CLAIMS

1. A method for expressing a plurality of target polypeptides at a high level, the method comprising:

(a) culturing a plurality of mammalian host cells, each comprising a plurality of recombinant polynucleotides, wherein

each recombinant polynucleotide comprises a promoter, a nucleotide sequence encoding a unique cell surface marker polypeptide, and a nucleotide sequence encoding a unique target polypeptide, wherein both the nucleotide sequence encoding the unique cell surface marker polypeptide and the nucleotide sequence encoding the unique target polypeptide are transcribed on the same mRNA,

wherein the culturing is under conditions that permit expression of each unique cell surface marker polypeptide on the surface of the mammalian host cells and expression of each unique target polypeptide;

(b) contacting the cultured mammalian host cells of step (a) with a plurality of detectable agents, each uniquely capable of binding one or more of the unique cell surface marker polypeptides expressed on the surface of the mammalian host cells;

(c) performing at least one round of fluorescence-activated cell sorting on the contacted cells from step (b), thereby selecting one or more mammalian host cells that are uniquely bound by at least one of the plurality of detectable agents;

(d) preparing one or more clonal populations of the mammalian host cells selected in step (c);

(e) analyzing one or more clonal populations from step (d) by detecting level of expression of at least two unique cell surface marker polypeptides on each of said clonal populations;

(f) selecting one or more clonal populations having high expression level of the at least two unique cell surface marker polypeptides; and

(g) culturing one or more clonal populations selected in step (f) under conditions that permit expression of the plurality of target polypeptides at a high level.

2. The method of claim 1, wherein step (c) comprises performing a single round of fluorescence-activated cell sorting on the contacted cells from step (b) with at least a first

detectable agent and a second detectable agent, thereby selecting one or more mammalian host cells that are uniquely bound by at least both the first detectable agent and the second detectable agent; or

wherein step (c) comprises

(c1) performing a first round of fluorescence-activated cell sorting on at least a first cell surface marker polypeptide, thereby selecting one or more mammalian host cells that are bound by at least a first detectable agent; and

(c2) performing a second round of fluorescence-activated cell sorting, on cells selected in step (c1), on at least a second cell surface marker polypeptide, thereby selecting one or more mammalian host cells that are bound by at least both the first detectable agent and the second detectable agent.

3. The method of claim 1 or 2, wherein:

(a) the step (e) is performed 7-28 days after step (d);

(b) the analyzing in step (e) comprises flow cytometry;

(c) the expression level of at least one of the plurality of unique cell surface marker polypeptides in step (f) is higher than a corresponding expression level of at least 70 % of the clonal populations analyzed in step (e);

(d) the expression level of each of the plurality of unique cell surface marker polypeptides in step (f) is higher than a corresponding expression level of at least 70 % of the clonal populations analyzed in step (e);

(e) the expression level of at least a first of the plurality of unique cell surface polypeptides in step (f) is higher than the expression level of at least a second of the plurality of unique cell surface polypeptides in step (f); and/or

(f) wherein the expression level of a first of the plurality of unique cell surface polypeptides in step (f) is higher than the expression level of a second of the plurality of unique cell surface polypeptides in step (f).

4. The method of any one of claims 1 to 3, further comprising:

(h) isolating at least a first unique target polypeptide and a second unique target polypeptide expressed in step (g) from the one or more selected clonal populations or from cell culture medium in which the one or more selected clonal populations are cultured.

5. The method of any one of claims 1 to 4, wherein the plurality of unique target polypeptides comprises 2 to 8 unique target polypeptides, optionally wherein the plurality of unique target polypeptides comprises 2 to 4 unique target polypeptides, the plurality of unique target polypeptides comprises 2 unique target polypeptides, the plurality of unique target polypeptides comprises 3 unique target polypeptides, or the plurality of unique target polypeptides comprises 4 unique target polypeptides.

