

ENGINEERED CELL PREPARATIONS FOR TREATMENT OF HYPOPHOSPHATASIA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Nos. 63/595,741, filed November 2, 2023, 63/623,768, filed January 22, 2024, 63/643,222, filed May 6, 2024, 63/660,968, filed June 17, 2024, and 63/698,944, filed September 25, 2024. The entirety of each of the priority applications is incorporated herein by reference.

BACKGROUND

[0002] Cell-based therapeutics are an emerging class of medicine that makes use of innate cellular machinery to combat disease. Unlike traditional treatment methods, cell therapies make use of cellular localization, migration, and proliferation within the body, which can translate to improved biodistribution and targeted delivery of therapeutics. Cell-based therapies have potential applications for a broad range of diseases, including those that have proved intractable or difficult to manage with traditional treatment options.

SUMMARY OF THE INVENTION

[0003] The present disclosure encompasses, among other things, compositions and methods comprising modified immune cells (e.g., B lineage cells). In some embodiments, modified immune cells (e.g., B lineage cells) comprise nucleic acid constructs comprising one or more nucleic acid sequences encoding an alkaline phosphatase (ALP) or fusion protein thereof. Methods of producing and using such modified immune cells are also provided.

[0004] The present disclosure also encompasses, among other things, nucleic acid constructs comprising one or more nucleic acid sequences encoding an ALP or fusion protein thereof (e.g., a fusion protein comprising ALP and a human immunoglobulin G fragment crystallizable region (IgG Fc; "ALP-Fc fusion protein") described herein) and methods of producing the same. The present disclosure provides, *inter alia*, engineered B lineage cell populations expressing an ALP or fusion protein thereof (e.g., an ALP-Fc fusion protein).

[0005] Among other things, in one aspect, the present disclosure provides methods of preparing an engineered B lineage cell population that express an alkaline phosphatase or fusion protein thereof. In some embodiments, methods comprise the steps of: (a) isolating primary B cells so that a primary B cell population is obtained, (b) activating the primary B cell population, and (c) during or after the activating step, engineering the primary B cell population by integrating a transgene encoding an alkaline phosphatase or fusion protein thereof, thereby generating an engineered B lineage cell population. In some embodiments, a fusion protein comprises an alkaline phosphatase and immunoglobulin fragment crystallizable region (ALP-Fc) fusion protein. In some embodiments, an ALP-Fc fusion protein comprises a Fc derived from human immunoglobulin G1 (IgG1) or human immunoglobulin G4 (IgG4). In some embodiments, an ALP-Fc fusion protein further comprises either an octa- or deca-aspartate tag.

[0006] In some embodiments, an ALP-Fc fusion protein further comprises one or more substitutions within an Fc region. In some embodiments, one or more substitutions within an Fc region comprise K392D, D399K, and K409D of IgG1 (numbered according to full-length IgG1 as shown, e.g., in Gunasekaran et al., J. Biol. Chem. 2010). In some other embodiments, one or more substitutions within an Fc region comprise K392D, D399K, and R409D of IgG4 (numbered according to full-length IgG4). In some embodiments, one or more substitutions within an Fc region comprise S228P, F234A, and/or L235A. In some embodiments, an ALP-Fc fusion protein further comprises a linker. In some embodiments, a linker comprises a tetra-glycine and mono-serine linker (GGGGS). In some embodiments, a linker comprises three GGGGS linkers continuously. In some embodiments, a linker comprises two GGGGS linkers continuously followed by a mono-glycine. In some embodiments, a linker comprises a leucine and a lysine. In some embodiments, an ALP-Fc fusion protein further comprises one or more substitutions within the ALP. In some embodiments, a transgene is integrated at a site selected from a CCR5 site, a IgH site, CD19, B2M, and a JCHAIN site. In some embodiments, an ALP or ALP-Fc fusion protein comprises or has an amino acid sequence of any one of SEQ ID NOs: 62-75, 91-94, 101, and 109-117. In some embodiments, an ALP or ALP-Fc fusion protein is encoded by a nucleic acid sequence comprising any one of SEQ ID NOs: 55-61, 87-90, 100, and 102-108. In some embodiments, methods further comprise a step of expanding engineered B lineage cell population described herein.

[0007] Among other things, in one aspect, the present disclosure provides methods of differentiating an engineered B lineage cell population into a population of plasmablasts. In some embodiments, a differentiation step comprises contacting an engineered B lineage cell population with culture media comprising: (a) IL-2, (b) IL-6, (c) IL-10, and/or (d) IL-15.

[0008] Among other things, in one aspect, the present disclosure provides methods of differentiating an engineered B lineage cell population into a population of plasma cells. In some embodiments, a differentiation step comprises contacting a population of plasmablasts with culture media comprising: (a) IL-6, (b) IL-15, and/or (c) IFN- α -2-beta (IFN α -2 β).

[0009] In some embodiments, the present disclosure provides methods of engineering B lineage cell populations. In some embodiments, a method further comprises a step of transferring an engineered B lineage cell population to cell culture media without human or bovine serum for about 24 hours. In some embodiments, a step of engineering further comprises introducing a expression cassette comprising a transgene into a primary B cell population.

[0010] In some embodiments, an expression cassette comprises: (a) a 5' homology arm that is at least 95% identical to a sequence 5' to a double-stranded break site, and (b) a 3' homology arm that is at least 95% identical to a sequence 3' to a double-stranded break site. In some embodiments, a 5' homology arm comprises or is: (i) a length about 375-850 base pairs in size, (ii) a PAM site or absence of a PAM site, and/or (iii) symmetrical or asymmetrical in length with a 3' homology arm. In some embodiments, a 3' homology arm comprises or is: (i) a length about 375-850 base pairs in size, (ii) a PAM site or absence of a PAM site, and/or (iii) symmetrical or asymmetrical in length with a 5' homology arm. In some embodiments, a 5' homology arm has at least 95% identity with one or more of SEQ ID NOs: 39-43, 50, 52, 54, 118, 121, and 123. In some embodiments, a 3' homology arm has at least 95% identity with one or more of SEQ ID NOs: 44-49, 51, 53, 119, 122, and 124.

[0011] In some embodiments, an expression cassette comprises a sequence with at least 95% identity with SEQ ID NO: 77. In some embodiments, an expression cassette further comprises one or more of: (a) a promoter sequence selected from SEQ ID NOs: 76, and 125-128, (b) a nucleic acid sequence encoding for alkaline phosphatase or a fusion protein thereof selected from SEQ ID NOs: 55-61, 87-90, 100, and 102-108, (c) an enhancer sequence of SEQ ID NOs:

95-99, and (d) a polyA sequence of SEQ ID NO: 78. In some embodiments, an expression cassette is or comprises an adeno-associated viral (AAV) vector.

[0012] In some embodiments, a step of engineering comprises contacting a primary B cell population with a targeted nuclease capable of introducing double-stranded breaks. In some embodiments, the targeted nuclease is or comprises a CRISPR-associated (Cas) protein, zinc finger nuclease (ZFN), transcription activator-like effector-based nuclease (TALEN), or meganuclease. In some embodiments, a Cas protein is or comprises Cas9, Cas12a, or Cas13a, or a variant thereof. In some embodiments, a Cas protein is complexed with a guide RNA (gRNA). In some embodiments, a gRNA is a single guide RNA (sgRNA). In some embodiments, a gRNA is or comprises a sequence with at least 95% sequence identity to any one of SEQ ID NOs: 79-86, and 129-132.

[0013] In some embodiments, a step of engineering further comprises electroporation of the primary B cell population; and/or transduction of a primary B cell population with an expression cassette. In some embodiments, electroporation is performed on a composition comprising: (a) a Cas protein complexed with a gRNA, and (b) a primary B cell population.

[0014] In another aspect, the present disclosure provides sites and transgenes for engineering of B lineage cell populations. In some embodiments, a JCHAIN site has at least 95% identity with one or more SEQ ID NOs: 80-82, and 129-130. In some embodiments, an IgH site has at least 95% identity with SEQ ID NO: 83. In some embodiments, an CD19 site has at least 95% identity with SEQ ID NO: 84-86. In some embodiments, a transgene has at least 95% identity with SEQ ID NO: 77.

[0015] In another aspect, the present disclosure provides populations of genetically modified B lineage cells. In some embodiments, a transgene is expressed from an endogenous CCR5, IgH, CD19, B2M, or JCHAIN locus. In some embodiments, cells express an ALP-Fc fusion protein and at least partially disrupt expression of the endogenous CCR5, IgH, CD19, B2M, or JCHAIN locus. In some embodiments, a population of genetically modified B lineage cells is or comprises a population of genetically modified plasma cells. In some embodiments, a population of genetically modified B lineage cells is or comprises a population of plasmablasts. In some embodiments, a population of genetically modified B lineage cells is or comprises a population of plasma cell precursors.

[0016] In another aspect, the present disclosure provides a pharmaceutical composition comprising one or more B lineage cells selected from a population of genetically modified B lineage cells. In some embodiments, a pharmaceutical composition comprises one or more B lineage cells selected from a population of genetically modified B lineage cells and further comprises one or more pharmaceutically acceptable excipients. In some embodiments, a method of administering a pharmaceutical composition, wherein the pharmaceutical composition comprises: (a) one or more B lineage cells selected from the population of genetically modified B lineage cells of any aspect or embodiment described herein, and (b) one or more pharmaceutically acceptable excipients, wherein the pharmaceutical composition is administered to a subject.

[0017] In another aspect, the present disclosure provides methods of treating a disease, disorder, or condition in a subject, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition, thereby treating the disease, disorder, or condition in the subject. In some embodiments, a pharmaceutical composition is administered intravenously or intraperitoneally. In some embodiments, a pharmaceutical composition is administered to an adult subject. In some embodiments, a pharmaceutical composition is administered to a pediatric subject. In some embodiments, a subject has hypophosphatasia (HPP).

[0018] In another aspect, the present disclosure provides methods of characterizing a population of genetically modified B lineage cells, the method comprising assessing one or more of (a)-(g) using an assay: (a) presence of a CD38 marker, (b) presence of a CD138 marker, (c) presence of at least 50% of a CD27 marker, (d) secretion of at least 0.5 pg/cell/day of IgG, (e) secretion of at least 2.5 pg/cell/day of IgM, (f) secretion of at least 0.5 pg/cell/day of IgD, and (g) secretion of at least 0.01 pg/cell/day of an alkaline phosphatase or fusion thereof. In some embodiments, an assay comprises or is one or more of: fluorescence-activated cell sorting (FACS), Western Blot, flow cytometry, enzyme-linked immunosorbent spot assay (ELISpot), and enzyme-linked immunosorbent assay (ELISA). In some embodiments, one or more B lineage cells selected from a population of genetically modified B lineage cells engraft within bone marrow of a subject. In some embodiments, a method of monitoring engraftment of genetically modified B lineage cells within a subject, comprising one or more of: bioluminescence, ELISpot,

flow cytometry, and enzyme-linked immunosorbent assay. In some embodiments, one or more B lineage cells selected from the population of genetically modified B lineage cells engraft within the bone marrow of the subject.

[0019] In some embodiments, a polynucleotide sequence for use in engineering a population of B lineage cells comprises one or more of: (i) a 5' homology arm that is or comprises SEQ ID NO: 39; (ii) a promoter that is or comprises SEQ ID NO: 76; (iii) a signal peptide encoded by a polynucleotide sequence that is or comprises SEQ ID NO: 143; (iv) a transgene that is or comprises SEQ ID NO: 139; (v) a terminator that is or comprises SEQ ID NO: 78; and/or (vi) a 3' homology arm that is or comprises SEQ ID NO: 44. In some embodiments, a polynucleotide sequence for use in engineering a population of B lineage cells encoding a polypeptide that comprises one or more of: (i) a signal peptide encoded that is or comprises SEQ ID NO: 144; (ii) a transgene that is or comprises SEQ ID NO: 147; and/or (iii) a linker encoded that is or comprises SEQ ID NO: 141. In some embodiments, a polynucleotide sequence is or comprises SEQ ID NO: 100. In some embodiments, a polynucleotide sequence is or comprises SEQ ID NO: 149. In some embodiments, a polynucleotide sequence encodes a polypeptide sequence that is or comprises SEQ ID NO: 101.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figures 1A-1B show assessment of alkaline phosphatase and immunoglobulin fusion proteins. Figure 1A shows Western blots of histidine-tagged alkaline phosphatase and immunoglobulin fusion protein (ALP-IgG1Fc-His) or guide alone control using either an anti-alkaline phosphatase antibody (left blot) or anti-IgG-CH1 antibody (right blot). Figure 1B shows Western blots of histidine purified unengineered control, alkaline phosphatase and immunoglobulin fusion protein (ALP-IgG1Fc-His), or alkaline phosphatase and immunoglobulin including K392D/D399K/K409D 3x substitutions (ALP-IgG1Fc-3xsubst-His) using either an anti-alkaline phosphatase antibody (top blot) or anti-IgG-CH1 antibody (bottom blot).

[0021] Figures 2A-2B show osteogenic stimulation protocols. Figure 2A shows a schematic of an osteogenic stimulation protocol involving osteoblast precursor (MC3T3) cells. MC3T3 cells are subjected or not subjected to pyrophosphate at days 6-21 and subsequently treated with either media alone control or supernatant from cultured engineered B lineage cells as

described herein. Figure 2B shows Alizarin Red S (ARS) staining images (left) and calcium levels (right) of osteoblast precursor cells (MC3T3) that underwent various treatment conditions including with or without pyrophosphate (PPi) and/or with 22 μ L of supernatant from cultured un-engineered B lineage cells as described herein.

[0022] Figure 3A-3B show assessment of ALP-Fc fusion proteins. Figure 3A shows a schematic of an osteogenic stimulation protocol involving osteoblast precursor (MC3T3) cells. These cells are subjected to pyrophosphate (PPi) and engineered B lineage cell derived alkaline phosphatase human immunoglobulin fusion protein with a deca-aspartate tag (ALP-Fc-D10) or alkaline phosphatase with a deca-aspartate tag (ALP-D10). Figure 3B shows ARS staining images (left) and a graph of calcium concentration (right) of osteogenic precursor cells treated with either 50, 250, or 500 ng/mL concentration of engineered B lineage cell derived ALP-D10 or ALP-Fc-D10.

[0023] Figure 4 shows a graph of mean calcium concentration of osteogenic precursor cells under osteogenic conditions treated with or without pyrophosphate and engineered B lineage cell derived ALP-D10, ALP-Fc-D10.

[0024] Figures 5A-5B shows assessment of engineered B lineage cell derived ALP-Fc. Figure 5A shows a graph of calcium concentration of osteogenic precursor cells under osteogenic conditions treated with or without pyrophosphate and different concentrations of engineered B lineage cell derived ALP-Fc. Figure 5B shows a graph of calcium concentration of osteogenic precursor cells under osteogenic conditions treated with or without pyrophosphate and different concentrations of recombinant human alkaline phosphatase ("rhALP").

[0025] Figure 6 shows a graph of a 4-Methylumbelliferyl phosphate (4-MUP) hydrolysis activity assay of recombinant ALP or engineered B lineage cell derived ALP fusion proteins (ALP-Fc, ALP-D10, or ALP-Fc-D10) as described herein.

[0026] Figure 7 shows a graph of enzymatic activity of ALP-IgG1Fc or ALP and IgG1 Fc with K392D, D399K, and K409D substitutions (ALP-IgG1-Fc-3xsubst).

[0027] Figure 8A-8B show assessment of engineered B lineage cells. Figure 8A shows a graph of plasma measurements of ALP or ALP-Fc over time in the NOG-IL-6 model treated with engineered B lineage cells as described herein. Figure 8B shows a graph of plasma human

immunoglobulin G (huIgG) over time in the NOG-IL-6 model treated with engineered B lineage cells as described herein.

[0028] Figures 9A-9D show assessment of ALP-Fc fusion proteins. Figure 9A shows a schematic of ALP Fc fusion proteins comprising a linker that comprises either a leucine and lysine linker (ALP-LK-IgG1Fc) or three continuous regions of tetra-glycine and mono-serine linker (ALP-GGGGS(x3)-IgG1Fc; also referred to herein as “long linker”). Figure 9B shows a graph of enzymatic activity of an ALP-Fc fusion protein comprising a linker that comprises leucine and lysine (LK) or three continuous regions of tetra-glycine and mono-serine (GGGGS(x3)). Figure 9C shows a graph of enzymatic activity of ALP-IgG1Fc or ALP and IgG1 Fc with K392D, D399K, and K409D substitutions (ALP-IgG1-Fc-3xsubst-His) comprising a linker comprising leucine and lysine (LK) or a linker comprising three continuous regions of tetra-glycine and mono-serine (GGGGS(x3)). Figure 9D shows a graph of enzymatic activity of ALP and IgG4 Fc with S288P, F234A, and L235A substitutions and deca-aspartate tag fusion protein (ALP-IgG4Fc-PAA-D10) or ALP, long linker (GGGGSx3) and IgG4 Fc S288P, F234A, L235A, K392D, D399K, and R409D substitutions and deca-aspartate tag fusion protein (ALP-LL-IgG4Fc-3xsubst-PAA-D10).

[0029] Figures 10A-10B show assessment of ALP-Fc fusion proteins. Figure 10A demonstrates secretion rate of ALP in B lineage cells engineered at either CCR5 or JCHAIN 31. Figure 10B demonstrates activity of ALP produced by B lineage cells engineered at either CCR5 or JCHAIN 31.

[0030] Figure 11 is a schematic demonstrating integration of a payload (e.g., expression cassette) at a target locus.

[0031] Figure 12 shows a schematic of: ALP-Fc fusion protein comprising human immunoglobulin G1 (hu-IgG1) Fc (left), soluble ALP (center), and ALP-Fc fusion protein comprising a human immunoglobulin G4 (hu-IgG4) and additional substitutions.

[0032] Figure 13 shows ALP-Fc fusion protein secretion levels from B lineage cells engineered to express ALP-Fc fusion proteins or variants thereof, such as codon-optimized variants (CO-1: GeneArt codon optimized variant, CO-2: alternative codon-optimized variant).

[0033] Figure 14 shows ALP-Fc fusion protein secretion levels from B lineage cells engineered to express ALP-Fc fusion proteins from a payload (c.g., expression cassette) comprising one or more signal peptides (SP-1: ALP signal peptide, SP-2: A1AT signal peptide).

[0034] Figure 15 shows ALP-Fc fusion protein secretion levels from B lineage cells engineered to express ALP-Fc fusion proteins at either the CCR5 locus or JCHAIN1 locus ("Locus B").

[0035] Figures 16A-16C shows assessment of ALP-Fc fusion proteins. Figure 16A shows a schematic of ALP-Fc fusion proteins with linkers of various lengths between ALP and IgG-Fc components. Figure 16B shows a crystal structure of ALP dimer, PDB#1HQ4. Figure 16C shows projected crystal structures for the following in order from left to right: ALP-Fc fusion protein, ALP-Short Linker ("SL")-Fc fusion protein, ALP-Long Linker ("LL")-Fc fusion protein, and overlay between ALP-SL-Fc and ALP-LL-Fc fusion proteins.

[0036] Figure 17 shows ALP-Fc fusion protein secretion levels from B lineage cells engineered to express ALP-Fc fusion proteins at either the CCR5 locus, JCHAIN173 locus ("Locus A"), or JCHAIN1 locus ("Locus B") which were compared to control electroporated plasma cells without vector ("EPC").

[0037] Figure 18 shows 4-Methylumbelliferyl phosphate (4-MUP) hydrolysis activity assay of B lineage cells engineered to express ALP fusion proteins at either the CCR5 locus, JCHAIN173 locus ("Locus A"), or JCHAIN1 locus ("Locus B") which were compared to control electroporated plasma cells without vector ("EPC"). A schematic demonstrating the 4-MUP assay method is shown in the top panel.

[0038] Figure 19 shows 4-Methylumbelliferyl phosphate (4-MUP) specific hydrolysis activity of B lineage cells engineered to express ALP fusion proteins at either the CCR5 locus, JCHAIN173 locus ("Locus A"), or JCHAIN1 locus ("Locus B") which were compared to a control electroporated plasma cells without vector ("EPC").

[0039] Figure 20 shows ALP-Fc fusion protein secretion levels from B lineage cells engineered to express ALP-Fc fusion proteins from the JCHAIN31 locus using different promoters.

[0040] Figure 21 shows ALP-Fc transgene integration from two different B lineage cell donor populations (LKP23185 and LKP23187) that were engineered with constructs described herein comprising either an MND, Fu_1, Fu_2, Fu_3, B29 promoter with or without an Eμ enhancer.

[0041] Figure 22 shows ALP-Fc protein secretion levels from two different donor populations of B lineage cells (LKP23185 and LKP23187) engineered with constructs described herein comprising either an MND, Fu_1, Fu_2, Fu_3, B29 promoter with or without an Eμ enhancer.

[0042] Figure 23 shows 4-Methylumbelliferyl phosphate (4-MUP) hydrolysis activity of two different donor populations of B lineage cells (LKP23185 and LKP23187) engineered with constructs described herein comprising either an MND, Fu_1, Fu_2, Fu_3, B29 promoter with or without an Eμ enhancer.

[0043] Figures 24A-24C show assessment of engineered B lineage cells. Figure 24A shows percentage of homology-directed repair (% HDR) for B lineage cells engineered with constructs described herein comprising an MND promoter with or without an Eμ enhancer. Figure 24B shows ALP-Fc protein secretion levels from B lineage cells engineered with constructs described herein comprising an MND promoter with or without an Eμ enhancer. Figure 24C shows 4-Methylumbelliferyl phosphate (4-MUP) hydrolysis activity of B lineage cells engineered with constructs described herein comprising an MND promoter with or without an Eμ enhancer.

[0044] Figures 25A-D depict assessment of B lineage cells engineered to express ALP-Fc (ALP-BCM). ALP-BCMs or control cells electroporated without vector were administered to immunodeficient mice. Figure 25A shows levels of secreted ALP-Fc isolated from mice to which ALP-BCMs were administered. Figure 25B shows assessment of ALP-Fc activity in serum isolated from mice to which ALP-BCMs were administered. Figure 25C shows assessment of human IgG secretion in mice to which ALP-BCMs were administered. Figure 25D shows weight assessment of mice to which ALP-BCMs were administered.

[0045] Figure 26 shows Western blots of histidine purified non-engineered control (Uneng), alkaline phosphatase and immunoglobulin fusion protein (ALP-IgG4Fc-His), or

alkaline phosphatase and immunoglobulin including K392D/D399K/K409D 3 substitutions (ALP-IgG4Fc-3xsubst-His) using an anti-ALP antibody.

[0046] Figure 27 shows Alizarin Red S (ARS) staining images (left) and calcium levels (right) of osteoblast precursor cells (MC3T3) cultured under osteogenic conditions for 21 days as indicated with media alone, PPI, 50 ng/mL of supernatant from cultured B lineage cells engineered to express ALP-Fc (ALP-BCM), or 50 ng/mL of supernatant from cultured control B lineage cells electroporated without vector (EPC).

[0047] Figures 28A-B depict assessment of B lineage cells engineered to express ALP-Fc (ALP-BCM). ALP-BCMs or control cells electroporated without vector were administered to immunodeficient mice. Figure 28A shows levels of secreted ALP-Fc isolated from mice to which ALP-BCMs were administered. Figure 28B shows assessment of hALP activity in serum isolated from mice to which ALP-BCMs were administered.

[0048] Figures 29A-B depict assessment of B lineage cells engineered to express ALP-Fc (ALP-BCM). ALP-BCMs or control cells electroporated without vector were administered to immunodeficient mice. Figure 29A shows levels of secreted ALP-Fc isolated from mice to which ALP-BCMs were administered. Figure 29B shows assessment of hALP activity in serum isolated from mice to which ALP-BCMs were administered.

[0049] Figure 30 depicts assessment of B lineage cells engineered to express ALP-Fc (ALP-BCM) after cryopreservation and expansion. ALP-BCMs or control cells electroporated without vector were administered to immunodeficient mice. Figure 30 shows levels of secreted ALP-Fc isolated from mice to which ALP-BCMs were administered.

[0050] Figures 31A-B depict assessment of B lineage cells engineered to express ALP-Fc (ALP-BCM) after cryopreservation and expansion. ALP-BCMs or control cells electroporated without vector were administered to immunodeficient mice. Figure 31A shows levels of secreted ALP-Fc isolated from mice to which ALP-BCMs were administered. Figure 31B shows assessment of hALP activity in serum isolated from mice to which ALP-BCMs were administered.

[0051] Figures 32A-B depict assessment of B lineage cells engineered to express ALP-Fc (ALP-BCM) through use of different expression cassettes. ALP-BCMs or control cells

electroporated without vector were administered to immunodeficient mice. Figure 32A shows levels of secreted ALP-Fc isolated from mice to which ALP-BCMs were administered. Figure 32B shows assessment of hALP activity in serum isolated from mice to which ALP-BCMs were administered.

[0052] Figures 33A-C depict assessment of B lineage cells engineered to express ALP-Fc (ALP-BCM) through use of different expression cassettes (p00215 and p00983) and viral transduction protocols (protocols 1 and 2). Figure 33A depicts percent targeted integration of ALP constructs into B-lineage cells. Figure 33B shows levels of ALP in supernatant of B lineage cells engineered to express ALP. Figure 33C depicts ALP activity of B-lineage cells engineered to express ALP.

[0053] Figures 34A-D depict assessment of B lineage cells engineered to express ALP constructs that do not comprise an Fc region. Figure 34A depicts percent targeted integration of ALP constructs into B-lineage cells. Figure 34B shows levels of ALP in supernatant of B lineage cells engineered to express ALP. Figure 34C depicts ALP activity of B-lineage cells engineered to express ALP. Figure 34D shows levels of secreted ALP isolated from mice to which ALP-BCMs were administered.

[0054] Figures 35A-C depict assessment of ALP constructs driven by Eu enhancers or variants thereof. Figure 35A depicts percent targeted integration of ALP constructs into B-lineage cells. Figure 35B shows levels of ALP in supernatant of B-lineage cells engineered to express ALP. Figure 35C depicts ALP activity of B-lineage cells engineered to express ALP.

[0055] Figures 36 depicts assessment of B lineage cells engineered to express ALP (ALP-BCM). ALP-BCMs or control cells electroporated without vector were administered to immunodeficient mice. Figure 36 shows levels of secreted hALP-Fc isolated from mice to which ALP-BCMs were administered.

[0056] Figures 37A-C depict assessment of ALP or ALP-Fc constructs driven by Eu enhancers or variants thereof. Figure 37A depicts percent targeted integration of ALP constructs into B-lineage cells. Figure 37B shows levels of ALP in supernatant of B-lineage cells engineered to express ALP. Figure 37C depicts ALP activity of B-lineage cells engineered to express ALP.

[0057] Figures 38A-C depict assessment of poly-aspartate-tagged ALP or untagged ALP in cells. Figure 38A depicts percent targeted integration of ALP constructs into B-lineage cells. Figure 38B depicts ALP activity of B-lineage cells engineered to express ALP constructs. Figure 38C shows Western blots of poly-aspartate-tagged alkaline phosphatase proteins (ALP-D8, ALP-D10), untagged ALP, or control using an anti-alkaline phosphatase antibody.

[0058] Figures 39A-C depict size exclusion chromatography analysis of ALP-Fc proteins. Figure 39A depicts chromatogram of ALP-Fc protein produced from fresh cells. Figure 39B depicts chromatogram of ALP-Fc protein produced from freeze-thawed cells. Figure 39C depicts a comparison of chromatogram properties of ALP-Fc proteins.

[0059] Figure 40 depicts a chromatogram of LC-MS analysis of ALP-Fc protein.

[0060] Figure 41 shows assessment of purified ALP-Fc protein binding to FcγRIIIa.

[0061] Figure 42 shows assessment of purified ALP-Fc protein binding to FcγRI.

[0062] Figure 43 shows assessment of purified ALP-Fc protein binding to FcRn.

[0063] Figure 44 depicts a quantification of sialic acids detected on purified ALP-Fc proteins.

[0064] Figure 45 shows assessment of purified ALP-Fc protein binding to FcγRIIIa

[0065] Figure 46 shows assessment of purified ALP-Fc protein binding to FcγRI.

[0066] Figure 47 shows assessment of purified ALP-Fc protein binding to FcRn.

[0067] Figure 48 depicts a quantification of sialic acids detected on purified ALP-Fc proteins.

[0068] Figures 49A-B depict assessment of ALP-Fc proteins purified from HEK cells after administration to mice. Figure 49A shows levels of secreted ALP-Fc isolated from mice to which ALP-Fc proteins were administered. Figure 49B shows assessment of ALP-Fc activity in serum isolated from mice to which ALP-Fc proteins were administered.

[0069] Figures 50A-B depict assessment of ALP-Fc proteins purified from HEK cells or B lineage cells engineered to express ALP-Fc. Figure 50A shows levels of secreted ALP-Fc isolated from mice to which ALP-Fc proteins were administered. Figure 50B shows assessment of ALP-Fc activity in serum isolated from mice to which ALP-Fc proteins were administered.

DEFINITIONS

[0070] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification. The publications and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

[0071] The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0072] *Approximately or about:* As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1 %, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0073] *Activation:* As used herein, the term “activation” refers to the state of a cell, for example a B cell that has been sufficiently stimulated to induce detectable cellular proliferation or has been stimulated to exert its effector function. Activation can also be associated with induced cytokine production, cell signaling, differentiation, and/or antigen processing and presentation.

[0074] *Administration:* As used herein, the term “administration” typically refers to the administration (e.g., of a composition or treatment) to a subject or system (e.g., that is or comprises one or more cells, tissues, organisms, etc), for example to achieve delivery of an agent that is, is included in, or is otherwise delivered or generated by, such composition or treatment. Those of ordinary skill in the art will be aware of a variety of routes that may, in appropriate circumstances, be utilized for administration to a subject, for example a human. For example, in some embodiments, administration may be ocular, oral, parenteral, topical, etc.. In some particular embodiments, administration may be bronchial (e.g., by bronchial instillation), buccal, dermal (which may be or comprise, for example, one or more of topical to the dermis,

intradermal, interdermal, transdermal, etc), enteral, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ (e. g. intrahepatic), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (e.g., by intratracheal instillation), vaginal, vitreal, etc. In some embodiments, administration may involve only a single dose. In some embodiments, administration may involve application of a fixed number of doses. In some embodiments, administration may involve dosing that is intermittent (e.g., a plurality of doses separated in time) and/or periodic (e.g., individual doses separated by a common period of time) dosing. In some embodiments, administration may involve continuous dosing (e.g., perfusion) for at least a selected period of time.

[0075] *Agent:* As used herein, the term “agent” (or “biological agent” or “therapeutic agent”), refers to a molecule that may be expressed, released, secreted or delivered to a target by a modified cell described herein. An agent includes, but is not limited to, a nucleic acid, an antibiotic, an anti-inflammatory agent, an antibody or fragments thereof, an antibody agent or fragments thereof, a growth factor, a cytokine, an enzyme, a protein (e.g., an RNase inhibitor), a peptide, a fusion protein, a synthetic molecule, an organic molecule (e.g., a small molecule), a carbohydrate, a lipid, a hormone, a microsome, a derivative or a variation thereof, and any combinations thereof. An agent may bind any cell moiety, such as a receptor, an antigenic determinant, or other binding site present on a target or target cell. An agent may diffuse or be transported into a cell, where it may act intracellularly.

[0076] *Alloantigen:* The term “alloantigen”, as used herein, refers to an antigen associated with allorecognition and/or graft rejection (e.g., an antigen against which a rejection immune response is directed). In general, alloantigens are agents that are present in or on tissue from one individual (e.g., a donor individual) of a particular species, but not in or on tissue from another individual (e.g., a recipient individual, for example who is genetically different from the donor individual) of the species, so that transfer of tissue from the donor individual to the recipient individual risks and/or results in a rejection immune response. In general, an antigen may be or include any chemical entity such as, for example, a small molecule, a nucleic acid, a polypeptide, a carbohydrate, a lipid, etc. In some embodiments, an alloantigen is or comprises a

polypeptide. A variety of polypeptides are known in the art whose amino acid sequences can vary between and among individuals of the same species such that they might act as alloantigens.

[0077] *Allogeneic*: As used herein, the term “allogeneic” refers to any material (e.g., a population of cells) derived from a different animal of the same species.

[0078] *Allorecognition*: The term “allorecognition”, as used herein, typically refers to an immune response mounted by the immune system of an individual (i.e., a recipient) who receives a tissue graft from another individual (i.e., a donor, who for example is genetically distinct from the recipient individual) of the same species, which immune response involves recognition of an alloantigen on the grafted tissue. Typically, allorecognition involves T cell recognition of the alloantigen. In many embodiments, T cells recognize an alloantigen peptide, for example, encoded by a polymorphic gene whose sequence differs between the donor and recipient individuals.

[0079] *Amelioration*: As used herein, refers to the prevention, reduction or palliation of a state, or improvement of the state of a subject. Amelioration includes, but does not require complete recovery or complete prevention of a disease, disorder or condition (e.g., radiation injury).

[0080] *Antigen*: As used herein, the term “antigen” or “Ag” refers to a molecule that is capable of provoking an immune response. This immune response may involve either antibody production, activation of specific immunologically-competent cells, or both. A skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA that comprises a nucleotide sequence or a partial nucleotide sequence encoding a protein that elicits an immune response encodes an “antigen” as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full-length nucleotide sequence of a gene. It is readily apparent that the present disclosure includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a “gene” at all. It is readily apparent that an antigen can be generated

synthesized or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid.

[0081] *Antibody agent:* As used herein, the term “antibody agent” (interchangeably referred to herein as “antibody”) refers to a polypeptide that may be expressed, released, secreted, or delivered to a target by a modified cell described herein. The polypeptide includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. In some embodiments, an antibody agent comprises an antibody. As is known in the art, antibodies as produced in nature are approximately 150 kD tetrameric agents comprising two identical heavy chain polypeptides (about 50 kD each) and two identical light chain polypeptides (about 25 kD each) that associate with each other into what is commonly referred to as a “Y-shaped” structure. Each heavy chain comprises at least four domains (each about 110 amino acids long) – an amino-terminal variable (VH) domain (located at the tips of the Y structure), followed by three constant domains: CH1, CH2, and the carboxy-terminal CH3 (located at the base of the Y’s stem). A short region, known as the “switch”, connects the heavy chain variable and constant regions. The “hinge” connects CH2 and CH3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain comprises two domains – an amino-terminal variable (VL) domain, followed by a carboxy-terminal constant (CL) domain, separated from one another by another “switch”. Intact antibody agent tetramers comprise two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and a tetramer is formed. Antibody agents are also glycosylated, typically on the CH2 domain. Each domain in a natural antibody has a structure characterized by an “immunoglobulin fold” formed from two beta sheets (e.g., 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as “complementarity determining regions” (CDR1, CDR2, and CDR3) and four somewhat invariant “framework” regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. The Fc region of

naturally-occurring antibodies binds to elements of the complement system, and also to receptors on effector cells, including, for example, effector cells that mediate cytotoxicity. Affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. In some embodiments, antibodies produced and/or utilized in accordance with the present disclosure (e.g., as a component of a CAR) include glycosylated Fc domains, including Fc domains with modified or engineered glycosylation. In some embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an “antibody agent”, whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody agent is polyclonal. In some embodiments, an antibody agent is monoclonal. In some embodiments, an antibody agent has constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, antibody agent sequence elements are humanized, primatized, chimeric, etc, as is known in the art. Moreover, the term “antibody agent”, as used herein, can refer in appropriate embodiments (unless otherwise stated or clear from context) to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. In some embodiments, an antibody agent may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody agent may contain a covalent modification (e.g., attachment of a glycan, a payload [e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc], or other pendant group [e.g., poly-ethylene glycol, etc].

[0082] *Autologous:* As used herein, the term “autologous” refers to any material derived from an individual to which it is later to be re-introduced into the same individual.

[0083] *Biologically active:* As used herein, refers to an observable biological effect or result achieved by an agent or entity of interest. For example, in some embodiments, a specific binding interaction is a biological activity. In some embodiments, modulation (e.g., induction, enhancement, or inhibition) of a biological pathway or event is a biological activity. In some embodiments, presence or extent of a biological activity is assessed through detection of a direct or indirect product produced by a biological pathway or event of interest.

[0084] *Biomarker:* The term “biomarker” is used herein, consistent with its use in the art, to refer to a to an entity, event, or characteristic whose presence, level, degree, type, and/or form, correlates with a particular biological event or state of interest, so that it is considered to be a “marker” of that event or state. To give but a few examples, in some embodiments, a biomarker may be or comprise a marker for a particular disease state, or for likelihood that a particular disease, disorder or condition may develop, occur, or reoccur. In some embodiments, a biomarker may be or comprise a marker for a particular disease or therapeutic outcome, or likelihood thereof. Thus, in some embodiments, a biomarker is predictive, in some embodiments, a biomarker is prognostic, in some embodiments, a biomarker is diagnostic, of the relevant biological event or state of interest. A biomarker may be or comprise an entity of any chemical class, and may be or comprise a combination of entities. For example, in some embodiments, a biomarker may be or comprise a nucleic acid, a polypeptide, a lipid, a carbohydrate, a small molecule, an inorganic agent (e.g., a metal or ion), or a combination thereof. In some embodiments, a biomarker is a cell surface marker. In some embodiments, a biomarker is intracellular. In some embodiments, a biomarker is detected outside of cells (e.g., is secreted or is otherwise generated or present outside of cells, e.g., in a body fluid such as blood, urine, tears, saliva, cerebrospinal fluid, etc. In some embodiments, a biomarker may be or comprise a genetic or epigenetic signature. In some embodiments, a biomarker may be or comprise a gene expression signature.

[0085] *Conservative sequence modifications:* As used herein, the term “conservative sequence modifications” refers to amino acid modifications that do not significantly affect or alter the binding characteristics of an antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody compatible with various embodiments by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which an amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine,

proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of an antibody can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for the ability to bind antigens using the functional assays described herein.

[0086] *Encoding:* As used herein, “encoding” refers to the inherent 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 polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[0087] *Engineered:* In general, the term “engineered” refers to the aspect of having been manipulated by the hand of man. For example, a polynucleotide is considered to be “engineered” when two or more sequences that are not linked together in that order in nature are manipulated by the hand of man to be directly linked to one another in an engineered polynucleotide and/or when a particular residue in a polynucleotide is non-naturally occurring and/or is caused through action of the hand of man to be linked with an entity or moiety with which it is not linked in nature. For example, in some embodiments described and/or utilized herein, an engineered polynucleotide comprises a regulatory sequence that is found in nature in operative association with a first coding sequence but not in operative association with a second coding sequence, is linked by the hand of man so that it is operatively associated with a second coding sequence. Comparably, a polypeptide may be considered to be “engineered” if encoded by or expressed from an engineered polynucleotide, and/or if produced other than natural expression in a cell. Analogously, a cell or organism is considered to be “engineered” if it has been subjected to a manipulation, so that its genetic, epigenetic, and/or phenotypic identity is altered relative to an appropriate reference cell such as otherwise identical cell that has not been

so manipulated. In some embodiments, such manipulation is or comprises a genetic manipulation, so that its genetic information is altered (e.g., new genetic material not previously present has been introduced, for example by transformation, mating, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or removed, for example by substitution or deletion mutation, or by mating protocols). In some embodiments, an engineered cell is one that has been manipulated so that it contains and/or expresses a particular agent of interest (e.g., a protein, a nucleic acid, and/or a particular form thereof) in an altered amount and/or according to altered timing relative to such an appropriate reference cell. As is common practice and is understood by those in the art, progeny of an engineered polynucleotide or cell are typically still referred to as “engineered” even though the actual manipulation was performed on a prior entity.

[0088] *Endogenous:* As used herein “endogenous” refers to any material from or produced inside a particular organism, cell, tissue or system.

[0089] *Excipient:* As used herein, refers to a non-therapeutic agent that may be included in a pharmaceutical composition, for example to provide or contribute to a desired consistency or stabilizing effect. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

[0090] *Exogenous:* As used herein, the term “exogenous” refers to any material introduced from or produced outside a particular organism, cell, tissue, or system.

[0091] *Expand:* As used herein, the term “expand” refers to increasing in number, as in an increase in the number of cells, for example, monocytes, macrophages, and/or dendritic cells. In one embodiment, monocytes, macrophages, or dendritic cells that are expanded *ex vivo* increase in number relative to the number originally present in a culture. In another embodiment, monocytes, macrophages, or dendritic cells that are expanded *ex vivo* increase in number relative to other cell types in a culture. In some embodiments, expansion may occur *in vivo*. The term “*ex vivo*,” as used herein, refers to cells that have been removed from a living organism, (e.g., a human) and propagated outside the organism (e.g., in a culture dish, test tube, or bioreactor).

[0092] *Expression:* As used herein, the term “expression” of a nucleic acid sequence refers to generation of any gene product from a nucleic acid sequence. In some embodiments, a gene product can be a transcript. In some embodiments, a gene product can be a polypeptide. In some embodiments, expression of a nucleic acid sequence involves one or more of the following: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end formation); (3) translation of an RNA into a polypeptide or protein; and/or (4) post-translational modification of a polypeptide or protein.

[0093] *Expression vector:* As used herein, the term “expression vector” refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses).

[0094] *Fragment:* As used herein, the terms “fragment” or “portion,” as used interchangeably herein, refers to a structure that includes a discrete portion of the whole, but lacks one or more moieties found in the whole structure. In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment consists of or comprises a characteristic structural element or moiety found in the whole. In some embodiments, a nucleotide fragment comprises or consists of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, or more monomeric units (e.g., nucleic acids) as found in the whole nucleotide. In some embodiments, a nucleotide fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more of the monomeric units (e.g., residues) found in the whole nucleotide. The whole material or entity may in some embodiments be referred to as the “parent” of the whole.

[0095] *Functional:* As used herein, a “functional” biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

[0096] *Gene product or expression product:* As used herein, the term “gene product” or “expression product” generally refers to an RNA transcribed from a gene (pre-and/or post-processing) or a polypeptide (pre- and/or post-modification) encoded by an RNA transcribed from a gene.

[0097] *Homology:* As used herein, the term “homology” refers to the overall relatedness between polymeric molecules, e.g., between nucleic acid molecules (e.g., DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% similar (e.g., containing residues with related chemical properties at corresponding positions). As will be understood by those skilled in the art, a variety of algorithms are available that permit comparison of sequences in order to determine their degree of homology, including by permitting gaps of designated length in one sequence relative to another when considering which residues “correspond” to one another in different sequences. Calculation of the percent homology between two nucleic acid sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-corresponding sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position; when a position in the first sequence is occupied by a similar nucleotide as the corresponding position in the second sequence, then the molecules are similar at that position. The percent homology between the two sequences is a function of the number of

identical and similar positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences.

[0098] *Identity:* As used herein, the term “identity” refers to the subunit sequence identity between two polymeric molecules particularly between two amino acid molecules, such as, between two polypeptide molecules. When two amino acid sequences have the same residues at the same positions; e.g., if a position in each of two polypeptide molecules is occupied by an Arginine, then they are identical at that position. The identity or extent to which two amino acid sequences have the same residues at the same positions in an alignment is often expressed as a percentage. The identity between two amino acid sequences is a direct function of the number of matching or identical positions; e.g., if half (e.g., five positions in a polymer ten amino acids in length) of the positions in two sequences are identical, the two sequences are 50% identical; if 90% of the positions (e.g., 9 of 10), are matched or identical, the two amino acids sequences are 90% identical.

[0099] *Immune cell:* As used herein, the term “immune cell,” refers to a cell that is involved in an immune response, e.g., promotion of an immune response. Examples of immune cells include, but are not limited to, plasma cells, plasmablasts, B-lymphocytes, macrophages, monocytes, dendritic cells, neutrophils, eosinophils, mast cells, platelets, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, or T-lymphocytes. A source of immune cells can be obtained from a subject. In some embodiments, immune cells comprise or are B lineage cells.

[0100] *Immune response:* As used herein the term “immune response” refers to a cellular and/or systemic response to an antigen that occurs when lymphocytes identify antigenic molecules as foreign and induce the formation of antibodies and/or activate lymphocytes to remove the antigen.

[0101] *Immunoglobulin:* As used herein, the term “immunoglobulin” or “Ig,” refers to a class of proteins that function as antibodies. Antibodies expressed by B cells are sometimes referred to as a BCR (B cell receptor) or antigen receptor. The five members included in this class of proteins are IgA, IgG, IgM, IgD, and IgE. IgA is the primary antibody that is present in body secretions, such as saliva, tears, breast milk, gastrointestinal secretions and mucus

secretions of the respiratory and genitourinary tracts. IgG is the most common circulating antibody. IgM is the main immunoglobulin produced in the primary immune response in most subjects. It is the most efficient immunoglobulin in agglutination, complement fixation, and other antibody responses, and is important in defense against bacteria and viruses. IgD is an immunoglobulin that has no known antibody function, but may serve as an antigen receptor. IgE is an immunoglobulin that mediates immediate hypersensitivity by causing release of mediators from mast cells and basophils upon exposure to allergen.

[0102] *Isolated:* As used herein, refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting) and/or otherwise previously associated, and/or (2) designed, produced, prepared, and/or manufactured by the hand of man. In some embodiments, a substance may be considered to be "isolated" if it is (or has been caused to be) free of or separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of other components (e.g., components with which it was previously associated). In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be considered "isolated" or even "pure", after having been combined with certain other components such as, for example, one or more carriers or excipients (e.g., buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of a substance is calculated without including such carriers or excipients. To give but one example, in some embodiments, a biological polymer such as a polypeptide or polynucleotide that occurs in nature is considered to be "isolated" when, a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; c) is expressed by or is otherwise in association with components from a cell or other expression system that is not of the species that produces it in nature. Thus, for instance, in some embodiments, a polypeptide that is chemically synthesized or is synthesized in a cellular system

different from that which produces it in nature is considered to be an "isolated" polypeptide. Alternatively or additionally, in some embodiments, a polypeptide that has been subjected to one or more purification techniques may be considered to be an "isolated" polypeptide to the extent that it has been separated from other components a) with which it is associated in nature; and/or b) with which it was associated when initially produced.

[0103] *Marker:* A marker, as used herein, refers to an entity or moiety whose presence or level is a characteristic of a particular state or event. In some embodiments, presence or level of a particular marker may be characteristic of presence or stage of a disease, disorder, or condition. To give but one example, in some embodiments, the term refers to a gene expression product that is characteristic of a particular tumor, tumor subclass, stage of tumor, etc. Alternatively or additionally, in some embodiments, a presence or level of a particular marker correlates with activity (or activity level) of a particular signaling pathway, for example that may be characteristic of a particular class of tumors. The statistical significance of presence or absence of a marker may vary depending upon a particular marker. In some embodiments, detection of a marker is highly specific in that it reflects a high probability that such tumor is of a particular subclass. Such specificity may come at the cost of sensitivity (i.e., a negative result may occur even if the tumor is a tumor that would be expected to express the marker). Conversely, markers with a high degree of sensitivity may be less specific than those with lower sensitivity. Those skilled in the art will appreciate that, in many embodiments, a useful marker need not distinguish with 100% accuracy.

[0104] *Modified:* As used herein, the term "modified" refers to a changed state or structure of a molecule or cell of the invention. Molecules may be modified in many ways, including chemically, structurally, and functionally. Cells may be modified through the introduction of nucleic acids.

[0105] *Modulating:* As used herein the term "modulating," refers to mediating a detectable increase or decrease in the level of a response and/or a change in the nature of a response in a subject compared with the level and/or nature of a response in the subject in the absence of a treatment or compound, and/or compared with the level and/or nature of a response in an otherwise identical but untreated subject. The term encompasses perturbing and/or

affecting a native signal or response thereby mediating a beneficial therapeutic response in a subject, preferably, a human.

