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(54) Title: **SIALIDASE-PD-L1-ANTIBODY FUSION PROTEINS AND METHODS OF USE THEREOF**

(57) Abstract: The invention relates generally to recombinant sialidase and anti-PD-L1 immunoglobulin antigen-binding domain fusion proteins. The invention also provides antibody conjugates including a sialidase and an anti-PD-L1 antibody or a portion thereof. The invention further relates to methods of using the sialidase fusion proteins or antibody conjugates for treating cancer.



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SIALIDASE-PD-L1-ANTIBODY FUSION PROTEINS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of, and priority to, U.S. Provisional Patent Application serial number 62/870,354, filed July 3, 2019, and U.S. Provisional Patent Application serial number 62/956,957, filed January 3, 2020, the entire disclosure of each of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates generally to recombinant sialidase fusion proteins and antibody conjugates, and their use in the treatment of cancer.

BACKGROUND

[0003] A growing body of evidence supports roles for glycans, and sialoglycans in particular, at various pathophysiological steps of tumor progression. Glycans regulate tumor proliferation, invasion, hematogenous metastasis and angiogenesis (Fuster *et al.* (2005) NAT. REV. CANCER 5(7): 526-42). The sialylation of cell surface glycoconjugates is frequently altered in cancers, resulting in the expression of sialylated tumor-associated carbohydrate antigens. The expression of sialylated glycans by tumor cells is often associated with increased aggressiveness and metastatic potential of a tumor.

[0004] It has recently become apparent that Siglecs (sialic acid-binding immunoglobulin-like lectins), a family of sialic acid binding lectins, play a role in cancer immune suppression by binding to hypersialylated cancer cells and mediating the suppression of signals from activating NK cell receptors, thereby inhibiting NK cell-mediated killing of tumor cells (Jandus *et al.* (2014) J. CLIN. INVEST. 124: 1810-1820; Läubli *et al.* (2014) PROC. NATL. ACAD. SCI. USA 111: 14211-14216; Hudak *et al.* (2014) NAT. CHEM. BIOL. 10: 69-75). Likewise, enzymatic removal of sialic acids by treatment with sialidase can enhance NK cell-mediated killing of tumor cells (Jandus, *supra*; Hudak, *supra*; Xiao *et al.* (2016) PROC. NATL. ACAD. SCI. USA 113(37): 10304-9).

[0005] Programmed death-ligand 1 (PD-L1) also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1) is a protein that in humans is encoded by the CD274 gene. Upregulation of PD-L1 may allow certain cancers to evade the host immune system. An analysis of 196 tumor specimens from patients with renal cell carcinoma found that high tumor

expression of PD-L1 was associated with increased tumor aggressiveness and a 4.5-fold increased risk of death. Many PD-L1 antibodies are in development as immuno-oncology therapies and are showing good results in clinical trials including NSCLC and melanoma. PD-L1 expression is detected in most human cancers, including bladder, breast, cervical, esophageal, gastric, kidney, lung, ovary and pancreatic cancer. High expression of PD-L1 is associated with reduced numbers of tumor infiltrating lymphocytes and poor prognosis. Anti-PD-L1 antibodies include atezolizumab, approved for use in, for example, urothelial carcinomas, non-small cell lung cancers (NSCLC), triple-negative breast cancers, and small cell lung cancers, durvalumab, approved for use in, for example, urothelial carcinomas, and NSCLCs, and avelumab, approved for use in Merkel cell carcinomas, urothelial carcinomas, and renal cell carcinomas.

[0006] Cancer immunotherapy with immune checkpoint inhibitors, including antibodies blocking the PD-1/PD-L1 pathway, has improved the outcome of many cancer patients. However, despite advances that have been made to date, many patients do not respond to currently available immune checkpoint inhibitors. Accordingly, there is still a need for effective interventions that overcome the immune suppressive tumor microenvironment and for treating cancers associated with hypersialylated cancer cells.

SUMMARY OF THE INVENTION

[0007] The invention is based, in part, upon the discovery that it is possible to produce fusion proteins containing a sialidase enzyme and an anti-PD-L1 immunoglobulin antigen-binding and/or antibody conjugates including a sialidase enzyme and an anti-PD-L1 antibody or a portion thereof. The fusion proteins and/or antibody conjugates have suitable substrate specificities and activities to be useful in removing sialic acid and/or sialic acid containing molecules from the surface of cancer cells, *e.g.*, PD-L1-expressing cancer cells, and/or removing sialic acid and/or sialic acid containing molecules from the tumor microenvironment, and/or reducing the concentration of sialic acid and/or sialic acid containing molecules in the tumor microenvironment.

[0008] Accordingly, in one aspect, the invention provides a fusion protein comprising (or consisting essentially of): (a) sialidase enzyme; and (b) an anti-PD-L1 immunoglobulin antigen-binding domain.

[0009] In certain embodiments, the sialidase is a human sialidase, *e.g.*, a recombinant mutant human sialidase. In certain embodiments, the sialidase comprises: (a) a substitution of a proline residue at a position corresponding to position 5 of wild-type human Neu2 (P5); (b) a substitution of a lysine residue at a position corresponding to position 9 of wild-type human

Neu2 (K9); (c) a substitution of a lysine residue at a position corresponding to position 44 of wild-type human Neu2 (K44); (d) a substitution of a lysine residue at a position corresponding to position 45 of wild-type human Neu2 (K45); (e) a substitution of a leucine residue at a position corresponding to position 54 of wild-type human Neu2 (L54); (f) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62); (g) a substitution of a glutamine residue at a position corresponding to position 69 of wild-type human Neu2 (Q69); (h) a substitution of an arginine residue at a position corresponding to position 78 of wild-type human Neu2 (R78); (i) a substitution of an aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 (D80); (j) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93); (k) a substitution of a glycine residue at a position corresponding to position 107 of wild-type human Neu2 (G107); (l) a substitution of a glutamine residue at a position corresponding to position 108 of wild-type human Neu2 (Q108); (m) a substitution of a glutamine residue at a position corresponding to position 112 of wild-type human Neu2 (Q112); (n) a substitution of a cysteine residue at a position corresponding to position 125 of wild-type human Neu2 (C125); (o) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126); (p) a substitution of an alanine residue at a position corresponding to position 150 of wild-type human Neu2 (A150); (q) a substitution of a cysteine residue at a position corresponding to position 164 of wild-type human Neu2 (C164); (r) a substitution of an arginine residue at a position corresponding to position 170 of wild-type human Neu2 (R170); (s) a substitution of an alanine residue at a position corresponding to position 171 of wild-type human Neu2 (A171); (t) a substitution of a glutamine residue at a position corresponding to position 188 of wild-type human Neu2 (Q188); (u) a substitution of an arginine residue at a position corresponding to position 189 of wild-type human Neu2 (R189); (v) a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (A213); (w) a substitution of a leucine residue at a position corresponding to position 217 of wild-type human Neu2 (L217); (x) a substitution of a glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 (E225); (y) a substitution of a histidine residue at a position corresponding to position 239 of wild-type human Neu2 (H239); (z) a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240); (aa) a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241); (bb) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242); (cc) a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244); (dd) a substitution of a

threonine residue at a position corresponding to position 249 of wild-type human Neu2 (T249); (ee) a substitution of an aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 (D251); (ff) a substitution of a glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 (E257); (gg) a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258); (hh) a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260); (ii) a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265); (jj) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2 (Q270); (kk) a substitution of a tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 (W292); (ll) a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301); (mm) a substitution of a tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 (W302); (nn) a substitution of a valine residue at a position corresponding to position 363 of wild-type human Neu2 (V363); or (oo) a substitution of a leucine residue at a position corresponding to position 365 of wild-type human Neu2 (L365); or a combination of any of the foregoing substitutions. For example, the sialidase may comprise a substitution of K9, P62, A93, Q216, A242, Q270, S301, W302, V363, or L365, or a combination of any of the foregoing substitutions.

[0010] In certain embodiments, in the sialidase: (a) the proline residue at a position corresponding to position 5 of wild-type human Neu2 is substituted by histidine (P5H); (b) the lysine residue at a position corresponding to position 9 of wild-type human Neu2 is substituted by aspartic acid (K9D); (c) the lysine residue at a position corresponding to position 44 of wild-type human Neu2 is substituted by arginine (K44R) or glutamic acid (K44E); (d) the lysine residue at a position corresponding to position 45 of wild-type human Neu2 is substituted by alanine (K45A), arginine (K45R), or glutamic acid (K45E); (e) the leucine residue at a position corresponding to position 54 of wild-type human Neu2 is substituted by methionine (L54M); (f) the proline residue at a position corresponding to position 62 of wild-type human Neu2 is substituted by asparagine (P62N), aspartic acid (P62D), histidine (P62H), glutamic acid (P62E), glycine (P62G), serine (P62S), or threonine (P62T); (g) the glutamine residue at a position corresponding to position 69 of wild-type human Neu2 is substituted by histidine (Q69H); (h) the arginine residue at a position corresponding to position 78 of wild-type human Neu2 is substituted by lysine (R78K); (i) the aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 is substituted by proline (D80P); (j) the alanine residue at a position corresponding to position 93 of wild-type human Neu2 is substituted by glutamic acid

(A93E) or lysine (A93K); (k) the glycine residue at a position corresponding to position 107 of wild-type human Neu2 is substituted by aspartic acid (G107D); (l) the glutamine residue at a position corresponding to position 108 of wild-type human Neu2 is substituted by histidine (Q108H); (m) the glutamine residue at a position corresponding to position 112 of wild-type human Neu2 is substituted by arginine (Q112R) or lysine (Q112K); (n) the cysteine residue at a position corresponding to position 125 of wild-type human Neu2 is substituted by leucine (C125L); (o) the glutamine residue at a position corresponding to position 126 of wild-type human Neu2 is substituted by leucine (Q126L), glutamic acid (Q126E), phenylalanine (Q126F), histidine (Q126H), isoleucine (Q126I), or tyrosine (Q126Y); (p) the alanine residue at a position corresponding to position 150 of wild-type human Neu2 is substituted by valine (A150V); (q) the cysteine residue at a position corresponding to position 164 of wild-type human Neu2 is substituted by glycine (C164G); (r) the arginine residue at a position corresponding to position 170 of wild-type human Neu2 is substituted by proline (R170P); (s) the alanine residue at a position corresponding to position 171 of wild-type human Neu2 is substituted by glycine (A171G); (t) the glutamine residue at a position corresponding to position 188 of wild-type human Neu2 is substituted by proline (Q188P); (u) the arginine residue at a position corresponding to position 189 of wild-type human Neu2 is substituted by proline (R189P); (v) the alanine residue at a position corresponding to position 213 of wild-type human Neu2 is substituted by cysteine (A213C), asparagine (A213N), serine (A213S), or threonine (A213T); (w) the leucine residue at a position corresponding to position 217 of wild-type human Neu2 is substituted by alanine (L217A) or valine (L217V); (x) the threonine residue at a position corresponding to position 249 of wild-type human Neu2 is substituted by alanine (T249A); (y) the aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 is substituted by glycine (D251G); (z) the glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 is substituted by proline (E225P); (aa) the histidine residue at a position corresponding to position 239 of wild-type human Neu2 is substituted by proline (H239P); (bb) the leucine residue at a position corresponding to position 240 of wild-type human Neu2 is substituted by aspartic acid (L240D), asparagine (L240N), or tyrosine (L240Y); (cc) the arginine residue at a position corresponding to position 241 of wild-type human Neu2 is substituted by alanine (R241A), aspartic acid (R241D), leucine (R241L), glutamine (R241Q), or tyrosine (R241Y); (dd) the alanine residue at a position corresponding to position 242 of wild-type human Neu2 is substituted by cysteine (A242C), phenylalanine (A242F), glycine (A242G), histidine (A242H), isoleucine (A242I), lysine (A242K), leucine (A242L), methionine (A242M), asparagine (A242N), glutamine (A242Q), arginine (A242R),

serine (A242S), valine (A242V), tryptophan (A242W), or tyrosine (A242Y); (ee) the valine residue at a position corresponding to position 244 of wild-type human Neu2 is substituted by isoleucine (V244I), lysine (V244K), or proline (V244P); (ff) the glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 is substituted by proline (E257P); (gg) the serine residue at a position corresponding to position 258 is substituted by cysteine (S258C); (hh) the leucine residue at a position corresponding to position 260 of wild-type human Neu2 is substituted by aspartic acid (L260D), phenylalanine (L260F), glutamine (L260Q), or threonine (L260T); (ii) the valine residue at a position corresponding to position 265 of wild-type human Neu2 is substituted by phenylalanine (V265F); (jj) the glutamine residue at a position corresponding to position 270 of wild-type human Neu2 is substituted by alanine (Q270A), histidine (Q270H), phenylalanine (Q270F), proline (Q270P), serine (Q270S), or threonine (Q270T); (kk) the tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 is substituted by arginine (W292R); (ll) the serine residue at a position corresponding to position 301 of wild-type human Neu2 is substituted by alanine (S301A), aspartic acid (S301D), glutamic acid (S301E), phenylalanine (S301F), histidine (S301H), lysine (S301K), leucine (S301L), methionine (S301M), asparagine (S301N), proline (S301P), glutamine (S301Q), arginine (S301R), threonine (S301T), valine (S301V), tryptophan (S301W), or tyrosine (S301Y); (mm) the tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 is substituted by alanine (W302A), aspartic acid (W302D), phenylalanine (W302F), glycine (W302G), histidine (W302H), isoleucine (W302I), lysine (W302K), leucine (W302L), methionine (W302M), asparagine (W302N), proline (W302P), glutamine (W302Q), arginine (W302R), serine (W302S), threonine (W302T), valine (W302V), or tyrosine (W302Y); (nn) the valine residue at a position corresponding to position 363 of wild-type human Neu2 is substituted by arginine (V363R); or (oo) the leucine residue at a position corresponding to position 365 of wild-type human Neu2 is substituted by glutamine (L365Q), histidine (L365H), isoleucine (L365I), lysine (L365K) or serine (L365S); or the sialidase comprises a combination of any of the foregoing substitutions. For example, the sialidase may comprise a substitution selected from K9D, P62G, P62N, P62S, P62T, A93E, Q126Y, A242F, A242W, A242Y, Q270A, Q270T, S301A, S301R, W302K, W302R, V363R, and L365I, or a combination of any of the foregoing substitutions.

[0011] In certain embodiments, the sialidase comprises: (a) a substitution or deletion of a methionine residue at a position corresponding to position 1 of wild-type human Neu2 (M1); (b) a substitution of a valine residue at a position corresponding to position 6 of wild-type human Neu2 (V6); (c) a substitution of an isoleucine residue at a position corresponding to position 187

of wild-type human Neu2 (I187); or (d) a substitution of a cysteine residue at a position corresponding to position 332 of wild-type human Neu2 (C332); or a combination of any of the foregoing substitutions.

[0012] In certain embodiments, in the sialidase: (a) the methionine residue at a position corresponding to position 1 of wild-type human Neu2 is deleted (Δ M1), is substituted by alanine (M1A), or is substituted by aspartic acid (M1D); (b) the valine residue at a position corresponding to position 6 of wild-type human Neu2 is substituted by tyrosine (V6Y); (c) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by lysine (I187K); or (d) the cysteine residue at a position corresponding to position 332 of wild-type human Neu2 is substituted by alanine (C332A); or the sialidase comprises a combination of any of the foregoing substitutions.

[0013] In certain embodiments, the sialidase comprises: (a) the M1D, V6Y, P62G, A93E, I187K, and C332A substitutions; (b) the M1D, V6Y, K9D, A93E, I187K, C332A, V363R, and L365I substitutions; (c) the M1D, V6Y, P62N, I187K, and C332A substitutions; (d) the M1D, V6Y, I187K, Q270A, S301R, W302K, and C332A substitutions; (e) the M1D, V6Y, P62S, I187K, Q270A, S301R, W302K, and C332A substitutions; (f) the M1D, V6Y, P62T, I187K, Q270A, S301R, W302K, and C332A substitutions; (g) the M1D, V6Y, P62N, I187K, Q270A, S301R, W302K, and C332A substitutions; (h) the M1D, V6Y, P62G, A93E, I187K, S301A, W302R, and C332A substitutions; (i) the M1D, V6Y, P62G, A93E, Q126Y, I187K, Q270T, and C332A substitutions; (j) the M1D, V6Y, P62G, A93E, Q126Y, I187K, and C332A substitutions; or (k) the M1D, V6Y, P62G, A93E, Q126Y, I187K, A242F, Q270T, and C332A substitutions.

[0014] In certain embodiments, the sialidase is selected from Neu1, Neu2, Neu3, and Neu4, *e.g.*, the sialidase is Neu2.

[0015] In certain embodiments, the sialidase has a different substrate specificity than the corresponding wild-type sialidase. For example, in certain embodiments the sialidase can cleave α 2,3, α 2,6, and/or α 2,8 linkages. In certain embodiments the sialidase can cleave α 2,3 and α 2,8 linkages.

[0016] In certain embodiments, the sialidase comprises any one of SEQ ID NOs: 48-62, 94, 97, 100, or 126, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 48-62, 94, 97, 100, or 126.

[0017] In certain embodiments, the sialidase comprises mutation or combination of mutations set forth in any one of Tables 5-12 or 14-27. In certain embodiments, the sialidase comprises a mutation or combination of mutations set forth in any one of Tables 1-4.

[0018] In certain embodiments, the fusion protein further comprises an immunoglobulin Fc domain. In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM Fc domain, *e.g.*, the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain, *e.g.*, the immunoglobulin Fc domain is derived from a human IgG1 Fc domain.

[0019] In certain embodiments, the anti-PD-L1 immunoglobulin antigen-binding domain is associated (for example, covalently or non-covalently associated) with a second anti-PD-L1 immunoglobulin antigen-binding domain to produce an anti-PD-L1 antigen-binding site.

[0020] In certain embodiments, the anti-PD-L1 immunoglobulin antigen-binding domain is derived from an antibody selected from avelumab, atezolizumab, durvalumab, MEDI4736, CS1001, KL-A167, CK-301, TQB2450, KN035, SHR-1316, STI-A1014, BGB-A333, MSB2311, HLX-20 and BMS-936559, *e.g.*, the anti-PD-L1 immunoglobulin antigen-binding domain is derived from avelumab.

[0021] In certain embodiments, the sialidase and the immunoglobulin Fc domain and/or the anti-PD-L1 immunoglobulin antigen-binding domain are linked by a peptide bond or an amino acid linker.

[0022] In certain embodiments, the fusion protein comprises any one of SEQ ID NOs: 65-75, 78, 81-89, 95, 96, 98, 99, 101, 102, 104, 106, 108, 110, 112, 114, 122-124, 127, or 128.

[0023] In another aspect, the invention provides an antibody conjugate comprising any of the foregoing fusion proteins. In certain embodiments, the antibody conjugate comprises a single sialidase. In other embodiments, the antibody conjugate comprises two sialidases, which can be the same or different. In certain embodiments the antibody conjugate comprises two identical sialidases. In certain embodiments, the antibody conjugate comprises a single anti-PD-L1 antigen-binding site. In other embodiments, the antibody conjugate comprises two anti-PD-L1 antigen-binding sites, which can be the same or different. In certain embodiments, the antibody conjugate comprises two identical anti-PD-L1 antigen-binding sites.

[0024] In certain embodiments, the antibody conjugate has a molecular weight from about 135 kDa to about 165 kDa, or the antibody conjugate has a molecular weight from about 215 kDa to about 245 kDa.

[0025] In certain embodiments, the antibody conjugate comprises: (a) a first polypeptide comprising an immunoglobulin light chain; (b) a second polypeptide comprising an immunoglobulin heavy chain; and (c) a third polypeptide comprising an immunoglobulin Fc

domain and a sialidase; wherein the first and second polypeptides are covalently linked together and the second and third polypeptides are covalently linked together, and wherein the first polypeptide and the second polypeptide together define an anti-PD-L1 antigen-binding site. The third polypeptide may, for example, comprise the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation. The first polypeptide may, for example, comprise SEQ ID NO: 65, the second polypeptide may, for example, comprise any one of SEQ ID NOs: 66, 104, or 124, and/or the third polypeptide may, for example, comprise any one of SEQ ID NOs: 67-73, 78, 81-87, 95, 96, 98, 99, 101, 102, 106, 108, 112, 122, 123, 127, or 128.

[0026] In certain embodiments, the antibody conjugate comprises: (a) a first polypeptide comprising a first immunoglobulin light chain; (b) a second polypeptide comprising a first immunoglobulin heavy chain and a first sialidase; (c) a third polypeptide comprising a second immunoglobulin heavy chain and a second sialidase; and (d) a fourth polypeptide comprising a second immunoglobulin light chain; wherein the first and second polypeptides are covalently linked together, the third and fourth polypeptides are covalently linked together, and the second and third polypeptides are covalently linked together, and wherein the first polypeptide and the second polypeptide together define a first anti-PD-L1 antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second anti-PD-L1 antigen-binding site. The second and third polypeptides may, for example, comprise the first and second immunoglobulin heavy chain and the first and second sialidase, respectively, in an N- to C-terminal orientation.

[0027] In certain embodiments, the antibody conjugate comprises: (a) a first polypeptide comprising a first sialidase, a first immunoglobulin Fc domain, and a first single chain variable fragment (scFv); and (b) a second polypeptide comprising a second sialidase, a second immunoglobulin Fc domain, and an optional second single chain variable fragment (scFv); wherein the first and second polypeptides are covalently linked together, and wherein the first scFv defines a first anti-PD-L1 antigen-binding site, and the second scFv, when present, defines a second anti-PD-L1 antigen-binding site. The first polypeptide may, for example comprise the first sialidase, the first immunoglobulin Fc domain, and the first scFv in an N- to C-terminal orientation. The second polypeptide may, for example, comprise the second sialidase, the second immunoglobulin Fc domain, and the optional second scFv in an N- to C-terminal orientation. The first polypeptide may, for example, comprise SEQ ID NO: 114, and/or the second polypeptide may, for example, comprise SEQ ID NO: 114.

[0028] In certain embodiments, the antibody conjugate comprises: (a) a first polypeptide comprising an immunoglobulin light chain; (b) a second polypeptide comprising an immunoglobulin heavy chain and a single chain variable fragment (scFv); and (c) a third polypeptide comprising an immunoglobulin Fc domain and a sialidase, wherein the first and second polypeptides are covalently linked together and the second and third polypeptides are covalently linked together, and wherein the immunoglobulin light chain and immunoglobulin heavy chain together define a first anti-PD-L1 antigen-binding site and the scFv defines a second anti-PD-L1 antigen-binding site. The second polypeptide may, for example comprise the immunoglobulin heavy chain and the scFv in an N- to C-terminal orientation. The third polypeptide may, for example, comprise the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation. The first polypeptide may, for example, comprise SEQ ID NO: 65, the second polypeptide may, for example, comprise SEQ ID NO: 110, and/or the third polypeptide may, for example, comprise any one of SEQ ID NOs: 78 or 106.

[0029] In another aspect, the invention provides an isolated nucleic acid comprising a nucleotide sequence encoding any of the foregoing fusion proteins or at least a portion of any of the foregoing antibody conjugates. In another aspect, the invention provides an expression vector comprising any of the foregoing nucleic acids. In another aspect, the invention provides a host cell comprising any of the foregoing expression vectors.

[0030] In another aspect, the invention provides a pharmaceutical composition comprising any of the foregoing fusion proteins or any of the foregoing antibody conjugates.

[0031] In another aspect, the invention provides a method of treating cancer in a subject in need thereof. The method comprises administering to the subject an effective amount of any of the foregoing fusion proteins, any of the foregoing antibody conjugates, or any of the foregoing pharmaceutical compositions.

[0032] In certain embodiments, the cancer is selected from non-small cell lung cancer (NSCLC), melanoma, bladder, breast, cervical, esophageal, gastric, kidney, lung, ovary, metastatic Merkel cell carcinoma (MCC), metastatic urothelial carcinoma (UC), and pancreatic cancer. For example, in certain embodiments, the cancer is NSCLC or melanoma.

[0033] These and other aspects and features of the invention are described in the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0034] The invention can be more completely understood with reference to the following drawings.

[0035] **FIGURE 1** depicts an SDS-PAGE gel showing recombinant human Neu1, Neu2, Neu3, and *Salmonella typhimurium* (St-sialidase) under non-reducing and reducing conditions. Monomer and dimer species are indicated.

[0036] **FIGURE 2** is a bar graph showing the enzymatic activity of recombinant human Neu1, Neu2, and Neu3.

[0037] **FIGURE 3** is a line graph showing enzymatic activity as a function of substrate concentration for recombinant human Neu2 and Neu3 at the indicated pH.

[0038] **FIGURE 4** depicts a schematic representation of an exemplary sialic acid biotinylated probe that can be used in phage display or yeast display screening for Neu2 variants.

[0039] **FIGURE 5** depicts an exemplary protocol that facilitates phage display screening of Neu2 variants.

[0040] **FIGURE 6** depicts an exemplary protocol that facilitates yeast display screening of Neu2 variants.

[0041] **FIGURE 7A** depicts an SDS-PAGE gel showing recombinant Neu2-Fc (wildtype) and Neu2-M106-Fc under non-reducing and reducing conditions. **FIGURE 7B** is an SEC-HPLC trace of Neu2-Fc (wildtype) and Neu2-M106-Fc. The monomer species has a retention time of 21 minutes.

[0042] **FIGURE 8** is a line graph depicting the enzymatic activity of Neu2 variant M106.

[0043] **FIGURE 9A** depicts an SDS-PAGE gel showing Neu2-M173-Fc under non-reducing and reducing conditions. **FIGURE 9B** is an SEC-HPLC trace of Neu2-M173-Fc. The monomer species has a retention time of 6.367 minutes. The monomer species has a purity of approximately 90% after purification by Protein A and CHT chromatography.

[0044] **FIGURE 10** depicts the enzyme activity of Neu2-M173-Fc, using 4-MU-Neu5Ac as the substrate, and fixing enzyme concentration to 2 µg/well.

[0045] **FIGURE 11A** depicts an SDS-PAGE gel showing Neu2-M106 under non-reducing (NR) and reducing (R) conditions. **FIGURE 11B** depicts a schematic representation of the Neu2 structure with the position of the R243 cleavage site indicated.

[0046] **FIGURE 12** depicts a reducing SDS-PAGE gel showing Neu2-M106 produced by a large or small scale expression with (+) or without (-) trypsin treatment.

[0047] **FIGURE 13** depicts an SDS-PAGE gel showing Neu2-M106 following incubation with trypsin and one of the protease inhibitors iron citrate (Fe Cit), aprotinin, AEBSF, leupeptin, or E-64 at the indicated concentrations.

[0048] **FIGURE 14** is a table depicting different mutations and combinations of mutations surrounding the trypsin cleavage site in Neu2.

[0049] **FIGURE 15A** depicts a reducing SDS-PAGE analysis of Neu2 variants with the indicated mutation at position A242 with or without trypsin treatment. Trypsin digestion was for 5 minutes at 4 °C using a 5,000% dilution of trypsin. The digestion was quenched by addition of SDS, and 2 µg of protein was loaded on the gel. **FIGURE 15B** depicts the enzymatic activity of Neu2 variants with the indicated mutation at position A242. **FIGURE 15C** is an SEC-HPLC trace of Neu2 variants with the indicated mutation at position A242. Neu2-M106 (the mutational background in which the mutations at position A242 were tested) is shown as a control.

[0050] **FIGURE 16** depicts a reducing SDS-PAGE analysis of the indicated Neu2 variants with or without trypsin treatment. Neu2-M106 is shown as a control. For example, Neu2-M255 was shown to have a greater than 10 fold improved trypsin resistance relative to Neu2-M106.

[0051] **FIGURES 17A-17I** depict schematic representations of certain antibody conjugate constructs containing a sialidase enzyme, *e.g.*, a human sialidase enzyme, and an anti-PD-L1 antigen binding site. For each antibody conjugate construct that contains more than one (*e.g.*, two) sialidase, each sialidase may be the same or different. For each antibody conjugate construct that contains more than one (*e.g.*, two) anti-PD-L1 antigen binding site, each anti-PD-L1 antigen binding site may be the same or different. For each antibody conjugate construct that contains an Fc domain, it is understood that the Fc domain can be a wild type Fc domain or can be an engineered Fc domain. For example, the Fc domain may be engineered to contain either a “knob” mutation, *e.g.*, T366Y, or a “hole” mutation, *e.g.*, Y407T, or both, to promote heterodimerization, or the Fc domain may be engineered to contain one or more modifications, *e.g.*, point mutations, to provide any other modified Fc domain functionality.

[0052] **FIGURE 18** depicts schematic representations of certain antibody conjugate constructs containing a sialidase enzyme, *e.g.*, a human sialidase enzyme, and an antigen binding site. For each antibody conjugate construct that contains more than one (*e.g.*, two) antigen binding site, each antigen binding site may be the same or different. For each antibody conjugate construct

that contains an Fc domain, it is understood that the Fc domain can be a wild type Fc domain or can be an engineered Fc domain. For example, the Fc domain may be engineered to contain either a “knob” mutation, *e.g.*, T366Y, or a “hole” mutation, *e.g.*, Y407T, or both, to promote heterodimerization, or the Fc domain may be engineered to contain one or more modifications, *e.g.*, point mutations, to provide any other modified Fc domain functionality.

[0053] FIGURES 19A-19D are schematic representations of exemplary fusion protein conjugates referred to as a Raptor antibody sialidase conjugate (**FIGURE 19A**), a Janus antibody sialidase conjugate (**FIGURE 19B**), a Lobster antibody sialidase conjugate (**FIGURE 19C**), and a Bunk antibody sialidase conjugate (**FIGURE 19D**).

[0054] FIGURE 20 depicts an SDS-PAGE gel showing purified recombinant human Janus # 1 (“Janus”) under non-reducing and reducing conditions.

[0055] FIGURE 21 shows an SEC-HPLC trace of purified Janus #1, showing approximately 85% monomer purity.

[0056] FIGURE 22 is a line graph showing the enzyme activity of Janus #1 using 4-MU-Neu5Ac as the substrate and measured as the level of fluorescence (AU) generated. Specifically, when Janus #1 is incubated with the fluorogenic substrate 4-MU-Neu5Ac, it cleaves the substrate, generating fluorescence which is indicative of the sialidase activity of the construct.

[0057] FIGURE 23A is an Octet binding graph showing the binding of avelumab to the PD-L1 antigen and **FIGURE 23B** is an octet binding graph showing the binding of Janus #1 (“Ave-Janus”) to the PD-L1 antigen. Kinetic binding experiments were carried out using ForteBio Octet. **FIGURE 23C** is a table showing equilibrium dissociation constant (KD), k_{on} , and k_{dis} for avelumab and Janus #1 (“Ave-Janus”).

[0058] FIGURE 24 depicts schematic representations of certain antibody conjugate constructs containing a sialidase enzyme and an anti-PD-L1 antigen binding site. Individual components of the constructs, including a *Salmonella typhimurium* sialidase (St-sia), a human Neu2 sialidase (Neu2), an immunoglobulin Fc domain including a T366Y knob mutation (Fc-knob), an immunoglobulin Fc domain including a Y407T hole mutation (Fc-hole), an anti-PD-L1 antigen-binding fragment (Fab), an anti-PD-L1 single-chain variable fragment (scFv) and a histidine tag (his-tag) are indicated. Constructs that correspond to Janus #1, Janus #2, Janus #3, Janus #4, Bunk #1, Bunk #2 and Lobster #1, as described in Examples 5 and 6, are indicated.

[0059] **FIGURE 25** is a line graph depicting the enzymatic activity of Janus #1, Janus #2, Lobster #1, Bunk #1, Bunk #2, and Neu2-M106-Fc ("M106 FC").

[0060] **FIGURE 26** depicts size exclusion chromatography (SEC) profiles of the indicated avelumab-based ASCs as well as the monomer purity of each.

5 [0061] **FIGURE 27** is an Octet binding graph showing the binding of avelumab and the indicated avelumab-based ASCs to PD-L1 antigen.

[0062] **FIGURE 28** is an Octet binding graph showing the binding of PD-L1 to the indicated avelumab-based ASCs.

10 [0063] **FIGURE 29** is a schematic representation of a cell-based PD-1/PD-L1 blockade bioassay.

[0064] **FIGURE 30** depicts the ability of the indicated avelumab-based ASCs to block the interaction of PD-L1 with PD-1 as measured using the assay depicted in **FIGURE 29**. Antibody blockade is indicated by an increase in luminescence. Relative light units (RLU) were calculated by subtracting background (substrate and media only) from assay wells. Fold induction was
15 calculated by dividing RLU (induced minus background) by RLU (no antibody control minus background). The EC50 for each ASC and avelumab is shown based on curve fitting fold induction data as determined by Prism.

[0065] **FIGURE 31** depicts the *in vivo* efficacy of the indicated avelumab-based ASCs at the indicated dose in a mouse A20 syngeneic subcutaneous tumor model. Tumor growth curves for
20 each mouse are depicted. Observed partial responses (PR) and complete responses (CR) are also indicated.

[0066] **FIGURE 32** depicts the *in vivo* efficacy of the indicated avelumab-based ASCs in a mouse A20 syngeneic subcutaneous tumor model. Tumor growth curves for each mouse are depicted. Triangles indicate dosing.

25

DETAILED DESCRIPTION

[0067] Various features and aspects of the invention are discussed in more detail below.

[0068] The invention relates to fusion proteins and/or antibody conjugates comprising a sialidase enzyme and an anti-PD-L1 antibody or portion thereof, *e.g.*, an immunoglobulin Fc domain
30 and/or an antigen-binding domain. The sialidase enzyme portion of the fusion protein and/or antibody conjugate may comprise at least one mutation relative to a wild-type sialidase. The

mutations, or combination of mutations, can improve the expression, activity or both the expression and activity of the sialidase to improve its use in cancer diagnosis and/or treatment.

[0069] The invention further relates to pharmaceutical compositions and methods of using fusion proteins and/or antibody conjugates to treat cancer.

5 **I. Sialidase anti-PD-L1 Fusion Proteins**

[0070] To promote the selective removal of sialic acids on hypersialylated cancer cells, *e.g.*, PD-L1 expressing cancer cells, and/or in the tumor microenvironment, it may be helpful to target a sialidase as described herein to such a cell or to such a tumor microenvironment. Additionally, in order to promote the removal of sialic acid by a sialidase in a subject, it may be helpful to extend the plasma half-life of the sialidase in the subject. These can be achieved by including the sialidase in a fusion protein and/or antibody conjugate (*e.g.*, a chemically conjugated conjugate).

[0071] Accordingly, the invention further provides fusion proteins comprising a sialidase enzyme, or a functional fragment thereof, and a portion or fragment of an anti-PD-L1 antibody, such as an immunoglobulin Fc domain (also referred to herein as an Fc domain), or an immunoglobulin antigen-binding domain (also referred to herein as an antigen-binding domain). In certain embodiments, the sialidase and anti-PD-L1 antibody or portion thereof (*e.g.*, immunoglobulin Fc domain or antigen-binding domain) are linked by a peptide bond or an amino acid linker.

[0072] As used herein, unless otherwise indicated, the term “fusion protein” is understood to refer to a single polypeptide chain comprising amino acid sequences based upon two or more separate proteins or polypeptide chains, where the two amino acid sequences may be fused together directly or via an intervening linker sequence, *e.g.*, via an intervening amino acid linker. A nucleotide sequence encoding such a fusion protein can, for example, be created using conventional recombinant DNA technologies.

[0073] In certain embodiments, a fusion protein comprises a tag, such as a Strep tag (*e.g.*, a Strep II tag), a His tag (*e.g.*, a 10x His tag), a myc tag, or a FLAG tag. The tag can be located on the C-terminus or the N-terminus of the fusion protein.

a. Sialidase Portion

[0074] As used herein, the term “sialidase” refers to any enzyme, or a functional fragment thereof, that cleaves a terminal sialic acid residue from a substrate, for example, a glycoprotein or a glycolipid. The term sialidase includes variants having one or more amino acid

substitutions, deletions, or insertions relative to a wild-type sialidase sequence, and/or fusion proteins or conjugates including a sialidase. Sialidases are also called neuraminidases, and, unless indicated otherwise, the two terms are used interchangeably herein. As used herein, the term “functional fragment” of a sialidase refers to fragment of a full-length sialidase that retains, for example, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the enzymatic activity of the corresponding full-length, naturally occurring sialidase. Sialidase enzymatic activity may be assayed by any method known in the art, including, for example, by measuring the release of sialic acid from the fluorogenic substrate 4-methylumbelliferyl-N-acetylneuraminic acid (4MU-NeuAc). In certain embodiments, the functional fragment comprises at least 100, 150, 200, 250, 300, 310, 320, 330, 340, 350, 360, or 370 consecutive amino acids present in a full-length, naturally occurring sialidase.

[0075] In certain embodiments, a sialidase portion of a sialidase-anti-PD-L1 fusion protein is derived from a eukaryotic sialidase, *e.g.*, a mammalian sialidase, *e.g.*, a human or mouse sialidase.

[0076] Four sialidases are encoded in the human genome: Neu1, Neu2, Neu3 and Neu4. Human Neu1 is a lysosomal neuraminidase enzyme which functions in a complex with beta-galactosidase and cathepsin A. The amino acid sequence of human Neu1 is depicted in SEQ ID NO: 7, and a nucleotide sequence encoding human Neu1 is depicted in SEQ ID NO: 23.

[0077] Human Neu2 is a cytosolic sialidase enzyme. The amino acid sequence of human Neu2 is depicted in SEQ ID NO: 1, and a nucleotide sequence encoding human Neu2 is depicted in SEQ ID NO: 24. Unless stated otherwise, as used herein, wild-type human Neu2 refers to human Neu2 having the amino acid sequence of SEQ ID NO: 1.

[0078] Human Neu3 is a plasma membrane sialidase with an activity specific for gangliosides. Human Neu3 has two isoforms: isoform 1 and isoform 2. The amino acid sequence of human Neu3, isoform 1 is depicted in SEQ ID NO: 8, and a nucleotide sequence encoding human Neu3, isoform 1 is depicted in SEQ ID NO: 25. The amino acid sequence of human Neu3, isoform 2 is depicted in SEQ ID NO: 9, and a nucleotide sequence encoding human Neu3, isoform 2 is depicted in SEQ ID NO: 34.

[0079] Human Neu4 has two isoforms: isoform 1 is a peripheral membrane protein and isoform 2 localizes to the lysosome lumen. The amino acid sequence of human Neu4, isoform 1 is depicted in SEQ ID NO: 10, and a nucleotide sequence encoding human Neu4, isoform 1 is depicted in SEQ ID NO: 26. The amino acid sequence of human Neu4, isoform 2 is depicted in

SEQ ID NO: 11, and a nucleotide sequence encoding human Neu4, isoform 2 is depicted in SEQ ID NO: 35.

[0080] Four sialidases have also been found in the mouse genome and are referred to as Neu1, Neu2, Neu3 and Neu4. The amino acid sequence of mouse Neu1 is depicted in SEQ ID NO: 38, and a nucleotide sequence encoding mouse Neu1 is depicted in SEQ ID NO: 42. The amino acid sequence of mouse Neu2 is depicted in SEQ ID NO: 39 and a nucleotide sequence encoding mouse Neu2 is depicted in SEQ ID NO: 43. The amino acid sequence of mouse Neu3 is depicted in SEQ ID NO: 40, and a nucleotide sequence encoding mouse Neu3 is depicted in SEQ ID NO: 44. The amino acid sequence of mouse Neu4 is depicted in SEQ ID NO: 41, and a nucleotide sequence encoding mouse Neu4 is depicted in SEQ ID NO: 45.

[0081] In certain embodiments, a sialidase portion of a sialidase-anti-PD-L1 fusion protein is derived from a prokaryotic sialidase. Exemplary prokaryotic sialidases include sialidases from *Salmonella typhimurium* and *Vibrio cholera*. The amino acid sequence of *Salmonella typhimurium* sialidase (St-sialidase) is depicted in SEQ ID NO: 30, and a nucleotide sequence encoding *Salmonella typhimurium* sialidase is depicted in SEQ ID NO: 6. The amino acid sequence of *Vibrio cholera* sialidase is depicted in SEQ ID NO: 36, and a nucleotide sequence encoding *Vibrio cholera* sialidase is depicted in SEQ ID NO: 37.

[0082] In certain embodiments, the sialidase portion of a sialidase-anti-PD-L1 fusion protein is a mutant sialidase, e.g., a recombinant mutant human sialidase. In certain embodiments, the recombinant mutant human sialidase has about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more than 100% of the enzymatic activity of a corresponding (or template) wild-type human sialidase.

[0083] In certain embodiments, the recombinant mutant human sialidase has the same substrate specificity as the corresponding wild-type human sialidase. In other embodiments, the recombinant mutant human sialidase has a different substrate specificity than the corresponding wild-type human sialidase. For example, in certain embodiments the recombinant mutant human sialidase can cleave $\alpha 2,3$, $\alpha 2,6$, and/or $\alpha 2,8$ linkages. In certain embodiments the sialidase can cleave $\alpha 2,3$ and $\alpha 2,8$ linkages.

[0084] In certain embodiments, the expression yield of the recombinant mutant human sialidase in mammalian cells, e.g., HEK293 cells, CHO cells, murine myeloma cells (NS0, Sp2/0), or human fibrosarcoma cells (HT-1080), e.g., HEK293 cells, is greater than about 10%, about 20%,

about 50%, about 75%, about 100%, about 150%, about 200%, about 250%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, about 900%, or about 1,000% of the expression yield of the corresponding wild-type human sialidase.

5 [0085] In certain embodiments, the recombinant mutant human sialidase has about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more than 100% of the enzymatic activity of a corresponding wild-type human sialidase, and the expression yield of the recombinant mutant human sialidase in mammalian cells, *e.g.*, HEK293 cells, is greater than about 10%, about 20%, about 50%,
10 about 75%, about 100%, about 150%, about 200%, about 250%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, about 900%, or about 1,000% of the expression yield of a corresponding wild-type human sialidase.

[0086] In certain embodiments, the amino acid sequence of the recombinant mutant human sialidase has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%,
15 or 99% sequence identity to the amino acid sequence of a corresponding wild-type human sialidase.

1. Substitution of Cysteine Residues

[0087] In certain embodiments, the recombinant mutant human sialidase comprises a substitution of at least one cysteine (cys, C) residue. It has been discovered that certain cysteine
20 residues in sialidases may inhibit expression of functional protein as a result of protein aggregation. Accordingly, in certain embodiments, the recombinant mutant human sialidase contains at least one mutation to remove a free cysteine (*e.g.*, for Neu1 (SEQ ID NO: 7), a mutation of, for example, one or more of C111, C117, C171, C183, C218, C240, C242, and C252; for Neu2 (SEQ ID NO: 1), a mutation of, for example, one or more of C125, C196, C219,
25 C272, C332, and C352; for Neu3 (SEQ ID NO: 8), a mutation of, for example, one or more of C7, C90, C99, C106, C127, C136, C189, C194, C226, C242, C250, C273, C279, C295, C356, C365, C368, C384, C383, C394, and C415; and for Neu4 (SEQ ID NO: 10), a mutation of, for example, one or more of C88, C125, C126, C186, C191, C211, C223, C239, C276, C437, C453, C480, and C481). Free cysteines can be substituted with any amino acid. In certain
30 embodiments, the free cysteine is substituted with serine (ser, S), isoleucine (iso, I), valine (val, V), phenylalanine (phe, F), leucine (leu, L), or alanine (ala, A). Exemplary cysteine substitutions in Neu2 include C125A, C125I, C125S, C125V, C196A, C196L, C196V, C272S, C272V, C332A, C332S, C332V, C352L, and C352V.

[0088] In certain embodiments, the recombinant mutant human sialidase comprises two or more cysteine substitutions. Exemplary double or triple cysteine substitutions in Neu2 include: C125S and C332S; C272V and C332A; C272V and C332S; C332A and C352L; C125S and C196L; C196L and C352L; C196L and C332A; C332A and C352L; and C196L, C332A and C352L.

5 **[0089]** In certain embodiments, the recombinant mutant human sialidase is a Neu2 sialidase and comprises the substitutions C322A and C352L (SEQ ID NO: 5).

[0090] In certain embodiments, the sialidase contains an amino acid substitution at 2, 3, 4, 5, or 6 cysteines typically present in a human sialidase, *e.g.*, Neu2 or Neu3.

10 **[0091]** In certain embodiments, the recombinant mutant human sialidase comprises a substitution or combination of substitutions corresponding to a substitution or combination of substitutions listed in **TABLE 1** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1)).

TABLE 1

Substitution(s)
C125A
C125I
C125S
C125V
C196A
C196L
C196V
C272S
C272V
C332A
C332S
C332V
C352L
C352V
C125S + C332S
C272V + C332A
C272V + C332S
C332A + C352L
C125S + C196L
C196L + C352L
C196L + C332A
C196L + C332A + C352L

2. Substitutions of Residues to Increase pI and/or Decrease Hydrophobicity

[0092] The isoelectric point (pI) of a protein is the pH at which the net charge is zero. The pI also generally indicates the pH at which the protein is least soluble, which may affect the ability to express and purify the protein. Generally, a protein has good solubility if its pI is greater than 2 units above the pH of the solution. Human Neu2 has a predicted pI of 7.5. Thus, human Neu2 is least soluble around neutral pH, which is undesirable because expression and physiological systems are at neutral pH. In contrast, the sialidase from *Salmonella typhimurium* (St-sialidase), which exhibits good solubility and recombinant expression, has a pI of 9.6. Accordingly, to increase expression of human Neu2 or the other human sialidases, a recombinant mutant human sialidase may be designed to contain one or more amino acid substitution(s) wherein the substitution(s) increase(s) the pI of the sialidase relative to a sialidase without the substitution. Additionally, decreasing the number of hydrophobic amino acids on the surface of a sialidase may improve expression of sialidase by, for example, reducing aggregation. Accordingly, to increase expression of human Neu2 or the other human sialidases, a recombinant mutant human sialidase may be designed to contain one or more amino acid substitution(s) wherein the substitution(s) decrease(s) the hydrophobicity of a surface of the sialidase relative to a sialidase without the substitution(s).

[0093] Accordingly, in certain embodiments, the recombinant mutant human sialidase comprises at least one amino acid substitution, wherein the substitution increases the isoelectric point (pI) of the sialidase and/or decreases the hydrophobicity of the sialidase relative to a sialidase without the substitution. This may be achieved by introducing one or more charged amino acids, for example, positively or negatively charged amino acids, into the recombinant sialidase. In certain embodiments, the amino acid substitution is to a charged amino acid, for example, a positively charged amino acid such as lysine (lys, K), histidine (his, H), or arginine (arg, R), or a negatively charged amino acid such as aspartic acid (asp, D) or glutamic acid (glu, E). In certain embodiments, the amino acid substitution is to a lysine residue. In certain embodiments, the substitution increases the pI of the sialidase to about 7.75, about 8, about 8.25, about 8.5, about 8.75, about 9, about 9.25, about 9.5, or about 9.75.

[0094] In certain embodiments, the amino acid substitution occurs at a surface exposed D or E amino acid, in a helix or loop, or in a position that has a K or R in the corresponding position of St-sialidase. In certain embodiments, the amino acid substitution occurs at an amino acid that is remote from the catalytic site or otherwise not involved in catalysis, an amino acid that is not conserved with the other human Neu proteins or with St-Sialidase or *Clostridium* NanH, or an

amino acid that is not located in a domain important for function (*e.g.*, an Asp-box or beta strand).

[0095] Exemplary amino acid substitutions in Neu2 that increase the isoelectric point (pI) of the sialidase and/or decrease the hydrophobicity of the sialidase relative to a sialidase without the substitution include A2E, A2K, D215K, V325E, V325K, E257K, and E319K. In certain embodiments, the recombinant mutant human sialidase comprises two or more amino acid substitutions, including, for example, A2K and V325E, A2K and V325K, E257K and V325K, A2K and E257K, and E257K and A2K and V325K.

[0096] In certain embodiments, the recombinant mutant human sialidase comprises a substitution or combination of substitutions corresponding to a substitution or combination of substitutions listed in **TABLE 2** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1)).

TABLE 2

Substitution(s)
A2K
E72K
D215K
E257K
V325K
A2K + E257K
A2K + V325E
A2K + V325K
E257K + V325K

3. Addition of N-terminal Peptides and N- or C-terminal Substitutions

[0097] It has been discovered that the addition of a peptide sequence of two or more amino acids to the N-terminus of a human sialidase can improve expression and/or activity of the sialidase. In certain embodiments, the peptide is at least 2 amino acids in length, for example, from 2 to 20, from 2 to 10, from 2 to 5, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length. In certain embodiments, the peptide may form, or have a propensity to form, an α -helix.

[0098] In mice, a Neu2 isoform (type B) found in thymus contains six amino acids not present in the canonical isoform of Neu2 found in skeletal muscle. In certain embodiments herein, the N-terminal six amino acids of the mouse thymus Neu2 isoform, MEDLRP (SEQ ID NO: 4), or variations thereof, can be added onto a human Neu, *e.g.*, human Neu2. In certain embodiments,

the recombinant mutant human sialidase comprises a peptide at least two amino acid residues in length covalently associated with an N-terminal amino acid of the sialidase. In certain embodiments the recombinant mutant human sialidase comprises the peptide MEDLRP (SEQ ID NO: 4) or EDLRP (SEQ ID NO: 3) covalently associated with an N-terminal amino acid of the sialidase. In certain embodiments, the sialidase may further comprise a cleavage site, *e.g.*, a proteolytic cleavage site, located between the peptide, *e.g.*, MEDLRP (SEQ ID NO: 4) or EDLRP (SEQ ID NO: 3), and the remainder of the sialidase. In certain embodiments, the peptide, *e.g.*, MEDLRP (SEQ ID NO: 4) or EDLRP (SEQ ID NO: 3), may be post-translationally cleaved from the remainder of the sialidase.

[0099] Alternatively to, or in combination with, the N-terminal addition, 1-5 amino acids of the 12 amino acid N-terminal region of the recombinant mutant human sialidase may be removed, *e.g.*, the N-terminal methionine can be removed. In certain embodiments, if the recombinant mutant human sialidase is Neu2, the N-terminal methionine can be removed, the first five amino acids (MASLP; SEQ ID NO: 12) can be removed, or the second through fourth amino acids (ASLP; SEQ ID NO: 13) can be removed.

[00100] In certain embodiments, 1-5 amino acids of the 12 amino acid N-terminal region of the recombinant mutant human sialidase are substituted with MEDLRP (SEQ ID NO: 4), EDLRP (SEQ ID NO: 3), or TVEKSVVF (SEQ ID NO: 14). For example, in certain embodiments, if the recombinant mutant human sialidase is Neu2, the amino acids MASLP (SEQ ID NO: 12), ASLP (SEQ ID NO: 13) or M are substituted with MEDLRP (SEQ ID NO: 4), EDLRP (SEQ ID NO: 3) or TVEKSVVF (SEQ ID NO: 14).

[00101] Human sialidases have a β -propeller structure, characterized by 6 blade-shaped β -sheets arranged toroidally around a central axis. Generally, hydrophobic interactions between the blades of a β -propeller, including between the N- and C-terminal blades, enhance stability. Accordingly, in order to increase expression of human Neu2 or the other human sialidases, a recombinant mutant human sialidase can be designed comprising an amino acid substitution that increases hydrophobic interactions and/or hydrogen bonding between the N- and C-terminal β -propeller blades of the sialidase.

[00102] Accordingly, in certain embodiments, the recombinant mutant human sialidase comprises a substitution of at least one wild-type amino acid residue, wherein the substitution increases hydrophobic interactions and/or hydrogen bonding between the N- and C-termini of the sialidase relative to a sialidase without the substitution. In certain embodiments, the wild-type amino acid is substituted with asparagine (asn, N), lysine (lys, K), tyrosine (tyr, Y),

phenylalanine (phe, F), or tryptophan (trp, W). Exemplary substitutions in Neu2 that increase hydrophobic interactions and/or hydrogen bonding between the N- and C-termini include L4N, L4K, V6Y, L7N, L4N and L7N, L4N and V6Y and L7N, V12N, V12Y, V12L, V6Y, V6F, or V6W. In certain embodiments, the sialidase comprises the V6Y substitution.

5 **[00103]** In certain embodiments, the recombinant mutant human sialidase comprises a combination of the above substitutions. For example, a recombinant mutant human Neu2 sialidase can comprise the additional amino acids MEDLRP (SEQ ID NO: 4), EDLRP (SEQ ID NO: 3), or TVEKSVVF (SEQ ID NO: 14) at the N-terminus and, in combination, can comprise at least one L4N, L4K, V6Y, L7N, L4N and L7N, L4N and V6Y and L7N, V12N, V12Y, V12L, 10 V6Y, V6F, or V6W substitution. In certain embodiments, the amino acids MASLP (SEQ ID NO: 12), ASLP (SEQ ID NO: 13) or M of a recombinant mutant human Neu2 sialidase are replaced with MEDLRP (SEQ ID NO: 4), EDLRP (SEQ ID NO: 3) or TVEKSVVF (SEQ ID NO: 14) and the recombinant mutant human Neu2 sialidase also comprises at least one L4N, L4K, V6Y, L7N, L4N and L7N, L4N and V6Y and L7N, V12N, V12Y, V12L, V6Y, V6F, or 15 V6W substitution.

[00104] In certain embodiments, the recombinant mutant human sialidase comprises a mutation or combination of mutations corresponding to a mutation or combination of mutations listed in **TABLE 3** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1)).

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TABLE 3

Mutation(s)
Substitute M at the N-terminus with EDLRP (SEQ ID NO: 3)
Substitute M at the N-terminus with MEDLRP (SEQ ID NO: 4)
Insert MEDLRP (SEQ ID NO: 4) at the N-terminus
Substitute MASLP (SEQ ID NO: 12) at the N-terminus with MEDLRP (SEQ ID NO: 4)
L4N
V6Y
L7N
V6F
V6W

[00105] Additionally, in certain embodiments, the sialidase comprises a substitution or deletion of an N-terminal methionine at the N-terminus of the sialidase. For example, in certain 25 embodiments, the sialidase comprises a substitution of a methionine residue at a position corresponding to position 1 of wild-type human Neu2 (SEQ ID NO: 1), *e.g.*, the methionine at a

position corresponding to position 1 of wild-type human Neu2 is substituted by alanine (M1A) or aspartic acid (M1D). In other embodiments, the sialidase comprises a deletion of a methionine residue at a position corresponding to position 1 (Δ M1) of wild-type human Neu2 (SEQ ID NO: 1).

- 5 [00106] In certain embodiments, the recombinant mutant human sialidase comprises a substitution or combination of substitutions corresponding to a substitution or combination of substitutions listed in **TABLE 4** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1)).

TABLE 4

Mutation(s)
Deletion of M1, V6Y, I187K
M1R, V6Y, I187K
M1H, V6Y, I187K
M1K, V6Y, I187K
M1D, V6Y, I187K
M1T, V6Y, I187K
M1N, V6Y, I187K
M1Q, V6Y, I187K
M1G, V6Y, I187K
M1A, V6Y, I187K
M1V, V6Y, I187K
M1L, V6Y, I187K
M1F, V6Y, I187K
M1Y, V6Y, I187K

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4. Substitutions of Residues to Decrease Proteolytic Cleavage

- [00107] It has been discovered that certain sialidases (*e.g.*, human Neu2) are susceptible to cleavage by a protease (*e.g.*, trypsin). As a result, proteolytic cleavage of the sialidase may occur during recombinant protein production, harvesting, purification, or formulation, during administration to a subject, or after administration to a subject. Accordingly, in certain embodiments, the recombinant mutant human sialidase comprises a substitution of at least one wild-type amino acid residue, wherein the substitution decreases cleavage of the sialidase by a protease (*e.g.*, trypsin) relative to a sialidase without the substitution.
- 15
- 20 [00108] In certain embodiments, incubation of the recombinant mutant human sialidase with a protease (*e.g.*, trypsin) results in from about 1% to about 50%, from about 1% to about

40%, from about 1%, to about 30%, from about 1% to about 20%, from about 1% to about 10%, from about 1% to about 5%, from about 5% to about 50%, from about 5% to about 40%, from about 5% to about 30%, from about 5% to about 20%, from about 5% to about 10%, from about 10% to about 50%, from about 10% to about 40%, from about 10% to about 30%, from about 10% to about 20%, from about 20% to about 50%, from about 20% to about 40%, from about 20% to about 30%, from about 30% to about 50%, from about 30% to about 40%, or from about 40% to about 50% of the proteolytic cleavage of a corresponding wild-type sialidase when incubated with the protease under the same conditions. In certain embodiments, incubation of the recombinant mutant human sialidase with a protease (*e.g.*, trypsin) results in less than 50%, less than 40%, less than 30%, less than 10%, less than 5%, less than 3%, less than 1%, or less than 0.5% of the proteolytic cleavage of a corresponding wild-type sialidase when incubated with the protease under the same conditions. Proteolytic cleavage can be assayed by any method known in the art, including for example, by SDS-PAGE as described in Example 4 herein.

[00109] Exemplary substitutions that increase resistance to proteolytic cleavage include:

(i) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (SEQ ID NO: 1), *e.g.*, a substitution by cysteine (A242C), phenylalanine (A242F), glycine (A242G), histidine (A242H), isoleucine (A242I), lysine (A242K), leucine (A242L), methionine (A242M), asparagine (A242N), glutamine (A242Q), arginine (A242R), serine (A242S), valine (A242V), tryptophan (A242W), or tyrosine (A242Y); (ii) a substitution of an arginine residue at a position corresponding to position 243 of wild-type human Neu2 (SEQ ID NO: 1), *e.g.*, a substitution by glutamic acid (R243E), histidine (R243H), asparagine (R243N), glutamine (R243Q), or lysine (R243K); (iii) a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (SEQ ID NO: 1), *e.g.*, a substitution by isoleucine (V244I), lysine (V244K), or proline (V244P); or (iv) a combination of any of the foregoing. In certain embodiments, the recombinant mutant human sialidase comprises a substitution selected from A242C, A242F, A242Y, and A242W. In certain embodiments, the recombinant mutant human sialidase comprises a substitution or a combination of substitutions corresponding to a substitution or combination of substitutions listed in **TABLE 5** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1)).

TABLE 5

Wild Type Human Neu2 (SEQ ID NO: 1) Amino Acid	Exemplary Substitution(s) at Specified Position(s)
A242	C, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, Y
R243	E, H, N, Q, K
V244	I, K, P

[00110] Additional exemplary substitutions that increase resistance to proteolytic cleavage (and/or increase expression yield and/or enzymatic activity) include: (i) a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (SEQ ID NO: 1), *e.g.*, a substitution by aspartic acid (L240D), asparagine (L240N), or tyrosine (L240Y); (ii) a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (SEQ ID NO: 1), *e.g.*, a substitution by cysteine (A213C), asparagine (A213N), serine (A213S), or threonine (A213T); (iii) a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (SEQ ID NO: 1), *e.g.*, a substitution by alanine (R241A), aspartic acid (R241D), leucine (R241L), glutamine (R241Q), or tyrosine (R241Y); (iv) a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (SEQ ID NO: 1), *e.g.*, a substitution by cysteine (S258C); (v) a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (SEQ ID NO: 1), *e.g.*, a substitution by aspartic acid (L260D), phenylalanine (L260F), glutamine (L260Q), or threonine (L260T); (vi) a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (SEQ ID NO: 1), *e.g.*, a substitution by phenylalanine (V265F); or (vii) a combination of any of the foregoing. It is contemplated that, in certain embodiments, a substitution or a combination of substitutions at these positions may improve hydrophobic and/or aromatic interaction between secondary structure elements in the sialidase (*e.g.*, between an α -helix and the nearest β -sheet) thereby stabilizing the structure and improving resistance to proteolytic cleavage.

[00111] In certain embodiments, the recombinant mutant sialidase comprises a mutation at position L240. In certain embodiments, the recombinant mutant sialidase comprises a combination of mutations at positions (i) A213 and A242, (ii) A213, A242, and S258, (iii) L240 and L260, (iv) R241 and A242, (v) A242 and L260, (vi) A242 and V265, or (vii) L240 and A242. In certain embodiments, the recombinant mutant human sialidase comprises a combination of substitutions selected from (i) A213C, A242F, and S258C, (ii) A213C and

A242F, (iii) A213T and A242F, (iv) R241Y and A242F, and (v) L240Y and A242F. In certain embodiments, the recombinant mutant human sialidase comprises a substitution or combination of substitutions corresponding to a substitution or combination of substitutions listed in **TABLE 6** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1)).

TABLE 6

Substitution(s)
A242C, V244P
A242R, V244R
A242R, V244H
A242Y, V244P
A242T, V244P
A242N, V244P
A213C, A242F
A213S, A242F
A213T, A242F
A213N, A242F
A213C, A242F, S258C
A242F, L260F
A242F, V265F
L240Y
L240Y, L260F
L240D, L260T
L240N, L260T
L240N, L260D
L240N, L260Q
L240Y, A242F
R241A, A242F
R241Y, A242F

5. Other Substitutions

[00112] In certain embodiments, the recombinant mutant human sialidase comprises at least one of the following substitutions: I187K, A328E, K370N, or H210N. In certain embodiments, a recombinant mutant human Neu2 comprises the substitution of the amino acids GDYDAPTHQVQW (SEQ ID NO: 15) with the amino acids SMDQGSTW (SEQ ID NO: 16) or

STDGGKTW (SEQ ID NO: 17). In certain embodiments, a recombinant mutant human Neu2 comprises the substitution of the amino acids PRPPAPEA (SEQ ID NO: 18) with the amino acids QTPLEAAC (SEQ ID NO: 19). In certain embodiments, a recombinant mutant human Neu2 comprises the substitution of the amino acids NPRPPAPEA (SEQ ID NO: 20) with the amino acids SQNDGES (SEQ ID NO: 21).

[00113] In certain embodiments, the recombinant mutant human sialidase comprises at least one substitution at a position corresponding to V212, A213, Q214, D215, T216, L217, E218, C219, Q220, V221, A222, E223, V224, E225, or T225.

[00114] In certain embodiments, the recombinant mutant human sialidase comprises an amino acid substitution at a position identified in **TABLE 7** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1). In certain embodiments, the sialidase comprises an amino acid substitution identified in **TABLE 7**. In certain embodiments, the sialidase comprises a combination of any amino acid substitutions identified in **TABLE 7**.

