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(57) Abstract: The present disclosure provides antigen-binding proteins which bind to DLK1; bispecific antigen-binding proteins which bind to DLK1 and a second antigen; and conjugates thereof. Related polypeptides, nucleic acids, vectors, host cells, and conjugates are further provided herein. Kits and pharmaceutical compositions comprising such entities are moreover provided. Also provided are methods of making an antigen-binding protein and methods of treating a subject having cancer.

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DLK1 ANTIBODIES AND METHODS OF TREATING CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/286,896, filed December 7, 2021; the entire contents of said application are incorporated herein in their entirety by this reference.

FIELD OF THE DISCLOSURE

[0002] The present disclosure relates, in general, to antibodies specific for Delta Like Non-Canonical Notch Ligand 1 (DLK1) and uses thereof to treat cancer.

BACKGROUND

[0003] Antibodies constitute powerful therapeutic agents characterized by limited side effects due to their ability to specifically target a distinct antigen on a cell, bacteria, virus, or toxin in connection with DLK1-associated disease. There is a clinical need to provide new antibodies, such as the antibodies described herein to address the medical needs of patients relating to DLK1-associated disease.

SUMMARY

[0004] Provided herein are antigen-binding proteins which bind to DLK1.

[0005] In various aspects, the antigen binding protein binds to DLK1 endogenously expressed by human cancer cells. In various instances, the antigen-binding proteins of the present disclosure inhibit tumor growth in a subject, e.g., a human, without any other moiety attached to the antigen-binding protein. In various instances, the antigen-binding proteins unconjugated to a heterologous moiety (e.g., unconjugated to any chemotherapeutic agent, drug or toxic moiety) inhibit tumor growth in a subject, e.g., a human.

[0006] In various aspects, the antigen-binding protein binds to DLK1 expressed by human cancer cells. Without being bound to a particular theory, the inhibiting action of the antigen-binding proteins provided herein allow such entities to be useful in methods of reducing tumor growth and treating a subject with a tumor or cancer. As further discussed herein, in various aspects, the antigen-binding protein is an antibody, antigen-binding antibody fragment thereof, or antibody protein product.

[0007] The present disclosure also provides antigen-binding proteins comprising at least 3, 4, 5, or all amino acid sequences of a specified group of amino acid sequences. In

various aspects, the antigen-binding proteins comprise at least 3, 4, 5, or 6 complementary determining region (CDR) amino acid sequences of DLK1 antibodies disclosed herein, e.g., Table 1 and Fig. 19-Fig. 30.

[0008] The present disclosure provides a bispecific antigen-binding protein that binds to DLK1 and a second antigen. The bispecific antigen-binding protein may comprise any one of the antigen-binding protein described here. The second antigen may be a cell surface protein, optionally a protein whose binding modulates immune response. In various embodiments, the second antigen is a cell surface protein expressed by a T cell, optionally a component of the T-cell receptor (TCR), for example CD3. In some embodiments, the second antigen is CD3. In some embodiments, the second antigen is CD3E. The bispecific antigen-binding protein may take any structure, e.g., diabody, TandAb (tandem diabody), BiTE (bispecific T cell engager), etc.

[0009] The present disclosure also provides a conjugate that comprises an antigen-binding protein or a bispecific antigen-binding protein and a heterologous moiety (e.g., a cytotoxic drug). The conjugate may comprise a cleavable linker or a noncleavable linker. The conjugate may have a various number of heterologous moiety (an agent) conjugated to the antigen-binding protein or a bispecific antigen-binding protein described herein, preferably 1-8 agents per protein or 3-8 agents per protein. The conjugate may be a site-specific conjugate. The conjugate may be a homogenous conjugate or a heterogeneous conjugate.

[0010] Related polypeptides, nucleic acids, vectors, host cells, and conjugates are further provided herein. Kits and pharmaceutical compositions comprising such entities are moreover contemplated.

[0011] Also provided are methods of making an antigen-binding protein. In various embodiments, the method comprises culturing a host cell comprising a nucleic acid encoding an antigen-binding protein or a polypeptide as described herein so as to express the antigen-binding protein or polypeptide.

[0012] Methods of treating a subject having cancer are additionally provided herein. In various embodiments, the method comprises administering to the subject the pharmaceutical composition of the present disclosure in an amount effective for treating the cancer in the subject.

[0013] Also provided are methods of treating a subject with a DLK1-expressing cancer comprising administering to the subject a pharmaceutical composition described herein. In various embodiments, the DLK1-expressing cancer expresses DLK1. Further contemplated is a method of inhibiting tumor growth in a subject, comprising administering to the subject a pharmaceutical composition described herein.

[0014] A method of reducing tumor size in a subject, or preventing the recurrence of cancer in a subject comprising administering to the subject a pharmaceutical composition described herein.

[0015] Also provided herein is a method of treating cancer in a subject diagnosed to be a low over-expresser of DLK1 comprising administering to the subject a pharmaceutical composition described herein.

[0016] In various embodiments, the administering induces apoptosis in tumor cells, for example in cells expressing DLK1. In various embodiments, the administration induces antibody-dependent cell-mediated cytotoxicity (ADCC) or Complement-dependent cytotoxicity (CDC), tumor necrosis and death or depletion of cells, and/or disruption of tumor cell adherence, each of which result tumor regression or slowing of tumor growth.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **FIG. 1** shows efficacy of a panel of anti-DLK1 chimeric mAbs in DLK1+ HEPG2 human hepatocellular cancer cell line xenografts. Fig. 1 represents a graph of tumor volume (mm^3) as a function of time (days) (**A**) or mean change in tumor volume (mm^3) at Day 23 (**B**) of tumors in mice bearing DLK1-positive human liver tumors (HepG2) after treatment weekly at 10 mg/kg with control human IgG1 antibody or 4 different anti-DLK1 chimeric antibodies (DLK1-547 (also called 04-547m), DLK1-548 (also called 04-548m), DLK1-557 (also called 04-557m) and DLK1-559 (also called 04-559m)) for 4 repeat doses, wherein the chimeric antibodies were produced by replacing the light chain and heavy chain variable regions of a human IgG1 with those of a mouse monoclonal anti-DLK1 antibody.

[0018] **FIG. 2** shows efficacy of a panel of humanized DLK1 mAbs in DLK1+ CORL279 human small cell lung cancer (SCLC) cell line xenografts. Fig. 2 represents a graph of tumor volume (mm^3) as a function of time (days) (**A**) or mean change in tumor volume (mm^3) at Day 21 (**B**) of tumors and percent change in body weight (**C**) in mice bearing DLK1-positive small cell lung carcinoma cells (COR-L279) after treatment weekly at 10 mg/kg with non-targeting IgG antibody control or 9 different anti-DLK1 humanized antibodies (DLK1-547-

h2, DLK1-548-h4, DLK1-557-h3, DLK1-557-f2, DLK1-559-h4, DLK1-559-f2, DLK1-561-f1 (also referred to as DLK1-561-F1 or 04-0561-F1), DLK1-562-h10 and DLK1-565-f2; note that "DLK1-" may be replaced with "04-" , "04-0", "mab-" or "DLK1-mab-" or be missing all together, which refers to the same humanized antibody, for example, DLK1-547-h2 is the same as 04-547-h2, 04-0547-h2, mab-547-h2, DLK1-mab-547-h2 and 562-h2) for 3 repeat doses.

[0019] **FIG. 3** shows selective tumor volume (mm^3) reduction of DLK1-positive (**A-C**) over DLK1-negative (**D-F**) human tumor xenografts following treatment with a DLK1-ADC comprising a humanized anti-DLK1-antibody, DLK1-561-f1, conjugated to cytotoxic monomethyl auristatin E (MMAE). Hu-IgG1 control is non-targeting, unconjugated human IgG1 control antibody. DLK1-positive human tumor cell lines: small cell lung carcinoma cell lines COR-L279 (**A**) and H524 (**C**), and rhabdomyosarcoma cell line JR-1 (also referred to as JR) (**B**); DLK1-negative human tumor cell lines: colorectal cancer cell lines SNU-C1 (also referred to as SNUC1) (**D**) and LS513 (**E**), and melanoma cell line M202 (**F**).

[0020] **FIG. 4** shows efficacy of humanized DLK1-ADCs in DLK1-positive CORL279 human SCLC cell line xenografts. Fig. 4 shows graphs of tumor volume (mm^3) as a function of time (**A**) and mean change in tumor volume (mm^3) at Day 13 (**B**) in mice and percent change in body weight (**C**) bearing human DLK1-positive human small cell lung carcinoma cell line COR-L279 after treatment weekly at 5 mg/kg with control IgG antibody or antibody drug conjugate (ADC) of humanized anti-DLK1 antibody conjugated to MMAE (DLK1-ADC-547-h2, DLK1-ADC-562-h10 and DLK1-ADC-561-f1) for 3 repeat doses. IgG is non-targeting, unconjugated control antibody.

[0021] **FIG. 5** shows that humanized DLK1-ADCs do not have anti-tumor activity in DLK1-negative M202 human melanoma cell line xenografts. Fig. 5 shows graphs of tumor volume (mm^3) as a function of time (**A**) and mean change in tumor volume (mm^3) at Day 31 (**B**) in mice bearing human DLK1-negative human melanoma cell line M202 after treatment weekly at 5 mg/kg with control IgG antibody or antibody drug conjugate (ADC) of humanized anti-DLK1 antibody conjugated to MMAE (DLK1-ADC-547-h2, DLK1-ADC-562-h10 and DLK1-ADC-561-f1) for 3 repeat doses. IgG is non-targeting, unconjugated control antibody.

[0022] **FIG. 6** summarizes biochemical, biophysical and cell biological properties of three lead humanized anti-DLK1 antibodies.

[0023] **FIG. 7** shows internalization of three humanized anti-DLK1 antibody-MMAE conjugates and one humanized anti-DLK1 antibody in a human DLK1-positive small cell lung carcinoma cell line (COR-L279).

[0024] **FIG. 8** summarizes effect of long-term storage at different temperatures on aggregation status and stability of humanized anti-DLK1 antibodies (04-0561-F1 and 04-0562-h10) as analyzed by size exclusion chromatography and non-reducing SDS-PAGE. Bottom set of panels shows non-reducing SDS-PAGE analysis following 5 weeks stored at the indicated temperature and concentration. Top panels: Long term storage (5 weeks) and temperature stress (RT or 37ºC) did not cause significant antibody aggregation and degradation (by SEC) at the concentrations of 10, 20, 50, or 75mg/ml. Specifically, after 5 weeks of storage at 37ºC, for 04-0561-F1, less than 3.5% of degradation (for all four concentrations) and ~4% of aggregation (only in 75mg/ml) were observed; whereas, for 04-562-h10, less than 8% of degradation was observed. Bottom panels: Long term storage (5 weeks) and temperature stress (RT or 37ºC) did not have significant effects on the antibody integrity (by non-reducing SDS-PAGE) at the concentrations of 10, 20, 50, or 75mg/ml. However, some antibody degradations were detected after 5 weeks storage at 37ºC at all concentrations (10, 20, 50, and 75mg/ml); 04-0561-F1 had less degradation level compared with 04-0562-h10.

[0025] **FIG. 9** shows effects of different temperatures and long-term storage on the stability of the DLK1 hAb 04-0547-h2 (10mg/ml) - antibody integrity assessment by non-reducing SDS PAGE and antibody aggregation by SEC (-80ºC vs 4ºC, RT, and 37ºC for 7 days). Fig. 9 shows SDS-PAGE analysis on stability of humanized anti-DLK1 antibody, 04-0547-h2, stored for 7 days at -80°C, 4°C, RT or 37°C with left side of each gel showing stored antibodies subjected to reducing condition and right side showing stored antibodies subjected to non-reducing condition just prior to electrophoresis (left panel). Aggregation and fragmentation status of the stored antibodies is analyzed by size exclusion chromatography as shown in the chromatogram and summarized below (**right panel**).

[0026] **FIG. 10** shows sequence alignment of human DLK1 (UniProtKB accession number P80370-1), crab-eating macaque DLK1 (UniProtKB accession number A0A2K5TMQ6), mouse DLK1 (UniProtKB accession number Q09163) and rat DLK1 (UniProtKB accession number O70534) protein sequences with yellow highlight indicating the extracellular domain of the human DLK1 transmembrane protein. Percent identity to

human DLK1 amino acid sequence and UniProtKB and NCBI accession numbers for the DLK1 proteins are indicated in the table below.

[0027] **FIG. 11** shows flow cytometry (**top half**) and KinExA (**bottom half**) assessment of the binding activity of three humanized anti-DLK1 antibodies (04-561-F1, 04-562-h10 and 04-547-h2) to HEK293T cells overexpressing human, monkey, mouse or rat DLK1 as a fusion protein to mGFP fluorescent protein.

[0028] **FIG. 12** shows time course of anti-DLK1 antibody internalization in cultured COR-L279 DLK1-positive human small cell lung carcinoma cells.

[0029] **FIG. 13** shows cellular internalization of anti-DLK1 antibody in cultured HEPG2 liver cancer cells. Negative controls – hIgG and no antibody. Number of seconds in each image indicates exposure time.

[0030] **FIG. 14** shows time course of humanized anti-DLK1 antibody internalization in cultured COR-L279 DLK1-positive human small cell lung carcinoma cells.

[0031] **FIG. 15** shows time course of humanized anti-DLK1 antibody internalization in cultured COR-L279 DLK1-positive human small cell lung carcinoma cells.

[0032] **FIG. 16** shows time course of humanized anti-DLK1 antibody internalization in cultured COR-L279 DLK1-positive human small cell lung carcinoma cells. Negative controls – non-targeting human IgG1 (hIgG1) and no antibody.

[0033] **FIG. 17** shows fluorescence associated with anti-DLK1 antibodies (red) 48 hrs from start of cellular internalization studies in of anti-DLK1 antibody in cultured COR-L279 DLK1-positive human small cell lung carcinoma cells. Negative controls – hIgG and no antibody.

[0034] **FIG. 18** shows time course of cellular internalization of 3 antibody-drug conjugates of humanized anti-DLK1 antibodies to MMAE and 1 non-ADC conjugated humanized anti-DLK1 antibody in cultured COR-L279 DLK1-positive human small cell lung carcinoma cells. Fig. 18 shows that all 3 anti-DLK1 ADCs bind to DLK1 well and are completely internalized after binding within 90 min.

[0035] **FIG. 19** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of the humanized anti-DLK1 antibody, 04-0547-h2, along with the lengths, positions and amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by Kabat and AbM methods.

[0036] **FIG. 20** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of the humanized anti-DLK1 antibody, 04-0561-F1, along with the lengths, positions and amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by Kabat and AbM methods.

[0037] **FIG. 21** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of the humanized anti-DLK1 antibody, 04-0562-h10, along with the lengths, positions and amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by Kabat and AbM methods.

[0038] **FIG. 22** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of the humanized anti-DLK1 antibody, 04-0548-h4, along with the lengths, positions and amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by Kabat and AbM methods.