[0106] *Nucleic acid:* As used herein, the term “nucleic acid” refers to a polymer of at least three nucleotides. In some embodiments, a nucleic acid comprises DNA. In some embodiments, a nucleic acid comprises RNA. In some embodiments, a nucleic acid is single stranded. In some embodiments, a nucleic acid is double stranded. In some embodiments, a nucleic acid comprises both single and double stranded portions. In some embodiments, a nucleic acid comprises a backbone that comprises one or more phosphodiester linkages. In some embodiments, a nucleic acid comprises a backbone that comprises both phosphodiester and non-phosphodiester linkages. For example, in some embodiments, a nucleic acid may comprise a backbone that comprises one or more phosphorothioate or 5'-N-phosphoramidite linkages and/or one or more peptide bonds, e.g., as in a “peptide nucleic acid”. In some embodiments, a nucleic acid comprises one or more, or all, natural residues (e.g., adenine, cytosine, deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine, guanine, thymine, uracil). In some embodiments, a nucleic acid comprises one or more, or all, non-natural residues. In some embodiments, a non-natural residue comprises a nucleoside analog (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some embodiments, a non-natural residue comprises one or more modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared to those in natural residues. In some embodiments, a nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or polypeptide. In some embodiments, a nucleic acid has a nucleotide sequence that comprises one or more introns. In some embodiments, a nucleic acid may be prepared by isolation from a natural source, enzymatic synthesis (e.g., by polymerization based on a complementary template, e.g., *in vivo* or *in vitro*, reproduction in a recombinant cell or system, or chemical synthesis. In some embodiments, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425,

450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long.

[0107] *Operably linked:* As used herein, the term “operably linked” refers to functional linkage between, for example, a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

[0108] *Payload:* In general, the term “payload”, as used herein, refers to an agent that may be delivered or transported by association with another entity. In some embodiments, such association may be or include a covalent linkage; in some embodiments such association may be or include non-covalent interaction(s). In some embodiments, association may be direct; in some embodiments, association may be indirect. The term “payload” is not limited to a particular chemical identity or type; for example, in some embodiments, a payload may be or comprise, for example, an entity of any chemical class including, for example, a lipid, a metal, a nucleic acid (e.g., a transgene), a polypeptide, a saccharide (e.g., a polysaccharide), small molecule, or a combination or complex thereof. In some embodiments, a payload may be or comprise a biological modifier, a detectable agent (e.g., a dye, a fluorophore, a radiolabel, etc.), a detecting agent, a nutrient, a therapeutic agent, etc., or a combination thereof. In some embodiments, a payload may be or comprise a cell or organism, or a fraction, extract, or component thereof. In some embodiments, a payload may be or comprise a natural product in that it is found in and/or is obtained from nature; alternatively or additionally, in some embodiments, the term may be used to refer to one or more entities that is man-made in that it is designed, engineered, and/or produced through action of the hand of man and/or is not found in nature. In some embodiments, an payload may be or comprise an agent in isolated or pure form; in some embodiments, such agent may be in crude form.

[0109] *Pharmaceutical composition:* As used herein, the term “pharmaceutical composition” refers to an active agent, formulated together with one or more pharmaceutically

acceptable carriers. In some embodiments, active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[0110] *Polynucleotide:* As used herein, the term “polynucleotide” refers to a chain of nucleotides. Furthermore, nucleic acids are polymers of nucleotides. Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are polynucleotides, which can be hydrolyzed into the monomeric “nucleotides.” The monomeric nucleotides can be hydrolyzed into nucleosides. As used herein polynucleotides include, but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR™, and the like, and by synthetic means.

[0111] *Polypeptide:* As used herein, the term “polypeptide” refers to any polymeric chain of residues (e.g., amino acids) that are typically linked by peptide bonds. In some embodiments, a polypeptide has an amino acid sequence that occurs in nature. In some embodiments, a polypeptide has an amino acid sequence that does not occur in nature. In some embodiments, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man. In some embodiments, a polypeptide may comprise or consist of natural amino acids, non-natural amino acids, or both. In some embodiments, a polypeptide may comprise or consist of only natural amino acids or only non-

natural amino acids. In some embodiments, a polypeptide may comprise D-amino acids, L-amino acids, or both. In some embodiments, a polypeptide may comprise only D-amino acids. In some embodiments, a polypeptide may comprise only L-amino acids. In some embodiments, a polypeptide may include one or more pendant groups or other modifications, e.g., modifying or attached to one or more amino acid side chains, at the polypeptide's N-terminus, at the polypeptide's C-terminus, or any combination thereof. In some embodiments, such pendant groups or modifications may be selected from the group consisting of acetylation, amidation, lipidation, methylation, pegylation, etc., including combinations thereof. In some embodiments, a polypeptide may be cyclic, and/or may comprise a cyclic portion. In some embodiments, a polypeptide is not cyclic and/or does not comprise any cyclic portion. In some embodiments, a polypeptide is linear. In some embodiments, a polypeptide may be or comprise a stapled polypeptide. In some embodiments, the term "polypeptide" may be appended to a name of a reference polypeptide, activity, or structure; in such instances it is used herein to refer to polypeptides that share the relevant activity or structure and thus can be considered to be members of the same class or family of polypeptides. For each such class, the present specification provides and/or those skilled in the art will be aware of exemplary polypeptides within the class whose amino acid sequences and/or functions are known; in some embodiments, such exemplary polypeptides are reference polypeptides for the polypeptide class or family. In some embodiments, a member of a polypeptide class or family shows significant sequence homology or identity with, shares a common sequence motif (e.g., a characteristic sequence element) with, and/or shares a common activity (in some embodiments at a comparable level or within a designated range) with a reference polypeptide of the class; in some embodiments with all polypeptides within the class). For example, in some embodiments, a member polypeptide shows an overall degree of sequence homology or identity with a reference polypeptide that is at least about 30-40%, and is often greater than about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more and/or includes at least one region (e.g., a conserved region that may in some embodiments be or comprise a characteristic sequence element) that shows very high sequence identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99%. Such a conserved region usually encompasses at least 3-4 and often up to 20 or more amino acids; in some embodiments, a conserved region encompasses at least one stretch of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous amino acids. In some

embodiments, a useful polypeptide may comprise or consist of a fragment of a parent polypeptide. In some embodiments, a useful polypeptide as may comprise or consist of a plurality of fragments, each of which is found in the same parent polypeptide in a different spatial arrangement relative to one another than is found in the polypeptide of interest (e.g., fragments that are directly linked in the parent may be spatially separated in the polypeptide of interest or vice versa, and/or fragments may be present in a different order in the polypeptide of interest than in the parent), so that the polypeptide of interest is a derivative of its parent polypeptide.

[0112] *Protein:* As used herein, the term “protein” refers to a polypeptide (i.e., a string of at least two amino acids linked to one another by peptide bonds). Proteins may include moieties other than amino acids (e.g., may be glycoproteins, proteoglycans, etc.) and/or may be otherwise processed or modified. Those of ordinary skill in the art will appreciate that a “protein” can be a complete polypeptide chain as produced by a cell (with or without a signal sequence), or can be a characteristic portion thereof. Those of ordinary skill will appreciate that a protein can sometimes include more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means. Polypeptides may contain L-amino acids, D-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, e.g., terminal acetylation, amidation, methylation, etc. In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof. The term “peptide” is generally used to refer to a polypeptide having a length of less than about 100 amino acids, less than about 50 amino acids, less than 20 amino acids, or less than 10 amino acids. In some embodiments, proteins are antibodies, antibody fragments, biologically active portions thereof, and/or characteristic portions thereof.

[0113] *Reference:* As used herein describes a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, animal, individual, population, sample, sequence or value of interest is compared with a reference or control agent, animal, individual, population, sample, sequence or value. In some embodiments, a reference or control is tested and/or determined substantially simultaneously with the testing or determination of interest. In some embodiments, a reference or control is a historical reference or control,

optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference or control is determined or characterized under comparable conditions or circumstances to those under assessment. Those skilled in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison to a particular possible reference or control.

[0114] *Response:* As used herein, a response to treatment may refer to a beneficial alteration in a subject's condition that occurs as a result of or correlates with treatment. In some embodiments, such alteration may be or comprise stabilization of a condition (e.g., prevention of deterioration that would have taken place in the absence of a treatment), amelioration of symptoms of a condition, and/or improvement in prospects for cure of a condition, etc. In some embodiments, the term "response" may refer to a response of a particular system or components thereof (e.g., of a particular cell, tissue, organism, or subject). Those skilled in the art will be aware of technologies available to assess a response of interest.

[0115] *Sample:* As used herein, the term "sample" typically refers to an aliquot of material obtained or derived from a source of interest, as described herein. In some embodiments, a source of interest is a biological or environmental source. In some embodiments, a source of interest may be or comprise a cell or an organism, such as a microbe, a plant, or an animal (e.g., a human). In some embodiments, a source of interest is or comprises biological tissue or fluid. In some embodiments, a biological tissue or fluid may be or comprise amniotic fluid, aqueous humor, ascites, bile, bone marrow, blood, breast milk, cerebrospinal fluid, cerumen, chyle, chime, ejaculate, endolymph, exudate, feces, gastric acid, gastric juice, lymph, mucus, pericardial fluid, perilymph, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum, semen, serum, smegma, sputum, synovial fluid, sweat, tears, urine, vaginal secretions, vitreous humor, vomit, and/or combinations or component(s) thereof. In some embodiments, a biological fluid may be or comprise an intracellular fluid, an extracellular fluid, an intravascular fluid (blood plasma), an interstitial fluid, a lymphatic fluid, and/or a transcellular fluid. In some embodiments, a biological fluid may be or comprise a plant exudate. In some embodiments, a biological tissue or sample may be obtained, for example, by aspirate, biopsy (e.g., fine needle or tissue biopsy), swab (e.g., oral, nasal, skin, or vaginal swab), scraping, surgery, washing or lavage (e.g., bronchoalveolar, ductal, nasal, ocular, oral, uterine, vaginal, or other washing or

lavage). In some embodiments, a biological sample is or comprises cells obtained from an individual. In some embodiments, a sample is a “primary sample” obtained directly from a source of interest by any appropriate means. In some embodiments, as will be clear from context, the term “sample” refers to a preparation that is obtained by processing (e.g., by removing one or more components of and/or by adding one or more agents to) a primary sample. For example, filtering using a semi-permeable membrane. Such a “processed sample” may comprise, for example nucleic acids or proteins extracted from a sample or obtained by subjecting a primary sample to one or more techniques such as amplification or reverse transcription of nucleic acid, isolation and/or purification of certain components, etc. In some embodiments, a sample may be a “crude” sample in that it has been subjected to relatively little processing and/or is complex in that it includes components of relatively varied chemical classes.

[0116] *Signal transduction pathway:* As used herein, the term “signal transduction pathway” refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. The phrase “cell surface receptor” includes molecules and complexes of molecules capable of receiving a signal and transmitting signal across the plasma membrane of a cell.

[0117] *Significant:* As used herein, the term “significant” typically refers to the context wherein the difference or relationship between two variables (e.g., sequence identity, protein production, spatiotemporal conditions, etc.) are certain and exist. Significance can be statistically measured (e.g., statistically significant) by various mathematical formulas and models as understood by one skilled in the art. These methods include, but are not limited to, student t-test, two-tailed test, analysis of variance (ANOVA), etc. Furthermore, significance can impart differences within structure and chemistry between two different entities. For example, a sample molecule may be compared to a reference molecule, and exhibits a structural difference from said reference molecule that is significant, e.g., in the presence or absence or in the level of one or more biological or chemical moieties as compared to the reference entity.

[0118] *Source:* The term “source” as used herein, typically refers to a context in which an agent of interest (e.g., that may be or comprise a carbohydrate, a lipid, a nucleic acid, a metal, polypeptide, a small molecule, or a combination thereof) may be found in nature, or from which

such agent can be or has been obtained (e.g., isolated). In some embodiments, a source may be or comprise a biological source (e.g., an organism, tissue, or cell, or sample thereof); in some embodiments, a source may be an environmental source. In some embodiments, a source may be or comprise a primary sample from an organism (e.g., which may be or comprise a tissue or fluid of such organism, and/or may be or comprise cell(s) of such organism). In some embodiments, an organism may be or comprise a prokaryotic organism (e.g., a bacterium) or a eukaryotic organism (e.g., a fungus or yeast, an insect, a mammal, a plant, a reptile, etc.). In some embodiments, an infectious agent such as a virus or phage may be considered an organism for purposes of this disclosure, and in particular with respect to being a source. In some embodiments, a source may be or comprise an engineered source, such as a cell line or culture, an in vitro system, etc.

[0119] *Subject:* As used herein, the term “subject” refers to an organism, for example, a mammal (e.g., a human, a non-human mammal, a non-human primate, a primate, a laboratory animal, a mouse, a rat, a hamster, a gerbil, a cat, or a dog). In some embodiments a human subject is an adult, adolescent, or pediatric subject. In some embodiments, a subject is suffering from a disease, disorder or condition, e.g., a disease, disorder, or condition that can be treated as provided herein, e.g., a cancer or a tumor listed herein. In some embodiments, a subject is susceptible to a disease, disorder, or condition; in some embodiments, a susceptible subject is predisposed to and/or shows an increased risk (as compared to the average risk observed in a reference subject or population) of developing the disease, disorder, or condition. In some embodiments, a subject displays one or more symptoms of a disease, disorder, or condition. In some embodiments, a subject does not display a particular symptom (e.g., clinical manifestation of disease) or characteristic of a disease, disorder, or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some embodiments, a subject is a patient. In some embodiments, a subject is an individual to whom diagnosis and/or therapy is and/or has been administered.

[0120] *Substantial identity:* As used herein, the term “substantial identity” refers to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be “substantially identical” if they contain identical residues in corresponding positions. As is well known in this art, amino

acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. In some embodiments, two sequences are considered to be substantially identical if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues. In the context of a CDR, reference to “substantial identity” typically refers to a CDR having an amino acid sequence at least 80%, preferably at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to that of a reference CDR.

[0121] *Substantially*: As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture a potential lack of completeness inherent in many biological and chemical phenomena.

[0122] *Substantially purified*: As used herein, the term “substantially purified”, for example as applied to a cell, refers to a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers simply to cell that have been separated from the cells with which they are naturally associated in their natural state. In some embodiments, the cells are cultured *in vitro*. In other embodiments, the cells are not cultured *in vitro*.

[0123] *Target*: As used herein, the term “target” refers to a cell, tissue, organ, or site within the body that is the subject of provided methods, systems, and/or compositions, for example, a cell, tissue, organ or site within a body that is in need of treatment or is preferentially bound by.

[0124] *Target Locus:* As used herein, the term “target locus” may refer to a specific site or location on a chromosome of interest. For example, a target locus may be a site to be “engineered” or “altered” by the hand of man. In some embodiments described and/or utilized herein, an engineered polynucleotide comprises homology to a target locus in order to allow further alterations at a specific site (e.g., CCR5 as a target locus, whose homologous sequence may be part of a guide RNA to result in incorporation of an edit via CRISPR/Cas -mediated gene editing). In some embodiments, target locus may interchangeably refer to a target gene of interest for manipulation by man. In some embodiments, such target locus manipulation is or comprises a genetic manipulation, so that its genetic information is altered (e.g., new genetic material not previously present has been introduced, for example by transformation, mating, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or removed, for example by substitution or deletion mutation, or by mating protocols).

[0125] *Target site:* As used herein, the term “target site” or “target sequence” refers to a genomic nucleic acid sequence that defines a portion of a nucleic acid to which a binding molecule may specifically bind under conditions sufficient for binding to occur.

[0126] *Therapeutic agent:* As used herein, the phrase “therapeutic agent” refers to an agent that, when administered to a subject, has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect. In some embodiments, a therapeutic agent is any substance that can be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition.

[0127] *Transfected:* As used herein, the term “transfected” or “transformed” or “transduced” refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

[0128] *Treat:* As used herein, the term “treat,” “treatment,” or “treating” refers to partial or complete alleviation, amelioration, delay of onset of, inhibition, prevention, relief, and/or reduction in incidence and/or severity of one or more symptoms or features of a disease,

disorder, and/or condition. In some embodiments, treatment may be administered to a subject who does not exhibit signs or features of a disease, disorder, and/or condition (e.g., may be prophylactic). In some embodiments, treatment may be administered to a subject who exhibits only early or mild signs or features of the disease, disorder, and/or condition, for example for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition. In some embodiments, treatment may be administered to a subject who exhibits established, severe, and/or late-stage signs of the disease, disorder, or condition.

[0129] *Variant:* As used herein in the context of molecules, e.g., nucleic acids, proteins, or small molecules, the term “variant” refers to a molecule that shows significant structural identity with a reference molecule but differs structurally from the reference molecule, e.g., in the presence or absence or in the level of one or more biological or chemical moieties as compared to the reference entity. In some embodiments, a variant also differs functionally from its reference molecule. In some embodiments, a variant differs structurally but performs the same or similar function (e.g., enzymatic function) as its reference molecule. In general, whether a particular molecule is properly considered to be a “variant” of a reference molecule is based on its degree of structural identity with the reference molecule. As will be appreciated by those skilled in the art, any biological or chemical reference molecule has certain characteristic structural elements. A variant, by definition, is a distinct molecule that shares one or more such characteristic structural elements but differs in at least one aspect from the reference molecule. To give but a few examples, a polypeptide may have a characteristic sequence element comprised of a plurality of amino acids having designated positions relative to one another in linear or three-dimensional space and/or contributing to a particular structural motif and/or biological function; a nucleic acid may have a characteristic sequence element comprised of a plurality of nucleotide residues having designated positions relative to one another in linear or three-dimensional space. In some embodiments, a variant polypeptide or nucleic acid may differ from a reference polypeptide or nucleic acid as a result of one or more differences in amino acid or nucleotide sequence and/or one or more differences in chemical moieties (e.g., carbohydrates, lipids, phosphate groups) that are covalently components of the polypeptide or nucleic acid (e.g., that are attached to the polypeptide or nucleic acid backbone). In some embodiments, a variant polypeptide or nucleic acid shows an overall sequence identity with a reference polypeptide or nucleic acid that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, or 99%. In some embodiments, a variant polypeptide or nucleic acid does not share at least one characteristic sequence element with a reference polypeptide or nucleic acid. In some embodiments, a reference polypeptide or nucleic acid has one or more biological activities. In some embodiments, a variant polypeptide or nucleic acid shares one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid lacks one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid shows a reduced level of one or more biological activities as compared to the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid is a truncated form of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide that is a truncated form of the reference polypeptide may demonstrate comparable, identical, or greater levels of one or more biological activities as compared to the reference polypeptide or nucleic acid. In some embodiments, a polypeptide or nucleic acid of interest is considered to be a “variant” of a reference polypeptide or nucleic acid if it has an amino acid or nucleotide sequence that is identical to that of the reference but for a small number of sequence alterations at particular positions.

[0130] *Vector:* As used herein, the term “vector” refers to a composition of matter that comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term “vector” includes an autonomously replicating plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

[0131] Throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For

example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

DETAILED DESCRIPTION

[0132] The present disclosure encompasses culturing and engineering of B lineage cell populations in order to express an alkaline phosphatase (ALP) or fusion protein thereof, e.g., a fusion protein comprising ALP and a human immunoglobulin G fragment crystallizable region (IgG Fc; an ALP-Fc fusion protein). In some embodiments, B lineage cell populations express a fusion protein comprising ALP and a human IgG1 Fc. In some embodiments, an IgG1 Fc of a fusion protein described herein comprises one or more substitutions. In some such embodiments, an IgG1 Fc comprises K392D, D399K, and/or K409D substitutions. In some embodiments, B lineage cell populations express a fusion protein comprising ALP and a human IgG4 Fc. In some embodiments, an IgG4 Fc of a fusion protein described herein comprises one or more substitutions. In some such embodiments, an IgG4 Fc comprises K392D, D399K, and/or R409D substitutions. In some embodiments, an ALP is or comprises tissue non-specific alkaline phosphatase (TNSALP). In some embodiments, an ALP is or comprises a catalytic domain of TNSALP. In some embodiments, an ALP comprises of a glycosyl-phosphatidylinositol (GPI) linkage. In some other embodiments, an ALP does not comprise of a glycosyl-phosphatidylinositol (GPI) linkage. In some embodiments, an ALP of a fusion protein described herein comprises one or more substitutions. In some embodiments, an Fc of ALP-Fc fusion protein comprises S288P, F234A, and/or L235A substitutions. In some embodiments, an ALP-Fc fusion protein further comprises a deca-aspartate (D10) tag. In some embodiments, an ALP-Fc fusion protein further comprises an octa-aspartate tag (D8). In some embodiments, an ALP-Fc fusion protein further comprises a linker between ALP and Fc components. In some embodiments, a linker comprises three continuous tetra-glycine and mono-serine (GGGGS) regions.

[0133] In some embodiments, an ALP-Fc fusion protein comprises an IgG1 Fc. In some embodiments, an ALP-Fc fusion protein comprises an IgG1 Fc and an D8 tag. In some embodiments, an ALP-Fc fusion protein comprises: (1) an IgG1 Fc comprising K392D, D399K,

and K409D substitutions, (2) linker comprising three continuous GGGGS regions, and (3) a D10 tag. In some embodiments, an ALP-Fc fusion protein comprises: (1) an IgG4 Fc comprising K392D, D399K, and R409D substitutions, (2) an Fc of ALP-Fc fusion protein comprising S288P, F234A, and L235A substitutions, (3) linker comprising three continuous GGGGS regions, and (4) a D10 tag.

[0134] In some embodiments, an ALP-Fc fusion protein comprises or has an amino acid sequence of any one of SEQ ID NOs: 62-75. In some embodiments, an ALP-Fc fusion protein is encoded by a nucleic acid sequence comprising any one of SEQ ID NOs: 55-61 and 87-90.

Hypophosphatasia

[0135] Hypophosphatasia (HPP) is a rare, heritable disease that is distinguished by one or more autosomal recessive or dominant loss-of-function mutations in the gene *ALPL*, which encodes tissue-nonspecific alkaline phosphatase (TNALP; a.k.a. liver/bone/kidney type ALP). Alkaline phosphatase (ALP) is important in dephosphorylating inorganic pyrophosphate (PPi) and releasing inorganic phosphate, which binds with calcium to form the building blocks for bones. Thus, ALP deficiency in osteoblasts and chondrocytes impairs skeletal mineralization, leading to symptoms of varying severity, from rickets or osteomalacia to almost complete absence of bone mineralization *in utero*. The current prevalence for HPP is unknown, but it is estimated that 1 in 100,000 live births and 1 in 200 Americans may be a carrier for HPP. HPP is also quite prevalent in other countries including Canada and Japan.

[0136] Subjects with persistently low ALP can experience debilitating symptoms that may progress with wide-ranging systemic complications. Dysfunctional bone mineralization leads to skeletal and dental deformities with manifestation that depends on both subtype and age. There are two notable subtypes of HPP including severe HPP and moderate HPP. Severe HPP is associated with deformed limbs, abnormally shaped chest/head, soft skulls, stunted growth, and life-threatening complications (still birth, brain pressure, seizures). Severe HPP is often diagnosed at perinatal and infantile stages. Moderate HPP, on the other hand, is associated with slow/stunted growth, delay in gross motor skills, bowed legs, enlarged joints, muscle weakness/pain, frequent bone injuries and fractures, and early tooth loss. Moderate HPP is often diagnosed in childhood or adulthood.

[0137] Current treatment methods are limited toward enzyme replace therapy, which is only currently approved for pediatric-onset HPP and requires burdensome dosing frequency of six times per week. Further, the current dosing routine is associated with intensely painful injection with serious side effects, including but not limited to, lipodystrophy. As result, new treatments and therapeutics are strongly needed. The present disclosure sets out to address these serious health concerns with compositions and methods provided herein.

[0138] The present disclosure provides methods and preparations of engineered B lineage cell populations that may be useful as a viable cell therapy option for treatment of HPP. In some embodiments, engineered B lineage cell populations prepared via methods disclosed herein express one or more therapeutic proteins, including alkaline phosphatase (ALP) or a fusion protein thereof, e.g., fusion proteins comprising ALP and a human immunoglobulin G fragment crystallizable region (IgG Fc), which are also referred to herein as “ALP-Fc fusion proteins.” In some embodiments, ALP-Fc fusion proteins comprise a Fc region derived from human immunoglobulin G1 (IgG1). In some embodiments, ALP-Fc fusion proteins comprise a Fc region derived from human immunoglobulin G4 (IgG4).

[0139] In some embodiments, ALP-Fc fusion proteins comprise one or more substitutions. In some embodiments, an IgG1 Fc of a fusion protein described herein comprises one or more substitutions. In some such embodiments, an IgG1 Fc comprises K392D, D399K, and/or K409D substitutions. In some embodiments, B lineage cell populations express a fusion protein comprising ALP and a human IgG4 Fc. In some embodiments, an IgG4 Fc of a fusion protein described herein comprises one or more substitutions. In some such embodiments, an IgG4 Fc comprises K392D, D399K, and/or R409D substitutions. In some embodiments, an ALP is or comprises tissue non-specific alkaline phosphatase (TNSALP). In some embodiments, an ALP is or comprises the catalytic domain of TNSALP. In some embodiments, an ALP of a fusion protein described herein comprises one or more substitutions. In some embodiments, an Fc of ALP-Fc fusion protein comprises S288P, F234A, and/or L235A substitutions. In some embodiments, an ALP-Fc fusion protein further comprises a deca-aspartate tag (D10). In some embodiments, an ALP-Fc fusion protein further comprises an octa-aspartate tag (D8). In some embodiments, an ALP-Fc fusion protein further comprises a linker between the ALP and Fc

components. In some embodiments, a linker comprises three continuous tetra-glycine and mono-serine (GGGGS) regions.

Cell therapy

[0140] Cell-based therapeutics (cell therapies) are an emerging class of medicine that makes use of innate cellular machinery to combat disease. Unlike many traditional treatment methods, cell therapies make use of cellular localization, migration, and proliferation within the body, which can translate to improved biodistribution and targeted delivery of therapeutics. Cell therapies also benefit from cellular ability to sense and respond to various extrinsic signals within a subject, including, *e.g.*, small molecules, other cells, physical forces, and/or marker proteins. Cellular persistence *in vivo* also enables cell therapies to survive, differentiate, function, etc. within a subject over extended time periods. These innate qualities may also lead to improved safety and efficacy for cell therapies as compared to other biologics or pharmaceutical compounds, providing long-lived, specifically targeted, adjustable, and/or responsive treatment for disease.

[0141] Cell-based therapies may have potential applications for a broad range of diseases, including those that have proved intractable or difficult to manage with traditional treatment options. Diseases that have been targeted for cell-based treatment include, *e.g.*, various cancers, autoimmune diseases, central nervous system (CNS) diseases, neurodegenerative disorders, inherited metabolic disorder, and cardiovascular diseases, among others. Cellular therapeutics offer an alternative over other treatment options for diseases where highly specific targeting (*e.g.*, to a particular tissue type, area of the body, etc.) and/or longer-term treatment efficacy (*e.g.*, enabling lower dosage frequency, single treatment options, etc.) are highly favored or necessary.

[0142] Cell therapies may employ a number of different cell types, which are typically modified to provide a therapeutic effect (*e.g.*, transgene expression, reprogrammed cellular targeting, etc.). Although many cell types have potential to provide some form of therapeutic effect, recent therapies have heavily favored adaptive immune cells such as T lymphocytes and B lymphocytes (referred to interchangeably herein as T cells and B cells, respectively). For example, chimeric antigen receptor T (CAR-T) cells have been engineered to treat various

cancers through recognition of one or more tumor cell markers, leading to cytotoxic destruction of tumor cells. CAR-T cells are also being adapted to treat infectious diseases (*e.g.*, HIV) through recognition of other targeted antigens. Recent engineering efforts are focused on enhancing CAR-T receptor functionality, reducing innate immune response to CAR-T, and developing allogeneic therapies that make use of donor cells.

B lineage cell therapies

[0143] While cell-based therapies present an exciting new avenue for treatment of diseases, there are numerous challenges including achievement of safe, specific, and long-term therapeutic changes within targeted cells or tissues while reducing off-target effects. Furthermore, immune tolerance of these cell-based therapies is essential in order to obviate any deleterious side effects (See Jeske et al. 2021, incorporated by reference herein in its entirety). In order to address these challenges, engineering of B lineage cells has also been an area of development for cell-based therapies, due to the natural role of B lineage cells in antibody production within the body while minimizing inflammation. Antibody-based treatments are an established, well-studied form of treatment for a number of diseases, including cancer, autoimmune diseases, and infectious diseases. Monoclonal antibodies can be produced to target antigens within the body, which is valuable for treatment of diseases where such antibodies cannot be induced through natural processes (*e.g.*, self-antigens for cancer and/or autoimmune diseases, antigens that fail to elicit natural immune response through infection and/or vaccination, etc.). Current antibody therapies require frequent administration and are costly to produce. Researchers have attempted to address these issues through use of gene therapy, which makes use of various techniques (*e.g.*, viral vectors, CRISPR/Cas9 editing) to deliver antibody payloads to endogenous cells in the body, leading to persistent antibody production within a subject. However, these approaches can result in low levels of antibody expression and a counter-active response by the subject's own immune system.

[0144] Furthermore, due to their minimal impact on a subject's native immune system and capacity to continually produce antibodies, B lineage cells are a highly desirable cell-based target to secrete other payloads including, but not limited to, enzymes, complement proteins, cytokines, cytokine receptors, chimeric antigen receptors (CARs), anti-fibrotic molecules, anti-

thrombotic molecules, antigens, both wild type and variant proteins, coagulation factors, glucose response elements, and fragments of antibodies, antigens, and proteins. Recent reports have described successful engineering of B lineage cells, for example, producing human B-cell activating factor (hBAFF) in a murine model. Excitingly, this work not only demonstrates successful production of hBAFF, but also engraftment in the bone marrow and viability of these engineered B lineage cells up to sixty days post-engraftment (See, Cheng et al. 2022, incorporated herein by reference in its entirety). B lineage cells are an attractive option for development of cell-based therapies and may potentially offer improved therapeutic effects (*e.g.*, payload delivery, targeting, long-term payload expression, reduced auto-immune response, etc.) as compared to traditional therapies (*e.g.*, antibody-based therapeutics, other cell therapies, etc.).

[0145] In some embodiments, a B lineage cell is a cell that expresses one or more B cell receptors (BCR) on a cell membrane. In some embodiments, a B lineage cell is a modified version or variant of a cell that expresses one or more B cell receptors (BCRs) on a cell membrane. In some embodiments, a B lineage cell is a naïve or memory B cell. In some embodiments, a B lineage cell is a cell derived from a naïve B cell (*e.g.*, activated B lineage cell, plasmablast, plasma cell) or a variant thereof. In some embodiments, a B lineage cell is an activated B lineage cell. In some embodiments, a B lineage cell is a plasmablast. In some embodiments, a B lineage cell is a plasma cell.

[0146] In some embodiments, a B lineage cell population comprise naïve B cells. In some embodiments, naïve B cell populations are used as reference cell populations. In some embodiments, naïve B cell populations express CD19 (CD19⁺). In some embodiments, expression of CD19 in naïve B cell populations is used as a reference to assist in characterization of other B lineage cell populations. In some embodiments, naïve B cell populations express CD20 (CD20⁺). In some embodiments, expression of CD20 in naïve B cell populations is used as a reference to assist in characterization of other B lineage cell populations. In some embodiments, naïve B cell populations express low amounts of CD27 (CD27^{lo}). In some embodiments, expression of CD27 in naïve B cell populations are as a reference to assist in characterization of other B lineage cell populations. In some embodiments, naïve B cell populations express low amounts of CD38 (CD38^{lo}). In some embodiments, expression of CD38 in naïve B cell populations are used as a reference to assist in characterization of other B lineage

cell populations. In some embodiments, naïve B cell populations express low amounts of CD138 (CD138^{lo}). In some embodiments, expression of CD138 in naïve B cell populations are used as a reference to assist in characterization of other B lineage cell population.

[0147] In some embodiments, a B lineage cell population comprises activated B lineage cells. In some embodiments, activated B lineage cell populations are used as reference cell populations. In some embodiments, activated B lineage cell populations are compared to a reference cell population (e.g., naïve B cell population). In some embodiments, activated B lineage cell populations express lower amounts of CD19 (CD19^{lo}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, activated B lineage cell populations express different (e.g., higher or lower) amounts of CD19 as compared to a reference cell population (e.g., differentiated B cell populations, etc.). In some embodiments, activated B lineage cell populations express lower amounts of CD20 (CD20^{lo}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, activated B lineage cell populations express different (e.g., higher or lower) amounts of CD20 as compared to a reference cell population (e.g., differentiated B cell populations, etc.). In some embodiments, activated B lineage cell populations express higher amounts of CD27 (CD27^{hi}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, activated B lineage cell populations express different (e.g., higher or lower) amounts of CD27 as compared to a reference cell population (e.g., differentiated B cell populations, etc.). In some embodiments, activated B lineage cell populations express lower amounts of CD38 (CD38^{lo}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, activated B lineage cell populations express different (e.g., higher or lower) amounts of CD38 as compared to a reference cell population (e.g., differentiated B cell populations, etc.). In some embodiments, activated B lineage cell populations express lower amounts of CD138 (CD138^{lo}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, activated B lineage cell populations express different (e.g., higher or lower) amounts of CD138 as compared to a reference cell population (e.g., differentiated B cell populations, etc.).

[0148] In some embodiments, a B lineage cell population may comprise plasmablast cells. In some embodiments, plasmablast cell populations are used as reference cell populations. In some embodiments, plasmablast cell populations are compared to a reference cell population

(e.g., naïve B cell population). In some embodiments, plasmablast cell populations express lower amounts of CD19 (CD19^{lo}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, plasmablast cell populations express different (e.g., higher or lower) amounts of CD19 as compared to a reference cell population (e.g., activated cell populations, plasma cell populations, etc.). In some embodiments, plasmablast cell populations express lower amounts of CD20 (CD20^{lo}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, plasmablast cell populations express different (e.g., higher or lower) amounts of CD20 as compared to a reference cell population (e.g., activated cell populations, plasma cell populations, etc.). In some embodiments, plasmablast cell populations express higher amounts of CD27 (CD27^{hi}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, plasmablast cell populations express different (e.g., higher or lower) amounts of CD27 as compared to a reference cell population (e.g., activated cell populations, plasma cell populations, etc.). In some embodiments, plasmablast cell populations express higher amounts of CD38 (CD38^{hi}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, plasmablast cell populations express different (e.g., higher or lower) amounts of CD38 as compared to a reference cell population (e.g., activated cell populations, plasma cell populations, etc.). In some embodiments, plasmablast cell populations express lower amounts of CD138 (CD138^{lo}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, plasmablast cell populations express different (e.g., higher or lower) amounts of CD138 as compared to a reference cell population (e.g., activated cell populations, plasma cell populations, etc.).

[0149] In some embodiments, a B lineage cell population comprises plasma cells. In some embodiments, plasma cell populations are used as reference cell populations. In some embodiments, plasma cell populations are compared to a reference cell population (e.g., naïve B cell population). In some embodiments, plasma cell populations express lower amounts of CD19 (CD19^{lo}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, plasma cell populations express different (e.g., higher or lower) amounts of CD19 as compared to a reference cell population (e.g., activated cell populations, plasmablast populations, etc.). In some embodiments, plasma cell populations express lower amounts of CD20 (CD20^{lo}) as compared to a reference cell population (e.g., naïve B cell populations). In some embodiments, plasma cell populations express different (e.g., higher or lower) amounts of

CD20 as compared to a reference cell population (*e.g.*, activated cell populations, plasmablast populations, etc.). In some embodiments, plasma cell populations express higher amounts of CD27 (CD27^{hi}) as compared to a reference cell population (*e.g.*, naïve B cell populations). In some embodiments, plasma cell populations express different (*e.g.*, higher or lower) amounts of CD27 as compared to a reference cell population (*e.g.*, activated cell populations, plasmablast populations, etc.). In some embodiments, plasma cell populations express higher amounts of CD38 (CD38^{hi}) as compared to a reference cell population (*e.g.*, naïve B cell populations). In some embodiments, plasma cell populations express different (*e.g.*, higher or lower) amounts of CD38 as compared to a reference cell population (*e.g.*, activated cell populations, plasmablast populations, etc.). In some embodiments, plasma cell populations express higher amounts of CD138 (CD138^{hi}) as compared to a reference cell population (*e.g.*, naïve B cell populations). In some embodiments, plasma cell populations express different (*e.g.*, higher or lower) amounts of CD138 as compared to a reference cell population (*e.g.*, activated cell populations, plasmablast populations, etc.).

Cell engineering

[0150] Production of engineered cells for various uses including, *e.g.*, cell therapies is an active area of development. Genomic and epigenomic modifications, synthetic biology, and application of biomaterials may be employed to generate engineered cells with desired properties for therapeutic applications. Selection of an appropriate method for generating engineered cells is typically dependent upon the desired output and effects of cell therapy and requires optimization for different cell types, transgenes of interest, etc. It is understood in the art that engineering methods that are effective for a particular cell type may be less effective (or not viable) for another cell type. Furthermore, engineering methods may vary depending upon whether a therapy requires cellular localization, expression of an endogenous or exogenous protein, removal of an endogenous protein, etc.

Genome editing

[0151] Genome editing tools can alter a cellular genome to produce a desired therapeutic effect, *e.g.*, expression of a therapeutic protein. Targeted nucleases (*e.g.*, Cas proteins, TALENs, ZFNs, etc.), viral vectors (*e.g.*, AAV, lentiviral, adenoviral, etc.), recombinases (*e.g.*, Cre recombinase, Flp recombinase, PhiC31 integrase, etc.), and other tools may be employed to provide genetic modifications. Gene editing efficiency may vary depending on, *e.g.*, cell type, desired function, and ease of delivery. Accordingly, editing methods often require extensive optimization to provide engineered cells with intended functionality for therapeutic applications.

B lineage cell engineering

[0152] Various B lineage cell engineering techniques are described in the art, including, *e.g.*, CRISPR/Cas9, AAV, lentiviral, and recombinase-based methods. These methods are generally employed to introduce a payload (*e.g.*, expression cassette, transgene, etc.) into naïve B cells in order to express a protein of interest. A payload may be designed for episomal expression, integration into a specific target locus (*e.g.*, endogenous target gene locus), or integration into a non-specific locus (*e.g.*, endogenous random or non-target gene locus). Payloads may be designed such that an endogenous target gene locus continues to produce functional protein and/or fulfill its natural function (non-disruptive integration). Payloads may also be designed to intentionally disrupt an endogenous target gene locus to produce lowered or non-detectable levels of functional protein and/or some amount of non-functional protein.

[0153] Methods for integration of a payload (*e.g.*, expression cassette comprising one or more transgenes) into an endogenous target locus may comprise site-specific cleavage with a targeted nuclease (*e.g.*, Cas protein, including Cas9), followed by integration of a transgene (*e.g.*, ALP or a fusion protein thereof, such as ALP-Fc fusion protein) through an endogenous repair pathway (*e.g.*, homologous recombination, homology-directed repair, etc.). In some embodiments, methods for integration of an expression cassette comprising an ALP or a fusion protein thereof (*e.g.*, an ALP-Fc fusion protein) encoding transgene comprise site-specific cleavage at a target locus (*e.g.*, CCR5) with a guide RNA / Cas9 complex, followed by integration of an ALP or a fusion protein thereof at the target locus through homologous recombination.

[0154] In some embodiments, a method of B lineage cell engineering is or comprises administration of a ribonucleoprotein (RNP) to a cell population. In some embodiments, a method of B lineage cell engineering is or comprises administration of a composition comprising a Cas protein complexed with guide RNA (gRNA) to a cell population. In some embodiments, a method of B lineage cell engineering is or comprises administration of a composition comprising a Cas9/guide RNA complex to a cell population. In some embodiments, a method of B lineage cell engineering is or comprises administration of a composition comprising a Cas9/guide RNA complex to a cell population. In some embodiments, a method of B lineage cell engineering is or comprises administration of a composition comprising a payload (*e.g.*, expression cassette comprising one or more transgenes) of interest to a cell population. In some embodiments, a method of B lineage cell engineering is or comprises administration of a composition comprising a payload (*e.g.*, expression cassette comprising one or more transgenes) to a cell population through use of a viral vector. In some embodiments, a method of B lineage cell engineering is or comprises administration of a composition comprising a payload (*e.g.*, expression cassette comprising one or more transgenes) encapsulated within an AAV capsid (*e.g.*, AAV2, AAV3, AAV5, AAV6, AAV8, etc.) to a cell population. In some embodiments, a method of B lineage cell engineering is or comprises administration of a composition comprising a transgene encapsulated within an AAV capsid (*e.g.*, AAV2, AAV3, AAV5, AAV6, AAV8, etc.) in combination with or in addition to administration of a composition comprising a Cas9/gRNA complex.

[0155] In some embodiments, a method of B lineage cell engineering comprises a step of electroporation to facilitate cellular uptake of one or more engineering components. In some embodiments, a method of B lineage cell engineering comprises a step of electroporation to facilitate cellular uptake of a Cas9/gRNA complex. In some embodiments, a method of B lineage cell engineering comprises a step of electroporation to facilitate cellular uptake of a Cas9/gRNA complex and a payload. In some embodiments, a method of B lineage cell engineering may comprise a step of electroporation to facilitate cellular uptake of a Cas9/gRNA complex and a payload (*e.g.*, expression cassette comprising one or more transgenes) encapsulated in an AAV capsid (AAV2, AAV3, AAV5, AAV6, AAV8, etc.). In some embodiments, a method of B lineage cell engineering comprises a step of electroporation to facilitate cellular uptake of a

Cas9/gRNA complex and a payload (*e.g.*, expression cassette comprising one or more transgenes) encapsulated in an AAV6 capsid.

[0156] In some embodiments, a method of B lineage cell engineering comprises a step of viral transduction to facilitate cellular uptake of a payload (*e.g.*, expression cassette comprising one or more transgenes). In some embodiments, a method of B lineage cell engineering comprises a step of viral transduction to facilitate cellular uptake of a payload (*e.g.*, transgene) encapsulated in an AAV capsid (AAV2, AAV3, AAV5, AAV6, AAV8, etc.). In some embodiments, a method of B lineage cell engineering comprises a step of viral transduction to facilitate cellular uptake of a payload (*e.g.*, expression cassette comprising one or more transgenes) encapsulated in an AAV6 capsid. In some embodiments, a method of B lineage cell engineering comprises one or more steps of: (i) electroporation to facilitate cellular uptake of a Cas9/gRNA complex; and (ii) viral transduction to facilitate cellular uptake of a payload encapsulated in an AAV capsid.

[0157] In some embodiments, a method of cell engineering is particularly effective for one type of cell (*e.g.*, T cell) and less effective for another type of cell (*e.g.*, B cell). In some embodiments, a method of B lineage cell engineering provides improved genome editing efficiency in B lineage cells as compared to other cell types (*e.g.*, T cell). In some embodiments, a method of B cell engineering provides at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% editing efficiency. In some embodiments, a method of B lineage cell engineering provides at least about 1E6, 1.5E6, 2E6, 2.5E6, 3E6, 3.5E6, 4E6, 4.5E6, 5E6, 5.5E6, 6E6, 6.5E6, 7E6, 7.5E6, 8E6, 8.5E6, 9E6, 9.5E6, 1E7, 1.5E7, 2E7, 2.5E7, 3E7, 3.5E7, 4E7, 4.5E7, or 5E7 edited B lineage cells.

[0158] In some embodiments, a method of B lineage cell engineering comprises a step of editing activated B cells. In some embodiments, a method of B lineage cell engineering comprises a step of editing B lineage cells after an activation step of about 1, 2, 3, 4, or 5 day(s). In some embodiments, a method of B lineage cell engineering comprises a step of editing B lineage cells after an activation step of 2 days. In some embodiments, a method of B lineage cell engineering comprises a step of editing B lineage cells after an activation step of about 1, 2, 3, 4, or 5 day(s) and expanding edited B lineage cells in the activation media for an additional period of about 1, 2, 3, 4, 5, 6, 7, or 8 days. In some embodiments, a method of B lineage cell

engineering comprises a step of editing B lineage cells after an activation step of 2 days and expanding edited B lineage cells in the activation media for an additional 6 days.

[0159] In some embodiments, a gRNA is or comprises a polynucleotide sequence in Table 8. In some embodiments, a gRNA is or comprises SEQ ID NO: 79. In some embodiments, a gRNA is or comprises SEQ ID NO: 80. In some embodiments, a gRNA is or comprises SEQ ID NO: 81. In some embodiments, a gRNA is or comprises SEQ ID NO: 82. In some embodiments, a gRNA is or comprises SEQ ID NO: 83. In some embodiments, a gRNA is or comprises SEQ ID NO: 84. In some embodiments, a gRNA is or comprises SEQ ID NO: 85. In some embodiments, a gRNA is or comprises SEQ ID NO: 86. In some embodiments, a gRNA is or comprises SEQ ID NO: 129. In some embodiments, a gRNA is or comprises SEQ ID NO: 130. In some embodiments, a gRNA is or comprises SEQ ID NO: 131. In some embodiments, a gRNA is or comprises SEQ ID NO: 132.

Payloads

[0160] Various methods described herein may be used for generation of engineered cells (*e.g.*, B lineage cell populations, etc.) comprising one or more payloads. In some embodiments, engineered cells (*e.g.*, B lineage cell populations, etc.) comprise a polynucleotide sequence encoding one or more payloads. In accordance with various aspects of the present disclosure, any of a variety of payloads may be used (*e.g.*, those with a therapeutic or monitoring purpose), alone or in combination. In some embodiments, a payload is or comprises a polynucleotide sequence encoding a peptide or polypeptide. In some embodiments, a payload is or comprises one or more transgenes. In some embodiments, a payload is or comprises one or more homology arm sequences. In some embodiments, a payload is or comprises a transgene flanked by one or more homology sequences.

[0161] In some embodiments, a payload may be or comprise a polynucleotide sequence, which comprises an expression cassette. In some embodiments, an expression cassette comprises one or more polynucleotide sequence elements (*e.g.*, promoters, enhancers, transgenes, termination elements, homology arms, biomarkers, signal peptide sequences, internal ribosome entry site elements, self-cleaving peptide sequences, ubiquitous chromatin opening element, etc.). In some embodiments, an expression cassette comprises one or more polynucleotide

sequence elements (*e.g.*, promoters, enhancers, transgenes, termination elements, homology arms, biomarkers, signal peptide sequences, internal ribosome entry site elements, self-cleaving peptide sequences, ubiquitous chromatin opening element, etc.) in a particular configuration and/or combination. In some embodiments, an expression cassette comprises one or more polynucleotide sequence elements (*e.g.*, promoters, enhancers, transgenes, termination elements, homology arms, biomarkers, signal peptide sequences, internal ribosome entry site elements, self-cleaving peptide sequences, ubiquitous chromatin opening element, etc.) in a particular configuration and/or combination in order to promote expression of a transgene in a cell population. In some embodiments, an expression cassette comprises one or more polynucleotide sequence elements (*e.g.*, promoters, enhancers, transgenes, termination elements, homology arms, biomarkers, signal peptide sequences, internal ribosome entry site elements, self-cleaving peptide sequences, ubiquitous chromatin opening element, etc.) in a particular configuration and/or combination in order to promote expression of a transgene in an engineered B lineage cell population.