6. The method of any one of claims 1 to 5, wherein

each recombinant polynucleotide comprises, from 5' to 3', the promoter, the nucleotide sequence encoding the unique cell surface marker polypeptide, and the nucleotide sequence encoding the unique target polypeptide;

each recombinant polynucleotide comprises 1 to 4 promoters, 1 to 4 nucleotide sequences each encoding a unique cell surface marker polypeptide, and 1 to 4 nucleotide sequences each encoding a unique target polypeptide, wherein one of the nucleotide sequences encoding a unique cell surface marker polypeptide is transcribed on the same mRNA as one of the nucleotide sequences encoding a unique target polypeptide;

each recombinant polynucleotide is a bicistronic polynucleotide comprising two promoters and two nucleotide sequences each encoding a unique target polypeptide, and further comprising one or two nucleotide sequences each encoding a unique cell surface marker polypeptide;

each recombinant polynucleotide is a bicistronic polynucleotide comprising two promoters and two nucleotide sequences each encoding a unique target polypeptide, and further comprising one or two nucleotide sequences each encoding a unique cell surface marker polypeptide;

each bicistronic polynucleotide comprises a single nucleotide sequence encoding a unique cell surface marker polypeptide;

each bicistronic polynucleotide comprises two nucleotide sequences each encoding a unique cell surface marker polypeptide, and wherein each one of the nucleotide sequences encoding a unique cell surface marker polypeptide is transcribed on the same mRNA as one of the nucleotide sequences encoding a unique target polypeptide;

each recombinant polynucleotide comprises, from 5' to 3', one of the from 1-4 promoters, one of the from 1-4 nucleotide sequences encoding a unique cell surface marker polypeptide, and one of the from 1-4 nucleotide sequences encoding a unique target polypeptide;

each recombinant polynucleotide comprises one promoter, one nucleotide sequence encoding a unique cell surface marker polypeptide, and one nucleotide sequence encoding a unique target polypeptide, wherein the nucleotide sequence encoding a unique cell surface marker polypeptide is transcribed on the same mRNA as the nucleotide sequence encoding a unique target polypeptide;

each recombinant polynucleotide further comprises an internal ribosome entry site (IRES) located 3' to the nucleotide sequence encoding the unique cell surface marker polypeptide and 5' to the nucleotide sequence encoding the unique target polypeptide; and/or

each recombinant polynucleotide further comprises an alternate (non-ATG) start codon for translation initiation of the nucleotide sequence encoding the unique cell surface marker polypeptide and an ATG start codon for translation initiation of the nucleotide sequence encoding the unique target polypeptide, optionally wherein each alternate (non-ATG) start codon is selected independently from the group consisting of CTG, GTG, TTG, ATT, ATA, and ACG, each alternate (non-ATG) start codon is selected independently from the group consisting of GTG and TTG, or wherein the nucleotide sequence encoding the unique cell surface marker polypeptide is devoid of any ATG triplets.

7. The method of any one of claims 1 to 6, wherein

at least a first recombinant polynucleotide comprises, from 5' to 3', a first promoter, a nucleotide sequence encoding a first unique cell surface marker polypeptide, and a nucleotide sequence encoding a first unique target polypeptide optionally wherein

the first recombinant polynucleotide further comprises an internal ribosome entry site (IRES) located 3' to the nucleotide sequence encoding the first unique cell surface marker polypeptide and 5' to the nucleotide sequence encoding the first unique target polypeptide, or

the first recombinant polynucleotide further comprises an alternate (non-ATG) start codon for translation initiation of the nucleotide sequence encoding the first unique cell surface marker polypeptide and an ATG start codon for translation initiation of the nucleotide sequence encoding the first unique target polypeptide; and

at least a second recombinant polynucleotide comprises, from 5' to 3', a second promoter, a nucleotide sequence encoding a second unique target polypeptide, and a nucleotide sequence encoding a second unique cell surface marker polypeptide, optionally wherein

the second recombinant polynucleotide further comprises an internal ribosome entry site (IRES) located 3' to the nucleotide sequence encoding the second unique target polypeptide and 5' to the nucleotide sequence encoding the second unique cell surface marker polypeptide; and/or

wherein each recombinant polynucleotide comprises, from 5' to 3', the promoter, the nucleotide sequence encoding the unique target polypeptide, and the nucleotide sequence encoding the unique cell surface marker polypeptide, optionally wherein each recombinant polynucleotide further comprises an internal ribosome entry site (IRES) located 3' to the nucleotide sequence encoding the unique target polypeptide and 5' to the nucleotide sequence encoding the unique cell surface marker polypeptide.

