulate plasma cell development

[0316] It was then investigated whether Cas9-induced gene disruption can be used to study gene roles in human plasma cell development and antibody production. An experimental workflow was developed that includes inducing NHEJ-mediated gene disruption in activated naïve human B cells using Cas9 RNPs and subsequently differentiating naïve B cells into plasma cells (CD19^{low}CD38^{high}CD27⁺CD138⁺) using a three-step plasma cell culture system (see **Figure 44A**). As a proof of concept, four genes encoding transcription factors previously reported to regulate plasma cell development in murine studies were studied: IRF4 and PRDM1/BLIMP1 are required for plasma cell differentiation (**Figure 44B**), while PAX5 and BACH2 antagonize plasma cell differentiation in mice (**Figure 44B**). CRISPR guides targeting each of these four genes were designed, transfected primary B cells with Cas9 RNP targeting each gene independently and subsequently induced *in vitro* plasma cell differentiation (**Figure 44A**). As a control, cells were also transfected with Cas9 RNPs targeting *CCR5*. Following differentiation, significantly lower percentages of CD19^{low}CD38^{high} and CD27⁺CD138⁺ plasma cells in cultures transfected with *PRDM1*- or *IRF4*-targeting RNPs compared to cells transfected with *CCR5*-targeting RNP or mock transfected cells were detected (mean indel percentages = 89%, 83% and 92% at the *PRDM1*, *IRF4* and *CCR5* target sites, respectively; **Figure 44C**, **44D**). As antibody secretion is a major function of plasma cells, IgM and IgG levels were measured in cell culture supernatants by enzyme-linked immunosorbent assay (ELISA) and found IgG to be significantly decreased in the *PRDM1*- and *IRF4*-targeted B cell cultures (**Figure 44E**). Together, as predicted, these data imply that both PRDM1 and IRF4 are required for human plasma cell differentiation and antibody production. Conversely, it was observed that there was significant increases in the percentages of plasma cells in cultures transfected with either *PAX5* or *BACH2*-targeting RNPs (mean indel percentages = 80% and 86% at the *PAX5* and *BACH2* target sites, respectively; **Figure 44C**, **44D**). Concomitant increases in IgM and IgG secretion in *PAX5*-targeted cultures were also observed (**Figure 44E**). Again, as predicted, these data indicate that Cas9-mediated disruption of *PAX5* or *BACH2* enhances human plasma cell differentiation. Further, the IgG/IgM ratio in *BACH2*-targeted B cell cultures was markedly lower than mock or *CCR5*-targeted cultures (**Figure 44E**, **Figure 45A**) and plasmablasts in *PAX5*-targeted cultures exhibited decreased surface CD19 expression (**Figure 45B**), both consistent with previous studies in mice. Taken

together, these results demonstrate that CRISPR/Cas9-induced gene disruption in primary B cells is useful for interrogating gene products that may modulate human plasma cell development and function.

Site-specific HDR at the PRDM1 locus using co-delivery of RNP and AAV donor template

[0317] HDR using ssODN donor templates is not suitable for delivering payloads larger than ~400 bases due to current limitations of the fidelity of ssODN synthesis. In contrast, adeno-associated virus (AAV) can package up to ~4.7 kilobases (kb) of ssDNA donor template. Many have used AAV to deliver candidate HDR templates leading to high levels of HDR in multiple cell types and at a variety of loci. Of note, there is no published data regarding the capacity of AAV to transduce primary human B cells. To investigate AAV transduction efficiency in human B cells, a self-complementary AAV (scAAV) with a green fluorescent protein (GFP) coding sequence driven by MND, a robust retroviral-derived ubiquitous promoter was designed. Activated B cells were transduced with this vector packaged using various serotypes and quantified GFP expression two days post transduction by flow cytometry. Regardless of serotype, it was observed that there was minimal loss of cell viability following viral exposure (**Figure 51A**). It was observed that the highest percentage of GFP⁺ cells (mean = 43%) and highest mean fluorescence intensity in B cells were transduced with AAV serotype 6 (**Figure 47A**).

[0318] Because the packaging size of single-stranded AAV (ssAAV) is greater than scAAV, the efficacy of HDR-mediated integration of larger payloads by delivering ssAAV into B cells was next assessed. Initially, an AAV6 donor template containing an MND-driven GFP cassette flanked by 400 bp homology arms at the PRDM1g-2 target site (PRDM1-GFP was designed; **Figure 47B**). The PAM was not included in the repair template, thereby rendering the repaired sequence non-cleavable by Cas9 (**Figure 47B**). After activation and electroporation with or without Cas9 RNP, B cells were transduced with AAV6 PRDM1-GFP at various MOI and cultured for 11 additional days with the same activating factors. Although it was initially observed that a 30% viability loss at the highest AAV dose, these cultures eventually recovered and exhibited comparable viabilities by day 11 (**Figure 46B**). At the highest AAV MOI, it was observed that there was persistent GFP

expression in ~10% of cells that received both the *PRDM1*-targeting RNP and AAV repair template, while 2% of cells that were treated with the AAV alone had persistent GFP expression (**Figure 47C**). In addition, cells were co-treated with the *PRDM1*-targeting RNP and a non-targeting AAV BFP control (MND-BFP without homology arms; BFP-noHA) and observed ~1% BFP expression at the endpoint (**Figure 47C**), indicating that the majority of GFP expression resulting from co-delivery of *PRDM1*-targeting RNP and *PRDM1*-GFP was likely driven by HDR-mediated integration. It was also found that higher AAV doses correlated with increased percentages of GFP⁺ cells. Finally, it was found that increasing homology arm lengths from 400bp to 1.0kb did not lead to higher levels of HDR (measured as persistent GFP expression; **Figure 48A, 48B**). Together, these data support the conclusion that co-delivery of Cas9 RNP and AAV can lead to efficient, targeted genomic integration of transgene, likely via the HDR pathway.

Generation of active FIX-secreting human plasma cells via HDR-mediated integration at the CCR5 locus

[0319] Engineering exogenous protein production in plasma cells may have therapeutic applications in protein deficiency diseases such as hemophilia B, which is caused by a deficiency of FIX. To assess whether primary B cells could be engineered to secrete active proteins with predicted functionality, HDR-mediated knock-in strategy was used to engineer *de novo* expression of FIX. Of note, our HDR studies targeting the *PRDM1* locus (described above) were anticipated to underrepresent HDR efficiencies due to the negative impact of *PRDM1* disruption on plasma B cell differentiation and the relative inefficiency of the guide used in the studies (*PRDM1*g-2; **Figure 42A**). Therefore, the *CCR5* locus as a “safe harbor” was targeted because: (a) it is not transcriptionally active in human B cells; (b) it is not required for plasma cell differentiation (**Figure 44A-D**); and (c) heterozygous and homozygous null mutations in *CCR5* are innocuous to human carriers. To generate plasma B cells capable of secreting exogenous human FIX, an AAV vector containing an MND-driven FIX (human codon-optimized FIX-R338L Padua variant) expression cassette was co-delivered, with the same flanking *CCR5* homology arms as described above (*CCR5*-FIX), into primary human B cells with or without *CCR5*-targeting RNP (**Figure 49A**). To also boost plasma cell differentiation in these cultures, *PAX5*-targeting RNPs were also co-

delivered in a subset of CCR5-targeted cells. Although it was observed that similar HDR rates of ~15-20% at the *CCR5* locus across conditions (**Figure 49B**), following plasma cell differentiation it was observed that increases in CD19^{low}CD38^{high} plasmablast percentages in the cultures that received both *CCR5*- and *PAX5*-targeting RNPs (**Figure 49C**). With or without delivery of *PAX5*-targeting RNPs, high levels of *de novo* FIX secretion were detected in the cultures (**Figure 49D**). In addition, dual-edited HDR cultures co-treated with both *CCR5*- and *PAX5*-targeting RNPs exhibited similar levels of FIX production (**Figure 49D**).

[0320] To assess whether the FIX produced by gene-edited plasma cells is active, cultures were supplemented with vitamin K1 to enable vitamin K-dependent post-translational carboxylation of FIX and subsequently measured FIX activity levels using a chromogenic assay. It was found that FIX activity was significantly higher in the vitamin K1-supplemented, gene-edited plasma cell cultures (**Figure 49E**). Despite low levels of activity in the BFP-noHA negative controls, this background was absent when FBS was completely removed from cultures, suggesting that this is likely non-specific assay background originating from murine FIX present in FBS. More importantly, FIX produced by gene-edited plasma cells exhibited high specific activity (~63 IU/mg protein) in a vitamin K-dependent manner (**Figure 49F**). These data demonstrate production of highly functional FIX via targeted HDR in gene-edited human B cells using Cas9 RNP and AAV and illustrate the possibility of multi-locus modifications to simultaneously drive plasma cell differentiation and *de novo* protein secretion.

Engineered BAFF secretion via HDR improves plasma cell survival in vitro

[0321] In order to use gene edited plasma cells in a therapeutic setting, their long-term survival will be required. One strategy to improve plasma cell survival is to engineer cells that secrete survival factors in an autocrine manner. As a first test of this approach, HDR-mediated knock-in strategy of the alternatives herein was used to engineer *de novo* expression of the B-cell activating factor (BAFF). Two AAV6 repair templates were designed: one contained MND-driven GFP and BAFF linked with a T2A self-cleaving peptide (CCR5-GFP-BAFF) and the other contained only an MND-BAFF expression cassette (CCR5-BAFF). Both donor constructs contained 800 bp homology arms around the

CCR5 guide target site (**Figure 51A**). Following RNP transfection and/or AAV transduction, cells were cultured using the three-step plasma cell culture system (**Figure 44A**). As in earlier *PRDM1*-targeting experiments, it was observed that persistent GFP expression (mean = 33% GFP⁺) only in the condition containing both *CCR5*-targeting RNP and AAV (**Figure 51B, Figure 50**). Regardless of the template used, it was observed that there was similar rates of HDR as quantified by digital PCR (~28-31% HDR; **Figure 51C**). While use of both donor templates led to BAFF secretion, B cells targeted by *CCR5*-BAFF (repair template solely containing BAFF) exhibited much higher levels of BAFF secretion (4.1 fold increase) relative to those targeted by *CCR5*-GFP-BAFF (template containing BAFF downstream of the T2A linkage) (**Figure 51D**). To determine if this exogenous BAFF is functional, cell expansion and plasmablast formation was assessed following genome editing and *in vitro* differentiation. In the *CCR5*-BAFF edited culture that contained the highest concentration of exogenous BAFF, increases in cell number was observed, viability and percentage of CD19^{low}CD38^{high} plasmablasts relative to controls that lacked Cas9 RNP (**Figure 51E, 51F**). This is consistent with previous studies showing that BAFF promotes both human and murine B cell survival and differentiation. Taken together, these results show that Cas9 RNP- and AAV-mediated genome editing can be used to generate functional, BAFF-secreting human plasma cells with improved *in vitro* survival.

[0322] Described herein are high rates of gene disruption in primary human B cells at multiple loci using CRISPR/Cas9 RNPs. This method is applied to modeling the impact of genetic changes on human plasma B cell differentiation. It is also demonstrated that the use of Cas9 RNP in combination with ssODN or AAV6 repair templates achieves high-efficiency HDR in B cells and to engineer secretion of functional and therapeutically relevant proteins. Importantly, gene-modified cells obtained a plasma cell phenotype following 13 days of culture (including 2 days pre-editing) *in vitro* and are detectable in humanized mice for an additional 3 weeks, providing evidence of the utility of this approach for adoptive cell therapies using engineered human plasma cells.

[0323] Cas9-mediated disruption of *PRDM1*, *IRF4*, *PAX5*, or *BACH2*, which either promote (*PRDM1*, *IRF4*) or inhibit (*PAX5*, *BACH2*) plasma cell differentiation in mice, led to human B cell phenotypes consistent with these regulatory roles. The ability to easily disrupt genes or induce single-base changes using Cas9 RNP with or without ssODN-

mediated HDR will facilitate further study of the impact of genetic changes on human B cell development. When combined with differentiation *in vitro*, this system could be used as a high-throughput model for studying genetic mutations that alter human B cell development, including somatic and/or germ-line mutations associated with a range of human B cell disorders (in B cell lymphoma and in autoimmune disorders including systemic lupus erythematosus); as well as a range of mechanistic studies designed to assess candidate genes identified by genome-wide association studies.

[0324] The development of a method for the efficient, site-specific introduction of transgenes via HDR opens the possibility of engineering plasma cells to act as autologous “cell factories,” capable of delivering sustained, high doses of therapeutic proteins to patients. As a proof of concept, plasma cells were engineered to secrete FIX. Deficiency of FIX is the cause of hemophilia B, a genetic disease characterized by blood clotting defects. Current treatment for hemophilia B is limited to protein replacement therapy, which is costly and non-curative. Recent gene therapy trials reported long-term FIX expression in patients after intravenous injection of a liver-tropic AAV8-FIX vector; however, pre-existing AAV neutralizing antibodies to all known AAV serotypes are prevalent in humans and greatly limit *in vivo* AAV transduction, making a significant group of patients with anti-AAV antibodies ineligible for treatment. In addition, systemic AAV delivery induces humoral immunity against AAV that prevents subsequent delivery, thus limiting AAV therapy to a single dose and/or necessitating use of alternative serotypes. Delivery of *ex vivo* gene-edited plasma cells producing FIX would remove the requirement for systemic AAV delivery and likely avoid limitations posed by AAV neutralizing antibodies.

[0325] In addition, immune responses to therapeutic proteins comprise a key unaddressed challenge. Based on evidence that B cell delivery can induce tolerance, an additional application of B cell editing technology would be to deliver smaller numbers of short-lived B cells producing FIX, or other immunogenic proteins, with the goal of inducing tolerance to the secreted product.

[0326] The engraftment studies contrast with the majority of those previously reported using human cells. In most other successful studies, human stem cells have been engrafted into neonatal humanized murine models, including co-engraftment with human liver and thymus (pelanta review). In these cases, CD34⁺ stem cells can differentiate into B

cells and even mature B cells. However, the majority of the engrafted cells remain in the transitional and/or immature developmental stages. Further, the demonstration that engineering mature B cells to express human BAFF confers a significant engraftment advantage parallels studies showing that access to human cytokines, including IL6, SIRP α , or T cell factors promote B cell maturation and the ability to respond to immunization in mice.

[0327] In summary, the alternatives herein, demonstrate high rates of locus-specific genome editing in primary human B cells. These engineered B cells retain the ability to differentiate into plasma cells *ex vivo* and secrete physiological doses of therapeutic proteins such as FIX. The ability to efficiently target specific loci enables us to drive B cell differentiation and secretory programs. Thus, creative multiplexing of gene disruption and HDR will provide ever more powerful tools to coordinately optimize protein secretion, cellular phenotype and long-term survival.

CRISPR/Cas9 reagents and ssODNs

[0328] crRNAs targeting the *CCR5*, *PRDM1*, *IRF4*, *PAX5*, *BACH2* loci were identified using the MIT CRISPR design tool and the Broad Institute sgRNA design tool and synthesized (IDT) containing phosphorothioate linkages and 2'O-methyl modifications. Single-stranded oligonucleotides (ssODNs) were commercially synthesized by IDT (Ultramer® DNA Oligonucleotides) with phosphorothioate linkages. crRNA and tracrRNA (IDT) hybrids were mixed with Cas9 nuclease (IDT) at a 1.2:1 ratio and delivered with or without ssODNs to cells by Neon electroporation (ThermoFisher Scientific).

Production of recombinant AAV vectors

[0329] The AAV vector, serotype helper and HgT1-adeno helper plasmids were transfected into HEK293T cells. Cells were harvested 48 hours later, lysed by 3 freeze-thaw cycles, and the cell lysate was treated with benzonase. Virions with recombinant AAV genomes were purified using an iodixanol density gradient. All multiplicity of infection (MOI) calculations were based on qPCR-based titers of AAV genomes using ITR specific primers and probe.

Samples and primary human B cell genome editing

[0330] Peripheral blood mononuclear cells (PBMCs) were collected from whole blood of consented donors and cryopreserved. CD19⁺ B cells were subsequently isolated by negative selection (Pan-B cell kit, Miltenyi Biotec) and cultured in Iscove's modified Dulbecco's medium (IMDM; Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) and 55 μ M beta-mercaptoethanol at $1-1.5 \times 10^6$ cells/ml. B cells were activated with 100 ng/ml of recombinant human MEGACD40L (Enzo Life Sciences), 1 μ g/ml of CpG oligodeoxynucleotide 2006 (Invitrogen), 50 ng/ml of IL2 (Peprotech), 50 ng/ml of IL-10 (Peprotech) and 10 ng/ml of IL15 (Peprotech) for two days. Cells were then electroporated with Cas9 RNP complexes. In some alternatives, cells are activated with 100 ng/ml of recombinant human MEGACD40L, 1 μ g/ml of CpG oligodeoxynucleotide 2006, 50 ng/ml of IL2, 50 ng/ml of IL10 and 10 ng/ml of IL15 for two days.

Flow cytometry

[0331] Flow cytometric analysis was done on an LSR II flow cytometer (BD Biosciences) and data were analyzed using FlowJo software (TreeStar). Flow cytometry gating for fluorescent proteins (**Figure 52A**), viability (**Figure 52B**) and immunophenotyping (**Figure 52C**) are described.

Plasma cell differentiation culture

[0332] Plasma cells were differentiated *in vitro* using a three-step culture system as previously described. CD19⁺ B cells were activated and gene-edited as outlined above and expanded for five days in the same cocktail. Following washing with 1X PBS, the cells were seeded in medium with IL-2 (50 ng/ml), IL-6 (50 ng/ml), IL-10 (50 ng/ml) and IL-15 (10 ng/ml) for three days. Next, the cells were washed with 1X PBS and seeded in medium with IL-6 (50 ng/ml), IL-15 (10 ng/ml) and human interferon- α 2B (100 U/ml, Sigma-Aldrich) for three days to stimulate plasma cell differentiation. In some alternatives, the cells were seeded in medium with IL-2 (50 ng/ml), IL-6 (50 ng/ml), IL-10 (50 ng/ml) and IL-15 (10 ng/ml) for three days. In some alternatives, the cells were washed with 1X PBS and seeded in medium with IL-6 (50 ng/ml), IL-15 (10 ng/ml) and human interferon- α 2B (100 U/ml, Sigma-Aldrich) for three days to stimulate plasma cell differentiation

ELISA

[0333] 3 days prior to measuring culture protein levels, cells were collected, washed with PBS and then resuspended in new culture medium at 1×10^6 cells/ml. After 3 days, the culture supernatant was collected and protein secretion levels were determined by enzyme-linked immunosorbent assay (ELISA) for IgG and IgM (Ready-SET-GO, eBioscience, San Diego, CA), BAFF (BAFF/BLyS/Quantikine ELISA Kit, R&D Systems, Minneapolis, MN) and FIX (FIX Human ELISA Kit, Abcam, Cambridge, MA).

FIX chromogenic assay

[0334] Activity of recombinant FIX expressed by gene-edited B cells was assessed using a chromogenic assay (Rox Factor IX, 900020). The procedure was performed following the manufacturer's instructions. Absorbance values were measured using a VICTOR3™ plate reader (PerkinElmer). A human normal pooled plasma standard was used to establish the calibration curve.

[0335] To assess FIX activity in edited plasma cell cultures, B cells were edited and differentiated *in vitro*. At day 8 post genome editing, FBS content was reduced from 10% to 2% of culture to reduce assay background. Insulin, transferrin, sodium selenite were added to cultures to maintain cell survival in the reduced FBS environment. Finally, in specified cultures that were supplemented with vitamin K1 5 µg/ml of vitamin K1 was added to the medium for generating active FIX via vitamin K-dependent carboxylation. 0.005% was used to increase solubility of vitamin K1.

NSG mouse transplant

[0336] NOD/SCID/gamma-c null (NSG) mice were purchased from Jackson Laboratories and maintained in a designated pathogen-free facility at the Seattle Children's Research Institute (SCRI). All animal studies were performed according to the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) standards, and were approved by the SCRI Institutional Animal Care and Use Committee (IACUC).

[0337] In the NSG transplant experiment, NSG mice were conditioned with 25 mg/kg of Busulfan (Selleckchem) via intraperitoneal injection. 24 hours after conditioning, 10×10^6 *in vitro* differentiated plasma B cells, either mock or edited BAFF-expressing, were delivered into each 6- to 8-week-old NSG recipient via retro-orbital infusion. Mice were bled

at day 10 and sacrificed at day 21 post infusion. All peripheral blood samples were collected in serum separator tubes for serum collection.

Statistical analysis and data availability

[0338] Statistical analyses were performed using Graphpad Prism 7 (GraphPad, San Diego, CA). Because there wasn't obvious skewing in any of the conditions and there was minimal variation within conditions, it is assumed all data followed a normal distribution. p values in multiple comparisons were calculated using one-way ANOVA with the Sidak correction; p values in comparisons between two groups were calculated using paired two-tailed t-test. Values from independent experiments are shown as means $\pm$ SEM. All sequencing data are accessible at the NCBI Sequence Read Archive through the following accession number: SRP113557.

Methods for making plasma cells or plasma cell precursors that express a macromolecule, such as a protein, protein mimetic or a peptide, and making a long lived plasma cell.

[0339] Described herein are methods for making long lived plasma cells. In some alternatives, these plasma cells can also express a desired macromolecule, such as a protein, an antibody, enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. These cells can then be used for treatment, amelioration, or inhibition of a disease such as cancer by e.g., utilizing immunotherapy.

[0340] The method of making a plasma cell or plasma cell precursor that expresses a molecule, such as a macromolecule is provided in several alternatives herein. The method comprises isolating B cells, developing the B cells, performing a first round of genome editing of the B cells for protein expression in absence of viral integration, expanding the B cells; and differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells or plasma cell precursors that express the molecule. In some alternatives, the B cells in step (a) comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma

cells. In some alternatives, the B cells in step (a) comprise memory B cells and/or naïve B cells. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the molecule is a macromolecule, such as a protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein is an enzyme. In some alternatives, the enzyme comprises a cofactor for enhanced enzymatic activity. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMK, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease

are delivered via translatable RNA or recombinant protein. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cell with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cell. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cell comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the step of increasing the proportion of gene edited B cells comprises the steps of: (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically

bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers. In some alternatives, the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers. In some alternatives, the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells. In some alternatives, the negative selecting of non-edited cells is performed by 6-thioguanine. In some alternatives, the method further comprises introducing nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by isolation of naïve or memory B cells. In some alternatives, the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15. In some alternatives, the plasmablast differentiation step is performed in the presence any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody,

neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment, amelioration, or inhibition of periodic fever/autoinflammatory syndromes or complement inhibitory proteins. In some alternatives, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibody or a binding fragment thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and cancer. In some alternatives, the antibody or binding fragment thereof is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding fragment of one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding fragment thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or binding portion thereof is specific for an antigen that is expressed in a virus, fungus, parasite or bacteria. In some alternatives, the protein comprises an antibody or portion thereof, wherein the antibody or portion thereof is specific for an antigen that is expressed in a viral, fungal, parasitic or bacterial infection. In some alternatives herein, the genome editing is performed by nonpathogenic AAV mediated editing by direct homologous recombination. In some alternatives herein, the protein comprises an enzyme, monoclonal antibody or a binding portion thereof, neutralizing antibodies or a binding portions thereof, therapeutic antibodies or binding portions thereof, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose

response element or a synthetic engineered protein. In some alternatives herein, the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives herein, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives herein, the protein is a receptor antagonist for treatment of periodic fever/autoinflammatory syndromes or complement inhibitory proteins. In some alternatives herein, the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor. In some alternatives herein, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives herein, the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives herein, the antibody is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives herein, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives herein, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives herein, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs), or a binding portion thereof. In some alternatives herein, the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection. In some alternatives herein, the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed by a virus, fungus, parasite or bacteria. In some alternatives herein, the protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed on a viral, fungal, parasitic or bacterial infection. In some alternatives herein, the method further comprises purifying the plasma cells or plasma cell precursors after step e) by positive selection against CD138. In some alternatives herein, purifying comprises selecting the plasma cells by an anti-CD138 antibody for targeted removal of the plasma cells. In some alternatives herein, the purifying comprises using anti-CD138 beads for plasma cell selection.

[0341] Novel aspects of the alternatives described herein can include, but are not limited to: (1) the use of blood-derived human B cells as a starting material for a plasma cell protein producing immunotherapy; (2) RNA- and protein-based transfection to facilitate delivery of candidate designer nucleases targeting a broad range of genetic loci in primary B

cells that include, but are not limited to zinc finger nucleases, transcription activator-like effector nucleases (TALEN), homing endonucleases (HEs), combined TALEN -HE proteins (megaTALs) and clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to the CAS nuclease; (3) transfection of long single-stranded DNA oligonucleotides or transduction with recombinant adeno-associated virus to facilitate efficient delivery of donor DNA templates carrying therapeutic expression cassettes into primary human B cells in order to facilitate efficient homologous recombination into a range of candidate genetic loci; (4) integrity measures that include, but are not limited to, methods to prevent somatic hypermutation of the B cell antibody locus during the engineering process including, but not limited, to disruption of the AID gene; (5) production enhancers that include, but are not limited to methods to introduce dimerizable drug-inducible activating proteins to enable selectable expansion of engineered human B cells *in vitro* or *in vivo*; (6) safety measures that include, but are not limited to, introduction of sequences from cell surface proteins including, but not limited to, the CD20 protein into B cells to enable targeted removal from recipients using rituxan or alternative therapeutic approaches; and (7) the use of a proprietary multi-step cytokine and co-culture based systems to facilitate differentiation of blood-derived B cells into long-lived plasma cells and their survival and expansion *in vitro*.

[0342] In some alternatives, the plasma cell or plasma cell precursor expresses a macromolecule, such as a protein, wherein the protein is used in a protocol such as immunotherapy, as envisioned below:

[0343] (1) Prophylactic or therapeutic protection from infection (viral, bacterial, or parasitic) following stem cell administration or solid-organ transplantation in pediatric and adult subjects including, but not limited to neutralizing antibodies that block influenza, parainfluenza, rhinovirus, Respiratory Syncytial Virus (RSV), HIV, pathogenic bacteria, and/or parasites.

[0344] (2) Protein replacement, enzyme replacement and rescue of enzyme deficiencies including, but not limited to Factor VIII (Hemophilia A), Factor IX (Hemophilia B), ADAMTS13 (Hereditary TTP), LIPA (lysosomal acid deficiency), SERPING1 (hereditary angioedema), SERPINA1 (alpha1 anti-trypsin deficiency), GLA (Fabry disease), and/or ALPL (Hypophosphatasia).

[0345] (3) Immune modulation via expressed cytokines, cytokine receptors, complement proteins or other inhibitory proteins including, but not limited to: Il1 receptor antagonist for the treatment or inhibition of periodic fever/autoinflammatory syndromes; complement inhibitory proteins (including Factor H, Factor I) for the treatment or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis; and/or C1 inhibitor for hereditary angioedema.

[0346] Anti-fibrotic molecules including, but not limited to SCGB1A1 for the treatment or inhibition of pulmonary fibrosis.

[0347] Therapeutic antibodies or a binding portion thereof for autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer including but not limited to: anti-IL-1 monoclonal antibodies or a binding portion thereof for treatment or inhibition of periodic fever/autoinflammatory syndromes; anti-TNF antibodies or a binding portion thereof for inflammatory arthritis/inflammatory bowel disease, anti-IL-33 antibodies or a binding portion thereof for the treatment or inhibition of asthma and anti-C5 antibodies or a binding portion thereof for treatment or inhibition of paroxysmal nocturnal hemoglobinuria/atypical HUS.

[0348] Anti-thrombotic molecules including, but not limited to APLN to block platelet function. Antithrombotic molecules are further described by Adam *et al.* ("Apelin: an antithrombotic factor that inhibits platelet function." Blood. 2016 Feb 18; 127(7):908-20.; incorporated by reference in its entirety herein).

[0349] (7) Glucose responses elements upstream of insulin for treatment or inhibition of diabetic conditions.

[0350] (8) Therapeutic monoclonal antibodies or a binding portion thereof for the treatment or inhibition of hyper-cholesterolemia, including anti-PCSK9 inhibitory antibodies or a binding portion thereof.

Methods for making a long lived plasma cell.

[0351] Methods for making a long lived plasma cell are also provided. Long lived plasma cells that express protein can survive in a subject in a need for a treatment or inhibition of a disease such as cancer and such approaches eliminate the need to provide administering of cells within a short time frame.

[0352] The method of making a long lived plasma cell, comprises: isolating B cells; activating the B cells; a first round of genome editing of the B cells for molecule expression in the absence of viral integration; expanding the B cells; and differentiating the B cells. In some alternatives, the B cells in the isolating step comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the molecule is a macromolecule, such as a protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein is an enzyme. In some alternatives, the enzyme comprises a cofactor for enhanced enzymatic activity. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA or protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cell. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, first round of genome editing comprises transducing the B cell with a recombinant adeno-associated virus vector for homologous recombination into a candidate genetic loci. In some alternatives, method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cell. In some

alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cell comprises disruption of an AID gene. In some alternatives, the method further comprises introducing production enhancers into the B cell or disruption of candidate loci within the B cell to enable selectable expansion of the B cells *in vitro* or *in vivo*. In some alternatives, the method further comprises introducing nucleic acid, wherein the nucleic acid encodes at least one cell surface protein. In some alternatives, the at least one cell surface protein is CD20. In some alternatives, the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values. In some alternatives, the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the isolating is performed by negative selection isolation of naïve or memory B cells. In some alternatives, the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis. In some alternatives, the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step. In some alternatives, the activation and proliferation step is performed in the presence any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15. In some alternatives, the plasmablast differentiation step is performed in the presence any combination of IL-2, IL-6, IL-10 and/or IL-15. In some alternatives, the plasma cell differentiation step is performed in the presence any combination of IL-6, IL-15, APRIL and/or IFN α . In some alternatives, the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein. In some alternatives, the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein. In some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In

some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for treatment, amelioration, or inhibition of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment, amelioration, or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody or binding portion thereof is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives herein, the genome editing is performed by nonpathogenic AAV mediated editing by direct homologous recombination. In some alternatives herein, the method further comprises purifying the plasma cells or plasma cell precursors by positive selection against CD138. In some alternatives herein, purifying comprises selecting the plasma cells by an anti-CD138 antibody for targeted removal of the plasma cells. In some alternatives herein, the purifying comprises using anti-CD138 beads for plasma cell selection.

[0353] In some alternatives, the plasma cell or plasma cell precursor that expresses a macromolecule, such as a protein, protein mimetic or peptide is used in a therapeutic protocol such as immunotherapy as envisioned below:

[0354] (1) Prophylactic or therapeutic protection from infection (viral, bacterial, or parasitic) following stem cell administration or solid-organ transplantation in pediatric and adult subjects including, but not limited to neutralizing antibodies that block influenza, parainfluenza, rhinovirus, Respiratory Syncytial Virus (RSV), HIV, pathogenic bacteria, and/or parasites.

[0355] (2) Protein replacement, enzyme replacement and rescue of enzyme deficiencies including, but not limited to Factor VIII (Hemophilia A), Factor IX (Hemophilia B), ADAMTS13 (Hereditary TTP), LIPA (lysosomal acid deficiency), SERPING1 (hereditary angioedema), SERPINA1 (alpha1 anti-trypsin deficiency), GLA (Fabry disease), and/or ALPL (Hypophosphatasia).

[0356] (3) Immune modulation via expressed cytokines, cytokine receptors, complement proteins or other inhibitory proteins including, but not limited to: IL1 receptor antagonist for the treatment or inhibition of periodic fever/autoinflammatory syndromes; complement inhibitory proteins (including Factor H, Factor I) for the treatment or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis; and/or C1 inhibitor for hereditary angioedema.

[0357] (4) Anti-fibrotic molecules including, but not limited to SCGB1A1 for the treatment or inhibition of pulmonary fibrosis.

[0358] (5) Therapeutic antibodies or a binding portion thereof for autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer including but not limited to: anti-IL1 monoclonal antibodies or a binding portion thereof for treatment or inhibition of periodic fever or autoinflammatory syndromes; anti-TNF antibodies or a binding portion thereof for inflammatory arthritis/inflammatory bowel disease, anti-IL-33 antibodies or a binding portion thereof for the treatment or inhibition of asthma and/or anti-C5 antibodies or a binding portion thereof for the treatment or inhibition of paroxysmal nocturnal hemoglobinuria/atypical HUS.

[0359] (6) Anti-thrombotic molecules including, but not limited to APLN to block platelet function. Antithrombotic molecules are further described by Adam *et al.* ("Apelin: an antithrombotic factor that inhibits platelet function." Blood. 2016 Feb 18; 127(7):908-20.; incorporated by reference in its entirety herein).

[0360] (7) Glucose responses elements upstream of insulin for the treatment or inhibition of diabetic conditions.

[0361] (8) Therapeutic monoclonal antibodies or a binding portion thereof for the treatment or inhibition of hyper-cholesterolemia, including anti-PCSK9 inhibitory antibodies or a binding portion thereof.

Plasma cells that express protein

[0362] Plasma cells plasma cell precursors that express macromolecules such as protein, protein mimetics or peptides are provided by the alternatives herein. Plasma cells are provided wherein the plasma cells are also manufactured by the alternative methods described herein. The plasma cell that expresses the macromolecule is long-lived and can be expected to survive within either the bone marrow or within the spleen. In some alternatives, the macromolecule is a protein, protein mimetic or a peptide. The plasma cells created as described herein can persist in non-dividing state within bone marrow survival niche without need for ongoing antigen exposure. They are also relatively resistant to immunosuppression/chemotherapy. Additionally, the plasma cells can be used in conjunction with CAR T cell therapy, which can be used in subjects in need that are suffering from a disease such as cancer, a bacterial or a viral infection so as to treat, ameliorate, or inhibit the disease, cancer or bacterial or viral infection. In some alternatives, the plasma cell expresses CD20.

[0363] In some alternatives, a plasma cell that expresses a molecule such as a macromolecule, is provided. In some alternatives, the molecule is a macromolecule, such as a protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein is an enzyme. In some alternatives, the enzyme comprises a cofactor for enhanced enzymatic activity. In some alternatives, the cell is derived from a B cell. In some alternatives, the B cells comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the protein comprises an enzyme, neutralizing antibody, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody, anti-thrombotic molecule, glucose response element or monoclonal antibody. In

some alternatives, the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1. In some alternatives, the enzyme is ADAMTS13, LIPA, GLA, or ALPL. In some alternatives, the protein is a receptor antagonist for the treatment or inhibition of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for treatment or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angioedema. In some alternatives, the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1. In some alternatives, the therapeutic antibodies or binding portions thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer. In some alternatives, the antibody or binding portion thereof is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies. In some alternatives, the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN. In some alternatives, the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof. In some alternatives, the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof. In some alternatives, the protein comprises a prodrug or a proenzyme. In some alternatives, the plasma cell expresses CD20 for removal from a subject.

[0364] The plasma cell or plasma cell precursor can be manufactured by any one of the alternative methods provided herein. The method of making a plasma cell or plasma cell precursor that expresses a molecule, such as a macromolecule is provided in several alternatives herein. In some alternatives, the molecule is a macromolecule, such as a protein, protein mimetic or peptide. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule is a prodrug. In some alternatives, the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids. In some alternatives, the molecule comprises a carbohydrate or lipid moiety. In some alternatives, the molecule comprises a cofactor. In some alternatives, the protein comprises a hydrophobic group for membrane localization. In some alternatives, the protein is acylated. In some alternatives, the protein is an enzyme. In some alternatives, the enzyme comprises a cofactor for enhanced enzymatic activity. The method comprises isolating B cells, developing the B cells, performing a first round of genome editing of the B cells for protein

expression in absence of viral integration, expanding the B cells; and differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells that express a protein. In some alternatives, the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation. In some alternatives, the B cell in the isolating step comprises B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells. In some alternatives, the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration. In some alternatives, performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid. In some alternatives, the B cells are subject derived or are allogeneic peripheral blood mononuclear cells. In some alternatives, the B cells are blood-derived human B cells. In some alternatives, the first round of genome editing is performed by an RNA and protein based transfection. In some alternatives, the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell. In some alternatives, the at least one genetic loci comprises JCHAIN, IGKC, IGMK, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1. In some alternatives, the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease. In some alternatives, the guide sequence comprises a sequence set forth in any one of SEQ ID NO: 2-13, 20-25 or 63-112. In some alternatives, the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9. In some alternatives, the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a CAS nuclease are delivered via translatable RNA or recombinant protein. In some alternatives, the

first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci. In some alternatives, the single stranded DNA oligonucleotides are unmodified. In some alternatives, the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates. In some alternatives, the first round of genome editing comprises transducing the B cell with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci. In some alternatives, the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary. In some alternatives, the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cell. In some alternatives, the method to prevent somatic hypermutation of an antibody locus in the B cell comprises disruption of an AID gene. In some alternatives, the method further comprises selectively increasing a proportion of gene edited B cells. In some alternatives, the step of increasing the proportion of gene edited B cells comprises: (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells. In some alternatives, the first round of genome editing further comprises techniques for homology-directed repair. In some alternatives, the second round of genome editing results in the excision of the IgM constant region. In some alternatives, the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells. In some alternatives, step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells. In some alternatives, the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the IgM positive cells are removed by negative selection. In some alternatives, the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1. In some alternatives, the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

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NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

WHAT IS CLAIMED IS:

1. A method of making plasma cells or plasma cell precursors that express a macromolecule, the method comprising:
 - (a) isolating B cells;
 - (b) developing the B cells;
 - (c) performing a first round of genome editing of the B cells for molecule expression in absence of viral integration;
 - (d) expanding the B cells; and
 - (e) differentiating the B cells, optionally, after step (c) or (d), thereby producing plasma cells or plasma cell precursors that express the macromolecule.
2. The method of Claim 1, wherein the macromolecule is a protein, protein mimetic or peptide.
3. The method of Claim 2, wherein the protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids.
4. The method of any one of Claims 1-3, wherein the macromolecule comprises a carbohydrate or lipid moiety.
5. The method of any one of Claims 1-4, wherein the macromolecule comprises a cofactor.
6. The method of any one of Claims 2-5, wherein the protein comprises a hydrophobic group for membrane localization.
7. The method of any one of Claims 2-6, wherein the protein is acylated.
8. The method of any one of Claims 2-7, wherein the protein comprises an enzyme, monoclonal antibody or a binding portion thereof, neutralizing antibodies or a binding portions thereof, therapeutic antibodies or binding portions thereof, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein.

9. The method of any one of Claims 2-8, wherein the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1.
10. The method of any one of Claims 2-9, wherein the enzyme is ADAMTS13, LIPA, GLA, or ALPL.
11. The method of any one of Claims 2-10, wherein the protein is a receptor antagonist for treatment of periodic fever/autoinflammatory syndromes or complement inhibitory proteins.
12. The method of Claim 11, wherein the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor.
13. The method of any one of Claims 2-8, wherein the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1.
14. The method of Claim 8, wherein the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer.
15. The method of Claim 8 or 14, wherein the antibody is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies.
16. The method of any one of Claims 2-8, wherein the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN.
17. The method of any one of Claims 2-8, wherein the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof.
18. The method of any one of Claims 2-8, wherein the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs), or a binding portion thereof.
19. The method of any one of Claims 2-8 or 18, wherein the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection.
20. The method of Claim 19, wherein the engineered protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed by a virus, fungus, parasite or bacteria.

21. The method of any one of Claims 2-8, wherein the protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed on a viral, fungal, parasitic or bacterial infection.
22. The method of any one of Claims 2 or 8-21, wherein the protein is an enzyme.
23. The method of any one of Claims 2 or 8-22, wherein the enzyme comprises a cofactor for enhanced enzymatic activity.
24. The method of any one of Claims 1-23, wherein the B cells in step (a) comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells.
25. The method of any one of Claims 1-24, wherein the B cells in step (a) comprise memory B cells and/or naïve B cells.
26. The method of any one of Claims 1-25, wherein the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation.
27. The method of Claim 26, wherein the B cell is arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell.
28. The method of any one of Claims 1-27, wherein performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid.
29. The method of any one of Claims 1-28, wherein the B cells are subject derived or are allogeneic peripheral blood mononuclear cells.
30. The method of any one of Claims 1-29, wherein the B cells are blood-derived human B cells.
31. The method of any one of Claims 1-30, wherein the first round of genome editing is performed by an RNA and protein based transfection.

32. The method of any one of Claim 31, wherein the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell.
33. The method of Claim 32, wherein the at least one genetic loci comprises JCHAIN, IGKC, IGMG, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1.
34. The method of Claim 32 or 33, wherein the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease.
35. The method of Claim 34, wherein the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9.
36. The method of any one of Claims 32-35, wherein the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to the CAS nuclease are delivered via translatable RNA or recombinant protein.
37. The method of any one of Claims 1-36, wherein the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci.
38. The method of Claim 37, wherein the single stranded DNA oligonucleotides are unmodified.
39. The method of Claim 37, wherein the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates.
40. The method of any one of Claims 1-39, wherein the first round of genome editing comprises transducing the B cell with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci.
41. The method of Claim 40, wherein the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary.

42. The method of any one of Claims 1-41, wherein the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cell.
43. The method of Claim 42, wherein the method to prevent somatic hypermutation of an antibody locus in the B cell comprises disruption of an AID gene.
44. The method of any one of Claims 1-43, wherein the method further comprises selectively increasing a proportion of gene edited B cells.
45. The method of Claim 44, wherein the increasing the proportion of gene edited B cells comprises: (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells.
46. The method of Claim 45, wherein the first round of genome editing further comprises techniques for homology-directed repair.
47. The method of Claims 45 or 46, wherein the second round of genome editing results in the excision of the IgM constant region.
48. The method of any one of Claims 45-47, wherein the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells
49. The method of any one of Claims 45-48, wherein step (a) of the step increasing the proportion of gene edited B cells further comprises removing IgM positive cells.
50. The method of any one of Claims 48 or 49, wherein the method further comprises activating the IgG1 positive cells and expanding the IgG1 positive cells, wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1.
51. The method of Claim 49 or 50, wherein the IgM positive cells are removed by negative selection.
52. The method of Claim 50 or 51, wherein the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1.

53. The method of any one of Claims 45-52, wherein the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers.
54. The method of any one of Claims 45-53, wherein the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers.
55. The method of any one of Claims 45-54, wherein the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells.
56. The method of Claim 55, wherein the negative selecting of non-edited cells is performed by 6-thioguanine.
57. The method of any one of Claims 1-56, further comprising introducing nucleic acid, wherein the nucleic acid encodes at least one cell surface protein.
58. The method of Claim 57, wherein the at least one cell surface protein is CD20.
59. The method of any one of Claims 37-58, wherein the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci.
60. The method of any one of Claims 37-59, wherein the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned lengths.
61. The method of any one of Claims 1-60, wherein the isolating is performed by isolation of early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells.
62. The method of any one of Claims 1-61, wherein the isolating is performed by isolation of naïve or memory B cells.
63. The method of Claim 62, wherein the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections.

64. The method of any one of Claims 1-63, wherein the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step.
65. The method of Claim 64, wherein the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15.
66. The method of Claims 64 or 65, wherein the plasmablast differentiation step is performed in the presence any combination of IL-2, IL-6, IL-10 and/or IL-15.
67. The method of any one of Claims 64-66, wherein the plasma cell differentiation step is performed in the presence any combination of IL-6, IL-15, APRIL and/or IFN α .
68. The method of any one of Claims 37-67, wherein the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein.
69. The method of any one of Claims 1-68, wherein the method further comprises purifying the plasma cells or plasma cell precursors after step e) by positive selection against CD138.
70. The method of Claim 69, wherein purifying comprises selecting the plasma cells by an anti-CD138 antibody for targeted removal of the plasma cells.
71. The method of Claim 69, wherein the purifying comprises using anti-CD138 beads for plasma cell selection.
72. A method of making a long lived plasma cell, the method comprising:
 - isolating B cells;
 - activating the B cells;
 - a first round of genome editing of the B cells for macromolecule expression in the absence of viral integration;
 - expanding the B cells; and
 - differentiating the B cells, thereby producing the long lived plasma cell.
73. The method of Claim 72, further comprising selecting the long lived plasma cell by CD138 selection of the plasma cells.
74. The method of Claim 72 or 73, wherein the macromolecule is a protein, protein mimetic or peptide.

75. The method of Claim 74, wherein the protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids.
76. The method of any one of Claims 72-75, wherein the macromolecule comprises a carbohydrate or lipid moiety.
77. The method of any one of Claims 72-76, wherein the macromolecule comprises a cofactor.
78. The method of any one of Claims 74-77, wherein the protein comprises a hydrophobic group for membrane localization.
79. The method of any one of Claims 74-78, wherein the protein is acylated.
80. The method of Claim 74-79, wherein the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein.
81. The method of Claim 74-80, wherein the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1.
82. The method of Claim 80, wherein the enzyme is ADAMTS13, LIPA, GLA, or ALPL.
83. The method of Claim 74-80, wherein the protein is a receptor antagonist for the treatment or inhibition of periodic fever or autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for the treatment or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angiodema.
84. The method of Claim 74-80, wherein the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1.
85. The method of Claim 80, wherein the therapeutic antibodies or a binding portion thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer.
86. The method of Claim 80, wherein the antibody or a binding portion thereof is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies.

87. The method of any one of Claims 74-80, wherein the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN.
88. The method of any one of Claims 74-80, wherein the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof.
89. The method of any one of Claims 72-88, wherein the B cells in the isolating step comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells.
90. The method of any one of Claims 72-89, wherein the B cells in the isolating step comprises naïve B cells and/or memory B cells.
91. The method of any one of Claims 72-90, wherein the B cells are subject derived or are allogeneic peripheral blood mononuclear cells.
92. The method of any one of Claims 72-91, wherein the B cells are blood-derived human B cells.
93. The method of any one of Claims 72-92, wherein the first round of genome editing is performed by an RNA or protein based transfection.
94. The method of any one of Claims 72-93, wherein the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic locus in the B cell.
95. The method of Claim 94, wherein the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease.
96. The method of Claim 95, wherein the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9.
97. The method of any one of Claims 72-96, wherein the first round of genome editing comprises transfecting single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci.

98. The method of any one of Claims 72-97, wherein first round of genome editing comprises transducing the B cell with a recombinant adeno-associated virus vector for homologous recombination into a candidate genetic loci.
99. The method of any one of Claims 72-98, wherein the method further comprises applying methods to prevent somatic hypermutation of an antibody locus and other target loci in the B cells.
100. The method of Claim 99, wherein the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene.
101. The method of any one of Claims 72-100, wherein the method further comprises introducing production enhancers into the B cells or disruption of candidate loci within the B cells to enable selectable expansion of the B cells *in vitro* or *in vivo*.
102. The method of any one of Claims 72-101, further comprising introducing nucleic acid, wherein the nucleic acid encodes at least one cell surface protein.
103. The method of Claim 102, wherein the at least one cell surface protein is CD20.
104. The method of any one of Claims 97-103, wherein the first round of genome editing further comprises cycling the cells for homologous recombination of the single stranded DNA oligonucleotides or the recombinant adeno-associated virus vector into the candidate genetic loci.
105. The method of any one of Claims 97-104, wherein the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values.
106. The method of any one of Claims 72-105, wherein the isolating is performed by negative selection isolation of hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells.

107. The method of any one of Claims 72-106, wherein the isolating is performed by negative selection isolation of naïve or memory B cells.
108. The method of Claim 107, wherein the naïve or memory B cells are from healthy donor PBMCs or cells collected by apheresis.
109. The method of any one of Claims 72-108, wherein the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step and a plasma cell differentiation step.
110. The method of Claim 109, wherein the activation and proliferation step is performed in the presence any combination of MCD40L, CpG, IL-2, IL-10 and/or IL-15.
111. The method of Claims 109 and 110, wherein the plasmablast differentiation step is performed in the presence any combination of IL-2, IL-6, IL-10 and/or IL-15.
112. The method of any one of Claims 109-111, wherein the plasma cell differentiation step is performed in the presence any combination of IL-6, IL-15, APRIL, and/or IFN α .
113. The method of any one of Claims 74-112, wherein the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein.
114. The method of any one of Claims 73-113 wherein the method further comprises purifying long lived plasma cells are purified by positive selection against CD138.
115. The method of Claim 114, wherein purifying comprises selecting the plasma cells by an anti-CD138 antibody for targeted removal of the plasma cells.
116. The method of Claim 114, wherein the purifying comprises using anti-CD138 beads for plasma cell selection.
117. A plasma cell that expresses a macromolecule, such as a heterologous protein, protein mimetic or a peptide.
118. The plasma cell of Claim 117, wherein the cell is a B cell or a B cell precursor.

119. The plasma cell of Claim 117 or 118, wherein the protein comprises an enzyme, neutralizing antibody or a binding portion thereof, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody or a binding portion thereof, anti-thrombotic molecule, glucose response element or monoclonal antibody or a binding portion thereof.
120. The plasma cell of Claim 119, wherein the protein is Factor VIII, Factor IX, SERPING1 or SERPINA1.
121. The plasma cell of Claim 119, wherein the enzyme is ADAMTS13, LIPA, GLA, or ALPL.
122. The plasma cell of Claim 119, wherein the protein is a receptor antagonist for the treatment or inhibition of periodic fever or autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for the treatment or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angioedema.
123. The plasma cell of Claim 119, wherein the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1.
124. The plasma cell of Claim 119, wherein the therapeutic antibodies or a binding portion thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer.
125. The plasma cell of Claim 119, wherein the antibody or a binding portion thereof is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies.
126. The plasma cell of Claim 119, wherein the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN.
127. The plasma cell of Claim 119, wherein the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof.
128. The plasma cell of Claim 119, wherein the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof.
129. The plasma cell of any one of Claims 117-128, further comprising at least one cell surface protein.

130. The plasma cell of Claim 129, wherein the at least one cell surface protein is CD20.
131. A composition comprising the plasma cell manufactured by any one of Claims 1-116 and a second B cell, wherein the second B cell secretes a macromolecule for inducing tolerance of a peptide for immunotherapeutic application or for inducing engraftment of the plasma cell manufactured by any one of Claims 1-116.
132. The composition of Claim 131, wherein the macromolecule expressed by the second B cell comprises IFN-alpha, BAFF, APRIL, IL-10 or IL-6.
133. A composition comprising the plasma cell manufactured by any one of Claims 1-116 or comprising the cell of any one of Claims 117-130.
134. A method of expressing a molecule, such as a macromolecule in a subject in need, the method comprising:
transplanting a plasma cell manufactured by any one of Claims 1-116, the plasma cell that expresses protein of any one of Claims 117-130, or the composition of any one of Claims 119 or 120 131-133 into the subject.
135. The method of Claim 134, wherein the administering is performed by adoptive transfer.
136. The method of any one of Claims 134-135, wherein the subject has received a stem cell administration or solid organ transplantation or is identified or selected as one to receive such therapies.
137. The method of any one of Claims 134-136, wherein the subject suffers from influenza, parainfluenza, rhinovirus, respiratory syncytial virus, HIV, or other pathogenic bacteria, virus, fungus or parasite infection.
138. The method of any one of Claims 134-137, wherein the subject suffers from an enzyme deficiency.
139. The method of any one of Claims 134-138, wherein the subject suffers from pulmonary fibrosis.
140. The method of any one of Claims 134-139, wherein the subject suffers from an autoimmune disorder, immune dysregulation or cancer.
141. The method of any one of Claims 134-140, wherein the subject suffers from diabetes.

142. The method of any one of Claims 134-141, wherein the subject suffers from hypercholesterolemia.
143. The method of any one of Claims 134-142, wherein the subject is also receiving CAR T-cell therapy or is identified or selected to receive such a therapy.
144. The method of any one of Claims 134-143, wherein the subject suffers from HIV.
145. The method of any one of Claims 134-144, further comprising monitoring of subject following plasma cell transplantation.
146. The method of Claim 145, wherein the subject is determined to no longer need treatment and the method further comprises administering rituxan or an anti-CD20 antibody for targeted removal of the plasma cells.
147. A method of ameliorating a disease in a subject in need, the method comprising:
administering a plasma cell manufactured by any one of Claims 1-116 or the plasma cell that expresses a molecule, such as a macromolecule manufactured by any one of Claims 117-130, or the composition of any one of Claims 131-133 into the subject in need.
148. The method of Claim 147, wherein the administering is performed by adoptive transfer.
149. The method of Claim 147 or 148, wherein the subject has received a stem cell administration or solid organ transplantation.
150. The method of any one of Claims 147-149, wherein the subject suffers from influenza, parainfluenza, rhinovirus, respiratory syncytial virus, HIV, or other pathogenic bacteria, virus, fungus or parasite infection.
151. The method of any one of Claims 147-150, wherein the subject suffers from an enzyme deficiency.
152. The method of any one of Claims 147-151, wherein the subject suffers from pulmonary fibrosis.
153. The method of any one of Claims 147-152, wherein the subject suffers from an autoimmune disorder, immune dysregulation or cancer.