[0162] In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding one or more signal peptides. In some embodiments, a polynucleotide sequence encoding a signal peptide is or comprises a sequence of ATGCCGTCTTCTGTCTCGTGGGGCATCCTCCTGCTGGCAGGCCTGTGCTGCCTGGTCC CTGTCTCCCTGGCT (SEQ ID NO: 143). In some embodiments, a polynucleotide sequence encoding a signal peptide is or comprises a sequence with at least 70% sequence identity to SEQ ID NO: 143. In some embodiments, a polynucleotide sequence encoding a signal peptide is or comprises a sequence with at least 75% sequence identity to SEQ ID NO: 143. In some embodiments, a polynucleotide sequence encoding a signal peptide is or comprises a sequence with at least 80% sequence identity to SEQ ID NO: 143. In some embodiments, a polynucleotide sequence encoding a signal peptide is or comprises a sequence with at least 85% sequence identity to SEQ ID NO: 143. In some embodiments, a polynucleotide sequence encoding a signal peptide is or comprises a sequence with at least 90% sequence identity to SEQ ID NO: 143. In some embodiments, a polynucleotide sequence encoding a signal peptide is or comprises a sequence with at least 95% sequence identity to SEQ ID NO: 143. In some embodiments, a polynucleotide sequence encoding a signal peptide is or comprises a sequence with at least 99% sequence identity to SEQ ID NO: 143. In some embodiments, a polynucleotide sequence

encoding a signal peptide consists of a polynucleotide sequence of SEQ ID NO: 143. In some embodiments, a signal peptide is or comprises a polypeptide sequence of MPSSVSWGILLLAGLCCCLVPVSLA (SEQ ID NO: 144). In some embodiments, a signal peptide is or comprises a polypeptide sequence with at least 70% sequence identity to SEQ ID NO: 144. In some embodiments, a signal peptide is or comprises a polypeptide sequence with at least 75% sequence identity to SEQ ID NO: 144. In some embodiments, a signal peptide is or comprises a polypeptide sequence with at least 80% sequence identity to SEQ ID NO: 144. In some embodiments, a signal peptide is or comprises a polypeptide sequence with at least 85% sequence identity to SEQ ID NO: 144. In some embodiments, a signal peptide is or comprises a polypeptide sequence with at least 90% sequence identity to SEQ ID NO: 144. In some embodiments, a signal peptide is or comprises a polypeptide sequence with at least 95% sequence identity to SEQ ID NO: 144. In some embodiments, a signal peptide is or comprises a polypeptide sequence with at least 99% sequence identity to SEQ ID NO: 144. In some embodiments, a signal peptide consists of a polypeptide sequence of SEQ ID NO: 144.

[0163] In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding one or more promoters (*e.g.*, MND, CMV, FEEK I, SFFV, PGK, EF-1a, B2M, etc.). In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding one or more exogenous promoters. In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding one or more endogenous promoters. In some embodiments, an expression cassette does not comprise one or more promoters. In some embodiments, an expression cassette does not comprise one or more exogenous promoters. In some embodiments, an expression cassette does not comprise one or more endogenous promoters. In some embodiments, a promoter is or comprises one or more polynucleotide sequences in Table 4. In some embodiments, a promoter is or comprises SEQ ID NO: 76. In some embodiments, a promoter is or comprises SEQ ID NO: 125. In some embodiments, a promoter is or comprises SEQ ID NO: 126. In some embodiments, a promoter is or comprises SEQ ID NO: 127. In some embodiments, a promoter is or comprises SEQ ID NO: 128.

[0164] In some embodiments, a promoter is or comprises a polynucleotide sequence of SEQ ID NO: 76. In some embodiments, a promoter is or comprises a polynucleotide sequence

having at least 70% sequence identity to SEQ ID NO: 76. In some embodiments, a promoter is or comprises a polynucleotide sequence having at least 75% sequence identity to SEQ ID NO: 76. In some embodiments, a promoter is or comprises a polynucleotide sequence having at least 80% sequence identity to SEQ ID NO: 76. In some embodiments, a promoter is or comprises a polynucleotide sequence having at least 85% sequence identity to SEQ ID NO: 76. In some embodiments, a promoter is or comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 76. In some embodiments, a promoter is or comprises a polynucleotide sequence having at least 95% sequence identity to SEQ ID NO: 76. In some embodiments, a promoter is or comprises a polynucleotide sequence having at least 99% sequence identity to SEQ ID NO: 76. In some embodiments, a promoter consists of a polynucleotide sequence of SEQ ID NO: 76.

[0165] In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding a translation initiation site (*e.g.*, Kozak consensus sequence, ribosomal binding site, etc.). In some embodiments, an expression cassette comprises one or more exogenous polynucleotide sequences encoding a translation initiation site. In some embodiments, an expression cassette comprises one or more endogenous polynucleotide sequences encoding a translation initiation site. In some embodiments, an expression cassette does not comprise one or more polynucleotide sequences encoding a translation initiation site. In some embodiments, an expression cassette does not comprise one or more exogenous polynucleotide sequences encoding a translation initiation site. In some embodiments, an expression cassette does not comprise one or more endogenous polynucleotide sequences encoding a translation initiation site.

[0166] In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding one or more enhancers (*e.g.*, WPRE, beta-globin, E μ etc.) or a variant thereof. In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding one or more exogenous enhancers. In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding one or more endogenous enhancers. In some embodiments, an enhancer may be viral (*e.g.*, WPRE, etc.) or non-viral. In some embodiments, an expression cassette does not comprise one or more enhancers. In some embodiments, an expression cassette does not comprise one or more

exogenous enhancers. In some embodiments, an expression cassette does not comprise one or more endogenous enhancers.

[0167] In some embodiments, one or more enhancers is an E μ enhancer or a variant thereof. In some embodiments, one or more enhancers is a murine E μ enhancer or a variant thereof. In some embodiments, one or more enhancers is a human E μ enhancer or a variant thereof. In some embodiments, one or more enhancers is a primate E μ enhancer or a variant thereof. In some embodiments, a E μ enhancer is a truncated, extended, and/or mutated version of a wild-type E μ enhancer. In some embodiments, an E μ enhancer may be or comprise a fusion with one or more additional sequence elements (*e.g.*, promoter, enhancer, signal peptide, polyA, etc.). In some embodiments, an expression cassette comprises an E μ enhancer in combination with another enhancer (*e.g.*, WPRE, beta-globin, E μ etc.). In some embodiments, an E μ enhancer may be oriented in a forward (*e.g.*, 5' to 3') or reverse (*e.g.*, 3' to 5') orientation within a payload. In some embodiments, an E μ enhancer may be in different positions within a payload. In some embodiments, an E μ enhancer may be upstream of (*e.g.*, 5' to) a transgene in a payload. In some embodiments, an E μ enhancer may be downstream of (*e.g.*, 3' to) a transgene in a payload. In some embodiments, an enhancer is or comprises a polynucleotide sequence in Table 9.

[0168] In some embodiments, an enhancer is or comprises a polynucleotide sequence of SEQ ID NO: 95. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 70% sequence identity to SEQ ID NO: 95. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 75% sequence identity to SEQ ID NO: 95. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 80% sequence identity to SEQ ID NO: 95. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 85% sequence identity to SEQ ID NO: 95. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO: 95. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 95% sequence identity to SEQ ID NO: 95. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 99% sequence identity to SEQ ID NO: 95. In some embodiments, an enhancer consists of a polynucleotide sequence of SEQ ID NO: 95.

[0169] In some embodiments, an enhancer is or comprises a polynucleotide sequence of SEQ ID NO: 96. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 70% sequence identity to SEQ ID NO: 96. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 75% sequence identity to SEQ ID NO: 96. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 80% sequence identity to SEQ ID NO: 96. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 85% sequence identity to SEQ ID NO: 96. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO: 96. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 95% sequence identity to SEQ ID NO: 96. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 99% sequence identity to SEQ ID NO: 96. In some embodiments, an enhancer consists of a polynucleotide sequence of SEQ ID NO: 96.

[0170] In some embodiments, an enhancer is or comprises a polynucleotide sequence of SEQ ID NO: 97. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 70% sequence identity to SEQ ID NO: 97. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 75% sequence identity to SEQ ID NO: 97. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 80% sequence identity to SEQ ID NO: 97. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 85% sequence identity to SEQ ID NO: 97. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO: 97. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 95% sequence identity to SEQ ID NO: 97. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 99% sequence identity to SEQ ID NO: 97. In some embodiments, an enhancer consists of a polynucleotide sequence of SEQ ID NO: 97.

[0171] In some embodiments, an enhancer is or comprises a polynucleotide sequence of SEQ ID NO: 98. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 70% sequence identity to SEQ ID NO: 98. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 75% sequence identity to SEQ ID NO: 98. In

some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 80% sequence identity to SEQ ID NO: 98. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 85% sequence identity to SEQ ID NO: 98. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO: 98. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 95% sequence identity to SEQ ID NO: 98. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 99% sequence identity to SEQ ID NO: 98. In some embodiments, an enhancer consists of a polynucleotide sequence of SEQ ID NO: 98.

[0172] In some embodiments, an enhancer is or comprises a polynucleotide sequence of SEQ ID NO: 99. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 70% sequence identity to SEQ ID NO: 99. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 75% sequence identity to SEQ ID NO: 99. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 80% sequence identity to SEQ ID NO: 99. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 85% sequence identity to SEQ ID NO: 99. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO: 99. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 95% sequence identity to SEQ ID NO: 99. In some embodiments, an enhancer is or comprises a polynucleotide sequence with at least 99% sequence identity to SEQ ID NO: 99. In some embodiments, an enhancer consists of a polynucleotide sequence of SEQ ID NO: 99.

[0173] In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding one or more terminators (*e.g.*, polyA, including, *e.g.*, BGH polyA, SV40 polyA, etc.). In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding one or more exogenous terminators. In some embodiments, an expression cassette comprises one or more polynucleotide sequences encoding one or more endogenous terminators. In some embodiments, an expression cassette does not comprise one or more terminators. In some embodiments, an expression cassette does not comprise one or more

exogenous terminators. In some embodiments, an expression cassette does not comprise one or more endogenous terminators.

[0174] In some embodiments, a terminator is or comprises a polynucleotide sequence of SEQ ID NO: 78. In some embodiments, a terminator is or comprises a polynucleotide sequence having at least 70% sequence identity to SEQ ID NO: 78. In some embodiments, a terminator is or comprises a polynucleotide sequence having at least 75% sequence identity to SEQ ID NO: 78. In some embodiments, a terminator is or comprises a polynucleotide sequence having at least 80% sequence identity to SEQ ID NO: 78. In some embodiments, a terminator is or comprises a polynucleotide sequence having at least 85% sequence identity to SEQ ID NO: 78. In some embodiments, a terminator is or comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 78. In some embodiments, a terminator is or comprises a polynucleotide sequence having at least 95% sequence identity to SEQ ID NO: 78. In some embodiments, a terminator is or comprises a polynucleotide sequence having at least 99% sequence identity to SEQ ID NO: 78. In some embodiments, a terminator consists of a polynucleotide sequence of SEQ ID NO: 78.

[0175] In some embodiments, an expression cassette comprises a polynucleotide sequence encoding a transgene (*e.g.*, ALP or a fusion protein thereof, such as an ALP-Fc fusion protein) or variant thereof. In some embodiments, an expression cassette comprises a polynucleotide sequence encoding ALP, or a variant thereof. In some embodiments, an expression cassette comprises a polynucleotide sequence encoding a functional ALP variant. In some embodiments, an expression cassette comprises a polynucleotide sequence encoding ALP or a fusion protein thereof (*e.g.*, an ALP-Fc fusion protein or a variant thereof). In some embodiments, an expression cassette comprises a polynucleotide sequence encoding a fusion protein comprising ALP and a human IgG1 Fc. In some embodiments, an IgG1 Fc of a fusion protein described herein comprises one or more substitutions. In some such embodiments, an IgG1 Fc comprises K392D, D399K, and/or K409D substitutions. In some embodiments, an expression cassette comprises a polynucleotide sequence encoding a fusion protein comprising ALP and a human IgG4 Fc. In some embodiments, an IgG4 Fc of a fusion protein described herein comprises one or more substitutions. In some such embodiments, an IgG4 Fc comprises K392D, D399K, and/or R409D substitutions. In some embodiments, an ALP is or comprises

tissue non-specific alkaline phosphatase (TNSALP). In some embodiments, an ALP is or comprises the catalytic domain of TNSALP. In some embodiments, an ALP of a fusion protein described herein comprises one or more substitutions. In some embodiments, an Fc of ALP-Fc fusion protein comprises S288P, F234A, and/or L235A substitutions. In some embodiments, an ALP-Fc fusion protein further comprises a deca-aspartate tag (D10). In some embodiments, an ALP-Fc fusion protein further comprises an octa-aspartate tag (D8). In some embodiments, an ALP-Fc fusion protein further comprises a linker between the ALP and Fc components.

[0176] In some embodiments, a linker is or comprises three continuous tetra-glycine and mono-serine (GGGS) regions. In some embodiments, a linker is encoded by a polynucleotide sequence that is or comprises CTGAAGGAGAGCAAGTACGGCCCCCCC (SEQ ID NO: 140). In some embodiments, a linker is encoded by a polynucleotide sequence having at least about 70% sequence identity to SEQ ID NO: 140. In some embodiments, a linker is encoded by a polynucleotide sequence having at least about 75% sequence identity to SEQ ID NO: 140. In some embodiments, a linker is encoded by a polynucleotide sequence having at least about 80% sequence identity to SEQ ID NO: 140. In some embodiments, a linker is encoded by a polynucleotide sequence having at least about 85% sequence identity to SEQ ID NO: 140. In some embodiments, a linker is encoded by a polynucleotide sequence having at least about 90% sequence identity to SEQ ID NO: 140. In some embodiments, a linker is encoded by a polynucleotide sequence having at least about 95% sequence identity to SEQ ID NO: 140. In some embodiments, a linker is encoded by a polynucleotide sequence having at least about 99% sequence identity to SEQ ID NO: 140. In some embodiments, a linker is encoded by a polynucleotide sequence consisting of SEQ ID NO: 140. In some embodiments, a linker comprises a polypeptide sequence that is or comprises LKESKYGPP (SEQ ID NO: 141). In some embodiments, a linker is or comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 141. In some embodiments, a linker is or comprises a polypeptide sequence having at least 75% sequence identity to SEQ ID NO: 141. In some embodiments, a linker is or comprises a polypeptide sequence having at least 80% sequence identity to SEQ ID NO: 141. In some embodiments, a linker is or comprises a polypeptide sequence having at least 85% sequence identity to SEQ ID NO: 141. In some embodiments, a linker is or comprises a polypeptide sequence having at least 90% sequence identity to SEQ ID NO: 141. In some embodiments, a linker is or comprises a polypeptide sequence having at least

95% sequence identity to SEQ ID NO: 141. In some embodiments, a linker is or comprises a polypeptide sequence having at least 99% sequence identity to SEQ ID NO: 141. In some embodiments, a linker consists of a polypeptide sequence of SEQ ID NO: 141.

[0177] In some embodiments, an expression cassette is or comprises a polynucleotide sequence in Table 2. In some embodiments, an expression cassette is or comprises SEQ ID NO: 55. In some embodiments, an expression cassette is or comprises SEQ ID NO: 56. In some embodiments, an expression cassette is or comprises SEQ ID NO: 57. In some embodiments, an expression cassette is or comprises SEQ ID NO: 58. In some embodiments, an expression cassette is or comprises SEQ ID NO: 59. In some embodiments, an expression cassette is or comprises SEQ ID NO: 60. In some embodiments, an expression cassette is or comprises SEQ ID NO: 61. In some embodiments, an expression cassette is or comprises SEQ ID NO: 87. In some embodiments, an expression cassette is or comprises SEQ ID NO: 88. In some embodiments, an expression cassette is or comprises SEQ ID NO: 89. In some embodiments, an expression cassette is or comprises SEQ ID NO: 90. In some embodiments, an expression cassette is or comprises SEQ ID NO: 100. In some embodiments, an expression cassette is or comprises SEQ ID NO: 102. In some embodiments, an expression cassette is or comprises SEQ ID NO: 103. In some embodiments, an expression cassette is or comprises SEQ ID NO: 104. In some embodiments, an expression cassette is or comprises SEQ ID NO: 105. In some embodiments, an expression cassette is or comprises SEQ ID NO: 106. In some embodiments, an expression cassette is or comprises SEQ ID NO: 107. In some embodiments, an expression cassette is or comprises SEQ ID NO: 108.

[0178] In some embodiments, an expression cassette is or comprises a polynucleotide sequence of SEQ ID NO: 100. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 70% sequence identity to SEQ ID NO: 100. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 75% sequence identity to SEQ ID NO: 100. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 80% sequence identity to SEQ ID NO: 100. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 85% sequence identity to SEQ ID NO: 100. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO:

100. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 95% sequence identity to SEQ ID NO: 100. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 99% sequence identity to SEQ ID NO: 100. In some embodiments, an expression cassette consists of a polynucleotide sequence of SEQ ID NO: 100.

[0179] In some embodiments, an expression cassette is or comprises a polynucleotide sequence of SEQ ID NO: 149. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 70% sequence identity to SEQ ID NO: 149. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 75% sequence identity to SEQ ID NO: 149. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 80% sequence identity to SEQ ID NO: 149. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 85% sequence identity to SEQ ID NO: 149. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 149. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 95% sequence identity to SEQ ID NO: 149. In some embodiments, an expression cassette is or comprises a polynucleotide sequence having at least 99% sequence identity to SEQ ID NO: 149. In some embodiments, an expression cassette consists of a polynucleotide sequence of SEQ ID NO: 149.

[0180] In some embodiments, an expression cassette encodes a polypeptide sequence that is or comprises SEQ ID NO: 101. In some embodiments, an expression cassette encodes a polypeptide sequence that is or comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 101. In some embodiments, an expression cassette encodes a polypeptide sequence that is or comprises a polypeptide sequence having at least 75% sequence identity to SEQ ID NO: 101. In some embodiments, an expression cassette encodes a polypeptide sequence that is or comprises a polypeptide sequence having at least 80% sequence identity to SEQ ID NO: 101. In some embodiments, an expression cassette encodes a polypeptide sequence that is or comprises a polypeptide sequence having at least 85% sequence identity to SEQ ID NO: 101. In some embodiments, an expression cassette encodes a polypeptide sequence that is or comprises a polypeptide sequence having at least 90% sequence

identity to SEQ ID NO: 101. In some embodiments, an expression cassette encodes a polypeptide sequence that is or comprises a polypeptide sequence having at least 95% sequence identity to SEQ ID NO: 101. In some embodiments, an expression cassette encodes a polypeptide sequence that is or comprises a polypeptide sequence having at least 99% sequence identity to SEQ ID NO: 101. In some embodiments, an expression cassette encodes a polypeptide sequence that consists of SEQ ID NO: 101.

[0181] In some embodiments, an expression cassette comprises a polynucleotide sequence that comprises one or more of:

- (i) a 5' homology arm that is or comprises SEQ ID NO: 39;
- (ii) a promoter that is or comprises SEQ ID NO: 76;
- (iii) a signal peptide encoded by a polynucleotide sequence that is or comprises SEQ ID NO: 143;
- (iv) a transgene that is or comprises SEQ ID NO: 139;
- (v) a terminator that is or comprises SEQ ID NO: 78; and/or
- (vi) a 3' homology arm that is or comprises SEQ ID NO: 44.

[0182] In some embodiments, an expression cassette comprises a polynucleotide sequence that comprises one or more of:

- (i) a 5' homology arm that is or comprises SEQ ID NO: 39;
- (ii) a promoter that is or comprises SEQ ID NO: 76;
- (iii) a signal peptide encoded by a polynucleotide sequence that is or comprises SEQ ID NO: 143;
- (iv) a transgene that is or comprises SEQ ID NO: 145;
- (v) a terminator that is or comprises SEQ ID NO: 78; and/or
- (vi) a 3' homology arm that is or comprises SEQ ID NO: 44.

[0183] In some embodiments, an expression cassette comprises a polynucleotide sequence that comprises one or more of:

- (i) a 5' homology arm that is or comprises SEQ ID NO: 39;
- (ii) a promoter that is or comprises SEQ ID NO: 76;
- (iii) a signal peptide encoded by a polynucleotide sequence that is or comprises SEQ ID NO: 143;
- (iv) a transgene that is or comprises SEQ ID NO: 146;
- (v) a terminator that is or comprises SEQ ID NO: 78; and/or
- (vi) a 3' homology arm that is or comprises SEQ ID NO: 44.

[0184] In some embodiments, an expression cassette comprises a polynucleotide sequence that comprises one or more of:

- (i) a 5' homology arm that is or comprises SEQ ID NO: 39;
- (ii) a promoter that is or comprises SEQ ID NO: 76;
- (iii) a signal peptide encoded by a polynucleotide sequence that is or comprises SEQ ID NO: 143;
- (iv) a transgene that is or comprises SEQ ID NO: 145;
- (v) a linker encoded by a polynucleotide that is or comprises SEQ ID NO: 140;
- (vi) a terminator that is or comprises SEQ ID NO: 78; and/or
- (vii) a 3' homology arm that is or comprises SEQ ID NO: 44.

[0185] In some embodiments, an expression cassette comprises a polynucleotide sequence that comprises one or more of:

- (i) a 5' homology arm that is or comprises SEQ ID NO: 39;
- (ii) a promoter that is or comprises SEQ ID NO: 76;
- (iii) a signal peptide encoded by a polynucleotide sequence that is or comprises SEQ ID NO: 143;
- (iv) a linker encoded by a polynucleotide that is or comprises SEQ ID NO: 140;
- (v) a transgene that is or comprises SEQ ID NO: 146;

(vi) a terminator that is or comprises SEQ ID NO: 78; and/or

(vii) a 3' homology arm that is or comprises SEQ ID NO: 44.

[0186] In some embodiments, an expression cassette comprises a polynucleotide sequence that comprises one or more of:

(i) a 5' homology arm that is or comprises SEQ ID NO: 39;

(ii) a promoter that is or comprises SEQ ID NO: 76;

(iii) a signal peptide encoded by a polynucleotide sequence that is or comprises SEQ ID NO: 143;

(iv) a transgene that is or comprises SEQ ID NO: 145;

(v) a linker encoded by a polynucleotide that is or comprises SEQ ID NO: 140;

(vi) a transgene that is or comprises SEQ ID NO: 146;

(vii) a terminator that is or comprises SEQ ID NO: 78; and/or

(viii) a 3' homology arm that is or comprises SEQ ID NO: 44.

[0187] In some embodiments, an expression cassette encodes a polypeptide that comprises one or more of:

(i) a signal peptide encoded that is or comprises SEQ ID NO: 144;

(ii) a transgene that is or comprises SEQ ID NO: 147; and/or

(iii) a linker encoded hat is or comprises SEQ ID NO: 141.

[0188] In some embodiments, an expression cassette encodes a polypeptide that comprises one or more of:

(i) a signal peptide encoded that is or comprises SEQ ID NO: 144;

(ii) a linker encoded that is or comprises SEQ ID NO: 141; and/or

(iii) a transgene that is or comprises SEQ ID NO: 148.

[0189] In some embodiments, an expression cassette encodes a polypeptide that comprises one or more of:

- (i) a signal peptide encoded that is or comprises SEQ ID NO: 144; and/or
- (iv) a transgene that is or comprises SEQ ID NO: 142.

[0190] In some embodiments, an expression cassette encodes a polypeptide that comprises one or more of:

- (i) a signal peptide encoded that is or comprises SEQ ID NO: 144;
- (ii) a transgene that is or comprises SEQ ID NO: 147;
- (iii) a linker encoded that is or comprises SEQ ID NO: 141; and/or
- (iv) a transgene that is or comprises SEQ ID NO: 148.

Transgenes

[0191] In some embodiments, a transgene is a corrective gene chosen to improve one or more signs and/or symptoms of a disease, disorder, or condition. In some embodiments, transgenes are functional versions of disease associated genes (i.e., gene isoform(s) which are associated with the manifestation or worsening of a disease, disorder or condition) found in a subject. In some embodiments, one or more transgenes are optimized versions of disease-associated genes found in a subject (e.g., codon optimized or expression-optimized variants). In some embodiments, transgenes are variants of disease-associated genes found in a subject (e.g., a functional gene fragment or variant thereof). In some embodiments, a transgene is a gene that causes expression of a peptide that is normally expressed in one or more healthy tissues.

[0192] In some embodiments, a transgene is a gene that causes expression of an altered protein with a gain- or loss-of-function mutation. In some embodiments, a transgene is a gene that causes expression of a fusion protein. In some embodiments, a transgene is a gene that causes expression of an antibody agent. In some embodiments, a transgene is a gene that causes expression of a multispecific antibody. In some embodiments, a transgene is a fragment of an antibody, antigen, or protein. In some embodiments, a transgene is a gene that causes expression of an enzyme (e.g., for enzyme replacement therapy). In some embodiments, a transgene is a gene that causes expression of a cytokine. In some embodiments, a transgene is a gene that causes expression of a cytokine receptor. In some embodiments, a transgene is a gene that causes expression of a chimeric antigen receptor (CAR). In some embodiments, a transgene is a gene

that causes expression of an anti-thrombotic molecule. In some embodiments, a transgene is a gene that causes expression of a coagulation factor. In some embodiments, a transgene is a gene that causes expression of a glucose response element. In some embodiments, a transgene is a gene that causes expression of a nanobody. In some embodiments, a transgene is a gene that causes expression of fusion protein or a variant thereof. In some embodiments, a transgene is a gene that causes expression of ALP or a fusion protein thereof. In some embodiments, a transgene is a gene that causes expression of ALP that comprises of a glycosylphosphatidylinositol (GPI) linkage. In some other embodiments, a transgene is a gene that causes expression of ALP that does not comprise of a glycosyl-phosphatidylinositol (GPI) linkage. In some embodiments, a transgene is a gene that causes expression of fusion protein comprising ALP and human immunoglobulin fragment crystallizable region ("ALP-Fc") or a variant thereof.

[0193] In some embodiments, a transgene is or comprises a gene encoding a functional nucleic acid. In some embodiments, a therapeutic agent is or comprises an agent that has a therapeutic effect upon a host cell or subject (including, *e.g.*, a ribozyme, guide RNA (gRNA), antisense oligonucleotide (ASO), miRNA, siRNA, and/or shRNA). For example, in some embodiments, a therapeutic agent promotes a biological process to treat a medical condition, *e.g.*, at least one symptom of a disease, disorder, or condition, such as Hypophosphatasia (HPP).

[0194] In some embodiments, transgene expression in a subject results substantially from integration at a target locus. In some embodiments, 75% or more (*e.g.*, 80% or more, 85% or more, 90% or more, 95% or more, 99% or more, 99.5% or more) of total transgene expression in a subject is from transgene integration at a target locus. In some embodiments, 25% or less (*e.g.*, 20% or less, 15% or less, 10% or less, 5% or less, 1% or less, 0.5% or less, 0.1% or less) of total transgene expression in a subject is from a source other than transgene integration at a target locus (*e.g.*, episomal expression, integration at a non-target locus).

[0195] In some embodiments, a transgene is or comprises a sequence having at least 80%, 85%, 90%, 95%, 99%, or 100% identity to a corresponding wild-type reference nucleotide sequence (*e.g.*, a wild-type gene sequence). In some embodiments, a transgene is or comprises a sequence having a least 80%, 85%, 90%, 95%, 99%, or 100% identity to a portion of a corresponding wild-type reference nucleotide sequence (*e.g.*, a wild-type gene sequence).

[0196] In some embodiments, a polynucleotide sequence encoding a transgene is or comprises one or more sequences in Table 5. In some embodiments, a polynucleotide sequence encoding a transgene is or comprises SEQ ID NO: 77. In some embodiments, an amino acid sequence encoding a transgene is or comprises SEQ ID NO: 133. In some embodiments, an amino acid sequence encoding a transgene is or comprises SEQ ID NO: 134. In some embodiments, an amino acid sequence encoding a transgene is or comprises SEQ ID NO: 135. In some embodiments, an amino acid sequence encoding a transgene is or comprises SEQ ID NO: 139. In some embodiments, a polynucleotide sequence encoding a transgene is or comprises one or more sequences in Table 6. In some embodiments, an amino acid sequence encoding a transgene is or comprises SEQ ID NO: 120. In some embodiments, an amino acid sequence encoding a transgene is or comprises SEQ ID NO: 136. In some embodiments, an amino acid sequence encoding a transgene is or comprises SEQ ID NO: 137. In some embodiments, an amino acid sequence encoding a transgene is or comprises SEQ ID NO: 138.

[0197] In some embodiments, a transgene is or comprises a polynucleotide sequence that is or comprises SEQ ID NO: 133. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 70% sequence identity to SEQ ID NO: 133. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 75% sequence identity to SEQ ID NO: 133. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 80% sequence identity to SEQ ID NO: 133. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 85% sequence identity to SEQ ID NO: 133. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 133. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 95% sequence identity to SEQ ID NO: 133. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 99% sequence identity to SEQ ID NO: 133. In some embodiments, a transgene consists of a polynucleotide sequence of SEQ ID NO: 133.

[0198] In some embodiments, a transgene is or comprises a polynucleotide sequence that is or comprises SEQ ID NO: 139. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 70% sequence identity to SEQ ID NO: 139. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 75%

sequence identity to SEQ ID NO: 139. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 80% sequence identity to SEQ ID NO: 139. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 85% sequence identity to SEQ ID NO: 139. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 139. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 95% sequence identity to SEQ ID NO: 139. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 99% sequence identity to SEQ ID NO: 139. In some embodiments, a transgene consists of a polynucleotide sequence of SEQ ID NO: 139.

[0199] In some embodiments, a transgene is or comprises a polynucleotide sequence that is or comprises SEQ ID NO: 145. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 70% sequence identity to SEQ ID NO: 145. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 75% sequence identity to SEQ ID NO: 145. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 80% sequence identity to SEQ ID NO: 145. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 85% sequence identity to SEQ ID NO: 145. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 145. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 95% sequence identity to SEQ ID NO: 145. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 99% sequence identity to SEQ ID NO: 145. In some embodiments, a transgene consists of a polynucleotide sequence of SEQ ID NO: 145.

[0200] In some embodiments, a transgene is or comprises a polynucleotide sequence that is or comprises SEQ ID NO: 146. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 70% sequence identity to SEQ ID NO: 146. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 75% sequence identity to SEQ ID NO: 146. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 80% sequence identity to SEQ ID NO: 146. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 85% sequence identity to SEQ ID NO: 146. In some embodiments, a transgene is or comprises a

polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 146. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 95% sequence identity to SEQ ID NO: 146. In some embodiments, a transgene is or comprises a polynucleotide sequence having at least 99% sequence identity to SEQ ID NO: 146. In some embodiments, a transgene consists of a polynucleotide sequence of SEQ ID NO: 146.

[0201] In some embodiments, a transgene encodes a polypeptide that is or comprises SEQ ID NO: 142. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 142. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 75% sequence identity to SEQ ID NO: 142. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 80% sequence identity to SEQ ID NO: 142. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 85% sequence identity to SEQ ID NO: 142. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 90% sequence identity to SEQ ID NO: 142. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 95% sequence identity to SEQ ID NO: 142. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 99% sequence identity to SEQ ID NO: 142. In some embodiments, a transgene encodes a polypeptide that consists of SEQ ID NO: 142.

[0202] In some embodiments, a transgene encodes a polypeptide that is or comprises SEQ ID NO: 147. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 147. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 75% sequence identity to SEQ ID NO: 147. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 80% sequence identity to SEQ ID NO: 147. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 85% sequence identity to SEQ ID NO: 147. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 90% sequence identity to SEQ ID NO: 147. In some embodiments, a

transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 95% sequence identity to SEQ ID NO: 147. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 99% sequence identity to SEQ ID NO: 147. In some embodiments, a transgene encodes a polypeptide that consists of SEQ ID NO: 147.

[0203] In some embodiments, a transgene encodes a polypeptide that is or comprises SEQ ID NO: 148. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 148. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 75% sequence identity to SEQ ID NO: 148. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 80% sequence identity to SEQ ID NO: 148. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 85% sequence identity to SEQ ID NO: 148. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 90% sequence identity to SEQ ID NO: 148. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 95% sequence identity to SEQ ID NO: 148. In some embodiments, a transgene encodes a polypeptide that is or comprises a polypeptide sequence having at least 99% sequence identity to SEQ ID NO: 148. In some embodiments, a transgene encodes a polypeptide that consists of SEQ ID NO: 148.

Homology arms

[0204] In some embodiments, a payload (*e.g.*, expression cassette) may comprise one or more flanking polynucleotide sequences with significant sequence homology to a target locus (*e.g.*, homology arms). In some embodiments, homology arms flank a polynucleotide sequence encoding a payload (*e.g.*, one homology arm is 5' to a payload (also referred to herein as a 5' homology arm) and one homology arm is 3' to a payload (also referred to herein as a 3' homology arm)). In some embodiments, homology arms direct site-specific integration of a payload.

[0205] In some embodiments, homology arms are between 400 and 1000 nt in length. In some embodiments, homology arms are between 800 and 1500 nt in length. In some

embodiments, homology arms are at least 500 nt in length. In some embodiments, homology arms are less than 1500 nt in length. In some embodiments, homology arms are 1000 nt in length. In some embodiments, homology arms are at least 50 nt in length. In some embodiments, homology arms are at least 600 nt in length. In some embodiments, homology arms are of the same length. In some embodiments, homology arms are not of the same length. In some embodiments, homology arms have at least 70% sequence homology to a target locus. In some embodiments, homology arms have at least 80% sequence homology to a target locus. In some embodiments, homology arms have at least 90% sequence homology to a target locus. In some embodiments, homology arms have at least 95% sequence homology to a target locus. In some embodiments, homology arms have at least 99% sequence homology to a target locus. In some embodiments, homology arms have 100% sequence homology to a target locus. In some embodiments, homology arms have at least 70% sequence identity to a target locus. In some embodiments, homology arms have at least 80% sequence identity to a target locus. In some embodiments, homology arms have at least 90% sequence identity to a target locus. In some embodiments, homology arms have at least 95% sequence identity to a target locus. In some embodiments, homology arms have at least 99% sequence identity to a target locus. In some embodiments, homology arms have 100% sequence identity to a target locus.

[0206] In some embodiments, constructs comprising homology arms provide rates of target site integration of at least 5%. In some embodiments, constructs comprising homology arms provide rates of target site integration of 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more. In some embodiments, constructs comprising homology arms provide rates of target site integration of 30% or more. In some embodiments, constructs comprising homology arms provide rates of target site integration of 35% or more. In some embodiments, a homology arm is or comprises a sequence in Table 1. In some embodiments, a homology arm is or comprises SEQ ID NO: 39. In some embodiments, a homology arm is or comprises SEQ ID NO: 40. In some embodiments, a homology arm is or comprises SEQ ID NO: 41. In some embodiments, a homology arm is or comprises SEQ ID NO: 42. In some embodiments, a homology arm is or comprises SEQ ID NO: 43. In some embodiments, a homology arm is or comprises SEQ ID NO: 44. In some embodiments, a homology arm is or comprises SEQ ID NO: 45. In some embodiments, a homology arm is or comprises SEQ ID NO: 46. In some embodiments, a homology arm is or comprises SEQ ID NO:

47. In some embodiments, a homology arm is or comprises SEQ ID NO: 48. In some embodiments, a homology arm is or comprises SEQ ID NO: 49. In some embodiments, a homology arm is or comprises SEQ ID NO: 50. In some embodiments, a homology arm is or comprises SEQ ID NO: 51. In some embodiments, a homology arm is or comprises SEQ ID NO: 52. In some embodiments, a homology arm is or comprises SEQ ID NO: 53. In some embodiments, a homology arm is or comprises SEQ ID NO: 54. In some embodiments, a homology arm is or comprises SEQ ID NO: 118. In some embodiments, a homology arm is or comprises SEQ ID NO: 119. In some embodiments, a homology arm is or comprises SEQ ID NO: 121. In some embodiments, a homology arm is or comprises SEQ ID NO: 122. In some embodiments, a homology arm is or comprises SEQ ID NO: 123. In some embodiments, a homology arm is or comprises SEQ ID NO: 124.

[0207] In some embodiments, a 5' homology arm is or comprises a polynucleotide sequence of SEQ ID NO: 39. In some embodiments, a 5' homology arm is or comprises a polynucleotide sequence having at least 70% sequence identity to SEQ ID NO: 39. In some embodiments, a 5' homology arm is or comprises a polynucleotide sequence having at least 75% sequence identity to SEQ ID NO: 39. In some embodiments, a 5' homology arm is or comprises a polynucleotide sequence having at least 80% sequence identity to SEQ ID NO: 39. In some embodiments, a 5' homology arm is or comprises a polynucleotide sequence having at least 85% sequence identity to SEQ ID NO: 39. In some embodiments, a 5' homology arm is or comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 39. In some embodiments, a 5' homology arm is or comprises a polynucleotide sequence having at least 95% sequence identity to SEQ ID NO: 39. In some embodiments, a 5' homology arm is or comprises a polynucleotide sequence having at least 99% sequence identity to SEQ ID NO: 39. In some embodiments, a 5' homology arm consists of a polynucleotide sequence of SEQ ID NO: 39.

[0208] In some embodiments, a 3' homology arm is or comprises a polynucleotide sequence of SEQ ID NO: 44. In some embodiments, a 3' homology arm is or comprises a polynucleotide sequence having at least 70% sequence identity to SEQ ID NO: 44. In some embodiments, a 3' homology arm is or comprises a polynucleotide sequence having at least 75% sequence identity to SEQ ID NO: 44. In some embodiments, a 3' homology arm is or comprises a polynucleotide sequence having at least 80% sequence identity to SEQ ID NO: 44. In some

embodiments, a 3' homology arm is or comprises a polynucleotide sequence having at least 85% sequence identity to SEQ ID NO: 44. In some embodiments, a 3' homology arm is or comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 44. In some embodiments, a 3' homology arm is or comprises a polynucleotide sequence having at least 95% sequence identity to SEQ ID NO: 44. In some embodiments, a 3' homology arm is or comprises a polynucleotide sequence having at least 99% sequence identity to SEQ ID NO: 44. In some embodiments, a 3' homology arm consists of a polynucleotide sequence of SEQ ID NO: 44.

Targeted Integration

[0209] In some embodiments, compositions disclosed herein direct integration of a payload (*e.g.*, a transgene) at a target locus (*e.g.*, an endogenous gene). In some embodiments, compositions and constructs provided herein direct integration of a payload at a target locus in a specific cell type (*e.g.*, naïve B cells, B lineage cell populations, etc.). In some embodiments, compositions and constructs provided herein direct integration of a payload (*e.g.*, expression cassette comprising a transgene encoding an ALP-Fc fusion protein) at a target locus in a specific cell type (*e.g.*, naïve B cells, B lineage cell populations, etc.). In some embodiments, a payload is or comprises a transgene or variant thereof encoding ALP or a fusion protein or a variant thereof, *e.g.*, an ALP-Fc fusion protein or a variant thereof.

[0210] In some embodiments, compositions and constructs provided herein direct integration of a payload at a target locus that is considered a safe-harbor site (*e.g.*, CCR5, AAVS1). In some embodiments, a target locus is selected from any genomic site appropriate for use with methods and compositions provided herein. In some embodiments, a target locus encodes a polypeptide. In some embodiments, a target locus encodes a polypeptide that is highly expressed in a subject (*e.g.*, a subject not suffering from a disease, disorder, or condition, or a subject suffering from a disease, disorder, or condition). In some embodiments, a target locus is selected from one or more of CD19, CD20, IGH, B2M, CCR5, JCHAIN, PAX5, IRF4, IRF8, BACH2, EZH2, XBP1, CARD11, PRDM1, and BAFF.

B lineage cell culture methods

[0211] Various methods for culturing and long-term maintenance of B lineage cells *in vitro* are described in the art (See, Rawlings et al. 1995, Rawlings et al 1997, and Fluckinger et al. 1998, each of which is incorporated by reference herein in its entirety). B lineage cell culturing conditions can significantly affect normal human B lineage development and for production of mature Ig-secreting B cells. Without wishing to be bound by any theory, it is generally thought that activation of B lineage cells *ex vivo* may be necessary for later genome editing approaches that make use of homology-directed repair (HDR), as required DNA repair proteins are present during the G₂/S phases of the cell cycle (See, Rogers and Cannon 2021, incorporated herein by reference in its entirety).

Activation of B lineage cells

[0212] Various technologies for activation of B lineage cells *in vitro* have been described (also interchangeably referred to as “activated B lineage cells”). Traditionally, B lineage cell activation and proliferation *in vitro* employed CD40L-expressing feeder cell layer systems. Such feeder cell systems were described as difficult to standardize and often unreliable for providing consistent levels of activation and proliferation of B lineage cells. Recent advances have shifted to protocols for *in vitro* activation and proliferation of B lineage cells in specific culture systems comprising cytokines or other components in the absence of feeder cells (See Jourdan et al., 2009 and Hartweger et al., 2019, each of which is incorporated herein by reference in its entirety).

[0213] In some embodiments, methods for B lineage cell activation comprise contacting cells with media comprising one or more components of the present disclosure. In some embodiments, methods for B lineage cell activation comprise contacting cells with media comprising one or more cytokines and/or oligonucleotides (*e.g.*, multimeric human CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG). In some embodiments, methods for B cell activation comprise contacting cells with media comprising at least about 5 ng/mL, 10 ng /mL, 15 ng/mL, 20 ng/mL, 25 ng/mL, 30 ng/mL, 35 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 55ng/mL, 60 ng/mL, 65 ng/mL, 70 ng/mL, 75 ng/mL, 80 ng/mL, 85 ng/mL, 90 ng/mL, 95 ng/mL, 100 ng/mL, 150 ng/mL, 200 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL, or 1 ug/mL of one or more cytokines and/or oligonucleotides (*e.g.*, multimeric human CD40L, IL-2, IL-10, IL-

15, IL-21 and/or CpG) . In some embodiments, methods for B lineage cell activation comprise contacting cells with media comprising at least about 0.1 ug/mL, 0.2 ug/mL, 0.3 ug/mL, 0.4 ug/mL, 0.5 ug/mL, 0.6 ug/mL, 0.7 ug/mL, 0.8 ug/mL, 0.9 ug/mL, 1 ug/mL, 1.5 ug/mL, 2 ug/mL, 2.5 ug/mL, 3 ug/mL, 3.5 ug/mL, 4 ug/mL, 4.5 ug/mL, or 5 ug/mL of one or more cytokines and/or oligonucleotides (*e.g.*, multimeric human CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG).

[0214] In some embodiments, methods for B lineage cell activation comprise a step of contacting cells with media comprising one or more components of the present disclosure for at least about 1, 2, 3, 4, 5, 6, or 7 days. In some embodiments, methods for B lineage cell activation comprise a step of contacting cells with media comprising one or more cytokines and/or oligonucleotides (*e.g.*, CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG) for at least about 1, 2, 3, 4, 5, 6, or 7 days. In some embodiments, methods for B lineage cell activation comprise a step of contacting cells with media comprising one or more cytokines and/or oligonucleotides (*e.g.*, CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG) for at least 2 days. In some embodiments, methods for B lineage cell activation comprise a step of contacting cells with media comprising one or more cytokines and/or oligonucleotides (*e.g.*, CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG) for at least 2 days, followed by a step of gene editing. In some embodiments, methods for B lineage cell activation comprise a step of contacting cells with media comprising one or more cytokines and/or oligonucleotides (*e.g.*, CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG) for at least 2 days, followed by a step of B lineage cell expansion. In some embodiments, methods for B lineage cell activation comprise a step of contacting cells with media comprising one or more cytokines and/or oligonucleotides (*e.g.*, CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG) for at least 2 days, followed by a step of B lineage cell expansion for at least about 1, 2, 3, 4, 5, 6, 7, or 8 days.

[0215] In some embodiments, methods described herein result in an activated B lineage cell population.

Differentiation of B cells into plasmablasts

[0216] Plasmablasts are short-lived, rapidly produced effector cells that are primarily present in an early antibody response and are one potential product of terminal B cell differentiation. Plasmablasts are capable of secreting antibodies, including IgM subtype

antibodies, in order to mount an immediate response to certain antigens in the body.

Differentiation of B cells into plasmablasts *in vitro* may be promoted through use of certain signaling molecules, including one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, and/or IL-15). A variety of methods for differentiation of B cells into plasmablasts are known, but not limited to, those outlined in WO/2018/170150, incorporated herein by reference in its entirety. Plasmablasts produced by such methods may be characterized as cells that are CD27⁺ / CD38⁺ / CD138⁻.

[0217] In some embodiments, methods for B cell differentiation into plasmablasts comprise contacting cells with media comprising one or more components. In some embodiments, methods for B cell differentiation into plasmablasts comprise contacting activated B lineage cells with media comprising one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, and/or IL-15). In some embodiments, methods for B cell differentiation into plasmablasts comprise contacting activated B lineage cells with media comprising at least about 0.5 ng/mL, 1 ng/mL, 1.5 ng/mL, 2 ng/mL, 2.5 ng/mL, 3 ng/mL, 3.5 ng/mL, 4 ng/mL, 4.5 ng/mL, 5 ng/mL, 10 ng/mL, 15 ng/mL, 20 ng/mL, 25 ng/mL, 30 ng/mL, 35 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 55 ng/mL, 60 ng/mL, 65 ng/mL, 70 ng/mL, 75 ng/mL, 80 ng/mL, 85 ng/mL, 90 ng/mL, 95 ng/mL, or ng/mL of one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, and/or IL-15).

[0218] In some embodiments, methods for B cell differentiation into plasmablasts comprise a step of contacting cells with media comprising one or more components of the present disclosure for at least about 1, 2, 3, or 4 days. In some embodiments, methods for B cell differentiation into plasmablasts comprise a step of contacting activated B lineage cells with media comprising one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, and/or IL-15) for at least about 1, 2, 3, or 4 days. In some embodiments, methods for B cell differentiation into plasmablasts comprise a step of contacting activated B lineage cells with media comprising one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, and/or IL-15) for at least 3 days. In some embodiments, methods for B cell differentiation into plasmablasts comprise a step of contacting activated B lineage cells with media comprising one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, and/or IL-15) for at least 3 days, followed by a step of plasmablast expansion.

Differentiation of plasmablasts into plasma cells

[0219] Although plasmablasts secrete more antibodies than naïve B cells, they are shorter-lived and secrete fewer antibodies than plasma cells (PCs). Long-lived plasma cells (LLPCs, used interchangeably throughout with plasma cells) localize to bone marrow in the body and are capable of secreting high levels of antibodies and surviving for decades in the absence of proliferation (See, Hammerland et al., 2017 and Khodadadi et al., 2019, both of which incorporated herewith in their entirety). Differentiation of plasmablasts to long-lived plasma cells can be triggered by certain events in the body, including, *e.g.*, activity of transcription factors Blimp-1/PRDM1 and IRF4. Differentiation of plasmablasts to plasma cells *in vitro* may be promoted through use of certain signaling molecules, including one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β). A variety of methods for differentiation of plasmablasts into plasma cells are known, but not limited to, those outlined in Jourdan et al. 2019 and WO/2018/170150 (each of which incorporated herein by reference in its entirety). Plasma cells produced by such methods may be characterized as cells that are CD27⁺ / CD38⁺ / CD138⁺.

[0220] In some embodiments, methods for plasmablast differentiation into plasma cells comprise contacting cells with media comprising one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β). In some embodiments, methods for plasmablast differentiation into plasma cells comprise contacting plasmablasts with media comprising one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β). In some embodiments, methods for plasmablast differentiation into plasma cells comprise contacting plasmablasts with media comprising at least about 0.5 ng/mL, 1 ng/mL, 1.5 ng/mL, 2 ng/mL, 2.5 ng/mL, 3 ng/mL, 3.5 ng/mL, 4 ng/mL, 4.5 ng/mL, 5 ng/mL, 10 ng /mL, 15 ng/mL, 20 ng/mL, 25 ng/mL, 30 ng/mL, 35 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 55 ng/mL, 60 ng/mL, 65 ng/mL, 70 ng/mL, 75 ng/mL, 80 ng/mL, 85 ng/mL, 90 ng/mL, 95 ng/mL, or ng/mL of one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β).

[0221] In some embodiments, methods for plasmablast differentiation into plasma cells comprise a step of contacting cells with media comprising one or more components of the present disclosure for at least about 1, 2, 3, or 4 days. In some embodiments, methods for plasmablast differentiation into plasma cells comprise a step of contacting plasmablasts with media comprising one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β) for at least about 1, 2, 3, or 4 days. In some embodiments, methods for plasmablast differentiation into plasma cells comprise a step of contacting plasmablasts with media comprising one or more cytokines (*e.g.*,

IL-6, IL-15, and/or IFN α -2 β) for at least 3 days. In some embodiments, methods for plasmablast differentiation into plasma cells comprise a step of contacting plasmablasts with media comprising one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β) for at least 3 days, followed by a step of cell isolation. In some embodiments, methods for plasmablast differentiation into plasma cells comprise a step of contacting plasmablasts with media comprising one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β) for at least 3 days, followed by a step of administration to a subject.

