

DNASE CO-EXPRESSION IN HOST CELLS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No.
5 63/580,228, filed September 1, 2023, the contents of which are hereby incorporated by reference
herein, in its entirety, for all purposes.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under CA197574 awarded by
10 National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE DISCLOSURE

[0003] The present disclosure provides host cells that allow for co-expression of an
endonuclease with a product of interest, e.g., a 3E10 antibody or antigen binding fragment
15 thereof, to improve expression and/or purification of the product of interest, e.g., a 3E10
antibody or antigen binding fragment thereof, from the host cell culture.

BACKGROUND OF THE DISCLOSURE

[0004] Therapeutic recombinant products are extensively used for human medical
20 purposes. Recombinant proteins can be expressed in various types of living organisms. CHO and
HEK cells are some of the most commonly used host cells for the manufacture of
biopharmaceuticals. Although using the host cells for the production of therapeutics has many
advantages, contamination of biopharmaceuticals with host cell DNA is a major concern for
manufacturers. Genomic DNA contamination in biopharmaceuticals has been considered as a
25 possible risk factor for patients receiving recombinant protein pharmaceuticals.

[0005] Thus, there is a need for biopharmaceutical manufacturers to monitor DNA
contamination and keep the quantity of impurities in bioproducts as low as a safety limit
suggested by the regulatory authorities such as FDA, which generally must be lower than 100 pg
of DNA per milligram of protein. Removal of unwanted DNA can be expensive, time
30 consuming, and reduce the efficiency of recovery of the biopharmaceutical protein.

[0006] Accordingly, what is needed in the art are approaches for limiting the contamination of biopharmaceutical protein preparations by host cell DNA.

SUMMARY OF THE DISCLOSURE

5 [0007] The present disclosure provides host cells that allow for co-expression of DNase with a product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof to improve expression and/or purification of the protein of interest from the host cell culture.

[0008] In some embodiments, the disclosure provides a eukaryotic host cell comprising (i) an exogenous nucleic acid sequence encoding an endonuclease operably linked to a first promoter
10 sequence and (ii) an exogenous nucleic acid sequence encoding a 3E10 antibody or antigen binding fragment thereof operably linked to a second promoter sequence.

[0009] In some embodiments, the host cell is selected from the group consisting of Chinese Hamster Ovary (CHO) cells, HEK 293 cells, CAP cells, bovine mammary epithelial cells, monkey kidney CV1 line transformed by SV40, baby hamster kidney cells, mouse sertoli cells,
15 monkey kidney cells, African green monkey kidney cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor, TRI cells, MRC 5 cells, FS4 cells, rat fibroblasts, MDBK cells, and human hepatoma line cells.

[0010] In some embodiments, the host cell is selected from the group consisting of a Chinese
20 Hamster Ovary (CHO) cell, an HEK 293 cell, and a CAP cell.

[0011] In some embodiments, the host cell line is a glutamine synthetase (GS) knockout cell line.

[0012] In some embodiments, wherein the host cell line is a dihydrofolate reductase (DHFR) knockout cell line.

25 [0013] In some embodiments, the endonuclease is a DNaseI endonuclease.

[0014] In some embodiments, the endonuclease is a human DNaseI endonuclease.

[0015] In some embodiments, the sequence encoding the endonuclease is further operably linked to a secretion signal sequence.

[0016] In some embodiments, the first promoter sequence is a weak promoter sequence.

[0017] In some embodiments, the first promoter sequence is a cytomegalovirus immediate early (CMV-IE) promoter sequence.

[0018] In some embodiments, the CMV-IE promoter sequence is a simian CMV-IE promoter (sCMV) sequence.

5 [0019] In some embodiments, the first promoter sequence is not a retroviral LTR promoter.

[0020] In some embodiments, the 3E10 antibody or antigen binding fragment thereof comprises (a) a heavy chain polypeptide or fragment thereof and (b) a light chain polypeptide or fragment thereof, and the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof comprises (1) a first exogenous nucleic acid sequence encoding
10 the heavy chain polypeptide or fragment thereof and (2) a second exogenous nucleic acid sequence encoding the heavy chain polypeptide or fragment thereof.

[0021] In some embodiments, the ratio of (i) the number of first exogenous nucleic acid sequences integrated into the host cell genome to (ii) the number of second exogenous nucleic acid sequences integrated into the host cell genome is from 1:2 to 2:1.

15 [0022] In some embodiments, the first exogenous nucleic acid sequence is operably linked to the second promoter sequence and the second exogenous nucleic acid sequence is operably linked to a third promoter sequence.

[0023] In some embodiments, the third promoter sequence has the same nucleotide sequence as the first promoter.

20 [0024] In some embodiments, the third promoter sequence has the same nucleotide sequence as the second promoter.

[0025] In some embodiments, the third promoter sequence has a different nucleotide sequence as the first promoter.

[0026] In some embodiments, the third promoter sequence has a different nucleotide
25 sequence as the second promoter.

[0027] In some embodiments, the third promoter sequence is a cytomegalovirus immediate early (CMV-IE) promoter sequence.

[0028] In some embodiments, the CMV-IE promoter sequence is a simian CMV-IE promoter (sCMV) sequence.

[0029] In some embodiments, the third promoter sequence is not a retroviral LTR promoter.

[0030] In some embodiments, the 3E10 antibody or antigen binding fragment thereof
5 comprises a monovalent, divalent, or multivalent single chain variable fragment (scFv).

[0031] In some embodiments, the 3E10 antibody or antigen binding fragment thereof
comprises an scFv-Fc polypeptide, a CrossMab polypeptide, a dual variable domain
immunoglobulin (DVD-Ig), a tandem double scFv, an (scFv)₂, a single-chain tandem fragment
variable (scTaFv) polypeptide, a single-chain fragment variable (scFv) polypeptide, a diabody, a
10 tandem diabody (TandAb), a Fabsc polypeptide, a modular IgG-scFv, or an F(ab')₂.

[0032] In some embodiments, the 3E10 antibody or antigen binding fragment thereof is
humanized.

[0033] In some embodiments, the 3E10 antibody or antigen binding fragment thereof is a
bivalent antibody or fragment thereof.

15 [0034] In some embodiments, the 3E10 antibody or antigen binding fragment thereof
comprises:

[0035] In some embodiments, a heavy chain variable region (VH) complementarity
determining region (CDR) 1 comprising the amino acid sequence of SEQ ID NO:58, a VH
CDR2 comprising the amino acid sequence of SEQ ID NO:59, and a VH CDR3 comprising the
20 amino acid sequence of SEQ ID NO:60; and

[0036] In some embodiments, a light chain variable region (VL) CDR1 comprising the
amino acid sequence of SEQ ID NO:61, a VL CDR2 comprising the amino acid sequence of
SEQ ID NO:62, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:63.

[0037] In some embodiments, the 3E10 antibody or antigen binding fragment thereof
25 comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO:64, a VH CDR2
comprising the amino acid sequence of SEQ ID NO:4, and a VH CDR3 comprising the amino
acid sequence of SEQ ID NO:5 and

[0038] In some embodiments, a VL CDR1 comprising the amino acid sequence of SEQ ID NO:9, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:10, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:11.

5 [0039] In some embodiments, the 3E10 antibody or antigen binding fragment thereof comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO:64, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:5, and

[0040] In some embodiments, a VL CDR1 comprising the amino acid sequence of SEQ ID NO:9, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:10, and a VL CDR3
10 comprising the amino acid sequence of SEQ ID NO:11.

[0041] In some embodiments, the 3E10 antibody or antigen binding fragment thereof, comprises a heavy chain variable region (VH) having an amino acid sequence that is at least 90% identical to SEQ ID NO:2 or SEQ ID NO:14 and a light chain variable region (VL) having an amino acid sequence that is at least 90% identical to SEQ ID NO:7.

15 [0042] In some embodiments, the 3E10 antibody or antigen binding fragment thereof, comprises a heavy chain variable region (VH) having an amino acid sequence that is at least 90% identical to SEQ ID NO:2 or SEQ ID NO:14 and a light chain variable region (VL) having an amino acid sequence that is at least 95% identical to SEQ ID NO:7.

[0043] In some embodiments, the 3E10 antibody or antigen binding fragment thereof,
20 comprises a heavy chain variable region (VH) having an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS:104-113 and a light chain variable region (VL) having an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:114-122.

[0044] In some embodiments, the second promoter sequence has the same nucleotide
25 sequence as the first promoter.

[0045] In some embodiments, the second promoter sequence has a different nucleotide sequence as the first promoter.

[0046] In some embodiments, the second promoter sequence is a cytomegalovirus immediate early (CMV-IE) promoter sequence.

[0047] In some embodiments, the CMV-IE promoter sequence is a simian CMV-IE promoter (sCMV) sequence.

[0048] In some embodiments, the third promoter sequence is not a retroviral LTR promoter.

[0049] In some embodiments, from 2 to 500 copies each of the exogenous nucleic acid sequence encoding the endonuclease and the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof are stably integrated into the genome of the host cell.

[0050] In some embodiments, from 5 to 500 copies each of the first exogenous nucleic acid sequence and at least a second exogenous nucleic acid sequence are stably integrated into the genome of the host cell.

[0051] In some embodiments, the ratio of (i) the number of exogenous nucleic acid sequences encoding the endonuclease to (ii) the number of exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:1 to 1:100.

[0052] In some embodiments, the ratio of (i) the number of exogenous nucleic acid sequences encoding the endonuclease to (ii) the number of exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:2 to 1:100.

[0053] In some embodiments, the exogenous nucleic acid sequence encoding the endonuclease and the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof are stably integrated into the genome of the host cell at a docking site.

[0054] In some embodiments, prior to integration of the exogenous nucleic acid sequence encoding the endonuclease and the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof into the docking site, the docking site comprised at least one dock site insertion element, and the exogenous nucleic acid sequence encoding the endonuclease and the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof were present in one or more transfer vector comprising at least one insertion element compatible with the at least one dock site insertion element.

[0055] In some embodiments, the exogenous nucleic acid sequence encoding the endonuclease is operably linked to a first polyadenylation sequence and the exogenous nucleic

acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is operably linked to a second polyadenylation sequence.

[0056] In some embodiments, the host cell genome comprises from 5 to 500 docking sites and each docking site comprises at least one dock site insertion element.

5 **[0057]** In some embodiments, the integrated docking sites are independently positioned throughout the host cell genome.

[0058] In some embodiments, the disclosure provides a cell culture comprising host cells as described herein.

[0059] In some embodiments, the disclosure provides a method for expressing a 3E10
10 antibody or antigen binding fragment thereof, the method comprising culturing a plurality of host cells, as described herein, in a culture medium under conditions for expression of expression of (i) the endonuclease from the exogenous nucleic acid sequence encoding the endonuclease and (ii) the 3E10 antibody or antigen binding fragment thereof.

[0060] In some embodiments, the culturing is performed under fed-batch conditions.

15 **[0061]** In some embodiments, the disclosure provides a cell culture supernatant comprising the 3E10 antibody or antigen binding fragment thereof prepared according to the methods described herein.

[0062] In some embodiments, the disclosure provides a cell culture supernatant with an antibody titer comprising at least 0.001 µg/mL, at least 0.01 µg/mL, at least 0.1 µg/mL, at least
20 0.125 µg/mL, at least 0.250 µg/mL, at least 0.5 µg/mL, at least 0.75 µg/mL, at least 1 µg/mL, at least 5 µg/mL, at least 10 µg/mL, at least 25 µg/mL, at least 50 µg/mL, or at least 100 µg/mL of the 3E10 antibody or antigen binding fragment thereof.

[0063] In some embodiments, less than 75%, or less than 70%, or less than 65%, or less than
25 60%, or less than 55%, or less than 50%, or less than 45%, or less than 40%, or less than 35%, or less than 30%, or less than 25%, or less than 20%, or less than 15%, or less than 10%, or less than 5%, or 0% of the 3E10 antibody or antigen binding fragment thereof is bound to nucleic acid.

[0064] In some embodiments, the disclosure provides a composition comprising a 3E10 antibody or antigen binding fragment thereof produced according to a method described herein.

In some embodiments, the disclosure provides a composition comprising a 3E10 antibody or antigen binding fragment thereof purified from a cell culture supernatant described herein.

[0065] In some embodiments, the disclosure provides a host cell culture comprising a eukaryotic host cell comprising an exogenous nucleic acid sequence encoding a 3E10 antibody or antigen binding fragment thereof operably linked to a promoter sequence, and a purified exogenously added endonuclease.

[0066] In some embodiments, the disclosure provides a method of making a host cell culture comprising a eukaryotic host cell comprising an exogenous nucleic acid sequence encoding a 3E10 antibody or antigen binding fragment thereof operably linked to a promoter sequence; and a purified, exogenous added endonuclease added to the host cell culture.

DESCRIPTION OF THE FIGURES

[0067] **FIG. 1A and B.** Human DNase 1 nucleic acid sequence and the flanking DNA cloning junctions (SEQ ID NO:139) in the final retrovector expression construct (FIG 1A) and DNase amino acid sequence (SEQ ID NO:140, FIG. 1B).

[0068] **FIG. 2.** Map of starting Retrovector pCS-newMCS-WPRE (new ori), 6187 bp.

[0069] **FIG. 3.** Map of Human DNase 1 "Pathway" Gene in GPEX[®] Vector pCS-CFSD1-WPRE (new ori), 6977 bp.

[0070] **FIG. 4A and B.** Sequence data for LC CDS in 207attB-GS-h3E10LC-WPRE. **FIG. 4A** provides the nucleic acid sequence (SEQ ID NO:141) while **FIG. 4B** provides the amino acid sequence (SEQ ID NO:142).

[0071] **FIG. 5.** Map of plasmid 207attB-GS-h3E10LC-WPRE.

[0072] **FIG. 6.** Sequence data HC CDS in 207attB-GS-h3E10HC-WPRE. **FIG. 6A** provides the nucleic acid sequence (SEQ ID NO:143) while **FIG. 6B** provides the amino acid sequence (SEQ ID NO:144).

[0073] **FIG. 7.** Map of plasmid 207attB-GS-h3E10HC-WPRE

[0074] **FIG. 8.** Sequence data for Pathway CDS in 215-puc19attB287-GS-Pathway-WPRE-TKpa. **FIG. 8A** provides the nucleic acid sequence (SEQ ID NO:145) while **FIG. 8B** provides the amino acid sequence (SEQ ID NO:146).

- [0075] FIG. 9. Map of plasmid 215-puc19attB287-GS-Pathway-WPRE-TKpa
- [0076] FIG. 10. SDS-PAGE results for pooled cells.
- [0077] FIG. 11. Graph showing viable cell density.
- [0078] FIG. 12. Graph showing percent viability of cells.
- 5 [0079] FIG. 13. Graph showing antibody titer.
- [0080] FIG. 14. Graph showing protein concentration (mg/ml) vs. clone number.
- [0081] FIG. 15. Graph showing Ambr15™ IVCD vs clone number.
- [0082] FIG. 16. Graph showing Ambr15™ rQp vs clone number.
- [0083] FIG. 17 illustrates amino acid sequences for the parent 3E10 monoclonal antibody.
- 10 [0084] FIGs 18A, 18B, and 18C illustrate amino acid sequences for the D31N variant (FIG. 18A), other CDR variants (FIG. 18B), and additionally contemplated CDR variants (FIG. 18C) of the 3E10 monoclonal antibody, in accordance with some embodiments of the present disclosure.
- [0085] FIG. 19 illustrates example charge-conserved CDR variants of the 3E10 monoclonal
15 antibody, in accordance with various embodiments of the present disclosure.
- [0086] FIG. 20 illustrates example CDR variants containing a combination of amino acid substitutions, charged-conserved amino acid substitutions, and rationally designed amino acid substitutions of the 3E10 monoclonal antibody, in accordance with various embodiments of the present disclosure.
- 20 [0087] FIG. 21 illustrates amino acid sequences of humanized 3E10 variable heavy (3E10-VH) domains, in accordance with various embodiments of the present disclosure.
- [0088] FIG. 22 illustrates amino acid sequences of mature humanized 3E10 heavy chains (3E10-HC), lacking a signal peptide, in accordance with various embodiments of the present disclosure.
- 25 [0089] FIG. 23 illustrates amino acid sequences of humanized 3E10 heavy chains (3E10-HC), in accordance with various embodiments of the present disclosure.

[0090] FIG. 24 illustrates amino acid sequences of humanized 3E10 variable light (3E10-VL) domains, in accordance with various embodiments of the present disclosure.

[0091] FIG. 25 illustrates amino acid sequences of mature humanized 3E10 light chains (3E10-LC), lacking a signal peptide, in accordance with various embodiments of the present disclosure.

[0092] FIG. 26 illustrates amino acid sequences of humanized 3E10 light chains (3E10-LC), in accordance with various embodiments of the present disclosure.

[0093] FIG. 27 illustrates a sequence alignment of examples of humanized 3E10 heavy chain variable regions, with CDRs underlined as indicated.

[0094] FIG. 28 illustrates a sequence alignment of examples of humanized 3E10 light chain variable regions, with CDRs and putative nuclear localization signals (NLS) underlined as indicated.

[0095] FIGs. 29A, 29B, 29C, 29D, and 29E collectively illustrate a sequence alignment of example of humanized di-scFv constructs of the 3E10 monoclonal antibody.

[0096] FIGs. 30A and 30B illustrate electrostatic surface potential renderings of a molecular model of a 3E10-scFv construct, revealing a putative Nucleic Acid Binding pocket (NAB1). FIG. 30A additionally shows predicted structural and electrostatic potential changes induced by amino acid substitutions at residue HC CDR1 residue 31. FIG. 30B is an illustration of molecular modeling of 3E10-scFv (Pymol) with NAB1 amino acid residues highlighted by punctate dots.

[0097] FIG. 31A illustrate the expression of IgG1 in the supernatant ($\mu\text{g/ml}$) 3E10 variants (D31N or V66) in the presence of exogenous DNase treatment.

[0098] FIG. 31B illustrate the SEAP expression (fold over untransfected cells) in the presence of exogenous DNase treatment.

DEFINITIONS

[0099] To facilitate understanding of the disclosure, a number of terms are defined below.

[00100] As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will also be understood that

the term “and/or” as used herein refers to and encompasses any and all possible combinations of one or more of the associated listed items. Unless the context requires otherwise, it will be further understood that the terms “includes,” “comprising,” or any variation thereof, when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof. Furthermore, to the extent that the terms “including,” “includes,” “having,” “has,” “with,” or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising.” Additionally, where the terms “comprising,” “including,” “includes,” “having,” “has,” “with,” or variants thereof are used in either the detailed description and/or the claims, alternatives reciting “consisting of” or “consisting essentially of” are intended to be encompassed within such disclosures.

[00101] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein.

[00102] Use of the term “about” is intended to describe values either above or below the stated value in a range of approx. +/- 10%.

[00103] As used herein, the term “antibody” refers to an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing through at least one antigen recognition site within the variable region of the immunoglobulin molecule. The term “antibody,” as used herein, is used in the broadest sense and encompasses monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen determination portion of an antibody, and antibody fragments (such as Fab, Fab', F(ab')₂, Fv fragments, scFv molecules), and any other modified immunoglobulin molecule comprising an antigen recognition site, so long as they exhibit one or more of the desired biological activities. In embodiments, “desired biological activity” of an antibody refers to the ability of the antibody to bind to its target antigen, e.g., a nucleic acid, e.g.,

DNA. In embodiments, “desired biological activity” can further include antibody binding to its target antigen and resulting in a measurable biological response which can be measured in vitro or in vivo. Such activity can be antagonistic or agonistic. In embodiments, “desired biological activity” of an antibody refers to the ability of the antibody to bind to a target, e.g., nucleic acid molecules. In embodiments, “desired biological activity” of an antibody refers to the ability of the antibody to bind to a cellular receptor, e.g., ENT2. In embodiments, “desired biological activity” of an antibody refers to the ability of the antibody to be internalized by a target cell. “Target antigen,” as used herein, refers to the molecule that is bound specifically by the antigen-binding domain comprising the variable regions of a given antibody. The term “specifically binds” refers to the binding of an antibody to its cognate antigen (e.g., a nucleic acid, e.g., DNA) while not significantly binding to other antigens.

[00104] Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses or isotypes, e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. “Isotype,” as used herein, refers to any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , γ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. *Cellular and Mol. Immunology*, 4th ed. (W.B. Saunders, Co., 2000). It should be understood that antibodies disclosed herein can also comprise hybrids of isotypes and/or subclasses.

[00105] Antibodies of the present disclosure are generally isolated or recombinant. “Isolated,” when used to describe the various polypeptides disclosed herein, refers to a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An “isolated antibody,” refers to an antibody which is substantially free of other antibodies having different antigenic specificities. As used herein, “recombinant antibody” refers to an antibody that is generated using recombinant nucleic acid techniques in exogenous host cells, and recombinant antibodies can be isolated as well.

[00106] “Native antibodies” are usually heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[00107] The term “constant domain” refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen-binding site. The constant domain contains the CH1, CH2 and CH3 domains (collectively, CH) of the heavy chain and the CHL (or CL) domain of the light chain.

[00108] The “variable region” or “variable domain” of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as “VH.” The variable domain of the light chain may be referred to as “VL.” These domains are generally the most variable parts of an antibody and contain the antigen-binding sites. The term “variable” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions (HVRs) or complementary determining regions (CDRs), both in the light-chain and the heavy-chain variable domains, that confer antigen specificity. A “variable heavy domain” pairs with a “variable light domain” to form an antigen-binding domain (ABD) that specifically binds a target antigen. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three CDRs/HVRs, which form loops connecting, and in some cases forming part of, the beta-

sheet structure. The CDRs/HVRs in each chain are held together in close proximity by the FR regions and, with the CDRs/HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[00109] The terms “hypervariable region,” “HVR,” “HV,” “complementary determining region,” and “CDR,” used interchangeably herein, refer to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs or CDRs; three in the VH (H1, H2, H3; or VH CDR1, VH CDR2, VH CDR3), and three in the VL (L1, L2, L3; or VL CDR1, VL CDR2, VL CDR3).

[00110] The “light chains” of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa (“κ”) and lambda (“λ”), based on the amino acid sequences of their constant domains.

[00111] Together, the CDRs of the VH and VL domains form an Fv region. In embodiments, a VH and a VL domain comprise the six CDRs of the ABD. In a “Fab” format, the variable heavy domain (VH; containing VH CDR1, VH CDR2, and VH CDR3) and the variable light domain (VL or VL; containing the VL CDR1, VL CDR2 and VL CDR3), comprise the set of 6 CDRs, with the C-terminus of the VH domain being attached to the N-terminus of the CH1 domain of the heavy chain and the C-terminus of the VL domain being attached to the N-terminus of the constant light domain (and thus forming the light chain). In an “scFv” format, the VH and VL domains are covalently attached, generally through the use of a linker (e.g., an “scFv linker”), into a single polypeptide sequence, which can have the N- to C-terminus arrangement of VH-linker-VL or VL-linker-VH. In general, the C-terminus of the scFv domain is attached to the N-terminus of the hinge in the second monomer.

[00112] “Fab” or “Fab region,” as used herein, refers to a polypeptide that comprises VH, CH1, VL, and CL immunoglobulin domains, generally on two different polypeptide chains (e.g., VH-CH1 on one chain and VL-CL on the other). Fab can refer to this region in isolation, or this region in the context of an antibody of the disclosure. In embodiments, a Fab comprises an Fv region in addition to CH1 CL domains.

[00113] Another part of the heavy chain is the hinge region. As used herein, “hinge,” “hinge region,” “antibody hinge region,” or “hinge domain” refers to the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody.

Structurally, the IgG CH1 domain ends at EU position 215, and the IgG CH2 domain begins at residue EU position 231. Thus, for IgG, the antibody hinge is herein defined to include positions 216 (E216 in IgG1) to 230 (p230 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some cases, a “hinge fragment” is used, which contains fewer amino acids at either or both of the N- and C-termini of the hinge domain.

[00114] “Heavy chain constant region,” as used herein, refers to the CH1-hinge-CH2-CH3 portion of an antibody or fragment thereof, excluding the variable heavy domain. In embodiments, the heavy chain constant region comprises amino acids 118-447 of human IgG1, in EU numbering. As used herein, “heavy chain constant region fragment” refers to a heavy chain constant region that contains fewer amino acids from either or both of the N- and C-termini but still retains the ability to form a dimer with another heavy chain constant region.

[00115] “Fv,” “Fv fragment,” or “Fv region,” as used herein, refers to a polypeptide that comprises VL and VH domains of an antibody binding domain. Fv regions can be formatted as both Fabs and scFvs, where the VL and VH domains are combined (e.g., by way of a linker, as discussed herein) to form an scFv.

[00116] “Fc,” “Fc region,” or “Fc domain,” as used herein, refers to a polypeptide comprising CH2-CH3 domains of an IgG molecule, and, in some cases, inclusive of the hinge. In EU numbering for human IgG1, the CH2-CH3 domain comprises amino acids 231 to 447, and the hinge is 216 to 230. Thus, the definition of “Fc domain” includes both amino acids 231-447 (CH2-CH3) and 216-447 (hinge-CH2-CH3) of IgG1, or fragments thereof. An “Fc fragment” in this context can contain fewer amino acids from either or both of the N- and C-termini but still retains the ability to form a dimer with another Fc domain or Fc fragment as can be detected using standard methods, generally based on size (e.g., non-denaturing chromatography, size exclusion chromatography, etc.). In embodiments, the disclosed antibodies comprise human Fc domains. In embodiments, the disclosed antibodies comprise Fc domains from human IgG1, IgG2, or IgG4.

[00117] A “variant Fc domain” contains amino acid modifications as compared to a parental Fc domain. Thus, a “variant human IgG1 Fc domain” is one that contains amino acid modifications (generally amino acid substitutions, although in the case of ablation variants, amino acid deletions are included) as compared to the human IgG1 Fc domain. In embodiments, variant Fc domains have at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identity to the corresponding parental human IgG Fc domain. In embodiments, the percent identity is calculated using the identity algorithms discussed below. In embodiments, the percent identity is calculated using the BLAST algorithm known in the art, using default parameters. In embodiments, variant Fc domains have from 1 to about 20 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) amino acid modifications as compared to the parental Fc domain. In embodiments, variant Fc domains retain the ability to form a dimer with Ir Fc domain as measured using known techniques as described herein, such as non-denaturing gel electrophoresis.

[00118] For all positions discussed in the present disclosure that relate to antibodies, unless otherwise noted, amino acid position numbering is according to the EU index. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof. See, *SEQUENCES OF IMMUNOLOGICAL INTEREST*, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al.; Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, the contents of which are incorporated herein by reference. In embodiments of the present disclosure, amino acid position numbering is according to the IMGT system.

[00119] The terms “full-length antibody,” “intact antibody” and “whole antibody” are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain an Fc region.

[00120] An “antibody fragment” comprises a portion of an intact antibody, preferably comprising the antigen-binding region thereof. Examples of antibody fragments include Fab,

Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[00121] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, e.g., the individual antibodies comprising the population are identical except for possible mutations, e.g., naturally occurring mutations, that can be present in minor amounts. Thus, the modifier “monoclonal” indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this disclosure. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

[00122] Antibodies herein specifically include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit one or more of the desired biological activities (see, e.g., U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). Typically, the variable region of both light and heavy chains corresponds to the variable region of antibodies derived from one species of mammals (e.g., mouse, rat, rabbit, etc.) with the desired specificity, affinity, and/or capability, while the

constant regions are homologous to the sequences of antibodies derived from another species of mammals (e.g., human) to avoid eliciting an immune response in that species. Chimeric antibodies include PRIMATTZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with the antigen of interest.

[00123] “Humanized” forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In embodiments, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a CDR/HVR of the recipient are replaced by residues from a CDR/HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications can be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. See, e.g., Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, e.g., Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409. Examples of methods used to generate humanized antibodies are described in U.S. Pat. 5,225,539 or 5,639,641, incorporated herein by reference in their entireties.

[00124] As used herein, the term “human antibody” refers to an antibody which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any technique known in the art. This definition of a human antibody includes intact or full-length antibodies, fragments thereof, and/or antibodies comprising at least one human heavy and/or light chain polypeptide. This definition of a human antibody specifically

excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 5 77 (1985); Boerner et al., *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, 10 e.g., immunized xenomice (see, e.g., U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[00125] A “species-dependent antibody” is one which has a stronger binding affinity for an antigen from a first mammalian species than it has for a homologue of that antigen from a second 15 mammalian species. Normally, the species-dependent antibody “binds specifically” to a human antigen (e.g., has a binding affinity (Kd) value of no more than about 1×10^{-7} M, preferably no more than about 1×10^{-8} M and preferably no more than about 1×10^{-9} M) but has a binding affinity for a homologue of the antigen from a second nonhuman mammalian species which is at 20 least about 50 fold, or at least about 500 fold, or at least about 1000 fold, weaker than its binding affinity for the human antigen. The species-dependent antibody can be any of the various types of antibodies as defined above, but preferably is a humanized or human antibody.

[00126] The expression “linear antibodies” refers to the antibodies described in Zapata et al. (1995 *Protein Eng.*, 8(10):1057-1062). Briefly, these antibodies comprise a pair of tandem Fd 25 segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen-binding regions. Linear antibodies can be bispecific or monospecific.

[00127] “Modification,” as used herein, refers to an amino acid substitution, insertion, deletion, and/or any other mutation in a polypeptide sequence.

[00128] “Variant protein,” or “protein variant,” or “variant,” as used herein refers to a protein 30 that differs from that of a parent protein by virtue of at least one amino acid modification. The

protein variant has at least one amino acid modification compared to the parent protein, yet not so many that the variant protein will not align with the parental protein using an alignment program such as that described below. In general, variant proteins (such as variant Fc domains, etc., described herein, are generally at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% identical to the parent polypeptide, using any alignment program known in the art, such as BLAST.

[00129] Sequence identity between two similar sequences (e.g., antibody variable domains) can be measured by algorithms such as that of Smith, T.F. & Waterman, M.S. (1981) "Comparison Of Biosequences," Adv. Appl. Math. 2:482 [local homology algorithm]; Needleman, S.B. & Wunsch, CD. (1970) "A General Method Applicable To The Search For Similarities In The Amino Acid Sequence Of Two Proteins," J. Mol. Biol. 48:443 [homology alignment algorithm]; Pearson, W.R. & Lipman, D.J. (1988) "Improved Tools For Biological Sequence Comparison," Proc. Natl. Acad. Sci. (U.S.A.) 85:2444 [search for similarity method]; or Altschul, S.F. et al, (1990) "Basic Local Alignment Search Tool," J. Mol. Biol. 215:403-10, the "BLAST" algorithm, see the webpage located at URL blast.ncbi.nlm.nih.gov/Blast.cgi. When using any of the aforementioned algorithms, the default parameters (for Window length, gap penalty, etc.) are used. Unless specifically stated otherwise, sequence identity is determined using the BLAST algorithm, using default parameters.

[00130] In embodiments, a parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the heavy constant domain or Fc region from IgG1, IgG2, IgG3 or IgG4, although human sequences with variants can also serve as "parent polypeptides." In embodiments, antibody sequences described herein have at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least

- about 99.5% sequence identity with a parent polypeptide sequence. Accordingly, “antibody variant” or “variant antibody” as used herein refers to an antibody that differs from a parent antibody by virtue of at least one amino acid modification; “IgG variant” or “variant IgG” as used herein refers to an IgG that differs from a parent IgG (e.g., from a human IgG sequence) by virtue of at least one amino acid modification; “immunoglobulin variant” or “variant immunoglobulin” as used herein refers to an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification; and “Fc variant” or “variant Fc” as used herein refers to an Fc that differs from a parent Fc, e.g., an Fc domain of human IgG1, IgG2, IgG3, or IgG4, by virtue of at least one amino acid modification.
- 10 **[00131]** “IgG subclass modification” or “isotype modification,” as used herein, refers to amino acid modifications that convert one amino acid of one IgG isotype to the corresponding amino acid in a different, aligned IgG isotype. For example, because IgG1 comprises a tyrosine and IgG2 a phenylalanine at EU position 296, a F296Y substitution in IgG2 is considered an IgG subclass modification.
- 15 **[00132]** “Non-naturally occurring modification” as used herein is meant an amino acid modification that is not isotypic. For example, because none of the human IgGs comprise a serine at position 434, the substitution 434S in IgG1, IgG2, IgG3, or IgG4 (or hybrids thereof) is considered a non-naturally occurring modification.
- [00133]** As used herein, “oligonucleotide” or “polynucleotide,” used interchangeably, refers to a linear polymer of natural or modified nucleoside monomers linked by phosphodiester bonds or analogs thereof. The term “oligonucleotide” usually refers to a shorter polymer, e.g., comprising from about 3 to about 100 monomers, and the term “polynucleotide” usually refers to longer polymers, e.g., comprising from about 100 monomers to many thousands of monomers, e.g., 10,000 monomers, or more. Oligonucleotides and polynucleotides can be natural or synthetic.
- 20 Oligonucleotides and polynucleotides can include deoxyribonucleosides, ribonucleosides, and/or non-natural analogs thereof. In embodiments, oligonucleotides or polynucleotides are capable of specifically binding to a target genome by way of a regular pattern of monomer-to-monomer interactions, such as Watson-Crick type of base pairing, base stacking, Hoogsteen or reverse Hoogsteen types of base pairing, or the like. As used herein, “functional nucleic acid” refers to a
- 25 nucleic acid having biological functions in vivo or in cells, such as enzymatic functions, catalytic
- 30

functions, or biologically inhibiting or enhancing functions (e.g., inhibition or enhancement of transcription or translation). In embodiments, examples of functional nucleic acids include, but are not limited to, siRNA, ASO, shRNA, miRNA (including pri-miRNA and pre-miRNA), nucleic acid aptamers (including RNA aptamers and DNA aptamers), ribozymes (including
5 deoxyribozymes), riboswitches, U1 adaptors, molecular beacons, and transcriptional factor-binding regions.

[00134] As used herein, a “3E10 antibody” refers to an antibody with a set of heavy chain CDRs (VH CDR1, VH CDR2, and VH CDR3), identified according to the Kabat system, comprising amino acid sequences that vary from SEQ ID NOS: 58, 59, and 60 by no more than
10 two amino acids each, respectively, a set of light chain CDRs (VL CDR1, VL CDR2, and VL CRD3) comprising amino acid sequences that vary from SEQ ID NOS: 61, 62, and 63 by no more than two amino acids each, respectively, that binds nucleic acids and is cell-penetrating at least when bound to a nucleic acid, as well as antigen-binding fragments thereof. As described herein, the 3E10 antigen is a polynucleotide.