[0039] **FIG. 23** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of the humanized anti-DLK1 antibody, 04-0557-F2, along with the lengths, positions and amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by Kabat and AbM methods.

[0040] **FIG. 24** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of the humanized anti-DLK1 antibody, 04-0557-h3, along with the lengths, positions and amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by Kabat and AbM methods.

[0041] **FIG. 25** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of the humanized anti-DLK1 antibody, 04-0559-F2, along with the lengths, positions and amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by Kabat and AbM methods.

[0042] **FIG. 26** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of the humanized anti-DLK1 antibody, 04-0559-h4,

along with the lengths, positions and amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by Kabat and AbM methods.

[0043] **FIG. 27** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of the humanized anti-DLK1 antibody, 04-0565-F2, along with the lengths, positions and amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by Kabat and AbM methods.

[0044] **FIG. 28** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of three humanized anti-DLK1 antibody, 04-0547-h2, 04-0561-F1 and 04-0562-h10, along with the amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by ImMunoGeneTics (IMGT) method and the name of the expression vector producing the antibody. The expression vector p04-0561-F1 was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209, on December 7, 2022, and assigned Accession Number _____ . This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

[0045] **FIG. 29** shows amino acid sequences of the heavy chain variable region (VH) and light chain variable region (VL) of five humanized anti-DLK1 antibody, 04-0548-h4, 04-0557-F2, 04-0559-F2, 04-0559-h4 and 04-0565-F2, along with the amino acid sequences of the complementary determining regions (CDRs) and framework regions (FRs) as defined by IMGT method and the name of the expression vector producing the antibody.

[0046] **FIG. 30** shows amino acid sequences of the human immunoglobulin light chain, kappa, constant region (IGHKC; UniProt ID: P01834) and human immunoglobulin heavy chain constant region gamma 1 (IGHG1; UniProt ID: P01857) and gamma 2 (IGHG2; UniProt ID: P01859) in some embodiments of the humanized anti-DLK1 antibodies of the invention.

[0047] **FIG. 31** shows nucleic acid sequences encoding the heavy chain variable region (VH) and light chain variable region (VL) for nine humanized anti-DLK1 antibodies,

04-0565-F2, 04-0562-h10, 04-0561-F1, 04-0559-h4, 04-0559-F2, 04-0557-h3, 04-0557-F2, 04-0548-h4 and 04-0547-h2.

[0048] **FIG. 32** shows nucleic acid sequences encoding the human immunoglobulin light chain, kappa, constant region (IGHKC) and human immunoglobulin heavy chain constant region gamma 1 (IGHG1) used in some embodiments of the invention to produce full length humanized anti-DLK1 antibody.

[0049] **FIG. 33** shows nucleic acid sequences encoding the heavy chain variable region (V_H) and light chain variable region (V_L) of a chimeric anti-DLK1 antibody, 04-0561-m, and humanized VH and VL in its humanized version, 04-0561-F1.

[0050] **FIG. 34** shows the epitope mapping data for humanized anti-DLK1 antibody, DLK1-561-F1, directly labeled with Alexa Fluor 647 (AF647) dye binding to control HEK293T cells or engineered HEK293T cells expressing mGFP fusion to a long form of human DLK1 protein as a full length protein (HEK293T DLK1 mGFP G06 (long form)) or a human-mouse chimeric DLK1 protein in which a protein segment containing one or more EGF-like domains from region 1 (834 HEK 293T DLK1HM1-mGFP), region 2 (835 HEK 293T DLK1HM2-mGFP), region 3 (836 HEK 293T DLK1HM3-mGFP), region 4 (837 HEK 293T DLK1HM4-mGFP), region 5 (850 HEK 293T DLK1HM5-mGFP), region 6 (851 HEK 293T DLK1HM6-mGFP), region 7 (848 HEK 293T DLK1HM7-mGFP), or region 8 (849 HEK 293T DLK1HM8-mGFP) of the human DLK1 protein is replaced with a corresponding region from the mouse DLK1 protein. DLK1-561-F1 antibody binds region to regions 6 and 7 (HM6 and HM7) of human DLK1 protein, corresponding to amino acid residues 55-113. Upper table: AF647 fluroescence of control and engineered HEK293T cells are reported for DLK1-561-F1-AF647 antibody as well as hIgG1-AF647 antibody control and no antibody control. Lower table: GFP fluroescence.

[0051] **FIG. 35** shows amino acid sequence of the long form of human DLK1 protein and regions used in swapping out a segment of human DLK1 with a corresponding segment of the mouse DLK1 protein (in aqua; top two bottom arrows within each aligned sequence set). Differences in amino acid sequence from the human DLK1 protein is also provided for mouse, rat and crab-eating macaque DLK1 proteins.

[0052] **FIG. 36** shows the 04-561-F1 antibody binding site in a predicted structure of human DLK1 protein long form (UniProtKB: P80370) and topology of long and short forms of human DLK1 protein. Fig. 36 shows (A) predicted structure and topology of the

long form of the human DLK1 protein (UniProtKB: P80370) and (B) long and short forms of human DLK1 protein. (A) Predicted structure, topology and location of amino acid number 24 and 169 are shown along with that of signal peptide and transmembrane domain followed by cytoplasmic domain for the long form of the human DLK1 protein (DLK-FL). Six EGF-like domains are found between amino acid numbers 24 and 245. DLK1-561-F1 antibody binds an epitope within EGF-like 2 and EGF-like 3 domains corresponding to amino acid number 55-113 based on epitope maping experiment results of Figure 34. (B) Diagram of the two known human isoforms of DLK1, the full-length cleavable isoform 1 (DLK1-FL) and the membrane-bound isoform 2 (DLK1-MB). The large bolt identified the ADAM17-mediated juxtamembrane cleavage site for the full-length ligand. Smaller bolts identify the other protease cleavage site.

[0053] **FIG. 37** shows flow cytometry assessment of the binding activity of DLK1-561-F1 (also called 561-F1) antibody, LegoChem 18A5 antibody, and human IgG1 (hIgG1) control antibody to parental HEK293T cells (HEK293T parental), HEK293 cells expressing the long form of human DLK1-mGFP fusion protein (HEK293T DLK1 mGFP G06 (long form)), or HEK293T expressing short form of human DLK1-mGFP fusion protein (HEK293T DLK1 Iso2-mGFP mass pop (short form)). Upper panel: (direct) flow cytometric analysis of cells bound to anti-DLK1 antibody conjugated to AF-647 fluorophore. Lower panel: (indirect) flow cytometric analysis of cells bound to anti-DLK1 antibody detected with a secondary anti-His epitope tag antibody which is conjugated to AF-647 fluorophore.

[0054] **FIG. 38** shows that the DLK1-561-F1 antibody binds both isoforms of the DLK1 protein, whereas LegoChem 18A5 antibody only binds to the long form. Fig. 38 shows binding curves and EC50 values for DLK1-561-F1 antibody and LegoChem 18A5 antibody.

[0055] **FIG. 39** shows that DLK1 antibody 561-F1 induces ADCC in the native and overexpressed models of DLK1 expression. Fig. 39 shows the ADCC potency of DLK1-561-F1 in (A) DLK1+ CORL279 and DLK1- M202 cells, and (B) HEK293 cells engineered to overexpress the short form of DLK1 (DLK-MB). NFAT activation, indicating the induced ADCC response, was assessed by determining Lucia luciferase activity in the supernatant. The data show that DLK1-561-F1 antibody induced ADCC in native and overexpressed models of DLK1 expression; whereas, LegoChem 18A5 antibody

and hIgG1 control antibody failed to induce ADCC response in native DLK1 expressing CORL279 cells and HEK293 cells overexpressing the short form of DLK1.

[0056] **FIG. 40** shows that DLK1 scFvs and BiTEs selectively bind DLK1$^+$ CORL279 cells but not DLK1$^-$ M202 cells. DLK1 scFvs are: 04-0547-h2scfv, 04-0561-F1scfv, and 04-0562-h10scfv, each of which further comprises a $(His)_6$ tag at its C-terminus. DLK1 BiTEs are: 04-0547-h2Bs, 04-0561-F1Bs, and 04-0562-h10Bs, each of which further comprises a $(His)_6$ tag at its C-terminus. The scFv and BiTE antibodies have a terminal $(His)_6$ epitope tag, which can be detected by a Alexa Fluor® 647-labeled anti-His tag secondary antibody (BioLegend catalog number: 652513). AF647 fluorescence of no antibody treated and only AF647 anti-His tag secondary antibody treated cells served as negative controls.

[0057] **FIG. 41** shows the anti-DLK1-CD3 bispecific antibodies (BiTEs) assessed for T-cell activation using Jurkat cells with NFAT-RE reporter. Co-incubation of DLK1+ cell lines (JR and CORL-279) but not DLK1- cell line (Raji) with 04-0547-h2Bs or 04-0561-F1Bs bispecific antibody and Jurkat T-cells from Promega's T Cell Activation Biosassy Kit (NFAT-RE J1621) resulted in significant T-cell activation. Anti-DLK1-CD3 BiTE, 04-0562-h10Bs, failed to induce T-cell activation. The BiTEs used herein further comprises a $(His)_6$ tag at its C-terminus.

DETAILED DESCRIPTION

[0058] The present disclosure describes an antigen binding protein against DLK1, to treat DLK1-expressing cancers.

[0059] *Antigen binding proteins*

[0060] Provided herein are antigen-binding proteins that bind to DLK1. The antigen-binding proteins of the present disclosure can take any one of many forms of antigen-binding proteins known in the art. In various embodiments, the antigen-binding proteins of the present disclosure take the form of an antibody, or antigen-binding antibody fragment, or an antibody protein product.

[0061] In various embodiments of the present disclosure, the antigen-binding protein comprises, consists essentially of, or consists of an antibody. As used herein, the term "antibody" refers to a protein having a conventional immunoglobulin format, comprising heavy and light chains, and comprising variable and constant regions. For example, an antibody may be an IgG which is a "Y-shaped" structure of two identical pairs of

polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). An antibody has a variable region and a constant region. In IgG formats, the variable region is generally about 100-110 or more amino acids, comprises three complementarity determining regions (CDRs), is primarily responsible for antigen recognition, and substantially varies among other antibodies that bind to different antigens. The constant region allows the antibody to recruit cells and molecules of the immune system. The variable region is made of the N-terminal regions of each light chain and heavy chain, while the constant region is made of the C-terminal portions of each of the heavy and light chains. (Janeway et al., "Structure of the Antibody Molecule and the Immunoglobulin Genes", Immunobiology: The Immune System in Health and Disease, 4th ed. Elsevier Science Ltd./Garland Publishing, (1999)).

[0062] The general structure and properties of CDRs of antibodies have been described in the art. Briefly, in an antibody scaffold, the CDRs are embedded within a framework in the heavy and light chain variable region where they constitute the regions largely responsible for antigen binding and recognition. A variable region typically comprises at least three heavy or light chain CDRs (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, Public Health Service N.I.H., Bethesda, Md.; see also Chothia and Lesk, 1987, J. Mol. Biol. 196:901-917; Chothia et al., 1989, Nature 342: 877-883), within a framework region (designated framework regions 1-4, FR1, FR2, FR3, and FR4, by Kabat et al., 1991; see also Chothia and Lesk, 1987, supra). CDRs can be annotated in various ways including the method according to Kabat, AbM, or IMGT. Accordingly, the CDRs of the same antibody can comprise different sequences, depending on which method was used to annotate the CDR sequences. Such is exemplified in the Tables presented herein. In related embodiments, the residues of the framework are altered. The heavy chain framework regions which can be altered lie within regions designated H-FR1, H-FR2, H-FR3 and H-FR4, which surround the heavy chain CDR residues, and the residues of the light chain framework regions which can be altered lie within the regions designated L-FR1, L-FR2, L-FR3 and L-FR4, which surround the light chain CDR residues. An amino acid within the framework region may be replaced, for example, with any suitable amino acid identified in a human framework or human consensus framework.

[0063] Antibodies can comprise any constant region known in the art. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgM has subclasses, including, but not limited to, IgM1 and IgM2. Embodiments of the present disclosure include all such classes or isotypes of antibodies. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, e.g., a human kappa- or lambda-type light chain constant region. The heavy chain constant region can be, for example, an alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant regions, e.g., a human alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. Accordingly, in various embodiments, the antibody is an antibody of isotype IgA, IgD, IgE, IgG, or IgM, including any one of IgG1, IgG2, IgG3 or IgG4. In various aspects, the antibody comprises a constant region comprising one or more amino acid modifications, relative to the naturally-occurring counterpart, in order to improve half-life/stability or to render the antibody more suitable for expression/manufacturability. In various instances, the antibody comprises a constant region wherein the C-terminal Lys residue that is present in the naturally-occurring counterpart is removed or clipped.

[0064] The antibody can be a monoclonal antibody. In some embodiments, the antibody comprises a sequence that is substantially similar to a naturally-occurring antibody produced by a mammal, e.g., mouse, rabbit, goat, horse, chicken, hamster, human, and the like. In this regard, the antibody can be considered as a mammalian antibody, e.g., a mouse antibody, rabbit antibody, goat antibody, horse antibody, chicken antibody, hamster antibody, human antibody, and the like. In certain aspects, the antigen-binding protein is an antibody, such as a human antibody.

[0065] In certain aspects, the antigen-binding protein is a chimeric antibody or a humanized antibody. The term "chimeric antibody" refers to an antibody containing domains from two or more different antibodies. A chimeric antibody can, for example, contain the constant domains from one species and the variable domains from a second, or more generally, can contain stretches of amino acid sequence from at least two species. A chimeric antibody also can contain domains of two or more different antibodies within the same species.

[0066] The term "humanized" when used in relation to antibodies refers to antibodies having at least CDR regions from a non-human source which are engineered to have a structure and immunological function more similar to true human antibodies than the original source antibodies. For example, humanizing can involve grafting a CDR from a non-human antibody, such as a mouse antibody, into a human antibody. Humanizing also can involve select amino acid substitutions to make a non-human sequence more similar to a human sequence. Information, including sequence information for human antibody heavy and light chain constant regions is publicly available through the Uniprot database as well as other databases well-known to those in the field of antibody engineering and production. For example, the IgG1 constant region is available from the Uniprot database as described below, incorporated herein by reference. Additionally, in another example, the IgG2 constant region is available from the Uniprot database as Uniprot number P01859, incorporated herein by reference.

[0067] Merely by way of example, the sequence for a murine immunoglobulin kappa light chain constant region or an immunoglobulin gamma-2A heavy chain constant region includes the following.

Name:	Sequence:
Immunoglobulin kappa constant, mouse (IGKC_MOUSE; UniProt ID: P01837)	RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI DGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNS YTCEATHKTSTSPIVKSFNRNEC
Ig gamma-2A chain C region, A allele, mouse (GCAA_MOUSE; UniProt ID: P01863)	AKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTW NSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNV AHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPP KIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTA QTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKD LPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMV TDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKL RVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

Merely by way of example, the sequences for human immunoglobulin kappa light chain constant region, human immunoglobulin lambda constant 2 light chain region, human immunoglobulin gamma 1 heavy chain constant region, and human immunoglobulin gamma 2 heavy chain constant region include the following.