Engineered cell preparations

[0222] The present disclosure describes engineered cell preparations comprising populations of cells modified to perform one or more desired functions. In some embodiments, engineered cell preparations are compositions comprising genetically modified immune cell populations (*e.g.*, B cell, T cell). In some embodiments, engineered cell preparations are compositions comprising genetically modified B lineage cell populations (*e.g.*, B cell, plasmablast, plasma cell). In some embodiments, engineered cell preparations are compositions comprising genetically modified plasmablast cell populations. In some embodiments, engineered cell preparations are compositions comprising genetically modified plasma cell populations.

[0223] In some embodiments, engineered cell preparations are genetically modified to express a payload (*e.g.*, transgene) of interest. In some embodiments, engineered cell preparations are genetically modified to express a transgene from an expression cassette (*e.g.*, comprising additional polynucleotide sequence elements).

[0224] In some embodiments, engineered cell preparations comprise genetically modified cells that express a transgene of interest (*e.g.*, therapeutic protein, antibody, etc.). In some embodiments, engineered cell preparations comprise genetically modified cells that express a transgene of interest (*e.g.*, therapeutic protein, antibody, etc.) from an endogenous gene locus. In some embodiments, engineered cell preparations comprise genetically modified cells that express a transgene of interest (*e.g.*, therapeutic protein, antibody, etc.) from an endogenous gene locus under control of an endogenous promoter. In some embodiments, engineered cell preparations comprise genetically modified cells that express a transgene of interest (*e.g.*, therapeutic protein, antibody, etc.) from an endogenous gene locus under control of an

exogenous promoter. In some embodiments, engineered cell preparations comprise genetically modified cells that express a transgene of interest (*e.g.*, therapeutic protein, antibody, etc.) from an endogenous gene locus without disrupting endogenous gene expression and/or function. In some embodiments, engineered cell preparations comprise genetically modified cells that express a transgene of interest (*e.g.*, therapeutic protein, antibody, etc.) from an endogenous gene locus and partially or fully disrupt endogenous gene expression and/or function.

[0225] In some embodiments, engineered cell preparations are genetically modified to express a transgene from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding one or more promoters. In some embodiments, engineered cell preparations are genetically modified to express a transgene from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding one or more enhancers. In some embodiments, engineered cell preparations are genetically modified to express a transgene from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding one or more terminators. In some embodiments, engineered cell preparations are genetically modified to express a transgene from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding one or more homology arms. In some embodiments, engineered cell preparations are genetically modified to express a transgene from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding one or more promoters, one or more enhancers, one or more terminators, and/or one or more homology arms. In some embodiments, engineered cell preparations are genetically modified to express an ALP-Fc fusion protein transgene, or variant thereof, from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding a MND promoter, a WPRE enhancer, a BGH polyA, a 5' homology arm, and a 3' homology arm. In some embodiments, engineered cell preparations are genetically modified to express an ALP-Fc fusion protein transgene, or variant thereof, from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding a MND promoter, a BGH polyA, a 5' homology arm, and a 3' homology arm. In some embodiments, engineered cell preparations are genetically modified to express an ALP-Fc fusion protein transgene, or variant thereof, from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide

sequences encoding a MND promoter, a WPRE enhancer, a SV40 polyA, a 5' homology arm, and a 3' homology arm. In some embodiments, engineered cell preparations are genetically modified to express an ALP-Fc fusion protein transgene, or variant thereof, from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding a MND promoter, a SV40 polyA, a 5' homology arm, and a 3' homology arm. In some embodiments, engineered cell preparations are genetically modified to express an ALP-Fc fusion protein transgene, or variant thereof, from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding an EF-1a promoter, a WPRE enhancer, a BGH polyA, a 5' homology arm, and a 3' homology arm. In some embodiments, engineered cell preparations are genetically modified to express an ALP-Fc fusion protein transgene, or variant thereof, from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding an EF-1a promoter, a BGH polyA, a 5' homology arm, and a 3' homology arm. In some embodiments, engineered cell preparations are genetically modified to express an ALP-Fc fusion protein transgene, or variant thereof, from an expression cassette, wherein the expression cassette further comprises one or more polynucleotide sequences encoding an EF-1a promoter, a SV40 polyA, a 5' homology arm, and a 3' homology arm.

Production

[0226] The present disclosure describes production of certain engineered B lineage cell preparations. As disclosed herein, in some embodiments, a B lineage cell preparation comprises both engineered and non-engineered cells. In some embodiments, an engineered B lineage cell population comprises plasmablasts. In some embodiments, an engineered B lineage cell population comprises plasma cells. In some embodiments, an engineered B lineage cell population comprises long-lived plasma cells. In some embodiments, an engineered B lineage cell population comprises plasmablasts, plasma cells, and/or long-lived plasma cells and/or any mixtures or combinations thereof disclosed herein.

Plasmablast preparation

[0227] In some embodiments, an engineered B lineage cell preparation comprises plasmablasts. As understood in the art, plasmablasts are rapidly produced and short-lived effector cells of the early antibody response. Plasmablasts can be generated from activated B lineage cells using methods described herein. In some embodiments, engineered plasmablasts may be generated from engineered activated B lineage cells.

[0228] In some embodiments, plasmablast cell populations may be contacted with media comprising one or more components of the present disclosure (*e.g.*, IL-6, IL-15, and/or IFN α -2 β) in order to initiate differentiation into plasma cell populations. In some embodiments, methods for plasmablast cell population differentiation into a plasma cell population comprise contacting plasmablast cell population with media comprising one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β). In some embodiments, methods for a plasmablast cell population differentiation into a plasma cell population comprise contacting plasmablast cell population with media comprising at least about 0.5 ng/mL, 1 ng/mL, 1.5 ng/mL, 2 ng/mL, 2.5 ng/mL, 3 ng/mL, 3.5 ng/mL, 4 ng/mL, 4.5 ng/mL, 5 ng/mL, 10 ng/mL, 15 ng/mL, 20 ng/mL, 25 ng/mL, 30 ng/mL, 35 ng/mL, 40 ng/mL, 45 ng/mL, 50 ng/mL, 55 ng/mL, 60 ng/mL, 65 ng/mL, 70 ng/mL, 75 ng/mL, 80 ng/mL, 85 ng/mL, 90 ng/mL, 95 ng/mL, or ng/mL of one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β).

[0229] In some embodiments, methods for plasmablast cell population differentiation into a plasma cell population comprise a step of contacting cells with media comprising one or more components of the present disclosure for at least about 1, 2, 3, or 4 days. In some embodiments, methods for plasmablast cell population differentiation into a plasma cell population comprise a step of contacting plasmablast cell population with media comprising one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β) for at least about 1, 2, 3, or 4 days. In some embodiments, methods for plasmablast cell population differentiation into a plasma cell population comprise a step of contacting plasmablast cell population with media comprising one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β) for at least 3 days. In some embodiments, methods for plasmablast cell population differentiation into a plasma cell population comprise a step of contacting plasmablast cell population with media comprising one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β) for at least 3 days, followed by a step of cell isolation. In some embodiments, methods for plasmablast cell population differentiation into a plasma cell

population comprise a step of contacting plasmablasts with media comprising one or more cytokines (*e.g.*, IL-6, IL-15, and/or IFN α -2 β) for at least 3 days, followed by a step of administration to a subject.

Plasma cell preparation

[0230] In some embodiments, an engineered B lineage cell preparation comprises a plasma cell population. In some embodiments, a plasma cell population comprises predetermined engineered plasma cell precursors (*e.g.*, plasma blasts) that, upon administration to a subject, further differentiates into a mature plasma cell population. As understood in the art, a mature plasma cell population comprises quiescent, non-dividing cells of the humoral immune response that are capable of secreting large amounts of antibodies. In some embodiments, a mature plasma cell population can comprise short-lived plasma cells and/or long-lived plasma cells (LLPCs) and/or any combination thereof.

Cryopreservation of cell populations

[0231] Cell therapies provide a number of potential advantages over traditional treatment options, at least in part due to the unique properties of viable cells within the body. However, it has been recognized by those in the art that successful clinical outcomes require that cell therapies be quickly and effectively delivered to a subject. Thus, it is essential to develop preservation and storage techniques to maintain a standardized efficacy until they can be transported and administered to a subject. Furthermore, it is equally crucial to recognize and identify conditions that will increase viability and function of the cell population, as each cell population has different structural considerations for preservation (See, Meneghel, 2020, incorporated herein by reference in its entirety). Numerous people have worked hard to try to address these concerns and develop useful preservation technologies, but these again are very cell-specific and can lead to various inadequacies. For example, there are reports describing significant drops in B lineage cell subpopulations when attempting to preserve over an extended duration (See, Ticha et al., 2021, incorporated herein by reference in its entirety). The present disclosure seeks to address these challenges in the field by providing novel culturing conditions, methodology and technologies that achieve effective cryopreservation of certain cell populations (*e.g.*, engineered B lineage cell populations).

[0232] Storage conditions are one of several considerations necessitated for cryopreservation. Short-term storage at lowered temperatures (*e.g.*, 2-4 days at 4°C) may not sufficiently preserve cell therapies such that they maintain normal metabolic processes, viability, and/or expression of any payload of interest. Accordingly, cell therapies often need stable storage at temperatures below -130°C, which prevents cellular metabolic changes. Cryopreservation processes are well-established for certain cell types, such as hematopoietic stem cells (HSCs) for transplantation, with over 47,000 procedures carried out in Europe in 2018 (See, Passweg et al, 2020, incorporated herein by reference in its entirety). In order to maintain consistency and reproducibility of cell therapies, cryopreservation protocols must be clear and precise, with specific requirements for each step of freezing, storage, and thawing prior to patient administration. Cryopreservation may also be used at multiple points in a process chain for cell therapy, *e.g.* multiple times between isolation of cells and patient administration.

[0233] Although various compositions and components for cryopreservation have been tested for a number of different cell types, optimal cryopreservation conditions may vary between cell therapies depending on specific requirements and features of each therapy. For example, in some embodiments, cryopreservation conditions that preserve activity of one type of cell therapy do not maintain activity of another type of cell therapy. Similarly, in some embodiments, cryopreservation conditions that preserve viability of a particular cell type do not maintain viability and/or activity of a cell therapy employing an engineered cell of the same cell type.

[0234] The present disclosure provides certain technologies that achieve effective cryopreservation of certain cell populations (*e.g.*, of certain B lineage cell populations).

Cryoprotective agents (CPAs)

[0235] Cryopreservation may cause a number of cellular injuries, including, *e.g.*, potentially harmful changes in cell morphology, characteristics (*e.g.*, adhesion, cell surface markers, protein expression profile), metabolic activity (*e.g.*, proliferative ability, potency), and/or function (*e.g.*, immunomodulation, signal responsiveness), and may also lead to cell death. Temperature changes associated with cryopreservation methods may cause an adverse response in cells, including, *e.g.*, activation of certain stress response pathways upon initial freezing and/or activation of apoptotic and necrotic pathways upon thawing. Ice crystal

formation in media during cryopreservative freezing can also cause significant cellular damage through, *e.g.*, physical interruption of cellular functions and processes and through solute exclusion and increased localized solute concentration, which may result in increased osmolarity and osmotic stress (See, Meneghel et al, 2020). Temperature changes may also lead to intracellular ice formation, which can cause cell damage and death.

[0236] In order to mitigate cellular injuries as a result of temperature changes, additive compounds known as cryoprotective agents (also referred to interchangeably herein as cryoprotectants) may be added to cell media. Cryoprotective agents (CPAs) have relatively low toxicity to cells, particularly at lower temperatures. CPAs may also exclude ice crystals and maintain a larger, unfrozen extracellular portion of media at certain temperatures, reducing osmotic effects and stress on cells (See, Meneghel et al, 2020). CPAs (*e.g.*, DMSO) may also promote cell dehydration, limiting probability of intracellular ice formation and interacting with / stabilizing cellular membranes (See, Gao et al, 2000). CPAs that diffuse through the outer cell membrane may also be capable of altering intracellular osmotic concentration, reducing and/or regulating intracellular ice formation, dehydration, etc (See, Whaley et al 2021). Mixtures of permeable and non-permeable CPAs may be employed to provide desired effects and can be optimized for particular cell types (*e.g.*, cell therapies) (See, Balci and Can 2013). Certain CPAs may also be optimized for exposure time, exposure temperature, loading time, loading temperature, etc., to balance cryoprotective effects with potential toxicity to cells. For example, permeable CPAs (*e.g.*, DMSO) may have a cell membrane permeability that is approximately 100 – 1000 times lower than that of water, requiring a minimum period of incubation with cells in order to allow equilibration of protectant (See, Mazur et al, 2004).

[0237] In some embodiments, cryopreserved cell preparations provided and/or utilized in accordance with the present disclosure (*e.g.*, cryopreserved B lineage cell preparation) comprise one or more CPAs disclosed herein. In some embodiments, cryopreserved cell preparations comprise one or more permeable CPAs. In some embodiments, cryopreserved cell preparations comprise DMSO. In some embodiments, cryopreserved cell preparations comprise glycerol. In some embodiments, cryopreserved cell preparations comprise ethylene glycol. In some embodiments, cryopreserved cell preparations comprise propylene glycol.

[0238] In some embodiments, cryopreserved cell preparations comprise one or more non-permeable CPAs. In some embodiments, cryopreserved cell preparations comprise glucose. In some embodiments, cryopreserved cell preparations comprise sucrose. In some embodiments, cryopreserved cell preparations comprise trehalose. In some embodiments, cryopreserved cell preparations comprise raffinose. In some embodiments, cryopreserved cell preparations comprise hydroxyethyl starch (HES). In some embodiments, cryopreserved cell preparations comprise polyvinylpyrrolidone (PVP). In some embodiments, cryopreserved cell preparations comprise polyvinyl alcohol (PVA). In some embodiments, cryopreserved cell preparations comprise polyethylene glycol (PEG). In some embodiments, cryopreserved cell preparations comprise dextran. In some embodiments, cryopreserved cell preparations comprise one or more albumin proteins. In some embodiments, cryopreserved cell preparations comprise human serum albumin (HSA). In some embodiments, cryopreserved cell preparations comprise blood serum. In some embodiments, cryopreserved cell preparations comprise poloxamers (*e.g.*, P188). In some embodiments, cryopreserved cell preparations comprise mannitol. In some embodiments, cryopreserved cell preparations comprise glutathione. In some embodiments, cryopreserved cell preparations comprise thioredoxin. In some embodiments, cryopreserved cell preparations comprise glutathione. In some embodiments, cryopreserved cell preparations comprise ascorbic acid. In some embodiments, cryopreserved cell preparations comprise mitoquinone. In some embodiments, cryopreserved cell preparations comprise salidroside. In some embodiments, cryopreserved cell preparations comprise resveratrol. In some embodiments, cryopreserved cell preparations comprise N-acetyl-L-cysteine. In some embodiments, cryopreserved cell preparations comprise catalase. In some embodiments, cryopreserved cell preparations comprise α -tocopheryl acetate. In some embodiments, cryopreserved cell preparations comprise lactated ringers. In some embodiments, cryopreserved cell preparations comprise dextrose.

[0239] In some embodiments, cryopreserved cell preparations comprise one or more permeable CPAs and one or more non-permeable CPAs. In some embodiments, cryopreserved cell preparations comprise DMSO and HSA. In some embodiments, cryopreserved cell preparations comprise lactated ringers and dextrose.

[0240] In some embodiments, cryoprotectants are added to cell preparations in a particular order to facilitate cryopreservation (*e.g.*, HSA followed by the addition of DMSO). In

some embodiments, one or more non-permeable CPAs is added first, followed by a permeable CPA. In some embodiments, one or more non-permeable CPAs is added first, followed by another non-permeable CPA. In some embodiments, one or more permeable CPAs is added first, followed by a non-permeable CPA. In some embodiments, one or more permeable CPAs is added first, followed by another permeable CPA.

[0241] In some embodiments, cryoprotectants are added to cell preparations at a particular temperature in order to facilitate cryopreservation. In some embodiments, cryoprotectants are added at a temperature of about -5 °C, -4 °C, -3 °C, -2 °C, -1 °C, 0 °C, 1 °C, 2 °C, 3 °C, 4 °C, 5 °C, 6 °C, 7 °C, 8 °C, 9 °C, or 10 °C. In some embodiments, cryoprotectants are added at a temperature of about 4 °C. In some embodiments, cryoprotectants are added at a temperature of about 10 °C, 11 °C, 12 °C, 13 °C, 14 °C, 15 °C, 16 °C, 17 °C, 18 °C, 19 °C, 20 °C, 21 °C, 22 °C, 23 °C, 24 °C, 25 °C, 26 °C, 27 °C, 28 °C, 29 °C, or 30 °C. In some embodiments, cryoprotectants are added at a temperature of about 20 °C. In some embodiments, cryoprotectants are added at a temperature of about 25 °C, 26 °C, 27 °C, 28 °C, 29 °C, 30 °C, 31 °C, 32 °C, 33 °C, 34 °C, 35 °C, 36 °C, 37 °C, 38 °C, 39 °C, 40 °C, 41 °C, 42 °C, 43 °C, 44 °C, or 45 °C. In some embodiments, cryoprotectants are added at a temperature of about 37 °C.

[0242] In some embodiments, cryoprotectants are added to cell preparations and allowed to equilibrate during an incubation period to facilitate cryopreservation. In some embodiments, cell preparations are incubated with cryoprotectants at a temperature of about 5 °C, -4 °C, -3 °C, -2 °C, -1 °C, 0 °C, 1 °C, 2 °C, 3 °C, 4 °C, 5 °C, 6 °C, 7 °C, 8 °C, 9 °C, or 10 °C. In some embodiments, cell preparations are incubated with cryoprotectants at a temperature of about 4 °C. In some embodiments, cell preparations are incubated with cryoprotectants at a temperature of about 10 °C, 11 °C, 12 °C, 13 °C, 14 °C, 15 °C, 16 °C, 17 °C, 18 °C, 19 °C, 20 °C, 21 °C, 22 °C, 23 °C, 24 °C, 25 °C, 26 °C, 27 °C, 28 °C, 29 °C, or 30 °C. In some embodiments, cell preparations are incubated with cryoprotectants at a temperature of about 20 °C. In some embodiments, cell preparations are incubated with cryoprotectants at a temperature of about 25 °C, 26 °C, 27 °C, 28 °C, 29 °C, 30 °C, 31 °C, 32 °C, 33 °C, 34 °C, 35 °C, 36 °C, 37 °C, 38 °C, 39 °C, 40 °C, 41 °C, 42 °C, 43 °C, 44 °C, or 45 °C. In some embodiments, cell preparations are incubated with cryoprotectants at a temperature of about 37 °C.

[0243] In some embodiments, cell preparations are incubated with cryoprotectants for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 minutes. In some embodiments, cell preparations are incubated with cryoprotectants for at least about 10 minutes. In some embodiments, cell preparations are incubated with cryoprotectants for at least about 30, 31, 32, 33, 34, 35, or 36 minutes. In some embodiments, cell preparations are incubated with cryoprotectants for at least about 36 minutes. In some embodiments, cell preparations are incubated with cryoprotectants for at least about 120, 121, 122, 123, 124, 125, 126, or 127 minutes. In some embodiments, cell preparations are incubated with cryoprotectants for at least about 127 minutes.

[0244] In some embodiments, cell preparations are exposed to cryoprotectants for a certain fixed time period prior to a freezing process. In some embodiments, cell preparations are exposed to cryoprotectants for no more than about 20, 21, 22, 23, 24, or 25 minutes. In some embodiments, cell preparations are exposed to cryoprotectants for no more than about 25 minutes. In some embodiments, cell preparations are exposed to cryoprotectants for no more than about 45, 46, 47, 48, 49, 50, or 51 minutes. In some embodiments, cell preparations are exposed to cryoprotectants for no more than about 51 minutes. In some embodiments, cell preparations are exposed to cryoprotectants for no more than about 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, or 142 minutes. In some embodiments, cell preparations are exposed to cryoprotectants for no more than about 142 minutes.

Freezing methods

[0245] Cooling rate is another factor for consideration when optimizing cryopreservation protocols. Appropriate freezing rates may vary between cell types and can also be affected by media components and cryoprotectants. Cellular injury during freezing may arise from both extracellular and intracellular ice formation and controlled cooling rate can affect when and where ice forms (See, Meneghel et al 2020). Relatively slow cooling rates can result in ice forming first within an extracellular media, excluding solutes from the ice crystals and leading to increased osmolarity and potential osmotic shock to the cells. These changes in osmolarity can also lead to partial dehydration of cells, which can provide a beneficial effect by reducing probability of intracellular ice formation and/or stabilizing cell membranes. Relatively high cooling rates may prevent cellular dehydration from occurring prior to freezing, leading to increased intracellular ice levels and cellular damage. The present disclosure, among other

things, recognizes that differences exist between various cell types in viability and function under different cooling rates. In order to address these cell-to-cell variations for cryopreservation, the present disclosure employs a process comprising of both heating and cooling phases in a process known as controlled cooling.

[0246] In some embodiments, a controlled cooling method can be used. In some embodiments, controlled cooling comprises a step of cooling a chamber until a cell preparation reaches about ± 1 -10°C. In some embodiments, controlled cooling comprises a step of cooling the chamber at a rate of ± 1 -25°C / minute until the cryopreservation cell preparation reaches about ± 1 -20°C. In some embodiments, controlled cooling comprises a step of cooling the chamber at a rate of ± 1 -25°C / minute until the chamber reaches about ± 1 -40°C. In some embodiments, controlled cooling comprises a step of heating the chamber at a rate of about ± 1 -10°C / minute until the chamber reaches about ± 1 -12°C. In some embodiments, controlled cooling comprises a step of cooling the chamber at a rate of ± 1 -10°C / minute until the chamber reaches ± 1 -40°C. In some embodiments, controlled cooling comprises a step of cooling the chamber at a rate of ± 1 -10°C / minute until the chamber reaches ± 1 -90°C.

[0247] In some embodiments, a controlled cooling method can be used. In some embodiments, controlled cooling comprises a step of cooling a chamber until a cell preparation reaches 4°C. In some embodiments, controlled cooling comprises a step of cooling the chamber at a rate of 1°C / minute until the cryopreservation cell preparation reaches -4°C. In some embodiments, controlled cooling comprises a step of cooling the chamber at a rate of 25°C / minute until the chamber reaches -40°C. In some embodiments, controlled cooling comprises a step of heating the chamber at a rate of 10°C / minute until the chamber reaches -12°C. In some embodiments, controlled cooling comprises a step of cooling the chamber at a rate of 1°C / minute until the chamber reaches -40°C. In some embodiments, controlled cooling comprises a step of cooling the chamber at a rate of 10°C / minute until the chamber reaches -90°C.

Storage methods

[0248] It is recognized by those in the art that large volumes of cells and tissues upon cryopreservation can be stored until the appropriate time and place for pre-clinical and clinical testing and delivery (See, Meneghel et al. 2020). Furthermore, cryopreservation and storage methods described herein provides numerous advantages over conventional short-term storage,

including but not limited to, availability of preparations of consistent viability and functionality, flexibility and predictability for delivery, less cellular waste, reproducibility, and minimization of contamination.

[0249] In some embodiments, B lineage cell preparations may be stored in sterile cryovials (*e.g.*, 1-50 ml). In some embodiments, such cryovials may be hermetically sealed. In some embodiments, B lineage cell preparations may be stored in a cryobag. In some embodiments, such cryobags may be hermetically sealed. In some embodiments, cryobags may additionally be stored within an overwrap bag. In some embodiments, cryobags may be stored in a metallic casing. In some embodiments, cryobags stored within an overwrap bag may be additionally stored in a metallic casing. In some embodiments, B lineage cell preparations may be stored in syringes.

[0250] In some embodiments, B lineage cell preparations are stored at -170 °C. In some embodiments, B lineage cell preparations are stored in ultra-cold freezers (*e.g.*, vapor-based liquid nitrogen-based freezer). In some embodiments, B lineage cell preparations are stored in liquid nitrogen-based containers. In some embodiments, B lineage cell preparations are stored in vapor phase above liquid nitrogen. In some embodiments, B lineage cell preparations are stored in cryogenic storage.

[0251] In some embodiments, B lineage cell preparations are transported through one or more methods to preserve a temperature of about -170°C. In some embodiments, B lineage cell preparations are transported in vapor phase above liquid nitrogen. In some embodiments, B lineage cell preparations are transported on dry ice.

Thawing methods

[0252] The present disclosure, among other things, describes cryopreserved engineered B lineage cell populations that can be used in both pre-clinical and clinical settings. Appropriate thawing rate must be considered in order to both successfully cryopreserved a cell population as well as for administration of said cell population. Thawing rate goes hand-in-hand with controlled cooling rate, as these are co-dependent processes. Rapid cooling will result in the amount of ice within the cryopreserved product to be lower than anticipated. As a circumstance, a relatively rapid rate of warming during thawing is required in order to obviate any potential cellular injuries to occur via further ice crystallization. Furthermore, this will minimize the level

of hypertonic stress that such cryopreserved cell populations experience upon thawing and will result in optimal viability (Sec, Meneghel et al. 2020). It is recognized that slower controlled cooling rates, *e.g.*, in cryobags, the proportion of extracellular ice will be greater. Thus, slower rates of warming during thawing would be necessary to reduced risk of ice recrystallization and reduced osmotic stress.

[0253] In some embodiments, the cryopreserved B lineage cell preparation is transferred from a cryogenic storage into a thawing mechanism. In some embodiments, a thawing mechanism is a dry heater. In some embodiments, a thawing mechanism is a water bath.

Characterization

[0254] The present disclosure provides various methods for characterization of engineered cell populations (*e.g.*, B lineage cell populations). In some embodiments, methods disclosed herein are used to segregate and/or characterize naïve B cell subpopulations within a B lineage cell population. In some embodiments, naïve B cell subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing naïve B cell subpopulations are employed during each step of a controlled cooling method. In some embodiments, methods of characterization are employed to segregate and/or characterize engineered naïve B cell subpopulations. In some embodiments, engineered naïve B cell subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing engineered naïve B cell subpopulations are employed during each step of a controlled cooling method.

[0255] In some embodiments, methods disclosed herein are used to segregate and/or characterize activated B lineage cell subpopulations within a B lineage cell population. In some embodiments, activated B lineage cell subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing activated B lineage cell subpopulations are employed during each step of a

controlled cooling method. In some embodiments, methods disclosed herein are used to segregate and/or characterize engineered activated B lineage cell subpopulations. In some embodiments, engineered activated B lineage cell subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing engineered activated B lineage cell subpopulations are employed during each step of a controlled cooling method.

[0256] In some embodiments, methods disclosed herein are used to segregate and/or characterize plasmablast cell subpopulations within a B lineage cell population. In some embodiments, plasmablast cell subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing plasmablast cell subpopulations are employed during each step of a controlled cooling method. In some embodiments, methods disclosed herein are used to segregate and/or characterize engineered plasmablast cell subpopulations. In some embodiments, engineered plasmablast cell subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing engineered plasmablast cell subpopulations are employed during each step of a controlled cooling method.

[0257] In some embodiments, methods disclosed herein are used to segregate and/or characterize plasma cell precursor subpopulations within a B lineage cell population. In some embodiments, plasma cell precursor subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing plasma cell precursor subpopulations are employed during each step of a controlled cooling method. In some embodiments, methods disclosed herein are used to segregate and/or characterize engineered plasma cell precursor subpopulations. In some embodiments, engineered plasma cell precursor subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of

characterizing engineered plasma cell precursor subpopulations are employed during each step of a controlled cooling method.

[0258] In some embodiments, methods disclosed herein are used to segregate and/or characterize plasma cell subpopulations within a B lineage cell population. In some embodiments, plasma cell subpopulations may be characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing plasma cell subpopulations are employed during each step of a controlled cooling method. In some embodiments, methods disclosed herein are used to segregate and/or characterize engineered plasma cell subpopulations. In some embodiments, engineered plasma cell subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing engineered plasma cell subpopulations are employed during each step of a controlled cooling method.

[0259] In some embodiments, methods disclosed herein are used to segregate and/or characterize short-lived plasma cell populations within a B lineage cell population. In some embodiments, short-lived plasma cell subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing short-lived plasma cell subpopulations are employed during each step of a controlled cooling method. In some embodiments, methods disclosed herein are used to segregate and/or characterize engineered short-lived plasma cell subpopulations. In some embodiments, engineered short-lived plasma cell subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing engineered short-lived plasma cell subpopulations are employed during each step of a controlled cooling method.

[0260] In some embodiments, methods disclosed herein may be used to segregate and/or characterize long-lived plasma cell subpopulations with a B lineage cell population. In some embodiments, long-lived plasma cell subpopulations are characterized through one or more of

flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing long-lived plasma cell subpopulations are employed during each step of a controlled cooling method. In some embodiments, methods disclosed herein are used to segregate and/or characterize engineered long-lived plasma cell subpopulations. In some embodiments, engineered long-lived plasma cell subpopulations are characterized through one or more of flow cytometry, fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and/or affinity chromatography. In some embodiments, one or more methods of characterizing engineered long-lived plasma cell subpopulations are employed during each step of a controlled cooling method.

Methods of Treatment

[0261] The present disclosure, among other things, provides methods of treating a disease, disorder, or condition (e.g., a disease, disorder, or condition described herein) in a subject comprising administering a pharmaceutical composition described herein. In some embodiments, a therapeutically effective amount of a pharmaceutical composition described herein is administered to a subject having a disease or disorder. Pharmaceutical compositions described herein can be for use in the manufacture of a medicament for treating a disease, disorder, or condition (e.g., a disease, disorder, or condition described herein) in a subject.

[0262] Pharmaceutical compositions described herein can comprise one or more B lineage cells selected from a population of genetically modified B lineage cells described herein. In some embodiments, B lineage cell populations are engineered to express or comprise a payload. In some embodiments, a payload is or comprises an expression cassette. In some embodiments, an expression cassette comprises a transgene encoding ALP or a fusion protein thereof, e.g., an ALP-Fc fusion protein.

[0263] A subject to be treated with methods described herein can be a mammal, e.g., a primate, e.g., a human (e.g., a patient having, or at risk of having, a disease, disorder, or condition described herein). In some embodiments, a subject has Hypophosphatasia (HPP). In some embodiments, a subject can be an adult subject. In some embodiments, engineered B lineage cells are administered to a pediatric subject.

[0264] Administration of pharmaceutical compositions described herein may be carried out in any convenient manner (e.g., injection, ingestion, transfusion, inhalation, implantation, or transplantation). In some embodiments, a pharmaceutical composition described herein is administered by injection or infusion. Pharmaceutical compositions described herein may be administered to a subject intravenously transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, or intraperitoneally. In some embodiments, a pharmaceutical composition described herein is administered parenterally (e.g., intravenously, subcutaneously, intraperitoneally, or intramuscularly). In some embodiments, a pharmaceutical composition described herein is administered by intravenous infusion or injection. In some embodiments, a pharmaceutical composition described herein is administered by intramuscular or subcutaneous injection.

[0265] In some embodiments, a pharmaceutical composition described herein is administered at a pharmaceutically suitable dosage to a subject. In some embodiments, a pharmaceutical composition described herein is administered monthly. In some embodiments, a pharmaceutical composition described herein is administered once every other month. In some embodiments, a pharmaceutical composition described herein is administered once every three months. In some embodiments, a pharmaceutical composition described herein is administered once every six months. In some embodiments, a pharmaceutical composition described herein is administered once a year. In some embodiments, a pharmaceutical composition described herein is administered to a subject having Hypophosphatasia (HPP). In some embodiments, a pharmaceutical composition described herein is administered to a subject with mild-to-severe HPP.

[0266] In some embodiments, the methods disclosed herein comprise measuring and/or monitoring treatment of Hypophosphatasia (HPP). In some embodiments, a sample is collected from a subject treated with a pharmaceutical composition described herein. In some embodiments, sample collection is or comprises venipuncture. In some embodiments, sample collection is or comprises tissue collection. In some embodiments, levels of an ALP-Fc fusion protein are measured from sample collection. In some embodiments, lipids are assessed from a collected sample.

[0267] In some embodiments, engraftment of one or more B lineage cells of a population of genetically modified B lineage cells described herein is measured in a non-clinical species (*e.g.*, hIL6-/NOG mice). In some embodiments, engraftment of one or more B lineage cells of a population of genetically modified B lineage cells described herein is measured in a clinical species (*e.g.*, a human subject). In some embodiments, engraftment is measured by use of bioluminescence. In some embodiments, engraftment is measured by use of ELISpot. In some embodiments, levels of plasma IgG are determined to measure engraftment. In some embodiments, levels of plasma IgM are determined to measure engraftment. In some embodiments, levels of a transgene (*e.g.*, ALP or a fusion protein thereof, such as ALP-Fc fusion protein) are determined to measure engraftment. In some embodiments, engraftment of one or more B lineage cells of an engineered B lineage cell population occurs in a non-clinical species (*e.g.*, hIL6-/NOG mice) with or without preconditioning of a non-clinical subject (*e.g.*, chemotherapy, immunosuppressive treatment, etc. of a subject prior to administering B lineage cells as described herein). In some embodiments, engraftment of one or more B lineage cells of an engineered B lineage cell population described herein occurs in a clinical species (*e.g.*, human subject) with or without preconditioning of a clinical subject (*e.g.*, chemotherapy, immunosuppressive treatment, etc. of a subject prior to administering B lineage cells as described herein).

[0268] In some embodiments, a B lineage cell population (*e.g.*, an engineered B lineage cell population) described herein is cryopreserved prior to administration to a subject. In some embodiments, a cryopreservation method is described herein and/or described in WO 2024/173502, which is incorporated herein by reference in its entirety.

EXAMPLES

[0269] The following examples are provided so as to describe to the skilled artisan how to make and use methods and compositions described herein and are not intended to limit the scope of the present disclosure.

Example 1: Materials and Methods

[0270] The present Example demonstrates in vitro assay methods that may be implemented to certain cell populations, including, but not limited to, engineered B lineage cell populations. Cell populations can comprise or be engineered B lineage cell populations that express an ALP or a fusion protein thereof, such as an ALP-Fc fusion protein. Engineered B lineage cell populations can comprise or be naïve B cells. Engineered B lineage cell populations can comprise or be engineered activated B lineage cells. Engineered B lineage cell populations may comprise or be engineered plasmablast. Engineered B lineage cell populations may comprise or be engineered plasma cells.

Culturing Methods (Serum variation)

[0271] The present application provides methods that result in engineered B lineage cell populations. These methods include, but are not limited to, serum variation methods described herein.

[0272] In some embodiments, B lineage cells are isolated and cultured in media comprising one or more cytokines and/or oligonucleotides (*e.g.*, CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG) for at least about 1, 2, 3, 4, 5, 6, or 7 days. In some embodiments, methods for B lineage cell activation comprise a step of contacting cells with media comprising one or more cytokines and/or oligonucleotides (*e.g.*, CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG) for at least 2 days. In some embodiments, methods for B lineage cell activation comprise a step of contacting cells with media comprising one or more cytokines and/or oligonucleotides (*e.g.*, CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG) for at least 2 days, followed by a step of gene editing.

[0273] In some embodiments, B lineage cells are contacted with media lacking serum (*e.g.*, human serum, bovine serum, horse serum, newborn calf serum, goat serum, rabbit serum, porcine serum, chicken serum, or a combination thereof) during gene editing. In some embodiments, B lineage cells subsequently undergo a wash step with base media (*e.g.*, Excellerate) with or without cytokines. In some embodiments, after gene editing, B lineage cells are contacted with media lacking serum (*e.g.*, human serum, bovine serum, horse serum, newborn calf serum, goat serum, rabbit serum, porcine serum, chicken serum, or a combination thereof) and then incubated at 37°C for up to 24 hours. In some embodiments, after gene editing,

B lineage cells are contacted with media substantially free of serum (*e.g.*, human serum, bovine serum, horse serum, newborn calf serum, goat serum, rabbit serum, porcine serum, chicken serum, or a combination thereof) and then incubated at 37°C for up to 24 hours. In some embodiments, B lineage cells after incubation are spun down, supernatant removed, and replated in media comprising serum (*e.g.*, human serum, bovine serum, horse serum, newborn calf serum, goat serum, rabbit serum, porcine serum, chicken serum, or a combination thereof). In some embodiments, B lineage cells are replated in media comprising one or more cytokines and/or oligonucleotides (*e.g.*, CD40L, IL-2, IL-10, IL-15, IL-21 and/or CpG) and expanded for at least 5 days.

Targeted mass spectrometry

[0274] Absolute ALP quantification was evaluated using targeted mass spectrometry. This technique employs tandem mass spectrometry (MS/MS) using high-resolution mass spectrometers, and enabled quantification of ALP-specific peptide sequences. In sum, samples were analyzed using single-point calibration against an ALP-specific standard isotopically labeled (SIL) peptide. For engineered B lineage cell supernatant, sample preparation began with reduction with Tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP) and heat followed by alkylation with iodoacetamide. Protein was then precipitated using methanol/chloroform, and total protein content was assessed with a BCA Protein assay. Subsequently, the protein sample was digested with trypsin and SIL peptides were spiked into the sample. Next, the tryptic peptides were fractionated using basic reverse phase liquid chromatography. The mobile phase consisted of 10 mM triethylamine bicarbonate at pH 7.5 (TEAB) in water as solvent A, and 10 mM TEAB in 90% acetonitrile as solvent B. The peptide fragments were then analyzed via selected reaction monitoring (SRM) on a mass spectrometer. Retention time was determined from prior analyses of synthetic peptide standards and found to be about 10.5 minutes. Sample peptide signal intensities were monitored against that of the SIL peptide. Area under the curve (AUC) for sample and SIL peptides were calculated in Skyline. Sample concentration was mathematically determined based on a ratio of SIL AUC/SIL concentration.

In vitro phenotypic assessment using osteogenic stimulation assay

[0275] A mouse preosteoblast cell line (MC3T3-E1 Subclone 4) was used to assess the ability of B lineage cells to influence *in vitro* mineralization. First, 175,000 MC3T3 cells were added to a 24-well cell culture plate in MC3T3 base media, which is composed of Alpha Minimum Essential Medium with nucleosides (α -MEM), 10% Fetal Bovine serum (FBS), and 1% Penicillin-Streptomycin. After 24 to 72 hours (about 3 days), osteogenesis was initiated by the addition of ascorbic acid and β -glycerophosphate to a final concentration of 200 μ M and 100 μ M, respectively. Following 6 days of osteogenic stimulation, cells were then treated with 5 μ M of inorganic pyrophosphate (PPi) to inhibit mineralization. To prevent the PPi-induced deficit mineralization, cells were also treated with the disclosed ALP and immunoglobulin fusion proteins described herein or other disclosed conditions. Media changes with appropriate supplementation were conducted three times weekly for the duration of the experiment.

[0276] After 21 days (about 3 weeks) of osteogenesis, mineralization was assessed by Alizarin Red S (ARS) staining or by total calcium levels. For ARS staining, cells were washed with PBS and subsequently fixed with 4% paraformaldehyde (PFA) for 15 minutes. After fixation, cells were washed 3X with diH₂O, allowing the water to sit for 5 to 10 minutes at each wash. Next, cells were submerged in ARS and incubated for 20 minutes at room temperature. Following staining, plates were washed three times with distilled water while gently rocking. Finally, fresh distilled water was added to prevent drying, and staining was imaged on a Cytation5 Plate Reader and Imager. Calcium deposition was measured using a colorimetric calcium assay. At first, calcium deposits were extracted by incubating the samples with 0.5 N hydrochloric acid (HCl) for 4 hours. HCl buffer was collected, and samples and standards then were plated in duplicate in a 96-well flat bottom plate. Chromogenic reagent and assay buffer were added to samples and standards. Plates developed for 5 to 10 minutes protected from light and read at 575 nm on a Cytation5 Plate Reader. Sample calcium levels were calculated using linear regression to a standard curve.

Quantification of Immunoglobulin G (IgG) and Immunoglobulin M (IgM)

[0277] MSD immunoassay plates were precoated with capture IgG and IgM antibodies on independent and well-defined spots. Next, the MSD plates were first blocked using 1X PBS with 1% BSA blocking buffer, prior to the addition of calibration standards, quality control

samples, and test samples. Mouse plasma or serum samples were diluted in diluent 100 to a minimum of 64-fold before adding to the MSD plate. All samples were incubated for two hours at room temperature, shaking at 700 rotations per minute (rpm), then the plate was washed, and sulfo-tag labelled anti-human/non-human primate (NHP) detection antibodies were added to all wells at 1:50 dilution in diluent 100 and incubated for two hours at room temperature, shaking at 700 rpm. Analytes in sample bound to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completed the sandwich. After another wash, read buffer was added to all wells, which provided an appropriate chemical environment for electrochemiluminescence. The plate was loaded onto the instrument, where voltage was applied to the plate electrodes and caused captured labels to emit light. The instrument measured the intensity of emitted light to provide quantitative measure of analytes in the sample. IgG and IgM concentrations were calculated by five parameter logistic regression model to appropriate reference protein standards.

Measurement of alkaline phosphatase (ALP) in B lineage cell population supernatant and mouse plasma

[0278] MSD streptavidin immunoassay plates were used to detect human ALP and ALP-Fc fusion proteins in mouse serum or plasma samples. Plates were first blocked using 1X PBS with 1% BSA blocking buffer and incubated for one hour room temperature shaking at 700 rpm. Then, plates were washed, and biotinylated anti-human ALP antibody was added to the plate and incubated for one hour room temperature shaking at 700 rpm. Plates were washed before adding standards, quality control samples, and test samples. Recombinant human ALP was used as a reference standard for ALP containing samples. For ALP-Fc containing samples, internal standards were generated. Mouse plasma or serum samples were diluted in diluent 100 to a minimum of 5-fold before adding to the MSD plate. All samples were incubated for one hour at room temperature, shaking at 700 rpm, then the plate was washed and sulfo-tag labelled anti-human ALP detection antibody was added to all wells. Next, samples were incubated for one hour at room temperature while shaking at 700 rpm. Analytes in sample bind to capture antibodies immobilized on the working electrode surface and recruitment of the detection antibodies by the bound analytes completed the sandwich. After another wash, read buffer is

added to all wells, which provides an appropriate chemical environment for electrochemiluminescence. The plate was loaded onto the instrument, where a voltage applied to the plate electrodes caused captured labels to emit light. The instrument measured the intensity of emitted light to provide quantitative measure of analytes in the sample. Human ALP and ALP-Fc fusion proteins concentrations were calculated by five parameter logistic regression model to appropriate reference protein standards.

[0279] Methods for quantifying cytokines on flow cytometers, such as Legendplex, was used per vendor recommendation to assess ALP or ALP-Fc fusion protein concentration in engineered B lineage cell culture supernatant or mouse plasma samples. Samples from relevant experiments were harvested and frozen at -80°C prior to testing. All samples and reagents were brought to room temperature prior to the initiation of the assay. ALP antibody coated capture beads, LEGENDPlex assay buffer, and standard/sample were added to a 96 well V-bottom plate. Plates were incubated (protected from light) for 2 hours at room temperature while shaking vigorously (at ~800 rpm). Following incubation, plates were washed twice by addition of LEGENDPlex wash buffer and centrifuged for 5 minutes at 250 g at 23°C. Supernatant was removed by flicking.

[0280] Next, biotinylated detection antibody was added and incubated (protected from light) at room temperature for one hour while shaking vigorously. Next, Streptavidin-Phycoerythrin (SA-PE) was added and incubated as described above for 30 minutes. Plates were washed twice with the LEGENDPlex wash buffer and centrifuged for 5 minutes at 250 g at 23°C. Subsequently, supernatant was removed by flicking. Capture beads were resuspended in wash buffer and read on a NovoCyte Penton Flow Cytometer. Data analysis was conducted in FlowJo and analyte signal intensity was assessed by median intensity of PE. Sample ALP or ALP-Fc concentration was calculated by four parameter logistic regression model to the appropriate reference protein standard.

[0281] Due to potential antibody binding alterations and molecular weight differences in ALP and ALP-Fc, ALP and ALP-Fc containing samples were analyzed using different reference standards. Recombinant human ALP was used as a reference standard for ALP containing samples. For ALP-Fc containing samples, an internal standard was generated and described herein. Bulk BCM cell culture supernatant containing ALP-Fc was harvested, aliquoted, banked,

and stored at -80°C. A thawed frozen aliquot was analyzed for absolute ALP quantification using targeted mass spectrometry (TMS; described above). Banked engineered B lineage cell supernatant containing a known amount of ALP-Fc (based on the TMS-derived concentration) was serially diluted and used as a reference standard for ALP-Fc BCM samples.

Immunoglobulin G (IgG) and Immunoglobulin M (IgM) ELISpot of engineered B lineage cell population supernatant

[0282] 96 well multiscreen filter plates were briefly washed with 35% ethanol for 40-60 seconds until the membrane turned gray from white. Plates were then washed 3 times with Dulbecco's PBS (dPBS), and subsequently coated with the capture antibody (anti-human IgG or anti-human IgM) at 10 µg/mL in PBS overnight at 4°C. Next day, the plates were washed 3 times with an automated plate washer. The antibody coated plates were then blocked against non-specific binding with Iscove's Modified Dulbecco's Medium (IMDM) with 1% FBS for 2 hours at 37°C. Next, cells were counted and added to the wells of the plate. The plates were incubated in an incubator at 37°C for 16-24 hours. Next day, the plates were washed for an additional 3 times. Plates were then blocked with 1% BSA at room temperature for 1 hour and washed 3 more times. Next, plate-bound hIgG or hIgM were detected by a secondary biotinylated antibody (anti-human IgG or anti-human IgM) at 1:1000 dilution in 1% BSA and were incubated for 2 hours at room temperature. At the end of incubation, plates were washed as previously described and then with exposed to streptavidin alkaline phosphatase at 1:5000 dilution in 1% BSA. Plates were subsequently incubated at room temperature for 1 hour before undergoing an additional 3 washes. 5-Bromo-4-Chloro-Indolyl-Phosphatase (BCIP) and Nitroblue Tetrazolium (NBT) was then added to the plates for 5-15 minutes, before being washed with tap water. Plates were then tapped onto a blotting paper and dried in the dark overnight. Once plates were dried, they were read on the AID plate reader and analyzed for the number of either human IgG or IgM producing spots.

ALP enzymatic activity analysis for engineered B lineage cell population supernatant

[0283] In order to evaluate the ALP activity of engineered B lineage cell populations as disclosed herein, enzymatic activity was assessed by measuring the hydrolysis of a synthetic substrate 4-methylumbelliferone phosphate (4-MUP), into its fluorescent product, 4-methylumbelliferone (4-MU). All reagents were brought to room temperature prior to the initiation of the assay. B lineage cell timed supernatant samples or standards were added to a black 384 well plate. The reaction was initiated by the addition of 4-MUP, then immediately placed into a Cytation5 plate reader set to 37°C. Fluorescent readings at 360/440 nm were taken in a kinetic fashion every minute for one hour. B lineage Cell-derived ALP enzymatic activity at a point of linearity (e.g., 15 minutes) was calculated by linear regression to a reference 4-MU product standard. In some instances, to enable representative comparison between ALP constructs, activity was normalized to percent HDR as determined by ddPCR.

[0284] Specific activities were also calculated to assess protein purity. For specific activity analyses, all reagents were brought to 37°C. The assay was initiated by addition of 4-MUP to B lineage cell derived samples. Plate fluorescence was read at 360/440 nm excitation/emission on a Cytation5 Plate Reader within a kinetic interval known to produce steady state kinetics. The amount of 4-MU generated was determined through linear regression against a 4-MU standard prepared in the same buffer as B lineage cell samples. Activity was converted to Units, where 1 unit is equal to 1 μ mole of 4-MUP hydrolyzed/minute. Finally, specific activity was calculated by dividing activity units by the amount of ALP assayed. Thus, specific activity is given in Units/mg.