8. The method of any one of claims 1 to 7, wherein at least one promoter is a β-actin promoter, each promoter is a β-actin promoter, at least one promoter is a hamster β-actin promoter, or each promoter is a hamster β-actin promoter.

9. The method of any one of claims 1 to 8, wherein

at least a first unique cell surface marker polypeptide is selected from the group consisting of CD20, CD52, CD59, and variants thereof;

at least a second unique cell surface marker polypeptide is selected from the group consisting of CD20, CD52, CD59, and variants thereof;

each unique cell surface marker polypeptide is selected from the group consisting of CD20, CD52, CD59, and variants thereof;

at least a first unique cell surface marker polypeptide is selected from the group consisting of human CD52 and variants thereof;

at least a second unique cell surface marker polypeptide is selected from the group consisting of human CD52 and variants thereof;

each unique cell surface marker polypeptide is selected from the group consisting of human CD52 and variants thereof;

at least a first unique cell surface marker polypeptide is human CD52; and/or

at least a second unique cell surface marker polypeptide is human CD52.

10. The method of any one of claims 1 to 9, wherein at least one unique cell surface marker polypeptide is a variant human CD52 comprising:

(a) the amino acid sequence set forth in SEQ ID NO: 19 ($GX_2NDTSQTSSPS$) wherein X_2 is selected from the group consisting of A, G, I, L, and V, optionally wherein X_2 is A;

(b) the amino acid sequence set forth in SEQ ID NO: 20 ($GQNX_4TSQTSSPS$) wherein X_4 is selected from the group consisting of A, G, I, L, and V, optionally wherein X_4 is A;

(c) the amino acid sequence set forth in SEQ ID NO: 21 ($GQNDTSX_7TSSPS$) wherein X_7 is selected from the group consisting of A, G, I, L, and V, optionally wherein X_7 is A;

(d) the amino acid sequence set forth in SEQ ID NO: 22 ($GQNDTSQX_8SSPS$) wherein X_8 is selected from the group consisting of A, G, I, L, and V, optionally wherein X_8 is A;

(e) the amino acid sequence set forth in SEQ ID NO: 23 ($GQNDTSQX_8X_9SPS$), wherein each of X_8 and X_9 is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_8 and X_9 is A;

(f) the amino acid sequence set forth in SEQ ID NO: 24 ($GQNDTSQX_8SX_{10}PS$), wherein each of X_8 and X_{10} is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_8 and X_{10} is A

(g) the amino acid sequence set forth in SEQ ID NO: 25 ($GQNDTSQX_8X_9X_{10}PS$), wherein each of X_8, X_9, and X_{10} is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_8, X_9, and X_{10} is A;

(h) the amino acid sequence set forth in SEQ ID NO: 26 ($GX_2NDTSQX_8X_9SPS$), wherein each of X_2, X_8, and X_9 is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_2, X_8, and X_9 is A;

(i) the amino acid sequence set forth in SEQ ID NO: 27 ($GX_2NDTSQX_8SX_{10}PS$), wherein each of X_2, X_8, and X_{10} is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_2, X_8, and X_{10} is A;

(j) the amino acid sequence set forth in SEQ ID NO: 28 ($GQNDTSX_7X_8X_9SPS$), wherein each of X_7, X_8, and X_9 is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_7, X_8, and X_9 is A; or

(k) the amino acid sequence set forth in SEQ ID NO: 29 ($GQNDTSX_7X_8SX_{10}PS$), wherein each of X_7, X_8, and X_{10} is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_7, X_8, and X_{10} is A.