[00135] As used herein, the term “cell-penetrating” refers to an antibody or antigen binding fragment thereof that can penetrate a cell, e.g., a mammalian cell, without the aid of an exogenous transport vehicle, such as a liposome, or a conjugated cell-penetrating peptide. With respect to 3E10 antibodies and antigen binding fragments thereof, the cell-penetrating antibody or antigen binding fragment thereof can penetrate a cell expressing an ENT2 receptor on its cell
20 surface in the presence of nucleic acids, e.g., non-covalently bound and/or conjugated to the 3E10 antibody or antigen binding fragment thereof, resulting in internalization of the 3E10 antibodies and antigen binding fragments thereof. In some embodiments, the cell-penetrating 3E10 antibody or antigen binding fragment thereof is conjugated to a functional molecule, e.g., a chemical agent, polynucleotide, or polypeptide. Although the cell-penetrating molecules are
25 generally referred to herein as “cell-penetrating antibodies,” it will be appreciated that fragments, including antigen-binding fragments, variants, binding proteins and fusion proteins such as scFv, di-scFv, tri-scFv, and other single chain variable fragments, and other cell-penetrating molecules disclosed herein are also expressly provided for use in compositions, conjugates, and methods disclosed herein. Autoantibodies against double-stranded deoxyribonucleic acid (dsDNA) are
30 frequently identified in the serum of patients with systemic lupus erythematosus (SLE) and are often implicated in disease pathogenesis. Therefore, in embodiments, cell-penetrating antibodies

(*e.g.*, cell-penetrating anti-DNA antibodies) can be derived or isolated from patients with SLE or animal models of SLE.

5 [00136] As used herein, the term "host cell" refers to any eukaryotic cell (*e.g.*, mammalian cells, avian cells, amphibian cells, plant cells, fish cells, and insect cells), whether located in vitro or in vivo.

[00137] As used herein, the term "cell culture" refers to any in vitro culture of cells. Included within this term are continuous cell lines (*e.g.*, with an immortal phenotype), primary cell cultures, finite cell lines (*e.g.*, non-transformed cells), and any other cell population maintained in vitro, including oocytes and embryos.

10 [00138] As used herein, the term "vector" refers to any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

15 [00139] As used herein, the term "genome" refers to the genetic material (*e.g.*, chromosomes) of an organism.

[00140] The term "nucleotide sequence of interest" refers to any nucleotide sequence (*e.g.*, RNA or DNA), the manipulation of which may be deemed desirable for any reason (*e.g.*, treat disease, confer improved qualities, expression of a protein of interest in a host cell, expression of a ribozyme, etc.), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (*e.g.*, reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product (*e.g.*, promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.).

25 [00141] As used herein, the term "product of interest" refers to a protein or nucleic acid product, such as a viral backbone or genome, encoded by a nucleic acid of interest. In some embodiments, a plurality of products of interest are expressed in a host cell.

[00142] As used herein, the term "protein of interest" refers to a protein encoded by a nucleic acid of interest.

[00143] As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," "DNA encoding," "RNA sequence encoding," and "RNA encoding" refer to the order or sequence of deoxyribonucleotides or ribonucleotides along a strand of deoxyribonucleic acid or ribonucleic acid. The order of these deoxyribonucleotides or ribonucleotides determines the order of amino acids along the polypeptide (protein) chain. The DNA or RNA sequence thus codes for the amino acid sequence.

[00144] The term "promoter," "promoter element," or "promoter sequence" as used herein, refers to a DNA sequence which when ligated to a nucleotide sequence of interest is capable of controlling the transcription of the nucleotide sequence of interest into mRNA. A promoter is typically, though not necessarily, located 5' (*i.e.*, upstream) of a nucleotide sequence of interest whose transcription into mRNA it controls, and provides a site for specific binding by RNA polymerase and other transcription factors for initiation of transcription.

[00145] Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription (Maniatis *et al.*, Science 236:1237 [1987]). Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect and mammalian cells, and viruses (analogous control elements, *i.e.*, promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset of cell types (for review see, Voss *et al.*, Trends Biochem. Sci., 11:287 [1986]; and Maniatis *et al.*, *supra*). For example, the SV40 early gene enhancer is very active in a wide variety of cell types from many mammalian species and has been widely used for the expression of proteins in mammalian cells (Dijkema *et al.*, EMBO J. 4:761 [1985]). Two other examples of promoter/enhancer elements active in a broad range of mammalian cell types are those from the human elongation factor 1 α gene (Uetsuki *et al.*, J. Biol. Chem., 264:5791 [1989]; Kim *et al.*, Gene 91:217 [1990]; and Mizushima and Nagata, Nuc. Acids. Res., 18:5322 [1990]) and the long terminal repeats of the Rous sarcoma virus (Gorman *et al.*, Proc. Natl. Acad. Sci. USA 79:6777 [1982]) and the human cytomegalovirus (Boshart *et al.*, Cell 41:521 [1985]).

[00146] As used herein, the term "promoter/enhancer" denotes a segment of DNA which contains sequences capable of providing both promoter and enhancer functions (*i.e.*, the functions provided by a promoter element and an enhancer element, see above for a discussion of these functions). For example, the long terminal repeats of retroviruses contain both promoter and enhancer functions. The enhancer/promoter may be "endogenous" or "exogenous" or "heterologous." An "endogenous" enhancer/promoter is one that is naturally linked with a given gene in the genome. An "exogenous" or "heterologous" enhancer/promoter is one that is placed in juxtaposition to a gene by means of genetic manipulation (*i.e.*, molecular biological techniques such as cloning and recombination) such that transcription of that gene is directed by the linked enhancer/promoter.

[00147] As used herein, the term "long terminal repeat" of "LTR" refers to transcriptional control elements located in or isolated from the U3 region 5' and 3' of a retroviral genome. As is known in the art, long terminal repeats may be used as control elements in retroviral vectors, or isolated from the retroviral genome and used to control expression from other types of vectors.

[00148] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "5'-A-G-T-3'," is complementary to the sequence "3'-T-C-A-5'." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

[00149] The terms "homology" and "percent identity" when used in relation to nucleic acids refers to a degree of complementarity. There may be partial homology (*i.e.*, partial identity) or complete homology (*i.e.*, complete identity). A partially complementary sequence is one that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid sequence and is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization

and the like) under conditions of low stringency. A substantially homologous sequence or probe (i.e., an oligonucleotide which is capable of hybridizing to another oligonucleotide of interest) will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous sequence to a target sequence under conditions of low stringency. This is not to say that

5 conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target which lacks even a partial degree of complementarity (e.g., less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary

10 target.

[00150] The terms "in operable combination," "in operable order," and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of amino acid sequences in

15 such a manner so that a functional protein is produced.

[00151] As used herein, the term "selectable marker" refers to a gene that encodes an enzymatic activity or other protein that confers the ability to grow in medium lacking what would otherwise be an essential nutrient; in addition, a selectable marker may confer resistance to an antibiotic or drug upon the cell in which the selectable marker is expressed.

20 **[00152]** As used herein, the term "retrovirus" refers to a retroviral particle which is capable of entering a cell (*i.e.*, the particle contains a membrane-associated protein such as an envelope protein or a viral G glycoprotein which can bind to the host cell surface and facilitate entry of the viral particle into the cytoplasm of the host cell) and integrating the retroviral genome (as a double-stranded provirus) into the genome of the host cell. The term "retrovirus" encompasses

25 Oncovirinae (*e.g.*, Moloney murine leukemia virus (MoMLV), Moloney murine sarcoma virus (MoMSV), and Mouse mammary tumor virus (MMTV), Spumavirinae, and Lentivirinae (*e.g.*, Human immunodeficiency virus, Simian immunodeficiency virus, Equine infection anemia virus, and Caprine arthritis-encephalitis virus; *See, e.g.*, U.S. Pat. Nos. 5,994,136 and 6,013,516, both of which are incorporated herein by reference).

[00153] As used herein, the term "retroviral vector" refers to a retrovirus that has been modified to express a gene of interest. Retroviral vectors can be used to transfer genes efficiently into host cells by exploiting the viral infectious process. Foreign or heterologous genes cloned (*i.e.*, inserted using molecular biological techniques) into the retroviral genome can be delivered efficiently to host cells that are susceptible to infection by the retrovirus. Through well-known genetic manipulations, the replicative capacity of the retroviral genome can be destroyed. The resulting replication-defective vectors can be used to introduce new genetic material to a cell but they are unable to replicate. A helper virus or packaging cell line can be used to permit vector particle assembly and egress from the cell. Such retroviral vectors comprise a replication-deficient retroviral genome containing a nucleic acid sequence encoding at least one gene of interest (*i.e.*, a polycistronic nucleic acid sequence can encode more than one gene of interest), a 5' retroviral long terminal repeat (5' LTR); and a 3' retroviral long terminal repeat (3' LTR).

[00154] As used herein, the term "lentivirus vector" refers to retroviral vectors derived from the Lentiviridae family (*e.g.*, human immunodeficiency virus, simian immunodeficiency virus, equine infectious anemia virus, and caprine arthritis-encephalitis virus) that are capable of integrating into non-dividing cells (*See, e.g.*, U.S. Pat. Nos. 5,994,136 and 6,013,516, both of which are incorporated herein by reference).

[00155] As used herein, the term "transposon" refers to transposable elements (*e.g.*, Tn5, Tn7, and Tn10) that can move or transpose from one position to another in a genome. In general, the transposition is controlled by a transposase. The term "transposon vector," as used herein, refers to a vector encoding a nucleic acid of interest flanked by the terminal ends of transposon. Examples of transposon vectors include, but are not limited to, those described in U.S. Pat. Nos. 6,027,722; 5,958,775; 5,968,785; 5,965,443; and 5,719,055, all of which are incorporated herein by reference.

[00156] As used herein, the term "adeno-associated virus (AAV) vector" refers to a vector derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, preferably the rep and/or cap genes, but retain functional flanking ITR sequences.

[00157] AAV vectors can be constructed using recombinant techniques that are known in the art to include one or more heterologous nucleotide sequences flanked on both ends (5' and 3') with functional AAV ITRs. In the practice of the disclosure, an AAV vector can include at least one AAV ITR and a suitable promoter sequence positioned upstream of the heterologous nucleotide sequence and at least one AAV ITR positioned downstream of the heterologous sequence. A "recombinant AAV vector plasmid" refers to one type of recombinant AAV vector wherein the vector comprises a plasmid. As with AAV vectors in general, 5' and 3' ITRs flank the selected heterologous nucleotide sequence.

[00158] As used herein, the term "adenoviral vector" refers to a non-enveloped double-stranded DNA vector comprising an adenovirus backbone.

[00159] As used herein, the term "purified" refers to molecules, either nucleic or amino acid sequences, that are removed from their normal environment, isolated or separated. An "isolated nucleic acid sequence" is therefore a purified nucleic acid sequence. "Substantially purified" molecules are at least 60% free, preferably at least 75% free, and more preferably at least 90% free from other components with which they are normally associated.

ABBREVIATIONS

[00160] AmpR= bacterial ampicillin resistance gene

attB= Bacterial Attachment Site

attP= Phage Attachment Site

attR= Recombined Upstream Attachmend Site

Backbone=Plasmid Backbone

CDS= Coding Sequence

EPR=MMLV Extended Packaging Region

GCI = Gene Copy Index

GS= Glutamine Synthetase

H or HC= Heavy Chain

hCMV= Human Cytomegalovirus immediate-early Promoter

I= intron

L or LC= Light Chain

MoMuSV 5'LTR= Moloney Murine Sarcoma Virus 5' Long Terminal Repeat

5 Neo= Neomycin resistance gene PA or PolyA= Polyadenylation signal

ProV SIN-LTR= Proviral Self- Inactivating Long Terminal Repeat

sCMV= Simian Cytomegalovirus immediate-early Promoter

SDS-PAGE= Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis

SIN-3'LTR= Self-Inactivation 3' Long Terminal Repeat

10 SV40= Simian Virus 40

TK= Thymidine Kinase

UTR= Untranslated Region

W or WPRE= Woodchuck Post-transcriptional Regulatory Element

15 DETAILED DESCRIPTION OF THE DISCLOSURE

[00161] The present disclosure provides host cells that allow for co-expression of an endonuclease, e.g., a DNase, with a product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, to improve expression and/or purification of the product of interest, from the host cell culture. In some embodiments, an expression construct encoding an endonuclease, e.g.,
 20 a DNase, and an expression construct(s) encoding one or more gene of interest are introduced into host cells at defined ratios. In some embodiments, the host cell lines contain multiple dock sites for insertion of the nucleic acid constructs. Cell lines containing multiple dock sites and expression constructs for use with the cells are described in WO2021247671 and WO2021247672, both of which are incorporated by reference herein in their entirety.

25 [00162] The present disclosure solves multiple problems by providing host cells that allow for co-expression of an endonuclease, e.g., a DNase, with one or more proteins of interest, e.g., a 3E10 antibody or antigen binding fragment thereof. In one aspect, co-expression of an

endonuclease, e.g., a DNase, with one or more products of interest allows for more efficient removal of host cell DNA from pharmaceutical preparations. In another aspect, co-expression of an endonuclease, e.g., a DNase, with one or more products of interest allows for more efficient production of the product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, especially where the product of interest may interact with host cell DNA. In both aspects, the inventors have surprisingly found that an endonuclease, e.g., a DNase, can be stably co-expressed in host cells lines.

[00163] Accordingly, in some embodiments, the present disclosure provides a eukaryotic host cell comprising a first exogenous nucleic acid sequence encoding an endonuclease, e.g., a DNase such as DNase I or DNase II, operably linked to a promoter sequence and at least a second exogenous nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, operably linked to a promoter sequence, wherein the first sequence encoding an endonuclease, e.g., a DNase such as DNase I or DNase II, and the at least second exogenous encoding the first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, are co-expressed in the host cell. In some embodiments, the present disclosure provides a host cell culture comprising the host cells.

[00164] In some embodiments, the host cells comprise multiple exogenous nucleic acids encoding multiple products of interest, for example, 2, 3, 4, 5, 6, 7, 8, 9 or 10 products of interest.

[00165] The present disclosure is not limited to the use of any particular endonuclease. In some embodiments, the endonuclease sequence incorporated into the construct encodes a DNase I (*i.e.*, a protein having DNase activity) having at least 80% sequence identity to SEQ ID NO:140. In some embodiments, the DNase sequence incorporated into the construct encodes a DNase having at least 90% sequence identity to SEQ ID NO:140. In some embodiments, the DNase sequence incorporated into the construct encodes a DNase having at least 95% sequence identity to SEQ ID NO:140. In some embodiments, the DNase sequence incorporated into the construct encodes a DNase having at least 98% sequence identity to SEQ ID NO:140. In some embodiments, the DNase sequence incorporated into the construct encodes a DNase having SEQ ID NO:140. In other embodiments, the DNase sequence incorporated into the construct has at least 80% sequence identity with the DNase encoding

portions of SEQ ID NO:139. In some embodiments, the DNase sequence incorporated into the construct has at least 90% sequence identity with the DNase encoding portions of SEQ ID NO:139. In some embodiments, the DNase sequence incorporated into the construct has at least 95% sequence identity with the DNase encoding portions of SEQ ID NO:139. In some
5 embodiments, the DNase sequence incorporated into the construct has at least 98% sequence identity with the DNase 1 encoding portions of SEQ ID NO:139. In some embodiments, the DNase 1 sequence incorporated into the construct is SEQ ID NO:139.

3E10 Antibodies and Antigen Binding Fragments Thereof

[00166] In general, an endonuclease, e.g., DNase 1 or DNase II, may be co-expressed with a
10 protein of interest which may be any pharmaceutical or industrial protein for which expression and production via a host culture is desired. In some embodiments, the protein of interest is a biopharmaceutical protein. In some embodiments, the protein of interest is a cell-penetrating immunoglobulin heavy and/or light chain. In some embodiments, the protein of interest is a cell-penetrating immunoglobulin fragment or single chain antibody. In some embodiments, where
15 expression of an immunoglobulin is desired, the host cell further comprises a third exogenous nucleic acid sequence encoding a second protein of interest operably linked to a promoter sequence and a secretion signal sequence, wherein the first protein of interest is an immunoglobulin light chain sequence and the second protein of interest is an immunoglobulin heavy chain sequence.

20 [00167] In some aspects, the present disclosure relates to the co-expression of an endonuclease, e.g., a DNase 1 or DNase II, with a 3E10 antibody or antigen binding fragment thereof. In some embodiments, the compositions and methods described herein increase the productivity and/or specific productivity of a cell culture expressing a 3E10 antibody or antigen binding fragment thereof. For example, as reported in Example 2, co-expression of a humanized
25 3E10 antibody and the endonuclease DNase 1 resulted in specific productivity of up to 5.1 picograms of the humanized 3E10 antibody per day per cell. In comparison, expression of the humanized 3E10 antibody in the same cell type, without co-expression of the endonuclease, resulted in a specific productivity of less than 5.0 picograms of the humanized 3E10 antibody per day per cell. In some embodiments, expression of the humanized 3E10 antibody in the same cell
30 type, without co-expression of the endonuclease, resulted in a specific productivity of less than

4.0 picograms of the humanized 3E10 antibody per day per cell. In some embodiments, expression of the humanized 3E10 antibody in the same cell type, without co-expression of the endonuclease, resulted in a specific productivity of less than 3.0 picograms of the humanized 3E10 antibody per day per cell. In some embodiments, expression of the humanized 3E10 antibody in the same cell type, without co-expression of the endonuclease, resulted in a specific productivity of less than 2.0 picograms of the humanized 3E10 antibody per day per cell. IN some embodiments, expression of the humanized 3E10 antibody in the same cell type, without co-expression of the endonuclease, resulted in a specific productivity of less than 1.0 picograms of the humanized 3E10 antibody per day per cell. Accordingly, in some embodiments, the endonuclease is co-expressed with a heavy chain, or fragment thereof, and a light chain, or fragment thereof, of a 3E10 antibody.

[00168] 3E10 antibodies or antigen binding fragments thereof useful in the compositions described herein include whole immunoglobulin, e.g., an intact antibody, of generally based on the IgG class, which has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgG, binding fragments thereof, and synthetic proteins, containing at least the nucleic acid binding variable domain. The sequence of the variable domains differs among the 3E10 antibodies and determine the binding kinetics and specificity for each particular 3E10 antibody. The variability is usually not evenly distributed through the variable domains of the 3E10 antibodies. It is typically concentrated in three segments called complementarity determining regions (CDRs), both in the heavy and light chain variable domains. The more highly conserved portions of the variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, adopting a beta-sheet configuration connected by three CDRs, which form connecting loops, and in some cases forming part of the beta-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions, and with the CDRs from the other chain, contribute to the formation of the nucleic acid binding site of antibody. Thus, the 3E10 antibodies or antigen binding fragment thereof typically contain at least the CDRs necessary to maintain DNA binding.

1. 3E10 Antibodies

[00169] Although generally referred to herein as “3E10” or “3E10 antibodies,” it will be appreciated that fragments, variants, and binding proteins, including antigen-binding fragments and fusion proteins, such as scFv, di-scFv, tr-scFv, and other single chain variable fragments,

and other cell-penetrating, nucleic acid transporting molecules disclosed herein, are encompassed by the phrase and are also expressly provided for use in compositions, conjugates, and methods disclosed herein. Thus, when describing a feature of a 3E10 antibody disclosed herein, that same feature is implicitly disclosed with respect to 3E10 antigen-binding fragments as well. In
5 embodiments, the antibodies and other binding proteins are also referred to herein as cell-penetrating.

[00170] In embodiments, a 3E10 antibody comprises VL CDRs of SEQ ID NOs: 61, 62, and 63 and VH CDRs of SEQ ID NOs: 58, 59, and 60. In embodiments, a 3E10 antibody comprises VL CDRs of SEQ ID NOs: 9, 10, and 11 and VH CDRs of SEQ ID NOs: 3, 4, and 5. In
10 embodiments, a 3E10 antibody comprises VL CDRs of SEQ ID NOs: 22, 23, and 24 and VH CDRs of SEQ ID NOs: 15, 17, and 18. In embodiments, a 3E10 antibody comprises VL CDRs of SEQ ID NOs: 9, 10, and 11 and VH CDRs of SEQ ID NOs: 16, 4, and 5. Other examples of 3E10 VL and VH CDR sequences are shown in Figures 17-20.

[00171] In embodiments, a 3E10 antibody has nucleic acid binding affinity.

15 [00172] In embodiments, a 3E10 antibody is competent for ENT2-mediated cell internalization when bound to nucleic acid.

[00173] In some aspects of the present disclosure, the antibody or antigen-binding fragment thereof is a murine, chimeric, humanized, or human antibody or antigen-binding fragment thereof.

20 [00174] In some aspects, a 3E10 construct of the present disclosure penetrates into cells and nuclei in an ENT2-dependent manner.

[00175] In embodiments, a polynucleotide is non-covalently bound to a 3E10 construct of the present disclosure, to help facilitate cellular internalization of the 3E10 construct. That is, in some embodiments, polynucleotides conjugated to the antibody (cargo polynucleotides) do not
25 interact with the nucleic acid-binding paratope of the antibody, and a second polynucleotide (e.g., carrier nucleic acid) is non-covalently complexed with the paratope to help facilitate internalization. In embodiments, the polynucleotide is precomplexed with the 3E10 construction prior to administering the 3E10 construct to a subject. In embodiments, the polynucleotide is an extracellular polynucleotide that is bound by the 3E10 construct at a site of interest in vivo, for

example, at a site of tumor ischemia and/or necrosis. In embodiments, the second polynucleotide is DNA. In embodiments, the polynucleotide is RNA.

[00176] In embodiments, a 3E10 construct disclosed herein comprises a VH and VL domain of a 3E10 antibody. In embodiments, the 3E10 construct comprises a VH CDR1, VH CDR2, VH
5 CDR3, VL CDR1, VL CDR2, and/or VL CDR3 of a 3E10 antibody.

[00177] In embodiments, the antibody or antigen-binding fragment thereof comprises a light chain variable region (VL) comprising an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:21. In embodiments, the antibody
10 or antigen-binding fragment thereof comprises a heavy chain variable region (VH) comprising an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:14. In embodiments, the antibody or antigen-binding fragment thereof comprises a full length light chain (LC) comprising an amino acid sequence that is at least about
15 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:20. In embodiments, the antibody or antigen-binding fragment thereof comprises a full length heavy chain (HC) comprising an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%,
20 or at least about 99% identical to SEQ ID NO:13.

[00178] In some aspects, the 3E10 antibody or antigen-binding fragment thereof can be transported into the cytoplasm and/or nucleus of the cells without the aid of a carrier or conjugate. For example, a monoclonal 3E10 antibody and active fragments thereof that are transported *in vivo* to the nucleus of mammalian cells without cytotoxic effect are disclosed in
25 U.S. Patent Nos. 4,812,397 and 7,189,396 to Richard Weisbart, the disclosures of which are incorporated by reference herein, in their entireties.

[00179] Amino acid sequences of 3E10 monoclonal antibodies and antigen-binding fragments thereof are known in the art. Example sequences of 3E10 heavy and light chains are provided below herein.

[00180] A murine version of the 3E10 antibody is described in Zack, et al., *Immunology and Cell Biology*, 72:513-520 (1994), the disclosure of which is incorporated by reference herein, in its entirety.

[00181] Amino acid variants of the 3E10 antibody are also known in the art, for example, as described in Zack, et al., *J. Immunol.*, 157(5):2082-8 (1996). For example, amino acid position 31, in CDR1 of the heavy chain variable region of 3E10, influences nucleic acid binding and the antibody's ability to penetrate nuclei. Substitution of the 'wild-type' (e.g., relative to the original murine antibody) aspartic acid by asparagine (the 'D31N' mutation) improves nucleic acid binding and nuclei penetration of the antibody, relative to the 'wild type' murine antibody. *See*, for example, Zack, et al., *Immunology and Cell Biology*, 72:513-520 (1994); Weisbart, et al., *J. Autoimmun.*, 11, 539-546 (1998); and Weisbart, *Int. J. Oncol.*, 25, 1867-1873 (2004) (which are incorporated by reference herein, in their entireties).

[00182] Sequences for 3E10 antibodies and antigen-binding fragments or variants thereof, with the D31N substitution, are disclosed herein. In some aspects, the 3E10 antibodies and antigen-binding fragments thereof disclosed herein include the D31N substitution. In some aspects, other amino acids are substituted at position 31 in the 3E10 antibodies and antigen-binding fragments thereof disclosed herein. For example, D31R, D31K, or D31R substitutions are incorporated in some aspects of the present disclosure.

[00183] Other 3E10 light chain sequences are known in the art. *See*, for example, Zack, et al., *J. Immunol.*, 15;154(4):1987-94 (1995); GenBank: L16981.1 - Mouse Ig rearranged L-chain gene, partial cds; GenBank: AAA65681.1 - immunoglobulin light chain, partial [*Mus musculus*]).

[00184] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. In embodiments, an antibody disclosed herein is an IgA, IgD, IgE, IgG, or IgM antibody, including any subtype or isotype thereof. In embodiments, an antibody disclosed herein is based on the IgG class. In embodiments, an antibody disclosed herein is based on one of the subclasses of IgG, including, but not limited to IgG1, IgG2, IgG3, and IgG4. In general,

IgG1, IgG2 and IgG4 are used more frequently than IgG3. It should be noted that IgG1 has different allotypes with polymorphisms at 356 (D or E) and 358 (L or M), and in embodiments, antibodies disclosed herein are based on IgG1 having D or E at position 356 and/or L or M at position 358.

- 5 **[00185]** The light chain generally comprises two domains, the variable light domain (containing the light chain CDRs and together with the variable heavy domains forming the Fv region), and a constant light chain region (often referred to as CL or C_κ). The heavy chain comprises a variable heavy domain and a constant domain, which includes a CH1-optional hinge-Fc domain comprising a CH2-CH3.
- 10 **[00186]** The hypervariable region of an antibody generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; “L” denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; “H” denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed.
- 15 Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those residues forming a hypervariable loop (e.g., residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) *J. Mol. Biol.* 196:901-917. Specific CDRs useful for the compositions, conjugates, and methods described herein are
- 20 described below.

[00187] As will be appreciated by those in the art, the exact numbering and placement of the CDRs can be different among different numbering systems. However, it should be understood that the disclosure of a variable heavy and/or variable light sequence includes the disclosure of the associated (inherent) CDRs. Accordingly, the disclosure of each variable heavy region is a disclosure of the VH CDRs (e.g., VH CDR1, VH CDR2 and VH CDR3) and the disclosure of each variable light region is a disclosure of the VL CDRs (e.g., VL CDR1, VL CDR2 and VL CDR3).

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[00188] Throughout the present disclosure, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) and the EU

30

numbering system for Fc regions (e.g., Kabat et al., supra (1991)). In some aspects, the present specification uses the IMGT system to define the complementarity determining regions (CDRs) provided herein.

[00189] The present disclosure provides a large number of different CDR sets. In this case, a
5 “full CDR set” comprises the three variable light CDRs, e.g., a VL CDR1, VL CDR2, and VL CDR3, and the three variable heavy CDRs, e.g. VH CDR1, VH CDR2, and VH CDR3. These can be part of a larger variable light or variable heavy domain, respectfully. In addition, as more fully outlined herein, the variable heavy and variable light domains can be on separate polypeptide chains, when a heavy and light chain is used (for example when Fabs are used), or
10 on a single polypeptide chain in the case of scFv sequences.

[00190] As noted above herein, the present disclosure refers to different antibody domains of a 3E10 antibody or antigen-binding fragment thereof. These domains include, but are not limited to, the Fc domain, the CH1 domain, the CH2 domain, the CH3 domain, the hinge domain, the heavy constant domain (CH1-hinge-Fc domain or CH1-hinge-CH2-CH3), the variable heavy
15 (VH) domain, the variable light (VL) domain, the light constant domain, Fab domains and scFv domains.

2. Humanized Antibodies

[00191] In certain aspects, the antibodies of the disclosure comprise a heavy chain variable region from a particular germline heavy chain immunoglobulin gene and/or a light chain variable
20 region from a particular germline light chain immunoglobulin gene. For example, such antibodies can comprise or consist of murine, chimeric, humanized, or human antibodies or antigen-binding fragments thereof comprising heavy or light chain variable regions that are “the product of” or “derived from” a particular germline sequence, e.g., that of the 3E10 antibody. A human antibody that is “the product of” or “derived from” a human germline immunoglobulin
25 sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (i.e., greatest % identity) to the sequence of the human antibody (using the methods outlined herein). A human antibody that is “the product of” or “derived from” a particular human germline immunoglobulin sequence can
30 contain amino acid differences as compared to the germline sequence, due to, for example,

naturally-occurring somatic mutations or intentional introduction of site-directed mutation.

However, a humanized antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the antibody as being derived from human sequences when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a humanized antibody can be at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a humanized antibody derived from a particular human germline sequence will display no more than 10-20 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the humanized antibody can display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

[00192] In one aspect, the parent antibody has been affinity matured, as is known in the art. Structure-based methods can be employed for humanization and affinity maturation, for example, as U.S. Patent Publication No. 2006/0008883, which is incorporated herein by reference. Selection based methods can be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, *J. Mol. Biol.* 294:151-162; Baca et al., 1997, *J. Biol. Chem.* 272(16):10678-10684; Rosok et al., 1996, *J. Biol. Chem.* 271(37): 22611-22618; Rader et al., 1998, *Proc. Natl. Acad. Sci. USA* 95: 8910-8915; Krauss et al., 2003, *Protein Engineering* 16(10):753-759, all of which are incorporated herein by reference. Other humanization methods can involve the grafting of only parts of the CDRs, including but not limited to methods described in U.S. Patent Publication No. 2001/0035606; Tan et al., 2002, *J. Immunol.* 169:1119-1125; De Pascalis et al., 2002, *J. Immunol.* 169:3076-3084, all of which are incorporated herein by reference.

3. Fc variants

[00193] In embodiments, one or more amino acid modifications can be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant can comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc

region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[00194] In embodiments, an Fc region variant possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays known in the art can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. To assess complement activation, a CDC assay can be performed (see, for example, Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[00195] In embodiments, an antibody provided herein can have reduced effector function and thus can comprise a substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[00196] In embodiments, an Fc region variant provided herein can have improved or diminished binding to FcRs. See, e.g., U.S. Pat. No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001), the disclosure of which are incorporated herein by reference, in their entireties.

[00197] In embodiments, an Fc region variant provided herein comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[00198] In embodiments, an Fc region variant provided herein comprises alterations that result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent

Cytotoxicity (CDC), e.g., as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

[00199] In embodiments, an Fc region variant provided herein comprises alterations that result in increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), e.g., as described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (U.S. Pat. No. 7,371,826).

[00200] In embodiments, an Fc region variant provided herein comprises “knob-in-hole” or “skew” variants, which refer to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation, as described in USSN 61/596,846, Ridgway et al, Protein Engineering 9(7):617 (1996); Atwell et al, J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety.

[00201] In embodiments, an Fc region variant provided herein comprises alterations described in Duncan & Winter, Nature 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351.

4. Antibody Fragments

[00202] In embodiments, the antibody comprises an antigen-binding fragment of a 3E10 antibody. In embodiments, the antigen-binding fragment retains the desired biological activity of a 3E10 antibody. In embodiments, the antigen-binding fragment retains at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% of the desired biological activity of a 3E10 antibody. In embodiments, the antigen-binding fragment retains the ability of the antibody to bind to its target antigen, e.g., a nucleic acid, e.g., DNA. In embodiments, the antigen-binding fragment retains the ability of the antibody

to bind to a cellular receptor, e.g., ENT2. In embodiments, the antigen-binding fragment retains the ability of the antibody to be internalized by a target cell.

[00203] In embodiments, the antibody comprises a single-chain fragment variable (scFv), a tandem double scFv, an (scFv)₂, a minibody, a VHH, an scFv-Fc, a CrossMab, a dual variable
5 domain immunoglobulin (DVD-Ig), a single-chain tandem fragment variable (scTaFv), a diabody, a tandem diabody (TandAb), a Fabsc, a modular IgG-scFv, a Fab, or an F(ab')₂.

[00204] In some embodiments, the endonuclease is co-expressed with a single chain variable fragment (scFv). An scFV is a small, genetically engineered antibody molecule composed of the variable regions of the heavy (VH) and light (VL) chain of a conventional antibody, joined by a
10 peptide linker. It retains the antigen-binding specificity of a full-length antibody but has a smaller size, making it easier to produce and modify for various applications. scFv can be used in therapeutic and diagnostic applications as well as in basic research for studying protein-protein interactions. In some embodiments, the scFv comprises 3E10 VH and VL CDRs.

[00205] In some embodiments, the endonuclease is co-expressed with a single-chain 3E10
15 fragment derived from a parent 3E10 antibody. In some embodiments, the single chain 3E10 fragment is an scFv-Fc polypeptide. An scFv-Fc polypeptide is a fusion protein made up of a single chain variable fragment (scFv) and a fragment crystallizable (Fc) region. The scFv part of the protein is derived from an antibody and binds to a specific antigen, while the Fc region provides stability and biological effector functions. This fusion protein can be used in therapeutic
20 applications for targeting specific diseases or as a research tool for studying protein-protein interactions. In some embodiments, the scFv-Fc comprises 3E10 VH and VL CDRs.

[00206] In some embodiments, the endonuclease is co-expressed with a bispecific 3E10 antibody or antigen binding fragment thereof. In some embodiments, one arm of the bispecific 3E10 antibody comprises 3E10 VH and VL CRDs. In some embodiments, the other arm of the
25 bispecific antibody has specificity for a cell surface antigen, e.g., a cell surface tumor antigen, for modulating the targeting of the bispecific antibody in vivo. For more information of bispecific antibodies see, for example, Ahamadi-Fesharaki R., et al., *Molecular Therapy Oncolytics*, 14:38-56 (2019), the disclosure of which is incorporated herein by reference in its entirety.

[00207] In some embodiments, the antibody is a CrossMab antibody a 3E10 antibody or
30 antigen binding fragment thereof. In the CrossMab format, complementary mutations are

introduced in the heavy chain constant region of each arm to generate so-called “holes and knobs,” resulting in preferred association between different arms, forming a heterodimer, rather than a homodimer of two of the same arms. The exact residues that are mutated in the heavy chain constant region of a CrossMab bispecific antibody to form “holes” and “knobs” can vary depending on the specific design and optimization goals of the antibody. For more information on CrossMab antibodies see, for example, Huang, J., et al., Journal of Biological Chemistry, 294(50):19001–10 (2019), the disclosure of which is incorporated herein by reference in its entirety.

[00208] In some embodiments, the antibody is a divalent, dual-variable domain immunoglobulin (DVD-Ig) of a 3E10 antibody or antigen binding fragment thereof. In the DVD-Ig format, each arm of the antibody contains two VH/VL pairs. In some embodiments, one of the VH/VL pairs comprises 3E10 VH and VL CDRs. For more information on DVD-Ig antibodies see, for example, Polson AG, et al., Journal of immunotherapy. 29(3):241-50 (2006) and U.S. Patent No. 7,612,181, the disclosures of which are incorporated herein by reference in their entireties.