ALP enzymatic activity analysis of blood products from mice dose dosed with ALP-BCM or ALP-BCM derived protein

[0285] To evaluate the ALP activity of engineered B lineage cell populations in BCM-dosed animals as disclosed herein, enzymatic activity was assessed by measuring the hydrolysis of a synthetic substrate 4-methylumbelliferone phosphate (4-MUP), into its fluorescent product, 4-methylumbelliferone (4-MU). All reagents were brought to room temperature prior to the initiation of the assay. Serum samples or standards were added to a black 384 well plate. The reaction was initiated by the addition of 4-MUP. Fluorescent readings at 360/440 nm were taken in a kinetic fashion every minute for one hour on a Cytation5 Plate Reader. Sample ALP

enzymatic activity was calculated by linear regression at a sample dilution that reaches linearity at 15 minutes to the activity of a reference 4-MU product standard. Activity was converted to Units, where 1 unit is equal to 1 μ mole of 4-MUP hydrolyzed/minute, then converted to Units/Liter of serum.

[0286] To evaluate the human-specific ALP activity of engineered B lineage cell populations in BCM-dosed animals as disclosed herein, enzymatic activity was assessed by measuring the hydrolysis of a synthetic substrate 4-methylumbelliferone phosphate (4-MUP), into its fluorescent product, 4-methylumbelliferone (4-MU) by ALP captured by a monoclonal antibody against human TNSALP. All reagents were brought to room temperature prior to the initiation of the reaction. Serum or plasma samples or standards were added to a black 384 well plate coated with anti-human ALP mAb. The reaction was initiated by the addition of 4-MUP to the washed plate. After 2 hours at 37°C (also protected from light) the plate fluorescence was read at 360/440 nm excitation/emission on a Cytation5 Plate Reader. B lineage Cell-derived ALP enzymatic activity was calculated by linear regression to the activity of a reference 4-MU product standard. Activity was converted to Units, where 1 unit is equal to 1 μ mole of 4-MUP hydrolyzed/minute, then converted to mUnits/Liter of sample.

Inference of CRISPR Editing (ICE)

[0287] Cells were collected 96 hours after electroporation from non-engineered control (reference template) and Ribonucleoprotein (RNP)-only engineered conditions. The Maxwell DNA extraction kit was used to extract genomic DNA and normalized to 16.5 ng/mL after quantitation on the NanoDrop Spectrometer. Primers specific to the CRISPR sgRNA site were designed such that the target cut site was ~200 bp downstream of the forward primer or ~200 bp upstream of the reverse primer (within a ~800 bp amplicon). PCR amplification was performed using Q5 HiFi Hot start 2x MasterMix and the recommended thermocycler program. A no-template control was included. Amplicon sizes were checked using the Lonza FlashGel system and shipped for Sanger Sequencing. Analysis of the Sanger sequences traces was performed using the Synthego ICE analytical method run off an internal server.

[0288] gCCR5_232 ICE Forward primer: GCAGCAAACCTTCCCTTCACTAC (SEQ ID NO: 1).

- [0289] gCCR5_232 ICE Reverse primer: AGGATTCCCGAGTAGCAGATGAC (SEQ ID NO: 2).
- [0290] gCCR5_232 ICE Sequencing primer: GGGTGGAACAAGATGGATTATC (SEQ ID NO: 3).
- [0291] gJchain_31 ICE Forward (and sequencing) primer: TTCAGACTTCAACCACAGTTG (SEQ ID NO: 4).
- [0292] gJchain3_1 ICE Reverse primer: AACCACAGACTCAGTGATAGG (SEQ ID NO: 5).
- [0293] gJchain_54 ICE Forward primer: TTGGCATTATAGAAAGGCC (SEQ ID NO: 6).
- [0294] gJchain_54 ICE Reverse (and sequencing) primer: AAAAGCCCTGGTTTCAAATTCATTG (SEQ ID NO: 7).
- [0295] gJtoIGHM_23 ICE Forward (and sequencing) primer: GAATGGCCACTCTAGGGCCTTTG (SEQ ID NO: 8).
- [0296] gJtoIGHM_23 ICE Reverse primer: TGATTTATGATGGTCAAAACGCAG (SEQ ID NO: 9).
- [0297] gB2M_3 ICE Forward primer: AGGACCTTCTCTGAGCTGTC (SEQ ID NO: 10).
- [0298] gB2M_3 ICE Reverse (and sequencing) primer: GAAACAGAGTCTCACTCTGG (SEQ ID NO: 11).
- [0299] gCD19_5 ICE Forward primer: GAAACAGAGTCTCACTCTGG (SEQ ID NO: 12).
- [0300] gCD19_5 ICE Reverse (and sequencing) primer: ACCAGGTTATAGAGCAGAGG (SEQ ID NO: 13).
- [0301] gCD19_109 ICE Forward (and sequencing) primer: CTAGTGGTGAAGGTGGAAGG (SEQ ID NO: 14).

[0302] gCD19_109 ICE Reverse primer: CTCATACTGAAGCCACGAAG (SEQ ID NO: 14).

[0303] gCD19_124 ICE Forward (and sequencing) primer: CTAGTGGTGAAGGTGGAAGG (SEQ ID NO: 15).

[0304] gCD19_124 ICE Reverse primer: CTCATACTGAAGCCACGAAG (SEQ ID NO: 16).

Droplet Digital Polymerase Chain Reaction (ddPCR)

[0305] ddPCR enables quantification of targeted integration efficiency as follows:

$$\frac{\text{copies}/\mu\text{L NHEJ-sensitive probe (FAM)} + \text{droplets (integration site)}}{\text{copies}/\mu\text{L NHEJ-insensitive probe (HEX)} + \text{droplets (reference amplicon)}} * 100 = \% \text{ Targeted integration}$$

[0306] For the integration site amplicon, one primer lies outside of a homology arm and the other lies inside the transgene. Thus, an amplicon was only generated when a genomic integration event has occurred. The reference amplicon was ideally a similar size and generated from either a distal region on the same locus or a reference gene. In this case, a similar size reference amplicon was generated from the CCRL2 reference gene. Amplicon primers were designed using the IDT PrimerQuest tool, and ddPCR probes were designed according to the following rules:

[0307] 1) Designed the FAM with its 5' end directly in the hotspot of the mutations, melting temperature (T_m) = 56-57°C;

[0308] 2) Designed the NHEJ-insensitive probe (HEX) within the amplicon where it is not likely to be mutated, T_m = 60°C;

[0309] 3) Designed primers to produce a 60-150 nt amplicon with 40-60% GC content;

[0310] 4) Primer length should be 18-24 nt with a GC content of 50-60%, T_m = 55°C (50-65°C acceptable); and

[0311] 5) Included two controls: no-template (excludes contamination) and transfection without gRNA (assess impact of endonuclease expression).

[0312] Reaction mix (including replicates) and ddPCR Supermix for Probes (no dUTP, BioRad) were allowed to equilibrate to room temperature. Droplets were generated using the Automated Droplet Generator following the manufacturer's instructions (BioRad). Reactions were then run in the C1000 Touch Thermal Cycler program recommended for the amplicon length. Samples were then read on a QX200 Droplet Reader (BioRad) and analyzed on 2D Amplitude (dot plot) for discrimination between negative, single, and double-positive droplets. Thresholding was done by calculating: $\text{Average of negative droplet intensity} + 0.1 \times (\text{Average of positive droplet intensity} - \text{average of negative droplet intensity}) = \text{Threshold}$

[0313] % Targeted integration was then calculated using the above equation based on the adjusted thresholding and the copies/uL of FAM and HEX.

[0314] The following CCR5 target gene primers and probes were used:

[0315] CCR5 ddPCR Forward primer: catcgcattgtctgagtagg (SEQ ID NO: 17);

[0316] CCR5 ddPCR Reverse primer: CAGTGGATCGGGTGTAAC (SEQ ID NO: 18); and

[0317] CCR5_ddPCR Probe (FAM): TCGGGAGCCTCTTGCTGGAAAATAGAA (SEQ ID NO: 19).

[0318] The following CCRL2 reference gene primers and probes were used:

[0319] CCRL2_ddPCR Forward primer: CCACATCAGAAGGAAGACTAC (SEQ ID NO: 20);

[0320] CCRL2_ddPCR Reverse primer: GCTGTATGAATCCAGGTCC (SEQ ID NO: 21);

[0321] CCRL2_ddPCR Probe (HEX): TGTTTCCTCCAGGATAAGGCAGCTGT (SEQ ID NO: 22);

[0322] CCRL2_ddPCR Forward primer (for pairing with gJCHAIN_54): ACTTCGGTGGAATGGTCAG (SEQ ID NO: 38);

[0323] CCRL2_ddPCR Reverse primer (for pairing with gJCHAIN_54): GCTGTATGAATCCAGGTCC (SEQ ID NO: 39); and

- [0324] CCRL2_ddPCR Probe (HEX) (for pairing with gJCHAIN_54):
TGTTTCCTCCAGGATAAGGCAGCTGT (SEQ ID NO: 40).
- [0325] The following JCHAIN reference gene primers and probes were used:
- [0326] gJchain_31_ddPCR Forward primer: catgcattgtctgagtagg (SEQ ID NO: 23);
- [0327] gJchain_31_ddPCR Reverse primer: GGCCTATTCTTAATCACATTCTG
(SEQ ID NO: 24);
- [0328] gJchain_31_ddPCR Probe (FAM):
TCATCTTGAAATAAGAACCACAGACTCAGTGA (SEQ ID NO: 25);
- [0329] gJchain_54_ddPCR Forward primer: catgcattgtctgagtagg (SEQ ID NO: 26);
- [0330] gJchain_54_ddPCR Reverse primer: GCAAAGCCACCTCAAATATG (SEQ ID
NO: 27);
- [0331] gJchain_54_ddPCR Probe (FAM):
TCAGACTGTGCTAGAGAATACGTACCATG (SEQ ID NO: 28);
- [0332] gJtoIGHM_23_ddPCR Forward primer: catgcattgtctgagtagg (SEQ ID NO: 29);
- [0333] gJtoIGHM_23_ddPCR Reverse primer: CCTCTTCACAACCAGAAGTG (SEQ
ID NO: 30); and
- [0334] gJtoIGHM_23_ddPCR Probe (FAM): TGCTTTGGCCTCAATTCCAGACAC
(SEQ ID NO: 31).
- [0335] The following CD19 reference gene primers and probes were used:
- [0336] gCD19_5_ddPCR Forward primer: catgcattgtctgagtagg (SEQ ID NO: 30);
- [0337] gCD19_5_ddPCR Reverse primer: CAGGAGAGGGAGAGAGATA (SEQ ID
NO: 31);
- [0338] gCD19_5_ddPCR Probe (FAM): TAGAGACAAAGACCCGCAGAGAGAG
(SEQ ID NO: 32);
- [0339] gCD19_109_ddPCR Forward primer: catgcattgtctgagtagg (SEQ ID NO: 33);

- [0340] gCD19_109_ddPCR Reverse primer: GCTCTAGGCTCAGCAATGA (SEQ ID NO: 34);
- [0341] gCD19_109_ddPCR Probe (FAM): GTGAGGTCTGGGACAGGGAGAGATA (SEQ ID NO: 35);
- [0342] gCD19_124_ddPCR Forward primer: catgcattgtctgagtagg (SEQ ID NO: 36);
- [0343] gCD19_124_ddPCR Reverse primer: GGACAGGGAGAGATAAGACAA (SEQ ID NO: 37); and
- [0344] gCD19_124_ddPCR Probe (FAM): GGTCTTTGGCCACACATACAGCTT (SEQ ID NO: 38).

Cell count and viability

[0345] Cells were sampled for the CellacaMx counter after thorough resuspension by gentle pipetting. Cells were gently mixed with the appropriate diluent of AOPI stain, loaded into a Nexcelom count plate, and analyzed for count and viability based on the acquired image and dilution factor.

Flow cytometry analysis

[0346] Following resuspension of the cell samples, 2×10^5 cells were labeled with fluorescently conjugated antibodies in the following manner for flow cytometric analysis. Samples were washed and stained with antibody cocktails focused on immune cell purity (markers: CD19, CD20, CD16, CD56, CD3, CD14, CD45), on B lineage cell phenotyping (markers: CD19, CD20, CXCR4, CD27, CD38, CD138, IgM, IgD), or class switch (markers: IgM, IgG, IgE, IgA). Cells were washed once in PBS, then stained with LIVE/DEAD Fixable Near-IR Dead Cell Stain (Life Technologies) for 10 minutes at room temperature. Cells were washed with Flow Cytometry Staining Buffer (eBioscience) and resuspended in Human TruStain FcX (BioLegend). After 5 minutes of Fc blocking at 4°C, samples were then resuspended in 100 µL of antibody cocktail master mix. Samples were incubated for 20 minutes at 4 °C in the dark, then washed two times before being resuspended in 400 µL staining buffer for acquisition on the

flow cytometer. Single color and FMO controls for voltage gating and compensation were also prepared. Following compensation as guided by the NovoCyt software prompt, >10,000 cellular events were acquired per sample. Files were saved and imported into FlowJo. Live cell singlets were further gated in this software, then population percentages, total events, and geometric mean fluorescence intensity values were exported, formatted, and statistically analyzed.

[0347] For intracellular class switch profiling, after viability staining, 4×10^5 cells were resuspended in fixation buffer. Following the last wash from viability staining, cells were suspended in fixation buffer (Invitrogen) and incubated at room temperature for 20 minutes. Cells were then washed in permeabilization buffer (Invitrogen) three times and resuspended in class switch antibody master mix (prepared in permeabilization buffer). Samples were incubated 20 minutes at room temperature protected from light, then washed 2 times with permeabilization buffer. Pellets were suspended in staining buffer as for fresh cell staining and run with forward/side-scatter gating adjusted for cell shrinkage after fixation.

Western blot and gel electrophoresis

[0348] Engineered B lineage cell supernatants were mixed one to one by volume with Laemmli sample buffer under non-reducing conditions and heated for 1 min at 90°C. Next, samples were run on 4-20% gradient TGX gels (Bio-Rad) at 200V to resolve protein products. Proteins were transferred to PVDF or nitrocellulose membranes (pre-stacked Trans-Blot Turbo Transfer Packs) using the Trans-Blot Turbo Transfer System (Bio-Rad) and manufacturer-recommended transfer conditions. Membranes were blocked for 2 hours in 0.1% Tween 20 in PBS (PBS-T) with 5% BSA at room temperature with gentle agitation. Primary antibodies (goat IgG anti-ALP or anti-IgG-CH1-biotin) in 1% BSA 0.1% PBS-T were incubated at room temperature with the membrane for 2 hours with gentle agitation. Membranes were washed with PBS-T for 30min. Secondary HRP conjugates (donkey anti-goat IgG-HRP or Streptavidin-HRP) in 1% BSA 0.1% PBS-T were incubated at room temperature with the membrane for 1 hour with gentle agitation. Membranes were washed with PBS-T for 45 minutes. Chemiluminescent detection of HRP was performed by soaking the membranes in Clarity Western ECL Substrate (Bio-Rad) for 2 minutes followed by imaging using the Bio-Rad ChemiDoc Imaging System.

De-N/O-glycosylation and intact mass spectrophotometry (MS)

[0349] Protein samples were treated with Protein Deglycosylation Mix II (New England BioLabs) overnight under a denaturing and reducing condition. Next, approximately 0.5 µg of the deglycosylated protein were then acidified with 1 % formic acid before being loaded onto an Agilent polymeric reversed phase (PLRP-S) column (2.1 x 50 mm, 8 µm). Notably the mobile phase component A was composed of 0.1% (v/v) formic acid in water, whereas mobile phase B was composed of 0.1% formic acid in acetonitrile. The acetonitrile gradient (25 to 55%) of mobile phase B was then delivered from 2 to 11 minutes at a flow rate of 0.25 mL/minute. On-line mass measurement was subsequently performed with an Agilent 6530 mass spectrometer (ESI-QTOF) in positive ion mode. Capillary and nozzle voltages were set at 4000 V and 2000 V, respectively. Desolvation and sheath gas temperatures were 350°C and 400°C, respectively, and the nitrogen gas flow was 12 L/minutes. The deconvoluted mass spectra were then calculated from the multiply charged raw data using BioConfirm software.

Analytical size-exclusion chromatography (SEC)

[0350] Approximately 30 µg of each sample was loaded onto a YMC high pressure liquid chromatography (HPLC) 300x8 mm S-5 µm 20 nm column on Waters Separation Module 2695. The mobile phase (300 mM NaCl, 20 mM sodium phosphate, pH 6.8) was delivered at the flow rate of 1.0 mL/min. UV absorbance was monitored at 280 nm.

FcγRIIIa binding assay

[0351] FcγRIIIa binding assay was performed using the Lumit FcγRIIIa (H131) Binding Immunoassay kit according to the manufacturer's instructions (Promega). Briefly, an 8-point, 4 fold titration series was generated for the human IgG negative control, human IgA positive control and test samples starting at 0.25 mg/mL. Next, 25 µL of prepared Tracer-LgBiT solution was added per well into the microplate wells, and 25 µL of control antibodies standards or protein samples were added to their respective wells. Next, 50 µL of the prepared FcγRIIIa (H131)-SmBiT solution was added per well. The plate was then covered with a foil plate seal and incubated with gentle mixing on a plate shaker (300 rpm) for 45 minutes at room

temperature. 25 μ L of prepared Lumit Detection Reagent per well was then added to the microplate. The plate was subsequently incubated at room temperature for 5 minutes and then read on a plate luminometer.

Fc γ RI binding assay

[0352] Fc γ RI binding assay was performed using the Lumit Fc γ RI Binding Immunoassay kit according to the manufacturer's instructions (Promega). Briefly, an 8-point, 4 fold titration series was generated for the human IgG negative control, human IgA positive control and test samples starting at 0.25 mg/mL. Next, 25 μ L of prepared Tracer-LgBiT solution was added per well into microplate wells and 25 μ L of the control antibodies standards or protein samples were added to their respective wells. 50 μ L of the prepared Fc γ RI-SmBiT solution was added per each reaction well. Next, the plate was covered with a foil plate seal and then incubated with gentle mixing on a plate shaker (300 rpm) for 60 minutes at room temperature. Next, 25 μ L of prepared Lumit Detection per well was added to the microplate. The plate was then incubated at room temperature for 5 minutes and read on a plate luminometer.

FcRn binding assay

[0353] FcRn binding assay was performed using the FcRn AlphaLISA Kit according to the manufacturer's instructions (Revvity). A 1x AlphaLISA MES buffer was prepared fresh using Milli-Q water and buffer concentrate. Next, serial dilutions of human IgG positive control, human IgA negative control antibody and protein samples were generated in 1x AlphaLISA MES buffer. To a 384 well plate, 10 μ L of control antibodies or test proteins were added to their appropriate wells and spun at 400xg for 1 minute. Next, 10 μ L of 800 ng/mL human FcRn was added to each well of the plate and spun at 400xg for 1 minute. 20 μ L Bead mix of human IgG conjugated acceptor beads and Streptavidin-donor beads were then added per well and spun at 400xg for 1 minute. The plate was then incubated for 90 minutes at 23°C in the dark and subsequently read on a plate reader.

Glycan profiling

[0354] First, N-linked glycans were released from the protein sample using PNGaseF (New England BioLabs) following the manufacturer's instruction, and were labeled with 2-aminobenzamide (2-AB) (Sigma-Aldrich). The released N-linked glycans were subsequently loaded onto a Waters XBridge Amide column (4.6 × 150 mm, 3.5 μm) with mobile phases A and B made of 0.05% trifluoroacetic acid in acetonitrile and 0.05% trifluoroacetic acid in water, respectively. Next, the water gradient (mobile phase B) of 28 to 42% was delivered from timepoints 5 to 42 minutes onto the column, then changed to water gradient of 42 to 90% from timepoint 42 to 43 minutes at a flow rate of 1.0 mL/minute. The 2-AB labeled glycans were then detected by fluorescence (330 nm excitation, 420 nm emission).

Example 2: Immunoglobulin substitutions prevent heterodimerization within engineered B lineage cells

[0355] The present Example demonstrates that B lineage cell populations engineered with one or more expression cassettes (*e.g.*, ALP or a fusion protein thereof, such as ALP-Fc fusion protein containing expression cassette) may integrate a polynucleotide sequence encoding for a transgene and express said transgene (*e.g.*, ALP or a fusion protein thereof, such as ALP-Fc fusion protein). Further, the present Example demonstrates that substitutions within the immunoglobulin fragment crystallizable region (Fc) may reduce undesirable heterodimer formation with endogenous Fc while maintaining enzymatic function.

[0356] B lineage cells were engineered using methods described herein with a histidine tagged alkaline phosphatase (ALP) and immunoglobulin fragment crystallizable region (ALP-IgG1Fc-His) or guide alone. Protein from engineered B lineage cell populations was then histidine purified and run on a Western blot using the methods described in **Example 1**. Use of anti-alkaline phosphatase antibody revealed two distinct bands in Western blot. Anti-IgG-CH1 antibody blotting revealed IgG heterodimerization, possibly corresponding to the secondary band in the anti-ALP blot (Figure 1A).

[0357] Various methods were tested to reduce heterodimer formation with ALP-Fc fusion proteins and endogenous Fc. One mutant ALP-Fc fusion protein comprised three substitutions (K392D/D399K/K409D) in the Fc domain (ALP-IgG1Fc-3xsubst). Both anti-ALP and anti-IgG CH1 Western blots showed reduced heterodimer formation in engineered B lineage

cells expressing the ALP-Fc fusion protein (Figure 1B). One mutant ALP-Fc fusion protein comprised three substitutions (K392D/D399K/K409D) in the Fc domain (ALP-IgG4Fc-3xsubst). Anti-ALP Western blot showed reduced heterodimer formation in engineered B lineage cells expressing ALP-IgG4Fc-3xsubst fusion protein (Figure 26).

[0358] In some embodiments, B lineage cells may be engineered to express a transgene which encodes an alkaline phosphatase (ALP) and immunoglobulin fragment crystallizable region fusion protein (ALP-Fc). In some embodiments, the Fc region is derived from human immunoglobulin 1 (IgG1Fc). In some embodiments, the Fc region is derived from human immunoglobulin 4 (IgG4Fc). In some embodiments, one or more substitutions may be introduced within the Fc region of the ALP-Fc fusion protein. In some embodiments, one or more substitutions within the Fc region comprise K392D, D399K, and/or K409D. In some embodiments, one or more substitutions within the IgG1Fc region comprise K392D, D399K, and/or K409D. In some embodiments, one or more substitutions within the IgG4Fc comprise K392D, D399K, and/or R409D. In some embodiments, incorporation of one or more substitutions within the Fc region of an ALP-Fc fusion protein may reduce formation of heterodimers with endogenous Fc. In some embodiments, incorporation of one or more substitutions within the Fc region of an ALP-Fc fusion protein may reduce formation of heterodimers with endogenous Fc while maintaining comparable enzymatic activity. In some embodiments, ALP-Fc fusions may be further optimized to reduce formation of heterodimers with endogenous Fc, while maintaining comparable enzymatic activity. In some embodiments, ALP-Fc fusions may be further optimized to reduce formation of heterodimers with endogenous Fc, while maintaining improving enzymatic activity.

[0359] Among other things, the present disclosure demonstrates that engineered B cells generated with compositions and methods described herein can express functional ALP-Fc fusion proteins. In some embodiments, the Fc region of the ALP-Fc fusion protein is derived from human immunoglobulin G1 (IgG1Fc). In some embodiments, the Fc region of the ALP-Fc fusion protein is derived from human immunoglobulin G4 (IgG4Fc). Further, the present disclosure demonstrates that substitutions within the Fc region, including but not limited to, K392D, D399K, and/or K409D of IgG1Fc or K392D, D399K, and/or R409D of IgG4Fc may reduce heterodimerization of the fusion protein with endogenous Fc.

Example 3: Engineered B lineage cell populations can integrate, express and/or secrete proteins of interest

[0360] The present Example demonstrates that B lineage cell populations engineered with one or more expression cassettes (*e.g.*, ALP or a fusion protein thereof, such as ALP-Fc fusion protein containing expression cassette) may integrate a polynucleotide sequence encoding for a transgene and express said transgene (*e.g.*, ALP-Fc fusion protein). Subjects treated with engineered cell populations described herein may demonstrate persistent levels of transgene expression (*e.g.*, tissue non-specific alkaline phosphatase) over an extended period of time (*e.g.*, one or more weeks, one or more months, etc.).

[0361] Osteogenic stimulation assay was performed with osteoblast precursor (MC3T3) cells, which were cultured in ascorbic acid and beta-glycerolphosphate over a time course of 21 days as described in Example 1 (Figure 2A). Osteoblast precursor cells were treated at days 6-21 with either media alone, media with pyrophosphate (PPi), or media containing cultured supernatant from un-engineered B lineage cells (Figure 2B). In order to evaluate if alkaline phosphatase activity is restored, osteogenic stimulation assay was performed (Figure 3A) involving osteoblast precursor cells (MC3T3) with 250 or 500 ng/mL of either ALP-Fc fusion proteins with a deca-aspartate tag (ALP-Fc-D10) or alkaline phosphatase with a deca-aspartate tag (ALP-D10) (Figure 3B). Osteogenic stimulation assay was performed involving osteoblast precursor (MC3T3) cells contacted with media alone, media with pyrophosphate (PPi), or 50 ng/mL of either media containing cultured supernatant from B lineage cells engineered to express ALP or electroporated control (EPC) B lineage cells. (Figure 27).

[0362] Mean calcium concentrations were measured (as described in Example 1) for osteoblast precursor cells that were treated with or without PPi, or treated with PPi and engineered B lineage cell derived alkaline phosphatase with a deca-aspartate tag ("BCM ALP-D10") or ALP-Fc fusion protein with a deca-aspartate tag ("BCM ALP-FC-D10") (Figure 4). Further rescue experiments were carried with osteoblast precursor cells treated with media alone, pyrophosphate alone ("Osteo PPi"), and differing concentrations (50, 250, or 500 ng/mL) of engineered B lineage cell derived ALP-Fc fusion protein (Figure 5A) and measured for calcium concentration. Additional calcium measurements, as described in Example 1, were carried out on

osteoblast precursors that were treated with the aforementioned media alone, pyrophosphate alone controls in addition to supernatant from cultured un-engineered B lineage cells, engineered B lineage cell derived ALP-Fc fusion protein, or recombinant human alkaline phosphatase (rhALP) (Figure 5B).

[0363] A 4-MUP activity assay was used to evaluate the enzymatic activity of recombinant ALP (rALP) and engineered B lineage cell derived ALP fusion proteins: ALP-Fc, ALP-D10, or ALP- Fc-D10 using methods described in Example 1 (Figure 6). Subsequently, ALP enzymatic activity was assessed for engineered B lineage cell derived alkaline phosphatase and immunoglobulin fragment crystallizable region fusion protein (ALP-IgG1Fc) or ALP-IgG1Fc with the K392D, D399K, and K409D substitutions (ALP-IgG1Fc-3xsubst) (Figure 7 and Figure 12).

[0364] Additional B lineage cell populations engineered using the constructs described herein targeting either endogenous CCR5 or JCHAIN 31 target locus were assessed for ALP secretion and activity via 4-MUP hydrolysis (Figure 10A, Figure 10B).

[0365] Experiments were conducted to evaluate B lineage cells engineered with ALP-D10 expression cassettes. Percent of targeted integration in vitro was measured using ddPCR as described in Example 1 (Figure 34A). In vitro ALP-D10 secretion was measured in supernatant of engineered B lineage cell populations using methods described in Example 1 (Figure 34B). Bulk in vitro ALP-D10 activity was measured in these engineered B lineage cell populations using a 4-MUP activity assay as described in Example 1 (Figure 34C).

[0366] Engineered B lineage cells expressing ALP-D10 were administered to NOG-IL6 mice. Levels of secreted ALP were measured in mice upon administration (day 0) of ALP-BCMs through day 84 post-administration (Figure 34D). Results were compared to NOG-IL6 mice to which control cells electroporated without vector were administered.

[0367] NOG-IL6 mice were either intravenously or intraperitoneally treated with engineered B lineage cells expressing ALP-Fc fusion protein and measured at days 7-14 post-engraftment for levels of either ALP or ALP-Fc in their serum (Figure 8A). Human immunoglobulin G was measured in mouse serum using methods described in Example 1. Results were compared to a media alone control (PlasmaLyte I.P.) (Figure 8B).

[0368] Engineered B lineage cells expressing ALP-Fc were administered to NOG-IL6 mice. Levels of secreted ALP-Fc were measured in mice upon administration (day 0) of ALP-BCMs through day 84 post-administration (Figure 25A). ALP-Fc was isolated at day 84 post-administration from mice to which ALP-BCMs were administered and assessed for activity in serum (Figure 25B). Levels of human immunoglobulin G were assessed in mice to which ALP-BCMs were administered using methods described in Example 1 (Figure 25C). Mouse weight was also assessed (Figure 25D). Results were compared to NOG-IL6 mice to which control cells electroporated without vector (EPC) were administered.

[0369] Further experiments involving B lineage cells engineered with HPPp0215 (ALP-LvL-IgG4Fc-D10) were administered to NOG-IL6 mice were performed. Levels of secreted ALP-Fc were measured in mice upon administration (day 0) of ALP-BCMs through at least 70 days post-administration (Figure 28A and Figure 29A). hALP activity was then assessed in different mouse donors post-administration (Figure 28B and Figure 29B). Results were compared to NOG-IL6 mice to which control cells electroporated without vector (EPC) were administered.

[0370] B lineage cells engineered with HPPp0215 (ALP-LvL-IgG4Fc-D10) were cryopreserved and expanded as described in Example 1 and were administered to NOG-IL6 mice. Levels of secreted ALP-Fc were measured in mice upon administration (day 0) of ALP-BCMs through day 14 post-administration (Figure 30). Results were compared to NOG-IL6 mice to which control cells electroporated without vector (EPC) were administered.

[0371] Cryopreserved B lineage cells engineered with HPPp0215 (ALP-LvL-IgG4Fc-D10) were administered to NOG-IL6 mice. Levels of secreted ALP-Fc were measured in mice upon administration (day 0) of ALP-BCMs through day 58 post-administration (Figure 31A). hALP activity was assessed at day 58 post-administration (Figure 31B). Results were compared to NOG-IL6 mice to which control cells electroporated without vector (EPC) were administered.

[0372] Among other things, the present disclosure demonstrates that engineered B cells expressing ALP or variants thereof (e.g., ALP-Fc fusion proteins) were effective in restoring bone mineralization (i.e., calcium levels). In some embodiments, the present disclosure demonstrates that engineered B lineage cells were able to express functional ALP-Fc fusion proteins. In some embodiments, the present disclosure demonstrates that cryopreserved

engineered B lineage cells were able to express functional ALP-Fc fusion proteins. In some embodiments, the present disclosure demonstrates that cryopreserved engineered B lineage cells expanded with various expansion processes were able to express functional ALP-Fc fusion proteins. In some embodiments, the present disclosure shows that engineered B lineage cells expressing ALP-Fc fusion proteins were capable of engrafting (e.g., in bone marrow) in a subject (e.g., mouse) for long-term protein expression. In some embodiments, the present disclosure demonstrates that engineered B lineage cells expressing ALP-Fc fusion proteins were capable of engrafting (e.g., in bone marrow) in a subject (e.g., mouse) and to produce and secrete functional ALP (e.g., in serum) for an extended time period. In some embodiments, the present disclosure demonstrates that engineered B lineage cells expressing ALP-IgG4-Fc-D10 fusion proteins were capable of engrafting (e.g., in bone marrow) in a subject (e.g., mouse) and to produce and secrete functional ALP (e.g., in serum) for an extended time period. In some embodiments, the present disclosure shows that treatment of a subject (e.g., mouse) with engineered B lineage cells expressing ALP-Fc fusion proteins does not result in adverse effects in the subject. In some embodiments, the present disclosure shows that treatment of a subject (e.g., mouse) with engineered B lineage cells expressing ALP-Fc fusion proteins could restore bone mineralization.

[0373] In some embodiments, such engineered B lineage cell populations are capable of producing clinically relevant doses of ALP or a fusion protein thereof, such as ALP-Fc fusion protein. In some embodiments, such engineered B lineage cell populations are capable of engraftment in a subject without pre-conditioning. In some embodiments, such engineered B lineage cell populations are capable of long-term expression of a protein (e.g., a protein described herein) in a subject.

Example 4: Engineered B lineage cell populations expressing ALP-Fc fusion proteins with variant linkers may have improved enzymatic activity

[0374] The present Example demonstrates that B lineage cell populations engineered with one or more expression cassettes (e.g., ALP or a fusion protein thereof, such as ALP-Fc fusion protein containing expression cassette) may integrate a polynucleotide sequence encoding for a transgene and express said transgene (e.g., ALP-Fc fusion protein). Further, the present Example demonstrates that integration of a 'long linker' (also interchangeably referred to herein

as “LL”) comprising three continuous regions of tetra-glycine and mono-serine (GGGGSx3) may improve enzymatic activity (Figure 16A). Further, the present Example demonstrates that integration of a linker comprising two continuous regions of tetra-glycine and mono-serine followed by a mono-glycine (GGGGSx2-G) may both improve enzymatic activity and allow for flexibility of ALP.

[0375] ALP-Fc fusion proteins comprising different linkers between the ALP and Fc domains were engineered. These ALP-Fc fusion proteins comprise: (1) a linker comprising a leucine and a lysine (LK), or (2) a linker comprising three continuous regions of tetra-glycine and mono-serine (GGGGSx3). As demonstrated in Figure 9A, ALP-Fc fusion proteins as described herein (ALP-IgG1Fc-3xsubst) were developed with either the LK or GGGGS(x3) linker. Comparison between the LK and GGGGS(x3) linkers revealed increased activity (Figure 9B). This is reflected in further evaluation of enzymatic activity of ALP-IgG1Fc and ALP-IgG1Fc fusion proteins with the K392D, D399K, and K409D substitutions (ALP-IgG1Fc-3xsubst) that further comprise the LK or GGGGS(x3) linker (Figure 9C). Additional enzymatic activity analysis involving ALP-Fc fusion proteins comprising a Fc derived from human immunoglobulin 4 (IgG4) and S288P, F234A, and L235A (PAA) substitutions was assessed with and without addition of the long linker (LL) and the K392D, D399K, and R409D substitutions (3xsubst) indicated a potential increase in enzymatic activity (Figure 9D). Thus, such increased enzymatic active may result from addition of a long linker to the ALP-Fc fusion proteins as described herein, and may also be in combination with substitutions within the Fc region (e.g., PAA and 3xsubst).

[0376] In some embodiments, design, testing, and/or selection of ALP-Fc fusion protein sequences may comprise various methods including, but not limited to, use of software (e.g., AI-assisted software, e.g., AlphaFold2). In some embodiments, such software (e.g., AlphaFold2) may provide information on projected protein structure and/or function. In some embodiments, such software may provide information to assist with selection of one or more linkers (e.g., linkers between domains, such as, e.g., ALP and Fc domains) (Figure 16B and Figure 16C).

[0377] ALP-Fc fusion proteins comprising different linkers between ALP and Fc domains were tested using software modeling. Linker length was systematically decreased, and

GGGGS(x2)-G and GGGGS(x2)-GS linkers were identified as allowing for flexibility of ALP in ALP-Fc fusion proteins while maintaining conformation.

[0378] In some embodiments, such engineered B lineage cell populations are capable of producing ALP or a fusion protein thereof, such as ALP-Fc fusion protein. In some embodiments, such engineered B lineage cell populations are capable of enzymatic activity. In some embodiments, ALP-Fc fusion proteins may further comprise of a linker. In some embodiments, a linker may comprise of three continuous regions of tetra-glycine and mono-serine (GGGGS). In some embodiments, incorporation of the linker comprising GGGGSx3 may improve enzymatic activity. In some embodiments, incorporation of the linker comprising GGGGSx3 may improve activity of the ALP in the ALP-Fc fusion protein. In some embodiments, a linker may comprise of two continuous regions of tetra-glycine and mono-serine (GGGGS) followed by an additional C-terminal mono-glycine (G). In some embodiments, a linker may comprise of two continuous regions of tetra-glycine and mono-serine (GGGGS) followed by an additional C-terminal mono-glycine and mono-serine (GS). In some embodiments, incorporation of the linker comprising GGGGSx2-G may allow for flexibility of ALP and improve activity of the ALP in the ALP-Fc fusion protein while maintaining conformation.

Example 5: Engineered B lineage cell populations expressing ALP-Fc fusion proteins with codon optimization and differing signal peptides may have improved secretion and enzymatic activity

[0379] The present Example demonstrates that B lineage cell populations engineered with one or more expression cassettes (e.g., comprising ALP or a fusion protein thereof, such as ALP-Fc fusion protein containing expression cassette) may integrate a polynucleotide sequence encoding for a transgene and express said transgene (e.g., ALP-Fc fusion protein). Further, the present Example demonstrates that expression cassettes may comprise one or more sequence elements (e.g., enhancers, promoters, etc.) providing enhanced integration, secretion, and/or activity of a transgene (e.g., ALP-Fc fusion protein) as compared to a reference (e.g., expression cassettes that do not comprise one or more sequence elements). In some embodiments, expression cassettes comprise one or more enhancers (e.g., E_μ) or variants thereof providing enhanced integration, secretion, and/or activity of a transgene (e.g., ALP-Fc fusion protein). In

some embodiments, expression cassettes may comprise a codon-optimized variant of a transgene (e.g., ALP-Fc) that provides enhanced integration, secretion, and/or activity of transgene (e.g., ALP-Fc fusion protein) as compared to a reference (e.g., expression cassettes that do not comprise a codon-optimized transgene variant). Further, the present Example demonstrates use of different gRNAs that may result in integration, secretion, and activity of transgenes (e.g., ALP-Fc fusion proteins) at one or more target loci (e.g., CCR5, JCHAIN (e.g., JCHAIN1 and JCHAIN173), etc.).

[0380] ALP-Fc fusion protein comprising different codon optimized regions were engineered using GeneArt ("CO-1"), or an alternative method ("CO-2"). B lineage cells were engineered with either CO-1, CO-2, or non-codon optimized (Unoptimized) expression cassettes as described in Example 1. ALP-Fc secretion from the supernatant of engineered B lineage cells was measured using LegendPlex assay as described within Example 1 (Figure 13). B lineage cells were engineered with expression cassettes comprising either ALP signal peptide ("SP-1") or A1AT signal peptide ("SP-2"). Measurements of ALP-Fc secretion from collected supernatant was analyzed using methods described herein (Figure 14).

[0381] B lineage cells were engineered with expression cassettes described herein using gRNAs targeting either CCR5 or JCHAIN1 ("Locus B") (Figure 11). ALP-Fc fusion protein secretion was measured from collected supernatant and assessed (Figure 15). Further assessment of ALP-Fc fusion protein production from B lineage cells engineered using guides targeting CCR5, JCHAIN173 ("Locus A"), or JCHAIN1 was conducted and compared to an electroporated control ("EPC") (Figure 17). Both Bulk (Figure 18) and Specific (Figure 19) ALP-Fc activity was measured in these engineered B lineage cell populations using a 4-MUP activity assay as described in Example 1.

[0382] Additional experiments were conducted to evaluate B lineage cells engineered with ALP-Fc expression cassettes comprising an EF-1 α or MND ("MND-V1") promoter. ALP-Fc secretion was measured in supernatant of engineered B lineage cell populations using the methods described in Example 1 (Figure 20).

[0383] Further experiments were conducted to evaluate two different donor populations of B lineage cells that were engineered with ALP-Fc expression cassettes comprising MND, Fu_1, Fu_2, Fu_3, or B29 promoter with or without an E μ enhancer element or a variant thereof.

Transgene integration was measured using methods described in Example 1 (Figure 21). ALP-Fc secretion was measured in supernatant of engineered B lineage cell populations using methods described in Example 1 (Figure 22). Bulk ALP-Fc activity was measured in these engineered B lineage cell populations using a 4-MUP activity assay as described in Example 1.

[0384] Additional experiments were conducted to evaluate B lineage cells engineered with ALP-Fc expression cassettes comprising a MND promoter with or without an E μ enhancer element. Percentage of homology-directed repair (% HDR) was measured using ddPCR as described in Example 1 (Figure 24A). ALP-Fc secretion was measured in supernatant of engineered B lineage cell populations using methods described in Example 1 (Figure 24B). Bulk ALP-Fc activity was measured in these engineered B lineage cell populations using a 4-MUP activity assay as described in Example 1 (Figure 24C).

[0385] Further experiments were conducted to evaluate B lineage cells engineered with ALP-Fc expression cassettes comprising a MND promoter with an E μ enhancer element or shortened variant thereof. Percent of targeted integration in vitro was measured using ddPCR as described in Example 1 (Figure 35A). In vitro ALP-Fc secretion was measured in supernatant of engineered B lineage cell populations using methods described in Example 1 (Figure 35B). Bulk in vitro ALP-Fc activity was measured in these engineered B lineage cell populations using a 4-MUP activity assay as described in Example 1 (Figure 35C).

[0386] Further experiments were conducted to evaluate B lineage cells engineered with ALP or ALP-Fc expression cassettes comprising a MND promoter with an E μ enhancer or variant thereof. Various codon optimized ALP or ALP-Fc fusion proteins were generated (CO1, CO2). Full-length or shortened E μ enhancer variants were selected from ENCODE or IMGT databases. Targeted integration was measured using methods described in Example 1 (Figure 37A). ALP-Fc secretion was measured in supernatant of engineered B lineage cell populations using methods described in Example 1 (Figure 37B). Bulk ALP activity was measured in these engineered B lineage cell populations using a 4-MUP activity assay as described in Example 1 (Figure 37C).

[0387] Further experiments were conducted to evaluate B lineage cells engineered with ALP-Fc expression cassettes comprising an MND promoter with or without a shortened E μ enhancer element. Percent of targeted integration in vitro was measured using ddPCR as

described in Example 1 (Figure 33A). In vitro ALP-Fc secretion was measured in supernatant of engineered B lineage cell populations using methods described in Example 1 (Figure 33B). Bulk in vitro ALP-Fc activity was measured in these engineered B lineage cell populations using a 4-MUP activity assay as described in Example 1 (Figure 33C).

[0388] Additional experiments were conducted to evaluate B lineage cells engineered with ALP-Fc expression cassettes comprising an MND promoter with a shortened E μ enhancer element or variant thereof in vivo. B lineage cells engineered with ALP-Fc expression cassettes comprising a MND promoter with a small E μ enhancer (p00983) or without a E μ enhancer (HPPp0215) were administered intravenously to NOG-IL6 mice. Levels of secreted ALP-Fc were measured in mice upon administration (day 0) of ALP-BCMs through day 35 post-administration (Figure 32A). hALP activity was assessed at day 35 post-administration (Figure 32B). Results were compared to NOG-IL6 mice to which control cells electroporated without vector were administered (“unengineered”).

[0389] Additional experiments were conducted to evaluate B lineage cells engineered with ALP-Fc expression cassettes comprising an MND promoter with a shortened E μ enhancer element or variant thereof in vivo. B lineage cells engineered with ALP-Fc expression cassettes comprising a MND promoter with a small E μ enhancer were administered intravenously to NOG-IL6 mice. Levels of secreted ALP-Fc were measured in mice upon administration (day 0) of ALP-BCMs through day 42 post-administration (Figure 36). Results were compared to NOG-IL6 mice to which control cells electroporated without vector were administered (“unengineered”).

[0390] Experiments were conducted to assess the impact of poly-aspartate tags in B lineage cells engineered with ALP expression cassettes without a poly-aspartate tag or comprising an octo-aspartate tag (D8) or deca-aspartate tag (D10). Percent of targeted integration in vitro was measured using ddPCR as described in Example 1 (Figure 38A). Bulk in vitro ALP activity was measured in these engineered B lineage cell populations using a 4-MUP activity assay as described in Example 1 (Figure 38B). The presence of ALP monomers and dimers was assessed by Western Blot (Figure 38C).

[0391] In some embodiments, expression cassettes may comprise one or more elements to increase expression and/or secretion of ALP-Fc fusion proteins, or variants thereof. In some

embodiments, expression cassettes may comprise one or more signal peptides (e.g., ALP signal peptide, A1AT signal peptide, etc.) that increase expression and/or secretion of ALP-Fc fusion proteins, or variants thereof. In some embodiments, expression cassettes may comprise a poly-aspartate tag. In some embodiments, expression cassettes may comprise one or more promoters (e.g., EF-1 α promoter, MND promoter, etc.) that increase expression and/or secretion of ALP-Fc fusion proteins, or variants thereof. In some embodiments, expression cassettes may comprise one or more enhancers (e.g., E μ) or variants thereof that increase transgene integration, expression, and/or secretion of ALP-Fc fusion proteins, or variants thereof. In some embodiments, expression cassettes may comprise one or more E μ enhancers or variants thereof that provide increased protein secretion. In some embodiments, expression cassettes may comprise one or more enhancers (e.g., E μ) or shortened variants thereof that alter transgene integration, expression, and/or secretion of ALP-Fc fusion proteins, or variants thereof. In some embodiments, expression cassettes may comprise one or more E μ enhancers or shortened variants thereof that provide altered protein secretion. In some embodiments, sequences encoding ALP-Fc fusion proteins, or variants thereof, are codon optimized (e.g., GeneArt codon optimization). In some embodiments, a particular target locus (e.g., CCR5, JCHAIN (e.g., JCHAIN1, JCHAIN173)) in B lineage cells may provide increased expression and/or secretion of one or more ALP-Fc fusions, or variants thereof as compared to a reference (e.g., B lineage cells engineered to expression ALP-Fc fusions from an alternative target locus).

Example 6: Immunoglobulin subclasses can impact properties of ALP fusion proteins in Engineered B lineage cell populations expressing ALP-Fc

[0392] The present Example demonstrates that ALP-Fc fusion proteins may exhibit desirable properties associated with increased ALP half-life in vivo. Further, the present Example demonstrates that B lineage cell populations engineered to express ALP-IgG4Fc may produce ALP fusion proteins with an extended half-life when compared to B lineage cell populations engineered to express ALP-IgG1Fc.

[0393] ALP-Fc fusion proteins were purified from cells expressing ALP-Fc expression cassettes as described in Example 1. ALP-IgG4Fc-D10 was purified from the supernatant of BCM engineered to express ALP-Fc and assessed via size exclusion chromatography as

described in Example 1 (Figure 39A). ALP-IgG4Fc-D10 was purified from the supernatant of BCM engineered to express ALP-Fc after three freeze-thaw cycles and assessed via size exclusion chromatography (Figure 39B). Size exclusion chromatography peaks for ALP-IgG4Fc-D10 from freeze-thaw and non-freeze-thaw BCMs were compared (Figure 39C).

[0394] ALP-Fc fusion proteins were purified from cells expressing ALP-Fc expression cassettes as described in Example 1. ALP-IgG4Fc-D10 was purified from the supernatant of BCM engineered to express ALP-Fc and assessed using mass spectrometry as described in Example 1 (Figure 40).

[0395] FcγRIIa binding assay was performed on ALP-Fc fusion proteins purified from ALP expression cassettes as described in Example 1. FcγIIa binding was measured on proteins produced from ALP-IgG4-D10, ALP-IgG4, ALP-IgG1-D10, IgG (positive control), or IgA (negative control) expression cassettes (Figure 41). FcγIIa binding was measured on proteins produced from ALP-IgG4-D10, ALP-IgG1-D10, IgG, or IgA expression cassettes (Figure 45).

[0396] FcγRI binding assay was performed on purified proteins produced from various ALP-Fc fusion protein constructs as described in Example 1. FcγRI binding was measured on proteins produced from ALP-IgG4-D10, ALP-IgG4, ALP-IgG1-D10, IgG (positive control), or IgA (negative control) expression cassettes (Figure 42). FcγRI binding was measured on proteins produced from ALP-IgG4-D10, ALP-IgG1-D10, or IgG expression cassettes (Figure 46).