11. The method of any one of claims 1 to 10, wherein at least a first unique cell surface marker polypeptide comprises a variant human CD52

(a) comprising the amino acid sequence set forth in SEQ ID NO: 19 ($GX_2NDTSQTSSPS$) wherein X_2 is selected from the group consisting of A, G, I, L, and V; and at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 20 ($GQNX_4TSQTSSPS$) and SEQ ID NO: 21 ($GQNDTSX_7TSSPS$) wherein each of X_4 and X_7 is independently selected from the group consisting of A, G, I, L, and V;

(b) comprising the amino acid sequence set forth in SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4 (GQNATSQTSSPS) and SEQ ID NO: 7 (GQNDTSATSSPS);

(c) consists of a variant human CD52 consisting of the amino acid sequence set forth in SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide consists of a variant human CD52 consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 4 (GQNATSQTSSPS) and SEQ ID NO: 7 (GQNDTSATSSPS)

(d) comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 4 (GQNATSQTSSPS);

(e) consists of a variant human CD52 consisting of the amino acid sequence set forth as SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide consists of a variant human CD52 consisting of the amino acid sequence set forth as SEQ ID NO: 4 (GQNATSQTSSPS);

(f) comprising the amino acid sequence set forth as SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 7 (GQNDTSATSSPS);

(g) consists of a variant human CD52 consisting of the amino acid sequence set forth as SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide consists of a variant human CD52 consisting of the amino acid sequence set forth as SEQ ID NO: 7 (GQNDTSATSSPS);

(h) comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 4 (GQNATSQTSSPS); and at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 7 (GQNDTSATSSPS);

(i) comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 20 ($GQNX_4TSQTSSPS$); at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 23 ($GX_2NDTSQX_8X_9SPS$); and at least a third unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 28 ($GQNDTSX_7X_8X_9SPS$), wherein each of X_4, X_7, X_8, and X_9 is independently selected from the group consisting of A, G, I, L, and V;

(j) comprising the amino acid sequence set forth as SEQ ID NO: 20 ($GQNX_4TSQTSSPS$); at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 27 ($GX_2NDTSQX_8SX_{10}PS$); and at least a third unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 29 ($GQNDTSX_7X_8SX_{10}PS$) wherein each of X_4, X_7, X_8, and X_{10} is independently selected from the group consisting of A, G, I, L, and V;

(k) comprising the amino acid sequence set forth as SEQ ID NO: 4 (GQNATSQTSSPS); at least a second unique cell surface marker polypeptide comprises a variant human CD52

comprising the amino acid sequence set forth as SEQ ID NO: 15 (GANDTSQAASPS); and at least a third unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 17 (GQNDTSAAASPS); or

(l) comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 4 (GQNATSQTSSPS); at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 16 (GANDTSQASAPS); and at least a third unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 18 (GQNDTSAASAPS).

12. The method of any one of claims 1 to 11, wherein at least one unique target polypeptide comprises a therapeutic polypeptide or a therapeutic protein, optionally wherein

at least one unique target polypeptide is a polypeptide of a multi-chain protein, wherein each unique target polypeptide is a polypeptide of a multi-chain protein,

at least one unique target polypeptide is a polypeptide of an antibody,

each unique target polypeptide is a polypeptide of an antibody,

at least one unique target polypeptide is a polypeptide of a crossover dual-variable domain (CODV) Ig-like protein,

each unique target polypeptide is a polypeptide of a crossover dual-variable domain (CODV) Ig-like protein,

at least one unique target polypeptide is a polypeptide of a CODV triabody, or

each unique target polypeptide is a polypeptide of a CODV triabody.

13. The method of any one of claims 1 to 12, wherein the recombinant mammalian host cell is a CHO cell.

14. An engineered mammalian host cell comprising a plurality of recombinant polynucleotides, wherein each recombinant polynucleotide comprises a promoter, a nucleotide sequence encoding a unique cell surface marker polypeptide, and a nucleotide sequence encoding a unique target polypeptide, wherein both the nucleotide sequence encoding the unique

cell surface marker polypeptide and the nucleotide sequence encoding the unique target polypeptide are transcribed on the same mRNA.

15. The engineered mammalian host cell of claim 15, wherein the plurality of unique target polypeptides comprises 2 to 8 unique target polypeptides.