154. The method of any one of Claims 147-153, wherein the subject suffers from diabetes.
155. The method of any one of Claims 147-154, wherein the subject suffers from hypercholesterolemia.
156. The method of any one of Claims 147-155, wherein the subject is already receiving a therapy for the disease or infection.
157. The method of any one of Claims 147-156, wherein the subject is selected to receive cancer therapy, diabetes therapy, HIV treatment, or viral therapy.
158. The method of any one of Claims 147-157, wherein the subject is also receiving CAR T-cell therapy or is selected to receive such a therapy.
159. The method of any one of Claims 147-158, further comprising monitoring of the subject following plasma cell administration.
160. The method of Claim 159, wherein the subject is determined to no longer need treatment and the method further comprises administering rituxan or an anti-CD20 antibody for targeted removal of the plasma cells.
161. A method of expressing a molecule, such as a macromolecule in a subject of need, the method comprising:
- (a) isolating B cells;
 - (b) developing the B cells;
 - (c) performing a first round of genome editing of the B cells for molecule expression in absence of viral integration;
 - (d) expanding the B cells; differentiating the B cells, optionally, after step (c) or (d), thereby producing differentiated B cells or plasma cells that express the molecule; and
 - (e) administering the differentiated B cells or plasma cells produced in step d) into the subject in need.
162. The method of Claim 161,
163. The method of any one of Claims 162-164, wherein the purifying comprises using anti-CD138 beads for plasma cell selection.
164. The method of any one of Claims 161-165, wherein the molecule is a macromolecule, such as a protein, protein mimetic or a peptide.

165. The method of any one of Claims 161-166, wherein the B cells comprise B cell precursors such as hematopoietic stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, T1 B cells, T2 B cells, marginal zone B cells, mature B cells, naïve B cells, plasmablast (short lived) cells, GC B cells, memory B cells, plasmablast cells and/or long lived plasma cells.
166. The method of any one of Claims 161-167, wherein the macromolecule, protein, protein mimetic, or peptide comprises natural amino acids and/or unnatural amino acids.
167. The method of any one of Claims 161-168, wherein the molecule comprises a carbohydrate or lipid moiety.
168. The method of any one of Claims 161-169, wherein the molecule comprises a cofactor.
169. The method of any one of Claims 161-170, wherein the protein comprises a hydrophobic group for membrane localization.
170. The method of any one of Claims 166-171, wherein the protein is acylated.
171. The method of any one of Claims 166-172, wherein the protein is an enzyme.
172. The method of Claim 173, wherein the enzyme comprises a cofactor for enhanced enzymatic activity.
173. The method of any one of Claims 161-174, wherein the developing of the B cells is performed after the B cells are arrested at a specific phase of development and/or activating the B cells such that the B cells are permissive for recombination without further B cell differentiation.
174. The method of Claim 175, wherein the B cells are arrested as an early pro-B cell, a late pro-B cell, a large pre-B cell, a small pre-B cell, an immature B cell, a T1 B cell, a T2 B cell, a marginal-zone B cell, a mature B cell or a memory B cell.
175. The method of any one of Claims 161-176, wherein performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration.
176. The method of any one of Claims 161-177, wherein performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid.

177. The method of any one of Claims 161-178, wherein the B cells are subject derived (autologous) or are allogeneic peripheral blood mononuclear cells.
178. The method of any one of Claims 161-179, wherein the B cells are blood-derived human B cells.
179. The method of any one of Claims 161-180, wherein the first round of genome editing is performed by an RNA and protein based transfection.
180. The method of Claim 181, wherein the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell.
181. The method of Claim 182, wherein the at least one genetic loci comprises JCHAIN, IGKC, IGMG, PON3, PRG2, FKBP11, SDC1, SLPI, DERL3, EDEM1, LY6C2, CRELD2, REXO2, PDIA4, PRDM1, CARD11, CCR5 or SDF2L1.
182. The method of Claim 182 or 183, wherein the nuclease is a zinc finger nuclease, transcription activator-like effector nuclease (TALEN), homing endonuclease (HEs), combined TALEN-HE protein (megaTALs) or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease.
183. The method of Claim 184, wherein the Cas nuclease comprises Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 or Cas9.
184. The method of any one of Claims 182-185, wherein the nuclease or synthetic guide RNAs targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to the CAS nuclease are delivered via translatable RNA or recombinant protein.
185. The method of any one of Claims 181-186, wherein the first round of genome editing comprises transfecting with single stranded DNA oligonucleotides for homologous recombination into a candidate genetic loci.
186. The method of Claim 187, wherein the single stranded DNA oligonucleotides are unmodified.
187. The method of Claim 188, wherein the single stranded DNA oligonucleotides are modified with 5' or 3' phosphorothioate linkages as repair templates.

188. The method of any one of Claims 181-189, wherein the first round of genome editing comprises transducing the B cells with a recombinant adeno-associated virus vector to serve as a donor template for homologous recombination into a candidate genetic loci.
189. The method of Claim 190, wherein the recombinant adeno-associated virus vector is single-stranded, double stranded or self-complementary.
190. The method of any one of Claims 161-191, wherein the method further comprises applying methods to prevent somatic hypermutation of an antibody locus in the B cells.
191. The method of Claim 192, wherein the method to prevent somatic hypermutation of an antibody locus in the B cells comprises disruption of an AID gene.
192. The method of any one of Claims 161-193, wherein the method further comprises selectively increasing a proportion of gene edited B cells.
193. The method of Claim 194, wherein the increasing the proportion of gene edited B cells comprises the steps of : (a) performing a second round of genome editing on the B cells to excise a region; (b) performing a third round of genome editing on the B cells, wherein the third round of genome editing results in expression of drug activatable growth enhancers; (c) RNA transfecting into the B cells short lived drug activatable growth enhancers; and (d) inserting genetic modifications that artificially induce non-transformative expansion of gene edited B cells.
194. The method of Claim 195, wherein the first round of genome editing further comprises techniques for homology-directed repair.
195. The method of Claims 195 or 196, wherein the second round of genome editing results in the excision of the IgM constant region.
196. The method of any one of Claims 195-197, wherein the second round of genome editing results in the B cells mimicking a class-switch to IgG1 positive cells
197. The method of any one of Claims 195-188, wherein step (a) further comprises removing IgM positive cells.
198. The method of any one of Claims 198 or 199, wherein the method further comprises, activating the IgG1 positive cells and expanding the IgG1 positive cells,

wherein the expanding is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1.

199. The method of Claim 199 or 200, wherein the IgM positive cells are removed by negative selection.
200. The method of Claim 200 or 201, wherein the expanding the IgG1 positive cells is performed by exogenous antigens that specifically bind the inserted surface-expressed IgG1.
201. The method of any one of Claims 195-202, wherein the drug activatable growth enhancers expressed after the third round of genome editing are rapamycin-inducible FKBP11 dimers.
202. The method of any one of Claims 195-203, wherein the activatable growth enhancers that are RNA transfected into the B cells of step (c) are rapamycin-inducible FKBP11 dimers.
203. The method of any one of Claims 195-204, wherein the inserting genetic modifications that artificially induce triggerable, non-transformative expansion of edited cells results in disruption of a HPRT locus and negative selection of non-edited cells.
204. The method of Claim 206, wherein the negative selecting of non-edited cells is performed by 6-thioguanine.
205. The method of any one of Claims 161-206, further comprising introducing nucleic acid, wherein the nucleic acid encodes at least one cell surface protein.
206. The method of Claim 207, wherein the at least one cell surface protein is CD20.
207. The method of any one of Claims 187-208, wherein the first round of genome editing further comprises cycling the B-cells for homologous recombination of the single stranded DNA oligonucleotides or recombinant adeno-associated virus into the candidate genetic loci.
208. The method of any one of Claims 206-209, wherein the single stranded DNA oligonucleotides or recombinant adeno-associated virus comprises a length of 0.2kb, 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb or 5kb or a length within a range defined by any two of the aforementioned values.

209. The method of any one of Claims 161-210, wherein the isolating is performed by isolation of naïve or memory B cells.
210. The method of Claim 211, wherein the isolating is performed from isolation from healthy donor PBMCs or cell apheresis collections.
211. The method of any one of Claims 161-212, wherein the differentiating step is performed in a three-step culture system comprising an activation and proliferation step, a plasmablast differentiation step, and a plasma cell differentiation step.
212. The method of Claim 213, wherein the activation and proliferation step is performed in the presence of any combination of MCD40L (CD40 trimer), CpG, IL-2, IL-10 and/or IL-15.
213. The method of Claims 213 or 214, wherein the plasmablast differentiation step is performed in the presence any combination of IL-2, IL-6, IL-10 and/or IL-15.
214. The method of any one of Claims 213-215, wherein the plasma cell differentiation step is performed in the presence any combination of IL-6, IL-15, APRIL and/or IFN α .
215. The method of any one of Claims 166-216, wherein the single stranded DNA oligonucleotides or the recombinant adenovirus comprises a sequence encoding the protein.
216. The method of any one of Claims 166-217, wherein the protein comprises an enzyme, monoclonal antibody, neutralizing antibodies, therapeutic antibodies, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, anti-thrombotic molecule, coagulation factor, glucose response element or a synthetic engineered protein.
217. The method of any one of Claims 166-218, wherein the protein is IFN-alpha, Factor VIII, Factor IX, SERPING1 or SERPINA1.
218. The method of Claim 218, wherein the enzyme is ADAMTS13, LIPA, GLA, or ALPL.
219. The method of anyone of Claims 166-220, wherein the protein is a receptor antagonist for the treatment or inhibition of periodic fever or auto-inflammatory syndromes or complement inhibitory proteins.

220. The method of Claim 221, wherein the complement inhibitory protein is Factor H, Factor I or a C1 inhibitor.
221. The method of anyone of Claims 166-218, wherein the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1.
222. The method of Claim 2218, wherein the therapeutic antibody or a binding portion thereof is specific for a protein or other molecule expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer.
223. The method of Claim 218, wherein the antibody or a binding portion thereof is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies.
224. The method of anyone of Claims 166-218, wherein the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN.
225. The method of anyone of Claims 166-218, wherein the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof.
226. The method of anyone of Claims 166-218, wherein the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof.
227. The method of anyone of Claims 166-218, wherein the protein is an engineered protein, wherein the engineered protein is protective in viral, fungal, parasitic or bacterial infection.
228. The method of Claim 229, wherein the engineered protein comprises an antibody or portion thereof, wherein the antibody or portion thereof is specific for an antigen that is expressed on a virus, fungus, parasite or bacteria.
229. The method of anyone of Claims 166-218, wherein the protein comprises an antibody or a binding portion thereof, wherein the antibody or a binding portion thereof is specific for an antigen that is expressed by a viral, fungal, parasitic or bacterial infection.
230. The method of any one of Claims 161-231, wherein the administering is performed by adoptive transfer.
231. The method of any one of Claims 161-232, wherein the subject has received a stem cell administration or solid organ transplantation.

232. The method of any one of Claims 161-233, wherein the subject suffers from influenza, parainfluenza, rhinovirus, respiratory syncytial virus, HIV, or other pathogenic bacteria, virus, fungus or parasite infection.
233. The method of any one of Claims 161-234, wherein the subject suffers from an enzyme deficiency.
234. The method of any one of Claims 161-235, wherein the subject suffers from pulmonary fibrosis.
235. The method of any one of Claims 161-236, wherein the subject suffers from an autoimmune disorder, immune dysregulation or cancer.
236. The method of any one of Claims 161-237, wherein the subject suffers from diabetes.
237. The method of any one of Claims 161-238, wherein the subject suffers from hypercholesterolemia.
238. The method of any one of Claims 161-239, wherein the subject is receiving therapy.
239. The method of any one of Claims 161-240, wherein the subject is selected to receive cancer therapy, diabetes therapy, HIV treatment, or viral therapy.
240. The method of any one of Claims 161-241, wherein the subject is also receiving CAR T-cell therapy or is selected to receive such a therapy.
241. The method of any one of Claims 161-242, further comprising monitoring of the subject following plasma cell transplantation.
242. The method of Claim 243, wherein the subject is determined to no longer need treatment and the method further comprises administering rituxan or an anti-CD20 antibody for targeted removal of the plasma cells.
243. A method of ameliorating a disease in a subject in need, the method comprising:
administering a plasma cell manufactured by any one of Claims 1-116 or the plasma cell that expresses a molecule, such as a macromolecule manufactured by any one of Claims 117-130, or the composition of any one of Claims 131-133 into the subject in need.

244. The method of Claim 245, wherein the administering is performed by adoptive transfer.
245. The method of Claim 245 or 246, wherein the subject has received a stem cell administration or solid organ transplantation.
246. The method of any one of Claims 245-247, wherein the subject suffers from influenza, parainfluenza, rhinovirus, respiratory syncytial virus, HIV, or other pathogenic bacteria, virus, fungus or parasite.
247. The method of any one of Claims 245-248, further comprising monitoring of the subject following plasma cell transplantation.
248. The method of Claim 249, wherein the subject is determined to no longer need treatment and the method further comprises administering rituxan or an anti-CD20 antibody for targeted removal of the plasma cells.
249. A method of enhancing immune tolerance to protein therapeutics in a subject in need, the method comprising:
administering a plasma cell manufactured by any one of Claims 1-116 or the plasma cell that expresses a molecule, such as a macromolecule manufactured by any one of Claims 117-130, or the composition of any one of Claims 131-133 into the subject in need.
250. The method of Claim 251, wherein the subject suffers from Hemophilia A, Hemophilia B, Hereditary TPP, lysosomal acid deficiency, hereditary angioedema, alpha1 anti-trypsin deficiency, Fabry disease or hypophosphatasia.
251. The method of any one of Claims 251-252, further comprising monitoring of the subject following plasma cell transplantation.
252. The method of Claim 253, wherein the subject is determined to no longer need treatment and the method further comprises administering rituxan or an anti-CD20 antibody for targeted removal of the plasma cells.
253. A method of inducing tolerance of a macromolecule secreted by a B cell in a subject in need *in vivo*, the method comprising:
A) isolating B cells;

- B) performing a first round of genome editing of the B cells for protein expression in absence of viral integration, wherein the performing is under conditions of rapid B cell expansion;
- C) expanding the B cells;
- D) differentiating the B cells, optionally, after step (c) or (d), thereby producing an activated B cell or naïve activated B cell that express the molecule; and
- E) providing or administering the B cell that expresses the macromolecule into a subject in need.
254. The method of Claim 255, wherein the method further comprises purifying the plasma cells of step D), wherein the purifying is performed after the differentiating step.
255. The method of Claim 256, wherein the purifying comprises positive selecting of cells against CD138.
256. The method of Claim 256 or 257, wherein the selecting the plasma cells is performed by an anti-CD138 antibody for targeted removal of the plasma cells.
257. The method of any one of Claims 256-258, wherein the purifying comprises using anti-CD138 beads for plasma cell selection.
258. The method of anyone of Claims 255-259, wherein the macromolecule, is a protein, protein mimetic or peptide.
259. The method of Claim 260, wherein the macromolecule comprises natural amino acids and/or unnatural amino acids.
260. The method of any one of Claims 260-261, wherein the protein is an enzyme.
261. The method of any one of Claims 255-262, wherein the method further comprises activating the B cells prior to gene editing, wherein activating is performed using at least one stimulant.
262. The method of Claim 263, wherein the at least one stimulant is CD40 ligand, CpG, IL-2, IL-10 and/or IL-15.
263. The method of Claim 264, wherein the CD40 ligand is artificially oligermized.
264. The method of Claim 265, wherein the CD40 ligand is oligermerized with two linked CD40L trimers.

265. The method of any one of Claims 263-266, wherein the cells are cultured for at least 13 days with the at least one stimulant.
266. The method of any one of Claims 255-267, wherein the cells are a naïve B cell phenotype, activated B cell or activated edited B cell after differentiation.
267. The method of any one of Claims 239-248, the cells are CD27-CD138-CD38^{low}/-CD19^{high}IgM⁺ IgD⁺ after differentiation.
268. The method of any one of Claims 255-269, wherein the providing or administering is performed by adoptive transfer.
269. The method of any one of Claims 255-270, wherein the subject has received a stem cell administration or solid organ transplantation.
270. The method of any one of Claims 255-271, wherein the subject suffers from influenza, parainfluenza, rhinovirus, respiratory syncytial virus, HIV, or other pathogenic bacteria, virus, fungus or parasite infection.
271. The method of any one of Claims 255-272, wherein the subject suffers from an enzyme deficiency.
272. The method of any one of Claims 255-273, wherein the subject suffers from pulmonary fibrosis.
273. The method of any one of Claims 255-274, wherein the subject suffers from an autoimmune disorder, immune dysregulation or cancer.
274. The method of any one of Claims 255-275, wherein the subject suffers from diabetes.
275. The method of any one of Claims 255-276, wherein the subject suffers from hypercholesterolemia.
276. The method of any one of Claims 255-277, wherein the subject is also receiving CAR T-cell therapy or is selected to receive such a therapy.
277. The method of any one of Claims 255-278, wherein the subject suffers from HIV.
278. The method of any one of Claims 255-279, wherein the subject suffers from Hemophilia A, Hemophilia B, Hereditary TPP, lysosomal acid deficiency, hereditary angioedema, alpha1 anti-trypsin deficiency, Fabry disease or hypophosphatasia.

279. The method of any one of Claims 255-280, wherein performing the first round of genome editing of the B cells for protein expression is performed in the absence of viral integration.
280. The method of any one of Claims 255-281, wherein performing the first round of genome editing of the B cells for protein expression is performed by introduction of a single stranded nucleic acid.
281. The method of any one of Claims 255-282, wherein the first round of genome editing is performed by an RNA and protein based transfection.
282. The method of Claim 283, wherein the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell.
283. The method of any one of Claims 255-284, wherein the first round of genome editing comprises delivering a nuclease, wherein the nuclease targets at least one genetic loci in the B cell and co-delivering the nuclease with a single stranded nucleic acid donor template.
284. The method of any one of Claims 255-285, wherein the nuclease is a synthetic guide RNA targeting clustered regularly interspersed short palindromic repeat DNA (CRISPR) coupled to a Cas nuclease.
285. The method of any one of Claims 255-286, further comprising monitoring of the subject following plasma cell transplantation.
286. The method of Claim 287, wherein the subject is determined to no longer need treatment and the method further comprises administering rituxan or an anti-CD20 antibody for targeted removal of the plasma cells.
287. A method of inducing tolerance of a peptide useful for immunotherapeutic application, secreted by a B cell in a subject in need *in vivo* and/or increasing engraftment of the B cell in a subject in need, the method comprising:
- A) isolating a first population of B cells;
 - B) performing a first round of genome editing of the first population of B cells for protein or peptide expression in absence of viral integration, wherein the performing is under conditions of rapid B cell expansion;
 - C) expanding the first population of B cells;

D) differentiating the first population of B cells, optionally, after step (c) or (d), thereby producing an activated first population of B cells or naïve activated first population of B cells that express the peptide;

E) providing or administering the first population of B cells that expresses the peptide into a subject in need; and

F) administering a second population of B cells that expresses a macromolecule for tolerance, survival of a B cell or engraftment.

288. The method of Claim 289, wherein the immunotherapeutic application is the treatment or amelioration of a cancer.

289. The method of Claim 290, wherein the cancer comprises a solid tumor.

290. The method of any one of Claims 289-291, wherein the peptide is an anti-PD1 antibody or a PDL-1 ligand.

291. The method of Claim 292, wherein the second B cell expresses BAFF, APRIL, IFN-alpha, IL-10 or IL-6.

292. The method of Claim 292, wherein the second B cell expresses a second peptide that is expressed and presented as a MHC class II molecule on a surface of the B cell.

293. The method of Claim 292, wherein the second B cell expresses a viral vector capsid protein.

294. The method of any one of Claims 289-295, further comprising monitoring of the subject following B cell transplantation.

295. The method of Claim 296, wherein the subject is determined to no longer need treatment and the method further comprises administering rituxan or an anti-CD20 antibody for targeted removal of the B cells.

296. The method of Claim 295, wherein the viral vector capsid protein comprises one or more of the following serotypes: AAV1 (VP1, VP2 and VP3), AAV2 (VP1, VP2 and VP3), AAV4 (VP1, VP2 and VP3), AAV5 (VP1, VP2 and VP3), AAV6 (VP1, VP2 and VP3), AAV1 (VP1, VP2 and VP3), AAV8 (VP1, VP2 and VP3), AAV1 (VP1, VP2 and VP3) and/or AAV9 (VP1, VP2 and VP3).

297. A method of inducing tolerance of a peptide for immunotherapeutic application, secreted by B cells in a subject *in vivo* and/or increasing engraftment or survival of the B cells in the subject, the method comprising:
- A) isolating a first population of B cells;
 - B) performing a first round of genome editing of the first population of B cells for protein or peptide expression in absence of viral integration, wherein the performing is under conditions of rapid B cell expansion;
 - C) expanding the first population of B cells;
 - D) differentiating the first population of B cells, optionally, after step (c) or (d), thereby producing an activated first population of B cells or naïve activated first population of B cells that express the protein or peptide;
 - E) providing or administering the first population of B cells that expresses the protein or peptide into a subject in need;
 - F) providing or administering a second population of B cells that expresses a macromolecule, wherein the second population of B cells allows tolerance of the protein or peptide and/or promotes engraftment and/or survival.
298. The method of Claim 297, wherein the first population of B cells and the second population of B cells are co-administered.
299. The method of Claim 297 or 298, wherein the macromolecule comprises BAFF, APRIL, IFN-alpha, IL-10 or IL-6.
300. The method of any one of Claims 297-299, wherein the protein comprises an enzyme, neutralizing antibody or a binding portion thereof, cytokine, cytokine receptor, complement protein, inhibitory protein, anti-fibrotic molecule, therapeutic antibody or a binding portion thereof, anti-thrombotic molecule, glucose response element or monoclonal antibody or a binding portion thereof.
301. The method of Claim 300, wherein the protein is SERPING1 or SERPINA1.
302. The method of Claim 300, wherein the protein is a receptor antagonist for the treatment or inhibition of periodic fever/autoinflammatory syndromes, complement inhibitory proteins (including Factor H, Factor I) for the treatment or inhibition of atypical hemolytic uremic syndrome/membranoproliferative glomerulonephritis or a C1 inhibitor for hereditary angioedema.

303. The method of Claim 302, wherein the protein is an anti-fibrotic molecule, wherein the anti-fibrotic molecule is SCGB1A1.
304. The method of Claim 302, wherein the protein comprises therapeutic antibodies or a binding portion thereof are specific for a protein expressed in autoimmune disorders, autoinflammatory disorders, immune dysregulation and/or cancer.
305. The method of Claim 302, wherein the antibody or a binding portion thereof is an anti-IL-1 monoclonal antibody, anti-TNF antibody, anti-IL-33 antibody or anti-C5 antibody or a binding portion of any one or more of these antibodies.
306. The method of Claim 302, wherein the protein is an anti-thrombotic molecule, wherein the anti-thrombotic molecule is APLN.
307. The method of Claim 302, wherein the protein comprises an anti-PCSK9 inhibitory antibody or a binding portion thereof.
308. The method of Claim 302, wherein the protein comprises broadly neutralizing HIV-1 antibodies (bNAbs) or a binding portion thereof.
309. The method of any one of Claims 299-308, further comprising monitoring of the subject following B cell administration.
310. The method of Claim 310, wherein the subject is determined to no longer need treatment and the method further comprises administering rituxan or an anti-CD20 antibody for targeted removal of the B cells.

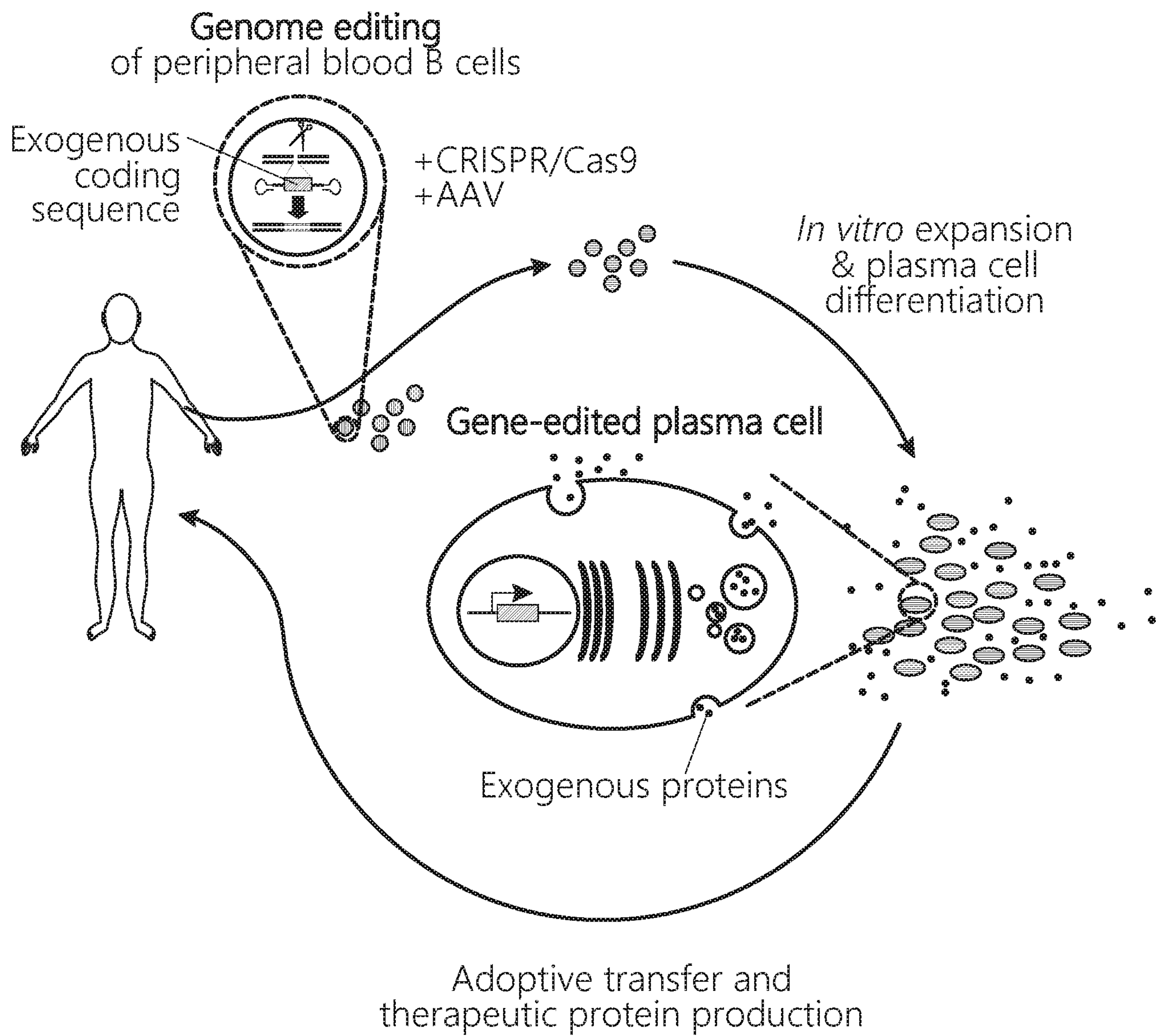


FIG. 1

Longevity

- Human influenza antibody titers persist for > 90 years
- Persist in non-dividing state within bone marrow survival niche without need for ongoing antigen exposure.
- Relatively resistant to immunosuppression/chemotherapy

Protein production

- Plasma cell: 2000-10,000 immunoglobulin molecules per second; ~50-340 pg protein/cell/day.
- Industrial cell-line protein production ~20-90 pg protein/cell/day

Potential for Ex vivo manipulation and generation**FIG. 2**

Candidate Nuclease Platforms

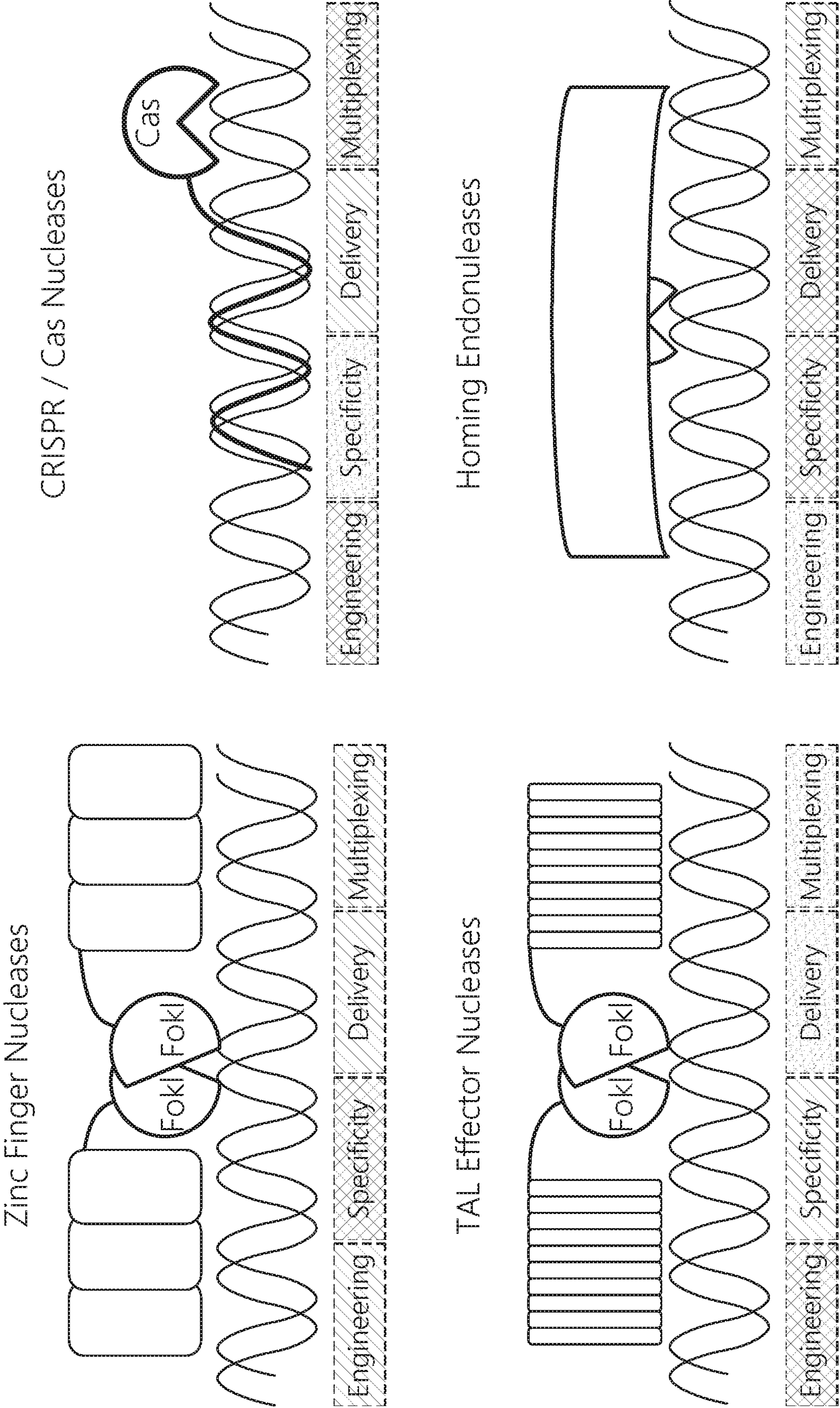


FIG. 3

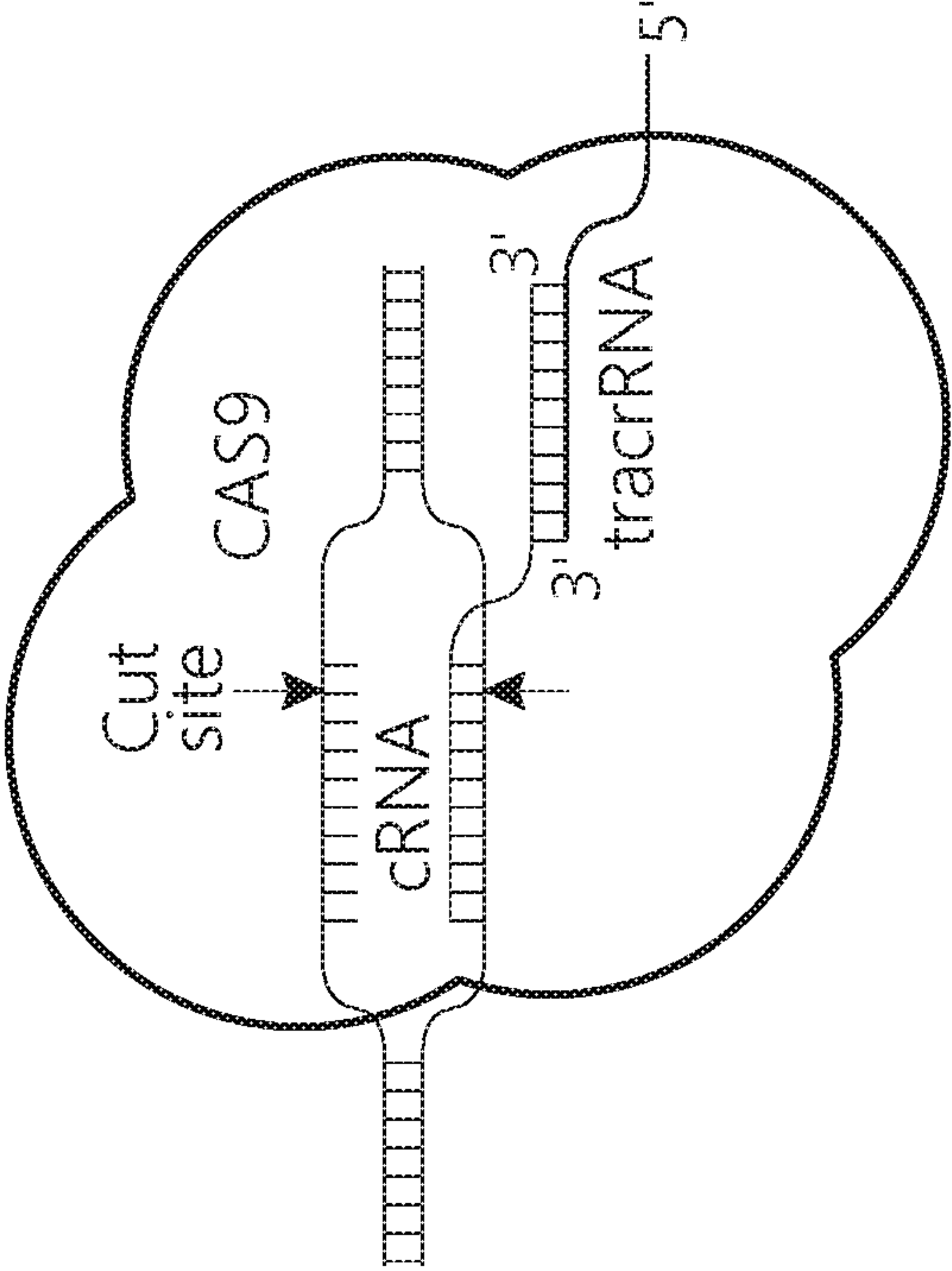
CRISPR EDITING OF PRIMARY B CELLS

	Plasmid Delivery (Incl. Sleeping beauty)	Lentiviral delivery	RNA (or RNP) + AAV co-delivery	RNP / ODN delivery
Ease	++++	+++	++	++++
Cost	+	++	++	+
Efficiency	+	+	+++ +++	+++ +++
Specificity	+	+	+++ +++	+++ +++

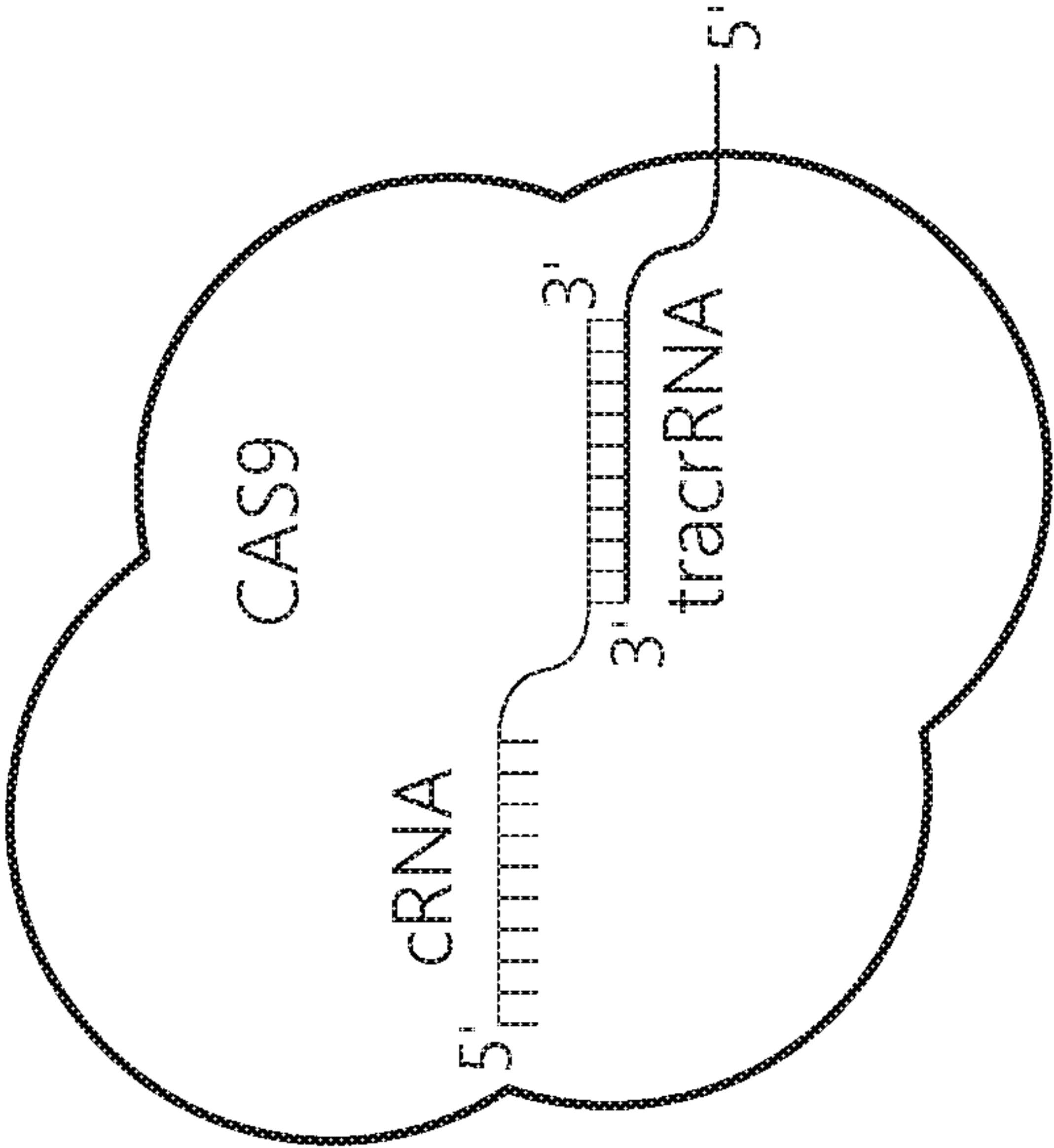
*Note that these assessments are B cell specific

FIG. 4

Cleave target



Transfect
primary B cells



Complex CAS9
and guides *in vitro*

FIG. 5

Day 5 Cleavage (PRDM1)

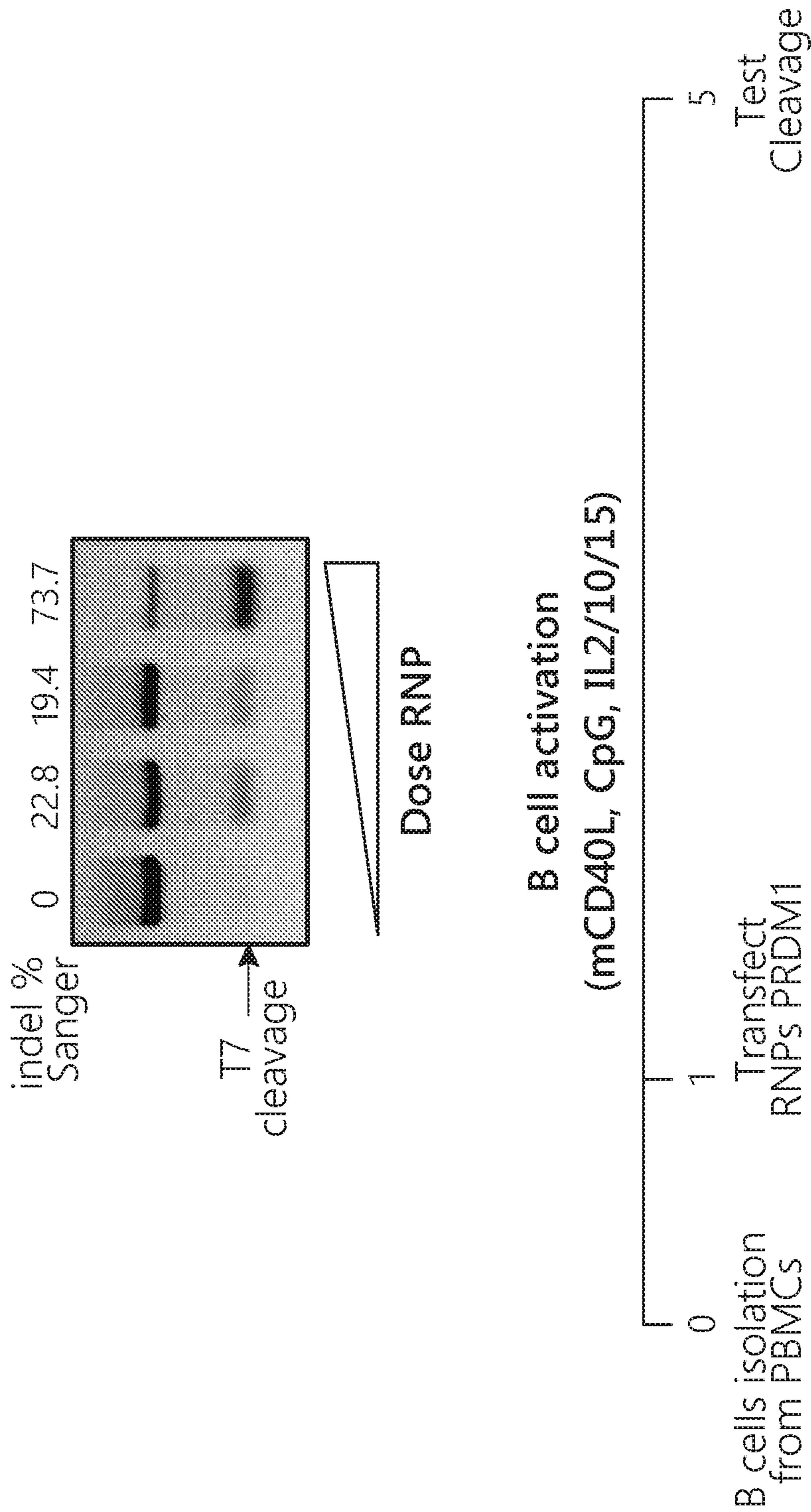


FIG. 6

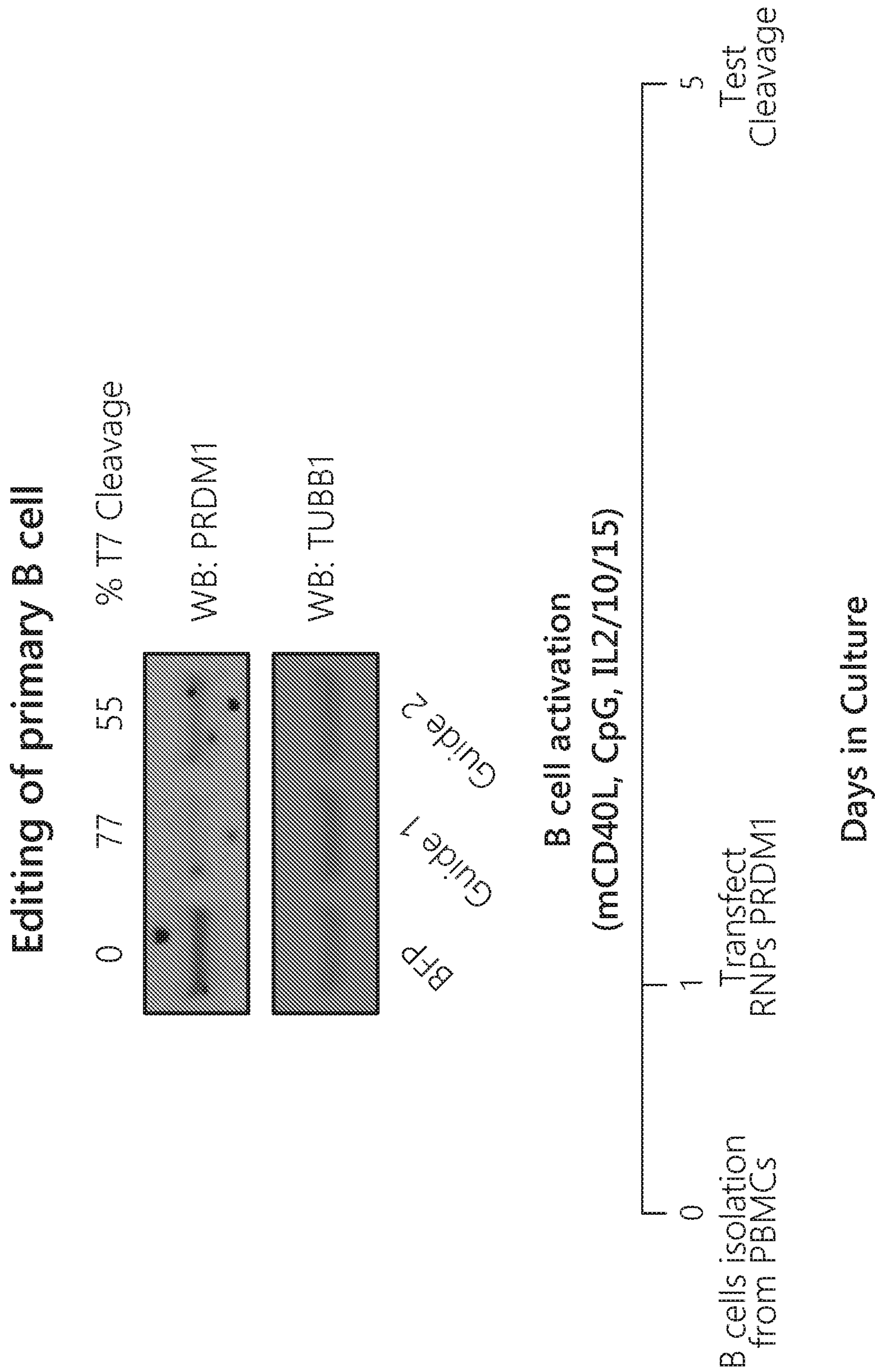
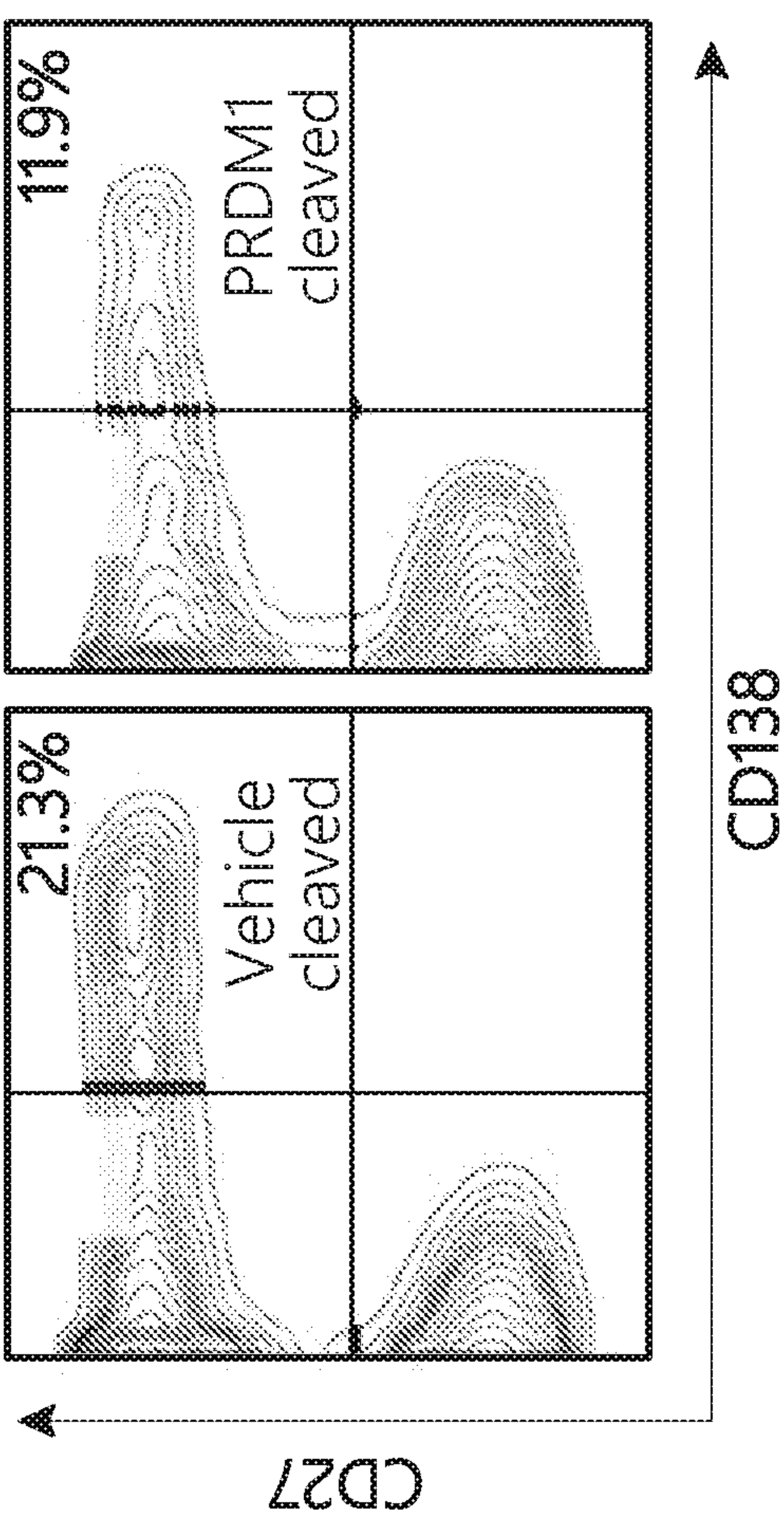


FIG. 7

PRDM1 disruption limits plasmablast generation

Day 9 Plasma Cell Differentiation



PRDM1 cleavage sanger sequencing	0	69%
Expected biallelic disruption	0	49%
Observed depletion CD38+/CD27+	0	44%

FIG. 8

Homologous-directed repair (HDR) using
single-stranded DNA templates

Repair template (short ssODN)