[00209] In embodiments, the antibody comprises a single-chain variable fragment (scFv). “Single chain Fv” or “scFv,” as used herein, refers to a VH domain covalently attached to a VL domain through a linker, e.g., a scFv linker as discussed herein, to form a continuous protein chain. A scFv domain can be in either arrangement from N- to C-terminus (i.e., VH-linker-VL or VL-linker-VH). In the sequences depicted in the sequence listing and in the figures herein, the order of the VH and VL domain is indicated in the name, e.g., H.X_L.Y means the N- to C-terminus arrangement is VH-linker-VL, and L.Y_H.X means the N- to C-terminus arrangement is VL-linker-VH.

[00210] In embodiments, an antigen-binding fragment of a 3E10 antibody or antigen-binding fragment thereof comprises a tandem double scFv.

[00211] In some embodiments, the antibody is a tandem double scFv (single-chain variable fragment) antibody. A tandem double scFv has two scFv domains linked in a linear fashion. In some embodiments, each scFv domain is derived from a different antibody and provides independent antigen-binding specificity. In some embodiments, one of the scFv domains comprises 3E10 VH and VL CDRs. For more information on tandem double scFvs see, for

example, Bossen C, et al., MAbs, 4(2):200-08 (2012), the disclosure of which is incorporated herein by reference in its entirety.

[00212] In some embodiments, the antibody is a dimeric scFv antibody (scFv)₂. A dimeric scFv antibody has two scFv domains linked in a dimeric arrangement. In some embodiments, each scFv domain is derived from a different antibody and provides independent antigen-binding specificity. In some embodiments, one of the scFv domains comprises 3E10 VH and VL CDRs. For more information on dimeric scFv antibodies see, for example, Llewellyn C, et al., Journal of immunological methods, 273(1-2):33-44 (2002), the disclosure of which is incorporated herein by reference in its entirety.

[00213] In some embodiments, the antibody comprises a scFv-Fc. An “scFv-Fc,” as meant herein, is a polypeptide that consists of a heavy and a light chain variable region of an antibody joined by a linker, which is followed by an Fc polypeptide chain of an antibody, optionally the Fc region of a human IgG antibody, such as an IgG1, IgG2, IgG3, or IgG4 antibody.

[00214] In embodiments, the antibody comprises a single-chain tandem fragment variable (scTaFv) antibody. A single-chain tandem fragment variable (scTaFv) antibody is a type of bispecific antibody that consists of two variable fragment (VH and VL) domains linked in a tandem arrangement. In some embodiments, one of the variable fragment domains comprises 3E10 VH and VL CDRs. For more information on scTaFv antibodies see, for example, Schramm C, et al., MAbs 5(3):442-49 (2013), the disclosure of which is incorporated herein by reference in its entirety.

[00215] In embodiments, the antibody comprises a VHH, also referred to as a nanobody. As used herein, the term “VHH” refers to a variable domain of heavy chain of heavy-chain antibody. A VHH is a molecule that can recognize an antigen through a single domain and is the smallest unit among antibody molecules that have been found to date. In embodiments, a VHH can include one or more variable domains of heavy chain derived from a heavy-chain antibody, and the number of the variable domains of heavy chain included in the VHH is not limited.

[00216] In embodiments, the antibody comprises a diabody. As used herein, “diabody” refers to a divalent antibody comprising two polypeptide chains, wherein each polypeptide chain is too short for a pair to form between two domains on the same chain such that each domain is paired with a complementary domain on another polypeptide chain (see, e.g., Holliger et al., 1993, Proc.

Natl. Acad. Sci. USA 90: 6444-48 and Poljak et al., 1994, Structure 2: 1121-23). If the two polypeptide chains of the diabody are identical, there will be two identical antigen-binding sites in the diabody resulting from their pairing. In embodiments, one of the antigen binding domains of the diabody comprises 3E10 VH and VL CDRs. For more information on diabodies see, for example, Hoogenboom HR, et al., Trends Biotechnol., 21(12):553-57 (2003), the disclosure of which is incorporated herein by reference in its entirety. Polypeptide chains of different sequences can be used to prepare diabodies with two different antigen-binding sites. Similarly, as used herein, “triabodies” and “tetraabodies” refer to antibodies that contain three and four polypeptide chains, respectively, and form three and four antigen-binding sites (which can be the same or different), respectively.

[00217] The term “minibody” is used to refer to an scFv-CH3 fusion protein that self-assembles into a bivalent dimer of 80 kDa (ScFv-CH3)₂.

[00218] In embodiments, the antibody comprises a tandem diabody (TandAb). A tandem diabody has two antigen-binding domains (VH and VL) linked in a tandem arrangement by a flexible peptide linker. In some embodiments, one of the antigen binding domains comprises 3E10 VH and VL CDRs. For more information on diabodies see, for example, Sidelmann JG, et al., Mol Immunol., 45(9):2597-607 (2008), the disclosure of which is incorporated herein by reference in its entirety.

[00219] In embodiments, the antibody thereof comprises a Fabsc. As used herein, a “Fabsc” format antibody molecule typically refers to a bispecific antibody molecule having a Fab fragment, which generally includes a hinge region, which is at the C-terminus of the Fab fragment linked to the N-terminus of a CH2 domain, of which the C-terminus is in turn linked to the N-terminus of a scFv fragment.

[00220] In embodiments, the antibody comprises a scFab. A scFab, also known as a single-chain fragment antigen binding (Fab), is a type of antibody fragment that combines the variable heavy chain (VH) and variable light chain (VL) domains into a single polypeptide chain, linked by a peptide linker. The domain structure of a Fabsc includes the variable domains of both the heavy chain and light chain (VH and VL), and a peptide linker that connects the two domains. In addition to the variable domains, a Fabsc also includes the constant domains of the light chain (CL) and the hinge region of the heavy chain. In some embodiments, one of the antigen binding

domains comprises 3E10 VH and VL CDRs. For more information on Fabscs see, for example, Kettner, C., et al., *Frontiers in Immunology*, 8(8):453 (2017), the disclosure of which is incorporated herein by reference in its entirety.

[00221] In some embodiments, the antibody comprises an IgG-scFv. An IgG-scFv is an antibody in which a scFv is fused to the light chain or heavy chain of an IgG. In some 5 embodiments, the scFv comprises 3E10 VH and VL CDRs. In some embodiments, the IgG comprises 3E10 VH and V LCDRs. In some embodiments, the antibody is an F(ab')₂.

5. 3E10 Sequences

[00222] The murine version of the 3E10 antibody is described in Zack, et al., *Immunology and 10 Cell Biology*, 72:513-520 (1994), which is incorporated by reference herein, in its entirety. Amino acid variants of the 3E10 antibody are also known in the art, as described in Zack et al., *J. Immunol.*, 157(5):2082-8 (1996). For example, amino acid position 31, in CDR1 of the heavy chain variable region of 3E10, influences nucleic acid binding. Substitution of the 'wild-type', e.g., relative to the original murine antibody, aspartic acid by asparagine (the 'D31N' mutation), 15 improves nucleic acid binding, relative to the 'wild type' murine antibody. See, Zack, et al., *Immunology and Cell Biology*, 72:513-520 (1994); Weisbart, et al., *J. Autoimmun.*, 11, 539-546 (1998); and Weisbart, *Int. J. Oncol.*, 25, 1867-1873 (2004), which are incorporated by reference herein, in their entireties.

[00223] Other 3E10 immunoglobulin light chain sequences are known in the art. See, Zack, et 20 al., *J. Immunol.*, 15;154(4):1987-94 (1995); GenBank: L16981.1 - Mouse Ig rearranged L-chain gene, partial cds; GenBank: AAA65681.1 - immunoglobulin light chain, partial [*Mus musculus*]).

[00224] As noted above herein, the 3E10 antibody or antigen binding fragment thereof of the present disclosure may refer to different antibody domains of a 3E10 antibody or antigen binding 25 fragment thereof. These domains include, but are not limited to, the Fc domain, the CH1 domain, the CH2 domain, the CH3 domain, the hinge domain, the heavy constant domain (CH1-hinge-Fc domain or CH1-hinge-CH2-CH3), the variable heavy domain, the variable light domain, the light constant domain, Fab domains and scFv domains.

[00225] In certain aspects, the 3E10 antibody or antigen binding fragment thereof of the 30 present disclosure comprises a heavy chain variable region from a particular germline heavy

chain immunoglobulin gene and/or a light chain variable region from a particular germline light chain immunoglobulin gene. For example, such proteins of interest can comprise or consist of murine, chimeric, humanized, or human antibodies or antigen binding fragments thereof comprising heavy or light chain variable regions that are "the product of" or "derived from" a particular germline sequence, e.g., that of the 3E10 antibody. A human antibody that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (i.e., greatest % identity) to the sequence of the human antibody (using the methods outlined herein). A human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence can contain amino acid differences as compared to the germline sequence, due to, for example, naturally occurring somatic mutations or intentional introduction of site-directed mutation. However, a humanized antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the antibody as being derived from human sequences when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a humanized antibody can be at least 95, 96, 97, 98 or 99%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a humanized antibody derived from a particular human germline sequence will display no more than 10-20 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the humanized antibody can display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

25 **[00226]** In some embodiments, the parent antibody has been affinity matured by methods well-known in the art. Structure-based methods may be employed for humanization and affinity maturation as described in US Publication US/2006/0008883 and which is incorporated herein by reference. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions which include, but are not limited to, methods described in Wu et al., 1999, *J. Mol. Biol.* 294:151-162; Baca et al., 1997, *J. Biol. Chem.* 272(16):10678-10684; Rosok et al., 1996, *J. Biol. Chem.* 271(37): 22611-22618; Rader et al., 1998, *Proc. Natl. Acad. Sci. USA*

95: 8910-8915; Krauss et al., 2003, *Protein Engineering* 16(10):753-759, all of which are incorporated herein by reference. Other humanization methods can involve the grafting of only parts of the CDRs, and include but are not limited to methods described in US Publication US/2001/0035606; Tan et al., 2002, *J. Immunol.* 169:1119-1125; De Pascalis et al., 2002, *J. Immunol.* 169:3076-3084, all of which are incorporated herein by reference.

[00227] In some embodiments, the 3E10 antibody or antigen binding fragment thereof described herein include CDR sequences corresponding to the parent 3E10 antibody. Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include a light chain variable region (VL) complementarity determining region (CDR) 1 comprising the amino acid sequence of 3E10-VL-CDR1 (SEQ ID NO:9), a VL CDR2 comprising the amino acid sequence of 3E10-VL-CDR2 (SEQ ID NO:10), a VL CDR3 comprising the amino acid sequence of 3E10-VL-CDR3 (SEQ ID NO:11), a heavy chain variable region (VH) CDR1 comprising the amino acid sequence of 3E10-VH-CDR1 (SEQ ID NO:3), a VH CDR2 comprising the amino acid sequence of 3E10-VH-CDR2 (SEQ ID NO:4), and a VH CDR3 comprising the amino acid sequence of 3E10-VH-CDR3 (SEQ ID NO:5).

[00228] In some embodiments, the protein of interest is a 3E10 antibody or antigen binding fragment thereof. In some embodiments, the 3E10 antibody or antigen binding fragment thereof described herein includes the (i) CDR sequences of a light chain variable region (VL) complementarity determining region (CDR) 1 comprising the amino acid sequence of 3E10-VL-CDR1m (SEQ ID NO:61), a VL CDR2 comprising the amino acid sequence of 3E10-VL-CDR2m (SEQ ID NO:62), a VL CDR3 comprising the amino acid sequence of 3E10-VL-CDR3m (SEQ ID NO:63) and heavy chain variable region (VH) CDR1 comprising the amino acid sequence of 3E10-VH-CDR1m (SEQ ID NO:58), a VH CDR2 comprising the amino acid sequence of 3E10-VH-CDR2m (SEQ ID NO:59), and a VH CDR3 comprising the amino acid sequence of 3E10-VH-CDR3m (SEQ ID NO:60); and (ii) that bind nucleic acids.

[00229] In some embodiments, the 3E10 antibody or antigen binding fragment thereof comprises an immunoglobulin heavy chain CDR1 with a D31N amino acid substitution. It is known in the art that mutation of aspartic acid at residue 31 of CDR1 to asparagine increases the cationic charge of this residue and enhanced nucleic acid binding (3E10-D31N). Additional exemplary CDR1 variants include mutation of aspartic acid at residue 31 to arginine (3E10-

D31R), of which molecular modeling indicates expansion cationic charge, and lysine at residue 31 (3E10-D31K), of which molecular modeling indicates a change in charge orientation. Thus, in some embodiments, the 3E10 antibody or antigen binding fragment thereof comprise a 3E10 antigen binding protein which include a D31R or D31K substitution. Additional exemplary
5 CDR variants include mutation of arginine at residue 96 to asparagine (3E10-R96N), and/or serine at residue 30 to aspartic acid (3E10-S30D), alone or in combination with D31N, D31R, or D31K. All of the sequences disclosed herein having the residue corresponding to 3E10-D31 or 3E10-N31, are expressly disclosed with a D31R, D31K, N31R, or N31K substitution. In some embodiments, the 3E10 antibody or nucleic acid binding fragment thereof comprise a heavy
10 chain variable region (VH) CDR1 comprising the amino acid sequence of NYGMH (SEQ ID NO:15).

[00230] In some embodiments, the 3E10 antibody or antigen binding fragment thereof comprises (a) a light chain variable region (VL) complementarity determining region (CDR) 1 comprising the amino acid sequence of RASKSVSTSSYSYMH (SEQ ID NO:22), (b) a VL
15 CDR2 comprising the amino acid sequence of YASYLES (SEQ ID NO:23), and (c) a VL CDR3 comprising the amino acid sequence of QHSREFPWT (SEQ ID NO:24), and (d) a heavy chain variable region (VH) CDR1 comprising the amino acid sequence of NYGMH (SEQ ID NO:15), (e) a VH CDR2 comprising the amino acid sequence of YISSGSSTIYYADTVKG (SEQ ID NO:17), and (f) a VH CDR3 comprising the amino acid sequence of RGLLLDY (SEQ ID
20 NO:18).

[00231] In some embodiments, the 3E10 antibody or antigen binding fragment thereof comprises a light chain variable region (VL) comprising an amino acid sequence that is identical to SEQ ID NO:21. In some embodiments, the 3E10 antibody or antigen binding fragment thereof comprise a heavy chain variable region (VH) comprising an amino acid sequence that is
25 identical to SEQ ID NO:14. In some embodiments, the 3E10 antibody or nucleic acid binding fragment thereof comprises a full-length light chain (LC) comprising an amino acid sequence that is identical to SEQ ID NO:20. In some embodiments, the 3E10 antibody or nucleic acid binding fragment thereof comprise a full-length heavy chain (HC) comprising an amino acid sequence that is identical to SEQ ID NO:13.

[00232] In some embodiments, the 3E10 antibody or antigen binding fragment thereof described herein include CDR sequences from a variant 3E10 antibody that includes a D31N amino acid substitution in the VH CDR1. Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include a light chain variable region (VL) complementarity determining region (CDR) 1 comprising the amino acid sequence of 3E10-VL-CDR1_D31N (SEQ ID NO:22), a VL CDR2 comprising the amino acid sequence of 3E10-VL-CDR2_D31N (SEQ ID NO:23), a VL CDR3 comprising the amino acid sequence of 3E10-VL-CDR3_D31N (SEQ ID NO:24), a heavy chain variable region (VH) CDR1 comprising the amino acid sequence of 3E10-VH-CDR1_D31N (SEQ ID NO:15), a VH CDR2 comprising the amino acid sequence of 3E10-VH-CDR2_D31N (SEQ ID NO:17), and a VH CDR3 comprising the amino acid sequence of 3E10-VH-CDR3_D31N (SEQ ID NO:18).

[00233] In some embodiments, the 3E10 antibody or antigen binding fragment thereof described herein refers to CDR sequences corresponding to the parent 3E10 antibody, optionally including a D31N amino acid substitution in the VH CDR1. Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include a light chain variable region (VL) complementarity determining region (CDR) 1 comprising the amino acid sequence of 3E10-VL-CDR1 (SEQ ID NO:9), a VL CDR2 comprising the amino acid sequence of 3E10-VL-CDR2 (SEQ ID NO:10), a VL CDR3 comprising the amino acid sequence of 3E10-VL-CDR3 (SEQ ID NO:11), a heavy chain variable region (VH) CDR1 comprising the amino acid sequence of 3E10-VH-CDR1a (SEQ ID NO:16), a VH CDR2 comprising the amino acid sequence of 3E10-VH-CDR2 (SEQ ID NO:17), and a VH CDR3 comprising the amino acid sequence of 3E10-VH-CDR3 (SEQ ID NO:18).

[00234] In some embodiments, the 3E10 antibody or antigen binding fragment thereof described herein include CDR sequences corresponding to the parent 3E10 antibody, with a known amino acid substitution in one or more CDR. Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof described herein include one or more amino acid substitution, relative to the CDR sequences of the parent 3E10 or 3E10-D31N variant, selected from a G to S substitution at position 5 of VH CDR2, a T to S substitution at position 14 of VH CDR2, an S to T substitution at position 5 of VL CDR1, an M to L substitution at position 14 of VL CDR1, an H to A substitution at position 15 of VL CDR1, and an E to Q substitution at position 6 of VL CDR2, as shown in Figure 18B.

[00235] Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include one or more CDR sequences shown in Figure 18B. For example, in some embodiments, the 3E10 antibody or antigen binding fragment thereof includes a VH CDR2 comprising the amino acid sequence of 3E10-VH-CDR2.1 (SEQ ID NO:26) or 3E10-VH-CDR2.2 (SEQ ID NO:27). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1-3, and VH CDRs 1 and 3 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1-3, and VH CDRs 1 and 3 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1-3, and VH CDRs 1 and 3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody or relative to the 3E10- D31N variant.

[00236] Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include VL CDR1 comprising the amino acid sequence of 3E10-VL-CDR1.1 (SEQ ID NO:28) or 3E10-VL-CDR1.2 (SEQ ID NO:29). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 2 and 3, and VH CDRs 1-3 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 2 and 3, and VH CDRs 1-3 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 2 and 3, and VH CDRs 1-3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody or relative to the 3E10- D31N variant.

[00237] Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include VL CDR2 comprising the amino acid sequence of 3E10-VL-CDR2.1 (SEQ ID NO:30). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1 and 3, and VH CDRs 1-3 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1 and 3, and VH CDRs 1-3 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1 and 3, and VH CDRs 1-3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody or relative to the 3E10- D31N variant.

[00238] While some of the amino acid substitutions described above are fairly conservative substitutions—e.g., an S to T substitution at position 5 of VL CDR1—other substitutions are to amino acids that have vastly different properties—e.g., an M to L substitution at position 14 of VL CDR1, an H to A substitution at position 15 of VL CDR1, and an E to Q substitution at position 6 of VL CDR2. This suggests, without being bound by theory, that at least these positions within the 3E10 CDR framework are tolerant to other amino acid substitutions.

[00239] Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof includes a conservative amino acid variation, e.g., as shown in Figure 18C. In some embodiments the 3E10 antibody or antigen binding fragment thereof comprises a VH CDR2 comprising the amino acid sequence of 3E10-VH-CDR2.3 (SEQ ID NO:31). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1-3, and VH CDRs 1 and 3 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1-3, and VH CDRs 1 and 3 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1-3, and VH CDRs 1 and 3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody or relative to the 3E10- D31N variant, e.g., as described herein.

[00240] Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include VL CDR1 comprising the amino acid sequence of 3E10-VL-CDR1.3 (SEQ ID NO:32). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 2 and 3, and VH CDRs 1-3 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 2 and 3, and VH CDRs 1-3 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 2 and 3, and VH CDRs 1-3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody or relative to the 3E10- D31N variant, e.g., as described herein.

[00241] Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include VL CDR2 comprising the amino acid sequence of 3E10-VL-CDR2.2 (SEQ ID NO:33). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1 and 3, and VH CDRs 1-3 according to the parent 3E10 antibody. In some

embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1 and 3, and VH CDRs 1-3 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1 and 3, and VH CDRs 1-3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody or relative to the 3E10- D31N variant, e.g., as described herein.

[00242] Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include VH CDR1 comprising the amino acid sequence of 3E10-VH-CDR1.c1 (SEQ ID NO:34), 3E10-VH-CDR1.c2 (SEQ ID NO:35), 3E10-VH-CDR1.c3 (SEQ ID NO:36), 3E10-VH-CDR1.c4 (SEQ ID NO:37), or 3E10-VH-CDR1.c5 (SEQ ID NO:38). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1-3, and VH CDRs 2 and 3 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1-3, and VH CDRs 2 and 3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody, e.g., as described herein.

[00243] Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include VH CDR2 comprising the amino acid sequence of 3E10-VH-CDR2.c1 (SEQ ID NO:39), 3E10-VH-CDR2.c2 (SEQ ID NO:40), or 3E10-VH-CDR2.c3 (SEQ ID NO:41). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1-3, and VH CDRs 1 and 3 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1-3, and VH CDRs 1 and 3 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1-3, and VH CDRs 1 and 3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody, e.g., as described herein.

[00244] Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include VH CDR3 comprising the amino acid sequence of 3E10-VH-CDR3.c1 (SEQ ID NO:42), 3E10-VH-CDR3.c2 (SEQ ID NO:43), or 3E10-VH-CDR3.c3 (SEQ ID NO:44). In some embodiments, the 3E10 antibody or antigen binding fragment thereof include VL CDRs 1-3, and VH CDRs 1 and 2 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1-3, and VH

CDRs 1 and 2 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further include VL CDRs 1-3, and VH CDRs 1 and 2 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody, e.g., as described herein.

5 **[00245]** Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include VL CDR1 comprising the amino acid sequence of 3E10-VL-CDR1.c1 (SEQ ID NO:45), 3E10-VL-CDR1.c2 (SEQ ID NO:46), 3E10-VL-CDR1.c3 (SEQ ID NO:47), 3E10-VL-CDR1.c4 (SEQ ID NO:48), 3E10-VL-CDR1.c5 (SEQ ID NO:49), or 3E10-VL-CDR1.c6 (SEQ ID NO:50). In some embodiments, the 3E10 antibody or antigen binding fragment thereof
10 further includes VL CDRs 2 and 3, and VH CDRs 1-3 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 2 and 3, and VH CDRs 1-3 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 2 and 3, and VH CDRs 1-3 having one or more amino acid substitutions relative to the CDRs of the parent
15 3E10 antibody, e.g., as described herein.

[00246] Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof includes VL CDR2 comprising the amino acid sequence of 3E10-VL-CDR2.c1 (SEQ ID NO:51). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 3, and VH CDRs 1-3 according to the parent 3E10 antibody. In some
20 embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 3, and VH CDRs 1-3 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 3, and VH CDRs 1-3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody, e.g., as described herein.

25 **[00247]** Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof includes VL CDR3 comprising the amino acid sequence of 3E10-VL-CDR3.c1 (SEQ ID NO:52), 3E10-VL-CDR3.c2 (SEQ ID NO:53), 3E10-VL-CDR3.c3 (SEQ ID NO:54), 3E10-VL-CDR3.c4 (SEQ ID NO:55), 3E10-VL-CDR3.c5 (SEQ ID NO:56), or 3E10-VL-CDR3.c6 (SEQ ID NO:57). In some embodiments, the 3E10 antibody or antigen binding fragment thereof
30 further includes VL CDRs 1 and 2, and VH CDRs 1-3 according to the parent 3E10 antibody. In

some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 2, and VH CDRs 1-3 according to the 3E10- D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 2, and VH CDRs 1-3 having one or more amino acid substitutions relative to the CDRs of the parent
5 3E10 antibody, e.g., as described herein.

[00248] It is also contemplated that the 3E10 antibody or antigen binding fragment thereof, as described herein, includes any combination of the 3E10 CDR amino acid substitutions described above.

[00249] Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment
10 thereof includes VH CDR1 comprising the amino acid sequence of 3E10-VH-CDR1m (SEQ ID NO:58). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1-3, and VH CDRs 2 and 3 according to the parent 3E10 antibody, as described in Figure 19. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1-3, and VH CDRs 2 and 3 having one or more amino acid
15 substitutions relative to the CDRs of the parent 3E10 antibody, e.g., as described herein and in Figure 19.

[00250] Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof includes VH CDR2 comprising the amino acid sequence of 3E10-VH-CDR2m (SEQ ID NO:59). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further
20 includes VL CDRs 1-3, and VH CDRs 1 and 3 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1-3, and VH CDRs 1 and 3 according to the 3E10-D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1-3, and VH CDRs 1 and 3 having one or more amino acid substitutions relative to the CDRs of the parent
25 3E10 antibody, e.g., as described herein.

[00251] Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof includes VH CDR3 comprising the amino acid sequence of 3E10-VH-CDR3m (SEQ ID NO:60). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further
30 includes VL CDRs 1-3, and VH CDRs 1 and 2 according to the parent 3E10 antibody. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs

1-3, and VH CDRs 1 and 2 according to the 3E10-D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1-3, and VH CDRs 1 and 2 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody, e.g., as described herein.

5 **[00252]** Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof includes VL CDR1 comprising the amino acid sequence of 3E10-VL-CDR1m (SEQ ID NO:61). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 2 and 3, and VH CDRs 1-3 according to the parent 3E10 antibody. In some
10 embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 2 and 3, and VH CDRs 1-3 according to the 3E10-D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 2 and 3, and VH CDRs 1-3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody, e.g., as described herein.

15 **[00253]** Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof includes VL CDR2 comprising the amino acid sequence of 3E10-VL-CDR2m (SEQ ID NO:62). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 3, and VH CDRs 1-3 according to the parent 3E10 antibody. In some
20 embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 3, and VH CDRs 1-3 according to the 3E10-D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 3, and VH CDRs 1-3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody, e.g., as described herein.

25 **[00254]** Similarly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof includes VL CDR3 comprising the amino acid sequence of 3E10-VL-CDR3m (SEQ ID NO:63). In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 2, and VH CDRs 1-3 according to the parent 3E10 antibody. In some
embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 2, and VH CDRs 1-3 according to the 3E10-D31N variant. In some embodiments, the 3E10 antibody or antigen binding fragment thereof further includes VL CDRs 1 and 2, and VH

CDRs 1-3 having one or more amino acid substitutions relative to the CDRs of the parent 3E10 antibody, e.g., as described herein.

[00255] In some embodiments, the 3E10 antibody or antigen binding fragment thereof described herein includes a VL CDR 1 comprising the amino acid sequence of 3E10-VL-
5 CDR1m (SEQ ID NO:61), a VL CDR2 comprising the amino acid sequence of 3E10-VL-
CDR2m (SEQ ID NO:62), a VL CDR3 comprising the amino acid sequence of 3E10-VL-
CDR3m (SEQ ID NO:63), a heavy chain variable region (VH) CDR1 comprising the amino acid
sequence of 3E10-VH-CDR1m (SEQ ID NO:58), a VH CDR2 comprising the amino acid
sequence of 3E10-VH-CDR2m (SEQ ID NO:59), and a VH CDR3 comprising the amino acid
10 sequence of 3E10-VH-CDR3m (SEQ ID NO:60).

[00256] In some embodiments, the 3E10 antibody or antigen binding fragment thereof described herein refers to CDR sequences having no more than one amino acid substitution relative to the parent 3E10 antibody optionally including a D31N amino acid substitution in the VH CDR1. Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment
15 thereof includes a VL CDR 1 comprising an amino acid sequence having no more than one
amino acid substitution relative to 3E10-VL-CDR1 (SEQ ID NO:9), a VL CDR2 comprising an
amino acid sequence having no more than one amino acid substitution relative to 3E10-VL-
CDR2 (SEQ ID NO:10), a VL CDR3 comprising an amino acid sequence having no more than
one amino acid substitution relative to 3E10-VL-CDR3 (SEQ ID NO:11), a heavy chain variable
20 region (VH) CDR1 comprising an amino acid sequence having no more than one amino acid
substitution relative to 3E10-VH-CDR1a (SEQ ID NO:16), a VH CDR2 comprising an amino
acid sequence having no more than one amino acid substitution relative to 3E10-VH-CDR2
(SEQ ID NO:4), and a VH CDR3 comprising an amino acid sequence having no more than one
amino acid substitution relative to 3E10-VH-CDR3 (SEQ ID NO:5).

[00257] In some embodiments, the 3E10 antibody or antigen binding fragment thereof described herein refers to CDR sequences having no more than two amino acid substitution relative to the parent 3E10 antibody optionally including a D31N amino acid substitution in the VH CDR1. Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment
25 thereof includes a VL CDR 1 comprising an amino acid sequence having no more than two
amino acid substitutions relative to 3E10-VL-CDR1 (SEQ ID NO:9), a VL CDR2 comprising an
30 amino acid substitutions relative to 3E10-VL-CDR1 (SEQ ID NO:9), a VL CDR2 comprising an

amino acid sequence having no more than two amino acid substitutions relative to 3E10-VL-CDR2 (SEQ ID NO:10), a VL CDR3 comprising an amino acid sequence having no more than two amino acid substitutions relative to 3E10-VL-CDR3 (SEQ ID NO:11), a heavy chain variable region (VH) CDR1 comprising an amino acid sequence having no more than two amino acid substitutions relative to 3E10-VH-CDR1a (SEQ ID NO:16), a VH CDR2 comprising an amino acid sequence having no more than two amino acid substitutions relative to 3E10-VH-CDR2 (SEQ ID NO:4), and a VH CDR3 comprising an amino acid sequence having no more than two amino acid substitutions relative to 3E10-VH-CDR3 (SEQ ID NO:5).

[00258] Other variants of a 3E10 antibody or variant thereof, or antigen-binding fragment thereof are also known in the art, as disclosed for example, in Zack, et al., *J. Immunol.*, 157(5):2082-8 (1996). For example, amino acid position 31 of the heavy chain variable region of 3E10 has been determined to be influential in the ability of the antibody and fragments thereof to penetrate nuclei and bind to DNA. A D31N mutation in CDR1 penetrates nuclei and binds DNA with much greater efficiency than the original antibody (Zack, et al., *Immunology and Cell Biology*, 72:513-520 (1994), Weisbart, et al., *J. Autoimmun.*, 11, 539-546 (1998); Weisbart, *Int. J. Oncol.*, 25, 1867-1873 (2004)). In some embodiments, the 3E10 antibody or variant thereof, or antigen-binding fragment thereof described herein has the D31N substitution.

[00259] In some embodiments, the 3E10 antibody or antigen binding fragment thereof is humanized. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Antibody humanization techniques generally involve the use of recombinant DNA technology to manipulate the DNA sequence encoding one or more polypeptide chains of an antibody molecule.

[00260] In some embodiments, the 3E10 antibody or antigen binding fragment thereof is a humanized antibody, or fragment thereof, known in the art. Examples of humanized See, for example US 10,221,250, US/2016/033324, US/2020/0216567, and US/2020/0216568 (each of which is specifically incorporated by reference herein, in its entirety).

[00261] In some embodiments, the disclosure provides any combination of the humanized VL and VH sequences disclosed herein, as well as VL and VH sequences having sequence identity

thereto, e.g., having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to a VH or VL sequence described herein.

[00262] In some embodiments, the humanized 3E10 antibody, or antigen-binding fragment thereof, comprises a light chain variable domain (3E10-VL) comprising an amino acid sequence that is at least 97% identical to an amino acid sequence selected from the group consisting of
5 3E10-VL-h1 (SEQ ID NO:86), 3E10-VL-h2 (SEQ ID NO:87), 3E10-VL-h3 (SEQ ID NO:88), 3E10-VL-h4 (SEQ ID NO:89), 3E10-VL-h5 (SEQ ID NO:90), and 3E10-VL-h6 (SEQ ID NO:91) and a heavy chain variable domain (3E10-VH) comprising an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of 3E10-
10 VH-h1 (SEQ ID NO:65), 3E10-VH-h2 (SEQ ID NO:66), 3E10-VH-h3 (SEQ ID NO:67), 3E10-VH-h4 (SEQ ID NO:68), 3E10-VH-h5 (SEQ ID NO:69), 3E10-VH-h6 (SEQ ID NO:70), and 3E10-VH-h7 (SEQ ID NO:71).

[00263] In some embodiments, the sequence of the 3E10-VL is at least 97% identical to 3E10-VL-h1 (SEQ ID NO:86). In some embodiments, the sequence of the 3E10-VL is at least
15 98% identical to 3E10-VL-h1 (SEQ ID NO:86). In some embodiments, the sequence of the 3E10-VL is at least 99% identical to 3E10-VL-h1 (SEQ ID NO:86). In some embodiments, the sequence of the 3E10-VL is 3E10-VL-h1 (SEQ ID NO:86).

[00264] In some embodiments, the sequence of the 3E10-VL is at least 97% identical to 3E10-VL-h2 (SEQ ID NO:87). In some embodiments, the sequence of the 3E10-VL is at least
20 98% identical to 3E10-VL-h2 (SEQ ID NO:87). In some embodiments, the sequence of the 3E10-VL is at least 99% identical to 3E10-VL-h2 (SEQ ID NO:87). In some embodiments, the sequence of the 3E10-VL is 3E10-VL-h2 (SEQ ID NO:87).

[00265] In some embodiments, the sequence of the 3E10-VL is at least 97% identical to 3E10-VL-h3 (SEQ ID NO:88). In some embodiments, the sequence of the 3E10-VL is at least
25 98% identical to 3E10-VL-h3 (SEQ ID NO:88). In some embodiments, the sequence of the 3E10-VL is at least 99% identical to 3E10-VL-h3 (SEQ ID NO:88). In some embodiments, the sequence of the 3E10-VL is 3E10-VL-h3 (SEQ ID NO:88).

[00266] In some embodiments, the sequence of the 3E10-VL is at least 97% identical to 3E10-VL-h4 (SEQ ID NO:89). In some embodiments, the sequence of the 3E10-VL is at least
30 98% identical to 3E10-VL-h4 (SEQ ID NO:89). In some embodiments, the sequence of the

3E10-VL is at least 99% identical to 3E10-VL-h4 (SEQ ID NO:89). In some embodiments, the sequence of the 3E10-VL is 3E10-VL-h4 (SEQ ID NO:89).

[00267] In some embodiments, the sequence of the 3E10-VL is at least 97% identical to 3E10-VL-h5 (SEQ ID NO:90). In some embodiments, the sequence of the 3E10-VL is at least
5 98% identical to 3E10-VL-h5 (SEQ ID NO:90). In some embodiments, the sequence of the 3E10-VL is at least 99% identical to 3E10-VL-h5 (SEQ ID NO:90). In some embodiments, the sequence of the 3E10-VL is 3E10-VL-h5 (SEQ ID NO:90).

[00268] In some embodiments, the sequence of the 3E10-VL is at least 97% identical to 3E10-VL-h6 (SEQ ID NO:91). In some embodiments, the sequence of the 3E10-VL is at least
10 98% identical to 3E10-VL-h6 (SEQ ID NO:91). In some embodiments, the sequence of the 3E10-VL is at least 99% identical to 3E10-VL-h6 (SEQ ID NO:91). In some embodiments, the sequence of the 3E10-VL is 3E10-VL-h6 (SEQ ID NO:91).