[0397] FcRn binding assay was performed on purified proteins produced from various ALP-Fc fusion protein constructs as described in Example 1. FcRn binding was measured on proteins produced from ALP-IgG4-D10, ALP-IgG4, ALP-IgG1-D10, IgG (positive control), or IgA (negative control) expression cassettes (Figure 43). FcRn binding was measured on proteins produced from ALP-IgG4-D10, ALP-IgG1-D10, IgA, or IgG expression cassettes (Figure 47).

[0398] N-glycan analysis was performed on purified proteins produced from various ALP-Fc fusion protein constructs as described in Example 1. Sialic acid residues were compared between ALP-IgG4-D10 and ALP-IgG1-D10 (Figure 44). Sialic acid residues were compared between ALP-IgG4-D10 and ALP-IgG1-D10 (Figure 48).

[0399] Alp-Fc levels were measured over time in serum from Tg32 mice dosed with purified proteins produced from ALP-Fc fusion protein constructs as described in Example 1.

ALP-Fc levels were measured in samples from mice dosed with ALP-IgG4Fc-D10, ALP-IgG4Fc and ALP-IgG1Fc-D10 purified from HEK293 cells (Figure 49A). ALP-Fc levels were measured in samples from mice dosed with ALP-IgG4Fc-D10 purified from HEK293 cells, ALP-IgG4Fc-D10 from BCM and ALP-IgG1Fc-D10 from HEK293 (Figure 50A).

[0400] Bulk ALP activity was measured over time in serum from Tg32 mice dosed with purified proteins produced from various ALP-Fc fusion protein constructs as described in Example 1. Bulk ALP activity was measured in samples from mice dosed with ALP-IgG4Fc-D10, ALP-IgG4Fc and ALP-IgG1Fc-D10 purified from HEK293 cells (Figure 49B). Bulk ALP activity was measured in samples from mice dosed with ALP-IgG4Fc-D10 purified from HEK293 cells, ALP-IgG4Fc-D10 from BCM and ALP-IgG1Fc-D10 from HEK293 (Figure 50B).

[0401] In some embodiments, the present disclosure demonstrates that purified ALP-IgG4Fc with substitutions in the Fc region (ALP-IgG4-Fc-PAA) exhibits similar biochemical spectra to ALP-IgG1-Fc. In some embodiments, the present disclosure demonstrates that purified ALP-IgG4Fc exhibits similar biochemical spectra when produced by fresh BCM or BCM after freeze-thaw cycles.

[0402] In some embodiments, the present disclosure demonstrates that ALP-IgG4Fc with substitutions in the Fc region (ALP-IgG4-Fc-PAA) exhibits higher binding to FcRn than ALP-IgG1-Fc. In some embodiments, the present disclosure demonstrates that ALP-IgG4-Fc-PAA shows lower binding to FcγRIIa than ALP-IgG1-Fc. In some embodiments, the present disclosure demonstrates that ALP-IgG4-Fc-PAA shows lower binding to FcγRI than ALP-IgG1-Fc. In some embodiments, the present disclosure demonstrates that ALP-IgG4-Fc-PAA contains increased sialic acid residues when compared to ALP-IgG1-Fc.

[0403] In some embodiments, the present disclosure demonstrates that ALP-IgG4Fc produced by HEK cells has comparable pharmacokinetics in serum to ALP-IgG1Fc. In some embodiment, the present disclosure demonstrates that ALP-IgG4Fc produced by BCM exhibits higher ALP levels and ALP activity over time in serum when compared to ALP-IgG1Fc.

[0404] In some embodiments, ALP-IgG4-Fc-PAA fusion proteins may be associated with decreased immune activation as compared to ALP-IgG1-Fc fusion proteins. In some embodiments, ALP-IgG4-Fc-PAA fusion proteins may be associated with increased cellular

Attorney Docket No. 2014034-0097

recycling as compared to ALP-IgG1-Fc fusion proteins. In some embodiments, ALP-IgG4-Fc-PAA fusion proteins may be associated with decreased endocytosis as compared to ALP-IgG1-Fc fusion proteins. In some embodiments, ALP-IgG4-Fc-PAA fusion proteins may evade receptor-mediated degradation. In some embodiments, ALP-IgG4-Fc-PAA fusion proteins may experience lower receptor-mediated degradation as compared to ALP-IgG1-Fc fusion proteins. In some embodiments, ALP-IgG4-Fc-PAA fusion proteins may have extended half-life in vivo as compared to ALP-IgG1-Fc fusion proteins.

EXEMPLARY SEQUENCES

Table 1 – Exemplary Homology Arms (HA)

Name	Nucleotide Sequence	SEQ ID NO
gCCR5_232 5' HA	ggtgctatagagcacaagattttatttggtgagatggtgcttcatgaattcccccaacagagccaagctctccatctagtggac aggggaagctagcagcaaaccttccttcaactacaaaacttcattgcttggccaaaagagagttaattcaatgtagacatctatg taggcaattaaaaacctattgatgtataaaacagttgcatcattgaggggcaactaaatacattctaggactttataaaagatcac tttttattatgcacagggtggaacaagatggattatcaagtgtcaagtcacatctatgacatcaattattatacatcggagccctg ccaaaaaatcaatgtgaagcaaatcgagcccgccctcctgcctcgcctactactggtgttcattcttggtttgggcaac atgctggtcatcctcatcctgataaaactgcaaaaggctgaagagcatgactgacatctacctgctcaacctggccatctctgac ctgttttcttcttactgtcccttctgggctcactatgctgccgccagtgaggactttggaataacaatgtgtcaactcttgaca	39
gJCHAIN_2 5' HA	tacaaaatcacagatttggagatatatacaaaaatacagatttggagatatatacaaaaatacagatttggagatatatacaaaa tacatatatttggagatatatacaaaaataTATATTTAAATCTGGTAGCTTGGTTAATTAAGGAC TAGTATAAAAAAATTAACATAAGATACACTTGGGACCTTAAAGGACTTTCAT TTCTCTCCCCCTTCCTTCATCTCTCCTCCTTCACTTACCTTCATTTATTATTCTA GGCATGACAAGTGTCAGATGTCTGATTGGAAGACTAATATGAATATATCTA GGTAATAAAATGGAAAATCCCTATTTCAACATTCTTAATGGAATCCCTTCCTT GAAATTATTATTAGTCTTCTGGTTTAAACATTTATATAGTGTAATGCAAAA TAAAAATGATTGTATCAAATTATGGTTTTTATTTGGATGCTTTGCAAGAAAA TACTTAAATGTGAGTCATACATTTTTCCAGTTCAGCATTTAAACTTCAGACT TCAACCACAGTTGTGATTGTTTTTAGTTTGTAGCTGCCTGGAGTGTTATTTT AAGAAAGCAGAAGCACCATCATTTGCACACT	40
gJCHAIN_31 5' HA	atacatatttggagatatatacaaaaataTATATTTAAATCTGGTAGCTTGGTTAATTAAGGAC TAGTATAAAAAAATTAACATAAGATACACTTGGGACCTTAAAGGACTTTCAT TTCTCTCCCCCTTCCTTCATCTCTCCTCCTTCACTTACCTTCATTTATTATTCTA GGCATGACAAGTGTCAGATGTCTGATTGGAAGACTAATATGAATATATCTA GGTAATAAAATGGAAAATCCCTATTTCAACATTCTTAATGGAATCCCTTCCTT GAAATTATTATTAGTCTTCTGGTTTAAACATTTATATAGTGTAATGCAAAA TAAAAATGATTGTATCAAATTATGGTTTTTATTTGGATGCTTTGCAAGAAAA TACTTAAATGTGAGTCATACATTTTTCCAGTTCAGCATTTAAACTTCAGACT TCAACCACAGTTGTGATTGTTTTTAGTTTGTAGCTGCCTGGAGTGTTATTTT AAGAAAGCAGAAGCACCATCATTTGCACACTCCTTATAGATCACACACCTT AACCTGACTTTTTTGTCTCCAGTTTTTCAGAAGAAGTGAAGTCAAGTTGAA GAACCATTGCTTTT	41
gJCHAIN_54 5' HA	TTTTCTAAAGAAAAAATCATTGGCATTTCATAGAAAGGCCAATTTCTCTTAAT AGTTCAATAAGTGGATTGATCTTATAAAAAGGCAGGTGTTCTTTGGAAAT GACAGACTCCAACATCAATTTTTTTAAAAAATTCCTCCTTCTTGTCACTATA AATAACTTGTTTAGACAGATATACAGTTGGGAATAAGCCTAACACAGTAGA AATTGCTGTATGGTGTAGATAAAACAATCATATTATCATATCATTAATTATA TTGCTTACTTTCAACTAATATATATTAAGATTGGAAAAATCCCATAGCTAT	42

Attorney Docket No. 2014034-0097

	ATTTTCTTTACTATTCTTTTAAAAAAGTTTCACTGTGTAGAGAACATATATGCATAAACATAGGTCAATTATATGTCTCCATTAGAAAAATAATAATTGGAAACATGTTCTAGA	
gJtoIGHM_23 3' HA	TGTAGGACTGCAAGATCGCTGCACAGCAGCGAATCGTGAAATATTTTCTTTAGAATTATGAGGTGCGCTGTGTGTCAACCTGCATCTTAAATTCCTTATTGGCTGGAAAAGAGAACTGTCGGAGTGGGTGAATCCAGCCAGGAGGGACGCGTAGCCCCGGTCTTGATGAGAGCAGGGTTGGGGGCAGGGGTAGCCCAGAAACGGTGGCTGCCGTCTGACAGGGGCTTAGGGAGGCTCCAGGACCTCAGTGCCTTGAAGCTGGTTTCCATGAGAAAAGGATTGTTTATCTTAGGAGGCATGCTTACTGTAAAAAGACAGGATATGTTTGAAGTGGCTTCTGAGAAAAATGGTTAAGAAATTATGACTTAAAAATGTGAGAGATTTTCAAGTATATTAATTTTAACTGTCCAAGTATTGAAATCTTATCATTTGATTAAACACCCAT	48
gCD19_5 3' HA	GAACCTCTAGTGGTGAAGGTGGAAGGTATGTCCAAAGGGCAGAAAGGGAAAGGATTGAGGCTGGAACCTTGAGTTGTGGCTGGGTGTCTTGGCTGAGTAACTTACCTCTCTGAGCCTCCATTTTCTTATTTGTAAAATTCAGGAAAGGGTTGGAAGGACTCTGCCGGCTCCTCCACTCCAGCTTTTGGAGTCTCTGCTCTATAACCTGGTGTGAGGAGTCGGGGGGCTTGGAGGTCCCCCACCCTATGCCACACCTCTCTCCCTCTCTCTCCACAGAGGGAGATAACGCTGTGCTGCAGTGCCTCAAGGGGACCTCAGATGGCCCCACTCAGCAGCTGACCTGGTCTCGGGAGTCCCCGCTTAAACCTTCTTAAAACTCAGCCTGGGGCTGCCAGGCCTGGGATCCACATGAGGCCCCCTGGCCATCTGGCTTTTCATCTTCAACGTCTCTCAACAGATGGGGGGCTTCTACCTGTGCCAGCCGGGGCCCCCTCTGAGAAGGCCTGGCAGCCTGGCTGGACAGTCAATGTGGAGGGCAGCGGTGAGGGCCGGGCTGGGGCAGGGGCAGGAGGAGAGAAGGGAGGCCACCATG	49
gCD19_5 5' HA	TCTTTTTTTTTTTGAACAGAGTCTCACTCTGGTGCCAGGCTGGAGTGCAGTGGTGTGATCTCGGCTCACTGCAACCTCCGCCTCCTGGTTTCCAGTGATTCCTTGCCCTCAGCCTCCTAAGTAGCTGGGATTACAGGTGTGCGCCACCATGCCCGGCTAATTTTTTTTTTTTTTTTGAAGAAGGAGTCTATGTGCCAGCATTGTTCTAGAGCACTTGCAATTAGTGGTGAACAACACGGTCTCTACTCCAAGGGGCTCACATTCTTGTGCAGAAAACAGAAATGAACAAATAAACACACAAGATCATTTCCCTGGTGTGAGAGCTGGGATGAAAATAAACAGCGTGGCAGGGAGAGGCAAGTGTTGTGAGTCTGGAGGGTTCCTGAGAAATGGGGCTGAGGCGTGACCACCGCTTCTCTCTGGGGGACTGCCTGCCGCCCCCGCAGACACCCATGGTTGAGTGCCCTCCAGGCCCTGCCTGCCCCAGCATCCCCTGCGCGAAGCTGGGTGCCCGGAGAGTCTGACCACCATGCCACCTCCTCGCCTCCTCTCTTCTCTCTTCTCACCCCATGGAAGTCAGG	50
gCD19_109 3' HA	AGGGCAGCGGTGAGGGCCGGGCTGGGGCAGGGGCAGGAGAGAGAAGGGAGGCCACCATGGACAGAAGAGGTCCGCGGCCACAATGGAGCTGGAGAGAGGGGCTGGAGGGATTGAGGGCGAACTCGGAGCTAGGTGGGCAGACTCCTGGGGCTCGTGGCTCAGTATGAGCTGCTTCTGTCCTTACCTCTCACTGCTTCTCTCTCTGCGGGTCTTTGICTCTATTTATCTCTGTCTTTGAGTCTCTATCTCTCCCTCTCCTGGGTGTCTCTGCATTTGGTTCTGGGTCTCTTCCAGGGGAGCTGTTCCGGTGGAATGTTTCGGACCTAGGTGGCCTGGGCTGTGGCCTGAAGAACAGGTCTCAGAGGGCCCCAGCTCCCCCTCCGGGAAGCTCATGAGCCCCAAGCTGTATGTGTGGGCCAAAGACCGCCCTGAGATCTGGGAGGGAGAGCCTCCGTGTCTCCACCGAGGGACAGCCTGAACCAGAGCCTCAGCCAGGGTATGGTGATGACTGGGGAGATGCCGGGAAGCGGGGTCCAGAGACAGAGGGGAGGGGAACTGAAGAGGTGAAACCCTGAGGATCAGG	51
gCD19_109 5' HA	ACCATGCCACCTCCTCGCCTCCTCTTCTTCTCTCTCTCCTCCTCCTCACCCCCATGGAAGTCAGGCCCCGAGGAACCTCTAGTGGTGAAGGTGGAAGGTATGTCCAAAGGGCAGAAAGGGAAGGGATTGAGGCTGGAAACTTGAGTTGTGGCTGGGTGTCCTTGGCTGAGTAACCTTACCCTCTCTGAGCCTCCATTTTCTTATTTGTAAAATTCAGGAAAGGGTTGGAAGGACTCTGCCGGCTCCTCCACTCCCAGCTTTGGAGTCCCTCTGCTCTATAACCTGGTGTGAGGAGTCGGGGGGCTTGGAGGTCCCACCCCATGCCCCACACCTCTCTCCCTCTCTCTCCACAGAGGGAGATAACG	52

Attorney Docket No. 2014034-0097

	CTGTGCTGCAGTGCCTCAAGGGGACCTCAGATGGCCCCACTCAGCAGCTGACCTGGTCTCGGGAGTCCCCGCTTAAACCCCTTCTTAAAACTCAGCCTGGGGCTGCCAGGCCTGGGAATCCACATGAGGCCCTGGCCATCTGGCTTTTCATCTCAACGTCTCTCAACAGATGGGGGGCTTCTACCTGTGCCAGCCGGGGCCCCCTCTGAGAAGGCCTGGCAGCCTGGCTGGACAGTCA	
gCD19_124 3' HA	ACCTCAGATGGCCCCACTCAGCAGCTGACCTGGTCTCGGGAGTCCCCGCTTAAACCCCTTCTTAAAACTCAGCCTGGGGCTGCCAGGCCTGGGAATCCACATGAGGCCCTGGCCATCTGGCTTTTCATCTTCAACGTCTCTCAACAGATGGGGGGCTTCTACCTGTGCCAGCCGGGGCCCCCTCTGAGAAGGCCTGGCAGCCTGGCTGGACAGTCAATGTGGAGGGCAGCGGTGAGGGCCGGGCTGGGGCAGGGGCAGGAGGAGAGAAGGGAGGCCACCATGGACAGAAGAGGTCCGCGGCCACAATGGAGCTGGAGAGAGGGGCTGGAGGGATTGAGGGCGAAACTCGGAGCTAGGTGGGCAGACTCCTGGGGCTTCGTGGCTTCAGTATGAGCTGCTTCCTGTCCCTACCTCTCACTGTCTTCTCTCTCTGCGGGTCTTTGTCTATTATCTCTGTCTTTGAGTCTCTATCTCTCTCCCTCTCCTGGGTGTCTCTGCATTTGGTCTGGGTCTCTTCCAGGGGAGCTGTTCCGGTGGAATGTTTCGGACCTAGGTGCGCTGGGCTGTGGCCTGAAGAACAGGTCCTCAGA	53
gCD19_124 5' HA	TGGTAGTGAGAGCTGGGATGAAAATAAAACAGCGTGGCAGGGAGGAGGCAAGTGTGTGAGTCTGGAGGGTTCCTGGAGAATGGGGCTGAGGCGTGACCCGCTTCTCTCTGGGGGGACTGCCTGCCGCCCGCAGACACCCATGGTGTGAGTGCCCTCCAGGCCCTGCCTGCCCCAGCATCCCCTGCGCGAAGCTGGGTGCCCCGAGAGTCTGACCACCATGCCACCTCCTCGCTCCTCTTCTTCTCTCTTCTTCACCCCCATGGAAGTCAGGCCCGAGGAACCTCTAGTGGTGAAGGTGGAAGGTATGTCCAAAGGGCAGAAAGGGAAGGGATTGAGGCTGGAAAC TTGAGTTGTGGCTGGGTGTCTTGGCTGAGTAACCTACCCTCTCTGAGCCTCATTCTCTTATTGTAAAATTCAGGAAAGGGTTGGAAGGACTCTGCCGGCTCTCCACTCCCAGCTTTTGGAGTCTCTGTCTATAACCTGGTGTGAGGAGTCGGGGGCTTGGAGGTCCCCCCACCCATGCCACACCTCTCTCCCTCTCTCTCCACAGAGGGAGATAACGCTGTGCTGCAGTGCCTC	54
Jenh_173 5' HA	CTGTAATCCCAGATACTCGGGAGCTGAGGCAGGAGAATTGCTTGAACCTGGGATACAGAGGTTGCAGTGAGCCAAGATCATGCCACTGCACTCCAGCCTGGGCGACAGAGTGAGACTCCATTTCAAAAAAGAGAGGCAAAAGCTGAATTTAAAAAAGAACCTCAAAATATGAAGAAGAACTTCATTCCAAATCTTACTAAAAATTAATAATACAAACCAAAATTGACTTCTTTTCTCGAACAGCTTTCCACATAGACTTTTAGGGTAGTCCTTCTGTGGTTAAATGTTTATGAGGAGAATTGCACCTGTGTGTGGTCAGCTTTTAAATAAACTTGCACCTGCAGTGGTTTTCATAGAATTGCTGTTTGAGAAATTACATGTCAGTGGTTTGACCATCACACAGAGCTATTTCCCTTACCATATCACCTGCCAGGCAGGTCTTAATTAAAAAGGGCCTACTTCCAAAAATTCATTTGAAAAAATCTGGTTAGATTTCATGGTAATCTCTCCAGGGACACAATAATAAGTAAGGTTTCCAGGATACCCTCAAACAAAAAGAATATGCTCCCTTCAATTCAT	118
Jenh_173 3' HA	CAGTGGTCTGGTCTTCTCTGCTTTAAGAAAACTTCTGATTTCTGGAGAGGCTTCTGCTCTAGTCAATTATGTCTGCTTTCCTTTTCTGTATATTATCTGCTAATTCCTGTTCAGAACTTTTTTAGTTTGTCAATTTTCTTACCATAAATTTATTTCTTTTCTTCTGTTTCATCTCATAAAAAACCTAAATCTTTCAAACCTAATCAAGTAGGTCTAAATGTCAATCCATTCTGTTCACCTTTTACTTTGTTCCTCATTGCTAATAAATTCATCTTGTATCTTGTATACCATTTTACTCAGCTGGCATATTACTTTATCACTTTTCTCATATGTGTGTAATTTATATTAATGTTTTCTAGATGCAGAGAGGTGTTCAAAAGAGCAATATCAGATGGCACCCCCAAAAAGGATATGAAAGAACCAAAAGGGAACCTCTAAAGATTTAGTTGGAAAAGTAGGTAAGAAAATACAATTTCTGTCTTAACCTCTCTATTCAGAGTGTTCATCATATGTAAGTGCCATACTAAGTTTCAATTTCCCTAATAACGAGAAAGCA TAAATCTGTGGTGAGGAATATCCA	119
gB2M_3 5' HA	AGGACCTTCTGAGCTGTCTCAGGATGCTTTTGGGACTATTTTCTTACCCAGAGAATGGAGAAACCTGCAGGGAATTCCCAAGCTGTAGTTATAAACAG	121

Attorney Docket No. 2014034-0097

	AAGTTCTCCTTCTGCTAGGTAGCATTCAAAGATCTTAATCTTCTGGGTTTCC GTTTTCTCGAATGAAAAATGCAGGTCCGAGCAGTtaactggctggggcaccattagcaagtc acttagcatctctggggccagctctgcaaagcgagggggCAGCCTTAATGTGCCTCCAGCCTGAAG TCCTAGAATGAGCGCCCGGTGTCCCAAGCTGGGGCGCGCACCCCAGATCGG AGGGCGCCGATGTACAGACAGCAAACCTACCCAGTCTAGTGCATGCCTTCT TAAACATCACGAGACTCTAAGAAAAGGAAACTGAAAACGGGAAAGTCCCT CTCTCTAACCTGGCACTGCGTGCCTGGCTTGGAGACAGGTGACGGTCCCTGC GGGCCTTGTCTGATTGGCTGGGCACGCGTTTAATATAAGTGGAGGCGTCG CGTGGCGGGCATTCCTGAAGCTGACAGCATTCGGGCGGAGATGTCTCGCT CCGTGGCCTTAG	
gB2M_3' HA	CTGTGCTCGCGTACTCTCTCTTCTGGCCTGGAGGCTATCCAGCGTGAGTC TCTCTACCTCCCGCTCTGGTCTTCTCTCCCGCTCTGCACCTCTGTGGC CCTCGCTGTGCTCTCTCGTCCGTGACTTCCCTTCTCCAAGTCTCCTTGGTG GCCCGCGTGGGGCTAGTCCAGGGCTGGATCTCGGGGAAGCGCGGGGTG GCCTGGGAGTGGGGAAGGGGTGCGCACCCGGGACGCGCTACTTGCCCC TTTCGGCGGGGAGCAGGGGAGACCTTTGGCCTACGGCGACGGGAGGGTTCG GGACAAAGTTTAGGGCGTCGATAAGCGTCAGAGCGCCGAGGTTGGGGGAG GGTTTCTCTTCCGCTCTTTCGCGGGGCTCTGGCTCCCCAGCGCAGCTGGA GTGGGGGACGGGTAGGCTCGTCCCAAGGCGCGGCGCTGAGGTTTGTGAAC GCGTGGAGGGGCGCTTGGGGTCTGGGGGAGGCGTCGCCCGGGTAAGCCTGT CTGCTGCGGCTCTGCTTCCCTTAGACTGGAGAGCTGTGGACTTCGTCTAGGC GCCCCGCTAAGTTCGCATGTCTAGCACCTCTGGGT	122
gJenh_1' 5' HA	gtctcctcctcattaaaataaaagctccttaagagcggaftgtctgttcttctgttccaagaacaa gcgcaggattgcctcaagtatattgattgaatgaaGGATGAATGGAGAACGTTTTT ACAAGGAGTTGAAAACCTGCCTCTAAGTTTATGTcttctaacaattagtc tcttttggataggctccatattgtcaatgtcctatttaaagagtagtatctagatacatattcaatagcaag tgaatctggacagtacaacaacagctgaacttaacattttcaataaatccacttaagatgataatct ctttttggcagctatgttacactattggctcaattaaagtgtacaatctcattttaaaaatatgtttctataaa gtaatgacttlcccatattlaaaattlaaacaagtlaggattlgggcatgagatttagcattggccctaagtl ttactctgtaactcagaccaacattacaatgtctcagattcttttaaatccagattcagaaccttgcaca ttagttcttctcagctttacagtatctgcaaacttgacacatg	123
GJenh_1' 3' HA	gatccttactgaagtcattaaagaaaatgttaattgggatagtgcttaagatagacttttgagcatacatctaaagtcctatcteta tcaataatcagtcattataaagcacattttgggtgcagtagtcatctcaagttaactcataatccatactatcacagactcaaa gtctgggtttatccataaggtacatgaataacttATCATAAAAGGCCAATCTCTCCTTTATTCTCT ATTTTAATGTCATCTCATTTCTCCCTTGAAAGCCTTCCCTAATATAAAAGG AGTCTTGATAGCAGCCTGGCATTTTGCCACTTGAACCTCTTAAAAGGGACGAT GATATTTTAAACAGGCTTCTTGGCCTTAAATCCAAAATGCAGCTGAAATCT CTTGATTTTCTTTTAAATGGATTAATTTCTACATTTAGTGTAATAATTACAT TTCCCATTTGTCCTTGAACCTTATATGGAAAACAAATTTACTAATAAATAG TACTGCTTAATCAGCTCTAAAATTAGGAGGGAAAAATATGAAGTCTACAAG AAACCTTGAATTAAATGTCAACCTGTAAATTCCAAGTAACCCAAA	124

Table 2 – Exemplary Expression Cassettes (Nucleotide Sequences)

Name	Nucleotide Sequence	SEQ ID NO
ALP-IgG1Fc (p0134)	atgccgtctctgtctctgtggggcatcctctctgtggcagcgctgtgtgctgctgctgctctctctgctctgctCTGGTGC CCGAGAAAAGAGAAGGACCCTAAGTATTGGAGAGATCAGGCCCAAGAGAC ACTGAAGTACGCCCTGGAACCTGCAGAACTGAACACCAACGTGGCAAAG AACGTGATCATGTTCTCGGCGACGGCATGGGCGTGTCCACAGTTACAGC CGCCAGAATCCTGAAGGGCCAGCTGCACCATAATCCTGGCGAAGAGACA CGGCTGGAAATGGACAAGTTCCTTCCTGGCCCTGAGCAAGACCTACAA	55

Attorney Docket No. 2014034-0097

	CACCAATGCTCAGGTGCCCCGATTCTGCCGGAACAGCCACAGCTTATCTGT GCGGCGTGAAGGCCAATGAGGGCACCGTTGGAGTGTCTGCCGCCACCGA GAGAAGCAGATGCAATACCACACAGGGCAACGAAGTGACCAGCATCCTG AGATGGGCCAAAGACGCCGGCAAGTCTGTGGGCATCGTGACCACCACCA GAGTGAACCACGCCACACCTAGCGCCGCTATGCTCACTCTGCCGACAGA GACTGGTACAGCGACAACGAGATGCCTCCTGAGGCTCTGTCTCAGGGCTG CAAGGATATCGCCTACCAGCTGATGCACAACATCCGGGACATTGATGTGA TCATGGGCGGAGGCCGGAAGTACATGTATCCCAAGAACAAGACCGACGT CGAGTACGAGAGCGACGAGAAGGCCAGAGGCACAAGACTGGATGGCCTG GACCTGGTGGATACCTGGAAGTCCTTCAAGCCCCGGTACAAGCACAGCCA CTTCATCTGGAACCGGACCGAGCTGCTGACCCTGGATCCTCACAATGTGG ACTACCTGCTGGGCTGTTCGAGCCCGCGATATGCAGTACGAGCTGAAC CGGAACAACGTGACAGACCCAGCCTGAGCGAGATGGTGGTTGTGGCCA TTCAGATCCTGCGGAAGAACCCCAAGGGATTCTTCTGCTGGTGGAAAGGC GGCAGGATCGATCACGGACACCATGAGGGAAAAGCCAAGCAGGCCCTGC ACGAGGCCGTCGAAATGGATAGAGCCATTGGCCAGGCCGGCAGCCTGAC AAGCTCTGAGGATACACTGACCGTGGTCACCGCCGATCACAGCCACGTGT TCACATTCGGCGGCTACACCCCTAGAGGGCAACAGCATCTTTGGACTGGCC CCTATGCTGAGCGACACCGACAAGAAGCCTTTACCGCCATCCTGTACGG CAACGGCCCTGGCTATAAGGTTGTGCGAGGCGAGAGGGAAAACGTGTCC ATGGTGGATTACGCCCACAACAACCTACCAGGCTCAGAGCGCCGTGCCTCT GAGACACGAAACACACGGCGGAGAAGATGTGGCCGTGTTACGAAGGGC CCCATGGCTCATTTGCTGCACGGCGTGCACGAGCAGAATTACGTGCCACA CGTGATGGCCTACGCCGCTGTATTGGAGCCAATCTGGGACATTGTGCCC CTGCCAGCAGCCTGAAGGACAAGACCCATACCTGTCCTCCATGTCCTGT CCAGAGCTGCTCGGAGGCCCTTCCGTGTTTCTGTTCCCTCCAAAGCCTAA GGACACCCTGATGATCAGCAGAACCCCTGAAGTGACCTGCGTGGTGGTG ATGTGTCCACGAGGATCCCAACCGTGAAGTTCAATTGGTACGTGGACGGC GTGGAAGTGCAACGCCAAGACCAAGCCTAGAGAGGAACAGTACAACA GCACCTACAGAGTGGTGTCTGTGCTGACCGTGTGACCAAGGATTGGCTG AACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCCTGCTC CTATCGAGAAAACCATCAGCAAGGCCAAGGGACAGCCCAGAGAACCCCA GGTGTACACACTGCCTCCAAGCCGGGAAGAGATGACCAAGAACCAGGTG TCCCTGACCTGCCTGGTCAAGGGCTTCTACCCCTCCGATATCGCCGTGGA ATGGGAGAGCAATGGCCAGCCAGAGAACAACCTACAAGACCACACCTCCT GTGCTGGACAGCGACGGCTCATTCTTTCTGTACAGCAAGCTGACGGGA CAAGAGCAGATGGCAGCAGGGCAATGTGTTACAGCTGCAGCGTGATGCAC GAAGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGTCTCCTGG CAAGTGA	
ALP-IgG1Fc (p0074)	ATGATTTCACCATTTCTTAGTACTGGCCATTGGCACCTGCCTTACTAACTCC CTGGTGCCCCGAGAAAAGAGAAGGACCCTAAGTATTGGAGAGATCAGGCCC AAGAGACACTGAAGTACGCCCTGGAACTGCAGAACTGAACACCAACGT GGCCAAGAACGTGATCATGTTCTCGGGCAGGGCATGGGCGTGTCCACAG TTACAGCCGCCAGAATCCTGAAGGGCCAGCTGCACCATAATCCTGGCGAA GAGACACGGCTGGAAATGGACAAGTTCCTATTCGTGGCCCTGAGCAAGA CCTACAACACCAATGCTCAGGTGCCCCGATTCTGCCGGAACAGCCACAGCT TATCTGTGCGGCGTGAAGGCCAATGAGGGCACCGTTGGAGTGTCTGCCGC CACCGAGAGAAGCAGATGCAATACCACACAGGGCAACGAAGTGACCAGC ATCCTGAGATGGGCCAAAGACGCCGGCAAGTCTGTGGGCATCGTGACCA CCACCAGAGTGAACCACGCCACCTAGCGCCGCTATGCTCACTCTGCC GACAGAGACTGGTACAGCGACAACGAGATGCCTCCTGAGGCTCTGTCTCA GGGCTGCAAGGATATCGCCTACCAGCTGATGCACAACATCCGGGACATTG ATGTGATCATGGGCGGAGGCCGGAAGTACATGTATCCCAAGAACAAGAC CGACGTTCGAGTACGAGAGCGACGAGAAGGCCAGAGGCACAAGACTGGAT GGCCTGGACCTGGTGGATACCTGGAAGTCCTTCAAGCCCCGGTACAAGCA CAGCCACTTCATCTGGAACCGGACCGAGCTGCTGACCCTGGATCCTCACA	56

Attorney Docket No. 2014034-0097

	ATGTGGACTACCTGCTGGGCTGTTCGAGCCCGGCGATATGCAGTACGAG CTGAACCGGAACAACGTGACAGACCCAGCCTGAGCGAGATGGTGGTTG TGGCCATTCAGATCCTGCGGAAGAACCCCAAGGGATTCTTCCTGCTGGTG GAAGGCGGCAGGATCGATCACGGACACCATGAGGGAAAAGCCAAGCAG GCCCTGCACGAGGCCGTCGAAATGGATAGAGCCATTGGCCAGGCCGGCA GCCTGACAAGCTCTGAGGATACACTGACCGTGGTCACCGCCGATCACAGC CACGTGTTTACATTGGGCGGTACACCCCTAGAGGCAACAGCATCTTTGG ACTGGCCCTATGCTGAGCGACACCGACAAGAAGCCTTTACCGCCATCC TGTACGGCAACGGCCCTGGCTATAAGGTTGTCGGAGGCGAGAGGGAAAA CGTGTCCATGGTGGATTACGCCCACAACAACCTACCAGGCTCAGAGCGCCG TGCTTCTGAGACACGAAACACACGGCGGAGAAGATGTGGCCGTGTTTCAG CAAGGGCCCCATGGCTCATTTGCTGCACGGCGTGCACGAGCAGAATTACG TGCCACACGTGATGGCCTACGCCGCTGTATTGGAGCCAATCTGGGACAT TGTGCCCTGCCAGCAGCCTGAAGGACAAGACCCATACCTGTCTCCATG TCCTGCTCCAGAGCTGCTCGGAGGCCCTTCCGTGTTTCTGTTCCCTCCAAA GCCTAAGGACACCCCTGATGATCAGCAGAACCCCTGAAGTGACCTGCGTG GTGGTGGATGTGTCCACAGGATCCCACCGTGAAGTTCAATTGGTACGT GGACGGCGTGGAAGTGACAACGCCAAGACCAAGCCTAGAGAGGAACA GTACAACAGCACCTACAGAGTGGTGTCTGTGCTGACCGTGTGCACCAAG ATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCT GCCTGCTCCTATCGAGAAAACCATCAGCAAGGCCAAGGGACAGCCAGA GAACCCAGGTGTACACACTGCCTCCAAGCCGGGAAGAGATGACCAAGA ACCAGGTGTCCCTGACCTGCCTGGTCAAGGGCTTCTACCCCTCCGATATC GCCGTGGAATGGGAGAGCAATGGCCAGCCAGAGAACAACCTACAAGACCA CACCTCCTGTGCTGGACAGCGACGGCTCATTCTTCTGTACAGCAAGCTG ACAGTGGACAAGAGCAGATGGCAGCAGGGCAATGTGTTTCAGCTGCAGCG TGATGCACGAAGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCCTG TCTCCTGGCAAGTGA	
ALP-IgG1Fc-D8 (p0042)	ATGATTTCACCATTCTTAGTACTGGCCATTGGCACCTGCCTTACTAACTCC TTAGTGCCAGAGAAAGAGAAAAGACCCCAAGTACTGGCGAGACCAAGCGC AAGAGACACTGAAATATGCCCTGGAGCTTCAGAAAGCTCAACACCAACGT GGCTAAGAATGTCATCATGTTCTGGGAGATGGGATGGGTGTCTCCACAG TGACGGCTGCCCGCATCCTCAAGGGTCAGCTCCACCACAACCCTGGGGAG GAGACCAGGCTGGAGATGGACAAGTTCCTTCTGTTGGCCCTCTCCAAGAC GTACAACACCAATGCCCAGGTCCCTGACAGTGCCGGCACCGCCACCGCCT ACCTGTGTGGGGTGAAGGCCAATGAGGGCACCGTGGGGGTAAAGCGCAGC CACTGAGCGTTCCCGGTGCAACACCAACCCAGGGGAACGAGGTCACTCC ATCCTGCGCTGGGCCAAGGACGCTGGGAAATCTGTGGGCATTGTGACCAC CACGAGAGTGAACCATGCCACCCCAAGCGCCGCTACGCCCACTCGGCTG ACCGGGACTGGTACTCAGACAACGAGATGCCCCCTGAGGCCTTGAGCCA GGGCTGTAAGGACATCGCCTACCAGCTCATGCATAACATCAGGGACATTG ACGTGATCATGGGGGGTGGCCGGAATACATGTACCCCAAGAATAAAAC TGATGTGGAGTATGAGAGTGACGAGAAAGCCAGGGGCACGAGGCTGGAC GGCCTGGACCTCGTTGACACCTGGAAGAGCTTCAAACCGAGATAACAAGC ACTCCCACTTCATCTGGAACCGCACGGAACCTCTGACCCCTGACCCCCAC AATGTGGACTACCTATTGGGTCTCTTCGAGCCAGGGGACATGCAGTACGA GCTGAACAGGAACAACGTGACGGACCCGTCACTCTCCGAGATGGTGGTG GTGGCCATCCAGATCCTGCGGAAGAACCCCAAGGCTTCTTCTGTGCTGGT GGAAGGAGGCAGAAATTGACCACGGGCACCATGAAGGAAAAGCCAAGCA GGCCCTGCATGAGGCGGTGGAGATGGACCGGGCCATCGGGCAGGCAGGC AGCTTGACCTCCTCGGAAGACACTCTGACCGTGGTCACTGCGGACCATTC CCACGTCTTCACATTTGGTGGATACACCCCGTGGCAACTCTATCTTTGG TCTGGCCCCCATGCTGAGTGACACAGACAAGAAGCCCTTCACTGCCATCC TGTATGGCAATGGGCCTGGCTACAAGGTGGTGGGCGGTGAACGAGAGAA TGTCTCCATGGTGGACTATGCTCACAACAACCTACCAGGCGCAGTCTGCTG TGCCCTGCGCCACGAGACCCACGGCGGGGAGGACGTGGCCGTCTTCTCC	57

Attorney Docket No. 2014034-0097

	AAGGGCCCCATGGCGCACCTGCTGCACGGCGTCCACGAGCAGAACTACG TCCCCACGTGATGGCGTATGCAGCCTGCATCGGGGCCAACCTCGGCCAC TGTGCTCCTGCCAGCTCGctgaagGACAAGACCCACACCTGCCCCCCTGCC CCGCCCCGAGCTGCTGGGCGGCCCCAGCGTGTTCCTGTTCCCCCCTAAG CCCAAGGACACCCTGATGATCAGCCGACCCCCGAGGTGACCTGCGTGGT GGTGGACGTGAGCCACGAGGACCCACCGTGAAAGTTCAACTGGTACGTG GACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGCGAGGAGCAGT ACAACAGCACCTACCGCGTGGTGAGCGTGCTGACCGTGCTGCACCAGGA CTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTG CCCGCCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGCG AGCCCCAGGTGTACACCCTGCCCCCAGCCGCGAGGAGATGACCAAGAA CCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCAGCGACATCG CCGTGGAGTGGGAGAGCAACGGCCAGCCCCGAGAACAATAACAAGACCAC CCCCCCGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGA CCGTGGACAAGAGCCGCTGGCAGCAGGGCAACGTGTTACGTGCAGCGT GATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTG AGCCCCGCAAGgacatcGATGATGATGATGATGATGATGACGAC	
ALP-LL (Long Linker)-IgG1Fc-3xsubst-D10 (p0127)	atgccgttcttctgtctgtgggcaatcctcctgctggaaggcctgtgctgectggtccctgtctcctggtctGGTGC CCGAGAAAGAGAAGGACCCTAAGTATTTGGAGAGATCAGGCCCAAGAGAC ACTGAAGTACGCCCTGGAACTGCAGAACTGAACACCAACGTGGCAAAG AACGTGATCATGTTCTCGGCGACGGCATGGGCGTGTCCACAGTTACAGC CGCCAGAATCCTGAAGGGCCAGCTGCACCATAATCCTGGCGAAGAGACA CGGCTGGAAATGGACAAGTTCCCATTCGTGGCCCTGAGCAAGACCTACAA CACCAATGCTCAGGTGCCCCGATTCTGCCGGAACAGCCACAGCTTATCTGT GCGGCGTGAAGGCCAATGAGGGCACCGTTGGAGTGTCTGCCGCCACCGA GAGAAGCAGATGCAATACCACACAGGGCAACGAAGTGACCAGCATCCTG AGATGGGCCAAAGACGCCGGCAAGTCTGTGGGCATCGTGACCACCACCA GAGTGAACCACGCCACACCTAGCGCCGCCTATGCTCACTCTGCCGACAGA GACTGGTACAGCGACAACGAGATGCCCTCCTGAGGCTCTGTCTCAGGGCTG CAAGGATATCGCCTACCAGCTGATGCACAACATCCGGGACATTGATGTGA TCATGGGCGGAGGCCGGAAGTACATGTATCCCAAGAACAAGCCGACGT CGAGTACGAGAGCGACGAGAAGGCCAGAGGCACAAGACTGGATGGCCTG GACCTGGTGGATACCTGGAAGTCCTTCAAGCCCCGGTACAAGCACAGCCA CTTCATCTGGAACCGGACCGAGCTGCTGACCCTGGATCCTCACAAATGTGG ACTACCTGCTGGGCTGTTCGAGCCCCGGCGATATGCAGTACGAGCTGAAC CGGAACAACGTGACAGACCCAGCCTGAGCGAGATGGTGGTGTGTGGCCA TTCAGATCCTGCGGAAGAACCCCAAGGGATTCTTCCTGCTGGTGGAAAGGC GGCAGGATCGATCACGGACACCATGAGGGAAAAGCCAAGCAGGCCCTGC ACGAGGCCGTGAAAATGGATAGAGCCATTGGCCAGGCCGGCAGCCTGAC AAGCTCTGAGGATACACTGACCGTGGTCAACGCCGATCACAGCCACGTGT TCACATTCGGCGGCTACACCCCTAGAGGCAACAGCATCTTTGGACTGGCC CCTATGCTGAGCGACACCGACAAGAAGCCTTTACCGCCATCCTGTACGG CAACGGCCCTGGCTATAAGGTTGTGCGAGGCGAGAGGGAAAAACGTGTCC ATGGTGGATTACGCCCACAACAATACTACCAGGCTCAGAGCGCCGTGCCTCT GAGACACGAAACACACGGCGGAGAAGATGTGGCCGTGTTACAGCAAGGGC CCCATGGCTCATTTGCTGCACGGCGTGCACGAGCAGAATTACGTGCCACA CGTGATGGCCTACGCCCGCTGTATTGGAGCCAATCTGGGACATTGTGCCC CTGCCAGCAGCggcgggcggggctccggcgggcggggctccggcgggcggggctccGACAAGAC CCACACATGCCCCCCTGCCCCGCCCCGAGCTGCTGGGCGGCCCCAGCG TGTTCTGTTCCCCCCTAAGCCCAAGGACACCCTGATGATCAGCCGACCC CCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCCACCG TGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGAC CAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTAGCGTG CTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCA AGGTGAGCAACAAGGCCCTGCCCCCCCCATCGAGAAGACCATCAGCAA GGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCCTGCCCCCAGC	58

Attorney Docket No. 2014034-0097

	CGCGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGG GCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCC CGAGAACAACCTAC _{gat} ACCACCCCCCGTGCT _{Gaaa} AGCGACGGCAGCTTC TTCCTGTACAGC _{gat} CTGACCGTGGACAAGAGCCGCTGGCAGCAGGGCAAC GTGTTACAGTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCA GAAGAGCCTGAGCCTGAGCCCCGGCAAGGATGATGATGATGATGATGAC GACGATGACTGA	
ALP-LL- IgG1Fc-3xsubst- D10 (p0137)	atgccgttctctgtctctgtgggcatctctctgtggcaggcctgtgctgectggctccctgtctccctggctCTGGTGC CCGAGAAAGAGAAGGACCCTAAGTATTGGAGAGATCAGGCCCAAGAGAC ACTGAAGTACGCCCTGGAACCTGCAGAACTGAACACCAACGTGGCAAAG AACGTGATCATGTTCTCGGCGACGGCATGGGCGTGTCCACAGTTACAGC CGCCAGAACTCTGAAGGGCCAGCTGCACCATAATCCTGGCGAAGAGACA CGGCTGGAATGGACAAGTTCCCATTCGTGGCCCTGAGCAAGACCTACAA CACCAATGCTCAGGTGCCCGATTCTGCCGGAACAGCCACAGCTTATCTGT GCCGCGTGAAAGGCCAATGAGGGCACCGTTGGAGTGTCTGCCGCCACCGA GAGAAGCAGATGCAATACCACACAGGGCAACGAAGTGACCAGCATCCTG AGATGGGCCAAAGACGCCGGCAAGTCTGTGGGCATCGTGACCACCACCA GAGTGAACCACGCCACACCTAGCGCCGCTATGCTCACTCTGCCGACAGA GACTGGTACAGCGACAACGAGATGCCTCCTGAGGCTCTGTCTCAGGGCTG CAAGGATATCGCCTACCAGCTGATGCACAACATCCGGGACATTGATGTGA TCATGGGCGGAGGCCGGAAGTACATGTATCCCAAGAAACAAGACCGACGT CGAGTACGAGAGCGACGAGAAGGCCAGAGGCACAAGACTGGATGGCCTG GACCTGGTGGATACCTGGAAGTCCTTCAAGCCCCGGTACAAGCACAGCCA CTTCATCTGGAACCGGACCGAGCTGCTGACCCTGGATCCTCACAATGTGG ACTACCTGCTGGGCTGTTCGAGCCCGGCGATATGCAGTACGAGCTGAAC CGGAACAACGTGACAGACCCAGCCTGAGCGAGATGGTGGTTGTGGCCA TTCAGATCCTGCGGAAGAACCCCAAGGGATTCTTCCTGCTGGTGGAAAGGC GGCAGGATCGATCACGGACACCATGAGGGAAAAGCCAAGCAGGCCCTGC ACGAGGCCGTGCAAAATGGATAGAGCCATTGGCCAGGCCGGCAGCCTGAC AAGCTCTGAGGATACACTGACCGTGGTCACCGCCGATCACAGCCACGTGT TCACATTCCGGCGGCTACACCCCTAGAGGCAACAGCATCTTTGGACTGGCC CCTATGTGAGCGACACCGACAAGAAGCCTTTCACCGCCATCCTGTACGG CAACGGCCCTGGCTATAAGGTTGTTCGGAGGCGAGAGGGAAAACGTGTCC ATGGTGGATTACGCCCCACAACAACCTACCAGGCTCAGAGCGCCGTGCCTCT GAGACACGAAACACACGGCGGAGAAGATGTGGCCGTGTTACAGCAAGGGC CCCATGGCTCATTGTCTGCACGGCGTGCACGAGCAGAATTACGTGCCACA CGTGATGGCCTACGCCGCTGTATTGGAGCCAATCTGGGACATTGTGCC CTGCCAGCAGC _{ggcg} ggcggggctccggcgggcgggctccggcgggcgggctccGACAAGAC CCACACATGCCCCCTGCCCCGCCCCGAGCTGCTGGGCGGCCCCAGCG TGTTCTCTGTTCCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGACCC CCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCACCG TGAAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGAC CAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGAGCGTG CTGACCGTGTGCACCAAGGACTGGCTGAACGGCAAGGAGTACAAGTGCA AGGTGAGCAACAAGGCCCTGCCCGCCCCCATCGAGAAGACCATCAGCAA GGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCTGCCCCCAGC CGCGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGGTGGTGAAGG GCTTCTACCCAGCGACA _{TCGCCGTGGAGTGGGAGAGCAACGGCCAGCC} CGAGAACAACCTAC _{gat} ACCACCCCCCGTGCT _{Gaaa} AGCGACGGCAGCTTC TTCCTGTACAGC _{gat} CTGACCGTGGACAAGAGCCGCTGGCAGCAGGGCAAC GTGTTACAGTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCA GAAGAGCCTGAGCCTGAGCCCCGGCAAG _{gacac} gacacGATGATGATGATGATGAT GACGACGATGACTGA	59
ALP-LL- IgG1Fc-3xsubst- D10 (p0140)	atgccgttctctgtctctgtgggcatctctctgtggcaggcctgtgctgectggctccctgtctccctggctCTGGTGC CCGAGAAAGAGAAGGACCCTAAGTATTGGAGAGATCAGGCCCAAGAGAC ACTGAAGTACGCCCTGGAACCTGCAGAACTGAACACCAACGTGGCAAAG	60