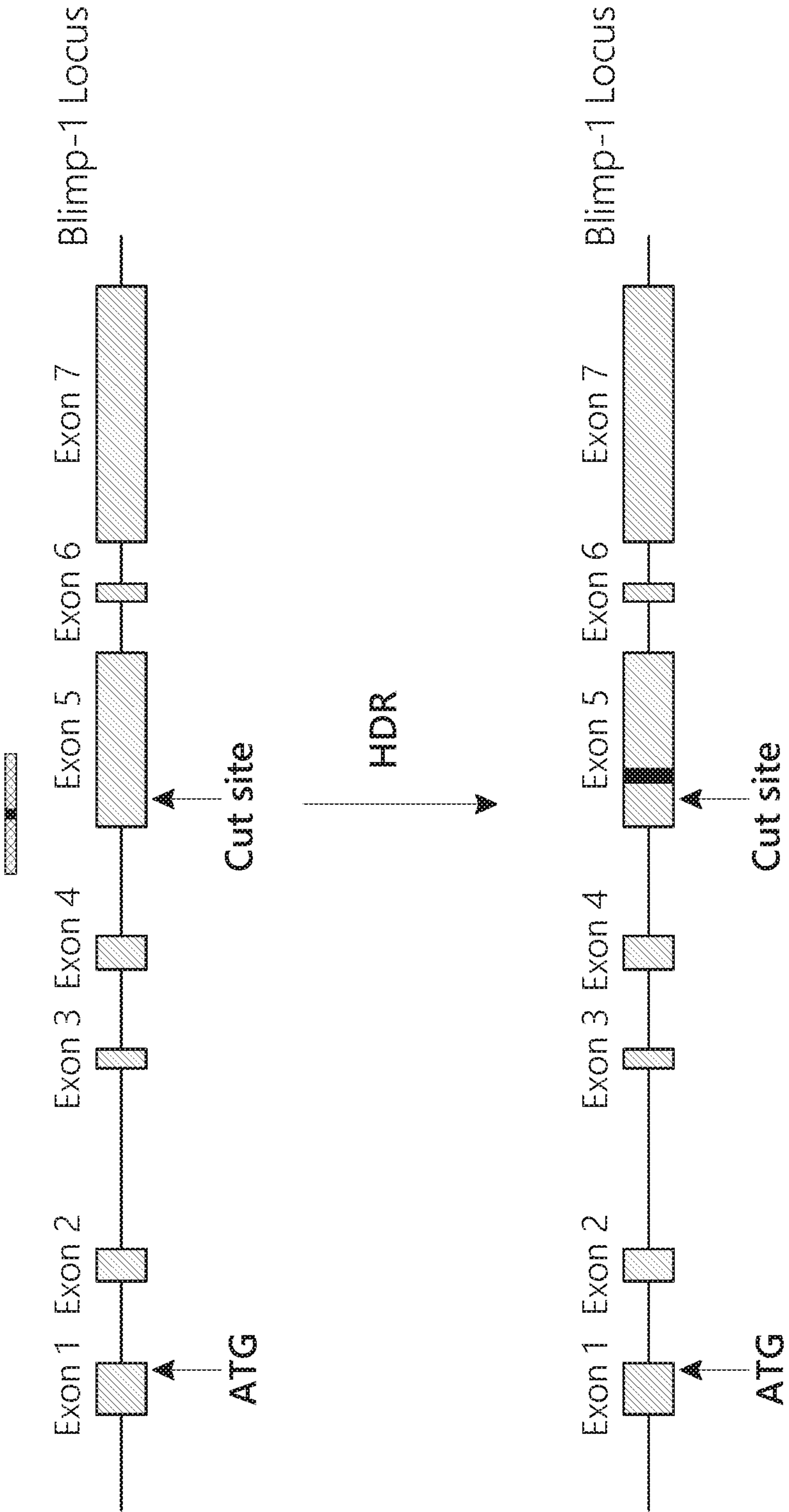
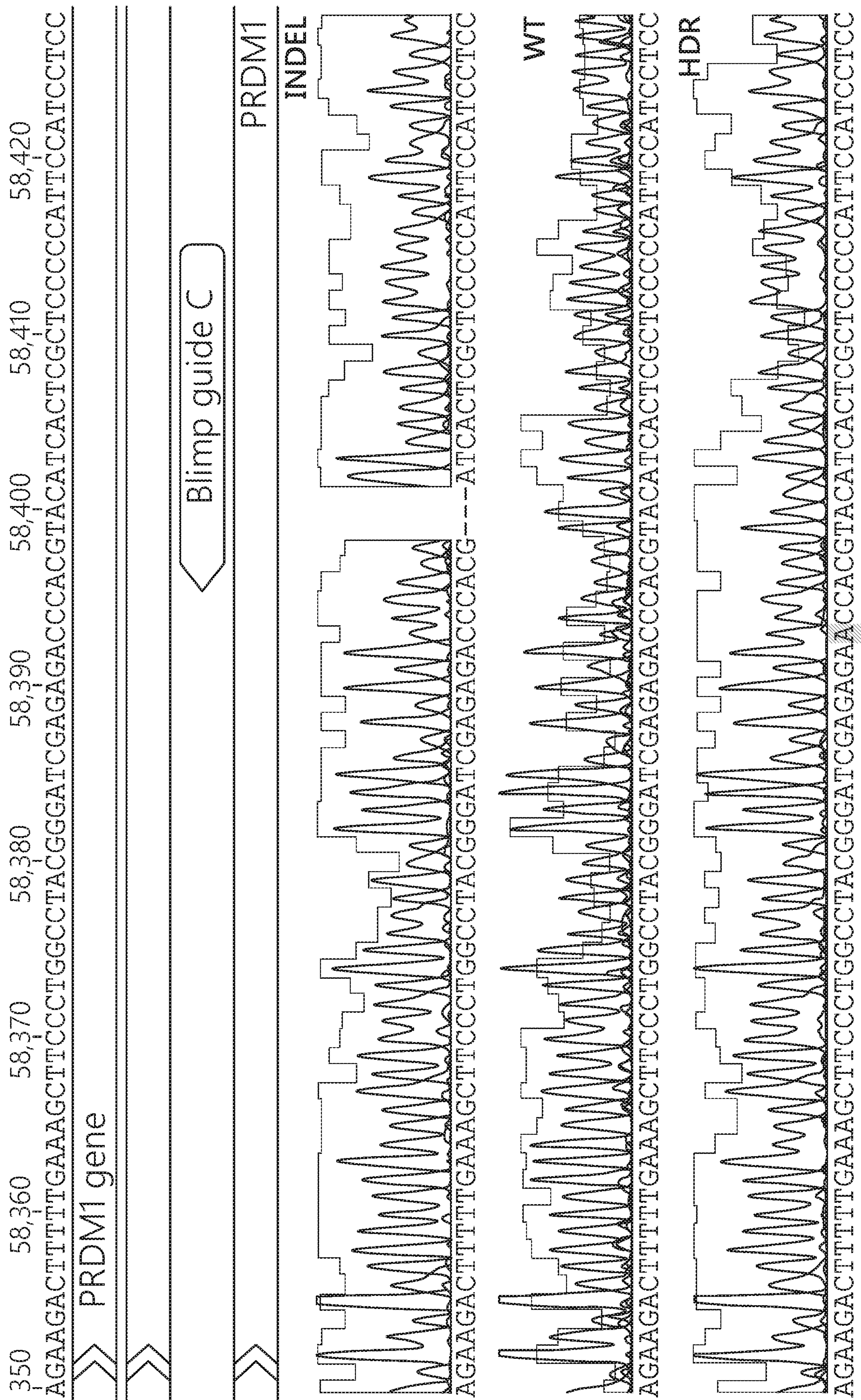


FIG. 9



OFFICE

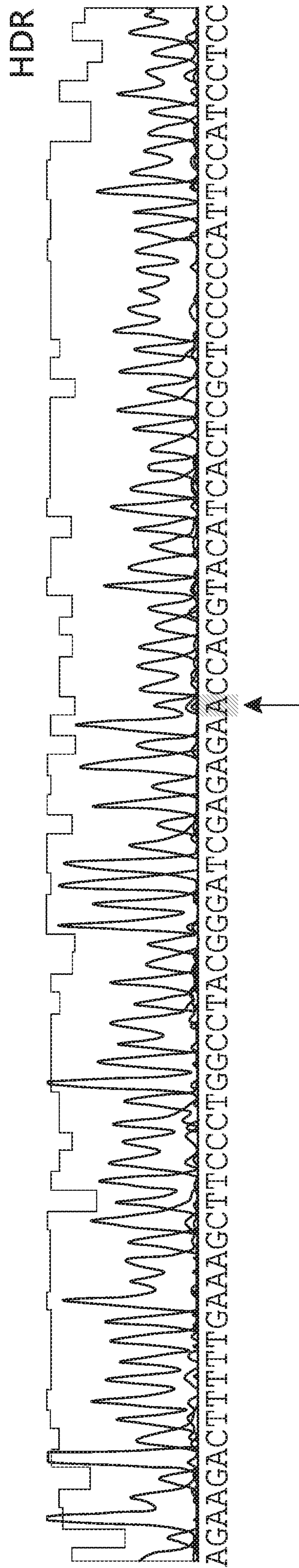
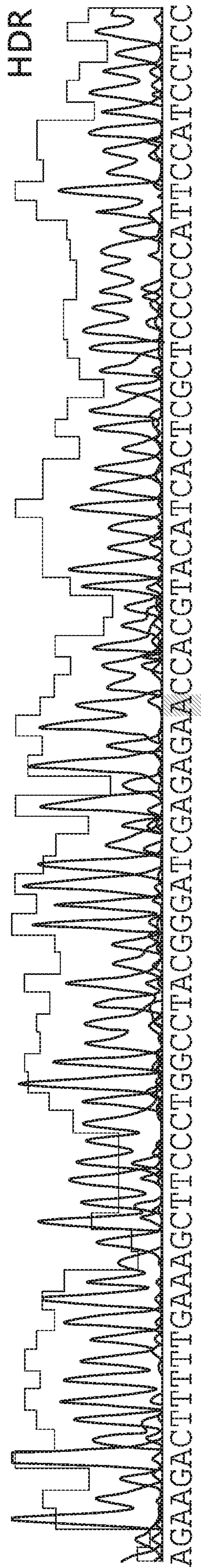
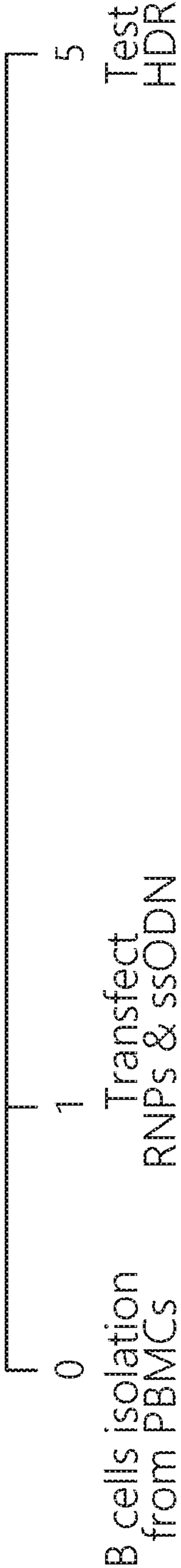


FIG. 10 (Continued)

Editing Condition	INDEL rate	HDR rate
Cas9:BlimpgC (2.5µg) + ODN (15pmol)	44% (11/25)	36% (9/25)
Cas9:BlimpgC (2.5µg) + ODN (30pmol)	24% (5/21)	38% (9/21)
Cas9:BlimpgC (5µg) + ODN (15pmol)	41% (21/51)	35% (18/51)
Cas9:BlimpgC (5µg) + ODN (30pmol)	40% (6/15)	33% (5/15)



Days in Culture

FIG. 10 (Continued)

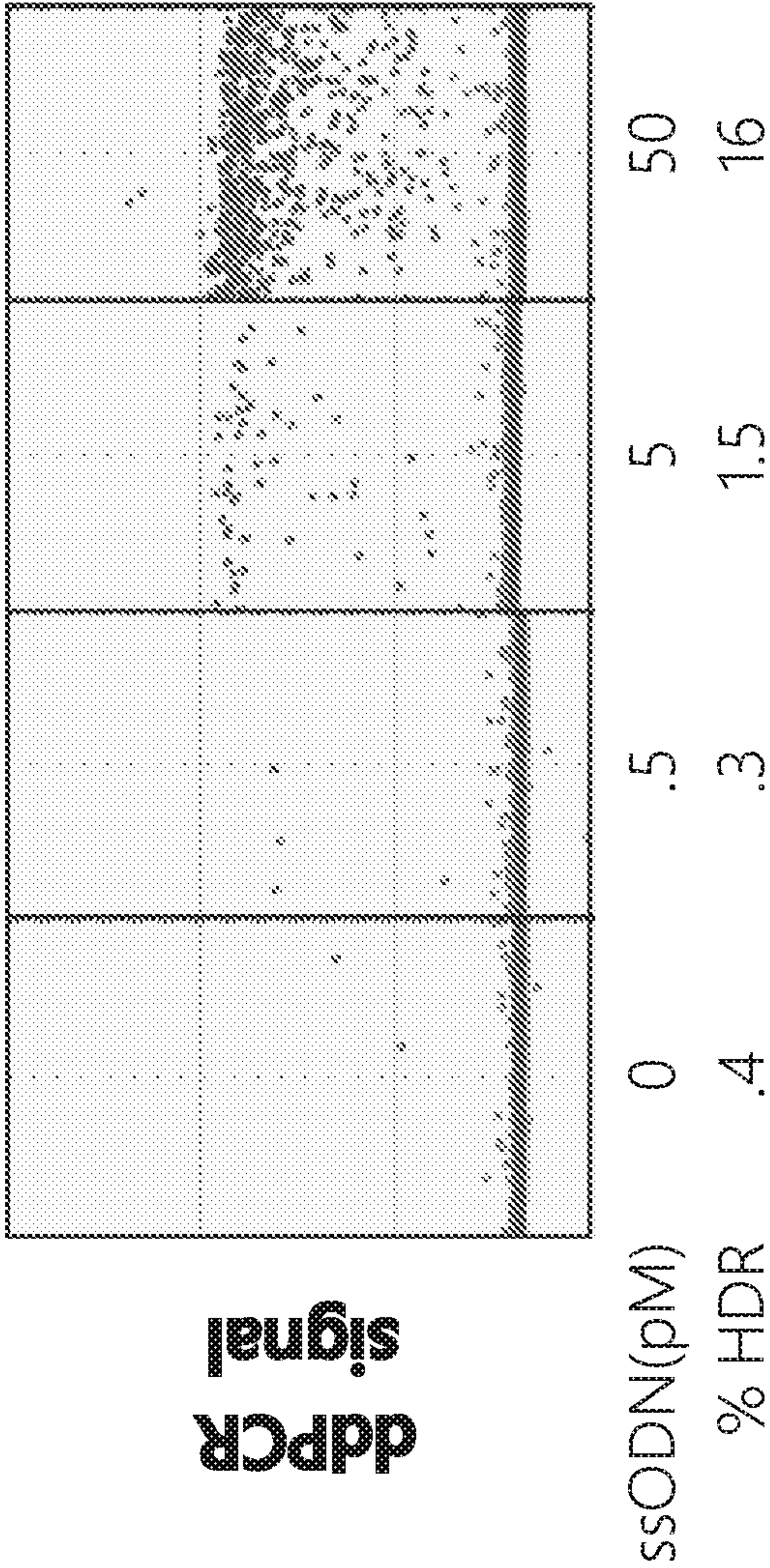


FIG. 11

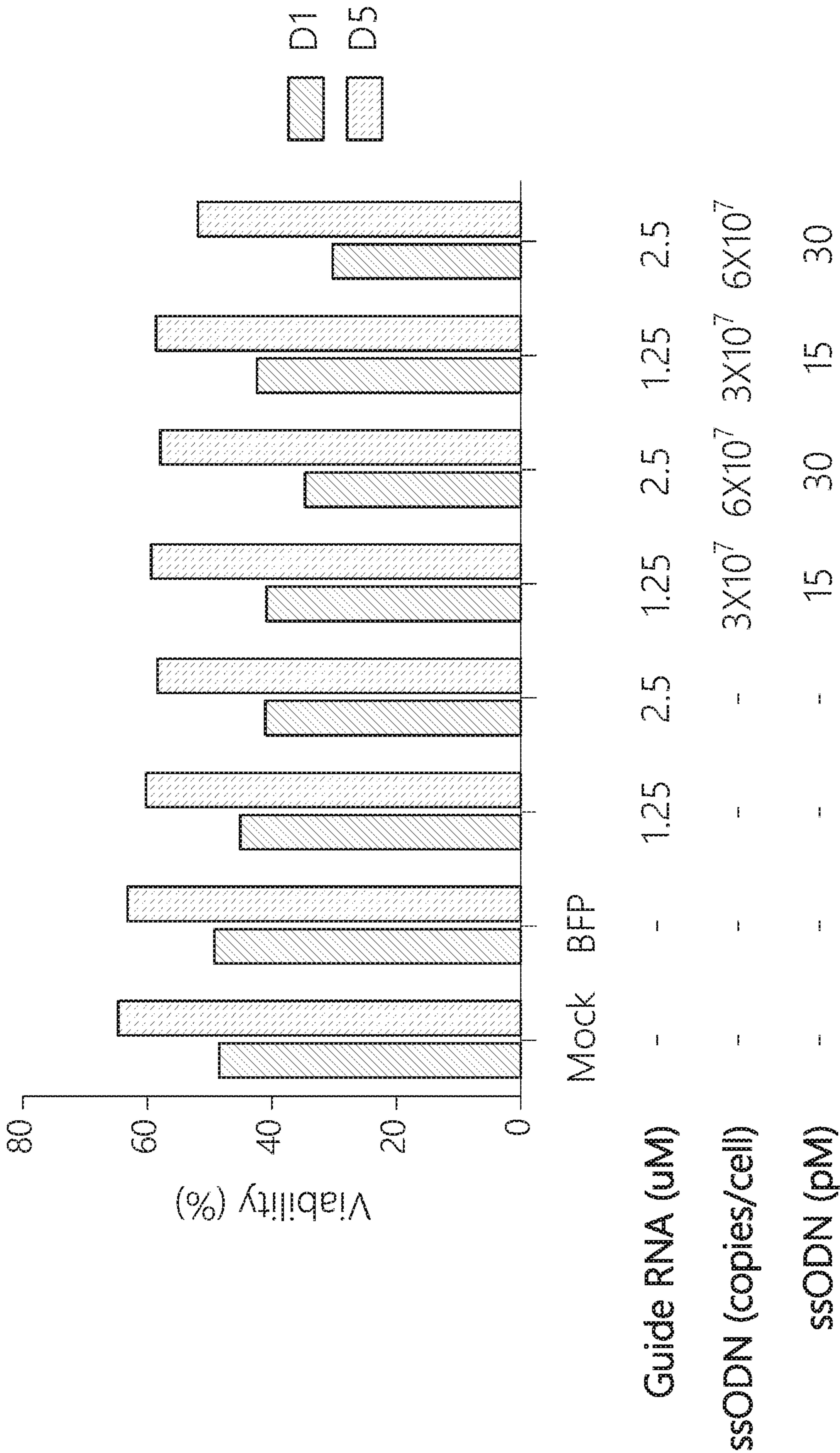


FIG. 12

Repair template (2kb ssODN)

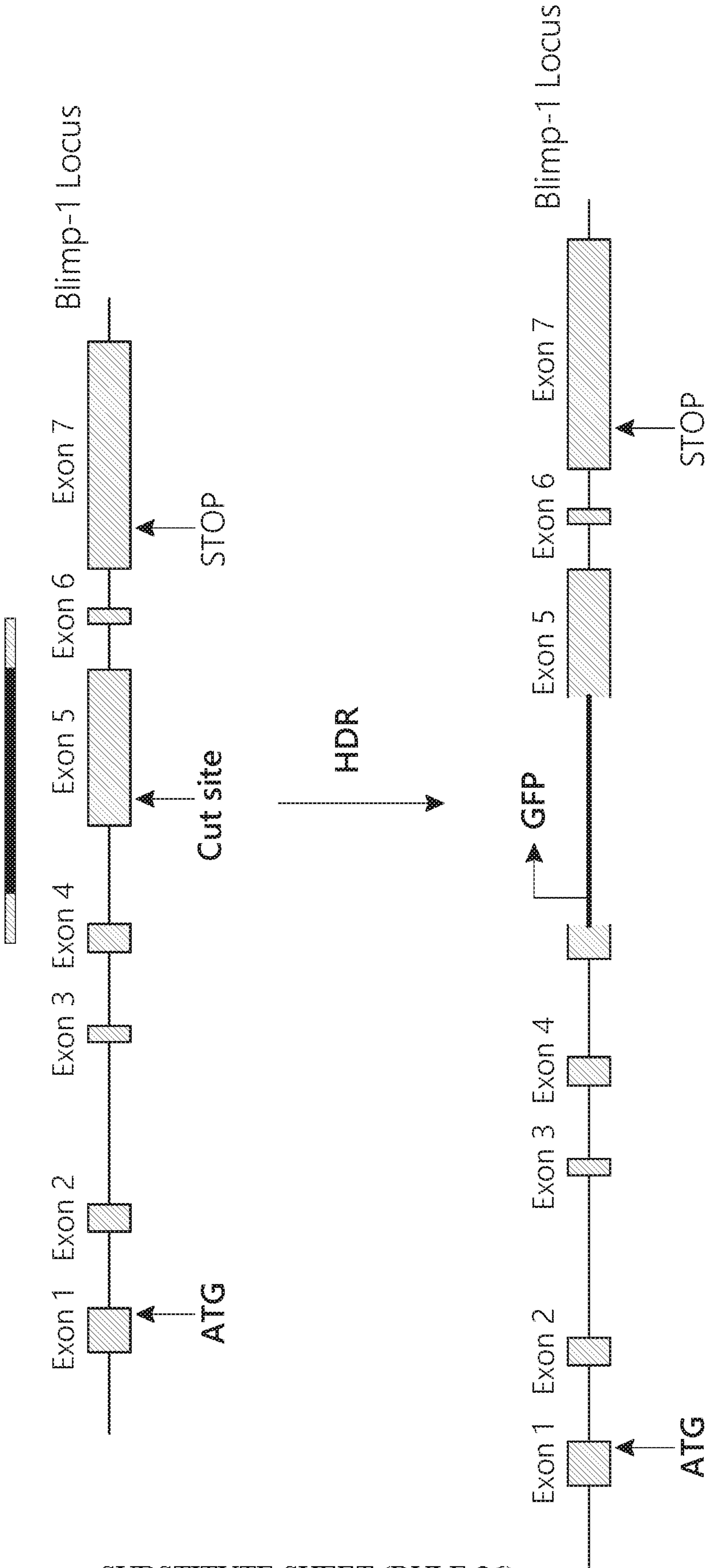


FIG. 13

Long template HDR is effective

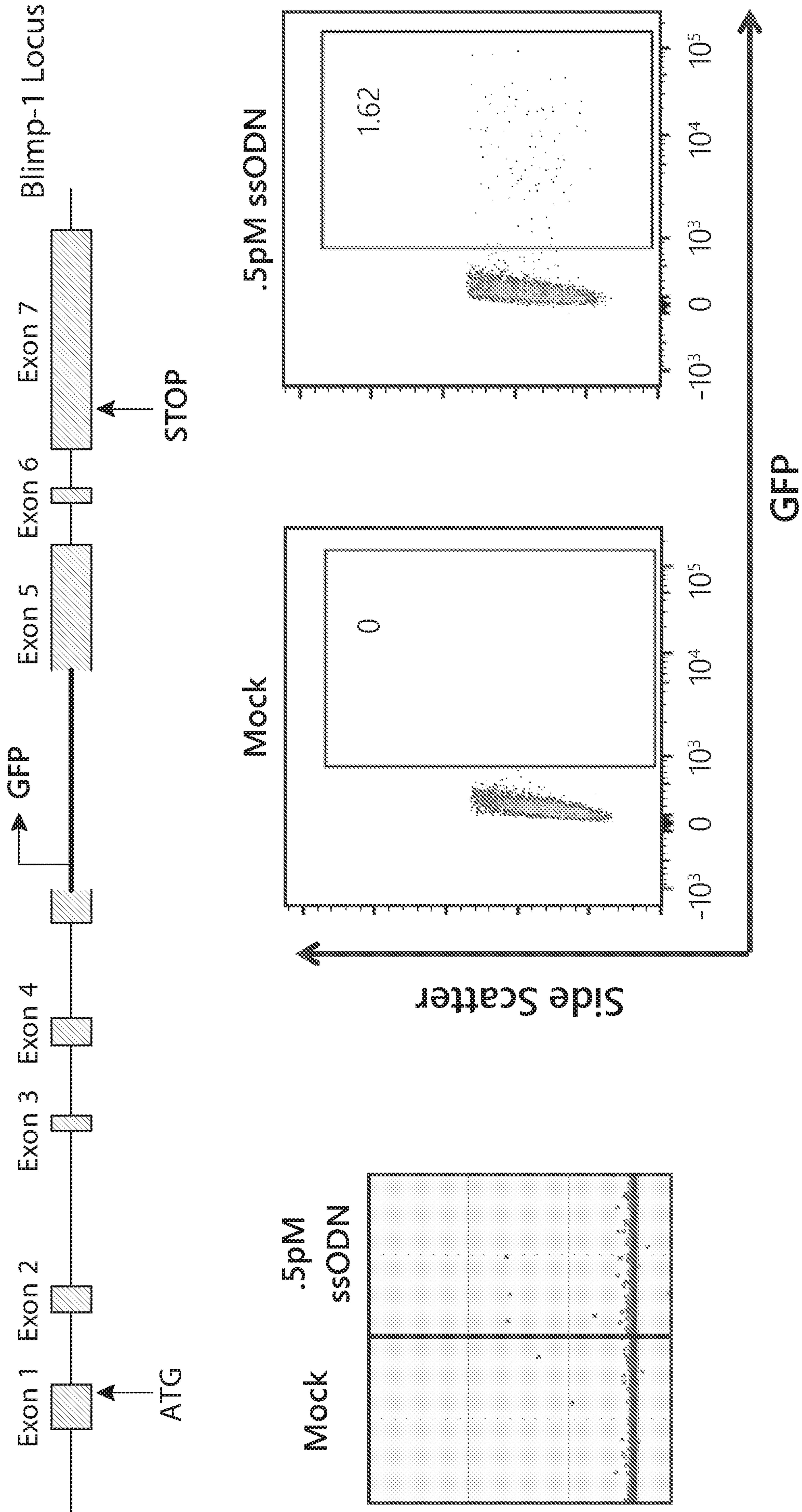
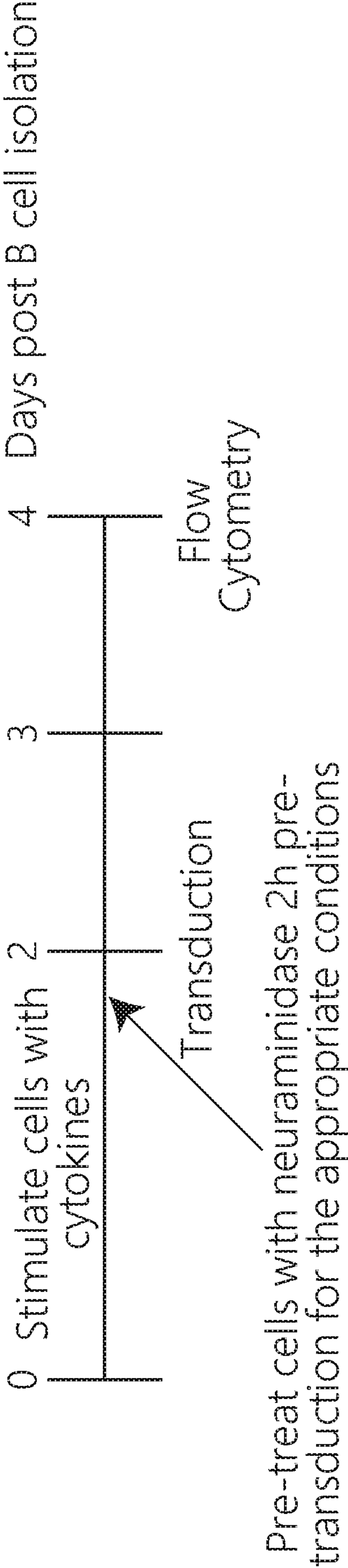


FIG. 14

Transduction 48h post isolation:



scAAV #1079	Titer (particles/ml)
1	9.50E+11
2	1.90E+12
2.5	1.30E+12
5	5.60E+12
6	2.00E+11
8	1.70E+12
9	2.00E+12
D-J	7.00E+11

FIG. 15

Transduction 48h Viability

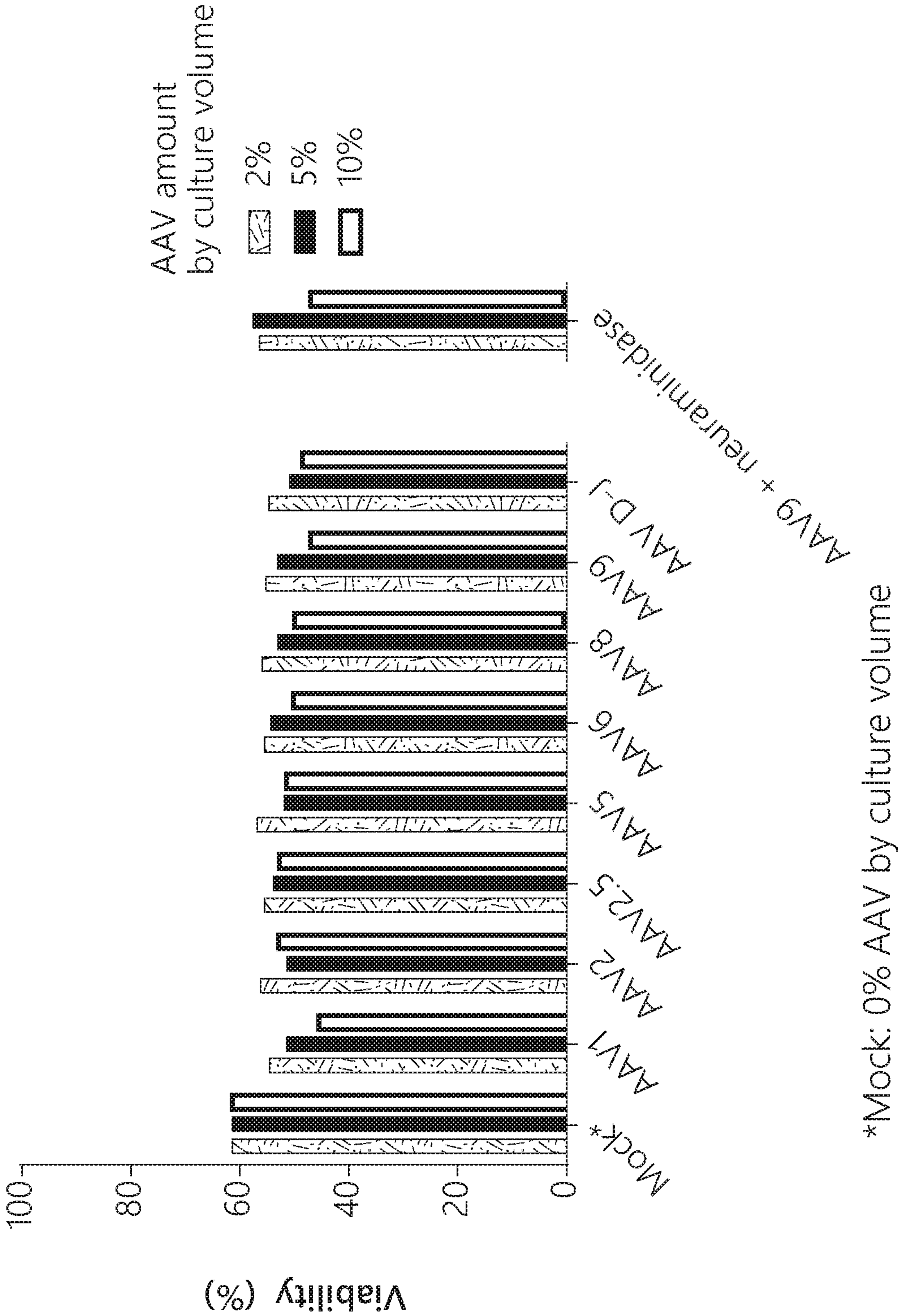


FIG. 15 (Continued)

%GFP with Different AAC Serotypes

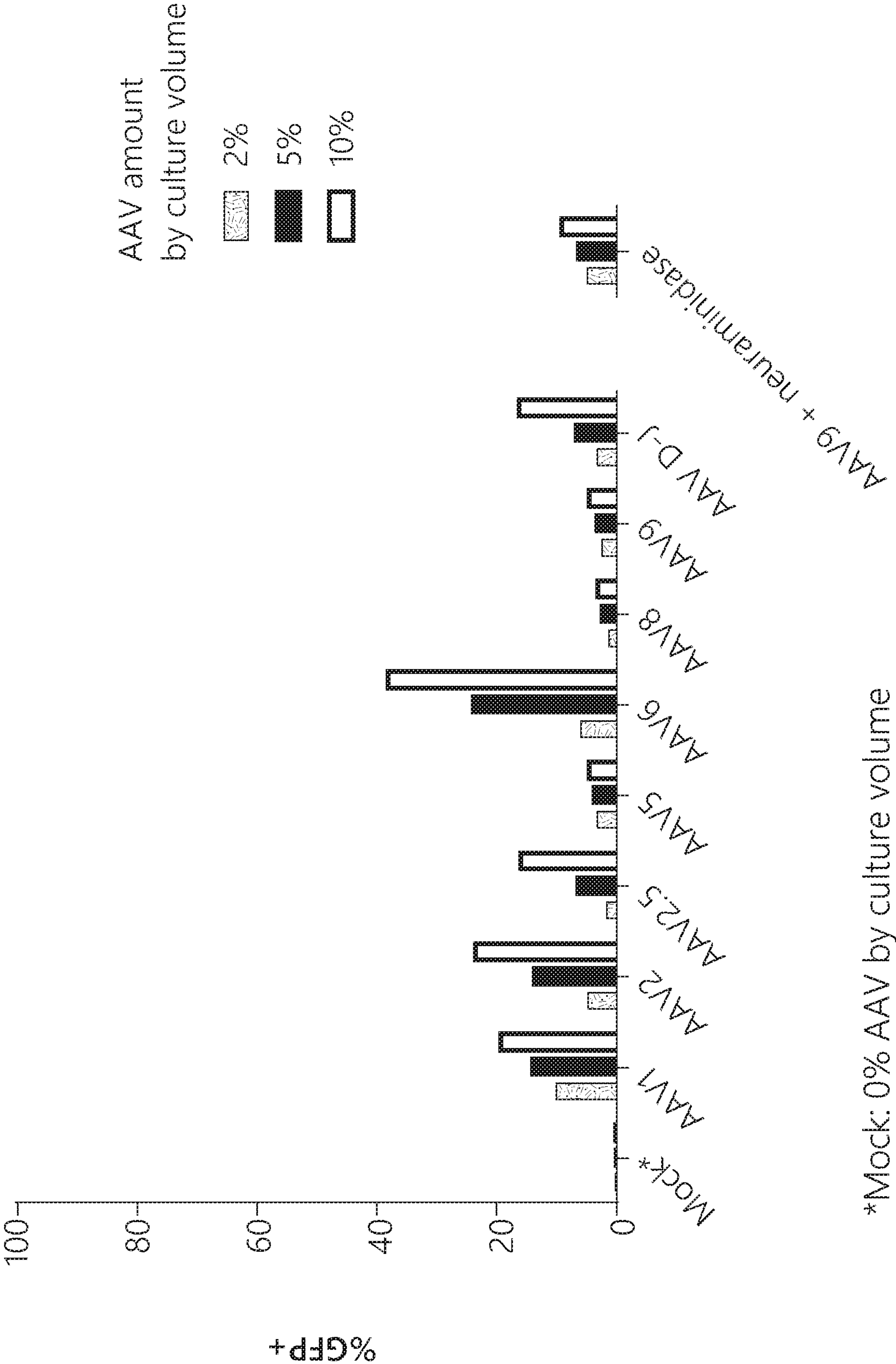
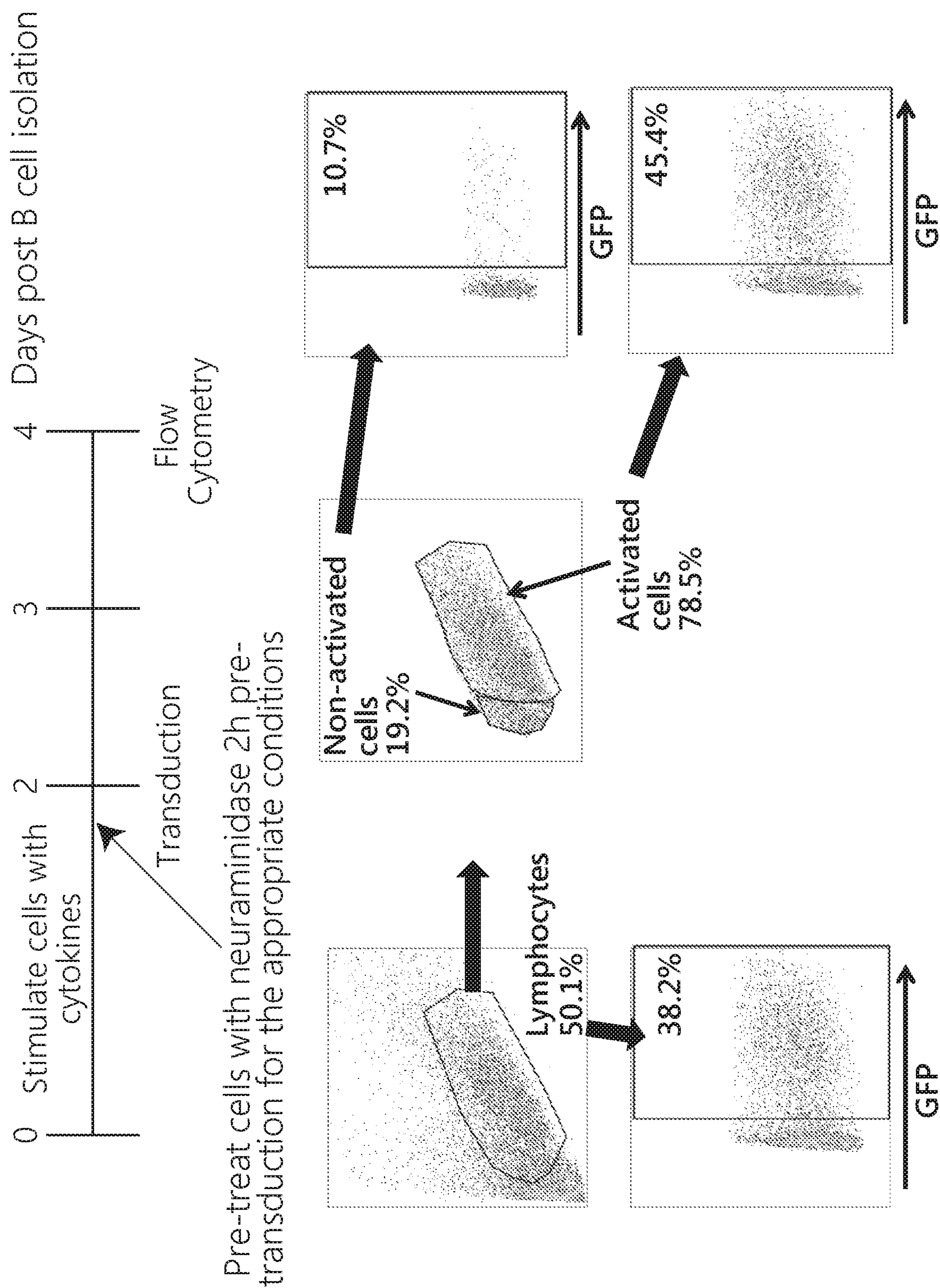


FIG. 15 (Continued)

915

Testing Primary B cell Transduction with AAV

Transduction 48h post isolation:



D2 post transduction

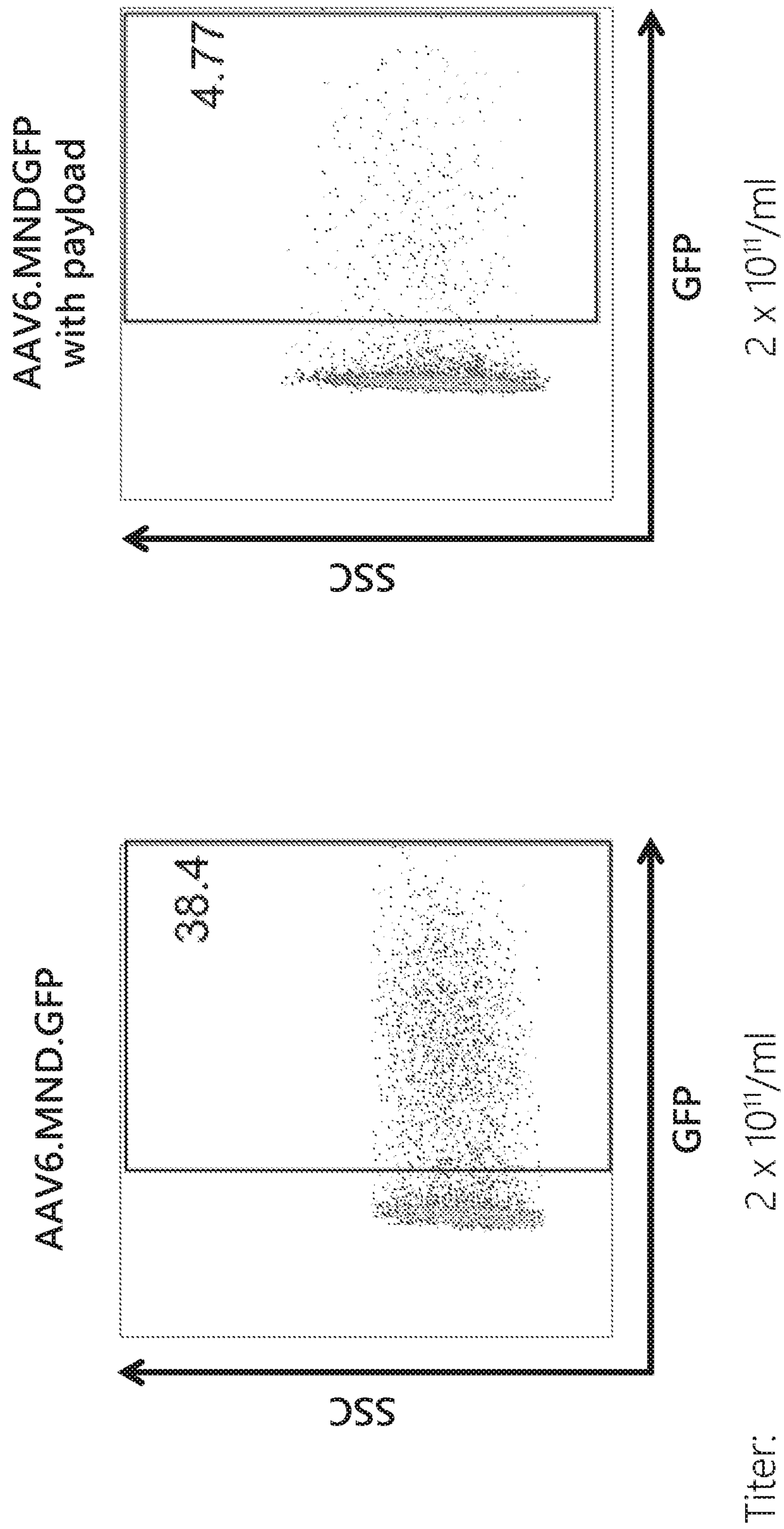


FIG. 17

Strategy: Homologous-directed repair
(HDR) using AAV repair templates

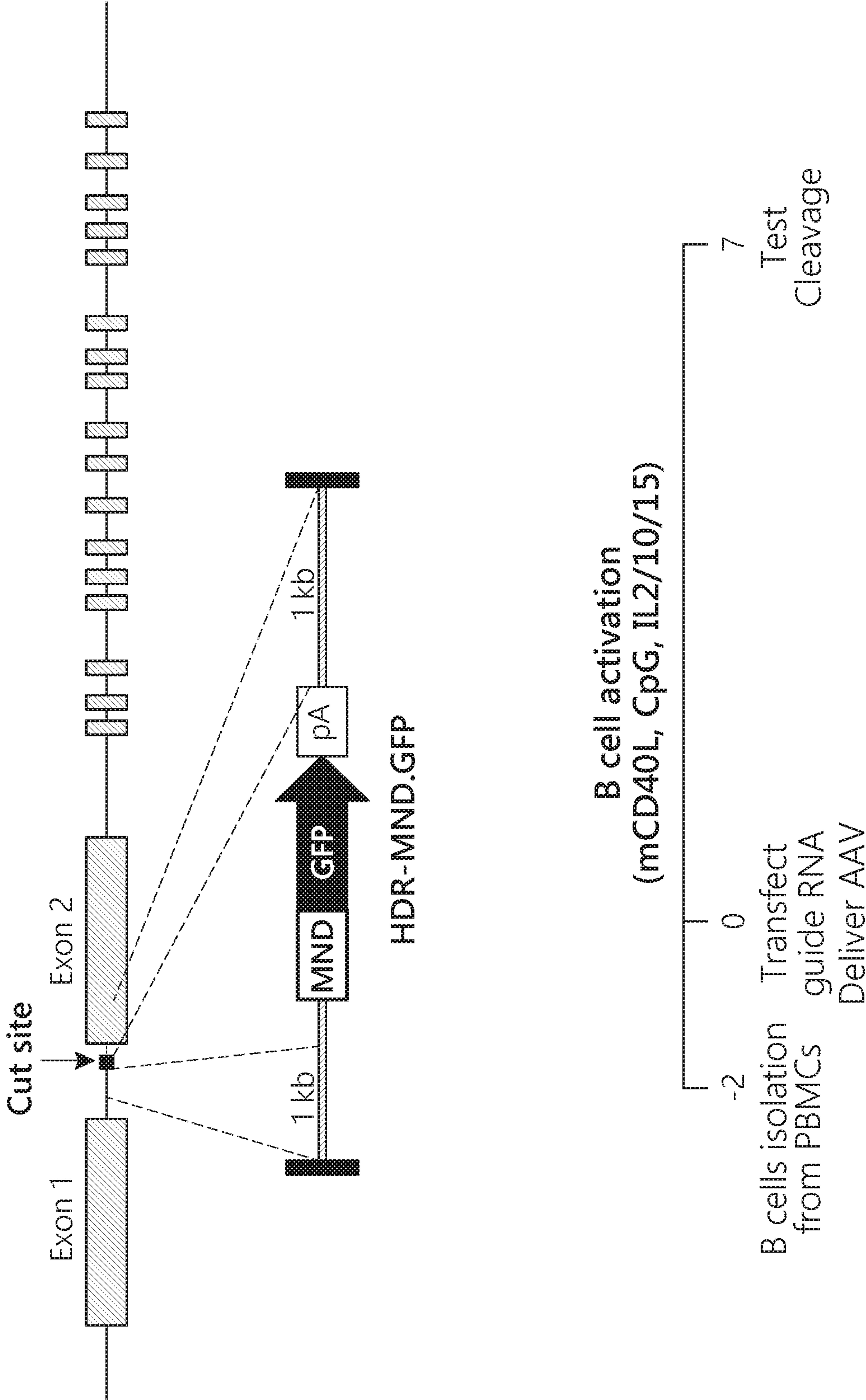


FIG. 18

CRISPR and AAV repair template co-delivery mediates HDR in primary B cells

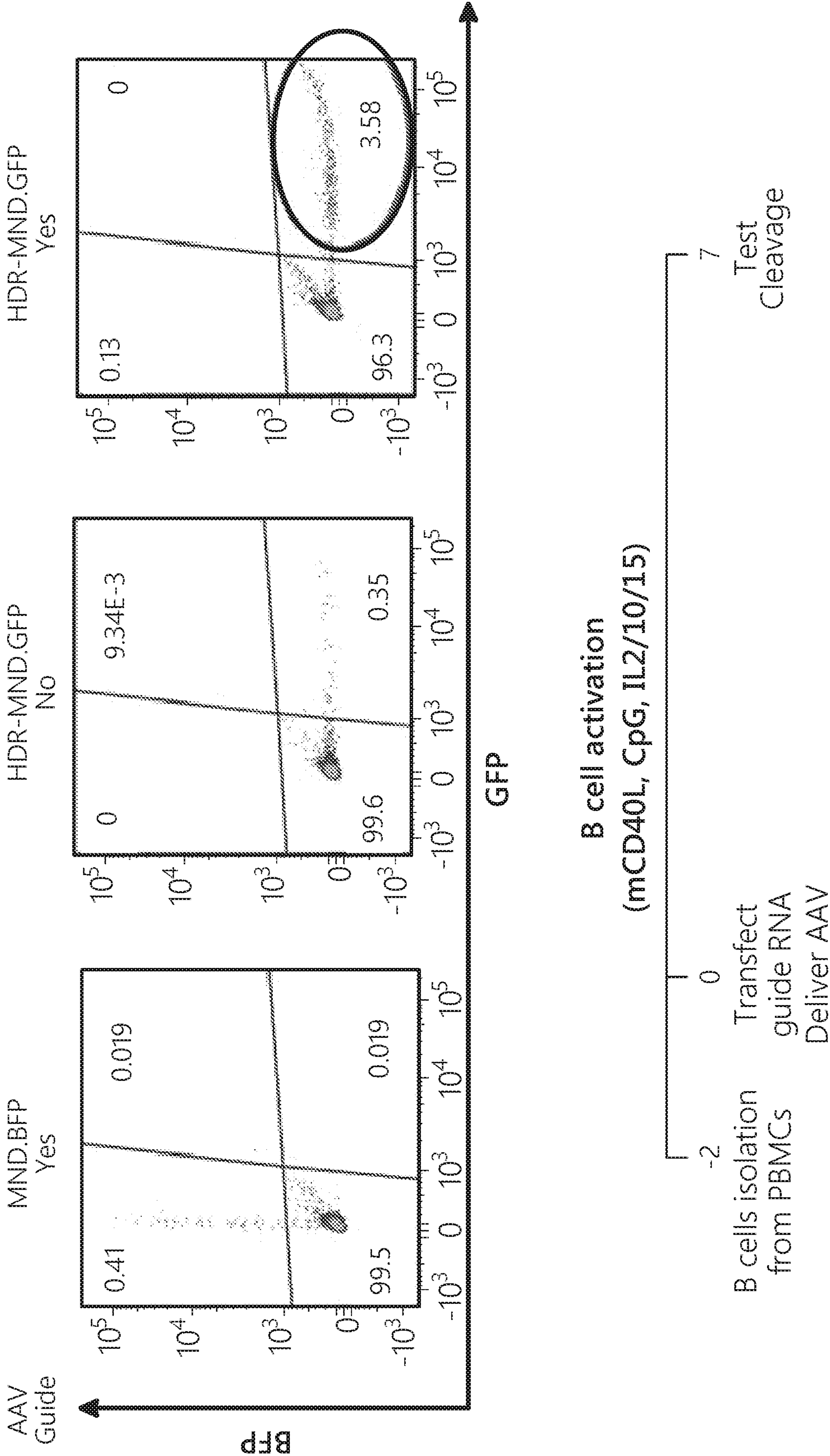
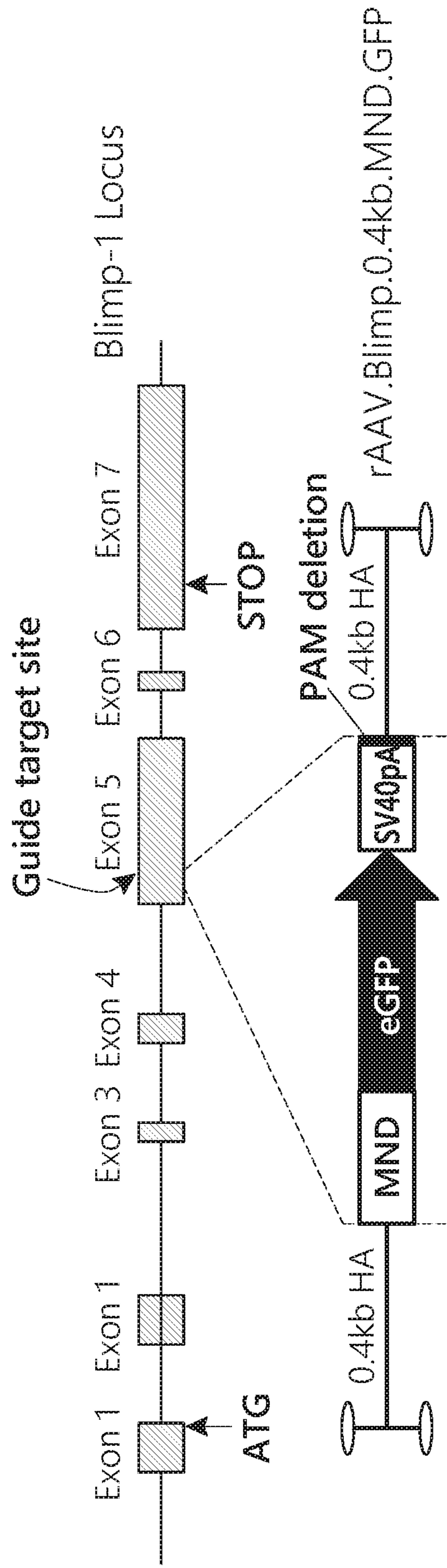
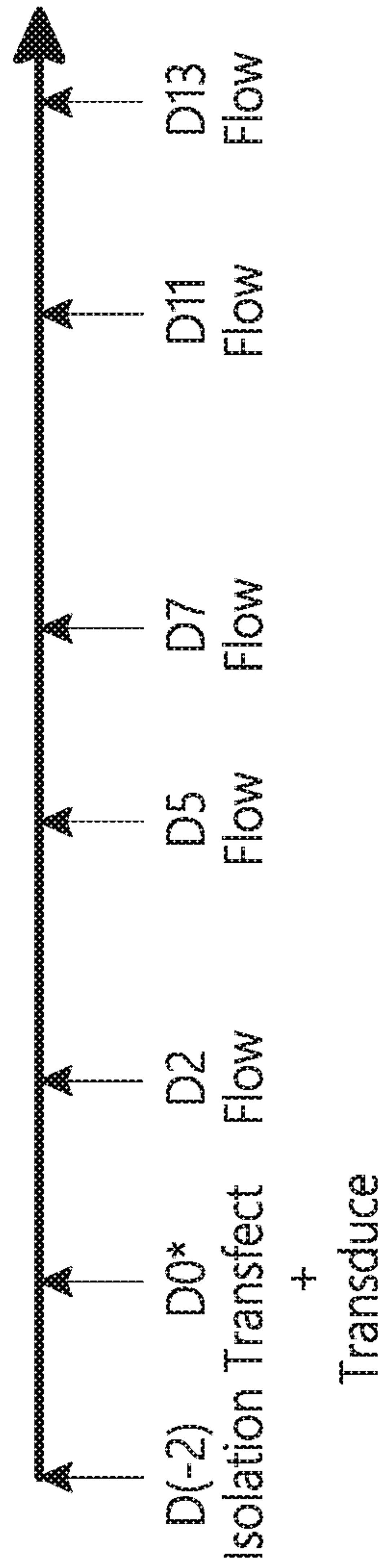


FIG. 19

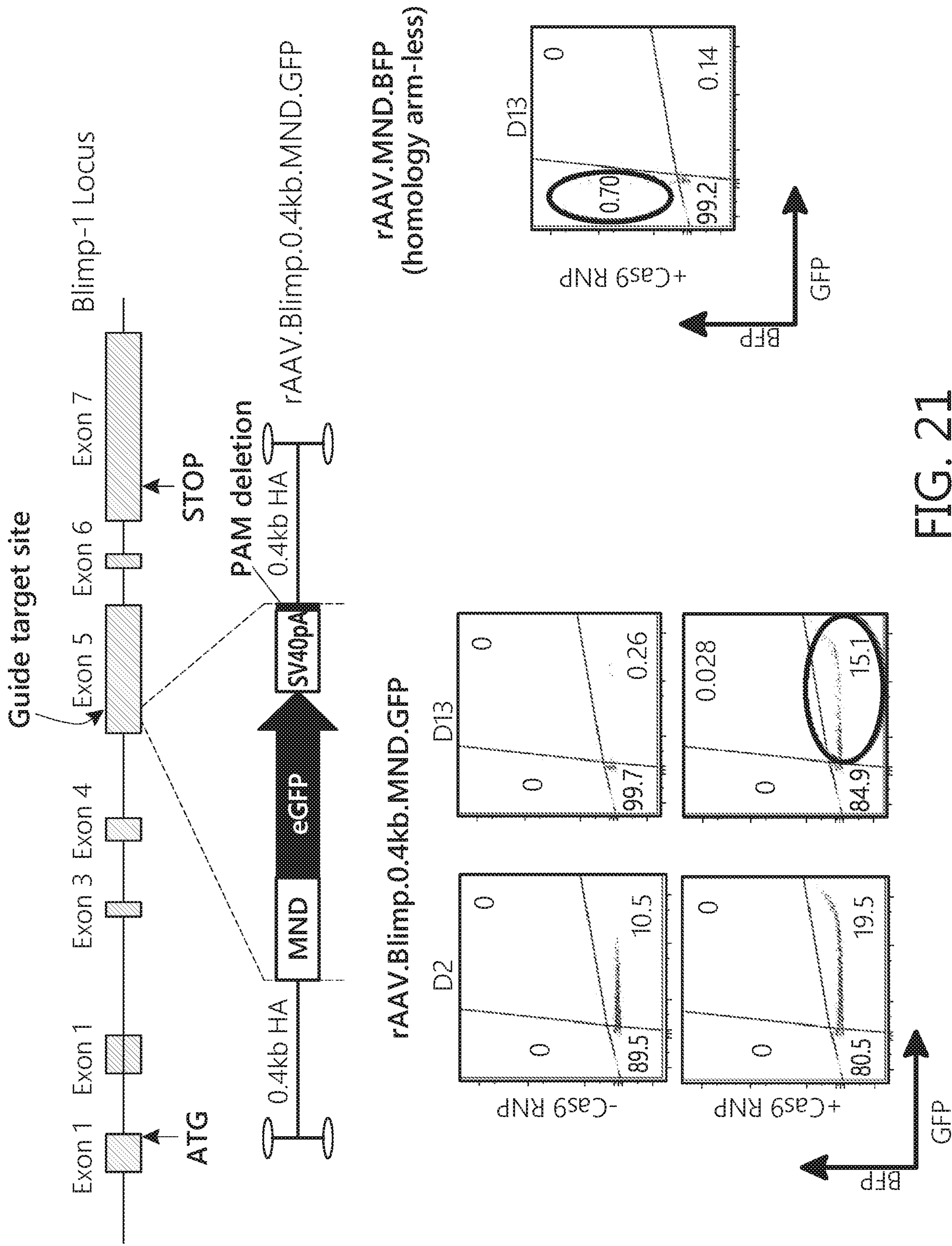


Experimental Setup



*AAV (20% culture volume) as added immediately after transfection.
5µg Cas9 protein complexed with crRNA:tracrRNA at a 1:1.2 molar ratio.