TABLE 7

Wild Type Human Neu2 (SEQ ID NO: 1) Amino Acid	Exemplary Substitution(s) at Specified Position(s)
M1	D
L4	S, T, Y, L, F, A, P, V, I, N, D, or H
P5	G
V6	Y
L7	F, Y, S, I, T, or N
K9	D
V12	L, A, P, V, N, D, or H
F13	S, N, R, K, T, G, D, E, or A
I22	S, N, R, K, T, G, D, E, A, Y, L, F, P, V, I, or H
A24	S, N, R, K, T, G, D, E, A, Y, L, F, P, V, I, or H
L34	S, T, Y, L, F, A, P, V, I, N, D, or H
A36	S, T, Y, L, F, A, P, V, I, N, D, or H
K44	R or E
K45	A, E, or R
L54	M

Wild Type Human Neu2 (SEQ ID NO: 1) Amino Acid	Exemplary Substitution(s) at Specified Position(s)
P62	H, G, N, T, S, F, I, D, or E
H64	F, Y, S, I, T, or N
Q69	H
R78	K
D80	P
P89	S, T, Y, L, F, A, P, V, I, N, D, H, or M
A93	E or K
G107	D
Q108	H
Q112	R or K
C125	Y, F, or L
Q126	E, F, H, I, L, or Y
A150	V
T156	R, N, D, C, G, H, I, L, F, S, Y, V, A, P, or T
F157	R, N, D, C, G, H, I, L, F, S, Y, V, A, or P
A158	R, N, D, C, G, H, I, L, F, S, Y, V, A, P, or T
V159	R, N, D, C, G, H, I, L, F, S, Y, V, A, or P
G160	R, N, D, C, G, H, I, L, F, S, Y, V, A, P, or T
P161	R, N, D, C, G, H, I, L, F, S, Y, V, A, or P
G162	R, N, D, C, G, H, I, L, F, S, Y, V, A, P, or T
H163	R, N, D, C, G, H, I, L, F, S, Y, V, A, or P
C164	R, N, D, C, G, H, I, L, F, S, Y, V, A, P, or T
L165	R, N, D, C, G, H, I, L, F, S, Y, V, A, or P
R170	P
A171	G
V176	R, N, D, C, G, H, I, L, F, S, Y, V, P, or A
P177	S, T, Y, L, F, A, P, V, I, N, D, or H
A178	S, T, Y, L, F, A, P, V, I, N, D, or H
L184	S, N, R, K, T, G, D, E, A, F, H, I, L, P, V, or Y
H185	S, N, R, K, T, G, D, E, or A
P186	S, N, R, K, T, G, D, E, A, F, H, I, L, P, V, or Y
I187	S, N, R, K, T, G, D, E, or A

Wild Type Human Neu2 (SEQ ID NO: 1) Amino Acid	Exemplary Substitution(s) at Specified Position(s)
Q188	P, S, N, R, K, T, G, D, E, or A
R189	P
P190	F, M, A, D, G, H, N, P, R, S, or T
I191	M, A, D, F, H, I, L, N, P, S, T, V, Y, E, G, K, or R
A194	S, T, Y, L, F, A, P, V, I, N, D, or H
A213	C, N, S, or T
L217	R, N, D, C, G, H, I, L, F, S, Y, or V
C219	R, N, D, C, G, H, I, L, F, S, Y, or V
A222	D
E225	P
H239	P
L240	D, N, or Y
R241	A, D, L, Q, or Y
A242	C, F, G, H, I, K, L, M, N, Q, R, S, V, W, or Y
V244	I or P
T249	A
D251	G
E257	P
S258	C
L260	D, F, Q, or T
V265	F
Q270	S, T, A, H, P, or F
G271	S, N, R, K, T, G, D, E, or A
C272	S, N, R, K, T, G, D, E, A, C, H, Y, F, H, L, P, or V
W292	R
S301	A, D, E, F, G, H, I, K, L, M, N, P, Q, T, V, W, Y, C, or R
W302	A, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, Y, or K
E319	D
V325	F, Y, S, I, T, N, A, D, H, L, P, or V
L326	F, Y, S, I, T, N, A, D, H, L, P, or V
L327	F, Y, S, I, T, N, A, D, H, L, P, or V

Wild Type Human Neu2 (SEQ ID NO: 1) Amino Acid	Exemplary Substitution(s) at Specified Position(s)
C332	A, D, G, H, N, P, R, S, or T
Y359	A or S
V363	R, S, T, Y, L, F, A, P, V, I, N, D, or H
L365	K, Q, F, Y, S, I, T, N, A, D, H, L, P, or V

[00115] For example, in certain embodiments, the recombinant mutant human sialidase comprises: (a) a substitution of a proline residue at a position corresponding to position 5 of wild-type human Neu2 (P5); (b) a substitution of a lysine residue at a position corresponding to position 9 of wild-type human Neu2 (K9); (c) a substitution of a lysine residue at a position corresponding to position 44 of wild-type human Neu2 (K44); (d) a substitution of a lysine residue at a position corresponding to position 45 of wild-type human Neu2 (K45); (e) a substitution of a leucine residue at a position corresponding to position 54 of wild-type human Neu2 (L54); (f) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62); (g) a substitution of a glutamine residue at a position corresponding to position 69 of wild-type human Neu2 (Q69); (h) a substitution of an arginine residue at a position corresponding to position 78 of wild-type human Neu2 (R78); (i) a substitution of an aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 (D80); (j) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93); (k) a substitution of a glycine residue at a position corresponding to position 107 of wild-type human Neu2 (G107); (l) a substitution of a glutamine residue at a position corresponding to position 108 of wild-type human Neu2 (Q108); (m) a substitution of a glutamine residue at a position corresponding to position 112 of wild-type human Neu2 (Q112); (n) a substitution of a cysteine residue at a position corresponding to position 125 of wild-type human Neu2 (C125); (o) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126); (p) a substitution of an alanine residue at a position corresponding to position 150 of wild-type human Neu2 (A150); (q) a substitution of a cysteine residue at a position corresponding to position 164 of wild-type human Neu2 (C164); (r) a substitution of an arginine residue at a position corresponding to position 170 of wild-type human Neu2 (R170); (s) a substitution of an alanine residue at a position corresponding to position 171 of wild-type human Neu2 (A171); (t) a substitution of a glutamine residue at a position corresponding to position 188 of wild-type human Neu2 (Q188); (u) a substitution of an arginine residue at a position corresponding to position 189 of wild-type

human Neu2 (R189); (v) a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (A213); (w) a substitution of a leucine residue at a position corresponding to position 217 of wild-type human Neu2 (L217); (x) a substitution of a glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 (E225); (y) a substitution of a histidine residue at a position corresponding to position 239 of wild-type human Neu2 (H239); (z) a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240); (aa) a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241); (bb) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242); (cc) a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244); (dd) a substitution of a threonine residue at a position corresponding to position 249 of wild-type human Neu2 (T249); (ee) a substitution of an aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 (D251); (ff) a substitution of a glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 (E257); (gg) a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258); (hh) a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260); (ii) a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265); (jj) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2 (Q270); (kk) a substitution of a tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 (W292); (ll) a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301); (mm) a substitution of a tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 (W302); (nn) a substitution of a valine residue at a position corresponding to position 363 of wild-type human Neu2 (V363); or (oo) a substitution of a leucine residue at a position corresponding to position 365 of wild-type human Neu2 (L365); or a combination of any of the foregoing substitutions. For example, the sialidase may comprise a substitution of K9, P62, A93, Q216, A242, Q270, S301, W302, V363, or L365, or a combination of any of the foregoing substitutions.

[00116] In certain embodiments, in the sialidase: (a) the proline residue at a position corresponding to position 5 of wild-type human Neu2 is substituted by histidine (P5H); (b) the lysine residue at a position corresponding to position 9 of wild-type human Neu2 is substituted by aspartic acid (K9D); (c) the lysine residue at a position corresponding to position 44 of wild-type human Neu2 is substituted by arginine (K44R) or glutamic acid (K44E); (d) the lysine

residue at a position corresponding to position 45 of wild-type human Neu2 is substituted by alanine (K45A), arginine (K45R), or glutamic acid (K45E); (e) the leucine residue at a position corresponding to position 54 of wild-type human Neu2 is substituted by methionine (L54M); (f) the proline residue at a position corresponding to position 62 of wild-type human Neu2 is substituted by asparagine (P62N), aspartic acid (P62D), histidine (P62H), glutamic acid (P62E), glycine (P62G), serine (P62S), or threonine (P62T); (g) the glutamine residue at a position corresponding to position 69 of wild-type human Neu2 is substituted by histidine (Q69H); (h) the arginine residue at a position corresponding to position 78 of wild-type human Neu2 is substituted by lysine (R78K); (i) the aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 is substituted by proline (D80P); (j) the alanine residue at a position corresponding to position 93 of wild-type human Neu2 is substituted by glutamic acid (A93E) or lysine (A93K); (k) the glycine residue at a position corresponding to position 107 of wild-type human Neu2 is substituted by aspartic acid (G107D); (l) the glutamine residue at a position corresponding to position 108 of wild-type human Neu2 is substituted by histidine (Q108H); (m) the glutamine residue at a position corresponding to position 112 of wild-type human Neu2 is substituted by arginine (Q112R) or lysine (Q112K); (n) the cysteine residue at a position corresponding to position 125 of wild-type human Neu2 is substituted by leucine (C125L); (o) the glutamine residue at a position corresponding to position 126 of wild-type human Neu2 is substituted by leucine (Q126L), glutamic acid (Q126E), phenylalanine (Q126F), histidine (Q126H), isoleucine (Q126I), or tyrosine (Q126Y); (p) the alanine residue at a position corresponding to position 150 of wild-type human Neu2 is substituted by valine (A150V); (q) the cysteine residue at a position corresponding to position 164 of wild-type human Neu2 is substituted by glycine (C164G); (r) the arginine residue at a position corresponding to position 170 of wild-type human Neu2 is substituted by proline (R170P); (s) the alanine residue at a position corresponding to position 171 of wild-type human Neu2 is substituted by glycine (A171G); (t) the glutamine residue at a position corresponding to position 188 of wild-type human Neu2 is substituted by proline (Q188P); (u) the arginine residue at a position corresponding to position 189 of wild-type human Neu2 is substituted by proline (R189P); (v) the alanine residue at a position corresponding to position 213 of wild-type human Neu2 is substituted by cysteine (A213C), asparagine (A213N), serine (A213S), or threonine (A213T); (w) the leucine residue at a position corresponding to position 217 of wild-type human Neu2 is substituted by alanine (L217A) or valine (L217V); (x) the threonine residue at a position corresponding to position 249 of wild-type human Neu2 is substituted by alanine (T249A); (y) the aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 is

substituted by glycine (D251G); (z) the glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 is substituted by proline (E225P); (aa) the histidine residue at a position corresponding to position 239 of wild-type human Neu2 is substituted by proline (H239P); (bb) the leucine residue at a position corresponding to position 240 of wild-type human Neu2 is substituted by aspartic acid (L240D), asparagine (L240N), or tyrosine (L240Y); (cc) the arginine residue at a position corresponding to position 241 of wild-type human Neu2 is substituted by alanine (R241A), aspartic acid (R241D), leucine (R241L), glutamine (R241Q), or tyrosine (R241Y); (dd) the alanine residue at a position corresponding to position 242 of wild-type human Neu2 is substituted by cysteine (A242C), phenylalanine (A242F), glycine (A242G), histidine (A242H), isoleucine (A242I), lysine (A242K), leucine (A242L), methionine (A242M), asparagine (A242N), glutamine (A242Q), arginine (A242R), serine (A242S), valine (A242V), tryptophan (A242W), or tyrosine (A242Y); (ee) the valine residue at a position corresponding to position 244 of wild-type human Neu2 is substituted by isoleucine (V244I), lysine (V244K), or proline (V244P); (ff) the glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 is substituted by proline (E257P); (gg) the serine residue at a position corresponding to position 258 is substituted by cysteine (S258C); (hh) the leucine residue at a position corresponding to position 260 of wild-type human Neu2 is substituted by aspartic acid (L260D), phenylalanine (L260F), glutamine (L260Q), or threonine (L260T); (ii) the valine residue at a position corresponding to position 265 of wild-type human Neu2 is substituted by phenylalanine (V265F); (jj) the glutamine residue at a position corresponding to position 270 of wild-type human Neu2 is substituted by alanine (Q270A), histidine (Q270H), phenylalanine (Q270F), proline (Q270P), serine (Q270S), or threonine (Q270T); (kk) the tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 is substituted by arginine (W292R); (ll) the serine residue at a position corresponding to position 301 of wild-type human Neu2 is substituted by alanine (S301A), aspartic acid (S301D), glutamic acid (S301E), phenylalanine (S301F), glycine (S301G), histidine (S301H), isoleucine (S301I), lysine (S301K), leucine (S301L), methionine (S301M), asparagine (S301N), proline (S301P), glutamine (S301Q), arginine (S301R), threonine (S301T), valine (S301V), tryptophan (S301W), or tyrosine (S301Y); (mm) the tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 is substituted by alanine (W302A), aspartic acid (W302D), glutamic acid (W302E), phenylalanine (W302F), glycine (W302G), histidine (W302H), isoleucine (W302I), lysine (W302K), leucine (W302L), methionine (W302M), asparagine (W302N), proline (W302P), glutamine (W302Q), arginine (W302R), serine (W302S), threonine (W302T), valine (W302V), or tyrosine (W302Y); (nn) the

valine residue at a position corresponding to position 363 of wild-type human Neu2 is substituted by arginine (V363R); or (oo) the leucine residue at a position corresponding to position 365 of wild-type human Neu2 is substituted by glutamine (L365Q), histidine (L365H), isoleucine (L365I), lysine (L365K) or serine (L365S); or the sialidase comprises a combination of any of the foregoing substitutions. For example, the sialidase may comprise a substitution selected from K9D, P62G, P62N, P62S, P62T, D80P, A93E, Q126H, Q126Y, R189P, H239P, A242T, Q270A, Q270S, Q270T, S301A, S301R, W302K, W302R, V363R, and L365I, or a combination of any of the foregoing substitutions.

[00117] In certain embodiments, the recombinant mutant human sialidase comprises a deletion of a leucine residue at a position corresponding to position 184 of wild-type human Neu2 (Δ L184), a deletion of a histidine residue at a position corresponding to position 185 of wild-type human Neu2 (Δ H185), a deletion of a proline residue at a position corresponding to position 186 of wild-type human Neu2 (Δ P186), a deletion of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (Δ I187), and a deletion of a glutamine residue at a position corresponding to position 184 of wild-type human Neu2 (Δ Q188), or a combination of any of the foregoing deletions.

[00118] In certain embodiments, the recombinant mutant human sialidase comprises an insertion between a threonine residue at a position corresponding to position 216 of wild-type human Neu2 and a leucine residue at a position corresponding to position 217 of wild-type human Neu2, for example, an insertion of an amino acid selected from S, T, Y, L, F, A, P, V, I, N, D, and H.

[00119] Additional exemplary sialidase mutations, and combinations of sialidase mutations, are described in International (PCT) Patent Application No. PCT/US2019/012207, filed January 3, 2019, including in the Detailed Description in the section entitled “I. Recombinant Human Sialidases,” and in the Examples in Examples 1, 2, 3, 4, 5, and 6.

6. Combinations of Substitutions

[00120] In certain embodiments, the recombinant mutant human sialidase comprises a combination of any of the mutations contemplated herein. For example, the recombinant mutant sialidase enzyme may comprise a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more of the mutations contemplated herein. It is contemplated that the recombinant mutant sialidase enzyme may comprise 1-15, 1-10, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-15, 2-10, 2-7, 2-6, 2-5, 2-4, 2-3, 3-15, 3-10, 3-7, 3-6, 3-5, or 3-4 of the mutations contemplated herein.

[00121] For example, the recombinant mutant sialidase enzyme may comprise a M1 deletion (Δ M1), M1A substitution, M1D substitution, V6Y substitution, K9D substitution, P62G substitution, P62N substitution, P62S substitution, P62T substitution, A93E substitution, I187K substitution, Q270A substitution, S301R substitution, W302K substitution, C332A substitution, V363R substitution, L365I substitution, or a combination of any of the foregoing.

[00122] In certain embodiments, the recombinant mutant sialidase enzyme comprises a M1 deletion (Δ M1), M1A substitution, M1D substitution, V6Y substitution, I187K substitution, C332A substitution, or a combination of any of the foregoing. For example, the recombinant mutant sialidase enzyme may comprise a combination of mutations selected from: M1A and V6Y; M1A and I187K; M1A and C332A; M1D and V6Y; M1D and I187K; M1D and C332A; Δ M1 and V6Y; Δ M1 and I187K; Δ M1 and C332A; V6Y and I187K; V6Y and C332A; I187K and C332A; M1A, V6Y, and I187K; M1A, V6Y, and C332A; M1A, I187K, and C332A; M1D, V6Y, and I187K; M1D, V6Y, and C332A; M1D, I187K, and C332A; Δ M1, V6Y, and I187K; Δ M1, V6Y, and C332A; Δ M1, I187K, and C332A; V6Y, I187K, and C332A; M1A, V6Y, I187K, and C332A; M1D, V6Y, I187K, and C332A; and Δ M1, V6Y, I187K, and C332A.

[00123] In certain embodiments, the recombinant mutant sialidase enzyme comprises (i) an amino acid substitution identified in **TABLE 7**, or a combination of any amino acid substitutions identified in **TABLE 7**, and (ii) an M1 deletion (Δ M1), M1A substitution, M1D substitution, V6Y substitution, I187K substitution, C332A substitution, or a combination of any of the foregoing. For example, the recombinant mutant sialidase enzyme may comprise (i) an amino acid substitution identified in **TABLE 7**, or a combination of any amino acid substitutions identified in **TABLE 7**, and (ii) a combination of mutations selected from: M1A and V6Y; M1A and I187K; M1A and C332A; M1D and V6Y; M1D and I187K; M1D and C332A; Δ M1 and V6Y; Δ M1 and I187K; Δ M1 and C332A; V6Y and I187K; V6Y and C332A; I187K and C332A; M1A, V6Y, and I187K; M1A, V6Y, and C332A; M1A, I187K, and C332A; M1D, V6Y, and I187K; M1D, V6Y, and C332A; M1D, I187K, and C332A; Δ M1, V6Y, and I187K; Δ M1, V6Y, and C332A; Δ M1, I187K, and C332A; V6Y, I187K, and C332A; M1A, V6Y, I187K, and C332A; M1D, V6Y, I187K, and C332A; and Δ M1, V6Y, I187K, and C332A.

[00124] In certain embodiments, the recombinant mutant sialidase enzyme comprises: (a) the M1D, V6Y, P62G, A93E, I187K, and C332A substitutions; (b) the M1D, V6Y, K9D, A93E, I187K, C332A, V363R, and L365I substitutions; (c) the M1D, V6Y, P62N, I187K, and C332A substitutions; (d) the M1D, V6Y, I187K, Q270A, S301R, W302K, and C332A substitutions; (e) the M1D, V6Y, P62S, I187K, Q270A, S301R, W302K, and C332A substitutions; (f) the M1D,

V6Y, P62T, I187K, Q270A, S301R, W302K, and C332A substitutions; (g) the M1D, V6Y, P62N, I187K, Q270A, S301R, W302K, and C332A substitutions; (h) the M1D, V6Y, P62G, A93E, I187K, S301A, W302R, and C332A substitutions; (i) the M1D, V6Y, P62G, A93E, Q126Y, I187K, Q270T, and C332A substitutions; (j) the M1D, V6Y, P62G, A93E, Q126Y, I187K, and C332A substitutions; or (k) the M1D, V6Y, P62G, A93E, Q126Y, I187K, A242F, Q270T, and C332A substitutions.

[00125] In certain embodiments, the recombinant mutant human sialidase comprises a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301) in combination with a substitution of a tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 (W302). For example, the recombinant mutant human sialidase may comprise a combination of substitutions corresponding to a combination of substitutions listed in a row of **TABLE 8** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1)). For example, the recombinant mutant human sialidase may comprise: the S301K and W302R substitutions; the S301K and W302K substitutions; or the S301A and W302S substitutions.

TABLE 8

Substitutions
S301A, W302R
S301A, W302S
S301A, W302T
S301K, W302S
S301N, W302S
S301T, W302S
S301T, W302T
S301T, W302R
S301A, W302A
S301K, W302R
S301K, W302T
S301N, W302T
S301K, W302K
S301P, W302R
S301P, W302S
S301P, W302T

[00126] In certain embodiments, the recombinant mutant human sialidase comprises a combination of substitutions corresponding to a combination of substitutions listed in a row of **TABLE 9** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1)).

TABLE 9

Substitutions
M1D, V6Y, P62G, I187K, C332A
M1D, V6Y, K9D, I187K, C332A, V363R, L365I
M1D, V6Y, P62G, A93E, I187K, C332A
M1D, V6Y, K9D, I187K, C332A, V363R, L365K
M1D, V6Y, K9D, I187K, C332A, V363R, L365S
M1D, V6Y, K9D, I187K, C332A, V363R, L365Q
M1D, V6Y, K9D, I187K, C332A, V363R, L365H
M1D, V6Y, A93K, I187K, C332A
M1D, V6Y, A93E, I187K, C332A
V6Y, I187K, W292R
V6Y, G107D, I187K
V6Y, C125L
C125L, I187K
V6Y, C125L, I187K
M1D, V6Y, K45A, I187K, C332A
M1D, V6Y, Q270A, I187K, C332A
M1D, V6Y, K44R, K45R, I187K, C332A
M1D, V6Y, Q112R, I187K, C332A
M1D, V6Y, Q270F, I187K, C332A
M1D, V6Y, I187K, S301R, W302K, C332A
M1D, V6Y, K44E, K45E, I187K, C332A
M1D, V6Y, I187K, L217V, C332A
M1D, V6Y, I187K, L217A, C332A
M1D, V6Y, K44E, K45E, I187K, S301R, W302K, C332A
M1D, V6Y, Q112R, I187K, S301R, W302K, C332A
M1D, V6Y, I187K, Q270A, S301R, W302K, C332A
M1D, V6Y, K44E, K45E, Q112R, I187K, C332A
M1D, V6Y, K44E, K45E, I187K, Q270A, C332A

Substitutions
M1D, V6Y, K45A, I187K, Q270A, C332A
M1D, V6Y, I187K, Q270H, C332A
M1D, V6Y, I187K, Q270P, C332A
M1D, V6Y, Q112K, I187K, C332A
M1D, V6Y, P62S, I187K, Q270A, S301R, W302K, C332A
M1D, V6Y, P62T, I187K, Q270A, S301R, W302K, C332A
M1D, V6Y, P62N, I187K, Q270A, S301R, W302K, C332A
V6Y, P62H, I187K
V6Y, Q108H, I187K
M1D, V6Y, P62H, I187K, C332A
M1D, V6Y, P62G, I187K, C332A
V6Y, P62G, I187K
M1D, V6Y, P62H, I187K
M1D, V6Y, Q108H, I187K
M1D, V6Y, P62N, I187K, C332A
M1D, V6Y, P62D, I187K, C332A
M1D, V6Y, P62E, I187K, C332A
V6Y, C164G, I187K, T249A
V6Y, C164G, I187K
V6Y, Q126L, I187K D251G
V6Y, L54M, Q69H, R78K, A171G, I187K
V6Y, P62T, I187K
V6Y, A150V, I187K
P5H, V6Y, P62S, I187K
V6Y, C164G, I187K
Q126Y, Q170T
Q126Y, A242F, Q270T
M1D, V6Y, P62G, A93E, Q126E, I187K, C332A
M1D, V6Y, P62G, A93E, Q126I, I187K, C332A
M1D, V6Y, P62G, A93E, Q126L, I187K, C332A
M1D, V6Y, P62G, A93E, Q126Y, I187K, C332A
M1D, V6Y, P62G, A93E, Q126F, I187K, C332A

Substitutions
M1D, V6Y, P62G, A93E, Q126H, I187K, C332A
M1D, V6Y, P62G, A93E, I187K, Q270S, C332A
M1D, V6Y, P62G, A93E, I187K, Q270T, C332A
M1D, V6Y, P62G, A93E, Q126Y, I187K, Q270T, C332A
M1D, V6Y, P62G, A93E, Q126Y, I187K, A242F, Q270T, C332A
M1D, V6Y, P62G, D80P, A93E, I187K, C332A
M1D, V6Y, P62G, A93E, R170P, I187K, C332A
M1D, V6Y, P62G, A93E, I187K, Q188P, C332A
M1D, V6Y, P62G, A93E, I187K, R189P, C332A
M1D, V6Y, P62G, A93E, I187K, E225P, C332A
M1D, V6Y, P62G, A93E, I187K, H239P, C332A
M1D, V6Y, P62G, A93E, I187K, E257P, C332A
M1D, V6Y, P62G, A93E, I187K, S301A, C332A
M1D, V6Y, P62G, A93E, I187K, S301D, C332A
M1D, V6Y, P62G, A93E, I187K, S301E, C332A
M1D, V6Y, P62G, A93E, I187K, S301F, C332A
M1D, V6Y, P62G, A93E, I187K, S301H, C332A
M1D, V6Y, P62G, A93E, I187K, S301K, C332A
M1D, V6Y, P62G, A93E, I187K, S301L, C332A
M1D, V6Y, P62G, A93E, I187K, S301M, C332A
M1D, V6Y, P62G, A93E, I187K, S301N, C332A
M1D, V6Y, P62G, A93E, I187K, S301P, C332A
M1D, V6Y, P62G, A93E, I187K, S301Q, C332A
M1D, V6Y, P62G, A93E, I187K, S301R, C332A
M1D, V6Y, P62G, A93E, I187K, S301T, C332A
M1D, V6Y, P62G, A93E, I187K, S301V, C332A
M1D, V6Y, P62G, A93E, I187K, S301W, C332A
M1D, V6Y, P62G, A93E, I187K, S301Y, C332A
M1D, V6Y, P62G, A93E, I187K, W302A, C332A
M1D, V6Y, P62G, A93E, I187K, W302D, C332A
M1D, V6Y, P62G, A93E, I187K, W302F, C332A
M1D, V6Y, P62G, A93E, I187K, W302G, C332A

Substitutions
M1D, V6Y, P62G, A93E, I187K, W302H, C332A
M1D, V6Y, P62G, A93E, I187K, W302I, C332A
M1D, V6Y, P62G, A93E, I187K, W302L, C332A
M1D, V6Y, P62G, A93E, I187K, W302M, C332A
M1D, V6Y, P62G, A93E, I187K, W302N, C332A
M1D, V6Y, P62G, A93E, I187K, W302P, C332A
M1D, V6Y, P62G, A93E, I187K, W302Q, C332A
M1D, V6Y, P62G, A93E, I187K, W302R, C332A
M1D, V6Y, P62G, A93E, I187K, W302S, C332A
M1D, V6Y, P62G, A93E, I187K, W302T, C332A
M1D, V6Y, P62G, A93E, I187K, W302V, C332A
M1D, V6Y, P62G, A93E, I187K, W302Y, C332A
M1D, V6Y, P62G, A93E, I187K, S301A, W302A, C332A
M1D, V6Y, P62G, A93E, I187K, S301A, W302R, C332A
M1D, V6Y, P62G, A93E, I187K, S301A, W302S, C332A
M1D, V6Y, P62G, A93E, I187K, S301A, W302T, C332A
M1D, V6Y, P62G, A93E, I187K, S301K, W302S, C332A
M1D, V6Y, P62G, A93E, I187K, S301K, W302R, C332A
M1D, V6Y, P62G, A93E, I187K, S301K, W302T, C332A
M1D, V6Y, P62G, A93E, I187K, S301N, W302S, C332A
M1D, V6Y, P62G, A93E, I187K, S301N, W302T, C332A
M1D, V6Y, P62G, A93E, I187K, S301T, W302R, C332A
Q126Y, Q270T
Q126Y, A242F, Q270T

[00127] In certain embodiments, the recombinant mutant human sialidase comprises the amino acid sequence of any one of SEQ ID NOs: 48-62, 94, 97, 100, or 126, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 48-62, 94, 97, 100, or 126.

5 **[00128]** In certain embodiments, the recombinant mutant human sialidase comprises the amino acid sequence of

X₁X₂SX₃X₄X₅LQX₆ESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASX₇X₈DEHAELIVX₉RRGD
YDAX₁₀THQVQWX₁₁AQEVVAQAX₁₂LDGHRSMNPCPLYDX₁₃QTGTLFLFFIAIPX₁₄X₁₅VTEX₁₆Q
QLQTRANVTRLX₁₇X₁₈VTSTDHGRTWSSPRDLTDAAIGPX₁₉YREWSTFAVGPGHX₂₀LQLHDX₂₁

RSLVVPAYAYRKLHPX₂₂QRPIPSAFX₂₃FLSHDHGRTWARGHFVAQDTX₂₄ECQVAEVETGEQRV
 VTLNARSHLRARVQAQX₂₅NX₂₆GLDFQX₂₇SQLVKKLVEPPX₂₈GX₂₉QGSVISFSPRSGPGSP
 AQX₃₀LLYTHPTHX₃₁X₃₂QRADLGAYLNPRPPAPEAWSEPX₃₃LLAKGSX₃₄AYSDDLQSMGTGPDGS
 PLFGX₃₅LYEANDYEEIX₃₆FX₃₇MFTLQKQAFPAEYLPQ

- 5 (SEQ ID NO: 47), wherein X₁ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Thr, Val, or not present, X₂ is Ala or Lys, X₃ is Asn or Leu, X₄ is Pro or His, X₅ is Phe, Trp, Tyr or Val, X₆ is Lys or Asp, X₇ is Lys, Arg, or Glu. X₈ is Lys, Ala, Arg, or Glu, X₉ is Leu or Met, X₁₀ is Pro, Asn, Asp, His, Glu, Gly, Ser or Thr, X₁₁ is Gln or His, X₁₂ is Arg or Lys, X₁₃ is Ala, Glu or Lys, X₁₄ is Gly or Asp, X₁₅ is Gln or His, X₁₆ is Gln, Arg, or Lys, X₁₇ is Ala, Cys, Ile, Ser,
 10 Val, or Leu, X₁₈ is Gln or Leu, X₁₉ is Ala or Val, X₂₀ is Cys or Gly, X₂₁ is Ala or Gly, X₂₂ is Arg, Ile, or Lys, X₂₃ is Ala, Cys, Leu, or Val, X₂₄ is Leu, Ala, or Val, X₂₅ is Thr or Ala, X₂₆ is Asp or Gly, X₂₇ is Glu or Lys, X₂₈ is Gln, Ala, His, Phe, or Pro, X₂₉ is Cys or Val, X₃₀ is Trp or Arg, X₃₁ is Ser or Arg, X₃₂ is Trp or Lys, X₃₃ is Lys or Val, X₃₄ is Ala, Cys, Ser, or Val, X₃₅ is Cys, Leu, or Val, X₃₆ is Val or Arg, and X₃₇ is Leu, Gln, His, Ile, Lys, or Ser, and the sialidase comprises at
 15 least one mutation relative to wild-type human Neu2 (SEQ ID NO: 1).

[00129] In certain embodiments, the recombinant mutant human sialidase comprises the amino acid sequence of

X₁ASLPX₂LQX₃ESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASKKDEHAELIVLRRGDYDAX₄
 THQVQWQAQEVVAQARLDGHRSMNPCPLYDX₅QTGTLFLFFIAIPGQVTEQQQLQTRANVTRLQ
 20 VTSTDHGRTWSSPRDLTDAAIQPAYREWSTFAVGPGHCLQLHADRARSRLVVPAYAYRKLHPX₆QRP
 IPSAFCFLSHDHGRTWARGHFVAQDTLEQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLD
 FQESQLVKKLVEPPX₇GCQGSVISFSPRSGPGSPAQWLLYTHPTHX₈X₉QRADLGAYLNPRPP
 APEAWSEPVLLAKGSX₁₀AYSDDLQSMGTGPDGSPLFGCLYEANDYEEIX₁₁FX₁₂MFTLQKQAFPAE
 YLPQ

- 25 (SEQ ID NO: 46), wherein X₁ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Thr, Val, or not present, X₂ is Phe, Trp, Tyr or Val, X₃ is Lys or Asp, X₄ is Pro, Asn, Asp, His, Glu, Gly, Ser or Thr, X₅ is Ala, Glu, or Lys, X₆ is Arg, Ile, or Lys, X₇ is Gln, Ala, His, Phe, or Pro, X₈ is Ser or Arg, X₉ is Trp or Lys, X₁₀ is Ala, Cys, Ser, or Val, X₁₁ is Val or Arg, and X₁₂ is Leu, Gln, His, Ile, Lys, or Ser, and the sialidase comprises at least one mutation relative to wild-
 30 type human Neu2 (SEQ ID NO: 1). In certain embodiments, X₁ is Ala, Asp, Met, or not present, X₂ is Tyr or Val, X₃ is Lys or Asp, X₄ is Pro, Asn, Gly, Ser or Thr, X₅ is Ala or Glu, X₆ is Ile or Lys, X₇ is Gln or Ala, X₈ is Ser or Arg, X₉ is Trp or Lys, X₁₀ is Ala or Cys, X₁₁ is Val or Arg, and X₁₂ is Leu or Ile.

[00130] In certain embodiments, the recombinant mutant human sialidase comprises the amino acid sequence of

X₁X₂SX₃X₄X₅LQX₆ESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASX₇X₈DEHAELIVX₉RRGD
 YDAX₁₀THQVQWX₁₁AQEVVAQAX₁₂LX₁₃GHRSMNPCPLYDX₁₄QTGTLFLFFIAIPX₁₅X₁₆VTEX₁₇
 QQQLQTRANVTRLX₁₈X₁₉VTSTDHGRTWSSPRDLTDAAIQPX₂₀YREWSTFAVGPGHX₂₁LQLHDX₂₂

X₂₃RSLVVPAYAYRKLHPX₂₄X₂₅X₂₆PIPSAFX₂₇FLSHDHGRTWARGHFVX₂₈QDTX₂₉ECQVAEVX
 30TGEQRVVTLNARSX₃₁X₃₂X₃₃X₃₄RX₃₅QAQX₃₆NX₃₇GLDFQX₃₈X₃₉QX₄₀VKKL
 X₄₁EPPPX₄₂GX₄₃QGSVISFPSRSGPGSPAQX₄₄LLYTHPTHX₄₅X₄₆QRADLGAYLNPRPPAPEA
 WSEPX₄₇LLAKGSX₄₈AYS DLQSMGTGPDGSP LFGX₄₉LYEANDYEEIX₅₀FX₅₁MFTLKQAFPAEYL
 PQ

(SEQ ID NO: 120), wherein X₁ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Thr, Val, or not present, X₂ is Ala or Lys, X₃ is Asn or Leu, X₄ is Pro or His, X₅ is Phe, Trp, Tyr or Val, X₆ is Lys or Asp, X₇ is Lys, Arg, or Glu, X₈ is Lys, Ala, Arg, or Glu, X₉ is Leu or Met, X₁₀ is Pro, Asn, Asp, His, Glu, Gly, Ser or Thr, X₁₁ is Gln or His, X₁₂ is Arg or Lys, X₁₃ is Asp or Pro, X₁₄ is Ala, Glu or Lys, X₁₅ is Gly or Asp, X₁₆ is Gln or His, X₁₇ is Gln, Arg, or Lys, X₁₈ is Ala, Cys, Ile, Ser, Val, or Leu, X₁₉ is Gln, Leu, Glu, Phe, His, Ile, Leu, or Tyr, X₂₀ is Ala or Val, X₂₁ is Cys or Gly, X₂₂ is Arg or Pro, X₂₃ is Ala or Gly, X₂₄ is Arg, Ile, or Lys, X₂₅ is Gln or Pro, X₂₆ is Arg or Pro, X₂₇ is Ala, Cys, Leu, or Val, X₂₈ is Ala, Cys, Asn, Ser, or Thr, X₂₉ is Leu, Ala, or Val, X₃₀ is Glu or Pro, X₃₁ is His or Pro, X₃₂ is Leu, Asp, Asn, or Tyr, X₃₃ is Arg, Ala, Asp, Leu, Gln, or Tyr, X₃₄ is Ala, Cys, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Val, Trp, or Tyr, X₃₅ is Val, Ile, or Lys, X₃₆ is Thr or Ala, X₃₇ is Asp or Gly, X₃₈ is Glu, Lys, or Pro, X₃₉ is Ser or Cys, X₄₀ is Leu, Asp, Phe, Gln, or Thr, X₄₁ is Val or Phe, X₄₂ is Gln, Ala, His, Phe, Pro, Ser, or Thr, X₄₃ is Cys or Val, X₄₄ is Trp or Arg, X₄₅ is Ser, Arg, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Thr, Val, Trp, or Tyr, X₄₆ is Trp, Lys, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, or Tyr, X₄₇ is Lys or Val, X₄₈ is Ala, Cys, Ser, or Val, X₄₉ is Cys, Leu, or Val, X₅₀ is Val or Arg, and X₅₁ is Leu, Gln, His, Ile, Lys, or Ser, and the sialidase comprises at least one mutation relative to wild-type human Neu2 (SEQ ID NO: 1).

[00131] In certain embodiments, the recombinant mutant human sialidase comprises the amino acid sequence of

X₁ASLPX₂LQX₃ESVFQSGAHAYRIPALLYLPQGQSLLAFAEQRASKKDEHAELIVLRRGDYDAX₄
 THQVQWQAQEVVAQARLDGHRSMNPCPLYDX₅QTGTLFLFFIAIPGQVTEQQQLQTRANVTRLXC
 6VTSTDHGRWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRSLVVPAYAYRKLHPX₇QR
 PIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEETGEQRVVTLNARSHLRX₈RVQAQSTNDGL
 30DFQESQLVKKLVEPPPX₉GCQGSVISFPSRSGPGSPAQWLLYTHPTHX₁₀X₁₁QRADLGAYLNPR
 PPAPEAWSEPVLAKGSX₁₂AYS DLQSMGTGPDGSP LFGCLYEANDYEEIX₁₃FX₁₄MFTLKQAFP
 AEYLPQ

(SEQ ID NO: 119), wherein X₁ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Thr, Val, or not present, X₂ is Phe, Trp, Tyr or Val, X₃ is Lys or Asp, X₄ is Pro, Asn, Asp, His, Glu, Gly, Ser or Thr, X₅ is Ala, Glu, or Lys, X₆ is Gln, Leu, Glu, Phe, His, Ile, Leu, or Tyr, X₇ is Arg, Ile, or Lys, X₈ is Ala, Cys, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Val, Trp, or Tyr, X₉ is Gln, Ala, His, Phe, Pro, Ser, or Thr, X₁₀ is Ser, Arg, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Thr, Val, Trp, or Tyr, X₁₁ is Trp, Lys, Ala, Asp, Glu, Phe, Gly,

His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, or Tyr, X₁₂ is Ala, Cys, Ser, or Val, X₁₃ is Val or Arg, and X₁₄ is Leu, Gln, His, Ile, Lys, or Ser, and the sialidase comprises at least one mutation relative to wild-type human Neu2 (SEQ ID NO: 1). In certain embodiments, X₁ is Ala, Asp, Met, or not present, X₂ is Tyr or Val, X₃ is Lys or Asp, X₄ is Pro, Asn, Gly, Ser or Thr, X₅ is Ala or Glu, X₆ is Gln or Tyr, X₇ is Ile or Lys, X₈ is Ala or Thr, X₉ is Gln, Ala, or Thr, X₁₀ is Ser, Arg, or Ala, X₁₁ is Trp, Lys, or Arg, X₁₂ is Ala or Cys, X₁₃ is Val or Arg, and X₁₄ is Leu or Ile.

[00132] In certain embodiments, the recombinant mutant human sialidase comprises a conservative substitution relative to a recombinant mutant human sialidase sequence disclosed herein. As used herein, the term “conservative substitution” refers to a substitution with a structurally similar amino acid. For example, conservative substitutions may include those within the following groups: Ser and Cys; Leu, Ile, and Val; Glu and Asp; Lys and Arg; Phe, Tyr, and Trp; and Gln, Asn, Glu, Asp, and His. Conservative substitutions may also be defined by the BLAST (Basic Local Alignment Search Tool) algorithm, the BLOSUM substitution matrix (*e.g.*, BLOSUM 62 matrix), or the PAM substitution matrix (*e.g.*, the PAM 250 matrix).

[00133] Sequence identity may be determined in various ways that are within the skill of a person skilled in the art, *e.g.*, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al.*, (1990) PROC. NATL. ACAD. SCI. USA 87:2264-2268; Altschul, (1993) J. MOL. EVOL. 36:290-300; Altschul *et al.*, (1997) NUCLEIC ACIDS RES. 25:3389-3402, incorporated by reference herein) are tailored for sequence similarity searching. For a discussion of basic issues in searching sequence databases *see* Altschul *et al.*, (1994) NATURE GENETICS 6:119-129, which is fully incorporated by reference herein. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The search parameters for histogram, descriptions, alignments, expect (*i.e.*, the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, (1992) PROC. NATL. ACAD. SCI. USA 89:10915-10919, fully incorporated by reference herein). Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at

every `wink.sup.th` position along the query); and `gapw=16` (sets the window width within which gapped alignments are generated). The equivalent `blastp` parameter settings may be `Q=9`; `R=2`; `wink=1`; and `gapw=32`. Searches may also be conducted using the NCBI (National Center for Biotechnology Information) BLAST Advanced Option parameter (*e.g.*: `-G`, Cost to open gap [Integer]: default = 5 for nucleotides/ 11 for proteins; `-E`, Cost to extend gap [Integer]: default = 2 for nucleotides/ 1 for proteins; `-q`, Penalty for nucleotide mismatch [Integer]: default = -3; `-r`, reward for nucleotide match [Integer]: default = 1; `-e`, expect value [Real]: default = 10; `-W`, wordsize [Integer]: default = 11 for nucleotides/ 28 for megablast/ 3 for proteins; `-y`, Dropoff (X) for blast extensions in bits: default = 20 for `blastn`/ 7 for others; `-X`, X dropoff value for gapped alignment (in bits): default = 15 for all programs, not applicable to `blastn`; and `-Z`, final X dropoff value for gapped alignment (in bits): 50 for `blastn`, 25 for others). ClustalW for pairwise protein alignments may also be used (default parameters may include, *e.g.*, Blosum62 matrix and Gap Opening Penalty = 10 and Gap Extension Penalty = 0.1). A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters `GAP=50` (gap creation penalty) and `LEN=3` (gap extension penalty). The equivalent settings in Bestfit protein comparisons are `GAP=8` and `LEN=2`.

b. Antibody Portion

[00134] As used herein, unless otherwise indicated, the term “antibody” is understood to mean an intact antibody (*e.g.*, an intact monoclonal antibody), or a fragment thereof, such as a Fc fragment of an antibody (*e.g.*, an Fc fragment of a monoclonal antibody), or an antigen-binding fragment of an antibody (*e.g.*, an antigen-binding fragment of a monoclonal antibody), including an intact antibody, antigen-binding fragment, or Fc fragment that has been modified, engineered, or chemically conjugated. Examples of antigen-binding fragments include Fab, Fab', (Fab')₂, Fv, single chain antibodies (*e.g.*, scFv), minibodies, and diabodies. Examples of antibodies that have been modified or engineered include chimeric antibodies, humanized antibodies, and multispecific antibodies (*e.g.*, bispecific antibodies). An example of a chemically conjugated antibody is an antibody conjugated to a toxin moiety.

[00135] In certain embodiments, the fusion protein comprises an immunoglobulin Fc domain. As used herein, unless otherwise indicated, the term “immunoglobulin Fc domain” refers to a fragment of an immunoglobulin heavy chain constant region which, either alone or in combination with a second immunoglobulin Fc domain, is capable of binding to an Fc receptor. An immunoglobulin Fc domain may include, *e.g.*, immunoglobulin CH2 and CH3 domains. An immunoglobulin Fc domain may include, *e.g.*, immunoglobulin CH2 and CH3 domains and an

immunoglobulin hinge region. Boundaries between immunoglobulin hinge regions, CH2, and CH3 domains are well known in the art, and can be found, *e.g.*, in the PROSITE database (available on the world wide web at prosite.expasy.org).

[00136] In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, and IgM Fc domain. A single amino acid substitution (S228P according to Kabat numbering; designated IgG4Pro) may be introduced to abolish the heterogeneity observed in recombinant IgG4 antibody. See Angal, S. *et al.* (1993) MOL. IMMUNOL. 30:105-108.

[00137] In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG1 isotype or another isotype that elicits antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement mediated cytotoxicity (CDC). In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG1 isotype (*e.g.*, SEQ ID NO: 31 or SEQ ID NO: 5).

[00138] In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG4 isotype or another isotype that elicits little or no antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement mediated cytotoxicity (CDC). In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG4 isotype.

[00139] In certain embodiments, the immunoglobulin Fc domain comprises either a “knob” mutation, *e.g.*, T366Y, or a “hole” mutation, *e.g.*, Y407T, for heterodimerization with a second polypeptide (residue numbers according to EU numbering, Kabat, E.A., *et al.* (1991) SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, FIFTH EDITION, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

[00140] In certain embodiments, the fusion protein comprises an immunoglobulin antigen-binding domain. The inclusion of such a domain may improve targeting of a fusion protein to a sialylated cancer cell, *e.g.*, a PD-L1 expressing cancer cell, and/or to the tumor microenvironment. As used herein, unless otherwise indicated, the term “immunoglobulin antigen-binding domain” refers to a polypeptide that, alone or in combination with another immunoglobulin antigen-binding domain, defines an antigen-binding site. Exemplary immunoglobulin antigen-binding domains include, for example, immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region, where the variable regions together define an antigen binding site, *e.g.*, an anti-PD-L1 antigen binding site.

[00141] In certain embodiments, the immunoglobulin antigen-binding domain is derived from an anti-PD-L1 antibody. Exemplary anti-PD-L1 antibodies are described, for example, in U.S. Patent Nos. 9,273,135, 7,943,743, 9,175,082, 8,741,295, 8,552,154, and 8,217,149. Exemplary

anti-PD-L1 antibodies include, atezolizumab (Tecentriq®, Genentech), durvalumab (AstraZeneca), MEDI4736, avelumab, CS1001 (CStone Therapeutics), KL-A167, CK-301 (Checkpoint Therapeutics), TQB2450, KN035, SHR-1316, STI-A1014, BGB-A333, MSB2311, HLX-20 and BMS-936559 by Bristol-Myers Squibb.

- 5 [00142] In certain embodiments, the immunoglobulin antigen-binding domain is derived from avelumab. The avelumab heavy chain amino acid sequence is depicted in SEQ ID NO: 63, and the avelumab light chain amino acid sequence is depicted in SEQ ID NO: 64. The amino acid sequence of an exemplary scFv derived from avelumab is depicted in SEQ ID NO: 125.

c. Linker

- 10 [00143] In certain embodiments, the sialidase portion of the fusion protein can be linked or fused directly to the anti-PD-L1 antibody portion (*e.g.*, immunoglobulin Fc domain and/or immunoglobulin antigen-binding domain) of the fusion protein. In other embodiments, the sialidase portion can be covalently bound to the anti-PD-L1 antibody portion by a linker.

- [00144] The linker may couple, with one or more natural amino acids, the sialidase, or
15 functional fragment thereof, and the antibody portions or fragments, where the amino acid (for example, a cysteine amino acid) may be introduced by site-directed mutagenesis. The linker may include one or more unnatural amino acids. It is contemplated that, in certain circumstances, a linker containing for example, one or more sulfhydryl reactive groups (*e.g.*, a maleimide) may covalently link a cysteine in the sialidase portion or the antibody portion that is
20 a naturally occurring cysteine residue or is the product of site-specific mutagenesis.

[00145] The linker may be a cleavable linker or a non-cleavable linker. Optionally or in addition, the linker may be a flexible linker or an inflexible linker.

- [00146] The linker should be a length sufficiently long to allow the sialidase and the antibody portions to be linked without steric hindrance from one another and sufficiently short to retain
25 the intended activity of the fusion protein. The linker preferably is sufficiently hydrophilic to avoid or minimize instability of the fusion protein. The linker preferably is sufficiently hydrophilic to avoid or minimize insolubility of the fusion protein. The linker should be sufficiently stable *in vivo* (*e.g.*, it is not cleaved by serum, enzymes, *etc.*) to permit the fusion protein to be operative *in vivo*.

- 30 [00147] The linker may be from about 1 angstroms (Å) to about 150 Å in length, or from about 1 Å to about 120 Å in length, or from about 5 Å to about 110 Å in length, or from about 10 Å to about 100 Å in length. The linker may be greater than about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13,

14, 15, 16, 17, 18, 19, 20, 25, 27, 30 or greater angstroms in length and/or less than about 110, 100, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, or fewer Å in length. Furthermore, the linker may be about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, and 120 Å in length.

5 **[00148]** In certain embodiments, the linker comprises a polypeptide linker that connects or fuses the sialidase portion of the fusion protein to the anti-PD-L1 antibody portion (*e.g.*, immunoglobulin Fc domain and/or immunoglobulin antigen-binding domain) of the fusion protein. For example, it is contemplated that a gene encoding a sialidase portion linked directly or indirectly (for example, via an amino acid containing linker) to an antibody portion can be
 10 created and expressed using conventional recombinant DNA technologies. For example, the amino terminus of a sialidase portion can be linked to the carboxy terminus of either the light or the heavy chain of an antibody portion. For example, for a Fab fragment, the amino terminus or carboxy terminus of the sialidase can be linked to the first constant domain of the heavy antibody chain (CH1). When a linker is employed, the linker may comprise hydrophilic amino
 15 acid residues, such as Gln, Ser, Gly, Glu, Pro, His and Arg. In certain embodiments, the linker is a peptide containing 1-25 amino acid residues, 1-20 amino acid residues, 2-15 amino acid residues, 3-10 amino acid residues, 3-7 amino acid residues, 4-25 amino acid residues, 4-20 amino acid residues, 4-15 amino acid residues, 4-10 amino acid residues, 5-25 amino acid residues, 5-20 amino acid residues, 5-15 amino acid residues, or 5-10 amino acid residues.
 20 Exemplary linkers include glycine and serine-rich linkers, *e.g.*, (GlyGlyPro)_n, or (GlyGlyGlyGlySer)_n, where n is 1-5. In certain embodiments, the linker comprises, consists, or consists essentially of GGGGS (SEQ ID NO: 121). In certain embodiments, the linker comprises, consists, or consists essentially of GGGGSGGGGS (SEQ ID NO: 90). In certain embodiments, the linker comprises, consists, or consists essentially of EPKSS (SEQ ID NO: 91).
 25 Additional exemplary linker sequences are disclosed, *e.g.*, in George *et al.* (2003) PROTEIN ENGINEERING 15:871–879, and U.S. Patent Nos. 5,482,858 and 5,525,491.

[00149] In certain embodiments, the fusion protein comprises the amino acid sequence of any one of SEQ ID NOs: 65-75, 78, 81-89, 95, 96, 98, 99, 101, 102, 104, 106, 108, 110, 112, 114, 122-124, 127, or 128, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%,
 30 98%, or 99% sequence identity to any one of SEQ ID NOs: 65-75, 78, 81-89, 95, 96, 98, 99, 101, 102, 104, 106, 108, 110, 112, 114, 122-124, 127, or 128.

d. Antibody Conjugates

[00150] The invention further provides antibody conjugates containing one or more of the fusion proteins disclosed herein. As used herein, unless otherwise indicated, the term “antibody conjugate” is understood to refer to an antibody, or a functional fragment thereof, that comprises antigen-binding activity (*e.g.*, anti-PD-L1 antigen-binding activity) and/or Fc receptor-binding activity, conjugated (*e.g.*, covalently coupled) to an additional functional moiety. In certain embodiments, the antibody or functional antibody fragment is conjugated to a sialidase enzyme, *e.g.*, a recombinant mutant human sialidase enzyme disclosed herein. In certain embodiments, an antibody conjugate comprises a single polypeptide chain. In certain embodiments, an antibody conjugate comprises two, three, four, or more polypeptide chains that are covalently or non-covalently associated together to produce a multimeric complex, *e.g.*, a dimeric, trimeric or tetrameric complex. For example, an antibody conjugate may comprise a first polypeptide (fusion protein) comprising a recombinant mutant human sialidase enzyme and an immunoglobulin heavy chain, and a second polypeptide comprising an immunoglobulin light chain, where, for example, the immunoglobulin heavy and light chains together define a single antigen-binding site, *e.g.*, an anti-PD-L1 antigen-binding site.

[00151] In certain embodiments, the antibody conjugate can include a single sialidase. In other embodiments, the antibody conjugate can include more than one (*e.g.*, two) sialidases. If more than one sialidase is included, the sialidases can be the same or different. In certain embodiments, the antibody conjugate can include a single anti-PD-L1 antigen-binding site. In other embodiments, the antibody conjugate can include more than one (*e.g.*, two) anti-PD-L1 antigen-binding sites. If two antigen-binding sites are used, they can be the same or different. In certain embodiments, the antibody conjugate comprises an immunoglobulin Fc fragment.

[00152] In certain embodiments, the antibody conjugate comprises one or two immunoglobulin heavy chains, or a functional fragment thereof. In certain embodiments, the antibody conjugate comprises one or two immunoglobulin light chains, or a functional fragment thereof. In certain embodiments, the antibody conjugate comprises a sialidase fused to the N- or C-terminus of an immunoglobulin heavy chain or an immunoglobulin light chain.

[00153] **FIGURE 17** depicts exemplary antibody conjugate constructs containing one or more sialidase enzymes. For example, in **FIGURE 17A**, a first anti-PD-L1 antigen-binding site is depicted as 10, a second anti-PD-L1 antigen-binding site is depicted as 20, a sialidase is depicted as 30, and a Fab is depicted as 40. In each of the constructs depicted in **FIGURES 17A-17I** it is understood that the Fc may optionally be modified in some manner, *e.g.* using Knobs-into-Holes

type technology, *e.g.*, as depicted by 50 in **FIGURE 17B**. Throughout **FIGURE 17** similar structures are depicted by similar schematic representations.

[00154] FIGURE 17A depicts antibody conjugate constructs comprising a first polypeptide comprising a first immunoglobulin light chain; a second polypeptide comprising a first immunoglobulin heavy chain; a third polypeptide comprising a second immunoglobulin heavy chain; and a fourth polypeptide comprising a second immunoglobulin light chain. The first and second polypeptides can be covalently linked together, the third and fourth polypeptides can be covalently linked together, and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define a first anti-PD-L1 antigen-binding site as depicted as 10, and the third polypeptide and the fourth polypeptide together define a second anti-PD-L1 antigen-binding site as depicted as 20. A sialidase enzyme as depicted as 30 can be conjugated to the N- or C-terminus of the first and second immunoglobulin light chain or the first and second immunoglobulin heavy chain.

[00155] FIGURE 17B depicts antibody conjugate constructs comprising a first polypeptide comprising a first immunoglobulin light chain; a second polypeptide comprising a first immunoglobulin heavy chain; a third polypeptide comprising a second immunoglobulin heavy chain; and a fourth polypeptide comprising a second immunoglobulin light chain. The first and second polypeptides can be covalently linked together, the third and fourth polypeptides can be covalently linked together, and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define a first anti-PD-L1 antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second anti-PD-L1 antigen-binding site. A sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin light chain or the first immunoglobulin heavy chain.

[00156] FIGURE 17C depicts antibody conjugate constructs comprising a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define an anti-PD-L1 antigen-binding site. A sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin light chain or the first immunoglobulin heavy chain.

[00157] **FIGURE 17D** depicts antibody conjugate constructs comprising a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain and a first sialidase enzyme. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. The third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation. In certain embodiments, the first polypeptide and the second polypeptide together define an anti-PD-L1 antigen-binding site. An optional second sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin light chain or the first immunoglobulin heavy chain.

[00158] **FIGURE 17E** depicts antibody conjugate constructs comprising a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain and a first sialidase enzyme. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. The third polypeptide comprises the immunoglobulin Fc domain and the sialidase in an N- to C-terminal orientation. In certain embodiments, the first polypeptide and the second polypeptide together define an anti-PD-L1 antigen-binding site. An optional second sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin light chain or the first immunoglobulin heavy chain.

[00159] **FIGURE 17F** depicts antibody conjugate constructs comprising a first polypeptide comprising a first immunoglobulin Fc domain, and a second polypeptide comprising a second immunoglobulin Fc domain. The first and second polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. A sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin Fc domain or to the N- or C-terminus of the second immunoglobulin Fc domain. An optional second sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin Fc domain or to the N- or C-terminus of the second immunoglobulin Fc domain.

[00160] **FIGURE 17G** depicts antibody conjugate constructs comprising a first polypeptide comprising an immunoglobulin light chain; and a second polypeptide comprising an immunoglobulin heavy chain variable region. The first and second polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define an anti-PD-L1

antigen-binding site. The sialidase enzyme can be conjugated to the N- or C-terminus of the immunoglobulin light chain or the immunoglobulin heavy chain variable region.

[00161] FIGURE 17H depicts antibody conjugate constructs comprising a first polypeptide comprising a first immunoglobulin Fc domain, and a second polypeptide comprising a second immunoglobulin Fc domain. The first and second polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. A sialidase enzyme can be conjugated to the N-terminus of the first immunoglobulin Fc domain or the second immunoglobulin Fc domain. An optional second sialidase enzyme can be conjugated to the N-terminus of the second immunoglobulin Fc domain or the first immunoglobulin Fc domain, respectively. A single chain variable fragment (scFv) can be conjugated to the C-terminus of the first immunoglobulin Fc domain or the second immunoglobulin Fc domain. An optional second single chain variable fragment (scFv) can be conjugated to the C-terminus of the first immunoglobulin Fc domain or the second immunoglobulin Fc domain, respectively.

[00162] FIGURE 17I depicts antibody conjugate constructs similar to those depicted in **FIGURE 17H** except that each scFv is replaced with an immunoglobulin antigen binding fragment, *e.g.*, an Fab. For example, **FIGURE 17I** depicts antibody conjugate constructs comprising a first polypeptide comprising a first immunoglobulin Fc domain, and a second polypeptide comprising a second immunoglobulin Fc domain. The first and second polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. A sialidase enzyme can be conjugated to the N-terminus of the first immunoglobulin Fc domain or the second immunoglobulin Fc domain. An optional second sialidase enzyme can be conjugated to the N-terminus of the second immunoglobulin Fc domain or the first immunoglobulin Fc domain, respectively. An antibody fragment (Fab) can be conjugated or fused to the C-terminus of the first immunoglobulin Fc domain or the second immunoglobulin Fc domain. An optional second antibody fragment (Fab) can be conjugated or fused to the C-terminus of the second immunoglobulin Fc domain or the first immunoglobulin Fc domain, respectively. In the case of a fusion, the C terminus of the Fc domain is linked (either by a bond or an amino acid linker) to a first polypeptide chain defining an anti-PD-L1 immunoglobulin antigen binding fragment. In the case of antibodies that have an antigen binding site defined by a single variable region, then this may be sufficient to impart binding affinity to a target antigen, *e.g.*, PD-L1. In other instances, *e.g.*, in the case of a human antibody, the first polypeptide chain defining an immunoglobulin antigen binding fragment can be conjugated (*e.g.*, covalently conjugated, *e.g.*, via a disulfide bond) to a second polypeptide chain defining an immunoglobulin antigen binding

fragment, there the two antigen binding fragments together define an antigen binding site for binding the target antigen, *e.g.*, PD-L1.

[00163] **FIGURE 18** depicts additional antibody conjugate constructs. For example,

FIGURE 18 depicts an antibody conjugate construct comprising a first polypeptide comprising

an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain and an scFv; and a third polypeptide comprising an immunoglobulin Fc domain and a first sialidase enzyme. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be

disulfide bonds. The second polypeptide comprises the heavy chain and the scFv in an N- to C-terminal orientation. The third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation. In certain embodiments, the first polypeptide and the second polypeptide together define a first antigen-binding site. In certain embodiments, the scFv defines a second antigen-binding site. **FIGURE 18** depicts an additional antibody

construct comprising a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain and a first sialidase enzyme, wherein a Fab fragment is conjugated to the N-terminus of the immunoglobulin heavy chain. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. The third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation. In certain embodiments, the first polypeptide and the second polypeptide together define a first antigen-binding site. In certain embodiments, the Fab fragment defines a second antigen-binding site.

In each of the constructs depicted in **FIGURE 18** it is understood that an scFv, when present, may be replaced with a Fab fragment, or a Fab fragment, when present, may be replaced with an scFv. In each of the constructs depicted in **FIGURE 18**, it is understood that the Fc may optionally be modified in some manner.

[00164] In certain embodiments, the antibody conjugate comprises a first polypeptide comprising a first immunoglobulin light chain; a second polypeptide comprising a first immunoglobulin heavy chain and a first sialidase; a third polypeptide comprising a second immunoglobulin heavy chain and a second sialidase; and a fourth polypeptide comprising a second immunoglobulin light chain. An example of this embodiment is shown in **FIGURE 19A**. The first and second polypeptides can be covalently linked together, the third and fourth polypeptides can be covalently linked together, and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain

embodiments, the first polypeptide and the second polypeptide together define a first anti-PDL-1 antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second anti-PD-L1 antigen-binding site. In certain embodiments, the second and third polypeptides comprise the first and second immunoglobulin heavy chain and the first and second sialidase, respectively, in an N- to C-terminal orientation. In certain embodiments, the second and third polypeptides comprise the first and second sialidase and the first and second immunoglobulin heavy chain, respectively, in an N- to C-terminal orientation.

[00165] In certain embodiments, the antibody conjugate comprises a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain and a sialidase. An example of this embodiment is shown in **FIGURE 19B**. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define an anti-PD-L1 antigen-binding site. In certain embodiments, the third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation, or the immunoglobulin Fc domain and the sialidase in an N- to C-terminal orientation.

[00166] In certain embodiments, the first polypeptide comprises the amino acid sequence of SEQ ID NO: 65, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 65. In certain embodiments, the second polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 66, 104, or 124, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 66, 104, or 124. In certain embodiments, the third polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 67-73, 78, 81-87, 95, 96, 98, 99, 101, 102, 106, 108, 112, 122, 123, 127, or 128, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 67-73, 78, 81-87, 95, 96, 98, 99, 101, 102, 106, 108, 112, 122, 123, 127, or 128.

[00167] In certain embodiments, the third polypeptide comprises the amino acid sequence of X₁X₂SX₃X₄X₅LQX₆ESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASX₇X₈DEHAELIVX₉RRGDYDAX₁₀THQVQWX₁₁AQEVVAQAX₁₂LDGHRSMNPCPLYDX₁₃QTGTLFLFFIAIPX₁₄X₁₅VTEX₁₆QQLQTRANVTRLX₁₇X₁₈VTSTDHGRTWSSPRDLTDAAIGPX₁₉YREWSTFAVGPGHX₂₀LQLHDX₂₁RSLVVPAYAYRKLHPX₂₂QRPIPSAFX₂₃FLSHDHGRTWARGHFVAQDTX₂₄ECQVAEVETGEQRVVTLNARSHLRARVQAQX₂₅NX₂₆GLDFQX₂₇SQLVKKLVEPPPX₂₈GX₂₉QGSVISFSPRSGPGSPAQX₃₀LLYTHPTHX₃₁X₃₂QRADLGAYLNPRPPAPEAWSEPX₃₃LLAKGSX₃₄AYSDDLQSMGTGPDGS

PLFGX₃₅LYEANDYEEIX₃₆FX₃₇MFTLKQAFPAEYLPQGGGGSGGGGSDKTHTCPPCPAPELLGG
 PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
 VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
 TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSQVMH
 5 EALHNHYTQKSLSLSPGK

(SEQ ID NO: 75), wherein X₁ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Thr, Val, or not present, X₂ is Ala or Lys, X₃ is Asn or Leu, X₄ is Pro or His, X₅ is Phe, Trp, Tyr or Val, X₆ is Lys or Asp, X₇ is Lys, Arg, or Glu. X₈ is Lys, Ala, Arg, or Glu, X₉ is Leu or Met, X₁₀ is Pro, Asn, Asp, His, Glu, Gly, Ser or Thr, X₁₁ is Gln or His, X₁₂ is Arg or Lys, X₁₃ is Ala, Glu or Lys, X₁₄ is Gly or Asp, X₁₅ is Gln or His, X₁₆ is Gln, Arg, or Lys, X₁₇ is Ala, Cys, Ile, Ser, Val, or Leu, X₁₈ is Gln or Leu, X₁₉ is Ala or Val, X₂₀ is Cys or Gly, X₂₁ is Ala or Gly, X₂₂ is Arg, Ile, or Lys, X₂₃ is Ala, Cys, Leu, or Val, X₂₄ is Leu, Ala, or Val, X₂₅ is Thr or Ala, X₂₆ is Asp or Gly, X₂₇ is Glu or Lys, X₂₈ is Gln, Ala, His, Phe, or Pro, X₂₉ is Cys or Val, X₃₀ is Trp or Arg, X₃₁ is Ser or Arg, X₃₂ is Trp or Lys, X₃₃ is Lys or Val, X₃₄ is Ala, Cys, Ser, or Val, X₃₅ is Cys, Leu, or Val, X₃₆ is Val or Arg, and X₃₇ is Leu, Gln, His, Ile, Lys, or Ser, and the sialidase comprises at least one mutation relative to wild-type human Neu2 (SEQ ID NO: 1).

[00168] In certain embodiments, the third polypeptide comprises the amino acid sequence of

X₁ASLPX₂LQX₃ESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASKKDEHAELIVLRRGDYDAX₄
 THQVQWQAQEVVAQARLDGHRSMNPCPLYDX₅QTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQ
 20 VTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVPAYAYRKLHPX₆QRP
 IPSAFCFLSHDHGRTWARGHFVAQDTLECCQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLD
 FQESQLVKKLVEPPX₇GCQGSVISFSPSRSGPGSPAQWLLYTHPTHX₈X₉QRADLGAYLNPRPP
 APEAWSEPVLLAKGSX₁₀AYSDDLQSMGTGPDGSPLFGCLYEANDYEEIX₁₁FX₁₂MFTLKQAFPAE
 YLPQGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 25 PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL
 DSDGSFFLTSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGK

(SEQ ID NO: 74), wherein X₁ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Thr, Val, or not present, X₂ is Phe, Trp, Tyr or Val, X₃ is Lys or Asp, X₄ is Pro, Asn, Asp, His, Glu, Gly, Ser or Thr, X₅ is Ala, Glu, or Lys, X₆ is Arg, Ile, or Lys, X₇ is Gln, Ala, His, Phe, or Pro, X₈ is Ser or Arg, X₉ is Trp or Lys, X₁₀ is Ala, Cys, Ser, or Val, X₁₁ is Val or Arg, and X₁₂ is Leu, Gln, His, Ile, Lys, or Ser, and the sialidase comprises at least one mutation relative to wild-type human Neu2 (SEQ ID NO: 1). In certain embodiments, X₁ is Ala, Asp, Met, or not present, X₂ is Tyr or Val, X₃ is Lys or Asp, X₄ is Pro, Asn, Gly, Ser or Thr, X₅ is Ala or Glu, X₆ is Ile or Lys, X₇ is Gln or Ala, X₈ is Ser or Arg, X₉ is Trp or Lys, X₁₀ is Ala or Cys, X₁₁ is Val or Arg, and X₁₂ is Leu or Ile.