16. The engineered mammalian host cell of claim 14 or 15, wherein each recombinant polynucleotide comprises, from 5' to 3', the promoter, the nucleotide sequence encoding the unique cell surface marker polypeptide, and the nucleotide sequence encoding the unique target polypeptide;

each recombinant polynucleotide comprises 1 to 4 promoters, 1 to 4 nucleotide sequences each encoding a unique cell surface marker polypeptide, and 1 to 4 nucleotide sequences each encoding a unique target polypeptide, wherein one of the nucleotide sequences encoding a unique cell surface marker polypeptide is transcribed on the same mRNA as one of the nucleotide sequences encoding a unique target polypeptide;

each recombinant polynucleotide is a bicistronic polynucleotide comprising two promoters and two nucleotide sequences each encoding a unique target polypeptide, and further comprising one or two nucleotide sequences each encoding a unique cell surface marker polypeptide;

each recombinant polynucleotide is a bicistronic polynucleotide comprising two promoters and two nucleotide sequences each encoding a unique target polypeptide, and further comprising one or two nucleotide sequences each encoding a unique cell surface marker polypeptide;

each bicistronic polynucleotide comprises a single nucleotide sequence encoding a unique cell surface marker polypeptide;

each bicistronic polynucleotide comprises two nucleotide sequences each encoding a unique cell surface marker polypeptide, and wherein each one of the nucleotide sequences encoding a unique cell surface marker polypeptide is transcribed on the same mRNA as one of the nucleotide sequences encoding a unique target polypeptide;

each recombinant polynucleotide comprises, from 5’ to 3’, one of the from 1-4 promoters, one of the from 1-4 nucleotide sequences encoding a unique cell surface marker polypeptide, and one of the from 1-4 nucleotide sequences encoding a unique target polypeptide;

each recombinant polynucleotide comprises one promoter, one nucleotide sequence encoding a unique cell surface marker polypeptide, and one nucleotide sequence encoding a unique target polypeptide, wherein the nucleotide sequence encoding a unique cell surface marker polypeptide is transcribed on the same mRNA as the nucleotide sequence encoding a unique target polypeptide;

each recombinant polynucleotide further comprises an internal ribosome entry site (IRES) located 3’ to the nucleotide sequence encoding the unique cell surface marker polypeptide and 5’ to the nucleotide sequence encoding the unique target polypeptide; and/or

each recombinant polynucleotide further comprises an alternate (non-ATG) start codon for translation initiation of the nucleotide sequence encoding the unique cell surface marker polypeptide and an ATG start codon for translation initiation of the nucleotide sequence encoding the unique target polypeptide, optionally wherein each alternate (non-ATG) start codon is selected independently from the group consisting of CTG, GTG, TTG, ATT, ATA, and ACG, each alternate (non-ATG) start codon is selected independently from the group consisting of GTG and TTG, or wherein the nucleotide sequence encoding the unique cell surface marker polypeptide is devoid of any ATG triplets.

17. The engineered mammalian host cell of any one of claims 14 to 16, wherein

at least a first recombinant polynucleotide comprises, from 5’ to 3’, a first promoter, a nucleotide sequence encoding a first unique cell surface marker polypeptide, and a nucleotide sequence encoding a first unique target polypeptide; optionally wherein

the first recombinant polynucleotide further comprises an internal ribosome entry site (IRES) located 3’ to the nucleotide sequence encoding the first unique cell surface marker polypeptide and 5’ to the nucleotide sequence encoding the first unique target polypeptide, or the first recombinant polynucleotide further comprises an alternate (non-ATG) start codon for translation initiation of the nucleotide sequence encoding the first unique cell surface marker polypeptide and an ATG start codon for translation initiation of the nucleotide sequence encoding the first unique target polypeptide; and

at least a second recombinant polynucleotide comprises, from 5’ to 3’, a second promoter, a nucleotide sequence encoding a second unique target polypeptide, and a nucleotide sequence encoding a second unique cell surface marker polypeptide, optionally wherein