Name:	Sequence:
Immunoglobulin kappa constant, human (IGKC_HUMAN; UniProt ID: 01834)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
Immunoglobulin lambda constant 2, human (IGLC2; UniProt ID: P0DOY2)	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAV TVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLT PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
Immunoglobulin heavy constant gamma 1, human (IGHG1_HUMAN ; UniProt ID: P01857)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
Immunoglobulin heavy constant gamma 2, human (IGHG2_HUMAN ; Uniprot ID: P01859)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYT CNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKV SNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFL

	YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK

[0068] An antibody can be cleaved into fragments by enzymes, such as, e.g., papain and pepsin. Papain cleaves an antibody to produce two Fab fragments and a single Fc fragment. Pepsin cleaves an antibody to produce a $F(ab')_2$ fragment and a pFc' fragment. In various aspects of the present disclosure, the antigen-binding protein of the present disclosure is an antigen-binding fragment of an antibody (a.k.a., antigen-binding antibody fragment, antigen-binding fragment, antigen-binding portion). In various instances, the antigen-binding antibody fragment is a Fab fragment or a $F(ab')_2$ fragment.

[0069] The architecture of antibodies has been exploited to create a growing range of alternative antibody formats that spans a molecular-weight range of at least about 12–150 kDa and has a valency (n) range from monomeric (n = 1), to dimeric (n = 2), to trimeric (n = 3), to tetrameric (n = 4), and potentially higher; such alternative antibody formats are referred to herein as "antibody protein products". Antibody protein products include those based on the full antibody structure and those that mimic antibody fragments which retain full antigen-binding capacity, e.g., scFvs, Fabs and VHH/VH (discussed below). The smallest antigen-binding fragment that retains its complete antigen binding site is the Fv fragment, which consists entirely of variable (V) regions. A soluble, flexible amino acid peptide linker is used to connect the V regions to a scFv (single chain fragment variable) fragment for stabilization of the molecule, or the constant (C) domains are added to the V regions to generate a Fab fragment [fragment, antigen-binding]. Both scFv and Fab fragments can be easily produced in host cells, e.g., prokaryotic host cells. Other antibody protein products include disulfide-bond stabilized scFv (ds-scFv), single chain Fab (scFab), as well as di- and multimeric antibody formats like dia-, tria- and tetra-bodies, or minibodies (miniAbs) that comprise different formats consisting of scFvs linked to oligomerization domains. The smallest fragments are VHH/VH of camelid heavy chain Abs as well as single domain Abs (sdAb). The building block that is most frequently used to create novel antibody formats is the single-chain variable (V)-domain antibody fragment (scFv), which comprises V domains from the heavy and light chain (VH and VL domain) linked by a peptide linker of ~15 amino acid residues. A peptibody or peptide-Fc fusion is yet another antibody protein product. The structure of a peptibody consists of a

biologically active peptide grafted onto an Fc domain. Peptibodies are well-described in the art. See, e.g., Shimamoto et al., mAbs 4(5): 586-591 (2012).

[0070] Other antibody protein products include a single chain antibody (SCA); a diabody; a triabody; a tetrabody; bispecific or trispecific antibodies, and the like. Bispecific antibodies can be divided into five major classes: BsIgG, appended IgG, bispecific antibody (BsAb) fragments, bispecific fusion proteins, and BsAb conjugates. See, e.g., Spiess et al., Molecular Immunology 67(2) Part A: 97-106 (2015).

[0071] In various aspects, the antigen-binding protein of the present disclosure comprises, consists essentially of, or consists of any one of these antibody protein products. In various aspects, the antigen-binding protein of the present disclosure comprises, consists essentially of, or consists of any one of an scFv, Fab VHH/VH, Fv fragment, ds-scFv, scFab, dimeric antibody, multimeric antibody (e.g., a diabody,, triabody, tetrabody), miniAb, peptibody VHH/VH of camelid heavy chain antibody, sdAb, diabody; a triabody; a tetrabody; a bispecific or trispecific antibody, BsIgG, appended IgG, BsAb fragment, bispecific fusion protein, and BsAb conjugate.

[0072] In various instances, the antigen-binding protein of the present disclosure is an antibody protein product in monomeric form, or polymeric, oligomeric, or multimeric form. In certain embodiments in which the antibody comprises two or more distinct antigen binding regions fragments, the antibody is considered bispecific, trispecific, or multi-specific, or bivalent, trivalent, or multivalent, depending on the number of distinct epitopes that are recognized and bound by the antibody.

[0073] In various embodiments, an anti-DLK1 antibody or antibody variant thereof is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a recombinant antibody, an antigen-binding antibody fragment, a single chain antibody, a monomeric antibody, a diabody, a triabody, a tetrabody, a Fab fragment, an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, and an IgG4 antibody.

[0074] In various aspects, the antigen-binding protein of the present disclosure is linked to a therapeutic agent. As described below, the therapeutic agent may be any known in the art, including, but not limited to, chemotherapeutic agents, cytokines and growth factors, cytotoxic agents, and the like. See "*Conjugates*" below.

[0075] In various aspects, any polypeptide of the present disclosure, e.g., an antigen-binding protein, may further comprise a heterologous peptide or polypeptide. In some embodiments, the heterologous peptide or polypeptide may be a marker or a tag that can be detected directly (e.g., GFP) or indirectly (e.g., using a secondary antibody that binds to the tag). Examples of detectable markers include various enzymes, prosthetic groups, and tags (e.g., a histidine tag, myc tag, flag tag, etc.). Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase. Examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin. Examples of bioluminescent materials include luciferase, luciferin, fluorescent protein (e.g., GFP, RFP, etc.), and aequorin.

[0076] *Afucosylated antibodies*

[0077] Many secreted proteins undergo post-translational glycosylation, a process by which sugar moieties (e.g., glycans, saccharides) are covalently attached to specific amino acids of a protein. In eukaryotic cells, two types of glycosylation reactions occur: (1) N-linked glycosylation, in which glycans are attached to the asparagine of the recognition sequence Asn-X-Thr/Ser, where "X" is any amino acid except proline, and (2) O-linked glycosylation in which glycans are attached to serine or threonine. Regardless of the glycosylation type (N-linked or O-linked), microheterogeneity of protein glycoforms exists due to the large range of glycan structures associated with each site (O or N).

[0078] All N-glycans have a common core sugar sequence: Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAcβ1-Asn-X-Ser/Thr ($Man_3GlcNAc_2Asn$) and are categorized into one of three types: (A) a high mannose (HM) or oligomannose (OM) type, which consists of two N-acetylglucosamine (GalNAc) moieties and a large number (e.g., 5, 6, 7, 8 or 9) of mannose (Man) residues (B) a complex type, which comprises more than two GlcNAc moieties and any number of other sugar types or (C) a hybrid type, which comprises a Man residue on one side of the branch and GlcNAc at the base of a complex branch.

[0079] N-linked glycans typically comprise one or more monosaccharides of galactose (Gal), N-acetylgalactosamine (GalNAc), galactosamine (GalN), glucose (GLc), N-acetylglucoasamine (ClcNAc), glucoasamine (GlcN), mannose (Man), N-Acetylmannosamine (ManNAc), Mannosamine (ManN), xylose (Xyl), N0Acetylneuraminic acid (Neu5Ac), N-Glycolylneuraminic acid (Neu5Gc), 2-keto-3-

doxynononic acid (Kdn), fucose (Fuc), Glucuronic acid (GLcA), Iduronic acid (IdoA), Galacturonic acid (Gal A), mannuronic acid (Man A).

[0080] N-linked glycosylation begins in the endoplasmic reticulum (ER), where a complex set of reactions result in the attachment of a core glycan structure made essentially of two GlcNAc residues and three Man residues. The glycan complex formed in the ER is modified by action of enzymes in the Golgi apparatus. If the saccharide is relatively inaccessible to the enzymes, it typically stays in the original HM form. If enzymes can access the saccharide, then many of the Man residues are cleaved off and the saccharide is further modified, resulting in the complex type N-glycans structure. For example, mannosidase-1 located in the cis-Golgi, can cleave or hydrolyze a HM glycan, while fucosyltransferase FUT-8, located in the medial-Golgi, fucosylates the glycan (Hanrue Imai- Nishiya (2007), BMC Biotechnology, 7:84).

[0081] Accordingly, the sugar composition and the structural configuration of a glycan structure varies, depending on the glycosylation machinery in the ER and the Golgi apparatus, the accessibility of the machinery enzymes to the glycan structure, the order of action of each enzyme and the stage at which the protein is released from the glycosylation machinery, among other factors.

[0082] In exemplary embodiments of the present disclosure, the antigen-binding proteins comprise an Fc polypeptide. The term “Fc polypeptide” as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. In exemplary aspects, the Fc polypeptide of the presently disclosed antigen-binding protein comprises a glycan. In various instances, the glycan lacks fucose or is afucosylated. In exemplary aspects, the antigen-binding protein comprises an afucosylated glycan. As used herein, the term "afucosylated glycan" or “afuco glycan” or “afucosylated glycoform” or “Afuc” refers to glycoforms which lack a core fucose, e.g., an α1,6-linked fucose on the GlcNAc residue involved in the amide bond with the Asn of the N-glycosylation site. Afucosylated glycoforms include, but are not limited to, A1G0, A2G0, A2G1a, A2G1b, A2G2, and A1G1M5. Additional afucosylated glycans include, e.g., A1G1a, G0[H3N4], G0[H4N4], G0[H5N4], FO-N[H3N3]. See, e.g., Reusch and Tejada, Glycobiology 25(12): 1325-1334 (2015).

[0083] The present disclosure also provides a composition, e.g., a pharmaceutical composition, comprising an antigen binding protein comprising an Fc polypeptide

comprising an afucosylated glycan. In exemplary aspects, at least or about 25% of the antigen-binding proteins present in the composition are antigen-binding proteins comprising an Fc polypeptide comprising an afucosylated glycan. In exemplary aspects, at least or about 25% of the antigen-binding proteins present in the composition are afucosylated. Optionally, at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% or more of the antigen-binding proteins present in the composition are afucosylated. Methods of producing compositions comprising antigen-binding proteins of a particular glycoprofile are known in the art. In exemplary embodiments, the antigen binding proteins are recombinant produced in cells that are genetically modified to alter the activity of an enzyme of the de novo pathway or the salvage pathway. In exemplary embodiments, the cells are genetically modified to alter the activity of any one or more of: a fucosyl-transferase (FUT, e.g.,FUT1, FUT2, FUT3, FUT4, FUT5, FUT6, FUT7, FUT8, FUT9), a fucose kinase, a GDP-fucose pyrophosphorylase, GDP-D-mannose-4,6-dehydratase (GMD), and GDP-keto-6-deoxymannose-3,5-epimerase, 4-reductase (FX). In exemplary embodiments, the cells are genetically modified to knock-out a gene encoding FX. See, e.g., International Patent Publication No. WO2017/079165 A1; Kanda et al., J Biotechnol 130, 2007, 300-310, Yamane-Ohunuki et al., Biotechnol Bioeng 87, 2004, 614-622, Malphettes et al., Biotechnol Bioeng 106, 2010, 774-783.

[0084] *Bispecific formats*

[0085] In exemplary aspects, the antigen-binding protein is bispecific and thus capable of binding two different and distinct antigens. In exemplary embodiments, the antigen binding protein is bispecific and binds to DLK1 and a second antigen.

[0086] In exemplary instances, the second antigen is a cell surface protein expressed by a T-cell. In exemplary aspects, the cell surface protein is a component of the T-cell receptor (TCR), for example, CD3. In exemplary instances, the second antigen is a costimulatory molecule which assists in T-cell activation, e.g., CD40 or 4-1BB (CD137). In exemplary aspects, the second antigen is an Fc receptor. In various aspects, the Fc receptor is a Fc gamma receptor, Fc-alpha receptor, Fc-epsilon receptor. In exemplary aspects, the Fc receptor is CD64 (Fc-gamma RI), CD32 (Fc-gamma RIIA), CD16A (Fc-gamma RIIIA), CD16b (Fc-gamma RIIIb), FcεRI, CD23 (Fc-epsilon RII), CD89 (Fc-epsilon RI), Fcα/μR, or FcRn. In exemplary aspects, the Fc receptor is CD16A. In exemplary instances, the second antigen is an immune checkpoint molecule, e.g., a protein involved in the immune

checkpoint pathway. The immune checkpoint pathway and molecules or proteins that function in it are known in the art. See, e.g., Pardoll, Nat Rev Genet 12(4): 252-264 (2012). In exemplary instances, the immune checkpoint molecule is A2AR, B7-H3, B7-H4, BTLA, CTLA4, IDO, KIR, LAG3, NOX2, PD-1, TIM3, VISTA, or SIGLEC7. Optionally, the immune checkpoint molecule is PD-1, LAG3, TIM3, or CTLA4.

[0087] Over fifty formats of bispecific antigen-binding proteins are known in the art, some of which are described in Kontermann and Brinkmann, Drug Discovery Today 20(7): 838-847 (2015); Zhang et al., Exp Hematol Oncol 6:12 (2017); Spiess et al., Mol Immunol.; 67(2 Pt A):95-106 (2015). In exemplary aspects, the bispecific antigen-binding protein of the present disclosure is made through chemical engineering, genetic engineering, or quadroma technology.