FIG. 20



Negative selection isolation of B cells
from healthy donor PBMCs

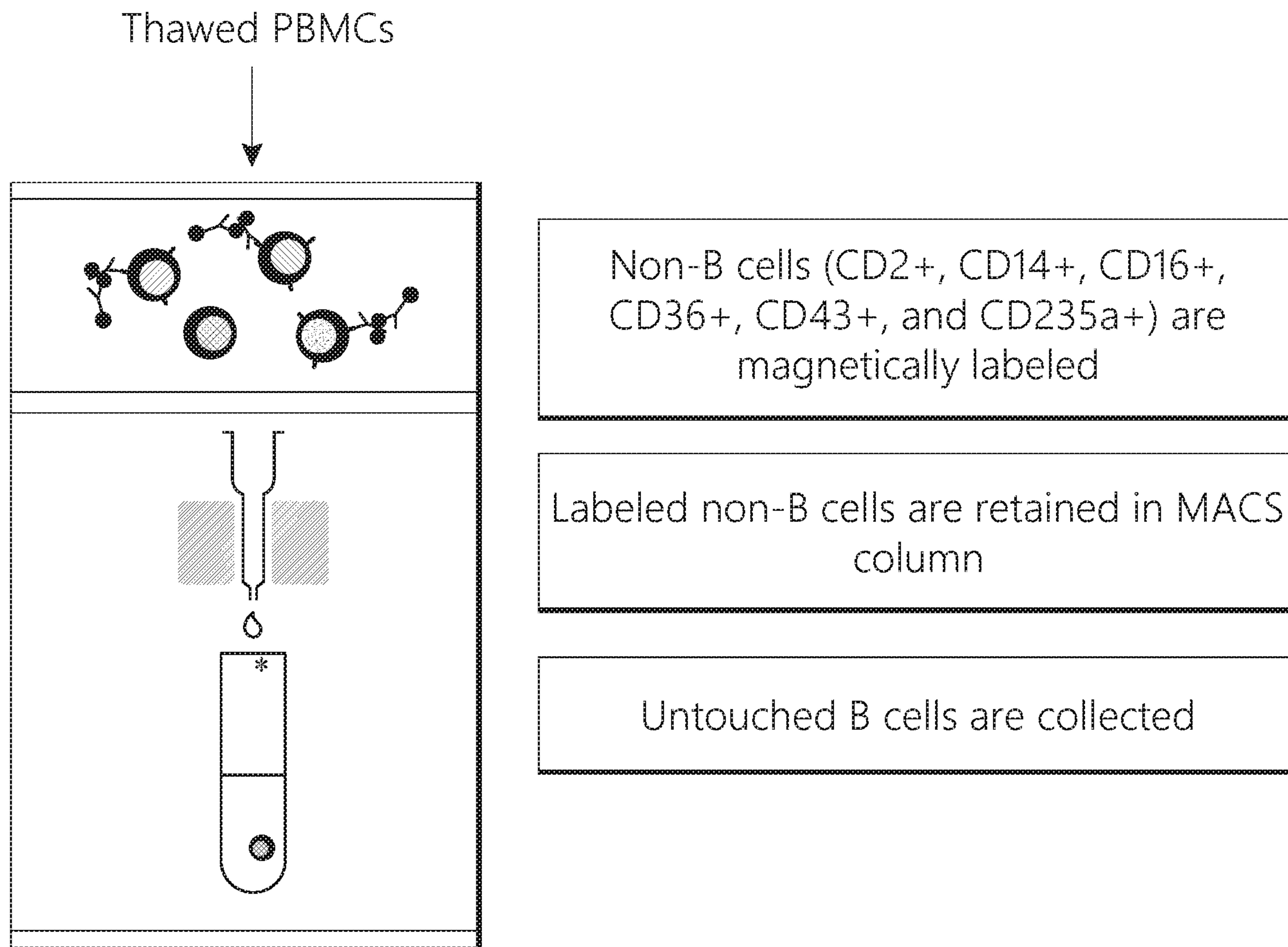
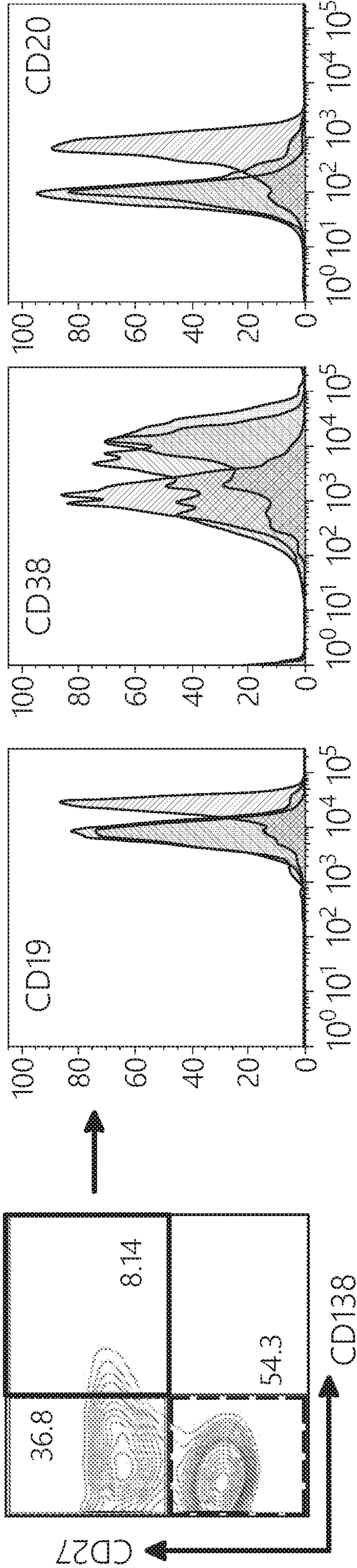


FIG. 22

Phenotype (D9 in culture)

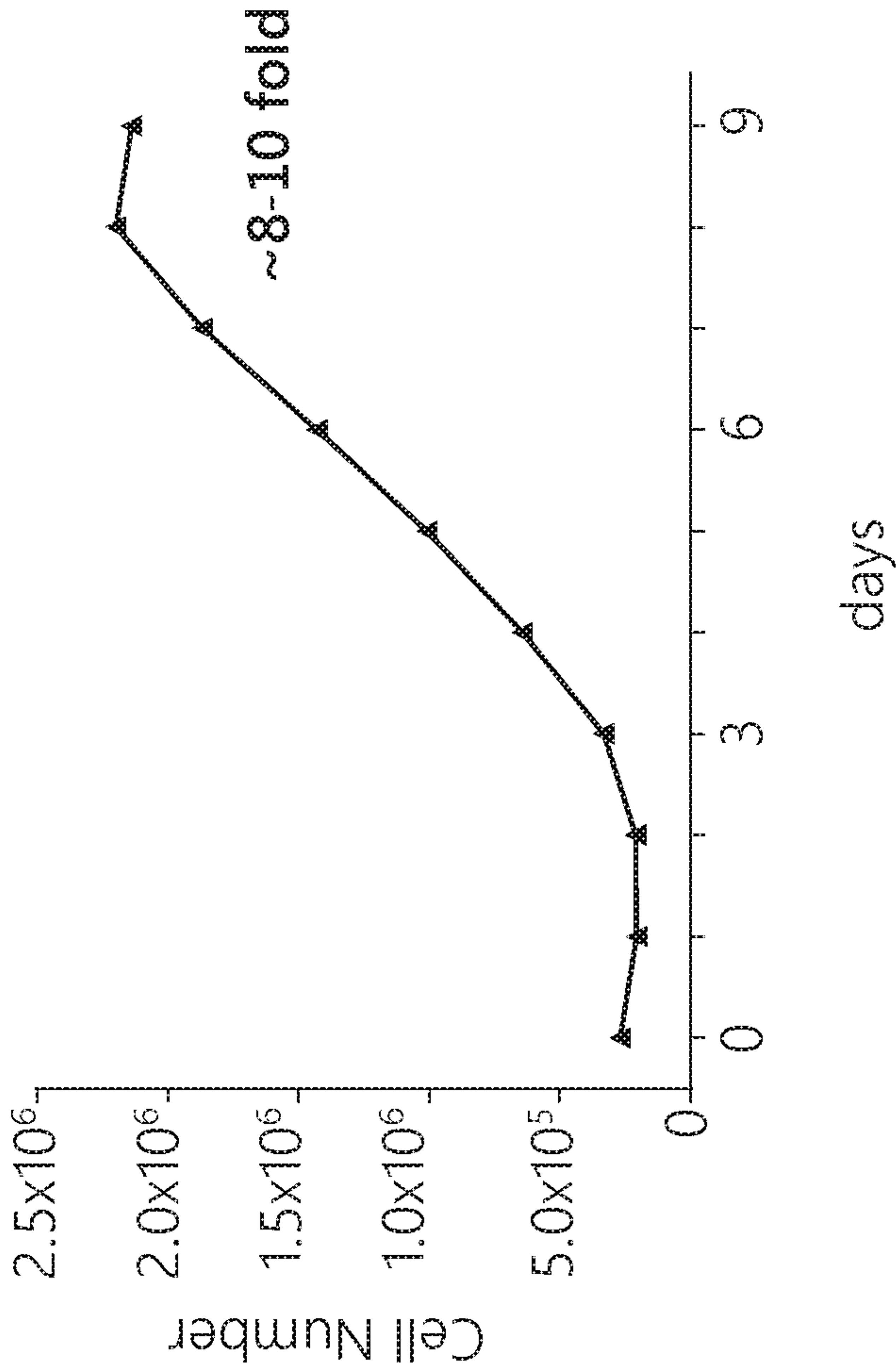


(Gating strategy: FSC/SSC > Singlets > Live/dead (AF350) > CD27/CD138)

Most cells remain CD138⁺CD27⁻ after 9 days in culture, and this population is primarily CD19^{high}CD38^{low}CD20⁺
Equivalent to Naïve and Active B cells

FIG. 23

Cell expansion curve



Viability by DAPI staining

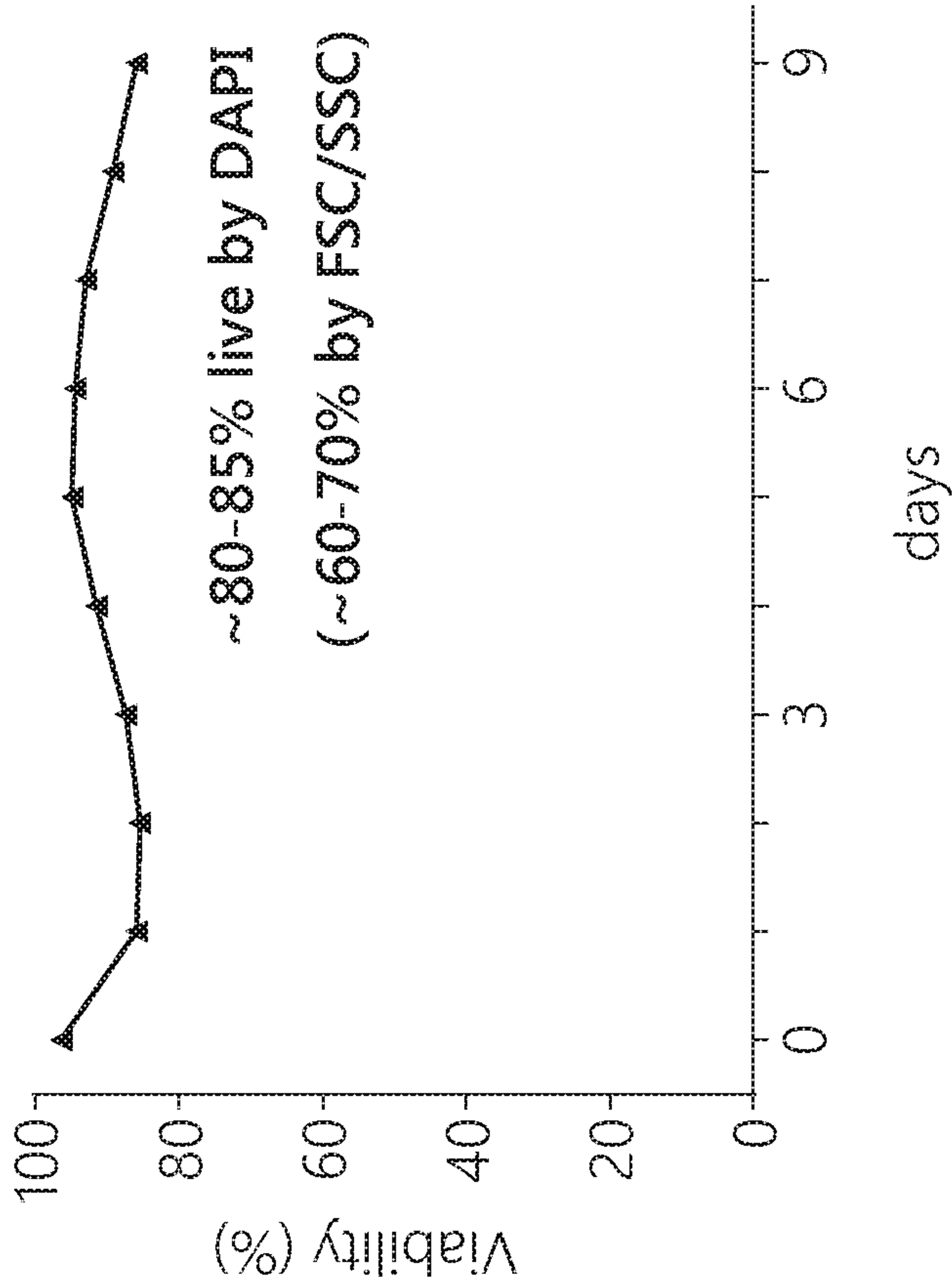
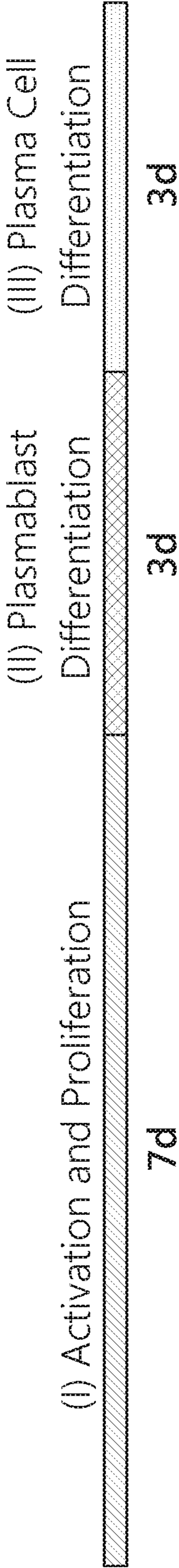


FIG. 24

3-step Culture System

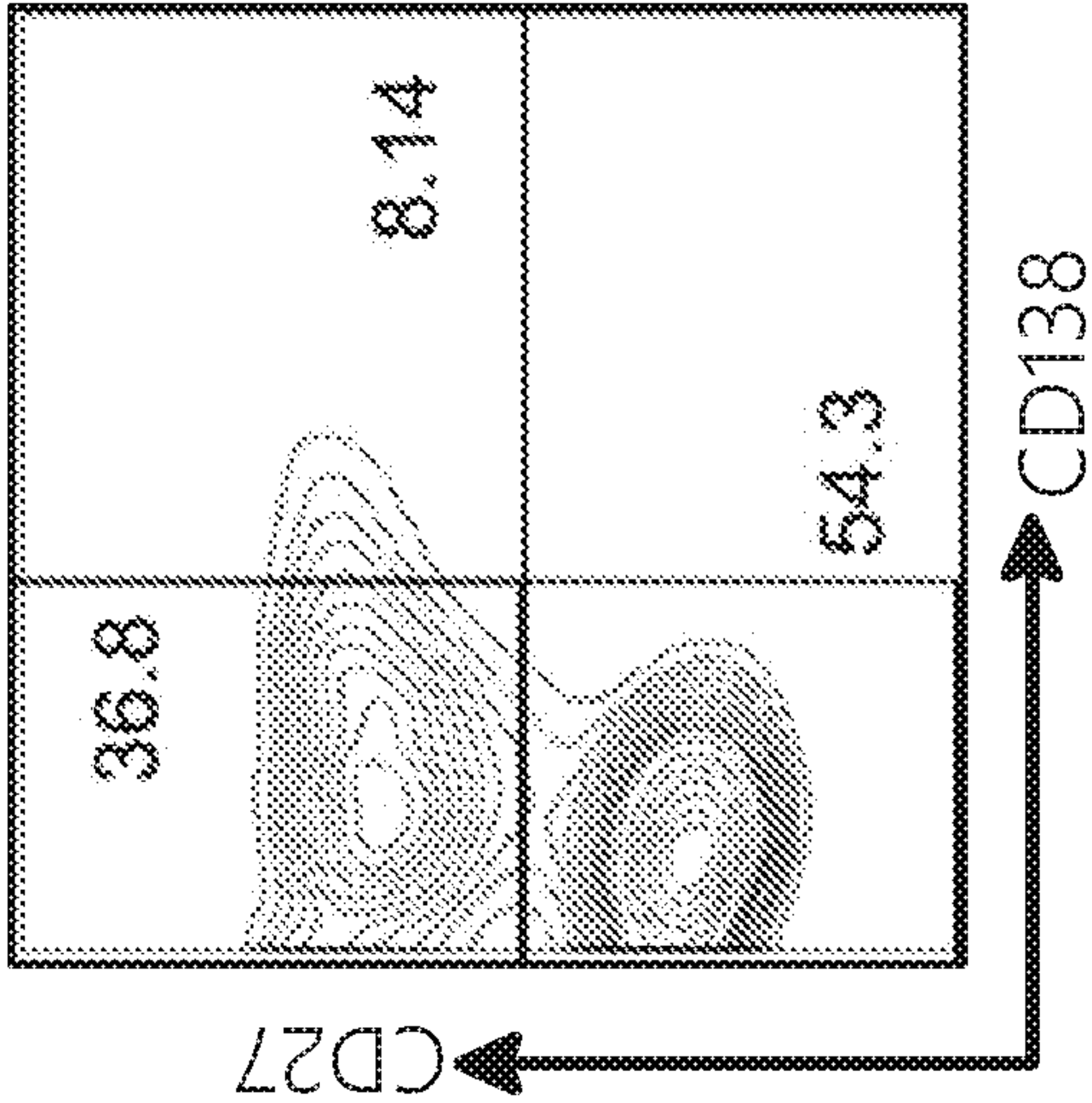


Phase (I): MCD40L + CpG + IL-2 + IL-10 + IL-15
Phase (II): IL-2 + IL-6 + IL-10 + IL-15
Phase (III): IL-6 + IL-15 + IFN α

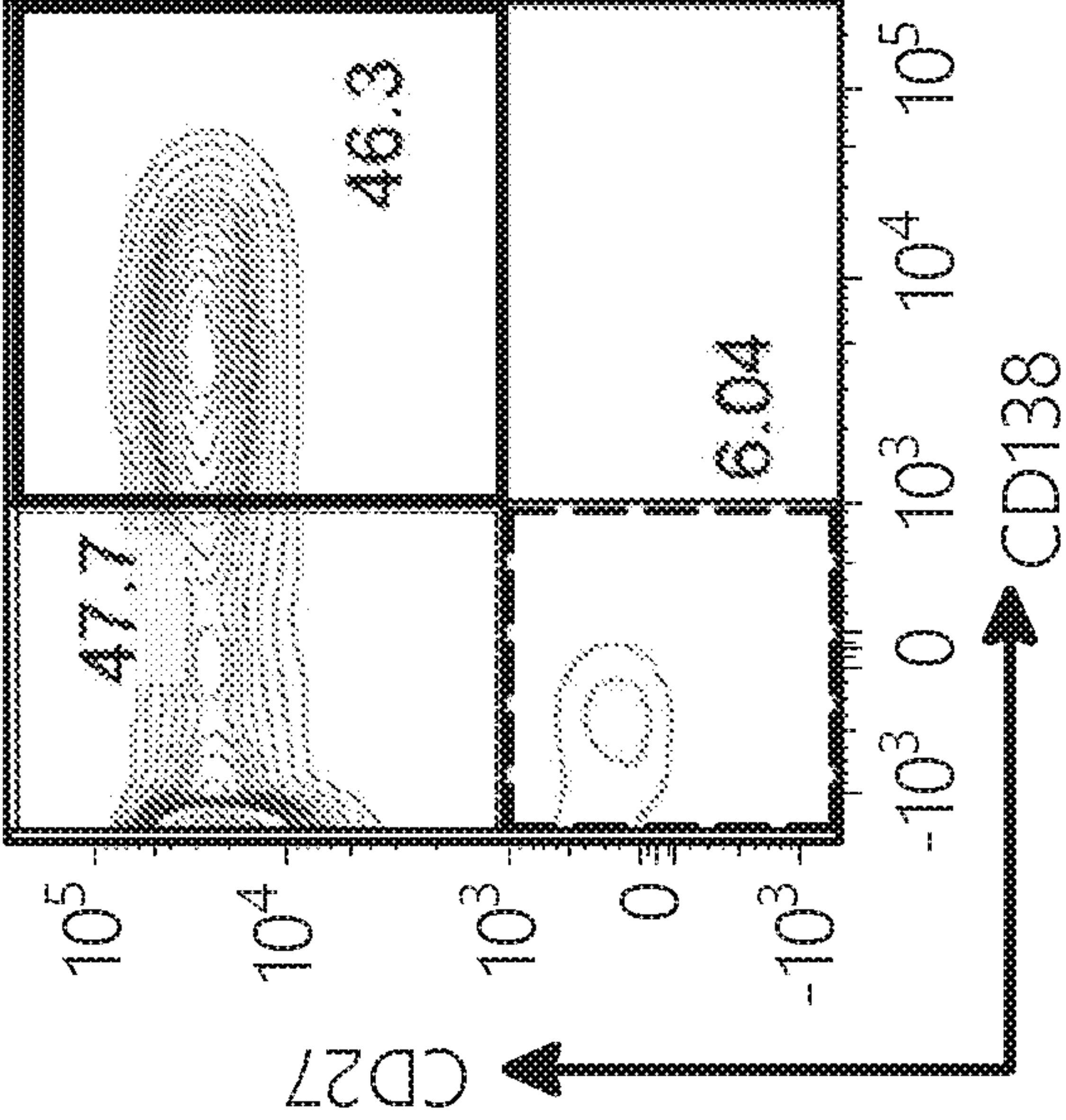
FIG. 25

Plasma cell differentiation

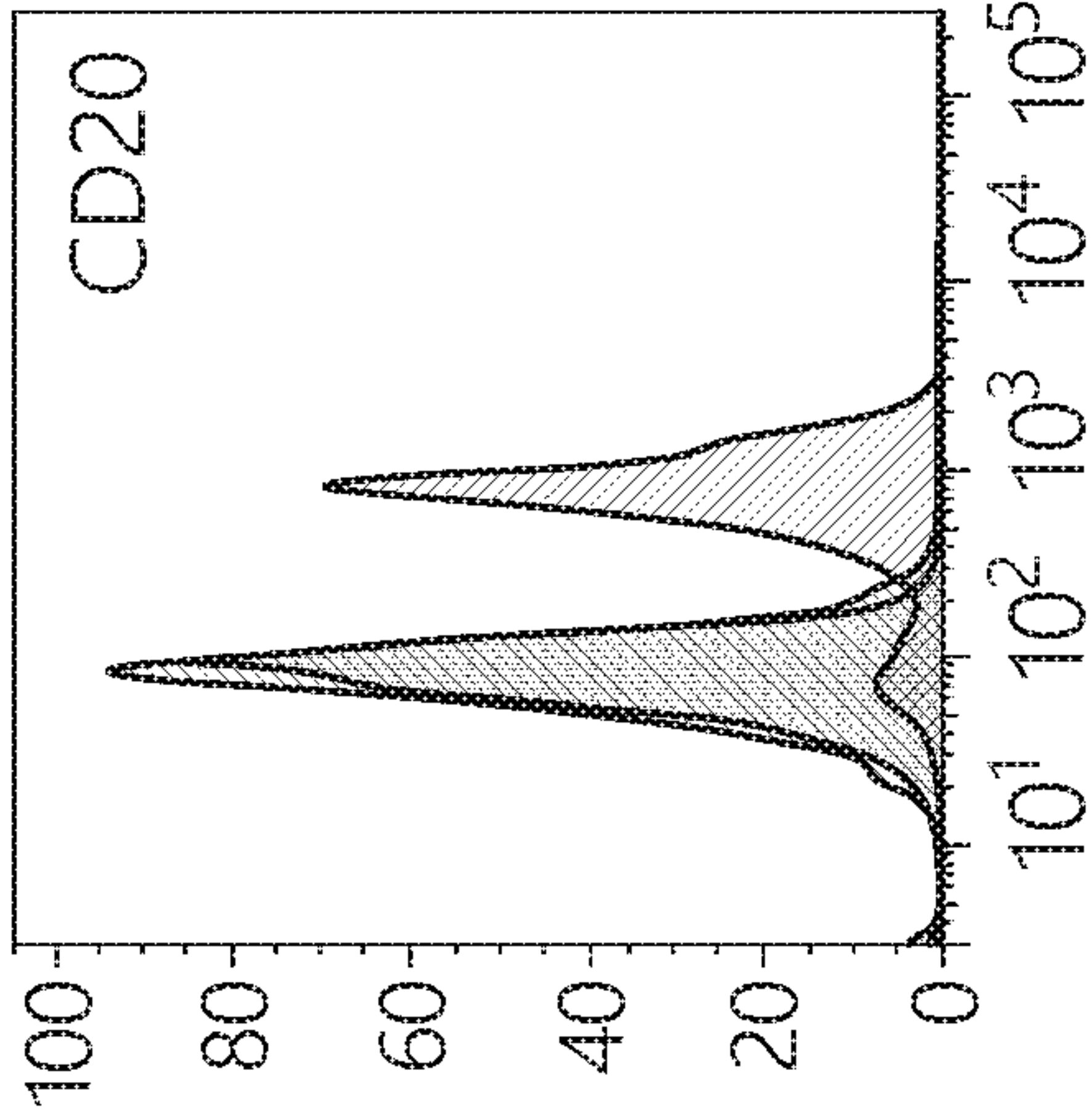
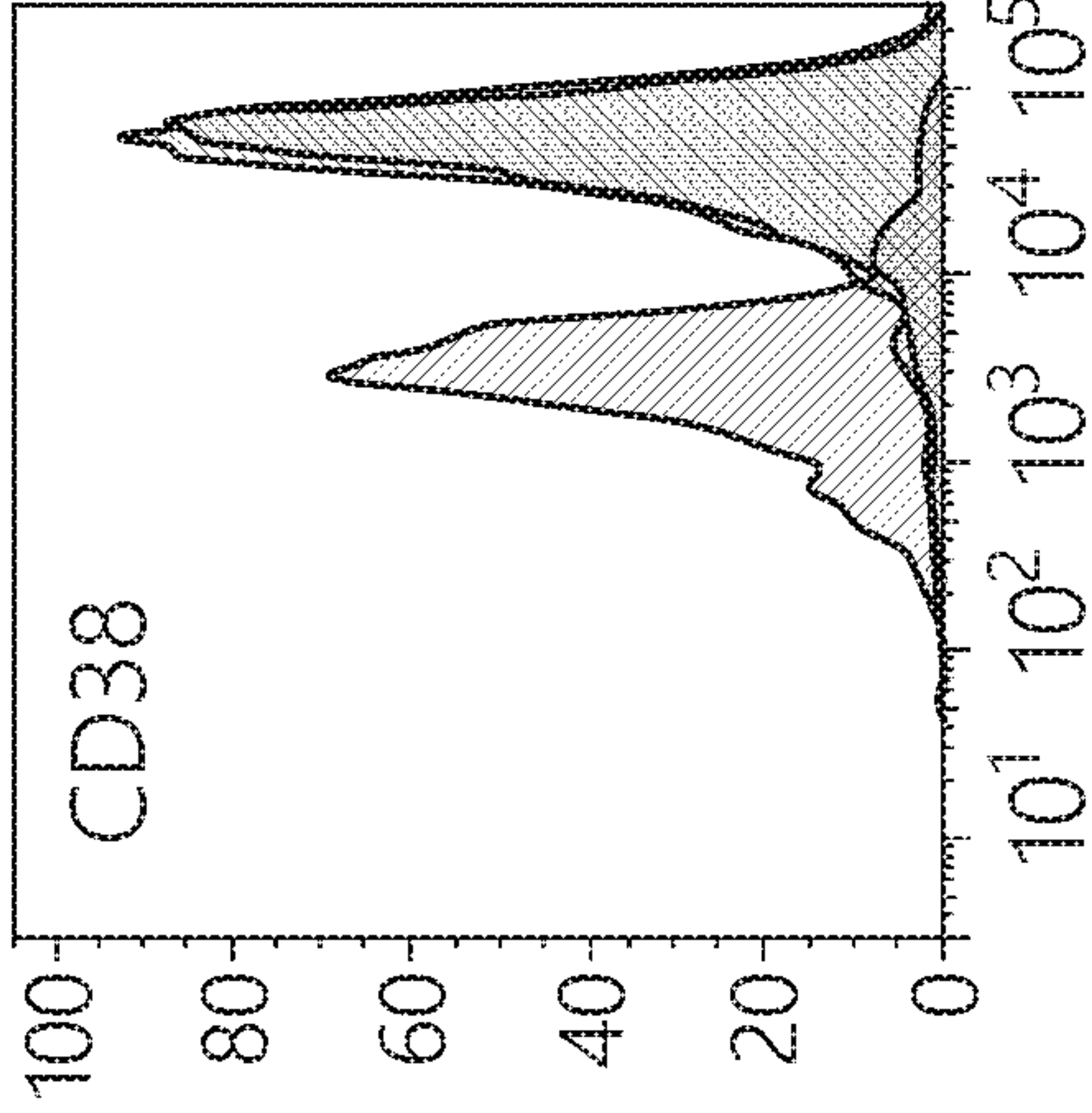
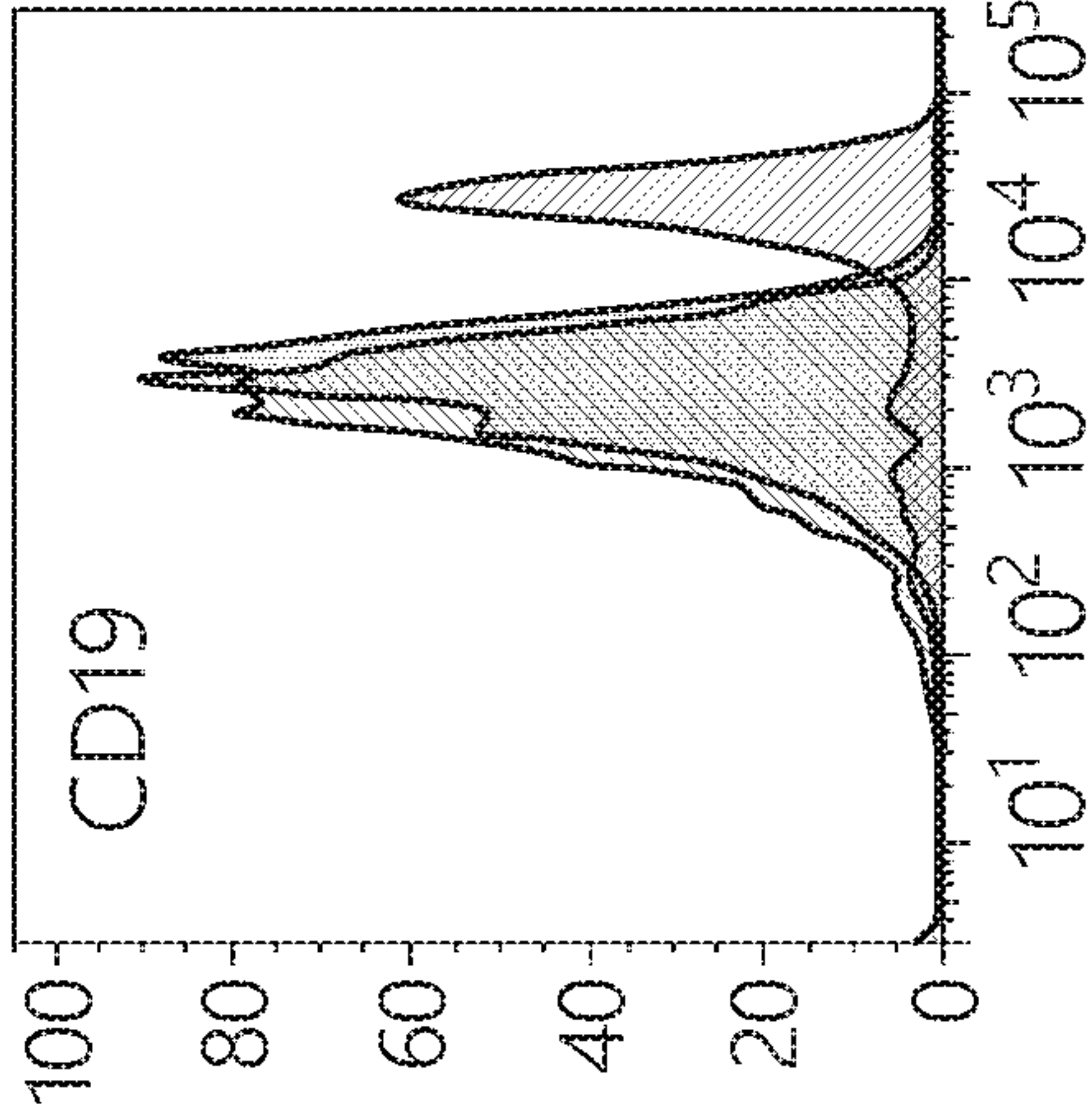
Pre-differentiation



Pre-differentiation (day 12)



(Gating strategy: FSC/SSC
> Singlets > Live/dead
(AF350) > CD27/CD138)
CD27⁺CD138⁺CD19^{neg} CD38⁺
CD138⁺ Human PC cells



CD27-CD138-
CD27+CD138-
CD27+CD138+

Viability by FSC/SSC on D12 is >60-80% (not shown)

FIG. 26

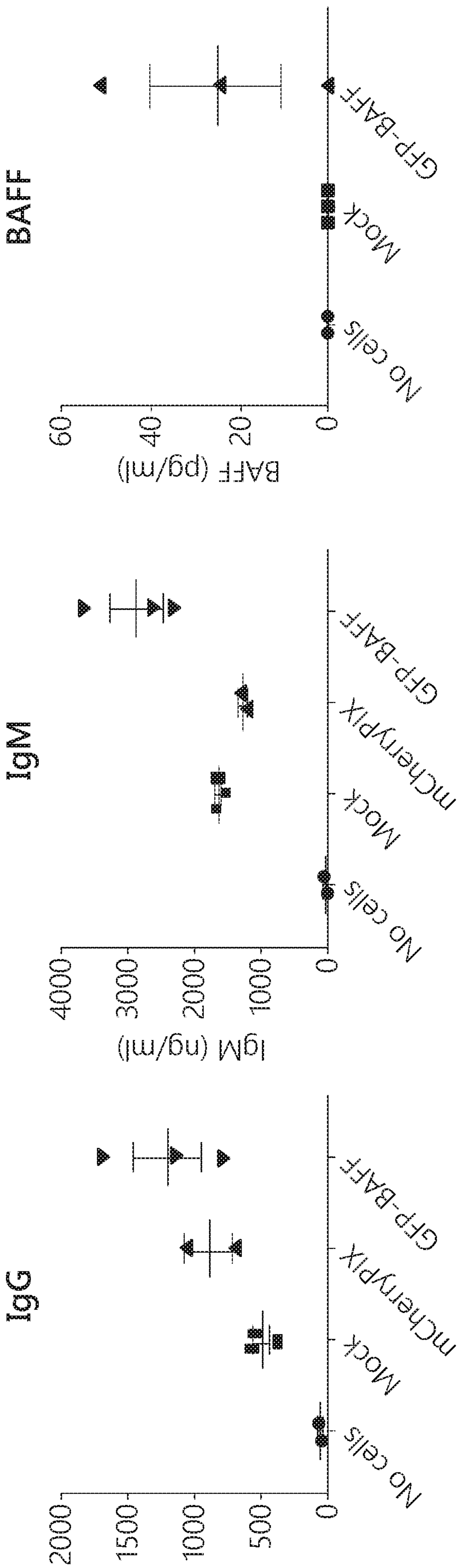


FIG. 27

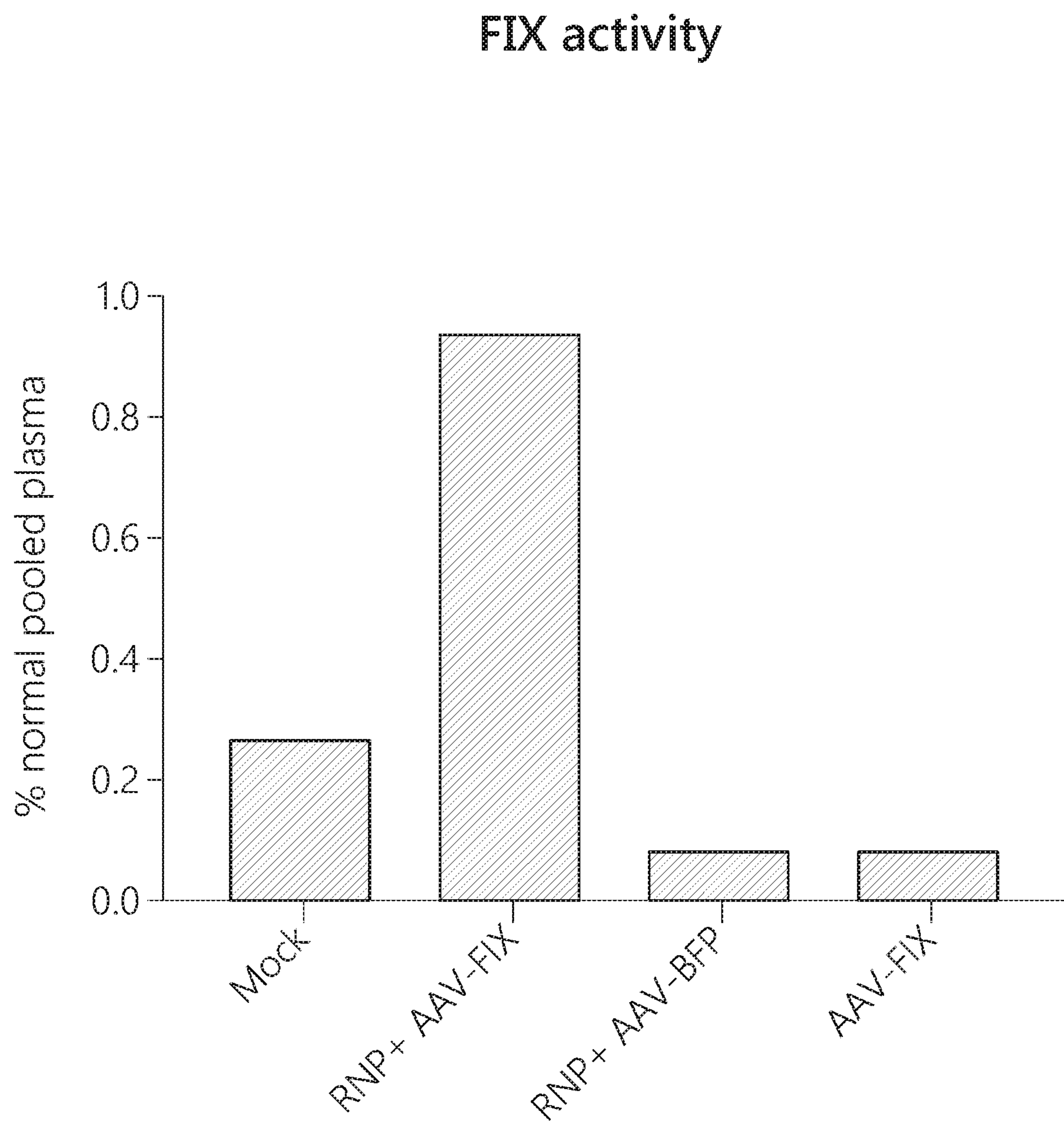
**FIG. 28**

FIG. 29A

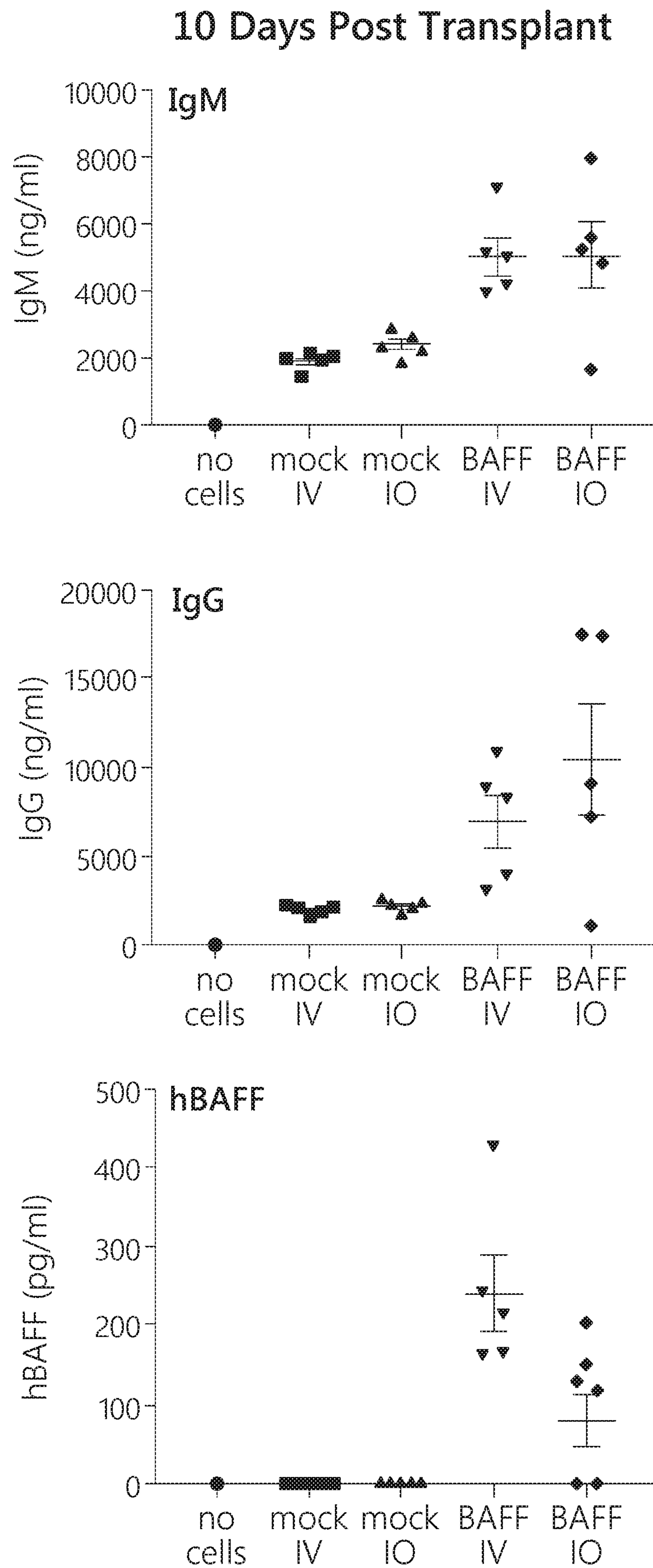
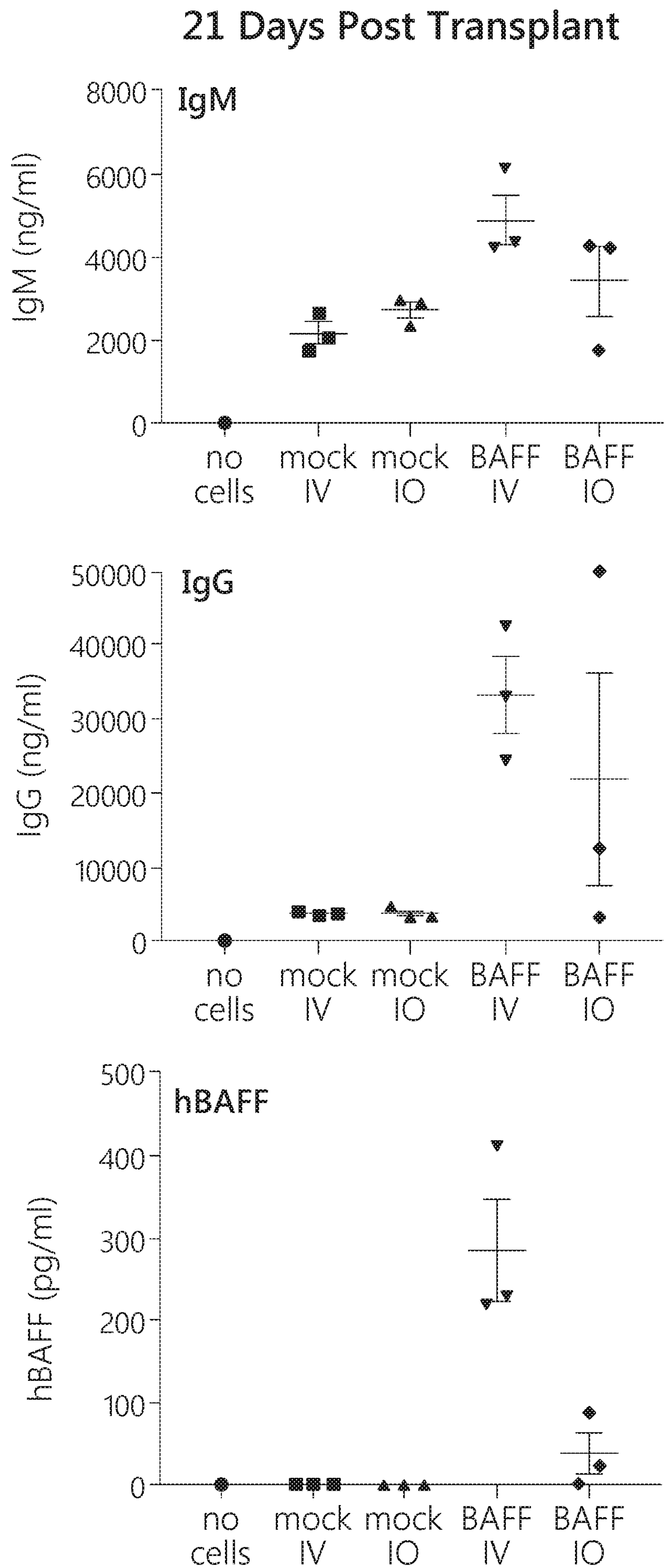


FIG. 29B



Experimental setup

Objectives:

- 1. Assess the impact of disruption on survival and differentiation
- 2. Combine gene disruption with HDR at CCR5 (FIX)