[00269] In some embodiments, the sequence of the 3E10-VH is at least 95% identical to 3E10-VH-h1 (SEQ ID NO:65). In some embodiments, the sequence of the 3E10-VH is at least
15 96% identical to 3E10-VH-h1 (SEQ ID NO:65). In some embodiments, the sequence of the 3E10-VH is at least 97% identical to 3E10-VH-h1 (SEQ ID NO:65). In some embodiments, the sequence of the 3E10-VH is at least 98% identical to 3E10-VH-h1 (SEQ ID NO:65). In some embodiments, the sequence of the 3E10-VH is at least 99% identical to 3E10-VH-h1 (SEQ ID NO:65). In some embodiments, the sequence of the 3E10-VH is 3E10-VH-h1 (SEQ ID NO:65).

20 [00270] In some embodiments, the sequence of the 3E10-VH is at least 95% identical to 3E10-VH-h2 (SEQ ID NO:66). In some embodiments, the sequence of the 3E10-VH is at least 96% identical to 3E10-VH-h2 (SEQ ID NO:66). In some embodiments, the sequence of the 3E10-VH is at least 97% identical to 3E10-VH-h2 (SEQ ID NO:66). In some embodiments, the sequence of the 3E10-VH is at least 98% identical to 3E10-VH-h2 (SEQ ID NO:66). In some
25 embodiments, the sequence of the 3E10-VH is at least 99% identical to 3E10-VH-h2 (SEQ ID NO:66). In some embodiments, the sequence of the 3E10-VH is 3E10-VH-h2 (SEQ ID NO:66).

[00271] In some embodiments, the sequence of the 3E10-VH is at least 95% identical to 3E10-VH-h3 (SEQ ID NO:67). In some embodiments, the sequence of the 3E10-VH is at least
30 96% identical to 3E10-VH-h3 (SEQ ID NO:67). In some embodiments, the sequence of the 3E10-VH is at least 97% identical to 3E10-VH-h3 (SEQ ID NO:67). In some embodiments, the

sequence of the 3E10-VH is at least 98% identical to 3E10-VH-h3 (SEQ ID NO:67). In some embodiments, the sequence of the 3E10-VH is at least 99% identical to 3E10-VH-h3 (SEQ ID NO:67). In some embodiments, the sequence of the 3E10-VH is 3E10-VH-h3 (SEQ ID NO:67).

[00272] In some embodiments, the sequence of the 3E10-VH is at least 95% identical to 3E10-VH-h4 (SEQ ID NO:68). In some embodiments, the sequence of the 3E10-VH is at least 96% identical to 3E10-VH-h4 (SEQ ID NO:68). In some embodiments, the sequence of the 3E10-VH is at least 97% identical to 3E10-VH-h4 (SEQ ID NO:68). In some embodiments, the sequence of the 3E10-VH is at least 98% identical to 3E10-VH-h4 (SEQ ID NO:68). In some embodiments, the sequence of the 3E10-VH is at least 99% identical to 3E10-VH-h4 (SEQ ID NO:68). In some embodiments, the sequence of the 3E10-VH is 3E10-VH-h4 (SEQ ID NO:68).

[00273] In some embodiments, the sequence of the 3E10-VH is at least 95% identical to 3E10-VH-h5 (SEQ ID NO:69). In some embodiments, the sequence of the 3E10-VH is at least 96% identical to 3E10-VH-h5 (SEQ ID NO:69). In some embodiments, the sequence of the 3E10-VH is at least 97% identical to 3E10-VH-h5 (SEQ ID NO:69). In some embodiments, the sequence of the 3E10-VH is at least 98% identical to 3E10-VH-h5 (SEQ ID NO:69). In some embodiments, the sequence of the 3E10-VH is at least 99% identical to 3E10-VH-h5 (SEQ ID NO:69). In some embodiments, the sequence of the 3E10-VH is 3E10-VH-h5 (SEQ ID NO:69).

[00274] In some embodiments, the sequence of the 3E10-VH is at least 95% identical to 3E10-VH-h6 (SEQ ID NO:70). In some embodiments, the sequence of the 3E10-VH is at least 96% identical to 3E10-VH-h6 (SEQ ID NO:70). In some embodiments, the sequence of the 3E10-VH is at least 97% identical to 3E10-VH-h6 (SEQ ID NO:70). In some embodiments, the sequence of the 3E10-VH is at least 98% identical to 3E10-VH-h6 (SEQ ID NO:70). In some embodiments, the sequence of the 3E10-VH is at least 99% identical to 3E10-VH-h6 (SEQ ID NO:70). In some embodiments, the sequence of the 3E10-VH is 3E10-VH-h6 (SEQ ID NO:70).

[00275] In some embodiments, the sequence of the 3E10-VH is at least 95% identical to 3E10-VH-h7 (SEQ ID NO:71). In some embodiments, the sequence of the 3E10-VH is at least 96% identical to 3E10-VH-h7 (SEQ ID NO:71). In some embodiments, the sequence of the 3E10-VH is at least 97% identical to 3E10-VH-h7 (SEQ ID NO:71). In some embodiments, the sequence of the 3E10-VH is at least 98% identical to 3E10-VH-h7 (SEQ ID NO:71). In some

embodiments, the sequence of the 3E10-VH is at least 99% identical to 3E10-VH-h7 (SEQ ID NO:71). In some embodiments, the sequence of the 3E10-VH is 3E10-VH-h7 (SEQ ID NO:71).

[00276] In some embodiments, the 3E10 antibody or antigen binding fragment thereof, described herein, includes a light chain (3E10-LC) comprising an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of 3E10-LC-h1m (SEQ ID NO:92), 3E10-LC-h2m (SEQ ID NO:93), 3E10-LC-h3m (SEQ ID NO:94), 3E10-LC-h4m (SEQ ID NO:95), 3E10-LC-h5m (SEQ ID NO:96), and 3E10-LC-h6m (SEQ ID NO:97) and a heavy chain (3E10-HC) comprising an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of 3E10-HC-h1m (SEQ ID NO: 72), 3E10-HC-h2m (SEQ ID NO: 73), 3E10-HC-h3m (SEQ ID NO: 74), 3E10-HC-h4m (SEQ ID NO:75), 3E10-HC-h5m (SEQ ID NO:76), 3E10-HC-h6m (SEQ ID NO:77), and 3E10-HC-h7m (SEQ ID NO:78).

[00277] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h1m (SEQ ID NO:92). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h1m (SEQ ID NO:92). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h1m (SEQ ID NO:92). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h1m (SEQ ID NO:92). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h1m (SEQ ID NO:92). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h1m (SEQ ID NO:92).

[00278] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h2m (SEQ ID NO:93). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h2m (SEQ ID NO:93). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h2m (SEQ ID NO:93). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h2m (SEQ ID NO:93). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h2m (SEQ ID NO:93). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h2m (SEQ ID NO:93).

[00279] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h3m (SEQ ID NO:94). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h3m (SEQ ID NO:94). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h3m (SEQ ID NO:94). In some embodiments, the

sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h3m (SEQ ID NO:94). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h3m (SEQ ID NO:94). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h3m (SEQ ID NO:94).

[00280] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h4m (SEQ ID NO:95). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h4m (SEQ ID NO:95). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h4m (SEQ ID NO:95). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h4m (SEQ ID NO:95). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h4m (SEQ ID NO:95). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h4m (SEQ ID NO:95).

[00281] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h5m (SEQ ID NO:96). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h5m (SEQ ID NO:96). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h5m (SEQ ID NO:96). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h5m (SEQ ID NO:96). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h5m (SEQ ID NO:96). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h5m (SEQ ID NO:96).

[00282] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h6m (SEQ ID NO:97). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h6m (SEQ ID NO:97). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h6m (SEQ ID NO:97). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h6m (SEQ ID NO:97). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h6m (SEQ ID NO:97). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h6m (SEQ ID NO:97).

[00283] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h1m (SEQ ID NO:72). In some embodiments, the sequence of the 3E10-HC is at least 96% identical to 3E10-HC-h1m (SEQ ID NO:72). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h1m (SEQ ID NO:72). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h1m (SEQ ID NO:72). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h1m (SEQ ID NO:72). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h1m (SEQ ID NO:72).

NO:72). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h1m (SEQ ID NO:72).

[00284] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h2m (SEQ ID NO:73). In some embodiments, the sequence of the 3E10-HC is at least
5 96% identical to 3E10-HC-h2m (SEQ ID NO:73). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h2m (SEQ ID NO:73). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h2m (SEQ ID NO:73). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h2m (SEQ ID NO:73). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h2m (SEQ
10 ID NO:73).

[00285] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h3m (SEQ ID NO:74). In some embodiments, the sequence of the 3E10-HC is at least 96% identical to 3E10-HC-h3m (SEQ ID NO:74). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h3m (SEQ ID NO:74). In some embodiments, the
15 sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h3m (SEQ ID NO:74). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h3m (SEQ ID NO:74). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h3m (SEQ ID NO:74).

[00286] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h4m (SEQ ID NO:75). In some embodiments, the sequence of the 3E10-HC is at least
20 96% identical to 3E10-HC-h4m (SEQ ID NO:75). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h4m (SEQ ID NO:75). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h4m (SEQ ID NO:75). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h4m (SEQ ID
25 NO:75). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h4m (SEQ ID NO:75).

[00287] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h5m (SEQ ID NO:76). In some embodiments, the sequence of the 3E10-HC is at least 96% identical to 3E10-HC-h5m (SEQ ID NO:76). In some embodiments, the sequence of the
30 3E10-HC is at least 97% identical to 3E10-HC-h5m (SEQ ID NO:76). In some embodiments, the

sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h5m (SEQ ID NO:76). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h5m (SEQ ID NO:76). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h5m (SEQ ID NO:76).

5 **[00288]** In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h6m (SEQ ID NO:77). In some embodiments, the sequence of the 3E10-HC is at least 96% identical to 3E10-HC-h6m (SEQ ID NO:77). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h6m (SEQ ID NO:77). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h6m (SEQ ID NO:77). In some
10 embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h6m (SEQ ID NO:77). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h6m (SEQ ID NO:77).

[00289] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h7m (SEQ ID NO:78). In some embodiments, the sequence of the 3E10-HC is at least
15 96% identical to 3E10-HC-h7m (SEQ ID NO:78). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h7m (SEQ ID NO:78). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h7m (SEQ ID NO:78). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h7m (SEQ ID NO:78). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h7m (SEQ ID
20 NO:78).

[00290] In some embodiments, the eukaryotic host cell provided herein comprises a 3E10 antibody or antigen binding fragment thereof comprising a light chain (3E10-LC) comprising an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of 3E10-LC-h1 (SEQ ID NO:98), 3E10-LC-h2 (SEQ ID NO:99), 3E10-LC-h3
25 (SEQ ID NO:100), 3E10-LC-h4 (SEQ ID NO:101), 3E10-LC-h5 (SEQ ID NO:102), and 3E10-LC-h6 (SEQ ID NO:103) and a heavy chain (3E10-HC) comprising an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of 3E10-HC-h1 (SEQ ID NO:79), 3E10-HC-h2 (SEQ ID NO:80), 3E10-HC-h3 (SEQ ID NO:81), 3E10-HC-h4 (SEQ ID NO:82), 3E10-HC-h5 (SEQ ID NO:83), 3E10-HC-h6 (SEQ ID NO:84), and
30 3E10-HC-h7 (SEQ ID NO:85).

[00291] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h1 (SEQ ID NO:98). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h1 (SEQ ID NO:98). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h1 (SEQ ID NO:98). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h1 (SEQ ID NO:98). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h1 (SEQ ID NO:98). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h1 (SEQ ID NO:98).

[00292] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h2 (SEQ ID NO:99). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h2 (SEQ ID NO:99). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h2 (SEQ ID NO:99). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h2 (SEQ ID NO:99). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h2 (SEQ ID NO:99). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h2 (SEQ ID NO:99).

[00293] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h3 (SEQ ID NO:100). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h3 (SEQ ID NO:100). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h3 (SEQ ID NO:100). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h3 (SEQ ID NO:100). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h3 (SEQ ID NO:100). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h3 (SEQ ID NO:100).

[00294] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h4 (SEQ ID NO:101). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h4 (SEQ ID NO:101). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h4 (SEQ ID NO:101). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h4 (SEQ ID NO:101). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h4 (SEQ ID NO:101). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h4 (SEQ ID NO:101).

[00295] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h5 (SEQ ID NO:102). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h5 (SEQ ID NO:102). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h5 (SEQ ID NO:102). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h5 (SEQ ID NO:102). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h5 (SEQ ID NO:102). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h5 (SEQ ID NO:102).

[00296] In some embodiments, the sequence of the 3E10-LC is at least 95% identical to 3E10-LC-h6 (SEQ ID NO:103). In some embodiments, the sequence of the 3E10-LC is at least 96% identical to 3E10-LC-h6 (SEQ ID NO:103). In some embodiments, the sequence of the 3E10-LC is at least 97% identical to 3E10-LC-h6 (SEQ ID NO:103). In some embodiments, the sequence of the 3E10-LC is at least 98% identical to 3E10-LC-h6 (SEQ ID NO:103). In some embodiments, the sequence of the 3E10-LC is at least 99% identical to 3E10-LC-h6 (SEQ ID NO:103). In some embodiments, the sequence of the 3E10-LC is 3E10-LC-h6 (SEQ ID NO:103).

[00297] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h1 (SEQ ID NO:79). In some embodiments, the sequence of the 3E10-HC is at least 96% identical to 3E10-HC-h1 (SEQ ID NO:79). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h1 (SEQ ID NO:79). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h1 (SEQ ID NO:79). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h1 (SEQ ID NO:79). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h1 (SEQ ID NO:79).

[00298] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h2 (SEQ ID NO:80). In some embodiments, the sequence of the 3E10-HC is at least 96% identical to 3E10-HC-h2 (SEQ ID NO:80). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h2 (SEQ ID NO:80). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h2 (SEQ ID NO:80). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h2 (SEQ ID NO:80). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h2 (SEQ ID NO:80).

[00299] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h3 (SEQ ID NO:81). In some embodiments, the sequence of the 3E10-HC is at least 96% identical to 3E10-HC-h3 (SEQ ID NO:81). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h3 (SEQ ID NO:81). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h3 (SEQ ID NO:81). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h3 (SEQ ID NO:81). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h3 (SEQ ID NO:81).

[00300] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h4 (SEQ ID NO:82). In some embodiments, the sequence of the 3E10-HC is at least 96% identical to 3E10-HC-h4 (SEQ ID NO:82). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h4 (SEQ ID NO:82). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h4 (SEQ ID NO:82). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h4 (SEQ ID NO:82). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h4 (SEQ ID NO:82).

[00301] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h5 (SEQ ID NO:83). In some embodiments, the sequence of the 3E10-HC is at least 96% identical to 3E10-HC-h5 (SEQ ID NO:83). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h5 (SEQ ID NO:83). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h5 (SEQ ID NO:83). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h5 (SEQ ID NO:83). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h5 (SEQ ID NO:83).

[00302] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h6 (SEQ ID NO:84). In some embodiments, the sequence of the 3E10-HC is at least 96% identical to 3E10-HC-h6 (SEQ ID NO:84). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h6 (SEQ ID NO:84). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h6 (SEQ ID NO:84). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h6 (SEQ ID NO:84). In some aspects, the sequence of the 3E10-HC is 3E10-HC-h6 (SEQ ID NO:84).

[00303] In some embodiments, the sequence of the 3E10-HC is at least 95% identical to 3E10-HC-h7 (SEQ ID NO:85). In some embodiments, the sequence of the 3E10-HC is at least

96% identical to 3E10-HC-h7 (SEQ ID NO:85). In some embodiments, the sequence of the 3E10-HC is at least 97% identical to 3E10-HC-h7 (SEQ ID NO:85). In some embodiments, the sequence of the 3E10-HC is at least 98% identical to 3E10-HC-h7 (SEQ ID NO:85). In some embodiments, the sequence of the 3E10-HC is at least 99% identical to 3E10-HC-h7 (SEQ ID NO:85). In some embodiments, the sequence of the 3E10-HC is 3E10-HC-h7 (SEQ ID NO:85).

[00304] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof described herein includes a light chain variable domain (3E10-VL) comprising an amino acid sequence that is at least 90% identical to an amino acid sequence selected from the group consisting of 3E10-VL-h1 (SEQ ID NO:86), 3E10-VL-h2 (SEQ ID NO:87), 3E10-VL-h3 (SEQ ID NO:88), 3E10-VL-h4 (SEQ ID NO:89), 3E10-VL-h5 (SEQ ID NO:90), and 3E10-VL-h6 (SEQ ID NO:91), where the light chain variable domain (3E10-VL) further comprises one or more amino acid residues selected from proline (Pro) at position 15, threonine (Thr) at position 22, tyrosine (Tyr) at position 49, Thr at position 74, asparagine (Asn) at position 76, alanine (Ala) at position 80, Asn at position 81, Thr at position 83, Asn at position 85, and valine (Val) at position 104, of the 3E10-VL according to Kabat numbering, and a set of 3E10-VL CDRs collectively having no more than 6 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VL-CDR1 (SEQ ID NO:9), 3E10-VL-CDR2 (SEQ ID NO:10), 3E10-VL-CDR3 (SEQ ID NO:11), and where the antibody includes a set of 3E10-VL CDRs collectively having no more than 6 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VL-CDR1 (SEQ ID NO:9), 3E10-VL-CDR2 (SEQ ID NO:10), 3E10-VL-CDR3 (SEQ ID NO:11).

[00305] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes a set of 3E10-VL CDRs comprising no more than 5 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VL-CDR1 (SEQ ID NO:9), 3E10-VL-CDR2 (SEQ ID NO:10), 3E10-VL-CDR3 (SEQ ID NO:11).

[00306] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes a set of 3E10-VL CDRs comprising no more than 4 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VL-CDR1 (SEQ ID NO:9), 3E10-VL-CDR2 (SEQ ID NO:10), 3E10-VL-CDR3 (SEQ ID NO:11).

[00307] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes a set of 3E10-VL CDRs comprising no more than 3 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VL-CDR1 (SEQ ID NO:9), 3E10-VL-CDR2 (SEQ ID NO:10), 3E10-VL-CDR3 (SEQ ID NO:11).

5 [00308] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes a set of 3E10-VL CDRs comprising no more than 2 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VL-CDR1 (SEQ ID NO:9), 3E10-VL-CDR2 (SEQ ID NO:10), 3E10-VL-CDR3 (SEQ ID NO:11).

[00309] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen
10 binding fragment thereof includes a set of 3E10-VL CDRs comprising no more than 1 amino acid substitution relative to the set of CDRs having the amino acid sequences of 3E10-VL-CDR1 (SEQ ID NO:9), 3E10-VL-CDR2 (SEQ ID NO:10), 3E10-VL-CDR3 (SEQ ID NO:11).

[00310] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes a set of 3E10-VL CDRs comprising a set of CDRs having the
15 amino acid sequences of 3E10-VL-CDR1 (SEQ ID NO:9), 3E10-VL-CDR2 (SEQ ID NO:10), 3E10-VL-CDR3 (SEQ ID NO:11).

[00311] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with a lysine (Lys) residue at position 49 of the 3E10-VL according to Kabat numbering.

20 [00312] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with a glutamic acid (Glu) residue at position 81 of the 3E10-VL according to Kabat numbering.

[00313] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with a proline (Pro) residue at position 15 of
25 the 3E10-VL according to Kabat numbering.

[00314] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with a valine (Val) residue at position 104, of the 3E10-VL according to Kabat numbering.

[00315] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof described herein includes a heavy chain variable domain (3E10-VH) comprising an amino acid sequence that is at least 90% identical to an amino acid sequence selected from the group consisting of 3E10-VH-h1 (SEQ ID NO:65), 3E10-VH-h2 (SEQ ID NO:66), 3E10-VH-h3 (SEQ ID NO:67), 3E10-VH-h4 (SEQ ID NO:68), 3E10-VH-h5 (SEQ ID NO:69), 3E10-VH-h6 (SEQ ID NO:70), and 3E10-VH-h7 (SEQ ID NO:71), where the heavy chain variable domain (3E10-VH) further comprises one or more amino acid residues selected from glutamine (Gln) at position 13, leucine (Leu) at position 18, arginine (Arg) at position 19, glycine (Gly) at position 42, serine (Ser) at position 49, Ser at position 77, tyrosine (Tyr) at position 79, Asn at position 82, Ala at position 84, Val at position 89, leucine (Leu) at position 108, Val at position 109, and Ser at position 113, of the 3E10-VH according to Kabat numbering, and where the antibody includes a set of 3E10-VH CDRs collectively having no more than 6 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VH-CDR1_D31N (SEQ ID NO:15), 3E10-VH-CDR2 (SEQ ID NO:4), and 3E10-VH-CDR3 (SEQ ID NO:5).

[00316] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes a set of 3E10-VH CDRs comprising no more than 5 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VH-CDR1_D31N (SEQ ID NO:15), 3E10-VH-CDR2 (SEQ ID NO:4), and 3E10-VH-CDR3 (SEQ ID NO:5).

[00317] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes a set of 3E10-VH CDRs comprising no more than 4 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VH-CDR1_D31N (SEQ ID NO:15), 3E10-VH-CDR2 (SEQ ID NO:4), and 3E10-VH-CDR3 (SEQ ID NO:5).

[00318] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes a set of 3E10-VH CDRs comprising no more than 3 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VH-CDR1_D31N (SEQ ID NO:15), 3E10-VH-CDR2 (SEQ ID NO:4), and 3E10-VH-CDR3 (SEQ ID NO:5).

[00319] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes set of 3E10-VH CDRs comprising no more than 2 amino acid substitutions relative to the a set of CDRs having the amino acid sequences of 3E10-VH-CDR1_D31N (SEQ ID NO:15), 3E10-VH-CDR2 (SEQ ID NO:4), and 3E10-VH-CDR3 (SEQ ID NO:5).

[00320] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes a set of 3E10-VH CDRs comprising no more than 1 amino acid substitution relative to the set of CDRs having the amino acid sequences of 3E10-VH-CDR1_D31N (SEQ ID NO:15), 3E10-VH-CDR2 (SEQ ID NO:4), and 3E10-VH-CDR3 (SEQ ID NO:5).

[00321] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof includes a set of 3E10-VH CDRs comprising no more than 5, 4, 3, 2, or 1 amino acid substitutions relative to the set of CDRs having the amino acid sequences of 3E10-VH-CDR1_D31N (SEQ ID NO:15), 3E10-VH-CDR2 (SEQ ID NO:4), and 3E10-VH-CDR3 (SEQ ID NO:5).

[00322] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with an arginine (Arg) residue at position 18 of the 3E10-VH according to Kabat numbering.

[00323] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with a (Lys) residue at position 19 of the 3E10-VH according to Kabat numbering.

[00324] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with an alanine (Ala) residue at position 49 of the 3E10-VH according to Kabat numbering.

[00325] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with a glutamine (Gln) residue at position 13, of the 3E10-VH according to Kabat numbering.

[00326] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with a leucine (Leu) residue at position 108, of the 3E10-VH according to the Kabat numbering.

5 [00327] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with a valine (Val) residue at position 109, of the 3E10-VH according to Kabat numbering.

[00328] In one aspect, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with a serine (Ser) residue at position 113, of the 3E10-VH according to Kabat numbering.

10 [00329] In some embodiments, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with a fragment crystallizable (Fc) region.

[00330] In some embodiments, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof with an Fc region selected from
15 a human IgG1 Fc, a human IgG2a Fc, a human IgG2b Fc, a human IgG3 Fc, and a human IgG4 Fc.

[00331] In some embodiments, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof comprising a heavy chain constant domain (CH).

20 [00332] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof comprises an Fc region selected from a human γ 1 CH1, a human γ 2 CH1, a human γ 3 CH1, and a human γ 4 CH1.

[00333] In some embodiments, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof comprising a light chain
25 constant domain (CL).

[00334] In some embodiments, the present disclosure provides a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof comprising an Fc region selected from the group consisting of a human λ CL and a human κ CL.

[00335] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof comprising a combination of a light chain variable domain (VL) and a heavy chain variable domain (VH) selected from 3E10-VL-h1 (SEQ ID NO:86) and 3E10-VH-h1 (SEQ ID NO:65), 3E10-VL-h1 (SEQ ID NO:86) and 3E10-VH-h2 (SEQ ID NO:66), 3E10-VL-h1 (SEQ ID NO:86) and 3E10-VH-h3 (SEQ ID NO:67), 3E10-VL-h1 (SEQ ID NO:86) and 3E10-VH-h4 (SEQ ID NO:68), 3E10-VL-h2 (SEQ ID NO:87) and 3E10-VH-h1 (SEQ ID NO:65), 3E10-VL-h2 (SEQ ID NO:87) and 3E10-VH-h2 (SEQ ID NO:66), 3E10-VL-h3 (SEQ ID NO:88) and 3E10-VH-h1 (SEQ ID NO:65), 3E10-VL-h5 (SEQ ID NO:90) and 3E10-VH-h5 (SEQ ID NO:69), 3E10-VL-h5 (SEQ ID NO:90) and 3E10-VH-h6 (SEQ ID NO:70), 3E10-VL-h6 (SEQ ID NO:91) and 3E10-VH-h5 (SEQ ID NO:69), and 3E10-VL-h6 (SEQ ID NO:91) and 3E10-VH-h6 (SEQ ID NO:70).

[00336] In some embodiments, a eukaryotic host cell comprising a 3E10 antibody or antigen binding fragment thereof comprises a combination of a light chain variable domain (VL) of 3E10-VL-h6 (SEQ ID NO:103) and a heavy chain variable domain (VH) of 3E10-VH-h6 (SEQ ID NO:70).

[00337] The 3E10 antibody or antigen binding fragment thereof, or humanized form thereof may comprise an scFv-Fc polypeptide, a CrossMab polypeptide, a dual variable domain immunoglobulin (DVD-Ig), a tandem double scFv, an (scFv)₂, a single-chain tandem fragment variable (scTaFv) polypeptide, a single-chain fragment variable (scFv) polypeptide, a diabody, a tandem diabody (TandAb), a Fabsc polypeptide, a modular IgG-scFv, or an F(ab')₂.

[00338] A deposit according to the terms of the Budapest Treaty of a hybridoma cell line producing monoclonal antibody 3E10 was received on September 6, 2000, and accepted by, American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209, USA, and given Patent Deposit Number PTA-2439. Thus, the antibody can have the same or different epitope specificity as monoclonal antibody 3E10 produced by ATCC No. PTA 2439 hybridoma.

[00339] In some embodiments, 3E10 antibody or antigen binding fragment thereof is a humanized antibody. As discussed herein, methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human, e.g., chimeric. These non-human amino acid

residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Antibody humanization techniques generally involve the use of recombinant DNA technology to manipulate the DNA sequence encoding one or more polypeptide chains of an antibody molecule.

5 **[00340]** Examples of humanized 3E10 sequences are discussed in WO 2015/106290, WO 2016/033324, WO 2019/018426, and WO/2019/018428, the disclosures of which are incorporated herein by reference in their entireties for all purposes, and specifically for their disclosure of humanized 3E10 sequences and methods for generating the same. Other examples of humanized 3E10 heavy chain variable regions (SEQ ID NOS: 104-113) and humanized 3E10
10 light chain variable regions (SEQ ID NOS: 114-122) are shown in FIGs. 27 and 28, respectively.

[00341] Molecular modeling of 3E10 (Pymol) revealed a putative Nucleic Acid Binding pocket (NAB1) as shown in FIGs 30A and 30B. Furthermore, FIG. 30B illustrates the molecular modeling of 3E10-scFv (Pymol) with NAB1 amino acid residues illustrated with punctate dots. In some embodiments, the disclosed 3E10 antibody or antigen binding fragment thereof include
15 some or all of the underlined NAB1 sequences. In some embodiments, 3E10 antibody or antigen binding fragment thereof include a variant sequence that has an altered ability of bind nucleic acids. In some embodiments, the mutations (e.g., substitutions, insertions, and/or deletions) in the NAB1 improve binding of the antibody to nucleic acids such as RNA. In some embodiments, the mutations are conservative substitutions. In some embodiments, the mutations
20 increase the cationic charge of the NAB1 pocket.

[00342] As exemplified herein, a mutation of aspartic acid at residue 31 of CDR1 to asparagine increased the cationic charge of this residue and enhanced nucleic acid binding and delivery in vivo (3E10-D31N). Mutation of aspartic acid at residue 31 of CDR1 to asparagine increased the cationic charge of this residue and enhanced nucleic acid binding and delivery in
25 vivo (3E10-D31N). Mutation of aspartic acid at residue 31 of CDR1 to arginine (3E10-D31R), further expanded the cationic charge while mutation to lysine (3E10-D31K) changed charge orientation (FIG. 30A).

[00343] Accordingly, in some embodiments, the 3E10 antibody or antigen binding fragment thereof include a substitution of aspartic acid at residue 31 of CDR1 to arginine (3E10-D31R),
30 which modeling indicates expands cationic charge, or lysine (3E10-D31K) which modeling

indicates changes charge orientation. Thus, in some embodiments, the 3E10 binding protein includes a D31R or D31K substitution. Accordingly, it is contemplated that all sequences disclosed herein having the residue corresponding to 3E10 D31 or N31 may include a D31R or D31K substitution therein.

5 [00344] The host cells may preferably comprise a plurality of integrated sequences. In some
embodiments, from at least 2 to at least 500 copies each of the first exogenous nucleic acid
sequence encoding an endonuclease and at least a second exogenous nucleic acid sequence
encoding the 3E10 antibody or antigen binding fragment thereof are stably integrated into the
genome of the host cell. In some embodiments, from no more than 1000 to no more than 2
10 copies each of the first exogenous nucleic acid sequence encoding an endonuclease and at least a
second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding
fragment thereof are stably integrated into the genome of the host cell. In some embodiments,
from ranges between 2 to 1000 copies each of the first exogenous nucleic acid sequence
encoding an endonuclease and at least a second exogenous nucleic acid sequence encoding the
15 3E10 antibody or antigen binding fragment thereof are stably integrated into the genome of the
host cell. In some embodiments, from 2 to 500 copies each of the first exogenous nucleic acid
sequence encoding an endonuclease and at least a second exogenous nucleic acid sequence
encoding the 3E10 antibody or antigen binding fragment thereof are stably integrated into the
genome of the host cell. In some embodiments, from 5 to 500 copies each of the first exogenous
20 nucleic acid sequence encoding an endonuclease and at least a second exogenous nucleic acid
sequence encoding the 3E10 antibody or antigen binding fragment thereof are stably integrated
into the genome of the host cell. In some embodiments, from 10 to 500 copies each of the first
exogenous nucleic acid sequence encoding an endonuclease and at least a second exogenous
nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof are
25 stably integrated into the genome of the host cell. In some embodiments, from 10 to 200 copies
each of the first exogenous nucleic acid sequence encoding an endonuclease and at least a second
exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment
thereof are stably integrated into the genome of the host cell.

[00345] The ratio of the integrated sequences in the host cell may be varied. In some
30 embodiments, the ratio of the first exogenous nucleic acid sequence (e.g., encoding an
endonuclease such as a DNase I or DNase II) to the second exogenous nucleic acid sequence

encoding the 3E10 antibody or antigen binding fragment thereof is from 1:1 to 1:10,000. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:1 to 1:1000. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:1 to 1:100. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease, i.e., DNase 1 or DNase II, to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:2 to 1:10,000. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence is from 1:2 to 1:1000. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:2 to 1:100. In some embodiments, the ratio of (i) the number of exogenous nucleic acid sequences encoding the endonuclease to (ii) the number of exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from at least 1:2 to at least 1:1000. In some embodiments, the ratio of (i) the number of exogenous nucleic acid sequences encoding the endonuclease to (ii) the number of exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is no more than 1:250 to no more than 1:2. In some embodiments, the ratio of (i) the number of exogenous nucleic acid sequences encoding the endonuclease to (ii) the number of exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof ranges between 1:2 to 1:250. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease, i.e., DNase 1 or DNase II, to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:5 to 1:10,000. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:5 to 1:1000. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:5 to 1:100. In some embodiments, the ratio of the first exogenous nucleic acid sequence

encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:1 to 1:50. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:2 to 1:50. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:5 to 1:50. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:1 to 1:20. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:2 to 1:20. In some embodiments, the ratio of the first exogenous nucleic acid sequence encoding an endonuclease to the second exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:5 to 1:20.

[00346] As mentioned above, in some embodiments, a second exogenous sequence encoding an antibody heavy chain and a third exogenous sequence encoding an antibody light chain are co-expressed with the endonuclease, i.e., the first exogenous nucleic acid sequence. In these embodiments, the ratios of the three integrated sequences in the host cell may also be varied. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:1:1 to 1:100:100. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:2:2 to 1:100:100. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:5:5 to 1:100:100. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:10:10 to 1:100:100. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:1:1 to 1:50:50. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:2:2 to 1:50:50. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:5:5 to 1:50:500. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence :

third exogenous sequence is from 1:10:10 to 1:50:50. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:1:1 to 1:20:20. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:2:2 to 1:20:20. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:5:5 to 1:20:20. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:10:10 to 1:20:20. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:2:2 to 1:15:15. In some embodiments, the ratio of the first exogenous sequence : second exogenous sequence : third exogenous sequence is from 1:5:5 to 1:15:15.

[00347] Similar ratios may be employed when 4, 5, 6, 7, 8, 9 or 10 products of interest are expressed.

Host Cells and Cell Culture Methods

[00348] In some embodiments, the present disclosure provides methods for production of a product(s) of interest. In these embodiments, the host cells as described above are cultured in a culture medium under conditions such that the first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, and/or two or more products of interest and/or an assembled complex thereof is produced. The product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, is then isolated or purified from the culture medium. In some embodiments, the product (or products) of interest is operably associated with a signal sequence so the product (or products) are secreted into the culture medium and are then isolated therefrom.

[00349] In some embodiments, the host cells (and cultures of host cells) are engineered to comprise a plurality of integrated docking sites. For example, in some embodiments, the genomes of the host cells of the present disclosure preferably comprise from 1 to 1000 integrated docking sites, each docking site comprising at least one dock site insertion element. In other embodiments, the genome of the host cells comprises from 1 to 500 integrated docking sites, each docking site comprising at least one dock site insertion element. In other embodiments, the genome of the host cells comprises at least 2 to at least 500 integrated docking sites, each docking site comprising at least one dock site insertion element. In other embodiments, the

genome of the host cells comprises no more than 1000 to no more than 2 integrated docking sites, each docking site comprising at least one dock site insertion element. In other embodiments, the genome of the host cells comprises a range between 2 to 1000 integrated docking sites, each docking site comprising at least one dock site insertion element. In other
5 embodiments, the genome of the host cells comprises from 5 to 500 integrated docking sites, each docking site comprising at least one dock site insertion element. In other embodiments, the genome of the host cells comprises from 5 to 250 integrated docking sites, each docking site comprising at least one dock site insertion element. In other embodiments, the genome of the host cell comprises from 5 to 250 integrated docking sites, each docking site comprising at least
10 one dock site insertion element. In other embodiments, the genome of the host cell comprises from 5 to 100 integrated docking sites, each docking site comprising at least one dock site insertion element. In other embodiments, the genome of the host cell comprises from 5 to 50 integrated docking sites, each docking site comprising at least one dock site insertion element. In some embodiments, the integrated docking sites are independent integrated docking sites that
15 are separated from one another and positioned at independent sites within the genome. For example, the integrated docking sites may preferably be spread across a number of chromosome sin the genome. In other embodiments, the integrated docking sites may be present as concatemers which comprise multiple copies of the same DNA sequence linked in series.