[0088] In exemplary aspects, the bispecific antigen-binding protein is constructed with some or all of the constant domains of an antibody. In exemplary aspects, the bispecific antigen-binding protein of the present disclosure comprises an Fc polypeptide and retains Fc-mediated effector functions. In various instances, the bispecific antigen-binding protein is a bispecific monoclonal antibody formed by, e.g., chemical cross-linking of two monoclonal antibodies (mabs), or by knob and hold technology. In exemplary aspects, the bispecific antigen-binding protein is made through "knobs-into-holes" technology in which H chain heterodimerization is forced by introducing different mutations into the two CH3 domains resulting in asymmetric antibodies. A "knob" mutation is made into one HC and a "hole" mutation is created in the other HC to promote heterodimerization. In exemplary aspects, the bispecific antigen-binding protein is a bispecifc antibody produced by quadroma technology which is based on the somatic fusion of two different hybridoma cells producing monoclonal antibodies with the desired specificity. Zhang et al., 2017, supra. In exemplary aspects, the bispecific antigen-binding protein is a crossMab, ortho-Fab IgG, DVD-Ig, two in one IgG, IgG-scFv and $scFv_2$-Fc (Kontermann and Brinkmann, 2015, supra. In various aspects, the bispecific antigen-binding protein is an Ig-scFv fusion wherein a new antigen-binding moiety is added to a full length IgG resulting in a fusion protein with tetravalency for two distinct antigens, e.g., IgG C-terminal scFv fusion and IgG N-terminal scFv fusion. In exemplary instances, the bispecific antigen-binding protein is a dual-variable-domain-IgG (DVD-IgG), wherein the LC and HC variable regions of an IgG specific for one antigen are fused to the N-terminal LC and HC variable regions of an

IgG specific for a second antigen through a linker to form a DVD-IgG. In exemplary aspects, the bispecific antigen-binding protein is a diabody-Fc fusion which involves the replacement of a Fab fragment of an IgG with a bispecific diabody

[0089] In alternative instances, the bispecific antigen-binding protein of the present disclosure does not comprise an Fc polypeptide. In exemplary aspects, the bispecific antigen-binding protein comprises the variable domains of each parental monoclonal antibody, and linkers are cloned and linked to form a single-chain bispecific antibody. In exemplary aspects, the bispecific antigen-binding protein is a tandem scFvs, diabody format, single-chain diabodies, tandem diabodies (TandAbs), dual-affinity retargeting molecules (DARTs), dock-and-lock (DNL), and nanobodies (Fan et al., J Hematol Oncol. 2015; 8:130). In various aspects, the bispecific antigen-binding protein is a bispecific $F(mab^1)_2$, an scFv, a bispecific diabody (BsDb), single-chain bispecific diabody (scBsDb), single-chain bispecific tandem variable domain (scBsTaFv), dock-and-lock trivalent Fab (DNL-(Fab)3), single-domain antibody (sdAb), or a bispecific single-domain antibody (BssdAb). In exemplary aspects, the bispecific antigen-binding protein is a tandem scFv comprising two scFv fragments linked by an extra peptide linker such as glycine-serine repeat motifs. Optionally, the tandem scFv comprises the structure: VL_A-linker1–VH_A-linker2–VH_B-linker3–VL_B (VL and VH derive from the single chain antibody fragment; A and B represent the parental monoclonal antibody A and B). In exemplary aspects, the bispecific antigen-binding protein is a TandAb which contains two pairs of VL and VH domains connected in a single polypeptide chain (Reusch et al., MAbs. 2015; 7(3):584-604). Two polypeptide products dimerize in a head-to-tail fashion, forming homodimers with large molecular weight (~105 kDa) upon expression. In exemplary aspects, the bispecific antigen-binding protein is one produced using crossMab technology which is described in PNAS 108(27): 11187-92 (2011). CrossMabs do not have any chemical linkers or connectors and are produced by a method that enforces correct light chain association in bispecific heterodimeric IgG antibodies. In exemplary aspects, the CrossMab is a bi- (1+1), tri- (2+1) and tetra-(2+2) valent bispecific crossMab, or is a non-Fc tandem antigen-binding fragment (Fab)-based crossMab. In exemplary instances, the crossMab is a $crossMab^{Fab}$, a $crossMab^{VH\text{-}VL}$, or a $crossMab^{CH1\text{-}CL}$.

[0090] In exemplary aspects, the bispecific antigen-binding protein comprises a single-domain antibody, or a nanobody, comprising a single monomeric variable antibody domain.

Optionally, the variable domain is based on the heavy chain variable domain. In alternative aspects, the variable domain is based on the light chain variable domain.

[0091] In exemplary aspects, the bispecific antigen-binding protein is a bispecific T cell engager or BiTE®. BiTEs are bivalent small molecules comprising only the variable regions of antibodies in the form of scFvs which are connected by flexible peptidic linkers. In exemplary aspects, the bispecific antigen-binding protein comprises an scFV comprising the LC and HC variable regions of the presently disclosed DLK1 antibodies and the LC and HC variable regions of a second antibody specific for a second antigen. In some embodiments, the BiTE comprises the LC and HC variable region of a second antibody specific for CD3. In some embodiments, the CD3 is CD3E.

[0092] In exemplary instances, the bispecific antigen-binding protein is a dual affinity retargeting (DART), which unlike BiTEs®, the covalent linkage between the two chains of DARTs limits the freedom of the antigen-binding sites. Therefore, DARTs are structurally compact and can form stable contacts between target and effector cells. The DART comprises two engineered Fv fragments which have their own VH exchanged with the VH of the other one. The inter-exchanged Fv domains advantageously releases variant fragments from the conformational constraint by the short linking peptide.

[0093] In exemplary aspects, the bispecific antigen binding protein is an HSABody comprising two scFvs fused to modified HSA. HSABodies are described in McDonagh et al., Mol Cancer Ther. 2012;11(3):582–93.

[0094] Accordingly, in exemplary aspects, the bispecific antigen-binding protein comprises an antigen binding fragment of any of the presently disclosed DLK1 antibodies. In exemplary aspects, the antigen binding fragment is a Fab. In exemplary aspects, the bispecific antigen-binding protein comprises an $F(ab)^{2'}$ of any of the presently disclosed DLK1 antibodies. In exemplary aspects, the bispecific antigen-binding protein comprises an scFv comprising the LC and HC variable regions of any of the presently disclosed DLK1 antibodies. In exemplary aspects, the scFv comprises the amino acid sequence of SEQ ID NO: 514 or 515. In various aspects, the antigen binding fragment is based on the heavy chain variable region and in other aspects, the antigen binding fragment is based on the light chain variable region. In exemplary aspects, the antigen binding fragment comprises at least part of both HC variable region and LC variable region. In exemplary aspects, the bispecific antigen-binding protein comprises at least one if not both of the LC or HC

variable regions of the presently disclosed DLK1 antibodies and at least one if not both of the LC and HC variable regions of a second antibody specific for a second antigen. In exemplary instances, the bispecific antigen-binding protein comprises an scFV comprising the LC and HC variable regions of the presently disclosed DLK1 antibodies and the LC and HC variable regions of a second antibody specific for a second antigen.

[0095] *Nucleic acids*

[0096] The present disclosure further provides nucleic acids comprising a nucleotide sequence encoding an antigen-binding protein of the present disclosure. By "nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, or modified forms thereof, which can be single-stranded or double- stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered inter-nucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. The nucleic acid can comprise any nucleotide sequence which encodes any of the antigen-binding proteins of the present disclosure.

[0097] The invention further provides nucleic acid molecules encoding the amino acid sequence corresponding to the antigen-binding proteins of the invention. In some embodiments, the nucleic acid molecule is a DNA (e.g., cDNA) or a hybrid thereof. Alternatively, the molecules is RNA or a hybrid thereof.

[0098] In some aspects, the nucleic acids of the present disclosure are recombinant. As used herein, the term "recombinant" refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be in vitro replication or in vivo replication.

[0099] Any nucleic acid of the present disclosure may be codon-optimized. Codon usage within a gene is helpful in determining the achievable protein expression levels. Certain sequences can be translated more readily by certain hosts, thus selecting the right codons for a given host may be necessary to maximizing expression. Methods of optimizing the codons are well known in the art. For example, there are many online tools, including

World Wide Web at codonstatsdb.unr.edu (see Subramanian et al. (2022) Mol Biol Evol 3;39(8):msac157.

[00100] The nucleic acids in some aspects are constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Sambrook et al., *supra*; and Ausubel et al., *supra*. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chIorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5- carboxymethylaminomethyl-2-thiouridme, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N^6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N -substituted adenine, 7-methylguanine, 5-methylammomethyluracil, 5- methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'- methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N^6-isopentenyladenine, uracil- 5-oxyacetic acid (v), wybutoxosine, pseudouratil, queosine, 2-thiocytosine, 5-methyl-2- thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3- (3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the present disclosure can be purchased from companies, such as Macromolecular Resources (Fort Collins, CO) and Synthegen (Houston, TX).

[00101] *Vector*

[00102] The nucleic acids of the present disclosure in some aspects are incorporated into a vector. In this regard, the present disclosure provides vectors comprising any of the presently disclosed nucleic acids. In various aspects, the vector is a recombinant expression vector. For purposes herein, the term "recombinant expression vector" means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide,

or peptide expressed within the cell. The vectors of the present disclosure are not naturally-occurring as a whole. However, parts of the vectors can be naturally-occurring. The presently disclosed vectors can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single- stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The vectors can comprise naturally-occurring or non-naturally-occurring internucleotide linkages, or both types of linkages. In some aspects, the altered nucleotides or non-naturally occurring internucleotide linkages do not hinder the transcription or replication of the vector.

[00103] The vector of the present disclosure can be any suitable vector, and can be used to transduce, transform or transfect any suitable host. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be a plasmid based expression vector. In various aspects, the vector is selected from the group consisting of the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJoIIa, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA). Bacteriophage vectors, such as λGTIO, λGTl 1, λZapII (Stratagene), λEMBL4, and λNMl 149, also can be used. Examples of plant expression vectors include pBIOl, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech). In some aspects, the vector is a viral vector, e.g., a retroviral vector. In various aspects, the vector is an adenovirus vector, an adeno-associated virus (AAV) vector, a Herpes Simplex Virus (HSV) vector, a Vesicular stomatitis virus (VSV) vector, vaccinia virus vector, or lentivirus vector. See, e.g., Howarth et al., Cell Biol. Toxicol. 26(1): 1-20 (2010). In various aspects, the vector is a baculovirus vector which infects arthropods, e.g., insects. In various aspects, the baculovirus vector is an Autographacalifornica multiple nuclear virus (AcMNPV) or a Bombyxmorinuclear polyhedrosis (BmNPV). See, e.g., Khan, Adv Pharm Bull 3(2): 257-263 (2013); Miller, Bioessays 11(4): 91-96 (1989); Atkinson et al., Pestic Sci 28: 215-224 (1990).

[00104] The vectors of the present disclosure can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., supra, and Ausubel et al., supra. Constructs of expression vectors, which are circular or linear, can be

prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from CoIEl, 2 μ plasmid, λ, SV40, bovine papilloma virus, and the like.

[00105] In some aspects, the vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA- based.

[00106] The vector can include one or more marker genes, which allow for selection of transformed or transfected hosts. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the presently disclosed expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[00107] The vector can comprise a native or normative promoter operably linked to the nucleotide sequence encoding the polypeptide (including functional portions and functional variants thereof), or to the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the polypeptide. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental- specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter, e.g., a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, and a promoter found in the long-terminal repeat of the murine stem cell virus.

[00108] *Host cells*

[00109] Provided herein are host cells comprising a nucleic acid or vector of the present disclosure. As used herein, the term "host cell" refers to any type of cell that can contain the presently disclosed vector and is capable of producing an expression product encoded by the nucleic acid (e.g., mRNA, protein). The host cell in some aspects is an adherent cell or a suspended cell, i.e., a cell that grows in suspension. The host cell in various aspects is a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be of any cell type, can originate from any type of tissue, and can be of any

developmental stage.

[00110] In various aspects, the antigen-binding protein is a glycosylated protein and the host cell is a glycosylation-competent cell. In various aspects, the glycosylation-competent cell is an eukaryotic cell, including, but not limited to, a yeast cell, filamentous fungi cell, protozoa cell, algae cell, insect cell, or mammalian cell. Such host cells are described in the art. See, e.g., Frenzel, et al., *Front Immunol* 4: 217 (2013). In various aspects, the eukaryotic cells are mammalian cells. In various aspects, the mammalian cells are non-human mammalian cells. In some aspects, the cells are Chinese Hamster Ovary (CHO) cells and derivatives thereof (e.g., CHO-K1, CHO pro-3), mouse myeloma cells (e.g., NS0, GS-NS0, Sp2/0), cells engineered to be deficient in dihydrofolatereductase (DHFR) activity (e.g., DUKX-X11, DG44), human embryonic kidney 293 (HEK293) cells or derivatives thereof (e.g., HEK293T, HEK293-EBNA), green African monkey kidney cells (e.g., COS cells, VERO cells), human cervical cancer cells (e.g., HeLa), human bone osteosarcoma epithelial cells U2-OS, adenocarcinomic human alveolar basal epithelial cells A549, human fibrosarcoma cells HT1080, mouse brain tumor cells CAD, embryonic carcinoma cells P19, mouse embryo fibroblast cells NIH 3T3, mouse fibroblast cells L929, mouse neuroblastoma cells N2a, human breast cancer cells MCF-7, retinoblastoma cells Y79, human retinoblastoma cells SO-Rb50, human liver cancer cells Hep G2, mouse B myeloma cells J558L, or baby hamster kidney (BHK) cells (Gaillet et al. 2007; Khan, Adv Pharm Bull 3(2): 257-263 (2013)).

[00111] For purposes of amplifying or replicating the vector, the host cell is in some aspects is a prokaryotic cell, e.g., a bacterial cell.

[00112] Also provided by the present disclosure is a population of cells comprising at least one host cell described herein. The population of cells in some aspects is a heterogeneous population comprising the host cell comprising vectors described, in addition to at least one other cell, which does not comprise any of the vectors. Alternatively, in some aspects, the population of cells is a substantially homogeneous population, in which the population comprises mainly host cells (e.g., consisting essentially of) comprising the vector. The population in some aspects is a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a vector, such that all cells of the population comprise the vector. In various embodiments of the present disclosure, the population of cells is a clonal population comprising host cells comprising a

vector as described herein.

[00113] *Manufacture methods*

[00114] Also provided herein are methods of producing an antigen-binding protein which binds to DLK1. In various embodiments, the method comprises culturing a host cell comprising a nucleic acid comprising a nucleotide sequence encoding the antigen-binding protein as described herein in a cell culture medium and harvesting the antigen-binding protein from the cell culture medium. The host cell can be any of the host cells described herein. In various aspects, the host cell is selected from the group consisting of: CHO cells, NS0 cells, COS cells, VERO cells, and BHK cells. In various aspects, the step of culturing a host cell comprises culturing the host cell in a growth medium to support the growth and expansion of the host cell. In various aspects, the growth medium increases cell density, culture viability and productivity in a timely manner. In various aspects, the growth medium comprises amino acids, vitamins, inorganic salts, glucose, and serum as a source of growth factors, hormones, and attachment factors. In various aspects, the growth medium is a fully chemically defined media consisting of amino acids, vitamins, trace elements, inorganic salts, lipids and insulin or insulin-like growth factors. In addition to nutrients, the growth medium also helps maintain pH and osmolality. Several growth media are commercially available and are described in the art. See, e.g., Arora, "Cell Culture Media: A Review" MATER METHODS 3:175 (2013).

[00115] In various aspects, the method comprises culturing the host cell in a feed medium. In various aspects, the method comprises culturing in a feed medium in a fed-batch mode. Methods of recombinant protein production are known in the art. See, e.g., Li et al., "Cell culture processes for monoclonal antibody production" MAbs 2(5): 466–477 (2010).

[00116] The method making an antigen-binding protein can comprise one or more steps for purifying the protein from a cell culture or the supernatant thereof and preferably recovering the purified protein. In various aspects, the method comprises one or more chromatography steps, e.g., affinity chromatography (e.g., protein A affinity chromatography), ion exchange chromatography, hydrophobic interaction chromatography. In various aspects, the method comprises purifying the protein using a Protein A affinity chromatography resin.