the second recombinant polynucleotide further comprises an internal ribosome entry site (IRES) located 3’ to the nucleotide sequence encoding the second unique target polypeptide and 5’ to the nucleotide sequence encoding the second unique cell surface marker polypeptide; and/or

wherein each recombinant polynucleotide comprises, from 5’ to 3’, the promoter, the nucleotide sequence encoding the unique target polypeptide, and the nucleotide sequence encoding the unique cell surface marker polypeptide, optionally wherein each recombinant polynucleotide further comprises an internal ribosome entry site (IRES) located 3’ to the nucleotide sequence encoding the unique target polypeptide and 5’ to the nucleotide sequence encoding the unique cell surface marker polypeptide.

18. The engineered mammalian host cell of any one of claims 14 to 17, wherein at least a first unique cell surface marker polypeptide is selected from the group consisting of CD20, CD52, CD59, and variants thereof

at least a second unique cell surface marker polypeptide is selected from the group consisting of CD20, CD52, CD59, and variants thereof;

each unique cell surface marker polypeptide is selected from the group consisting of CD20, CD52, CD59, and variants thereof;

at least a first unique cell surface marker polypeptide is selected from the group consisting of human CD52 and variants thereof;

at least a second unique cell surface marker polypeptide is selected from the group consisting of human CD52 and variants thereof;

each unique cell surface marker polypeptide is selected from the group consisting of human CD52 and variants thereof;

at least a first unique cell surface marker polypeptide is human CD52; and/or

at least a second unique cell surface marker polypeptide is human CD52.

19. The engineered mammalian host cell of any one of claims 14 to 18, wherein at least a first unique cell surface marker polypeptide is a variant of CD52 comprising:

(a) the amino acid sequence set forth in SEQ ID NO: 19 ($GX_2NDTSQTSSPS$) wherein X_2 is selected from the group consisting of A, G, I, L, and V, optionally wherein X_2 is A;

(b) the amino acid sequence set forth in SEQ ID NO: 20 ($GQNX_4TSQTSSPS$) wherein X_4 is selected from the group consisting of A, G, I, L, and V, optionally wherein X_4 is A;

(c) the amino acid sequence set forth in SEQ ID NO: 21 ($GQNDTSX_7TSSPS$) wherein X_7 is selected from the group consisting of A, G, I, L, and V, optionally wherein X_7 is A;

(d) the amino acid sequence set forth in SEQ ID NO: 22 ($GQNDTSQX_8SSPS$) wherein X_8 is selected from the group consisting of A, G, I, L, and V, optionally wherein X_8 is A;

(e) the amino acid sequence set forth in SEQ ID NO: 23 ($GQNDTSQX_8X_9SPS$), wherein each of X_8 and X_9 is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_8 and X_9 is A;

(f) the amino acid sequence set forth in SEQ ID NO: 24 ($GQNDTSQX_8SX_{10}PS$), wherein each of X_8 and X_{10} is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_8 and X_{10} is A

(g) the amino acid sequence set forth in SEQ ID NO: 25 ($GQNDTSQX_8X_9X_{10}PS$), wherein each of X_8, X_9, and X_{10} is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_8, X_9, and X_{10} is A;

(h) the amino acid sequence set forth in SEQ ID NO: 26 ($GX_2NDTSQX_8X_9SPS$), wherein each of X_2, X_8, and X_9 is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_2, X_8, and X_9 is A;

(i) the amino acid sequence set forth in SEQ ID NO: 27 ($GX_2NDTSQX_8SX_{10}PS$), wherein each of X_2, X_8, and X_{10} is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_2, X_8, and X_{10} is A;

(j) the amino acid sequence set forth in SEQ ID NO: 28 ($GQNDTSX_7X_8X_9SPS$), wherein each of X_7, X_8, and X_9 is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_7, X_8, and X_9 is A; or

(k) the amino acid sequence set forth in SEQ ID NO: 29 ($GQNDTSX_7X_8SX_{10}PS$), wherein each of X_7, X_8, and X_{10} is independently selected from the group consisting of A, G, I, L, and V, optionally wherein each of X_7, X_8, and X_{10} is A.