[00169] In certain embodiments, the third polypeptide comprises the amino acid sequence of

X₁X₂SX₃X₄X₅LQX₆ESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASX₇X₈DEHAELIVX₉RRGD
 YDAX₁₀THQVQWX₁₁AQEVVAQAX₁₂LDGHRSMNPCPLYDX₁₃QTGTLFLFFIAIPX₁₄X₁₅VTEX₁₆Q

QLQTRANVTRLX₁₇X₁₈VTSTDHGRTWSSPRDLTDAAIGPX₁₉YREWSTFAVGPGHX₂₀LQLHDX₂₁
 RSLVVPAYAYRKLHPX₂₂QRP I PSAFX₂₃FLSHDHGRTWARGHFVAQDTX₂₄ECQVAEVETGEQRV
 VTLNARSHLRARVQAQX₂₅NX₂₆GLDFQX₂₇SQLVKKLVEPPPX₂₈GX₂₉QGSVISFSPRSGPGSP
 AQX₃₀LLYTHPTHX₃₁X₃₂QRADLGAYLNPRPPAPEAWSEPX₃₃LLAKGSX₃₄AYSDLQSMGTGPDGS
 5 PLFGX₃₅LYEANDYEEIX₃₆FX₃₇MFTLKQAFPAEYLPQX₃₈DKTHTCPPCPAPELLGGPSVFLFPP
 KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
 HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
 PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFCFSVMHEALHNHYT
 QKSLSLSPGK

- 10 (SEQ ID NO: 89), wherein X₁ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe,
 Thr, Val, or not present, X₂ is Ala or Lys, X₃ is Asn or Leu, X₄ is Pro or His, X₅ is Phe, Trp, Tyr
 or Val, X₆ is Lys or Asp, X₇ is Lys, Arg, or Glu. X₈ is Lys, Ala, Arg, or Glu, X₉ is Leu or Met,
 X₁₀ is Pro, Asn, Asp, His, Glu, Gly, Ser or Thr, X₁₁ is Gln or His, X₁₂ is Arg or Lys, X₁₃ is Ala,
 Glu or Lys, X₁₄ is Gly or Asp, X₁₅ is Gln or His, X₁₆ is Gln, Arg, or Lys, X₁₇ is Ala, Cys, Ile, Ser,
 15 Val, or Leu, X₁₈ is Gln or Leu, X₁₉ is Ala or Val, X₂₀ is Cys or Gly, X₂₁ is Ala or Gly, X₂₂ is Arg,
 Ile, or Lys, X₂₃ is Ala, Cys, Leu, or Val, X₂₄ is Leu, Ala, or Val, X₂₅ is Thr or Ala, X₂₆ is Asp or
 Gly, X₂₇ is Glu or Lys, X₂₈ is Gln, Ala, His, Phe, or Pro, X₂₉ is Cys or Val, X₃₀ is Trp or Arg, X₃₁
 is Ser or Arg, X₃₂ is Trp or Lys, X₃₃ is Lys or Val, X₃₄ is Ala, Cys, Ser, or Val, X₃₅ is Cys, Leu, or
 Val, X₃₆ is Val or Arg, X₃₇ is Leu, Gln, His, Ile, Lys, or Ser, and X₃₈ is GGGGSGGGGS (SEQ
 20 ID NO: 90) or EPKSS (SEQ ID NO: 91), and the sialidase comprises at least one mutation
 relative to wild-type human Neu2 (SEQ ID NO: 1).

[00170] In certain embodiments, the third polypeptide comprises the amino acid sequence of
 X₁ASLPX₂LQX₃ESVFQSGAHAYRIPALLYLPQGQSLLAFAEQRASKKDEHAELIVLRRGDYDAX₄
 THQVQWQAQEVVAQARLDGHRSMNPCPLYDX₅QTGTFLFLFFIAIPGQVTEQQQLQTRANVTRLQC
 25 VTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDXRARSRLVVPAYAYRKLHPX₆QRP
 IPSAFCFLSHDHGRTWARGHFVAQDTLEECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLD
 FQESQLVKKLVEPPPX₇GCQGSVISFSPRSGPGSPAQWLLYTHPTHX₈X₉QRADLGAYLNPRPP
 APEAWSEPVLLAKGSX₁₀AYSDLQSMGTGPDGSPLFGCLYEANDYEEIX₁₁FX₁₂MFTLKQAFPAE
 YLPQGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 30 PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
 L DSDGSFFLTSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

- (SEQ ID NO: 88), wherein X₁ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe,
 Thr, Val, or not present, X₂ is Phe, Trp, Tyr or Val, X₃ is Lys or Asp, X₄ is Pro, Asn, Asp, His,
 35 Glu, Gly, Ser or Thr, X₅ is Ala, Glu, or Lys, X₆ is Arg, Ile, or Lys, X₇ is Gln, Ala, His, Phe, or
 Pro, X₈ is Ser or Arg, X₉ is Trp or Lys, X₁₀ is Ala, Cys, Ser, or Val, X₁₁ is Val or Arg, X₁₂ is Leu,
 Gln, His, Ile, Lys, or Ser, and X₁₃ is GGGGSGGGGS (SEQ ID NO: 90) or EPKSS (SEQ ID NO:
 91), and the sialidase comprises at least one mutation relative to wild-type human Neu2 (SEQ ID
 NO: 1). In certain embodiments, X₁ is Ala, Asp, Met, or not present, X₂ is Tyr or Val, X₃ is Lys

or Asp, X₄ is Pro, Asn, Gly, Ser or Thr, X₅ is Ala or Glu, X₆ is Ile or Lys, X₇ is Gln or Ala, X₈ is Ser or Arg, X₉ is Trp or Lys, X₁₀ is Ala or Cys, X₁₁ is Val or Arg, and X₁₂ is Leu or Ile.

[00171] In certain embodiments, the third polypeptide comprises the amino acid sequence of

X₁X₂SX₃X₄X₅LQX₆ESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASX₇X₈DEHAELIVX₉RRGD
 YDAX₁₀THQVQWX₁₁AQEVVAQAX₁₂LX₁₃GHRSMNPCPLYDX₁₄QTGTFLFLFFIAIPX₁₅X₁₆VTEX₁₇
 QQLQTRANVTRLX₁₈X₁₉VTSTDHGRTWSSPRDLTDAAIGPX₂₀YREWSTFAVGPGHX₂₁LQLHDX₂₂
 X₂₃RSLVVPAYAYRKLHPX₂₄X₂₅X₂₆PIPSAFX₂₇FLSHDHGRTWARGHFVX₂₈QDTX₂₉ECQVAEVX₃₀
 TGEQRVVTLNARSX₃₁X₃₂X₃₃X₃₄RX₃₅QAQSX₃₆NX₃₇GLDFQX₃₈X₃₉QX₄₀VKKL
 X₄₁EPPPX₄₂GX₄₃QGSVISFPSRSGPGSPAQX₄₄LLYTHPTHX₄₅X₄₆QRADLGAYLNPRPPAPEA
 WSEPX₄₇LLAKGSX₄₈AYSDLQSMGTGPDGSPFGX₄₉LYEANDYEEIX₅₀FX₅₁MFTLKQAFPAEYL
 PQX₅₂DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
 EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLT
 S KLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK

(SEQ ID NO: 117), wherein X₁ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Thr, Val, or not present, X₂ is Ala or Lys, X₃ is Asn or Leu, X₄ is Pro or His, X₅ is Phe, Trp, Tyr or Val, X₆ is Lys or Asp, X₇ is Lys, Arg, or Glu, X₈ is Lys, Ala, Arg, or Glu, X₉ is Leu or Met, X₁₀ is Pro, Asn, Asp, His, Glu, Gly, Ser or Thr, X₁₁ is Gln or His, X₁₂ is Arg or Lys, X₁₃ is Asp or Pro, X₁₄ is Ala, Glu or Lys, X₁₅ is Gly or Asp, X₁₆ is Gln or His, X₁₇ is Gln, Arg, or Lys, X₁₈ is Ala, Cys, Ile, Ser, Val, or Leu, X₁₉ is Gln, Leu, Glu, Phe, His, Ile, Leu, or Tyr, X₂₀ is Ala or Val, X₂₁ is Cys or Gly, X₂₂ is Arg or Pro, X₂₃ is Ala or Gly, X₂₄ is Arg, Ile, or Lys, X₂₅ is Gln or Pro, X₂₆ is Arg or Pro, X₂₇ is Ala, Cys, Leu, or Val, X₂₈ is Ala, Cys, Asn, Ser, or Thr, X₂₉ is Leu, Ala, or Val, X₃₀ is Glu or Pro, X₃₁ is His or Pro, X₃₂ is Leu, Asp, Asn, or Tyr, X₃₃ is Arg, Ala, Asp, Leu, Gln, or Tyr, X₃₄ is Ala, Cys, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Val, Trp, or Tyr, X₃₅ is Val, Ile, or Lys, X₃₆ is Thr or Ala, X₃₇ is Asp or Gly, X₃₈ is Glu, Lys, or Pro, X₃₉ is Ser or Cys, X₄₀ is Leu, Asp, Phe, Gln, or Thr, X₄₁ is Val or Phe, X₄₂ is Gln, Ala, His, Phe, Pro, Ser, or Thr, X₄₃ is Cys or Val, X₄₄ is Trp or Arg, X₄₅ is Ser, Arg, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Thr, Val, Trp, or Tyr, X₄₆ is Trp, Lys, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, or Tyr, X₄₇ is Lys or Val, X₄₈ is Ala, Cys, Ser, or Val, X₄₉ is Cys, Leu, or Val, X₅₀ is Val or Arg, X₅₁ is Leu, Gln, His, Ile, Lys, or Ser, X₅₂ is GGGGS (SEQ ID NO: 121), GGGGSGGGGS (SEQ ID NO: 90), or EPKSS (SEQ ID NO: 91), and the sialidase comprises at least one mutation relative to wild-type human Neu2 (SEQ ID NO: 1).

[00172] In certain embodiments, the third polypeptide comprises the amino acid sequence of

X₁ASLPX₂LQX₃ESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASKKDEHAELIVLRRGDYDAX₄
 THQVQWQAQEVVAQARLDGHRSMNPCPLYDX₅QTGTFLFLFFIAIPGQVTEQQQLQTRANVTRLCX
₆VTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPX₇QR
 PIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRX₈RVQAQSTNDGL
 DFQESQLVKKLVEPPPX₉GCQGSVISFPSRSGPGSPAQWLLYTHPTHX₁₀X₁₁QRADLGAYLNPR

PPAPEAWSEPVLLAKGSX₁₂AYS DLQSMGTGPDGSPLFGCLYEANDYEEIX₁₃FX₁₄MFTTLKQAFPAEYLPQX₁₅DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF

(SEQ ID NO: 116), wherein X₁ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Thr, Val, or not present, X₂ is Phe, Trp, Tyr or Val, X₃ is Lys or Asp, X₄ is Pro, Asn, Asp, His, Glu, Gly, Ser or Thr, X₅ is Ala, Glu, or Lys, X₆ is Gln, Leu, Glu, Phe, His, Ile, Leu, or Tyr, X₇ is Arg, Ile, or Lys, X₈ is Ala, Cys, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Val, Trp, or Tyr, X₉ is Gln, Ala, His, Phe, Pro, Ser, or Thr, X₁₀ is Ser, Arg, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Thr, Val, Trp, or Tyr, X₁₁ is Trp, Lys, Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, or Tyr, X₁₂ is Ala, Cys, Ser, or Val, X₁₃ is Val or Arg, X₁₄ is Leu, Gln, His, Ile, Lys, or Ser, X₁₅ is GGGGS (SEQ ID NO: 121), GGGGSGGGGS (SEQ ID NO: 90), or EPKSS (SEQ ID NO: 91), and the sialidase comprises at least one mutation relative to wild-type human Neu2 (SEQ ID NO: 1). In certain embodiments, X₁ is Ala, Asp, Met, or not present, X₂ is Tyr or Val, X₃ is Lys or Asp, X₄ is Pro, Asn, Gly, Ser or Thr, X₅ is Ala or Glu, X₆ is Gln or Tyr, X₇ is Ile or Lys, X₈ is Ala or Thr, X₉ is Gln, Ala, or Thr, X₁₀ is Ser, Arg, or Ala, X₁₁ is Trp, Lys, or Arg, X₁₂ is Ala or Cys, X₁₃ is Val or Arg, and X₁₄ is Leu or Ile.

[00173] In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 66, and the third polypeptide comprises SEQ ID NO: 67. In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 66, and the third polypeptide comprises SEQ ID NO: 68. In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 66, and the third polypeptide comprises SEQ ID NO: 69. In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 66, and the third polypeptide comprises SEQ ID NO: 70. In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 66, and the third polypeptide comprises SEQ ID NO: 71. In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 66, and the third polypeptide comprises SEQ ID NO: 72. In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 66, and the third polypeptide comprises SEQ ID NO: 73. In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 66, and the third polypeptide comprises SEQ ID NO: 78. In certain

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embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 104, and the third polypeptide comprises SEQ ID NO: 108. In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 124, and the third polypeptide comprises SEQ ID NO: 122. In certain
5 embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 124, and the third polypeptide comprises SEQ ID NO: 123.

[00174] In certain embodiments, the antibody conjugate comprises a first polypeptide comprising a first sialidase, a first immunoglobulin Fc domain, and a first single chain variable fragment (scFv) (it is also understood that the scFv may be replaced by a first polypeptide chain
10 of an immunoglobulin antigen binding fragment, *e.g.*, Fab fragment); and a second polypeptide comprising a second sialidase, a second immunoglobulin Fc domain, and a second single chain variable fragment (scFv) (it is also understood that the scFv may be replaced by a second polypeptide chain of an immunoglobulin antigen binding fragment, *e.g.*, Fab fragment). An example of this embodiment is shown in **FIGURE 19C**. The first and second polypeptides can
15 be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first scFv defines a first anti-PD-L1 antigen-binding site, and the second scFv defines a second anti-PD-L1 antigen-binding site. In certain embodiments, the first polypeptide comprises the first sialidase, the first immunoglobulin Fc domain, and the first scFv in an N- to C-terminal orientation. In certain embodiments, the first polypeptide comprises the first scFv,
20 the first immunoglobulin Fc domain, and the first sialidase in an N- to C-terminal orientation. In certain embodiments, the second polypeptide comprises the second sialidase, the second immunoglobulin Fc domain, and the second scFv in an N- to C-terminal orientation. In certain embodiments, the second polypeptide comprises the second scFv, the second immunoglobulin Fc domain, and the second sialidase in an N- to C-terminal orientation.

[00175] In certain embodiments, the first polypeptide comprises the amino acid sequence of SEQ ID NO: 114, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 114. In certain embodiments, the second polypeptide comprises the amino acid sequence of SEQ ID NO: 114, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 114. In
25 certain embodiments, the first and second polypeptide comprise SEQ ID NO: 114.

[00176] In certain embodiments, the antibody conjugate comprises: a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain and a single chain variable fragment (scFv) (it is also understood

that the scFv may be replaced by a first polypeptide chain of an immunoglobulin antigen binding fragment, *e.g.*, Fab fragment); and a third polypeptide comprising an immunoglobulin Fc domain and a sialidase. An example of this embodiment is shown in **FIGURE 19D**. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define a first anti-PD-L1 antigen-binding site (*i.e.*, the immunoglobulin light chain and immunoglobulin heavy chain together define a first anti-PD-L1 antigen-binding site). In certain embodiments, the scFv defines a second anti-PD-L1 antigen-binding site. In certain embodiments, the second polypeptide comprises the immunoglobulin heavy chain and the scFv in an N- to C-terminal orientation, or the scFv and the immunoglobulin heavy chain in an N- to C-terminal orientation. In certain embodiments, the third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation, or the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.

[00177] In certain embodiments, the first polypeptide comprises the amino acid sequence of SEQ ID NO: 65, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 65. In certain embodiments, the second polypeptide comprises the amino acid sequence of SEQ ID NO: 110, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 110. In certain embodiments, the third polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 78 or 106, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 78 or 106. In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 110, and the third polypeptide comprises SEQ ID NO: 78. In certain embodiments, the first polypeptide comprises SEQ ID NO: 65, the second polypeptide comprises SEQ ID NO: 110, and the third polypeptide comprises SEQ ID NO: 106.

[00178] In certain embodiments, the antibody conjugate has a molecular weight from about 135 kDa to about 165 kDa, *e.g.*, about 140 kDa. In other embodiments, the antibody conjugate has a molecular weight from about 215 kDa to about 245 kDa, *e.g.*, about 230 kDa.

[00179] In certain embodiments, the antibody conjugate comprises two polypeptides that each comprise an immunoglobulin Fc domain, and the first polypeptide has either a “knob” mutation, *e.g.*, T366Y, or a “hole” mutation, *e.g.*, Y407T, for heterodimerization with the second polypeptide, and the second polypeptide has either a respective “knob” mutation, *e.g.*, T366Y, or

a “hole” mutation, *e.g.*, Y407T, for heterodimerization with the first polypeptide (residue numbers according to EU numbering, Kabat, E.A., *et al.* (1991) *supra*). For example, in certain embodiments, the antibody comprises two polypeptides that each comprise an immunoglobulin Fc domain derived from human IgG1 Fc domain, and the first polypeptide comprises a Y407T mutation (*e.g.*, the first polypeptide comprises SEQ ID NO: 32 or SEQ ID NO: 92), and the second polypeptide comprises a T366Y mutation (*e.g.*, the second polypeptide comprises SEQ ID NO: 33 or SEQ ID NO: 93).

[00180] As used herein, the term “multispecific antibody” is understood to mean an antibody that specifically binds to at least two different antigens, *i.e.*, an antibody that comprises at least two antigen-binding sites that bind to at least two different antigens. As used herein, the term “bispecific antibody” is understood to mean an antibody that specifically binds to two different antigens, *i.e.*, an antibody that comprises two antigen-binding sites each of which bind to separate and distinct antigens. In other words, a first binding site binds a first antigen and a second binding site binds a second, different antigen. A multispecific or bispecific antibody may, for example, be a human or humanized antibody, and/or be a full length antibody or an antibody fragment (*e.g.*, a F(ab')₂ bispecific antibody).

[00181] The present invention encompasses antibody conjugates comprising antibody fragments, which may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. For a review of certain antibody fragments, see Hudson *et al.* (2003) *supra*.

[00182] In certain embodiments, the antibody conjugate or fusion protein can be covalently or non-covalently associated with a biological modifier, wherein the biological modifier can be used to enhance the solubility of the antibody, increase binding specificity, decrease immunogenicity or toxicity or modify the pharmacokinetic profile of the antibody. For example, the biological modifier can be used to increase the molecular weight of the antibody to increase its circulating half-life.

[00183] It is contemplated that the antibody conjugate or fusion protein may be covalently bound to one or more (for example, 2, 3, 4, 5, 6, 8, 9, 10 or more) biological modifiers that may comprise linear or branched polymers. Exemplary biological modifiers may include, for example, a variety of polymers, such as those described in U.S. Patent No. 7,842,789. Particularly useful are polyalkylene ethers such as polyethylene glycol (PEG) and derivatives thereof (for example, alkoxy polyethylene glycol, for example, methoxypolyethylene glycol, ethoxypolyethylene glycol and the like); block copolymers of polyoxyethylene and

polyoxypropylene (Pluronics); polymethacrylates; carbomers; and branched or unbranched polysaccharides which comprise the saccharide monomers such as D-mannose, D- and L-galactose, fucose, fructose, D-xylose, L-arabinose, and D-glucuronic acid.

5 **[00184]** In other embodiments, the biological modifier can be a hydrophilic polyvinyl polymer such as polyvinyl alcohol and polyvinylpyrrolidone (PVP)-type polymers. The biological modifier can be a functionalized polyvinylpyrrolidone, for example, carboxy or amine functionalized on one (or both) ends of the polymer (as available from PolymerSource). Alternatively, the biological modifier can include Poly N-(2-hydroxypropyl)methacrylamide (HPMA), or functionalized HPMA (amine, carboxy, *etc.*), Poly(N-isopropylacrylamide) or
 10 functionalized poly(N-isopropylacrylamide). Alternatively, the biological modifier can include Poly N-(2-hydroxypropyl)methacrylamide (HPMA), or functionalized HPMA (amine, carboxy, *etc.*), Poly(N-isopropylacrylamide) or functionalized poly(N-isopropylacrylamide). The modifier prior to conjugation need not be, but preferably is, water soluble, but the final conjugate should be water soluble.

15 **[00185]** In general, the biological modifier may have a molecular weight from about 2 kDa to about 5 kDa, from about 2 kDa to about 10 kDa, from about 2 kDa to about 20 kDa, from about 2 kDa to about 30 kDa, from about 2 kDa to about 40 kDa, from about 2 kDa to about 50 kDa, from about 2 kDa to about 60 kDa, from about 2 kDa to about 70 kDa, from about 2 kDa to about 80 kDa, from about 2 kDa to about 90 kDa, from about 2 kDa to about 100 kDa, from
 20 about 2 kDa to about 150 kDa, from about 5 kDa to about 10 kDa, from about 5 kDa to about 20 kDa, from about 5 kDa to about 30 kDa, from about 5 kDa to about 40 kDa, from about 5 kDa to about 50 kDa, from about 5 kDa to about 60 kDa, from about 5 kDa to about 70 kDa, from about 5 kDa to about 80 kDa, from about 5 kDa to about 90 kDa, from about 5 kDa to about 100 kDa, from about 5 kDa to about 150 kDa, from about 10 kDa to about 20 kDa, from about 10 kDa to
 25 about 30 kDa, from about 10 kDa to about 40 kDa, from about 10 kDa to about 50 kDa, from about 10 kDa to about 60 kDa, from about 10 kDa to about 70 kDa, from about 10 kDa to about 80 kDa, from about 10 kDa to about 90 kDa, from about 10 kDa to about 100 kDa, from about 10 kDa to about 150 kDa, from about 20 kDa to about 30 kDa, from about 20 kDa to about 40 kDa, from about 20 kDa to about 50 kDa, from about 20 kDa to about 60 kDa, from about 20
 30 kDa to about 70 kDa, from about 20 kDa to about 80 kDa, from about 20 kDa to about 90 kDa, from about 20 kDa to about 100 kDa, from about 20 kDa to about 150 kDa, from about 30 kDa to about 40 kDa, from about 30 kDa to about 50 kDa, from about 30 kDa to about 60 kDa, from about 30 kDa to about 70 kDa, from about 30 kDa to about 80 kDa, from about 30 kDa to about 90 kDa, from about 30 kDa to about 100 kDa, from about 30 kDa to about 150 kDa, from about

40 kDa to about 50 kDa, from about 40 kDa to about 60 kDa, from about 40 kDa to about 70 kDa, from about 40 kDa to about 80 kDa, from about 40 kDa to about 90 kDa, from about 40 kDa to about 100 kDa, from about 40 kDa to about 150 kDa, from about 50 kDa to about 60 kDa, from about 50 kDa to about 70 kDa, from about 50 kDa to about 80 kDa, from about 50 kDa to about 90 kDa, from about 50 kDa to about 100 kDa, from about 50 kDa to about 150 kDa, from about 60 kDa to about 70 kDa, from about 60 kDa to about 80 kDa, from about 60 kDa to about 90 kDa, from about 60 kDa to about 100 kDa, from about 60 kDa to about 150 kDa, from about 70 kDa to about 80 kDa, from about 70 kDa to about 90 kDa, from about 70 kDa to about 100 kDa, from about 70 kDa to about 150 kDa, from about 80 kDa to about 90 kDa, from about 80 kDa to about 100 kDa, from about 80 kDa to about 150 kDa, from about 90 kDa to about 100 kDa, from about 90 kDa to about 150 kDa, or from about 100 kDa to about 150 kDa.

[00186] It is contemplated that the antibody conjugate or fusion protein is attached to about 10 or fewer polymer molecules (*e.g.*, 9, 8, 7, 6, 5, 4, 3, 2, or 1), each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D.

[00187] Although a variety of polymers can be used as biological modifiers, it is contemplated that the antibody conjugates or fusion proteins described herein may be attached to polyethylene glycol (PEG) polymers. In one embodiment, the antibody conjugate or fusion protein described herein is covalently attached to at least one PEG having an actual MW of at least about 20,000 D. In another embodiment, the antibody conjugate or fusion protein described herein is covalently attached to at least one PEG having an actual MW of at least about 30,000 D. In another embodiment, the antibody conjugate or fusion protein described herein is covalently attached to at least one PEG having an actual MW of at least about 40,000 D. In certain embodiments, the PEG is methoxyPEG(5000)-succinimidylpropionate (mPEG-SPA), methoxyPEG(5000)-succinimidylsuccinate (mPEG-SS). Such PEGs are commercially available from Nektar Therapeutics or SunBiowest.

[00188] Attachment sites on an antibody conjugate or fusion protein for a biological modifier include the N-terminal amino group and epsilon amino groups found on lysine residues, as well as other amino, imino, carboxyl, sulfhydryl, hydroxyl or other hydrophilic groups. The polymer may be covalently bonded directly to the antibody conjugate or fusion protein with or without the known use of a multifunctional (ordinarily bifunctional) crosslinking agent using chemistries and used in the art. For example, sulfhydryl groups can be derivatized by coupling to

maleimido-substituted PEG (*e.g.* alkoxy-PEG amine plus sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate), or PEG-maleimide commercially available from Shearwater Polymers, Inc., Huntsville, Ala.).

II. Methods of Making a Fusion Protein or Antibody Conjugate

5 [00189] Methods for producing fusion proteins, *e.g.*, those disclosed herein, antibodies, or antibody conjugates, *e.g.*, those disclosed herein, are known in the art. For example, DNA molecules encoding light chain variable regions and/or heavy chain variable regions can be synthesized chemically or by recombinant DNA methodologies. For example, the sequences of the antibodies can be cloned from hybridomas by conventional hybridization techniques or
10 polymerase chain reaction (PCR) techniques, using the appropriate synthetic nucleic acid primers. The resulting DNA molecules encoding the variable regions of interest can be ligated to other appropriate nucleotide sequences, including, for example, constant region coding sequences, and expression control sequences, to produce conventional gene expression constructs (*i.e.*, expression vectors) encoding the desired antibodies. Production of defined gene
15 constructs is within routine skill in the art.

[00190] Nucleic acids encoding desired fusion proteins, and/or antibody conjugates can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Exemplary host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) cells, HeLa cells,
20 baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and myeloma cells that do not otherwise produce IgG protein. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the immunoglobulin light and/or heavy chain variable regions.

[00191] Specific expression and purification conditions will vary depending upon the
25 expression system employed. For example, if a gene is to be expressed in *E. coli*, it is first cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, *e.g.*, Trp or Tac, and a prokaryotic signal sequence. The expressed protein may be secreted. The expressed protein may accumulate in refractile or inclusion bodies, which can be harvested after disruption of the cells by French press or sonication. The refractile bodies
30 then are solubilized, and the protein may be refolded and/or cleaved by methods known in the art.

[00192] If the engineered gene is to be expressed in eukaryotic host cells, *e.g.*, CHO cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion

signal, a poly A sequence, and a stop codon. Optionally, the vector or gene construct may contain enhancers and introns. In embodiments involving fusion proteins comprising an antibody or portion thereof, the expression vector optionally contains sequences encoding all or part of a constant region, enabling an entire, or a part of, a heavy or light chain to be expressed.

5 The gene construct can be introduced into eukaryotic host cells using conventional techniques.

[00193] The host cells express a fusion protein and/or antibody conjugate comprising a sialidase and V_L or V_H fragments, V_L - V_H heterodimers, V_H - V_L or V_L - V_H single chain polypeptides, complete heavy or light immunoglobulin chains, or portions thereof, each of which may be attached to a moiety having another function (*e.g.*, cytotoxicity). In some embodiments
10 involving fusion proteins and/or antibody conjugates, a host cell is transfected with a single vector expressing a polypeptide expressing a sialidase and an entire, or part of, a heavy chain (*e.g.*, a heavy chain variable region) or a sialidase and a light chain (*e.g.*, a light chain variable region), or a polypeptide expressing an entire, or part of, a heavy chain (*e.g.*, a heavy chain variable region) or a light chain (*e.g.*, a light chain variable region). In some embodiments, a
15 host cell is transfected with a single vector encoding (a) a polypeptide comprising a heavy chain variable region and a polypeptide comprising a light chain variable region, or (b) an entire immunoglobulin heavy chain and an entire immunoglobulin light chain, wherein in (a) or in (b), the polypeptide may also comprise a sialidase. In some embodiments, a host cell is co-transfected with more than one expression vector (*e.g.*, one expression vector expressing a
20 polypeptide comprising an entire, or part of, a heavy chain or heavy chain variable region, optionally comprising a sialidase fused thereto, and another expression vector expressing a polypeptide comprising an entire, or part of, a light chain or light chain variable region, optionally comprising a sialidase fused thereto).

[00194] A polypeptide comprising a fusion protein, *e.g.*, a fusion protein comprising an
25 immunoglobulin heavy chain variable region or light chain variable region, can be produced by growing (culturing) a host cell transfected with an expression vector encoding such a variable region, under conditions that permit expression of the polypeptide. Following expression, the polypeptide can be harvested and purified or isolated using techniques known in the art, *e.g.*, affinity tags such as glutathione-S-transferase (GST) or histidine tags.

30 **[00195]** In embodiments in which a fusion protein and/or antibody conjugate is produced, a sialidase fused to a monoclonal antibody, Fc domain, or an antigen-binding domain of the antibody, can be produced by growing (culturing) a host cell transfected with: (a) an expression vector that encodes a complete or partial immunoglobulin heavy chain, and a separate expression

vector that encodes a complete or partial immunoglobulin light chain; or (b) a single expression vector that encodes both chains (*e.g.*, complete or partial heavy and light chains), under conditions that permit expression of both chains. The sialidase will be fused to one or more of the chains. The intact fusion protein and/or antibody conjugate can be harvested and purified or isolated using techniques known in the art, *e.g.*, Protein A, Protein G, affinity tags such as glutathione-S-transferase (GST) or histidine tags. It is within ordinary skill in the art to express the heavy chain and the light chain from a single expression vector or from two separate expression vectors.

[00196] In certain embodiments, in order to express a protein, *e.g.*, a fusion protein, as a secreted protein, a native N-terminal signal sequence of the protein is replaced, *e.g.*, with MDMRVPAQLLGLLLLWLPGARC (SEQ ID NO: 28). In certain embodiments, to express a protein, *e.g.*, a fusion protein, as a secreted protein, an N-terminal signal sequence, *e.g.*, MDMRVPAQLLGLLLLWLPGARC (SEQ ID NO: 28), is added. Additional exemplary N-terminal signal sequences include signal sequences from interleukin-2, CD-5, IgG kappa light chain, trypsinogen, serum albumin, and prolactin. In certain embodiments, in order to express a protein, *e.g.*, a fusion protein, as a secreted protein, a C terminal lysosomal signal motif, *e.g.*, YGTL (SEQ ID NO: 29) is removed.

[00197] Methods for reducing or eliminating the antigenicity of antibodies and antibody fragments are known in the art. When the antibodies are to be administered to a human, the antibodies preferably are “humanized” to reduce or eliminate antigenicity in humans. Preferably, each humanized antibody has the same or substantially the same affinity for the antigen as the non-humanized mouse antibody from which it was derived.

[00198] In one humanization approach, chimeric proteins are created in which mouse immunoglobulin constant regions are replaced with human immunoglobulin constant regions. See, *e.g.*, Morrison *et al.*, 1984, PROC. NAT. ACAD. SCI. 81:6851-6855, Neuberger *et al.*, 1984, NATURE 312:604-608; U.S. Patent Nos. 6,893,625 (Robinson); 5,500,362 (Robinson); and 4,816,567 (Cabilly).

[00199] In an approach known as CDR grafting, the CDRs of the light and heavy chain variable regions are grafted into frameworks from another species. For example, murine CDRs can be grafted into human FRs. In some embodiments, the CDRs of the light and heavy chain variable regions of an antibody are grafted into human FRs or consensus human FRs. To create consensus human FRs, FRs from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence. CDR grafting is described in U.S.

Patent Nos. 7,022,500 (Queen); 6,982,321 (Winter); 6,180,370 (Queen); 6,054,297 (Carter); 5,693,762 (Queen); 5,859,205 (Adair); 5,693,761 (Queen); 5,565,332 (Hoogenboom); 5,585,089 (Queen); 5,530,101 (Queen); Jones *et al.* (1986) NATURE 321: 522-525; Riechmann *et al.* (1988) NATURE 332: 323-327; Verhoeyen *et al.* (1988) SCIENCE 239: 1534-1536; and Winter (1998) FEBS LETT 430: 92-94.

[00200] In an approach called “SUPERHUMANIZATION[™],” human CDR sequences are chosen from human germline genes, based on the structural similarity of the human CDRs to those of the mouse antibody to be humanized. See, *e.g.*, U.S. Patent No. 6,881,557 (Foote); and Tan *et al.*, 2002, J. IMMUNOL. 169:1119-1125.

[00201] Other methods to reduce immunogenicity include “reshaping,” “hyperchimerization,” and “veneering/resurfacing.” See, *e.g.*, Vaswami *et al.*, 1998, ANNALS OF ALLERGY, ASTHMA, & IMMUNOL. 81:105; Roguska *et al.*, 1996, PROT. ENGINEER 9:895-904; and U.S. Patent No. 6,072,035 (Hardman). In the veneering/resurfacing approach, the surface accessible amino acid residues in the murine antibody are replaced by amino acid residues more frequently found at the same positions in a human antibody. This type of antibody resurfacing is described, *e.g.*, in U.S. Patent No. 5,639,641 (Pedersen).

[00202] Another approach for converting a mouse antibody into a form suitable for medical use in humans is known as ACTIVMAB[™] technology (Vaccinex, Inc., Rochester, NY), which involves a vaccinia virus-based vector to express antibodies in mammalian cells. High levels of combinatorial diversity of IgG heavy and light chains can be produced. See, *e.g.*, U.S. Patent Nos. 6,706,477 (Zauderer); 6,800,442 (Zauderer); and 6,872,518 (Zauderer). Another approach for converting a mouse antibody into a form suitable for use in humans is technology practiced commercially by KaloBios Pharmaceuticals, Inc. (Palo Alto, CA). This technology involves the use of a proprietary human “acceptor” library to produce an “epitope focused” library for antibody selection. Another approach for modifying a mouse antibody into a form suitable for medical use in humans is HUMAN ENGINEERING[™] technology, which is practiced commercially by XOMA (US) LLC. See, *e.g.*, International (PCT) Publication No. WO 93/11794 and U.S. Patent Nos. 5,766,886 (Studnicka); 5,770,196 (Studnicka); 5,821,123 (Studnicka); and 5,869,619 (Studnicka).

[00203] Any suitable approach, including any of the above approaches, can be used to reduce or eliminate human immunogenicity of an antibody.

[00204] In addition, it is possible to create fully human antibodies in mice. Fully human mAbs lacking any non-human sequences can be prepared from human immunoglobulin transgenic

mice by techniques referenced in, *e.g.*, Lonberg *et al.*, NATURE 368:856-859, 1994; Fishwild *et al.*, NATURE BIOTECHNOLOGY 14:845-851, 1996; and Mendez *et al.*, NATURE GENETICS 15:146-156, 1997. Fully human monoclonal antibodies can also be prepared and optimized from phage display libraries by techniques referenced in, *e.g.*, Knappik *et al.*, J. MOL. BIOL. 296:57-86, 2000; and Krebs *et al.*, J. IMMUNOL. METH. 254:67-84 2001).

[00205] The present invention encompasses fusion proteins comprising antibody fragments, which may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. For a review of certain antibody fragments, see Hudson *et al.* (2003) NAT. MED. 9:129-134.

[00206] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.* (1992) JOURNAL OF BIOCHEMICAL AND BIOPHYSICAL METHODS 24:107-117; and Brennan *et al.* (1985) SCIENCE 229:81). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter *et al.* (1992) BIO/TECHNOLOGY 10:163-167). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')₂ fragments with increased *in vivo* half-life comprising salvage receptor binding epitope residues are described in U.S. Patent No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). See U.S. Patent Nos. 5,571,894 and 5,587,458.

[00207] Methods for making bispecific antibodies are known in the art. See Milstein and Cuello (1983) NATURE 305:537, International (PCT) Publication No. WO93/08829, and Traunecker *et al.* (1991) EMBO J., 10:3655. For further details of generating bispecific antibodies see, for example, Suresh *et al.* (1986) METHODS ENZYMOL. 121:210. Bispecific antibodies include cross-linked or "heteroconjugate" or "heterodimer" antibodies. For example, one of the antibodies in the heterodimer can be coupled to avidin, the other to biotin. Heterodimer antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

[00208] Examples of heterodimeric or asymmetric IgG-like molecules include but are not limited to those obtained with the following technologies or using the following formats: Triomab/Quadroma, Knobs-into-Holes, CrossMabs, electrostatically-matched antibodies, LUZ-Y, Strand Exchange Engineered Domain body, Biclonic and DuoBody.

5 [00209] Advantages of using antibody fragments (*e.g.*, F(ab) and F(ab')₂ fragments) include the elimination of non-specific binding between Fc portions of antibodies and Fc receptors on cells (such as macrophages, dendritic cells, neutrophils, NK cells and B cells). In addition, they may be able to penetrate tissues more efficiently due to their smaller size.

[00210] Heterodimeric antibodies, or asymmetric antibodies, allow for greater flexibility and
10 new formats for attaching a variety of drugs to the antibody arms. One of the general formats for creating a heterodimeric antibody is the “knobs-into-holes” format. This format is specific to the heavy chain part of the constant region in antibodies. The “knobs” part is engineered by replacing a small amino acid with a larger one, which fits into a “hole”, which is engineered by replacing a large amino acid with a smaller one. What connects the “knobs” to the “holes” are
15 the disulfide bonds between each chain. The “knobs-into-holes” shape facilitates antibody dependent cell mediated cytotoxicity. Single chain variable fragments (scFv) are connected to the variable domain of the heavy and light chain via a short linker peptide. The linker is rich in glycine, which gives it more flexibility, and serine/threonine, which gives it specificity. Two different scFv fragments can be connected together, via a hinge region, to the constant domain of
20 the heavy chain or the constant domain of the light chain. This gives the antibody bispecificity, allowing for the binding specificities of two different antigens. The “knobs-into-holes” format enhances heterodimer formation but doesn't suppress homodimer formation.

[00211] Several approaches to support heterodimerization have been described, for example in International (PCT) Publication Nos. WO96/27011, WO98/050431, WO2007/110205,
25 WO2007/147901, WO2009/089004, WO2010/129304, WO2011/90754, WO2011/143545, WO2012/058768, WO2013/157954, and WO2013/096291, and European Patent Publication No. EP1870459. Typically, in the approaches known in the art, the CH₃ domain of the first heavy chain and the CH₃ domain of the second heavy chain are both engineered in a complementary manner so that the heavy chain comprising one engineered CH₃ domain can no longer
30 homodimerize with another heavy chain of the same structure (*e.g.* a CH₃-engineered first heavy chain can no longer homodimerize with another CH₃-engineered first heavy chain; and a CH₃-engineered second heavy chain can no longer homodimerize with another CH₃-engineered second heavy chain). Thereby the heavy chain comprising one engineered CH₃ domain is forced

to heterodimerize with another heavy chain comprising the CH₃ domain, which is engineered in a complementary manner. As a result, the CH₃ domain of the first heavy chain and the CH₃ domain of the second heavy chain are engineered in a complementary manner by amino acid substitutions, such that the first heavy chain and the second heavy chain are forced to heterodimerize, whereas the first heavy chain and the second heavy chain can no longer homodimerize (*e.g.*, for steric reasons).

III. Pharmaceutical Compositions

[00212] For therapeutic use, a fusion protein and/or antibody conjugate preferably is combined with a pharmaceutically acceptable carrier. The term “pharmaceutically acceptable” as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00213] The term “pharmaceutically acceptable carrier” as used herein refers to buffers, carriers, and excipients suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable carriers include any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see, *e.g.*, Martin, Remington’s Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA [1975]. Pharmaceutically acceptable carriers include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

[00214] In certain embodiments, a pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In such embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine

tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as glucose, mannose or dextrans); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants (see, *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990)).

[00215] In certain embodiments, a pharmaceutical composition may contain nanoparticles, *e.g.*, polymeric nanoparticles, liposomes, or micelles (See Anselmo *et al.* (2016) *BIOENG. TRANSL. MED.* 1: 10-29).

[00216] In certain embodiments, a pharmaceutical composition may contain a sustained- or controlled-delivery formulation. Techniques for formulating sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. Sustained-release preparations may include, *e.g.*, porous polymeric microparticles or semipermeable polymer matrices in the form of shaped articles, *e.g.*, films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides, copolymers of L-glutamic acid and gamma ethyl-L-glutamate, poly (2-hydroxyethyl-inethacrylate), ethylene vinyl acetate, or poly-D(-)-3-hydroxybutyric acid. Sustained release compositions may also include liposomes that can be prepared by any of several methods known in the art.

[00217] Pharmaceutical compositions containing a sialidase fusion protein or an antibody conjugate disclosed herein can be presented in a dosage unit form and can be prepared by any suitable method. A pharmaceutical composition should be formulated to be compatible with its intended route of administration. Examples of routes of administration are intravenous (IV), intradermal, inhalation, transdermal, topical, transmucosal, intrathecal and rectal administration.

In certain embodiments, a sialidase fusion protein or an antibody conjugate disclosed herein is administered by IV infusion. In certain embodiments, a sialidase fusion protein or an antibody conjugate disclosed herein is administered by intratumoral injection. Useful formulations can be prepared by methods known in the pharmaceutical art. For example, see *Remington's*

5 *Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990). Formulation components suitable for parenteral administration include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as
10 acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.

[00218] For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should
15 be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof.

[00219] In certain embodiments, a pharmaceutical composition may contain a stabilizing agent. In certain embodiments, the stabilizing agent is a cation, such as a divalent cation. In certain
20 embodiments, the cation is calcium or magnesium. The cation can be in the form of a salt, such as calcium chloride (CaCl₂) or magnesium chloride (MgCl₂).

[00220] In certain embodiments, the stabilizing agent is present in an amount from about 0.05 mM to about 5 mM. For example, the stabilizing agent may be present in an amount of from about 0.05 mM to about 4 mM, from about 0.05 mM to about 3 mM, from about 0.05 mM to
25 about 2 mM, from about 0.05 mM to about 1 mM, from about 0.05 mM to about 0.5 mM, from about 0.5 mM to about 4 mM, from about 0.5 mM to about 3 mM, from about 0.5 mM to about 2 mM, from about 0.5 mM to about 1 mM, from about 1 mM to about 4 mM, from about 1 mM to about 3 mM, of from about 1 mM to about 2 mM.

[00221] Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished
30 by any suitable method, *e.g.*, filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

[00222] The compositions described herein may be administered locally or systemically. Administration will generally be parenteral administration. In a preferred embodiment, the pharmaceutical composition is administered subcutaneously and in an even more preferred embodiment intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions.

[00223] Generally, a therapeutically effective amount of active component, for example, a fusion protein and/or antibody conjugate, is in the range of 0.1 mg/kg to 100 mg/kg, *e.g.*, 1 mg/kg to 100 mg/kg, 1 mg/kg to 10 mg/kg. The amount administered will depend on variables such as the type and extent of disease or indication to be treated, the overall health of the patient, the *in vivo* potency of the antibody, the pharmaceutical formulation, and the route of administration. The initial dosage can be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue-level. Alternatively, the initial dosage can be smaller than the optimum, and the daily dosage may be progressively increased during the course of treatment. Human dosage can be optimized, *e.g.*, in a conventional Phase I dose escalation study designed to run from 0.5 mg/kg to 20 mg/kg. Dosing frequency can vary, depending on factors such as route of administration, dosage amount, serum half-life of the fusion protein and/or antibody conjugate, and the disease being treated. Exemplary dosing frequencies are once per day, once per week and once every two weeks. A preferred route of administration is parenteral, *e.g.*, intravenous infusion. In certain embodiments, a fusion protein and/or antibody conjugate is lyophilized, and then reconstituted in buffered saline, at the time of administration.

IV. Therapeutic Uses

[00224] The compositions and methods disclosed herein can be used to treat various forms of cancer in a subject or inhibit cancer growth in a subject. The invention provides a method of treating a cancer in a subject. The method comprises administering to the subject an effective amount of a sialidase anti-PD-L1 fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody conjugate disclosed herein, either alone or in a combination with another therapeutic agent to treat the cancer in the subject. The term “effective amount” as used herein refers to the amount of an active agent (*e.g.*, fusion protein according to the present invention) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route.

[00225] As used herein, “treat”, “treating” and “treatment” mean the treatment of a disease in a subject, *e.g.*, in a human. This includes: (a) inhibiting the disease, *i.e.*, arresting its development;

and (b) relieving the disease, *i.e.*, causing regression of the disease state. As used herein, the terms “subject” and “patient” refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably includes humans.

[00226] Examples of cancers include solid tumors, soft tissue tumors, hematopoietic tumors and metastatic lesions. Examples of hematopoietic tumors include, leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), *e.g.*, transformed CLL, diffuse large B-cell lymphomas (DLBCL), follicular lymphoma, hairy cell leukemia, myelodysplastic syndrome (MDS), a lymphoma, Hodgkin’s disease, a malignant lymphoma, non-Hodgkin’s lymphoma, Burkitt’s lymphoma, multiple myeloma, or Richter’s Syndrome (Richter’s Transformation). Examples of solid tumors include malignancies, *e.g.*, sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting head and neck (including pharynx), thyroid, lung (small cell or non-small cell lung carcinoma (NSCLC)), breast, lymphoid, gastrointestinal (*e.g.*, oral, esophageal, stomach, liver, pancreas, small intestine, colon and rectum, anal canal), genitals and genitourinary tract (*e.g.*, renal, urothelial, bladder, ovarian, uterine, cervical, endometrial, prostate, testicular), CNS (*e.g.*, neural or glial cells, *e.g.*, neuroblastoma or glioma), or skin (*e.g.*, melanoma and metastatic Merkel cell carcinoma (MCC)).

[00227] In certain embodiments the cancer is an epithelial cancer, *e.g.*, an epithelial cancer that upregulates the expression of sialylated glycans. Exemplary epithelial cancers include, but are not limited to, endometrial cancer, colon cancer, ovarian cancer, cervical cancer, vulvar cancer, uterine cancer or fallopian tube cancer, breast cancer, prostate cancer, lung cancer, pancreatic cancer, urinary cancer, bladder cancer, head and neck cancer, oral cancer and liver cancer. Epithelial cancers also include carcinomas, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatousum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic

carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniformi carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhus carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, and carcinoma villosum.

[00228] In certain embodiments the cancer is selected from lung bronchioloalveolar carcinoma (BAC), bladder cancer, a female genital tract malignancy (*e.g.*, uterine serous carcinoma, endometrial carcinoma, vulvar squamous cell carcinoma, and uterine sarcoma), an ovarian surface epithelial carcinoma (*e.g.*, clear cell carcinoma of the ovary, epithelial ovarian cancer, fallopian tube cancer, and primary peritoneal cancer), breast carcinoma, non-small cell lung cancer (NSCLC), a male genital tract malignancy (*e.g.*, testicular cancer), retroperitoneal or peritoneal carcinoma, gastroesophageal adenocarcinoma, esophagogastric junction carcinoma, liver hepatocellular carcinoma, esophageal and esophagogastric junction carcinoma, cervical cancer, cholangiocarcinoma, pancreatic adenocarcinoma, extrahepatic bile duct adenocarcinoma, a small intestinal malignancy, gastric adenocarcinoma, cancer of unknown primary (CUP), colorectal adenocarcinoma, esophageal carcinoma, prostatic adenocarcinoma, kidney cancer, head and neck squamous carcinoma, thymic carcinoma, non-melanoma skin cancer, thyroid carcinoma (*e.g.*, papillary carcinoma), a head and neck cancer, anal carcinoma, non-epithelial ovarian cancer (non-EOC), metastatic urothelial carcinoma (UC), uveal melanoma, malignant pleural mesothelioma, small cell lung cancer (SCLC), a central nervous system cancer, a neuroendocrine tumor, and a soft tissue tumor.

[00229] In certain embodiments, the cancer is melanoma, non-small cell lung cancer, colon cancer, breast cancer, bladder cancer, or kidney cancer.

[00230] In certain embodiments, the cancer is an adenocarcinoma. In certain embodiments, the cancer is a metastatic cancer. In certain embodiments, the cancer is a refractory cancer.

5 [00231] In certain embodiments, the cancer is resistant to or non-responsive to treatment with an antibody, *e.g.*, an antibody with ADCC activity, *e.g.*, avelumab.

[00232] In certain embodiments, the cancer is a PD-L1-expressing cancer, *e.g.*, the cancer comprises cells that express PD-L1. An analysis of 196 tumor specimens from patients with renal cell carcinoma found that high tumor expression of PD-L1 was associated with increased
10 tumor aggressiveness and a 4.5-fold increased risk of death. High expression of PD-L1 is associated with reduced numbers of tumor infiltrating lymphocytes and poor prognosis. In certain embodiments, the PD-L1 status of a cancer can be determined using immunohistochemistry staining protocols, such as DAKO 22C3 and VENTANA SP142 FDA approved protocols, which are used as companion diagnostics for anti-PD-L1 antibodies
15 pembrolizumab, durvalumab, atezolizumab, and avelumab.

[00233] The methods and compositions described herein can be used alone or in combination with other therapeutic agents and/or modalities. The term administered “in combination,” as used herein, is understood to mean that two (or more) different treatments are delivered to the subject during the course of the subject’s affliction with the disorder, such that the effects of the
20 treatments on the patient overlap at a point in time. In certain embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as “simultaneous” or “concurrent delivery.” In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In certain embodiments of either case, the treatment is more effective
25 because of combined administration. For example, the second treatment is more effective, *e.g.*, an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In certain embodiments, delivery is such that the reduction in a symptom, or other parameter
30 related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[00234] In certain embodiments, a method or composition described herein, is administered in combination with one or more additional therapies, *e.g.*, surgery, radiation therapy, or administration of another therapeutic preparation. In certain embodiments, the additional therapy may include chemotherapy, *e.g.*, a cytotoxic agent. In certain embodiments the additional therapy may include a targeted therapy, *e.g.* a tyrosine kinase inhibitor, a proteasome inhibitor, or a protease inhibitor. In certain embodiments, the additional therapy may include an anti-inflammatory, anti-angiogenic, anti-fibrotic, or anti-proliferative compound, *e.g.*, a steroid, a biologic immunomodulator, a monoclonal antibody, an antibody fragment, an aptamer, an siRNA, an antisense molecule, a fusion protein, a cytokine, a cytokine receptor, a bronchodilator, a statin, an anti-inflammatory agent (*e.g.* methotrexate), or an NSAID. In certain embodiments, the additional therapy may include a combination of therapeutics of different classes.

[00235] In certain embodiments, a method or composition described herein is administered in combination with a second checkpoint inhibitor. The checkpoint inhibitor may, for example, be selected from a PD-1 antagonist, a second PD-L1 antagonist, CTLA-4 antagonist, adenosine A2A receptor antagonist, B7-H3 antagonist, B7-H4 antagonist, BTLA antagonist, KIR antagonist, LAG3 antagonist, TIM-3 antagonist, VISTA antagonist or TIGIT antagonist.

[00236] In certain embodiments, the checkpoint inhibitor is a PD-1 or a second PD-L1 inhibitor. PD-1 is a receptor present on the surface of T-cells that serves as an immune system checkpoint that inhibits or otherwise modulates T-cell activity at the appropriate time to prevent an overactive immune response. Cancer cells, however, can take advantage of this checkpoint by expressing ligands, for example, PD-L1, that interact with PD-1 on the surface of T-cells to shut down or modulate T-cell activity. Exemplary PD-1/PD-L1 based immune checkpoint inhibitors include antibody based therapeutics. Exemplary treatment methods that employ PD-1/PD-L1 based immune checkpoint inhibition are described in U.S. Patent Nos. 8,728,474 and 9,073,994, and EP Patent No. 1537878B1, and, for example, include the use of anti-PD-1 antibodies. Exemplary anti-PD-1 antibodies are described, for example, in U.S. Patent Nos. 8,952,136, 8,779,105, 8,008,449, 8,741,295, 9,205,148, 9,181,342, 9,102,728, 9,102,727, 8,952,136, 8,927,697, 8,900,587, 8,735,553, and 7,488,802. Exemplary anti-PD-1 antibodies include, for example, nivolumab (Opdivo®, Bristol-Myers Squibb Co.), pembrolizumab (Keytruda®, Merck Sharp & Dohme Corp.), PDR001 (Novartis Pharmaceuticals), and pidilizumab (CT-011, Cure Tech). Exemplary anti-PD-L1 antibodies are described, for example, in U.S. Patent Nos. 9,273,135, 7,943,743, 9,175,082, 8,741,295, 8,552,154, and 8,217,149.

Exemplary anti-PD-L1 antibodies include, atezolizumab (Tecentriq®, Genentech), durvalumab (AstraZeneca), MEDI4736, avelumab, and BMS 936559 (Bristol Myers Squibb Co.).

[00237] In certain embodiments, a method or composition described herein is administered in combination with a CTLA-4 inhibitor. In the CTLA-4 pathway, the interaction of CTLA-4 on a T-cell with its ligands (*e.g.*, CD80, also known as B7-1, and CD86) on the surface of an antigen presenting cells (rather than cancer cells) leads to T-cell inhibition. Exemplary CTLA-4 based immune checkpoint inhibition methods are described in U.S. Patent Nos. 5,811,097, 5,855,887, 6,051,227. Exemplary anti-CTLA-4 antibodies are described in U.S. Patent Nos. 6,984,720, 6,682,736, 7,311,910; 7,307,064, 7,109,003, 7,132,281, 6,207,156, 7,807,797, 7,824,679, 8,143,379, 8,263,073, 8,318,916, 8,017,114, 8,784,815, and 8,883,984, International (PCT) Publication Nos. WO98/42752, WO00/37504, and WO01/14424, and European Patent No. EP 1212422 B1. Exemplary CTLA-4 antibodies include ipilimumab or tremelimumab.

[00238] In certain embodiments, a method or composition described herein is administered in combination with a CTLA-4 inhibitor, *e.g.*, a CTLA-4 inhibitor disclosed herein.

[00239] In certain embodiments, a method or composition described herein is administered in combination with an IDO inhibitor. Exemplary IDO inhibitors include 1-methyl-D-tryptophan (known as indoximod), epacadostat (INCB24360), navoximod (GDC-0919), and BMS-986205.

[00240] Exemplary cytotoxic agents that can be administered in combination with a method or composition described herein include, for example, antimicrotubule agents, topoisomerase inhibitors, antimetabolites, protein synthesis and degradation inhibitors, mitotic inhibitors, alkylating agents, platinating agents, inhibitors of nucleic acid synthesis, histone deacetylase inhibitors (HDAC inhibitors, *e.g.*, vorinostat (SAHA, MK0683), entinostat (MS-275), panobinostat (LBH589), trichostatin A (TSA), mocetinostat (MGCD0103), belinostat (PXD101), romidepsin (FK228, depsipeptide)), DNA methyltransferase inhibitors, nitrogen mustards, nitrosoureas, ethylenimines, alkyl sulfonates, triazenes, folate analogs, nucleoside analogs, ribonucleotide reductase inhibitors, vinca alkaloids, taxanes, epothilones, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis and radiation, or antibody molecule conjugates that bind surface proteins to deliver a toxic agent. In one embodiment, the cytotoxic agent that can be administered with a method or composition described herein is a platinum-based agent (such as cisplatin), cyclophosphamide, dacarbazine, methotrexate, fluorouracil, gemcitabine, capecitabine, hydroxyurea, topotecan, irinotecan, azacytidine, vorinostat, ixabepilone, bortezomib, taxanes (*e.g.*, paclitaxel or docetaxel), cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide,

tenoposide, vincristine, vinblastine, vinorelbine, colchicin, anthracyclines (*e.g.*, doxorubicin or epirubicin) daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, adriamycin, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, ricin, or maytansinoids.

5 **[00241]** The invention also provides a method of increasing the expression of HLA-DR, CD86, CD83, IFN γ , IL-1b, IL-6, TNF α , IL-17A, IL-2, or IL-6 in a cell, tissue, or subject. The method comprises contacting the cell, tissue, or subject with an effective amount of a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody conjugate disclosed herein. In certain embodiments, the cell is selected from a dendritic cell and a peripheral blood
10 mononuclear cell (PBMC).

[00242] In certain embodiments, expression of HLA-DR, CD86, CD83, IFN γ , IL-1b, IL-6, TNF α , IL-17A, IL-2, or IL-6 in the cell, tissue, or subject is increased by at least about 10%, at least about 20%, at least about 50%, at least about 75%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 400%, at
15 least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, or at least about 1,000%, relative to a similar or otherwise identical cell or tissue that has not been contacted with the fusion protein or antibody conjugate. Gene expression may be measured by any suitable method known in the art, for example, by ELISA, or by Luminex multiplex assays.

20 **[00243]** The invention also provides a method of promoting infiltration of immune cells into a tumor in a subject in need thereof. The method comprises administering to the subject an effective amount of a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody conjugate disclosed herein. In certain embodiments, the immune cells are T-cells, *e.g.*, CD4⁺ and/or CD8⁺ T-cells, *e.g.*, CD69⁺CD8⁺ and/or GzmB⁺CD8⁺ T-cells. In certain embodiments,
25 the immune cells are natural killer (NK) cells.

[00244] In certain embodiments, the infiltration of immune cells into the tumor in the subject is increased by at least about 10%, at least about 20%, at least about 50%, at least about 75%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at
30 least about 800%, at least about 900%, or at least about 1,000%, relative to a similar or otherwise identical tumor and/or subject that has not been administered the fusion protein or antibody conjugate. Infiltration of immune cells into a tumor may be measured by any suitable method known in the art, for example, antibody staining.

[00245] The invention also provides a method of increasing the number of circulating natural killer (NK) cells in a subject in need thereof. The method comprises administering to the subject an effective amount of a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody conjugate disclosed herein, so as to increase the number of circulating NK cells relative to prior to administration of the fusion protein, antibody conjugate or pharmaceutical composition.

[00246] In certain embodiments, the number of circulating NK cells in the subject is increased by at least about 10%, at least about 20%, at least about 50%, at least about 75%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, or at least about 1,000%, relative to a similar or otherwise identical subject that has not been administered the fusion protein or antibody conjugate. Circulating NK cells in a subject may be measured by any suitable method known in the art, for example, antibody staining.

[00247] The invention also provides a method of increasing the number of T-cells in the draining lymph node in a subject in need thereof. The method comprises administering to the subject an effective amount of a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody conjugate disclosed herein, so as to increase the number of T-cells in the draining lymph node relative to prior to administration of the fusion protein, antibody conjugate or pharmaceutical composition. In certain embodiments, the immune cells are T-cells, *e.g.*, CD4+ and/or CD8+ T-cells.

[00248] In certain embodiments, the number of T-cells in the draining lymph node in the subject is increased by at least about 10%, at least about 20%, at least about 50%, at least about 75%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, or at least about 1,000%, relative to a similar or otherwise identical subject that has not been administered the fusion protein or antibody conjugate. T-cells in the draining lymph node in a subject may be measured by any suitable method known in the art, for example, antibody staining.

[00249] The invention also provides a method of increasing expression of Cd3, Cd4, Cd8, Cd274, Ctl4, Icos, Pdcd1, Lag3, Il6, Il1b, Il2, Ifng, Ifna1, Mx1, Gzmb, Cxcl9, Cxcl12, and/or Ccl5 in a cell, tissue, or subject. The method comprises contacting the cell, tissue, or subject with an effective amount of a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or

antibody conjugate disclosed herein, so as to increase the expression of Cd3, Cd4, Cd8, Cd274, Ctl4, Icos, Pdcd1, Lag3, Il6, Il1b, Il2, Ifng, Ifna1, Mx1, Gzmb, Cxcl9, Cxcl12, and/or Ccl5 relative to the cell, tissue or subject prior to contact with the fusion protein, antibody conjugate or pharmaceutical composition.

5 [00250] In certain embodiments, expression of Cd3, Cd4, Cd8, Cd274, Ctl4, Icos, Pdcd1, Lag3, Il6, Il1b, Il2, Ifng, Ifna1, Mx1, Gzmb, Cxcl9, Cxcl12, and/or Ccl5 in the cell, tissue, or subject is increased by at least about 10%, at least about 20%, at least about 50%, at least about 75%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%,
10 at least about 800%, at least about 900%, or at least about 1,000%, relative to a similar or otherwise identical cell, tissue, or subject that has not been contacted with the fusion protein or antibody conjugate. Gene expression may be measured by any suitable method known in the art, for example, by ELISA, Luminex multiplex assays, or Nanostring technology.

[00251] The invention also provides a method of removing sialic acid from a cell or tissue. The
15 method comprises contacting the cell or tissue with an effective amount of a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody conjugate disclosed herein. The invention also provides a method of removing sialic acid from a cell in a subject, the method comprising administering to the subject an effective amount of a pharmaceutical composition comprising a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody
20 conjugate disclosed herein, thereby to remove sialic acid from the cell.

[00252] In certain embodiments, the cell is tumor cell, dendritic cell (DC) or monocyte. In certain embodiments, the cell is a monocyte, and the method results in increased expression of an MHC-II molecule (*e.g.*, HLA-DR) on the monocyte. In certain embodiments, expression of an MHC-II molecule in the cell or tissue is increased by at least about 10%, at least about 20%,
25 at least about 50%, at least about 75%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, or at least about 1,000%, relative to a similar or otherwise identical cell or tissue that has not been contacted with the fusion protein and/or antibody conjugate. Gene expression may be measured by any suitable
30 method known in the art, for example, by ELISA, by Luminex multiplex assays, or by flow cytometry.

[00253] The invention also provides a method of enhancing phagocytosis of a tumor cell. The method comprises contacting the tumor cell with a fusion protein and/or antibody conjugate,

e.g., a fusion protein or antibody conjugate disclosed herein, in an amount effective to remove sialic acid from the tumor cell, thereby enhancing phagocytosis of the tumor cell. In certain embodiments, the disclosure relates to a method of increasing phagocytosis of a tumor cell in a subject, the method comprising administering to the subject an effective amount of a

5 pharmaceutical composition a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody conjugate disclosed herein, in an amount effective to remove sialic acid from the tumor cell, thereby to increase phagocytosis of the tumor cell.

[00254] In certain embodiments, phagocytosis is increased by at least about 10%, at least about 20%, at least about 50%, at least about 75%, at least about 100%, at least about 150%, at least
10 about 200%, at least about 250%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, or at least about 1,000%, relative to a similar or otherwise identical tumor cell or population of tumor cells that has not or have not been contacted with the fusion protein and/or antibody conjugate.

Phagocytosis may be measured by any suitable method known in the art.

15 [00255] The invention also provides a method of activating a dendritic cell (DC). The method comprises contacting the DC with a tumor cell that has been treated with a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody conjugate disclosed herein. In certain embodiments, the disclosure relates to a method of activating a dendritic cell (DC) or a population of DCs in a subject, the method comprising administering to the subject an amount of
20 a pharmaceutical composition comprising a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody conjugate disclosed herein, effective to remove sialic acid from a tumor cell in the subject, thereby to activate the DC or the population of DCs in the subject.

[00256] In certain embodiments, activation of the DC or a population of DCs is increased by at least about 10%, at least about 20%, at least about 50%, at least about 75%, at least about 100%,
25 at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, or at least about 1,000%, relative to a similar or otherwise identical DC or population of DCs that has not or have not been contacted with a tumor cell that has been treated with the fusion protein and/or antibody conjugate. Activation may be measured by any suitable
30 method known in the art.

[00257] The invention also provides a method of reducing Siglec-15 binding activity, thereby to increase anti-tumor activity in a tumor microenvironment, the method comprising contacting a T cell with a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody

conjugate disclosed herein. In certain embodiments, the disclosure relates to a method of reducing Siglec-15 binding activity, thereby to increase anti-tumor activity in a tumor microenvironment of a patient, the method comprising administering to the subject an effective amount of a pharmaceutical composition comprising a fusion protein and/or antibody conjugate, *e.g.*, a fusion protein or antibody conjugate disclosed herein, thereby to increase anti-tumor activity (*e.g.*, T cell activity) in the subject.

[00258] In certain embodiments, Siglec-15 binding activity is reduced by at least about 10%, at least about 20%, at least about 50%, at least about 75%, or about 100%, relative to Siglec-15 that has not or have not been contacted with the fusion protein and/or antibody conjugate.

10 Binding may be measured by any suitable method known in the art.

[00259] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

[00260] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

[00261] Further, it should be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present invention, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present invention and/or in methods of the present invention, unless otherwise understood from the context. In other words, within this application, embodiments have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and invention(s). For example, it will be appreciated that all features described and depicted herein can be applicable to all aspects of the invention(s) described and depicted herein.

[00262] It should be understood that the expression “at least one of” includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression “and/or” in connection with three or more recited objects should be understood to have the same meaning unless otherwise understood from the context.

[00263] The use of the term “include,” “includes,” “including,” “have,” “has,” “having,” “contain,” “contains,” or “containing,” including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

[00264] Where the use of the term “about” is before a quantitative value, the present invention also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term “about” refers to a $\pm 10\%$ variation from the nominal value unless otherwise indicated or inferred.

[00265] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present invention remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[00266] The use of any and all examples, or exemplary language herein, for example, “such as” or “including,” is intended merely to illustrate better the present invention and does not pose a limitation on the scope of the invention unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present invention.

EXAMPLES

[00267] The following Examples are merely illustrative and are not intended to limit the scope or content of the invention in any way.

Example 1

[00268] This example describes the construction of recombinant human sialidases (Neu1, Neu2, and Neu3).

[00269] The human sialidases Neu1, Neu2, Neu3 (isoform 1), and Neu4 (isoform 1) were expressed as secreted proteins with a 10xHis tag. To express Neu1 as a secreted protein, the native N terminal signal peptide (MTGERPSTALPDRRWGPRILGFWGGCRVWVFAAIFLLLSLAASWSKA; SEQ ID NO: 27) was replaced by MDMRVPAQLLGLLLLWLPGARC (SEQ ID NO: 28), and the C terminal

lysosomal signal motif (YGTL; SEQ ID NO: 29) was removed. To express Neu2, Neu3, and Neu4 as secreted proteins, the N terminal signal peptide MDMRVPAQLLGLLLLWLPGARC (SEQ ID NO: 28) was added to each.