[00117] In various embodiments, the method further comprises steps for formulating the purified protein, etc., thereby obtaining a formulation comprising the purified protein. Such steps are described in Formulation and Process Development Strategies for Manufacturing, eds. Jameel and Hershenson, John Wiley & Sons, Inc. (Hoboken, NJ), 2010.

[00118] In various aspects, the antigen-binding protein linked to a polypeptide and the antigen-binding protein is part of a fusion protein. Thus, the present disclosure further provides methods of producing a fusion protein comprising an antigen-binding protein which binds to DLK1. In various embodiments, the method comprises culturing a host cell comprising a nucleic acid comprising a nucleotide sequence encoding the fusion protein as described herein in a cell culture medium and harvesting the fusion protein from the cell culture medium.

[00119] *Conjugates*

[00120] The present disclosure also provides antigen-binding proteins attached, linked or conjugated to a second moiety (e.g., a heterologous moiety, a conjugate moiety). Accordingly, the present disclosure provides a conjugate comprising an antigen-binding protein and a heterologous moiety. As used herein, the term "heterologous moiety" is synonymous with "conjugate moiety" and refers to any molecule (chemical or biochemical, naturally-occurring or non-coded) which is different from the antigen-binding proteins of the present disclosure. Various heterologous moieties include, but are not limited to, a polymer, a carbohydrate, a lipid, a nucleic acid, an oligonucleotide, a DNA or RNA, an amino acid, peptide, polypeptide, protein, therapeutic agent, (e.g., a cytotoxic agent, cytokine), or a diagnostic agent.

[00121] In some embodiments, the heterologous moiety is a detectable marker. Examples of detectable markers include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate (FITC), rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin (PE); an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I,

^{131}I, ^{35}S, or ^{3}H. As used herein, the term "labeled", with regard to the antibody, is intended to encompass direct labeling of the antibody by coupling (*i.e.*, physically linking) a detectable substance, such as a radioactive agent or a fluorophore (*e.g.* fluorescein isothiocyanate (FITC) or phycoerythrin (PE) or indocyanine (Cy5)) to the antibody, as well as indirect labeling of the antibody by reactivity with a detectable substance. For example, an antibody may be labeled with a nucleic acid sequence that may be amplified and detected, or an antisense oligonucleotide to reduce expression of a particular gene, such that expression can then be detected and measured.

[00122] In some embodiments, the heterologous moiety is a polymer. The polymer can be branched or unbranched. The polymer can be of any molecular weight. The polymer in some embodiments has an average molecular weight of between about 2 kDa to about 100 kDa (the term "about" indicating that in preparations of a water soluble polymer, some molecules will weigh more, some less, than the stated molecular weight). The average molecular weight of the polymer is in some aspect between about 5 kDa and about 50 kDa, between about 12 kDa to about 40 kDa or between about 20 kDa to about 35 kDa.

[00123] In some embodiments, the polymer is modified to have a single reactive group, such as an active ester for acylation or an aldehyde for alkylation, so that the degree of polymerization can be controlled. The polymer in some embodiments is water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. In some embodiments, when, for example, the composition is used for therapeutic use, the polymer is pharmaceutically acceptable. Additionally, in some aspects, the polymer is a mixture of polymers, e.g., a co-polymer, a block co-polymer.

[00124] In some embodiments, the polymer is selected from the group consisting of: polyamides, polycarbonates, polyalkylenes and derivatives thereof including, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polymers of acrylic and methacrylic esters, including poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate), polyvinyl polymers including polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, poly(vinyl acetate), and polyvinylpyrrolidone, polyglycolides,

polysiloxanes, polyurethanes and co-polymers thereof, celluloses including alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, and cellulose sulphate sodium salt, polypropylene, polyethylenes including poly(ethylene glycol), poly(ethylene oxide), and poly(ethylene terephthalate), and polystyrene.

[00125] A particularly preferred water-soluble polymer for use herein is polyethylene glycol (PEG). As used herein, polyethylene glycol is meant to encompass any of the forms of PEG that can be used to derivatize other proteins, such as mono-(C1-C10) alkoxy- or aryloxy-polyethylene glycol. PEG is a linear or branched neutral polyether, available in a broad range of molecular weights, and is soluble in water and most organic solvents.

[00126] In some embodiments, the heterologous moiety is a carbohydrate. In some embodiments, the carbohydrate is a monosaccharide (e.g., glucose, galactose, fructose), a disaccharide (e.g., sucrose, lactose, maltose), an oligosaccharide (e.g., raffinose, stachyose), a polysaccharide (a starch, amylase, amylopectin, cellulose, chitin, callose, laminarin, xylan, mannan, fucoidan, galactomannan.

[00127] In some embodiments, the heterologous moiety is a lipid. The lipid, in some embodiments, is a fatty acid, eicosanoid, prostaglandin, leukotriene, thromboxane, N-acyl ethanolamine), glycerolipid (e.g., mono-, di-, tri-substituted glycerols), glycerophospholipid (e.g., phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine), sphingolipid (e.g., sphingosine, ceramide), sterol lipid (e.g., steroid, cholesterol), prenol lipid, saccharolipid, or a polyketide, oil, wax, cholesterol, sterol, fat-soluble vitamin, monoglyceride, diglyceride, triglyceride, a phospholipid.

[00128] In some embodiments, the heterologous moiety is a therapeutic agent. The therapeutic agent can be any of those known in the art. Examples of therapeutic agents that are contemplated herein include, but are not limited to, natural enzymes, proteins derived from natural sources, recombinant proteins, natural peptides, synthetic peptides, cyclic peptides, antibodies, receptor agonists, cytotoxic agents, immunoglobins, beta-adrenergic blocking agents, calcium channel blockers, coronary vasodilators, cardiac glycosides, antiarrhythmics, cardiac sympathomemetics, angiotensin converting enzyme (ACE) inhibitors, diuretics, inotropes, cholesterol and triglyceride reducers, bile acid sequestrants,

fibrates, 3-hydroxy-3-methylgluteryl (HMG)-CoA reductase inhibitors, niacin derivatives, antiadrenergic agents, alpha-adrenergic blocking agents, centrally acting antiadrenergic agents, vasodilators, potassium-sparing agents, thiazides and related agents, angiotensin II receptor antagonists, peripheral vasodilators, antiandrogens, estrogens, antibiotics, retinoids, insulins and analogs, alpha-glucosidase inhibitors, biguanides, meglitinides, sulfonylureas, thizaolidinediones, androgens, progestogens, bone metabolism regulators, anterior pituitary hormones, hypothalamic hormones, posterior pituitary hormones, gonadotropins, gonadotropin-releasing hormone antagonists, ovulation stimulants, selective estrogen receptor modulators, antithyroid agents, thyroid hormones, bulk forming agents, laxatives, antiperistaltics, flora modifiers, intestinal adsorbents, intestinal anti-infectives, antianorexic, anticachexic, antibulimics, appetite suppressants, antiobesity agents, antacids, upper gastrointestinal tract agents, anticholinergic agents, aminosalicylic acid derivatives, biological response modifiers, corticosteroids, antispasmodics, 5-HT_4 partial agonists, antihistamines, cannabinoids, dopamine antagonists, serotonin antagonists, cytoprotectives, histamine H2-receptor antagonists, mucosal protective agent, proton pump inhibitors, H. pylori eradication therapy, erythropoieses stimulants, hematopoietic agents, anemia agents, heparins, antifibrinolytics, hemostatics, blood coagulation factors, adenosine diphosphate inhibitors, glycoprotein receptor inhibitors, fibrinogen-platelet binding inhibitors, thromboxane-A_2 inhibitors, plasminogen activators, antithrombotic agents, glucocorticoids, mineralcorticoids, corticosteroids, selective immunosuppressive agents, antifungals, drugs involved in prophylactic therapy, AIDS-associated infections, cytomegalovirus, non-nucleoside reverse transcriptase inhibitors, nucleoside analog reverse transcriptse inhibitors, protease inhibitors, anemia, Kaposi's sarcoma, aminoglycosides, carbapenems, cephalosporins, glycopoptides, lincosamides, macrolies, oxazolidinones, penicillins, streptogramins, sulfonamides, trimethoprim and derivatives, tetracyclines, anthelmintics, amebicies, biguanides, cinchona alkaloids, folic acid antagonists, quinoline derivatives, Pneumocystis carinii therapy, hydrazides, imidazoles, triazoles, nitroimidzaoles, cyclic amines, neuraminidase inhibitors, nucleosides, phosphate binders, cholinesterase inhibitors, adjunctive therapy, barbiturates and derivatives, benzodiazepines, gamma aminobutyric acid derivatives, hydantoin derivatives, iminostilbene derivatives, succinimide derivatives, anticonvulsants, ergot alkaloids, antimigrane preparations, biological response modifiers, carbamic acid eaters, tricyclic derivatives, depolarizing agents, nondepolarizing agents,

neuromuscular paralytic agents, CNS stimulants, dopaminergic reagents, monoamine oxidase inhibitors, COMT inhibitors, alkyl sulphonates, ethylenimines, imidazotetrazines, nitrogen mustard analogs, nitrosoureas, platinum-containing compounds, antimetabolites, purine analogs, pyrimidine analogs, urea derivatives, antracyclines, actinomycinds, camptothecin derivatives, epipodophyllotoxins, taxanes, vinca alkaloids and analogs, antiandrogens, antiestrogens, nonsteroidal aromatase inhibitors, protein kinase inhibitor antineoplastics, azaspirodecanedione derivatives, anxiolytics, stimulants, monoamind reuptake inhibitors, selective serotonin reuptake inhibitors, antidepressants, benzisooxazole derivatives, butyrophenone derivatives, dibenzodiazepine derivatives, dibenzothiazepine derivatives, diphenylbutylpiperidine derivatives, phenothiazines, thienobenzodiazepine derivatives, thioxanthene derivatives, allergenic extracts, nonsteroidal agents, leukotriene receptor antagonists, xanthines, endothelin receptor antagonist, prostaglandins, lung surfactants, mucolytics, antimitotics, uricosurics, xanthine oxidase inhibitors, phosphodiesterase inhibitors, metheamine salts, nitrofuran derivatives, quinolones, smooth muscle relaxants, parasympathomimetic agents, halogenated hydrocarbons, esters of amino benzoic acid, amides (e.g. lidocaine, articaine hydrochloride, bupivacaine hydrochloride), antipyretics, hynotics and sedatives, cyclopyrrolones, pyrazolopyrimidines, nonsteroidal anti-inflammatory drugs, opioids, para-aminophenol derivatives, alcohol dehydrogenase inhibitor, heparin antagonists, adsorbents, emetics, opoid antagonists, cholinesterase reactivators, nicotine replacement therapy, vitamin A analogs and antagonists, vitamin B analogs and antagonists, vitamin C analogs and antagonists, vitamin D analogs and antagonists, vitamin E analogs and antagonists, vitamin K analogs and antagonists.

[00129] The antigen-binding proteins of the present disclosure can be conjugated to one or more cytokines and growth factors that are effective in inhibiting tumor metastasis, and wherein the cytokine or growth factor has been shown to have an antiproliferative effect on at least one cell population. Such cytokines, lymphokines, growth factors, or other hematopoietic factors include, but are not limited to: M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IFN, TNFα, TNF1, TNF2, G-CSF, Meg-CSF, GM-CSF, thrombopoietin, stem cell factor, and erythropoietin. Additional growth factors for use herein include angiogenin, bone morphogenic protein-1, bone morphogenic protein-2, bone morphogenic protein-3, bone morphogenic protein-4, bone morphogenic protein-5, bone morphogenic protein-6,

bone morphogenic protein-7, bone morphogenic protein-8, bone morphogenic protein-9, bone morphogenic protein-10, bone morphogenic protein-11, bone morphogenic protein-12, bone morphogenic protein-13, bone morphogenic protein-14, bone morphogenic protein-15, bone morphogenic protein receptor IA, bone morphogenic protein receptor IB, brain derived neurotrophic factor, ciliary neutrophic factor, ciliary neutrophic factor receptor α, cytokine-induced neutrophil chemotactic factor 1, cytokine-induced neutrophil, chemotactic factor 2 α, cytokine-induced neutrophil chemotactic factor 2 β, β endothelial cell growth factor, endothelin 1, epithelial-derived neutrophil attractant, glial cell line-derived neutrophic factor receptor α 1, glial cell line-derived neutrophic factor receptor α 2, growth related protein, growth related protein α, growth related protein β, growth related protein γ, heparin binding epidermal growth factor, hepatocyte growth factor, hepatocyte growth factor receptor, insulin-like growth factor I, insulin-like growth factor receptor, insulin-like growth factor II, insulin-like growth factor binding protein, keratinocyte growth factor, leukemia inhibitory factor, leukemia inhibitory factor receptor α, nerve growth factor nerve growth factor receptor, neurotrophin-3, neurotrophin-4, pre-B cell growth stimulating factor, stem cell factor, stem cell factor receptor, transforming growth factor α, transforming growth factor β, transforming growth factor β1, transforming growth factor β1.2, transforming growth factor β2, transforming growth factor β3, transforming growth factor β5, latent transforming growth factor β1, transforming growth factor β binding protein I, transforming growth factor β binding protein II, transforming growth factor β binding protein III, tumor necrosis factor receptor type I, tumor necrosis factor receptor type II, urokinase-type plasminogen activator receptor, and chimeric proteins and biologically or immunologically active fragments thereof.

[00130] In some embodiments, the conjugate comprises an antigen-binding protein as described herein and a cytotoxic agent. The cytotoxic agent is any molecule (chemical or biochemical) which is toxic to a cell. In some aspects, when a cytotoxic agent is conjugated to an antigen-binding protein of the present disclosure, the results obtained are synergistic. That is to say, the effectiveness of the combination therapy of an antigen-binding protein and the cytotoxic agent is synergistic, i.e., the effectiveness is greater than the effectiveness expected from the additive individual effects of each. Therefore, the dosage of the cytotoxic agent can be reduced and thus, the risk of the toxicity problems and other side effects is concomitantly reduced. In some embodiments, the cytotoxic agent is a

chemotherapeutic agent. Chemotherapeutic agents are known in the art and include, but not limited to, platinum coordination compounds, topoisomerase inhibitors, antibiotics, antimitotic alkaloids and difluoronucleosides, as described in U.S. Pat. No. 6,630,124.

[00131] In some embodiments, the chemotherapeutic agent is a platinum coordination compound. The term "platinum coordination compound" refers to any tumor cell growth inhibiting platinum coordination compound that provides the platinum in the form of an ion.