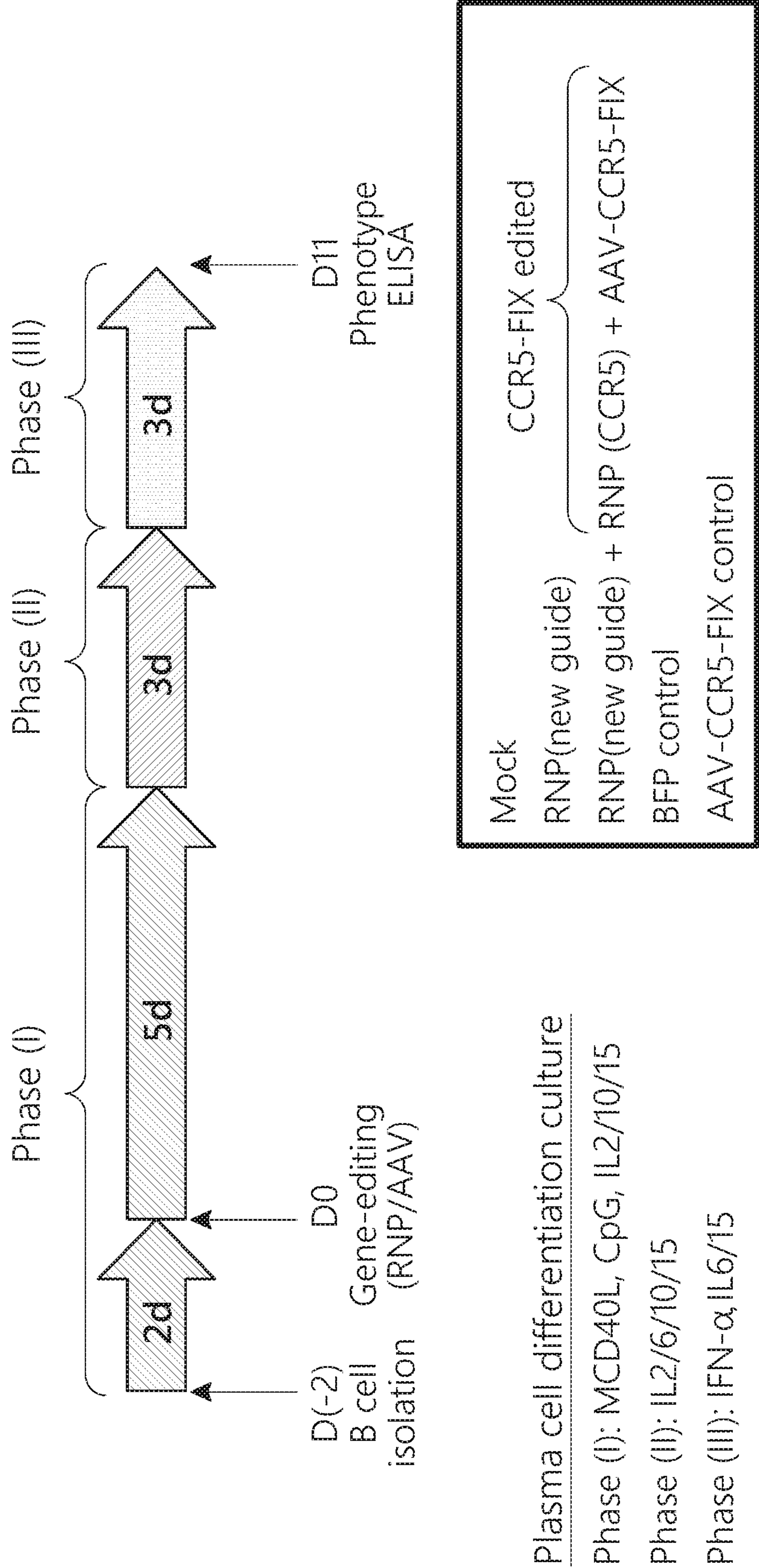


FIG. 30

Long-lived human antibody production in mice

Group	Editing condition	# mice (F)	Route	# B cells/animal
1	Mock unedited	5	Retro-orbital injection	10E+06
2	CCR5-RNP + AAV-GFP-BAFF	5	Retro-orbital injection	10E+06
3	Mock unedited	5	Intra-osseous injection	10E+06
4	CCR5-RNP + AAV-GFP-BAFF	5	Intra-osseous injection	10E+06

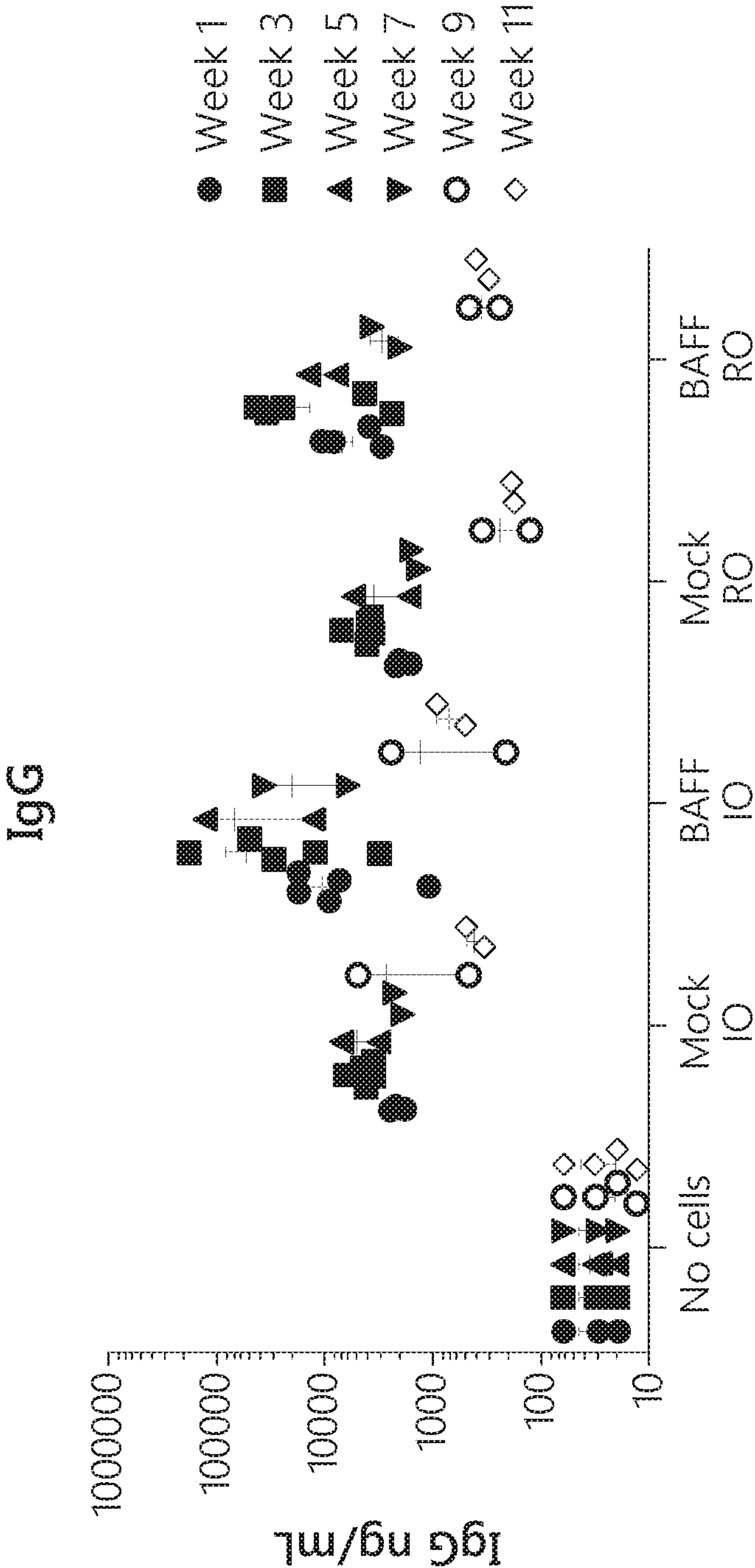


FIG. 31

Expression of human IL6 in mice confers a growth advantage for transplanted ex vivo differentiated human plasma

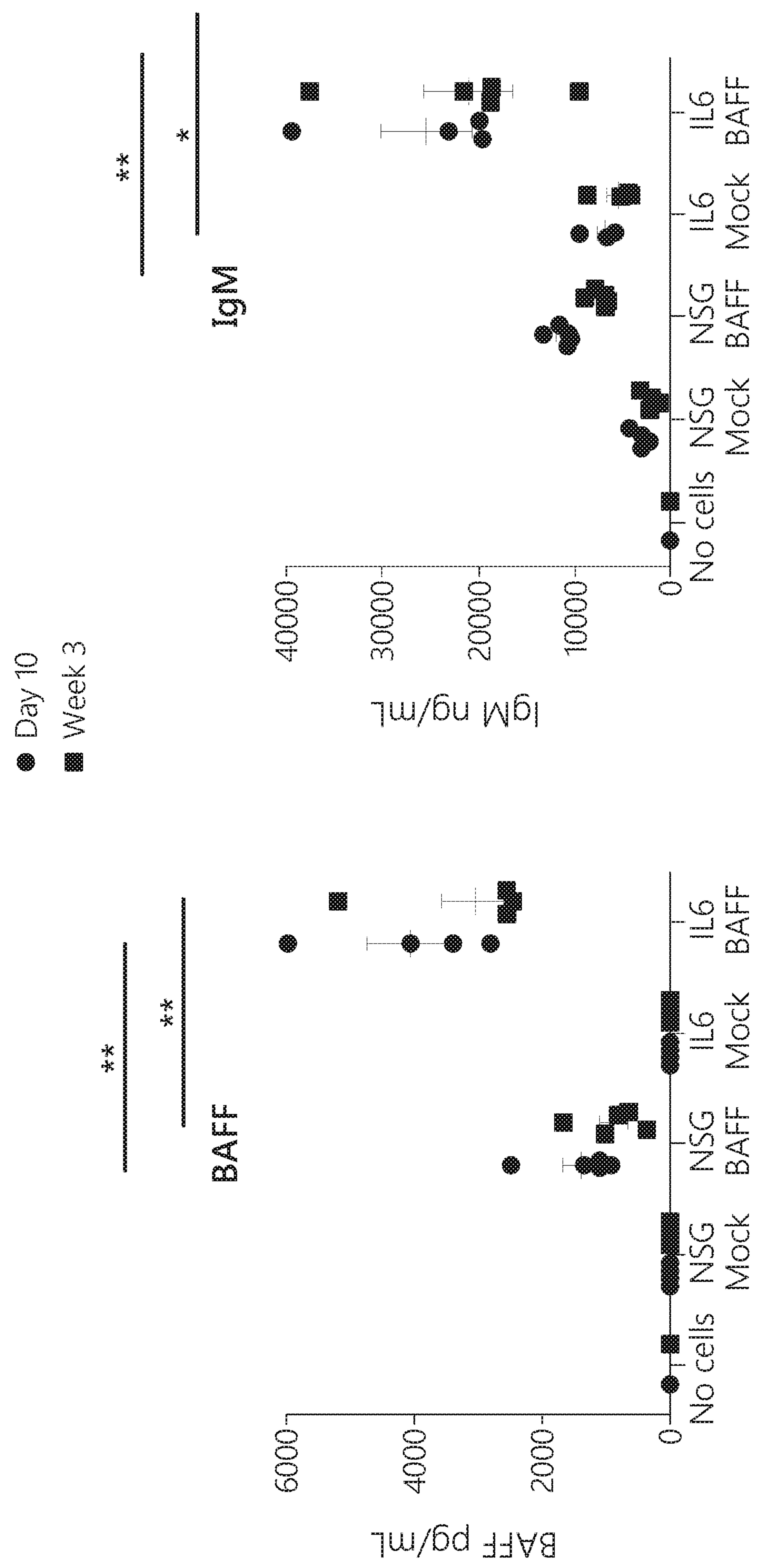


FIG. 32

High IL6 expression correlates with high plasma cell secretion

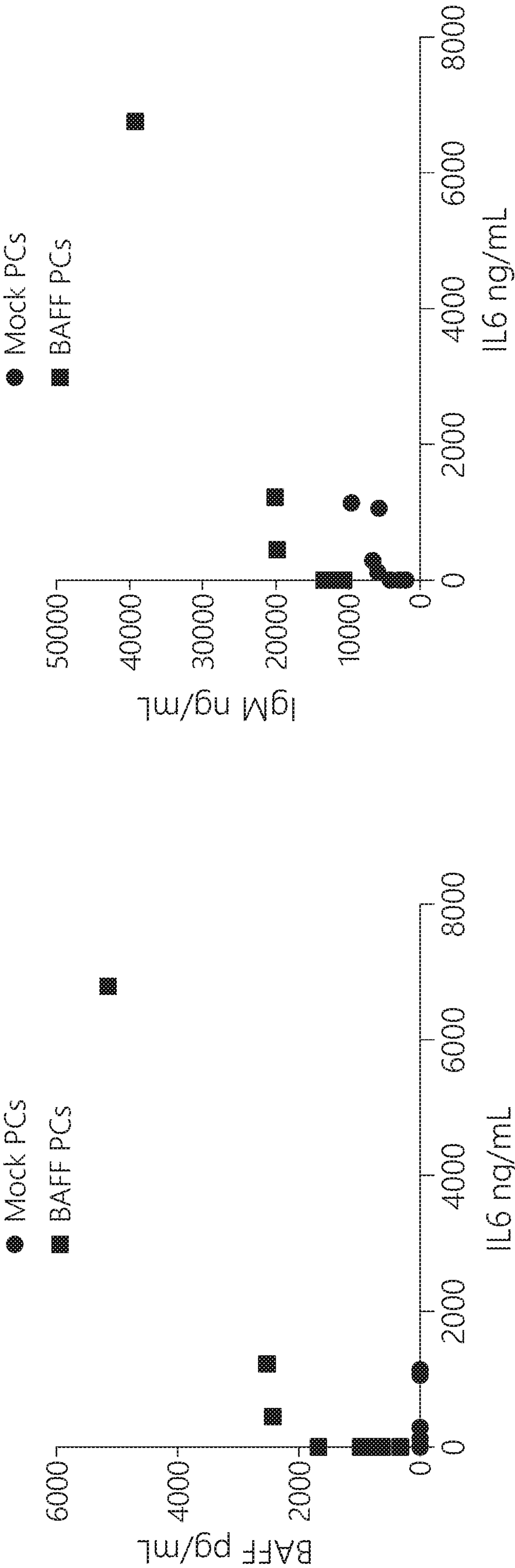


FIG. 33

In vitro human APRIL treatment correlates with increased plasma
call antibody secretion *in vivo*

- Mock
- Mega-April

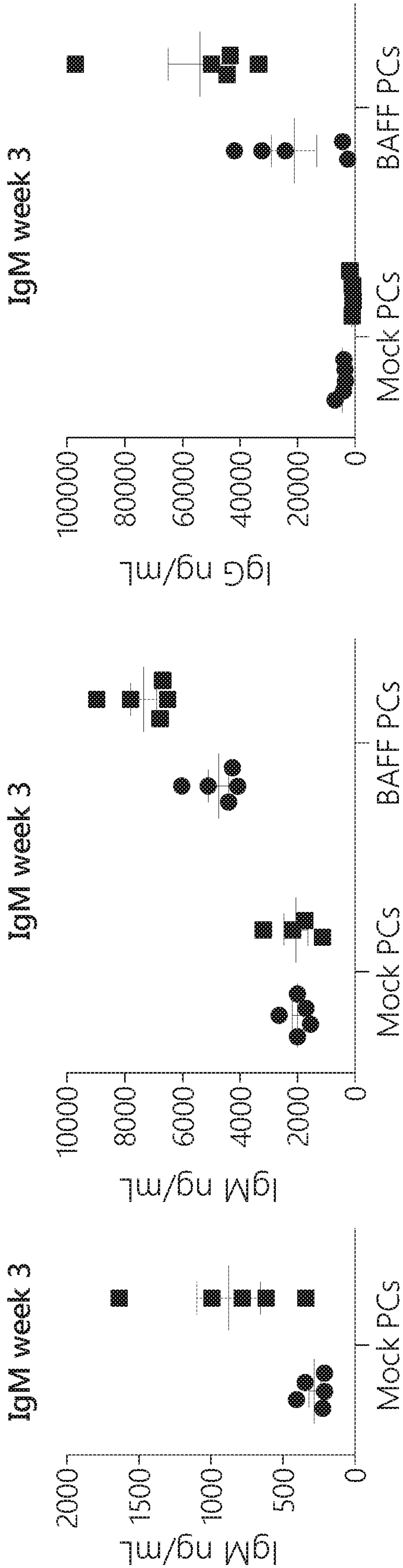


FIG. 34

Gene disruption and protein expression

Guide to Target Candidate loci - highly expressed genes predicted to impact protein secretion

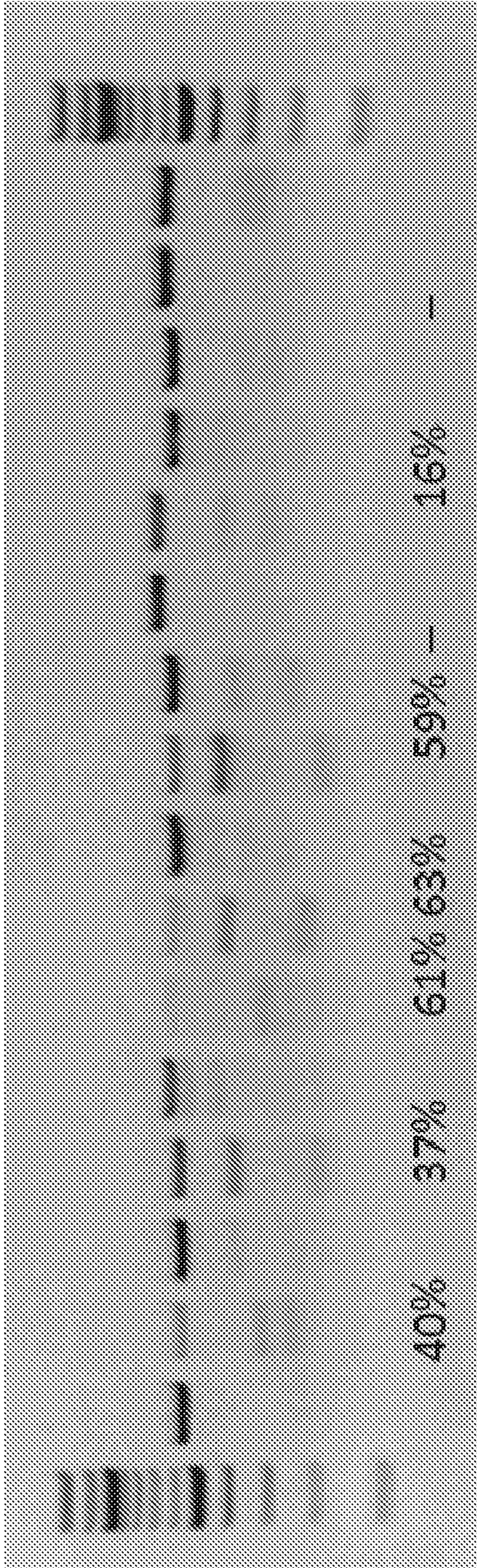
- Highly expressed in plasma cells based on RNA expression from single cell sequencing data
- Secreted proteins - extracellular or ER/Golgi
- Proteins that are not involved in vesicular transport/protein secretion

001	SLPI-2sense	}	Secreted protease inhibitor
002	SLPI-1sense		
003	IGKC-sense	}	Kappa light chain constant region
004	IGKC-antisense		
005	IGMC-sense	}	IgM heavy chain constant region
006	IGMC-antisense		
007	JCHAIN-1antisense	}	Jchain
008	JCHAIN2-sense		
009	JCHAIN-4antisense		
010	PON3-2antisense	}	Paraoxonase 3 - secreted protein that associates with HDL
011	PON3-3sense		
012	PRG2-3antisense	}	Pro eosinophil major basic protein - secreted protein that binds HSPGs
013	PRG2-3antisense		
014	CRELD2-3sense	}	ER protein
015	CRELD2-4antisense		

FIG. 35

Target of abundant plasma cell genes using CRISPR

<u>T7E1</u>	<u>SLPI-2</u>	<u>SLPI-1</u>	<u>IGKC</u>	<u>IGMC</u>	<u>JCHAIN-1</u>	<u>JCHAIN-2</u>	<u>JCHAIN-4</u>								
-	001	-	002	-	003	004	-	005	006	-	007	-	008	-	009



<u>PON3</u>	<u>PON3</u>	<u>PRG2</u>	<u>CRELD2</u>	<u>CRELD2</u>
- 010	- 011	- 012 013	- 014	- 015

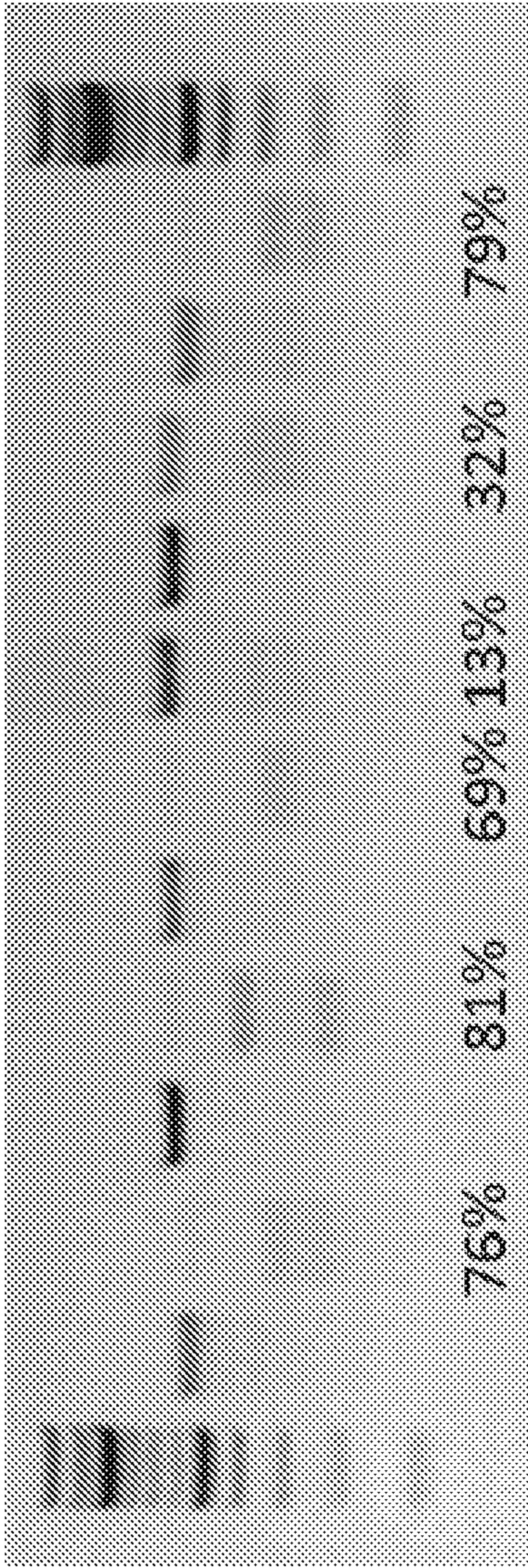
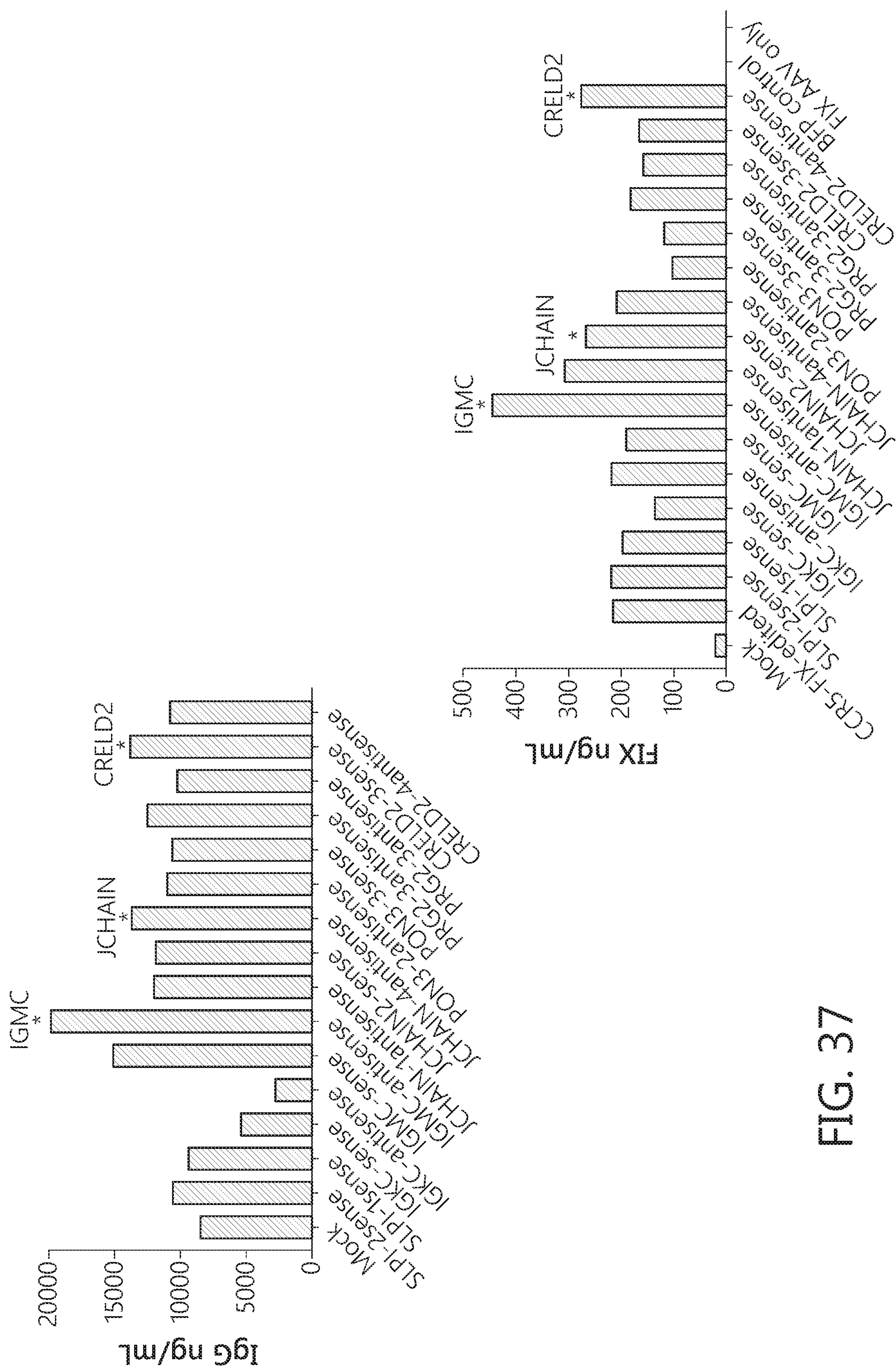


FIG. 36



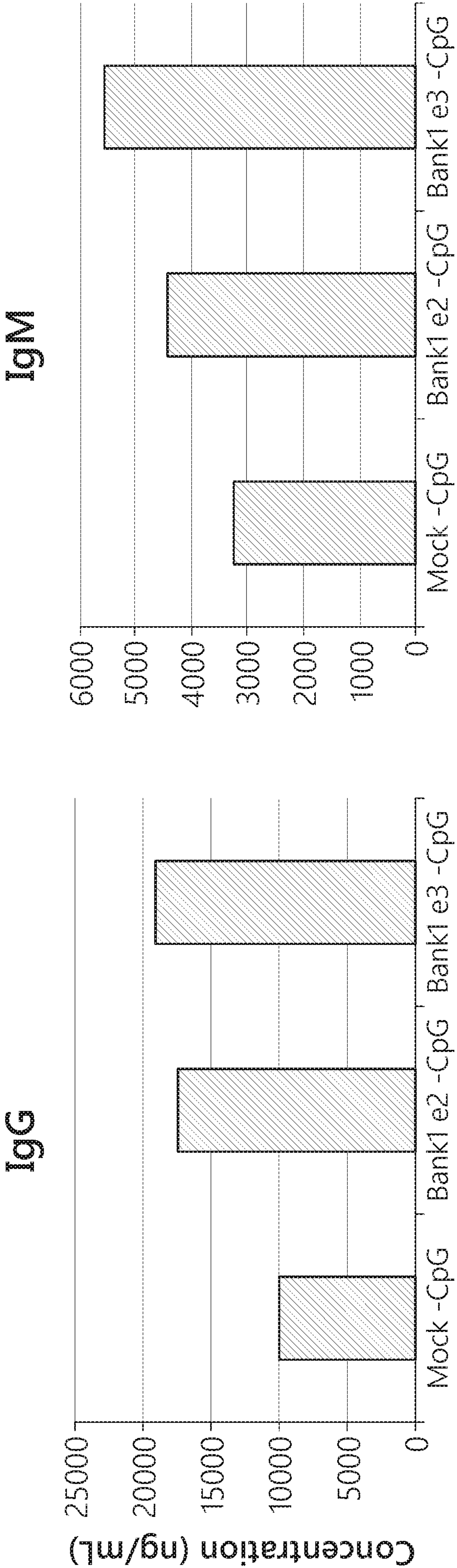


FIG. 38

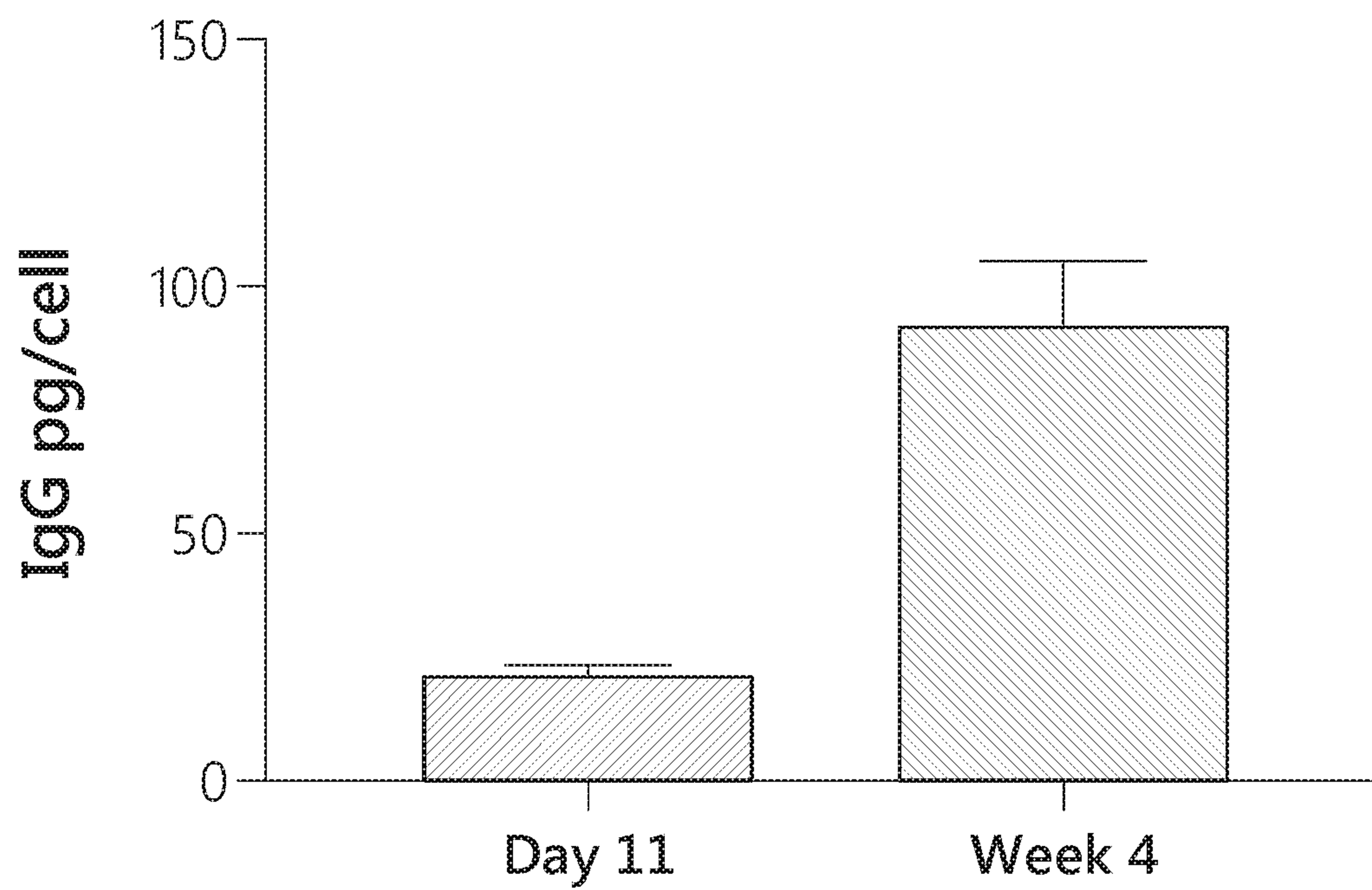


FIG. 39

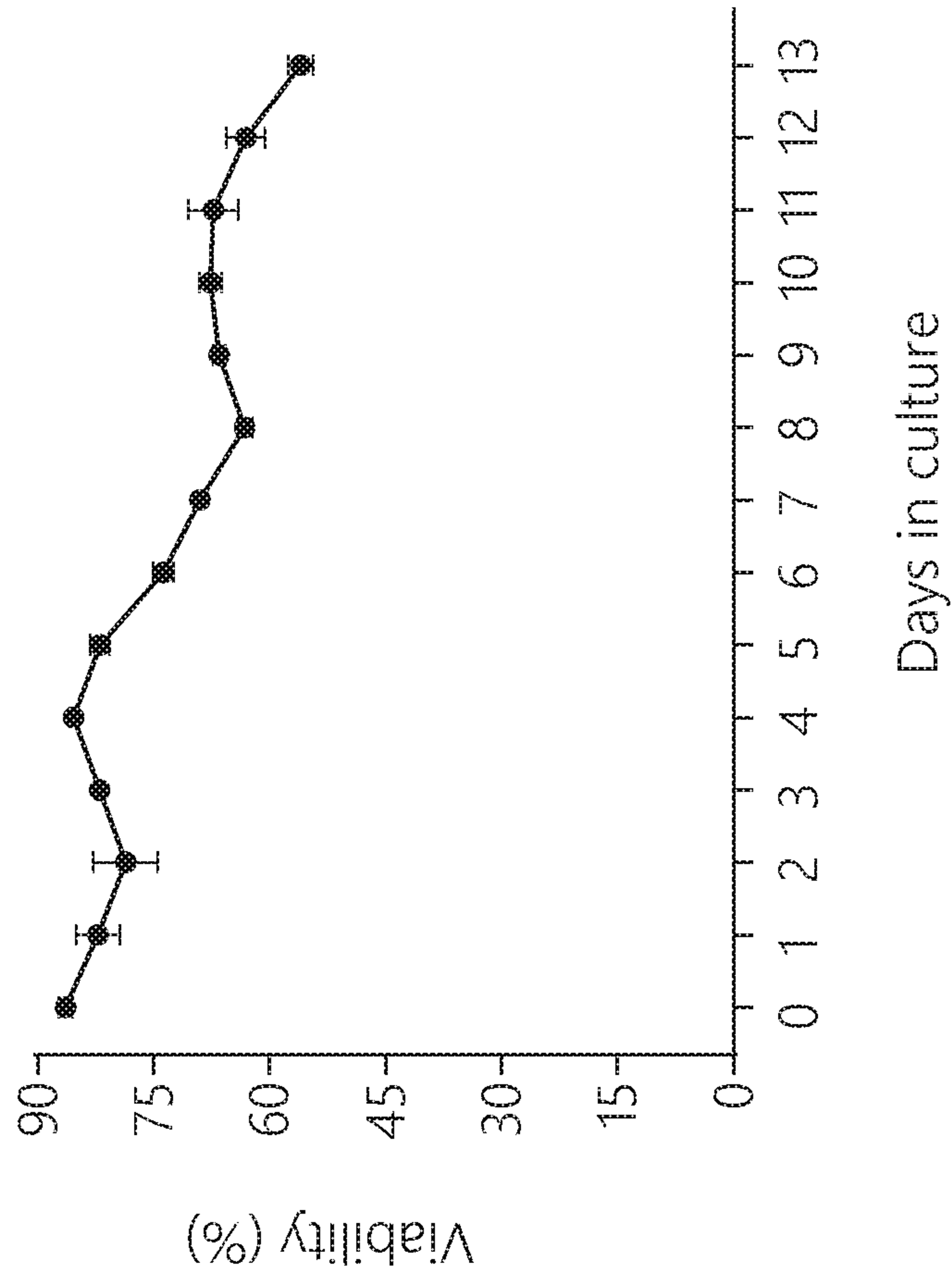


FIG. 40B

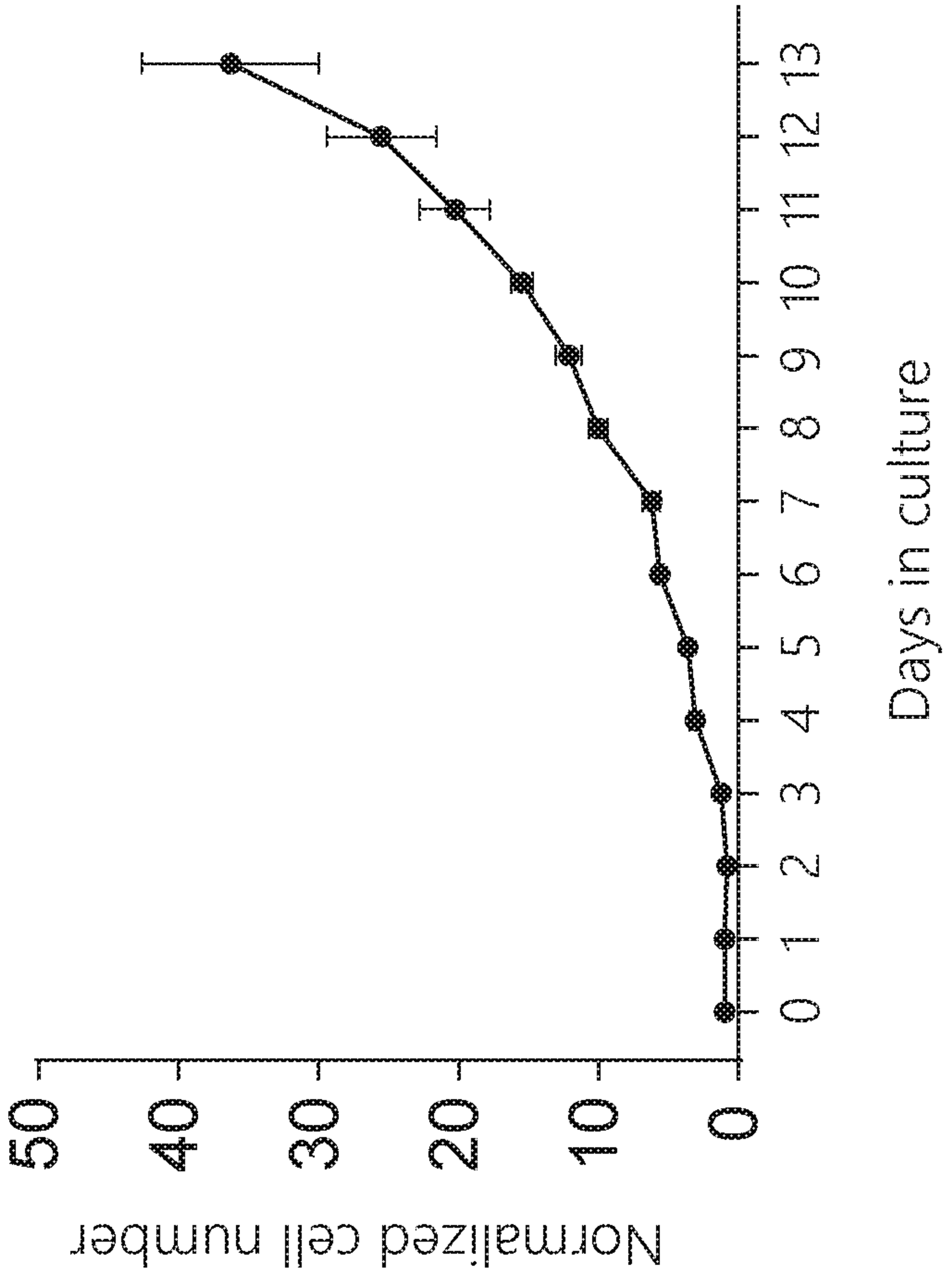


FIG. 40A

FIG. 40C

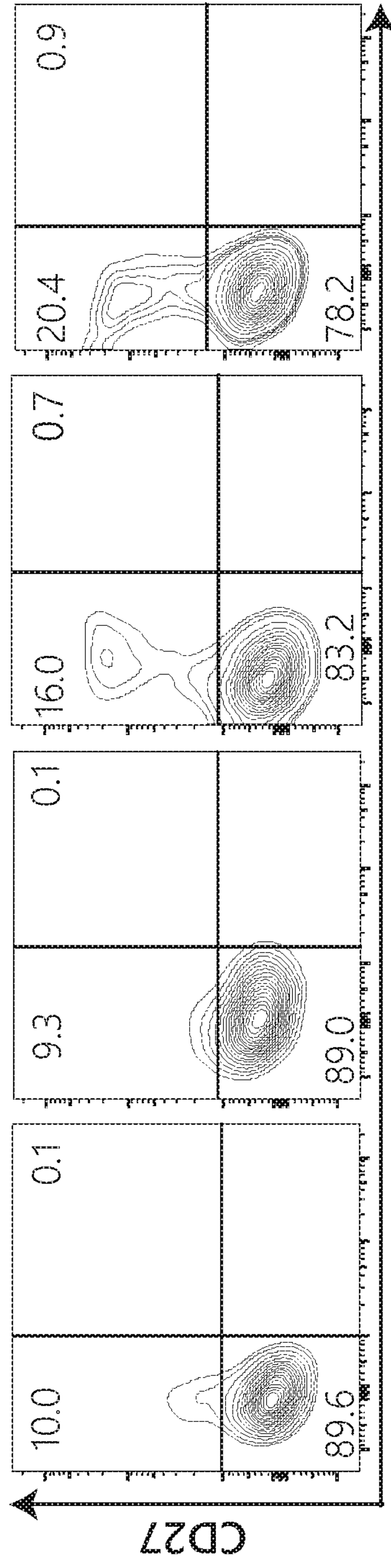
Time in culture:

0 days

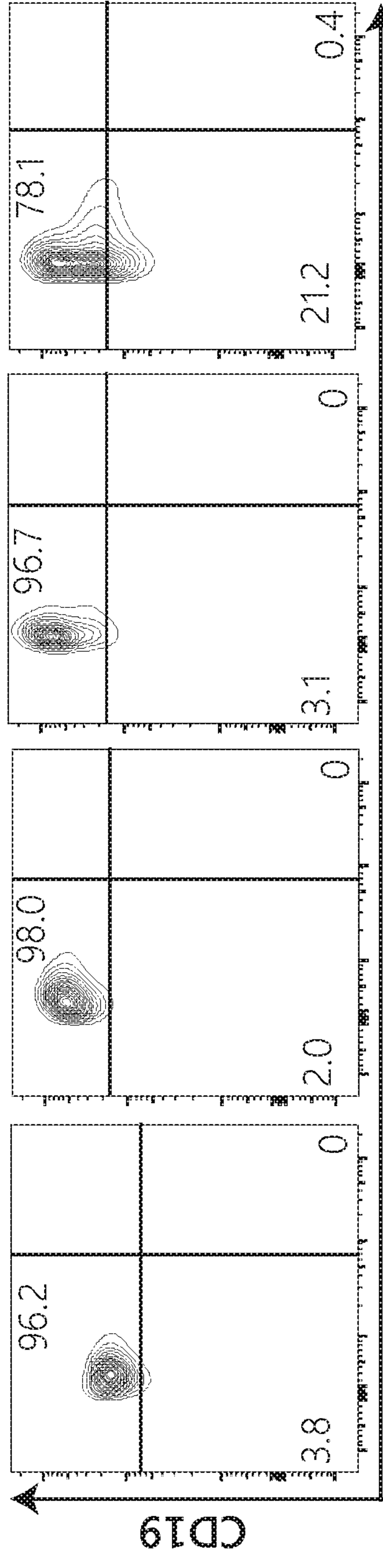
2 days

8 days

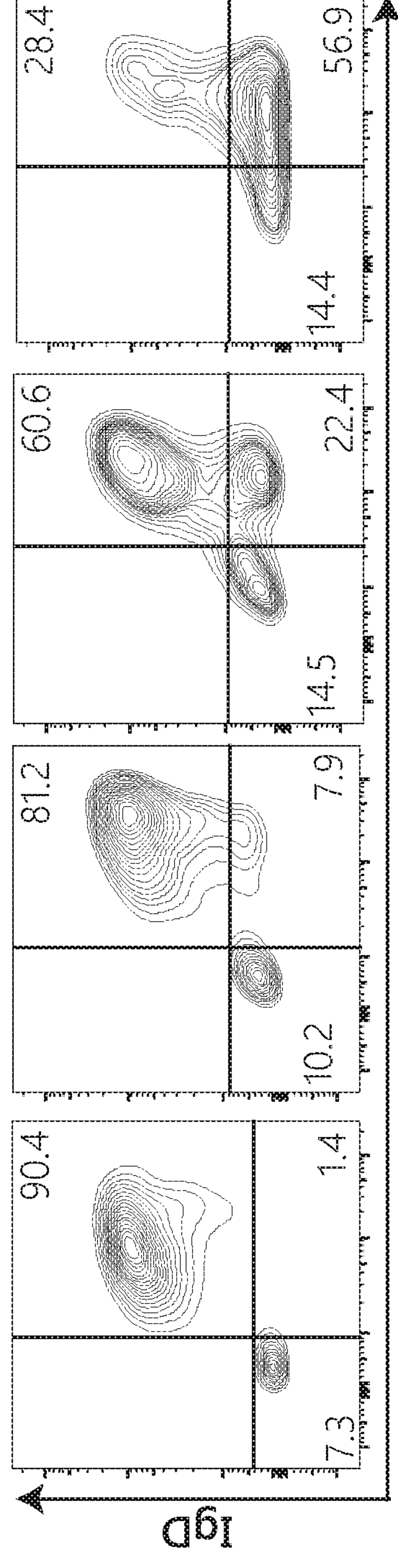
13 days



CD138



CD38



IgM

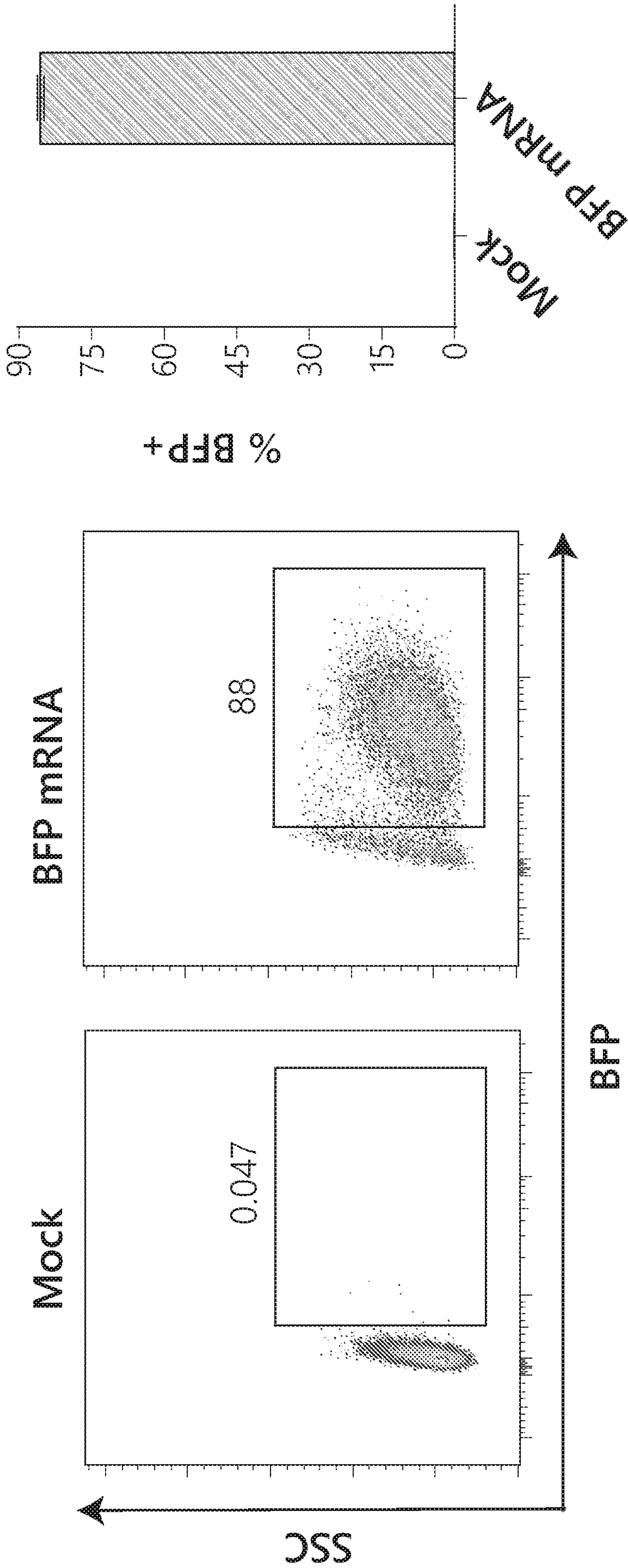


FIG. 41

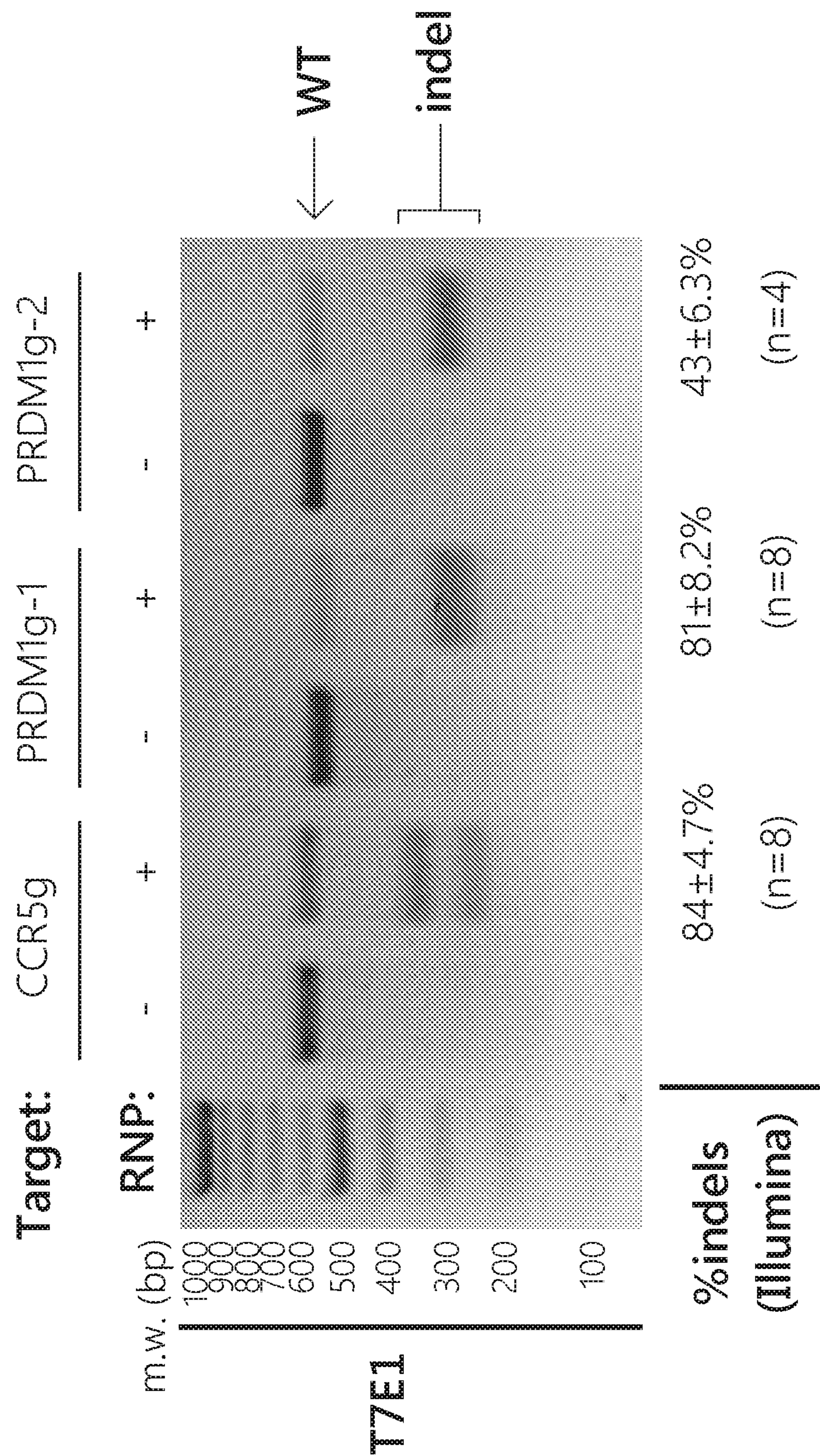


FIG. 42A

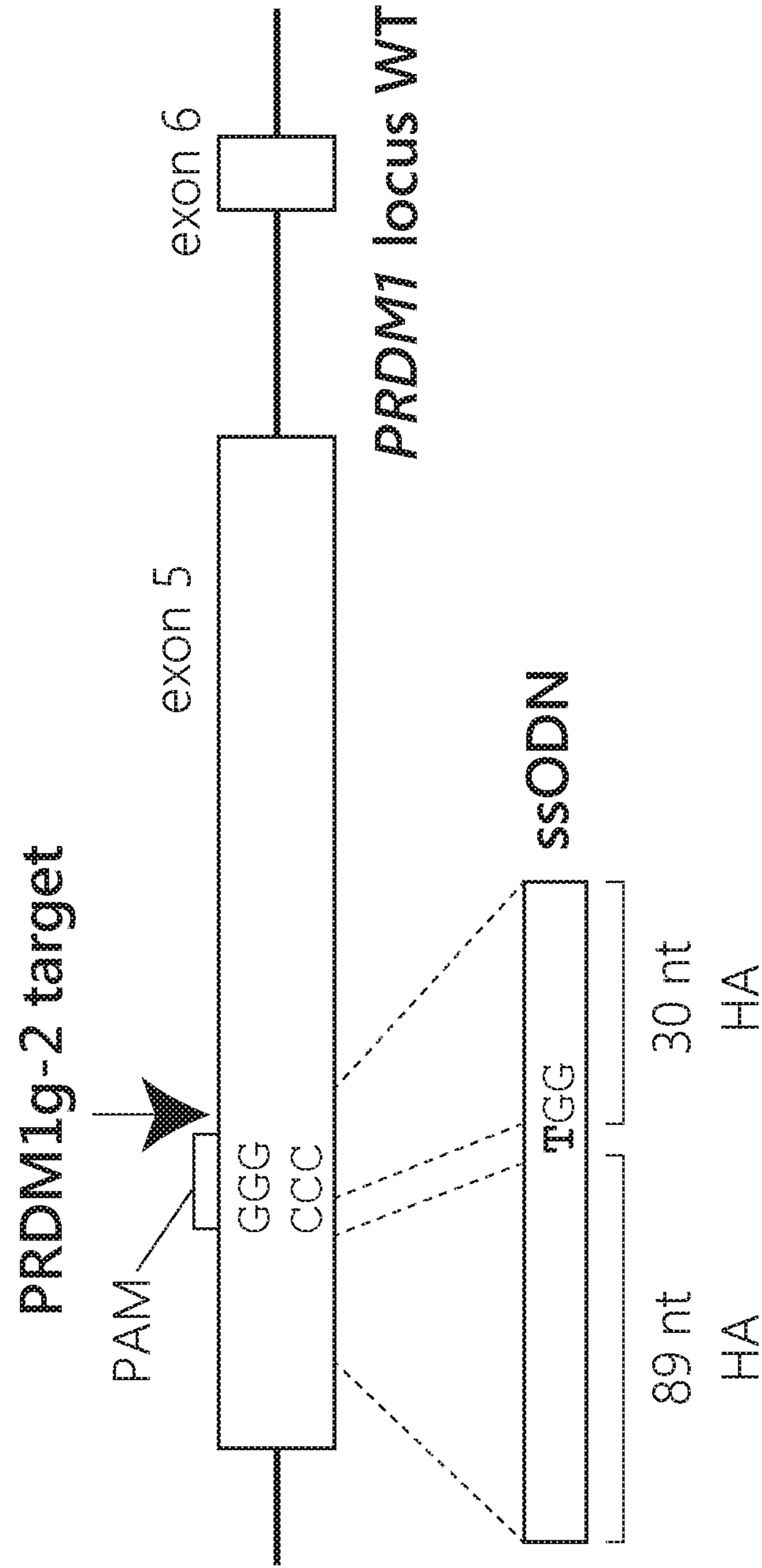


FIG. 42B

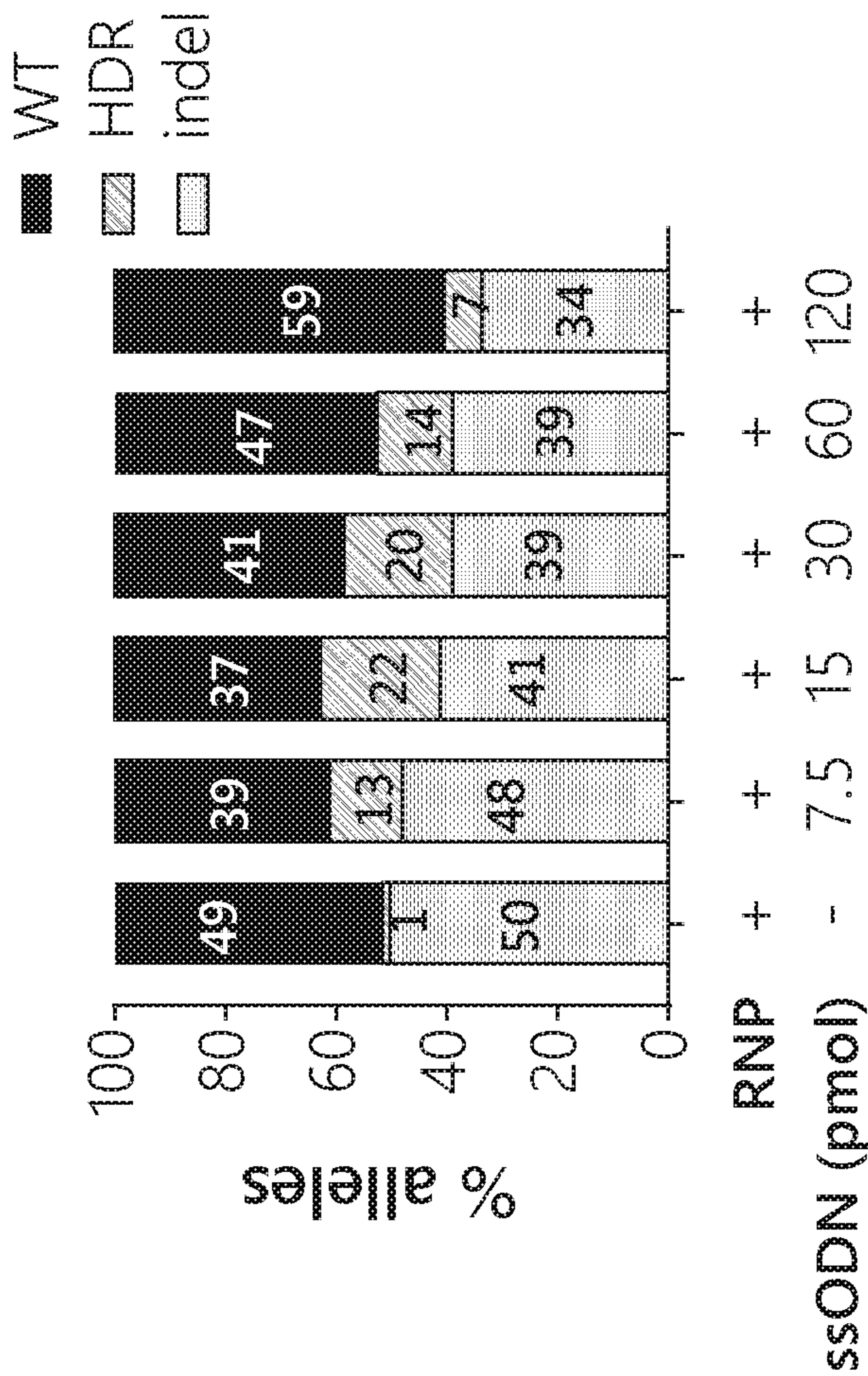


FIG. 42D

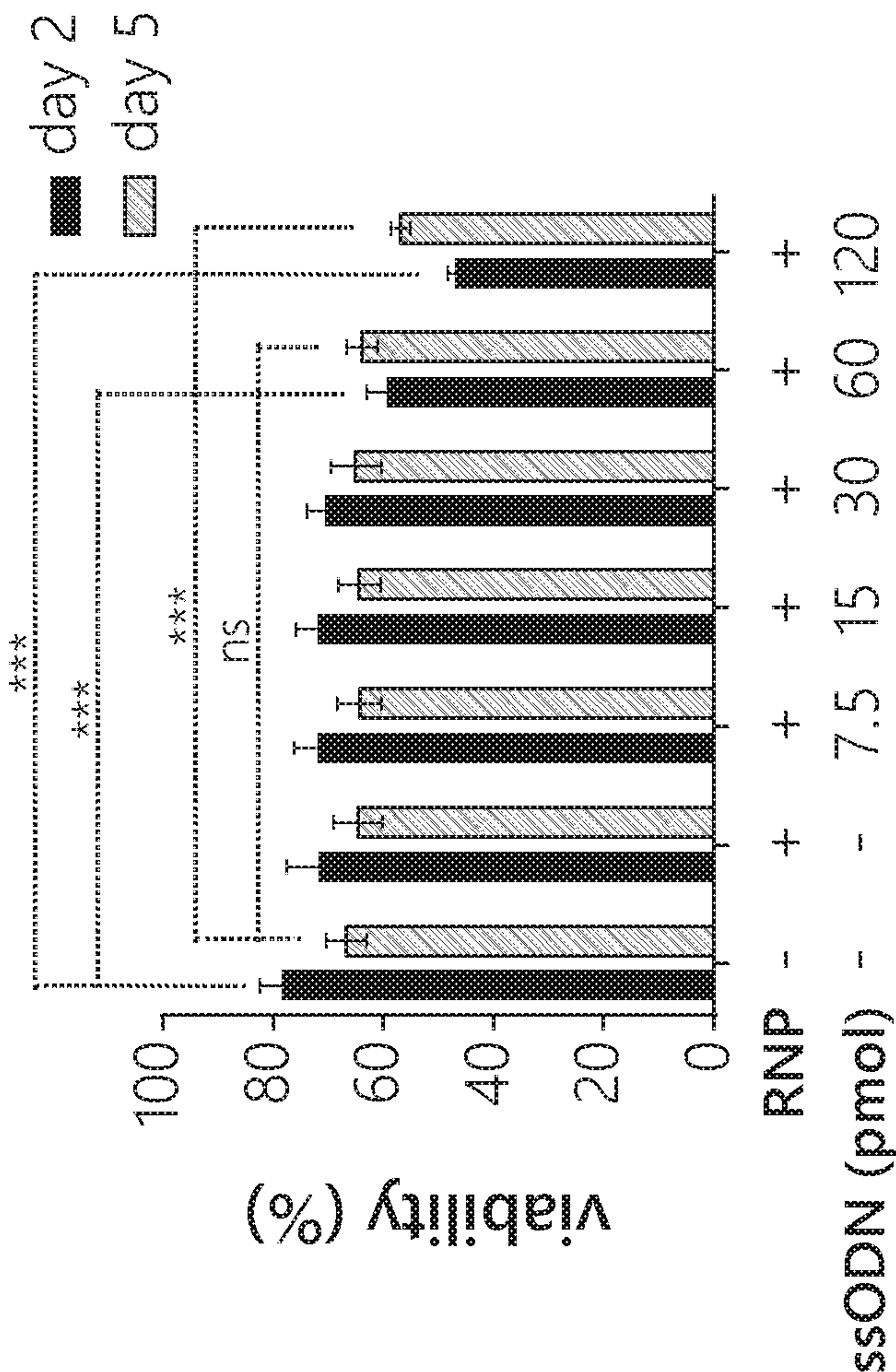


FIG. 42C

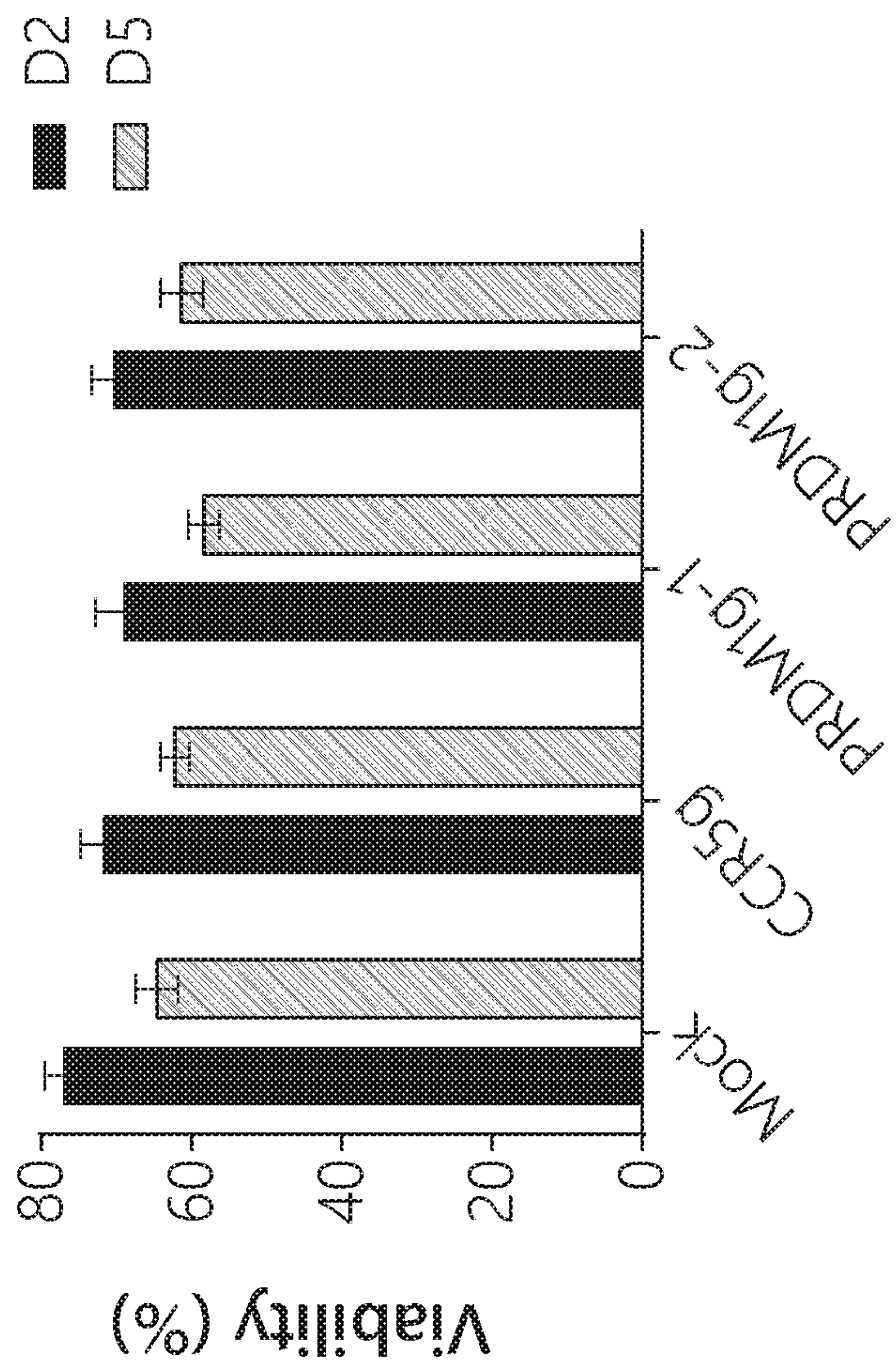


FIG. 43B

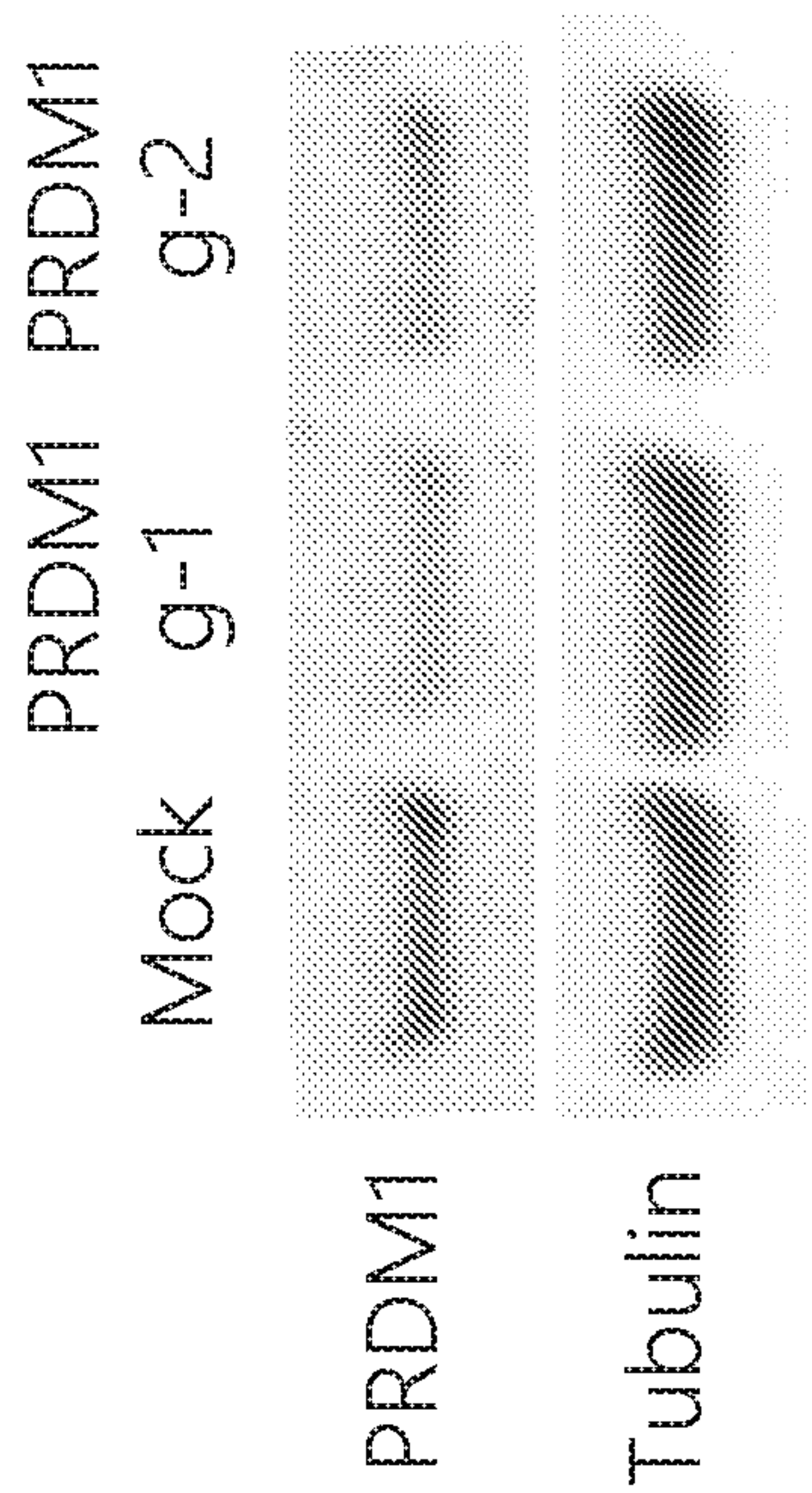


FIG. 43A

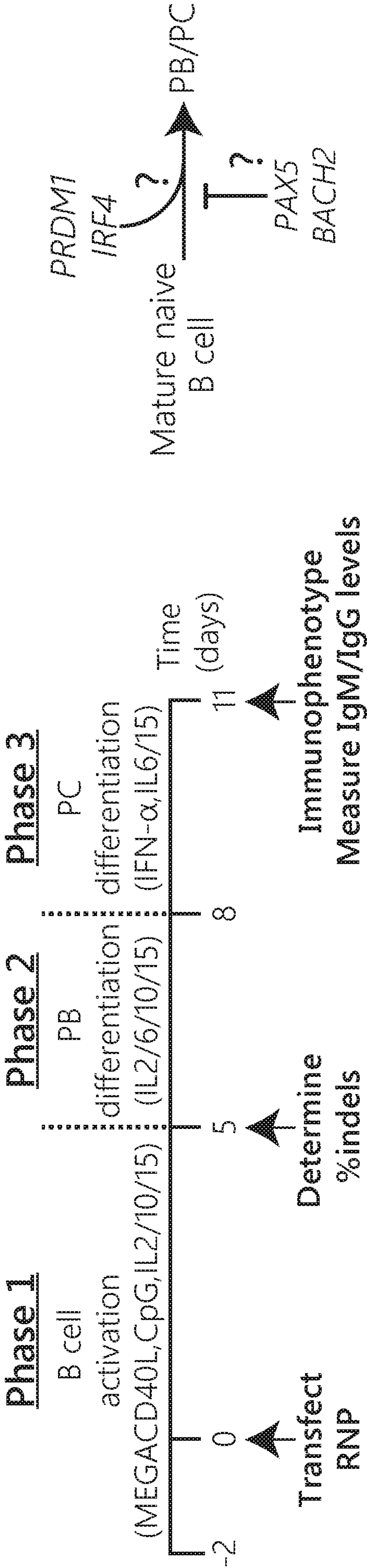


FIG. 44B

FIG. 44A

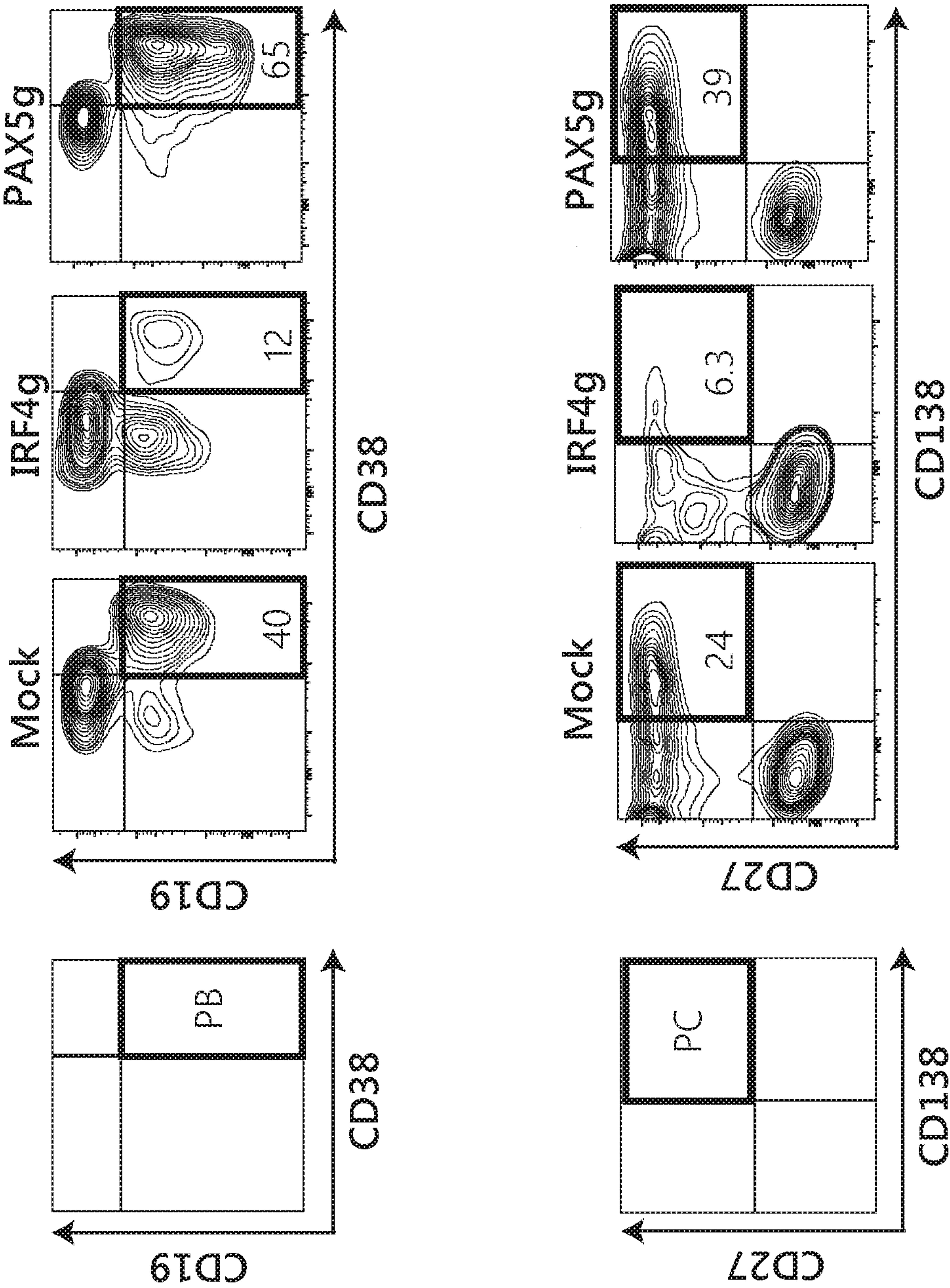
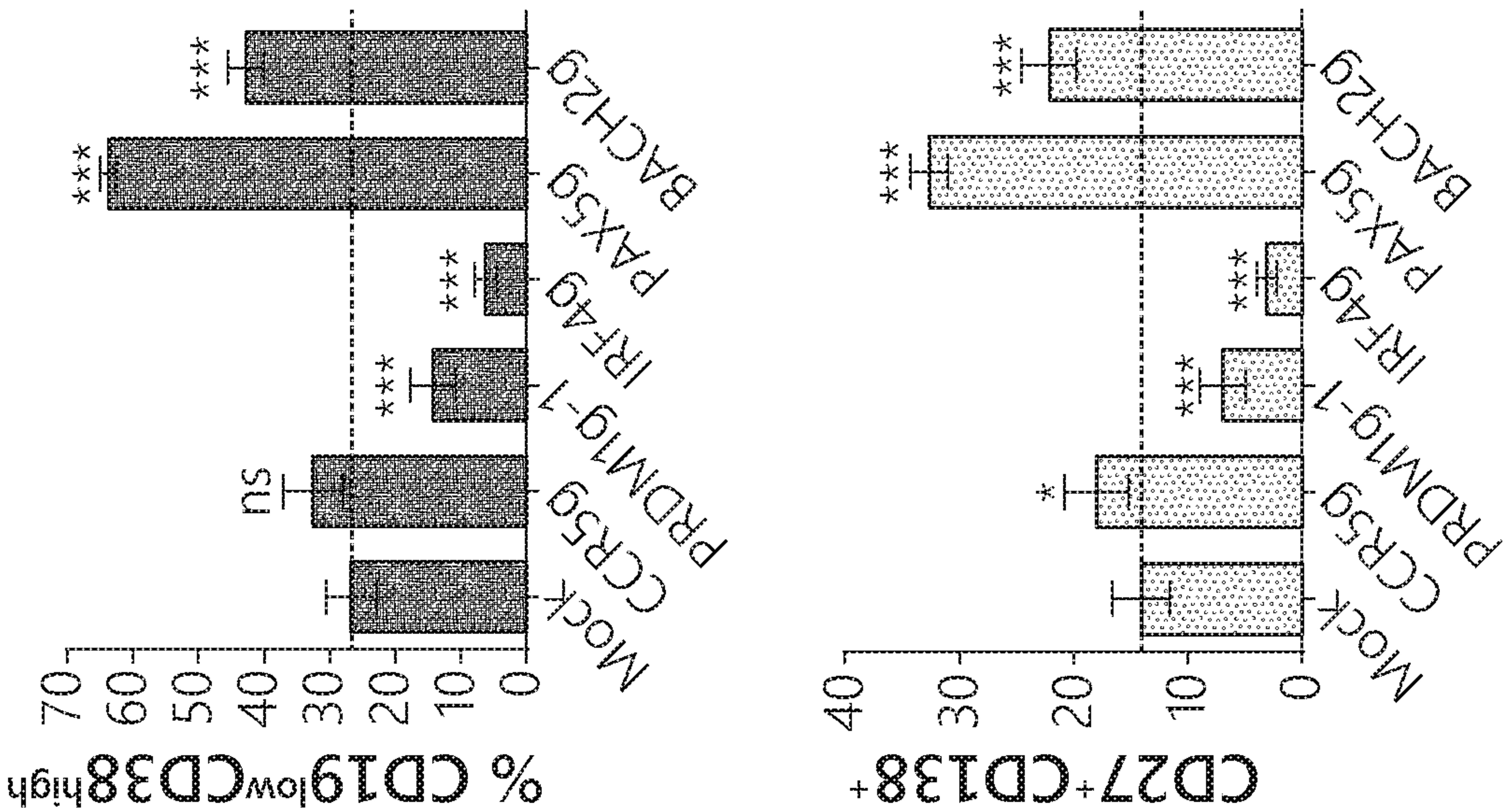


FIG. 44C

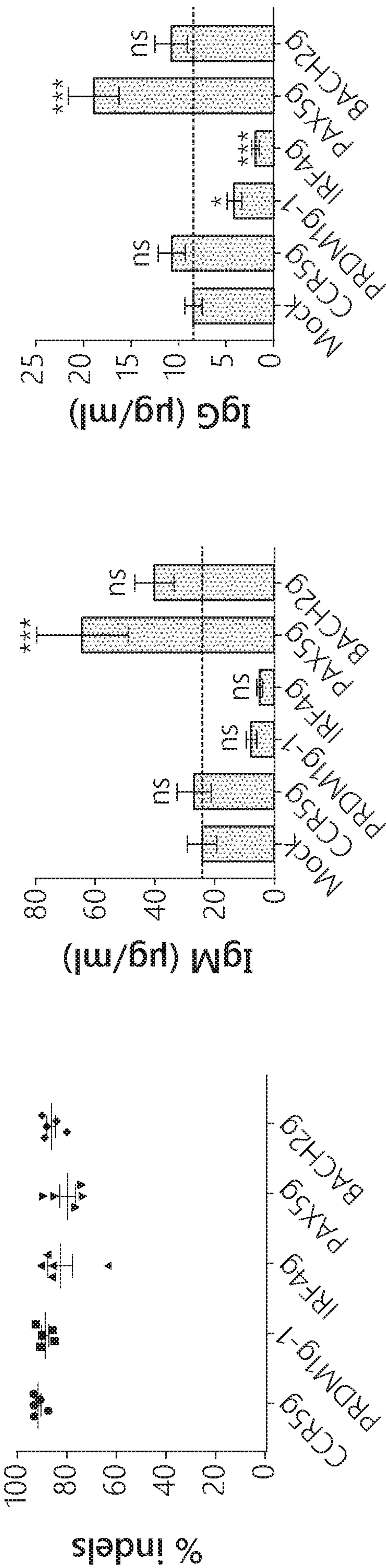


FIG. 44D

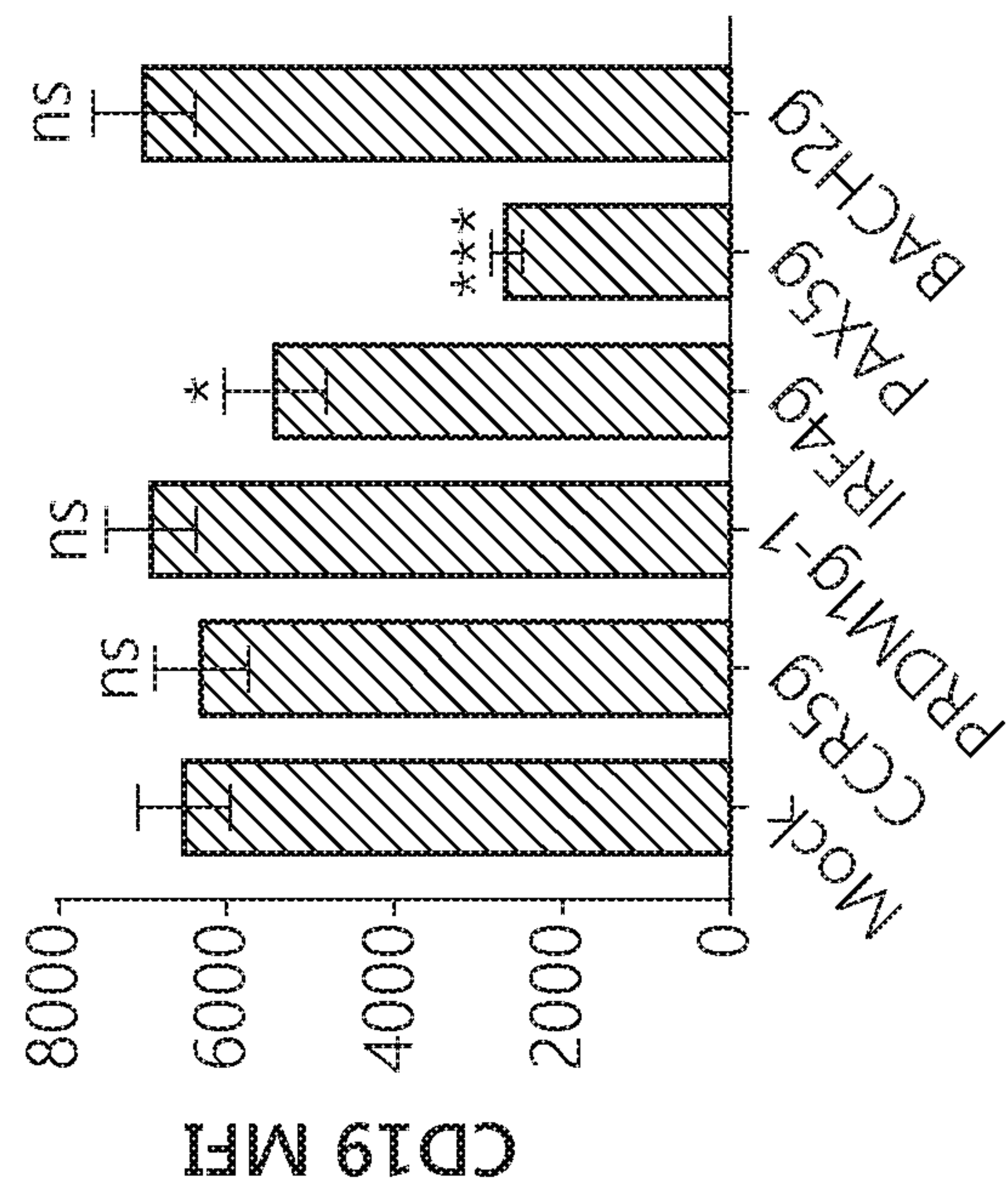


FIG. 45C

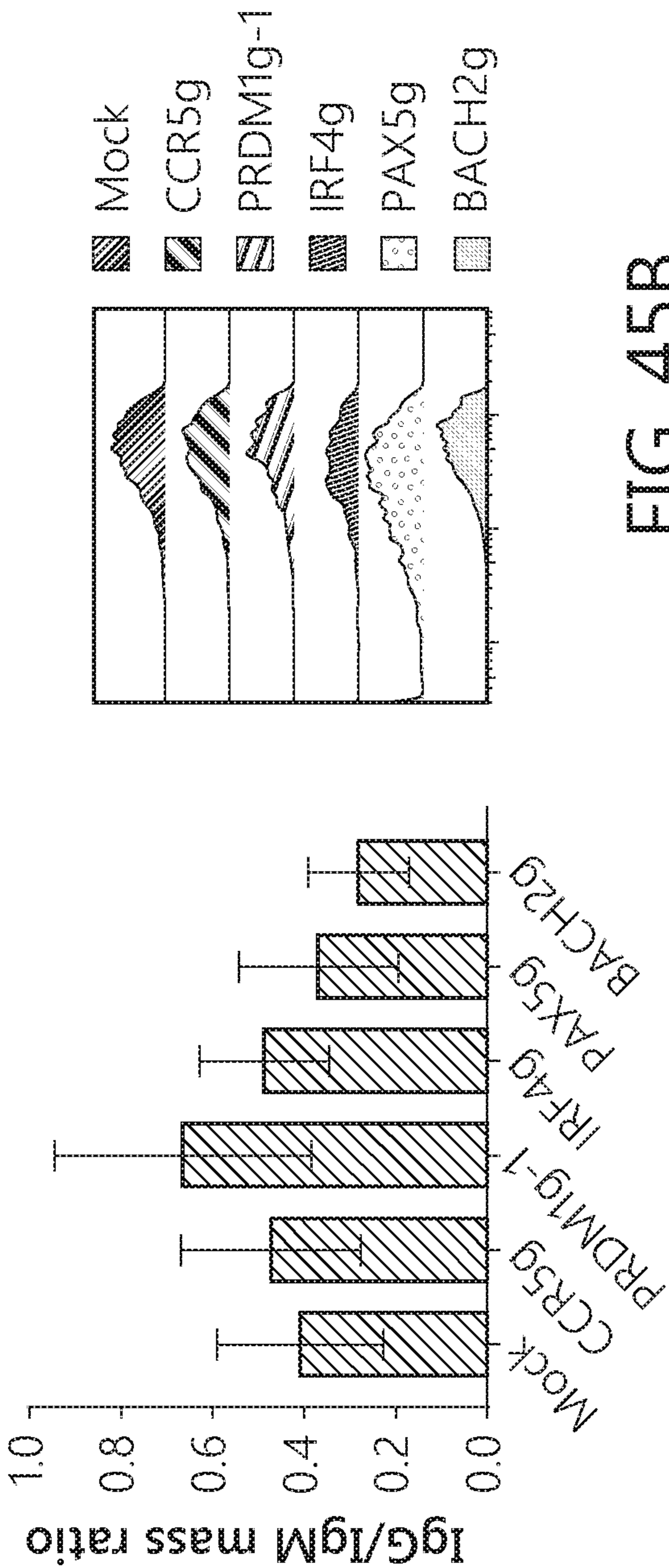


FIG. 45B

FIG. 45A

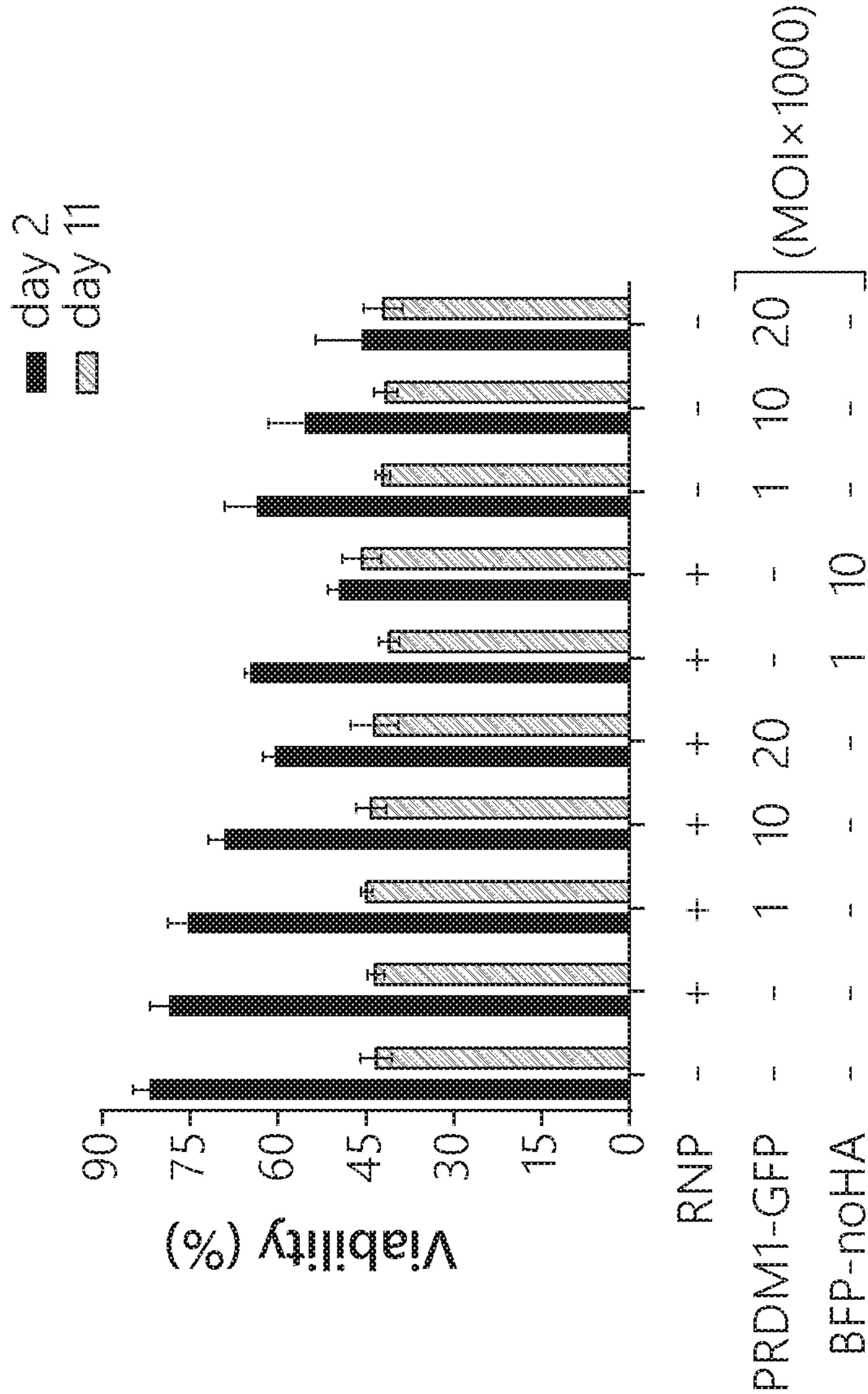


FIG. 46B

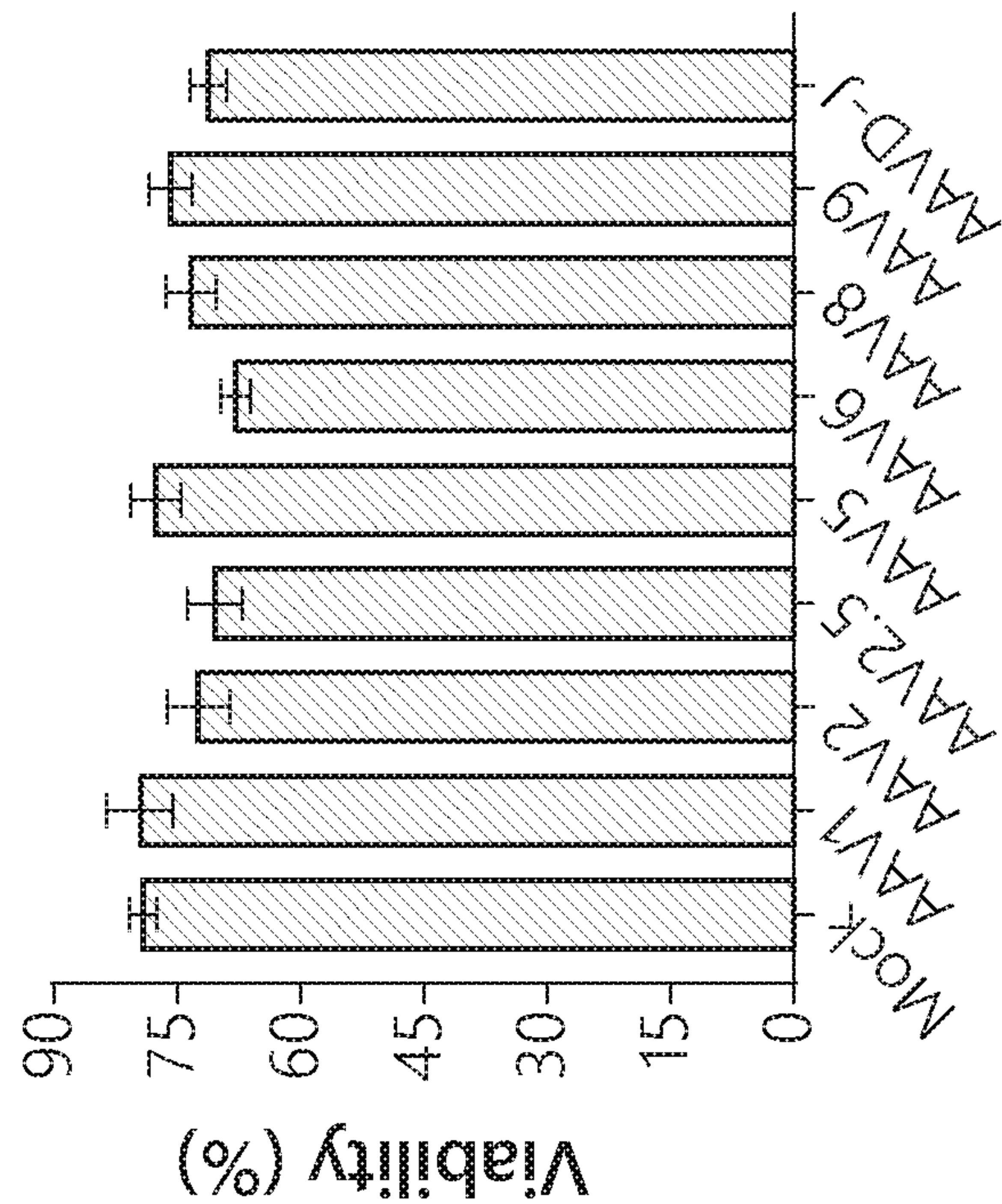


FIG. 46A

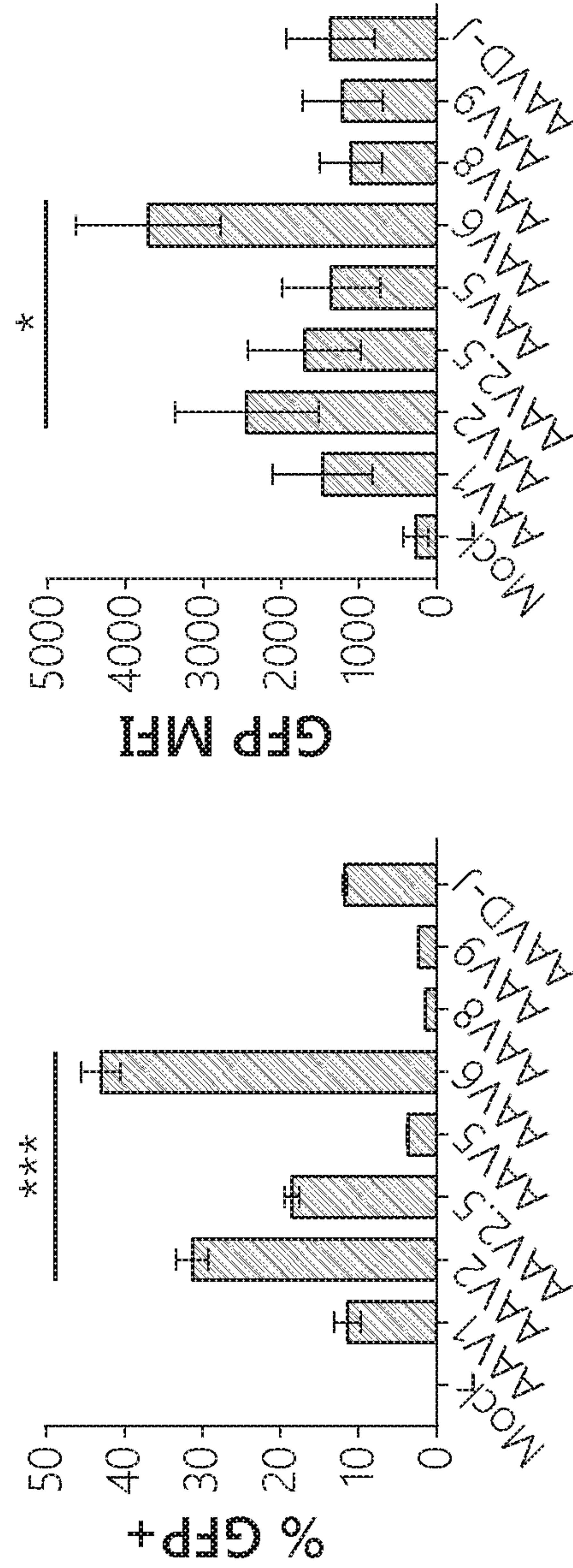


FIG. 47A

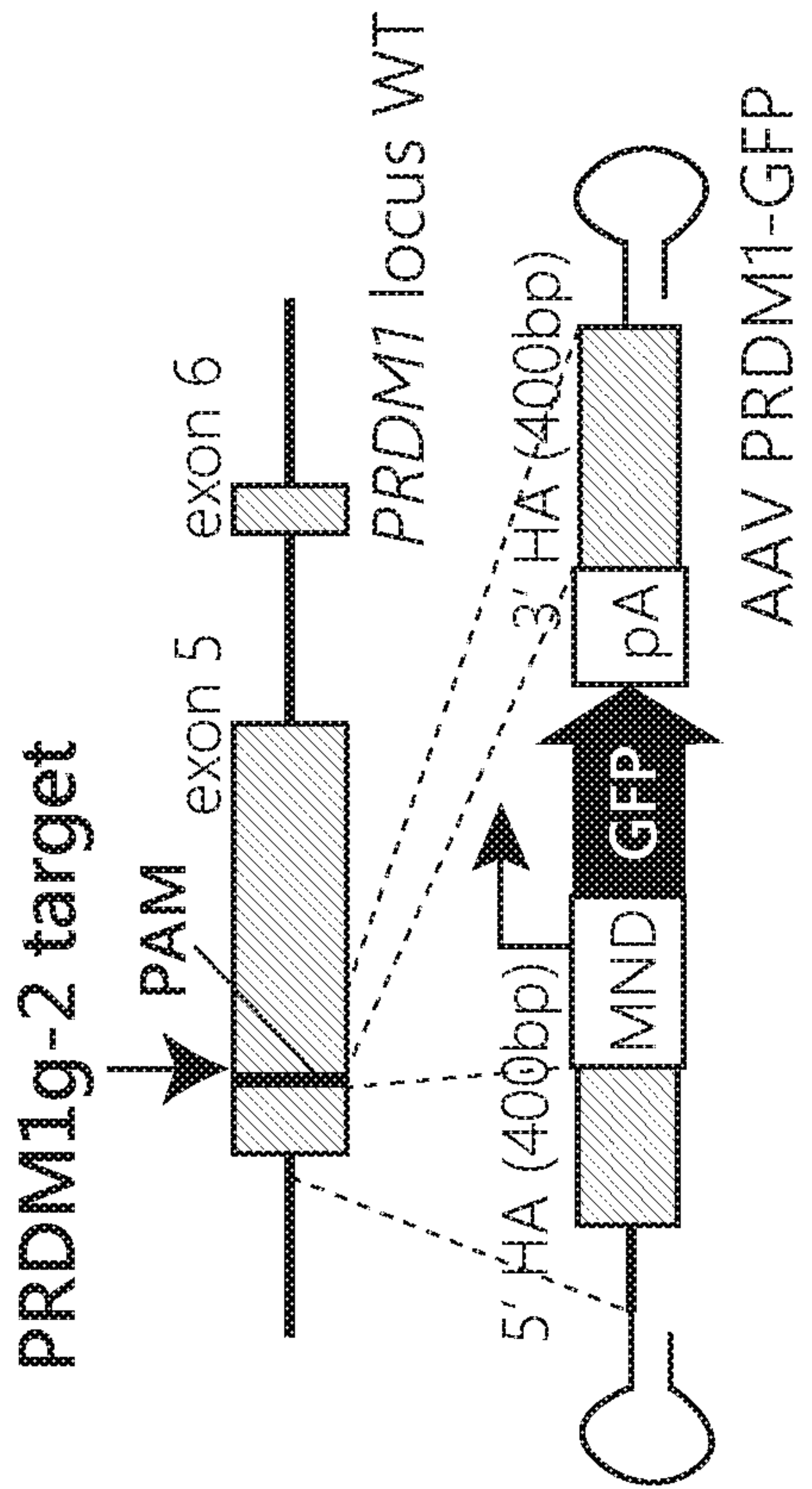
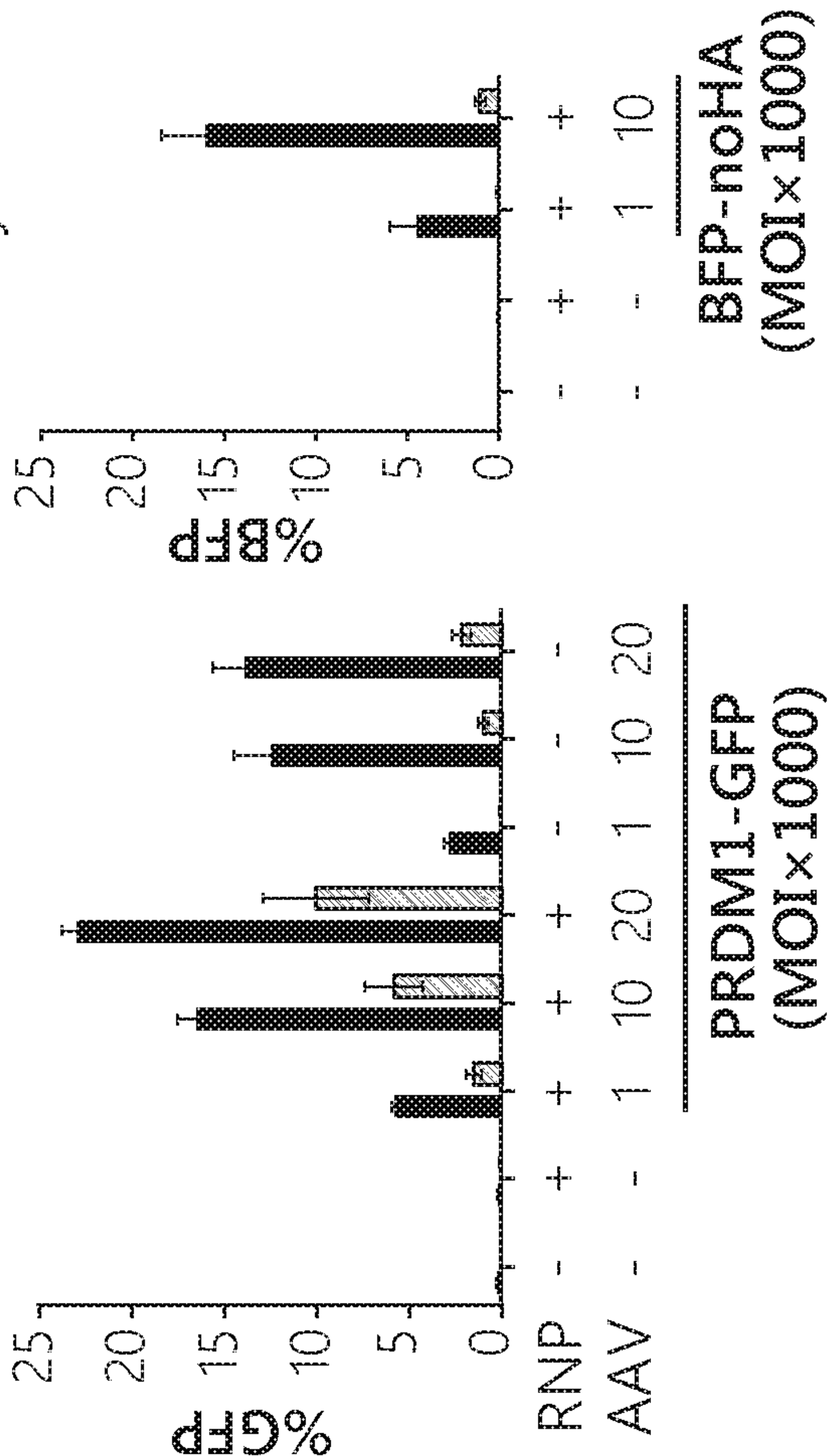
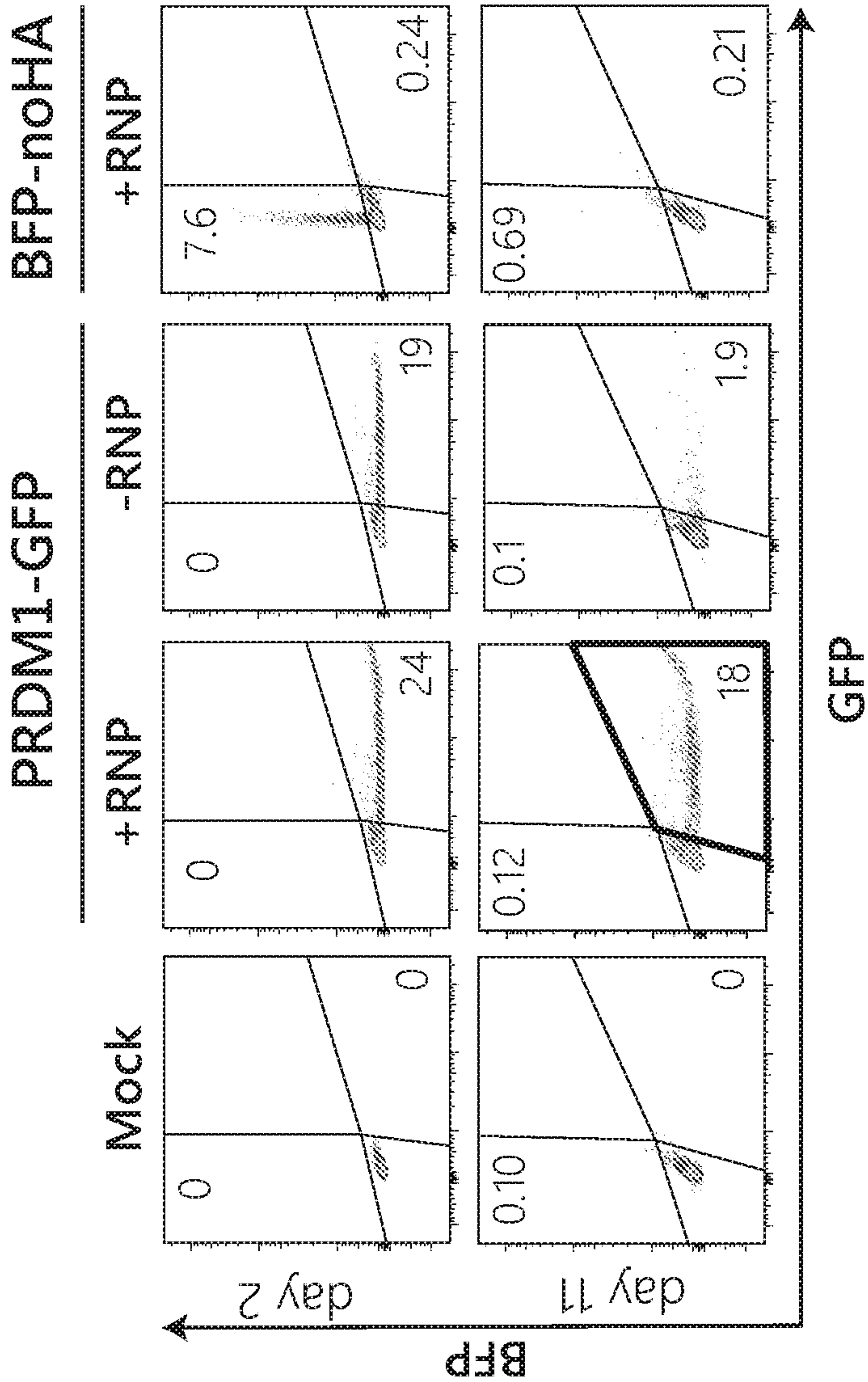