[00270] Sialidases were expressed in a 200 mL transfection of HEK293F human cells in 24-well plates using the pCEP4 mammalian expression vector with an N-terminal 6xHis tag. Sialidases were purified using Ni-NTA columns, quantified with a UV-Vis spectrophotometer (NanoDrop), and examined by SDS-PAGE as shown in **FIGURE 1**. Neu1 expressed well, with a yield of ~3 µg/ml, and was present primarily in a monomeric form. Neu2 and Neu3 expression each gave yields of ~0.15 µg/mL and each were present primarily in a dimeric form. Neu4 had no detectable expression yield as measured by NanoDrop. Bacterial sialidase from *Salmonella typhimurium* (St-sialidase; SEQ ID NO: 30), which was used as a positive control for expression, gave a comparable yield to Neu1, and was present primarily in a monomeric form.

[00271] The activity of the recombinantly expressed sialidases was assayed by measuring the release of sialic acid from the fluorogenic substrate 4-methylumbelliferyl-N-acetylneuraminic acid (4MU-NeuAc). As shown in **FIGURE 2**, Neu1 has no detectable activity above a no-enzyme control, which is consistent with previous reports indicating that Neu1 is inactive unless it is in complex with beta-galactosidase and protective protein/cathepsin A (PPCA). Neu2 and Neu3 were active. An enzyme kinetics assay was performed with Neu2 and Neu3. A fixed concentration of enzyme at 1 nM was incubated with fluorogenic substrate 4MU-NeuAc at concentrations ranging from 4000 µM to 7.8 µM. Assays were conducted at both acidic (pH 5.6) and neutral (pH 7) conditions. As shown in **FIGURE 3**, both Neu2 and Neu3 were active at acidic and neutral conditions and showed enzyme kinetics that were comparable to those previously reported.

[00272] Most of the recombinantly expressed sialidases ran as aggregates or dimers on a non-reducing SDS-PAGE gel. Subsequent treatment with the reducing agent dithiothreitol (DTT) resulted in a monomeric form of the enzyme that ran at 42 kDa on a reducing SDS-PAGE gel (**FIGURE 1**).

Example 2

[00273] This example describes the construction of recombinant human sialidases with mutations that increase expression and/or activity of the sialidase.

A. Rational Design

[00274] Structural and sequence analysis identified residues A93 and P62 of Neu2 as candidates for substitutions to increase solubility and/or expression. In particular, a comparison of homologous sialidase sequences showed a preference for D or E amino acid residues at positions corresponding to position 93 of Neu2, and a preference for G amino acid residues at positions corresponding to position 62 of Neu2.

[00275] The beta-propeller family of proteins are usually stabilized by extensive hydrogen bonding interactions at the N- and C-termini of the protein. A structural analysis revealed that Neu2, which is a member of the beta-propeller family, appears to lack these stabilizing interactions. In contrast, sialidases from *Salmonella typhimurium* and *Micromonospora viridifaciens* (the bacterial sialidase most homologous to human Neu2) have extensive hydrogen bonding interactions at their N- and C-termini. Accordingly, residues K9, V363, and L365 of Neu2 were mutated to promote hydrogen bonding between the N- and C-termini of Neu2.

B. Phage Display

[00276] Neu2 was expressed in a phage display system allowing for screening of Neu2 variants for both expression level and resistance to heat denaturation. Neu2 with V6Y and I187K substitutions was used as a template for library preparation. Designed phage display libraries 1, 2, and 3 are depicted in TABLES 10-12, respectively. Each library included all of the possible combinations of the mutations depicted. A fourth library included random mutations generated by error prone PCR.

TABLE 10 - Phage Library 1

Wild-Type Neu2 Amino Acid(s)	Design	Substituting Amino Acids	Codon Usage
184-188 LHPIQ	Adjust length of 5 wild-type residues from 2 to 5 and substitute each residue with one of 9 polar amino acids	S, N, R, K, T, G, D, E, A	RVM
P190, I191	Substitute with one of 5 non-polar amino acids	F, I, L, M, V	NTK
C219	Substitute with one of 12 polar or nonpolar amino acids	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
L217	Substitute with one of 12 polar or nonpolar amino acids	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
T216 and L217	Insert one of 12 polar or nonpolar amino acids between the wild-type amino acids	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
G271	Substitute with one of 9 polar amino acids	S, N, R, K, T, G, D, E, A	RVM

Wild-Type Neu2 Amino Acid(s)	Design	Substituting Amino Acids	Codon Usage
C272	Substitute with one of 9 polar amino acids	S, N, R, K, T, G, D, E, A	RVM

TABLE 11 – Phage Library 2

Wild-Type Neu2 Amino Acid(s)	Design	Substituting Amino Acids	Codon Usage
156-165 TFAVGPGHCL	Substitute with one of 12 hydrophobic or polar amino acids	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
V176	Substitute with one of 12 hydrophobic or polar amino acids	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
P177	Substitute with one of 12 hydrophobic or polar amino acids	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
A178	Substitute with one of 12 hydrophobic or polar amino acids	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
A194	Substitute with one of 12 hydrophobic or polar amino acids	S, T, Y, L, F, A, P, V, I, N, D, H	NHT

TABLE 12 – Phage Library 3

Wild-Type Neu2 Amino Acid(s)	Design	Substituting Amino Acids	Codon Usage
F13	Substitute with one of 9 polar amino acids chain AAs	S, N, R, K, T, G, D, E, A	RMV
L4	Substitute with one of 12 hydrophobic or polar amino acids	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
L7, V12	Substitute with one of 6 polar or aromatic amino acids	F, Y, S, I, T, N	WHT
I22, A24	Substitute with one of 9 polar amino acids	S, N, R, K, T, G, D, E, A	RMV
V325, L326, and L327	Substitute with one of 6 polar or aromatic amino acids	F, Y, S, I, T, N	WHT
L365	Substitute with one of 6 polar or aromatic amino acids	F, Y, S, I, T, N	WHT
P89	Substitute with one of 5 non-polar amino acids	F, I, L, M, V	NTK
L34, A36, and V363	Substitute with one of 12 hydrophobic or polar amino acids	S, T, Y, L, F, A, P, V, I, N, D, H	NHT

[00277] The codon usage columns in **TABLES 10-12** represent degenerate codon codes used in the design of the library, where the first, second, and third positions of a given codon encoding an amino acid are as shown in **TABLE 13** and as described in Mena *et al.* (2005) *PROTEIN ENG DES SEL.* 18(12):559-61.

TABLE 13

Degenerate Codon Code	Mixed bases
R	A, G
Y	C, T/U
M	A, C
K	G, T/U
S	G, C
W	A, T
H	A, C, T/U
B	G, C, T (or U)
V	A, C, G
D	A, G, T/U
N	A, C, G, T/U

[00278] The phage display libraries were screened for binding to a conformation-specific antibody and/or a sialic acid biotinylated probe after heating to enrich for thermal stability and expression. The sialic acid biotinylated probe and its synthesis is depicted in **FIGURE 4**. An exemplary phage display screening procedure is depicted in **FIGURE 5**. Briefly, phage libraries expressing the desired Neu2 variants were generated. Phage were screened for binding to immobilized anti-Neu2 antibody and/or sialic acid biotinylated probe. Following washing to remove unbound phage, bound phage were eluted from the antibody or probe and analyzed as appropriate.

C. Yeast display

[00279] Neu2 was also expressed in a yeast display system allowing for screening of Neu2 variants for both expression level and resistance to heat denaturation. Neu2 with V6Y and I187K substitutions was used as a template for library preparation. Designed yeast display libraries 1a, 1b, 1c, 1d, 2a, 2b, 2c, 3a, 3b, and 3c are depicted in **TABLES 14-23**, respectively. Each library included all of the possible combinations of the mutations depicted. Five additional sublibraries were generated by error prone PCR, at an approximate average rate of 1, 2, 3, 4, and 5 substitutions per enzyme.

TABLE 14 -Yeast Library 1a

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
L184	All	NNK
H185	All	NNK
P186	None (Wild-type)	
K187	None (Wild-type)	
Q188	None (Wild-type)	
P190	A, D, G, H, I, L, N, P, R, S, T, V	VNT
I191	A, D, F, H, I, L, N, P, S, T, V, Y	NHT
C219	None (Wild-type)	
G271	All	NNK
C272	All	NNK

Total Diversity: 2.30E+07

TABLE 15 - Yeast Library 1b

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
L184	A, D, F, H, I, L, N, P, S, T, V, Y	NHT
H185	All	NNK
P186	A, D, F, H, I, L, N, P, S, T, V, Y	NHT
K187	None (Wild-type)	
Q188	None (Wild-type)	
P190	A, D, G, H, I, L, N, P, R, S, T, V	VNT
I191	A, D, F, H, I, L, N, P, S, T, V, Y	NHT
C219	None (Wild-type)	
G271	A, D, E, G, K, N, R, S, T	RVM
C272	A, C, D, G, H, N, P, R, S, T	NVT

Total Diversity: 4.11E+07

TABLE 16 -Yeast Library 1c

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
L184	A, D, F, H, I, L, N, P, S, T, V, Y	NHT
H185	A, D, E, G, K, N, R, S, T	RVM
P186	A, D, F, H, I, L, N, P, S, T, V, Y	NHT
K187	A, D, E, G, K, N, R, S, T	RVM
Q188	None (Wild-type)	
P190	F, L, I, V	NTT
I191	A, D, F, H, I, L, N, P, S, T, V, Y	NHT
C219	None (Wild-type)	
G271	A, D, E, G, K, N, R, S, T	RVM
C272	A, D, F, H, L, P, S, V, Y	BHT

Total Diversity: 4.53E+07

TABLE 17 -Yeast Library 1d

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
L184	A, D, F, H, L, P, S, V, Y	BHT
H185	A, D, E, G, K, N, R, S, T	RVM
P186	A, D, F, H, L, P, S, V, Y	BHT
K187	A, D, E, G, K, N, R, S, T	RVM
Q188	A, D, F, H, L, P, S, V, Y	BHT
P190	None (Wild-type)	
I191	A, D, E, G, K, N, R, S, T	RVM
C219	None (Wild-type)	
G271	A, D, E, G, K, N, R, S, T	RVM
C272	A, D, F, H, L, P, S, V, Y	BHT

Total Diversity: 4.30E+07

TABLE 18 -Yeast Library 2a

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
T156	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
F157	None (Wild-type)	
A158	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
V159	None (Wild-type)	
G160	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
P161	None (Wild-type)	
G162	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
H163	None (Wild-type)	
C164	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
L165	None (Wild-type)	
V176	L, V, P, A, H, D	SHT
P177	L, V, P, A, H, D	SHT
A178	L, V, P, A, H, D	SHT
A194	L, V, P, A, H, D	SHT

Total Diversity: 3.22E+08

TABLE 19 -Yeast Library 2b

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
T156	None (Wild-type)	
F157	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
A158	None (Wild-type)	
V159	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
G160	None (Wild-type)	
P161	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
G162	None (Wild-type)	
H163	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
C164	None (Wild-type)	
L165	R, N, D, C, G, H, I, L, F, S, Y, V	NDT
V176	L, V, P, A, H, D	SHT

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
P177	L, V, P, A, H, D	SHT
A178	L, V, P, A, H, D	SHT
A194	L, V, P, A, H, D	SHT

Total Diversity: 3.22E+08

TABLE 20 -Yeast Library 2c

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
T156	A, D, G, H, N, P, R, S, T	VVC
F157	A, D, F, H, L, P, S, V, Y	BHT
A158	A, D, G, H, N, P, R, S, T	VVC
V159	A, D, F, H, L, P, S, V, Y	BHT
G160	A, D, G, H, N, P, R, S, T	VVC
P161	A, D, F, H, L, P, S, V, Y	BHT
G162	A, D, G, H, N, P, R, S, T	VVC
H163	A, D, F, H, L, P, S, V, Y	BHT
C164	A, D, G, H, N, P, R, S, T	VVC
L165	A, D, F, H, L, P, S, V, Y	BHT
V176	None (Wild-type)	
P177	None (Wild-type)	
A178	None (Wild-type)	
A194	None (Wild-type)	

Total Diversity: 3.49E+09

5

TABLE 21 -Yeast Library 3a

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
L4	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
L7	F, Y, S, I, T, N	WHT
V12	None (Wild-type)	
F13	None (Wild-type)	
I22	S, N, R, K, T, G, D, E, A	RMV
A24	S, N, R, K, T, G, D, E, A	RMV
L34	None (Wild-type)	
A36	None (Wild-type)	
H64	F, Y, S, I, T, N	WHT
P89	F, I, L, V	NTT
C164	None (Wild-type)	
V325	F, Y, S, I, T, N	WHT
L326	F, Y, S, I, T, N	WHT
L327	F, Y, S, I, T, N	WHT
C332	None (Wild-type)	
V363	S, T, Y, L, F, A, P, V, I, N, D, H	NHT

Total Diversity: 3.63E+08

TABLE 22 -Yeast Library 3b

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
L4	None (Wild-type)	
L7	F, Y, S, I, T, N	WHT
V12	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
F13	S, N, R, K, T, G, D, E, A	RMV
I22	S, N, R, K, T, G, D, E, A	RMV
A24	S, N, R, K, T, G, D, E, A	RMV
L34	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
A36	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
H64	None (Wild-type)	
P89	None (Wild-type)	
C164	A, G, S, T	RST
V325	None (Wild-type)	
L326	None (Wild-type)	
L327	None (Wild-type)	
C332	A, D, G, H, N, P, R, S, T	VVC
V363	None (Wild-type)	

Total Diversity: 2.72E+08

TABLE 23 -Yeast Library 3c

Wild-Type Neu2 Amino Acid(s)	Substituting Amino Acids	Codon Usage
L4	None (Wild-type)	
L7	None (Wild-type)	
V12	None (Wild-type)	
F13	None (Wild-type)	
I22	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
A24	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
L34	None (Wild-type)	
A36	None (Wild-type)	
H64	F, Y, S, I, T, N	WHT
P89	S, T, Y, L, F, A, P, V, I, N, D, H	NHT
C164	A, G, S, T	RST
V325	A, D, F, H, L, P, S, V, Y	BHT
L326	A, D, F, H, L, P, S, V, Y	BHT
L327	A, D, F, H, L, P, S, V, Y	BHT
C332	A, D, G, H, N, P, R, S, T	VVC
V363	None (Wild-type)	

Total Diversity: 2.72E+08

5

[00280] The codon usage columns in **TABLES 14-23** represent degenerate codon codes used in the design of the library, where the first, second, and third positions of a given codon encoding an amino acid are as shown herein above in **TABLE 13** and as described in Mena *et al.* (2005)

10 PROTEIN ENG DES SEL. 18(12):559-61.

[00281] The yeast display libraries were screened for binding to a conformation-specific antibody and/or a sialic acid biotinylated probe after heating to enrich for thermal stability and expression. An exemplary yeast display screening procedure is depicted in **FIGURE 6**. Briefly, a plasmid library encoding for the desired Neu2 variants, and yeast cells expressing the desired Neu2 variants on the surface, were generated. Yeast cells were heat shocked and then screened for binding to anti-Neu2 antibody and/or sialic acid biotinylated probe on magnetic beads. The magnetic beads were isolated to remove unbound cells, and bound cells were further analyzed for Neu2 affinity, activity, and stability as appropriate.

D. Results

[00282] Mutant sialidases including mutations identified using the rational design, phage display, and yeast display approaches described in this Example were expressed as secreted proteins with a C-terminal human Fc tag in Expi293F cells using the pCEP4 mammalian expression vector. Expression was assayed using a ForteBio Octet with anti-human Fc sensors and Western blot and enzymatic activity was assayed using the fluorogenic substrate 4MU-NeuAc as described above.

[00283] Expression and activity levels for the mutant sialidases are shown in **TABLE 24**. In **TABLE 24**, enzymatic activity is indicated as “+++,” which denotes activity >2 fold higher than wild-type Neu2, “++,” which denotes activity comparable to wild-type Neu2, “+,” which denotes activity lower than wild-type Neu2, or “-,” which denotes no detectable activity, and expression is indicated as “++++,” which denotes expression > 15 fold higher than wildtype-Neu2, “+++,” which denotes expression > 6 fold higher than wild-type Neu2, “++,” which denotes expression 2-5 fold higher than wild-type Neu2, “+,” which denotes expression comparable to wild-type Neu2, or “-,” which denotes no detectable expression.

TABLE 24

Identifier	Mutation(s)	Activity	Expression
Neu2-M104	M1D, V6Y, P62G, I187K, C332A	+	++++
Neu2-M105	M1D, V6Y, K9D, I187K, C332A, V363R, L365I	+	+++
Neu2-M106	M1D, V6Y, P62G, A93E, I187K, C332A	++	++++
Neu2-M107	M1D, V6Y, K9D, I187K, C332A, V363R, L365K	+	++++
Neu2-M108	M1D, V6Y, K9D, I187K, C332A, V363R, L365S	+	++++
Neu2-M109	M1D, V6Y, K9D, I187K, C332A, V363R, L365Q	+	++++

Identifier	Mutation(s)	Activity	Expression
Neu2-M110	M1D, V6Y, K9D, I187K, C332A, V363R, L365H	+	+++
Neu2-M111	M1D, V6Y, A93K, I187K, C332A	++	++
Neu2-M112	M1D, V6Y, A93E, I187K, C332A	++	++
Neu2-M113	V6Y, I22N, C125Y, I187K, S301C, E319D	-	+++
Neu2-M114	V6Y, P62T, C125F, I187K, A222D	-	+++
Neu2-M115	V6Y, I187K, W292R	+	+++
Neu2-M116	V6Y, G107D, I187K	+	+++
Neu2-M117	C125L	+	+
Neu2-M118	V6Y, C125L	+	++
Neu2-M119	C125L, I187K	++	++
Neu2-M120	V6Y, C125L, I187K	+	+++
Neu2-M121	M1D, V6Y, K45A, I187K, C332A	++	++
Neu2-M122	M1D, V6Y, Q270A, I187K, C332A	++	+++
Neu2-M123	M1D, V6Y, K44R, K45R, I187K, C332A	+	++
Neu2-M124	M1D, V6Y, Q112R, I187K, C332A	+	++
Neu2-M125	M1D, V6Y, Q270F, I187K, C332A	+	++
Neu2-M126	M1D, V6Y, I187K, S301R, W302K, C332A	++	+++
Neu2-M127	M1D, V6Y, K44E, K45E, I187K, C332A	++	+
Neu2-M128	M1D, V6Y, I187K, L217V, C332A	+	++
Neu2-M129	M1D, V6Y, I187K, L217A, C332A	+	++
Neu2-M130	M1D, V6Y, I187K, C332A, Y359A	-	+++
Neu2-M131	M1D, V6Y, I187K, C332A, Y359S	-	+++
Neu2-M132	M1D, V6Y, K44E, K45E, I187K, S301R, W302K, C332A	++	++
Neu2-M133	M1D, V6Y, Q112R, I187K, S301R, W302K, C332A	++	+++
Neu2-M134	M1D, V6Y, I187K, Q270A, S301R, W302K, C332A	++	+++
Neu2-M135	M1D, V6Y, K44E, K45E, Q112R, I187K, C332A	+	+
Neu2-M136	M1D, V6Y, K44E, K45E, I187K, Q270A, C332A	++	++
Neu2-M137	M1D, V6Y, K45A, I187K, Q270A, C332A	++	+++
Neu2-M138	M1D, V6Y, I187K, Q270H, C332A	++	+++
Neu2-M139	M1D, V6Y, I187K, Q270P, C332A	+	+++
Neu2-M140	M1D, V6Y, Q112K, I187K, C332A	++	++
Neu2-M141	M1D, V6Y, P62S, I187K, Q270A, S301R, W302K, C332A	+	+++
Neu2-M142	M1D, V6Y, P62T, I187K, Q270A, S301R, W302K, C332A	+	+++

Identifier	Mutation(s)	Activity	Expression
Neu2-M143	M1D, V6Y, P62N, I187K, Q270A, S301R, W302K, C332A	+	+++
Neu2-M144	V6Y, P62H, I187K	+	+++
Neu2-M145	V6Y, Q108H, I187K	+	++
Neu2-M146	M1D, V6Y, P62H, I187K, C332A	++	+++
Neu2-M147	M1D, V6Y, P62G, I187K, C332A	++	++
Neu2-M148	V6Y, P62G, I187K	+	++
Neu2-M149	M1D, V6Y, P62H, I187K	++	++
Neu2-M150	M1D, V6Y, Q108H, I187K	++	++
Neu2-M151	M1D, V6Y, P62F, I187K, C332A	+	-
Neu2-M152	M1D, V6Y, P62I, I187K, C332A	+	-
Neu2-M153	M1D, V6Y, P62N, I187K, C332A	++	+++
Neu2-M154	M1D, V6Y, P62D, I187K, C332A	++	+
Neu2-M155	M1D, V6Y, P62E, I187K, C332A	++	+++
Neu2-M156	V6Y, C164G, I187K, T249A	+	+
Neu2-M157	V6Y, C164G, I187K	+	+
Neu2-M158	V6Y, Q126L, I187K D251G	+	++
Neu2-M159	V6Y, L54M, Q69H, R78K, A171G, I187K	+	+
Neu2-M160	V6Y, P62T, I187K	+	++
Neu2-M161	V6Y, A150V, I187K	+	++
Neu2-M162	P5H, V6Y, P62S, I187K	+	++
Neu2-M163	V6Y, C164G, I187K	+	+

[00284] To confirm these results, Neu2-M106 (with amino acid sequence SEQ ID NO: 48, encoded by nucleotide sequence SEQ ID NO: 80) was expressed and purified with a protein A column. **FIGURE 7A** is an image of an SDS-PAGE gel showing recombinant wildtype human Neu2 and Neu2 variant M106 (each with a C-terminal human Fc tag) under non-reducing and reducing conditions. **FIGURE 7B** is an SEC-HPLC trace for recombinant wildtype human Neu2 and Neu2 variant M106 (each with a C-terminal human Fc tag). While Neu2-Fc had a yield of 0.3 mg/liter following protein-A purification, and monomer content of 7% as determined by SEC, Neu2-M106 had a yield of 20 mg/liter, and a monomer content of 85%.

[00285] The enzyme kinetics of Neu2-M106 were assayed by measuring the release of sialic acid from the fluorogenic substrate 4-methylumbelliferyl-N-acetylneuraminic acid (4MU-NeuAc) as described above. A fixed concentration of enzyme at 2 µg/well was incubated with fluorogenic substrate 4MU-NeuAc at concentrations ranging from 4mM to 0.03µM. **FIGURE 8** depicts the enzyme activity of Neu2 variant M106. Enzymatic activity of Neu2-M106 was comparable to wildtype Neu2, with a K_M determined to be 230µM.

[00286] Together, these results show that the mutations identified by rational design, phage display, and/or yeast display approaches described herein can increase stability and/or expression of a sialidase.

Example 3

5 [00287] This example describes the construction of recombinant human sialidases with mutations that increase expression and/or activity of the sialidase.

[00288] Unless indicated otherwise, mutant sialidases in this Example were expressed as secreted proteins with a C-terminal human Fc tag in Expi293F cells using the pCEP4 mammalian expression vector. Expression was assayed using a ForteBio Octet with anti-human
10 Fc sensors and Western blot and enzymatic activity was assayed using the fluorogenic substrate 4MU-NeuAc as described above.

[00289] Mutant Neu2 sialidases were constructed including rationally designed substitutions at position Q126. Inspection of the Neu2 crystal structure revealed that mutation of Q126 may increase interactions with neighboring amino acid residues.

15 [00290] Additional mutant Neu2 sialidases were constructed including rationally designed substitution at position Q270. Inspection of the Neu2 crystal structure revealed that mutation of Q270 to certain amino acids may stabilize interactions with R237 and stabilize binding in the substrate pocket.

[00291] Additional mutant Neu2 sialidases were constructed including a substitution of an
20 amino acid residue in a beta turn with a proline (for example D80P, R189P, and/or H239P substitutions). Substitution with a proline at these positions may, for example, stabilize the protein by influencing local protein folding.

[00292] Expression and activity levels for the resulting mutant sialidases are shown in **TABLE 25**. In **TABLE 25**, enzymatic activity is indicated as “++,” which denotes activity comparable
25 to wild-type Neu2, “+,” which denotes activity lower than wild-type Neu2, or “-,” which denotes no detectable activity, and expression is indicated as “+++++”, which denotes expression > 40 fold higher than wildtype-Neu2, “++++”, which denotes expression > 15 fold higher than wildtype-Neu2, “+++,” which denotes expression > 6 fold higher than wild-type Neu2, “++,” which denotes expression 2-5 fold higher than wild-type Neu2, “+,” which denotes expression
30 comparable to wild-type Neu2, or “-,” which denotes no detectable expression.

TABLE 25

Identifier	Mutation(s)	Activity	Expression
Neu2-M164	M1D, V6Y, P62G, A93E, Q126E, I187K, C332A	++	+++++
Neu2-M165	M1D, V6Y, P62G, A93E, Q126I, I187K, C332A	++	+++++
Neu2-M166	M1D, V6Y, P62G, A93E, Q126L, I187K, C332A	+	+++++
Neu2-M167	M1D, V6Y, P62G, A93E, Q126Y, I187K, C332A	+	+++++
Neu2-M168	M1D, V6Y, P62G, A93E, Q126F, I187K, C332A	+	+++++
Neu2-M169	M1D, V6Y, P62G, A93E, Q126H, I187K, C332A	++	++++
Neu2-M170	M1D, V6Y, P62G, A93E, I187K, Q270S, C332A	++	++++
Neu2-M171	M1D, V6Y, P62G, A93E, I187K, Q270T, C332A	++	++++
Neu2-M172	M1D, V6Y, P62G, A93E, Q126Y, I187K, Q270T, C332A	++	+++++
Neu2-M173	M1D, V6Y, P62G, A93E, Q126Y, I187K, A242F, Q270T, C332A	++	+++++
Neu2-M174	M1D, V6Y, P62G, D80P, A93E, I187K, C332A	++	++++
Neu2-M175	M1D, V6Y, P62G, A93E, R170P, I187K, C332A	++	+++
Neu2-M176	M1D, V6Y, P62G, A93E, I187K, Q188P, C332A	++	++++
Neu2-M177	M1D, V6Y, P62G, A93E, I187K, R189P, C332A	++	++++
Neu2-M178	M1D, V6Y, P62G, A93E, I187K, E225P, C332A	+	++++
Neu2-M179	M1D, V6Y, P62G, A93E, I187K, H239P, C332A	++	++++
Neu2-M180	M1D, V6Y, P62G, A93E, I187K, E257P, C332A	++	+++

[00293] To confirm these results, Neu2-M173 (with amino acid sequence SEQ ID NO: 100, encoded by nucleotide sequence SEQ ID NO: 118) was expressed with a C-terminal human Fc tag and purified with a protein A and a ceramic hydroxyapatite (CHT) column. **FIGURE 9A** is an image of an SDS-PAGE gel showing Neu2-M173-Fc (with a C-terminal human Fc tag) under non-reducing and reducing conditions. **FIGURE 9B** is an SEC-HPLC trace for Neu2-M173-Fc (with a C-terminal human Fc tag). Neu2-M173-Fc had a yield of 120 mg/liter, and a monomer content of 90%.

[00294] The enzyme kinetics of Neu2-M173-Fc were assayed by measuring the release of sialic acid from the fluorogenic substrate 4-methylumbelliferyl-N-acetylneuraminic acid (4MU-NeuAc) as described above. A fixed concentration of enzyme at 2 μ g/well was incubated with fluorogenic substrate 4MU-NeuAc at concentrations ranging from 4mM to 0.03 μ M. **FIGURE 10** depicts the enzyme activity of Neu2-M173-Fc. Enzymatic activity of Neu2-M173-Fc was comparable to wildtype Neu2, with a K_M determined to be 230 μ M.

[00295] Additional mutant Neu2 sialidases were constructed including rationally designed substitutions at positions S301 and/or W302. Mutations of S301 and/or W302 may influence interactions with neighboring amino acid residues and/or substrate.

[00296] Expression and activity levels for the mutant sialidases are shown in **TABLE 26**. In **TABLE 26**, enzymatic activity is indicated as “++,” which denotes activity comparable to wild-type Neu2, “+,” which denotes activity lower than wild-type Neu2, or “-,” which denotes no detectable activity, and expression is indicated as “+++++,” which denotes expression > 40 fold higher than wildtype-Neu2, “++++,” which denotes expression > 15 fold higher than wildtype-Neu2, “+++,” which denotes expression > 6 fold higher than wild-type Neu2, “++,” which denotes expression 2-5 fold higher than wild-type Neu2, “+,” which denotes expression comparable to wild-type Neu2, or “-,” which denotes no detectable expression.

TABLE 26

Identifier	Mutation(s)	Activity	Expression
Neu2-M182	M1D, V6Y, P62G, A93E, I187K, S301A, C332A	++	++++
Neu2-M183	M1D, V6Y, P62G, A93E, I187K, S301D, C332A	++	++++
Neu2-M184	M1D, V6Y, P62G, A93E, I187K, S301E, C332A	++	+++
Neu2-M185	M1D, V6Y, P62G, A93E, I187K, S301F, C332A	+	++++
Neu2-M186	M1D, V6Y, P62G, A93E, I187K, S301H, C332A	+	++++
Neu2-M187	M1D, V6Y, P62G, A93E, I187K, S301K, C332A	++	+++++
Neu2-M188	M1D, V6Y, P62G, A93E, I187K, S301L, C332A	++	++++
Neu2-M189	M1D, V6Y, P62G, A93E, I187K, S301M, C332A	++	++++
Neu2-M190	M1D, V6Y, P62G, A93E, I187K, S301N, C332A	++	++++
Neu2-M191	M1D, V6Y, P62G, A93E, I187K, S301P, C332A	++	++++

Identifier	Mutation(s)	Activity	Expression
Neu2-M192	M1D, V6Y, P62G, A93E, I187K, S301Q, C332A	++	+++
Neu2-M193	M1D, V6Y, P62G, A93E, I187K, S301R, C332A	++	+++++
Neu2-M194	M1D, V6Y, P62G, A93E, I187K, S301T, C332A	++	++++
Neu2-M195	M1D, V6Y, P62G, A93E, I187K, S301V, C332A	++	++++
Neu2-M196	M1D, V6Y, P62G, A93E, I187K, S301W, C332A	+	++++
Neu2-M197	M1D, V6Y, P62G, A93E, I187K, S301Y, C332A	++	++++
Neu2-M198	M1D, V6Y, P62G, A93E, I187K, W302A, C332A	+	++++
Neu2-M199	M1D, V6Y, P62G, A93E, I187K, W302D, C332A	+++	+++
Neu2-M200	M1D, V6Y, P62G, A93E, I187K, W302F, C332A	+	++++
Neu2-M201	M1D, V6Y, P62G, A93E, I187K, W302G, C332A	++	++++
Neu2-M202	M1D, V6Y, P62G, A93E, I187K, W302H, C332A	+	++++
Neu2-M203	M1D, V6Y, P62G, A93E, I187K, W302I, C332A	+	++++
Neu2-M204	M1D, V6Y, P62G, A93E, I187K, W302L, C332A	++	++++
Neu2-M205	M1D, V6Y, P62G, A93E, I187K, W302M, C332A	+	++
Neu2-M206	M1D, V6Y, P62G, A93E, I187K, W302N, C332A	+	++++
Neu2-M207	M1D, V6Y, P62G, A93E, I187K, W302P, C332A	-	++++
Neu2-M208	M1D, V6Y, P62G, A93E, I187K, W302Q, C332A	++	++++
Neu2-M209	M1D, V6Y, P62G, A93E, I187K, W302R, C332A	++	+++++
Neu2-M210	M1D, V6Y, P62G, A93E, I187K, W302S, C332A	++	++++
Neu2-M211	M1D, V6Y, P62G, A93E, I187K, W302T, C332A	++	++++
Neu2-M212	M1D, V6Y, P62G, A93E, I187K, W302V, C332A	+	++++
Neu2-M213	M1D, V6Y, P62G, A93E, I187K, W302Y, C332A	+	++++
Neu2-M214	M1D, V6Y, P62G, A93E, I187K, S301A, W302A, C332A	++	++++
Neu2-M215	M1D, V6Y, P62G, A93E, I187K, S301A, W302R, C332A	+	++++

Identifier	Mutation(s)	Activity	Expression
Neu2-M216	M1D, V6Y, P62G, A93E, I187K, S301A, W302S, C332A	++	++++
Neu2-M217	M1D, V6Y, P62G, A93E, I187K, S301A, W302T, C332A	++	++++
Neu2-M218	M1D, V6Y, P62G, A93E, I187K, S301K, W302S, C332A	+	++++
Neu2-M219	M1D, V6Y, P62G, A93E, I187K, S301K, W302R, C332A	++	+++++
Neu2-M219	M1D, V6Y, P62G, A93E, I187K, S301K, W302T, C332A	++	++++
Neu2-M220	M1D, V6Y, P62G, A93E, I187K, S301N, W302S, C332A	+	++++
Neu2-M221	M1D, V6Y, P62G, A93E, I187K, S301N, W302T, C332A	++	++++
Neu2-M222	M1D, V6Y, P62G, A93E, I187K, S301T, W302R, C332A	+	++++

Example 4

[00297] This example describes the construction of recombinant human sialidases with mutations that reduce proteolytic cleavage.

5 **[00298]** Neu2-M106 (as described in Example 2, and with amino acid sequence SEQ ID NO: 48) was expressed as an Fc-fused single chain protein using a CHO cell expression system in a large scale (10 L) high cell density production and purified with a protein A column. The resulting protein was analyzed by SDS-PAGE. Results are shown in **FIGURE 11**. Under reducing conditions, the protein included a mixture of full length (70 kDa, approx. 50%) and
10 cleaved (40 kDa and 30 kDa, approx. 50%) fractions. However, in non-reducing conditions, there was no cleavage and the protein remained as a single chain (**FIGURE 11**). Additionally, when Neu2-M106 was expressed on a smaller scale (with a shorter duration of cell culture and lower cell density) there was no cleavage and the protein remained as a single chain. A preliminary mass spectrometry analysis showed that the 40 kDa and 30 kDa molecular weight
15 fractions observed under reducing conditions following large scale production are a result of cleavage between amino acid residues R243 and V244 of the sialidase. The enzymatic activity of cleaved Neu2-M106 was similar to that of uncleaved Neu2-M106.

[00299] It was hypothesized that the cleavage of Neu2-M106 could be due to the activity of intracellular proteases released as a result of cell lysis during protein production, harvesting, and/or purification. To test this hypothesis, cleaved Neu2-M106 (prepared using the large scale-
20 production described above that results in cleavage) and uncleaved Neu2-M106 (prepared using the smaller scale production described above that does not result in cleavage) were both

incubated with trypsin and analyzed by SDS-PAGE under reducing conditions (**FIGURE 12**). Briefly, trypsin digestion reactions were performed by incubation of trypsin (5 μ L, 0.005% solution in PBS) with Neu2-M106 (25 μ L, 0.25 mg/mL in PBS pH 8.0) for 5 minutes on ice. Reactions were stopped by addition of LDS gel loading buffer (5 μ L) and run on a reducing SDS-PAGE gel to observe trypsin mediated cleavage. The SDS-PAGE analysis showed that incubation of the uncleaved Neu2-M106 with trypsin resulted in the same cleavage pattern as that of the cleaved Neu2-M106. Additionally, incubation of the cleaved Neu2-M106 with trypsin resulted in increased intensity of the bands corresponding to the cleavage products.

[00300] Neu2-M106 was also incubated with trypsin in the presence of various protease inhibitors. Briefly, trypsin digestion reactions were performed by incubation of trypsin (0.005%) with Neu2-M106 (0.5 mg/mL) and protease inhibitor for 5 minutes on ice. Reactions were stopped by addition of LDS gel loading buffer and run on a reducing SDS-PAGE gel to observe trypsin mediated cleavage. Inhibitors used included iron citrate (at 0.3 and 5 mM), aprotinin (at 5,000 and 20,000 U/mL), AEBSF (at 0.1 and 1 mM), leupeptin (at 1 and 10 μ M) or E-64 (at 1 and 10 μ M). As seen in **FIGURE 13**, protease inhibitors reduced the extent of trypsin cleavage.

[00301] Together, these results confirm that cleavage of Neu2-M106 following large-scale production is due to trypsin or a member of a similar class of proteases.

[00302] We next attempted to rationally design recombinant human sialidases with mutations that increase resistance to trypsin cleavage.

[00303] Unless indicated otherwise, in the remainder of this Example mutant sialidases were expressed as secreted proteins with a C-terminal human Fc tag in Expi293F cells (on a 50 mL scale) using the pCEP4 mammalian expression vector. The resulting protein was purified using a Protein A column. Expression was assayed using a ForteBio Octet with anti-human Fc sensors and Western blot and enzymatic activity was assayed using the fluorogenic substrate 4MU-NeuAc as described above. Protease cleavage was assayed by SDS-PAGE as described above.

[00304] First, R243 was mutated to different polar/charged amino acids such as K, E, H, N and Q. However, these mutations of R243 resulted in complete loss of activity and reduction in expression yields (R243 is also a conserved amino acid among similar sialidases).

[00305] Next, various amino residues surrounding the cleavage site were mutated and tested for expression, activity and trypsin cleavage resistance. Substitutions and combinations of substitutions that were tested are shown in **FIGURE 14**. All mutations were tested in a Neu2-M106 background (i.e., including M1D, V6Y, P62G, A93E, I187K, and C332A substitutions).

[00306] Most of the mutant sialidases depicted in **FIGURE 14** expressed well, however only two of the mutant sialidases (including V244I or A242C mutations) were active. The A242C mutation resulted in greater than 10 fold improved trypsin resistance and slightly lower activity (both relative to Neu2-M106). However, having an unpaired cysteine could be a potential liability, so, A242 was mutated to all 19 other amino acids and assayed for activity and trypsin resistance. As shown in **FIGURE 15**, mutation of A242 to aromatic amino acids such as F, W and Y resulted in a dramatic improvement in trypsin cleavage resistance compared to Neu2-M106 (**FIGURE 15A**) and similar enzymatic activity to Neu2-M106 (**FIGURE 15B**). SEC analysis showed that proteins containing each of these mutations had a similar pattern to that of Neu2-M106 and more than 95% monomer content (**FIGURE 15C**).

[00307] Structural analysis showed that replacing A242 with an aromatic amino acid could provide additional hydrophobic or stacking interactions to L260 and V265 (nonpolar amino acids located in the vicinity of the A242). Therefore, L260 and V265 were also mutated to phenylalanine. Along with these mutations several other rationally designed mutants, which could provide extra stability, for example by increasing stacking interactions, were also tested for expression, activity, and protease resistance.

[00308] Select results are shown in **FIGURE 16**. As shown in **FIGURE 16**, the combination of R241Y and A242F mutations (Neu2-M255) resulted in the most resistance to trypsin cleavage (a greater than 10 fold improved trypsin resistance relative to Neu2-M106).

[00309] Expression, activity, and protease resistance levels for the mutant sialidases are shown in **TABLE 27**. In **TABLE 27**, enzymatic activity is indicated as “++,” which denotes activity comparable to wild-type Neu2, “+,” which denotes activity lower than wild-type Neu2, or “-,” which denotes no detectable activity, and expression is indicated as “+++++”, which denotes expression > 40 fold higher than wildtype-Neu2, “++++”, which denotes expression > 15 fold higher than wildtype-Neu2, “+++”, which denotes expression > 6 fold higher than wild-type Neu2, “++,” which denotes expression 2-5 fold higher than wild-type Neu2, “+,” which denotes expression comparable to wild-type Neu2, or “-,” which denotes no detectable expression. Protease/trypsin resistance is indicated as “+++,” which denotes resistance > 10 fold higher than Neu2-M106; “++,” which denotes resistance ≥ 5 fold higher than Neu2-M106, “+,” which denotes resistance comparable to Neu2-M106, or “-,” which denotes resistance lower than Neu2-M106. NT = not tested.

TABLE 27

Identifier	Mutation(s)	Activity	Expression	Protease resistance
Neu2-M223	M1D, V6Y, P62G, A93E, I187K, L240Y, C332A	++	+++++	+
Neu2-M224	M1D, V6Y, P62G, A93E, I187K, A242C, C332A	+	+++	+++
Neu2-M225	M1D, V6Y, P62G, A93E, I187K, A242D, C332A	-	++++	-
Neu2-M226	M1D, V6Y, P62G, A93E, I187K, A242E, C332A	-	+++	-
Neu2-M227	M1D, V6Y, P62G, A93E, I187K, A242F, C332A	++	++++	++
Neu2-M228	M1D, V6Y, P62G, A93E, I187K, A242G, C332A	+	+++	+
Neu2-M229	M1D, V6Y, P62G, A93E, I187K, A242H, C332A	+	+++	+
Neu2-M230	M1D, V6Y, P62G, A93E, I187K, A242I, C332A	+	+++	+
Neu2-M231	M1D, V6Y, P62G, A93E, I187K, A242K, C332A	+	++++	-
Neu2-M232	M1D, V6Y, P62G, A93E, I187K, A242L, C332A	+	+++	+
Neu2-M233	M1D, V6Y, P62G, A93E, I187K, A242M, C332A	+	+++	+
Neu2-M234	M1D, V6Y, P62G, A93E, I187K, A242N, C332A	+	++++	++
Neu2-M235	M1D, V6Y, P62G, A93E, I187K, A242Q, C332A	+	+++	++
Neu2-M236	M1D, V6Y, P62G, A93E, I187K, A242R, C332A	+	++++	-
Neu2-M237	M1D, V6Y, P62G, A93E, I187K, A242S, C332A	+	+++	-
Neu2-M238	M1D, V6Y, P62G, A93E, I187K, A242T, C332A	-	+++	+
Neu2-M239	M1D, V6Y, P62G, A93E, I187K, A242V, C332A	+	+++	+
Neu2-M240	M1D, V6Y, P62G, A93E, I187K, A242W, C332A	++	++++	++
Neu2-M241	M1D, V6Y, P62G, A93E, I187K, A242Y, C332A	++	++++	++
Neu2-M242	M1D, V6Y, P62G, A93E, I187K, A242F, L260F, C332A	++	++++	++
Neu2-M243	M1D, V6Y, P62G, A93E, I187K, A242F, V265F, C332A	+	++++	++
Neu2-M244	M1D, V6Y, P62G, A93E, I187K, A242F, A213C, C332A	+	++++	++
Neu2-M245	M1D, V6Y, P62G, A93E, I187K, A242F, A213S, C332A	+	++++	++
Neu2-M246	M1D, V6Y, P62G, A93E, I187K, A242F, A213T, C332A	++	++++	++

Identifier	Mutation(s)	Activity	Expression	Protease resistance
Neu2-M247	M1D, V6Y, P62G, A93E, I187K, A242F, A213N, C332A	+	++	NT
Neu2-M248	M1D, V6Y, P62G, A93E, I187K, A242F, A213C, S258C, C332A	++	++++	++
Neu2-M249	M1D, V6Y, P62G, A93E, I187K, L240Y, L260F, C332A	++	+++++	+
Neu2-M250	M1D, V6Y, P62G, A93E, I187K, L240D, L260T, C332A	+	+	NT
Neu2-M251	M1D, V6Y, P62G, A93E, I187K, L240N, L260D, C332A	+	++	NT
Neu2-M252	M1D, V6Y, P62G, A93E, I187K, L240N, L260T, C332A	++	++++	+
Neu2-M253	M1D, V6Y, P62G, A93E, I187K, L240N, L260Q, C332A	++	++++	+
Neu2-M254	M1D, V6Y, P62G, A93E, I187K, R241A, A242F, C332A	++	+++	++
Neu2-M255	M1D, V6Y, P62G, A93E, I187K, R241Y, A242F, C332A	++	+++	+++
Neu2-M256	M1D, V6Y, P62G, A93E, Q126Y, I187K, L240Y, A242F, Q270T, C332A	++	+++++	++
Neu2-M257	M1D, V6Y, P62G, A93E, I187K, V244I, C332A	++	++++	+
Neu2-M258	M1D, V6Y, P62G, A93E, I187K, A242C, V244K, C332A	+	+++	++

Example 5

[00310] This example describes the construction of an anti-PD-L1 antibody sialidase conjugate (ASC) based on the Janus format.

- 5 **[00311]** The architecture for four exemplary types of antibody sialidase conjugates (ASCs) is depicted in **FIGURE 19**. The first type of ASC, referred to as “Raptor,” includes an antibody (with two heavy chains and two light chains) with a sialidase fused at the C-terminus of each heavy chain of the antibody (**FIGURE 19A**). The second type of ASC, referred to as “Janus,” contains one antibody arm (with one heavy chain and one light chain), and one sialidase-Fc fusion with a sialidase fused at the N-terminus of one arm of the Fc. Each Fc domain polypeptide in the Janus ASC contains either the “knob” (T366Y) or “hole” (Y407T) mutation for heterodimerization (residue numbers according to EU numbering, Kabat, E.A., *et al.* (1991) *supra*) (**FIGURE 19B**). The third type of ASC, referred to as “Lobster,” contains two Fc domain polypeptides each with a sialidase fused at the N-terminus of the Fc and a scFv fused at the C-terminus of the Fc (**FIGURE 19C**). The fourth type of ASC, referred to as “Bunk,” contains one antibody arm (with one heavy chain and one light chain) with an scFv fused at the
- 10
- 15

C-terminus of one arm of the Fc, and one sialidase-Fc fusion arm with a sialidase fused at the N-terminus of the other arm of the Fc. Each Fc domain polypeptide in the Bunk ASC contains either the “knob” (T366Y) or “hole” (Y407T) mutation for heterodimerization (residue numbers according to EU numbering, Kabat, E.A., *et al.* (1991) *supra*) (**FIGURE 19D**).

5 **[00312]** A Janus Antibody Sialidase Conjugate (ASC) was constructed using human Neu2 containing M1D, V6Y, P62G, A93E, I187K, and C332A mutations and the variable region of avelumab, an anti-PD-L1 antibody. This Janus ASC (referred to as Janus #1, and including a first polypeptide chain with amino acid sequence SEQ ID NO: 64, encoded by nucleotide sequence SEQ ID NO: 76, a second polypeptide chain with amino acid sequence SEQ ID NO: 66, encoded by nucleotide sequence SEQ ID NO: 77, and a third polypeptide chain with amino acid sequence SEQ ID NO: 78, encoded by nucleotide sequence SEQ ID NO: 103) was expressed and characterized for purity using SDS-PAGE and enzymatic activity using 4MU-NeuAc as described below.

15 **[00313]** Janus #1 was expressed in a 600 mL transfection of Expi293 human cells using the pCEP4 mammalian expression vector. The ASC was purified using protein A followed by cation exchange chromatography, quantified with a UV-Vis spectrophotometer (NanoDrop), and examined by SDS-PAGE as shown in **FIGURE 20**. Janus #1 expressed with a yield of 34 mg with good purity after purification (see, e.g., **FIGURE 21**).

20 **[00314]** The activity of Janus #1 was assayed by measuring the release of sialic acid from the fluorogenic substrate 4-methylumbelliferyl-N-acetylneuraminic acid (4MU-NeuAc). Specifically, an enzyme kinetics assay was performed by incubating a fixed concentration of enzyme at 1 nM with fluorogenic substrate 4MU-NeuAc at concentrations ranging from 4000 μ M to 7.8 μ M. As shown in **FIGURE 22**, Janus #1 was active, causing the release of sialic acid which generated fluorescence. Assays were conducted at pH 5.6.

25 **[00315]** **FIGURE 23** shows a binding assay (ForteBio octet binding) between Avelumab (**FIGURE 23A**) or Janus #1 (**FIGURE 23B**) and recombinant human PD-L1. Janus #1 had a comparable K_d to Avelumab (1.28nM vs. 0.75 nM) as well as a comparable K_{on} (1.29×10^5 vs. 3.31×10^5 (1/Ms)) and K_{dis} (1.39×10^{-4} vs. 2.35×10^{-4} (1/s)) (**FIGURE 23C**).

Example 6

30 **[00316]** This example describes the construction of additional anti-PD-L1 antibody sialidase conjugates (ASCs) based on the Janus format, and the construction of anti-PD-L1 ASCs based on the Bunk and Lobster formats.

[00317] A Janus ASC was constructed using Neu2 with M1D, V6Y, P62G, A93E, I187K, and C332A mutations and the variable region of avelumab. This Janus ASC is referred to as Janus #2, and includes a first polypeptide chain with amino acid sequence SEQ ID NO: 64, encoded by nucleotide sequence SEQ ID NO: 76, a second polypeptide chain with amino acid sequence SEQ ID NO: 66, encoded by nucleotide sequence SEQ ID NO: 77, and a third polypeptide chain with amino acid sequence SEQ ID NO: 112, encoded by nucleotide sequence SEQ ID NO: 113.

Janus #2 has the same amino acid sequence as Janus #1 described in Example 5 except for a different linker sequence between the sialidase and Fc in the third polypeptide chain, where in Janus #1 the linker was GGGGS (SEQ ID NO: 121) and in Janus #2 the linker was

GGGGSGGGGS (SEQ ID NO: 90).

[00318] Another Janus ASC was constructed using a bacterial sialidase from *Salmonella typhimurium* (St-sialidase) and the variable region of avelumab. This Janus ASC is referred to as Janus #3, and includes a first polypeptide chain with amino acid sequence SEQ ID NO: 64, encoded by nucleotide sequence SEQ ID NO: 76, a second polypeptide chain with amino acid sequence SEQ ID NO: 66, encoded by nucleotide sequence SEQ ID NO: 77, and a third polypeptide chain with amino acid sequence SEQ ID NO: 106, encoded by nucleotide sequence SEQ ID NO: 107. Janus #3 also contains a C-terminal Histidine repeat sequence (His-tag) for potential purification.

[00319] Another Janus ASC was constructed using St-sialidase and the variable region of avelumab. This Janus ASC is referred to as Janus #4, and includes a first polypeptide chain with amino acid sequence SEQ ID NO: 64, encoded by nucleotide sequence SEQ ID NO: 76, a second polypeptide chain with amino acid sequence SEQ ID NO: 104, encoded by nucleotide sequence SEQ ID NO: 105, and a third polypeptide chain with amino acid sequence SEQ ID NO: 108, encoded by nucleotide sequence SEQ ID NO: 109. Janus #4 has an Asn297 to Gly (N297G) mutation in the Fc domain to remove an N-linked glycosylation site. Janus #4 also contains a C-terminal His-tag for potential purification.

[00320] A Bunk ASC was constructed using Neu2 with M1D, V6Y, P62G, A93E, I187K, and C332A mutations, the variable region of avelumab, and an avelumab-based scFv. This Bunk ASC is referred to as Bunk #1, and includes a first polypeptide chain with amino acid sequence SEQ ID NO: 64, encoded by nucleotide sequence SEQ ID NO: 76, a second polypeptide chain with amino acid sequence SEQ ID NO: 110, encoded by nucleotide sequence SEQ ID NO: 111, and a third polypeptide chain with amino acid sequence SEQ ID NO: 78, encoded by nucleotide sequence SEQ ID NO: 103.

[00321] Another Bunk ASC was constructed using St-sialidase, the variable region of avelumab, and an avelumab-based scFv. This Bunk ASC is referred to as Bunk #2, and includes a first polypeptide chain with amino acid sequence SEQ ID NO: 64, encoded by nucleotide sequence SEQ ID NO: 76, a second polypeptide chain with amino acid sequence SEQ ID NO: 110, encoded by nucleotide sequence SEQ ID NO: 111, and a third polypeptide chain with amino acid sequence SEQ ID NO: 106, encoded by nucleotide sequence SEQ ID NO: 107. Bunk #2 also contains a C-terminal His-tag for potential purification.

[00322] A Lobster ASC was constructed using Neu2 with M1D, V6Y, P62G, A93E, I187K, and C332A mutations and an avelumab-based scFv. This Lobster ASC is referred to as Lobster #1, and includes a first polypeptide with an amino acid sequence of SEQ ID NO: 114, encoded by nucleotide sequence SEQ ID NO: 115, and a second polypeptide with an amino acid sequence of SEQ ID NO: 114, encoded by nucleotide sequence SEQ ID NO: 115.

[00323] A depiction of each of the constructs described in this Example is shown in **FIGURE 24**.

[00324] Janus #2, Janus #3, Janus #4, Bunk #1, Bunk #2, and Lobster #1 were each expressed in a 600 mL transfection of Expi293 human cells using the pCEP4 mammalian expression vector. The ASCs were purified using protein A followed by cation exchange chromatography, quantified with a UV-Vis spectrophotometer (NanoDrop), and examined by SDS-PAGE. Janus #1 was expressed and purified as described in Example 5, and Neu2-M106-Fc was expressed and purified as described in Example 2.

[00325] The activity of Janus #1, Janus #2, Bunk #1, Bunk #2, Lobster #1, and Neu2-M106-Fc was assayed by measuring the release of sialic acid from the fluorogenic substrate 4-methylumbelliferyl-N-acetylneuraminic acid (4MU-NeuAc). Specifically, an enzyme kinetics assay was performed by incubating a fixed concentration of enzyme at 1 nM with fluorogenic substrate 4MU-NeuAc at concentrations ranging from 2000 μ M to 7.8 μ M. As shown in **FIGURE 25**, the tested ASCs were all active, causing the release of sialic acid which generated fluorescence.

[00326] Monomer purity of Janus #2, Janus #3, Bunk #2, and Lobster #1 was assayed by Size Exclusion Chromatography (SEC). As shown in **FIGURE 26**, the tested ASCs had a percent homogeneity ranging from 80 to 100%.

[00327] The binding kinetics of Janus #2, Janus #3, Bunk #1, Bunk #2, and Lobster #1 for recombinant human PD-L1 was determined by ForteBio octet binding. Avelumab was used as a control. Monovalent binding kinetics experiments, where the ASC was adsorbed onto the sensor

and PD-L1 was titrated in, are shown in **FIGURE 27**. Avidity driven binding experiments, where PD-L1 was adsorbed onto the sensor and the ASC was titrated in, are shown in **FIGURE 28**. Kinetic parameters are shown in **TABLE 28**. As can be seen, all ASCs demonstrated comparable binding kinetics to avelumab.

TABLE 28

	KD (M)	kon(1/Ms)	kdis(1/s)
Avelumab	7.54E-10	3.20E+05	2.34E-04
Janus #3	5.56E-10	3.58E+05	1.88E-04
Janus #2	7.47E-10	3.02E+05	2.12E-04
Bunk #2	9.44E-10	3.24E+05	2.73E-04
Lobster #1	5.88E-10	2.50E+05	1.25E-04

Example 7

[00328] This example describes the testing of anti-PD-L1 antibody sialidase conjugates (ASCs) in a cell-based assay measuring the disruption of human PD-L1 binding to PD-1.

[00329] Janus #2, Janus #3, Bunk #1, Bunk #2, and Lobster #1 were expressed and purified as described in Example 6. The avelumab-based ASCs were tested in the PD-1/PD-L1 Blockade Bioassay (Promega, Cat. Nos. J1250 and J1255) according to manufacturer's instructions. In brief, the avelumab-based ASCs were co-cultured with (i) engineered CHO-K1 cells expressing human PD-L1 and TCR activating protein and (ii) Jurkat T cells expressing human PD-1, TCR and a luciferase reporter driven by an NFAT response element. A schematic of the assay is depicted in **FIGURE 29**. Absent intervention, PD-L1 interacting with PD-1 inhibits TCR-mediated luminescence. Blockade of the PD-L1/ PD-1 interaction, for instance using avelumab or avelumab-based ASCs, results in a luminescent signal. A luciferase substrate was added after 6 hours of incubation and luminescence was measured. Relative light units (RLU) were calculated by subtracting background (substrate and media only) from assay wells. Fold induction was calculated by dividing RLU (induced minus background) by RLU (no antibody control minus background). As can be seen in **FIGURE 30**, the tested avelumab-based ASCs disrupted the PD-L1/PD-1 interaction, resulting in a dose dependent increase in luminescence. EC50s of the ASCs were comparable to the EC50 for avelumab.

Example 8

[00330] This Example describes the *in vivo* administration of anti-PD-L1 antibody sialidase conjugates (ASCs) containing human sialidases.

[00331] Avelumab-based Janus ASCs can be tested in a mouse syngeneic tumor model injected with a murine cancer cell line either expressing endogenous PD-L1 or engineered to express human PD-L1, as avelumab binds human and mouse PD-L1 with similar affinity. Female BALB/c mice, 6-8 weeks of age, are inoculated subcutaneously in the right lower flank region with either the parent murine cell line or human PD-L1 expressing tumor cells for tumor development. Mice are randomly allocated to 4 groups of 5 animals each when tumors reach 50-100 mm³, mean ~ 75-100 mm³.

[00332] Mice are treated via intraperitoneal injection of 10 mg/kg of either avelumab-based Janus ASC, avelumab, or vehicle control, and tumor volume (mm³) is recorded. Mean tumor volumes for the individual mice for the indicated treatments are determined. Complete Responses (CR, defined as regression below the limit of palpitation at any point during the study) are also determined.

[00333] It is expected that avelumab-based Janus ASC will reduce tumor volume and increase the number of CRs as compared to vehicle control. It is also expected that avelumab-based Janus ASC will reduce tumor volume comparably or better than avelumab.

Example 9

[00334] This example describes *in vivo* testing of avelumab-based ASCs in an A20 syngeneic mouse model. Mouse A20 cells express endogenous mouse PD-L1 which is bound by avelumab and avelumab-based ASCs. Female Balb/c mice, 5-6 weeks of age, were inoculated subcutaneously in the right lower flank region with murine A20 B cell lymphoma cells in matrigel (1:1 by volume). Mice were randomly allocated into groups of 8 mice when tumors reached approximately 100 mm³ (the average tumor volume of each group ranged from 86 to 90 mm³). **TABLE 29** describes the various arms of the study. Mice were treated intraperitoneally with 1-30 mg/kg of Janus #3 (as described in Example 6), avelumab, or antibody isotype control twice a week for a total of 5 doses. Tumor volumes and body weights were recorded three times a week.

TABLE 29

Group	# Mice	Treatment	Dose	Schedule
1	8	Isotype control	10 mg/kg	Twice weekly for 5 doses
2	8	Avelumab	5 mg/kg	Twice weekly for 5 doses
3	8	Avelumab	10 mg/kg	Twice weekly for 5 doses
4	8	Janus #3	1 mg/kg	Twice weekly for 5 doses
5	8	Janus #3	5 mg/kg	Twice weekly for 5 doses

Group	# Mice	Treatment	Dose	Schedule
6	8	Janus #3	10 mg/kg	Twice weekly for 5 doses
7	8	Janus #3	30 mg/kg	Twice weekly for 5 doses

[00335] **FIGURE 31** depicts the tumor growth in each mouse in each of the groups. Complete responders (CR) and partial responders (PR) for each group are shown. As can be seen, Janus #3 demonstrated a dose dependent increase in PR and CR.

[00336] Mice with tumors that demonstrated CR were grouped and rechallenged with murine A20 cells (all approximately 12 weeks of age) and compared to naïve control mice injected with A20 cells of either 6 or 12 weeks of age. Tumor volumes and body weights were recorded three times a week. While tumors grew as expected in both the 6 week and 12 week naïve mice, no substantial tumor growth was observed in the rechallenged mice.

Example 10

[00337] This example describes *in vivo* testing of avelumab-based ASCs in an A20 syngeneic mouse model. Mouse A20 cells express endogenous mouse PD-L1 which is bound by avelumab and avelumab-based ASCs. Female Balb/c mice, 5-6 weeks of age, were inoculated subcutaneously in the right lower flank region with murine A20 B cell lymphoma cells in matrigel (1:1 by volume). Mice were randomly allocated into groups of 8 mice when tumors reached approximately 100 mm³ (the average tumor volume of each group ranged from 84 to 92 mm³). **TABLE 30** describes the various arms of the study. Mice were treated intraperitoneally with 10-13.5 mg/kg Janus #2, Janus #3, Janus #4, Bunk #2, Lobster #1 (as described in Example 6), avelumab, or antibody isotype control twice a week for a total of 6 doses. Tumor volumes and body weights were recorded three times a week.

TABLE 30

Group	Treatment	Route/dose	Schedule
1	Isotype	10 mg/kg IP	Twice weekly for 3 weeks
2	Avelumab	10 mg/kg IP	
3	Janus #3	10 mg/kg IP	
4	Janus #4	10 mg/kg IP	
5	Bunk #2	12 mg/kg IP	
6	Janus #2	10 mg/kg IP	
7	Lobster #1	13.5 mg/kg IP	

[00338] **FIGURE 32** depicts the results of tumor growth in each mouse in each of the groups. The avelumab-based ASCs demonstrated varying degrees of efficacy.