[00132] In some embodiments, the platinum coordination compound is cis-diamminediaquoplatinum (II)-ion; chloro(diethylenetriamine)-platinum(II)chloride; dichloro(ethylenediamine)-platinum(II), diammine(1,1-cyclobutanedicarboxylato) platinum(II) (carboplatin); spiroplatin; iproplatin; diammine(2-ethylmalonato)-platinum(II); ethylenediaminemalonatoplatinum(II); aqua(1,2-diaminodyclohexane)-sulfatoplatinum(II); (1,2-diaminocyclohexane)malonatoplatinum(II); (4-caroxyphthalato)(1,2-diaminocyclohexane)platinum(II); (1,2-diaminocyclohexane)-(isocitrato)platinum(II); (1,2-diaminocyclohexane)cis(pyruvato)platinum(II); (1,2-diaminocyclohexane)oxalatoplatinum(II); ormaplatin; and tetraplatin.

[00133] In some embodiments, cisplatin is the platinum coordination compound employed in the compositions and methods of the present invention. Cisplatin is commercially available under the name PLATINOL™ from Bristol Myers-Squibb Corporation and is available as a powder for constitution with water, sterile saline or other suitable vehicle. Other platinum coordination compounds suitable for use in the present invention are known and are available commercially and/or can be prepared by conventional techniques. Cisplatin, or cis-dichlorodiammineplatinum II, has been used successfully for many years as a chemotherapeutic agent in the treatment of various human solid malignant tumors. More recently, other diamino-platinum complexes have also shown efficacy as chemotherapeutic agents in the treatment of various human solid malignant tumors. Such diamino-platinum complexes include, but are not limited to, spiroplatinum and carboplatinum. Although cisplatin and other diamino-platinum complexes have been widely used as chemotherapeutic agents in humans, they have had to be delivered at high dosage levels that can lead to toxicity problems such as kidney damage.

[00134] In some embodiments, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerases are enzymes that are capable of altering DNA topology in eukaryotic cells.

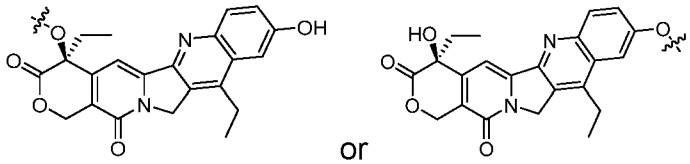
They are critical for cellular functions and cell proliferation. Generally, there are two classes of topoisomerases in eukaryotic cells, type I and type II. Topoisomerase I is a monomeric enzyme of approximately 100,000 molecular weight. The enzyme binds to DNA and introduces a transient single-strand break, unwinds the double helix (or allows it to unwind), and subsequently reseals the break before dissociating from the DNA strand. Various topoisomerase inhibitors have recently shown clinical efficacy in the treatment of humans afflicted with ovarian, cancer, esophageal cancer or non-small cell lung carcinoma.

[00135] In some aspects, the topoisomerase inhibitor is camptothecin or a camptothecin analog. Camptothecin is a water-insoluble, cytotoxic alkaloid produced by Camptotheca accuminata trees indigenous to China and Nothapodytes foetida trees indigenous to India. Camptothecin exhibits tumor cell growth inhibiting activity against a number of tumor cells. Compounds of the camptothecin analog class are typically specific inhibitors of DNA topoisomerase I. By the term "inhibitor of topoisomerase" is meant any tumor cell growth inhibiting compound that is structurally related to camptothecin. Compounds of the camptothecin analog class include, but are not limited to; topotecan, irinotecan and 9-amino-camptothecin.

[00136] In additional embodiments, the cytotoxic agent is any tumor cell growth inhibiting camptothecin analog claimed or described in: U.S. Pat. No. 5,004,758, issued on Apr. 2, 1991 and European Patent Application Number 88311366.4, published on Jun. 21, 1989 as 20' Publication Number EP 0 321 122; U.S. Pat. No. 4,604,463, issued on Aug. 5, 1986 and European Patent Application Publication Number EP 0 137 145, published on Apr. 17, 1985; U.S. Pat. No. 4,473,692, issued on Sep. 25, 1984 and European Patent Application Publication Number EP 0 074 256, published on Mar. 16, 1983; U.S. Pat. No. 4,545,880, issued on Oct. 8, 1985 and European Patent Application Publication Number EP 0 074 256, published on Mar. 16, 1983; European Patent Application Publication Number EP 0 088 642, published on Sep. 14, 1983; Wani et al., J. Med. Chem., 29, 2358-2363 (1986); Nitta et al., Proc. 14th International Congr. Chemotherapy, Kyoto, 1985, Tokyo Press, Anticancer Section 1, p. 28-30, especially a compound called CPT-11. CPT-11 is a camptothecin analog with a 4-(piperidino)-piperidine side chain joined through a carbamate linkage at C-10 of 10-hydroxy-7-ethyl camptothecin. CPT-11 is currently undergoing human clinical trials and is also referred to as irinotecan; Wani et al, J. Med. Chem., 23, 554 (1980); Wani et. al., J. Med. Chem., 30, 1774 (1987); U.S. Pat. No. 4,342,776, issued

on Aug. 3, 1982; U.S. patent application Ser. No. 581,916, filed on Sep. 13, 1990 and European Patent Application Publication Number EP 418 099, published on Mar. 20, 1991; U.S. Pat. No. 4,513,138, issued on Apr. 23, 1985 and European Patent Application Publication Number EP 0 074 770, published on Mar. 23, 1983; U.S. Pat. No. 4,399,276, issued on Aug. 16, 1983 and European Patent Application Publication Number 0 056 692, published on Jul. 28, 1982; the entire disclosure of each of which is hereby incorporated by reference. All of the above-listed compounds of the camptothecin analog class are available commercially and/or can be prepared by conventional techniques including those described in the above-listed references. The topoisomerase inhibitor may be selected from the group consisting of topotecan, irinotecan and 9-aminocamptothecin.

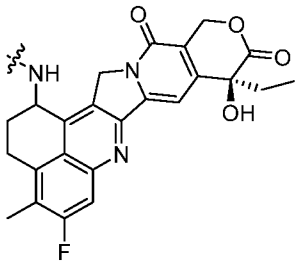
[00137] In some embodiments, the camptothecin analog is an active metabolite of irinotecan (CPT-11). In some such embodiments, the camptothecin analog is 7-ethyl-10-hydroxycamptothecin (SN-38). As a metabolite, SN-38 is formed by hydrolysis of irinotecan by carboxylesterases. In some embodiments, SN-38 has one of the following structures:



SN-38 has been described in U.S. patent application Ser. No. 7,999,083; U.S. patent application Ser. No. 8,080,250; U.S. patent application Ser. No. 8,759,496; U.S. patent application Ser. No. 8,999,344; U.S. patent application Ser. No. 10,195,288; and U.S. patent application Ser. No. 9,808,537.

[00138] In some embodiments, the camptothecin analog is exatecan methanesulfonate. Exatecan methanesulfonate is a water-soluble camptothecin (CPT) that exhibits more potent topoisomerase I inhibitory activity and antitumor activity than other CPT analogs. In addition, exatecan is effective against p-glycoprotein (P-gp)-mediated multi-drug resistant cells.

[00139] In some embodiments, the camptothecin analog is deruxtecan (Dxd), a potent derivative of exatecan, which has 10-fold higher topoisomerase I inhibitory potency than SN-38. In some embodiments, Dxd has the following structure:



Dxd has been described in U.S. patent application Ser. No. 6,407,115; U.S. patent application Ser. No. 10,195,288; U.S. patent application Ser. No. 9,808,537; and U.S. patent application Ser. No. 6,407,115.

[00140] The preparation of numerous compounds of the camptothecin analog class (including pharmaceutically acceptable salts, hydrates and solvates thereof) as well as the preparation of oral and parenteral pharmaceutical compositions comprising such a compounds of the camptothecin analog class and an inert, pharmaceutically acceptable carrier or diluent, is extensively described in U.S. Pat. No. 5,004,758, issued on Apr. 2, 1991 and European Patent Application Number 88311366.4, published on Jun. 21, 1989 as Publication Number EP 0 321 122, the teachings of which are incorporated herein by reference.

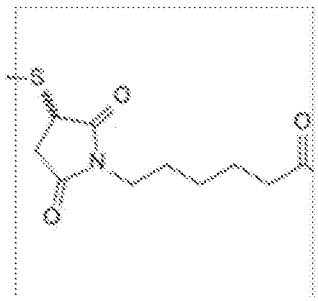
[00141] In still yet other embodiments of the invention, the chemotherapeutic agent is an antibiotic compound. Suitable antibiotic include, but are not limited to, doxorubicin, mitomycin, bleomycin, daunorubicin and streptozocin.

[00142] In some embodiments, the chemotherapeutic agent is an antimitotic alkaloid. In general, antimitotic alkaloids can be extracted from Cantharanthus roseus, and have been shown to be efficacious as anticancer chemotherapy agents. A great number of semi-synthetic derivatives have been studied both chemically and pharmacologically (see, O. Van Tellingen et al, Anticancer Research, 12, 1699-1716 (1992)). The antimitotic alkaloids of the present invention include, but are not limited to, vinblastine, vincristine, vindesine, Taxol and vinorelbine. The latter two antimitotic alkaloids are commercially available from Eli Lilly and Company, and Pierre Fabre Laboratories, respectively (see, U.S. Pat. No. 5,620,985). In some embodiments, the antimitotic alkaloid is vinorelbine.

[00143] In other embodiments of the invention, the chemotherapeutic agent is a difluoronucleoside. 2'-deoxy-2',2'-difluoronucleosides are known in the art as having antiviral activity. Such compounds are disclosed and taught in U.S. Pat. Nos. 4,526,988 and 4,808614. European Patent Application Publication 184,365 discloses that these same

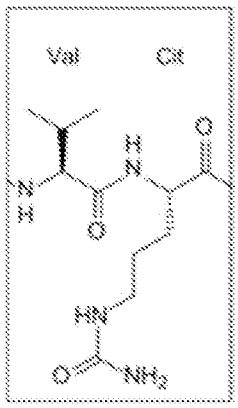
difluoronucleosides have oncolytic activity. In certain aspects, the 2'-deoxy-2',2'-difluoronucleoside used in the compositions and methods of the present invention is 2'-deoxy-2',2'-difluorocytidine hydrochloride, also known as gemcitabine hydrochloride. Gemcitabine is commercially available or can be synthesized in a multi-step process as disclosed and taught in U.S. Pat. Nos. 4,526,988, 4,808,614 and 5,223,608, the teachings of which are incorporated herein by reference.

[00144] In various aspects, the chemotherapeutic agent is an anti-mitotic agent which inhibits cell division by blocking tubulin polymerization, destabilizing microtubules, or altering microtubule dynamics, *e.g.,* maytansinoid or a derivative thereof (*e.g.,* DM1 or DM4), auristatin or a derivative thereof. In various instances, the chemotherapeutic agent is an auristatin. For instance, the auristatin is in some aspects, dolastatin, Monomethyl auristatin E (MMAE), Monomethyl auristatin E (MMAE), or PF-06380101. Auristatins are described in the art. See, e.g., Maderna, A.; *et al.*, Mol Pharmaceutics 12(6): 1798-1812 (2015). In various aspects, the conjugate comprises an antibody of the present disclosure in combination with MMAE. Optionally, the conjugate comprises a linker. In some aspects, the linker comprises a cleavable linking moiety. In various instances, the conjugate comprises an antibody of the present disclosure linked to an attachment group which is linked to a cathepsin-cleavable linker, which in turn is linked to a spacer which is linked to MMAE. In aspects, the attachment group is attached to the antibody via a Cys residue of the Fc region of the antibody. In exemplary aspects, the attachment group comprises the structure of Formula I:



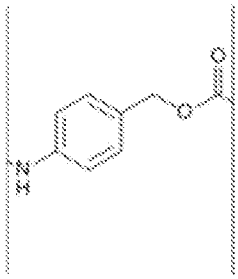
[Formula I]

In exemplary aspects, the cathepsin cleavable linker comprises the structure of Formula II:



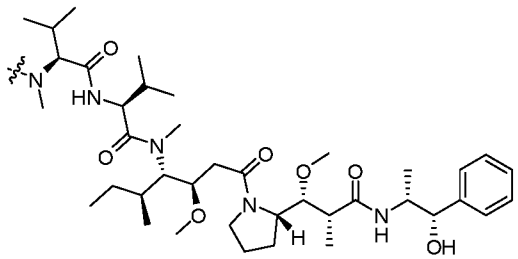
[Formula II].

In exemplary aspects, the spacer comprises the structure of Formula III:



[Formula III].

[00145] In some embodiments, MMAE has the following structure:



[00146] The present disclosure also provides conjugates comprising an antigen-binding protein of the present disclosure linked to a polypeptide, such that the conjugate is a fusion protein. Therefore, the present disclosure provides fusion proteins comprising an antigen-binding protein of the present disclosure linked to a polypeptide. In various embodiments, the polypeptide is a diagnostic label, e.g., a fluorescent protein, such as green fluorescent protein, or other tag, e.g., Myc tag. In various aspects, the polypeptide is one of the cytokines, lymphokines, growth factors, or other hematopoietic factors listed above.

[00147] *Linkers*

[00148] In some embodiments, the conjugate is directly linked to the heterologous moiety. In alternative embodiments, the conjugate comprises a linker that joins the compound of the present disclosure to the heterologous moiety. In some aspects, the linker

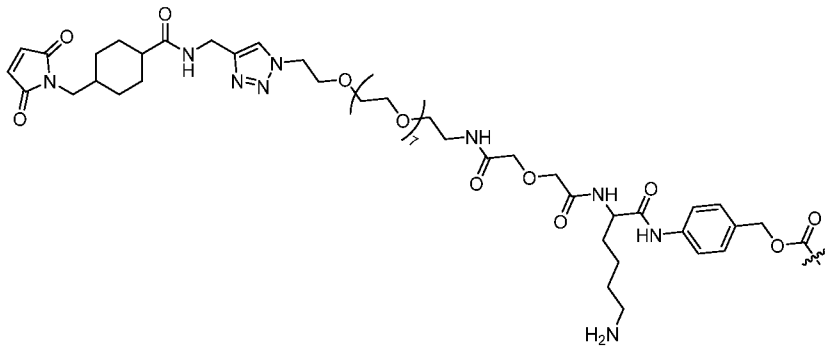
comprises a chain of atoms from 1 to about 60, or 1 to 30 atoms or longer, 2 to 5 atoms, 2 to 10 atoms, 5 to 10 atoms, or 10 to 20 atoms long. In some embodiments, the chain atoms are all carbon atoms. In some embodiments, the chain atoms in the backbone of the linker are selected from the group consisting of C, O, N, and S. Chain atoms and linkers can be selected according to their expected solubility (hydrophilicity) so as to provide a more soluble conjugate. In some embodiments, the linker provides a functional group that is subject to cleavage by an enzyme or other catalyst or hydrolytic conditions found in the target tissue or organ or cell. In some embodiments, the length of the linker is long enough to reduce the potential for steric hindrance. In some embodiments, the linker is an amino acid or a peptidyl linker. Such peptidyl linkers can be any length. Various linkers are from about 1 to 50 amino acids in length, 5 to 50, 3 to 5, 5 to 10, 5 to 15, or 10 to 30 amino acids in length.