[00350] The integrated docking sites preferably comprise one or more insertion elements
20 (which may be termed a “dock site insertion element.” The dock site insertion elements are preferably nucleic acid sequences that facilitate insertion of a nucleic acid sequence encoding a protein of interest at the dock site. Nucleic acid constructs that can be inserted into the dock sites in the host cells of the present disclosure are described in detail below.

[00351] The present disclosure is not limited to the use of any particular insertion elements.
25 Indeed the use of a variety of insertion elements is contemplated. In some embodiments, the insertion element is a recombinase dock site insertion element. Recombinase dock site insertion elements are nucleic acid sequences that are recognized and utilized by recombinase enzymes.

[00352] For example, in some embodiments, the recombinase dock site insertion element
30 comprises an attachment site (att). In some embodiments, the attachment site is attP. These attachment sites are utilized by the PhiC31 integrase, which is a recombinase enzyme and which

can be provided in the host cell via a vector in embodiments. These dock sites serve as acceptors for integration of nucleic acid constructs comprising an attB attachment site. In other embodiments, attR and attL attachment sites are utilized

[00353] In other embodiments, the recombinase dock site insertion element comprises an Flp
5 Recombination Target (FRT) site. These sites are utilized by the enzyme flippase, which is a recombinase enzyme and which can be provided in the host cell via a vector in embodiments. These dock sites serve as acceptors for integration of nucleic acid constructs comprising at the FRT site.

[00354] In other embodiments, the recombinase dock site insertion element comprises a LoxP
10 site. These sites are utilized by the Cre recombinase which can be provided in the host cell via a vector in embodiments. These dock sites serve as acceptors for integration of nucleic acid constructs comprising the LoxP site.

[00355] In other embodiments, the insertion element is an HDR (homology directed repair)
dock site insertion element. HDR dock site insertion elements are nucleic acid sequences that
15 provide an area of homology (a “homology arm”) that base pair with corresponding homology arms on the nucleic acid construct that is inserted at the site. These systems are preferably used with endonucleases that introduce double stranded breaks at a targeted site or sites, preferably flanked by the homology arms. In some embodiments, the HDR dock site insertion element is an AAVS1 safe harbor locus. In these embodiments, the dock site is used utilized by the Rep 78
20 endonuclease (nickase) which may be introduced into the host cell via a vector. The Rep 78 protein nickase promotes site-specific integration of nucleic acid sequences bearing homology arms corresponding to the AAVS1 safe harbor locus.

[00356] In other embodiments, the HDR dock site insertion element comprises one or more
homology arms that are exogenous sequences of from 30 to 1000 base pairs in length. These
25 dock sites are preferably used in conjunction with CRISPR gene editing systems. In some embodiments, the dock site further comprises one or more sequences that are homologous to guide RNA sequences. In these embodiments, the nucleic acid construct that is inserted at the dock site preferably comprises homology arms that are homologous to and base pair with the homology arms in the dock site. For utilization with CRISPR gene editing systems, a CRISPR
30 gene editing system-compatible nuclease is introduced into the host cell. The CRISPR gene

editing system-compatible nuclease may be a wild-type endonuclease that creates a double-stranded break at a position determined by the guide RNA (and within the docking site) or a mutated nuclease (i.e., a nickase) that creates a single stranded break at a staggered positions within the dock site defined by two guide RNAs. Suitable nucleases are described in detail below in the discussion of nucleic acid expression constructs.

[00357] In some embodiments, the docking site may preferably comprise a suitable promoter so that a promoter trap scheme is utilized when suitable nucleic acid constructs are introduced at the docking site. Suitable promoters include, but are not limited to, SIN-LTR, SV40, EF1 α , *E. coli* lac, *E. coli* trp, phage lambda PL, phage lambda PR, T3, T7, cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, alpha-lactalbumin, and mouse metallothionein-I promoter sequences. In some embodiments the promoter sequence is oriented at the dock site so that the promoter will drive expression from an inserted nucleic acid construct. In some embodiments, the promoter is oriented 5' to the docking site. In some embodiments, the promoter is a SIN LTR. In these embodiments, the SIN-LTR and EPR are positioned 5' to the dock site and a SIN LTR is positioned 3' to the dock site.

[00358] The docking sites may be introduced into any suitable host cell line. Suitable host cell lines include, but are not limited to, Chinese hamster ovary cells (CHO-K1, ATCC CCL-61); bovine mammary epithelial cells (ATCC CRL 10274; bovine mammary epithelial cells); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture; see, e.g., Graham et al., J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; rat fibroblasts (208F cells); MDBK cells (bovine kidney cells); CAP (CEVEC's Amniocyte Production) cells; and a human hepatoma line (Hep G2).

[00359] In some embodiments, the host cells are modified so that they are deficient, or are naturally deficient, in an enzyme activity that is required for growth or survival of the cells in the presence of a selection agent and which is provided by the selectable marker. For example, Chinese Hamster Ovary (CHO) cells have been modified to be deficient for GS. In some
5 embodiments where vector includes a GS selectable marker, the host cell line is deficient in GS. In some embodiments, the GS deficient host cell line is the CHOZN® GS^{-/-} cell line available from Merck KGaA. In other embodiments, where the selectable marker is, for example, DHFR, the cell line may preferably be deficient for DHFR activity (i.e., DHFR⁻). Suitable DHFR⁻ cell lines include but are not limited to CHO-DG44 and derivatives thereof.

10 [00360] The docking site sequences may be introduced into the host cells by any suitable genome modification system. In some embodiments, the docking sites are incorporated into the host cells via the use of integrating vectors. The use of integrating vectors to introduce high copy numbers of a sequence of interest, such as a docking site, is described in detail in US Pat. Nos. 6,852,510 and 7,332,333 as well as US Publ. Nos. 2003/0092882, 2003/0224415,
15 2004/0235173 and 2005/0100952, all which are incorporated herein by reference in their entirety.

[00361] According to the present disclosure, host cells such as those described above are transduced or transfected with integrating vectors comprising a dock site under conditions such that multiple copies of the dock site are integrated into the genome of the host cell. Examples of
20 integrating vectors include, but are not limited to, retroviral vectors, lentiviral vectors, adeno-associated viral vectors, and transposon vectors.

[00362] In some embodiments, nucleic acid constructs for expression of a product of interest, e.g., a 3E10 antibody and antigen binding fragment thereof, are introduced into the host cell lines containing multiple docking sites. As discussed above, in embodiments, the nucleic acid
25 constructs preferably comprise nucleic acid sequences (which may be termed “expression construct insertion elements”) that are compatible with the dock site insertion elements as described above.

[00363] Accordingly, in some embodiments, the present disclosure provides nucleic acid expression constructs for use in expressing a protein of interest in a host cell, and in particular to
30 expression of two or more proteins of interest where the nucleic acid expression constructs

encoding the two or more proteins of interest are integrated into the genome of the host cell at desired ratios as described in detail above.

[00364] In some embodiments, where the dock site does not comprise a promoter, the nucleic acid expression constructs, for example, comprise the following elements in operable

5 association, most preferably in 5' to 3' order:

first promoter sequence - selectable marker sequence - second promoter sequence -
nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or
antigen binding fragment thereof, - poly A signal sequence.

10 first promoter sequence - selectable marker sequence - second promoter sequence -
nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or
antigen binding fragment thereof, - poly A signal sequence.

first promoter sequence - selectable marker sequence - second promoter sequence -
nucleic acid sequence encoding a third product of interest, e.g., a 3E10 antibody or
antigen binding fragment thereof, - poly A signal sequence.

15 first promoter sequence - selectable marker sequence - second promoter sequence -
nucleic acid sequence encoding a fourth product of interest, e.g., a 3E10 antibody or
antigen binding fragment thereof, - poly A signal sequence.

[00365] In some embodiments, where the dock site comprises an exogenous promoter, the nucleic acid expression constructs, for example, comprise the following elements in operable

20 association, most preferably in 5' to 3' order:

selectable marker sequence - second promoter sequence - nucleic acid sequence
encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment
thereof, - poly A signal sequence.

25 selectable marker sequence - second promoter sequence - nucleic acid sequence
encoding a second product of interest, e.g., a 3E10 antibody or antigen binding
fragment thereof, - poly A signal sequence.

selectable marker sequence - second promoter sequence - nucleic acid sequence
encoding a third product of interest, e.g., a 3E10 antibody or antigen binding
fragment thereof, - poly A signal sequence.

selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a fourth product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - poly A signal sequence.

[00366] In some embodiments, the constructs of the disclosure do not comprise a poly A
5 signal sequence between the selectable marker sequence and second promoter sequence. The present disclosure is not limited to any particular mechanism of action. Indeed, an understanding of the mechanism of action is not necessary to practice the present disclosure. Nevertheless, constructs which lack a poly A signal sequence after the selectable marker have been found to provide for better selection and production of the protein of interest in host cell cultures. In still
10 other embodiments, the selectable marker is adjacent to the second promoter. In still other embodiments, the second promoter is adjacent to the nucleic acid sequence encoding the first protein of interest. In this context, the term “adjacent” means that there is no intervening functional element or intron between the listed components.

[00367] In some embodiments, the nucleic acid expression constructs further comprises at
15 least one expression construct insertion element at a position or positions selected from the group consisting of 5' to the first promoter, 3' to the poly A signal sequence, between the first promoter and the poly A signal sequence, between the selectable marker and the second promoter sequence, and both 5' to the first promoter and 3' to the poly A signal sequence. Suitable constructs are shown in the following non-limiting examples:

20 expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second (i.e., internal) promoter sequence - nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - poly A signal sequence

25 first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - poly A signal sequence – expression
30 construct insertion element

5 expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - poly A signal sequence – expression construct insertion element.

10 first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence)- selectable marker sequence - expression construct insertion element - second promoter sequence - nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - poly A signal sequence.

15 expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second (i.e., internal) promoter sequence - nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - poly A signal sequence

20 first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - poly A signal sequence – expression construct insertion element

25 expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - poly A signal sequence – expression construct insertion element.

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first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence)- selectable marker sequence - expression construct insertion element - second promoter sequence - nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - poly A signal sequence.

[00368] In some embodiments, the constructs may include nucleic acid sequences encoding multiple products of interest, for example 2, 3, 4 or 5 (or more) products of interest. Suitable constructs for expressing two products of interest are shown in the following nonlimiting examples. These expression constructs may be used at different ratios in conjunction with expression constructs encoding an additional third product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, or as exemplified below, third and fourth products of interest.

expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second (i.e., internal) promoter sequence - nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - WPRE (optional) - poly A signal sequence - third promoter sequence or IRES - nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - WPRE (optional) - poly A signal sequence

first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - WPRE (optional) - poly A signal sequence - third promoter sequence - intron (optional) - nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - WPRE (optional) - poly A signal sequence - expression construct insertion element

expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable

marker sequence - second promoter sequence - nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - WPRE (optional) – poly A signal sequence – third promoter sequence – intron (optional) - nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE (optional) - poly A signal sequence – expression construct insertion element.

first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - expression construct insertion element - second promoter sequence - nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - WPRE – poly A signal sequence – third promoter sequence or IRES - nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE - poly A signal sequence

expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE (optional) – poly A signal sequence – third promoter sequence - nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE (optional) - poly A signal sequence – expression construct insertion element.

expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a first product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - WPRE (optional) – poly A signal sequence – third promoter sequence – intron- nucleic acid sequence encoding a second product of interest, e.g., a 3E10 antibody or antigen binding

fragment thereof, – WPRE (optional) - poly A signal sequence – expression construct insertion element.

5 expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second (i.e., internal) promoter sequence - nucleic acid sequence encoding a third product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE (optional) – poly A signal sequence – third promoter sequence or IRES
10 - nucleic acid sequence encoding a fourth product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE (optional) - poly A signal sequence

first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a third product of interest, e.g., a 3E10
15 antibody or antigen binding fragment thereof, - WPRE (optional) – poly A signal sequence – third promoter sequence – intron (optional) - nucleic acid sequence encoding a fourth product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE (optional) - poly A signal sequence – expression construct insertion element

20 expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a third product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - WPRE (optional) – poly A signal sequence – third promoter sequence – intron (optional) -
25 nucleic acid sequence encoding a fourth product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE (optional) - poly A signal sequence – expression construct insertion element.

30 first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - expression construct insertion element - second promoter sequence - nucleic acid sequence encoding a third

product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - WPRE – poly A signal sequence – third promoter sequence or IRES - nucleic acid sequence encoding a fourth product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE - poly A signal sequence

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expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a third product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE (optional) – poly A signal sequence – third promoter sequence - nucleic acid sequence encoding a fourth product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE (optional) - poly A signal sequence – expression construct insertion element.

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expression construct insertion element - first promoter sequence (optional depending on whether the dock site already comprises an exogenous promoter sequence) - selectable marker sequence - second promoter sequence - nucleic acid sequence encoding a third product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, - WPRE (optional) – poly A signal sequence – third promoter sequence – intron- nucleic acid sequence encoding a fourth product of interest, e.g., a 3E10 antibody or antigen binding fragment thereof, – WPRE (optional) - poly A signal sequence – expression construct insertion element.

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[00369] In some embodiments, the mixtures of different constructs are utilized. In some embodiments, the mixture of different constructs may comprise constructs as described above and constructs starting with the internal or second promoter (i.e., starting after and not including the selectable marker). It is contemplated that by using mixtures of constructs, some which do not include selectable markers, that higher insertion rates may be achieved.

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[00370] In some embodiments, the expression construct insertion elements are elements that find use in conjunction with or are recognized by transposons, integrases, recombinases or CRISPR systems. Suitable insertion elements include, but are not limited to, inverted terminal

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repeats, integrase attachment sites (att), and homologous recombination arms which in the context of the constructs described herein can be described as homologous recombination insertion elements.

[00371] The nucleic acid constructs may be utilized with many different vectors and vectors
5 systems. These vectors and vectors system may preferably be used to introduce the nucleic acid expression constructs into the host cells described above. Suitable vectors and vectors systems include, but are not limited to, viral gene insertion technologies such as retroviral, lentiviral and AAV systems as well as non-viral gene insertion technologies such as transposase, recombinase, integrase or CRISPR gene insertion. Specific examples of technologies/enzymes that can be
10 used with nucleic acid constructs of the present disclosure include piggyback transposase systems, sleeping beauty transposase systems, Mos1 transposase systems, Tol2 transposase systems, Leapin transposase systems, Lambda recombinase systems, FLP/FRT systems, Cre/Lox systems, MMLV integrase systems, Rep 78 integrase systems and CRISPR systems which can include nucleases or nickases as well as guide sequences. In some embodiments, the system is a
15 nucleic acid integration system with the proviso that the system is not a retroviral or lentiviral systems utilizing a retroviral or lentiviral LTR.

[00372] As discussed above, in some embodiments, the expression construct insertion element comprises an attachment site (att). In some particular embodiments, the attachment site is attB. These attachment sites are utilized by the PhiC31 integrase, which is a recombinase enzyme and
20 which can be provided in the host cell via a vector in embodiments. These sites facilitate integration of the nucleic acid constructs into a dock site comprising attP attachment site. In other embodiments, attR and attL attachment sites may be utilized.

[00373] In other embodiments, the expression construct insertion element comprises an FLP Recombination Target (FRT) site. These sites are utilized by the enzyme flippase, which is a
25 recombinase enzyme and which can be provided in the host cell via a vector in embodiments. These sites serve facilitate integration of nucleic acid constructs into dock sites comprising corresponding FRT sites.

[00374] In other embodiments, the expression construct insertion element comprises a LoxP site. These sites are utilized by the Cre recombinase which can be provided in the host cell via a

vector in embodiments. These sites facilitate integration of nucleic acid constructs into dock sites comprising corresponding LoxP sites.

- [00375] In other embodiments, the expression construct insertion element is an HDR (homology directed repair) expression construct insertion element. HDR expression construct insertion elements are nucleic acid sequences that provide an area of homology (a “homology arm”) that base pair with corresponding homology arms in the dock site. These systems are preferably used with endonucleases that introduce double stranded breaks at a targeted site or sites, preferably flanked by the homology arms. In some embodiments, the HDR expression construct insertion element comprises AAVS1 safe harbor locus homology arms. In these embodiments, the expression construct is specifically integrated in a dock site comprising the AAVS1 safe harbor locus. The integration is facilitated by the Rep 78 endonuclease (nickase) which may be introduced into the host cell via a vector. The Rep 78 protein nickase promotes site-specific integration of nucleic acid sequences bearing homology arms corresponding to the AAVS1 safe harbor locus.
- [00376] In other embodiments, the HDR expression construct insertion element comprises one or more homology arms that are exogenous sequences of from 30 to 1000 base pairs in length. These expression constructs are preferably used in conjunction with CRISPR gene editing systems. In these embodiments, the nucleic acid construct is inserted at dock sites that comprise homology arms that are homologous to and base pair with the homology arms in the nucleic acid construct. For utilization with CRISPR gene editing systems, a CRISPR gene editing system-compatible nuclease is introduced into the host cell. The CRISPR gene editing system-compatible nuclease may be a wild-type endonuclease that creates a double-stranded break at a position determined by the guide RNA (and within the docking site) or a mutated nuclease (i.e., a nickase) that creates a single stranded break at a staggered positions within the dock site defined by two guide RNAs. Suitable nucleases are described in detail below in the discussion of nucleic acid expression constructs.

- [00377] As discussed above, integration at the dock sites generally requires expression of an exogenous enzyme in the host cell. Suitable enzymes include, but are not limited to, recombinases (including integrases), endonucleases, and nickases. Accordingly, in some embodiments, host cells of the present disclosure comprise an exogenous nucleic acid sequence

(or expression construct) for expression of a recombinase (including integrases), an endonuclease, and a nickase. In some embodiments, constructs for expressing the exogenous enzymes may be stably integrated into the genome of the host cell. In other embodiments, vectors for expressing the exogenous enzymes are transiently introduced into the host cell, for example with an extrachromosomal vector such as a plasmid.

[00378] In some embodiments, both the vectors comprising exogenous enzyme and the vectors comprising the nucleic acid constructs for expression of the protein of interest are transiently introduced into the host cell, for example by transfection. In some embodiments, the ratio of the vectors encoding the exogenous enzyme to the gene of interest vectors is from 1:1000 to 1:10. In some more embodiments, the ratio is from 1:100 to 1:750. In some still more embodiments, the ratio is from 1:400 to 1:600. This is surprising as the literature for other integrase systems generally indicates that a higher level of vector encoding the exogenous enzyme to the gene of interest construct is required.

[00379] In some embodiments, the integrase is the phiC31 integrase (BioCat GmbH, Heidelberg, DE or System Biosciences, Palo Alto, CA)). The phiC31 integrase is a sequence-specific recombinase encoded within the genome of the bacteriophage phiC31. The phiC31 integrase mediates recombination between two 34 base pair sequences termed attachment sites (att), one found in the phage and the other in the host. This serine integrase has been shown to function efficiently in many different cell types including mammalian cells. In the presence of phiC31 integrase, an attB- containing donor plasmid can be unidirectional integrated into a target genome through recombination at sites with sequence similarity to the native attP site (termed pseudo-attP sites). phiC31 integrase can integrate a plasmid of any size, as a single copy, and requires no cofactors. The integrated transgenes are stably expressed and heritable.

[00380] Other suitable recombinase-based systems include CRISPR gene editing systems, CRE-Lox, FLP-FRT, and lambda recombinase systems.

[00381] Cre-Lox recombination is a site-specific recombinase technology, used to carry out deletions, insertions, translocations and inversions at specific sites in the DNA of cells. It allows the DNA modification to be targeted to a specific cell type or be triggered by a specific external stimulus. It is implemented both in eukaryotic and prokaryotic systems. The Cre-lox recombination system has been particularly useful to help neuroscientists to study the brain in

which complex cell types and neural circuits come together to generate cognition and behaviors. The system consists of a single enzyme, Cre recombinase, that recombines a pair of short target sequences called the Lox sequences. This system can be implemented without inserting any extra supporting proteins or sequences. The Cre enzyme and the original Lox site called the LoxP sequence are derived from bacteriophage P1. See, e.g., Targeted integration of DNA using mutant lox sites in embryonic stem cells. Araki, et al. Nucleic Acids Res, Feb 1997, Vol. 25, Issue 4, pp. 868-872; High-Resolution Labeling and Functional Manipulation of Specific Neuron Types in Mouse Brain by Cre-Activated Viral Gene Expression. Kuhlman, et al. PLoS One, Apr 2008, Vol. 3, e2005; When reverse genetics meets physiology: the use of site-specific recombinases in mice. Tronche, et al. FEBS Letters, Aug 2002, Vol. 529, Issue 1, pp. 116-121.

[00382] The FLP-FRT recombination system is another site-directed recombination technology very conceptually similar to Cre-lox, with flippase (Flp) and the short flippase recognition target (FRT) site being analogous to Cre and loxP, respectively. See, e.g., Candice et al., Cre/loxP, Flp/FRT Systems and Pluripotent Stem Cell Lines (2012) Topics in Current Genetics, vol 23. The FLP-FRT technology can be an effective alternative to Cre-lox, and has also been used in conjunction with it, allowing for two separate recombination events to be controlled in parallel.

[00383] The nucleic acid constructs of the present disclosure may be used in conjunction with CRISPR homologous recombination (HDR) systems. HDR is initiated by the presence of double strand breaks (DSBs) in DNA. The CRISPR/Cas9 system is preferably used to create targeted double stranded breaks via a guide RNA sequence so that the nucleic acid construct of the disclosure can be inserted. See, e.g., Zhang et al., Efficient precise knockin with a double cut HDR donor after CRISPR/Cas9-mediated double-stranded DNA cleavage (2017) Genome Biol. 18:35; Mali et al., Cas9 as a versatile tool for engineering biology. Nature Methods 10, 957-963 (2013); Mali et al., RNA-Guided Human Genome Engineering via Cas9. Science 339(6121), 823-826 (2013); Ran et al., Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell, 155(2), 479-480(2013). Suitable guide RNA sequences (gRNAs) may be designed as is known in the art. In some embodiments, CRISPR systems for HDR utilize either one or two guide sequences. In some embodiments, when one guide RNA sequence is utilized, a nuclease such as a Cas9 nuclease which makes a single double stranded break guided by the guide RNA sequence is used. In some embodiments, when two guide sequences are

utilized, a nickase, which can be a mutated Cas9 nuclease which only makes single stranded breaks in the target DNA sequence guided by each of the guide RNA sequences is used. The single stranded breaks are preferably positioned at staggered points on different strands (i.e., the sense and antisense strands) of the target DNA sequence. This arrangement generally improves HDR efficiency.

[00384] In general, “CRISPR system” refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated (“Cas”) genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a “direct repeat” and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a “spacer” in the context of an endogenous CRISPR system), or other sequences and transcripts from a CRISPR locus. In some embodiments, one or more elements of a CRISPR system is derived from a type I, type II, or type III CRISPR system. In some embodiments, one or more elements of a CRISPR system is derived from a particular organism comprising an endogenous CRISPR system, such as *Streptococcus pyogenes*. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence (also referred to as a protospacer in the context of an endogenous CRISPR system). In the context of formation of a CRISPR complex, “target sequence” refers to a sequence to which a guide sequence is designed to have complementarity, where hybridization between a target sequence and a guide sequence promotes the formation of a CRISPR complex. Full complementarity is not necessarily required, provided there is sufficient complementarity to cause hybridization and promote formation of a CRISPR complex. A target sequence may comprise any polynucleotide, such as DNA or RNA polynucleotides. In some embodiments, a target sequence is located in the nucleus or cytoplasm of a cell. In some embodiments, the target sequence may be within an organelle of a eukaryotic cell, for example, mitochondrion or chloroplast. A sequence or template that may be used for recombination into the targeted locus comprising the target sequences is referred to as an “editing template” or “editing polynucleotide” or “editing sequence”. In aspects of the disclosure, an exogenous template polynucleotide may be referred to as an editing template. In an aspect of the disclosure the recombination is homologous recombination.

[00385] Typically, in the context of an endogenous CRISPR system, formation of a CRISPR complex (comprising a guide sequence hybridized to a target sequence and complexed with one or more Cas proteins) results in cleavage of one or both strands in or near (e.g. within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the target sequence. Without wishing to be bound by theory, the tracr sequence, which may comprise or consist of all or a portion of a wild-type tracr sequence (e.g. about or more than about 20, 26, 32, 45, 48, 54, 63, 67, 85, or more nucleotides of a wild-type tracr sequence), may also form part of a CRISPR complex, such as by hybridization along at least a portion of the tracr sequence to all or a portion of a tracr mate sequence that is operably linked to the guide sequence. In some embodiments, the tracr sequence has sufficient complementarity to a tracr mate sequence to hybridize and participate in formation of a CRISPR complex. As with the target sequence, it is believed that complete complementarity is not needed, provided there is sufficient to be functional. In some embodiments, the tracr sequence has at least 50%, 60%, 70%, 80%, 90%, 95% or 99% of sequence complementarity along the length of the tracr mate sequence when optimally aligned. In some embodiments, one or more vectors driving expression of one or more elements of a CRISPR system are introduced into a host cell such that expression of the elements of the CRISPR system direct formation of a CRISPR complex at one or more target sites. For example, a Cas enzyme, a guide sequence linked to a tracr-mate sequence, and a tracr sequence could each be operably linked to separate regulatory elements on separate vectors. Alternatively, two or more of the elements expressed from the same or different regulatory elements, may be combined in a single vector, with one or more additional vectors providing any components of the CRISPR system not included in the first vector. CRISPR system elements that are combined in a single vector may be arranged in any suitable orientation, such as one element located 5' with respect to ("upstream" of) or 3' with respect to ("downstream" of) a second element. The coding sequence of one element may be located on the same or opposite strand of the coding sequence of a second element, and oriented in the same or opposite direction. In some embodiments, a single promoter drives expression of a transcript encoding a CRISPR enzyme and one or more of the guide sequence, tracr mate sequence (optionally operably linked to the guide sequence), and a tracr sequence embedded within one or more intron sequences (e.g. each in a different intron, two or more in at least one intron, or all in a single intron). In some embodiments, the CRISPR enzyme, guide sequence,

tracr mate sequence, and tracr sequence are operably linked to and expressed from the same promoter.

[00386] Non-limiting examples of Cas proteins useful in the present disclosure include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, homologs thereof, or modified versions thereof. These enzymes are known; for example, the amino acid sequence of *S. pyogenes* Cas9 protein may be found in the SwissProt database under accession number Q99ZW2. In some embodiments, the unmodified CRISPR enzyme has DNA cleavage activity, such as Cas9. In some embodiments the CRISPR enzyme is Cas9, and may be Cas9 from *S. pyogenes* or *S. pneumoniae*. In some embodiments, the CRISPR enzyme directs cleavage of one or both strands at the location of a target sequence, such as within the target sequence and/or within the complement of the target sequence. In some embodiments, the CRISPR enzyme directs cleavage of one or both strands within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, 200, 500, or more base pairs from the first or last nucleotide of a target sequence. In some embodiments, a vector encodes a CRISPR enzyme that is mutated to with respect to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence. For example, an aspartate-to-alanine substitution (D10A) in the RuvC I catalytic domain of Cas9 from *S. pyogenes* converts Cas9 from a nuclease that cleaves both strands to a nickase (cleaves a single strand). Other examples of mutations that render Cas9 a nickase include, without limitation, H840A, N854A, and N863A. In aspects of the disclosure, nickases may be used for genome editing via homologous recombination.

[00387] In some embodiments, the HDR insertion element comprises AAVS1 safe harbor locus homology arms and are used in conjunction with Rep 78 endonuclease (nickase). The adeno-associated virus serotype 2 (AAV2) Rep 78 protein is a strand-specific endonuclease (nickase) that promotes site-specific integration of transgene sequences bearing homology arms corresponding to the AAVS1 safe harbor locus. See, e.g., Ramachandra et al., Efficient recombinase-mediated cassette exchange at the AAVS1 locus in human embryonic stem cells using baculoviral vectors (2011) *Nucleic Acids Research*, 39(16):e107; WO1998027207).

[00388] As indicated above, in some embodiments, the nucleic acid constructs of the present disclosure comprise an optional first and a second promoter sequence. The first and second promoter sequences may be the same or different. Suitable first and second promoter sequences include, but are not limited to the MMLV LTR promoter, the MoMuSV LTR promoter, the RSV LTR promoter, the SIN LTR promoter, the SV40 promoter, cytomegalovirus (CMV) immediate early promoter, herpes simplex virus (HSV) thymidine kinase promoter, alpha-lactalbumin promoter, mouse metallothionein-I promoter, dihydrofolate reductase promoter, the β -actin promoter, phosphoglycerol kinase (PGK) promoter, and the EF1 α promoter sequences, and combinations thereof. In some embodiments, the first promoter sequence is not a retroviral LTR promoter, *i.e.*, the first promoter is promoter sequence other than a retroviral LTR promoter sequence. However, when the promoter is a retroviral promoter sequence, it may be a SIN (self-inactivating) LTR promoter sequence. *See, e.g.*, co-pending application PCT/US2019/064423, which is incorporated herein by reference in its entirety. Suitable Sin LTR promoters are known in the art and are prepared by removing either all or a portion of the U3 region of the LTR.

[00389] As described in PCT/US2019/064423, in some embodiments the first promoter which drives selectable marker is a weak promoter. In some embodiments, a weak promoter is a promoter, preferably a constitutive promoter, that has activity that equal to or less than the activity of the SIN LTR promoter in a host of interest (e.g., a CHO cell) when operably linked to a selectable marker sequence. In still other embodiments, a weak promoter is a promoter, preferably a constitutive promoter, that has activity that equal to or less than the activity of the human Ubiquitin C (UBC) promoter in a host of interest (e.g., a CHO cell) when operably linked to a selectable marker sequence. Suitable methods for assessing promoter strength are known in the art. *See, e.g.*, Dandindorj et al. (2014) A Comparative Analysis of Constitutive Promoters Located in Adeno-Associated Viral Vectors, PLoS One 9(8): e106472; Zhang and Baum (2005) Evaluation of Viral and Mammalian Promoters for Use in Gene Delivery to Salivary Glands Mol. Ther. 12(3):528-536; Qin et al. (2010) Systematic Comparison of Constitutive Promoters and the Doxycycline-Inducible Promoter PLoS 5(5): e10611; Jeyaseelan et al. (2001) Real-time detection of gene promoter activity: quantitation of toxin gene transcription, Nucleic Acids Research. 29 (12): 58e–58. In some embodiments, weak promoters have been altered to reduce promoter activity. Accordingly, in some embodiments, the present disclosure provides vector(s) for expression of a protein of interest comprising a nucleic acid sequence encoding a selectable

marker in operable association with a first weak promoter sequence or promoter sequence that has been altered to reduce promoter activity as compared to a non-altered or wild-type version of the first promoter sequence and a nucleic acid sequence encoding the protein of interest operably linked to a second promoter sequence. The SIN LTR promoter sequence is one such example.

- 5 Other promoter sequences described above may also be altered to reduce activity and provide a weak promoter or the weak promoter may be naturally occurring weak promoter such as the UBC promoter.

- [00390] In some embodiments, the nucleic acid constructs include a selectable marker. Suitable selectable markers include but are not limited to glutamine synthetase (GS),
10 dihydrofolate reductase (DHFR) and the like. These genes are described in U.S. Pat. Nos. 5,770,359; 5,827,739; 4,399,216; 4,634,665; 5,149,636; and 6,455,275; all of which are incorporated herein by reference. In some embodiments, the selectable marker that is utilized is compatible with a host cell line that is deficient in the production of the enzyme encoded by the selectable marker nucleic acid sequence. Suitable host cell lines are described in more detail
15 below. In other embodiments, the selectable marker is an antibiotic resistance marker, i.e., a gene that produces a protein that provides cells expressing this protein with resistance to an antibiotic. Suitable antibiotic resistance markers include genes that provide resistance to neomycin (neomycin resistance gene (neo)), hygromycin (hygromycin B phosphotransferase gene), puromycin (puromycin N-acetyl-transferase), and the like.

- 20 [00391] In other embodiments of the present disclosure, where secretion of the protein of interest is desired, the nucleic acid constructs include a signal peptide sequence in operable association with the protein of interest. The sequences of several suitable signal peptides are known to those in the art, including, but not limited to, those derived from tissue plasminogen activator, human growth hormone, lactoferrin, alpha-casein, and alpha-lactalbumin.

- 25 [00392] In other embodiments of the present disclosure, the nucleic acid constructs include an RNA export element (See, e.g., U.S. Pat. Nos. 5,914,267; 6,136,597; and 5,686,120; and WO99/14310, all of which are incorporated herein by reference) either 3' or 5' to the nucleic acid sequence encoding the protein of interest. It is contemplated that the use of RNA export elements allows high levels of expression of the protein of interest without incorporating splice
30 signals or introns in the nucleic acid sequence encoding the protein of interest.

[00393] In still other embodiments, the nucleic acid constructs include at least one internal ribosome entry site (IRES) sequence. The sequences of several suitable IRES's are available, including, but not limited to, those derived from foot and mouth disease virus (FMDV), encephalomyocarditis virus, and poliovirus. The IRES sequence can be interposed between two transcriptional units (e.g., nucleic acids encoding different proteins of interest or subunits of a multi-subunit protein such as an antibody) to form a polycistronic sequence so that the two transcriptional units are transcribed from the same promoter.

[00394] In some embodiments, the nucleic acid constructs are incorporated into a nucleic acid expression vector. Vectors include, but are not limited to, nucleic acid molecules that are single-stranded, double-stranded, or partially double-stranded; nucleic acid molecules that comprise one or more free ends, no free ends (e.g., circular); nucleic acid molecules that comprise DNA, RNA, or both; and other varieties of polynucleotides known in the art. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Another type of vector is a viral vector, wherein virally-derived DNA or RNA sequences are present in the vector for packaging into a virus (e.g. retroviruses, replication defective retroviruses, adenoviruses, replication defective adenoviruses, and adeno-associated viruses). Viral vectors also include polynucleotides carried by a virus for transfection into a host cell. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g. bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors." Common expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. Other suitable vectors include, but are not limited to, cosmids and Yeast Artificial Chromosomes.

[00395] Accordingly, suitable nucleic acid expression vectors include, but are not limited to, transposon vectors as described above, as well as plasmid vectors, retroviral vectors, lentiviral vectors, AAV vectors, phage vectors, etc). It is contemplated that any vector may be used as long as it is replicable and viable in the host. In embodiments, the vectors are mammalian expression vectors that comprise among other elements described herein an origin of replication,

a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences.