FIG. 47B



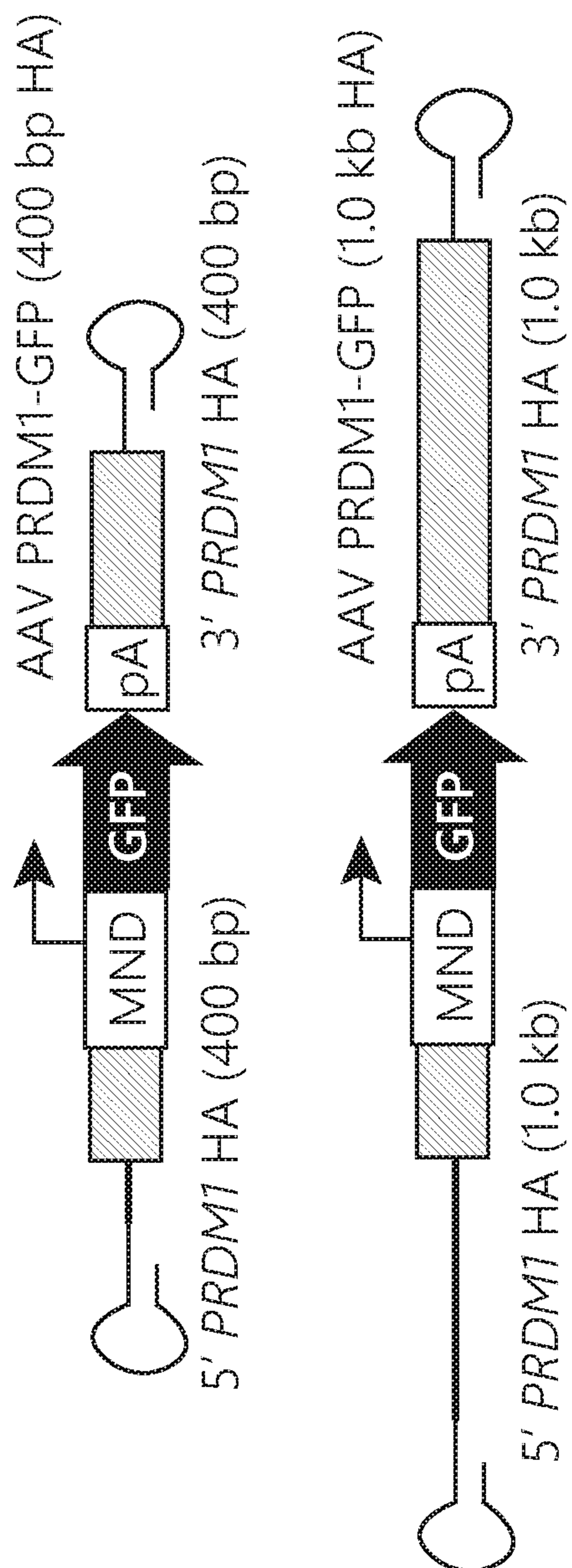
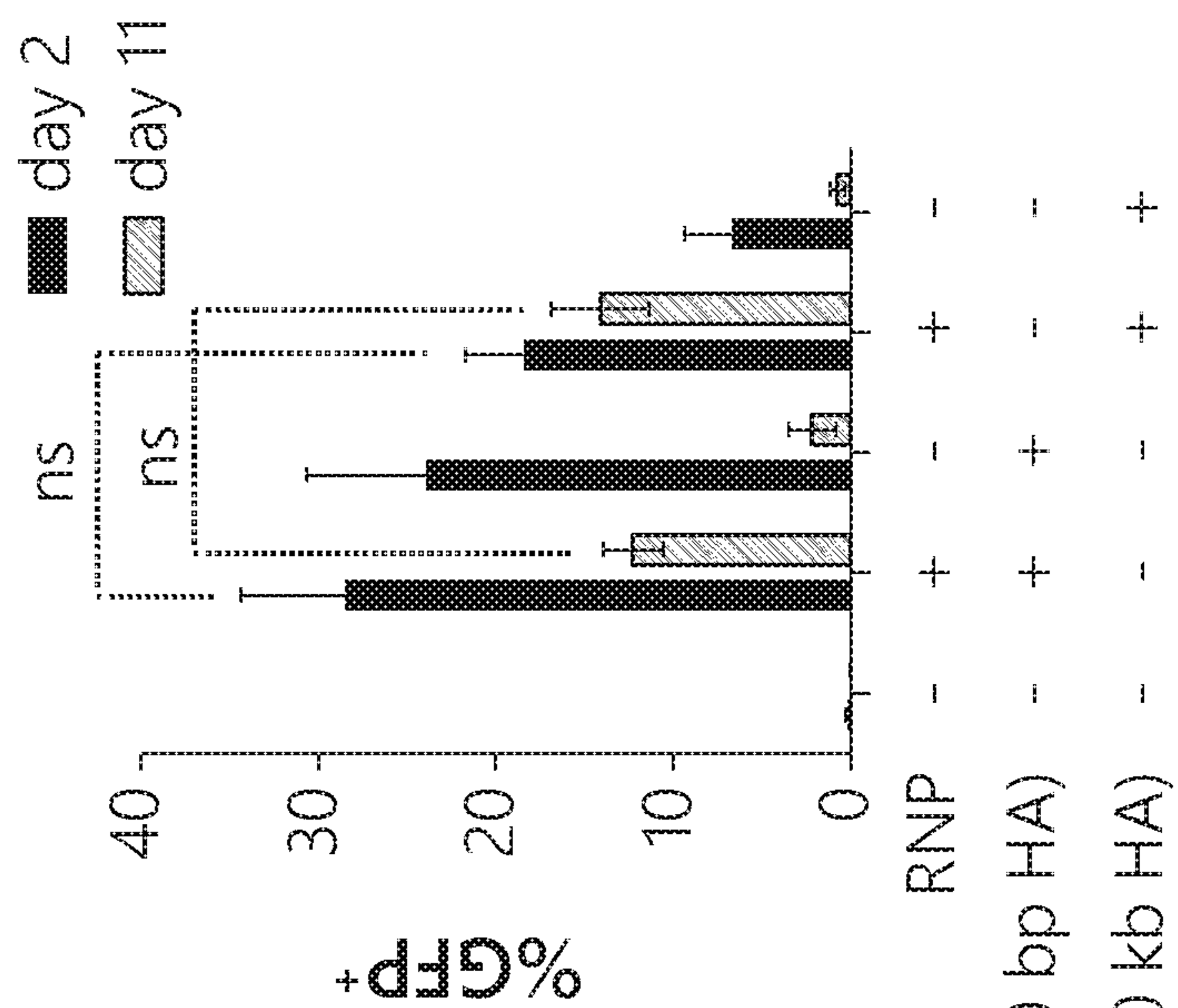


FIG. 48A



A vertical sequence of six mathematical symbols: ∞ , $+$, $\leq$, $\geq$, and two horizontal lines.

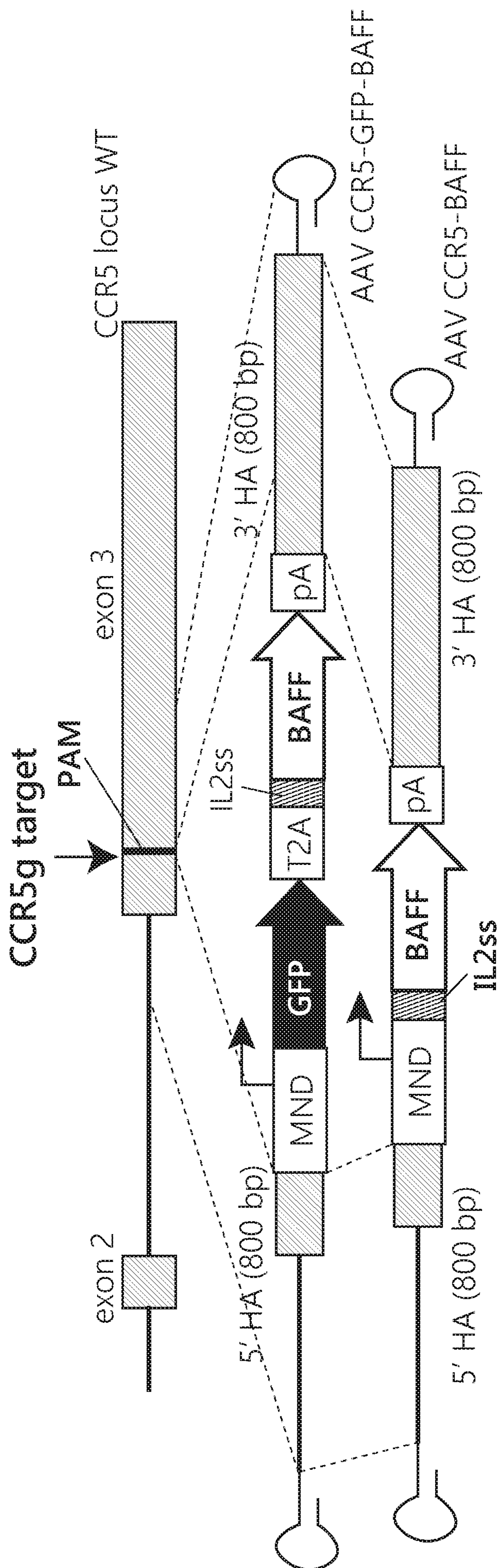


FIG. 49A

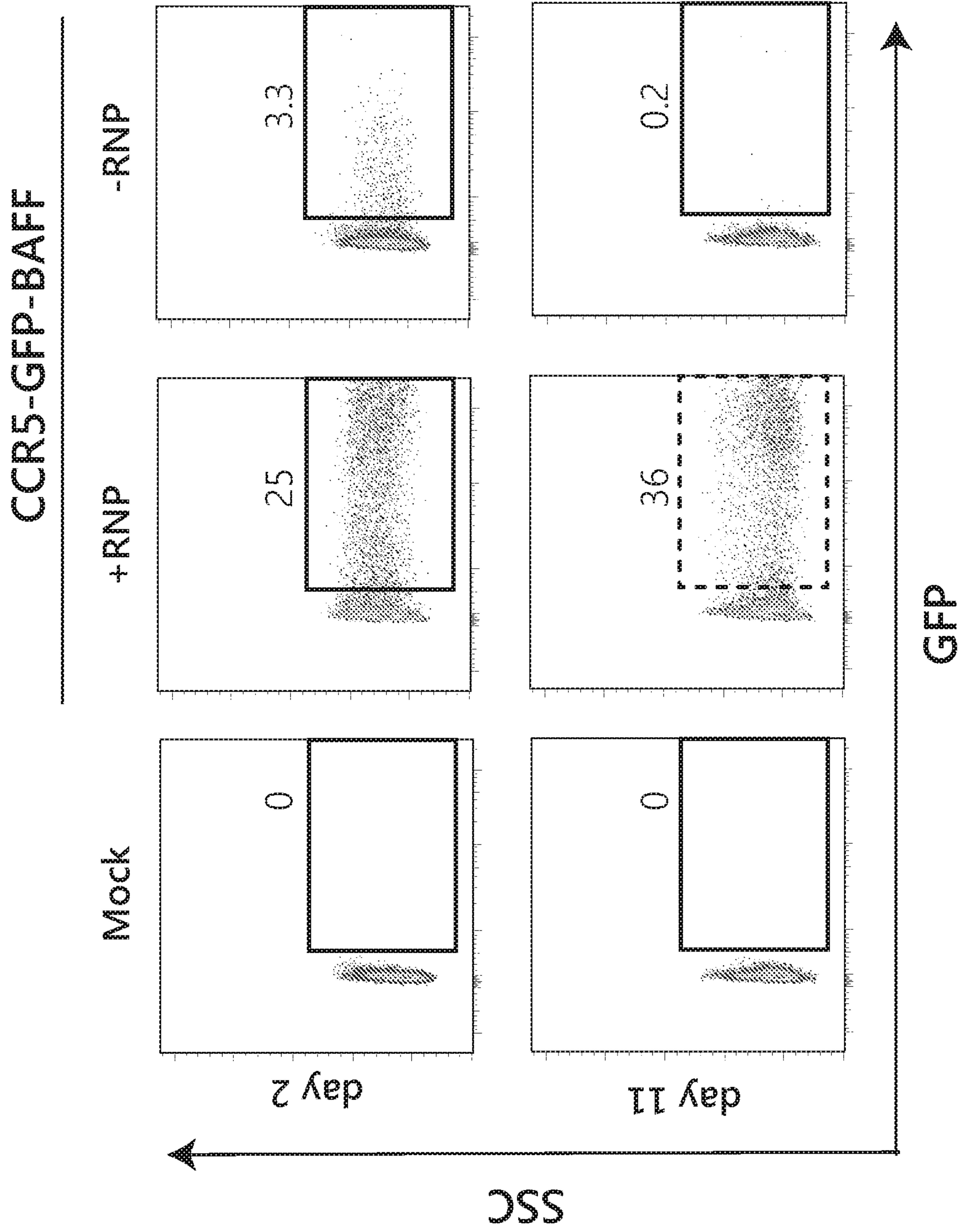


FIG. 49B

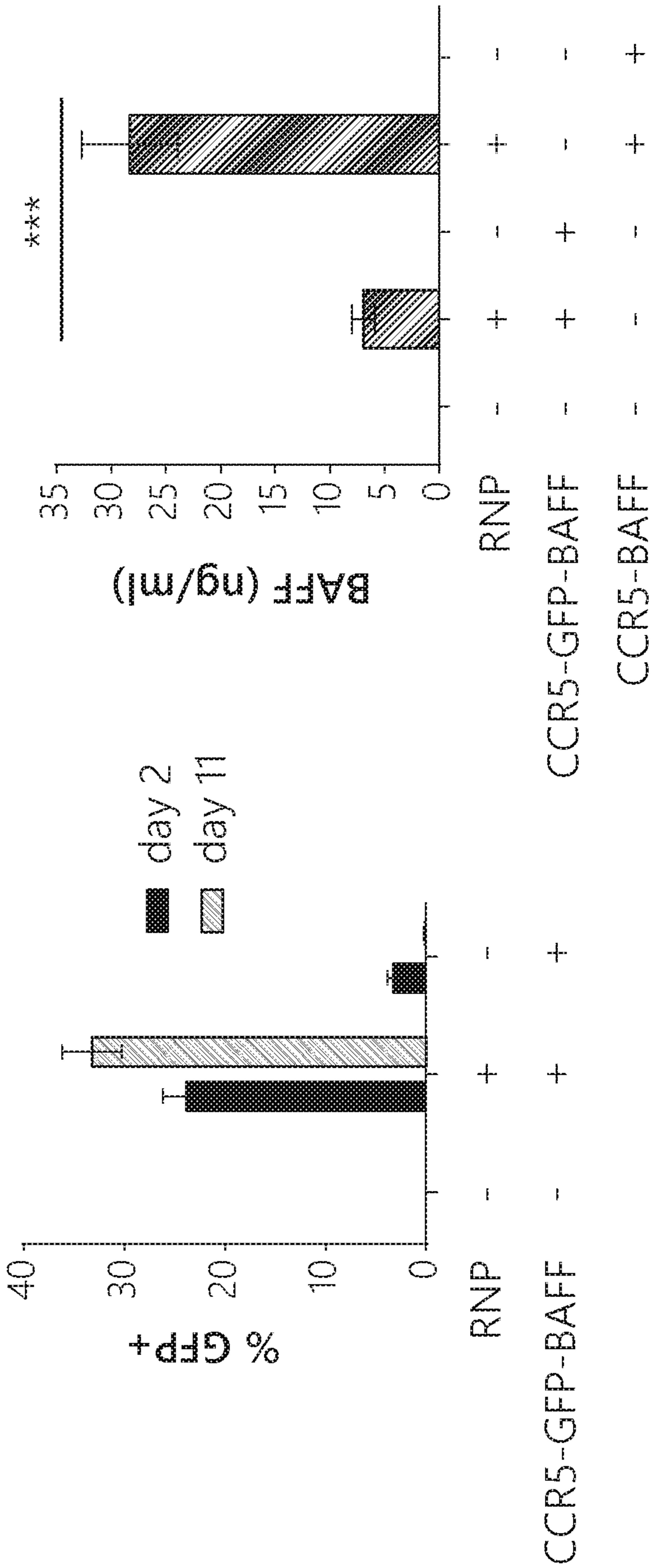


FIG. 49C

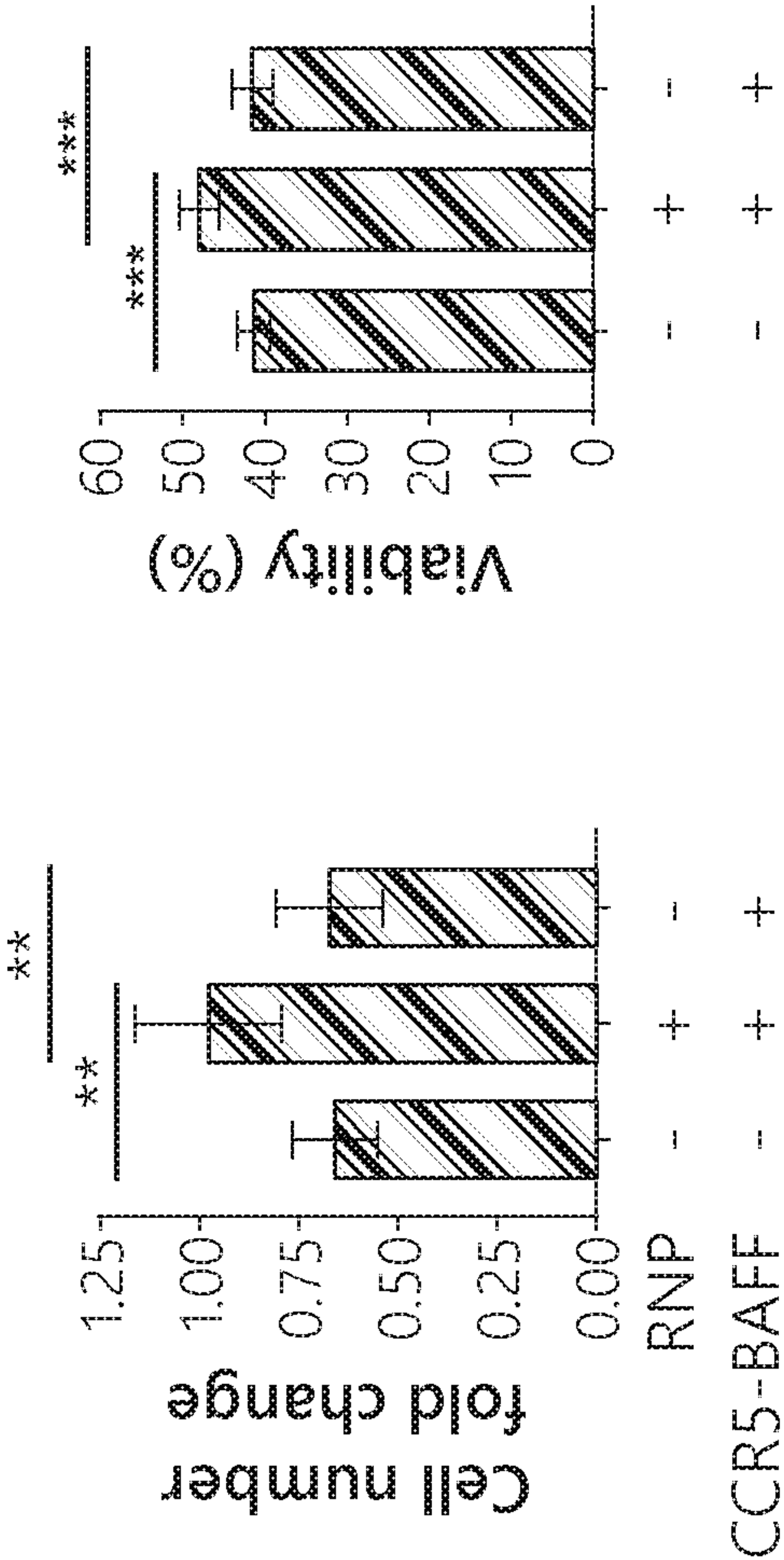


FIG. 49D

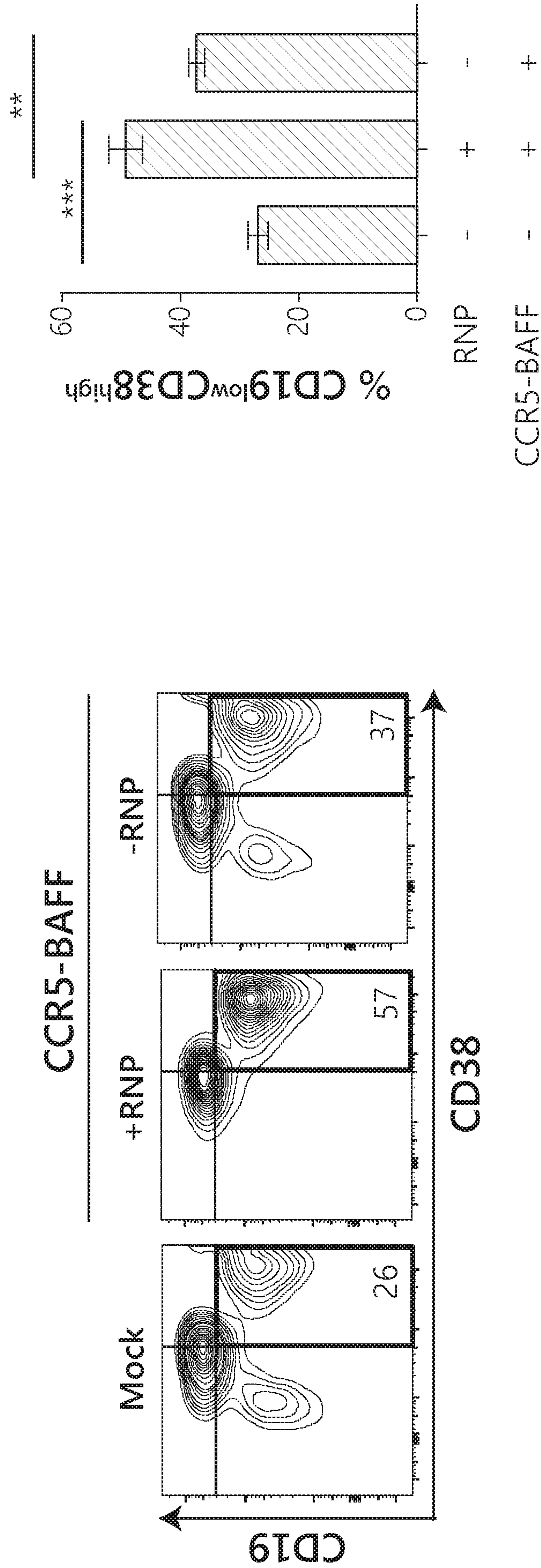


FIG. 49E

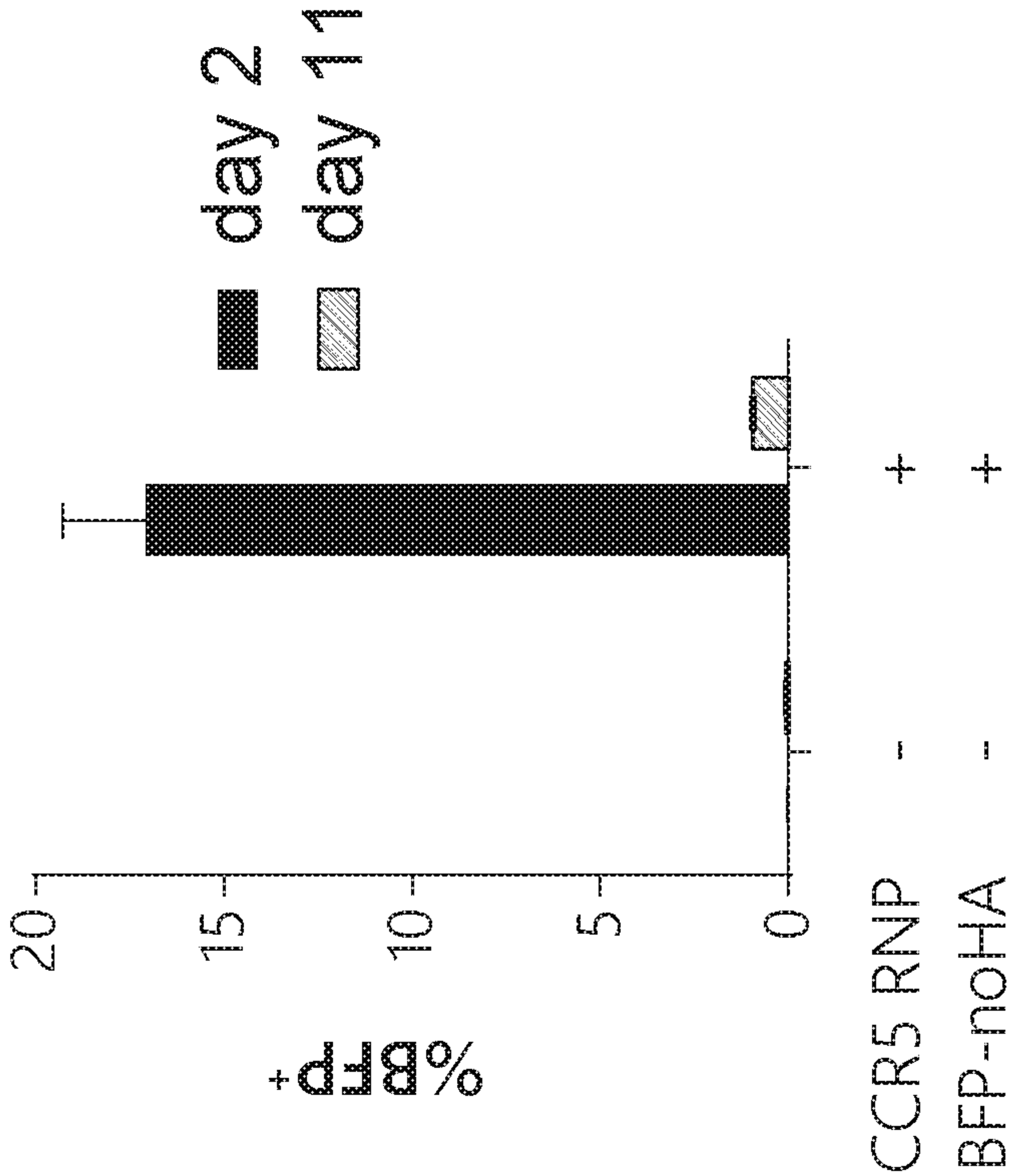
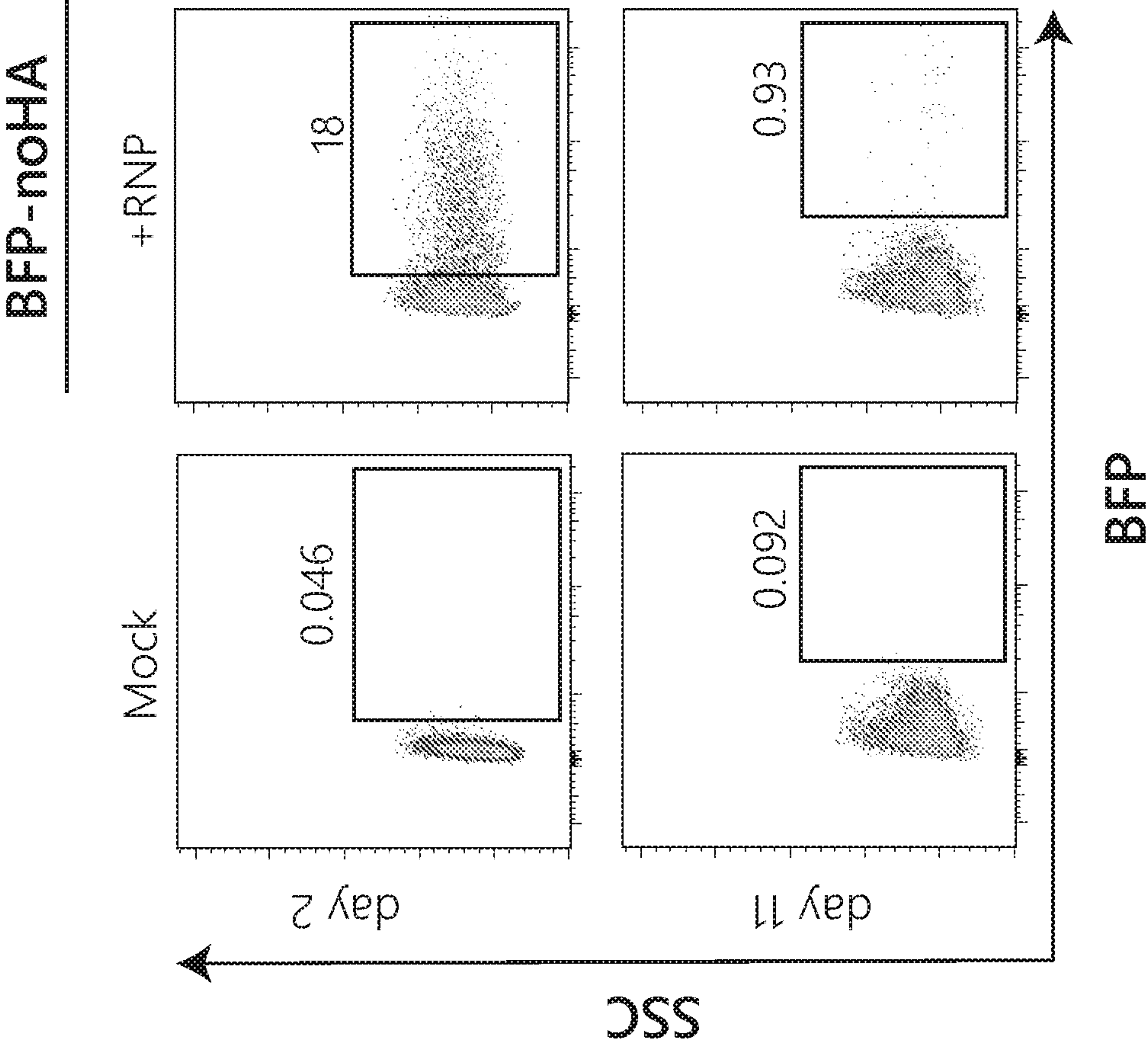