Attorney Docket No. 2014034-0097

	AACGTGATCATGTTCTCGGCGACGGCATGGGCGTGTCACAGTTACAGC CGCCAGAATCCTGAAGGGCCAGCTGCACCATAATCCTGGCGAAGAGACA CGGCTGGAAATGGACAAGTTCCCATTCGTGGCCCTGAGCAAGACCTACAA CACCAATGCTCAGGTGCCCCGATTCTGCCGGAACAGCCACAGCTTATCTGT GCGGCGTGAAGGCCAATGAGGGCACCGTTGGAGTGTCTGCCGCCACCGA GAGAAGCAGATGCAATACCACACAGGGCAACGAAGTGACCAGCATCCTG AGATGGGGCCAAAGACGCCGGCAAGTCTGTGGGCATCGTGACCACCACCA GAGTGAACCACGCCACACCTAGCGCCGCCTATGCTCACTCTGCCGACAGA GACTGGTACAGCGACAACGAGATGCCTCCTGAGGCTCTGTCTCAGGGCTG CAAGGATATCGCCTACCAGCTGATGCACAACATCCGGGACATTGATGTGA TCATGGGCGGAGGCCGGAAGTACATGTATCCCAAGAACAAGACCGACGT CGAGTACGAGAGCGACGAGAAGGCCAGAGGCACAAGACTGGATGGCCTG GACCTGGTGGATACCTGGAAGTCCTTCAAGCCCCGGTACAAGCACAGCCA CTTCATCTGGAACCGGACCGAGCTGCTGACCCTGGATCCTCACAAATGTGG ACTACCTGCTGGGCGTGTTCGAGCCCGGCGATATGCAGTACGAGCTGAAC CGGAACAACGTGACAGACCCAGCCTGAGCGAGATGGTGGTTGTGGCCA TTCAGATCCTGCGGAAGAACCCCAAGGGATTCTTCCTGCTGGTGGAAAGGC GGCAGGATCGATCACGGACACCATGAGGGAAAAGCCAAGCAGGGCCCTGC ACGAGGCCGTGCAATGGATAGAGCCATTGGCCAGGCCGGCAGCCTGAC AAGCTCTGAGGATACACTGACCGTGGTACCGCGATCACAGCCACGTGT TCACATTCGGCGGCTACACCCCTAGAGGCAACAGCATCTTTGGACTGGCC CCTATGCTGAGCGACACCGACAAGAAGCCTTTCACCGCCATCCTGTACGG CAACGGCCCTGGCTATAAGGTTGTTCGGAGGCGAGAGGGAAAACGTGTCC ATGGTGGATTACGCCCACAACAACCTACCAGGCTCAGAGCGCCGTGCCTCT GAGACAGAAACACACGGCGGAGAAGATGTGGCCGTGTTACGCAAGGGC CCCATGGCTCATTGTCTGCACGGCGTGCACGAGCAGAATTACGTGCCACA CGTGATGGCCTACGCCGCTGTATTGGAGCCAATCTGGGACATTGTGCCC CTGCCAGCAGCggcgggggggtccggcgggggggtccggcgggggggtccGACAAGAC CCACACATGCCCCCCTGCCCCGCCCCGAGCTGCTGGGCGGCCCCAGCG TGTTCTGTTCCTGTTCCCCCCTAAGCCCAAGGACACCCTGATGATCAGCCGACC CCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCAACCG TGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGAC CAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGAGCGTG CTGACCGTGTCTGCACAGGACTGGCTGAACGGCAAGGAGTACAAGTGCA AGGTGAGCAACAAGGCCCTGCCCCCCCCATCGAGAAGACCATCAGCAA GGCCAAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCTGCCCCCAGC CGCGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCCTGGTGAAGG GCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCC CGAGAACAACCTACgatACCACCCCCCGTGTGCTGaaaAGCGACGGCAGCTTC TTCCTGTACAGCgatCTGACCGTGGACAAGAGCCGCTGGCAGCAGGGCAAC GTGTTAGCTGCAGCGTGTATGCACGAGGCCCTGCACAACCACTACACCCA GAAGAGCCTGAGCCTGAGCCCCGGCAAGgacatcGATGATGATGATGATGAT GACGACGATGACTGA	
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Attorney Docket No. 2014034-0097

	TCATGGGCGGAGGCCGGAAGTACATGTATCCCAAGAACAAGACCGACGT CGAGTACGAGAGCGACGAGAAGGCCAGAGGCACAAGACTGGATGGCCTG GACCTGGTGGATACCTGGAAGTCCTTCAAGCCCCGGTACAAGCACAGCCA CTTCATCTGGAACCGGACCGAGCTGCTGACCTGGATCCTCACAATGTGG ACTACCTGCTGGGCCTGTTTCGAGCCCGGCGATATGCAGTACGAGCTGAAC CGGAACAACGTGACAGACCCAGCCTGAGCGAGATGGTGGTGTGGCCA TTCAGATCCTGCGGAAGAACCCCAAGGGATTCTTCCTGCTGGTGGAAGGC GGCAGGATCGATCACGGACACCATGAGGGAAAAGCCAAGCAGGCCCTGC ACGAGGCCGTGCAAATGGATAGAGCCATTGGCCAGGCCGGCAGCCTGAC AAGCTCTGAGGATACACTGACCGTGGTCACCGCCGATCACAGCCACGTGT TCACATTGCGCGGCTACACCCCTAGAGGCAACAGCATCTTTGGAAGTGGCC CCTATGCTGAGCGACACCGACAAGAAGCCTTTACCGCCATCCTGTACGG CAACGGCCCTGGCTATAAGGTTGTTCGGAGGCGAGAGGGAAAACGTGTCC ATGGTGGATTACGCCCCACAACAACCTACCAGGCTCAGAGCGCCGTGCCTCT GAGACACGAAACACACGGCGGAGAAGATGTGGCCGTGTTACAGCAAGGGC CCCATGGCTCATTTGCTGCACGGCGTGCACGAGCAGAATTACGTGCCACA CGTGATGGCCTACGCCCGCTGTATTGGAGCCAATCTGGGACATTGTGCC CTGCCAGCAGCgggcgcgggcggtccggcgcgggcggtccggcgcgggcggtccTGTCTCCA TGTCTGCTCCAGAGgcccgcGGCGGACCCCTCCGTGTTCTGTCTTCTCCAAA GCCTAAGGACACCCCTGATGATCAGCAGAAACCCCTGAAGTGACCTGCGTG GTGGTGGACGTGTCCCAAGAGGATCCTGAGGTGCAGTTCAATTGGTACGT GGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACA GTTCAACAGCACCTACAGAGTGGTGTCTGTGCTGACCGTGTGCACCAAGG ATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGGCCT GCCTAGCAGCATCGAGAAAACCATCAGCAAGGCCAAGGGACAGGCCAGA GAACCCAGGTGTACACACTGCCTCCAAGCCAAGAGGAAATGACCAAGA ACCAGGTGTCCCTGACCTGCCTGGTCAAGGGCTTCTACCCTTCCGATATC GCCGTGGAATGGGAGAGCAATGGCCAGCCAGAGAACAACCTACGACACCA CACCTCCAGTGCTGAAGTCCGACGGCTCATTTCTTCTGTACTCCGACCTGA CAGTGGACAAGAGCAGATGGCAAGAGGGCAACGTGTTCTCTGACGCGT GATGCACGAAGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGT CTCTGGGCAAAGATGATGATGATGATGATGACGACGATGACTGA	
ALP-Fc GeneArt Codon Optimized 1 (p0164)	atgccgtctctgtctctgtggggcatctctctgtggcaggcctgtgtgctggtccctgtctccctggtctCTGGTGC CCGAGAAAGAGAAGGACCCCTAAGTATTGGAGAGATCAGGCCCAAGAGAC ACTGAAGTACGCCCTGGAACCTGCAGAACTGAACACCAACGTGGCAAAG AACGTGATCATGTTTCTCGGCGACGGCATGGGCGTGTCCACAGTTACAGC CGCCAGAAATCCTGAAGGGCCAGCTGCACCATAATCCTGGCGAAGAGACA CGGCTGGAATGGACAAGTTCCCATTCGTGGCCCTGAGCAAGACCTACAA CACCAATGCTCAGGTGCCCCGATTCTGCCGGAACAGCCACAGCTTATCTGT GCGGCGTGAAGGCCAATGAGGGCACCGTTGGAGTGTCTGCCGCCACCGA GAGAAGCAGATGCAATACCACACAGGGCAACGAAGTGACCAGCATCCTG AGATGGGCCAAAGACGCCCGCAAGTCTGTGGGCATCGTGACCAACCA GAGTGAACCACGCCACCTAGCGCCGCCTATGCTCACTCTGCCGACAGA GACTGGTACAGCGACAACGAGATGCCTCCTGAGGCTCTGTCTCAGGGCTG CAAGGATATCGCCTACCAGCTGATGCACAACATCCGGGACATTGATGTGA TCATGGGCGGAGGCCGGAAGTACATGTATCCCAAGAACAAGACCGACGT CGAGTACGAGAGCGACGAGAAGGCCAGAGGCACAAGACTGGATGGCCTG GACCTGGTGGATACCTGGAAGTCCTTCAAGCCCCGGTACAAGCACAGCCA CTTCATCTGGAACCGGACCGAGCTGCTGACCTGGATCCTCACAATGTGG ACTACCTGCTGGGCCTGTTTCGAGCCCGGCGATATGCAGTACGAGCTGAAC CGGAACAACGTGACAGACCCAGCCTGAGCGAGATGGTGGTGTGGCCA TTCAGATCCTGCGGAAGAACCCCAAGGGATTCTTCCTGCTGGTGGAAGGC GGCAGGATCGATCACGGACACCATGAGGGAAAAGCCAAGCAGGCCCTGC ACGAGGCCGTGCAAATGGATAGAGCCATTGGCCAGGCCGGCAGCCTGAC AAGCTCTGAGGATACACTGACCGTGGTCACCGCCGATCACAGCCACGTGT TCACATTGCGCGGCTACACCCCTAGAGGCAACAGCATCTTTGGAAGTGGCC	87

Attorney Docket No. 2014034-0097

	CCTATGCTGAGCGACACCGACAAGAAGCCTTTCACCGCCATCCTGTACGG CAACGGCCCTGGCTATAAGGTTGTCTGGAGGCGAGAGGGAAAAACGTGTCC ATGGTGGATTACGCCCCACAACAATAACAGGCTCAGAGCGCCGTGCCTCT GAGACACGAAACACACGGCGGAGAAGATGTGGCCGTGTTACAGCAAGGGC CCCATGGCTCATTTGCTGCACGGCGTGACGAGCAGAATTACGTGCCACA CGTGATGGCCTACGCCGCTGTATTGGAGCCAATCTGGGACATTGTGCCC CTGCCAGCAGCggcggcggeggctccCTGAAGGACAAGACCCACACATGCCCC CCTGCCCCGCCCCGAGCTGTCTGGGCGGCCCCAGCGTGTTCCTGTTCCTCC CCCAAGCCCAAGGACACCCCTGATGATCAGCCGCACCCCGAGGTGACCT GCGTGGTGGTGGACGTGAGCCACGAGGACCCACCCGTGAAGTTCAACTG GTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGCGAG GAGCAGTACAACAGCACCTACCGCGTGGTGAGCGTGTCTGACCGTGTCTGC ACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAA GGCCCTGCCCGCCCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAG CCCCGCGAGCCCCAGGTGTACACCTGCCCCCAGCCGCGAGGAGATGA CCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGC GACATCGCCGTGGAGTGGGAGAGCAACGCCAGCCCCGAGAACAATACTACg atACCACCCCCCGTGTGaaaAGCGACGGCAGCTTCTTCCTGTACAGCgat CTGACCGTGGACAAGAGCCGTGGCAGCAGGGCAACGTGTTCAGCTGCA GCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAG CCTGAGCCCCGGCAAAGacacGATGATGATGATGATGATGACGACGATGAC TGA	
ALP-Fe GeneArt Codon Optimized 2 (p0168)	atgccgttcttctgtctgtgggcatctctctgtggaaggcctgtgtgctggtctctctctgtctctctgtgctCTGGTCC CCGAGAAGGAGAAGGACCCCCAAGTACTGGCGGGACCAGGCGCAGGAGA CCCTGAAGTACGCGCTGGAGCTGCAGAAGCTGAACACCAACGTTCGCGAA GAACGTATCATGTTCCTGGGCGACGGCATGGGCGTCAGCACCGTCACCG CGGCGCGGATCCTGAAGGGCCAGCTGCACCACAACCCCGCGCAGGAGAC CCGGCTGGAGATGGACAAGTTCCCTTCGTTCGCGCTGAGCAAGACCTACA ACACCAACGCGCAGGTCCCCGACAGCGCGGGCACCCGCGACCCGCGTACCT GTGCGGCGTCAAGGCGAACGAGGGCACCGTTCGGCGTCAGCGCGGGCAGC GAGCGGAGCCGTTGCAACACCACCCAGGGCAACGAGGTACACGATCC TGCGGTGGGCGAAGGACGCGGGCAAGAGCGTCGGCATCGTCACCACCAC CCGGGTCAACCACGCGACCCCCAGCGCGGCGTACGCGCACAGCGCGGAC CGGGACTGGTACAGCGACAACGAGATGCCCCCGAGGCGCTGAGCCAGG GCTGCAAGGACATCGCGTACCAGCTGATGCACAACATCCGGGACATCGA CGTCATCATGGGCGGCGCCGGAAGTACATGTACCCCAAGAACAAGACC GAGCTCGAGTACGAGAGCGACGAGAAGGCGCGGGGCACCCGGCTGGACG GCCTGGACCTGGTTCGACACCTGGAAGAGCTTCAAGCCCCGGTACAAGCA CAGCCACTTCATCTGGAACCGGACCGAGCTGCTGACCCTGGACCCCCACA ACGTGCGACTACCTGTGGGCTGTTCGAGCCCCGGCGACATGCAGTACGAG CTGAACCGGAACAACGTACCGACCCCCAGCCTGAGCGAGATGGTTCGTCTG TCGCGATCCAGATCCTGCGGAAGAACCCCAAGGGCTTCTTCCTGTCTGGTC GAGGGCGGCGGATCGACCACGGCCACCACGAGGGCAAGGCGAAGCAG GCGCTGCACGAGGCGGTTCGAGATGGACCGGGCGATCGGCCAGGCGGGCA GCCTGACCAGCAGCGAGGACACCCGTGACCGTTCGTACCCGCGGACACAG CCACGTCTTCACCTTCGGCGGCTACACCCCCGGGGCAACAGCATCTTCG GCCTGGCGCCCATGTGAGCGACACCGACAAGAAGCCCTTCACCGGATC CTGTACGGCAACGCCCCGGCTACAAGGTCTGTGCGCGGCGAGCGGGAGA ACGTACGATGGTTCGACTACGCGCACAACTACCAGGCGCAGAGCGC GGTCCCCCTGCGGCACGAGACCCACGGCGGGCAGGACGTTCGCGGTCTTC AGCAAGGGCCCCATGGCGCACCTGTGACCGCGTCCACGAGCAGAACT ACGTCCCCACGTATGGCGTACGCGGCGTGCATCGGCGCGAACCTGGGC CACTGCGCGCCCCGAGCAGCGCGCGCGCGCGCAGCCTGAAGGACAAGA CCCACACCTGCCCCCCTGCCCGCGCCCGAGCTGTGGGCGGCCCCAGC GTCTTCCTGTTCCCCCCAAGCCCAAGGACACCTGATGATCAGCCGAC CCCCGAGGTACCTGCGTCTGTCGACGTACGCCACGAGGACCCACCG	88

Attorney Docket No. 2014034-0097

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Attorney Docket No. 2014034-0097

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Attorney Docket No. 2014034-0097

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Attorney Docket No. 2014034-0097

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Attorney Docket No. 2014034-0097

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Attorney Docket No. 2014034-0097

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	GGACTGGTACAGCGACAACGAGATGCCCCCCGAGGCGCTGAGCCAGGGC TGCAAGGACATCGCGTACCAGCTGATGCACAACATCCGGGACATCGACG TCATCATTGGGCGGCGGCCGGAAGTACATGTACCCCAAGAACAAGACCGA CGTCGAGTACGAGAGCGACGAGAAGGCGCGGGGACCCGGCTGGAGCGGC CTGGACCTGGTTCGACACCTTGAAGAGCTTCAAGCCCGGTACAAGCACA GCCACTTCATCTTGAACCGGACCGAGCTGCTGACCCTGGACCCCCAAC GTCGACTACCTGCTGGGCTGTTCGAGCCCGGCGACATGCAGTACGAGCT GAACCGGAACAACGTACCGACCCAGCCTGAGCGAGATGGTCGTCGTC GCGATCCAGATCCTGCGGAAGAACCCCAAGGGCTTCTTCCTGCTGGTCTGA GGGCGGCCGATCGACACCGGCCACACGAGGGCAAGGCGAAGCAGGC GCTGCACGAGGCGCTCGAGATGGACCGGGCGATCGGCGAGGCGGCGAGC CTGACCAGCAGCGAGGACACCCTGACCGTCTGCACCGCGGACCACAGCC ACGTCCTTACCTTCGGCGGCTACACCCCGGGGCAACAGCATCTTCGGC CTGGCGCCCATGTCTGAGCGACACCGACAAGAAGCCCTTACCGCGATCCT GTACGGCAACGGCCCCGGCTACAAGGTCGTCGGCGGCGAGCGGGAGAAC GTCAGCATGGTTCGACTACGCGCACAACTACCAGCGCAGAGCGCGG TCCCCCTGCGGCACGAGACCCACGGCGGCGAGGACGTCGCGCTTTCAGC AAGGGCCCCATGGCGCACCTGCTGCACGGCGTCCACGAGCAGAACTACG TCCCCACGTCATGGCGTACGCGGCGTGCATCGGCGCGAACCTGGGCCAC TGCGCGCCCGGAGCGACGATGACGATGACGATGACGATGACGATTGAct gtgcttctagtgtccagccatctgtgttggccctccccgtgcttctgacctgtggaaggtgccaactccccgttctt cttaataaaatgaggaatgcatcgatgtctgagtaggtgtcattcttctgtgggggtgggggtggggcaggacagca agggggaggttggaagagaaatagcagcatgctgggactctattttataggtcttctgtgacttcttctcatcact cttgacaatcgataggtacctgtgctgtctcatgctgtgtttgtttaaaagccaggacgtcacctttgggtgtgtgaaa gtgtgatacttgggtgtgctgtgtgttgcgtctcccaggaaatcatctttaccagatctcaaaaagaaggtcttattacac ctgcagctctctatttccatacagtcagtatcaattctggaagaattccagacattaaagatagtcatttgggtgctgtctg ccgctgctgtcatgtgtcatctgtactcgggaatcctaaaactctgtcttgggtgtcgaatgagaagaagagcagagg cgtgtgaggttatcttcacatcatgattgtttatttcttcttgggctcctacaacattgtcttctctgaacacctccagg aattcttggcctgaataattgcagtagctctaa	
HPPp0156	atacatatttgtgagatatatacaaaataTATATTTAAATCTGGTAGCTTGGTTAATTAAGG ACTAGTATAAAAAATTAACATAAGATACACTTGGGACCTTAAAGGACTTT CATTTCTCTCCCTTCCTTCATCTCTCCTCCTTCACTTACCTTCATTTATTAT TCTAGGCATGACAAGTGTCTGAGATGTCTGATTTGAAGACTAATATGAATAT ATCTAGGTAATTAATAATGGAAAATCCCTATTTCAACATTCTTAATGGAATC CTTCCTTGAAAATTATTATTAGTCTTCTGGTTTAAACATTTATATAGTGTA ATGCAAAAATAAAAATGATTGTATCAAATTATGGTTTTTATTGGATGCTT GCAAGAAAATACTTAAATGTGAGTCATACATTTTCCAGTTCAGCATTTA AACTTCAGACTTCAACCAACAGTTGTGATTGTTTTAGTTTGTGTAGCTGCCT GGAGTGTATTATTTAAGAAAGCAGAAGCACCATTATGACACACTCTTAT AGATCACACACCTTAACCCTGACTTTTTTTTGCTCCAGTTTTTCAGAAGAAG TGAAGTCAAGTTGAAGAACCATTTGCTTTTgaacagttggaacagcagaatatgggccaac aggatatctgtgtgaagcagttctgccccggctcaggggccaagaacagttggaacagcagaatatgggccaacaggga tatctgtgtgaagcagttctgccccggctcaggggccaagaacagatggtccccagatgcggtcccgccctcagcagttt tagaagaacctcagatgtttccagggtgccccaaaggacclgaatgacctgtgcttatttgaactaaccatcagttgct tctctgcttctgctgcgcttctgctccccgagctctatataagcagagctgtttagtgaacccgtcagcagtcgcaactgc cgcttctgtctctgtgggcatctctctgctggcaggcctgtgctgctggttctctgttctcctggtCTGGTGCC GAGAAAGAGAAGGACCTTAAGTATTGGAGAGATCAGGCCCAAGAGACAC TGAAGTACGCCCTTGAACITGCAGAAACITGAACACCAACGTGGCAAAGAA CGTGATCATGTTCTCGGCGACGGCATGGGCGTGTCCACAGTTACAGCCG CCAGATCTGTAAAGGCCAGCTGCACCATAATCTTGGCGAAGAGACAGC GCTGGAAATGGACAAGTTCCCATTCGTGGCCCTGAGCAAGACCTACAACA CCAATGCTCAGGTGCCCCGATTCTGCCGGAACAGCCACAGCTTATCTGTGC GGCGTGAAGGCCAATGAGGGCACCGTTGGAGTGTCTGCCGCCACCGAGA GAAGCAGATGCAATACCACACAGGGCAACGAAAGTGACCAGCATCCTGAG ATGGGCCAAAGACGCCCGGAAGTCTGTGGGCATCGTGACCACCACAGA GTGAACCAACGCCACACTAGCGCCGCTATGCTCACTCTGCCGACAGAGA GAG	107

Attorney Docket No. 2014034-0097

	CTGGTACAGCGACAACGAGATGCCTCCTGAGGCTCTGTCTCAGGGCTGCA AGGATATCGCCTACCAGCTGATGCACAACATCCGGGACATTGATGTGATC ATGGGCGGAGGCCGGAAGTACATGTATCCCAAGAACAAAGACCGACGTCG AGTACGAGAGCGACGAGAAGGCCAGAGGCACAAGACTGGATGGCCTGGA CCTGGTGGATACCTGGAAGTCCTTCAAGCCCCGGTACAAGCACAGCCACT TCATCTGGAACCGGACCGAGCTGCTGACCCTGGATCCTCACAATGTGGAC TACCTGCTGGGCCTGTTCGAGCCCCGGCGATATGCAGTACGAGCTGAACCG GAACAACGTGACAGACCCAGCCTGAGCGAGATGGTGGTTGTGGCCATTG AGATCCTGCGGAAGAACCCCAAGGGATTCTTCCTGCTGGTGGAAGGCGGC AGGATCGATCACGGACACCATGAGGGAAAAGCCAAGCAGGCCCTGCACG AGGCCGTGCAAAATGGATAGAGCCATTGGCCAGGCCGGCAGCCTGACAAG CTCTGAGGATACACTGACCGTGGTCACCGCCGATCACAGCCACGTGTTCA CATTGCGCGGCTACACCCCTAGAGGCAACAGCATCTTTGGACTGGCCCCCT ATGCTGAGCGACACCGACAAGAAGCCTTTCACCGCCATCCTGTACGGCAA CGGCCCTGGCTATAAGGTTGTTCGGAGGCGAGAGGGAAAAACGTGTCCATG GTGGATTACGCCCACAACAACCTACCAGGCTCAGAGCGCCGTGCCTCTGAG ACACGAAACACACGGCGGAGAAGATGTGGCCGTGTTACAGCAAGGGCCCC ATGGCTCATTTGCTGCACGGCGTGCACGAGCAGAATTACGTGCCACACGT GATGGCCTACGCCGCCTGTATTGGAGCCAATCTGGGACATTGTGCCCTG CCAGCAGCctgaagGAGAGCAAGTACGGCCCCCCTGTCTCCATGTCTGC TCCAGAGgcccgcGGCGGACCCCTCCGTGTTCTGTTTCTCCAAAGCCTAAG GACACCCCTGATGATCAGCAGAACCCCTGAAGTGACCTGCGTGGTGGTGA CGTGTCCCAAGAGGATCCTGAGGTGCAGTTCAATTGGTACGTGGACGGCG TGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTTCAACAG CACCTACAGAGTGGTGTCTGTGCTGACCGTGTGCACACAGGATTGGCTGA ACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGGCCTGCCTAGCAG CATCGAGAAAACCATCAGCAAGGCCAAGGGACAGCCAGAGAACCCAG GTGTACACACTGCCTCCAAGCCAAGAGGAAATGACCAAGAACCAGGTGT CCCTGACCTGCCTGGTCAAGGGCTTCTACCCCTCCGATATCGCCGTGGAA TGGGAGAGCAATGGCCAGCCAGAGAACAACCTACGACACCACACCTCCAG TGCTGAAGTCCGACGGCTCATTCTTTCTGTACTCCGACCTGACAGTGGAC AAGAGCAGATGGCAAGAGGGCAACGTGTTCTCTCGACGCGTGTGCACG AAGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGTCTCTGGGC AAATGActgtgccttctagttgccagccatctgtgtttgcccctccccgtgccttcttgacctggaaggtgccact cccactgtccttcttaataaaataggaaattgcatcgcattgtctgagtaggtgtcattctattctgggggtgggggtggg gcaggacagcaagggggaggattgggaagagaatagcaggcatgctggggaAGTCCTGGCGGTTTTT ATTAAGGCTGTTCATGTGAAAGGTATGTGATATTTAGAAAAATGATCCAG ATCAAAGGAAAAATATAGGACAGATCTGTTTTTCAGTTTTAAACATTTAT GCCTATGCTTTGGTTGGCCAGTTAGCCAGCTACTTTTTCCTAACATGCTTT CATCTTTCTATGGCAGTTGTCTTTACATAGATAAGACTCTTTTATTTCTTTT TTCTCTTATCTTACTTACTTGTTTGCCTACTACAACTAGAGAATAAGAAA TTAGCTAACTACAGATAGTCTGCTTATATGAATGTCCATATTGAATCTTA AAAATCTATAATCCTTCCCTTGAGTCTCTTAAATTAATATTCTGGAGAAAT GAAATAATAATTACTGTAAGTACAACATGGAATGCATTGGGATTTTATTT CCCCAAAGAGGCTGGTATIGGTTTTAACAGCTTTCCTGGATCTGATCCAC AGATTTATTATGACCCTTTAAACAATGTTTAAATGTTATACAGGTTGCCTATA AATTGATGACTGAACTTTGTATTGTTTACTGATCTTCAAACAACCTT CAGAAAAATGGAATACATTCTGAATT	
HPPp0182	CTGTAATCCCAGATACTCGGGAGCTGAGGCAGGAGAATTGCTTGAACCTG GGATACAGAGGTTGCAGTGAGCCAAGATCATGCCACTGCACTCCAGCCTG GGCGACAGAGTGAGACTCCATTTCAAAAAAAAAAACAGAGGCAAAAGCT GAATTTAAAAAAAAAAAAACCCCTCAAAATATGAAGAAGAACTTCATTCCA AATTCCTTACTAAAAATTAATAAATAACAAACCAAAAAATTGACTTCTTTT CTCGAACAGCTTTCCACATAGACTTTTAGGGTAGTCCTTCTGTGGTTAAAT GTTTATGAGGAGAATTGCACCTGTGTTGTGGTCAGCTTTTAAATAAACTTG CACCTGCAGTGGTTTTTCATAGAATTGCTGTTGAGAAATTACATGTCAGT	108

Attorney Docket No. 2014034-0097

GGTTTGACCATCACACAGAGCTATTTCCTTCACCATATCACCTGCCAGG CAGGTCCTAATTTAAAAAGGGCCTACTTCCAAAAATTCATTTGAAAAAAA ATCTGGTTAGATTTCATGGTAATCTCTCCAGGGACACAATAATAAGTAAG GTTTCCAGGATACCCCTCAAACAAAAAGAATATGCTCCCTTCAATTCATga acagttggaacagcagaatatgggccaacaggatatctgtgtaagcagttcctgccccggtcaggccaagaacag ttggaacagcagaatatgggccaacaggatatctgtgtaagcagttcctgccccggtcaggccaagaacagatggt ccccagatgagggtccccccctcagcagtttctagagaaccatcagatgtttccagggtgccccaggacctgaatgacc ctgtgcttatttgaactaaccaatcagttcgttctcgttctgttcgcgcgttctgctccccgagctctatataagcagagc tcgttttagtaaccgtcagatgccaccatgccgtcttctgtctgtggtggcctcctcctgctgagcagcctgtgtgctgctgg tccctgtctcctggtCTGGTCCCCGAGAAGGAGAAGGACCCCCAAGTACTGGCGG GACCAGGCGCAGGAGACCCTGAAGTACGCGCTGGAGCTGCAGAAGCTGA ACACCAACGTCGCGAAGAACGTCATCATGTTCTGGGCGACGGCATGGG CGTCAGCACCGTCACCGCGGCGCGGATCCTGAAGGGCCAGCTGCACCAC AACCCCGGCGAGGAGACCCGGCTGGAGATGGACAAGTTCCTTCGTCG CGTGAGCAAGACCTACAACACCAACGCGCAGGTCCCCGACAGCGCGGG CACCGCGACCGCTACCTGTGCGGCGTCAAGGCGAACGAGGGCACCGTC GGCGTCAGCGCGGCGACCGAGCGGAGCCGGTGCAACACCACCCAGGGCA ACGAGGTCAACAGCATCCTGCGGTGGGCGAAGGACGCGGGCAAGAGCGT CGGCATCGTCAACACACCCGGGTCAACCACGCGACCCCCAGCGCGGCGT ACGCGCACAGCGCGGACCCGGGACTGGTACAGCGACAACGAGATGCCCCC CGAGGCGCTGAGCCAGGGCTGCAAGGACATCGCGTACCAGCTGATGCAC AACATCCGGGACATCGACGTCATCATGGGCGGCGGCCGGAAGTACATGT ACCCCAAGAACAAAGACCGACGTCGAGTACGAGAGCGACGAGAAGGCGC GGGGCACCCGGCTGGACGGCTGGACCTGGTTCGACACCTGGAAGAGCTT CAAGCCCCGTACAAGCACAGCCACTTCATCTGGAACCGGACCGAGCTG CTGACCCTGGACCCCCACAACGTCGACTACCTGCTGGGCCTGTTTCGAGCC CGGCGACATGCAGTACGAGCTGAACCGGAACAACGTCACCGACCCCAGC CTGAGCGAGATGGTTCGTTCGTTCGCGATCCAGATCCTGCGGAAGAACCCCA AGGGCTTCTTCTGCTGGTTCGAGGGCGGCCGGATCGACCACGGCCACCAC GAGGGCAAGGCGAAGCAGGCGCTGCACGAGGCGGTTCGAGATGGACCGG GCGATCGGCCAGGCGGGCAGCCTGACCAGCAGCGAGGACACCCCTGACCG TCGTACCCGCGGACCACAGCCACGTCTTCACCTTCGGCGGCTACACCCCC CGGGGCAACAGCATCTTCGGCCTGGCGCCCATGCTGAGCGACACCGACA AGAAGCCCTTACCCGCGATCCTGTACGGCAACGGCCCCGGCTACAAGGTC GTCGGCGGCGAGCGGGAGAACGTCAGCATGGTTCGACTACGCGCACACA ACTACAGGCGCAGAGCGCGTCCCCCTGCGGCACGAGACCCACGGCGG CGAGGACGTCGCGGTCTTCAGCAAGGGCCCCATGGCGCACCTGCTGCACG GCGTCCACGAGCAGAACTACGTCCCCACGTCATGGCGTACGCGGCGTGC ATCGGCGCGAACCTGGGCCACTGCGCGCCCCGCGAGCAGCCTGAAGGAGA GCAAGTACGGCCCCCCTGCCCCCCTGCCCCGCGCCGAGGCGGCGGGC GGCCCCAGCGTCTTCTGTTCCTGTTCCCCCAAGCCCAAGGACACCTGATGAT CAGCCGGACCCCCGAGGTACCTGCGTCGTTCGTACGTCAGCCAGGAG GACCCCGAGGTCCAGTTCAACTGGTACGTCGACGGCGTCGAGGTCCACAA CGCGAAGACCAAGCCCCGGGAGGAGCAGTTCAACAGCACCTACCGGGTC GTCAGCGTCTGACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGT ACAAGTGCAAGGTACGCAACAAGGGCCTGCCAGCAGCATCGAGAAGAC CATCAGCAAGGCGAAGGGCCAGCCCCGGGAGCCCCAGGTCTACACCTG CCCCCAGCCAGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCC TGGTCAAGGGCTTCTACCCAGCGACATCGCGGTTCGAGTGGGAGAGCAA CGGCCAGCCGAGAACAACTACGACACCACCCCCCGTCTGAAGAGC GACGGCAGCTTCTTCTGTACAGCGACCTGACCGTCGACAAGAGCCGGT GCAGGAGGGCAACGTCTCAGCTGCAGCGTCATGCACGAGGCGCTGCAC AACCCTACACCCAGAAGAGCCTGAGCCTGAGCCTGGGCAAGGACATCG ACGATGACGATGACGATGACGATGACGATGACGATGactgtgcttctagtgcagccatctgtttt gccccctccctgcttctgacctgaaggtgccactccactgtcttctctataaataaggaaattgcacgcac tgtctgagttagtgtcattctatttgggggtgggggtgggcaggacagcaagggggaggattgggaagagaatagca	
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Attorney Docket No. 2014034-0097

	TGGTCAAGGGCTTCTACCCAGCGACATCGCGGTCGAGTGGGAGAGCAA CGGCCAGCCCGAGAACAACACTACGACACCAACCCCCCGTCTGAAGAGC GACGGCAGCTTCTTCTGTACAGCGACCTGACCGTCGACAAGAGCCGGTG GCAGGAGGGCAACGTCTTCAGCTGCAGCGTCATGCACGAGGCGCTGCAC AACCCTACACCCAGAAGAGCCTGAGCCTGAGCCTGGGCAAGGACATCG ACGATGACGATGACGATGACGATGACGATGactgtgcttctagtccagccatctgtgtt gccccctcccgctgcttcttgacctggaaggtgccaactccactgcttctcctaataaaataggaaattgcatgcat tgtctagtagtgctcattctattctgggggtgggtggggcaggacagcaagggggaggattgggaagagaatagca ggcatgctgggga	
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Table 3 – Exemplary Expression Cassettes (Amino Acid Sequences)

Name	Amino Acid Sequence	SEQ ID NO
ALP-IgG1Fc (p0134)	MPSSVSWGILLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIA YQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL DGLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVLDYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSDITLVVTAADHSHVFTFGGYTPRGNSIFGLAPMLS DIDKKPFTAILYGNPGYKVVGGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSL KDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPT VKFNWYVDGVEVHNATKPKREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALPAIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTITPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCS VMHEALHNHYTQKSLSLSPGK	62
ALP-IgG1Fc (Secreted)	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTV TAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYL CGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRV NHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIA YQLMHNIRDIDVIM GGGRKMYMPKNKTDVEYESDEKARGTRL DGLDLVDTWKSFKPRYKHSFIW NRTELLTLDPHNVLDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRK NPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSDITLV VTAADHSHVFTFGGYTPRGNSIFGLAPMLS DTDKKPFTAILYGNPGYKVVGG GERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMALLHGVHE QNYVPHVMAYAACIGANLGHCAPASSLKDKTHTCPPCPAPELLGGPSVFLF PKPKDTLMISRTPEVTCVVVDVSHEDPTVKFNWYVDGVEVHNATKPKREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTITPPVLD SDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK	63
ALP-IgG1Fc (p0074)	MISPFLVLAIGTCLTNSLVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAK NVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNT NAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWA KDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIA YQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL DGLDLVD TWKSFKPRYKHSFIWNRTELLTLDPHNVLDYLLGLFEPGDMQYELNRNNVTD PSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDR AIGQAGSLTSSDITLVVTAADHSHVFTFGGYTPRGNSIFGLAPMLS DTDKKP FTAILYGNPGYKVVGGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDV AVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSLKDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPTVKFNWYV	64

Attorney Docket No. 2014034-0097

	DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHLEALHNHYTQKSLSLSPGK	
ALP-IgG1Fc (Secreted)	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFVVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSDITLTVTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNGPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSLKDKTHTCPCPAPELLGGPSVFLFPKPKDITLMISRTPEVTCVVVDVSHEDPTVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHLEALHNHYTQKSLSLSPGK	65
ALP-IgG1Fc-D8 (p0042)	MISPFVLAIPTCLTNSLVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFVVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSDITLTVTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNGPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSLKDKTHTCPCPAPELLGGPSVFLFPKPKDITLMISRTPEVTCVVVDVSHEDPTVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHLEALHNHYTQKSLSLSPGKDIDDDDDDDDD	66
ALP-IgG1Fc-D8 (Secreted)	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFVVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSDITLTVTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNGPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSLKDKTHTCPCPAPELLGGPSVFLFPKPKDITLMISRTPEVTCVVVDVSHEDPTVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHLEALHNHYTQKSLSLSPGKDIDDDDDDDDD	67
ALP-LL-IgG1Fc-3xsubst-D10 (p0127)	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFVVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHE	68

Attorney Docket No. 2014034-0097

	AVEMDRAIGQAGSLTSSSEDTLTVVTDADHSHVFTFGGYTPRGNSIFGLAPMLS DTDCKPFTAILYGNPGYKVVGGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSG GGGSGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPTVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYDTPPVLKSDGSFFLYSDLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKDDDDDDDDDD	
ALP-LL- IgG1Fc-3xsubst- D10 (Secreted)	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTV TAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYL CGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRV NHATPSAAYAHSADRDWYSDNEMPEALSQGCKDIAYQLMHNIRDIDVIMG GGRKMYMPKNKTDVEYESDEKARGTRLDDGLDLDVDTWKSFKPRYKHSFIW NRTELLTLDPHNVLDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRK NPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSSEDTLTV VTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDCKPFTAILYGNPGYKVVGG ERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMALLHGVHE QNYVPHVMAYAACIGANLGHCAPASSGGGGSGGGSGGGGSDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPTVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYDTPPVLKSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGKDDDDDDDDDD	69
ALP-LL- IgG1Fc-3xsubst- D10 (p0137)	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRLDD GLDLDVDTWKSFKPRYKHSFIWNRTELLTLDPHNVLDYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKPNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSSEDTLTVVTDADHSHVFTFGGYTPRGNSIFGLAPMLS DTDCKPFTAILYGNPGYKVVGGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSG GGGSGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPTVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYDTPPVLKSDGSFFLYSDLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKDIDDDDDDDDDDD	70
ALP-LL- IgG1Fc-3xsubst- D10 (Secreted)	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTV TAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYL CGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRV NHATPSAAYAHSADRDWYSDNEMPEALSQGCKDIAYQLMHNIRDIDVIMG GGRKMYMPKNKTDVEYESDEKARGTRLDDGLDLDVDTWKSFKPRYKHSFIW NRTELLTLDPHNVLDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRK NPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSSEDTLTV VTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDCKPFTAILYGNPGYKVVGG ERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMALLHGVHE QNYVPHVMAYAACIGANLGHCAPASSGGGGSGGGSGGGGSDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPTVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYDTPPVLKSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGKDIDDDDDDDDDDD	71

Attorney Docket No. 2014034-0097

ALP-LL-IgG1Fc-3xsubst-D10 (p0140)	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKYMYPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKPNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSDTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLS DTDKKPFTAILYGNPGYKVVGGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSG GGGSGGGSGGGGSDKTHTCPPELPGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPTVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYDTPPVKSDGSFFLYSDLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKDIDDDDDDDDDDD	72
ALP-LL-IgG1Fc-3xsubst-D10 (p0140) (Secreted)	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTV TAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYL CGVKANEGTVGVSAATERSRCNTTQNEVTSILRWAKDAGKSVGIVTTTRV NHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNIRDIDVIMG GGRKYMYPKNKTDVEYESDEKARGTRLGLDLVDTWKSFKPRYKHSFIW NRTELLTLDPHNVYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRK NPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSDTLTV VTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNPGYKVVGG ERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMALLHGVHE QNYVPHVMAYAACIGANLGHCAPASSGGGGSGGGSGGGGSDKTHTCPPE LPGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPTVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYDTPPVKSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGKDIDDDDDDDDDDD	73
ALP-LL-IgG4Fc-3xsubst-PAA-D10 (p0126)	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKYMYPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKPNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSDTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLS DTDKKPFTAILYGNPGYKVVGGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSG GGGSGGGSGGGGSCPPCAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYDTPPVKSDGSFFLYSDLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLGLKDDDDDDDDDDDD	74
ALP-LL-IgG4Fc-3xsubst-PAA-D10 (Secreted)	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTV TAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYL CGVKANEGTVGVSAATERSRCNTTQNEVTSILRWAKDAGKSVGIVTTTRV NHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNIRDIDVIMG GGRKYMYPKNKTDVEYESDEKARGTRLGLDLVDTWKSFKPRYKHSFIW NRTELLTLDPHNVYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRK NPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSDTLTV VTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNPGYKVVGG ERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMALLHGVHE QNYVPHVMAYAACIGANLGHCAPASSGGGGSGGGSGGGGSCPPCAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHN	75

Attorney Docket No. 2014034-0097

	AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YDTPPVLKSDGSFFLYSDLTVDKSRWQEGNVFSCSVMEALHNHYTQKSL SLSLGKDDDDDDDDDD	
ALP-Fc GeneArt Codon Optimized 1 (p0164)	MPSSVSWGILLAGLCCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPEAL SQGCKDIA YQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSEDTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLS DTDCKPFTAILYGNPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSG GGGSLKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPTVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYDTPPVLKSDGSFFLYSDLTVDKSRWQQGN VFSCSVMEALHNHYTQKSLSLSPGKDIDDDDDDDDDDD	91
ALP-Fc GeneArt Codon Optimized 2 (p0168)	MPSSVSWGILLAGLCCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPEAL SQGCKDIA YQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSEDTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLS DTDCKPFTAILYGNPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSG GGGSLKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPTVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYDTPPVLKSDGSFFLYSDLTVDKSRWQQGN VFSCSVMEALHNHYTQKSLSLSPGKDIDDDDDDDDDDD	92
ALP-Fc with ALP signal peptide (p0100)	MISPFLVLAIGTCLTNSLVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAK NVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNT NAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWA KDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPEALSQGCKDIA YQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRLGLDLVD TWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTD PSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDR AIGQAGSLTSSEDTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDCKP FTAILYGNPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVA VFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPAS	93
ALP-Fc with A1AT signal peptide (p095)	MPSSVSWGILLAGLCCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPEAL SQGCKDIA YQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSEDTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLS DTDCKPFTAILYGNPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPAS	94

Attorney Docket No. 2014034-0097

ALP-IgG4Fc (p0215)	MPSSVSWGILLLAGLCCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSDITLTVVTAADHSHVFTFGGYTPRGNSIFGLAPMLS DITDKKPFTAILYGNPGYKVVGGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSL KESKYGPCCPPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNATKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYDTTPPVLKSDGSFFLYSDLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLGLKDIIDDDDDDDDDDD	101
HPPp0155	MPSSVSWGILLLAGLCCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSDITLTVVTAADHSHVFTFGGYTPRGNSIFGLAPMLS DITDKKPFTAILYGNPGYKVVGGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSL KESKYGPCCPPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNATKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYDTTPPVLKSDGSFFLYSDLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLGLKDDDDDDDDDDDD	109
HPPp0257	MPSSVSWGILLLAGLCCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSDITLTVVTAADHSHVFTFGGYTPRGNSIFGLAPMLS DITDKKPFTAILYGNPGYKVVGGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASD DDDDDDDDDD	110
p00983	MPSSVSWGILLLAGLCCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVYLLGLFEPGDMQYEL NRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHE AVEMDRAIGQAGSLTSSDITLTVVTAADHSHVFTFGGYTPRGNSIFGLAPMLS DITDKKPFTAILYGNPGYKVVGGGERENVSMVDYAHNNYQAQSAVPLRHET HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSL KESKYGPCCPPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNATKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYDTTPPVLKSDGSFFLYSDLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLGLKDIIDDDDDDDDDDD	111

Attorney Docket No. 2014034-0097

PLA256	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNV D YLLGLFEPGDMQYEL NRNNVTDPSLSEM V V V A I Q I L R K N P K G F F L L V E G G R I D H G H H E G K A K Q A L H E AVEMDRAIGQAGSLTSS EDTLT V V T A D H S H V F T F G G Y T P R G N S I F G L A P M L S D T D K K P F T A I L Y G N G P G Y K V V G G E R E N V S M V D Y A H N N Y Q A Q S A V P L R H E T HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSL KESKYGPCCPPCAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYDTTPPV L K S D G S F F L Y S D L T V D K S R W Q E G N V F S C SVMHEALHNHYTQKSLSLGLKIDDDDDDDDDDD	112
HPPp0275	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNV D YLLGLFEPGDMQYEL NRNNVTDPSLSEM V V V A I Q I L R K N P K G F F L L V E G G R I D H G H H E G K A K Q A L H E AVEMDRAIGQAGSLTSS EDTLT V V T A D H S H V F T F G G Y T P R G N S I F G L A P M L S D T D K K P F T A I L Y G N G P G Y K V V G G E R E N V S M V D Y A H N N Y Q A Q S A V P L R H E T HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASD DDDDDDDDDD	113
HPPp0156	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNV D YLLGLFEPGDMQYEL NRNNVTDPSLSEM V V V A I Q I L R K N P K G F F L L V E G G R I D H G H H E G K A K Q A L H E AVEMDRAIGQAGSLTSS EDTLT V V T A D H S H V F T F G G Y T P R G N S I F G L A P M L S D T D K K P F T A I L Y G N G P G Y K V V G G E R E N V S M V D Y A H N N Y Q A Q S A V P L R H E T HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSL KESKYGPCCPPCAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYDTTPPV L K S D G S F F L Y S D L T V D K S R W Q E G N V F S C SVMHEALHNHYTQKSLSLGLK	114
HPPp0182	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFV ALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNE VTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEAL SQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRL GLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNV D YLLGLFEPGDMQYEL NRNNVTDPSLSEM V V V A I Q I L R K N P K G F F L L V E G G R I D H G H H E G K A K Q A L H E AVEMDRAIGQAGSLTSS EDTLT V V T A D H S H V F T F G G Y T P R G N S I F G L A P M L S D T D K K P F T A I L Y G N G P G Y K V V G G E R E N V S M V D Y A H N N Y Q A Q S A V P L R H E T HGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSL KESKYGPCCPPCAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYDTTPPV L K S D G S F F L Y S D L T V D K S R W Q E G N V F S C SVMHEALHNHYTQKSLSLGLKIDDDDDDDDDDD	115

Attorney Docket No. 2014034-0097

ALP-IgG4Fc-long linker-3xsubst-S228P-F234A-L235A-D10-3	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTV TAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYL CGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRV NHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIA YQLMHNIRDIDVIMG GGRKMYMPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSFIW NRTELLTLDPHNV DYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRK NPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSD TLTV VTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNPGYKVVGG ERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMALLHGVHE QNYVPHVMAYAACIGANLGHCAPASSGGGGSGGGSGSCPPCAPEAAGGP SVFLFPPKPKDTLMISRTPVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG QPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYDT TPPV LKSDGSFFLYSDLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSL GKDDDDDDDDDD	116
ALP-IgG4Fc-long linker-3xsubst-S228P-F234A-L235A-D10-4	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTV TAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYL CGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRV NHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIA YQLMHNIRDIDVIMG GGRKMYMPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSFIW NRTELLTLDPHNV DYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRK NPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSD TLTV VTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNPGYKVVGG ERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMALLHGVHE QNYVPHVMAYAACIGANLGHCAPASSGGGGSGGGSGSCPPCAPEAAGGP SVFLFPPKPKDTLMISRTPVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG QPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYDT TPPV LKSDGSFFLYSDLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSL GKDDDDDDDDDD	117