[00396] Suitable plasmid vectors that may be adapted to incorporate the nucleic acid constructs of the present disclosure include specific plasmids systems for transposon vectors, FLP-FLT systems, Cre-lox systems, CRISPR-Cas9 systems, recombinase systems and integrase systems as well as plasmid vectors derived from pCIneo, pVAX1, pACT, Gateway plasmids, pAdvantage, pBIND, pG5luc, pTNT, pTarget, pCat3, pSI, pCMV, pSV and the like.

[00397] In some embodiments, the present disclosure provides host cells and host cell culture wherein the host cells express the protein of interest from the nucleic acid constructs described above. In some embodiments, the host cells are mammalian host cells. A number of mammalian host cell lines are known in the art. In general, these host cells are capable of growth and survival when placed in either monolayer culture or in suspension culture in a medium containing the appropriate nutrients and growth factors, as is described in more detail below.

Typically, the cells are capable of expressing and secreting large quantities of a particular protein of interest into the culture medium. Examples of suitable mammalian host cells include, but are not limited to Chinese hamster ovary cells (CHO-K1, ATCC CCL-61); bovine mammary epithelial cells (ATCC CRL 10274; bovine mammary epithelial cells); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture; see, e.g., Graham et al., J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; rat fibroblasts (208F cells); MDBK cells (bovine kidney cells); CAP (CEVEC's Amniocyte Production) cells; and a human hepatoma line (Hep G2).

[00398] In some embodiments, the host cells are modified so that they are deficient, or are naturally deficient, in an enzyme activity that is required for growth or survival of the cells in the presence of a selection agent and which is provided by the selectable marker. For example, Chinese Hamster Ovary (CHO) cells have been modified to be deficient for GS. In some
5 embodiments where vector includes a GS selectable marker, the host cell line is deficient in GS. In some embodiments, the GS deficient host cell line is the CHOZN® GS^{-/-} cell line available from Merck KGaA. In other embodiments, where the selectable marker is, for example, DHFR, the cell line may preferably be deficient for DHFR activity (i.e., DHFR⁻). Suitable DHFR⁻ cell lines include but are not limited to CHO-DG44 and derivatives thereof.

10 [00399] The nucleic acid constructs and vectors of the present disclosure may be introduced into host cells by any suitable means such as by transfection, transformation or transduction. In some embodiments, after transfection or transduction, the cells are allowed to multiply, and are then trypsinized and re-plated. Individual colonies are then selected to provide clonally selected cell lines. In still further embodiments, the clonally selected cell lines are screened by Southern
15 blotting or PCR assays to verify that the desired number of integration events has occurred. It is also contemplated that clonal selection allows the identification of superior protein producing cell lines. In other embodiments, the cells are not clonally selected following transfection.

[00400] In some embodiments, the nucleic acid constructs encoding different proteins of interest are introduced into the host cells, for example by transfection or electroporation. The
20 nucleic acid constructs encoding different proteins of interest can be introduced into the host cells at the same time or in a serial manner (e.g., a nucleic acid construct encoding a first protein of interest is introduced, a period of time is allowed to pass, and then a nucleic acid construct encoding a second protein of interest is introduced).

[00401] In some embodiments of the present disclosure, following transformation of a suitable
25 host strain and growth of the host strain to an appropriate cell density in media, the protein of interest is secreted during culture of the host cells. In some embodiments where amplifiable markers are utilized, it is contemplated that culture of transduced host cells in a medium comprising an inhibitor of the gene. Suitable inhibitors include, but are not limited to methotrexate for inhibition of DHFR and methionine sulfoximine (Msx) or phosphinothricin
30 for inhibition of GS. It is contemplated that as concentrations of these inhibitors are increased in

a cell culture system, cells with higher copy numbers of the amplifiable marker (and thus the genes or genes of interest) or which contain higher-producing insertions are selected.

[00402] Accordingly, the host cells containing vectors as described above are preferably cultured according to methods known in the art. Suitable culture conditions for mammalian cells are well known in the art (See e.g., J. Immunol. Methods (1983) 56:221-234 [1983], Animal Cell Culture: A Practical Approach 2nd Ed., Rickwood, D. and Hames, B. D., eds. Oxford University Press, New York [1992]).

[00403] The host cell cultures of the present disclosure are prepared in a media suitable for the particular cell being cultured. Commercially available media such as ActiPro media (HyClone), ExCell Advanced Fed Batch Medium (SAFC), Ham's F10 (Sigma, St. Louis, MO), Minimal Essential Medium (MEM, Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM, Sigma) are exemplary nutrient solutions. Suitable media are also described in U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 5,122,469; 4,560,655; and WO 90/03430 and WO 87/00195; the disclosures of which are herein incorporated by reference. Any of these media may be supplemented as necessary with serum, hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as gentamycin (gentamicin), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range) lipids (such as linoleic or other fatty acids) and their suitable carriers, and glucose or an equivalent energy source. In some embodiments where selectable markers such as GS are utilized, for example, the media will lack glutamine. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art.

[00404] The present disclosure also contemplates the use of a variety of culture systems (e.g., petri dishes, 96 well plates, roller bottles, and bioreactors) for the transfected host cells. For example, the transfected host cells can be cultured in a perfusion system. Perfusion culture refers to providing a continuous flow of culture medium through a culture maintained at high cell density. The cells are suspended and do not require a solid support to grow on. Generally, fresh nutrients must be supplied continuously with concomitant removal of toxic metabolites and,

ideally, selective removal of dead cells. Filtering, entrapment and micro-capsulation methods are all suitable for refreshing the culture environment at sufficient rates.

[00405] As another example, in some embodiments a fed batch culture procedure can be employed. In fed batch culture, generally the host cells, e.g., mammalian host cells, and culture medium are supplied to a culturing vessel initially and additional culture nutrients are fed, continuously or in discrete increments, to the culture during culturing, with or without periodic cell and/or product harvest before termination of culture. The fed batch culture can include, for example, a semi-continuous fed batch culture, wherein periodically whole culture (including cells and medium) is removed and replaced by fresh medium. Fed batch culture is distinguished from simple batch culture in which all components for cell culturing (including the cells and all culture nutrients) are supplied to the culturing vessel at the start of the culturing process. Fed batch culture can be further distinguished from perfusion culturing insofar as the supernatant is not removed from the culturing vessel during the process (in perfusion culturing, the cells are restrained in the culture by, e.g., filtration, encapsulation, anchoring to microcarriers etc. and the culture medium is continuously or intermittently introduced and removed from the culturing vessel). In some embodiments, the batch cultures are performed in roller bottles.

[00406] Further, the cells of the culture may be propagated according to any scheme or routine that may be suitable for the particular host cell and the particular production plan contemplated. Therefore, the present disclosure contemplates a single step or multiple step culture procedure. In a single step culture, the host cells are inoculated into a culture environment and the processes of the instant disclosure are employed during a single production phase of the cell culture. Alternatively, a multi-stage culture is envisioned. In the multi-stage culture cells may be cultivated in a number of steps or phases. For instance, cells may be grown in a first step or growth phase culture wherein cells, possibly removed from storage, are inoculated into a medium suitable for promoting growth and high viability. The cells may be maintained in the growth phase for a suitable period of time by the addition of fresh medium to the host cell culture.

[00407] Fed batch or continuous cell culture conditions are devised to enhance growth of the mammalian cells in the growth phase of the cell culture. In the growth phase cells are grown under conditions and for a period of time that is maximized for growth. Culture conditions, such

as temperature, pH, dissolved oxygen (dO₂) and the like, are those used with the particular host and will be apparent to the ordinarily skilled artisan. Generally, the pH is adjusted to a level between about 6.5 and 7.5 using either an acid (e.g., CO₂) or a base (e.g., Na₂CO₃ or NaOH). A suitable temperature range for culturing mammalian cells such as CHO cells is between about 30° to 38°C and a suitable dO₂ is between 5-90% of air saturation.

[00408] Following the polypeptide production phase, the polypeptide of interest is recovered from the culture medium using techniques that are well established in the art. The protein of interest preferably is recovered from the culture medium as a secreted polypeptide (e.g., the secretion of the protein of interest is directed by a signal peptide sequence), although it also may be recovered from host cell lysates. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The polypeptide thereafter is purified from contaminant soluble proteins and polypeptides, with the following procedures being exemplary of suitable purification procedures: by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and protein A Sepharose columns to remove contaminants such as IgG. A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification. Additionally, the protein of interest can be fused in frame to a marker sequence that allows for purification of the protein of interest. Non-limiting examples of marker sequences include a hexa-histidine tag, which may be supplied by a vector, preferably a pQE-9 vector, and a hemagglutinin (HA) tag. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (See e.g., Wilson et al., *Cell*, 37:767 [1984]). One skilled in the art will appreciate that purification methods suitable for the polypeptide of interest may require modification to account for changes in the character of the polypeptide upon expression in recombinant cell culture.

[00409] In some embodiments, the nucleic acid constructs are incorporated into systems. In some embodiments, the systems comprise multiple nucleic acid constructs or vectors as described above which are intended for introduction into a host cell. In other embodiments, the systems comprise one or more multiple nucleic acid constructs or vectors as described above which are intended for introduction into a host cell in addition to a nucleic acid or vector that encodes an enzyme that is necessary for incorporation of the nucleic acid constructs into a host

cell genome. Exemplary enzymes include, but are not limited to, transposases for use with transposon vector systems, integrases for use in systems which utilize integration sequences such as the PhiC31 system, MMLV systems, and the like, recombinases for use in vector systems such as Cre-lox, FLP-FRT and the like, and Cas9 nucleases for use in CRISPR based systems.

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EXAMPLES

[00410] The disclosure provides a unique way of combining the expression of Human DNase 1 and another product in a cell production system to allow for removal of DNA from the final product that is to be produced. The level of production of DNase may need to be varied depending on the product and its potential use. Human DNase 1 could be substituted for any other DNase or many other endonuclease molecules.

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Example 1: Production of CHO Cells Producing Human DNase 1

[00411] The full-length Human DNase 1 coding DNA sequence was identified from NCBI Genbank and the following publication (Proc. Natl. Acad. Sci. USA, Vol. 87, pp. 9188-9192, December 1990), including the endogenous signal peptide region. The Human DNase 1 gene was designed, the flanking cloning sequences adding an optimized Kozak translation initiation sequence and added Hind III cloning site at the 5' terminus and Xho I cloning site at the 3' terminus of the human DNase 1 gene sequence for expedited expression cloning. See FIG. 1A for nucleic acid sequence (SEQ ID NO:139) and FIG. 1B for protein sequence (SEQ ID NO:140). This sequence was also codon optimized for improved expression. This full-length CDS encoding human DNase 1 with the added flanking sequences was synthesized and cloned into pUC57 vector.

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[00412] The synthesized plasmid was digested with Hind III and Xho I restriction enzymes to release the Human DNase 1 encoding DNA fragment that was gel-purified and ligated into the pCS-newMCS-WPREplasmid digested with the same two enzymes. See FIGs. 2 and 3 for plasmid maps. The final clone was sequenced through the Human DNase 1 CDS to verify the congruity to the predicted DNA sequence, named pCS-CFSD1-WPRE (new ori).

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Table 1. Features of Starting Retrovector pCS-newMCS-WPRE (new ori)

Component	Description	Function/Notes
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5' hCMV-MoMuSV LTR (R-U5) bp 142 – 766 hCMV promoter bp 767 – 911 MoMuSV R-U5	A fusion of the human CMV promoter to the R–U5 regions of the Moloney Murine Sarcoma Virus 5' LTR	The human cytomegalovirus IE promoter has strong constitutive activity in most mammalian cells. It is used to create high titer of retrovector particles when transfected into packaging cells. The hCMV promoter is lost after the packaging cell step.
Extended packaging region bp 981 - 1790	MoMuLV/SV packaging region from the LTR through a mutated ATG site in the MLV Gag gene	Packaging region allows creation of retrovector particles by allowing RNA to associate with MLV Gag/Pol gene products.
sCMV promoter bp 1822 - 2424	The immediate early promoter from simian CMV	Alternative strong constitutive promoter to drive expression of product gene.
New MCS bp 2425 - 2533	New Multiple cloning site constructed from synthesized oligonucleotides	Series of restriction enzyme recognition sites to allow cloning of product gene.
WPRE bp 2589 – 3183	A fragment from the woodchuck Hepatitis B virus Pol gene	Region that is thought to aid export of unspliced RNA and improve protein expression.
3' LTR bp 3227 - 3820	The 3' LTR from MoMuLV	Functions as a Poly A signal for RNA. Allows reverse transcription and DNA insertion of retrovector into mammalian cells from retrovector particles.
Plasmid backbone – <i>E. coli</i> origin of replication and b-lactamase gene for ampicillin resistance bp 3821 – 6187 bp 1 - 148	Basic <i>E. coli</i> plasmid sequences	Allows selection of plasmid containing bacteria in <i>E. coli</i> and replication of DNA in <i>E. coli</i> . These regions are lost after transfecting plasmids into packaging cells and creating retrovector particles.

Table 2. Features of pCS-CFSD1-WPRE (new ori)

Component	Description	Function/Notes
5' hCMV-MoMuSV LTR (R-U5) bp 142 – 766 hCMV promoter bp 767 – 911 MoMuSV R-U5	A fusion of the human CMV promoter to the R–U5 regions of the Moloney Murine Sarcoma Virus 5' LTR	The human cytomegalovirus IE promoter has strong constitutive activity in most mammalian cells. Used to create high titer of retrovector particles when transfected into packaging cells. The hCMV promoter is lost after the packaging cell step.

Extended packaging region bp 981 - 1790	MoMuLV/SV packaging region from the LTR through a mutated ATG site in the MLV Gag gene	Packaging region allows creation of retrovector particles by allowing RNA to associate with MoMuLV Gag/Pol gene products.
sCMV promoter bp 1828 - 2492	The immediate early promoter from simian CMV	Alternative strong constitutive promoter to drive expression of product gene.
Human DNase 1 gene bp 2510 - 3358	Human DNase 1 gene CDS	Synthetic Human DNase 1 CDS
WPRE bp 3377 - 3977	A fragment from the woodchuck hepatitis B virus Pol gene	Region that is thought to aid export of unspliced RNA and improve protein expression.
3' LTR bp 4017 - 4610	The 3' LTR from MoMuLV	Functions as a Poly A signal for RNA. Allows reverse transcription and DNA insertion of retrovector into mammalian cells from retrovector particles.
Plasmid backbone – <i>E. coli</i> origin of replication and b-lactamase gene for ampicillin resistance bp 5913 < 6772 bp 1 - 148	Basic <i>E. coli</i> plasmid sequences	Allows selection of plasmid containing bacteria in <i>E. coli</i> and replication of DNA in <i>E. coli</i> . These regions are lost after transfecting plasmids into packaging cells and creating retrovector particles.

[00413] 1.1 Summary of development of DNase 1 expressing clones.

[00414] Chinese Hamster Ovary (CHO-S) production cell lines were made by three rounds of transduction of the CHO-S parental cell line with retrovector made from the Human DNase 1 expression gene construct. The pooled populations were expanded for cryopreservation after each transduction.

[00415] The (3X) pooled population was diluted into 96-well cell culture plates to establish clonal cell lines that originated from single cells. The clonal cell lines were screened by Pico Green DNA assay for protein titer. The top thirteen clones were expanded and tested in triplicate T175 flasks for overgrowth productivity and were cryopreserved. The QC analyses of the clonal cell lines were performed after cryopreservation. The QC tests included viability, gene copy index, retrovector component, bioburden, and mycoplasma.

[00416] 1.2 Materials.

[00417] The plasmid for expression of Vesicular Stomatitis Virus (Indiana) envelope glycoprotein (pHCMV-G) was originally developed by Pangenix (San Diego, CA) and was prepared by Bayou Biolabs (Harahan, LA). The retrovector plasmid was prepared in house by endotoxin free maxi-prep (Qiagen, Valencia, CA) using the manufacturer's protocol. Quality control analyses included determination of DNA concentration and sequencing of the protein coding region and cloning junctions. DNA sequencing was performed by ACGT, Inc. (Wheeling, IL) using primers manufactured by Invitrogen (Carlsbad, CA).

[00418] The retrovector packaging cell line 293GP was developed by Pangenix (San Diego, CA), and has been characterized and master cell banked at BioReliance (Rockville, MD) for CPS-M. The suspension-adapted Chinese Hamster Ovary cell line (CHO-S) was received from GIBCO Life Technologies Inc. (Rockville, MD, Catalog #11619012), and has been characterized and master cell banked at BioReliance (Rockville, MD) for CPS-M. DF medium is Dulbecco's Modified Eagle's Medium (HyClone, Catalog # SH30243) plus 10% Fetal Bovine Serum (FBS) from HyClone (Catalog # SH30070). DFP medium is DF medium plus 10 µg/mL phleomycin. PF CHO Liquid Soy Medium (PF CHO LS) is purchased from HyClone (Catalog # SH30359).

[00419] 1.3 Methods.

[00420] 1.3.1 Retrovector production.

[00421] 293GP cells were cultivated in DFP medium and then passaged to 16 T150 flasks using trypsin (HyClone Catalog # SH30042). Two hours prior to transfection, the flasks were changed to 25 mL of DF medium. Transfection was performed following SOP STM-CEL-0325 using 864 µg of retrovector construct DNA plasmid pCS-CFSD1-WPRE (new ori), and 54 µg of expression plasmid for Vesicular Stomatitis Virus envelope glycoprotein. The plasmid solutions were combined with 1:10 TE for a total volume of 17.47 mL and 2.52 mL of 2M CaCl₂, and then precipitated by dropwise addition into 19.92 mL of 2X HBS solution. Then, 2.5 mL of suspension was added to each of the 16 T150 flasks and incubated on the cells for six hours at 37°C in a 5% CO₂ atmosphere. Growth at 37°C ± 1°C in a 5% ± 1% CO₂ atmosphere will be referred to as standard conditions from this point forward.

[00422] After six hours, the culture medium was replaced with 20 mL of fresh DF medium. The flasks were incubated under standard conditions until the second day after transfection. The medium was collected from the 16 T150 flasks and filtered through a 0.45 micron filter and then

a 0.2 micron filter. The retrovector was concentrated from the 320 mL of harvested medium by centrifugation in a Beckman J-30I centrifuge with a JA-30.5 rotor at 18,750 rpm (40,000 x G) for 90 minutes at 4°C. The supernatant was aspirated from the centrifuge tubes and the pelleted material in each tube was resuspended in 25 µL of PF CHO LS medium. The concentrated
5 vector was used for the CHO-S transduction step.

[00423] 1.3.2 Transduction of CHO-S cells with retrovectors

[00424] Parental CHO-S cells were established in culture and prepared for transduction following procedure SOP STM-CEL-0350. A suspension of 4×10^4 viable CHO-S cells was prepared in 5 mL of PF CHO LS medium with 8 µg/mL polybrene. This cell suspension was
10 incubated under standard conditions for a minimum of two hours prior to the addition of the retrovector. Immediately prior to the addition of retrovector, the cell suspension was centrifuged for four minutes at 1500 rpm (500 x G) in a tabletop centrifuge (Beckman Coulter Allegra 6 with a GH 3.8 rotor) and the supernatant was removed without disturbing the cell pellet. The retrovector was added to the CHO-S cell pellet, mixed and was incubated under standard
15 conditions.

[00425] After one day, 5 mL of PF CHO LS medium was added to the tube containing the cell-retrovector mixture and mixed. This mixture was then centrifuged for four minutes at 1500 rpm (500 x G) in a tabletop centrifuge. The supernatant containing any residual retrovector was removed and another wash and spin were performed. The supernatant was removed without
20 disturbing the cell pellet, and the cells were resuspended in 2 mL PF CHO LS medium. The cell suspension was transferred to a 12-well cell culture plate and expanded through consecutive passages into successively larger cell culture flasks using PF CHO LS medium.

[00426] Each subsequent transduction was performed following the same methods as before using cells in culture from the previous transduction. The pooled population from each
25 transduction was expanded and cryopreserved. In addition, a sample of cells was submitted for gene copy index testing.

[00427] 1.3.3 Establishment of clonal cell lines

[00428] Clonal selection was performed on an aliquot of sCHO-S/sC-CFSD1-R (3X) cells at passage level 6. The cells were diluted to 0.5 and 0.75 viable cells per 200 µL in PF CHO LS

medium with 2% FBS. The addition of FBS allowed cells to grow as adherent cultures and assisted in the growth of founder colonies originating from single cells. Twenty 96-well plates were seeded with 200 μ L per well of cell suspension for each of the dilutions.

[00429] 1.3.4 Selection and testing of top clonal cell lines.

5 [00430] The seeded 96-well plates were incubated under standard conditions and were observed microscopically on two different days for the development of colonies originating from single parental cells (SOP STM-CEL-0330). Media was collected on Day 14 from 218 wells in which single colonies were observed. The media samples were screened by an activity assay to determine protein titer. Media was replaced with PF CHO LS without FBS and cells were
10 cultured in PF CHO LS without FBS from this point on. The top 24 clones were selected based on Human DNase 1 titer. Eleven clones did not survive the transition from adherent to suspension and were discarded. The remaining 13 clones were expanded for productivity testing in an overgrowth study. Triplicate T175 flasks were seeded with 300,000 viable cells per mL in 50 mL working volume of PF CHO LS. Viable cell densities (VCD) were determined on day
15 three and samples were collected for protein analysis by an activity assay on days three and 14. The cultures were terminated on day 14.

[00431] 1.4 Results and discussion.

[00432] The Human DNase 1 expression cell lines were created by performing multiple rounds of transduction of the sCHO-S parental cell line using retrovector made with construct
20 DNA plasmid for the expression of pCS-CFSD1-WPRE (new ori). The pooled population was expanded for cryopreservation after each transduction and a sample of cells was submitted for gene copy analysis. The names used to identify the sCHO-S cell lines after each round of transduction and the gene copy index results are shown.

Table 3. Gene Copy Index Results of the Human DNase 1 Expression Cell Lines

Transduction Date	Cell Line	Gene Copy Index
08/31/06	sCHO-S/sC-CFSD1-R (1X)	3.70
09/07/06	sCHO-S/sC-CFSD1-R (2X)	5.83
09/14/06	sCHO-S/sC-CFSD1-R (3X)	5.37

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[00433] Each transduction created a population of cells with the Human DNase 1 genes inserted in differing numbers and locations in the production cell line. Clonal selection by limited dilution plating was performed to identify candidate clonal cell lines for production. The pooled population of sCHO-S/sC-CFSD1-R (3X) cells was diluted to 0.5 and 0.75 viable cells per 200 μ L media and plated in 96-well cell culture plates to establish clonal cell lines that originated from single cells. Forty 96-well plates were screened twice microscopically to identify 218 wells with a single colony.

[00434] In the clonal selection procedure, the number of wells containing single cells corresponds with the expected number based on seeding density; however, the number of wells with two or more cell colonies is usually higher than the number of wells with single cells. The screening process is very stringent and as such, any well that contains a colony slightly outside the 'norm' is classified as a 2+ cell-colony well (i.e., a larger than normal colony, satellite cells, irregular shaped colony, floating cells). Due to this selection process, a number of what are probably single cell colonies are categorized as > 1 cell colonies per well.

Table 4. Microscopic Clonal Screening Results

Plate Numbers	Dilution (Cells/Well)	Wells With No Cells	Wells With 1 Cell	Wells With >1 Cell	Totals
1 to 20	0.5	1451	77	392	1920
21 to 40	0.75	1177	141	602	1920
	Totals	2628	218	994	3840

[00435] Fourteen days post seeding, media samples were collected from the 96-well plates and screened by an activity assay for protein production. The top 24 clones from the 96-well plate samples ranged from 32 to 47 μ g/mL of Human DNase 1 protein.

Table 5. Activity Assay Results from 96-Well Plates

Rank	Clone Number	Titer (μ g/mL)
1	3*	47
2	106*	45
3	128	43
4	44	42

Rank	Clone Number	Titer (µg/mL)
5	1	41
6	117*	41
7	20	39
8	110*	38
9	57	37
10	35	37
11	127*	36
12	66	35
13	97*	34
14	85	34
15	40	34
16	134*	34
17	37*	34
18	114*	34
19	48	34
20	36	33
21	30*	33
22	74	33
23	32*	32
24	98	32

*Discarded due to poor growth.

[00436] The top 13 clones were set up in triplicate T175 flasks for overgrowth productivity testing.

Table 6. Productivity Results of the Top 12 Clones in Triplicate T175 Flasks

Clone Number	Day 3 Average VCD [x 10 ⁵ cells/mL]	Day 3 Average Protein [μg/mL]	Day 3 Average PCD	Day 14 Average Protein [μg/mL]
1*	9.75	21	12.04	47
20*	11.18	21	11.09	43
35	7.60	15	9.77	42
36	15.33	9	3.82	36
40	13.78	11	5.08	38
44	16.30	16	6.69	36
48	14.71	5	2.31	29
66*	14.01	23	10.64	46
74	15.37	8	3.32	36
85*	8.79	21	12.84	42
98*	9.93	29	16.67	44
128	12.75	14	6.84	41
Average	12.46	16	8.43	40

The top five clones based on titer (then PCD) are indicated (*).

[00437] 1.5 Conclusion.

[00438] Clonal cell lines were made by performing limited dilution cloning of the sCHO-S/sC-CFSD1-R (3X) pooled population. Twelve clonal cell lines were screened by overgrowth analysis. Protein titers were determined by ELISA. The average maximum protein level of the top 12 clones was 40 mg/L. The top five clones, #1, 20, 66, 85 and 98 were primarily selected based on titer. These top five clonal lines had maximum expression levels ranging from 42 mg/L for clone #85 to 47 mg/L for clone #1. The mean PCD of the top 12 clones was 8.43 on day three. The PCD for the top five clones ranged from 10.64 for clone #66 to 16.67 for clone #98. The clones produced high levels of Human DNase 1 and the production of the product showed no effects on CHO cell growth or behavior. Upon further cell culture optimization levels of protein production reach as high as 2 g/L in fed-batch culture.

Example 2: Production of CHO cells expressing Human DNase 1 and an antibody product

[00439] The presence of residual DNA during the culture of cells, may in some instances inhibit the production of particular recombinant proteins or in other instances bind so tightly to the recombinant protein product that prevents effective removal of the DNA during purification of the product. The co-expression of Human DNase 1 or other nucleases with these types of products, may improve product expression and product purity. In this experiment we co-expressed Human DNase 1 at different levels with an antibody product to determine if this approach could be successful. The GPEX Lightning technology was utilized to co-express Human DNase 1, an antibody heavy chain and an antibody light chain in CHO cells.

[00440] 2.1 Summary.

[00441] Based on the obtained antibody sequence information, Catalent designed coding DNA sequences (CDS) for optimal expression of the antibody light chain (LC) and heavy chain (HC) of GMAB, as well as the Human DNase 1 gene. Each of the three genes were then cloned into the GPEX[®] Lightning expression vector. The LC and HC CDS in the expression vector (plasmid) were each confirmed by DNA sequencing.

[00442] Nine independent production cell lines were made by performing a single round of transfections of the 1F7 CHO parental cell line with the gene constructs developed to express GMAB and Human DNase 1. The nine pooled populations underwent selection via removal of glutamine and were expanded for gene copy index value (GCIV) testing and cryopreservation. The CHOZn/GA04-LC/HC/DNase pooled population #6 cell line exhibited the most desirable ratio of GCI values between LC, HC, and DNase 1, and was further tested in a fed-batch productivity study.

[00443] The CHOZn/GA04-LC/HC/DNase pooled population #6 was processed through a single round of clonal selection using the Beacon[®] platform to establish clonal cell lines that originated from a single cell expressing the protein of interest. The clones reported herein have an average calculated probability of monoclonality of > 99%. The Top 12 clones were grown in a fed-batch productivity culture study under generic conditions to delineate the highest GMAB-producing cell lines.

[00444] Clones #31, 37, and 1328 were identified as the top three Master Cell Bank (MCB) candidates, with Clone #11 as a backup candidate, based on protein production and cell line quality.

[00445] 2.2 DNA constructions and cloning.

[00446] 2.2.1 Materials and methods.

[00447] Based on starting antibody sequence information, we designed CDS for optimal expression of the antibody LC and HC. The unique CDS for the GMAB LC and HC were designed by Catalent using proprietary Triplet-Fix® codon optimization technology and public access sources including NCBI and IMGT websites. Unique restriction sites for cloning the complete LC and HC CDS into Catalent expression vectors were added on the 5' and 3' ends of the CDS. Additionally, a Kozak sequence for efficient protein expression and two tandem stop codons to prevent translational read-throughs were introduced at the 5' and 3' ends, respectively. Human DNase 1 "Pathway" was to be co-expressed with the antibody chains to potentially enhance their expression and secretion. The Human DNase 1 CDS was subcloned into Lightning expression vector as described.

[00448] Each designed CDS was synthesized by IDT. For each full-length CDS, cloning was performed in a similar manner. The IDT gBlock® or the parental vector was digested with HindIII and XhoI (HC and Pathway) or NotI and BglII (LC) restriction endonucleases and the released CDS was ligated into the GPEX® Lightning expression vector that had also been digested with the same two corresponding restriction endonucleases. The ligation reactions were performed according to the manufacturer's procedure using NEBuilder HiFi DNA Assembly Master Mix. The Lightning expression vector 207puc19attB287-GS-NewMCS-WPRE-TKpa was previously generated at Catalent.

[00449] 2.2.2 Results.

[00450] A clone was confirmed to encode full-length GMAB LC and HC CDS and the DNase Pathway CDS. The new plasmid encoding each of the three CDS were named and designated respectively: 207attB-GS-h3E10LC-WPRE and, 207attB-GS-h3E10HC-WPRE, and 215-puc19attB287-GS-Pathway-WPRE-TKpa. All three CDS and the flanking DNA cloning junctions in the final expression constructs are shown. The cloning restriction sites are also shown. The expression vectors were DNA-sequenced through all three CDS and the cloning junctions to exclude mutations.

[00451] FIG. 4A provides the nucleic acid sequence for the h3E10LC insert (SEQ ID NO:141) while FIG. 4B provides the amino acid sequence for the h3E10LC insert (SEQ ID NO:142). FIG. 5 provides the plasmid map for plasmid 207attB-GS- h3E10LC -WPRE. FIG. 6A provides the nucleic acid sequence for h3E10HC insert (SEQ ID NO:143) while FIG. 6B provides the amino acid sequence for h3E10HC insert (SEQ ID NO:144). FIG. 7 provides the plasmid map for plasmid 207attB-GS-h3E10HC-WPRE. FIG. 8A provides the nucleic acid sequence for the 1008-215-Pathway-207 insert (SEQ ID NO:145) while FIG. 8B provides the amino acid sequence for the 1008-215-Pathway-207 insert (SEQ ID NO:146). FIG. 9 provides the plasmid map for plasmid 215-puc19attB287-GS-Pathway-WPRE-TKpa. Tables 7, 8 and 9 provide a summary of the features of these expression vectors.

Table 7. Features of Expression Vector 207attB-GS-h3E10LC-WPRE

Component	Description	Function/Notes
attB287 bp 402-690	A recombination site of site-specific serine recombinase qC31	Binds specifically to one of the recombinase domains and participates in site specific recombination
Glutamine Synthetase (GS) CDS bp 699-1820	CHO GS gene	Enables selection of high producers in GS-deficient cells lines
sCMV promoter bp 1822-2494	The immediate early promoter from simian CMV	A strong constitutive promoter to drive expression of product gene in mammalian cells
h3E10 Light Chain bp 2513-3226	h3E10 Light Chain CDS including signal peptide	Synthesized CDS cloned by restriction digestion
WPRE bp 3247-3847	Woodchuck Post-transcriptional Regulatory Element from the woodchuck Hepatitis B virus	Region that is thought to aid export of unspliced RNA and improve protein expression.
TK PA bp 3848-4119	Herpes simplex virus thymidine kinase polyA sequence	Functions as a Poly A signal for RNA. Encodes transcription termination signal and can add poly A tail to RNA
Plasmid backbone – <i>E. coli</i> origin of replication and β -lactamase gene for ampicillin resistance bp 5305-6165 bp 1-401 and 6166-6365	Basic <i>E. coli</i> plasmid sequences	Allows selection of plasmid containing bacteria in <i>E. coli</i> and replication of DNA in <i>E. coli</i> .

Table 8. Features of Expression Vector 207attB-GS-h3E10HC-WPRE

Component	Description	Function/Notes
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attB287 bp 402-690	A recombination site of site-specific serine recombinase ϕ C31	Binds specifically to one of the recombinase domains and participates in site specific recombination
Glutamine Synthetase (GS) CDS bp 699-1820	CHO GS gene	Enables selection of high producers in GS-deficient cells lines
sCMV promoter bp 1822-2494	The immediate early promoter from simian CMV	A strong constitutive promoter to drive expression of product gene in mammalian cells
h3E10 Heavy Chain bp 2519-3913	h3E10 Heavy Chain CDS including signal peptide	Synthesized CDS cloned by restriction digestion
WPRE bp 3929-4529	Woodchuck Post-transcriptional Regulatory Element from the woodchuck Hepatitis B virus	Region that is thought to aid export of unspliced RNA and improve protein expression.
TK PA bp 4530-4801	Herpes simplex virus thymidine kinase polyA sequence	Functions as a Poly A signal for RNA. Encodes transcription termination signal and can add poly A tail to RNA
Plasmid backbone – <i>E. coli</i> origin of replication and β -lactamase gene for ampicillin resistance bp 5987-6847 bp 1-401 and 6848-7047	Basic <i>E. coli</i> plasmid sequences	Allows selection of plasmid containing bacteria in <i>E. coli</i> and replication of DNA in <i>E. coli</i> .

Table 9. Features of Expression Vector 215-puc19attB287-GS-Pathway-WPRE-TKpa

Component	Description	Function/Notes
attB287 bp 402-690	A recombination site of site-specific serine recombinase ϕ C31	Binds specifically to one of the recombinase domains and participates in site specific recombination
Glutamine Synthetase (GS) CDS bp 699-1820	CHO GS gene	Enables selection of high producers in GS-deficient cells lines
sCMV promoter bp 1822-2494	The immediate early promoter from simian CMV	A strong constitutive promoter to drive expression of product gene in mammalian cells
Pathway DNase bp 2519-3367	Pathway CDS including signal peptide	CDS cloned by restriction digestion
WPRE bp 3386-3986	Woodchuck Post-transcriptional Regulatory Element from the woodchuck Hepatitis B virus	Region that is thought to aid export of unspliced RNA and improve protein expression.

TK PA bp 3987-4258	Herpes simplex virus thymidine kinase polyA sequence	Functions as a Poly A signal for RNA. Encodes transcription termination signal and can add poly A tail to RNA
Plasmid backbone – <i>E. coli</i> origin of replication and β -lactamase gene for ampicillin resistance bp 5444-6304 bp 1-401 and 6305-6504	Basic <i>E. coli</i> plasmid sequences	Allows selection of plasmid containing bacteria in <i>E. coli</i> and replication of DNA in <i>E. coli</i> .