INCORPORATION BY REFERENCE

5 [00339] The entire disclosure of each of the patent and scientific documents referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[00340] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be
10 considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

SEQUENCE LISTING

[00341] SEQ ID NO: 1:

5 MASLPVLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPIQRPI
 SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQVLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
 WSEPVLAKGSCAYSIDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

10 **[00342]** SEQ ID NO: 2:

15 MEDLRPMATCPVLQKETLFRITGVHAYRIPALLYLKKQKTLAFAEKRASTDEHAELIVLRRGS
 YNEATNRVKWQPEEVVTQAQLEGHRSMNPCPLYDKQTKTLFLFFIAVPGRVSEHHQLHTKVNVT
 RLCCVSSTDHGRTWSPIQDLTETTIGSTHQEWATFAVGPGHCLQLRNPAGSLLVPAYAYRKLHP
 AQKPTPFACFISLDHGHTWKLGNFVAENSLECQVAEVTGAQRMVYLNARSFLGARVQAQSPN
 DGLDFQDNRVVSKLVEPPHGGCHGSVVAFHNPISKPHALDTWLLYTHPTDSRNRNTNLGVYLNQMP
 LDPTAWSEPTLLAMGICAYSIDLQNMGGPDGSPQFGCLYESGNYEEIIFLIFTLTKQAFPTVFDA
 Q

[00343] SEQ ID NO: 3:

20 EDLRP

[00344] SEQ ID NO: 4:

MEDLRP

[00345] SEQ ID NO: 5:

25 DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
 YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
 DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00346] SEQ ID NO: 6:

30 ACAGTGGAAAAGTCCGTGGTGTTCAGGCCGAGGGCGAGCACTTCACCGACCAGAAAGGCAATA
 CCATCGTCGGCTCTGGCAGCGGCGGCACCACCAAGTACTTTAGAATCCCCGCCATGTGCACCAC
 CAGCAAGGGCACCATTGTGGTGTTCGCCGACGCCAGACACAACACCGCCAGCGATCAGAGCTTC
 ATCGATACCGCTGCCGCCAGATCTACCGATGGCGGCAAGACCTGGAACAAGAAGATCGCCATCT
 ACAACGACCGCGTGAACAGCAAGCTGAGCAGAGTGATGGACCCTACCTGCATCGTGGCCAACAT
 35 CCAGGGCAGAGAAACCATCCTGGTCATGGTCGGAAAGTGGAACAACAACGATAAGACCTGGGGC
 GCCTACAGAGACAAGGCCCTGATACCGATTGGGACCTCGTGCTGTACAAGAGCACCGATGACG
 GCGTGACCTTCAGCAAGGTGGAACAACATCCACGACATCGTGACCAAGAAGCGCACCATCTC
 TGCCATGCTCGGCGGCGTTGGATCTGGCCTGCAACTGAATGATGGCAAGCTGGTGTTCCTCGTG
 CAGATGGTCCGAACAAGAATATCACCACCGTGCTGAATACCAGCTTCATCTACAGCACCGACG
 40 GCATCACATGGTCCCTGCCTAGCGGCTACTGTGAAGGCTTTGGCAGCGAGAACAACATCATCGA
 GTTCAACGCCAGCCTGGTCAACAACATCCGGAACAGCGGCCTGCGGAGAAGCTTCGAGACAAAG
 GACTTCGGAAGACGTGGACCGAGTTTCCTCCAATGGACAAGAAGGTGGACAACCGGAACCACG
 GCGTGACGGGAGCACAATCACAATCCCTAGCGGCAACAACACTGGTGGCCGCTCACTCTAGCGC

CCAGAACAAAGAACAAACGACTACACCAGAAGCGACATCAGCCTGTACGCCCACAACCTGTACAGC
GGCGAAGTGAAGCTGATCGACGACTTCTACCCCAAAGTGGGCAATGCCAGCGGAGCCGGCTACA
GCTGTCTGAGCTACCGGAAAAATGTGGACAAAGAAACCCTGTACGTGGTGTACGAGGCCAACGG
CAGCATCGAGTTTCAGGACCTGAGCAGACATCTGCCCCGTGATCAAGAGCTACAAC

5

[00347] SEQ ID NO: 7:

ENDFGLVQPLVTMEQLLWVSGRQIGSVDTFRIPLITATPRGTLFAFAEARKMSSSDEGAKFIAL
RRSMDQGSTWSPTAFIVNDGDVPDGLNLGAVVSDVETGVVFLFYSLCAHKAGCQVASTMLVWSK
DDGVSWSTPRNLSLDIGTEVFAPGPGSGIQKQREPRKGRLIVCGHGTLELDGVFCLLSDDHGAS
10 WRYGSGVSGIPYGQPKQENDFNPDECQPYELPDGSSVINARNQNNYHCHCRIVLRSYDACDTRL
PRDVTFDPELVDPVVAAGAVVTSSGIVFFSNPAHPEFRVNLTLRWSFSNGTSWRKETVQLWPGP
SGYSSLATLEGSMGDGEEQAPQLYVLYEKGRNHYTESISVAKISV

[00348] SEQ ID NO: 8:

15 MEEVTTCSFNSPLFRQEDDRGITYRIPALLYIPPTHTFLAFAEKRSTRRDEDALHLVLRRLRI
GQLVQWGPKPLMEATLPGHRTMNPVWEQKSGCVFLFFICVRGHVTERQQIVSGRNAARLCF
IYSQDAGCSWSEVRDLTEEVIGSELKHWATFAVGPGHGIQLQSGRLVIPAYTTYIPSWFFCFQL
PCKTRPHSLMIYSDDLGVTHHGRILRPMVTVECEVAEVTGRAGHPVLYCSARTPNRCRAEALS
TDHGEGFQRLALSRQLCEPPHGCQGSVVSFRPLEIPHRCQDSSSKDAPTIQQSSPGSSRLREEE
20 AGTPSESWLLYSHPTSRSKQVRDLGIYLNQTPLEAACWSRPWILHCGPCGYSDLAALEEEGLFGC
LFECGKQCECEQIAFRLFTHREILSHLQGDCTSPGRNPSQFKN

[00349] SEQ ID NO: 9:

MRPADLPPRPMEESSPASSAPTETEEPGSSAEVMEEVTTCSFNSPLFRQEDDRGITYRIPALLY
25 IPPTHTFLAFAEKRSTRRDEDALHLVLRRLRIIGQLVQWGPKPLMEATLPGHRTMNPVWEQ
KSGCVFLFFICVRGHVTERQQIVSGRNAARLCFIYSQDAGCSWSEVRDLTEEVIGSELKHWATF
AVGPGHGIQLQSGRLVIPAYTTYIPSWFFCFQLPCKTRPHSLMIYSDDLGVTHHGRILRPMVT
VECEVAEVTGRAGHPVLYCSARTPNRCRAEALSTDHGEGFQRLALSRQLCEPPHGCQGSVVSFR
PLEIPHRCQDSSSKDAPTIQQSSPGSSRLREEEAGTPSESWLLYSHPTSRSKQVRDLGIYLNQTP
30 LEAACWSRPWILHCGPCGYSDLAALEEEGLFGCLFECGKQCECEQIAFRLFTHREILSHLQGDC
TSPGRNPSQFKN

[00350] SEQ ID NO: 10:

MGVPRTPSRTVLFERERTGLTYRVPSLLPVPPGPTLLAFVEQRLSPDDSHAHRLVLRRLTAGG
35 SVRWGALHVLGTAALAEHRSMNCPVHDAGTGTVFLFFIAVLGHTPEAVQIATGRNAARLCCVA
SRDAGLSWGSARDLTEEAIGGAVQDWATFAVGPGHGVQLPSGRLLVPAYTYRVDRRECFGKICR
TSPHSFAFYSDDHGRTWRCGGLVPNLRSGECQLAAVDGGQAGSFLYCNARSPGSRVQALSTDE
GTSFLPAERVASLPETAWGCGSIVGFAPAPNRPDDSWSVGPGSPLQPPLLGPGVHEPPEEA
AVDPRGGQVPGGPFSLRLQPRGDGPRQPGPRPGVSGDVGSWTLALPMPFAAPPQSPTWLLYSHPV
40 GRRARLHMGIKLSQSPLDPRSWTEPWVIYEGPSGYSDLASIGPAPEGGLVFACLYESGARTSYD
EISFCTFSLREVLNVPASPKPPNLGDKPRGCCWPS

[00351] SEQ ID NO: 11:

45 MMSSAAFPRWLSMGVPRTPSRTVLFERERTGLTYRVPSLLPVPPGPTLLAFVEQRLSPDDSHAH
RLVLRRLTAGGSRWALHVLGTAALAEHRSMNCPVHDAGTGTVFLFFIAVLGHTPEAVQIA
TGRNAARLCCVASRDAGLSWGSARDLTEEAIGGAVQDWATFAVGPGHGVQLPSGRLLVPAYTYR

VDRRECFGKICRTSPHSFAFYSDDHGRTWRCGGLVPNLRSGECQLAAVDGGQAGSFLYCNARSP
LGSRVQALSTDEGTSFLPAERVASLPETAWGCQGSIVGFAPAPAPNRPRDDSWSVGPGSPLQPPL
LPGVHEPPEEA AVDPRGGQVPGGPF SRLQPRGDGPRQPGPRPGVSGDVGSWTLALPMPFAAPP
QSPTWLLYSHPVGRRARLHMGIRLSQSPLDPRSWTEPWVIYEGPSGYSDLASIGPAPEGGLVFA
5 CLYESGARTSYDEISFCTFSLREVLENPASPKPPNLGDKPRGCCWPS

[00352] SEQ ID NO: 12:

MASLP

[00353] SEQ ID NO: 13:

10 ASLP

[00354] SEQ ID NO: 14:

TVEKSVVF

[00355] SEQ ID NO: 15:

GDYDAPTHQVQW

15 **[00356]** SEQ ID NO: 16:

SMDQGSTW

[00357] SEQ ID NO: 17:

STDGGKTW

[00358] SEQ ID NO: 18:

20 PRPPAPEA

[00359] SEQ ID NO: 19:

QTPLEAAC

[00360] SEQ ID NO: 20:

NPRPPAPEA

25 **[00361]** SEQ ID NO: 21:

SQNDGES

[00362] SEQ ID NO: 22:

LSHSLST

30 **[00363]** SEQ ID NO: 23:

GAGAACGACTTTTGGACTGGTGCAGCCTCTGGTCACCATGGAACAGCTGCTGTGGGTTTCCGGCA
GACAGATCGGCAGCGTGGACACCTTCAGAATCCCTCTGATCACCGCCACACCTAGAGGCACCCT
GCTGGCCTTTTGCCGAGGCCAGAAAGATGAGCAGCTCTGACGAGGGCGCCAAGTTTATTGCCCTG
AGGCGGTCTATGGACCAGGGCTCTACATGGTCCCCTACCGCCTTCATCGTGAACGATGGCGACG
5 TCCCCGATGGCCTGAATCTGGGAGCTGTGGTGTCCGATGTGGAAACCGGCGTGGTGTTCCTGTT
CTACAGCCTGTGTGCCACAAGGCCGGTTGTCAGGTGGCCAGCACAAATGCTCGTGTGGTCCAAG
GACGACGGCGTGTCTGGTCTACCCCTAGAAACCTGAGCCTGGACATCGGCACCGAAAGTGTGTG
CTCCAGGACCTGGCTCTGGCATCCAGAAGCAGAGAGAGCCCAGAAAGGGCAGACTGATCGTGTG
TGGCCACGGCACCCTTGAGAGAGATGGCGTTTTCTGCCTGCTGAGCGACGATCATGGCGCCTCT
10 TGGAGATACGGCAGCGGAGTGTCTGGAATCCCTTACGGCCAGCCTAAGCAAGAGAACGATTTCA
ACCCCGACGAGTGCCAGCCTTACGAGCTGCCTGATGGCAGCGTCGTGATCAACGCCCGGAACCA
GAACAACCTACCACTGCCACTGCCGGATCGTGCTGAGAAGCTACGACGCCTGCGATACCCTGCGG
CCTAGAGATGTGACCTTCGATCCTGAGCTGGTGGACCCTGTTGTTGCCGCTGGTGCCGTCGTGA
CATCTAGCGGCATCGTGTCTTCAGCAACCCTGCTCACCCCGAGTTCAGAGTGAATCTGACCCT
15 GCGGTGGTCTTTCAGCAATGGCACAAGCTGGCGGAAAGAAACCGTGCAGCTTTGGCCTGGACCT
AGCGGCTACTCTTCTCTGGCTACACTGGAAGGCAGCATGGACGGCGAAGAACAGGCCCTCAGC
TGTACGTGCTGTACGAGAAGGGCAGAAACCACTACACCGAGAGCATCAGCGTGGCCAAGATCAG
CGTT

[00364] SEQ ID NO: 24:

ATGGCCAGCCTGCCTGTGCTGCAGAAAGAAAGCGTGTTCCAGTCTGGCGCCCACGCCTACAGAA
TTCCCGCTCTGCTGTATCTGCCAGGCCAGCAGTCTCTGCTGGCTTTTCGCTGAACAGCGGGCCAG
CAAGAAGGATGAGCACGCCGAACCTGATCGTGCTGCGGAGAGGCGATTACGACGCCCCCTACACAT
CAGGTGCAGTGGCAGGCTCAAGAGGTGGTGGCTCAGGCTAGACTGGACGGCCACAGATCTATGA
ACCCCTGTCTCTGTACGATGCCAGACCGGCACACTGTTTCTGTTCTTTATCGCTATCCCCGG
25 CCAAGTGACCGAGCAGCAGCAGCTGCAGACAAGAGCCAACGTGACCAGACTGTGTCAAGTGACC
TCCACCGACCACGGCAGAACCTGGTCTAGCCCTAGAGATCTGACCGACGCCGCCATCGGACCTG
CCTATAGAGAGTGGTCCACCTTCGCCGTTGGACCTGGACACTGTCTCCAGCTGCACGACAGGGC
TAGATCTCTGGTGGTGCCTACGCCTATAGAAAGCTGCACCCCATCCAGCGGCCTATTCTCT
AGCGCCTTCTGCTTTCTGAGCCACGATCACGGCAGGACATGGGCCAGAGGACATTTCTGTTGGCCC
30 AGGACACACTGGAATGCCAGGTGGCCGAAGTGGAACCGGCGAGCAGAGAGTCGTGACCCTGAA
CGCCAGATCTCACCTGAGAGCCAGAGTGCAGGCCAGAGCACAAACGACGGCCTGGATTTCCAA
GAGAGCCAGCTGGTCAAGAACTGGTGGAACTCCTCCACAGGGCTGTGAGGGAAGCGTGATCA
GCTTTCCATCTCCTAGAAGCGGCCCTGGCTCTCCTGCTCAGTGGCTGCTGTATACACACCCAC
ACACAGCTGGCAGAGAGCCGATCTGGGCGCCTACCTGAATCCTAGACCTCCTGCTCCTGAGGCT
35 TGGAGCGAACCTGTTCTGCTGGCCAAGGGCAGCTGTGCCTACAGCGATCTGCAGTCTATGGGCA
CAGGCCCTGATGGCAGCCCTCTGTTTGGCTGTCTGTACGAGGCCAACGACTACGAAGAGATCGT
GTTCTGATGTTACCCCTGAAGCAGGCCTTTCCAGCCGAGTACCTGCCTCAA

[00365] SEQ ID NO: 25:

ATGGAGGAAGTGACCACCTGTAGCTTCAACAGCCCTCTGTTCCGGCAAGAGGACGACCGGGGCA
TCACCTACAGAATCCCTGCTCTGCTGTACATCCCTCCTACACACACCTTTCTGGCCTTCGCCGA
GAAGCGGAGCACCAGACGAGATGAAGATGCCCTGCACCTGGTGTGAGAAGAGGCCTGAGAATC
GGACAGCTGGTGCAGTGGGGACCTCTGAAGCCTCTGATGGAAGCCACACTGCCCGGCCACAGAA
CCATGAATCCTTGTCTGTGTGGGAGCAGAAAAGCGGCTGCGTGTTCTGTTCTTCATCTGCGT
45 GCGGGGCCACGTGACCGAGAGACAGCAAATCGTGTCCGGCAGAAACGCCGCCAGACTGTGCTTC

ATCTACAGCCAGGATGCCGGCTGCTCTTGGAGCGAAGTTCGGGATCTGACCGAAGAAGTGATCG
GCAGCGAGCTGAAGCACTGGGCCACATTTGCTGTTGGCCCTGGCCACGGAATCCAGCTGCAATC
TGGCAGACTGGTCATCCCCGCCTACACCTACTATATCCCCAGCTGGTTCTTCTGCTTCCAACTG
CCTTGCAAGACCCGGCCTCACAGCCTGATGATCTACAGCGACGATCTGGGCGTGACATGGCACC
5 ACGGCAGACTGATCAGACCCATGGTCACCGTGGGAATGCGAGGTGGCCGAAGTGACAGGCAGAGC
TGGACACCCTGTGCTGTACTGCTCTGCCAGAACACCCAACCGGTGTAGAGCCGAGGCTCTGTCT
ACAGATCACGGCGAGGGCTTTTCAGAGACTGGCCCTCTCTAGACAGCTGTGCGAACCTCCTCATG
GCTGTCAAGGCAGCGTGGTGTCTTCAGACCTCTGGAAATCCCTCACCGGTGCCAGGACAGCAG
CTCTAAGGATGCCCCCTACCATCCAGCAGTCTAGCCCTGGCAGCAGCCTGAGACTGGAAGAGGAA
10 GCCGGAACACCTAGCGAGAGCTGGCTGCTGTACTCTCACCCCACCAGCAGAAAGCAGAGAGTGG
ACCTGGGCATCTACCTGAATCAGACCCCTCTGGAAGCCGCCTGTTGGAGCAGACCTTGGATTCT
GCACTGTGGCCCTTGCGGCTACTCTGATCTGGCCGCTCTGGAAGAAGAGGGCCTGTTGCGCTGC
CTGTTTGAGTGCGGCACAAAGCAAGAGTGCGAGCAGATCGCCTTCCGGCTGTTACCCACAGAG
AGATCCTGAGCCATCTGCAGGGCGACTGCACAAGCCCAGGCAGAAATCCCAGCCAGTTC AAGAG
15 CAAC

[00366] SEQ ID NO: 26:

ATGGGCGTGCCCAGAACACCCAGCAGAACCGTGCTGTTTCGAGAGAGAGAGGACCGGCCTGACCT
ACAGAGTGCCTTCTCTGCTGCCTGTGCCTCCTGGACCTACACTGCTGGCCTTCGTGGAACAGAG
ACTGAGCCCCGATGATTCTCACGCCCACAGACTGGTGCTGAGAAGAGGAACACTGGCTGGCGGC
20 TCTGTTAGATGGGGAGCACTGCATGTGCTGGGCACAGCTGCTCTTGCCGAGCACAGATCCATGA
ATCCCTGTCCTGTGCACGACGCCGGAACCGGCACAGTGTTTCTGTTCTTTATCGCCGTGCTGGG
CCACACACCTGAGGGCCGTTCAAATTGCCACCGGCAGAAATGCCGCCAGACTGTGTTGTGTGGCC
TCCAGAGATGCCGGCCTGTCTTGGGGATCTGCCAGAGATCTGACCGAGGAAGCCATTGGCGGAG
CCGTTTCAGGATTGGGCCACATTTGCTGTTGGACCTGGACACGGCGTGACGCTGCCAAGTGGTAG
25 ACTGCTGGTGCCTGCCTACACATACAGAGTGGATCGGAGAGAGTGCTTCGGAAAGATCTGCCGG
ACAAGCCCTCACAGCTTCGCCTTCTACTCCGACGATCACGGCCGGACTTGAGAGATGTGGTGGCC
TGGTGCCTAATCTGAGAAGCGGCGAATGTCAACTGGCCGCCGTTGATGGTGGACAGGCTGGCAG
CTTCCTGTACTGCAACGCCAGATCTCCTCTGGGCTCTAGAGTGACAGGCCCTGTCTACCGATGAG
GGCACCAAGTTTTCTGCCCCGCCGAAAGAGTTGCCTCTCTGCCTGAAACAGCCTGGGGCTGTCAGG
30 GCTCTATCGTGGGATTTCTGCTCCTGCTCCAAACAGACCCCGGGACGATTCTTGGAGTGTGCG
CCCTGGATCTCCACTGCAGCCTCCATTGCTTGGACCAGGCGTTCACGAGCCACCTGAAGAGGCT
GCCGTTGATCCTAGAGGCGGACAAGTTCCCTGGCGGCCCTTTTAGCAGACTGCAGCCAAGAGGCG
ACGGCCCTAGACAACCTGGACCAAGACCTGGCGTCAGCGGAGATGTTGGCTCTTGGACACTGGC
CCTGCCTATGCCTTTTGCCGCTCCTCCTCAGTCTCCTACCTGGCTGCTGTACTCTCACCCCTGTT
35 GGCAGACGGGCCAGACTGCACATGGGCATCAGACTGTCTCAGAGCCCTCTGGACCCCAGAAGCT
GGACAGAGCCTTGGGTCACTATGAGGGCCCTAGCGGCTACAGCGATCTGGCCTCTATTGGCCC
AGCTCCTGAAGGCGGACTGGTGTTCGCTTGTCTGTATGAGAGCGGCGCCAGAACCAGCTACGAC
GAGATCAGCTTCTGCACCTTCAGCCTGCGCGAGGTGCTGGAAAATGTGCCCCGCTCTCCTAAGC
CTCCTAACCTGGGCGATAAGCCTAGAGGCTGTTGCTGGCCATCT

[00367] SEQ ID NO: 27:

MTGERPSTALPDRRWGPRILGFWGGCRVWVFAAIFLLLSLAASWSKA

[00368] SEQ ID NO: 28:

MDMRVPAQLLGLLLLWLPGARC

[00369] SEQ ID NO: 29:

YGTL

[00370] SEQ ID NO: 30:

5 MTVEKSVVFKAEGEHFTDQKGNTIVGSGSGGTTKYFRIPAMCTTSKGTIVVFADARHNTASDQS
FIDTAAARSTDGCKTWNKKIAIYNDRVNSKLSRVMPTCIVANIQGRETIILVMVGKWNNDKTW
GAYRDKAPDTDWDLVLYKSTDDGVTFISKVETNIHDIIVTKNGTISAMLGGVGSGLQLNDGKLVFP
VQMVRTKNITTVLNTSFIYSTDGITWSLPSGYCEGFGSENNIIEFNASLVNNIRNSGLRRSFET
KDFGKTWTEFPPMDKKVDNRNHGVQGSTITIPSGNKLVAHSSAQNKNNDYTRSDISLYAHNLY
10 SGEVKLIDDFYPKVGNASGAGYSCLSYRKNVDKETLYVVYEANGSIEFQDLRHLPLVIKSYN

[00371] SEQ ID NO: 31:

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
15 REPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[00372] SEQ ID NO: 32:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
20 YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLT
DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[00373] SEQ ID NO: 33:

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV
25 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYITLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

30 [00374] SEQ ID NO: 34:

ATGAGACCTGCGGACCTGCCCCGCGCCCCATGGAAGAATCCCCGGCGTCCAGCTCTGCCCCGA
CAGAGACGGAGGAGCCGGGGTCCAGTGCAGAGGTCATGGAAGAAGTGACAACATGCTCCTTCAA
CAGCCCTCTGTTCCGGCAGGAAGATGACAGAGGGATTACCTACCGGATCCCAGCCCTGCTCTAC
35 ATACCCCCCACCCACACCTTCTGGCCTTTGCAGAGAAGCGTTCTACGAGGAGAGATGAGGATG
CTCTCCACCTGGTGCTGAGGCGAGGGTTGAGGATTGGGCAGTTGGTACAGTGGGGGCCCCCTGAA
GCCACTGATGGAAGCCACACTACCGGGGCATCGGACCATGAACCCCTGTCTGTATGGGAGCAG
AAGAGTGGTTGTGTGTTCTTCTTCATCTGTGTGCGGGGCCATGTACAGAGCGTCAACAGA
TTGTGTCAGGCAGGAATGCTGCCCCGCTTTGCTTCATCTACAGTCAGGATGCTGGATGTTTCATG
GAGTGAGGTGAGGGACTTGACTGAGGAGGTCATTGGCTCAGAGCTGAAGCACTGGGCCACATTT
40 GCTGTGGGCCCAGGTCATGGCATCCAGCTGCAGTCAGGGAGACTGGTCATCCCTGCGTATACCT
ACTACATCCCTTCTGTTCTTTTGCTTCAGCTACCATGTAAAACCAGGCCTCATTCTCTGAT
GATCTACAGTGATGACCTAGGGGTCACATGGCACCATGGTAGACTCATTAGGCCCATGGTTACA
GTAGAATGTGAAGTGGCAGAGGTGACTGGGAGGGCTGGCCACCCTGTGCTATATTGCAGTGCCC
GGACACCAAACAGGTGCCGGGCAGAGGCGCTCAGCACTGACCATGGTGAAGGCTTTCAGAGACT
45 GGCCCTGAGTCGACAGCTCTGTGAGCCCCACATGGTTGCCAAGGGAGTGTGGTAAGTTTCCGG

CCCCTGGAGATCCCACATAGGTGCCAGGACTCTAGCAGCAAAGATGCACCCACCATTTCAGCAGA
 GCTCTCCAGGCAGTTCCTACTGAGGCTGGAGGAGGAAGCTGGAACACCGTCAGAATCATGGCTCTT
 GTACTCACACCCAACCAGTAGGAAACAGAGGGTTGACCTAGGTATCTATCTCAACCAGACCCCC
 TTGGAGGCTGCCTGCTGGTCCCCGCCCTGGATCTTGCACTGTGGGCCCTGTGGCTACTCTGATC
 5 TGGCTGCTCTGGAGGAGGAGGGCTTGTGGGTGTTTGTGGTGAATGTGGGACCAAGCAAGAGTG
 TGAGCAGATTGCCTTCCGCCTGTTTACACACCGGGAGATCCTGAGTCACCTGCAGGGGGACTGC
 ACCAGCCCTGGTAGGAACCCAAGCCAATTCAAAAGCAAT

[00375] SEQ ID NO: 35:

10 ATGATGAGCTCTGCAGCCTTCCCAAGGTGGCTGAGCATGGGGGTCCCTCGTACCCCTTCACGGA
 CAGTGCTCTTCGAGCGGGAGAGGACGGCCTGACCTACCGCGTGCCCTCGCTGCTCCCCGTGCC
 CCCCCGGGCCACCCCTGCTGGCCTTTGTGGAGCAGCGGCTCAGCCCTGACGACTCCACGCCCCAC
 CGCCTGGTGCTGAGGAGGGGCACGCTGGCCGGGGGCTCCGTGCGGTGGGGTGCCCTGCACGTGC
 TGGGGACAGCAGCCCTGGCGGAGCACCGGTCCATGAACCCCTGCCCTGTGCACGATGCTGGCAC
 15 GGGCACCGTCTTCTCTTCTTCATCGCGGTGCTGGGCCACACGCCTGAGGCCGTGCAGATCGCC
 ACGGGAAGGAACGCCGCGCGCCTCTGCTGTGTGGCCAGCCGTGACGCCGGCCTCTCGTGGGGCA
 GCGCCCGGGACCTCACCGAGGAGGCCATCGGTGGTGCCGTGCAGGACTGGGCCACATTCGCTGT
 GGGTCCCCGGCCACGGTGTGCAGCTGCCCTCAGGCCGCCTGCTGGTACCCGCCTACACCTACCGC
 GTGGACCGCCGAGAGTGTTTTGGCAAGATCTGCCGACCAGCCCTCACTCCTTCGCCTTCTACA
 20 GCGATGACCACGGCCGCACCTGGCGCTGTGGAGGCCTCGTGCCCAACCTGCGCTCAGGCGAGTG
 CCAGCTGGCAGCGGTGGACGGTGGGCAGGCCGGCAGCTTCCTCTACTGCAATGCCCCGAGCCCA
 CTGGGCAGCCGTGTGCAGGCGCTCAGCACTGACGAGGGCACCTCCTTCCTGCCCGCAGAGCGCG
 TGGCTTCCCTGCCCGAGACTGCCTGGGGCTGCCAGGGCAGCATCGTGGGCTTCCCAGCCCCCGC
 CCCCCAACAGGCCACGGGATGACAGTTGGTCAGTGGGCCCCGGGAGTCCCCTCCAGCCTCCACTC
 25 CTCGGTCTTGAGTCCACGAACCCCCAGAGGAGGCTGCTGTAGACCCCCGTGGAGGCCAGGTGC
 CTGGTGGGCCCTTCAGCCGTCTGCAGCCTCGGGGGGATGGCCCCAGGCAGCCTGGCCCCAGGCC
 TGGGGTCAGTGGGGATGTGGGGTCCTGGACCCTGGCACTCCCCATGCCCTTTGCTGCCCCGCCC
 CAGAGCCCCACGTGGCTGCTGTACTCCCACCCAGTGGGGCGCAGGGCTCGGCTACACATGGGTA
 TCCGCTTGAGCCAGTCCCCGCTGGACCCGCGCAGCTGGACAGAGCCCTGGGTGATCTACGAGGG
 30 CCCCAGCGGCTACTCCGACCTGGCGTCCATCGGGCCGGCCCCCTGAGGGGGGCTGGTTTTTGCC
 TGCCTGTACGAGAGCGGGGCCAGGACCTCCTATGATGAGATTTCCCTTTTGTACATTCTCCCTGC
 GTGAGGTCCTGGAGAACGTGCCCGCCAGCCCCAAACCGCCCAACCTTGGGGACAAGCCTCGGGG
 GTGCTGCTGGCCCTCC

35 [00376] SEQ ID NO: 36:

MRFKNVKKTALMLAMFGMATSSNAALFDYNATGDTEFDSAPAKQGWMQDNTNNGSGVLTNADGMP
 AWLVQIGIGRAQWTYSLSTNQHAQASSFGWRMTTEMKVLSSGMITNYYANGTQRVLP I I SL DSS
 GNLVVEFEGQTGRTVLATGTAATEYHKFELVFLPGSNPSASFYFDGKLIRDNIQPTASKQNMIV
 WNGSSNTDGVAAAYRDIKFEIQGDVIFRGPDRIPSIVASSVTPGVVTAFAEKRVGGDPGALS
 40 TNDI I TRTSRDGGITWDTELNLTEQINVSDEFDFSDPRPIYDPSNTVLVS YARWPTDAAQNGD
 RIKPWMPNGIFYSVYDVASGNWQAPIDVTDQVKERSFQIAGWGGSELYRRNTSLNSQQDWQSN
 KIRIVDGAANQIQVADGSRKYVVTL SIDESGGLVANLNGVSAPI I LQSEHAKVHSFHDYELQYS
 ALNHTTTTLFVDGQQITTWAGEVSQENNIQFGNADAQIDGRLHVQKIVLTQQGHNLVEFDFAYLA
 QQTPVEVEKDLEKLGWTKIKTGNTMSLYGNASVNP GP GHGITLTRQQNISGSQNGRLIYPAIVLD
 45 RFFLNVMISIYSDDGGSNWQTGSTLPIPFWRKSSSILETLEPSEADMVELQNGDLLLTARLDFNQ
 IVNGVNYSRQQFLSKDGGITWSLLEANNANVFSNISTGTVDASITRFEQSDGSHFLFTNPQG
 NPAGTNGRQNLGLWFSFDEGVTWKGP IQLVNGASAYS D IYQLDSENAIVIVETDNSNMRI LRMP
 ITLLKQKLTLSON

[00377] SEQ ID NO: 37:

TTGTCAATCAAGATGACTTCACAACGAAGAAGAGCATCGATTCAACAAGGAAACAGATTCTAATA
TAAAGGGAGTAGATATGCGTTTCAAAAACGTAAAGAAAACCGCTTTAATGCTTGCAATGTTTCGG
TATGGCGACAAGCTCAAACGCCGCACTTTTTGACTATAACGCAACGGGTGACACTGAGTTTGAC
5 AGTCCAGCCAAACAGGGATGGATGCAAGACAACACGAATAATGGCAGCGGCGTTTTAACCAATG
CAGATGGAATGCCCCGCTTGGTTGGTGCAAGGTATTGGAGGGAGAGCTCAATGGACATATTCTCT
CTCTACTAATCAACATGCCCAAGCATCAAGTTTCGGTTGGCGAATGACGACAGAAATGAAAGTG
CTCAGTGGTGAATGATCACAACACTACTACGCCAACGGCACTCAGCGTGTCTTACCCATCATTT
CATTAGATAGCAGTGGTAACTTAGTTGTTGAGTTTGAAGGGCAAACCTGGACGCACCGTTTTTGGC
10 AACCGGCACAGCAGCAACGGAATATCATAAATTTGAATTGGTATTCCTTCCTGGAAGTAACCCA
TCCGCTAGCTTTTACTTCGATGGCAAACCTCATTCGTGACAACATCCAGCCGACTGCATCAAAAC
AAAATATGATCGTATGGGGGAATGGCTCATCAAATACGGATGGTGTGCGCCGCTTATCGTGATAT
TAAGTTTGAAATTCAAGGCGACGTCATCTTCAGAGGCCAGACCGTATACCGTCCATTGTAGCA
AGTAGCGTAACACCAGGGGTGGTAACCGCATTTGCAGAGAAACGTGTGGGGGGAGGAGATCCCG
15 GTGCTCTGAGTAATACCAATGACATAATCACTCGTACCTCACGAGATGGCGGTATAACTTGGGA
TACCGAGCTCAACCTCACTGAGCAAATCAATGTCAGTGATGAGTTTGATTTCTCCGATCCTCGG
CCTATCTATGATCCTTCCTCCAATACGGTTCCTTGTCTCTTATGCTCGATGGCCGACCGATGCCG
CTCAAAACGGAGATCGAATAAAACCATGGATGCCAAACGGTATTTTTTACAGCGTCTATGATGT
TGCATCAGGGAACCTGGCAAGCGCCTATCGATGTTACCGATCAGGTGAAAGAACGCAGTTTCCAA
20 ATCGCTGGTTGGGGTGGTTCAGAGCTGTATCGCCGAAATACCAGCCTAAATAGCCAGCAAGACT
GGCAATCAAACGCTAAGATCCGAATTGTTGATGGTGCAGCGAACCAGATACAAGTTGCCGATGG
TAGCCGAAAATATGTTGTCACACTGAGTATTGATGAATCAGGTGGTCTAGTCGCTAATCTAAAC
GGTGTTAGTGCTCCGATTATCCTGCAATCTGAACACGCAAAGGTACACTCTTTCCATGACTACG
AACTTCAATATTCGGCGTTAAACCACACCACAACGTTATTCGTGGATGGTCAGCAAATCACAAC
25 TTGGGCTGGCGAAGTATCGCAGGAGAACAACATTCAGTTTGGTAATGCGGATGCCCAAATTGAC
GGCAGACTGCATGTGCAAAAAATGTTCTCACACAGCAAGGCCATAACCTCGTGGAGTTTGATG
CTTTCTATTTAGCACAGCAAACCCCTGAAGTAGAGAAAGACCTTGAAAAGCTTGGTTGGACAAA
AATTAAACGGGCAACACCATGAGTTTGTATGGAAATGCCAGTGTCAACCCAGGACCGGGTCAT
GGCATCACCTTACTCGACAACAAAATATCAGTGGCAGCCAAAACGGCCGCTTGATCTACCCAG
30 CGATTGTGCTTGATCGTTTCTTCTTGAACGTCATGTCTATTTACAGTGATGATGGCGGTTCAAA
CTGGCAAACCGGTTCAACACTCCCTATCCCTTTTCGCTGGAAGAGTTCGAGTATCCTAGAACT
CTCGAACCTAGTGAAGCTGATATGGTTGAACTCCAAAACGGTGATCTACTCCTTACTGCACGCC
TTGATTTTTAACCAAATCGTTAATGGTGTGAACTATAGCCACGCCAGCAATTTTTGAGTAAAGA
TGGTGAATCACGTGGAGCCTACTTGAGGCTAACAACGCTAACGTCTTTAGCAATATCAGTACT
35 GGTACCGTTGATGCTTCTATTACTCGGTTTCGAGCAAAGTGACGGTAGCCATTTCTTACTCTTTA
CTAACCACAAAGGAAACCTGCGGGGACAAATGGCAGGCAAAATCTAGGCTTATGGTTTAGCTT
CGATGAAGGGGTGACATGGAAAGGACCAATTCAACTTGTTAATGGTGCATCGGCATATTCTGAT
ATTTATCAATTGGATTTCGGAAAATGCGATTGTCTATTGTTGAAACGGATAATTCAAATATGCGAA
TTCTTCGTATGCCTATCACATTGCTAAAACAGAAGCTGACCTTATCGCAAACTAA

40

[00378] SEQ ID NO: 38:

MVGADPTRPRGPLSYWAGRRGQGLAAIFLLLVSAESEARAEDDFSLVQPLVTMEQLLWVSGKQ
IGSVDTFRITPLITATPRGTLLEFAEARKKSASDEGAKFIAMRRSTDQGSTWSSTAFIVDDGEAS
45 DGLNLGAVVNDVDTGIVFLIYTLCAHKVNCQVASTMLVWSKDDGISWSPPRNLSVDIGTEMFAP
GPGSGIQKQREPGKGRILIVCGHGTLELDGVFCLLSDDHGASWHYGTGVSIGIPFGQPKHDFNP
DECQPYELPDGSVIINARNQNNYHCRICRIVLRSYDACDTLRPRDVTFDPELVDPVVAAGALATS
SGIVFFSNPAHPEFRVNLTLRWSFSNGTSWLKERVQVWPGPSGYSSLTALENSTDGKKQPPQLF
VLYEKGLNRYTESISMVKISVYGT

50

[00379] SEQ ID NO: 39:

MTVQPSPWFSDLRPMATCPVLQKETLFRITGVHAYRIPALLYLKKQKTLAFAEKRAKSTDEHAE
LIVLRRGSYNEATNRVKWQPEEVVTQAQLEGHRSMNPCPLYDKQTKTLFLFFIAVPGRVSEHHQ
5 LHTKVNVTSLCCVSSTDHGRTWSPIQDLTETTIGSTHQEWATFAVGPGHCLQLRNPAGSLLVPA
YAYRKLHPAQKPTPFAFCFISLDHGHTWKLGNFVAENSLECVAEVGTGAQRMVYLNARSFLGA
RVQAQSPNDGLDFQDNRVSKLVEPPHGCHGSVVAFHNPISKPHALDTWLLYTHPTDSRNRNL
GVYLNQMPDLPTAWSEPTLLAMGICAYSDLQNMGGQPDGSPQFGCLYESGNYEEIIIFLIFTLKQ
10 AFPTVFDAQ

[00380] SEQ ID NO: 40:

MEEVPPYSLSSTLFQQEEQSGVTYRIPALLYLPPTHTFLAFAEKRTSVRDEDAACLVLRRLMK
GRSVQWGPQRLMEATLPGHRTMNPVWEKNTGRVYLFFICVRGHVTERCQIVWGKNAARLCF
15 LCSEDAGCSWGEVKDLTEEVIKSEVKRWATFAVGPGHGIQLHSGRLIIPAYAYYVSRWFLCFAC
SVKPHSLMIYSDDFGVTWHHGKFIQVGTGECQVAEVAGTAGNPVLYCSARTPSRFRAEAFSTD
SGGCFQKPTLNPQLHEPRTGCQGSVVSFRPLKMPNTYQDSIGKGAPATQKCPLLDSPLEVEKGA
ETPSATWLLYSHPTSKRKRINLGIYYNRNPLEVNCWSRPWILNRGPSGYSDLAHVVEQDLVACL
20 FECGEKNEYERIDFCLFSDHEVLSCEDCTSPSSD

[00381] SEQ ID NO: 41:

METAGAPFCFHVDSLVPCTSYWKVMGPTRVPRRTVLFQORERTGLTYRVPALLCVPPRPTLLAFAE
QRLSPDDSHAHRLVLRRLTLTRGSRWGTLSVLETAVLEEHRSMNPCPVLDEHSGTIFLFFIAV
25 LGHTPEAVQIATGKNAARLCCVTSCDAGLTWGSVRDLTEEAIGAALQDWATFAVGPGHGVQLRS
GRLLVPAITYHVDRRECFCGKICWTSPHSLAFYSDDHGISWHCGGLVPLNLSGECQLAAVDGDFL
YCNARSPLGNRVQALSADGTSFLPGELVPTLAETARGCQGSIVGFLAPPSIEPQDDRWGTGSPR
NTPHSPCFNLRVQESSGEGARGLLERWMPRLPLCYPQSRSPENHGLEPGSDGDKTSWTPECPMS
SDSMLQSPTWLLYSHYPAGRRARLHMGIIYLSRSPLDPHSWTEPWIIYEGPSGYSDLAFLGPMPGA
30 SLVFACLFESGTRTSYEDISFCLFSLADVLENVPTGLEMLSLRDKAQGHWCPS

[00382] SEQ ID NO: 42:

GGGTCACATGCTGATGGACTAATTGGAGTCGCGGCAGCGCGGGCTGCGGCCCCCAAGGGGAGGG
35 GTCGGAGTGACGTGCGCGCTTTTAAAGGGCCGAGGTGAGCTGACGGCTTGCCACCGGTGACCAG
TTCTTGACAGGGATCGCCGGGAGCTATGGTGGGGGCAGACCCGACCAGACCCCGGGGACCGCT
GAGCTATTGGGCGGGCGCTCGGGGTGAGGGGCTCGCAGCGATCTTCTGCTCCTGGTGTCCGCG
GCGGAATCCGAGGCCAGGGCAGAGGATGACTTCAGCCTGGTGCAGCCGCTGGTGACCATGGAGC
AGCTGCTGTGGGTGAGCGGGAAGCAGATCGGCTCTGTAGACACTTTCCGCATCCCGCTCATCAC
40 AGCCACCCCTCGGGGCACGCTCCTGGCCTTCGCTGAGGCCAGGAAAAATCTGCATCCGATGAG
GGGGCCAAGTTCATCGCCATGAGGAGGTCCACGGACCAGGGTAGCACGTGGTCTCTACAGCCT
TCATCGTAGACGATGGGGAGGCCTCCGATGGCCTGAACCTGGGCGCTGTGGTGAACGATGTAGA
CACAGGGATAGTGTTCCTTATCTATAACCTCTGTGCTCACAAGGTCAACTGCCAGGTGGCCTCT
ACCATGTTGGTTTGGAGTAAGGACGACGGCATTTCCTGGAGCCCACCCCGGAATCTCTGTGG
45 ATATTGGCACAGAGATGTTTGCCCCTGGACCTGGCTCAGGCATTCAGAAACAGCGGGAGCCTGG
GAAGGGCCGGCTCATTGTGTGTGGACACGGGACGCTGGAGCGAGATGGGGTCTTCTGTCTCCTC
AGTGATGACCACGGTGCCTCCTGGCACTACGGCACTGGAGTGAGCGGCATTCCCTTTGGCCAGC
CCAAACACGATCACGATTTCAACCCCGACGAGTGCCAGCCCTACGAGCTTCCAGATGGCTCGGT
CATCATCAACGCCCGGAACCAGAATAACTACCATTGCCGCTGCAGGATCGTCTCCGCAGCTAT
50 GACGCCTGTGACACCCTCAGGCCCCGGGATGTGACCTTCGACCCTGAGCTCGTGGACCCTGTGG
TAGCTGCAGGAGCACTAGCCACCAGCTCCGGCATTGTCTTCTTCTCCAATCCAGCCCACCCTGA
GTTCCGAGTGAACCTGACCCTGCGCTGGAGTTTCAGCAATGGTACATCCTGGCAGAAGGAGAGG

GTCCAGGTGTGGCCGGGACCCAGCGGCTACTCGTCCCTGACAGCCCTGGAAAACAGCACGGATG
GAAAGAAGCAGCCCCCGCAGCTGTTTCGTTCTGTACGAGAAAGGCCTGAACCGGTACACCGAGAG
CATCTCCATGGTCAAAATCAGCGTCTACGGCACGCTCTGAGCCCCGTGCCCAAAGGACACCAAG
TCCTGGTCTGCTGACTTCACAGCTCTCTGGACCATCTGCAGAGGGTGCCTGAAACACAGCTCTTC
5 CTCTGAACTCTGACCTTTTGCAACTTCTCATCAACAGGGAAGTCTCTTCGTTATGACTTAACAC
CCAGCTTCCTCTCGGGGCAGGAAGTCCCTCCGTCACCAAGAGCACTTTTTTCCAGTATGCTGGG
GATGGCCCCCTGTCCATTCTCTTCCAGGACAACGGAGCTGTGCCTTTCTGGGACAGGATGGGGGA
GGGGCTCCCCCTGGAGAGATGAACAGATACGAACTCAGGGAAGTGAAGGCCCGGTGTCCTAG
GGTACAAAGGCAGGTACTAGATGTGATTGCTGAAAGTCCCCAGGGCAGAGTGTCTTTTCAGAGC
10 AAGGATAAGCACACCTACGTGTGCACCTTTGATTATTTATGAATCGAAATATTTGTAACCTTAA
ATTTTTGATGCAGAAAAAGCGTTTGTGGAGTCTGTGGTTCTGTCTGCTCACGCCTTCCCAATTG
CCTCCTGGAGAGACAGGAAGGCAGCTGGAAGAGGAGCCGATGTACTTACTGGGAAGCAGAAACC
CCTAGATTCCATCCTGGCTGCTGCTGTTTGCAAGTGTCAAAGATGGGGGGGCGTGTTTATATTT
TATATTTCTAAGATGGGGTGGCATAGGAAATAGGGAACAGATGTGTAAAACCAGATGGGAAGGA
15 CAGTCTGTGAGAAAGGAGCAAGCAGTTGCTGCAGGTGTGGGAGAGCAAAGCCCTTCTCCACGTG
GAAAGAGCCCAGATGGACGCTAAGCATGTTGGGCACCTGTAACCCCGCACTCGCTGGACTGACG
GTGTAGCTCAGTGGTGGAGCTAGTACTTGAACGCCTAAGACTCTGGGTTTCACTCCTTGGGGGG
GGGGGTATGTGTTTATTGAGAGGAAGGTGTACGTACTGTAGGTCAGAGGACAGCTTACTGGAGT
TGTCTCTCTCCTTACGCTGTGAGTCTGTGGAATGACCTCAGGTGTGAGAGTTGGGGGCAGGT
20 GCCTTTGCCAGCTGAGCCATCTTGCTGTCTCTGCTTTAATTTAAAAAAAAAAAAAAAAAAGAAT
ATTAAGGTCTGAGGGATTCGGGCTGCGTTCATTTCAATTAGAGGGTCATATTTCTTTTGACATT
TCTTCTCTAAGAAATGTTAAGATCATTTGTTCTGTGTGATAGAGGTATAGCTCCATTGTATGTC
AGCAGTGAGGGATCCTGTGCATTTTATCCAGAGTTTGTACGGTGTCTAGGGGCTGCTAGTGCA
GCCCAGTGCTAAACACTTCAGCATGCACAAGGCCTCAATCAGTGCATGCATGTGCACACACACA
25 CAGACACACACGTACACACTGACACAGGTACACAAATACACACTGGCCACATGTACACATCGA
CTCACAGGTACACAGACCCACTTTGACACACATATACACAGACACAAACGCCTGGCACACACA
TATACACAGGCACACATGGATAGATGGACACACGTGTACACATACACACACACAGAAATACA
AATGTTTCAAGTTTTCTAAAAAAAAAAAAAATTAGAGACGTGTTGACTTCATTTTTAGCAAAAATC
CTGTCTATGTATCTTAAAGTGGATTGAACCCACTATGTAGCCCAGGCTGGCCTCCAAATGGGCAT
30 CCTTCTGCCTCAGTCTCCCGAGGGCTAGGATAACAGGAGTATGCCATCACACCTGGCTAATAGA
AATTTTCAAAATTGTTTGTGTTGAAGGTGACTCTTACTATATTGCCTAACTGATCTCCAGTTTCGT
GAAATCCTCCTGCCTCAGAACCAGGACTGTCAATATAACCCACCAAGACAGGCCAACATTCACA
ATTGATTGTTAGTTTGTGGTCTGAATCAAGGTCTTATACTGTAGCCCAGGCTAGCCCGGAATAC
ACGATATCTCCAGTGCTTCAGATCCTCAGTTCTAATAAGCATGGCCACATCCATGTTTAACTG
35 CAAATTTGATGTTACCATGGTTTGGTTTGGTTTGGTTTGGTTTGGTTTGGTTTGGTTTGGTTT
TTGGCCATTTTTTTTTTCTCATGCTGAGGCCTTGTGCTCTCAAGTTGGGGAGACAGCATGGAGG
GTAGCTGCAACTGTAACCCAGTTCCAGGGGACCTGACACCCTCTGGCCTCCACAAGTATTAGG
CACATCTGTGGTGCACAGACATACAATCAGGCAAAATATTCATACACATAAAATAAAATAATTT
AAAACAAAAGCAAAATCAGGACCTAAGAAAAAAATCTATTCTGATTCTTTTATGTTTTGTTT
40 GTATTTTATCAAGACAGGGTTGTTTCTCTGTATAGCCCTGGCTGTCTTGGAATTCAGTCTGTAG
ACCAGGCTGGCCTCAAACTCAGAAATCCTCCTGCCTTTGCCTTCCAAGTGCTGGAATTAAAGGC
ATGCGCCACC

[00383] SEQ ID NO: 43:

45 GACATGACCCAAACGGCCCCCTGGCTGCAAGGTAATATCGGAAGTTGACTAAGAATGGACGCCCC
ACCACTGACTGACCCGCCCCCTGAGTCTGAGATTGGACTTGTCTCTGGATACAGTCATACTTTG
AGGTACTACAAGTTAGAACTGTTAGGTTACTCAGTTTCACTCCATGACAGTCCAACCTTCTCCA
TGGTTTTTCCGATCTCAGGCCCATGGCGACCTGCCCTGTCCTGCAGAAGGAGACACTGTTCCGCA
50 CAGGCGTCCATGCTTACAGAATCCCTGCTCTGCTCTACCTGAAGAAGCAGAAGACCCTGCTGGC
CTTTGCGGAAAAGCGAGCCAGCAAGACGGATGAGCACGCAGAGTTGATTGTCCTGAGAAGAGGA
AGCTACAACGAAGCCACCAACCGTGTCAAGTGGCAGCCTGAGGAAGTGGTGACCCAAGCCCAGC

TGGAAGGCCACCGCTCCATGAATCCATGTCCCTTGTATGACAAGCAAACAAAGACCCCTCTTCCT
TTTCTTCATCGCTGTCCCTGGGCGTGTATCAGAACATCATCAGCTCCACACTAAGGTAAATGTC
ACACGGCTGTGCTGTGTGTCAGCAGCACTGACCATGGGAGGACCTGGAGCCCCATCCAGGACCTCA
CAGAGACCACCATTTGGCAGCACTCATCAGGAATGGGCCACATTTGCTGTGGGTCCTGGGCATTG
5 TCTGCAGCTGCGGAACCCAGCTGGGAGCCTGCTGGTACCTGCTTATGCCTACCGGAAACTGCAC
CCTGCTCAGAAGCCTACCCCTTTGCCTTCTGCTTCATCAGCCTTGACCATGGGCACACATGGA
AACTAGGCAACTTTGTGGCTGAAAACCTCACTGGAGTGCCAGGTGGCTGAGGTTGGCACTGGAGC
TCAGAGGATGGTATATCTCAATGCTAGGAGCTTCCTGGGAGCCAGGGTCCAGGCACAAAGTCCT
AATGATGGTCTGGATTTCCAGGACAACCGGGTAGTGAGTAAGCTTGTAGAGCCCCCCCCACGGGT
10 GTCATGGAAGTGTGGTTGCCTTCCACAACCCCATCTCTAAGCCACATGCCTTAGACACATGGCT
TCTTTATACACACCCTACAGACTCCAGGAATAGAACCAACCTGGGTGTGTACCTAAACCAGATG
CCACTAGATCCCACAGCCTGGTCAGAGCCCACCCTGCTGGCCATGGGCATCTGTGCCTACTCAG
ACTTACAGAACATGGGGCAAGGCCCTGATGGCTCCCCACAGTTTGGGTGTCTGTATGAATCAGG
TAACTATGAAGAGATCATTTTCCTCATATTCACCCTGAAGCAAGCTTTCCTCCACTGTATTTGAT
15 GCCCAGTGATCTCAGTGCACGTGGCCCAAAGGGCTTCCTTGTGCTTCAAACACCCATCTCTCT
TTGCTTCCAGCATCCTCTGGACTCTTGAGTCCAGCTCTTGGGTAACTTCCTCAGGAGGATGCAG
AGAATTTGGTCTCTTGACTCTCTGCAGGCCTTATTGTTTCAGCCTCTGGTTCTCTTTTCAGCCC
AGAAATCAAAGGAGCCTGGCTTTCTCAGCCTGTTGGCAGGGCAGGTGGGGACAGTATATATAG
AGGCTGCCATTCTGCATGTCGGTTGTCACTATGCTAGTTTAACTGCCTGTTTCCCATGCCTA
20 GTGTTTGAATGAGTATTAATAAAATATCCAACCCAGCCCATTCTTCCTGGAAAAAA

[00384] SEQ ID NO: 44:

ACTGCGCGGTGAAGGGGCGTGGCCTGGCCGGGGAGGTTGACACCCAGACGCTGCTCTCAGTCCT
25 CTGGCGCCTGCTCCCCAGCGCATTCCTTCTGCTCCTGGGATATTTGTCTCATTACTGCCAGTTC
TTGCGCAGCGGTCACTGGGTTTCGTTTCAGCGTCTGTGGTTTCTGTGCTGTTATCCAGTCTCCA
TCGCCCCAGCTCAGCTTCAGGCCTTCTTCCGAGACTCCACGGGAGAGCCCAGAGAGCCTCCGGA
GCCGAAGCCATGGAGGAAGTCCCACCCTACTCCCTCAGCAGCACCTGTTCCAGCAGGAAGAAC
AGAGTGGGGTGACCTACCGGATCCCAGCCCTGCTGTACCTTCCTCCCACCCACACCTTCCTGGC
30 CTTTGCAGAGAAGCGGACCTCAGTCAGAGATGAGGATGCTGCCTGCCTGGTGCTCAGACGAGGG
CTGATGAAGGGGCGCTCTGTACAGTGGGGCCCCCAACGGCTACTGATGGAGGCCACATTACCTG
GGCATCGCACCATGAACCCCTGCCCTGTGTGGGAGAAAAATACTGGCCGTGTGTACCTGTTTTT
CATCTGTGTGCGGGGCCATGTTACTGAGAGGTGCCAGATTGTGTGGGGCAAAAATGCCGCCCCGT
CTCTGCTTCCCTTTGCAGTGAAGATGCCGGCTGCTCTTGGGGTGAAGTGAAAGACTTGACCGAGG
35 AGGTCATTGGCTCAGAGGTGAAGCGCTGGGCCACATTTGCTGTGGGCCCAGGTCATGGCATCCA
GCTACACTCGGGAAGGCTGATCATCCCCGCCTATGCCTACTATGTCTCACGTTGGTTTCTCTGC
TTTGCGTGTTTCAGTCAAGCCCCATTCCCTGATGATCTACAGTGATGACTTTGGAGTCACATGGC
ACCATGGCAAGTTCATTGAGCCCCAGGTGACAGGGGAGTGCCAAGTGGCCGAAGTGGCTGGGAC
GGCTGGTAACCCCTGTGCTCACTGCAGTGCCCGAACACCAAGCCGATTTTCGAGCAGAGGCTTTTA
40 GTACTGATAGTGGTGGCTGCTTTCAGAAGCCAACCCCTGAACCCACAACCTCCATGAGCCTCGAAC
CGGCTGCCAAGGTAGTGTAGTGAGCTTCCGGCCTTTGAAGATGCCAAATACCTATCAAGACTCA
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GATTAACCTAGGCATCTACTACAACCGGAACCCCTTGGAGGTGAACTGCTGGTCCCCGCCCGTGG
45 ATCTTGAACCGTGGGCCCAGTGGCTACTCTGATCTGGCTGTTGTGGAAGAACAGGACTTGGTGG
CGTGTTTGTGTTGAGTGTGGGGAGAAGAATGAGTATGAGCGGATTGACTTCTGTCTGTTTTTCAGA
CCATGAGGTCCTGAGCTGTGAAGACTGTACCAGCCCTAGTAGCGACTAAAGCCAAATCAAGACG
GATGAGTGAGGCCCAGCTTCCCACAGAAAGGAATGGCAGCTACAGCCAGGGTAACAGAGGTCTC
TGATGTCTAGAGAAAACCTCTAAAACTAATAATCTGCTCCTTGAATTTTTTCACTTTTCCCTTC
50 AATGAGCATGGTGAAAATTGTGCCATATCTTACATAACGAGGCTCTTGAAGTGGGAGTTTGAAT
CTCTTCTCTTCCCATTAAGAGGAGAGGCCATGTGCTCGCTTCGCGTTTCGACAAAGCCTGGATTC
TGATCTTGAGTGGAAGCCACAGGCTTGTCTTTTCCAATGGTTCACTGCTCACCTGAGTATTAGG

5 TGATGTGTAGGTGCCTTGGCCAGAAGAAAGATCTGTGTTGTTGTATTTTTTTTAAATTTATTTAT
TTACTATATGTAAGTACACTGCAGCTGTCTTCAGACACACCAGAAGAGGGCGTCAGATCTCATT
AGAGATGGTTGTGAGCCACCATGTGGTTGCTGGGATTTGAACTCAGGACCTTCAGAAGAGCAGT
CAGTGCTCTTAATACTACTGAGCCATCTCTCAAGCCCCGCATTGCTGTATTTTTTAATAAGAAAAAT
10 GCCCTTATCCTTCCAATAATGCCTGGAGCTGTACAAATTCTCTGTCTTAGAAGACTTGAGAAAG
CAGAACTGTAAGGTCAGATGCTTTCTCCAGCCTTGATGCTGTGTTCCACCTTCCCTTCCTCATC
CAGAAAACAGTTACTAGGGAGAAAATGAGAAACCCATGCCAGCTGCCCTTGATGATGGTTGATA
ACGGTGCTTATTGCTTTTGATGTCATTACCTCTGTTAGAGATGAATCAGAGTCAGAGGTCCTTA
GCTGCATCCACCCATTTCCAGGGGGACATTCTAACACTGCTGAACAGTCAGCTAAAATGAGAGC
15 TGTGTGTCTTAGCCTGATTCCAGGTTAGTCATGATGCTTCCTGGAGCTGGGCTTTTATCTAATC
CCAGGAGCCATCTAGGGGAGGCTCAGAGCTAGCAGGTGATCTTCCTGAGATGGTTTCACCGTGA
CAGGTGAACCATGAGCCCTTCCAAGCAAGGCCAAAGGACAACATTATAGGAAAGATTTCTAGTA
TTAATATGCCTTTTCTCTGTGTGTGTACTGTCTTGTAGTGATGCTATATAGACAAATAGATGAT
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20 ACTTTGTAAACCAGGCTGGCCTCGATCTCAGAAATCCGCCTGCCTCTGCCTCCCGAGTGCTGGG
ATTAAAGGTGTGCACCACCACACCTTAATGATGATCCTATAAGTATTCCTAAAATTATACTAGT
AATTATTAACCTCCTTTATAATAGGACTGCTATTAAAGCCCTCGCTGATATGAAAACCTACAGTGA
GAACTCTGCCAGTCTTCACATGTCATAATTACTTCTGAGATAGAAAGCAGGCATTTACAACCTTA
GAACACATTTCTTAGAGCTGTAAAACAATTAAGTAGAGGTCATAAAAGGGAATGAAAGATTTAT
25 TGTAGGTGCTAGGACAGAACATAAAATATTGACTGGGCTTATCTATATGAAACTTCATTGTTAA
CTTTTACACAAGAATTATGGTTTTTAACTTTTCACTGTAACCTGCGGAGCTAGTGACAGAAGAGAA
ATGTCTAGTTAGATAACTACTCTTAATGGAAATTCACATAAACATCTGTTGCCATCTTCTTTTT
GAATTTATGTTTAACTTGTGAATGTTTGAATTAGACACTACGCGAGCACATAGAAAATAAAGA
ACTAAGCGTGAA

25

[00385] SEQ ID NO: 45:

30 GGACAGTGTGCATCACGGAGCTTGTGGCCCAGACTGTGCCTGGCAGACCCAGAGGACCTAAGGC
TTGGCTCTAGTGGTGGTCAGCACAGCCCTCGGTGGTCTGCGGAGCCTGATATTGCTTTACGTAA
GGGCTGTTCTGCTGTGCATCTCCTGTGTCTGAAGCTATTCGCCATGGAGACTGCTGGAGCTCCC
TTCTGCTTCCATGTGGACTCCCTGGTACCTTGCTCCTACTGGAAGGTTATGGGGCCCACGCGTG
TTCCCAGGAGAACGGTGCTCTTCCAGAGGGAAAGGACGGGCCTGACCTACCGTGTGCCTGCGTT
ACTCTGTGTGCCTCCCAGGCCTACTCTGCTGGCCTTCGCGAACAGCGACTTAGCCCTGATGAC
35 TCCCATGCCACCGCCTGGTGCTACGGAGGGGCACGCTGACCAGGGGCTCAGTGCGGTGGGGCA
CTCTGAGTGTACTGGAGACTGCAGTACTGGAGGAGCACAGGTCTATGAACCTTGCCCGGTGCT
GGATGAGCACTCTGGTACCATCTTCCTCTTCTTCAATTGCCGTGCTGGGCCACACACCGGAGGCC
GTGCAAATCGCCACTGGCAAGAACGCTGCTCGCCTCTGCTGTGTGACCAGCTGTGACGCTGGCC
TCACCTGGGGCAGTGTTTCGAGATCTCACTGAGGAAGCCATTGGTGCTGCATTGCAGGACTGGGC
CACCTTTGCTGTGGGTCCGGGCCATGGAGTTCAGCTGCGCTCGGGTCGCTGCTTGTTCCTGCT
40 TACACCTATCATGTGGACCGACGGGAATGTTTTGGCAAGATCTGCTGGACCACTCCCCACTCCT
TGGCATTCTACAGTGATGATCATGGGATCTCCTGGCATTGTGGAGGCCTTGTGCCAACCTACG
CTCTGGAGAGTGCCAACTGGCTGCGGTAGATGGAGACTTTCTCTACTGTAATGCTCGAAGCCCT
CTGGGTAAACCGTGTGCAGGCACTGAGTGCTGATGAAGGCACGTCCTTCCTACCAGGGGAGCTGG
TGCCCTACATTGGCAGAGACGGCTCGTGGTTGCCAGGGTAGCATTGTGGGCTTCCTAGCTCCACC
45 CTCAATCGAGCCTCAGGATGACCGGTGGACAGGGAGTCTTAGGAACACCCACATTCCCCATGC
TTCAATCTCAGAGTACAGGAGTCTTCGGGGGAAGGTGCCAGAGGTCTTCTTGAACGTTGGATGC
CCAGGTTGCCTCTCTGCTACCCACAGTCCCGGAGCCCAGAGAATCATGGCCTAGAGCCTGGGTC
AGATGGAGATAAGACATCCTGGACTCCGGAATGTCCTATGTCCTCTGATTCCATGCTTCAGAGC
CCACATGGCTACTATATTCCCACCCAGCAGGGCGTAGAGCTCGGCTCCACATGGGAATCTACC
50 TGAGCCGATCCCCCTTGGATCCCCACAGCTGGACAGAGCCCTGGGTGATCTATGAGGGCCCCAG
TGGCTACTCTGACCTTGCCCTTCTTGGGCCTATGCCTGGGGCATCCCTGGTTTTTGCCTGTCTG
TTTGAGAGCGGGACCAGGACTTCCTATGAAGACATTTCTTTTTGCTTGTCTCACTGGCGGATG

TCCTGGAGAATGTGCCCACTGGCTTAGAGATGCTAAGTCTCAGGGATAAGGCTCAGGGGGCATTG
CTGGCCCTCTTGATGGCCTCACCCCTCTCGTAGCCGCCTGGAGAGGAAGGGTAGACTATATAGAG
GAGGTTAGGGGTAGGTCAGCATGATGCTAGGATGGAGAGAGCTCTGTCCCCTCGTGGATGGTGG
TGGTGA CTACCCGGGGGGCCAGCTGCTTTCTGAGTGCAAATGAGAAAAATAAAGAGCTGCGCT
5 GTGACTTTTCTTTCCACATCAAAGCTTGGGTGTCAGTGCTTTAGCTTGATGCTCTGATCACCAT
GCAAATCTTCCACCGGCGCCTTGCTCAGCTTTTCATATCCCAAGGGTGCCTGGGAGGAAGGCAAC
AGGGACAGTGGACATCACTGCACCACTTTCCACGACCCTGTGTGCCAACCTCAGCCACTTTGAA
ACATGCTGATGACTGAGGTCTGTTCACTTTCTTAATTTCAAGCAGGAGAAGCAGGTGGGGAGC
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10 CCATGTGCCTACTTCTTTACCCTTCTCTAGAGAGGGCTCTTGTCCTGTCAGAGCTGTTTTCTCC
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CTCTGTAGATCAGGCTGACCTTGAGTTCAAAGCTCCATCTGCCTCTACTTCTCACATTACTGTG
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GATTATAAAAACAGTCTGTGTGGGCTGGAGTGATGGCTTACTCAGTAAAGCACTTGCCATGGAA
15 TCTGGGCAATCTGAGTTTCATTTTTCAGCATCCTGTAAAAATCCCAATTTGATGGTGTACTTGTA
ATGTCAGCATGGAGAGGCAGAGATAGGTAAGTTCCTCAAGACTCTTTGAACCGACAGCTTGCC
TCACTGGCACATTCCAGGTCTCAGTGAGAGACCCTGCCTCAAATAACAAAGAAAGAGCTGCTGA
AGAGTGGGTGAGAGTTGACCTCTGATCTCCGGAAGTATATGATACACACCCGTGCATGCACTCT
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20 AGATTTAGAAAGCTATACATAAAAAAAATGACCTAAAGAAAAATCTTTACTGTTCTGGGCACT
ATCCCTATCAAACCACTGTGTTCTTTGGCCAAGCCTTGGGGTGGACACTGTTTTGAGGTGGGTC
CTGTTATCTCCACTAGGTAGTGGAGTTTTGTGTGACACTAACTGGGTCTTAAAGCTGTCTTTAA
GGCCATCAGGAGCTACTGACTTGCCCTGCCTCAGCAGAGCATATCCTGAAGGTCGGGGTTAAGTC
TCCTTCCCGAGCGAGTTGCCTTCCAGTGGGCCCTGGACTCCTAGGTCCTCAGCGCTCATCAGC
25 TGCCAAGGACTCTGAGGGAATGTCCTCTGACTGTGGCCCCGAAAGGTAGGGGAGGGGGATGTGC
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ACAGACTGGGCGAGCTTTAAAGGAAGGAGGTTGATATTGGTTCCCACTTCTGGGGATCATGGTT
GAGCAGCCTTGTCTGATGATGGTTGTCTTGATGGTAGATCGTGAGGTAGTTGATGAAGGTATGA
CATGGTGAGAACTCTGTGTGTGTGTGTTATTTTCTCTGTGTTCTACCTATACATCTATCTATG
30 TATATATGTATCTATCTATCTACCTGGAGGCTGGAGAGATAGCTTAGTGGTTAAGAACATTTGT
TGTTCTTGCATAGTCCTGGATTTAAATTTTCAGCACCCACATGGCAGCTCACAAACAACCCATAA
ATCCAGTTTCAGAGGATCCAACCTCTGATATACCATGTCAGCCAGAGCAGACACGGCTGAAGGT
GGTTTGATCCCCGTATGGAGAGGTGACAATTGGGAAGAGAGAAAGATCAACTTAACCATGCAAG
GAACAGGAAGTTAAATACTGAACAGGGAAGGTAAAGGCAGGAAGTAGATGTAGAGGGCAAATCA
35 ATGAAACCCAAACATAACCCAAATTACGCTAAACACACACTGACATGCCAATTAAAAGGACAAAT
TGGCTCCACTGGCAAAACCAAACAGACACTGAAGATCCAACAGTCACATGCCAACTACCGCG
GAGGGAGACAGACACAGAGAAGACCGTGACAGACACTTGGACACTCTTGAGAGTGGATGTGCAG
GAAGAGAGCTCTGCCAGTGGAGAAGAAAGCACTCAGAAGAAAGTGACAGCAGCTGTAAATTTGT
ATTCTGCTAATGTTATGTTCCAAAGTTGAAAGCAAAATTTGTACCAATTCATAAGAACAAACAGG
40 CTGACTCTCAGTTGTGACTGAACGTCTCTCAGTAACTGACGGGGCGAGCAGGCCAAAGGAGAGT
CGGCTCAGAAGGGTGCATAGCCACGCCAAATCAAATAAGCAAGTACAACCGGCAGGCTCTATTT
CTAGCACAAAGGGGTCTGTGCCTCATTCTGTGCTTGGGTGAGAGCTTGGGTCTCTCATTTGGAT
GTAAGTGGTGTAGTGGAGAAGCAGGAAATAATCCGGAGCGCATATTTTGATTTTAAACATAAGTG
CTGATTTGGGAGGGAGTTTTGTCAAATTGTGTTTTTACAATGTTTTTTTTTTTTTAAATGATGC
45 TTTTTTGTAAAGTGACAAATGTGATATAAGATTGGTTCTGCTACATTCTATATAAAGT
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TATGTAATAATTAATATTAGAAGAAAATATAATTTATTTGAATATAAAAAAAAAAAAAAAAAA

[00386] SEQ ID NO: 46:

50

X₁ASLPX₂LQX₃ESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAX₄
THQVQWQAQEVVAQARLDGHRSMNPCPLYDX₅QTGTLFLFFIAIPGQVTEQQQLQTRANVTRLQ

VTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARS LVVPAYAYRKLHPX₆QRP
 IPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLD
 FQESQLVKKLVEPPX₇GCQGSVISFPSRSGPGSPAQWLLYTHPTHX₈X₉QRADLGAYLNPRPP
 APEAWSEPVLLAKGSX₁₀AYSDLQSMGTGPDGSPLFGCLYEANDYEEIX₁₁FX₁₂MFTLKQAFPAE
 YLPQ

[00387] SEQ ID NO: 47:

X₁X₂SX₃X₄X₅LQX₆ESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASX₇X₈DEHAELIVX₉RRGD
 YDAX₁₀THQVQWX₁₁AQEVVAQAX₁₂LDGHRSMNPCPLYDX₁₃QTGTLFLFFIAIPX₁₄X₁₅VTEX₁₆Q
 QLQTRANVTRLX₁₇X₁₈VTSTDHGRTWSSPRDLTDAAIGPX₁₉YREWSTFAVGPGHX₂₀LQLHDX₂₁
 RSLVVPAYAYRKLHPX₂₂QRPIPSAFX₂₃FLSHDHGRTWARGHFVAQDTX₂₄ECQVAEVETGEQRV
 VTLNARSHLRARVQAQX₂₅NX₂₆GLDFQX₂₇SQLVKKLVEPPX₂₈GX₂₉QGSVISFPSRSGPGSP
 AQX₃₀LLYTHPTHX₃₁X₃₂QRADLGAYLNPRPPAPEAWSEPX₃₃LLAKGSX₃₄AYSDLQSMGTGPDGS
 PLFGX₃₅LYEANDYEEIX₃₆FX₃₇MFTLKQAFPAEYLPQ

[00388] SEQ ID NO: 48:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASKKDEHAELIVLRRGDYDAGTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARS LVVPAYAYRKLHPKQRP
 IPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQLVKKLVEPPPQGCQGSVISFPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
 WSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQ

[00389] SEQ ID NO: 49:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASKKDEHAELIVLRRGDYDAPTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARS LVVPAYAYRKLHPKQRP
 IPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQLVKKLVEPPPQGCQGSVISFPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
 WSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIRFIMFTLKQAFPAEYLPQ

[00390] SEQ ID NO: 50:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASKKDEHAELIVLRRGDYDANTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARS LVVPAYAYRKLHPKQRP
 IPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQLVKKLVEPPPQGCQGSVISFPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
 WSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQ

[00391] SEQ ID NO: 51:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASKKDEHAELIVLRRGDYDAPTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARS LVVPAYAYRKLHPKQRP
 IPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQLVKKLVEPPPAGCQGSVISFPSRSGPGSPAQWLLYTHPTHKQRADLGAYLNPRPPAPEA
 WSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQ

[00392] SEQ ID NO: 52:

DASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDASTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
5 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPAGCQGSVISFSPRSGPGSPAQWLLYTHPTHRQKQADLGAYLNPRPPAPEA
WSEPVLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

10 [00393] SEQ ID NO: 53:

DASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDATH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
15 SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPAGCQGSVISFSPRSGPGSPAQWLLYTHPTHRQKQADLGAYLNPRPPAPEA
WSEPVLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

[00394] SEQ ID NO: 54:

20 DASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDANTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
25 ESQLVKKLVEPPPAGCQGSVISFSPRSGPGSPAQWLLYTHPTHRQKQADLGAYLNPRPPAPEA
WSEPVLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

[00395] SEQ ID NO: 55:

30 ASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTHQ
VQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVTS
TDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
AFCFLSHDHGRTWARGHFVAQDTLECQVAEVEETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
SQLVKKLVEPPPQGCQGSVISFSPRSGPGSPAQWLLYTHPTHSWQADLGAYLNPRPPAPEA
35 SEPVLAKGSCAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

[00396] SEQ ID NO: 56:

MASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
40 SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPQGCQGSVISFSPRSGPGSPAQWLLYTHPTHSWQADLGAYLNPRPPAPEA
WSEPVLAKGSCAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

[00397] SEQ ID NO: 57:

45 ASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTHQ
VQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVTS
TDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
AFCFLSHDHGRTWARGHFVAQDTLECQVAEVEETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ

SQLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEAW
SEPVLLAKGSAAYSIDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

[00398] SEQ ID NO: 58:

5 DASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRWTWSSPRDLTDAAGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRWTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
10 ESQVLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLLAKGSAAYSIDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

[00399] SEQ ID NO: 59:

DASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
15 STDHGRWTWSSPRDLTDAAGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRWTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQVLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLLAKGSCAYSIDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

[00400] SEQ ID NO: 60:

AASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRWTWSSPRDLTDAAGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRWTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
25 ESQVLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLLAKGSAAYSIDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

[00401] SEQ ID NO: 61:

MASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
30 STDHGRWTWSSPRDLTDAAGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRWTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQVLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLLAKGSAAYSIDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ
35

[00402] SEQ ID NO: 62:

AASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRWTWSSPRDLTDAAGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
40 SAFCFLSHDHGRWTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQVLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLLAKGSCAYSIDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

[00403] SEQ ID NO: 63:

45 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSGGITFYADTV
KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARIKLGTVTTVTDYWGQGTLLTVSSASTKGPSV

5 FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
 SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
 SRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
 EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSP
 GK

[00404] SEQ ID NO: 64:

10 QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIDVSNRPSGVSNRF
 SGSKSGNTASLTISGLQAEDEADYICSSYTSSSTRVFGTGTKVTVLGQPKANPTVTLFPPSSEE
 LQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSH
 RSYSCQVTHEGSTVEKTVAPTECS

[00405] SEQ ID NO: 65:

15 QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIDVSNRPSGVSNRF
 SGSKSGNTASLTISGLQAEDEADYICSSYTSSSTRVFGTGTKVTVLGQPKANPTVTLFPPSSEE
 LQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSH
 RSYSCQVTHEGSTVEKTVAPTECS

20 **[00406] SEQ ID NO: 66:**

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSSGGITFYADTV
 KGRFTISRDNSTLYLQMNLSRAEDTAVYYCARIKLGTVTTVDYWGQGLTVTVSSASTKGPSV
 FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
 25 SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
 SRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
 EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEW
 ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSP
 GK

30 **[00407] SEQ ID NO: 67:**

DASLPYLQKESVVFQSGAHAYRIPALLYLPQGQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 35 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLVVPAYAYRKLHPKQRP
 SAFCFSLSHDHGRTWARGHFVAQDTLEQVAEVEETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQLVKKLVEPPPQGCQGSVISFPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
 WSEPVLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQGGGG
 SGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 40 PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 TSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK

[00408] SEQ ID NO: 68:

45 DASLPYLQDES VVFQSGAHAYRIPALLYLPQGQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLVVPAYAYRKLHPKQRP
 SAFCFSLSHDHGRTWARGHFVAQDTLEQVAEVEETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ

ESQLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVL LAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIRFIMFTLTKQAFPAEYLPQGGGG
SGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
5 PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
TSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

[00409] SEQ ID NO: 69:

10 DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDANTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGR TWSSPRDLTDA AIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
SAFCFLSHDHGR TWARGHFVAQDTLE CQVAE VETGEQ RVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVL LAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQGGGG
15 SGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
TSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

20 [00410] SEQ ID NO: 70:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGR TWSSPRDLTDA AIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
SAFCFLSHDHGR TWARGHFVAQDTLE CQVAE VETGEQ RVVTLNARSHLRARVQAQSTNDGLDFQ
25 ESQLVKKLVEPPPAGCQGSVISFSPSRSGPGSPAQWLLYTHPTHRKQRADLGAYLNPRPPAPEA
WSEPVL LAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQGGGG
SGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
30 TSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

[00411] SEQ ID NO: 71:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDASTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
35 STDHGR TWSSPRDLTDA AIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
SAFCFLSHDHGR TWARGHFVAQDTLE CQVAE VETGEQ RVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPAGCQGSVISFSPSRSGPGSPAQWLLYTHPTHRKQRADLGAYLNPRPPAPEA
WSEPVL LAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQGGGG
SGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
40 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
TSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

[00412] SEQ ID NO: 72:

45 DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDATH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGR TWSSPRDLTDA AIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
SAFCFLSHDHGR TWARGHFVAQDTLE CQVAE VETGEQ RVVTLNARSHLRARVQAQSTNDGLDFQ

ESQLVKKLVEPPPAGCQGSVISFSPSRSGPGSPAQWLLYTHPTHRQKQADLGLAYLNPRPPAPEA
 WSEPVLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQGGGG
 SGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 5 PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 TSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[00413] SEQ ID NO: 73:

DASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASKKDEHAELIVLRRGDYDANTH
 10 QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRWTWSSPRDLTDAAGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
 SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQLVKKLVEPPPAGCQGSVISFSPSRSGPGSPAQWLLYTHPTHRQKQADLGLAYLNPRPPAPEA
 WSEPVLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQGGGG
 15 SGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 TSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[00414] SEQ ID NO: 74:

X₁ASLPX₂LQX₃ESVVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASKKDEHAELIVLRRGDYDAX₄
 THQVQWQAQEVVAQARLDGHRSMNPCPLYDX₅QTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQ
 VTSTDHGRWTWSSPRDLTDAAGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPX₆QRP
 25 IPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLD
 FQESQLVKKLVEPPPX₇GCQGSVISFSPSRSGPGSPAQWLLYTHPTHX₈X₉QADLGLAYLNPRPP
 APEAWSEPVLAKGSX₁₀AYSDDLQSMGTGPDGSPLFGCLYEANDYEEIX₁₁FX₁₂MFTLTKQAFPAE
 YLPQGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 30 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 DSDGSFFLTSTKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[00415] SEQ ID NO: 75:

X₁X₂SX₃X₄X₅LQX₆ESVVFQSGAHAYRIPALLYLPGQQSLLAFQAEQRASX₇X₈DEHAELIVX₉RRGD
 YDAX₁₀THQVQWX₁₁AQEVVAQAX₁₂LDGHRSMNPCPLYDX₁₃QTGTLFLFFIAIPX₁₄X₁₅VTEX₁₆Q
 QLQTRANVTRLX₁₇X₁₈VTSTDHGRWTWSSPRDLTDAAGPX₁₉YREWSTFAVGPGHX₂₀LQLHDX₂₁
 RSLVVPAYAYRKLHPX₂₂QRPISAFX₂₃FLSHDHGRTWARGHFVAQDTX₂₄ECQVAEVETGEQRV
 40 VTLNARSHLRARVQAQX₂₅NX₂₆GLDFQX₂₇SQLVKKLVEPPPX₂₈GX₂₉QGSVISFSPSRSGPGSP
 AQX₃₀LLYTHPTHX₃₁X₃₂QADLGLAYLNPRPPAPEAWSEPX₃₃LLAKGSX₃₄AYSDDLQSMGTGPDGS
 PLFGX₃₅LYEANDYEEIX₃₆FX₃₇MFTLTKQAFPAEYLPQGGGGSGGGGSDKTHTCPPCPAPELLGG
 PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
 VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
 45 TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSTKLTVDKSRWQQGNVFCFSVMH
 EALHNHYTQKSLSLSPGK

[00416] SEQ ID NO: 76:

CAATCTGCTCTTACACAGCCTGCCAGCGTGTCCGGATCTCCTGGCCAGAGCATCACCATCAGCT
 GTACCGGCACCAGCTCTGATGTCGGCGGCTACAATTACGTGTCCTGGTATCAGCAGCACCCCGG

CAAGGCCCCCTAAGCTGATGATCTACGACGTGTCCAACAGACCCAGCGGCGTGTCCAATAGATTC
TCCGGCAGCAAGAGCGGCAACACCGCCAGCCTGACAATTAGCGGACTGCAGGCCGAGGACGAGG
CCGATTACTACTGTAGCAGCTACACCAGCTCCAGCACCAGAGTGTTTGGCACCGGCACAAAAGT
GACCGTGCTGGGCCAGCCTAAGGCCAATCCTACCGTGACACTGTTCCCTCCAAGCAGCGAGGAA
5 CTGCAGGCTAACAAGGCCACACTCGTGTGCCTGATCAGCGACTTTTATCCTGGCGCCGTGACCG
TGGCCTGGAAGGCTGATGGATCTCCAGTGAAAGCCGGCGTGGAACCACCAAGCCTAGCAAGCA
GAGCAACAACAAATACGCCGCCAGCAGCTACCTGAGCCTGACACCTGAGCAGTGGAAGTCCCAC
AGATCCTACAGCTGCCAAGTGACCCACGAGGGCAGCACCGTGGAACCAACAGTGGCCCCCTACCG
AGTGCTCT

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[00417] SEQ ID NO: 77:

GAGGTGCAGCTGCTGGAATCTGGCGGAGGACTTGTTTCAGCCTGGCGGCTCTCTGAGACTGTCTT
GTGCCGCCAGCGGCTTCACCTTCAGCAGCTATATCATGATGTGGGTCCGACAGGCCCTGGCAA
15 AGGCCTTGAATGGGTGTCCAGCATCTATCCCAGCGGCGGCATCACCTTTTACGCCGACACAGTG
AAGGGCAGATTACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCC
TGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAGAATCAAGCTGGGCACCGTGACCACCGT
GGATTATTGGGGACAGGGCACCCCTGGTCACCGTGTCTGCTAGCACCAAGGGCCCCTCCGTC
TTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCA
20 AGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGCA
CACCTTCCCCGGCTGTCTTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG
TGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAACTCACACATGCCACCGTGCCCAGCACC
TGAACCTCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAGGACACCCCTCATGATC
25 TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGT
TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA
CAACAGCACGTACCGTGTGGTCAGCGTCCCTACCGTCCCTGCACCAGGACTGGCTGAATGGCAAG
GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAG
CCAAAGGGCAGCCCCGAGAACCACAGGTCTACACCCTGCCCCCATCCCGGGAGGAGATGACCAA
30 GAACCAGGTACGCTGTACTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGG
GAGAGCAATGGGCAGCCGGAGAACAATAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT
CCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG
GGTAAA

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[00418] SEQ ID NO: 78:

DASLPYLQKESVVFQSGAHAYRIPALLYLPQGQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLQVVT
40 STDHGRTWSSPRDLTDAAGPAYREWSTFAVPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPQGCQGSVISFPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQGGGG
SDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
45 VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLTSLKLT
VDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

[00419] SEQ ID NO: 79:

50 GATGCATCTCTGCCTTACCTGCAGAAAGAAAGCGTGTTCCAGTCTGGCGCCCACGCCTACAGAA
TTCCCGCTCTGCTGTATCTGCCAGGCCAGCAGTCTCTGCTGGCTTTCGCTGAACAGCGGGCCAG

CAAGAAGGATGAGCACGCCGAACCTGATCGTGCTGCGGAGAGGCGATTACGACGCCGGCAGACAT
CAGGTGCAGTGGCAGGCTCAAGAGGTGGTGGCTCAGGCTAGACTGGACGGCCACAGATCTATGA
ACCCCTGTCCTCTGTACGATGAACAGACCGGCACACTGTTTCTGTTCTTTATCGCTATCCCCGG
CCAAGTGACCGAGCAGCAGCAGCTGCAGACAAGAGCCAACGTGACCAGACTGTGTCAAGTGACC
5 TCCACCGACCACGGCAGAACCTGGTCTAGCCCTAGAGATCTGACCGACGCCGCCATCGGACCTG
CCTATAGAGAGTGGTCCACCTTCGCCGTTGGACCTGGACACTGTCTCCAGCTGCACGACAGGGC
TAGATCTCTGGTGGTGCCTGCCTACGCCTATAGAAAGCTGCACCCCCAACAGCGGCCTATTTCCT
AGCGCCTTCTGCTTTCTGAGCCACGATCACGGCAGGACATGGGCCAGAGGACATTTTCGTGGCCC
AGGACACACTGGAATGCCAGGTGGCCGAAGTGGAACCGGGCGAGCAGAGAGTCTGTGACCCTGAA
10 CGCCAGATCTCACCTGAGAGCCAGAGTGCAGGCCAGAGCACAAACGACGGCCTGGATTTCCAA
GAGAGCCAGCTGGTCAAGAACTGGTGGAACTCCTCCACAGGGCTGTCAGGGAAGCGTGATCA
GCTTTCCATCTCCTAGAAGCGGCCCTGGCTCTCCTGCTCAGTGGCTGCTGTATACACACCCAC
ACACAGCTGGCAGAGAGCCGATCTGGGCGCCTACCTGAATCCTAGACCTCCTGCTCCTGAGGCT
TGGAGCGAACCTGTTCTGCTGGCCAAGGGCAGCGCTGCCTACAGCGATCTGCAGTCTATGGGCA
15 CAGGCCCTGATGGCAGCCCTCTGTTTGGCTGTCTGTACGAGGCCAACGACTACGAAGAGATCGT
GTTTCCTGATGTTACCCCTGAAGCAGGCCTTTCCAGCCGAGTACCTGCCTCAAGGCGGAGGCGGA
TCCGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCT
TCCTCTTCCCCCAAACCCAAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGT
GGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
20 GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG
TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA
AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTCTACACCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGG
TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA
25 CTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCACTAGCAAGCTCACC
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
ACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

[00420] SEQ ID NO: 80:

30 GATGCATCTCTGCCTTACCTGCAGAAAGAAAGCGTGTTCCAGTCTGGCGCCCCACGCCTACAGAA
TTCCCGCTCTGCTGTATCTGCCAGGCCAGCAGTCTCTGCTGGCTTTTCGCTGAACAGCGGGCCAG
CAAGAAGGATGAGCACGCCGAACCTGATCGTGCTGCGGAGAGGCGATTACGACGCCGGCAGACAT
CAGGTGCAGTGGCAGGCTCAAGAGGTGGTGGCTCAGGCTAGACTGGACGGCCACAGATCTATGA
35 ACCCTGTCCTCTGTACGATGAACAGACCGGCACACTGTTTCTGTTCTTTATCGCTATCCCCGG
CCAAGTGACCGAGCAGCAGCAGCTGCAGACAAGAGCCAACGTGACCAGACTGTGTCAAGTGACC
TCCACCGACCACGGCAGAACCTGGTCTAGCCCTAGAGATCTGACCGACGCCGCCATCGGACCTG
CCTATAGAGAGTGGTCCACCTTCGCCGTTGGACCTGGACACTGTCTCCAGCTGCACGACAGGGC
TAGATCTCTGGTGGTGCCTGCCTACGCCTATAGAAAGCTGCACCCCCAACAGCGGCCTATTTCCT
40 AGCGCCTTCTGCTTTCTGAGCCACGATCACGGCAGGACATGGGCCAGAGGACATTTTCGTGGCCC
AGGACACACTGGAATGCCAGGTGGCCGAAGTGGAACCGGGCGAGCAGAGAGTCTGTGACCCTGAA
CGCCAGATCTCACCTGAGAGCCAGAGTGCAGGCCAGAGCACAAACGACGGCCTGGATTTCCAA
GAGAGCCAGCTGGTCAAGAACTGGTGGAACTCCTCCACAGGGCTGTCAGGGAAGCGTGATCA
GCTTTCCATCTCCTAGAAGCGGCCCTGGCTCTCCTGCTCAGTGGCTGCTGTATACACACCCAC
45 ACACAGCTGGCAGAGAGCCGATCTGGGCGCCTACCTGAATCCTAGACCTCCTGCTCCTGAGGCT
TGGAGCGAACCTGTTCTGCTGGCCAAGGGCAGCGCTGCCTACAGCGATCTGCAGTCTATGGGCA
CAGGCCCTGATGGCAGCCCTCTGTTTGGCTGTCTGTACGAGGCCAACGACTACGAAGAGATCGT
GTTTCCTGATGTTACCCCTGAAGCAGGCCTTTCCAGCCGAGTACCTGCCTCAA

[00421] SEQ ID NO: 81:

5 DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
10 SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPQGCQGSVISFSPRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQEPKS
SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSLKLT
VDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK

[00422] SEQ ID NO: 82:

15 DASLPYLQDES VFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
20 ESQVLKKLVEPPPQGCQGSVISFSPRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIRFIMFTLKQAFPAEYLPQEPKS
SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSLKLT
25 VDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK

[00423] SEQ ID NO: 83:

30 DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDANTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPQGCQGSVISFSPRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQEPKS
35 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSLKLT
VDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK

[00424] SEQ ID NO: 84:

40 DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPAGCQGSVISFSPRSGPGSPAQWLLYTHPTHKQRADLGAYLNPRPPAPEA
45 WSEPVLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQEPKS
SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSLKLT
VDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK

[00425] SEQ ID NO: 85:

5 DASLPYLQKESV FQSGAHAYRI PALLYLPGQQSLLAF AEQRASKKDEHAELIVLRRGDYDASTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGT LFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
 10 SAFCFLSHDHGRTWARGHFVAQDTLE CQVAE VETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQLVKKLVEPPPAGCQGSVISFPSPRSGPGSPAQWLLYTHP THRKQRADLGAYLNPRPPAPEA
 WSEPVL LAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQEPKS
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
 15 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLT SKLT
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00426] SEQ ID NO: 86:

15 DASLPYLQKESV FQSGAHAYRI PALLYLPGQQSLLAF AEQRASKKDEHAELIVLRRGDYDATH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGT LFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
 20 SAFCFLSHDHGRTWARGHFVAQDTLE CQVAE VETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQLVKKLVEPPPAGCQGSVISFPSPRSGPGSPAQWLLYTHP THRKQRADLGAYLNPRPPAPEA
 WSEPVL LAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQEPKS
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
 25 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLT SKLT
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00427] SEQ ID NO: 87:

30 DASLPYLQKESV FQSGAHAYRI PALLYLPGQQSLLAF AEQRASKKDEHAELIVLRRGDYDANTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGT LFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
 35 SAFCFLSHDHGRTWARGHFVAQDTLE CQVAE VETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQLVKKLVEPPPAGCQGSVISFPSPRSGPGSPAQWLLYTHP THRKQRADLGAYLNPRPPAPEA
 WSEPVL LAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQEPKS
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
 40 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLT SKLT
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00428] SEQ ID NO: 88:

40 X₁ASLPX₂LQX₃ESV FQSGAHAYRI PALLYLPGQQSLLAF AEQRASKKDEHAELIVLRRGDYDAX₄
 THQVQWQAQEVVAQARLDGHRSMNPCPLYDX₅QTGT LFLFFIAIPGQVTEQQQLQTRANVTRLCQ
 VTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPX₆QRP
 IPSAFCFLSHDHGRTWARGHFVAQDTLE CQVAE VETGEQRVVTLNARSHLRARVQAQSTNDGLD
 45 FQESQLVKKLVEPPPX₇GCQGSVISFPSPRSGPGSPAQWLLYTHP THX₈X₉QRADLGAYLNPRPP
 APEAWSEPVL LAKGSX₁₀AYS DLQSMGTGPDGSPLFGCLYEANDYEEIX₁₁FX₁₂MFTLTKQAFPAE
 YLPQX₁₃DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 TSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00429] SEQ ID NO: 89:

5 X₁X₂SX₃X₄X₅LQX₆ESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASX₇X₈DEHAELIVX₉RRGD
 YDAX₁₀THQVQWX₁₁AQEVVAQAX₁₂LDGHRSMNPCPLYDX₁₃QTGTLFLFFIAIPX₁₄X₁₅VTEX₁₆Q
 10 QLQTRANVTRLX₁₇X₁₈VTSTDHGRTWSSPRDLTDAAIGPX₁₉YREWSTFAVGPGHX₂₀LQLHDRX₂₁
 RSLVVPAYAYRKLHPX₂₂QRPIPSAFX₂₃FLSHDHGRTWARGHFVAQDTX₂₄ECQVAEVETGEQRV
 VTLNARSHLRARVQAQXS₂₅NX₂₆GLDFQX₂₇SQLVKKLVEPPPX₂₈GX₂₉QGSVISFSPRSGPGSP
 AQX₃₀LLYTHPTHX₃₁X₃₂QRADLGAYLNPRPPAPEAWSEPX₃₃LLAKGSX₃₄AYSDDLQSMGTGPDGS
 15 PLFGX₃₅LYEANDYEEIX₃₆FX₃₇MFTLKQAFPAEYLPQX₃₈DKTHTCPPCPAPELLGGPSVFLFPP
 KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
 HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
 PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSLKLTVDKSRWQQGNVFSCSVMHEALHNHYT
 QKSLSLSPGK

[00430] SEQ ID NO: 90:

GGGGSGGGGS

[00431] SEQ ID NO: 91:

EPKSS

[00432] SEQ ID NO: 92:

25 EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
 DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
 30 YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLT
 SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00433] SEQ ID NO: 93:

35 DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
 YTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
 DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00434] SEQ ID NO: 94:

40 DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
 45 SAFCFLSHDHGRTWARGHFVAQDTLEQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQVLVKKLVEPPPQGCQGSVISFSPRSGPGSPAQWLLYTHPTHARQADLGAYLNPRPPAPEA
 WSEPVLLAKGSAAYSDDLQSMGTGPDGSPFLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQ

[00435] SEQ ID NO: 95:

50 DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
 STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP

SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHARQADLGAYLNPRPPAPEA
WSEPVLLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQGGGG
SGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
5 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
TSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[00436] SEQ ID NO: 96:

10 DASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
15 ESQLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHARQADLGAYLNPRPPAPEA
WSEPVLLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQEPKS
SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLT
20 VDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[00437] SEQ ID NO: 97:

25 DASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPTGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQADLGAYLNPRPPAPEA
WSEPVLLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ
30

[00438] SEQ ID NO: 98:

35 DASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPTGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQADLGAYLNPRPPAPEA
WSEPVLLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQGGGG
SGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
40 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
TSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[00439] SEQ ID NO: 99:

45 DASLPYLQKESVVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARSLLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
50 ESQLVKKLVEPPPTGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQADLGAYLNPRPPAPEA
WSEPVLLAKGSAAYSDDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQEPKS
SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE

VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLT
VDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

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[00440] SEQ ID NO: 100:

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DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARS LVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRFRVQAQSTNDGLDFQ
ESQLVKKLVEPPPTGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEP VLLAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQ

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[00441] SEQ ID NO: 101:

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DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARS LVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRFRVQAQSTNDGLDFQ
ESQLVKKLVEPPPTGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEP VLLAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQGGGG
SGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
TSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

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[00442] SEQ ID NO: 102:

30

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
STDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHADRARS LVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRFRVQAQSTNDGLDFQ
ESQLVKKLVEPPPTGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEP VLLAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQEPKS
SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLT
VDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

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[00443] SEQ ID NO: 103:

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GATGCATCTCTGCCTTACCTGCAGAAAGAAAGCGTGTTCCAGTCTGGCGCCACGCCTACAGAA
TTCCCGCTCTGCTGTATCTGCCAGGCCAGCAGTCTCTGCTGGCTTTTCGCTGAACAGCGGGCCAG
CAAGAAGGATGAGCACGCCGAACCTGATCGTGCTGCGGAGAGGCGATTACGACGCCGGCACACAT
CAGGTGCAGTGGCAGGCTCAAGAGGTGGTGGCTCAGGCTAGACTGGACGGCCACAGATCTATGA
ACCCCTGTCTCTGTACGATGAACAGACCGGCACACTGTTTCTGTTCTTTATCGCTATCCCCGG
CCAAGTGACCGAGCAGCAGCAGCTGCAGACAAGAGCCAACGTGACCAGACTGTGTCAAGTGACC
TCCACCGACCACGGCAGAACCTGGTCTAGCCCTAGAGATCTGACCGACGCCGCCATCGGACCTG
CCTATAGAGAGTGGTCCACCTTCGCCGTTGGACCTGGACACTGTCTCCAGCTGCACGACAGGGC
TAGATCTCTGGTGGTGCCTGCCTACGCCTATAGAAAGCTGCACCCCAAACAGCGGCCTATTCTCT

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AGCGCCTTCTGCTTTCTGAGCCACGATCACGGCAGGACATGGGCCAGAGGACATTTCTGTTGGCCC
AGGACACACTGGAATGCCAGGTGGCCGAAGTGGAACCGGCGAGCAGAGAGTCGTGACCCTGAA
CGCCAGATCTCACCTGAGAGCCAGAGTGCAGGCCAGAGCACAAACGACGGCCTGGATTTCCAA
GAGAGCCAGCTGGTCAAGAACTGGTGGAACTCCTCCACAGGGCTGTCAGGGAAGCGTGATCA
5 GCTTTCCATCTCCTAGAAGCGGCCCTGGCTCTCCTGCTCAGTGGCTGCTGTATACACACCCAC
ACACAGCTGGCAGAGAGCCGATCTGGGCGCCTACCTGAATCCTAGACCTCCTGCTCCTGAGGCT
TGGAGCGAACCTGTTCTGCTGGCCAAGGGCAGCGCTGCCTACAGCGATCTGCAGTCTATGGGCA
CAGGCCCTGATGGCAGCCCTCTGTTTGGCTGTCTGTACGAGGCCAACGACTACGAAGAGATCGT
GTTCTGATGTTACCCCTGAAGCAGGCCTTTCCAGCCGAGTACCTGCCTCAAGGCGGAGGCGGA
10 TCCGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCT
TCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGT
GGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG
TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA
15 AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTCTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG
TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA
CTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCCTACTAGCAAGCTCACC
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
20 ACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

[00444] SEQ ID NO: 104:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSGGITFYADTV
25 KGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARIKLGTVTTVDYWGQGLTVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEW
30 ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSP
GK

[00445] SEQ ID NO: 105:

GAGGTGCAGCTGCTGGAATCTGGCGGAGGACTTGTTTCAGCCTGGCGGCTCTCTGAGACTGTCTT
GTGCCGCCAGCGGCTTCACCTTCAGCAGCTATATCATGATGTGGGTCCGACAGGCCCTGGCAA
AGGCCTTGAATGGGTGTCCAGCATCTATCCCAGCGGCGGCATCACCTTTTACGCCGACACAGTG
AAGGGCAGATTACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCC
TGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAGAATCAAGCTGGGCACCGTGACCACCGT
40 GGATTATTGGGGACAGGGCACCCCTGGTCACCGTGTCTATCTGCTAGCACCAAGGGCCCCTCCGTC
TTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCA
AGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGCA
CACCTTCCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG
45 TGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAACTCACACATGCCACCGTGCCAGCACC
TGAACCTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAGGACACCCTCATGATC
TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGT
TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA
CGGTAGCACGTACCGTGTGGTCAGCGTCTCACCCTGTCACCAGGACTGGCTGAATGGCAAG
50 GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAG
CCAAAGGGCAGCCCCGAGAACCACAGGTCTACACCCTGCCCCCATCCCGGGAGGAGATGACCAA
GAACCAGGTGACCTGTACTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGG

GAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT
CCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG
GGTAAA

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[00446] SEQ ID NO: 106:

10 TVEKSVVFKAEGEHFTDQKGNTIVGSGSGGTTKYFRIPAMCTTSKGTIVVFADARHNTASDQSF
IDTAAARSTDGGKTWNKKIAIYNDRVNSKLSRVMPTCIVANIQGRETILVMVGKWNNDKWTG
AYRDKAPDTDWDLVLYKSTDDGVTF SKVETNIHDI VTKNGTISAMLGGVGSGLQLNDGKLVFPV
QMVRTKNITTVLNTSFIYSTDGITWSLPSGYCEGFGSENNIIIEFNASLVNNIRNSGLRRSFETK
DFGKTWTEFPMPDKKVDNRNHGVQGSTITIPSGNKLVAAHSSAQNKNNNDYTRSDISLYAHNLYS
GEVKLIDDFYPKVGNASGAGYSCLSYRKNVDKETLYVVYEANGSIEFQDL SRHLPVIKSYNGGG
15 GSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LTSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGKSGGGGSHHHHHHHH

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[00447] SEQ ID NO: 107:

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ACAGTGGAAAAGTCCGTGGTGTTC AAGGCCGAGGGCGAGCACTTCACCGACCAGAAAGGCAATA
CCATCGTCGGCTCTGGCAGCGGCGGCACCACCAAGTACTTTAGAATCCCCGCCATGTGCACCAC
CAGCAAGGGCACCATTGTGGTGTTCGCCGACGCCAGACACAACACCGCCAGCGATCAGAGCTTC
ATCGATACCGCTGCCGCCAGAAGTACAGACGGCGGCAAGACCTGGAACAAGAAGATCGCCATCT
25 ACAACGACCGCGTGAACAGCAAGCTGAGCAGAGTGATGGACCCTACCTGCATCGTGGCCAAACAT
CCAGGGCAGAGAAACCATCCTGGTCATGGTCGGAAGTGGAACAACAACGATAAGACCTGGGGC
GCCTACAGAGACAAGGCCCTGATACCGATTGGGACCTCGTGCTGTATAAGAGCACCGACGACG
GCGTGACCTTCAGCAAGGTGGAACAACATCCACGACATCGTGACCAAGAACGGCACCATCTC
TGCCATGCTCGGCGGCGTTGGATCTGGCCTGCAACTGAATGATGGCAAGCTGGTGTTC CCGTG
30 CAGATGGTCCGAACAAAGAACATCACCACCGTGCTGAATACCAGCTTCATCTACTCCACCGACG
GCATCACATGGTCCCTGCCTAGCGGCTACTGTGAAGGCTTTGGCAGCGAGAACAACATCATCGA
GTTCAACGCCAGCCTGGTCAACAACATCCGGAACAGCGGCCTGCGGAGAAGCTTCGAGACAAAG
GACTTCGGAAGACGTGGACCGAGTTTCTCCTCAATGGACAAGAAGGTGGACAACCGGAACCACG
GCGTGACGGGCGAGCACAATCACAATCCCTAGCGGCAACAACTGGTGGCCGCTCACTCTAGCGC
35 CCAGAACAAGAACAACGATTACACCAGAAGCGACATCAGCCTGTACGCCCACAACCTGTACTCC
GGCGAAGTGAAGCTGATCGACGACTTCTACCCCAAAGTGGGCAATGCCAGCGGAGCCGGCTACA
GCTGTCTGAGCTACCGGAAAAATGTGGACAAAGAAACCTGTACGTGGTGTACGAGGCCAACGG
CAGCATCGAGTTTCAGGACCTGAGCAGACATCTGCCCCGTGATCAAGAGCTACAATGGCGGAGGT
GGAAGTGGCGGAGGCGGATCCGACAAACTCACACATGCCACCGTGCCAGCACCTGAACTCC
40 TGGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGAC
CCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG
TACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
CGTACCGTGTGGTCAGCGTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGG
45 CAGCCCCGAGAACCACAGGTCTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGG
TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAA
TGGGCAGCCGGAGAACAAC TACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC
CTCACTAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCG
TGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAAG
50 CGGCGGAGGCGGATCTCATCATCACCATCATCACCATCAC

[00448] SEQ ID NO: 108:

TVEKSVVFKAEGEHFTDQKGNTIVGSGSGGTTKYFRIPAMCTTSKGTIVVFADARHNTASDQSF
 IDTAAARSTDGGKTWNKKIAIYNDRVNSKLSRVMPTCIVANIQGRETILVMVGKWNNDKTWG
 5 AYRDKAPDTDWDLVLYKSTDDGVTFSKVTNIHDIIVTKNGTISAMLGGVGSGGLQNDGKLVFPV
 QMVRTKNITTVLNTSFIYSTDGITWSLPSGYCEGFGSENNIIEFNASLVNNIRNSGLRRSFETK
 DFGKWTWTEFPPMDKKVDNRNHGVQGSTITIPSGNKLVAAHSSAQNKNNDYTRSDISLYAHNLYS
 GEVKLIDDFYPKVGNASGAGYSCLSYRKNVDKETLYVVYEANGSIEFQDLSRHLPVIKSYNGGG
 GSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNW
 10 YVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
 QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
 LTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGGGGSHHHHHHHH

[00449] SEQ ID NO: 109:

15 ACAGTGGAAAAGTCCGTGGTGTTC AAGGCCGAGGGCGAGCACTTCACCGACCAGAAAGGCAATA
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 CAGCAAGGGCACCATTGTGGTGTTCGCCGACGCCAGACACAACACCGCCAGCGATCAGAGCTTC
 ATCGATACCGCTGCCGCCAGAAGTACAGACGGCGGCAAGACCTGGAACAAGAAGATCGCCATCT
 20 ACAACGACCGCGTGAACAGCAAGCTGAGCAGAGTGATGGACCCTACCTGCATCGTGGCCAACAT
 CCAGGGCAGAGAAACCATCCTGGTCATGGTCGGAAGTGGAACAACAACGATAAGACCTGGGGC
 GCCTACAGAGACAAGGCCCTGATACCGATTGGGACCTCGTGCTGTATAAGAGCACCGACGACG
 GCGTGACCTTCAGCAAGGTGGAACAACAACATCCACGACATCGTGACCAAGAACGGCACCATCTC
 TGCCATGCTCGGCGGCGTTGGATCTGGCCTGCAACTGAATGATGGCAAGCTGGTGTTC CCGTG
 25 CAGATGGTCCGAACAAGAACATCACCACCGTGCTGAATACCAGCTTCATCTACTCCACCGACG
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 GTTCAACGCCAGCCTGGTCAACAACATCCGGAACAGCGGCCTGCGGAGAAGCTTCGAGACAAAG
 GACTTCGGAAGACGTGGACCGAGTTTCCTCCAATGGACAAGAAGGTGGACAACCGGAACCACG
 GCGTGACGGCAGCACAAATCACAATCCCTAGCGGCAACAACCTGGTGGCCGCTCACTCTAGCGC
 30 CCAGAACAAGAACAACGATTACACCAGAAGCGACATCAGCCTGTACGCCCACAACCTGTACTCC
 GGCGAAGTGAAGCTGATCGACGACTTCTACCCCAAAGTGGGCAATGCCAGCGGAGCCGGCTACA
 GCTGTCTGAGCTACCGGAAAAATGTGGACAAAGAAACCCTGTACGTGGTGTACGAGGCCAACGG
 CAGCATCGAGTTTCAGGACCTGAGCAGACATCTGCCCCGTGATCAAGAGCTACAATGGCGGAGGT
 GGAAGTGGCGGAGGCGGATCCGACAAAACCTCACACATGCCACCGTGCCCAGCACCTGAACTCC
 35 TGGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGAC
 CCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG
 TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGGTAGCA
 CGTACCGTGTGGTCAGCGTCTCACCCTGCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
 GTGCAAGGTCTCCAACAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGGCCAAAGGG
 40 CAGCCCCGAGAACCACAGGTCTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGG
 TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAA
 TGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC
 CTCACTAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCG
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 45 CGGCGGAGGCGGATCTCATCATCACCATCATCACCATCAC

[00450] SEQ ID NO: 110:

50 EVQLLESGLLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSSGGITFYADTV
 KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARIKLGTVTTVDYWGQGLTVTVSSASTKGPSV
 FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
 SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI

SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLVCLVKGFYPSDIAVEW
ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSP
GKGGGSGGGGSGGGGSGSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPK
5 LMIYDVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTRVFGTGKVTVLG
QPKAGGGGSGGGGSGGGGSEVQLLESGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGL
EWVSSIYPSGGITFYADTVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARIKLGTVTTVDY
WGQGTLLTVSS

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[00451] SEQ ID NO: 111:

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GAGGTGCAGCTGCTGGAATCTGGCGGAGGACTTGTTCAGCCTGGCGGCTCTCTGAGACTGTCTT
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AGGCCTTGAATGGGTGTCCAGCATCTATCCCAGCGGCGGCATCACCTTTTACGCCGACACAGTG
AAGGGCAGATTACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCC
TGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAGAATCAAGCTGGGCACCGTGACCACCGT
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TTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCA
AGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCTCTGACCAGCGGCGTGCA
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TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG
TGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAACTCACACATGCCCACCGTGCCCAGCACC
TGAACCTCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAGGACACCCCTCATGATC
TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGT
TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA
CAACAGCACGTACCGTGTGGTTCAGCGTCTCACCCTGCTGCACCAGGACTGGCTGAATGGCAAG
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CCAAAGGGCAGCCCCGAGAACCACAGGTCTACACCCTGCCCCCATCCCGGGAGGAGATGACCAA
GAACCAGGTACGCTGTACTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGG
GAGAGCAATGGGCAGCCGGAGAACAATAACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT
CCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTAAGCTTGTCTCCG
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CACAGCCTGCCAGCGTGTCCGGATCTCCTGGCCAGAGCATCACCATCAGCTGTACCGGCACCAG
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CTGATGATCTACGACGTGTCCAACAGACCCAGCGGCGTGTCCAATAGATTCTCCGGCAGCAAGA
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CAGCCTAAGGCCGGTGGAGGTGGGTCTGGAGGGGGTGGATCTGGAGGTGGCGGATCGGAGGTGC
AGCTGCTGGAATCTGGCGGAGGACTTGTTCAGCCTGGCGGCTCTCTGAGACTGTCTTGTGCCGC
CAGCGGCTTCACCTTCAGCAGCTATATCATGATGTGGGTCCGACAGGCCCTGGCAAAGGCCCTT
GAATGGGTGTCCAGCATCTATCCCAGCGGCGGCATCACCTTTTACGCCGACACAGTGAAGGGCA
GATTCACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGAGC
CGAGGACACCGCGTGTACTACTGCGCCAGAATCAAGCTGGGCACCGTGACCACCGTGGATTAT
TGGGGACAGGGCACCCCTGGTCACCGTGTCTATCT

[00452] SEQ ID NO: 112:

50

DASLPYLQKESVFSQGAHAYRIPALLYLPQGQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQGTGLFLFFIAIPGQVTEQQQLQTRANVTRLCQVT
STDHGRTWSSPRDLTDAAGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP

SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPQGCQGSVISFSPSPRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLLAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQGGGG
SGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
5 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
TSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

10 [00453] SEQ ID NO: 113:

GATGCATCTCTGCCTTACCTGCAGAAAGAAAGCGTGTTCAGTCTGGCGCCCACGCCTACAGAA
TTCCCGCTCTGCTGTATCTGCCAGGCCAGCAGTCTCTGCTGGCTTTCGCTGAACAGCGGGCCAG
CAAGAAGGATGAGCACGCCGAACCTGATCGTGCTGCGGAGAGGCGATTACGACGCCGGCACACAT
15 CAGGTGCAGTGGCAGGCTCAAGAGGTGGTGGCTCAGGCTAGACTGGACGGCCACAGATCTATGA
ACCCCTGTCTCTGTACGATGAACAGACCGGCACACTGTTTCTGTTCTTTATCGCTATCCCCGG
CCAAGTGACCGAGCAGCAGCAGCTGCAGACAAGAGCCAACGTGACCAGACTGTGTCAAGTGACC
TCCACCGACCACGGCAGAACCTGGTCTAGCCCTAGAGATCTGACCGACGCCGCCATCGGACCTG
CCTATAGAGAGTGGTCCACCTTCGCCGTTGGACCTGGACACTGTCTCCAGCTGCACGACAGGGC
20 TAGATCTCTGGTGGTGCCTACGCCTATAGAAAGCTGCACCCCAAACAGCGGCCCTATTCTT
AGCGCCTTCTGCTTTCTGAGCCACGATCACGGCAGGACATGGGCCAGAGGACATTTCTGGCCCC
AGGACACACTGGAATGCCAGGTGGCCGAAGTGGAACCGGCGAGCAGAGAGTCGTGACCCTGAA
CGCCAGATCTCACCTGAGAGCCAGAGTGCAGGCCAGAGCACAAACGACGGCCTGGATTTCCTAA
GAGAGCCAGCTGGTCAAGAACTGGTGGAACTCCTCCACAGGGCTGTCAGGGAAGCGTGATCA
25 GCTTTCCATCTCCTAGAAGCGGCCCTGGCTCTCCTGCTCAGTGGCTGCTGTATACACACCCAC
ACACAGCTGGCAGAGAGCCGATCTGGGCGCCTACCTGAATCCTAGACCTCCTGCTCCTGAGGCT
TGGAGCGAACCTGTTCTGCTGGCCAAGGGCAGCGCTGCCTACAGCGATCTGCAGTCTATGGGCA
CAGGCCCTGATGGCAGCCCTCTGTTTGGCTGTCTGTACGAGGCCAACGACTACGAAGAGATCGT
GTTCTTGATGTTTACCCTGAAGCAGGCCTTTCCAGCCGAGTACCTGCCTCAAGGCGGAGGTGGA
30 AGTGGCGGAGGCGGATCCGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGG
GGGGACCGTCAGTCTTCCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCC
TGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTAC
GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGT
ACCGTGTGGTCAGCGTCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
35 CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAG
CCCCGAGAACCACAGGTCTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCA
GCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGG
GCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTC
ACTAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGA
40 TGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

[00454] SEQ ID NO: 114:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
45 QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLQV
STDHGRTWSSPRDLTDAAGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPKQRP
SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPQGCQGSVISFSPSPRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEPVLLAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLTKQAFPAEYLPQEPKS
50 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT

VDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSPGKGGGSGGGGSGGGGSQSALTQPASVSGS
PGQSITISCTGTSSDVGGYNYVSWYQOHPGKAPKLMYDVSNRPSGVSNRFSGSKSGNTASLTI
SGLQAEDEADYYCSSYTSSSTRVFGTGTKVTVLGQPKAGGGGSGGGGSGGGGSEVQLLESGGGL
VQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSGGITFYADTVKGRFTISRDN
5 KNTLYLQMNSLRAEDTAVYYCARIKLGTVTTVDYWGQGLTVTVSS

[00455] SEQ ID NO: 115:

GATGCATCTCTGCCTTACCTGCAGAAAGAAAGCGTGTTCAGTCTGGCGCCACGCCTACAGAA
10 TTCCCGCTCTGCTGTATCTGCCAGGCCAGCAGTCTCTGCTGGCTTTCGCTGAACAGCGGGCCAG
CAAGAAGGATGAGCACGCCGAACCTGATCGTGCTGCGGAGAGGCGATTACGACGCCGGCACACAT
CAGGTGCAGTGGCAGGCTCAAGAGGTGGTGGCTCAGGCTAGACTGGACGGCCACAGATCTATGA
ACCCCTGTCCTCTGTACGATGAACAGACCGGCACACTGTTTCTGTTCTTTATCGCTATCCCCGG
CCAAGTGACCGAGCAGCAGCAGCTGCAGACAAGAGCCAACGTGACCAGACTGTGTCAAGTGACC
15 TCCACCGACCACGGCAGAACCTGGTCTAGCCCTAGAGATCTGACCGACGCCGCCATCGGACCTG
CCTATAGAGAGTGGTCCACCTTCGCCGTTGGACCTGGACACTGTCTCCAGCTGCACGACAGGGC
TAGATCTCTGGTGGTGCCTGCCTACGCCTATAGAAAGCTGCACCCCAAACAGCGGCCCTATTCT
AGCGCCTTCTGCTTTCTGAGCCACGATCACGGCAGGACATGGGCCAGAGGACATTTCTGTTGGCC
AGGACACACTGGAATGCCAGGTGGCCGAAGTGGAACCGGCGAGCAGAGAGTCGTGACCCTGAA
20 CGCCAGATCTCACCTGAGAGCCAGAGTGCAGGCCAGAGCACAAACGACGGCCTGGATTTCCTAA
GAGAGCCAGCTGGTCAAGAACTGGTGGAACTCCTCCACAGGGCTGTCAGGGAAGCGTGATCA
GCTTTCATCTCCTAGAAGCGGCCCTGGCTCTCCTGCTCAGTGGCTGCTGTATACACACCCAC
ACACAGCTGGCAGAGAGCCGATCTGGGCGCCTACCTGAATCCTAGACCTCCTGCTCCTGAGGCT
TGGAGCGAACCTGTTCTGCTGGCCAAGGGCAGCGCTGCCTACAGCGATCTGCAGTCTATGGGCA
25 CAGGCCCTGATGGCAGCCCTCTGTTTGGCTGTCTGTACGAGGCCAACGACTACGAAGAGATCGT
GTTCTGATGTTACCCCTGAAGCAGGCCTTTCAGCCGAGTACCTGCCTCAAGAGCCCAAATCT
TCTGACAAAACCTCACACATGCCACCGTGCCACGACCTGAACTCCTGGGGGGACCGTCAGTCT
TCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGT
GGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
30 GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG
TCCTCACCGTCTGTCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA
AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTCTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG
TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA
35 CTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACC
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
ACAACCACTACACGCAGAAGAGCCTAAGCTTGTCTCCGGGTAAAGGAGGCGGAGGATCTGGCGG
AGGTGGAAGTGGCGGAGGCGGATCTCAATCTGCTCTTACACAGCCTGCCAGCGTGTCCGGATCT
CCTGGCCAGAGCATCACCATCAGCTGTACCGGCACCAGCTCTGATGTGCGGCGGCTACAATTACG
40 TGTCTTGGTATCAGCAGCACCCCGGCAAGGCCCTAAGCTGATGATCTACGACGTGTCCAACAG
ACCCAGCGGCGTGTCCAATAGATTCTCCGGCAGCAAGAGCGGCAACACCGCCAGCCTGACAATT
AGCGGACTGCAGGCCGAGGACGAGGCCGATTACTACTGTAGCAGCTACACCAGCTCCAGCACCA
GAGTGTTTGGCACCGGCACAAAAGTGACCGTGCTGGGCCAGCCTAAGGCCGGTGGAGGTGGGTC
TGGAGGGGGTGGATCTGGAGGTGGCGGATCGGAGGTGCAGCTGCTGGAATCTGGCGGAGGACTT
45 GTTCAGCCTGGCGGCTCTCTGAGACTGTCTTGTGCCCGCAGCGGCTTCACCTCAGCAGCTATA
TCATGATGTGGGTCCGACAGGCCCTGGCAAAGGCCTTGAATGGGTGTCCAGCATCTATCCCAG
CGGCGGCATCACCTTTTACGCCGACACAGTGAAGGGCAGATTCACCATCAGCCGGGACAACAGC
AAGAACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCG
CCAGAATCAAGCTGGGCACCGTGACCACCGTGGATTATTGGGGACAGGGCACCCCTGGTCACCGT
50 GTCATCT

[00456] SEQ ID NO: 116:

5 X₁ASLPX₂LQX₃ESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAX₄
 THQVQWQAQEVVAQARLDGHRSMNPCPLYDX₅QTGTLFLFFIAIPGQVTEQQQLQTRANVTRLX₆
 VTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPX₇QR
 PIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRX₈RVQAQSTNDGL
 10 DFQESQLVKKLVEPPPX₉GCQGSVISFSPSRSGPGSPAQWLLYTHPTHX₁₀X₁₁QRADLGAYLNPR
 PPAPAEAWSEPVLAKGSX₁₂AYSDDLQSMGTGPDGSPFGCLYEANDYEEIX₁₃FX₁₄MFTLTKQAF
 AEYLPQX₁₅DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
 GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
 FLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00457] SEQ ID NO: 117:

15 X₁X₂SX₃X₄X₅LQX₆ESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASX₇X₈DEHAELIVX₉RRGD
 YDAX₁₀THQVQWX₁₁AQEVVAQAX₁₂LX₁₃GHRSMNPCPLYDX₁₄QTGTLFLFFIAIPX₁₅X₁₆VTEX₁₇
 QQLQTRANVTRLX₁₈X₁₉VTSTDHGRTWSSPRDLTDAAIGPX₂₀YREWSTFAVGPGHX₂₁LQLHDX₂₂
 X₂₃RSLVVPAYAYRKLHPX₂₄X₂₅X₂₆PIPSAFX₂₇FLSHDHGRTWARGHFVX₂₈QDTX₂₉ECQVAEVX
 20 X₃₀TGEQRVVTLNARSX₃₁X₃₂X₃₃X₃₄RX₃₅QAQX₃₆NX₃₇GLDFQX₃₈X₃₉QX₄₀VKKL
 X₄₁EPXPX₄₂GX₄₃QGSVISFSPSRSGPGSPAQX₄₄LLYTHPTHX₄₅X₄₆QRADLGAYLNPRPPAPEA
 WSEPX₄₇LLAKGSX₄₈AYSDDLQSMGTGPDGSPFGX₄₉LYEANDYEEIX₅₀FX₅₁MFTLTKQAFPAEYL
 PQX₅₂DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
 25 EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTS
 KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00458] SEQ ID NO: 118:

30 gatGCATCTCTGCCTTACCTGCAGAAAGAAAGCGTGTTCAGTCTGGCGCCCACGCCTACAGAA
 TTCCCGCTCTGCTGTATCTGCCAGGCCAGCAGTCTCTGCTGGCTTTTCGCTGAACAGCGGGCCAG
 CAAGAAGGATGAGCACGCCGAAGTATCGTGTCTGCGGAGAGGCGATTACGACGCCggcACACAT
 CAGGTGCAGTGGCAGGCTCAAGAGGTGGTGGCTCAGGCTAGACTGGACGGCCACAGATCTATGA
 ACCCCTGTCTCTGTACGATgaaCAGACCGGCACACTGTTTCTGTTCTTTATCGCTATCCCCGG
 35 CCAAGTGACCGAGCAGCAGCAGCTGCAGACAAGAGCCAACGTGACCAGACTGTGTtacGTGACC
 TCCACCGACCACGGCAGAACCTGGTCTAGCCCTAGAGATCTGACCGACGCCGCCATCGGACCTG
 CCTATAGAGAGTGGTCCACCTTCGCCGTGGACCTGGACACTGTCTCCAGCTGCACGACAGGGC
 TAGATCTCTGGTGGTGCCTACGCCTATAGAAAGCTGCACCCCAAACAGCGGCCTATTCTCT
 AGCGCCTTCTGCTTTCTGAGCCACGATCACGGCAGGACATGGGCCAGAGGACATTTCTGTGGCCC
 40 AGGACACACTGGAATGCCAGGTGGCCGAAGTGGAACCGGCGAGCAGAGAGTCGTGACCCTGAA
 CGCCAGATCTCACCTGAGATTGAGAGTGCAGGCCAGAGCACAAACGACGGCCTGGATTTCCAA
 GAGAGCCAGCTGGTCAAGAACTGGTGGAACTCCTCCAaccGGCTGTCAGGGAAGCGTGATCA
 GCTTTCCATCTCCTAGAAGCGGCCCTGGCTCTCCTGCTCAGTGGCTGCTGTATACACACCCAC
 ACACAGCTGGCAGAGAGCCGATCTGGGCGCCTACCTGAATCCTAGACCTCCTGCTCCTGAGGCT
 45 TGGAGCGAACCTGTTCTGCTGGCCAAGGGCAGCgctGCCTACAGCGATCTGCAGTCTATGGGCA
 CAGGCCCTGATGGCAGCCCTCTGTTTGGCTGTCTGTACGAGGCCAACGACTACGAAGAGATCGT
 GTTCTGATGTTACCCCTGAAGCAGGCCTTTCCAGCCGAGTACCTGCCTCAA

[00459] SEQ ID NO: 119:

5 X₁X₂SX₃X₄X₅LQX₆ESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASX₇X₈DEHAELIVX₉RRGD
 YDAX₁₀THQVQWX₁₁AQEVVAQAX₁₂LX₁₃GHRSMNPCPLYDX₁₄QTGTLFLFFIAIPX₁₅X₁₆VTEX₁₇
 10 QQLQTRANVTRLX₁₈X₁₉VTSTDHGRWTWSSPRDLTDAAIGPX₂₀YREWSTFAVGPGHX₂₁LQLHDX₂₂
 X₂₃RSLVVPAYAYRKLHPX₂₄X₂₅X₂₆PIPSAFX₂₇FLSHDHGRTWARGHFVX₂₈QDTX₂₉ECQVAEVX
 30 TGEQRVVTLNARSX₃₁X₃₂X₃₃X₃₄RX₃₅QAQX₃₆NX₃₇GLDFQX₃₈X₃₉QX₄₀VKKL
 X₄₁EPPPX₄₂GX₄₃QGSVISFSPSRSGPGSPAQX₄₄LLYTHPTHX₄₅X₄₆QRADLGAYLNPRPPAPEA
 WSEPX₄₇LLAKGSX₄₈AYSDDLQSMGTGPDGSPFLFGX₄₉LYEANDYEEIX₅₀FX₅₁MFTLKQAFPAEYL
 10 PQ

[00460] SEQ ID NO: 120:

15 X₁ASLPX₂LQX₃ESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAX₄
 THQVQWQAQEVVAQARLDGHRSMNPCPLYDX₅QTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCX
 6VTSTDHGRWTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDDRARSLLVVPAYAYRKLHPX₇QR
 PIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRX₈RVQAQSTNDGL
 DFQESQLVKKLVEPPPX₉GCQGSVISFSPSRSGPGSPAQWLLYTHPTHX₁₀X₁₁QRADLGAYLNPR
 20 PPAPEAWSEPVLAKGSX₁₂AYSDDLQSMGTGPDGSPFLFGCLYEANDYEEIX₁₃FX₁₄MFTLKQAFP
 AEYLPQ

[00461] SEQ ID NO: 121:

25 GGGGS

[00462] SEQ ID NO: 122:

30 DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
 STDHGRWTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDDRARSLLVVPAYAYRKLHPKQRP
 35 SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQ
 ESQVLVKKLVEPPPTGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
 WSEPVLAKGSAAYSDDLQSMGTGPDGSPFLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQEPKS
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
 40 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00463] SEQ ID NO: 123:

40 DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAGTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
 STDHGRWTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDDRARSLLVVPAYAYRKLHPKQRP
 45 SAFCFLSHDHGRTWARGHFVAQDTLECQVAEVEVETGEQRVVTLNARSHLRFRVQAQSTNDGLDFQ
 ESQVLVKKLVEPPPTGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
 WSEPVLAKGSAAYSDDLQSMGTGPDGSPFLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQEPKS
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
 50 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00464] SEQ ID NO: 124:

EVQLLES GGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSSGGITFYADTV
KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARIKLGTVTTVDYWGQGT LVTVSSASTKGPSV
5 FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLT SKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSP
10 GK

[00465] SEQ ID NO: 125:

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLM IYDVSNRPSGVSNRF
15 SGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTRVFGTGT KVTVLGQPKAGGGGSGGGGSGGG
GSEVQLLES GGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSSGGITFYAD
TVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARIKLGTVTTVDYWGQGT LVTVSS

[00466] SEQ ID NO: 126:

DASLPYLQKESV FQSGAHAYRIPALLYLP GQQSLLAF AEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGT LFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
STDHGR TWSSPRDLTDA AIGPAYREWSTFAVGPGHCLQLH DRARSLVVPAYAYRKLHPKQRP I P
25 SAFCFLSHDHGR TWARGHFVAQDTLE CQVAE VETGEQ RVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEP VLLAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTL KQAFPAEYLPQ

[00467] SEQ ID NO: 127:

DASLPYLQKESV FQSGAHAYRIPALLYLP GQQSLLAF AEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGT LFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
STDHGR TWSSPRDLTDA AIGPAYREWSTFAVGPGHCLQLH DRARSLVVPAYAYRKLHPKQRP I P
SAFCFLSHDHGR TWARGHFVAQDTLE CQVAE VETGEQ RVVTLNARSHLRARVQAQSTNDGLDFQ
35 ESQLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEP VLLAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTL KQAFPAEYLPQEPKS
SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLT SKLT
40 VDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

[00468] SEQ ID NO: 128:

DASLPYLQKESV FQSGAHAYRIPALLYLP GQQSLLAF AEQRASKKDEHAELIVLRRGDYDAGTH
QVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGT LFLFFIAIPGQVTEQQQLQTRANVTRLCYVT
45 STDHGR TWSSPRDLTDA AIGPAYREWSTFAVGPGHCLQLH DRARSLVVPAYAYRKLHPKQRP I P
SAFCFLSHDHGR TWARGHFVAQDTLE CQVAE VETGEQ RVVTLNARSHLRARVQAQSTNDGLDFQ
ESQLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEA
WSEP VLLAKGSAAYS DLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTL KQAFPAEYLPQGGGG
SGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWY
50 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
TSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

WHAT IS CLAIMED IS:

1. A fusion protein comprising:
 - (a) a sialidase enzyme; and
 - 5 (b) an anti-PD-L1 immunoglobulin antigen-binding domain.
2. The fusion protein of claim 1, wherein the sialidase is a human sialidase.
3. The fusion protein of claim 1 or 2, wherein the sialidase is a recombinant mutant human sialidase.
4. The fusion protein of claim 3, wherein the sialidase comprises:
 - 10 (a) a substitution of a proline residue at a position corresponding to position 5 of wild-type human Neu2 (P5);
 - (b) a substitution of a lysine residue at a position corresponding to position 9 of wild-type human Neu2 (K9);
 - (c) a substitution of a lysine residue at a position corresponding to position 44 of wild-type human Neu2 (K44);
 - 15 (d) a substitution of a lysine residue at a position corresponding to position 45 of wild-type human Neu2 (K45);
 - (e) a substitution of a leucine residue at a position corresponding to position 54 of wild-type human Neu2 (L54);
 - 20 (f) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62);
 - (g) a substitution of a glutamine residue at a position corresponding to position 69 of wild-type human Neu2 (Q69);
 - (h) a substitution of an arginine residue at a position corresponding to position 78 of wild-type human Neu2 (R78);
 - 25 (i) a substitution of an aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 (D80);
 - (j) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93);
 - 30 (k) a substitution of a glycine residue at a position corresponding to position 107 of wild-type human Neu2 (G107);
 - (l) a substitution of a glutamine residue at a position corresponding to position 108 of wild-type human Neu2 (Q108);

- (m) a substitution of a glutamine residue at a position corresponding to position 112 of wild-type human Neu2 (Q112);
- (n) a substitution of a cysteine residue at a position corresponding to position 125 of wild-type human Neu2 (C125);
- 5 (o) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126);
- (p) a substitution of an alanine residue at a position corresponding to position 150 of wild-type human Neu2 (A150);
- (q) a substitution of a cysteine residue at a position corresponding to position 164 of wild-type human Neu2 (C164);
- 10 (r) a substitution of an arginine residue at a position corresponding to position 170 of wild-type human Neu2 (R170);
- (s) a substitution of an alanine residue at a position corresponding to position 171 of wild-type human Neu2 (A171);
- 15 (t) a substitution of a glutamine residue at a position corresponding to position 188 of wild-type human Neu2 (Q188);
- (u) a substitution of an arginine residue at a position corresponding to position 189 of wild-type human Neu2 (R189);
- (v) a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (A213);
- 20 (w) a substitution of a leucine residue at a position corresponding to position 217 of wild-type human Neu2 (L217);
- (x) a substitution of a glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 (E225);
- 25 (y) a substitution of a histidine residue at a position corresponding to position 239 of wild-type human Neu2 (H239);
- (z) a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240);
- (aa) a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241);
- 30 (bb) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242);
- (cc) a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244);

- (dd) a substitution of a threonine residue at a position corresponding to position 249 of wild-type human Neu2 (T249);
- (ee) a substitution of an aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 (D251);
- 5 (ff) a substitution of a glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 (E257);
- (gg) a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258);
- (hh) a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260);
- 10 (ii) a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265);
- (jj) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2 (Q270);
- 15 (kk) a substitution of a tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 (W292);
- (ll) a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301);
- (mm) a substitution of a tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 (W302);
- 20 (nn) a substitution of a valine residue at a position corresponding to position 363 of wild-type human Neu2 (V363); or
- (oo) a substitution of a leucine residue at a position corresponding to position 365 of wild-type human Neu2 (L365);
- 25 or a combination of any of the foregoing substitutions.