[00149] A variety of suitable linkers are known in the art. The linker can be cleavable (a cleavable linker), e.g., under physiological conditions, e.g., under intracellular conditions, such that cleavage of the linker releases the drug in the intracellular environment. Alternatively, the linker can be cleavable under extracellular conditions, *e.g.,* outside the tumor cells or in the vicinity of the tumor mass, such that cleavage of the linker releases the drug that permeates preferentially inside the tumor cells. In other embodiments, the linker is not cleavable (a non-cleavable linker), and the drug is released, for example, by antibody degradation.

[00150] The linker can be bonded to a chemically reactive group on the antibody moiety, e.g., to a free amino, imino, hydroxyl, thiol, or carboxyl group (e.g., to the N- or C-terminus, to the epsilon amino group of one or more lysine residues, to the free carboxylic acid group of one or more glutamic acid or aspartic acid residues, to the sulfhydryl group of one or more cysteinyl residues, or to the hydroxyl group of one or more serine or threonine residues). The site to which the linker is bound can be a natural residue in the amino acid sequence of the antibody moiety, or it can be introduced into the antibody moiety, e.g., by DNA recombinant technology (e.g., by introducing a cysteine or protease cleavage site in the amino acid sequence) or by protein biochemistry (e.g., reduction, pH adjustment, or proteolysis). The site to which the linker is bound can also be a non-natural amino acids. The site to which the linker is bound can also be a glycan on the antibody.

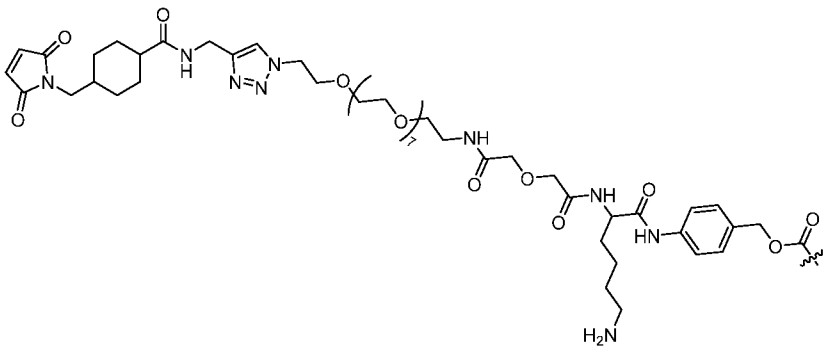
[00151] Typically, the linker is substantially inert under conditions for which the two groups it is connecting are linked. The term "bifunctional crosslinking agent," "bifunctional linker" or "crosslinking agent" refers to a modifying agent that possess two reactive groups at each end of the linker, such that one reactive group can be first reacted with the cytotoxic compound to provide a compound bearing the linker moiety and a second reactive group, which can then react with the antibody. Alternatively, one end of the bifunctional crosslinking agent can be first reacted with the antibody to provide an antibody bearing a linker moiety and a second reactive group, which can then react with the cytotoxic compound. The linking moiety may contain a chemical bond that allows for the release of the cytotoxic moiety at a particular site. Suitable chemical bonds are well known in the art and include disulfide bonds, thioether bonds, acid labile bonds, photolabile bonds, protease/peptidase labile bonds, and esterase labile bonds. See, for example, U.S. Patent Nos. 5,208,020; 5,475,092; 6,441,163; 6,716,821; 6,913,748; 7,276,497; 7,276,499; 7,368,565; 7,388,026 and 7,414,073. In some embodiments, the bonds are disulfide bonds, thioether, and/or protease/peptidase labile bonds. Other linkers that can be used in the present invention include non-cleavable linkers, such as those described in detail in US 20050169933, charged linkers, or hydrophilic linkers, such as those described in US 2009/0274713, US 2010/0129314, and WO 2009/134976, each of which is expressly incorporated herein by reference.

[00152] In some embodiments, the linker is a hydrophilic linker that confers hydrophilicity to the conjugate. In some embodiments, the hydrophilic linker comprises polyethylene glycol (PEG). In some embodiments, the hydrophilic linker is CLA2. In some embodiments, the CLA2 linker has the following structure:



CLA2 has been described in U.S. Patent Nos. 8,080,250; 8,759,496; and 10,195,288.

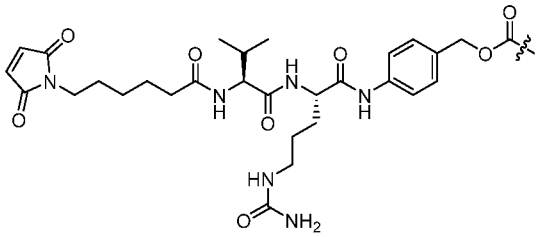
[00153] In some embodiments, the hydrophilic linker is CL2E. In some embodiments, the CL2E has the following structure:



CL2E has been described in U.S. Patent Nos. 8,080,250; 8,759,496; and 10,195,288.

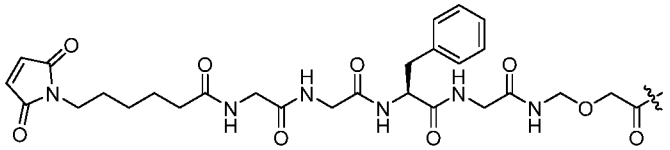
[00154] In some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (e.g., within a lysosome or endosome or caveolea). The linker can be, e.g., a peptide linker that is cleaved by an intracellular or extracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptide linker comprises at least two, at least three, at least four, or at least five amino acids long.

[00155] In some embodiments, the peptide linker is VC-PAB, comprising valine and citruline residues. In some such embodiments, the peptide linker is MC-VC-PAB. In some embodiments, the MC-VC-PAB linker has the following structure:



MC-VC-PAB has been described in U.S. Patent Nos. 7,659,241; 7,829,531; 6,884,869; 6,214,345; and 6,214,345.

[00156] In some embodiments, the peptide linker is maleimidocaproyl glycine-glycine-phenylalanine-glycine (MC-GGFG). In some embodiments, the MC-GGFG linker has the following structure:



MC-GGFG has been described in U.S. Patent Nos. 9,808,537 and 10,195,288.

[00157] In other embodiments, the cleavable linker is pH-sensitive, i.e., sensitive to hydrolysis at certain pH values. In some embodiments, the pH-sensitive linker is

hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (e.g., a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used (see, e.g., US Patent Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville et al, 1989, Biol. Chem. 264: 14653-14661). Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, e.g., a thioether attached to the therapeutic agent via an acylhydrazone bond (see, e.g., US Patent No. 5,622,929).

[00158] In other embodiments, the linker is cleavable under reducing conditions (e.g., a disulfide linker). Bifunctional crosslinking agents that enable the linkage of an antibody with cytotoxic compounds via disulfide bonds include, but are not limited to, N-succinimidyl-4-(4-nitropyridyl-2-dithio)butanoate, N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl-4-(2-pyridyldithio)butanoate (SPDB), N-succinimidyl-4-(2-pyridyldithio)-2-sulfo butanoate (sulfo-SPDB). Sulfo-SPDB is described, e.g., in US Patent 8,236,319, incorporated herein by reference. Alternatively, crosslinking agents that introduce thiol groups such as 2-iminothiolane, homocysteine thiolactone, or S-acetylsuccinic anhydride can be used. In other embodiments, the linker may contain a combination of one or more of the peptide, pH-sensitive, or disulfide linkers described previously.

[00159] "Heterobifunctional crosslinking agents" are bifunctional crosslinking agents having two different reactive groups. Heterobifunctional crosslinking agents containing both an amine-reactive N-hydroxysuccinimide group (NHS group) and a carbonyl-reactive hydrazine group can also be used to link cytotoxic compounds with an antibody. Examples of such commercially available heterobifunctional crosslinking agents include succinimidyl 6-hydrazinonicotinamide acetone hydrazone (SANH), succinimidyl 4-hydrazidoterephthalate hydrochloride (SHTH) and succinimidyl hydrazinium nicotinate hydrochloride (SHNH). Conjugates bearing an acid-labile linkage can also be prepared using a hydrazine-bearing benzodiazepine derivative of the present invention. Examples of bifunctional crosslinking agents that can be used include succinimidyl-p-formyl benzoate (SFB) and succinimidyl-p-formylphenoxyacetate (SFPA).

[00160] The linkers described herein may be used in any combination with the heterologous moiety described herein. In addition, the linkers described herein can have any chemical reactive moieties (e.g., maleimide, cysteine, etc.) that can react with any part (e.g., an amino acid, disulfide bond, carbohydrate (e.g., those from the post-translational modification), etc.) of the antigen-binding protein of the present disclosure. Often, lysines or cysteines (e.g., cysteines from the reduced disulfide bonds (e.g., from interchain or intrachain disulfide bonds of the antibody or antigen-binding protein) or an engineered unpaired cysteine) on an antibody or an antigen-binding protein have been used as a site for conjuation. All of the above-listed linkers and heterologous moiety described herein are available commercially and/or can be prepared by conventional techniques including those described in the above-listed references.

[00161] *Conjugation*

[00162] The heterologous moiety-to-antigen-binding protein ratio (HAR) represents the number of a heterologous moiety linked per antigen-binding molecule. In some embodiments, the HAR ranges from 1 to 15, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2. In some embodiments, the HAR ranges from 2 to 10, 2 to 9, 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4 or 2 to 3. In other embodiments, the HAR is about 2, about 2.5, about 3, about 4, about 5, or about 6. In some embodiments, the HAR ranges from about 2 to about 4. The HAR may be characterized by conventional means such as mass spectrometry, UV/Vis spectroscopy, ELISA assay, and/or HPLC.

[00163] In some embodiments, the conjugates are heterogeneous conjugates (also referred to as "conventional"), wherein the antigen-binding proteins are conjugated to a different number of the heterologous moiety. In some embodiments, the heterogeneous conjugates follow a Gaussian distribution or quasi-Gaussian distribution of the conjugates, wherein the distribution centers on the average heterologous moiety loading value with some antigen-binding proteins conjugated with higher than average and some antigen-binding proteins conjugated with lower than the average.

[00164] In some embodiments, the conjugates are homogeneous conjugates, wherein the substantial percentage of the antigen-binding proteins are conjugated to a defined number of the heterologous moiety. In some embodiments, the homogeneous conjugates comprise the HAR of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the homogeneous conjugates comprise the HAR of 2, 4, 6, or 8. In preferred embodiments, the homogeneous

conjugates comprise the HAR of 4. In other preferred embodiments, the homogeneous conjugates comprise the HAR of 2. In some embodiments, the homogeneous conjugates comprise greater than or equal to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 percent conjugates with the defined HAR. In some embodiments, the homogeneous conjugates comprise about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 percent conjugates with the defined HAR. In some embodiments, the homogeneous conjugates comprise at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 percent conjugates with the defined HAR. In some embodiments, the homogeneous conjugates comprise the HAR distribution that is not Gaussian or quasi-Gaussian distribution. In some embodiments, the homogeneity of the homogeneous conjugates is determined by a chromatogram, *e.g.*, HPLC or any suitable chromatography. In some embodiments, the chromatogram is a HIC chromatogram. The homogeneous conjugate may be generated by a site-specific conjugation.

[00165] In some embodiments, the heterologous moiety is conjugated to the antigen-binding protein (e.g., antibody) in a site-specific manner. Various site-specific conjugation methods are known in the art, e.g., thiomab or TDC or conjugation at an unpaired cysteine residue (Junutula *et al.* (2008) *Nat. Biotechnol.* 26:925-932; Dimasi *et al.* (2017) *Mol. Pharm.* 14:1501-1516; Shen *et al.* (2012) *Nat. Biotechnol.* 30:184-9); thiol bridge linker (Behrens *et al.* (2015) *Mol. Pharm.* 12:3986-98); conjugation at glutamine using a transglutaminase (Dennler *et al.* (2013) *Methods Mol. Bio.* 1045:205-15; Dennler *et al.* (2014) *Bioconjug Chem.* 25:569-78); conjugation at engineered unnatural amino acid residues (Axup *et al.* (2012) *Proc Natl Acad Sci U.S.A.* 104-16101-6; Tian *et al.* (2014) *Proc Natl Acad Sci U.S.A.* 111:1766-71; VanBrunt *et al.* (2015) *Bioconjug Chem* 26:2249-60; Zimmerman *et al.* (2014) *Bioconjug Chem* 25:351-61); selenocysteine conjugation (Li *et al.* (2017) *Cell Chem Biol* 24:433-442); glycan-mediated conjugation (Okeley *et al.* (2013) *Bioconjug Chem* 24:1650-5); conjugation at galactose or GalNAc analogues (Ramakrishnan and Qasba (2002) *J Biol Chem* 277:20833-9; van Geel *et al.* (2015) *Bioconjug Chem* 26:2233-42); via glycan engineering (Zhou *et al.* (2014) *Bioconjug Chem* 25:510-20; Tang *et al.* (2017) *Nat Protoc* 12:1702-1721); via a short peptide tag, such as engineering a glutamine tag or sortase A-mediated transpeptidation (Strop *et al.* (2013)

Chem Biol 20:161-7; Beerli *et al.* (2015) *PLoS One* 10:e0131177); and via an aldehyde tag (Wu *et al.* (2009) *Proc Natl Acad Sci U.S.A.* 106:3000-5).

[00166] *Unpredictability of conjugate (e.g., ADC)*

[00167] It is not possible to predict in advance, simply based on an antibody profile, or a drug payload profile, which antibody-drug conjugates will be sufficiently safe and effective for clinical applications. For example, a particular drug payload may function perfectly well when conjugated to an antibody directed to one target, but it may not work nearly as well when conjugated to an antibody directed to a different target, or even to a different antibody directed to the same target. Why different antibody-drug conjugates display different anti-tumor activity in vivo is not sufficiently well understood to allow accurate predictions in the design of new antibody-drug conjugates. It is speculated that an unpredictable interplay of many factors play a role. These factors may include, for example, the binding affinity of an antibody-drug conjugate to a target antigen, the ability of the conjugate to penetrate solid tumors, as well as the half-life in circulation for proper exposure to tumors without causing toxicity.

[00168] The complexity and unpredictability is well demonstrated by antibody affinity alone. Antibodies or antibody-drug conjugates with high affinity track with better cellular uptake, which leads to a higher level of the cytotoxic payloads released inside the cells. Higher affinity is also known to enhance the antibody-dependent cellular cytotoxicity (ADCC). All these attributes favor the cell killing property of antibody-drug conjugates. However, it is also known that high affinity of an antibody or antibody-drug conjugate can prevent efficient tumor penetration via an "antigen barrier effect," suggesting that in order to achieve a strong anti-tumor activity in vivo, affinity of the antibody-drug conjugate has to be just right: not too high or not too low. To date, it is not known how to predict what will be the most efficient or effective level of affinity for an antibody-drug conjugate.