[00452] 2.3 Cell line development

[00453] 2.3.1 Materials and methods.

[00454] Next, parental GPEx Lightning 1F7 CHO cells were established in culture and prepared for transfection. EX-CELL[®] Advanced CHO Fed-Batch medium supplemented with 6 mM L-glutamine was used as the base media for culture maintenance. Immediately prior to the addition of transfection reagents and DNA, a required amount of 1F7 parental cell suspension was centrifuged for five minutes at 500 x g in a tabletop centrifuge (Sorvall Legend XT with TX-750 rotor). As much supernatant as possible was removed without disturbing the cell pellet. Cells were then washed with 5 mL of CHOgro expression media, spun at 500 x g in a tabletop centrifuge, and aspirated a second time. Fresh CHOgro expression media was then added to the cell pellet to achieve a final suspension of 200 x 10⁵ viable 1F7 cells in 2 mL of CHOgro expression medium. The LC/HC/DNAse 207puc19attB287-GS-NewMCS-WPRE-TKpa plasmids were combined at ratios of 1:1:1, 5:5:1, and 10:10:1 (LC:HC:DNAse) with the total amount of DNA remaining constant across the 3 DNA preparations. DNA plasmids and the recombinase DNA plasmid were mixed in Optipro media at a ratio of 500:1 in a tube (tube #1). The expifectamine reagent was diluted in Optipro media in a second tube (tube #2). Tubes #1 and 2 were mixed, allowed to sit for 1–5 min, and then added to the 1F7 cells (30 x 10⁵) in a 50 mL culture vessel and then shaken at 250 rpm and 37 °C. Three independent transfections were performed resulting in three individual pooled populations.

[00455] After 2–4 hours of incubation, the cell-transfection reagent mixture was centrifuged for five minutes at 500 x g in a tabletop centrifuge. The supernatant was removed, and the transfected cells were suspended in 10 mL of EX-CELL[®] Advanced CHO Fed-Batch medium supplemented with 2% ACF and 6 mM L-glutamine. The transfected cells recovered for three days post-transfection before the selection process was initiated.

After the cells had fully recovered from the transfection process, cultures were centrifuged for five minutes at 500 x g in a tabletop centrifuge; all media was aspirated without disturbing the pellet, and cells were resuspended in 10 mL of EX-CELL[®] Advanced CHO Fed-Batch medium without L-Glutamine or ACF. The cultures were then counted at least every other day to monitor the viable cell density, the percent viability, and the doubling time. Generally, the transfected cells exhibited a decline in viability from the beginning of selection until Day 10 post-transfection (Nadir). After the minimum viability was achieved, the selection process was complete, and successfully transfected cells began to recover. Cultures recovered for another 10 days until the viability fully recovered and the doubling time stabilized.

[00456] The pooled cell populations from transfection were expanded and cryopreserved. A supernatant sample was taken for protein titer analysis by BioHT and protein quality analysis by SDS-PAGE. A sample of the cells was submitted for gene copy index (GCIV) analysis and residual recombinase analysis.

[00457] 2.3.2 Generation of GMAB expression cell line.

[00458] Next, the GMAB expression cell lines were created by performing one round of transfection of the 1F7 parental cell line with the 3 DNA plasmids. The names used to identify each of the nine 1F7 cell lines after transfection, along with the GCIV results are shown. See Table 10. The SDS-PAGE results are also shown. See Fig. 10.

Table 10. Transfection of the GMAB Expression Cell Lines

Date	DNA Lot Number	Ratio (LC:HC:DNase)	Cell Line Name After Transfection	LC GCIV	HC GCIV	DNA se GCIV
22Apr2022	GA01.0004.01192 2,	1:1:1	CHOZn/GA04-LC/HC/DNase Pool #1	3.95	3.7	4.31
	GA01.0005.01192 2,		CHOZn/GA04-LC/HC/DNase Pool #2	4.05	3.93	4.38
	1008.0215.04082 2		CHOZn/GA04-LC/HC/DNase Pool #3	3.79	3.47	4.05
		5:5:1	CHOZn/GA04-LC/HC/DNase Pool #4	4.69	4.42	2.80

			CHOZn/GA04-LC/HC/DNAse Pool #5	4.59	4.11	2.60
			CHOZn/GA04-LC/HC/DNAse Pool #6	4.52	4.45	2.75
		10:10:1	CHOZn/GA04-LC/HC/DNAse Pool #7	4.68	4.43	1.64
			CHOZn/GA04-LC/HC/DNAse Pool #8	4.58	4.41	1.59
			CHOZn/GA04-LC/HC/DNAse Pool #9	4.88	4.77	1.75

[00459] 2.4 Fed-batch production from a pooled cell population.

[00460] 2.4.1 Materials and methods.

[00461] Cell line CHOZn/GA04-LC/HC/DNAse pooled population #6 was scaled up for initial protein production by fed-batch analysis in 500mL Shake Flask seeded at 6.0×10^5 viable cells/mL in 120 mL of ActiPro medium supplemented with 4% ps307.

[00462] Viable cell density (VCD) and viability was determined every day after Day 2. Protein titer was determined by BioHT IgG analysis. Specific protein production (picograms produced per cell per day, pcd) was determined by taking the slope of the linear fit curve of protein concentration (in pg/nL) plotted against integral cell density (cells*day/nL). The culture was terminated when the viability fell below 90%, but did not exceed Day 14. See FIGs. 11-13.

[00463] 2.4.2. Results.

[00464] The gels showed that each of the three protein products (LC/HC/DNAse 1) were expressed in the expected ratio based on the plasmid transfection. The production of DNAse 1 by the cells does not appear to inhibit the production of functional antibody at high levels. These cells behaved similarly to a standard CHO cell line just producing an antibody. The CHOZn/GA04-LC/HC/DNAse pooled population reached a peak viable cell density on Day 10 of 254×10^5 cells/mL, and was harvested on Day 14 with a viability of 96.4%. The cell line produced 5.1 picograms of antibody/cell/day (pg/c/d).

[00465] The concentration of antibody in the supernatant is at least 0.001 µg/mL, at least 0.01 µg/mL, at least 0.1 µg/mL, at least 0.125 µg/mL, at least 0.250 µg/mL, at least 0.5 µg/mL, at least 0.75 µg/mL, at least 1 µg/mL, at least 5 µg/mL, at least 10 µg/mL, at least 25 µg/mL, at least 50 µg/mL, or at least 100 µg/mL. In some embodiments, the concentration of antibody in the supernatant is between 0.001 µg/mL and 100 µg/mL. In some embodiments, the concentration of antibody in the supernatant is between 0.01 µg/mL and 50 µg/mL. In some embodiments, the concentration of antibody in the supernatant is between 0.01 µg/mL and 50 µg/mL. In some embodiments, the concentration of antibody in the supernatant is between 0.1 µg/mL and 25 µg/mL. In some embodiments, the concentration of antibody in the supernatant is between 0.125 µg/mL and 10 µg/mL. In some embodiments, the concentration of antibody in the supernatant is between 0.250 µg/mL and 5 µg/mL. In some embodiments, the concentration of antibody in the supernatant is between 0.5 µg/mL and 1 µg/mL. In some embodiments, the concentration of antibody in the supernatant is between 0.75 µg/mL and 1 µg/mL.

[00466] 2.5 Clonal selection with the Beacon Instrument.

[00467] 2.5.1 Materials and methods.

[00468] One round of clonal selection was performed using the Beacon instrument (Berkeley Lights Inc (BLI), Emeryville, CA) to identify candidate clonal cell line. The Beacon optofluidic platform uses OptoSelect™ Opto-Electric Positioning technology (light cages) to place single cells into 1 nL pens on chips. The instrument pens, cultures, assays, images, ranks, and then exports high-expressing clonal cell lines. CHOZn/GA04-LC/HC/DNAse cells were seeded at 1–2 million cells per mL in a volume of 10 mL in dynamic culture, 1 day prior to loading on the Beacon instrument that contained G12.1 medium supplemented with 6 mM L-glutamine and 0.25 g/L PS307 with 2.5% animal component free (ACF) supplement. The viable cell density of the cell suspension upon loading was 38.2×10^5 cells/mL and the viability was 99.9%. The penning efficiency (percentage of pens containing single cells) was calculated to be 80.7% (2837/3516). Each pen was verified manually by an operator to ensure a single cell was present. Cells were then cultured on the chip for three days in G12.1 medium supplemented with 6 mM L-glutamine and 0.25 g/L PS307 with 2.5% ACF supplement. It was confirmed that no pens were overgrown (not more than 50% full). The percent on chip clonal expansion (OCCE; percentage of pens containing 4 or more cells by Day 3) was calculated to be 96.3% (2731/2837).

[00469] On Day 3 of the culture, an in-pen diffusion assay using Spotlight Hu3™ (BLI) was performed, and parameters were set and applied for the selection of colonies to be exported. Clones fitting the criteria were ranked by titer score. The Top 96 colonies were exported into optical 96-well plates (VWR, Cat. #82050-772) with 160 µL per well of G12.1 culture medium with 6 mM L-glutamine and 0.25 g/L PS307 with 2.5% ACF supplement.

[00470] Colonies were cultured for eleven days and imaged via the Operetta Imaging system (Perkin Elmer) at Days 3, 7, 9, and 10 post-export for the purposes of monitoring growth. Clones were expanded from 96-well plates into 24-well plates (VWR, Cat. #82050-846) and then into 6-well plates. Clones were submitted for gene copy index value (GCIV) analysis. Subsequently, the clones were expanded into shaking culture in 50mL bioreactor tubes, and then up to a volume of 120 mL in 500mL shake flasks for cryopreservation. Cell counts were performed at each passage of dynamic culture. Twenty-three vials of each cell line were prepared for cryopreservation; three vials of each clone were used for QC testing. The QC tests included viability upon thaw, MET (bioburden), and mycoplasma.

Table 11. Beacon® Parameters Used for Selection of Clones for Export

Primary selection		
Parameter	Specification	Justification
# cells in pen on Day 0	One single cell as confirmed by Beacon instrument and verified by operator	Colonies must originate from a single cell
# of cells in pen on Day of export	At least 8 cells	Selection of colonies with adequate growth rates and the export of sufficient cells
doubling time	16–32 hours	Selection of colonies with adequate growth rates
Secondary selection		
Parameter	Specification	Justification
Protein score	Top 96	Expected highest protein producing clones

[00471] 2.5.2 Clonal analysis

[00472] The transfection process creates a population of cells with the GMAB CDS inserted in variable quantities and locations in the production cell line. During clonal selection, a total of approximately 2731 colonies were evaluated.

[00473] The Top 96 clones ranked by AU score were exported into optical well 96-well plates. These clones were observed for growth using the Operetta imaging system (Perkin Elmer) on Days 3, 7, 9, and 10 post-export. Of the Top 96 clones, a total of 74 scaled up successfully from 96-well plates, to 24-well plates, and then to 6-well plates. The Top 48 clones selected by GCI values were next evaluated in a fed-batch productivity study.

Table 12. Beacon Results – Top 71 Clones

Clone Number	AU Titer Score	LC GCIV	HC GCIV	DNase GCIV	attR GCIV
77	0.02978	4.56	5.00	3.07	6.23
404	0.02931 8	4.77	5.47	1.91	6.09
1730	0.02911 4	5.21	6.00	3.00	7.02
1702	0.02883 1	5.06	5.36	3.88	6.88
1258	0.02851 6	5.03	5.14	3.10	6.54
1455	0.02833 5	5.59	5.67	3.63	6.80
1640	0.02832 9	4.92	5.59	3.45	6.90
1410	0.02802 9	4.51	4.78	2.99	6.26
11	0.02766 2	5.75	5.42	3.62	6.87
1476	0.02729 6	4.76	5.42	2.53	5.57
500	0.02724 9	5.45	5.51	3.51	7.07
1750	0.02715 9	4.77	5.09	2.90	6.59

Clone Number	AU Titer Score	LC GCIV	HC GCIV	DNase GCIV	attR GCIV
1360	0.02712 8	4.63	4.85	3.21	6.36
1040	0.02699 8	4.96	5.64	2.91	6.52
1239	0.02658 2	4.43	5.07	3.60	6.61
1081	0.02634 3	5.08	5.34	3.83	6.67
201	0.02634 2	4.93	5.35	3.18	6.66
1219	0.02628 7	3.90	4.77	1.97	5.66
141	0.02624 2	4.70	5.31	3.93	6.51
28	0.02555 6	5.14	5.42	3.91	6.57
61	0.02346 3	4.79	5.45	3.20	6.58
63	0.02300 8	5.74	5.93	4.02	7.01
18	0.02293 3	5.06	5.66	2.99	6.76
34	0.02210 6	4.80	5.39	3.05	6.66
4	0.02167 4	5.18	5.74	3.98	6.79
31	0.02163 1	5.82	5.88	3.84	7.11
37	0.02154 7	4.91	5.59	3.77	6.72

Clone Number	AU Titer Score	LC GCIV	HC GCIV	DNase GCIV	attR GCIV
54	0.02118	4.91	5.59	3.38	6.44
52	0.02076 7	4.26	4.61	2.24	5.60
22	0.02064	5.14	5.67	2.42	6.57
46	0.02047	4.31	5.28	2.91	6.22
16	0.02037 8	4.68	5.18	2.77	6.54
27	0.02012 6	5.02	4.97	3.16	6.54
15	0.01991 2	4.19	5.04	2.03	6.11
8	0.01959 3	4.86	5.07	2.78	6.11
60	0.01908 4	6.15	6.97	4.32	6.74
50	0.01889	5.72	5.88	2.80	6.56
32	0.01888 8	4.83	5.64	3.21	6.63
39	0.01860 2	5.24	5.59	3.71	6.86
49	0.01845 2	4.72	4.76	2.30	6.40
419	0.03696 4	5.41	5.76	3.58	6.91
1646	0.02978 6	5.55	5.63	3.88	7.16
1324	0.02926 2	5.24	6.16	2.75	6.84
1333	0.02878 2	3.90	5.09	1.79	5.53

Clone Number	AU Titer Score	LC GCIV	HC GCIV	DNase GCIV	attR GCIV
1528	0.02799 2	5.63	5.57	3.90	6.92
816	0.02796 9	3.44	4.63	2.30	5.38
380	0.02711 2	4.95	5.45	3.17	6.72
1328	0.02673 4	5.39	5.51	3.83	6.98
66	0.02632 6	4.86	5.50	3.53	6.72
265	0.02609 9	5.53	6.07	3.40	6.28
1747	0.02587 3	4.73	5.74	3.27	6.96
453	0.02563 9	5.87	6.02	0.93	6.59
1153	0.02503	5.47	5.81	3.28	6.80
317	0.02500 8	6.56	6.66	4.60	7.01
7	0.02458 5	4.75	5.10	2.35	6.49
31	0.02276 2	5.02	5.58	2.67	6.65
16	0.02268	3.76	4.65	2.65	5.76
10	0.02267 6	4.85	5.25	3.47	6.53
15	0.02155 8	4.83	5.14	3.58	6.44
12	0.02117 5	5.13	5.16	3.57	6.62

Clone Number	AU Titer Score	LC GCIV	HC GCIV	DNase GCIV	attR GCIV
52	0.02000 6	5.05	5.05	3.31	6.66
5	0.01987 6	5.82	6.84	4.51	7.01
53	0.01982 1	5.30	5.75	3.70	6.61
34	0.01975 2	3.92	4.31	1.34	5.60
3	0.01950 9	4.59	5.11	3.15	6.47
23	0.01948 7	4.52	5.29	2.41	6.38
51	0.01871 8	5.94	7.00	4.50	6.79
4	0.01864 3	4.09	5.01	2.47	5.91
46	0.01830 9	5.28	5.52	3.90	6.90
28	0.01751 1	5.16	5.13	3.15	6.45
2	0.01702 9	4.98	5.20	3.04	6.06
1074	0.02980 0	5.80	6.66	3.32	6.68
1067	0.02549 8	5.74	6.35	4.27	6.99
48	0.01915 3	4.96	5.80	2.37	6.56

[00474] 2.5.3 Productivity study in 24-well plate

[00475] The Top 71 clones as ranked by GCI values were tested in a dynamic batch culture in 24-well Deep Well Plates and evaluated for VCD and protein titer on Days 3 and 6 to identify the Top 12 clones for cryopreservation and further characterization in a microbioreactor (Ambr® 15; Sartorius AG) study. The VCD, viability, and titer for the 48 clones were determined. See Table 13.

Table 13. 24-well Plate Specific Production Rates and Harvest Data

Ranking	Clone	pcd	VCD Day 6 (x 10 ⁵ cells/mL)	Max Titer (mg/L)
1	11	4.55	172.3	171.8
2	31	4.57	146.9	142.0
3	37	4.84	104.7	133.8
4	28	3.56	162.6	129.9
5	1476	3.06	212.2	129.7
6	1730	2.96	181.5	116.0
7	5	4.12	123.7	108.9
8	419	3.55	145.8	101.7
9	66	3.17	141.0	93.3
10	1328	3.14	119.2	81.6
11	12	3.27	113.3	81.3
12	317	2.86	105.0	61.9

[00476] 2.5.4 Testing and Selection of the Clonal Cell Lines in an Ambr15™.

[00477] The selected Top 12 clonal cell lines were tested for productivity in an Ambr15™ miniature bioreactor system. The Ambr15™ mimics the operational parameters of classical bioreactors (2 L and 10 L) at a smaller scale (10–15 mL). The automated workstation is comprised of blocks of 12 disposable microbioreactors with independent environmental (aeration and pH), feed, and sampling controls for each culture. The clones were evaluated in ActiPro medium with Cell Boost™ 7a/7b feed and ExCell Advance Fed-Batch medium with Feed1/4FEED, with a temperature shift to 34 °C occurring on Day 6 for both the ActiPro and Fed-Batch media cultures. Cultures were terminated when the viability fell below 90% and did not exceed Day 14.

[00478] For scale-up, cells were used from the CHOZn/GA04-LC/HC/DNAse cell line for Clones #5, 11, 12, 28, 31, 37, 66, 317, 419, 1328, 1476, and 1730. The cells were maintained in Actipro medium supplemented with 0.25 g/L PS307; cells from each of the clonal cell lines were then adapted to Fed-Batch medium (with no supplements). Three passages were performed on the Top 12 clones in ActiPro medium or Fed-Batch medium prior to seeding in the Ambr15™ vessels.

[00479] The clones were inoculated in Ambr15™ vessels at cell densities of 6.0×10^5 cells/mL or 10.0×10^5 cells/mL. Samples were taken daily for VCD, viability, and metabolite levels (glucose, lactate, and ammonia). Titer was measured on days 4, 8, 12, 14, or harvest.

Table 14. Ambr15™ Experimental Design

Culture Station	Clone	Medium	Feed	Feed Strategy	Seeding Density (x 10 ⁵ cells/mL)	Temp Shift (°C)
CS1-1	5	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS1-2	11	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS1-3	12	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS1-4	28	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS1-5	31	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS1-6	37	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS1-7	66	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS1-8	317	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS1-9	419	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34

Culture Station	Clone	Medium	Feed	Feed Strategy	Seeding Density (x 10 ⁵ cells/mL)	Temp Shift (°C)
CS1-10	1328	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS1-11	1476	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS1-12	1730	ActiPro	Cell Boost™ 7a/7b	4% (7a), 0.4% (7b)	6.0	34
CS2-1	5	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-2	11	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-3	12	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-4	28	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-5	31	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-6	37	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-7	66	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-8	317	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-9	419	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-10	1328	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-11	1476	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34
CS2-12	1730	ActiPro	Cell Boost™ 7a/7b	5% (7a), 0.5% (7b)	10.0	34

Culture Station	Clone	Medium	Feed	Feed Strategy	Seeding Density (x 10⁵ cells/mL)	Temp Shift (°C)
CS3-1	5	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-2	11	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-3	12	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-4	28	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-5	31	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-6	37	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-7	66	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-8	317	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-9	419	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-10	1328	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-11	1476	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS3-12	1730	Fed-Batch	Feed1/4FEED	7.5% feed blend	6.0	34
CS4-1	5	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34
CS4-2	11	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34
CS4-3	12	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34

Culture Station	Clone	Medium	Feed	Feed Strategy	Seeding Density (x 10 ⁵ cells/mL)	Temp Shift (°C)
CS4-4	28	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34
CS4-5	31	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34
CS4-6	37	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34
CS4-7	66	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34
CS4-8	317	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34
CS4-9	419	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34
CS4-10	1328	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34
CS4-11	1476	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34
CS4-12	1730	Fed-Batch	Feed1/4FEED	7.5% feed blend	10.0	34

[00480] The results comparing the clone number relative to the cumulative cell density (CCD) are shown, the clone number relative to the titer i, and the clone number relative to rQp. Based on the analysis of the Ambr15™ production data, the Top 4 clonal candidates are Clones #31, 37, 1328, and 11 based on titer production (BioHT). On average, Fed-Batch conditions yielded higher titers than Actipro conditions, however the highest overall titer was observed in ActiPro. Further optimization of these conditions and other culture conditions could result in additional protein production.

[00481] 2.6 Overall conclusions

[00482] The results indicate that co-expression of a nuclease in this example Human DNase 1 and a second recombinant protein or antibody is possible. The production for the

antibody in these cell lines was at commercially viable levels. Based on an activity assay being performed on media harvested from the culture, the Human DNase 1 is active and capable of cleaving DNA in the environment of cell culture media. We have shown the level of DNase 1 being produced can be adjusted in order to obtain the desired effect for that particular product.

[00483] All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the disclosure will be apparent to those skilled in the art without departing from the scope and spirit of the disclosure. Although the disclosure has been described in connection with specific embodiments, it should be understood that the disclosure as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the disclosure that are obvious to those skilled in the field of this disclosure are intended to be within the scope of the following claims.

Example 3: A Comparison of Stable CHO Cell Pools Expressing the 3E10 Antibody with and without Human DNase 1

[00484] The GPEx Lightning Process was used to generate two stable cell pools expressing the 3E10 antibody. One cell pool, PP5, was designed only to express the heavy and light chain of the 3E10 antibody. The second cell pool, PP6, was designed to express the heavy and light chain of the 3E10 antibody and the human DNase 1 enzyme. After generating the two stable cell pools, they were tested for heavy and light chain gene copy index values and for productivity performance in fed-batch culture.

Table 15. Heavy and Light Chain Gene Copy Index for PP5 and PP6

Stable Cell Pool	Heavy Chain Gene Copy Index	Light Chain Gene Copy Index
PP5	4.79	5.04
PP6	4.44	4.71

[00485] The results indicated that the gene copy index values are similar for both the PP5 and PP6 cell pools. Fed-batch productivity studies were performed for each of the cell pools at Day 14. A comparison of the results for PP5 and PP6 is shown below:

Table 16. Fed-batch Productivity Studies for PP5 and PP6 at Day 14

Stable Cell Pool	Titer (mg/L) Day 14
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PP5	289
PP6	910

[00486] The results indicated that 3E10 antibody expression alone produced much lower-than-expected titers for an antibody product and the cells producing the antibody showed poorer growth characteristics when compared to cells producing a traditional antibody product. Since the 3E10 antibody binds to nucleic acids forming a complex that may be internalized by cells, it was hypothesized that DNA from dead cells in culture may be binding to secreted antibody and entering cells causing issues with expression and cell growth. It was further hypothesized that removal of DNA by having the cells secrete human DNase 1 in addition to the 3E10 antibody may improve antibody production and cell health. The comparison of the two cell pools, PP5 and PP6, support these hypotheses. The GPEx Lightning technology gene copy index is directly correlated to product titer. For the two stable pool cell lines, the PP5 (the pool without DNase 1) would be expected to produce more antibody than PP6 (the pool with DNase 1) since it has a slightly higher transgene number for both heavy and light chain genes. The opposite result was observed, with the PP6 cell pool producing 3-fold higher antibody levels than PP5 cell pool. Also, the cell growth behavior of the PP6 cell pool is more like CHO cells producing other types of antibodies than the PP5 cell pool. These results indicate that the DNase 1 co-expression approach is a much more viable method to produce the 3E10 antibody than the traditional method of just heavy and light chain expression in CHO cells.

Example 4: The use of exogenous DNase to achieve 3E10 expression in a mammalian cell culture

[00487] In an experiment, a purified exogenous endonuclease enzyme, e.g., deoxyribonuclease-I (DNase I), was added directly to the culture medium of a mammalian cell culture of cells transiently or stably transfected for expression of a 3E10 antibody or fragments thereof. Specifically, purified DNase I was added at a concentration of 0-10µg/ml directly into cell culture medium of HEK293 cells transiently transfected with plasmids encoding either a chimeric or a humanized antibody variant of 3E10 one day after transfection. As a control, a mutational variant, designated R92, that lacks the potential to bind nucleic acid, was included in the experiment. To assess the effect of DNase treatment on protein secretion, a plasmid encoding the secreted alkaline phosphatase protein (SEAP) was included. At time of cell harvest, supernatants were evaluated for production of antibodies. As shown in the Figure 31A, significant expression of IgG1 in the supernatant (µg/ml) was observed for 3E10 variants (D31N or V66) in the presence of the exogenous

DNase treatment. Reduced expression of IgG1 in the supernatant ($\mu\text{g/ml}$) was detected in untreated samples (without DNase) for these variants, and the R92 control was shown to express IgG1 in the supernatant regardless of treatment. It was further observed that administering DNase to transfected cells did not significantly affect the expression and secretion of SEAP (Figure , further supporting a favorable effect of DNase on the expression and retention of secreted anti-DNA binding 3E10 antibody. Dosing in transiently transfected cells may be more frequent if the incubation period is longer than 3-4 days. Furthermore, more optimal concentrations of DNase I may be 5-10 $\mu\text{g/ml}$, 10-25 $\mu\text{g/ml}$, 25-50 $\mu\text{g/ml}$, 50-100 $\mu\text{g/ml}$ or greater than 100 $\mu\text{g/ml}$. This methodology is also applicable to stably expressed production cell lines for 3E10 and derivative antibodies. DNase may be administered at these concentrations once or at regular intervals to ensure sustained presence of DNase in the medium throughout the culture period (e.g., up to 14-21 days).

CLAIMS

What is claimed is:

1. A eukaryotic host cell comprising (i) an exogenous nucleic acid sequence encoding an endonuclease operably linked to a first promoter sequence and (ii) an exogenous nucleic acid sequence encoding a 3E10 antibody or antigen binding fragment thereof operably linked to a second promoter sequence.
2. The host cell of claim 1, wherein the host cell is selected from the group consisting of Chinese Hamster Ovary (CHO) cells, HEK 293 cells, CAP cells, bovine mammary epithelial cells, monkey kidney CV1 line transformed by SV40, baby hamster kidney cells, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor, TRI cells, MRC 5 cells, FS4 cells, rat fibroblasts, MDBK cells, and human hepatoma line cells.
3. The host cell of claim 1, wherein the host cell is selected from the group consisting of a Chinese Hamster Ovary (CHO) cell, an HEK 293 cell, and a CAP cell.
4. The host cell line of claim 1 or 2, wherein the host cell line is a glutamine synthetase (GS) knockout cell line.
5. The host cell line of any one of claims 1 to 3, wherein the host cell line is a dihydrofolate reductase (DHFR) knockout cell line.
6. The host cell of any one of claims 1 to 5, wherein the endonuclease is a DNase I endonuclease.
7. The host cell of claim 6, wherein the endonuclease is a human DNase I endonuclease.
8. The host cell of any one of claims 1 to 5, wherein the endonuclease is DNase II endonuclease.
9. The host cell of claim 8, wherein the endonuclease is a human DNase II endonuclease.

10. The host cell of any one of claims 1 to 9, wherein the sequence encoding the endonuclease is further operably linked to a secretion signal sequence.
11. The host cell of any one of claims 1 to 10, wherein the first promoter sequence is a weak promoter sequence.
12. The host cell of any one of claims 1 to 10, wherein the first promoter sequence is a cytomegalovirus immediate early (CMV-IE) promoter sequence.
13. The host cell of claim 12, wherein the CMV-IE promoter sequence is a simian CMV-IE promoter (sCMV) sequence.
14. The host cell of any one of claims 1 to 11, wherein the first promoter sequence is not a retroviral LTR promoter.
15. The host cell of any one of claims 1 to 14, wherein:
the 3E10 antibody or antigen binding fragment thereof comprises (a) a heavy chain polypeptide or fragment thereof and (b) a light chain polypeptide or fragment thereof; and
the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof comprises (1) a first exogenous nucleic acid sequence encoding the heavy chain polypeptide or fragment thereof and (2) a second exogenous nucleic acid sequence encoding the light chain polypeptide or fragment thereof.
16. The host cell of claim 15, wherein the ratio of (i) the number of first exogenous nucleic acid sequences integrated into the host cell genome to (ii) the number of second exogenous nucleic acid sequences integrated into the host cell genome is from 1:2 to 2:1.
17. The host cell of claim 15 or 16, wherein the first exogenous nucleic acid sequence is operably linked to the second promoter sequence and the second exogenous nucleic acid sequence is operably linked to a third promoter sequence.
18. The host cell of claim 17, wherein the third promoter sequence has the same nucleotide sequence as the first promoter.

19. The host cell of claim 17 or 18, wherein the third promoter sequence has the same nucleotide sequence as the second promoter.
20. The host cell of claim 17, wherein the third promoter sequence has a different nucleotide sequence as the first promoter.
21. The host cell of claim 17 or 20, wherein the third promoter sequence has a different nucleotide sequence as the second promoter.
22. The host cell of any one of claims 17 to 21, wherein the third promoter sequence is a cytomegalovirus immediate early (CMV-IE) promoter sequence.
23. The host cell of claim 22, wherein the CMV-IE promoter sequence is a simian CMV-IE promoter (sCMV) sequence.
24. The host cell of any one of claims 17 to 21, wherein the third promoter sequence is not a retroviral LTR promoter.
25. The host cell of any one of claims 1 to 24, wherein the 3E10 antibody or antigen binding fragment thereof comprises a monovalent, divalent, or multivalent single chain variable fragment (scFv).
26. The host cell of any one of claims 1 to 24, wherein the 3E10 antibody or antigen binding fragment thereof comprises an scFv-Fc polypeptide, a CrossMab polypeptide, a dual variable domain immunoglobulin (DVD-Ig), a tandem double scFv, an (scFv)₂, a single-chain tandem fragment variable (scTaFv) polypeptide, a single-chain fragment variable (scFv) polypeptide, a diabody, a tandem diabody (TandAb), a Fabsc polypeptide, a modular IgG-scFv, or an F(ab')₂.
27. The host cell of any one of claims 1 to 26, wherein the 3E10 antibody or antigen binding fragment thereof is humanized.
28. The host cell of any one of claims 1 to 27, wherein the 3E10 antibody or antigen binding fragment thereof is a bivalent antibody or fragment thereof.

29. The host cell of any one of claims 1 to 28, wherein the 3E10 antibody or antigen binding fragment thereof is a 3E10 antibody or antigen binding fragment thereof.

30. The host cell of claim 29, wherein the 3E10 antibody or antigen binding fragment thereof comprises:

a heavy chain variable region (VH) complementarity determining region (CDR) 1 comprising the amino acid sequence of SEQ ID NO:58, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:59, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:60; and

a light chain variable region (VL) CDR1 comprising the amino acid sequence of SEQ ID NO:61, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:62, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:63.

31. The host cell of claim 29, wherein the 3E10 antibody or antigen binding fragment thereof comprises:

a VH CDR1 comprising the amino acid sequence of SEQ ID NO:64, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:4, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:5; and

a VL CDR1 comprising the amino acid sequence of SEQ ID NO:9, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:10, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:11.

32. The host cell of claim 29, wherein the 3E10 antibody or antigen binding fragment thereof comprises:

a VH CDR1 comprising the amino acid sequence of SEQ ID NO:64, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:5; and

a VL CDR1 comprising the amino acid sequence of SEQ ID NO:9, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:10, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:11.

33. The host cell of any one of claims 29 to 32, wherein the 3E10 antibody or cell-penetrating variant thereof, comprises a heavy chain variable region (VH) having an amino acid sequence that is at least 90% identical to SEQ ID NO:2 or SEQ ID NO:14 and a light

chain variable region (VL) having an amino acid sequence that is at least 90% identical to SEQ ID NO:7.

34. The host cell of any one of claims 29 to 32, wherein the 3E10 antibody or cell-penetrating variant thereof, comprises a heavy chain variable region (VH) having an amino acid sequence that is at least 90% identical to SEQ ID NO:2 or SEQ ID NO:14 and a light chain variable region (VL) having an amino acid sequence that is at least 95% identical to SEQ ID NO:7.

35. The host cell of any one of claims 29 to 32, wherein the 3E10 antibody or cell-penetrating variant thereof, comprises a heavy chain variable region (VH) having an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS:104-113 and a light chain variable region (VL) having an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:114-122.

36. The host cell of any one of claims 1 to 35, wherein the second promoter sequence has the same nucleotide sequence as the first promoter.

37. The host cell of any one of claims 1 to 35, wherein the second promoter sequence has a different nucleotide sequence as the first promoter.

38. The host cell of any one of claims 1 to 37, wherein the second promoter sequence is a cytomegalovirus immediate early (CMV-IE) promoter sequence.

39. The host cell of claim 38, wherein the CMV-IE promoter sequence is a simian CMV-IE promoter (sCMV) sequence.

40. The host cell of any one of claims 1 to 37, wherein the third promoter sequence is not a retroviral LTR promoter.

41. The host cell of any one of claims 1 to 40, wherein from 2 to 500 copies each of the exogenous nucleic acid sequence encoding the endonuclease and the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof are stably integrated into the genome of the host cell.

42. The host cell of any one of claims 15 to 40, wherein from 5 to 500 copies each of the first exogenous nucleic acid sequence and at least a second exogenous nucleic acid sequence are stably integrated into the genome of the host cell.
43. The host cell of any one of claims 1 to 42, wherein the ratio of (i) the number of exogenous nucleic acid sequences encoding the endonuclease to (ii) the number of exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:1 to 1:100.
44. The host cell of any one of claims 1 to 42, wherein the ratio of (i) the number of exogenous nucleic acid sequences encoding the endonuclease to (ii) the number of exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is from 1:2 to 1:100.
45. The host cell of any one of claims 1 to 44, wherein the exogenous nucleic acid sequence encoding the endonuclease and the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof are stably integrated into the genome of the host cell at a docking site.
46. The host cell of claim 45, wherein, prior to integration of the exogenous nucleic acid sequence encoding the endonuclease and the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof into the docking site:
the docking site comprised at least one dock site insertion element; and
the exogenous nucleic acid sequence encoding the endonuclease and the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof were present in one or more transfer vector comprising at least one insertion element compatible with the at least one dock site insertion element.
47. The host cell of any one of claims 1 to 46, wherein the exogenous nucleic acid sequence encoding the endonuclease is operably linked to a first polyadenylation sequence and the exogenous nucleic acid sequence encoding the 3E10 antibody or antigen binding fragment thereof is operably linked to a second polyadenylation sequence.