5. The fusion protein of claim 3 or 4, wherein the sialidase comprises:

- (a) a substitution of a proline residue at a position corresponding to position 5 of wild-type human Neu2 (P5);
- (b) a substitution of a lysine residue at a position corresponding to position 9 of wild-type human Neu2 (K9);
- 30 (c) a substitution of a lysine residue at a position corresponding to position 44 of wild-type human Neu2 (K44);
- (d) a substitution of a lysine residue at a position corresponding to position 45 of wild-type human Neu2 (K45);

- (e) a substitution of a leucine residue at a position corresponding to position 54 of wild-type human Neu2 (L54);
- (f) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62);
- 5 (g) a substitution of a glutamine residue at a position corresponding to position 69 of wild-type human Neu2 (Q69);
- (h) a substitution of an arginine residue at a position corresponding to position 78 of wild-type human Neu2 (R78);
- 10 (i) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93);
- (j) a substitution of a glycine residue at a position corresponding to position 107 of wild-type human Neu2 (G107);
- (k) a substitution of a glutamine residue at a position corresponding to position 108 of wild-type human Neu2 (Q108);
- 15 (l) a substitution of a glutamine residue at a position corresponding to position 112 of wild-type human Neu2 (Q112);
- (m) a substitution of a cysteine residue at a position corresponding to position 125 of wild-type human Neu2 (C125);
- 20 (n) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126);
- (o) a substitution of an alanine residue at a position corresponding to position 150 of wild-type human Neu2 (A150);
- (p) a substitution of a cysteine residue at a position corresponding to position 164 of wild-type human Neu2 (C164);
- 25 (q) a substitution of an alanine residue at a position corresponding to position 171 of wild-type human Neu2 (A171);
- (r) a substitution of a leucine residue at a position corresponding to position 217 of wild-type human Neu2 (L217);
- 30 (s) a substitution of a threonine residue at a position corresponding to position 249 of wild-type human Neu2 (T249);
- (t) a substitution of an aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 (D251);
- (u) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2 (Q270);

- (v) a substitution of a tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 (W292);
- (w) a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301);
- 5 (x) a substitution of a tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 (W302);
- (y) a substitution of a valine residue at a position corresponding to position 363 of wild-type human Neu2 (V363); or
- 10 (z) a substitution of a leucine residue at a position corresponding to position 365 of wild-type human Neu2 (L365);
- or a combination of any of the foregoing substitutions.
6. The fusion protein of claim 4 or 5, wherein the sialidase comprises a substitution of K9, P62, A93, Q216, A242, Q270, S301, W302, V363, or L365, or a combination of any of the foregoing substitutions.
- 15 7. The fusion protein of any one of claims 4-6, wherein the sialidase comprises a substitution of K9, P62, A93, Q270, S301, W302, V363, or L365, or a combination of any of the foregoing substitutions.
8. The fusion protein of any one of claims 4-7, wherein, in the sialidase:
- 20 (a) the proline residue at a position corresponding to position 5 of wild-type human Neu2 is substituted by histidine (P5H);
- (b) the lysine residue at a position corresponding to position 9 of wild-type human Neu2 is substituted by aspartic acid (K9D);
- (c) the lysine residue at a position corresponding to position 44 of wild-type human Neu2 is substituted by arginine (K44R) or glutamic acid (K44E);
- 25 (d) the lysine residue at a position corresponding to position 45 of wild-type human Neu2 is substituted by alanine (K45A), arginine (K45R), or glutamic acid (K45E);
- (e) the leucine residue at a position corresponding to position 54 of wild-type human Neu2 is substituted by methionine (L54M);
- (f) the proline residue at a position corresponding to position 62 of wild-type human Neu2 is substituted by asparagine (P62N), aspartic acid (P62D), histidine (P62H), glutamic acid (P62E), glycine (P62G), serine (P62S), or threonine (P62T);
- 30 (g) the glutamine residue at a position corresponding to position 69 of wild-type human Neu2 is substituted by histidine (Q69H);

- (h) the arginine residue at a position corresponding to position 78 of wild-type human Neu2 is substituted by lysine (R78K);
- (i) the aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 is substituted by proline (D80P);
- 5 (j) the alanine residue at a position corresponding to position 93 of wild-type human Neu2 is substituted by glutamic acid (A93E) or lysine (A93K);
- (k) the glycine residue at a position corresponding to position 107 of wild-type human Neu2 is substituted by aspartic acid (G107D);
- 10 (l) the glutamine residue at a position corresponding to position 108 of wild-type human Neu2 is substituted by histidine (Q108H);
- (m) the glutamine residue at a position corresponding to position 112 of wild-type human Neu2 is substituted by arginine (Q112R) or lysine (Q112K);
- (n) the cysteine residue at a position corresponding to position 125 of wild-type human Neu2 is substituted by leucine (C125L);
- 15 (o) the glutamine residue at a position corresponding to position 126 of wild-type human Neu2 is substituted by leucine (Q126L), glutamic acid (Q126E), phenylalanine (Q126F), histidine (Q126H), isoleucine (Q126I), or tyrosine (Q126Y);
- (p) the alanine residue at a position corresponding to position 150 of wild-type human Neu2 is substituted by valine (A150V);
- 20 (q) the cysteine residue at a position corresponding to position 164 of wild-type human Neu2 is substituted by glycine (C164G);
- (r) the arginine residue at a position corresponding to position 170 of wild-type human Neu2 is substituted by proline (R170P);
- (s) the alanine residue at a position corresponding to position 171 of wild-type human Neu2 is substituted by glycine (A171G);
- 25 (t) the glutamine residue at a position corresponding to position 188 of wild-type human Neu2 is substituted by proline (Q188P);
- (u) the arginine residue at a position corresponding to position 189 of wild-type human Neu2 is substituted by proline (R189P);
- 30 (v) the alanine residue at a position corresponding to position 213 of wild-type human Neu2 is substituted by cysteine (A213C), asparagine (A213N), serine (A213S) or threonine (A213T);
- (w) the leucine residue at a position corresponding to position 217 of wild-type human Neu2 is substituted by alanine (L217A) or valine (L217V);

- (x) the threonine residue at a position corresponding to position 249 of wild-type human Neu2 is substituted by alanine (T249A);
- (y) the aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 is substituted by glycine (D251G);
- 5 (z) the glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 is substituted by proline (E225P);
- (aa) the histidine residue at a position corresponding to position 239 of wild-type human Neu2 is substituted by proline (H239P);
- (bb) the leucine residue at a position corresponding to position 240 of wild-type human Neu2 is substituted by aspartic acid (L240D), asparagine (L240N), or tyrosine (L240Y);
- 10 (cc) the arginine residue at a position corresponding to position 241 of wild-type human Neu2 is substituted by alanine (R241A), aspartic acid (R241D), leucine (R241L), glutamine (R241Q), or tyrosine (R241Y);
- 15 (dd) the alanine residue at a position corresponding to position 242 of wild-type human Neu2 is substituted by cysteine (A242C), phenylalanine (A242F), glycine (A242G), histidine (A242H), isoleucine (A242I), lysine (A242K), leucine (A242L), methionine (A242M), asparagine (A242N), glutamine (A242Q), arginine (A242R), serine (A242S), valine (A242V), tryptophan (A242W), or tyrosine (A242Y);
- 20 (ee) the valine residue at a position corresponding to position 244 of wild-type human Neu2 is substituted by isoleucine (V244I) or proline (V244P);
- (ff) the glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 is substituted by proline (E257P);
- (gg) the serine residue at a position corresponding to position 258 is substituted by cysteine (S258C);
- 25 (hh) the leucine residue at a position corresponding to position 260 of wild-type human Neu2 is substituted by aspartic acid (L260D), phenylalanine (L260F), glutamine (L260Q), or threonine (L260T);
- (ii) the valine residue at a position corresponding to position 265 of wild-type human Neu2 is substituted by phenylalanine (V265F);
- 30 (jj) the glutamine residue at a position corresponding to position 270 of wild-type human Neu2 is substituted by alanine (Q270A), histidine (Q270H), phenylalanine (Q270F), proline (Q270P), serine (Q270S), or threonine (Q270T);
- (kk) the tryptophan residue at a position corresponding to position 292 of wild-type human

Neu2 is substituted by arginine (W292R);

- (ll) the serine residue at a position corresponding to position 301 of wild-type human Neu2 is substituted by alanine (S301A), aspartic acid (S301D), glutamic acid (S301E), phenylalanine (S301F), histidine (S301H), lysine (S301K), leucine (S301L), methionine (S301M), asparagine (S301N), proline (S301P), glutamine (S301Q), arginine (S301R), threonine (S301T), valine (S301V), tryptophan (S301W), or tyrosine (S301Y);
 - (mm) the tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 is substituted by alanine (W302A), aspartic acid (W302D), phenylalanine (W302F), glycine (W302G), histidine (W302H), isoleucine (W302I), lysine (W302K), leucine (W302L), methionine (W302M), asparagine (W302N), proline (W302P), glutamine (W302Q), arginine (W302R), serine (W302S), threonine (W302T), valine (W302V), or tyrosine (W302Y);
 - (nn) the valine residue at a position corresponding to position 363 of wild-type human Neu2 is substituted by arginine (V363R); or
 - (oo) the leucine residue at a position corresponding to position 365 of wild-type human Neu2 is substituted by glutamine (L365Q), histidine (L365H), isoleucine (L365I), lysine (L365K) or serine (L365S);
- or the sialidase comprises a combination of any of the foregoing substitutions.

9. The fusion protein of any one of claims 4-8, wherein, in the sialidase:

- (a) the proline residue at a position corresponding to position 5 of wild-type human Neu2 is substituted by histidine (P5H);
- (b) the lysine residue at a position corresponding to position 9 of wild-type human Neu2 is substituted by aspartic acid (K9D);
- (c) the lysine residue at a position corresponding to position 44 of wild-type human Neu2 is substituted by arginine (K44R) or glutamic acid (K44E);
- (d) the lysine residue at a position corresponding to position 45 of wild-type human Neu2 is substituted by alanine (K45A), arginine (K45R), or glutamic acid (K45E);
- (e) the leucine residue at a position corresponding to position 54 of wild-type human Neu2 is substituted by methionine (L54M);
- (f) the proline residue at a position corresponding to position 62 of wild-type human Neu2 is substituted by asparagine (P62N), aspartic acid (P62D), histidine (P62H), glutamic acid (P62E), glycine (P62G), serine (P62S), or threonine (P62T);
- (g) the glutamine residue at a position corresponding to position 69 of wild-type human

Neu2 is substituted by histidine (Q69H);

(h) the arginine residue at a position corresponding to position 78 of wild-type human Neu2 is substituted by lysine (R78K);

(i) the alanine residue at a position corresponding to position 93 of wild-type human Neu2 is substituted by glutamic acid (A93E) or lysine (A93K);

(j) the glycine residue at a position corresponding to position 107 of wild-type human Neu2 is substituted by aspartic acid (G107D);

(k) the glutamine residue at a position corresponding to position 108 of wild-type human Neu2 is substituted by histidine (Q108H);

(l) the glutamine residue at a position corresponding to position 112 of wild-type human Neu2 is substituted by arginine (Q112R) or lysine (Q112K);

(m) the cysteine residue at a position corresponding to position 125 of wild-type human Neu2 is substituted by leucine (C125L);

(n) the glutamine residue at a position corresponding to position 126 of wild-type human Neu2 is substituted by leucine (Q126L);

(o) the alanine residue at a position corresponding to position 150 of wild-type human Neu2 is substituted by valine (A150V);

(p) the cysteine residue at a position corresponding to position 164 of wild-type human Neu2 is substituted by glycine (C164G);

(q) the alanine residue at a position corresponding to position 171 of wild-type human Neu2 is substituted by glycine (A171G);

(r) the leucine residue at a position corresponding to position 217 of wild-type human Neu2 is substituted by alanine (L217A) or valine (L217V);

(s) the threonine residue at a position corresponding to position 249 of wild-type human Neu2 is substituted by alanine (T249A);

(t) the aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 is substituted by glycine (D251G);

(u) the glutamine residue at a position corresponding to position 270 of wild-type human Neu2 is substituted by alanine (Q270A), histidine (Q270H), phenylalanine (Q270F) or proline (Q270P);

(v) the tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 is substituted by arginine (W292R);

(w) the serine residue at a position corresponding to position 301 of wild-type human Neu2 is substituted by arginine (S301R);

- (x) the tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 is substituted by lysine (W302K);
- (y) the valine residue at a position corresponding to position 363 of wild-type human Neu2 is substituted by arginine (V363R); or
- 5 (z) the leucine residue at a position corresponding to position 365 of wild-type human Neu2 is substituted by glutamine (L365Q), histidine (L365H), isoleucine (L365I), lysine (L365K) or serine (L365S);
- or the sialidase comprises a combination of any of the foregoing substitutions.

10 10. The fusion protein of claim 8 or 9, wherein the sialidase comprises a substitution selected from K9D, P62G, P62N, P62S, P62T, A93E, Q126Y, A242F, A242W, A242Y, Q270A, Q270T, S301A, S301R, W302K, W302R, V363R, and L365I, or a combination of any of the foregoing substitutions.

15 11. The fusion protein of any one of claims 8-10, wherein the sialidase comprises a substitution selected from K9D, P62G, P62N, P62S, P62T, A93E, Q270A, S301R, W302K, V363R, and L365I, or a combination of any of the foregoing substitutions.

12. The fusion protein of any one of claims 1-11, wherein the sialidase comprises:

- (a) a substitution or deletion of a methionine residue at a position corresponding to position 1 of wild-type human Neu2 (M1);
- 20 (b) a substitution of a valine residue at a position corresponding to position 6 of wild-type human Neu2 (V6);
- (c) a substitution of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187); or
- (d) a substitution of a cysteine residue at a position corresponding to position 332 of wild-type human Neu2 (C332);
- 25 or a combination of any of the foregoing substitutions.

13. The fusion protein of claim 12, wherein, in the sialidase:

- (a) the methionine residue at a position corresponding to position 1 of wild-type human Neu2 is deleted (Δ M1), is substituted by alanine (M1A), or is substituted by aspartic acid (M1D);
- 30 (b) the valine residue at a position corresponding to position 6 of wild-type human Neu2 is substituted by tyrosine (V6Y);
- (c) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by lysine (I187K); or

- (d) the cysteine residue at a position corresponding to position 332 of wild-type human Neu2 is substituted by alanine (C332A);
or the sialidase comprises a combination of any of the foregoing substitutions.

14. The fusion protein of claim 13, wherein the sialidase comprises:

- 5 (a) the M1D, V6Y, P62G, A93E, I187K, and C332A substitutions;
(b) the M1D, V6Y, K9D, A93E, I187K, C332A, V363R, and L365I substitutions;
(c) the M1D, V6Y, P62N, I187K, and C332A substitutions;
(d) the M1D, V6Y, I187K, Q270A, S301R, W302K, and C332A substitutions;
(e) the M1D, V6Y, P62S, I187K, Q270A, S301R, W302K, and C332A substitutions;
10 (f) the M1D, V6Y, P62T, I187K, Q270A, S301R, W302K, and C332A substitutions;
(g) the M1D, V6Y, P62N, I187K, Q270A, S301R, W302K, and C332A substitutions;
(h) the M1D, V6Y, P62G, A93E, I187K, S301A, W302R, and C332A substitutions;
(i) the M1D, V6Y, P62G, A93E, Q126Y, I187K, Q270T, and C332A substitutions;
(j) the M1D, V6Y, P62G, A93E, Q126Y, I187K, and C332A substitutions; or
15 (k) the M1D, V6Y, P62G, A93E, Q126Y, I187K, A242F, Q270T, and C332A substitutions.

15. The fusion protein of claim 13 or 14, wherein the sialidase comprises:

- (a) the M1D, V6Y, P62G, A93E, I187K, and C332A substitutions;
(b) the M1D, V6Y, K9D, A93E, I187K, C332A, V363R, and L365I substitutions;
20 (c) the M1D, V6Y, P62N, I187K, and C332A substitutions;
(d) the M1D, V6Y, I187K, Q270A, S301R, W302K, and C332A substitutions;
(e) the M1D, V6Y, P62S, I187K, Q270A, S301R, W302K, and C332A substitutions;
(f) the M1D, V6Y, P62T, I187K, Q270A, S301R, W302K, and C332A substitutions; or
(g) the M1D, V6Y, P62N, I187K, Q270A, S301R, W302K, and C332A substitutions.

25 16. The fusion protein of any one of claims 1-15, wherein the sialidase is selected from Neu1, Neu2, Neu3, and Neu4.

17. The fusion protein of claim 16, wherein the sialidase is Neu2.

18. The fusion protein of any one of claims 1-17, wherein the sialidase has a different substrate specificity than the corresponding wild-type sialidase.

30 19. The fusion protein of claim 18, wherein the sialidase can cleave α 2,3, α 2,6, and/or α 2,8 linkages.

20. The fusion protein of claim 19, wherein the sialidase can cleave α 2,3 and α 2,8 linkages.

21. The fusion protein of any one of claims 1-20, wherein the sialidase comprises any one of SEQ ID NOs: 48-62, 94, 97, 100, or 126.
22. The fusion protein of any one of claims 1-21, wherein the sialidase comprises any one of SEQ ID NOs: 48-54.
- 5 23. The fusion protein of any one of claims 1-22, wherein the sialidase comprises a mutation set forth in any one of Tables 5-12 or 14-27.
24. The fusion protein of any one of claims 1-23, wherein the sialidase comprises a mutation set forth in any one of Tables 1-4.
25. The fusion protein of any one of claims 1-24, wherein the fusion protein further comprises an
10 immunoglobulin Fc domain.
26. The fusion protein of claim 25, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM Fc domain.
27. The fusion protein of claim 26, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain.
- 15 28. The fusion protein of claim 27, wherein the immunoglobulin Fc domain is derived from a human IgG1 Fc domain.
29. The fusion protein of any one of claims 1-28, wherein the anti-PD-L1 immunoglobulin antigen-binding domain is associated with a second anti-PD-L1 immunoglobulin antigen-binding domain to produce an anti-PD-L1 antigen-binding site.
- 20 30. The fusion protein of any one of claims 1-29, wherein the anti-PD-L1 immunoglobulin antigen-binding domain is derived from an antibody selected from avelumab, atezolizumab, durvalumab, MEDI4736, CS1001, KL-A167, CK-301, TQB2450, KN035, SHR-1316, STI-A1014, BGB-A333, MSB2311, HLX-20 and BMS-936559.
31. The fusion protein of claim 30, wherein the anti-PD-L1 immunoglobulin antigen-binding
25 domain is derived from avelumab.
32. The fusion protein of any one of claims 1-31, wherein the sialidase and the immunoglobulin Fc domain and/or the anti-PD-L1 immunoglobulin antigen-binding domain are linked by a peptide bond or an amino acid linker.
33. The fusion protein of any one of claims 1-32, wherein the fusion protein comprises any one
30 of SEQ ID NOs: 65-75, 78, 81-89, 95, 96, 98, 99, 101, 102, 104, 106, 108, 110, 112, 114, 122-124, 127, or 128.

34. The fusion protein of any one of claims 1-33, wherein the fusion protein comprises any one of SEQ ID NOs: 65-75, 78, or 81-89.
35. An antibody conjugate comprising the fusion protein of any one of claims 1-34.
36. The antibody conjugate of claim 35, wherein the antibody conjugate comprises a single
5 sialidase.
37. The antibody conjugate of claim 35, wherein the antibody conjugate comprises two sialidases.
38. The antibody conjugate of claim 37, wherein the two sialidases are identical.
39. The antibody conjugate of any one of claims 35-38, wherein the antibody conjugate
10 comprises a single anti-PD-L1 antigen-binding site.
40. The antibody conjugate of any one of claims 35-38, wherein the antibody conjugate comprises two anti-PD-L1 antigen-binding sites.
41. The antibody conjugate of claim 40, wherein the two anti-PD-L1 antigen-binding sites are identical.
- 15 42. The antibody conjugate of any one of claims 35-41, wherein the antibody conjugate has a molecular weight from about 135 kDa to about 165 kDa.
43. The antibody conjugate of any one of claims 35-41, wherein the antibody conjugate has a molecular weight from about 215 kDa to about 245 kDa.
44. The antibody conjugate of any one of claims 35-43, wherein the antibody conjugate
20 comprises:
- (a) a first polypeptide comprising an immunoglobulin light chain;
 - (b) a second polypeptide comprising an immunoglobulin heavy chain; and
 - (c) a third polypeptide comprising an immunoglobulin Fc domain and a sialidase;
- 25 wherein the first and second polypeptides are covalently linked together and the second and third polypeptides are linked together, and wherein the first polypeptide and the second polypeptide together define an anti-PD-L1 antigen-binding site.
45. The antibody conjugate of claim 44, wherein the third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.
46. The antibody conjugate of claim 44 or 45, wherein the first polypeptide comprises SEQ ID
30 NO: 65.

47. The antibody conjugate of any one of claims 44-46, wherein the second polypeptide comprises SEQ ID NO: 66, 104, or 124.

48. The antibody conjugate of any one of claims 44-47, wherein the third polypeptide comprises any one of SEQ ID NOs: 67-73, 78, 81-87, 95, 96, 98, 99, 101, 102, 106, 108, 112, 122, 123, 127, or 128.

49. The antibody conjugate of any one of claims 35-43, wherein the fusion protein comprises:

- (a) a first polypeptide comprising a first immunoglobulin light chain;
 - (b) a second polypeptide comprising a first immunoglobulin heavy chain and a first sialidase;
 - (c) a third polypeptide comprising a second immunoglobulin heavy chain and a second sialidase; and
 - (d) a fourth polypeptide comprising a second immunoglobulin light chain;
- wherein the first and second polypeptides are covalently linked together, the third and fourth polypeptides are covalently linked together, and the second and third polypeptides are covalently linked together, and wherein the first polypeptide and the second polypeptide together define a first anti-PD-L1 antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second anti-PD-L1 antigen-binding site.

50. The antibody conjugate of claim 49, wherein the second and third polypeptides comprise the first and second immunoglobulin heavy chain and the first and second sialidase, respectively, in an N- to C-terminal orientation.

51. The antibody conjugate of any one of claims 35-43, wherein the fusion protein comprises:

- (a) a first polypeptide comprising a first sialidase, a first immunoglobulin Fc domain, and a first single chain variable fragment (scFv); and
 - (b) a second polypeptide comprising a second sialidase, a second immunoglobulin Fc domain, and a second single chain variable fragment (scFv);
- wherein the first and second polypeptides are covalently linked together, and wherein the first scFv defines a first anti-PD-L1 antigen-binding site, and the second scFv defines a second anti-PD-L1 antigen-binding site.

52. The antibody conjugate of claim 51, wherein the first polypeptide comprises the first sialidase, the first immunoglobulin Fc domain, and the first scFv in an N- to C-terminal orientation, and the second polypeptide comprises the second sialidase, the second

immunoglobulin Fc domain, and the second scFv in an N- to C-terminal orientation.

53. The antibody conjugate of claim 51 or 52, wherein the first polypeptide comprises SEQ ID NO: 114.

54. The antibody conjugate of any one of claims 51-53, wherein the second polypeptide comprises SEQ ID NO: 114.

55. The antibody conjugate of any one of claims 35-43, wherein the antibody conjugate comprises:

- (a) a first polypeptide comprising an immunoglobulin light chain;
- (b) a second polypeptide comprising an immunoglobulin heavy chain and a single chain variable fragment (scFv); and
- (c) a third polypeptide comprising an immunoglobulin Fc domain and a sialidase; wherein the first and second polypeptides are covalently linked together and the second and third polypeptides are covalently linked together, and wherein the immunoglobulin light chain and immunoglobulin heavy chain together define a first anti-PD-L1 antigen-binding site and the scFv defines a second anti-PD-L1 antigen-binding site.

56. The antibody conjugate of claim 55, wherein the second polypeptide comprises the immunoglobulin heavy chain and the scFv in an N- to C-terminal orientation, and the third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.

57. The antibody conjugate of claim 55 or 56, wherein the first polypeptide comprises SEQ ID NO: 65.

58. The antibody conjugate of any one of claims 55-57, wherein the second polypeptide comprises SEQ ID NO: 110.

59. The antibody conjugate of any one of claims 55-58, wherein the third polypeptide comprises any one of SEQ ID NOs: 78 or 106.

60. An isolated nucleic acid comprising a nucleotide sequence encoding the fusion protein of any one of claims 1-34, or at least a portion of the antibody conjugate of any one of claims 35-59.

61. An expression vector comprising the nucleic acid of claim 60.

62. A host cell comprising the expression vector of claim 61.

63. A pharmaceutical composition comprising the fusion protein of any one of claims 1-34 or the antibody conjugate of any one of claims 35-59.

64. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of the fusion protein of any one of claims 1-34, the antibody conjugate of any one of claims 35-59, or the pharmaceutical composition of claim 63.

65. The method of claim 64, wherein the cancer is selected from NSCLC, melanoma, bladder, breast, cervical, esophageal, gastric, kidney, lung, ovary, metastatic Merkel cell carcinoma (MCC), metastatic urothelial carcinoma (UC), and pancreatic cancer.

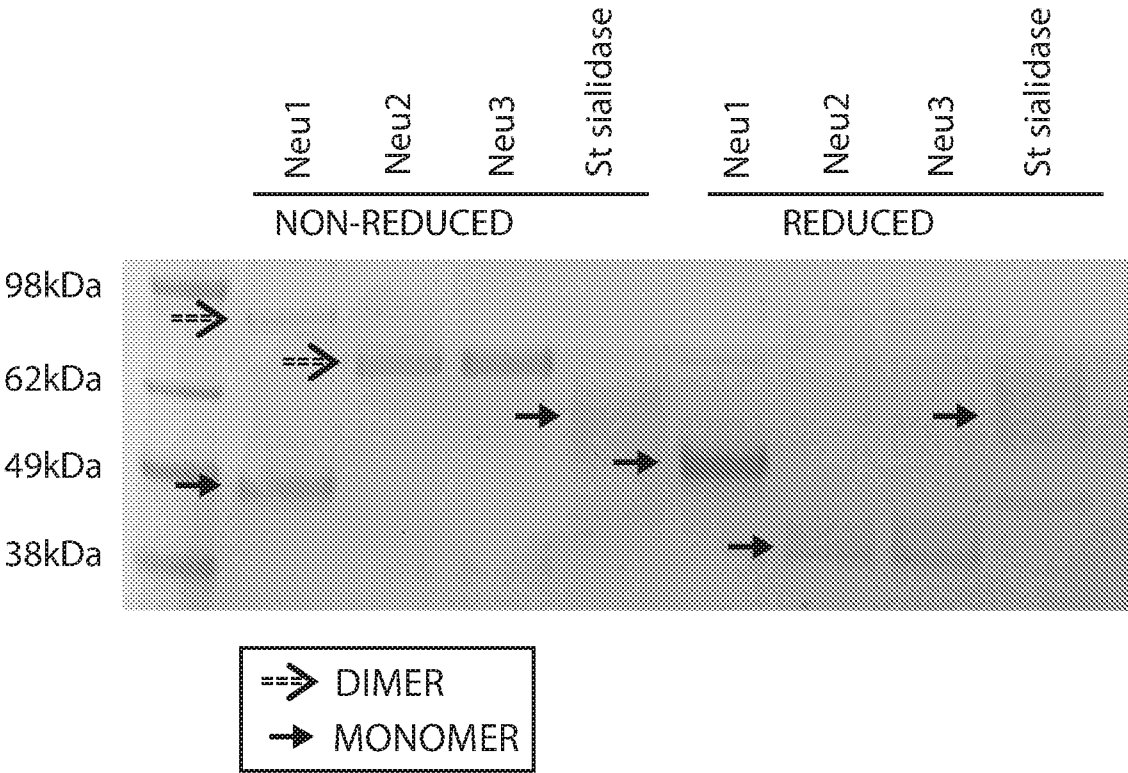


FIGURE 1

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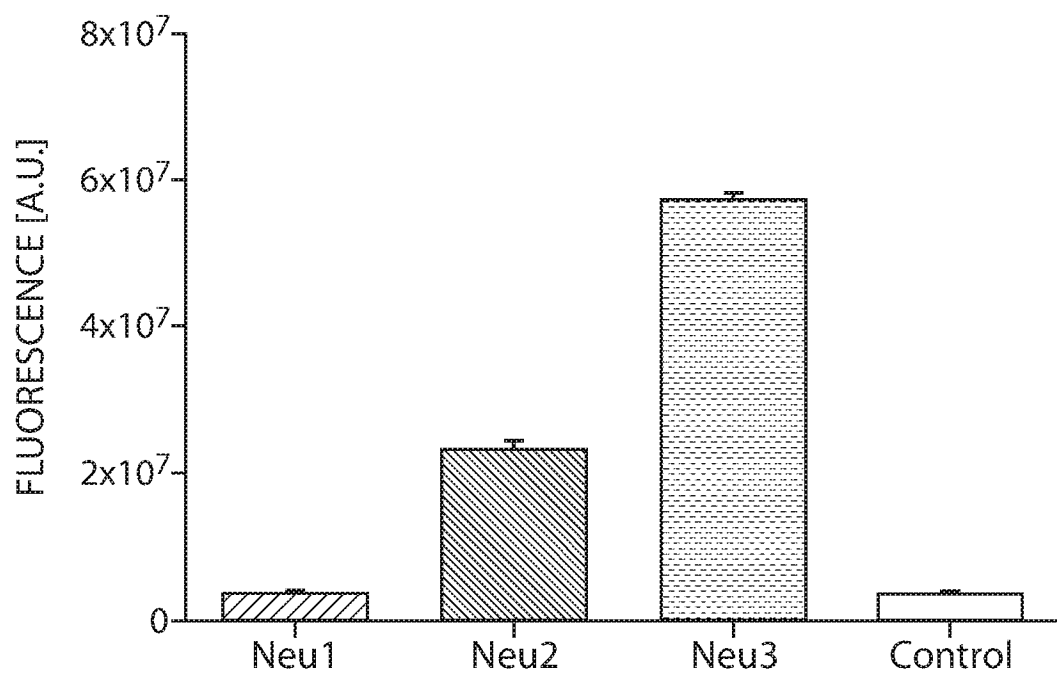


FIGURE 2

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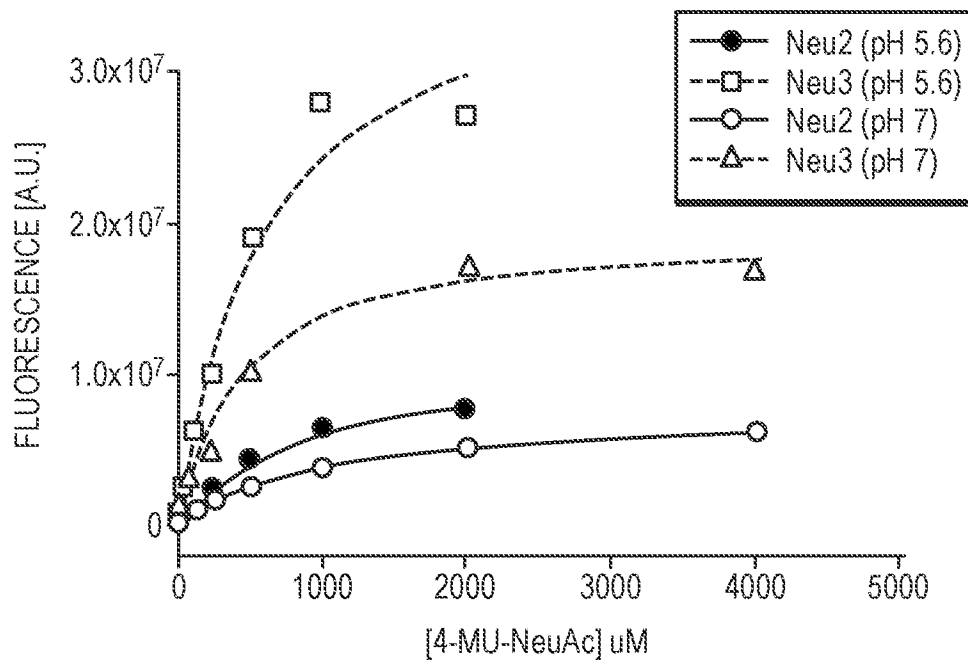


FIGURE 3

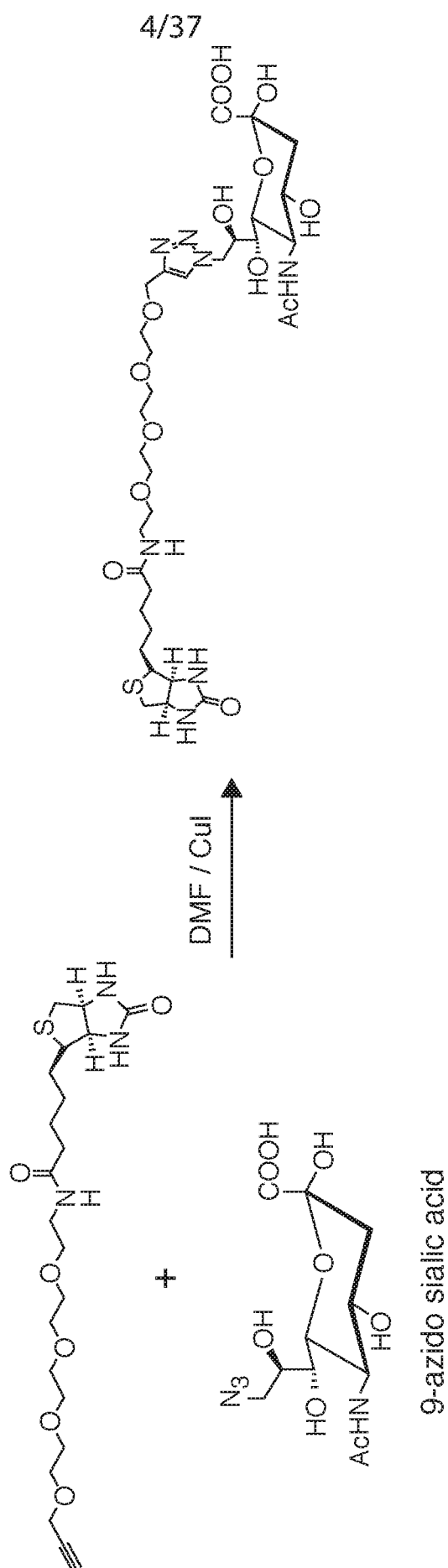


FIGURE 4

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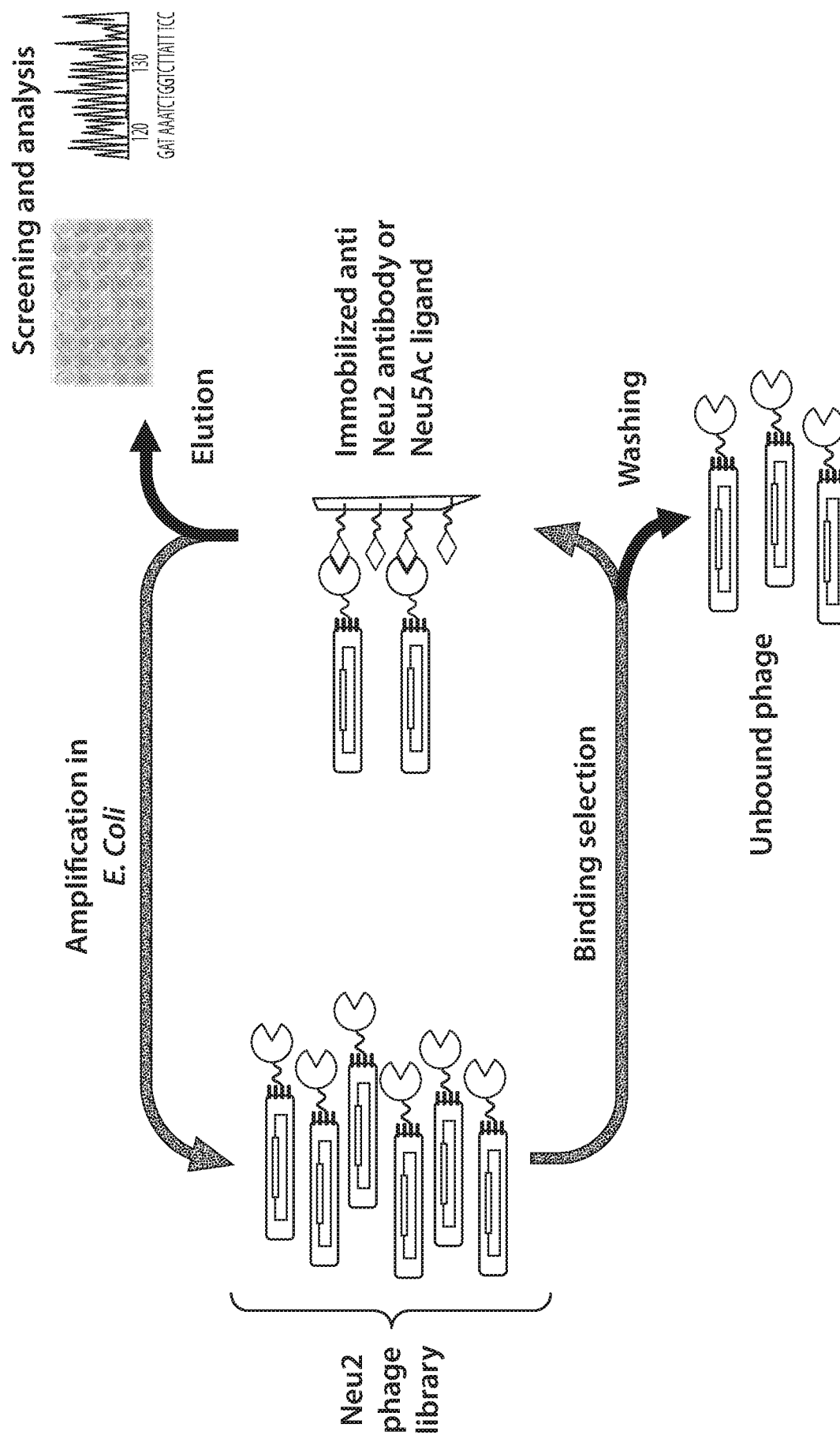


FIGURE 5

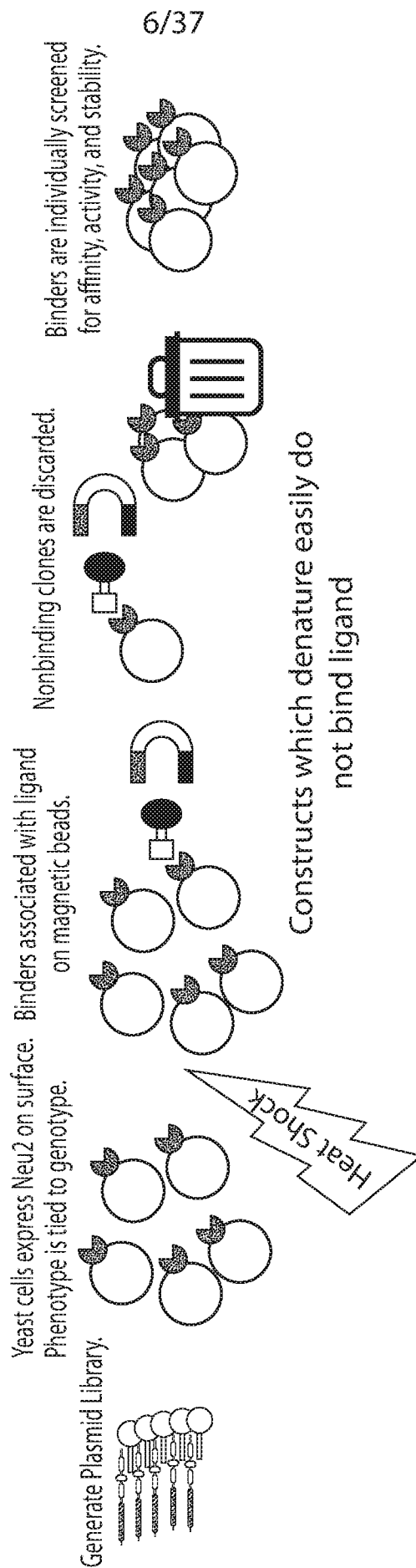


FIGURE 6

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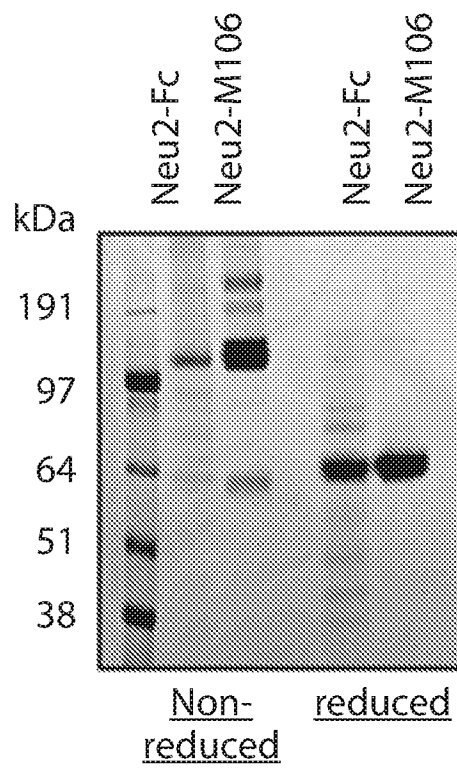


FIGURE 7A

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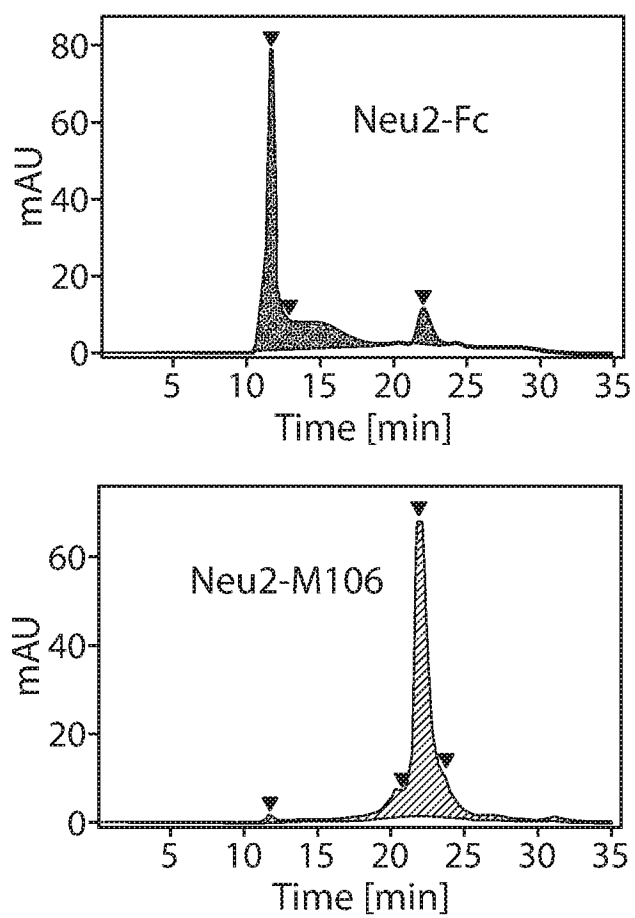


FIGURE 7B

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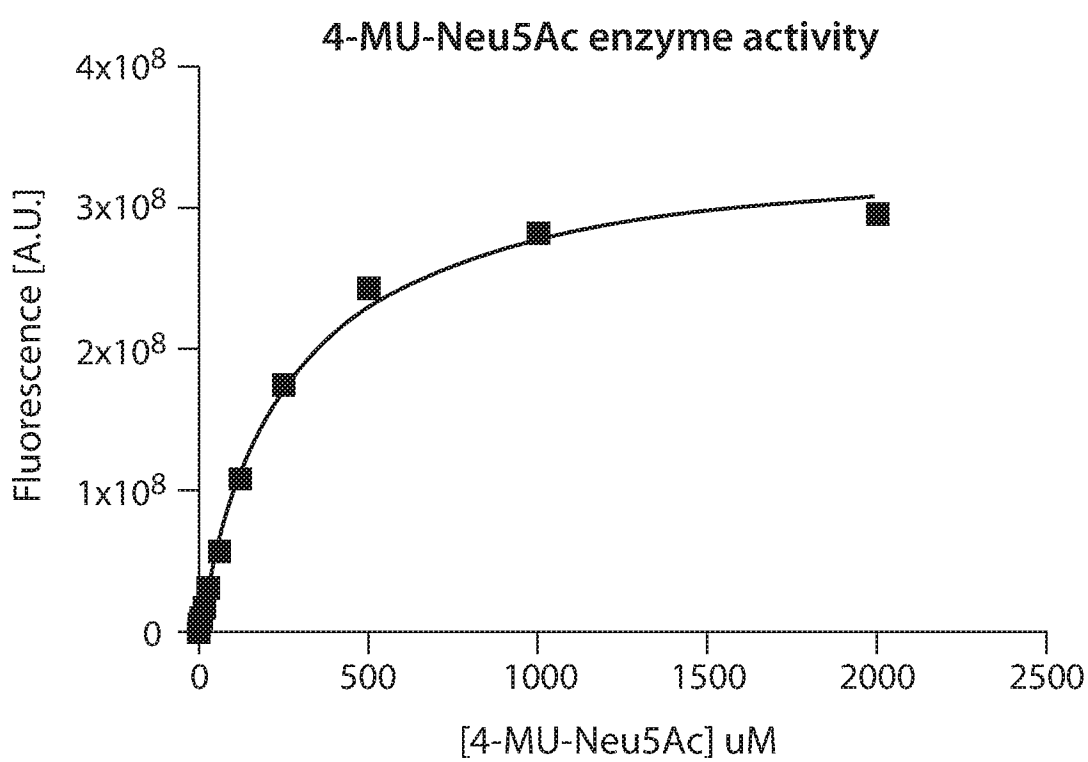


FIGURE 8

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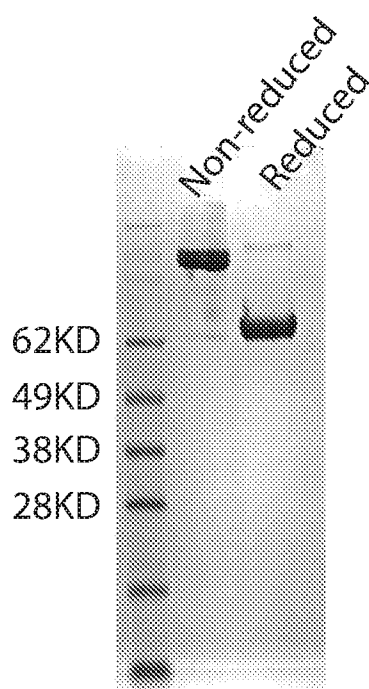


FIGURE 9A

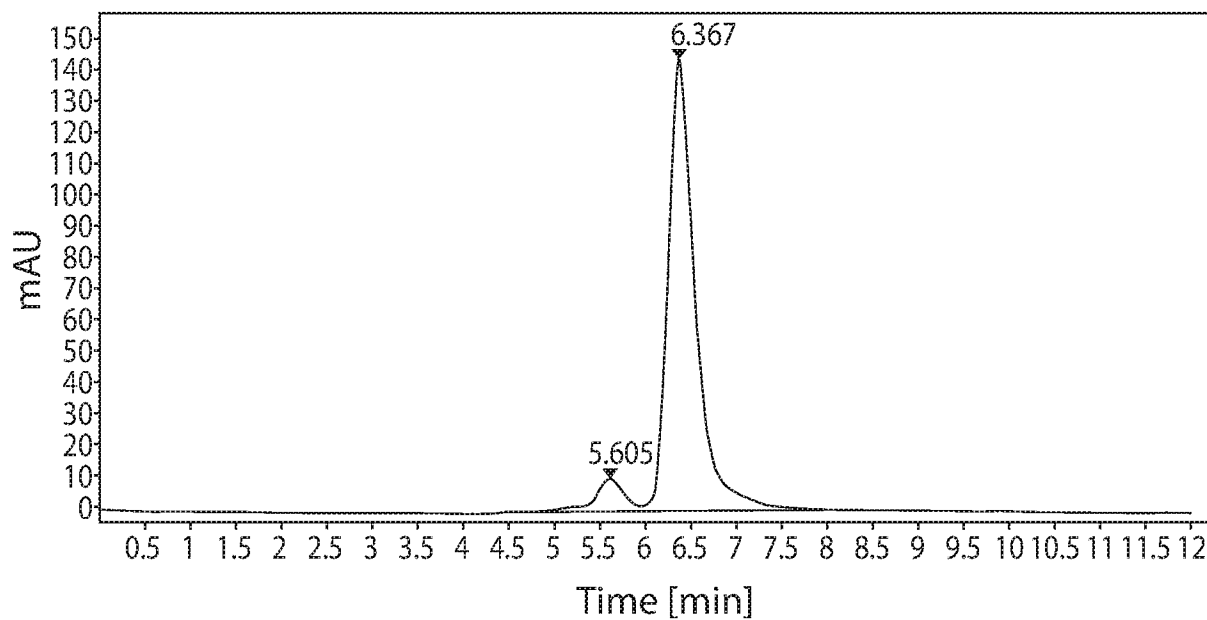


FIGURE 9B

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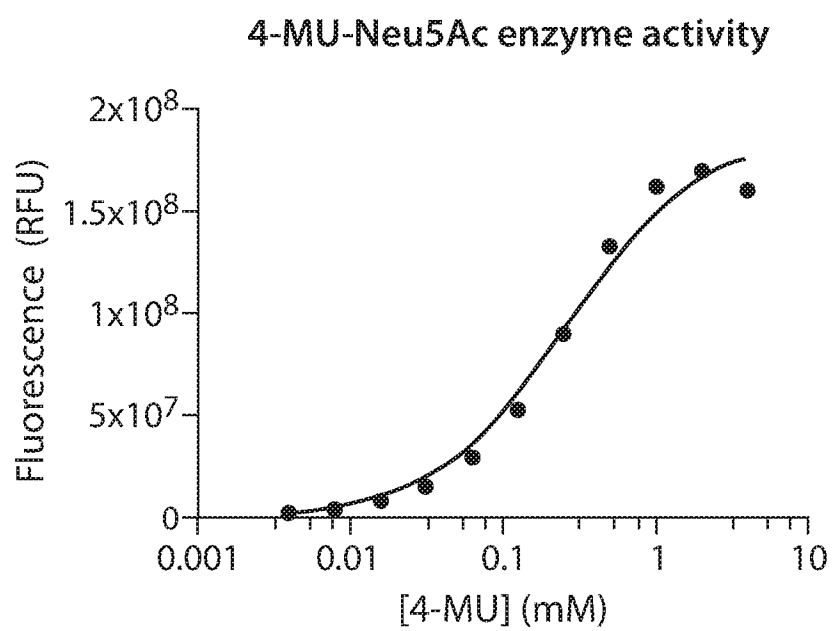


FIGURE 10

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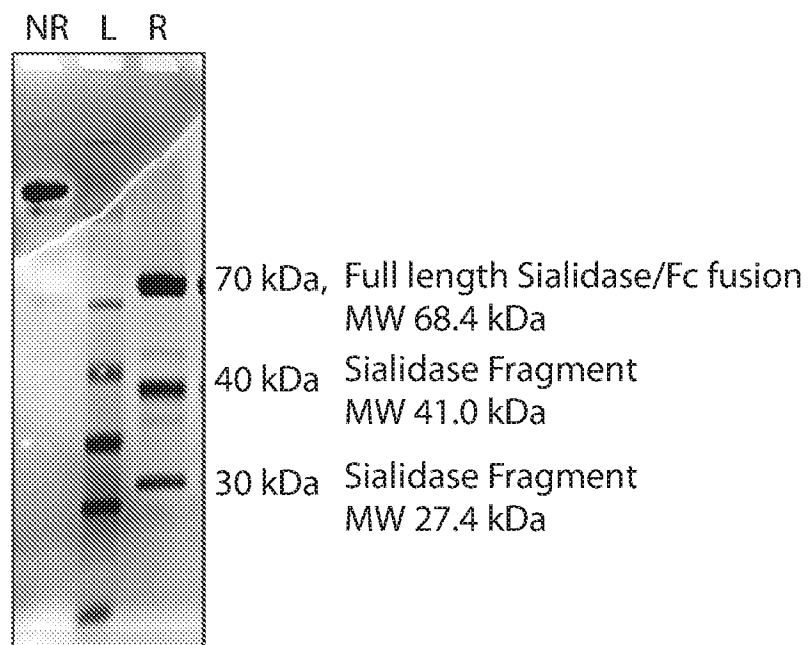


FIGURE 11A

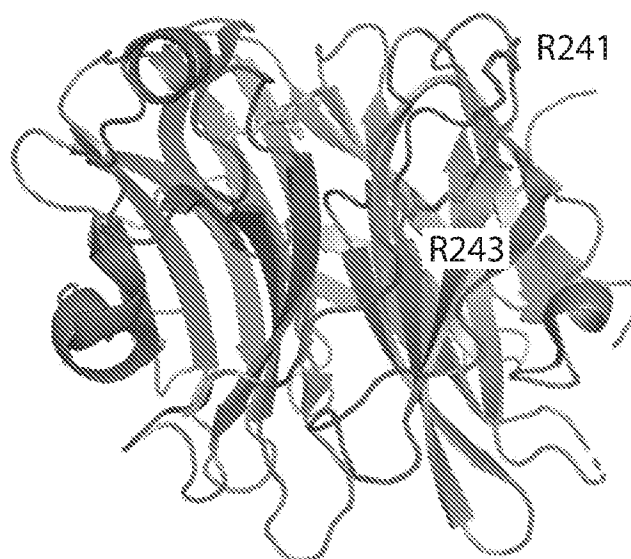


FIGURE 11B

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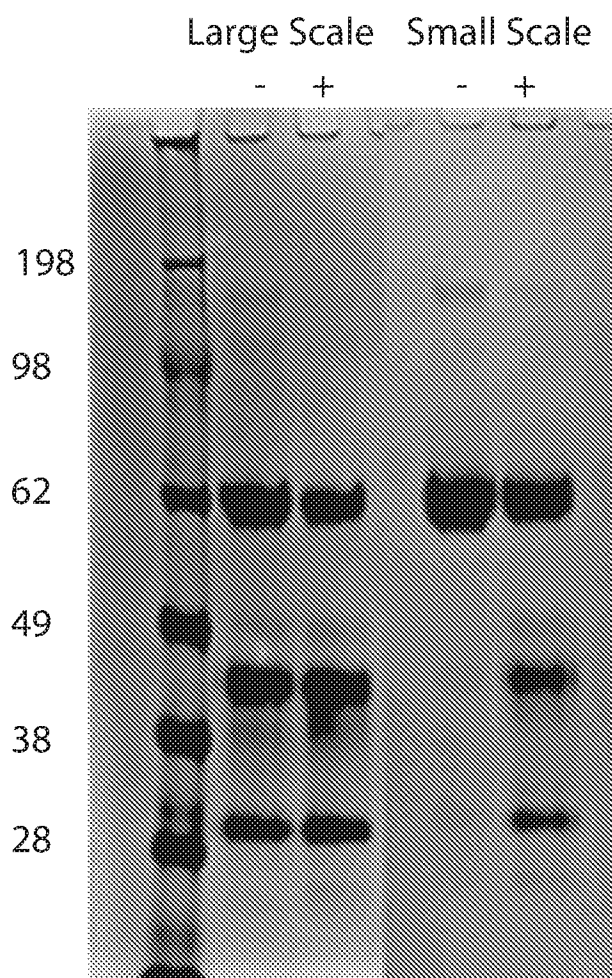


FIGURE 12

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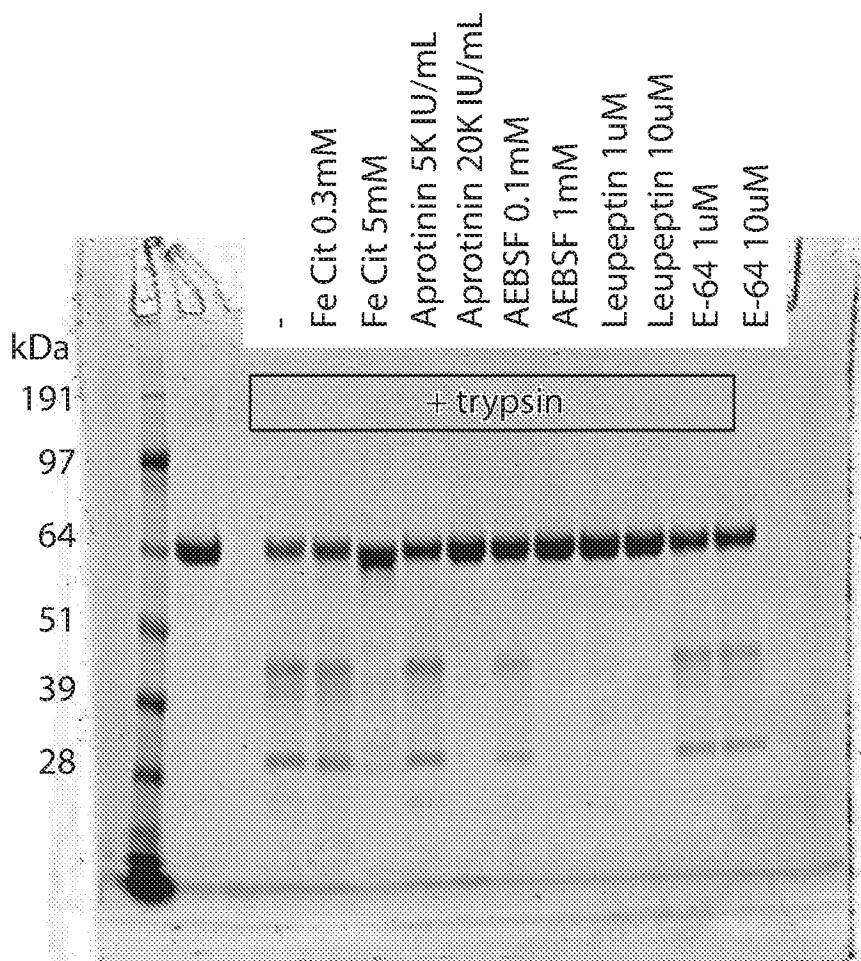
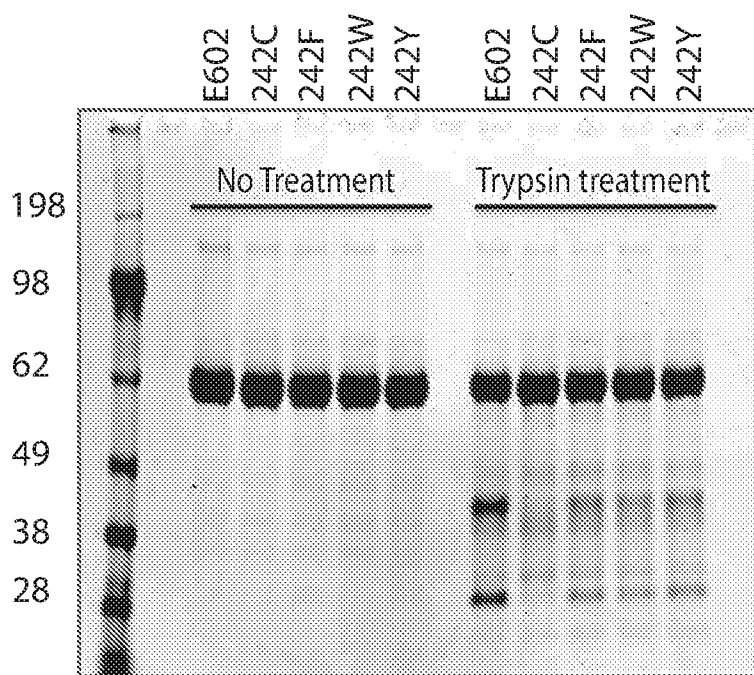


FIGURE 13

		P2	P1	P1'		
240	241	242	243	244	245	
L	R	A	R	V	Q	
		C				
			K			
				I		
				P		
		C		K		
		R		R		
		R		H		
		T		P		
		Y		P		
		N		P		

FIGURE 14

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SDS PAGE reduced
FIGURE 15A

Activity by titration with 0.5 mM 4-MU

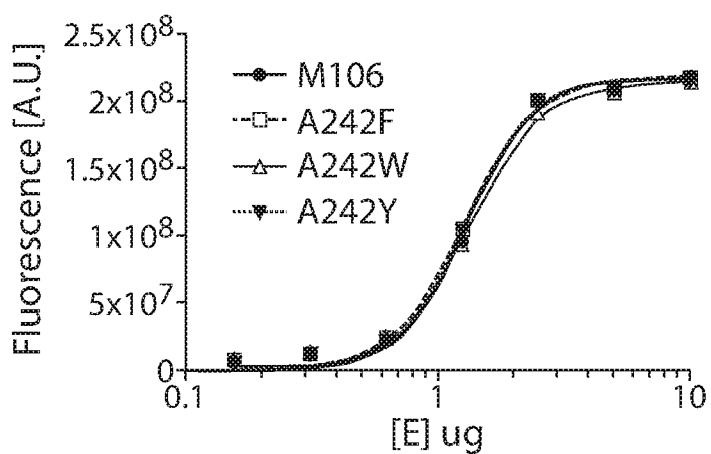


FIGURE 15B

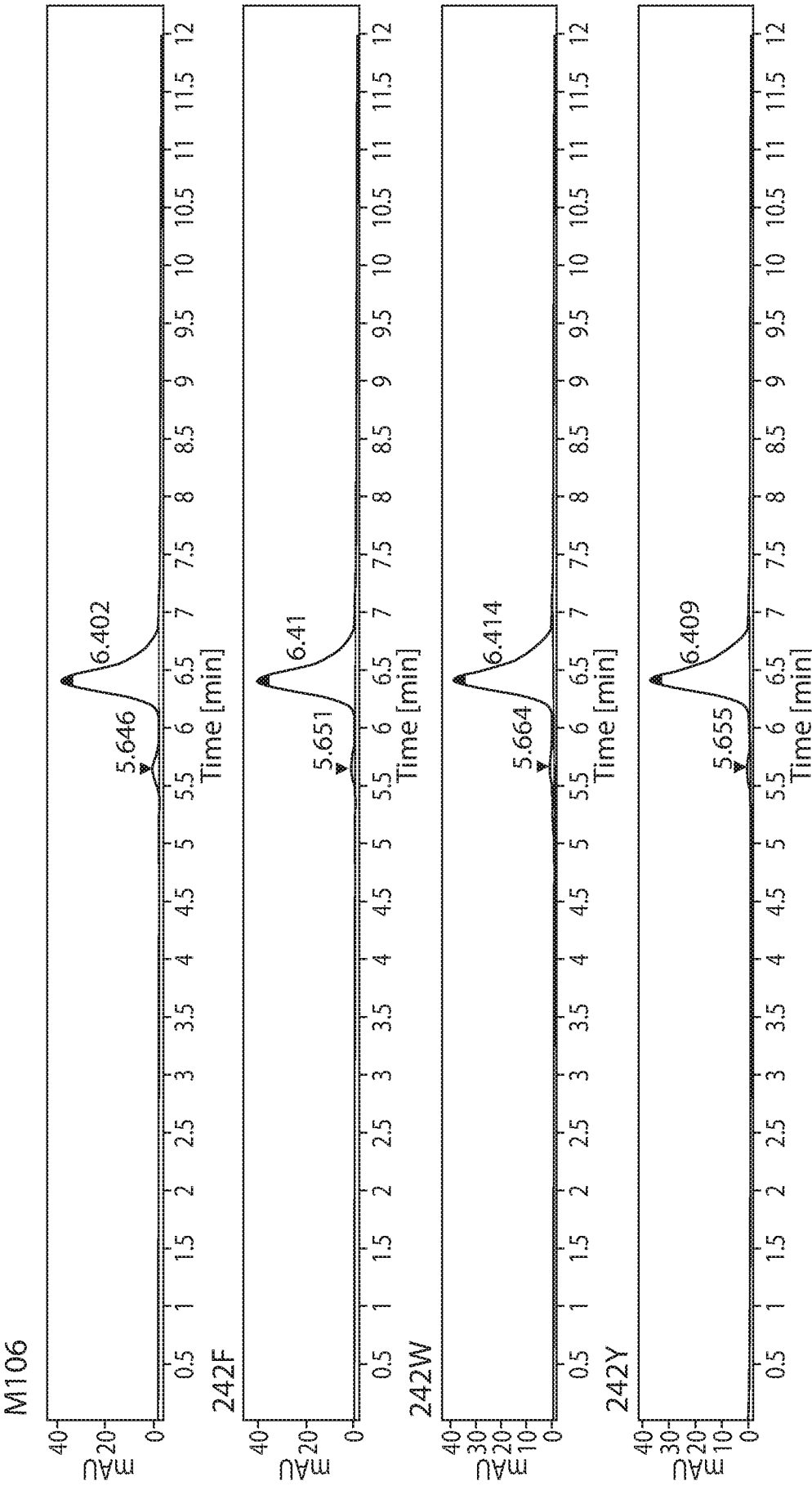


FIGURE 15C

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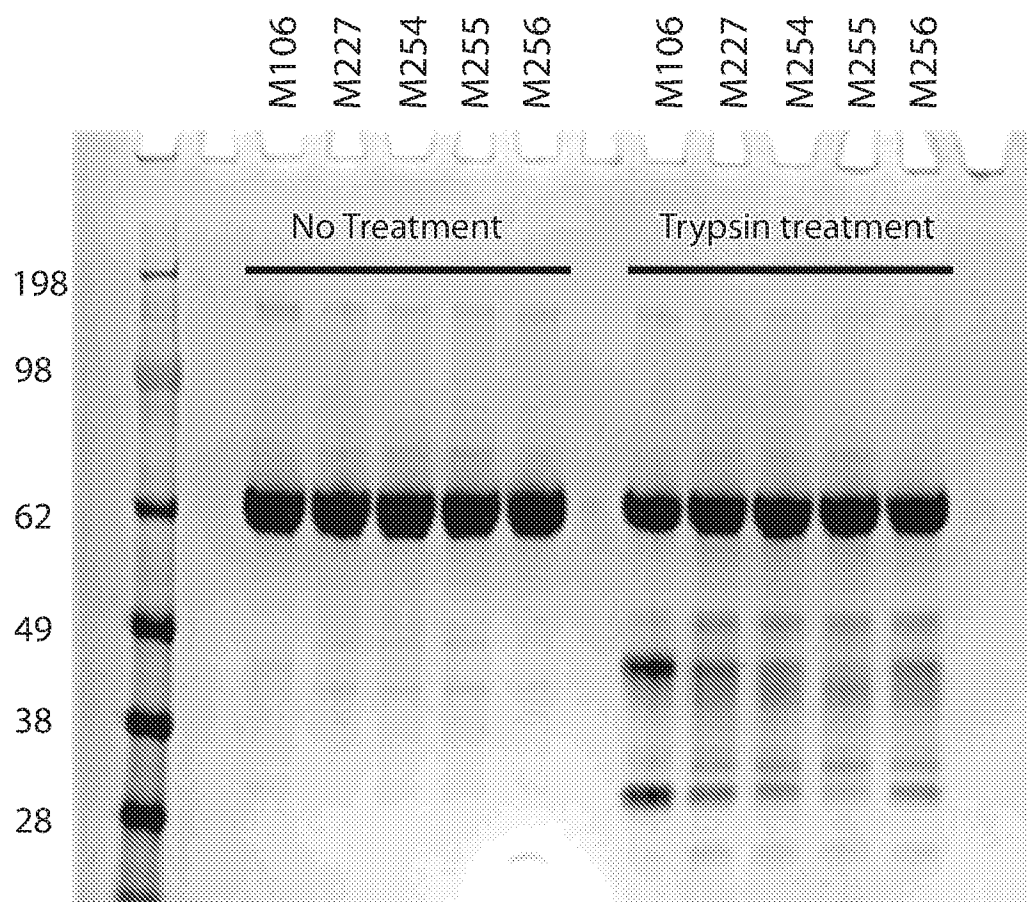