20. The engineered mammalian host cell of any one of claims 14 to 19, wherein at least one unique cell surface marker polypeptide is a variant human CD52 comprising:

(a) the amino acid sequence set forth in SEQ ID NO: 19 ($GX_2NDTSQTSSPS$) wherein X_2 is selected from the group consisting of A, G, I, L, and V

(b) comprising the amino acid sequence set forth in SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4 (GQNATSQTSSPS) and SEQ ID NO: 7 (GQNDTSATSSPS);

(c) consists of a variant human CD52 consisting of the amino acid sequence set forth in SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide consists of a variant human CD52 consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 4 (GQNATSQTSSPS) and SEQ ID NO: 7 (GQNDTSATSSPS)

(d) comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 4 (GQNATSQTSSPS);

(e) consists of a variant human CD52 consisting of the amino acid sequence set forth as SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide consists of a variant human CD52 consisting of the amino acid sequence set forth as SEQ ID NO: 4 (GQNATSQTSSPS);

(f) comprising the amino acid sequence set forth as SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 7 (GQNDTSATSSPS);

(g) consists of a variant human CD52 consisting of the amino acid sequence set forth as SEQ ID NO: 2 (GANDTSQTSSPS); and at least a second unique cell surface marker polypeptide consists of a variant human CD52 consisting of the amino acid sequence set forth as SEQ ID NO: 7 (GQNDTSATSSPS);

(h) comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 4 (GQNATSQTSSPS); and at least a second unique cell surface marker

polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 7 (GQNDTSATSSPS);

(i) comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 20 (GQNX_4TSQTSSPS); at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 23 (GX_2NDTSQX_8X_9SPS); and at least a third unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 28 (GQNDTS$X_7X_8X_9$SPS), wherein each of X_4, X_7, X_8, and X_9 is independently selected from the group consisting of A, G, I, L, and V;

(j) comprising the amino acid sequence set forth as SEQ ID NO: 20 (GQNX_4TSQTSSPS); at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 27 (GX_2NDTSQX_8SX_{10}PS); and at least a third unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 29 (GQNDTSX_7X_8SX_{10}PS) wherein each of X_4, X_7, X_8, and X_{10} is independently selected from the group consisting of A, G, I, L, and V;

(k) comprising the amino acid sequence set forth as SEQ ID NO: 4 (GQNATSQTSSPS); at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 15 (GANDTSQAASPS); and at least a third unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 17 (GQNDTSAAASPS); or

(l) comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 4 (GQNATSQTSSPS); at least a second unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 16 (GANDTSQASAPS); and at least a third unique cell surface marker polypeptide comprises a variant human CD52 comprising the amino acid sequence set forth as SEQ ID NO: 18 (GQNDTSAASAPS).

21. The engineered mammalian host cell of any one of claims 14 to 21, wherein at least one unique target polypeptide comprises a therapeutic polypeptide or a therapeutic protein, optionally wherein

at least one unique target polypeptide is a polypeptide of a multi-chain protein, wherein each unique target polypeptide is a polypeptide of a multi-chain protein,

at least one unique target polypeptide is a polypeptide of an antibody,

each unique target polypeptide is a polypeptide of an antibody,

at least one unique target polypeptide is a polypeptide of a crossover dual-variable domain (CODV) Ig-like protein,

each unique target polypeptide is a polypeptide of a crossover dual-variable domain (CODV) Ig-like protein,

at least one unique target polypeptide is a polypeptide of a CODV triabody, or

each unique target polypeptide is a polypeptide of a CODV triabody.

22. An isolated variant human CD52 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 19 ($GX_2NDTSQTSSPS$) wherein X_2 is selected from the group consisting of A, G, I, L, and V;

SEQ ID NO: 20 ($GQNX_4TSQTSSPS$) wherein X_4 is selected from the group consisting of A, G, I, L, and V; or

SEQ ID NO: 22 ($GQNDTSQX_8SSPS$) wherein X_8 is selected from the group consisting of A, G, I, L, and V.