48. The host cell of any one of claims 45 to 47, wherein the host cell genome comprises from 5 to 500 docking sites and each docking site comprises at least one dock site insertion element.
49. The host cell of claim 48, wherein the integrated docking sites are independently positioned throughout the host cell genome.
50. A cell culture comprising host cells of any one of claims 1 to 49.
51. A method for expressing a 3E10 antibody or antigen binding fragment thereof, the method comprising:
culturing a plurality of host cells according to any one of claims 1 to 49 in a culture medium under conditions for expression of (i) the endonuclease from the exogenous nucleic acid sequence encoding the endonuclease and (ii) the 3E10 antibody or antigen binding fragment thereof.
52. The method of claim 51, wherein the culturing is performed under fed-batch conditions.
53. A cell culture supernatant comprising the 3E10 antibody or antigen binding fragment thereof prepared according to the method of claim 51.
54. A cell culture supernatant comprising at least an antibody titer of at least 0.001 µg/mL, at least 0.01 µg/mL, at least 0.1 µg/mL, at least 0.125 µg/mL, at least 0.250 µg/mL, at least 0.5 µg/mL, at least 0.75 µg/mL, at least 1 µg/mL, at least 5 µg/mL, at least 10 µg/mL, at least 25 µg/mL, at least 50 µg/mL, or at least 100 µg/mL of the 3E10 antibody or antigen binding fragment thereof.
55. The cell culture of claim 53 or 54, wherein less than 75%, or less than 70%, or less than 65%, or less than 60%, or less than 55%, or less than 50%, or less than 45%, or less than 40%, or less than 35%, or less than 30%, or less than 25%, or less than 20%, or less than 15%, or less than 10%, or less than 5%, or 0% of the 3E10 antibody or antigen binding fragment thereof is bound to nucleic acid.

56. A composition comprising a 3E10 antibody or antigen binding fragment thereof produced according to the method of claim 51 or 52.
57. A composition comprising a 3E10 antibody or antigen binding fragment thereof purified from a cell culture supernatant of any one of claims 53-55.
58. A cell culture comprising a eukaryotic host cell comprising an exogenous nucleic acid sequence encoding a 3E10 antibody or antigen binding fragment thereof operably linked to a promoter sequence and a purified, exogenous added endonuclease.
59. The eukaryotic host cell of claim 58, wherein the eukaryotic host cell is selected from the group consisting of Chinese Hamster Ovary (CHO) cells, HEK 293 cells, CAP cells, bovine mammary epithelial cells, monkey kidney CV1 line transformed by SV40, baby hamster kidney cells, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor, TRI cells, MRC 5 cells, FS4 cells, rat fibroblasts, MDBK cells, and human hepatoma line cells.
60. The host cell of claim 59, wherein the host cell is selected from the group consisting of a Chinese Hamster Ovary (CHO) cell, an HEK 293 cell, and a CAP cell.
61. The host cell of claims 58 or 59, wherein the host cell line is a glutamine synthetase (GS) knockout cell line.
62. The host cell of any one of claims 58 to 60, wherein the host cell line is a dihydrofolate reductase (DHFR) knockout cell line.
63. The host cell of claim 58, wherein the exogenous nucleic acid sequence encoding a 3E10 antibody or antigen binding fragment is stably integrated into the host genome.
64. The host cell of claim 58, wherein the exogenous nucleic acid sequence encoding a 3E10 antibody or antigen binding fragment is expressed on one or more DNA plasmids transiently transfected into the cells.

65. The host cell of claim 64, wherein the DNA plasmids are transiently transfected into the cells by electroporation, PEI, lipofection, or calcium phosphate precipitation.
66. The host cell of any one of claims 58 to 65, wherein the purified, exogenously added endonuclease is a DNase I endonuclease.
67. The host cell of claim 66, wherein the purified, exogenously added endonuclease is a human DNase I endonuclease.
68. The host cell of claim 66 or 67, wherein the purified, exogenously added endonuclease is added at least once to a host cell culture at a concentration of 10 µg/ml.
69. The host cell of claim 66 or 67, wherein the purified, exogenously added endonuclease is added more than once to a host cell culture at a concentration of 10 µg/ml.
70. The host cell of claim 66 or 67, wherein the purified, exogenously added endonuclease is added a concentration of about 5-10 µg/ml, of about 10-25 µg/ml, of about 25-50 µg/ml, of about 50-100 µg/ml, or greater than 100 µg/ml.
71. A method of making a host cell culture comprising:
- a. a eukaryotic host cell comprising an exogenous nucleic acid sequence encoding a 3E10 antibody or antigen binding fragment thereof operably linked to a promoter sequence; and
 - b. a purified, exogenous added endonuclease added to the host cell culture.
72. The method claim 71, wherein the eukaryotic host cell is selected from the group consisting of Chinese Hamster Ovary (CHO) cells, HEK 293 cells, CAP cells, bovine mammary epithelial cells, monkey kidney CV1 line transformed by SV40, baby hamster kidney cells, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor, TRI cells, MRC 5 cells, FS4 cells, rat fibroblasts, MDBK cells, and human hepatoma line cells.
73. The host cell of claim 72, wherein the host cell is selected from the group consisting of a Chinese Hamster Ovary (CHO) cell, an HEK 293 cell, and a CAP cell.

74. The host cell of claims 71 or 72, wherein the host cell line is a glutamine synthetase (GS) knockout cell line.
75. The host cell of any one of claims 71 to 73, wherein the host cell line is a dihydrofolate reductase (DHFR) knockout cell line.
76. The host cell of claim 71, wherein the exogenous nucleic acid sequence encoding a 3E10 antibody or antigen binding fragment is stably integrated into the host genome.
77. The host cell of claim 71, wherein the exogenous nucleic acid sequence encoding a 3E10 antibody or antigen binding fragment is expressed on one or more DNA plasmids transiently transfected into the cells.
78. The host cell of claim 77, wherein the DNA plasmids are transiently transfected into the cells by electroporation, PEI, lipofection, or calcium phosphate precipitation.
79. The host cell of any one of claims 71 to 78, wherein the purified, exogenously added endonuclease is a DNase I endonuclease.
80. The host cell of claim 79, wherein the purified, exogenously added endonuclease is a human DNase I endonuclease.
81. The host cell of claim 79 or 80, wherein the purified, exogenously added endonuclease is added at least once to a host cell culture at a concentration of 10 µg/ml.
82. The host cell of claim 79 or 80, wherein the purified, exogenously added endonuclease is added more than once to a host cell culture at a concentration of 10 µg/ml.
83. The host cell of claim 79 or 80, wherein the purified, exogenously added endonuclease is added a concentration of about 5-10 µg/ml, of about 10-25 µg/ml, of about 25-50 µg/ml, of about 50-100 µg/ml, or greater than 100 µg/ml.
84. A eukaryotic host cell comprising (i) a plurality of copies of a first exogenous nucleic acid sequence integrated into the genome of the host cell, the first exogenous nucleic acid sequence encoding a first polypeptide and (ii) one or more copies of a second exogenous

nucleic acid sequence integrated into the genome of the host cell, the second exogenous nucleic acid sequence encoding a second polypeptide, wherein:

the ratio of (a) the number of copies of the first exogenous nucleic acid sequence to (b) the number of copies of the second exogenous nucleic acid sequence is at least 2:1; and the first polypeptide is a 3E10 polypeptide.

85. The eukaryotic host cell of claim 84, wherein the first polypeptide comprises a 3E10 heavy chain polypeptide or fragment thereof and the second polypeptide comprises a 3E10 light chain polypeptide or fragment thereof.

86. The eukaryotic host cell of claim 84, wherein the first polypeptide comprises a 3E10 light chain polypeptide or fragment thereof and the second polypeptide comprises a 3E10 heavy chain polypeptide or fragment thereof.

87. The eukaryotic host cell of claim 84, wherein the first polypeptide comprises a 3E10 heavy chain polypeptide or fragment thereof and the second polypeptide comprises an endonuclease.

88. The eukaryotic host cell of claim 84, wherein the first polypeptide comprises a 3E10 light chain polypeptide or fragment thereof and the second polypeptide comprises an endonuclease.

89. The host cell of claim 87 or 88, wherein the endonuclease is a DNase I endonuclease.

90. The host cell of claim 89, wherein the endonuclease is a human DNase I endonuclease.

91. The host cell of any one of claims 87 or 88, wherein the endonuclease is a DNase II endonuclease.

92. The host cell of claim 91, wherein the endonuclease is a human DNase II endonuclease.

93. The host cell of any one of claims 84 to 92, wherein the host cell encodes a 3E10 antibody or antigen binding fragment thereof.

94. The host cell of claim 93, wherein the 3E10 antibody or antigen binding fragment thereof comprises:

a heavy chain variable region (VH) complementarity determining region (CDR) 1 comprising the amino acid sequence of SEQ ID NO:58, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:59, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:60; and

a light chain variable region (VL) CDR1 comprising the amino acid sequence of SEQ ID NO:61, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:62, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:63.

95. The host cell of claim 93, wherein the 3E10 antibody or antigen binding fragment thereof comprises:

a VH CDR1 comprising the amino acid sequence of SEQ ID NO:64, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:4, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:5; and

a VL CDR1 comprising the amino acid sequence of SEQ ID NO:9, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:10, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:11.

96. The host cell of claim 93, wherein the 3E10 antibody or antigen binding fragment thereof comprises:

a VH CDR1 comprising the amino acid sequence of SEQ ID NO:64, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:5; and

a VL CDR1 comprising the amino acid sequence of SEQ ID NO:9, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:10, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:11.

97. The host cell of any one of claims 93 to 96, wherein the 3E10 antibody or cell-penetrating variant thereof, comprises a heavy chain variable region (VH) having an amino acid sequence that is at least 90% identical to SEQ ID NO:2 or SEQ ID NO:14 and a light chain variable region (VL) having an amino acid sequence that is at least 90% identical to SEQ ID NO:7.

98. The host cell of any one of claims 93 to 96, wherein the 3E10 antibody or cell-penetrating variant thereof, comprises a heavy chain variable region (VH) having an amino acid sequence that is at least 90% identical to SEQ ID NO:2 or SEQ ID NO:14 and a light chain variable region (VL) having an amino acid sequence that is at least 95% identical to SEQ ID NO:7.

99. The host cell of any one of claims 93 to 96, wherein the 3E10 antibody or cell-penetrating variant thereof, comprises a heavy chain variable region (VH) having an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS:104-113 and a light chain variable region (VL) having an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:114-122.

100. The host cell of any one of claims 84 to 99, wherein the ratio of (a) the number of copies of the first exogenous nucleic acid sequence to (b) the number of copies of the second exogenous nucleic acid sequence is at least 3:1, at least 5:1, at least 10:1, at least 25:1, at least 50:1, at least 100:1, at least 250:1, at least 500:1, or at least 1000:1.

101. The host cell of any one of claims 84 to 99, wherein the ratio of (a) the number of copies of the first exogenous nucleic acid sequence to (b) the number of copies of the second exogenous nucleic acid sequence is from 2:1 to 1000:1, from 2:1 to 500:1, from 2:1 to 250:1, from 2:1 to 100:1, from 2:1 to 50:1, from 2:1 to 25:1, from 2:1 to 10:1, or from 2:1 to 5:1.

102. A cell culture comprising host cells of any one of claims 84 to 101.

103. A method for expressing a 3E10 antibody or antigen binding fragment thereof, the method comprising:

culturing a plurality of host cells according to any one of claims 84 to 101 in a culture medium under conditions for expression of (i) the endonuclease from the exogenous nucleic acid sequence encoding the endonuclease and (ii) the 3E10 antibody or antigen binding fragment thereof.

104. The method of claim 103, wherein the culturing is performed under fed-batch conditions.

105. A cell culture supernatant comprising the 3E10 antibody or antigen binding fragment thereof prepared according to the method of claim 103 or 104.

106. A cell culture supernatant comprising at least an antibody titer of at least 0.001 µg/mL, at least 0.01 µg/mL, at least 0.1 µg/mL, at least 0.125 µg/mL, at least 0.250 µg/mL, at least 0.5 µg/mL, at least 0.75 µg/mL, at least 1 µg/mL, at least 5 µg/mL, at least 10 µg/mL, at least 25 µg/mL, at least 50 µg/mL, or at least 100 µg/mL of a 3E10 antibody or antigen binding fragment thereof.

107. The cell culture of claim 105 or 106, wherein less than 75%, or less than 70%, or less than 65%, or less than 60%, or less than 55%, or less than 50%, or less than 45%, or less than 40%, or less than 35%, or less than 30%, or less than 25%, or less than 20%, or less than 15%, or less than 10%, or less than 5%, or 0% of the 3E10 antibody or antigen binding fragment thereof is bound to nucleic acid.

108. A composition comprising a 3E10 antibody or antigen binding fragment thereof produced according to the method of claim 103 or 104.

109. A composition comprising a 3E10 antibody or antigen binding fragment thereof purified from a cell culture supernatant of any one of claims 105-107.

110. A method comprising:

introducing at least first nucleic acid constructs encoding a first protein or nucleic acid of interest and second nucleic acid constructs encoding a second protein or nucleic acid of interest at a ratio of first nucleic acid constructs to second nucleic acid constructs of from 1 to 1000:1 into a host cell having genome comprising from 1 to 500 integrated docking sites, each docking site comprising at least one dock site insertion element and the nucleic acid constructs each comprising at least one insertion element compatible with the at least one dock site insertion element in the integrated docking sites, under conditions such that the nucleic acid expression constructs are inserted at the dock sites at a ratio of first nucleic acid constructs to second nucleic acid constructs of at least 1:1, wherein the first protein of interest or the second protein of interest comprises (i) a 3E10 heavy chain or CDR-containing fragment thereof, or (ii) a 3E10 light chain or CDR-containing fragment thereof.

111. The method of claim 110, wherein the ratio of first nucleic acid constructs to second nucleic acid constructs is from 2:1 to 1000:1.
112. The method of claim 110, wherein the ratio of first nucleic acid constructs to second nucleic acid constructs is from 5:1 to 500:1.
113. The method of claim 110, wherein the ratio of first nucleic acid constructs to second nucleic acid constructs is from 10:1 to 200:1.
114. The method of claim 110, wherein the ratio of first nucleic acid constructs to second nucleic acid constructs is from 10:1 to 100:1.
115. The method of any one of claims 110 to 114, wherein the first protein of interest is a 3E10 heavy chain or CDR-containing fragment thereof and the second protein of interest is a 3E10 light chain or CDR-containing fragment thereof.
116. The method of any one of claims 110 to 114, wherein the first protein of interest comprises (i) a 3E10 heavy chain or CDR-containing fragment thereof, or (ii) a 3E10 light chain or CDR-containing fragment thereof, and the second protein of interest is an endonuclease
117. The method of any one of claims 110 to 116, wherein only first nucleic acid constructs and second nucleic acid constructs are introduced into the host cell.
118. The method of any one of claims 110 to 116, further comprising introducing a third nucleic acid construct encoding a third protein of interest at a ratio of first nucleic acid construct or second nucleic acid construct to third nucleic acid construct selected from the group consisting of at least 1:1, at least 2:1, from 2:1 to 1000:1, from 5:1 to 500:1, from 10:1 to 200:1, and from 10:1 to 100:1.
119. The method of claim 118, further comprising introducing a fourth nucleic acid construct encoding a fourth protein of interest at a ratio of first nucleic acid construct, second nucleic acid construct, or third nucleic acid construct to the fourth nucleic construct selected from the group consisting of at least 1:1, at least 2:1, from 2:1 to 1000:1, from 5:1 to 500:1, from 10:1 to 200:1, and from 10:1 to 100:1.

120. The method of claim 119, further comprising introducing a fifth nucleic acid construct encoding a fifth protein of interest at a ratio of first nucleic acid construct, second nucleic acid construct, third nucleic acid construct or fourth nucleic construct to the fifth nucleic acid construct selected from the group consisting of at least 1:1, at least 2:1, from 2:1 to 1000:1, from 5:1 to 500:1, from 10:1 to 200:1, and from 10:1 to 100:1.

121. The method of any one of claims 110 to 120, wherein the at least first and second nucleic acid constructs further comprise at least the following elements in operable association in 5' to 3' order:

an internal promoter sequence;

a nucleic acid sequence encoding the first protein of interest or second protein that is operably linked to the internal promoter; and

a poly A signal sequence.

122. The method of claim 121, wherein the at least first and second nucleic acid constructs comprise a selectable marker sequence.

123. The method of claim 122, wherein the at least first and second nucleic acid constructs comprise different selectable marker sequences.

124. The method of claim 121, wherein one of the first and second nucleic acid constructs comprises a selectable marker sequence and the other of the first and second nucleic acid constructs does not comprise a selectable marker sequence.

125. The method of any one of claims 121 to 122, wherein the selectable marker sequences are 5' to the internal promoter sequence and are operably linked to a 5' promoter sequence.

126. The method of any one of claims 121-124, wherein the nucleic acid construct comprises an extending packaging region (EPR) between the 5' promoter and the selectable marker.

127. The method of claim 126, wherein the EPR comprises multiple potential Kozak sequences and/or ATG translation start sites.

128. The method of any one of claims 121 to 127, wherein the promoter sequence is selected from the group consisting of SIN-LTR, SV40, EF1 α , *E. coli* lac, *E. coli* trp, phage lambda PL, phage lambda PR, T3, T7, cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, alpha-lactalbumin, and mouse metallothionein-I promoter sequences.
129. The method of any one of claims 121 to 127, wherein the first promoter sequence is a weak promoter sequence.
130. The method of any one of claims 121 to 129, wherein the first promoter sequence is not a retroviral LTR promoter.
131. The method of any one of claims 121 to 130, wherein the integrated docking sites further comprise an exogenous promoter.
132. The method of claim 131, wherein the exogenous promoter is selected from the group consisting of SIN-LTR, SV40, EF1 α , *E. coli* lac, *E. coli* trp, phage lambda PL, phage lambda PR, T3, T7, cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, alpha-lactalbumin, and mouse metallothionein-I promoter sequences.
133. The method of claim 132, wherein the promoter is a retroviral LTR.
134. The method of claim 133, wherein the retroviral LTR is a SIN LTR.
135. The method of any one of claims 110 to 134, wherein the nucleic acid expression constructs are provided in a vector.
136. The method of claim 135, wherein the vector is a plasmid vector.
137. The method of any one of claims 135 to 136, wherein the vector is transiently introduced into the host cell.
138. The method of any one of claims 110 to 137, wherein the host cell line comprises a nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site.

139. The method of claim 138, wherein the nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is transiently introduced into the host cell.

140. The method of claims 138 or 139, wherein the nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is provided in a vector.

141. The method of claim 140, wherein the vector is a plasmid vector.

142. The method of any one of claims 138 to 141, wherein the ratio of the ratio of the nucleic acid constructs encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site to the nucleic acid expression constructs encoding a first protein of interest that are transiently introduced into the host cell is from 1:1000 to 1:10.

143. The method of any one of claims 138 to 141, wherein the enzyme is selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.

144. The method of any one of claims 138 to 143, wherein the nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is provided in a vector.

145. The method of any one of claims 138 to 144, wherein the host cell genome comprises from 5 to 500 integrated docking sites, each docking site comprising at least one dock site insertion element.

146. The method of any one of claims 138 to 145, wherein the host cell genome comprises from 5 to 250 integrated docking sites, each docking site comprising at least one dock site insertion element.

147. The method of any one of claims 110 to 146, wherein the host cell genome comprises from 5 to 100 integrated docking sites, each docking site comprising at least one dock site insertion element.

148. The method of any one of claims 110 to 147, wherein the integrated docking sites are independently positioned throughout the host cell genome.

149. The method of any one of claims 110 to 148, wherein the dock site insertion element is targeted by enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.

150. The method of any one of claims 110 to 149, wherein the dock site insertion element is selected from the group consisting of a recombinase dock site insertion element and a HDR dock site insertion element.

151. The method of claim 150, wherein the dock site insertion element is a recombinase dock site insertion element.

152. The method of claim 151, wherein the recombinase dock site insertion element comprises an attachment site (att).

153. The method of claim 152, wherein the attachment site (att) is selected from the group consisting of attB and attP and attR and attL.

154. The method of claim 151, wherein the recombinase dock site insertion element comprises a LoxP sequence.

155. The method of claim 151, wherein the recombinase dock site insertion element is a Flp Recombination Target (FRT) site.

156. The method of claim 150, wherein the dock site insertion element is a HDR dock site insertion element.

157. The method of claim 156, wherein the HDR dock site insertion element comprises one or two dock site homology arms.

158. The method of claim 157, wherein the HDR dock site insertion element further comprises one or more sequences homologous to a guide RNA sequence.

159. The method of any of claims 157 to 158, wherein the dock site homology arms are from about 30 to 1000 bases in length.
160. The method of claim 159, wherein the integrase dock site insertion element comprises an AAVS1 safe harbor locus sequence.
161. The method of any one of claims 110 to 160, wherein each docking site is flanked by exogenous integrating vector sequences.
162. The method of claim 161, wherein the exogenous integrating vector sequences are selected from the group consisting of viral vector sequences and transposon vector sequences.
163. The method of any one of claims 110 to 162, wherein the docking sites each further comprise a sequence encoding a selectable marker operably linked to a promoter.
164. The method of any one of claims 110 to 163, wherein the host cell further comprises an expression construct encoding an exogenous enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.
165. The method of claim 164, wherein the expression construct encoding an exogenous enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase is provided in an episomal expression vector.
166. The method of claim 165, wherein the expression construct encoding an exogenous enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase is integrated into the host cell genome.
167. The method of any one of claims 110 to 166, wherein the dock site insertion element is positioned to facilitate cassette exchange.
168. The method of any one of claims 110 to 167, wherein each docking site comprises two dock site insertion elements.
169. The method of claim 168, wherein the two dock site insertion elements are positioned to facilitate cassette exchange.

170. The method of any one of claims 168 to 169, wherein the two dock site insertion elements flank sequences encoding a selectable marker, an enzyme, or a combination thereof.
171. The method of any one of claims 168 to 169, wherein the nucleic acid expression constructs further comprise a signal peptide sequence operably linked to the first protein of interest.
172. The method of claim 171, wherein the signal peptide sequence is selected from the group consisting of tissue plasminogen activator, human growth hormone, lactoferrin, alpha-casein and alpha-lactalbumin signal peptide sequences.
173. The method of any one of claims 110 to 172, wherein the nucleic acid expression constructs further comprise a protein purification marker sequence.
174. The method of claim 173, wherein the protein purification marker sequence is a hexahistidine tag or a hemagglutinin (HA) tag.
175. The method of any one of claims 110 to 174, wherein the host cell is selected from the group consisting of Chinese Hamster Ovary (CHO) cells, HEK 293 cells, CAP cells, bovine mammary epithelial cells, monkey kidney CV1 line transformed by SV40, baby hamster kidney cells, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor, TRI cells, MRC 5 cells, FS4 cells, rat fibroblasts, MDBK cells and human hepatoma line cells.
176. The method of claim 175, wherein the host cell is selected from the group consisting of a Chinese Hamster Ovary (CHO) cells, a HEK 293 cells and a CAP cells.
177. The method of any one of claims 175 to 176, wherein the host cell is a GS knockout cell line.
178. The method of any one of claims 175 to 176, wherein the host cell is a DHFR knockout cell line.
179. A cell culture comprising host cells made the method on any of claims 110 to 178.

180. A process for producing a protein of interest comprising culturing host cells according to claim 179 under conditions that the protein of interest is expressed and purifying the protein of interest from the host cell culture.

181. The process of claim 180, wherein the host cells are grown in a medium comprising an inhibitor of the selectable marker.

182. The process of claim 181, wherein the selectable marker is GS and the inhibitor is phosphinothricin or methionine sulfoximine (Msx).

183. The process of claim 181, wherein the selectable marker is DHFR and the inhibitor is methotrexate.

184. A host cell comprising:

 a plurality of docking sites integrated into the genome of the host cell, each docking site comprising at least one dock site insertion element;

 at least integrated first nucleic acid constructs comprising at least one insertion element compatible with the dock site insertion element and encoding a first protein or nucleic acid of interest, and

 at least integrated second nucleic acid constructs comprising at least one insertion element compatible with the dock site insertion and encoding a second protein or nucleic acid of interest, wherein:

 the first protein of interest or the second protein of interest comprises (i) a 3E10 heavy chain or CDR-containing fragment thereof, or (ii) a 3E10 light chain or CDR-containing fragment thereof, and

 the at least integrated first nucleic acid constructs and the at least second integrated nucleic acid constructs are integrated at the plurality of docking sites at a ratio of first nucleic acid constructs to second nucleic acid constructs of at least 1:1.

185. The host cell of claim 184, wherein the ratio of first nucleic acid constructs to second nucleic acid constructs is from 2:1 to 1000:1.

186. The host cell of claim 184, wherein the ratio of first nucleic acid constructs to second nucleic acid constructs is from 5:1 to 500:1.

187. The host cell of claim 184, wherein the ratio of first nucleic acid constructs to second nucleic acid constructs is from 10:1 to 200:1.

188. The host cell of claim 184, wherein the ratio of first nucleic acid constructs to second nucleic acid constructs is from 10:1 to 100:1.

189. The method of any one of claims 184 to 188, wherein the first protein of interest is a 3E10 heavy chain or CDR-containing fragment thereof and the second protein of interest is a 3E10 light chain or CDR-containing fragment thereof.

190. The method of any one of claims 184 to 188, wherein the first protein of interest comprises (i) a 3E10 heavy chain or CDR-containing fragment thereof, or (ii) a 3E10 light chain or CDR-containing fragment thereof, and the second protein of interest is an endonuclease

191. The host cell of any one of claims 184 to 190, wherein only first nucleic acid constructs and second nucleic acid constructs are introduced into the host cell.

192. The host cell of any one of claims 184 to 191, further comprising a third nucleic acid construct encoding a third protein of interest at a ratio of first nucleic acid construct or second nucleic acid construct to third nucleic acid construct selected from the group consisting of at least 1:1, at least 2:1, from 2:1 to 1000:1, from 5:1 to 500:1, from 10:1 to 200:1, and from 10:1 to 100:1.

193. The host cell of claim 192, further comprising a fourth nucleic acid construct encoding a fourth protein of interest at a ratio of first nucleic acid construct, second nucleic acid construct, or third nucleic acid construct to the fourth nucleic construct selected from the group consisting of at least 1:1, at least 2:1, from 2:1 to 1000:1, from 5:1 to 500:1, from 10:1 to 200:1, and from 10:1 to 100:1.

194. The host cell of claim 193, further comprising a fifth nucleic acid construct encoding a fifth protein of interest at a ratio of first nucleic acid construct, second nucleic acid construct, third nucleic acid construct or fourth nucleic construct to the fifth nucleic acid

construct selected from the group consisting of at least 1:1, at least 2:1, from 2:1 to 1000:1, from 5:1 to 500:1, from 10:1 to 200:1, and from 10:1 to 100:1.

195. The host cell of any one of claims 184 to 194, wherein the at least first and second nucleic acid constructs further comprise at least the following elements in operable association in 5' to 3' order:

an internal promoter sequence;

a nucleic acid sequence encoding the first protein of interest or second protein that is operably linked to the internal promoter; and

a poly A signal sequence.

196. The host cell of claim 195, wherein the at least first and second nucleic acid constructs comprise a selectable marker sequence.

197. The host cell of claim 196, wherein the at least first and second nucleic acid constructs comprise different selectable marker sequences.

198. The host cell of claim 196, wherein one of the first and second nucleic acid constructs comprises a selectable marker sequence and the other of the first and second nucleic acid constructs does not comprise a selectable marker sequence.

199. The host cell of any one of claims 195 to 198, wherein the selectable marker sequences are 5' to the internal promoter sequence and are operably linked to a 5' promoter sequence.

200. The host cell of any one of claims 195 to 198, wherein the nucleic acid construct comprises an extending packaging region (EPR) between the 5' promoter and the selectable marker.

201. The host cell of claim 200, wherein the EPR comprises multiple potential Kozak sequences and/or ATG translation start sites.

202. The host cell of any one of claims 195 to 201, wherein the promoter sequence is selected from the group consisting of SIN-LTR, SV40, EF1 α , *E. coli* lac, *E. coli* trp, phage lambda PL, phage lambda PR, T3, T7, cytomegalovirus (CMV) immediate early, herpes

simplex virus (HSV) thymidine kinase, alpha-lactalbumin, and mouse metallothionein-I promoter sequences.

203. The host cell of any one of claims 195 to 202, wherein the first promoter sequence is a weak promoter sequence.

204. The host cell of any one of claims 195 to 203, wherein the first promoter sequence is not a retroviral LTR promoter.

205. The host cell of any one of claims 195 to 204, wherein the integrated docking sites further comprise an exogenous promoter.

206. The host cell of claim 205, wherein the exogenous promoter is selected from the group consisting of SIN-LTR, SV40, EF1 α , *E. coli* lac, *E. coli* trp, phage lambda PL, phage lambda PR, T3, T7, cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, alpha-lactalbumin, and mouse metallothionein-I promoter sequences.

207. The host cell of claim 206, wherein the promoter is a retroviral LTR.

208. The host cell of claim 207, wherein the retroviral LTR is a SIN LTR.

209. The host cell of any one of claims 184 to 208, wherein the nucleic acid expression constructs are provided in a vector.

210. The host cell of claim 209, wherein the vector is a plasmid vector.

211. The host cell of any one of claims 184 to 210, wherein the host cell comprises a nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site.

212. The host cell of claim 211, wherein the nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is provided in a vector.

213. The host cell of claim 211, wherein the vector is a plasmid vector.

214. The host cell of any one of claims 210 to 213, wherein the ratio of the ratio of the nucleic acid constructs encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site to the nucleic acid expression constructs encoding a first protein of interest that are transiently introduced into the host cell is from 1:1000 to 1:10.
215. The host cell of any one of claims 210 to 214, wherein the enzyme is selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.
216. The host cell of any one of claims 210 to 215, wherein the nucleic acid construct encoding an enzyme that facilitates insertion of the nucleic acid expression construct at the dock site is provided in a vector.
217. The host cell of any one of claims 184 to 216, wherein the host cell genome comprises from 5 to 500 integrated docking sites, each docking site comprising at least one dock site insertion element.
218. The host cell of any one of claims 184 to 217, wherein the host cell genome comprises from 5 to 250 integrated docking sites, each docking site comprising at least one dock site insertion element.
219. The host cell of any one of claims 184 to 218, wherein the host cell genome comprises from 5 to 100 integrated docking sites, each docking site comprising at least one dock site insertion element.
220. The host cell of any one of claims 184 to 219, wherein the integrated docking sites are independently positioned throughout the host cell genome.
221. The host cell of any one of claims 184 to 220, wherein the dock site insertion element is targeted by enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.
222. The host cell of any one of claims 184 to 221, wherein the dock site insertion element is selected from the group consisting of a recombinase dock site insertion element and a HDR dock site insertion element.

223. The host cell of claim 222, wherein the dock site insertion element is a recombinase dock site insertion element.
224. The host cell of claim 223, wherein the recombinase dock site insertion element comprises an attachment site (att).
225. The host cell of claim 224, wherein the attachment site (att) is selected from the group consisting of attB and attP and attR and attL.
226. The host cell of claim 223, wherein the recombinase dock site insertion element comprises a LoxP sequence.
227. The host cell of claim 223, wherein the recombinase dock site insertion element is a Flp Recombination Target (FRT) site.
228. The host cell of claim 222, wherein the dock site insertion element is a HDR dock site insertion element.
229. The host cell of claim 228, wherein the HDR dock site insertion element comprises one or two dock site homology arms.
230. The host cell of claim 229, wherein the HDR dock site insertion element further comprises one or more sequences homologous to a guide RNA sequence.
231. The host cell of any of claims 229 to 230, wherein the dock site homology arms are from about 30 to 1000 bases in length.
232. The host cell of claim 221, wherein the integrase dock site insertion element comprises an AAVS1 safe harbor locus sequence.
233. The host cell of any one of claims 184 to 232, wherein each docking site is flanked by exogenous integrating vector sequences.
234. The host cell of claim 233, wherein the exogenous integrating vector sequences are selected from the group consisting of viral vector sequences and transposon vector sequences.

235. The host cell of any one of claims 184 to 234, wherein the docking sites each further comprise a sequence encoding a selectable marker operably linked to a promoter.
236. The host cell of any one of claims 184 to 235, wherein the host cell further comprises an expression construct encoding an exogenous enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase.
237. The host cell of claim 236, wherein the expression construct encoding an exogenous enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase is provided in an episomal expression vector.
238. The host cell of claim 236, wherein the expression construct encoding an exogenous enzyme selected from the group consisting of an integrase, a recombinase, a nuclease and a nickase is integrated into the host cell genome.
239. The host cell of any one of claims 184 to 238, wherein the dock site insertion element is positioned to facilitate cassette exchange.
240. The host cell of any one of claims 184 to 239, wherein each docking site comprises two dock site insertion elements.
241. The host cell of claim 240, wherein the two dock site insertion elements are positioned to facilitate cassette exchange.
242. The host cell of any one of claims 240 to 241, wherein the two dock site insertion elements flank sequences encoding a selectable marker, an enzyme, or a combination thereof.
243. The host cell of any one of claims 184 to 242, wherein the nucleic acid expression constructs further comprise a signal peptide sequence operably linked to the first protein of interest.
244. The host cell of claim 243, wherein the signal peptide sequence is selected from the group consisting of tissue plasminogen activator, human growth hormone, lactoferrin, alpha-casein and alpha-lactalbumin signal peptide sequences.

245. The host cell of any one of claims 184 to 244, wherein the nucleic acid expression constructs further comprise a protein purification marker sequence.
246. The host cell of claim 245, wherein the protein purification marker sequence is a hexahistidine tag or a hemagglutinin (HA) tag.
247. The host cell of any one of claims 184 to 246, wherein the host cell is selected from the group consisting of Chinese Hamster Ovary (CHO) cells, HEK 293 cells, CAP cells, bovine mammary epithelial cells, monkey kidney CV1 line transformed by SV40, baby hamster kidney cells, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor, TRI cells, MRC 5 cells, FS4 cells, rat fibroblasts, MDBK cells and human hepatoma line cells.
248. The host cell of claim 247, wherein the host cell is selected from the group consisting of a Chinese Hamster Ovary (CHO) cells, a HEK 293 cells and a CAP cells.
249. The host cell of any one of claims 247 to 248, wherein the host cell is a GS knockout cell line.
250. The host cell of any one of claims 247 to 248, wherein the host cell is a DHFR knockout cell line.
251. A cell culture comprising host cells of any of claims 184 to 250.
252. A process for producing a protein of interest comprising culturing host cells according to any of claims 184 to 250 under conditions that the protein(s) of interest are expressed and purifying the protein(s) of interest from the host cell culture.
253. The process of claim 252, wherein the host cells are grown in a medium comprising an inhibitor of the selectable marker.
254. The process of claim 252, wherein the selectable marker is GS and the inhibitor is phosphinothricin or methionine sulfoximine (Msx).

255. The process of claim 252, wherein the selectable marker is DHFR and the inhibitor is methotrexate.