FIGURE 16

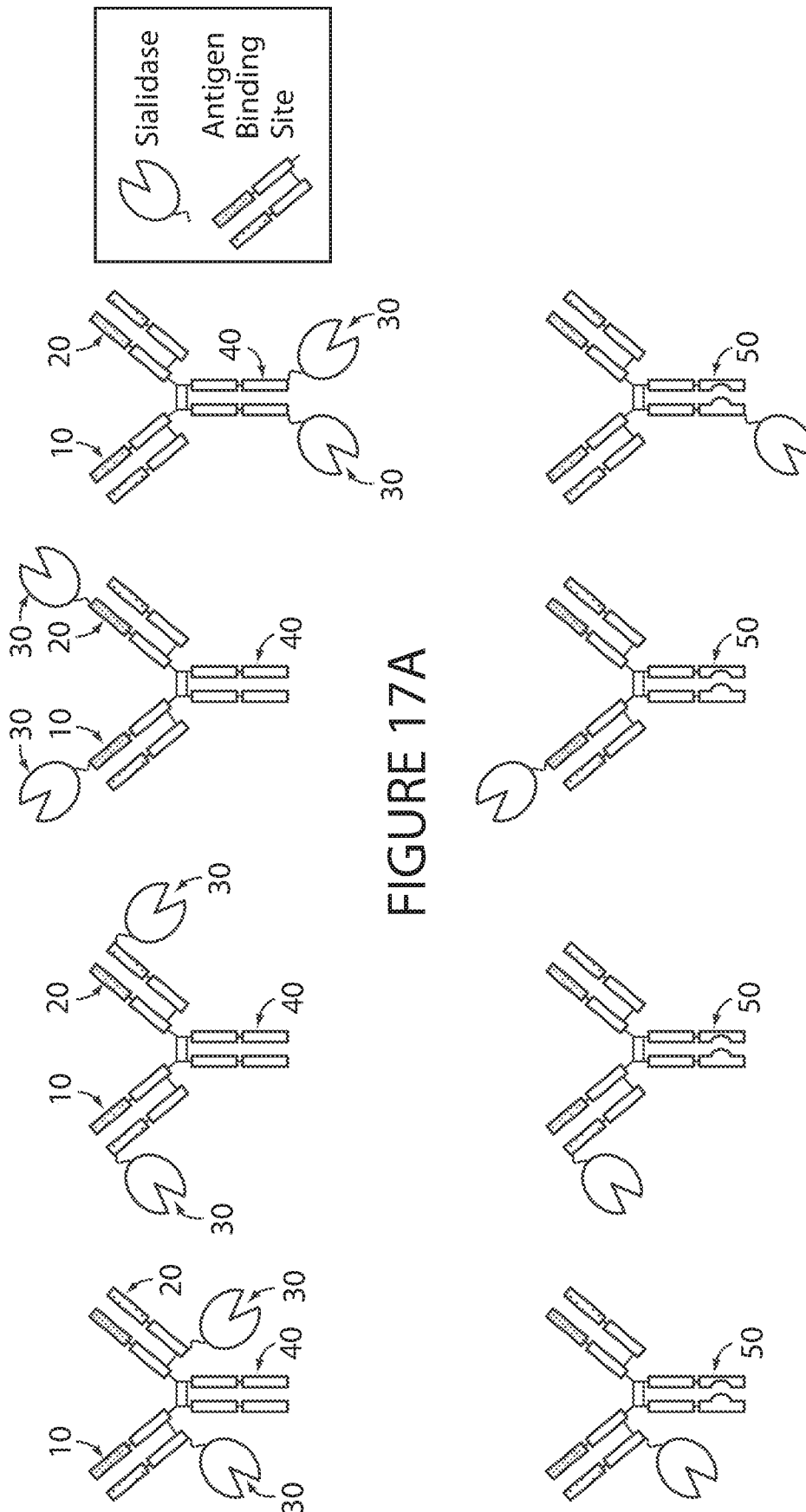


FIGURE 17A

FIGURE 17B

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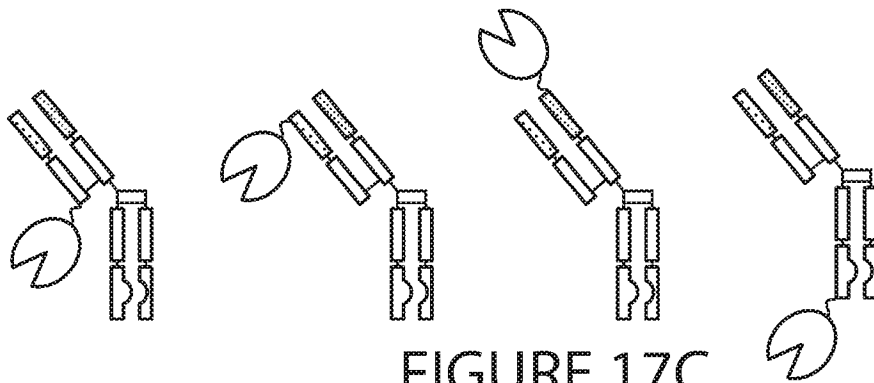


FIGURE 17C

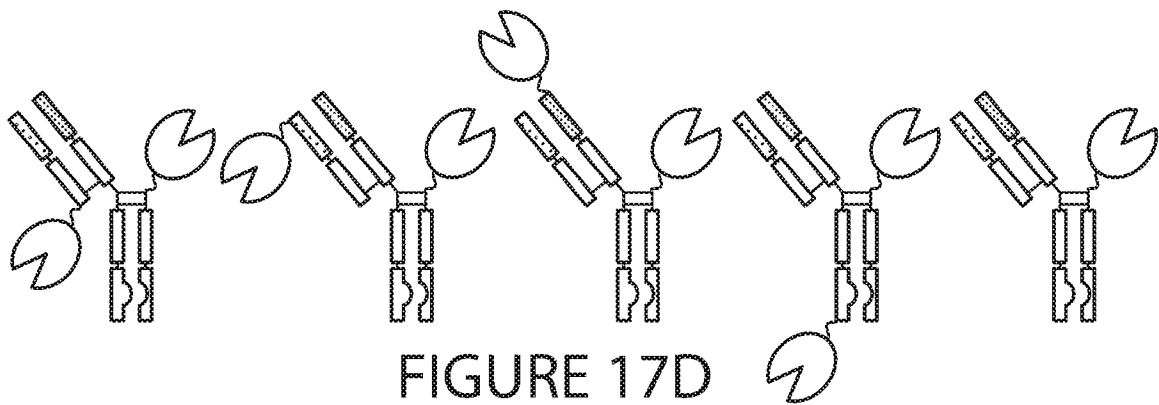


FIGURE 17D

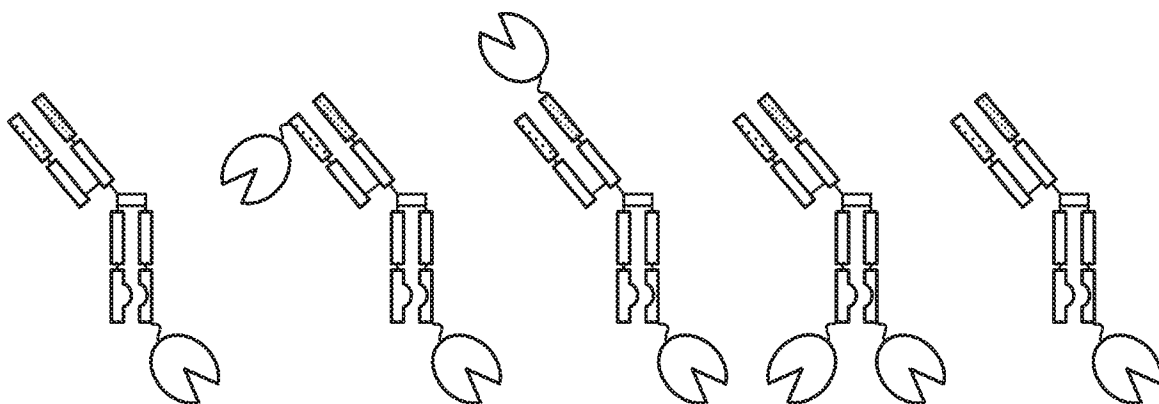


FIGURE 17E

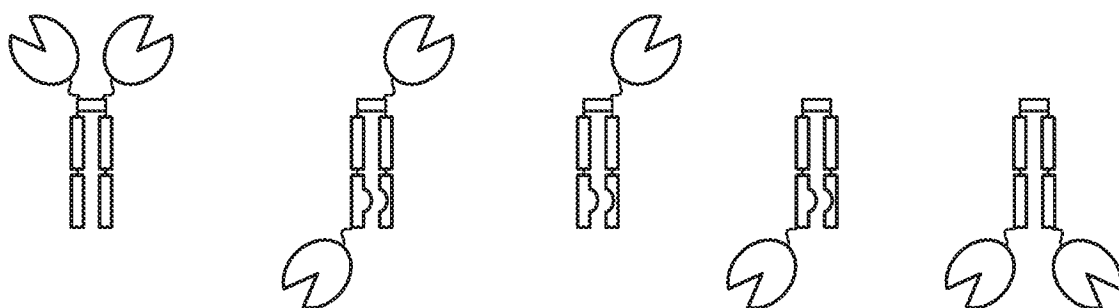


FIGURE 17F

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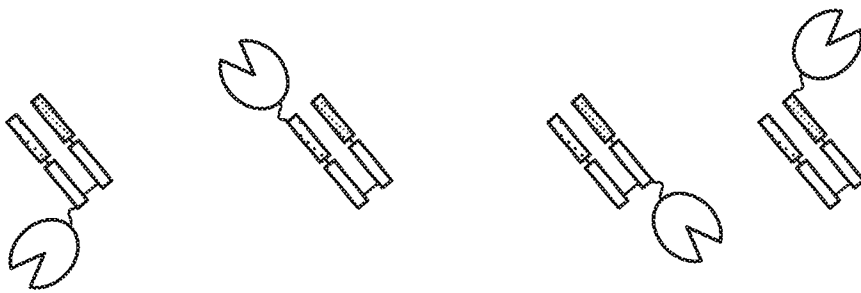


FIGURE 17G

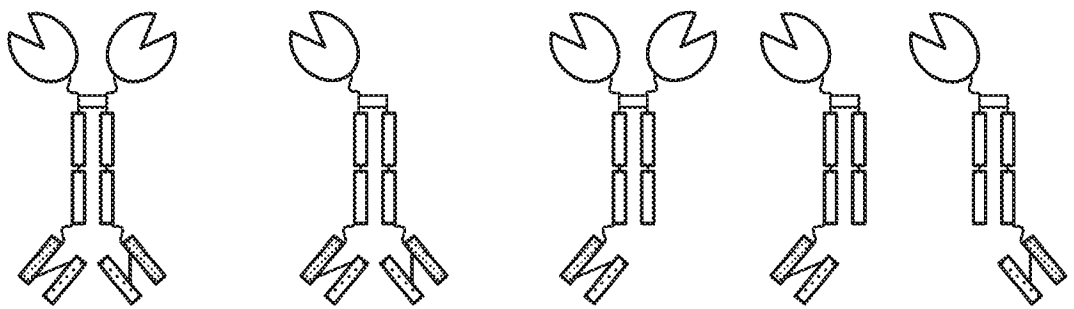


FIGURE 17H

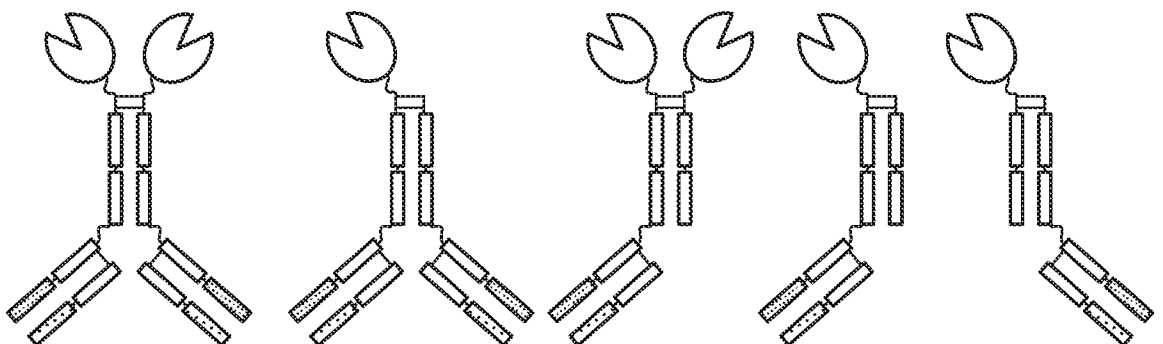


FIGURE 17I

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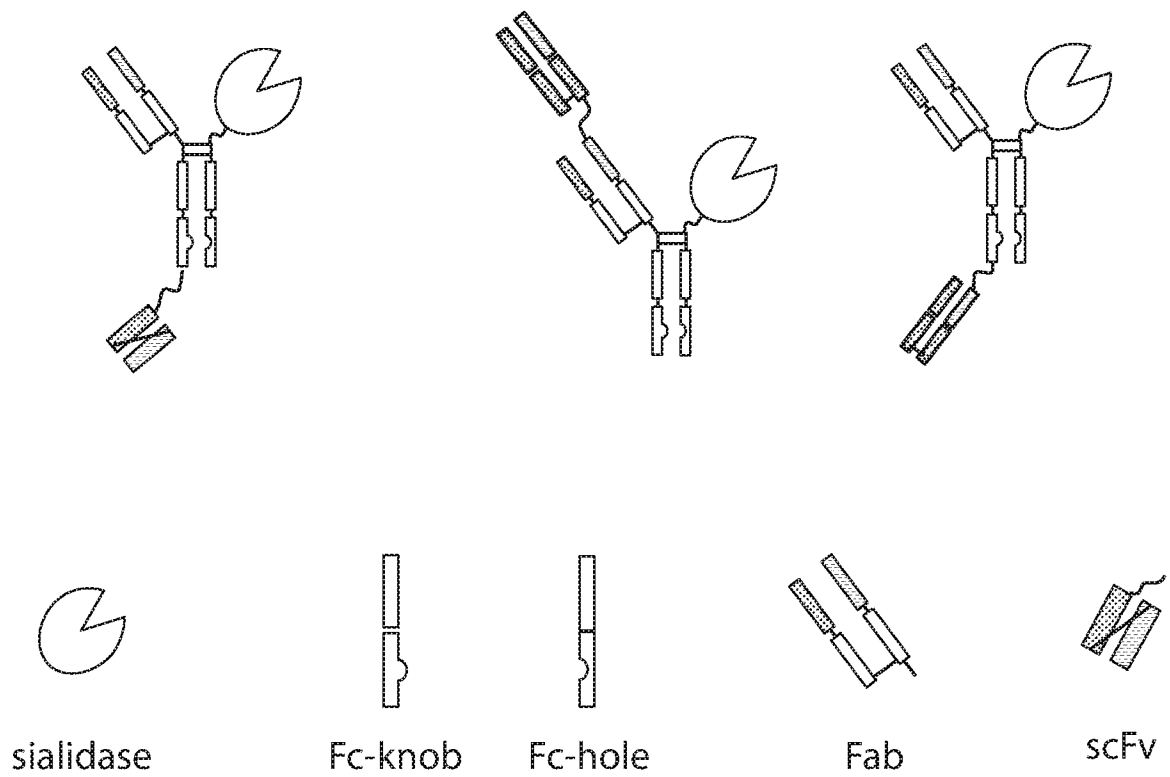


FIGURE 18

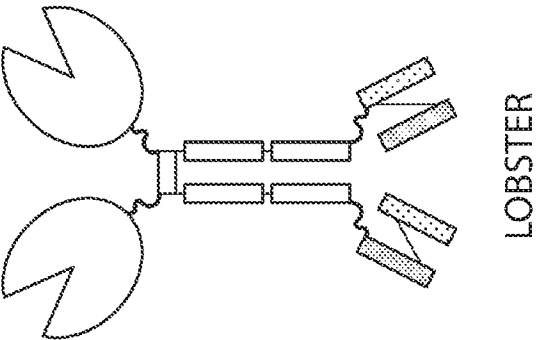


FIGURE 19C

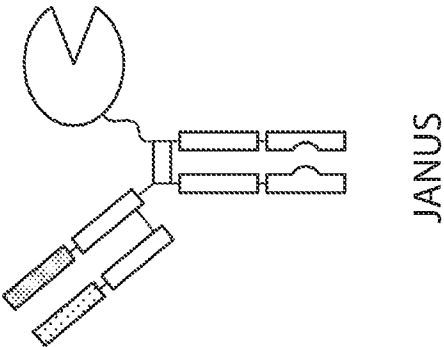


FIGURE 19B

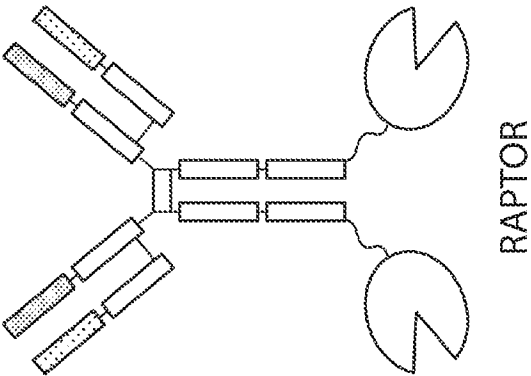


FIGURE 19A

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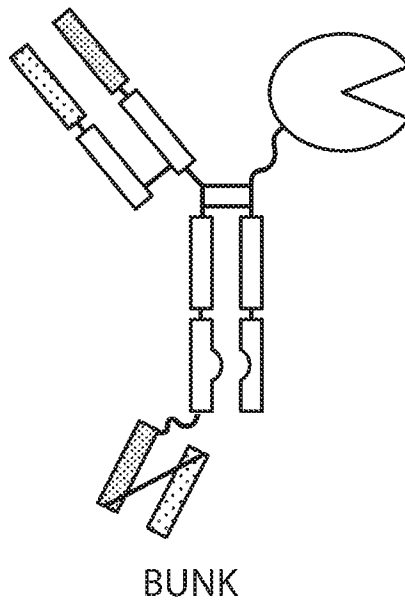


FIGURE 19D

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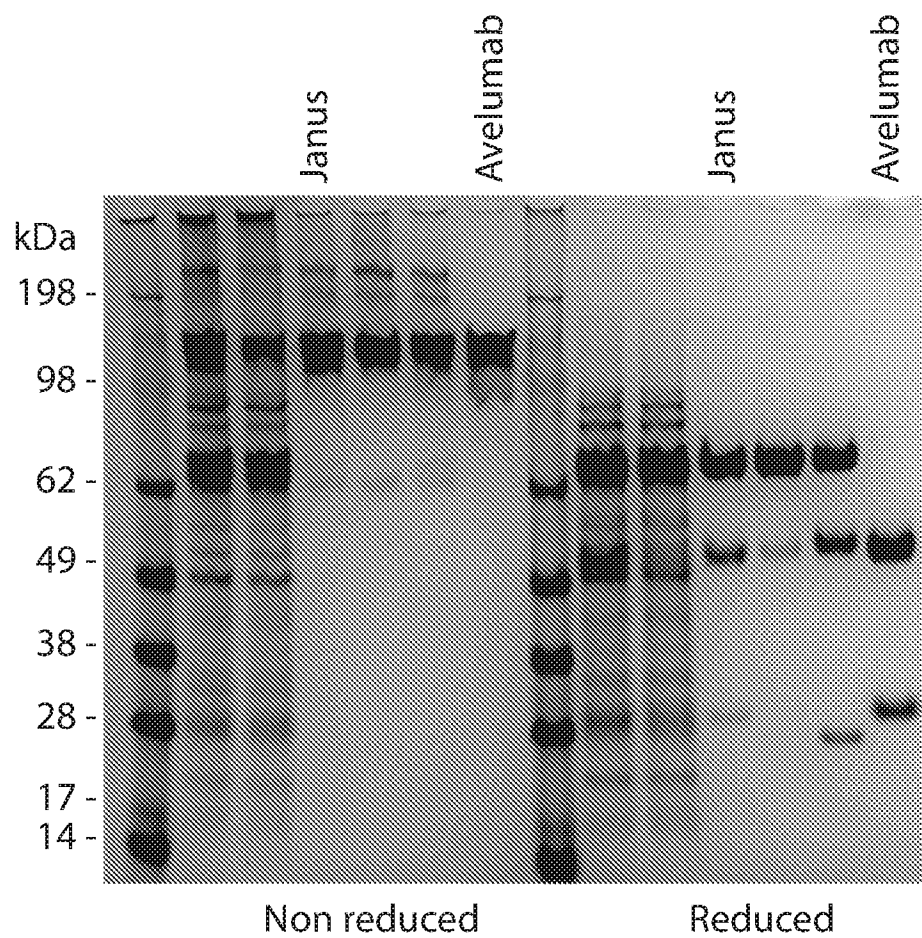


FIGURE 20

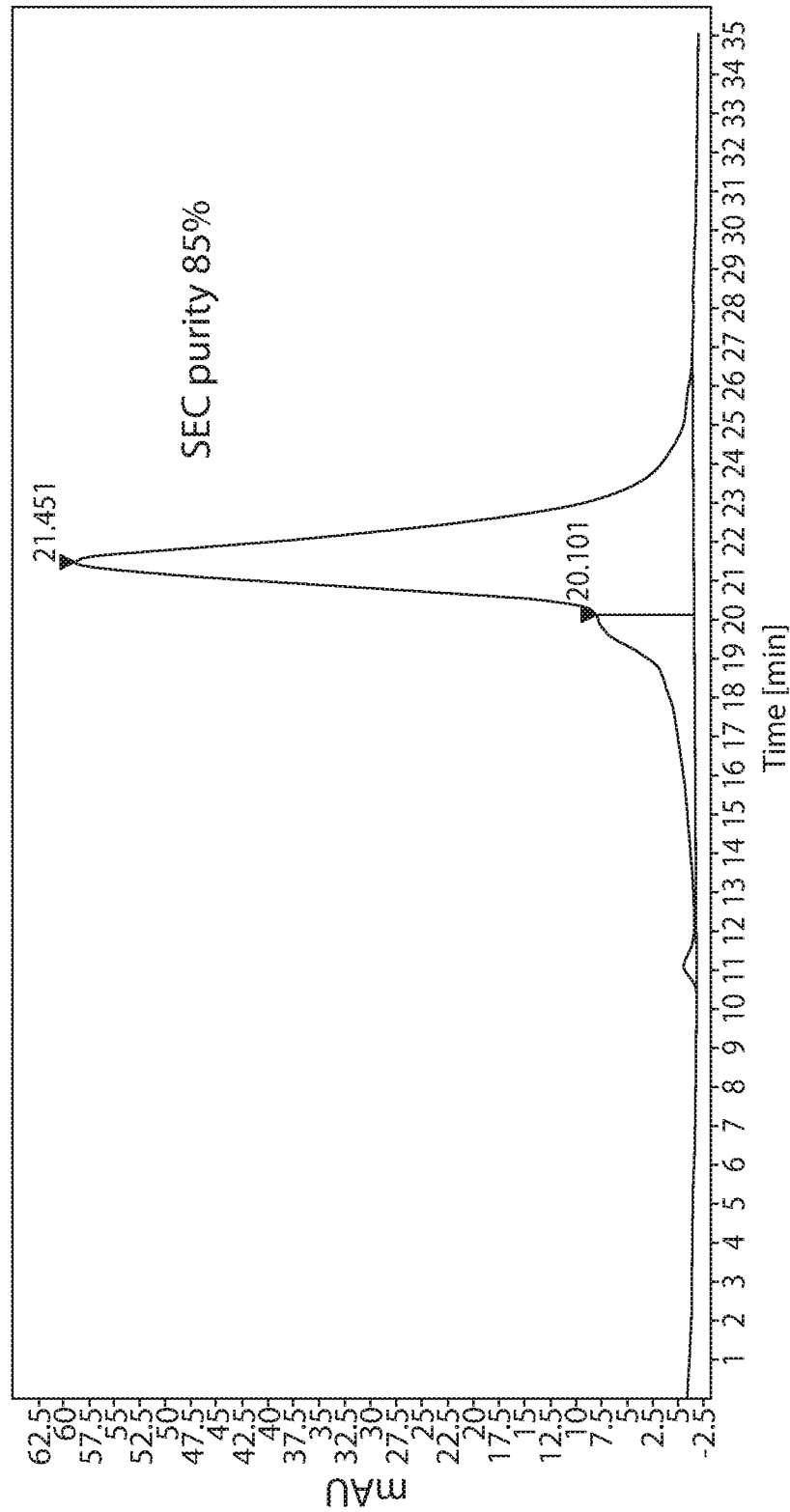


FIGURE 21

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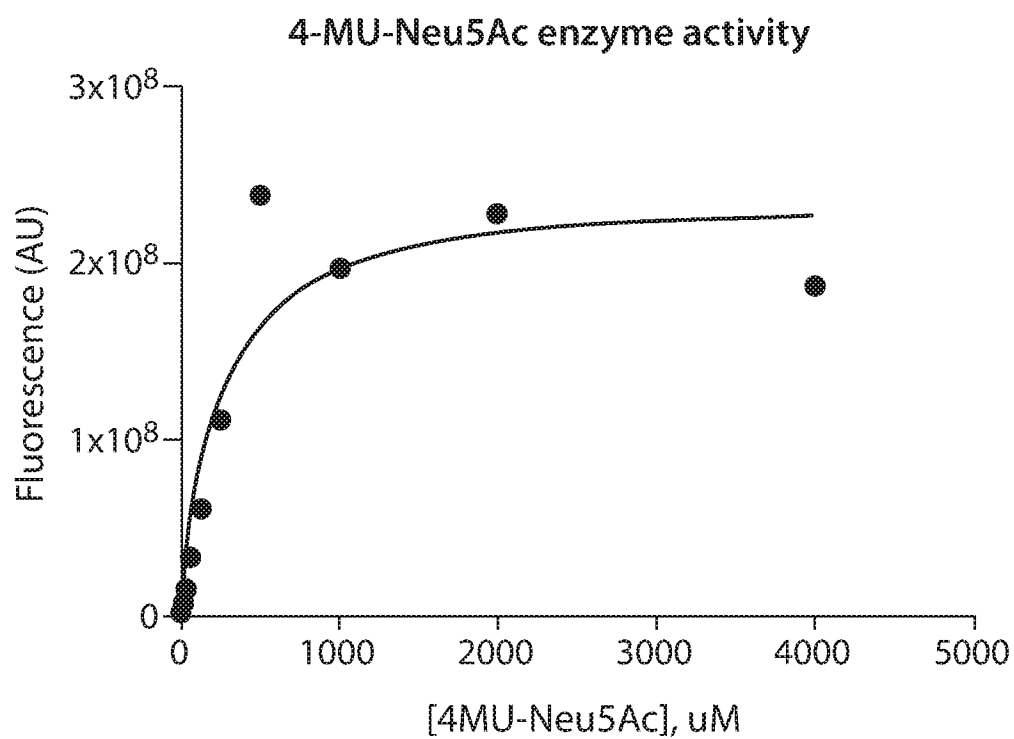


FIGURE 22

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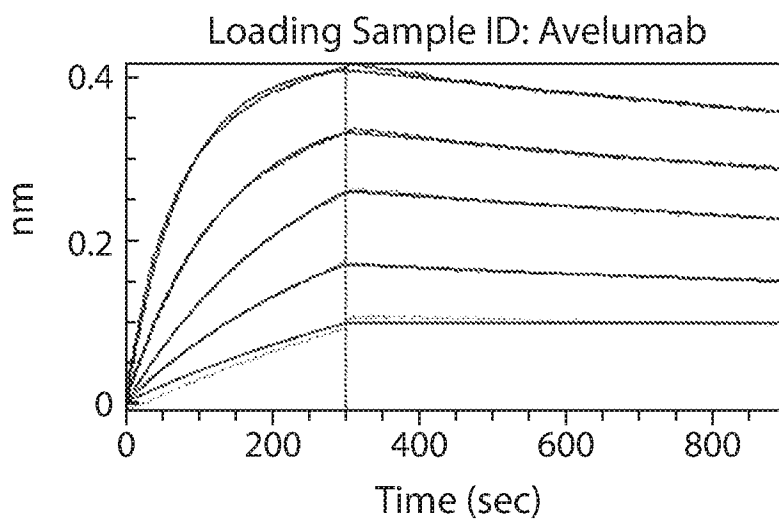


FIGURE 23A

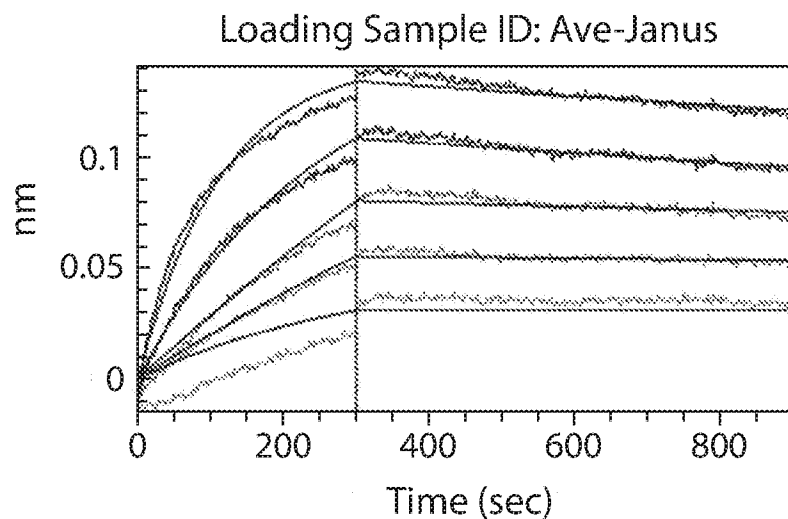


FIGURE 23B

	KD (M)	kon(1/Ms)	kdis(1/s)
Ave-Janus	1.28E-09	1.29E+05	1.39E-04
Avelumab	7.47E-10	3.31E+05	2.35E-04

FIGURE 23C

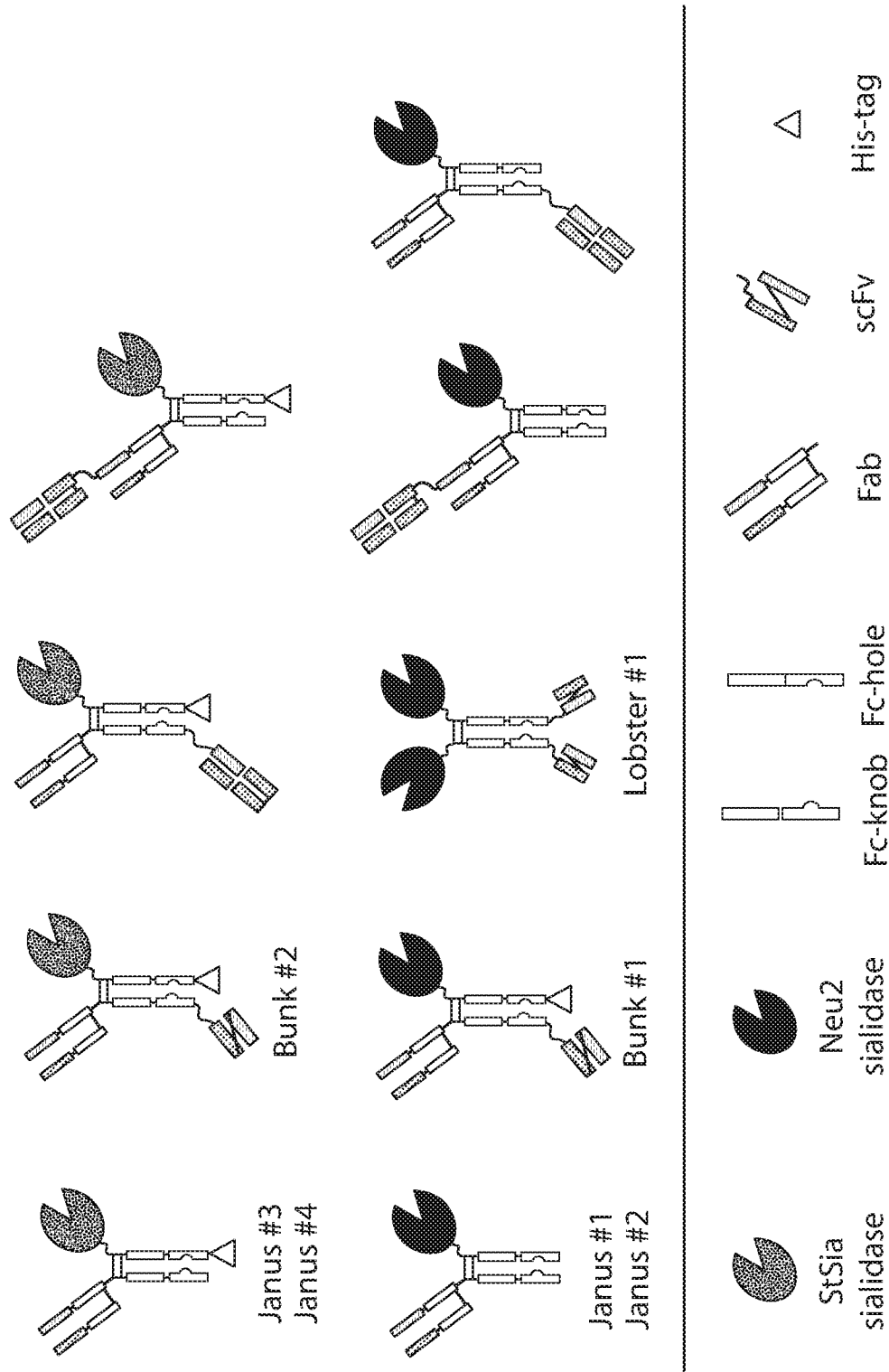


FIGURE 24

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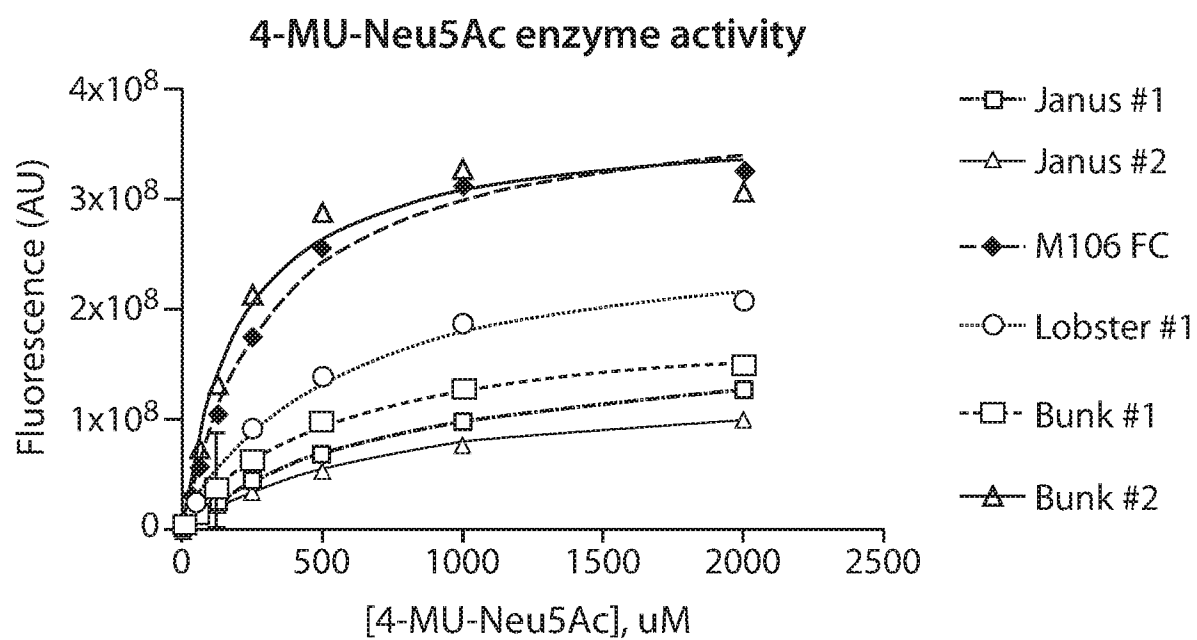


FIGURE 25

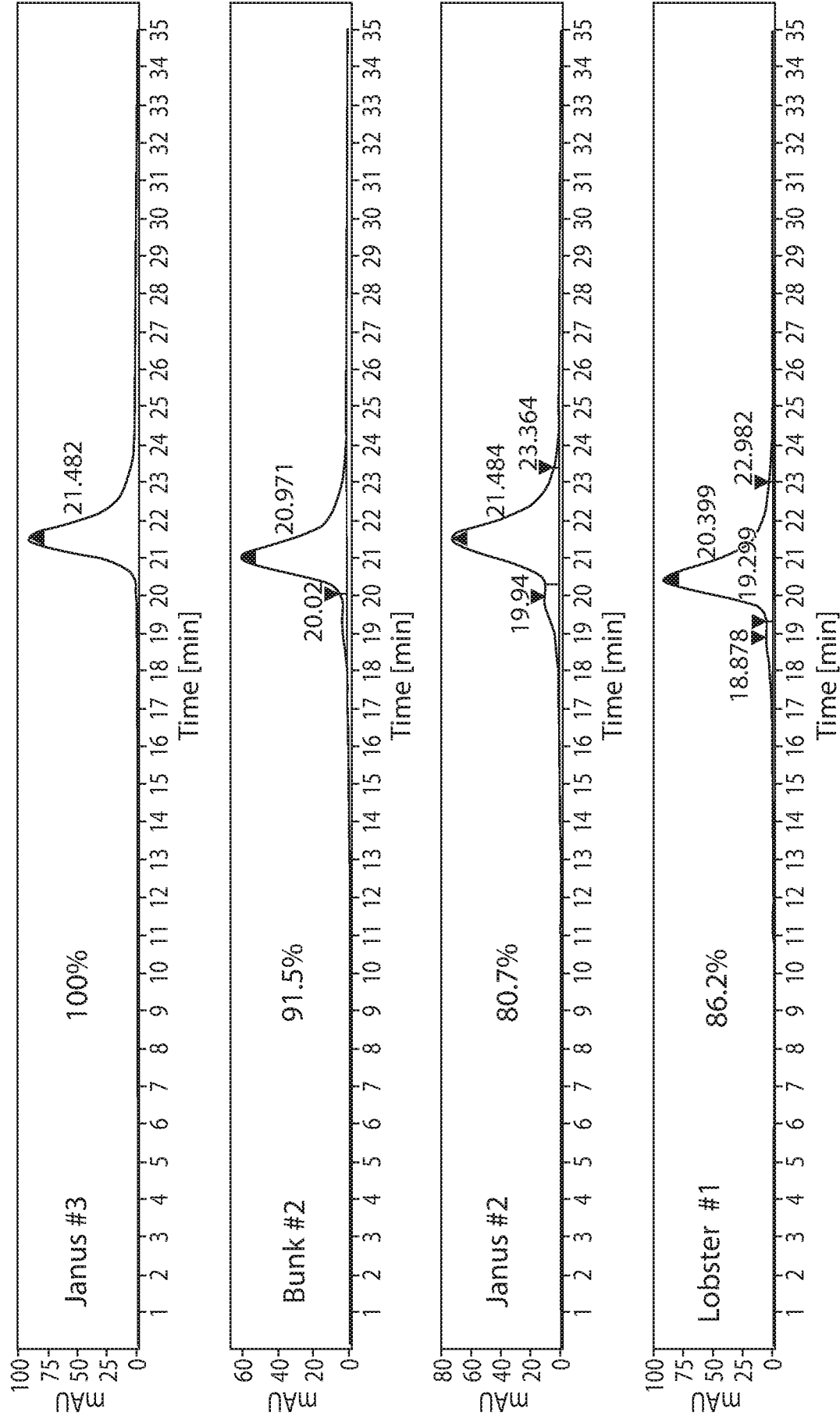


FIGURE 26

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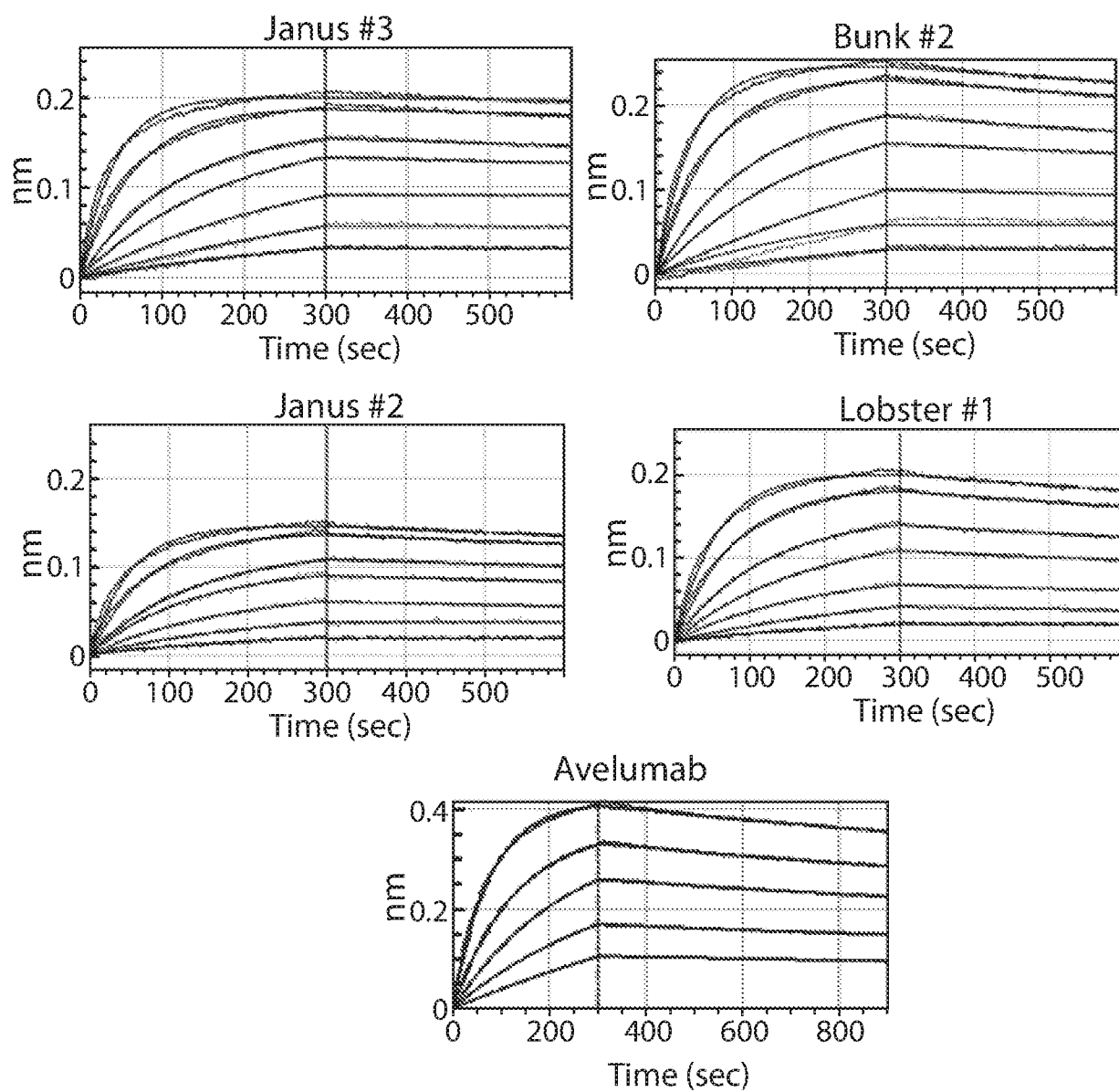


FIGURE 27

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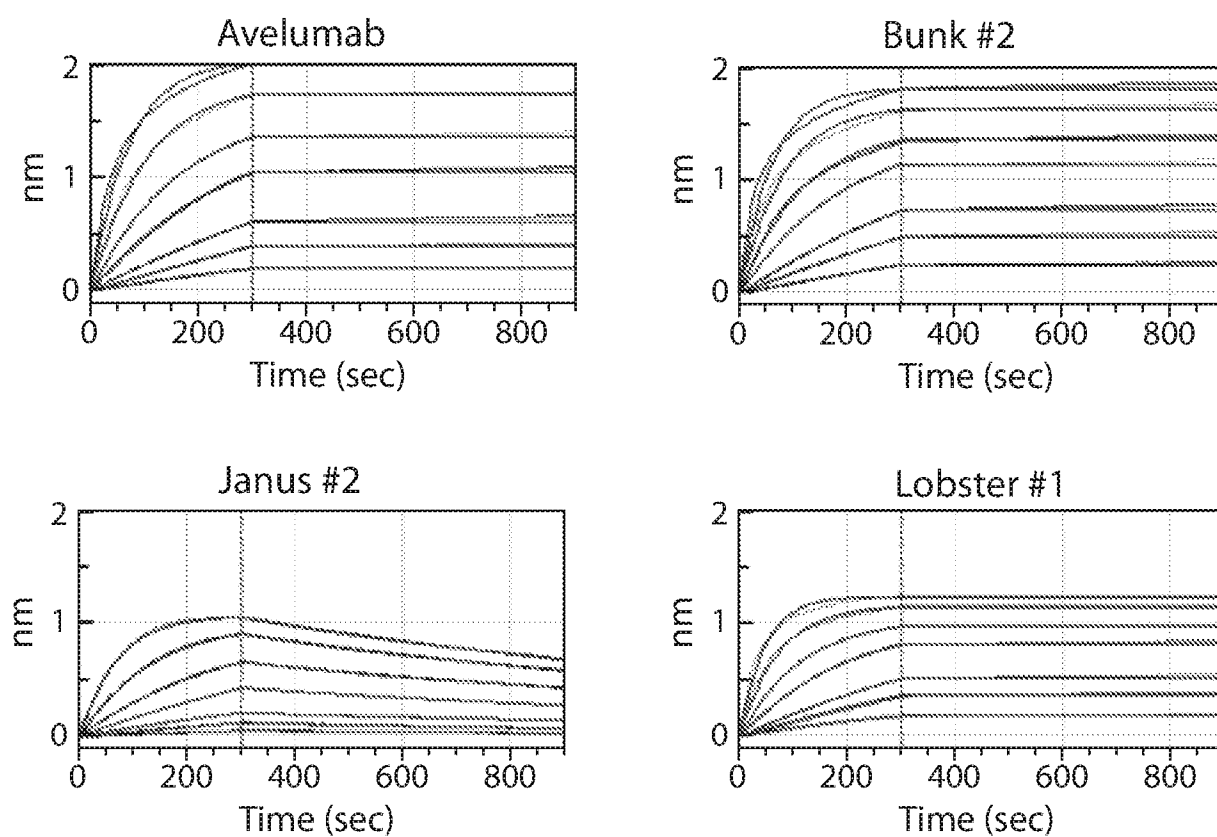


FIGURE 28

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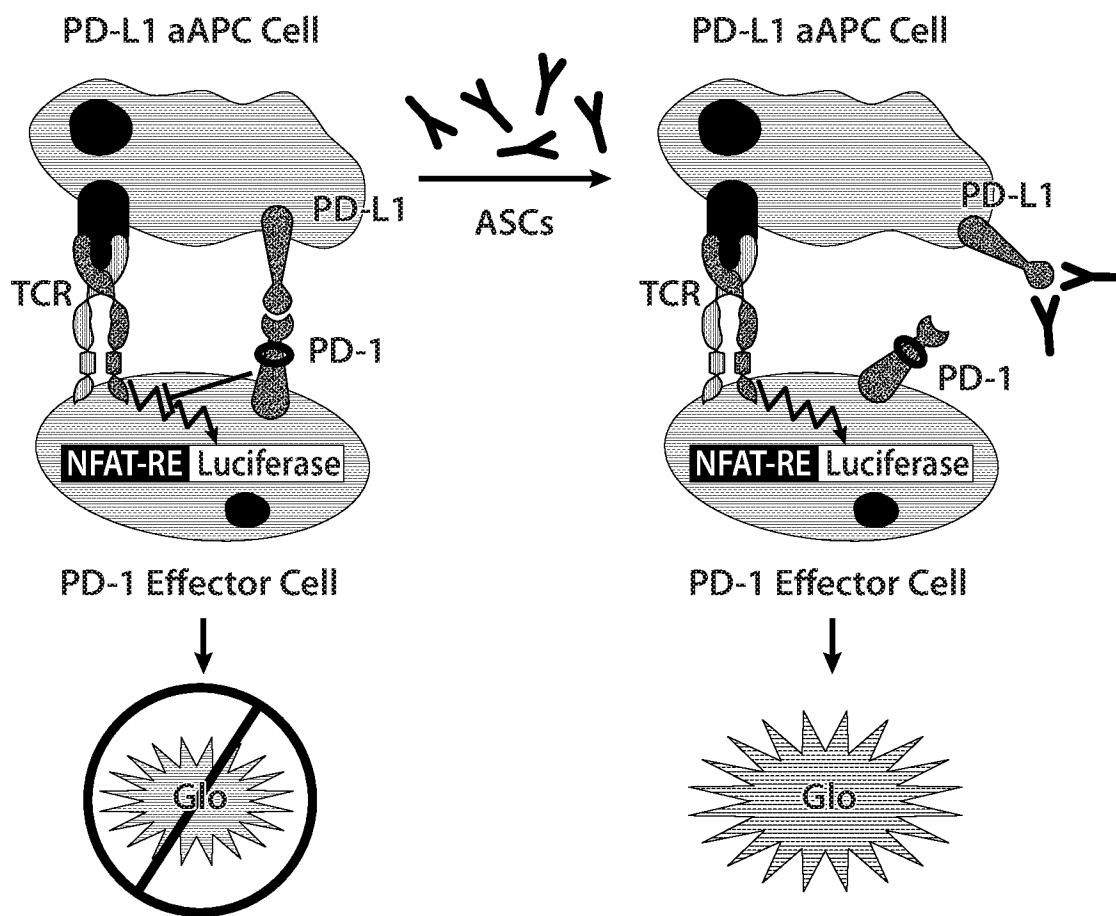


FIGURE 29

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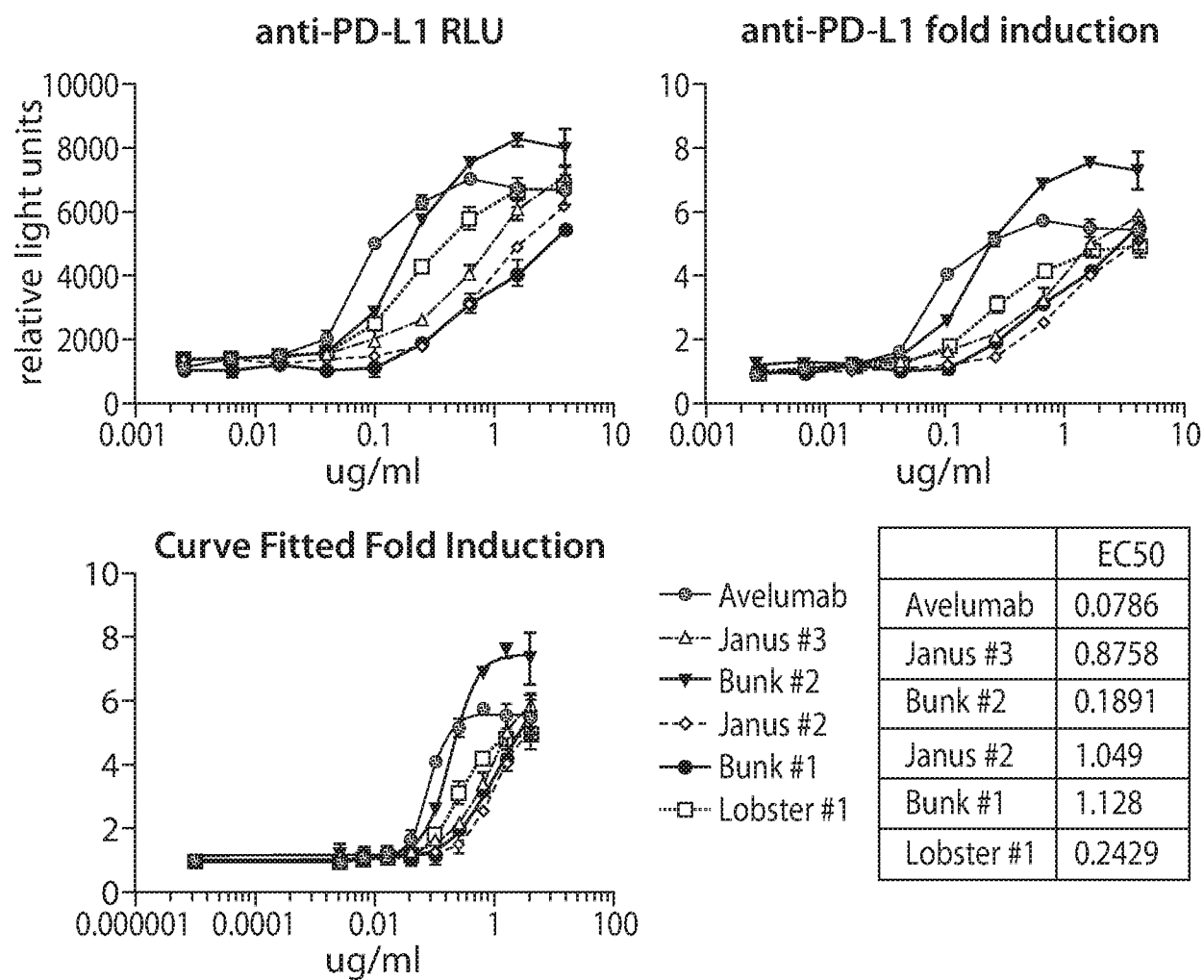


FIGURE 30

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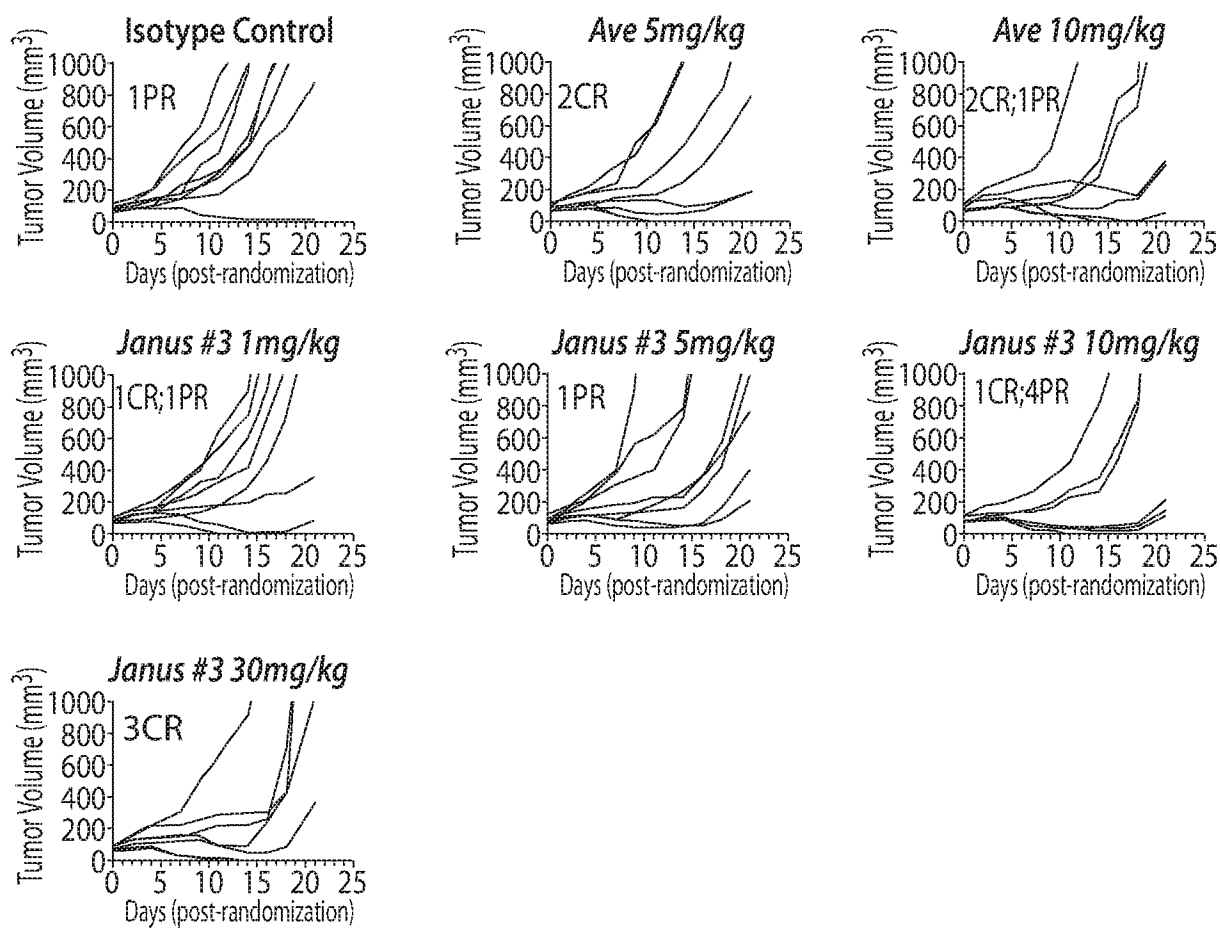


FIGURE 31

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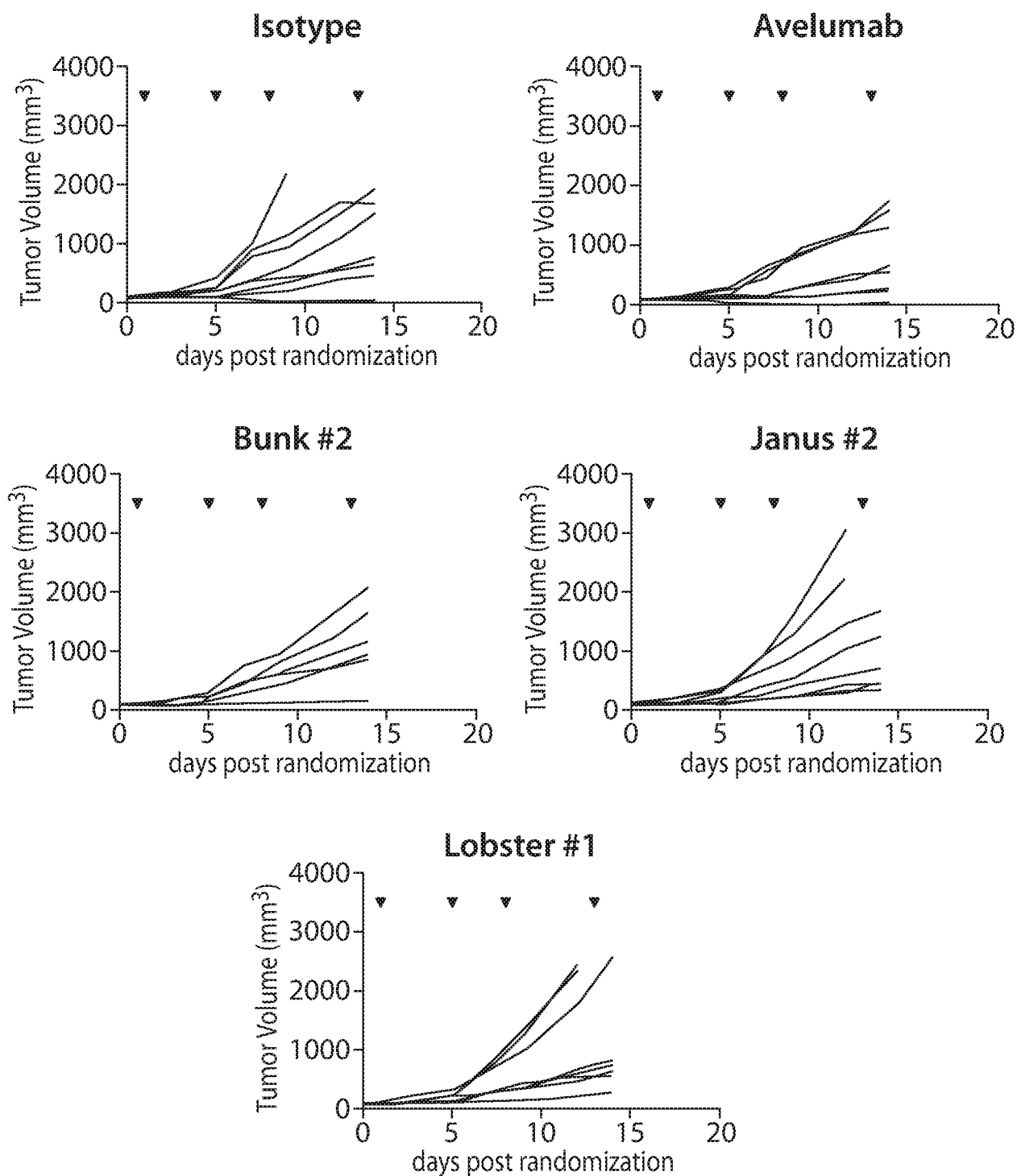


FIGURE 32

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/40815

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 19/00, A61P 35/00, C07K 16/32, A61K 38/47, A61K 47/68, C07K 16/28 (2020.01)

CPC - C12N 9/2402, C07K 16/2827, C12Y 302/01018, C07K 2317/732, C07K 2319/035, C07K 2319/33, C07K 2319/74, A61K 39/39558, A61K 47/6815, A61K 47/6855, A61K 47/6863, A61K 47/6869, C12N 15/63, C07K 2317/569, A61K 39/395

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2018/006034 A1 (GRAY) 4 January 2018 (04.01.2018) Abstract; Claim 1; Claim 3; Claim 17; Claim 36; p18, ln 28; p43, ln 18-20	1-2
Y	US 2019/0177416 A1 (NANOMAB TECHNOLOGY LIMITED) 13 June 2019 (13.06.2019) Abstract; para [0004]; para [0080]	1-2

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

* Special categories of cited documents:

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

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

6 November 2020

Date of mailing of the international search report

15 DEC 2020

Name and mailing address of the ISA/US

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P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Lee Young

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/40815

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 5-65
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
—Please see continuation in first extra sheet —

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-2, limited to a fusion protein with human sialidase Neu2

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US 20/40815

Continuation of Box No. III. Observations where unity of invention is lacking.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+, Claims 1-4, directed to a sialidase fusion protein. The fusion protein will be searched to the extent that the fusion protein encompasses (a) a sialidase enzyme; and (b) an anti-PD-L1 immunoglobulin antigen-binding domain, wherein the sialidase is a human sialidase Neu2 (note, this is the first claimed sialidase for the inventive fusion protein, a non-recombinant wild-type sialidase). It is believed that claims 1-2 encompass this first named invention, and thus these claims will be searched without fee to the extent that the fusion protein encompasses (a) a sialidase enzyme; and (b) an anti-PD-L1 immunoglobulin antigen-binding domain, wherein the sialidase is a human sialidase Neu2. Additional fusion proteins containing additional sialidase polypeptide(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected fusion proteins containing additional sialidase polypeptide(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a fusion protein containing a mutant sialidase comprising a recombinant mutant human sialidase with (a) a substitution of a proline residue at a position corresponding to position 5 of wildtype human Neu2 (P5)(claims 1-4).

The inventions listed as Group I+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

The inventions of Group I+ each include the special technical feature of a unique amino acid sequence. Each amino acid sequence encodes a unique peptide, and is considered a distinct technical feature.

Common technical features

No technical features are shared between the sialidase amino acid sequences of Group I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Group I+ were considered to share the technical features of including:
a fusion protein comprising:

(a) a sialidase enzyme; and

(b) an anti-PD-L1 immunoglobulin antigen-binding domain, these shared technical features are previously disclosed by WO 2018/006034 A1 to Gray (hereinafter 'Gray') in view of US 2019/0177416 A1 to Nanomab Technology Limited (hereinafter 'Nanomab').

Gray teaches a fusion protein comprising:

(a) a sialidase enzyme and an antibody (Claim 1 - 'A conjugate, comprising:

a targeting moiety that binds to a cell surface molecule of a target cell; and a target cell surface-editing enzyme.'; Claim 17 - 'The conjugate of Claim 16, wherein the conjugate is a fusion protein.'; Claim 3 - 'The conjugate of Claim 2, wherein the targeting moiety is an antibody.'; Claim 36 - 'The conjugate of any one of Claims 1 to 35, wherein the target cell surface- editing enzyme is a sialidase.'). Gray does not teach specifically teach sialidase fusion protein with (b) an anti-PD-L1 immunoglobulin antigen-binding domain.

Nanomab teaches fusion proteins with anti-PD-L1 antibody (Abstract - 'Provided in the present invention are a type of anti-human PD-L1 specific nanobodies and VHH chains thereof, coding sequences of the foregoing nanobodies or VHH chains thereof,'; para [0080] - 'The invention also provides other proteins or fusion expression products having the nanobodies of the invention. Specifically, the present invention includes any protein or protein conjugate and fusion expression product (i.e. immunoconjugate and fusion expression product) having a heavy chain containing a variable region'). It would have been obvious to one of ordinary skill in the art that the anti-PD-L1 antibody of Nanomab could be used to generate a fusion protein with sialidase according to Gray, to enable tumor cell surface editing according to Gray because Nanomab teaches PD-L1/PD-1 axis promotes escape of tumor cells from immune surveillance (para [0004] - 'However, in the occurrence of tumors, PD-L1 expressed by tumor cells combined with PD-1 can promote tumor immune escape by inhibiting lymphocytes').

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of Item 4 above: claims 5-65 are held unsearchable because they are not drafted in accordance with the second and third sentences of Rule 6.4(a).