Table 4 – Exemplary Promoters

Name	Nucleotide Sequence	SEQ ID NO
MND	gaacagtggaaacagcagaatatgggccaacaggatatctgtgtaagcagtctctccccggctcaggccaagaa cagttggaacagcagaatatgggccaacaggatatctgtgtaagcagtctctccccggctcaggccaagaaacag atggtccccagatcggtccccccctcagcagttctagagaacctcagatgttccagggtcccccaaggacctgaa atgacctgtgcttattgaactaaccatcagtcgcttctgcttctgctgctgcttctgcttctccccgagctctatataag cagagctcgtttagtaaccgctcagatc	76
B29	GACCCCAGCTGTGCTGCCCAAGCTGGGCGACCGCCAAACCTTAGCGGCC CAGCTGACAAAAGCCTGCCCTCCCCAGGGTCCCCGGAGAGCTGGTGCC TCCCCTGGGTCCCAATTTGCATGGCAGGAAGGGGCCTGGTGAGGAAGAG GCGGGGAGGGGACAGGCTGCAGCCGGTGCAGTTACACGTTTTCTCCAA GGAGCCTCGGACGTTGTCACGGGTTTGGGGTTCGGGGACAGAGCC	125
Fu_1	TGTTTGTCAGTAACCTACAAATAGTGTCTAGTAACCAATATAGGACAATA AGATGAGGAAGTGATATCCCTGTGGCTTCCCAATAGTACGACTAAAGGT GCAGCTGTGCTCAGTTAGTTATAGCATGTAGATTTGCATCTAGTAATGGA CACGTGATTGTATCTCTTAGAATCATTTATAAACCAGGTCCTTGCAGTGA GATCTGAAATACATCAGATCAGCATGGGCATCAAGATGatggacttgcca	126
Fu_2	TCGTGGTTGATGGAAATGTAAAGTAGATAAGGTAAGGGGATTAAACATAA TCAACATATTAGACCGTGCGAAGGTATACACCCAACGATCTGACTGTTT ATTTAAGCTTAGAGAAGGGGGAAGTGTATTGATTGATTGATTACAGAA	127

Attorney Docket No. 2014034-0097

	CGGATCTAGTTGTCAGTAACTTACTGATAAAAGCTCACACTAAGCTGAG AAGCTCCATCCTCTTCTCATAGAGCCTCCATCAGAGCATGatcctctcagct	
Fu_3	TGGGGGAGCAACCCATCAAAAGTGTCAGTAACATTGCGCAACCTCTCCA GACCACTCTGCTAATTTCTCATACAATCAAAATCTCCACCAAATTTGGAC ACTACCCGAAGGGGGAAGTAATAAGCCCACATTTGCATGAGGACAGCTA ACCCTAAGCTCCATCCCCTGGAGTTGTTTAATAATCTGATCATACACACT CCAACAGTCATTTCTGGTCAGGAGACGTTGTAGAAATGattacaggcatg	128

Table 5 – Exemplary Transgene (Nucleotide Sequences)

Name	Nucleotide Sequence	SEQ ID NO
ALP-Fc CDS	ATGATTTTACCATTCTTAGTACTGGCCATTGGCACCTGCCTTACTAACTC CCTGGTGCCCGAGAAAGAGAAGGACCCTAAGTATTGGAGAGATCAGGC CCAAGAGACACTGAAGTACGCCCTGGAACCTGCAGAACTGAACACCAA CGTGGCCAAGAACGTGATCATGTTCTCGGCGACGGCATGGGCGTGTCC ACAGTTACAGCCGCCAGAATCCTGAAGGGCCAGCTGCACCATAATCCTG GCGAAGAGACACGGCTGGAAATGGACAAGTTCCTTCTGTTGGCCCTGAG CAAGACCTACAACACCAATGCTCAGGTGCCCGATTCTGCCGGAACAGCC ACAGCTTATCTGTGCGGCGTGAAGGCCAATGAGGGCACCGTTGGAGTGT CTGCCGCCACCGAGAGAAGCAGATGCAATACCACACAGGGCAACGAAG TGACCAGCATCCTGAGATGGGCCAAAGACGCCGGCAAGTCTGTGGGCAT CGTGACCACCACCAGAGTGAACCACGCCACACCTAGCGCCGCCTATGCT CACTCTGCCGACAGAGACTGGTACAGCGACAACGAGATGCCTCCTGAGG CTCTGTCTCAGGGCTGCAAGGATATCGCCTACCAGCTGATGCACAACAT CCGGGACATTGATGTGATCATGGGCGGAGGCCGGAAGTACATGTATCCC AAGAACAAGACCGACGTCGAGTACGAGAGCGACGAGAAGGCCAGAGGC ACAAGACTGGATGGCTTGGACCTGGTGGATACCTGGAAGTCCTTCAAGC CCCGGTACAAGCACAGCCACTTCATCTGGAACCGGACCGAGCTGCTGAC CCTGGATCCTACAATGTGGACTACCTGCTGGGCCCTGTTGAGCCCCGGC GATATGCAGTACGAGCTGAACCGGAACAACGTGACAGACCCCCAGCCTG AGCGAGATGGTGGTTGTGGCCATTAGATCCTGCGGAAGAACCCCAAGG GATTCTTCTGTGTTGGTGAAGGCGGCAGGATCGATCACGGACACCATGA GGGAAAAAGCCAAGCAGGCCCTGCACGAGGCCGTCGAAATGGATAGAGC CATTGGCCAGGCCGGCAGCCTGACAAGCTCTGAGGATACACTGACCGTG GTCACCGCCGATCACAGCCACGTGTTACATTTCGGCGGCTACACCCCTA GAGGCAACAGCATCTTTGGACTGGCCCTATGCTGAGCGACACCGACAA GAAGCCTTTCACCGCCATCCTGTACGGCAACGGCCCTGGCTATAAGGTT GTCGGAGGCGAGAGGGAAAACGTGTCCATGGTGGATTACGCCCAACAAC AACTACCAGGCTCAGAGCGCCGTGCCTCTGAGACACGAAACACACGGCG GAGAAGATGTGGCCGTGTTAGCAAGGGCCCCATGGCTCATTTGCTGCA CGGCGTGACAGAGCAGAATTACGTGCCACACGTGATGGCCTACGCCGCC TGTATTGGAGCCAATCTGGGACATTGTGCCCTGCCAGCAGCCTGAAGG ACAAGACCCATACCTGTCTCCATGTCTGCTCCAGAGCTGCTCGGAGG CCCTTCCGTGTTCTGTTCCTCCAAAGCCTAAGGACACCCGTGATGATCA GCAGAACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCCACGAGGA TCCCACCGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAAC GCCAAGACCAAGCCTAGAGAGGAACAGTACAACAGCACCTACAGAGTG GTGTCTGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAGAGT ACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCTATCGAGAAAAC CATCAGCAAGGCCAAGGGACAGCCAGAGAACCCAGGTGTACACACT GCCTCCAAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGC CTGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGGGAGAGCA ATGGCCAGCCAGAGAACAACCTACAAGACCACACCTCCTGTGCTGGACAG	77

Attorney Docket No. 2014034-0097

HPPp0257	gaacagttggaacagcagaatatgggccaacaggatatctgtgtaagcagttcctgccccggctcaggccaagaa cagttggaacagcagaatatgggccaacaggatatctgtgtaagcagttcctgccccggctcaggccaagaaacag atggtccccagatgcggtcccgccctcagcagtttctagagaacctcagatgtttccagggtgccccaggacctgaa atgacctgtgcctatttgaactaaccaatcagttcgttctcgttctgttcgcgcgttctgtctcccgagctctatataag cagagctcgtttagtgaaccgtcagatgccaccatgccgttctgtctcgtgggcatcctctctgtggcaggcctgtg ctgcctgtgccctgtctccctggctCTGGTCCCCGAGAAAGGAGAAGGACCCCAAGTACT GGCGGGACCCAGGCGCAGGAGACCCCTGAAGTACGCGCTGGAGCTGCAGA AGCTGAACACCAACGTCGCGAAGAACGTCATCATGTTCTGGGCGACGG CATGGGCGTCAGCACCGTCACCGCGGCGCGGATCCTGAAGGGCCAGCTG CACCACAACCCCGGCGAGGAGACCCGGCTGGAGATGGACAAGTTCCTT TCGTGCGCTGAGCAAGACCTACAACACCAACGCGCAGGTCCCCGACAG CGCGGGCACCGCGACCGCGTACCTGTGCGGCGTCAAGGCGAACGAGGG CACCGTCGGCGTCAGCGCGGCGACCGAGCGGAGCCGGTGCAACACCCAC CCAGGGCAACGAGGTCAACAGCATCCTGCGGTGGGCGAAGGACCGCGG CAAGAGCGTCGGCATCGTCACCAACACCCGGGTCAACCACGCGACCCCC AGCGCGGCGTACGCGCACAGCGCGGACCGGGACTGGTACAGCGACAAC GAGATGCCCCCGAGGCGCTGAGCCAGGGCTGCAAGGACATCGCGTACC AGCTGATGCACAACATCCGGGACATCGACGTCATCATGGGCGGCGGCCG GAAGTACATGTACCCCAAGAACAAGACCGACGTCGAGTACGAGAGCGA CGAGAAGGCGCGGGGCACCCGGCTGGACGGCTGGACCTGGTTCGACAC CTGGAAGAGCTTCAAGCCCCGGTACAAGCACAGCCACTTCATCTGGAAC CGGACCGAGCTGCTGACCCTGGACCCCAACAACGTCGACTACCTGCTGG GCCTGTTTCGAGCCCGGCGACATGCAGTACGAGCTGAACCGGAACAACGT CACCGACCCAGCCTGAGCGAGATGGTCTGTCGTCGCGATCCAGATCCTG CGGAAGAACCCCAAGGGCTTCTTCTGCTGGTTCGAGGGCGGCGGATCG ACCACGGCCACACGAGGGCAAGGCGAAGCAGGCGCTGCACGAGGCGG TCGAGATGGACCGGGCGATCGGCCAGGCGGGCAGCCTGACCAGCAGCG AGGACACCCTGACCGTCGTCACCGCGGACCACAGCCACGTCCTTACCTT CGGCGGCTACACCCCGGGGCAACAGCATCTTCGGCCTGGCGCCCATG CTGAGCGACACCGACAAGAAGCCCTTACCGCGATCCTGTACGGCAACG GCCCCGCTACAAGTTCGTCGGCGGCGAGCGGGAGAACGTCAGCATGG TCGACTACGCGCACACAACACTACCAGGCGCAGAGCGCGGTCCCCCTGCG GCACGAGACCCACGGCGGCGAGGACGTCGCGGTCTTCAGCAAGGGCCC CATGGGCGACCTGCTGCACGGCGTCCACGAGCAGAACTACGTCCCCAC GTCATGGCGTACGCGGCGTGCATCGGCGCGAACCTGGGCCACTGCGCGC CCGCGAGCGACGATGACGATGACGATGACGATGACGATGACGATgtagtgtgctttagtgc ccagccatctgtttgttgcctccctcgtgcttctgacctggaaggtgccactccactgtcttcttaataaaatg aggaaattgcacgcattgtctgtagtggtgtcattctattctgtgggggtgggtggggcaggacagcaagggggagg attgggaagagaatagcaggcatgctgggga	134
HPPp0160 (ALP-LL- IgG1Fc-3xsubst- D10)	gaacagttggaacagcagaatatgggccaacaggatatctgtgtaagcagttcctgccccggctcaggccaagaa cagttggaacagcagaatatgggccaacaggatatctgtgtaagcagttcctgccccggctcaggccaagaaacag atggtccccagatgcggtcccgccctcagcagtttctagagaacctcagatgtttccagggtgccccaggacctgaa atgacctgtgcctatttgaactaaccaatcagttcgttctcgttctgttcgcgcgttctgtctcccgagctctatataag cagagctcgtttagtgaaccgtcagatgccaccatgccgttctgtctcgtgggcatcctctctgtggcaggcctgtg ctgcctgtgccctgtctccctggctCTGGTGGCCGAGAAAGAGAAGGACCCCTAAGTATT GGAGAGATCAGGCCCAAGAGACACTGAAGTACGCCCTGGAAGTGCAGA AACTGAACACCAACGTCGGCAAAGAACGTGATCATGTTCTCGGCGACGG CATGGGCGTGTCCACAGTTACAGCCGCCAGAATCCTGAAGGGCCAGCTG CACCATAATCCTGGCGAAGAGACACGGCTGGAAATGGACAAGTTCCTAT TCGTGGCCCTGAGCAAGACCTACAACACCAATGCTCAGGTGCCGATTC TGCCGGAACAGCCACAGCTTATCTGTGCGGCGTGAAGGCCAATGAGGGC ACCGTTGGAGTGTCTGCCGCCACCGAGAGAAGCAGATGCAATACCACAC AGGGCAACGAAGTGACCAGCATCCTGAGATGGGCCAAAGACGCCGGCA AGTCTGTGGGCATCGTGACCACCACAGAGTGAACCACGCCACACCTAG CGCCGCTATGCTCACTCTGCCGACAGAGACTGGTACAGCGACAACGAG ATGCCTCCTGAGGCTCTGTCTCAGGGCTGCAAGGATATCGCCTACCAGCT	135

Attorney Docket No. 2014034-0097

	GATGCACAACATCCGGGACATTGATGTGATCATGGGCGGAGGCCGGAA GTACATGTATCCCAAGAACAAGACCGACGTCGAGTACGAGAGCGACGA GAAGGCCAGAGGCACAAGACTGGATGGCTGGACCTGGTGGATACCTG GAAGTCCTTCAAGCCCCGGTACAAGCACAGCCACTTCATCTGGAACCGG ACCGAGCTGCTGACCCTGGATCCTCACAATGTGGACTACCTGCTGGGCC TGTTCGAGCCCGCGATATGCAGTACGAGCTGAACCGGAACAACGTGAC AGACCCAGCCTGAGCGAGATGGTGGTTGTGGCCATTACAGATCCTGCGG AAGAACCCCAAGGGATTCTTCTGCTGGTGGAAAGGCGGCAGGATCGATC ACGGACACCATGAGGGAAGCCAAAGCAGGCCCTGCACGAGGCCGTGCG AAATGGATAGAGCCATTGGCCAGGCCGGCAGCCTGACAAGCTCTGAGG ATACACTGACCGTGGTCACCGCCGATCACAGCCACGTGTTACATTTCGG CGGTACACCCCTAGAGGCAACAGCATCTTTGGACTGGCCCTATGCTG AGCGACACCGACAAGAAGCCTTTCACCGCCATCCTGTACGGCAACGGCC CTGGCTATAAGGTTGTGCGAGGCGAGAGGGAAAACGTGTCCATGGTGG TTACGCCCAACAACACTACCAGGCTCAGAGCGCCGTGCCTCTGAGACAC GAAACACACGGCGGAGAAGATGTGGCCGTGTTACGCAAGGGCCCCATG GCTCATTGTGTCACGGCGTGCACGAGCAGAATTACGTGCCACACGTGA TGGCCTACGCCGCTGTATTGGAGCCAATCTGGGACATTTGTGCCCTGCC AGCAGCggcggcgccgctccggcgccgctccggcgccgctccGACAAGACCCAC ACATGCCCCCTGCCCCGCCCCGAGCTGCTGGGCGGCCCAAGCGTGT TCCTGTTCCCCCAAGCCCAAGGACACCTGATGATCAGCCGACCCCC CGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCACCGTG AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACC AAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGGAGCGTG CTGACCGTGTGCAACAGGACTGGCTGAACGGCAAGGAGTACAAGTGCA AGGTGAGCAACAAGGCCCTGCCCCGCCCCATCGAGAAGACCATCAGCA AGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCTGCCCCCAG CCGCGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAA GGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAG CCCGAGAACAACCTACgatACCACCCCCCGTGTGaaaAGCGACGGCAGC TTCTTCCTGTACAGCgatCTGACCGTGGACAAGAGCCGCTGGCAGCAGGG CAACGTGTTACGCTGCAGCGTGTATGCACGAGGCCCTGCACAACCACTAC ACCCAGAAGAGCCTGAGCCTGAGCCCCGGCAAGGATGATGATGATGAT GATGACGACGATGACTGActgtgcttctagtgcagccatctgtgttgcctccccgtgcttct tgacctggaaggtgccaactccactgtcttcttaataaaatgaggaaattgcacgcatgtctgagtaggtgctattt attctgggggtggggtggggcaggacagcaaggggaggattgggaagagaatagcaggcatgctgggga	
HPPp0215 (transgene only)	CTGGTCCCCGAGAAGGAGAGAAGGACCCCAAGTACTGGCGGGACCAAGCG CAGGAGACCTGAAGTACGCGCTGGAGCTGCAGAAGCTGAACACCAAC GTCGCGAAGAACGTATCATGTTCCTGGGCGACGGCATGGGCGTCAGCA CCGTACCCGCGGCGCGGATCCTGAAGGGCCAGCTGCACCACAACCCCGG CGAGGAGACCCGGCTGGAGATGGACAAGTTCCCTTCGTCGCGCTGAGC AAGACCTACAACACCAACGCGCAGGTCCCCGACAGCGCGGGCACCGCG ACCGCGTACCTGTGCGGCGTCAAGGCGAACGAGGGCACCGTCGGCGTCA GCGCGGCGACCGAGCGGAGCCGGTGCAACACCACCCAGGGCAACGAGG TCACCAGCATCCTGCGGTGGGCGAAGGACGCGGGCAAGAGCGTCGGCA TCGTACCAACACCCGGGTCAACCACGCGACCCCCAGCGCGGCGTACGC GCACAGCGCGGACCCGGACTGGTACAGCGACAACGAGATGCCCCCCGA GGCGCTGAGCCAGGGCTGCAAGGACATCGCGTACCAAGCTGATGCAAC ATCCGGGACATCGACGTATCATGGGCGGCGGCGGGAAGTACATGTACC CCAAGAACAAGACCGACCTCGAGTACGAGAGCGACGAGAAGGCGCGGG GCACCCGGCTGGACGGCTGGACCTGGTTCGACACCTGGAAGAGCTTCAA GCCCCGGTACAAGCACAGCCACTTCATCTGGAACCGGACCGAGCTGCTG ACCTTGGACCCCCACAACGTGCGACTACCTGCTGGGCTGTTCGAGCCCCG GCGACATGCAGTACGAGCTGAACCGGAACAACGTACCGACCCAGCCT GAGCGAGATGGTTCGTCGCGATCCAGATCCTGCGGAAGAACCCCAAG GGCTTCTTCCTGCTGGTTCGAGGGCGGCCGATCGACCACGGCCACCAG	139

	AGGGCAAGGCGAAGCAGGCGCTGCACGAGGCGGTTCGAGATGGACCGGG CGATCGGCCAGGCGGGCAGCCTGACCAGCAGCGAGGACACCCCTGACCG TCGTACCCGCGGACCACAGCCACGTCTTACCTTCGGCGGGCTACACCCCC CGGGGCAACAGCATCTTCGGCCTGGCGCCCATGCTGAGCGACACCGACA AGAAGCCCTTCACCGCGATCCTGTACGGCAACGGCCCCGGCTACAAGGT CGTCGGCGGCGAGCGGGAGAACGTACGATGGTCGACTACGCGCACAA CAACTACCAGGCGCAGAGCGCGGTCCCCCTGCGGCACGAGACCCACGGC GGCGAGGACGTTCGCGGTCTTCAGCAAGGGCCCCATGGCGCACCTGCTGC ACGGCGTCCACGAGCAGAACTACGTCCCCACGTTCATGGCGTACGCGGC GTGCATCGGCGGAACCTGGGCCACTGCGCGCCCGCGAGCAGCCTGAAG GAGAGCAAGTACGGCCCCCCTGCCCCCCTGCCCCGCGCCCGAGGCGG CGGGCGGCCCCAGCGTCTTCCTGTTCCTCCCCCAAGCCCAAGGACACCT GATGATCAGCCGGACCCCCGAGGTACCTGCGTTCGTCGACGTTCAGC CAGGAGGACCCCGAGGTCCAGTTCAACTGGTACGTTCGACGCGCTCGAGG TCCACAACGCGAAGACCAAGCCCCGGGAGGAGCAGTTCAACAGCACCT ACCGGGTCGTACGCTCCTGACCGTCTGCACCAGGACTGGCTGAACGG CAAGGAGTACAAGTGCAAGGTACAGCAACAAGGGCCTGCCAGCAGCAT CGAGAAGACCATCAGCAAGGCGAAGGGCCAGCCCCGGGAGCCCCAGGT CTACACCTGCCCCCAGCCAGGAGGAGATGACCAAGAACCAGGTACG CTGACCTGCCTGGTCAAGGGCTTCTACCCACGCGACATCGCGGTTCGAGT GGGAGAGCAACGGCCAGCCCGAGAACAACCTACGACACCAACCCCCCG TCCTGAAGAGCGACGGCAGCTTCTTCCTGTACAGCGACCTGACCGTCGA CAAGAGCCGCTGGCAGGAGGGCAACGTCTTCAGCTGCAGCGTCATGCAC GAGGCGCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCTGG GCAAGGACATCGACGATGACGATGACGATGACGATGACGAT	
HPPp0215 (ALP)	CTGGTCCCCGAGAAGGAGAAGGACCCCAAGTACTGGCGGGACCAAGCG CAGGAGACCCTGAAGTACGCGCTGGAGCTGCAGAAGCTGAACACCAAC GTCGCGAAGAACGTTCATCATGTTCTGGGCGACGGCATGGGCGTCAGCA CCGTACCCGCGGCGCGGATCCTGAAGGGCCAGCTGCACCACAACCCCGG CGAGGAGACCCGGCTGGAGATGGACAAGTTCCCTTCGTTCGCGCTGAGC AAGACCTACAACACCAACGCGCAGGTCCCCGACAGCGCGGGCACCGCG ACCGCGTACCTGTGCGGCGTCAAGGCGAACGAGGGCACCGTCGGCGTCA GCGCGGCGACCGAGCGGAGCCGGTGCAACACCAACCCAGGGCAACGAGG TCACCAGCATCCTGCGGTGGGCGAAGGACGCGGGCAAGAGCGTCGGCA TCGTACCAACCAACCCGGGTCAACCACGCGACCCCCAGCGCGGCGTACGC GCACAGCGCGGACCCGGGACTGGTACAGCGACAACGAGATGCCCCCCGA GGCGCTGAGCCAGGGCTGCAAGGACATCGCGTACCAGCTGATGCACAAC ATCCGGGACATCGACGTTCATCATGGGCGGCGGCGGGAAGTACATGTACC CCAAGAACAAGACCGACGTTCGAGTACGAGAGCGACGAGAAGGCGCGGG GCACCCGGCTGGACGGCCTGGACCTGGTCGACACCTGGAAGAGCTTCAA GCCCCGGTACAAGCACAGCCACTTCATCTGGAACCCGACCGAGCTGCTG ACCTGGACCCCAACAACGTTCGACTACCTGCTGGGCCTGTTCGAGCCCCG GCGACATGCAGTACGAGCTGAACCGGAACAACGTACCGACCCAGCCT GAGCGAGATGGTCGTTCGTGCGATCCAGATCCTGCGGAAGAACCCCAAG GGCTTCTTCTGCTGGTCGAGGGCGGCGGATCGACCAACGGCCACCAAG AGGGCAAGGCGAAGCAGGCGCTGCACGAGGCGGTTCGAGATGGACCGGG CGATCGGCCAGGCGGGCAGCCTGACCAGCAGCGAGGACACCTGACCG TCGTACCCGCGGACCAACAGCCACGTCTTCACCTTCGGCGGCTACACCCCC CGGGGCAACAGCATCTTCGGCCTGGCGCCCATGCTGAGCGACACCGACA AGAAGCCCTTCACCGCGATCCTGTACGGCAACGGCCCCGGCTACAAGGT CGTCGGCGGCGAGCGGGAGAACGTACGATGGTCGACTACGCGCACAA CAACTACCAGGCGCAGAGCGCGGTCCCCCTGCGGCACGAGACCCACGGC GGCGAGGACGTTCGCGGTCTTCAGCAAGGGCCCCATGGCGCACCTGCTGC ACGGCGTCCACGAGCAGAACTACGTCCCCACGTTCATGGCGTACGCGGC GTGCATCGGCGGAACCTGGGCCACTGCGCGCCCGCGAGCAGC	145

HPPp0215 (Fc)	TGCCCCCCTGCCCCGCGCCCGAGGCGGGCGGGCGGCCCCAGCGTCTTCC TGTTCCCCCCCCAAGCCCAAGGACACCTGATGATCAGCCGGACCCCCGA GGTCACCTGCGTCGTCGTCGACGTCAGCCAGGAGGACCCCGAGGTCCAG TTCAACTGGTACGTCGACGGCGTCGAGGTCCACAACGCGAAGACCAAGC CCCGGGAGGAGCAGTTCAACAGCACCTACCGGGTCGTCAGCGTCCTGAC CGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTC AGCAACAAGGGCCTGCCCAGCAGCATCGAGAAGACCATCAGCAAGGCG AAGGGCCAGCCCCGGGAGCCCCAGGTCTACACCCTGCCCCCAGCCAGG AGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAAGGGCTT CTACCCAGCGACATCGCGGTGAGTGGGAGAGCAACGGCCAGCCCCGA GAACA ACTACGACACCACCCCCCGTCC TGAAGAGCGACGGCAGCTTC TTCCTGTACAGCGACCTGACCGTCGACAAGAGCCGGTGGCAGGAGGGCA ACGTCTTCAGCTGCAGCGTCATGCACGAGGCGCTGCACAACCACTACAC CCAGAAGAGCCTGAGCCTGAGCCTGGGCAAG	146
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Table 6 – Exemplary Transgene (Amino Acid Sequences)

Name	Amino Acid Sequence	SEQ ID NO
ALP-Fc	MISPFVLVAIGTCLNSLVPEKEKDPKYWRDQAQETLKYALELQKLNTNVA KNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPFVALSKTY NTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILR WAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGC KDIA YQLMHNIRDIDVIMGGGRKYMYPKNKTDVEYESDEKARGTRLDGLD LVDTWKSFKPRYKHSFIWNRTELLTLDPHNV DYLGLFEPGDMQYELNR NNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEA VEMDRAIGQAGSLTSSD TLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLSD TDKKPFTAILYGNPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETH GGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGHCAPASSL KDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPT VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALPAIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK	120
HPPp0215	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPF VALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQG NEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPP EALSQGCKDIA YQLMHNIRDIDVIMGGGRKYMYPKNKTDVEYESDEKARG TRLDGLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNV DYLGLFEPGDM QYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAK QALHEAVEMDRAIGQAGSLTSSD TLTVVTADHSHVFTFGGYTPRGNSIFGL APMLSDTDKKPFTAILYGNPGYKVVGGERENVSMVDYAHNNYQAQSAV PLRHETHGGEDVAVFSKGPMALLHGVHEQNYVPHVMAYAACIGANLGH CAPASSLKESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYDTTPPVLKSDGSFFLYSLD TVDKS RWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKDIDDDDDDDDDDD	136
HPPp0257	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQ KLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLEMDKFPF VALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQG NEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPP EALSQGCKDIA YQLMHNIRDIDVIMGGGRKYMYPKNKTDVEYESDEKARG	137

	TRLDGLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSDRTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPM AHL LHGVHEQNYVPHVMAY AACIGANLGH CAPASDDDDDDDDDD	
HPPp0160 (ALP-LL-IgG1Fc-3xsubst-D10)	MPSSVSWGILLLAGLCLVPVSLALVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLMDKFPFVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPEALSQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSDRTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPM AHL LHGVHEQNYVPHVMAY AACIGANLGH CAPASSGGGGSGGGSGGGSGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPTVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYDTPPV LKSDGSFFLYSDLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGKDDDDDDDDDD	138
HPPp0215 (transgene only)	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLMDKFPFVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPEALSQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSDRTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPM AHL LHGVHEQNYVPHVMAY AACIGANLGH CAPASSLKESKYGPCCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYDTPPV LKSDGSFFLYSDLTVDKSRWQEGNV FSCSV MHEALHNHYTQKSLSLGLKDDDDDDDDDDDDDDDD	142
HPPp0215 (ALP)	LVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHNPGEETRLMDKFPFVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPEALSQGCKDIAYQLMHNIRDIDVIMGGGRKMYMPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSDRTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPM AHL LHGVHEQNYVPHVMAY AACIGANLGH CAPASS	147
HPPp0215 (Fc)	CPPCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYDTPPV LKSDGSFFLYSDLTVDKSRWQEGNV FSCSV MHEALHNHYTQKSLSLGLK	148

Table 7 – Exemplary Terminator Sequences

Name	Nucleotide Sequence	SEQ ID NO
BGH polyA	ctgtgccttctagttgccagccatctgttgttgcctcccccgtgccttcttgaccctggaaggtgccactcccactgtc ctttcctaataaaatgaggaaattgcatcgattgtctgagtaggtgctattctattctgggggtgggggtgggcaggac agcaagggggaggattgggaagagaatagcaggcatgctgggga	78

Table 8 – Exemplary Guide RNA Sequences

Name	Nucleotide Sequence	SEQ ID NO
gCCR5_232	CAATGTGTCAACTCTTGACA	79
gJCHAIN_2	GTTAAGGTGTGTGATCTATA	80
gJCHAIN_31	AAGAACCATTTGCTTTTCTG	81
gJCHAIN_54	TCTCACCACCATATACGAGT	82
gJtolGHM_23	GTCTCAGGAGCGGTGTCTGT	83
gCD19_5	TTCACCACTAGAGGTTCCCTC	84
gCD19_109	GCCTGGCTGGACAGTCAATG	85
gCD19_124	CTGTGCTGCAGTGCCTCAAG	86
gJCHAINenh_1 73	GAAGGACCAGACCACTGCAA	129
gJCHAINenh_1	GCAAACCTTGACACATGCCAT	130
gJenh_1	GCAAACCTTGACACATGCCAT	131
gB2M_3	GAGTAGCGCGAGCACAGCTA	132

Table 9 – Exemplary Enhancers

Name	Nucleotide Sequence	SEQ ID NO
Murine Eu Enhancer (Full-length)	ctagataattgcattcatTTAAAAAAGTCTTCTCCTAAAAATGAATACTCAGAAAGTGGTCTGAAAAAGATTGTGAAGC cgTTTTgaccagaatgtcaaaagtcttaataagtaaggcaaaacaactaaaaagatcatgaacaagtcactgtaaat gcttcgggtattgaaaagaattgaatggagaccaataatcagagggaagaataatagatgaattttaagaagttttctaaa tatattagaataaagacactaaagtcctcaatttctacataacctaaTTTgaaatgaattctaaatcatTTtagaagtcg ataaacttaagtttgggaaactagaactactcaagctaaaataaaaggTgaactcaataagttaaaagaggacctctcc agtttcggctgaatcctcaactattttagaatgcaaattaccaggTggtgttttgcTcagcctggacttcggtttggtgg ggctggacagagTgtttcaaaaccacttctcaaccacagctacaagttacctagTgtttatttccctcccaaatag ccttgccacatgacctgttctcTgccagctgtgcaggtgttctggtctgacTggccatttTgactccaactcaacattgct caattcatttaaaatattgaaactaatttattattgttaaaagtcagttctgaaataggttatgagagagcctcactccattcc tcggttaaaacttaagtaatacagttctacacaacaagacctcaaaactgattgacaagaattttgacatttaaaaaatga gtactgaaaaccctctcacattttaaaagtcacagtatttaactatttctcTggaaccaacttaagagtaaaagcaacattct ctaatttccatacacatactctgtgttcttTgaaagctggactttTgcaggctccaccagacctcg	95
Murine EcoRI	gaattctaatacatTTtagaagtcgataaaacttaagtttgggaaactagaactactcaagctaaaataaaaggTtgaact caataagTtaaaagaggacctctcagtttcggctgaatcctcaactattttagaatgcaaattaccaggTgtgttttgc tcagcctggacttctcggttTgtgggctggacagagTgtttcaaaaccacttcttcaaacacagctacaagtttacctagt ggtttatttccctcccaaatagccttTgccacatgacctgttctcTgccagctgtgcag	96
Murine ENCODE	aactagaactactcaagctaaaataaaaggTtgaactcaataagTtaaaagaggacctctccagtttcggctgaatcctca actattttagaatgcaaattaccaggTgtgtgttttgcTcagcctggacttcggtttggTgggctggacagagTgtttca	97

	aaaccacttctcaaacacagctacaagttacctagtggtttatttccctccccaatagccttgccacatgacctgctt cctgccagctgctgcagggtgttctggtctgacggccatctgactccaactcaacattgctcaattcatttaaaaatttga aacttaa	
Human IMGT	TAAGCTAAGATTGGAAATGAAATTCAGATAGTTAAAAAAGCCTTTTCA GTTTCGGTCAGCCTCGCCTTATTTTAGAAACGCAAATTGTCCAGGTGTTG TTTTGCTCAGTAGAGCACTTTCAGATCTGGGCCTGGGCAAAACCACCTCT TCACAACCAGAAGTGATAAATTTACCAATTGTGTTTTTTTGCTTCCTAAA ATAGACTCTCGCGGTGACCTGCTTCCTGCCACCTGCTGTGGGTGCCGGAG ACCCCATGCAGCCATCTTGACTCTAATTCATCATCTGCTTCCAGCTTCG CTCAATTAATTAATAAATAA	98
Human ENCODE	AAGATTGGAAATGAAATTCAGATAGTTAAAAAAGCCTTTTCAGTTTCG GTCAGCCTCGCCTTATTTTAGAAACGCAAATTGTCCAGGTGTTGTTTGC TCAGTAGAGCACTTTCAGATCTGGGCCTGGGCAAAACCACCTCTTCACA ACCAGAAGTGATAAATTTACCAATTGTGTTTTTTTGCTTCCTAAAATAGA CTCTCGCGGTGACCTGCTTCCTGCCACCTGCTGTGGGTGCCGGAGACCCC CATGCAGCCATCTTGACTCTAATTCATCATCTGCTTCCAGCTTCGCTCAA TTAATTAATAAATAAATAAATTGATTTATGATGGTCAAAACGCAGTCCCGC AT	99

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EQUIVALENTS

[0405] It is to be appreciated by those skilled in the art that various alterations, modifications, and improvements to the present disclosure will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of the present disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawing are by way of example only and any invention described in the present disclosure if further described in detail by the claims that follow.

[0406] Those skilled in the art will appreciate typical standards of deviation or error attributable to values obtained in assays or other processes described herein. The publications, websites and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference in their entireties.

CLAIMS

1. A method of preparing an engineered B lineage cell population that expresses an alkaline phosphatase or fusion protein thereof, the method comprising the steps of:
 - (a) isolating primary B cells so that a primary B cell population is obtained;
 - (b) activating the primary B cell population; and
 - (c) during or after the activating step, engineering the primary B cell population by integrating a transgene encoding an alkaline phosphatase (ALP) or fusion protein thereof, thereby generating an engineered B lineage cell population.
2. The method of claim 1, wherein the fusion protein comprises ALP and an immunoglobulin fragment crystallizable region (ALP-Fc) fusion protein.
3. The method of claim 2, wherein the ALP-Fc fusion protein comprises a Fc derived from human immunoglobulin G1 (IgG1).
4. The method of claim 2, wherein the ALP-Fc fusion protein comprises a Fc derived from human immunoglobulin G4 (IgG4).
5. The method of any one of claims 1-4, wherein the ALP-Fc fusion protein further comprises either an octa- or deca-aspartate tag.
6. The method of any one of claims 2-5, wherein the ALP-Fc fusion protein further comprises one or more substitutions within the Fc region.
7. The method of claim 6, wherein the one or more substitutions within the Fc region comprise K392D, D399K, and K409D of IgG1.
8. The method of claim 6, wherein the one or more substitutions within the Fc region comprise K392D, D399K, and R409D of IgG4.
9. The method of any one of claims 2-8, wherein the ALP-Fc fusion protein further comprises a linker.
10. The method of claim 9, wherein the linker comprises a tetra-glycine and mono-serine linker (GGGGS).
11. The method of claim 9, wherein the linker comprises leucine and lysine.
12. The method of any one of claims 2-11, wherein the ALP-Fc fusion protein further comprises one or more substitutions within the ALP.

13. The method of claim 12, wherein the one or more substitutions within the Fc of ALP fusion protein comprise S228P, F234A, and/or L235A.
14. The method of any one of claims 2-13, wherein the ALP-Fc fusion protein further comprise a deca-aspartate tag (D10).
15. The method of any one of claims 1-14, wherein the transgene is integrated at a site selected from a CCR5 site, a IgH site, CD19, B2M, and a JCHAIN site.
16. The method of any one of claims 1-15, wherein the ALP or fusion protein thereof comprises or has an amino acid sequence of any one of SEQ ID NOs: 62-75, 91-94, 101, 109-117, 136, 142, and 147.
17. The method of any one of claims 1-15, wherein the ALP or fusion protein thereof is encoded by a nucleic acid sequence comprising any one of SEQ ID Nos: 55-61, 87-90, 100, 102-108, 139, and 145.
18. The method of any one of claims 1-17, further comprising a step of expanding the engineered B lineage cell population.
19. The method of any one of claims 1-18, further comprising a step of differentiating the engineered B lineage cell population into a population of plasmablasts.
20. The method of claim 19, wherein the differentiation step comprises contacting the engineered B lineage cell population with culture media comprising:
 - (a) IL-2;
 - (b) IL-6;
 - (c) IL-10; and/or
 - (d) IL-15.
21. The method of claim 19 or 20, further comprising a step of differentiating the population of plasmablasts into a population of plasma cells.
22. The method of claim 21, wherein the differentiation step comprises contacting the population of plasmablasts with culture media comprising:
 - (a) IL-6;
 - (b) IL-15; and/or
 - (c) IFN- α -2-beta (IFN α -2 β).

23. The method of any one of the above claims, further comprising a step of transferring the engineered B lineage cell population to cell culture media without human or bovine serum for about 24 hours.
24. The method of any one of the above claims, wherein the step of engineering further comprises introducing an expression cassette comprising the transgene into the primary B cell population.
25. The method of claim 24, wherein the expression cassette comprises:
 - (a) a 5' homology arm that is at least 95% identical to a sequence 5' to the double-stranded break site; and
 - (b) a 3' homology arm that is at least 95% identical to a sequence 3' to the double-stranded break site.
26. The method of claim 24, wherein the 5' homology arm comprises or is:
 - (i) a length about 375-850 base pairs in size;
 - (ii) a PAM site or absence of a PAM site; and/or
 - (iii) symmetrical or asymmetrical in length with the 3' homology arm.
27. The method of claim 25 or 26, wherein the 3' homology arm comprises or is:
 - (i) a length about 375-850 base pairs in size,
 - (ii) a PAM site or absence of a PAM site, and/or
 - (iii) symmetrical or asymmetrical in length with the 5' homology arm.
28. The method of claim 25, wherein the 5' homology arm has at least 95% identity with one or more of SEQ ID NOs: 39-43, 50, 52, 54, 118, 121, and 123.
29. The method of claim 25, wherein the 3' homology arm has at least 95% identity with one or more of SEQ ID NOs: 44-49, 51, 53, 119, 122, and 124.
30. The method of claim 25, wherein the expression cassette is or comprises a polynucleotide sequence with at least 70% sequence identity to SEQ ID NO: 139.
31. The method of claim 25, wherein the expression cassette is or comprises a polynucleotide sequence with at least 70% sequence identity to SEQ ID NO: 100.
32. The method of claim 25, wherein the expression cassette is or comprises a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO: 100.
33. The method of claim 25, wherein the expression cassette is or comprises a polynucleotide sequence that encodes a polypeptide with at least 70% sequence identity to SEQ ID NO: 101.

34. The method of claim 25, wherein the expression cassette is or comprises a polynucleotide sequence that encodes a polypeptide with at least 90% sequence identity to SEQ ID NO: 101.
35. The method of claim 25, wherein the expression cassette comprises one or more of:
- (a) a promoter sequence selected from SEQ ID NOs: 76, and 125-128;
 - (b) a nucleic acid sequence encoding for ALP or a fusion protein thereof selected from SEQ ID NOs: 55-61, 87-90, 100, 102-108, 139, and 145;
 - (c) an enhancer sequence selected from SEQ ID NOs: 95-99; and
 - (d) a polyA sequence of SEQ ID NO: 78.
36. The method of any one of claims 24-35, wherein the expression cassette is or comprises an adeno-associated viral (AAV) vector.
37. The method of any one of the above claims, wherein the step of engineering comprises contacting the primary B cell population with a targeted nuclease capable of introducing double-stranded breaks.
38. The method of claim 37, wherein the targeted nuclease is or comprises a CRISPR-associated (Cas) protein, zinc finger nuclease (ZFN), transcription activator-like effector-based nuclease (TALEN), or meganuclease.
39. The method of claim 38, wherein the Cas protein is or comprises Cas9, Cas12a, or Cas13a, or a variant thereof.
40. The method of claim 39, wherein the Cas protein is complexed with a guide RNA (gRNA).
41. The method of claim 40, wherein the gRNA is a single guide RNA (sgRNA).
42. The method of claim 40, wherein the gRNA is or comprises a sequence with at least 95% sequence identity to any one of SEQ ID NOs: 79-86, and 129-132.
43. The method of any one of claims 1-42, wherein the step of engineering comprises electroporation of the primary B cell population.
44. The method of claim 43, wherein the electroporation is performed on a composition comprising:
- (a) the Cas protein complexed with the gRNA; and
 - (b) the primary B cell population.

45. The method of any one of claims 1-44, wherein the step of engineering comprises: transduction of the primary B cell population with an expression cassette.
46. The method of any one of claims 1-44, wherein the JCHAIN site has at least 95% identity with one or more SEQ ID NOs: 80-82, and 129-130.
47. The method of any one of claims 1-44, wherein the IgH site has at least 95% identity with SEQ ID NO: 83.
48. The method of any one of claims 1-44, wherein the CD19 site has at least 95% identity with one or more of SEQ ID NO: 84-86.
49. The method of any one of claims 1-44, wherein the transgene is or comprises a polynucleotide sequence with at least 70% sequence identity to any one of SEQ ID NOs: 77 and 133-135.
50. The method of any one of claims 1-44, wherein the transgene is or comprises a polynucleotide sequence with at least 90% sequence identity to any one of SEQ ID NOs: 77 and 133-135.
51. The method of any one of claims 1-44, wherein the transgene is or comprises a polynucleotide sequence encoding a polypeptide with at least 70% sequence identity to any one of SEQ ID NOs: 120 and 136-138.
52. The method of any one of claims 1-44, wherein the transgene is or comprises a polynucleotide sequence encoding a polypeptide with at least 90% sequence identity to any one of SEQ ID NO: 120 and 136-138.
53. A population of genetically modified B lineage cells comprising a transgene sequence encoding an alkaline phosphatase (ALP) or fusion protein thereof.
54. The population of genetically modified B lineage cells of claim 53, wherein the fusion protein comprises an ALP and an immunoglobulin fragment crystallizable region (ALP-Fc) fusion protein.
55. The population of genetically modified B lineage cells of claim 53 or 54, wherein the transgene is expressed from an endogenous CCR5, IgH, CD19, B2M, or JCHAIN locus.
56. The population of genetically modified B lineage cells of claim 55, wherein the cells express an ALP-Fc fusion protein and at least partially disrupt expression of the endogenous CCR5, IgH, CD19, B2M, or JCHAIN locus.

57. The population of any one of claims 53-56, wherein the population of genetically modified B lineage cells is or comprises a population of genetically modified plasma cells.
58. The population of any one of claims 53-56, wherein the population of genetically modified B lineage cells is or comprises a population of plasmablasts.
59. The population of any one of claims 53-56, wherein the population of genetically modified B lineage cells is or comprises a population of plasma cell precursors.
60. The population of any one of claims 53-59, wherein the transgene is or comprises a polynucleotide sequence with at least 70% sequence identity to SEQ ID NO: 139.
61. The population of any one of claims 53-60, wherein the transgene is or comprises a polynucleotide sequence with at least 90% sequence identity to SEQ ID NO: 139.
62. The population of any one of claims 53-61, wherein the transgene is or comprises a polynucleotide sequence encoding a polypeptide with at least 70% sequence identity to SEQ ID NO: 142.
63. The population of any one of claims 53-62, wherein the transgene is or comprises a polynucleotide sequence encoding a polypeptide with at least 90% sequence identity to SEQ ID NO: 142.
64. A pharmaceutical composition comprising one or more B lineage cells selected from the population of genetically modified B lineage cells of any of the above claims, and one or more pharmaceutically acceptable excipients.
65. A method of administering a pharmaceutical composition, wherein the pharmaceutical composition comprises:
- (a) one or more B lineage cells selected from the population of genetically modified B lineage cells of any one of the above claims; and
 - (b) one or more pharmaceutically acceptable excipients;
- wherein the pharmaceutical composition is administered to a subject.
66. A method of treating a disease, disorder, or condition in a subject, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition of claim 53, thereby treating the disease, disorder, or condition in the subject.
67. The method of claim 65 or 66, wherein the pharmaceutical composition is administered intravenously or intraperitoneally.

68. The method of any one of claims 65-67, wherein the pharmaceutical composition is administered to an adult subject.
69. The method of any one of claims 65-67, wherein the pharmaceutical composition is administered to a pediatric subject.
70. The method of any one of claims 65-69, wherein the subject has hypophosphatasia (HPP).
71. A method of characterizing a population of genetically modified B lineage cells of any one of claims 58-63, comprising assessing one or more of (a)-(g) using an assay:
- (a) presence of a CD38 marker;
 - (b) presence of a CD138 marker;
 - (c) presence of at least 50% of a CD27 marker;
 - (d) secretion of at least 0.5 pg/cell/day of IgG;
 - (e) secretion of at least 2.5 pg/cell/day of IgM;
 - (f) secretion of at least 0.5 pg/cell/day of IgD; and
 - (g) secretion of at least 0.01 pg/cell/day of an alkaline phosphatase or fusion thereof.
72. The method of claim 71, wherein the assay comprises or is one or more of: fluorescence-activated cell sorting (FAC-sort), Western Blot, flow cytometry, enzyme-linked immunosorbent spot assay (ELISpot), electrochemiluminescence, and enzyme-linked immunosorbent assay (ELISA).
73. The method of claim 71 or 72, wherein one or more B lineage cells selected from the population of genetically modified B lineage cells engraft within bone marrow of a subject.
74. A polynucleotide sequence for use in engineering a population of B lineage cells comprising one or more of:
- (i) a 5' homology arm that is or comprises SEQ ID NO: 39;
 - (ii) a promoter that is or comprises SEQ ID NO: 76;
 - (iii) a signal peptide encoded by a polynucleotide sequence that is or comprises SEQ ID NO: 143;
 - (iv) a transgene that is or comprises SEQ ID NO: 139;
 - (v) a terminator that is or comprises SEQ ID NO: 78; and/or

- (vi) a 3' homology arm that is or comprises SEQ ID NO: 44.
75. A polynucleotide sequence for use in engineering a population of B lineage cells encoding a polypeptide that comprises one or more of:
- (i) a signal peptide encoded that is or comprises SEQ ID NO: 144;
 - (ii) a transgene that is or comprises SEQ ID NO: 147; and/or
 - (iii) a linker encoded that is or comprises SEQ ID NO: 141.
76. The polynucleotide of any one of claims 74-75, wherein the polynucleotide sequence is or comprises SEQ ID NO: 100.
77. The polynucleotide of any one of claims 74-75, wherein the polynucleotide sequence is or comprises SEQ ID NO: 149.
78. The polynucleotide of any one of claims 74-75, wherein the polynucleotide sequence encodes a polypeptide sequence that is or comprises SEQ ID NO: 101.