FIG. 50

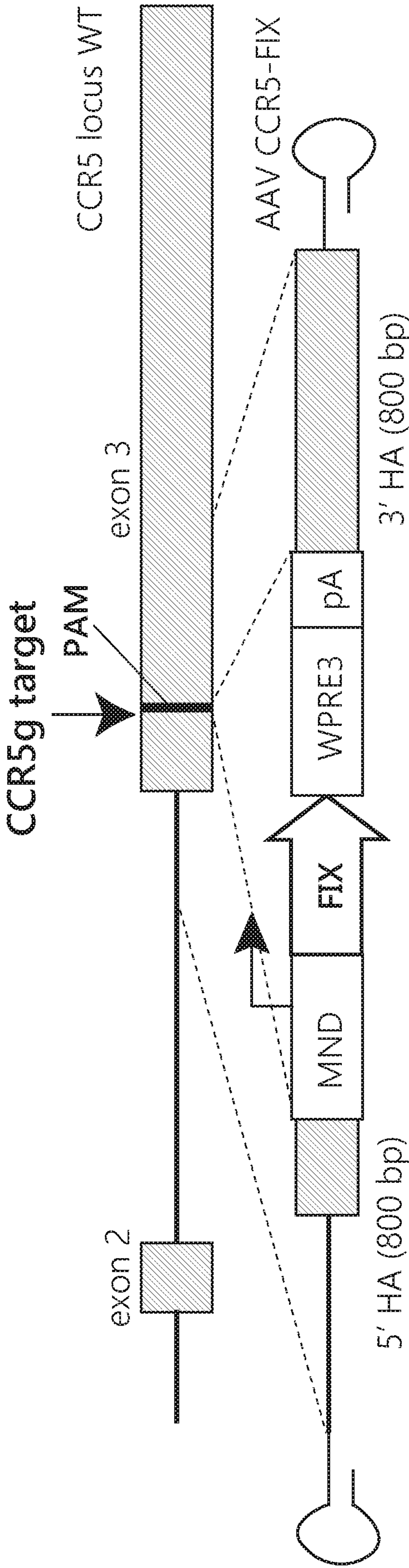


FIG. 51A

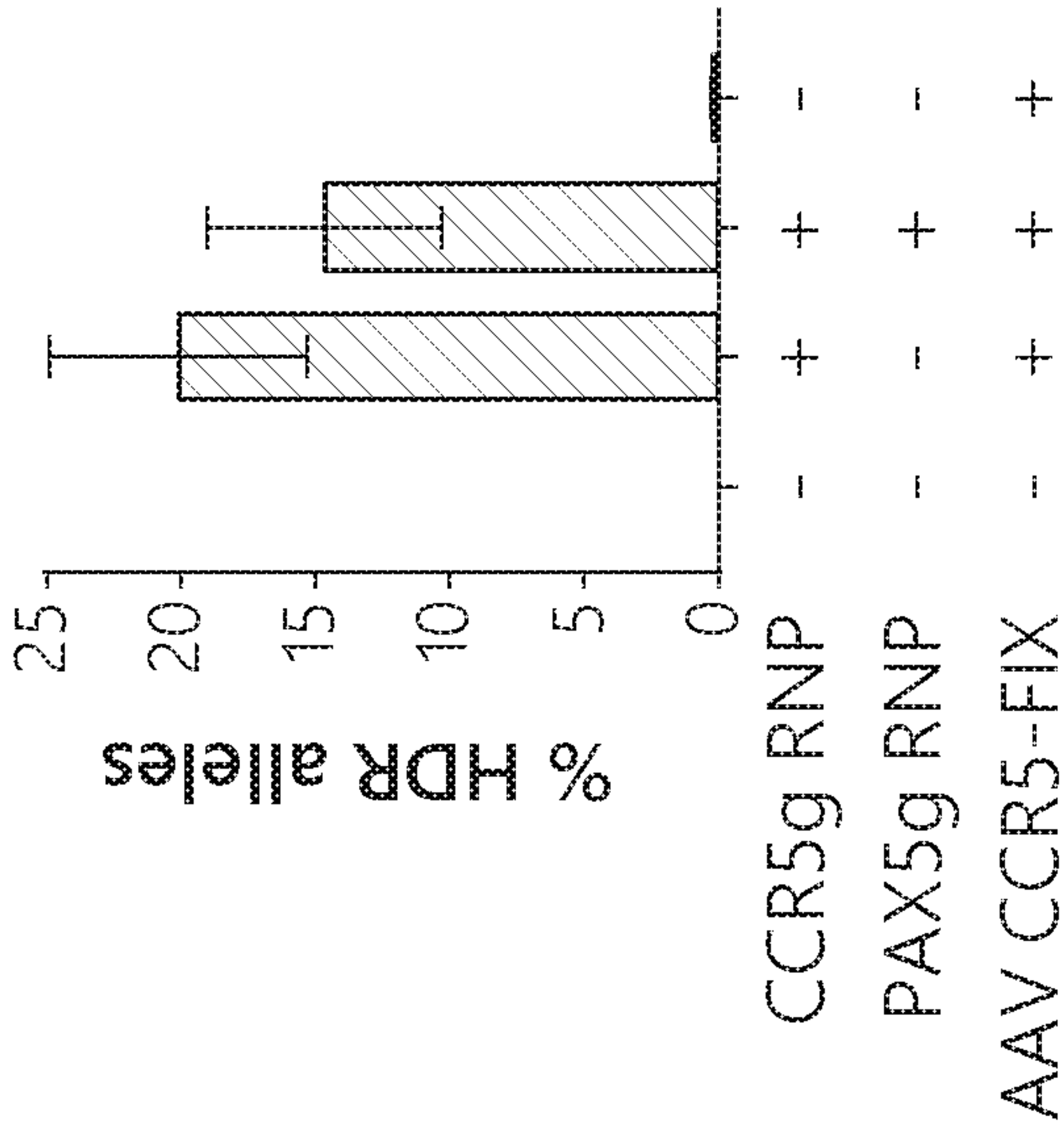


FIG. 51B

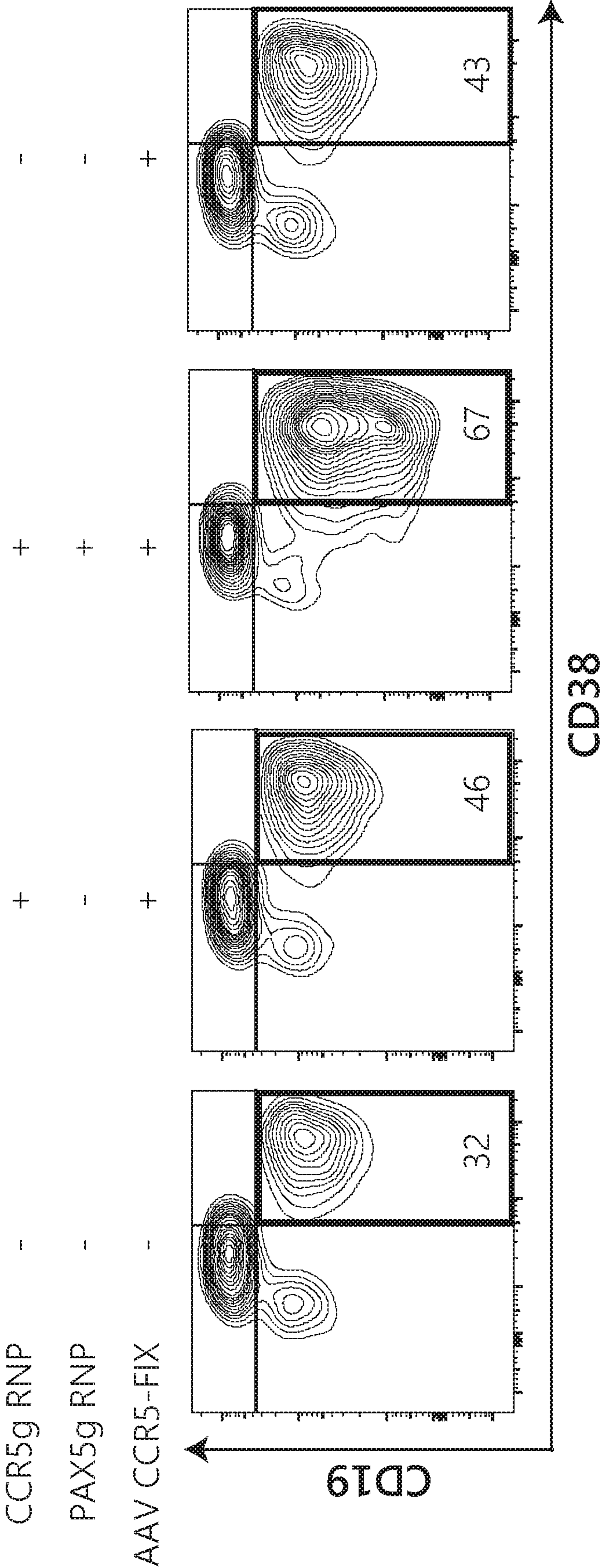


FIG. 51C

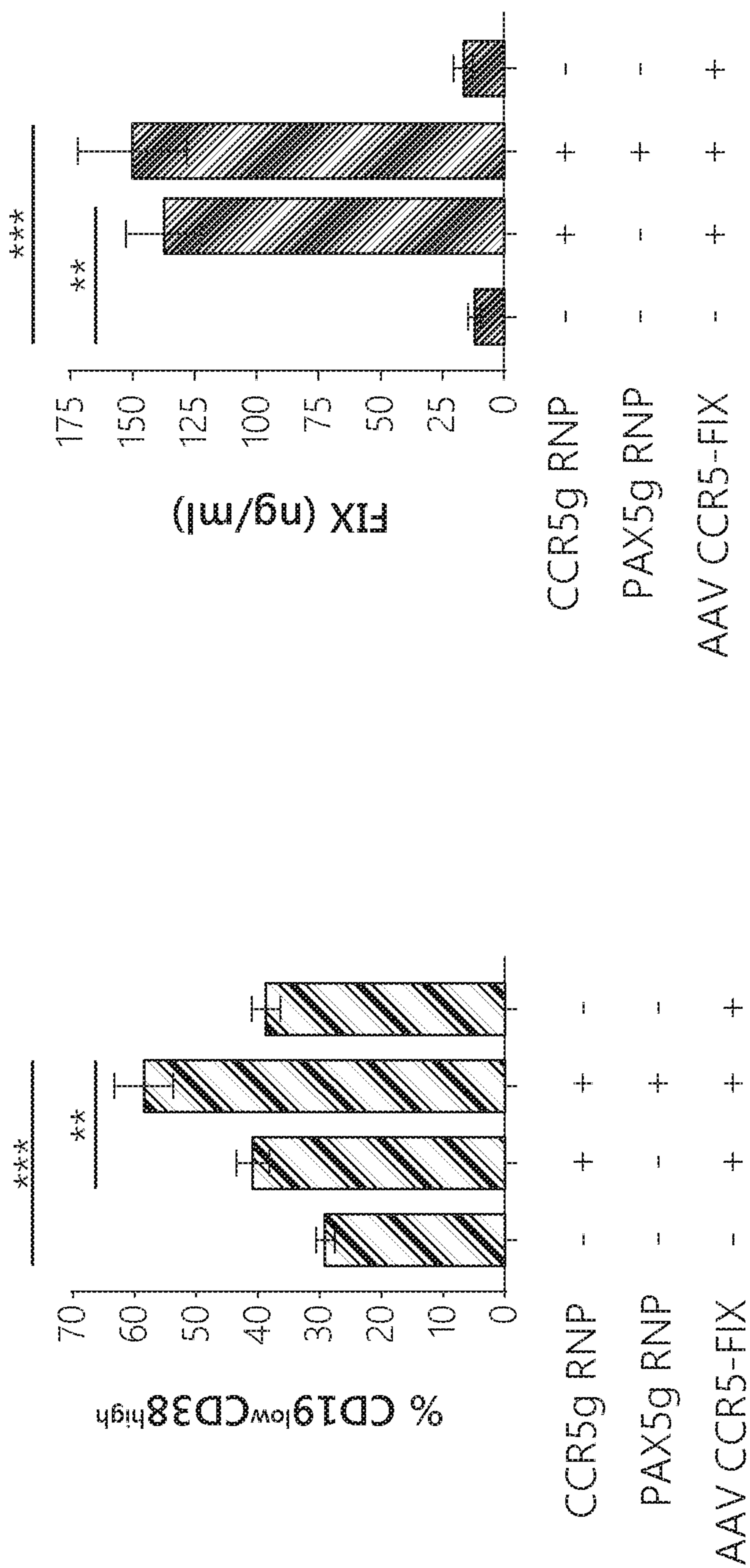


FIG. 51D

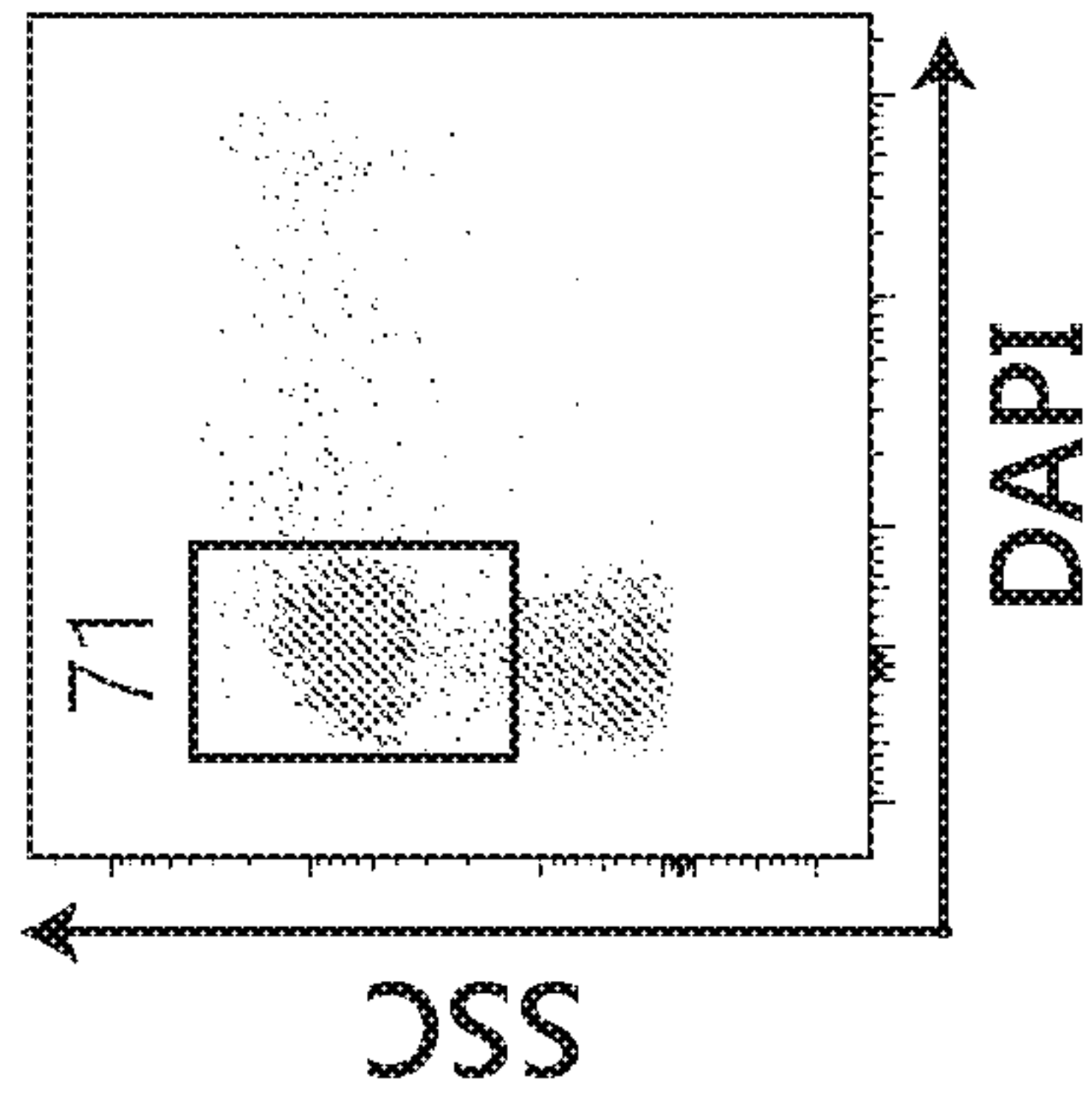


FIG. 52B

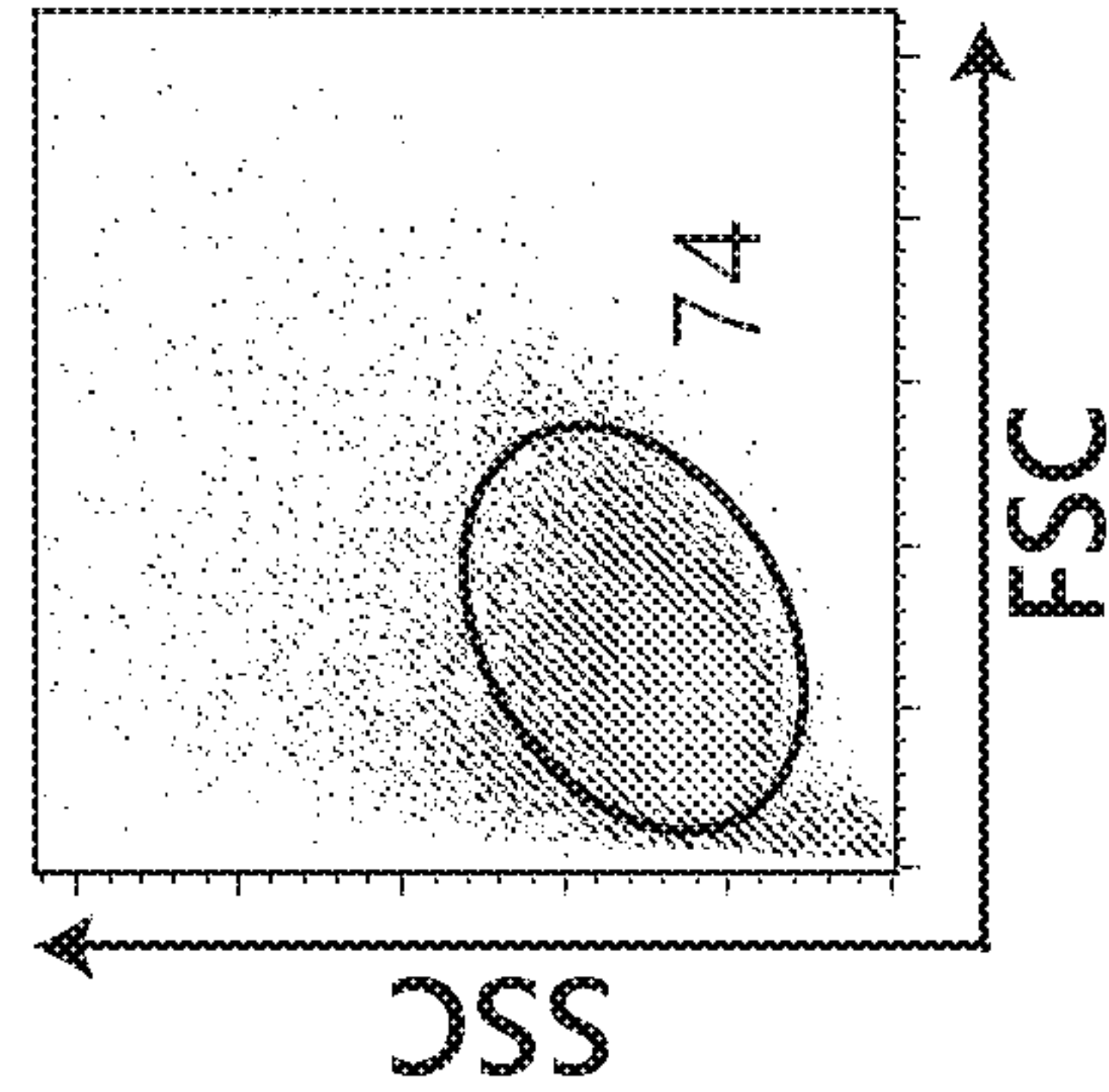
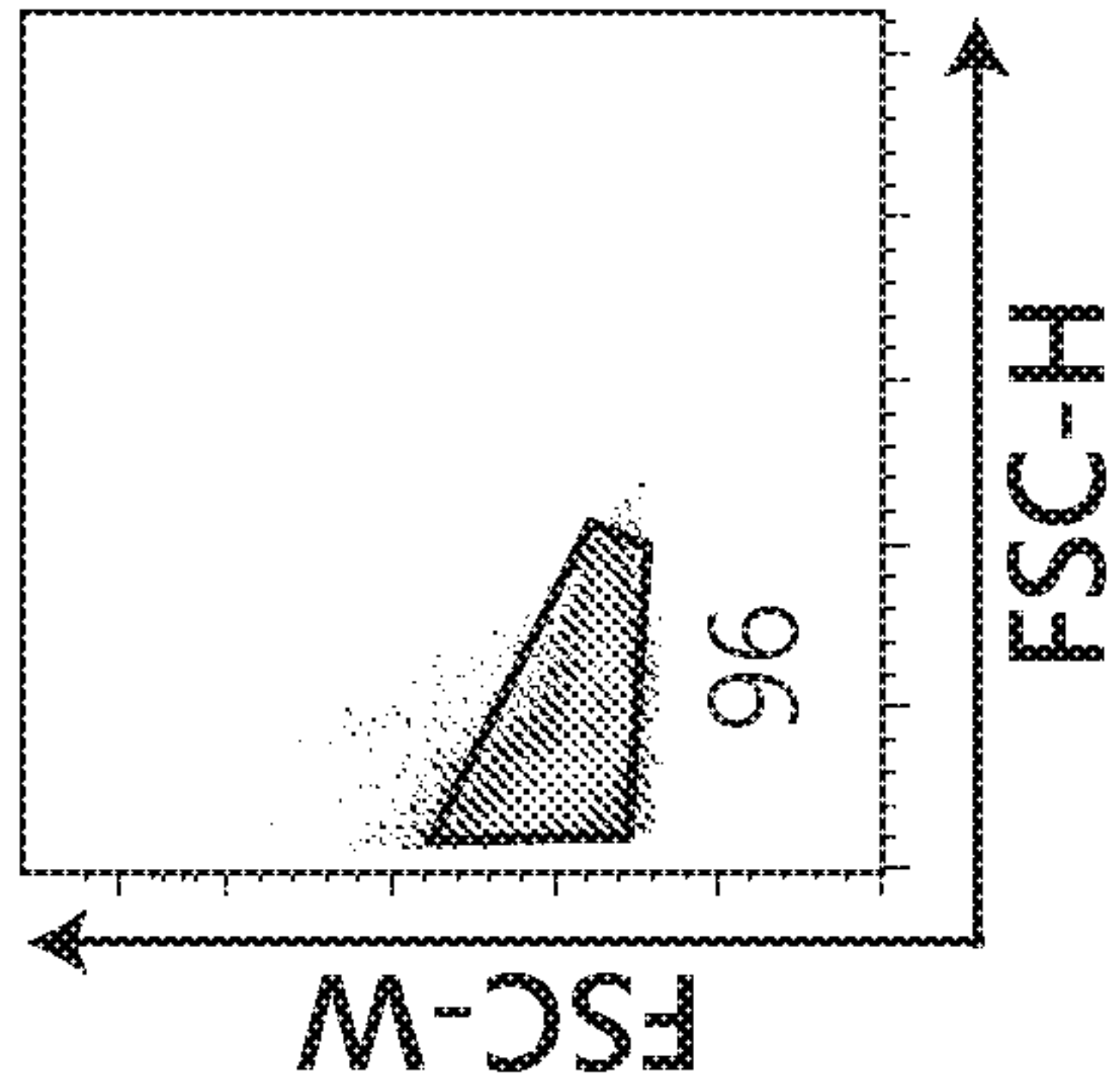
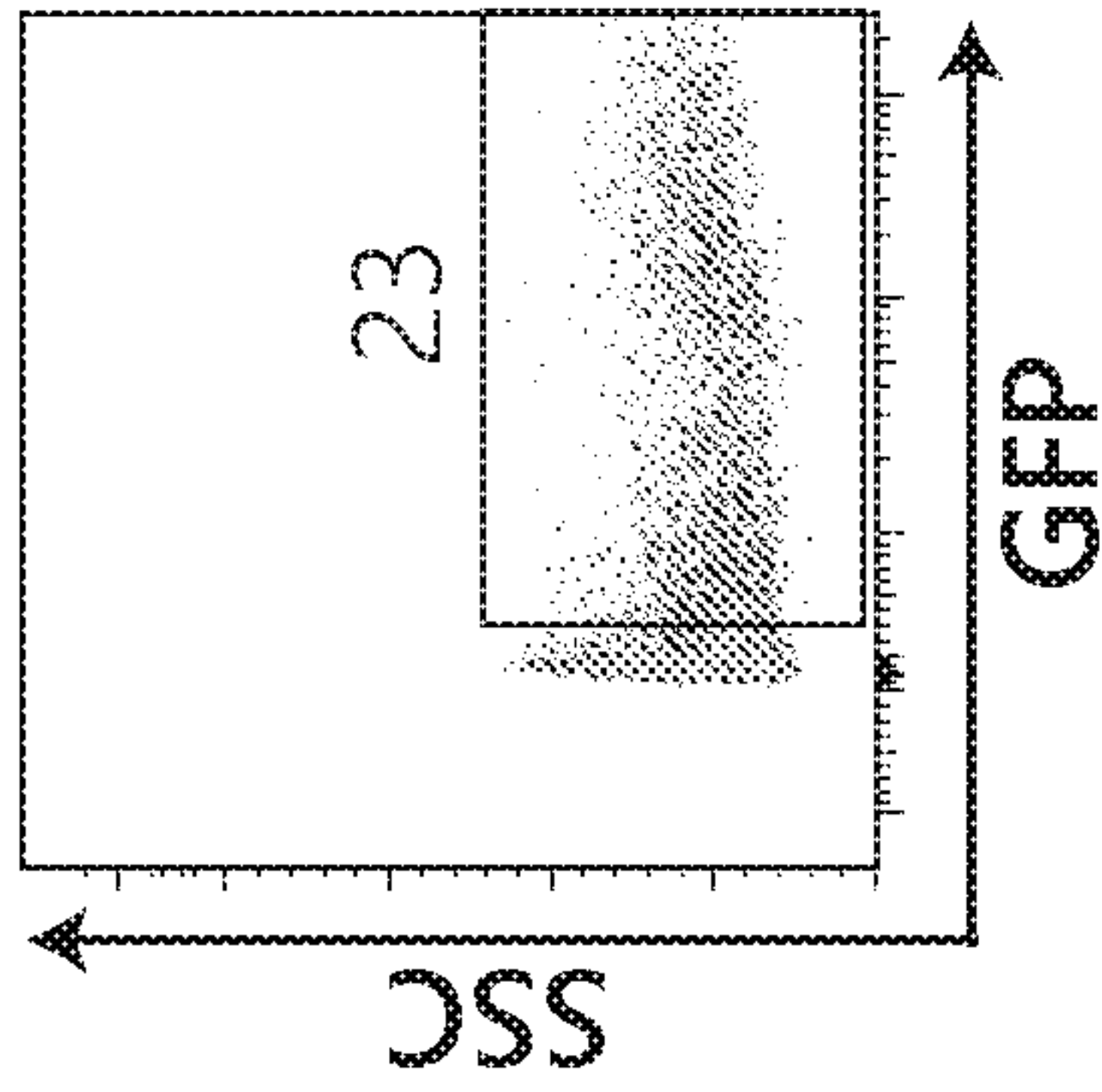


FIG. 52A

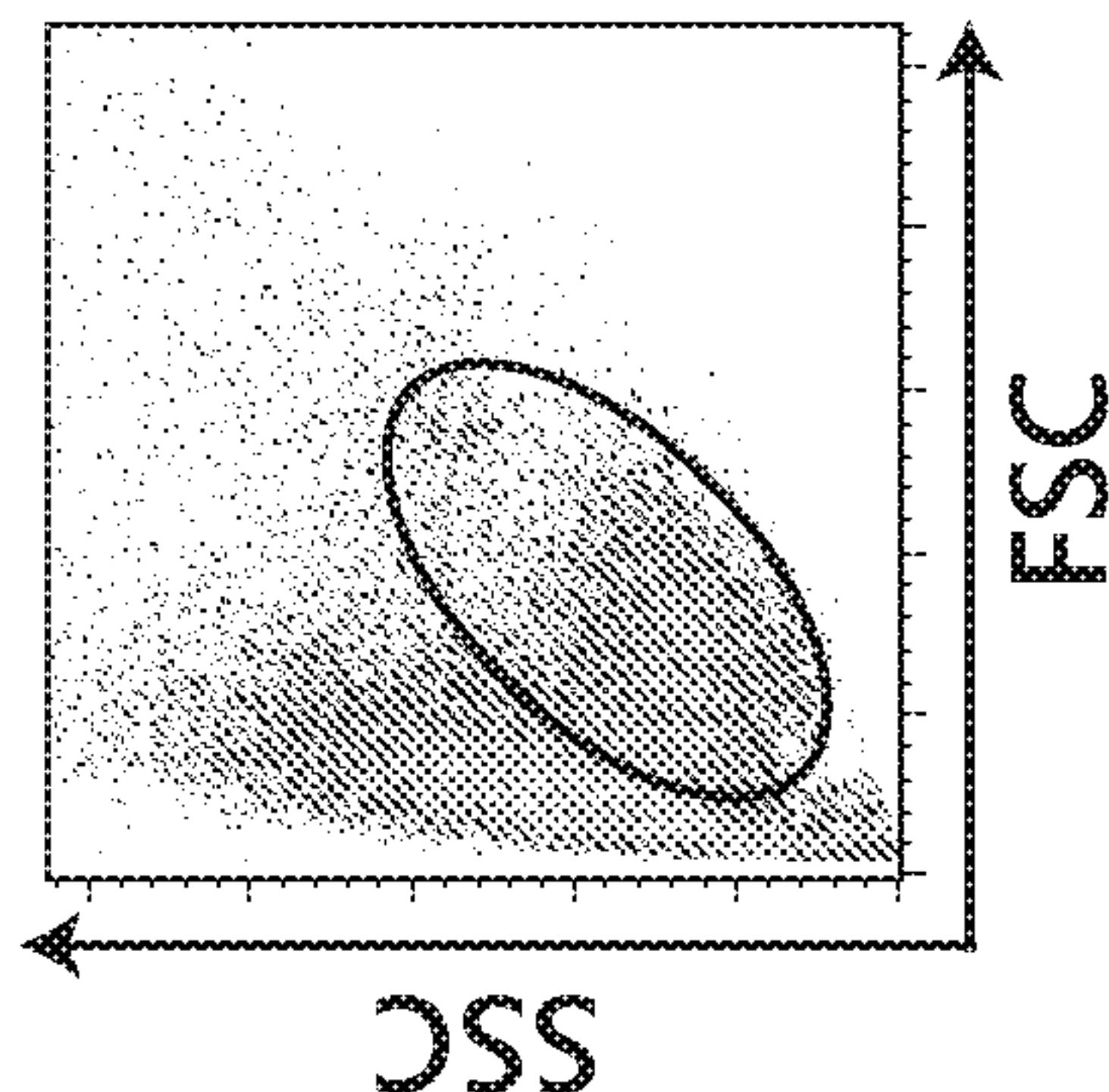
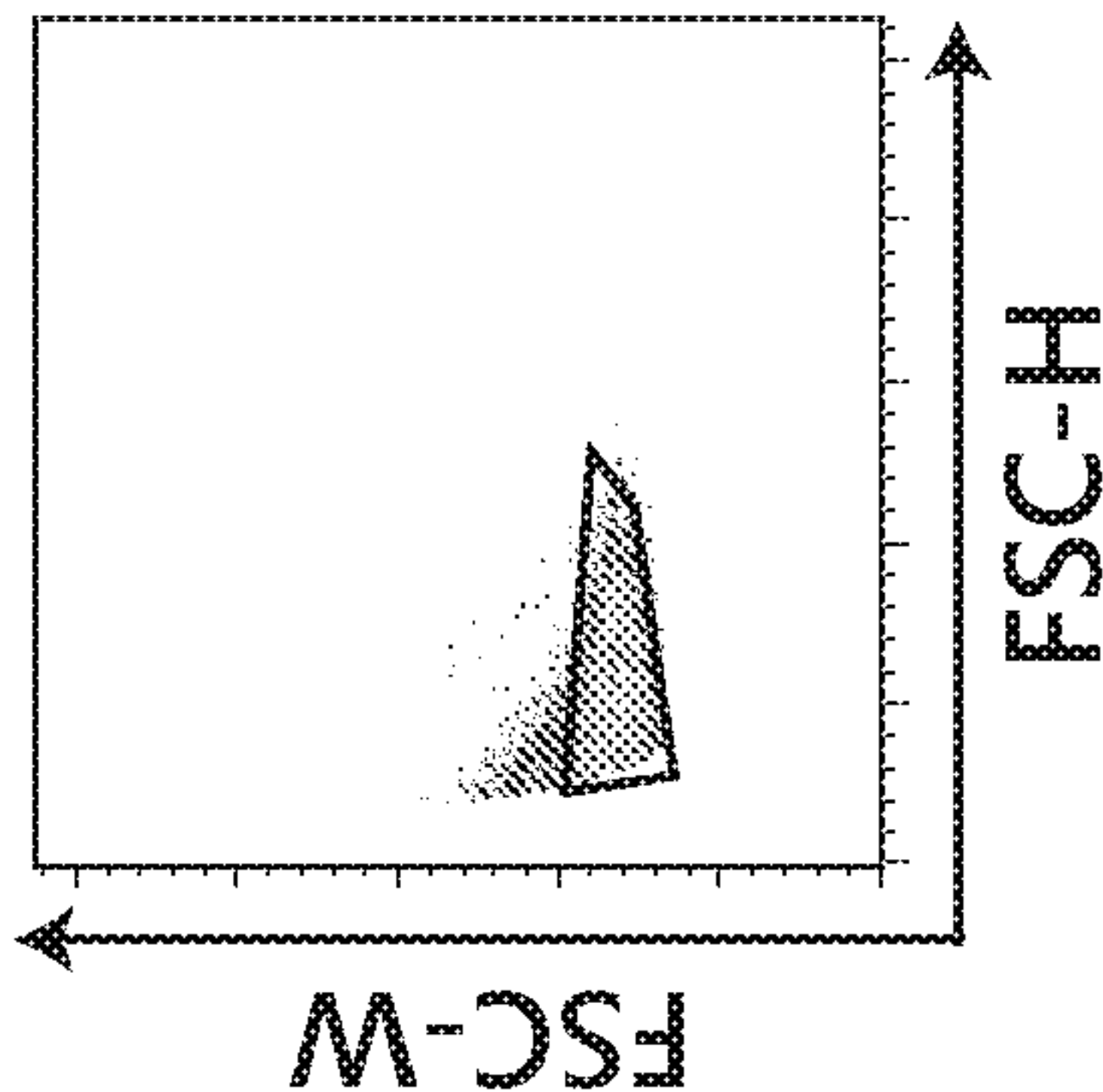
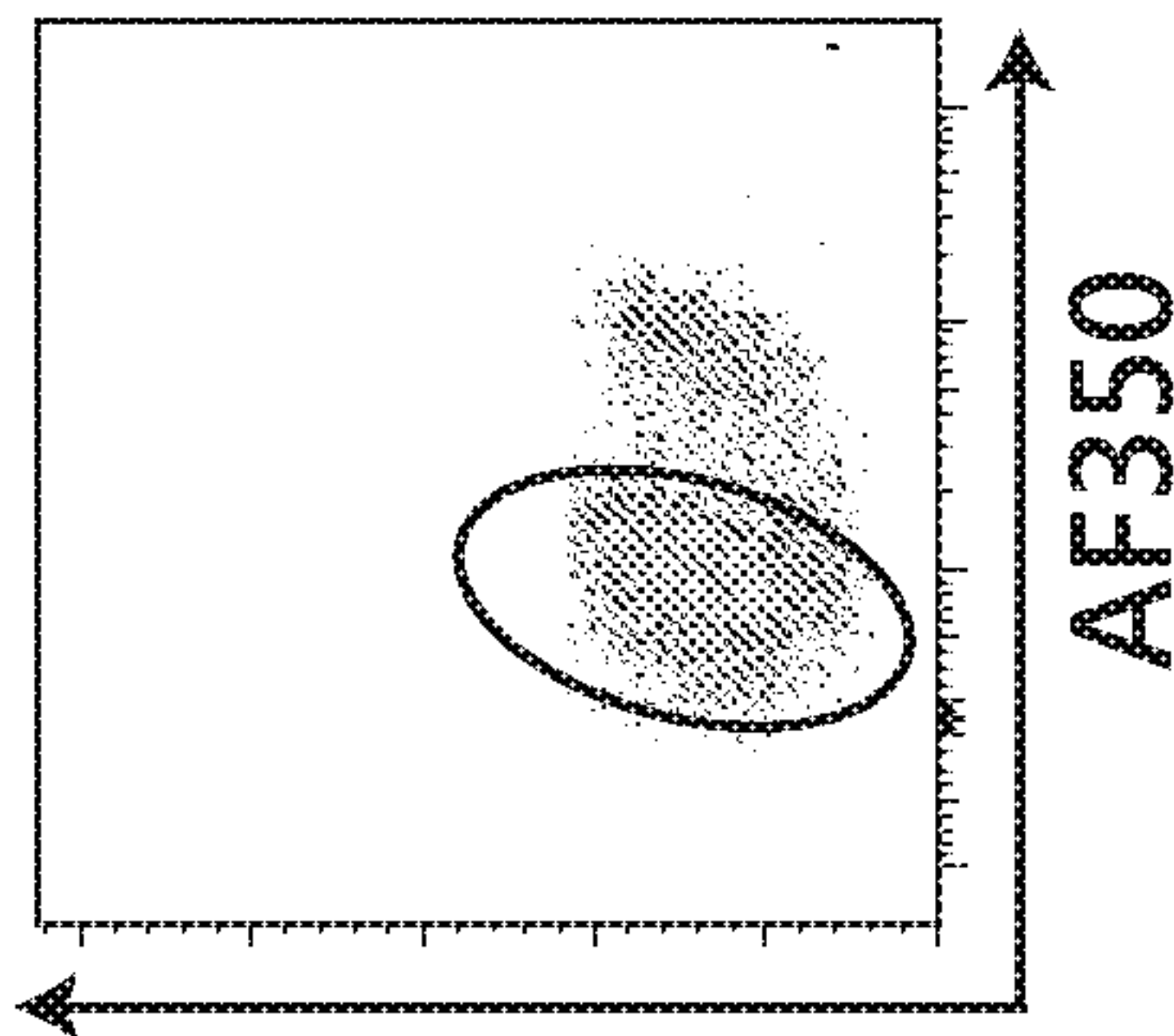
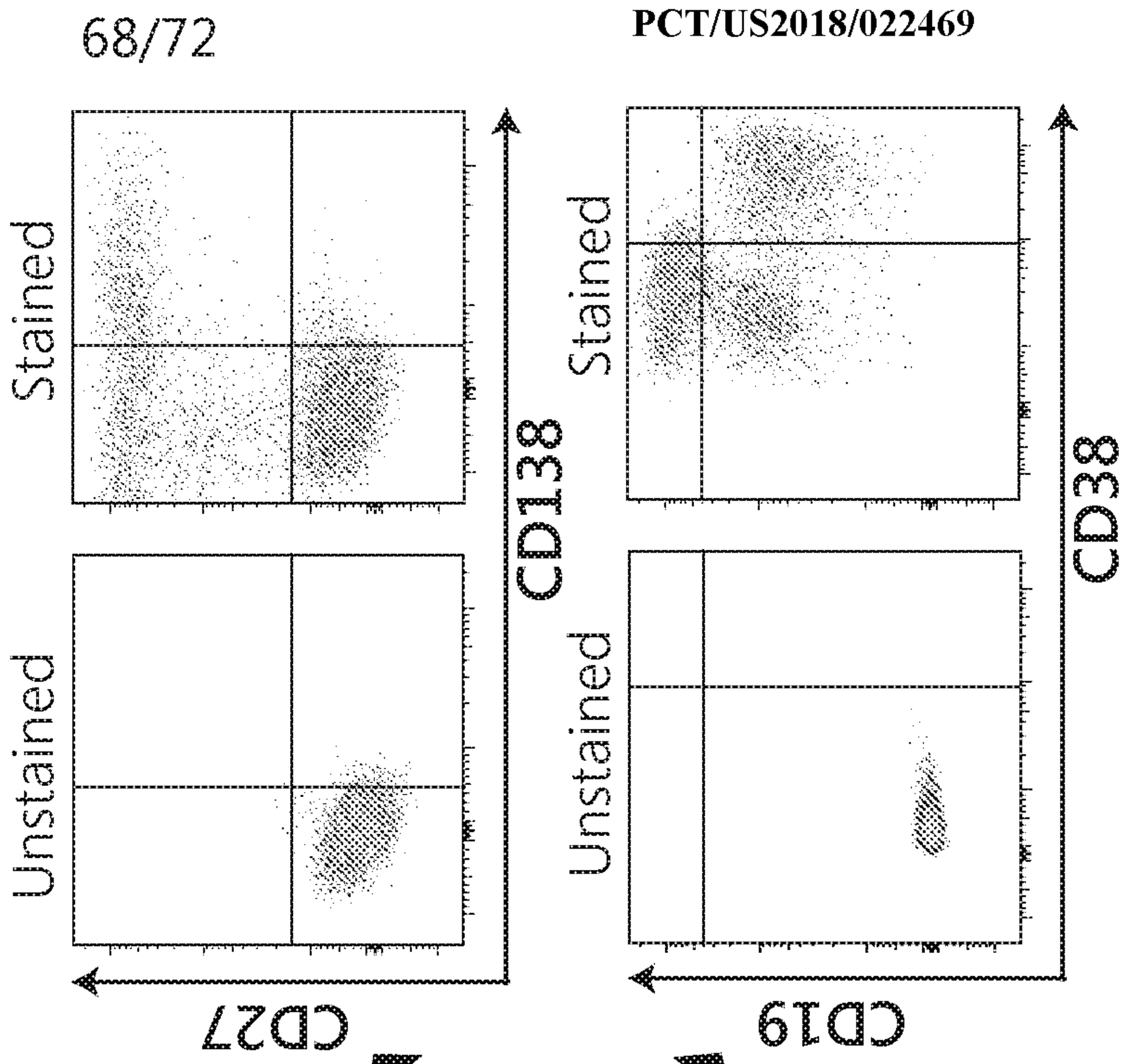
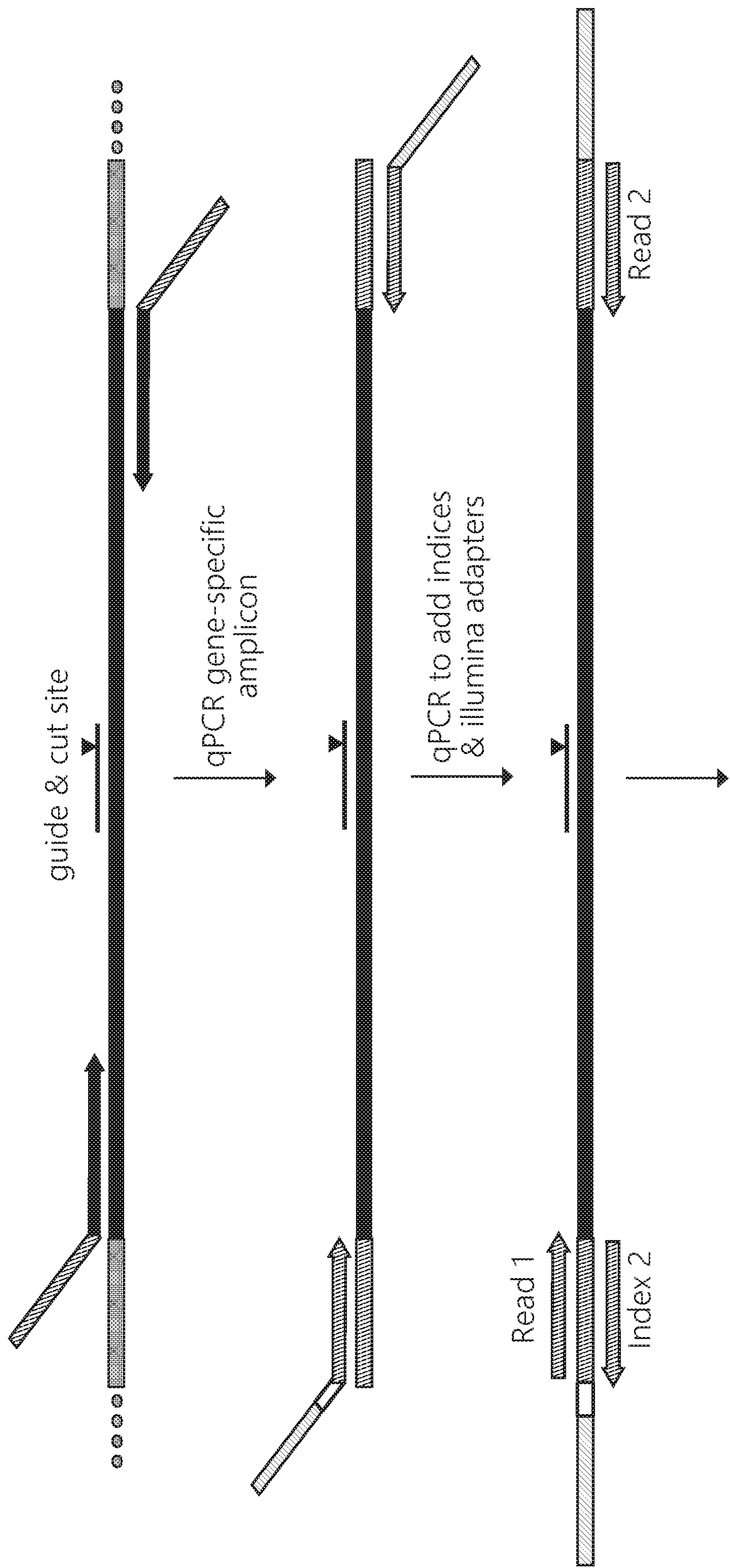


FIG. 52C



1. Pool samples: RNP gene disruption (CCR5g, PRDM1g-1, PRDM1g-2, IRF4g, PAX5g, BACH2g) and HDR studies
2. Sequence using NexSeq Mid-kit (150bp)
3. Demultiplex, align sequences & assess indel/HDR rates (CRISPResso)

FIG. 53A

Indel size distribution
IRF4g

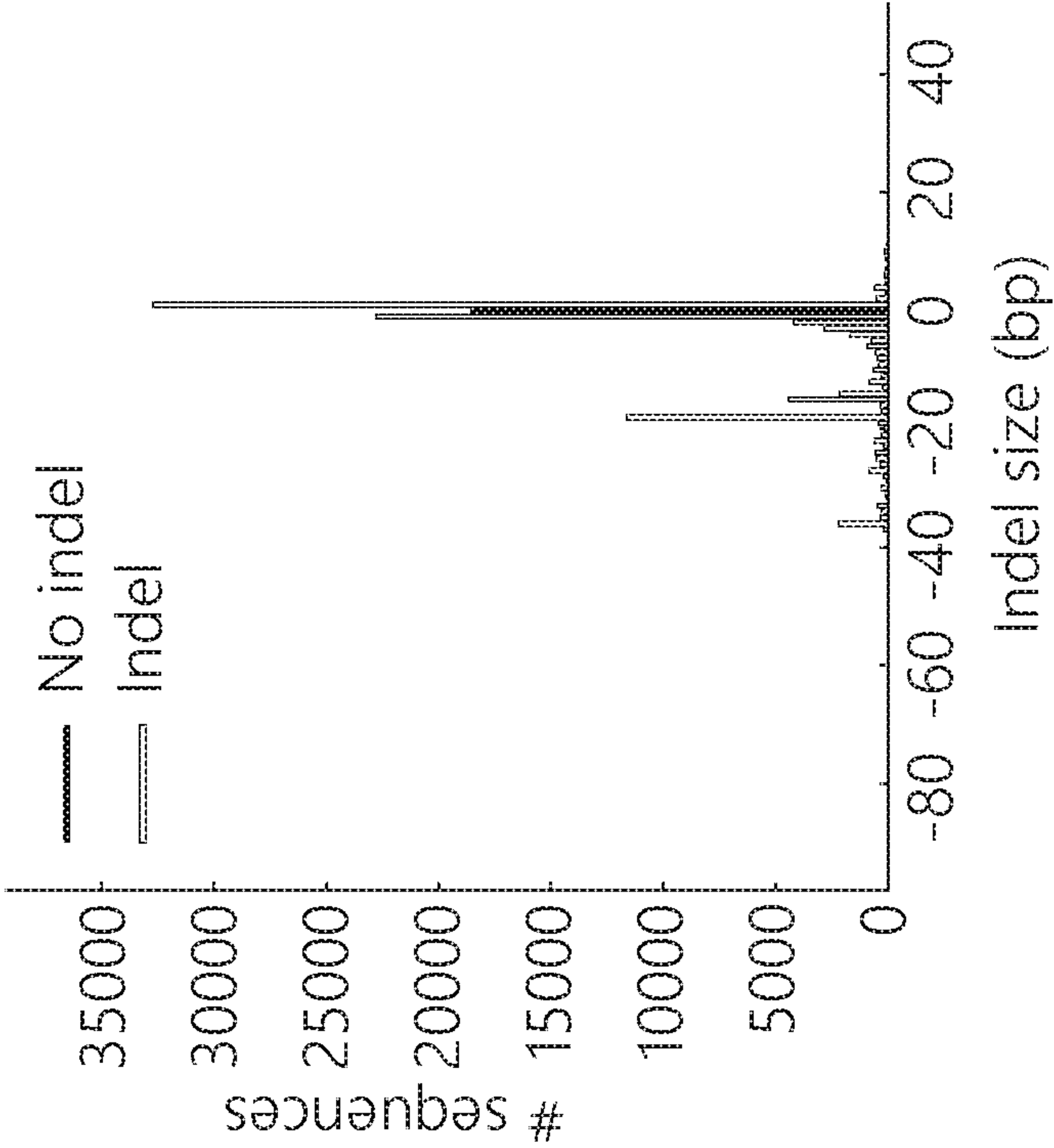


FIG. 53D

Mutation position
IRF4g

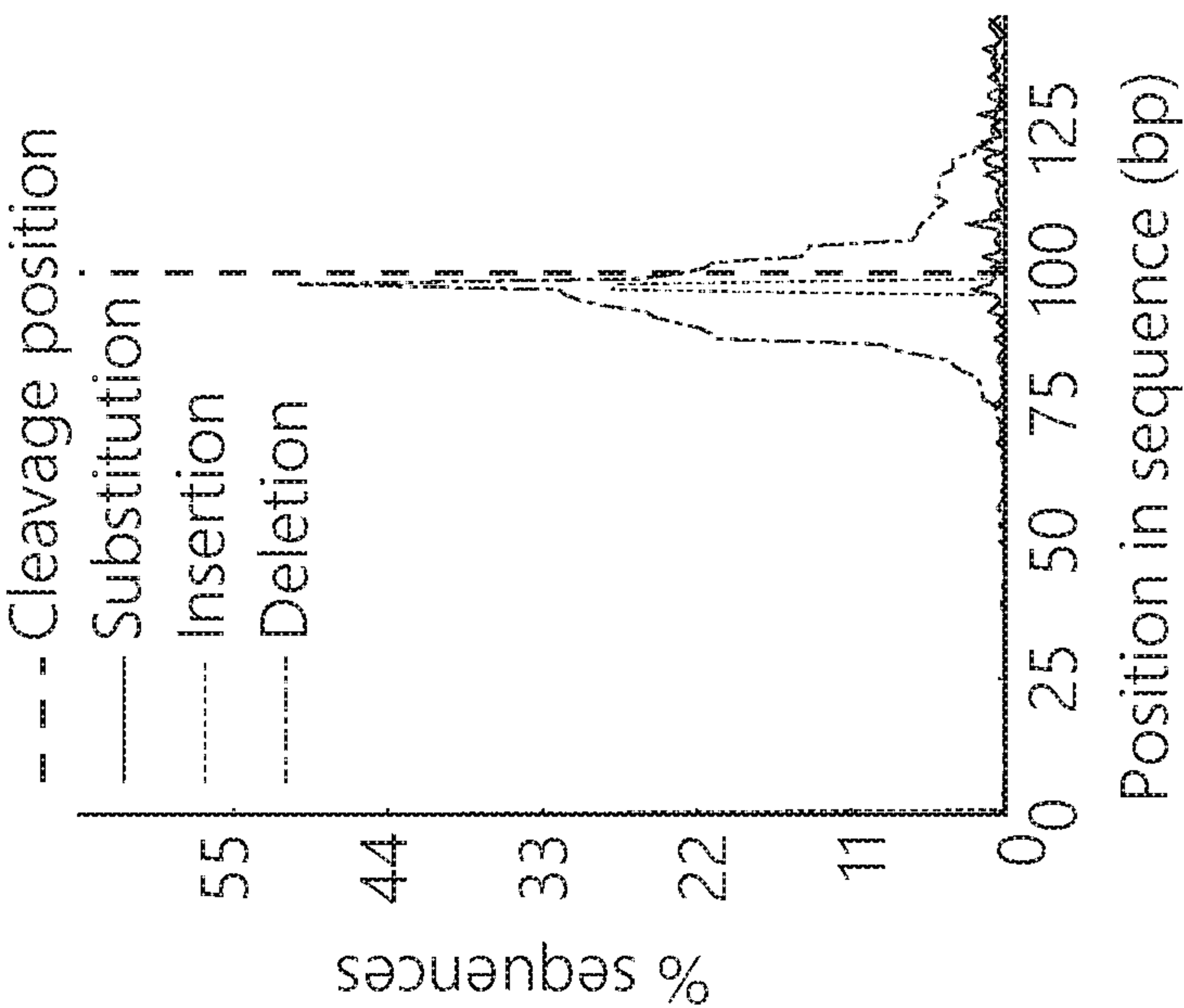


FIG. 53C

Indel frequency
IRF4g

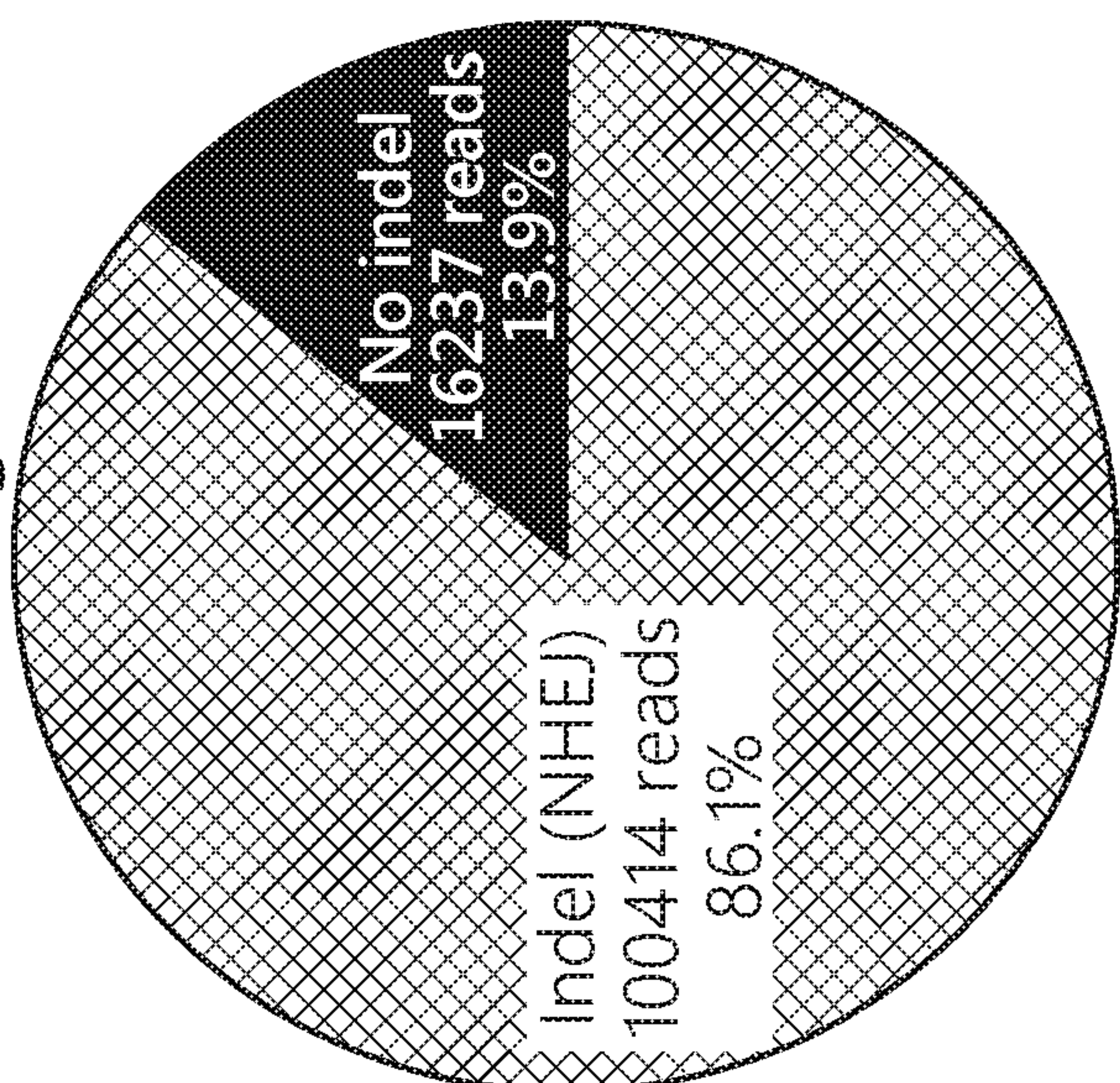


FIG. 53B

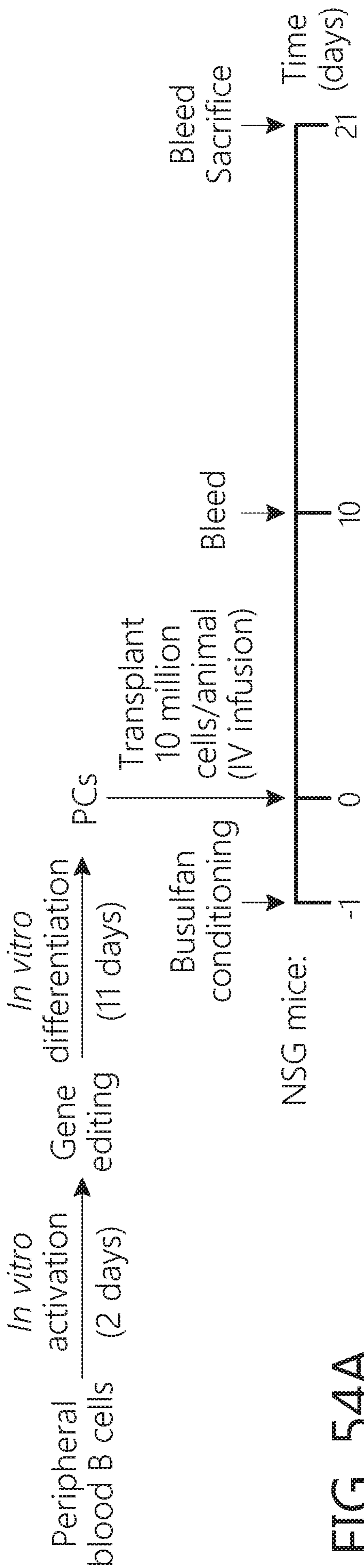


FIG. 54A

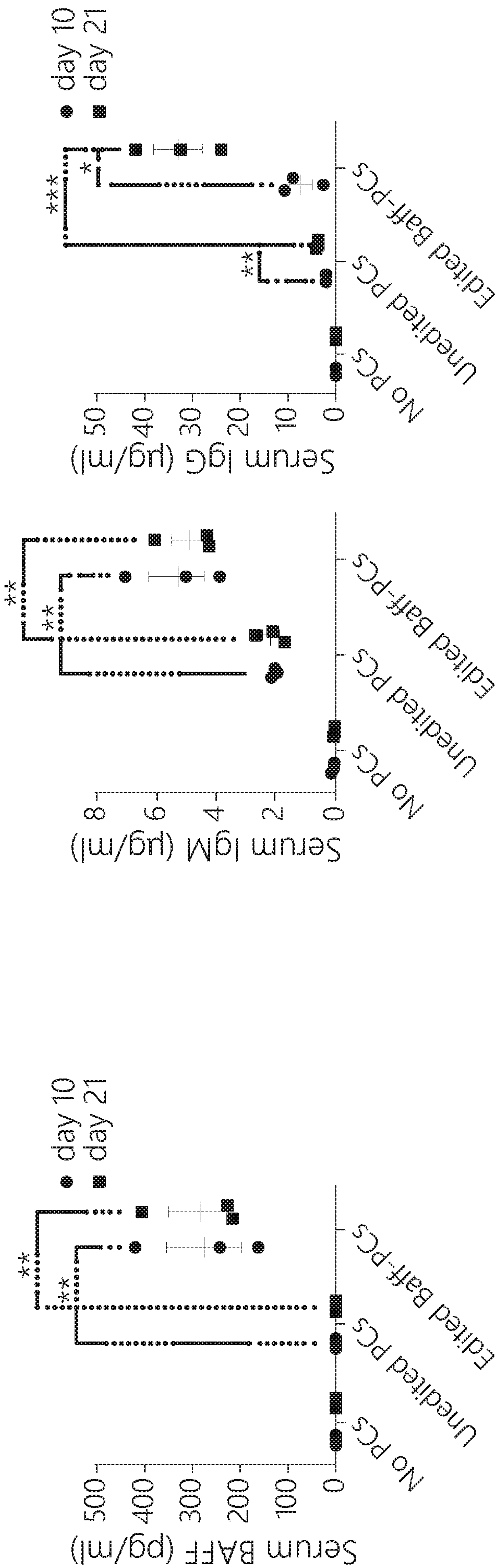


FIG. 54B

FIG. 54C

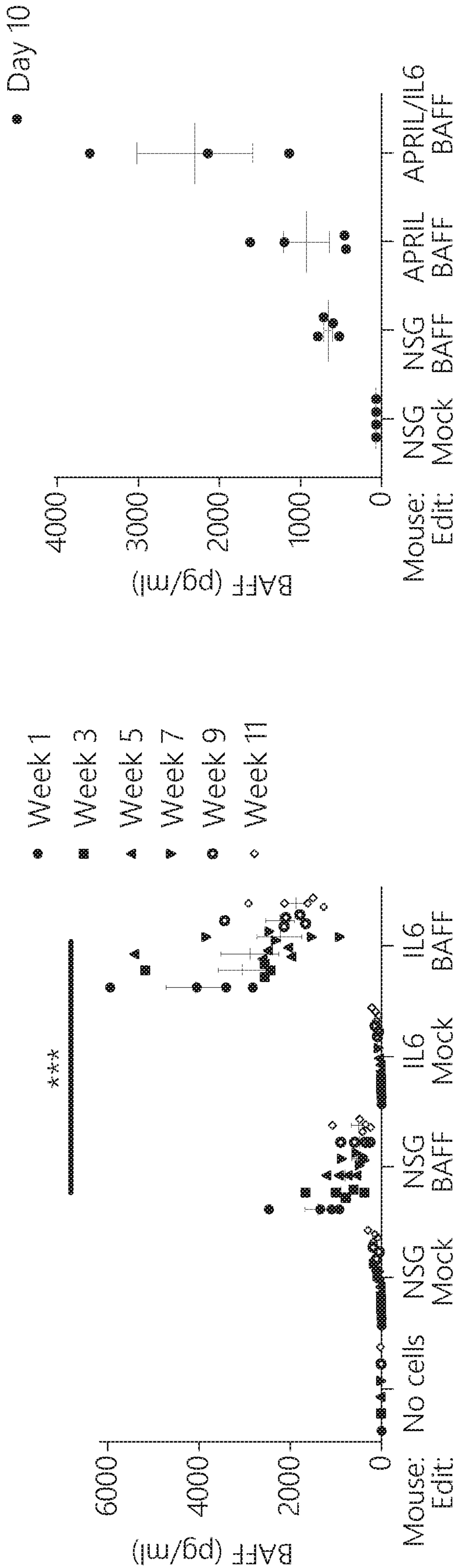


FIG. 55

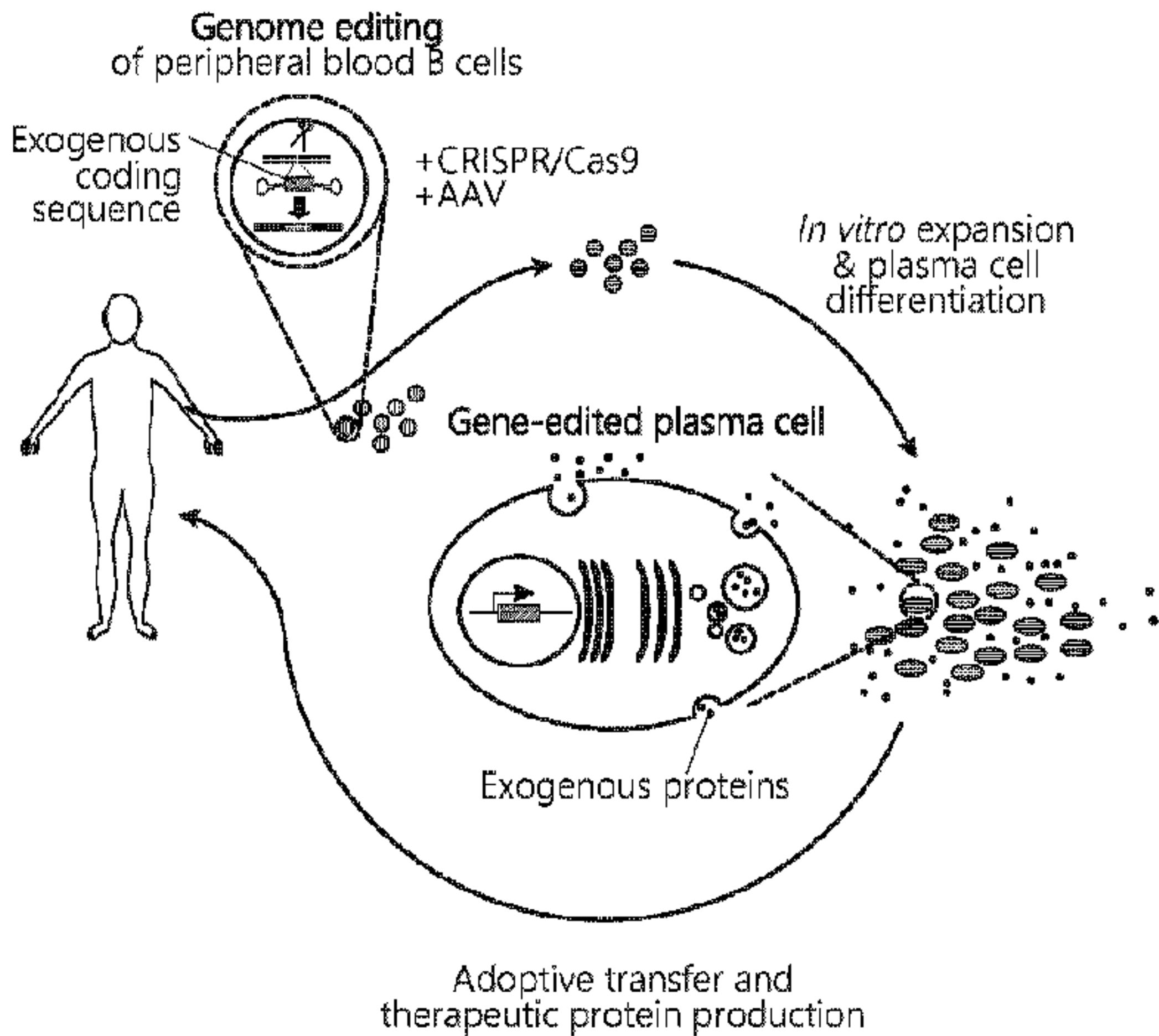


FIG. 1