[00169] In addition, *in vivo* anti-tumor activity cannot be predicted by the mechanism of linkers and payloads alone. For example, O. Ab et al, *Mol. Cancer Ther.* 14(&):1605-1613 (2015) demonstrated that, when tested in pre-clinical cancer models, the same antibody conjugated to the same anti-tubulin toxin via different linkers exhibited dramatically different anti-tumor activity. This example is particularly surprising because the chemical structures of the two linkers are very similar. Moreover, the linker present in the superior conjugate contained a hydrophilic moiety. Hydrophilic metabolites are generally less

membrane-permeable, and are thought to be slower in efflux from the lysosomes (the site of conjugate degradation), leading to a delay in the anti-tubulin activity of the released payload. This finding argues for an "ideal" kinetics of payload delivery, but to date, there is no insight into what constitutes such kinetics. Adding to this complexity is the open question of whether ideal kinetics of payload delivery, even if defined for a particular cell type, would apply to all cell types. Thus, it is not possible to predict the most effective *in vivo* anti-tumor activity merely from the chemical composition of the linker or payload.

[00170] *Compositions, Pharmaceutical Compositions and Formulations*

[00171] Compositions comprising an antigen-binding protein, a nucleic acid, a vector, a host cell, or a conjugate as presently disclosed are provided herein. The compositions in some aspects comprise the antigen-binding proteins in isolated and/or purified form. In some aspects, the composition comprises a single type (e.g., structure) of an antigen-binding protein of the present disclosure or comprises a combination of two or more antigen-binding proteins of the present disclosure, wherein the combination comprises two or more antigen-binding proteins of different types (e.g., structures).

[00172] In some aspects, the composition comprises agents which enhance the chemico-physico features of the antigen-binding protein, e.g., via stabilizing the antigen-binding protein at certain temperatures, e.g., room temperature, increasing shelf life, reducing degradation, e.g., oxidation protease mediated degradation, increasing half-life of the antigen-binding protein, etc. In some aspects, the composition comprises any of the agents disclosed herein as a heterologous moiety or conjugate moiety, optionally in admixture with the antigen-binding proteins of the present disclosure or conjugated to the antigen-binding proteins.

[00173] In various aspects of the present disclosure, the composition additionally comprises a pharmaceutically acceptable carrier, diluents, or excipient. In some embodiments, the antigen-binding protein, a nucleic acid, a vector, a host cell, or a conjugate as presently disclosed (hereinafter referred to as "active agents") is formulated into a pharmaceutical composition comprising the active agent, along with a pharmaceutically acceptable carrier, diluent, or excipient. In this regard, the present disclosure further provides pharmaceutical compositions comprising an active agent which is intended for administration to a subject, e.g., a mammal.

[00174] In some embodiments, the active agent is present in the pharmaceutical composition at a purity level suitable for administration to a patient. In some embodiments, the active agent has a purity level of at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99%, and a pharmaceutically acceptable diluent, carrier or excipient. In some embodiments, the compositions contain an active agent at a concentration of about 0.001 to about 30.0 mg/ml.

[00175] In various aspects, the pharmaceutical compositions comprise a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

[00176] The pharmaceutical composition can comprise any pharmaceutically acceptable ingredients, including, for example, acidifying agents, additives, adsorbents, aerosol propellants, air displacement agents, alkalizing agents, anticaking agents, anticoagulants, antimicrobial preservatives, antioxidants, antiseptics, bases, binders, buffering agents, chelating agents, coating agents, coloring agents, desiccants, detergents, diluents, disinfectants, disintegrants, dispersing agents, dissolution enhancing agents, dyes, emollients, emulsifying agents, emulsion stabilizers, fillers, film forming agents, flavor enhancers, flavoring agents, flow enhancers, gelling agents, granulating agents, humectants, lubricants, mucoadhesives, ointment bases, ointments, oleaginous vehicles, organic bases, pastille bases, pigments, plasticizers, polishing agents, preservatives, sequestering agents, skin penetrants, solubilizing agents, solvents, stabilizing agents, suppository bases, surface active agents, surfactants, suspending agents, sweetening agents, therapeutic agents, thickening agents, tonicity agents, toxicity agents, viscosity-increasing agents, water-absorbing agents, water-miscible cosolvents, water softeners, or wetting agents. *See, e.g.*, the *Handbook of Pharmaceutical Excipients*, Third Edition, A. H. Kibbe (Pharmaceutical Press, London, UK, 2000), which is incorporated by reference in its entirety. *Remington's Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), which is incorporated by reference in its entirety.

[00177] In various aspects, the pharmaceutical composition comprises formulation materials that are nontoxic to recipients at the dosages and concentrations employed. In specific embodiments, pharmaceutical compositions comprising an active agent and one or more pharmaceutically acceptable salts; polyols; surfactants; osmotic balancing agents; tonicity agents; anti-oxidants; antibiotics; antimycotics; bulking agents; lyoprotectants; anti-foaming agents; chelating agents; preservatives; colorants; analgesics; or additional pharmaceutical agents. In various aspects, the pharmaceutical composition comprises one or more polyols and/or one or more surfactants, optionally, in addition to one or more excipients, including but not limited to, pharmaceutically acceptable salts; osmotic balancing agents (tonicity agents); anti-oxidants; antibiotics; antimycotics; bulking agents; lyoprotectants; anti-foaming agents; chelating agents; preservatives; colorants; and analgesics.

[00178] In certain embodiments, the pharmaceutical composition can 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 dextrins); 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, 18″ Edition, (A. R. Genrmo, ed.), 1990, Mack Publishing Company.

[00179] The pharmaceutical compositions can be formulated to achieve a physiologically compatible pH. In some embodiments, the pH of the pharmaceutical composition can be for example between about 4 or about 5 and about 8.0 or about 4.5 and about 7.5 or about 5.0 to about 7.5. In various embodiments, the pH of the pharmaceutical composition is between 5.5 and 7.5.

[00180] The present disclosure provides methods of producing a pharmaceutical composition. In various aspects, the method comprises combining the antigen-binding protein, conjugate, fusion protein, nucleic acid, vector, host cell, or a combination thereof, with a pharmaceutically acceptable carrier, diluent, or excipient.

[00181] *Routes of Administration*

[00182] With regard to the present disclosure, the active agent, or pharmaceutical composition comprising the same, can be administered to the subject via any suitable route of administration. For example, the active agent can be administered to a subject via parenteral, nasal, oral, pulmonary, topical, vaginal, or rectal administration. The following discussion on routes of administration is merely provided to illustrate various embodiments and should not be construed as limiting the scope in any way.

[00183] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The term, "parenteral" means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous. The active agent of the present disclosure can be administered with a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol or hexadecyl alcohol, a glycol, such as propylene glycol or polyethylene glycol, dimethylsulfoxide,

glycerol, ketals such as 2,2- dimethyl-l53-dioxolane-4-methanol, ethers, poly(ethyleneglycol) 400, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

[00184] Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[00185] Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-β-aminopropionates, and 2-alkyl -imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[00186] The parenteral formulations in some embodiments contain from about 0.5% to about 25% by weight of the active agent of the present disclosure in solution. Preservatives and buffers can be used. In order to minimize or eliminate irritation at the site of injection, such compositions can contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations in some aspects are presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections,

immediately prior to use. Extemporaneous injection solutions and suspensions in some aspects are prepared from sterile powders, granules, and tablets of the kind previously described.

[00187] Injectable formulations are in accordance with the present disclosure. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)).

[00188] *Dosages*

[00189] The active agents of the disclosure are believed to be useful in methods of inhibiting tumor growth, as well as other methods, as further described herein, including methods of treating or preventing cancer. For purposes of the disclosure, the amount or dose of the active agent administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject or animal over a reasonable time frame. For example, the dose of the active agent of the present disclosure should be sufficient to treat cancer as described herein in a period of from about 1 to 4 minutes, 1 to 4 hours or 1 to 4 weeks or longer, e.g., 5 to 20 or more weeks, from the time of administration. In certain embodiments, the time period could be even longer. The dose will be determined by the efficacy of the particular active agent and the condition of the animal (e.g., human), as well as the body weight of the animal (e.g., human) to be treated.

[00190] Many assays for determining an administered dose are known in the art. For purposes herein, an assay, which comprises comparing the extent to which cancer is treated upon administration of a given dose of the active agent of the present disclosure to a mammal among a set of mammals, each set of which is given a different dose of the active agent, could be used to determine a starting dose to be administered to a mammal. The extent to which cancer is treated upon administration of a certain dose can be represented by, for example, the extent of tumor regression achieved with the active agent in a mouse xenograft model. Methods of assaying tumor regression are known in the art.

[00191] The dose of the active agent of the present disclosure also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular active agent of the present disclosure. Typically, the

attending physician will decide the dosage of the active agent of the present disclosure with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, active agent of the present disclosure to be administered, route of administration, and the severity of the condition being treated. By way of example and not intending to limit the present disclosure, the dose of the active agent of the present disclosure can be about 0.0001 to about 1 g/kg body weight of the subject being treated/day, from about 0.0001 to about 0.001 g/kg body weight/day, or about 0.01 mg to about 1 g/kg body weight/day.

[00192] *Controlled Release Formulations*

[00193] In some embodiments, the active agents described herein can be modified into a depot form, such that the manner in which the active agent of the present disclosure is released into the body to which it is administered is controlled with respect to time and location within the body (see, for example, U.S. Patent No. 4,450,150). Depot forms of active agents of the present disclosure can be, for example, an implantable composition comprising the active agents and a porous or non-porous material, such as a polymer, wherein the active agent is encapsulated by or diffused throughout the material and/or degradation of the non-porous material. The depot is then implanted into the desired location within the body of the subject and the active agent is released from the implant at a predetermined rate.

[00194] The pharmaceutical composition comprising the active agent in certain aspects is modified to have any type of *in vivo* release profile. In some aspects, the pharmaceutical composition is an immediate release, controlled release, sustained release, extended release, delayed release, or bi-phasic release formulation. Methods of formulating peptides for controlled release are known in the art. See, for example, Qian et al., *J Pharm* 374: 46-52 (2009) and International Patent Application Publication Nos. WO 2008/130158, WO2004/033036; WO2000/032218; and WO 1999/040942.

[00195] The instant compositions can further comprise, for example, micelles or liposomes, or some other encapsulated form, or can be administered in an extended release form to provide a prolonged storage and/or delivery effect.

[00196] *Use*

[00197] The antigen-binding proteins of the present disclosure are useful for inhibiting tumor growth. Without being bound to a particular theory, the inhibiting action of the

antigen-binding proteins provided herein allow such entities to be useful in methods of treating cancer.

[00198] Accordingly, provided herein are methods of inhibiting tumor growth in a subject and methods of reducing tumor size in a subject. In various embodiments, the methods comprise administering to the subject the pharmaceutical composition of the present disclosure in an amount effective for inhibiting tumor growth or reducing tumor size in the subject. In various aspects, the growth of an ovarian tumor, melanoma tumor, bladder tumor, or endometrial tumor is inhibited. In various aspects, the size of an ovarian tumor, melanoma tumor, bladder tumor, or endometrial tumor is reduced.

[00199] As used herein, the term "inhibit" or "reduce" and words stemming therefrom may not be a 100% or complete inhibition or reduction. Rather, there are varying degrees of inhibition or reduction of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the antigen-binding proteins of the present disclosure may inhibit tumor growth or reduce tumor size to any amount or level. In various embodiments, the inhibition provided by the methods of the present disclosure is at least or about a 10% inhibition (e.g., at least or about a 20% inhibition, at least or about a 30% inhibition, at least or about a 40% inhibition, at least or about a 50% inhibition, at least or about a 60% inhibition, at least or about a 70% inhibition, at least or about a 80% inhibition, at least or about a 90% inhibition, at least or about a 95% inhibition, at least or about a 98% inhibition). In various embodiments, the reduction provided by the methods of the present disclosure is at least or about a 10% reduction (e.g., at least or about a 20% reduction, at least or about a 30% reduction, at least or about a 40% reduction, at least or about a 50% reduction, at least or about a 60% reduction, at least or about a 70% reduction, at least or about a 80% reduction, at least or about a 90% reduction, at least or about a 95% reduction, at least or about a 98% reduction).

[00200] Additionally provided herein are methods of treating a subject with cancer, e.g., DLK1-expressing cancer. In various embodiments, the method comprises administering to the subject the pharmaceutical composition of the present disclosure in an amount effective for treating the cancer in the subject.

[00201] For purposes herein, the cancer of the methods disclosed herein can be any cancer, e.g., any malignant growth or tumor caused by abnormal and uncontrolled cell division that may spread to other parts of the body through the lymphatic system or the

blood stream. The cancer in some aspects is one selected from the group consisting of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, gastrointestinal carcinoid tumor, Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer (e.g., renal cell carcinoma (RCC)), small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, ureter cancer, and urinary bladder cancer. In particular aspects, the cancer is selected from the group consisting of: head and neck, ovarian, cervical, bladder and oesophageal cancers, pancreatic, gastrointestinal cancer, gastric, breast, endometrial and colorectal cancers, hepatocellular carcinoma, glioblastoma, bladder, lung cancer, e.g., non-small cell lung cancer (NSCLC), bronchioloalveolar carcinoma. In various aspects, the cancer is pancreatic cancer, gastrointestinal cancer, bladder cancer, colon cancer, lung cancer, liver cancer, endometrial cancer. In various aspects, the cancer is any cancer characterized by moderate to high expression of DLK1. In various aspects, the cancer is acute myeloid leukemia, large B-cell lymphoma, stomach cancer, prostate cancer, melanoma, colon cancer, rectal cancer, bladder cancer, cervical cancer, liver cancer, breast cancer, kidney clear cell carcinoma, head and neck cancer, sarcoma, kidney chromophobe cancer, lower grade glioma, adrenocortical cancer, glioblastoma, kidney papillary cell carcinoma, lung squamous cell carcinoma, thyroid cancer, lung adenocarcinoma, pancreatic cancer, endometroid cancer, uterine carcinsarcoma, or ovarian cancer. In various aspects, the cancer is selected from pancreatic cancer, gastrointestinal cancer, bladder cancer, colon cancer, lung cancer, liver cancer, ovarian cancer, endometrioid cancer, uterine cancer, lung cancer, gastric cancer, breast cancer Head and Neck Squamous Cell Carcinoma (HNSCC) cancer, and cervical cancer.

[00202] As used herein, the term "treat," as well as words related thereto, do not necessarily imply 100% or complete treatment. Rather, there are varying degrees of

treatment of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the methods of treating cancer of the present disclosure can provide any amount or any level of treatment. Furthermore, the treatment provided by the method of the present disclosure can include treatment of one or more conditions or symptoms or signs of the cancer being treated. Also, the treatment provided by the methods of the present disclosure can encompass slowing the progression of the cancer. For example, the methods can treat cancer by virtue of enhancing the T cell activity or an immune response against the cancer, reducing tumor or cancer growth, reducing metastasis of tumor cells, increasing cell death of tumor or cancer cells, and the like. In various aspects, the methods treat by way of delaying the onset or recurrence of the cancer by at least 1 day, 2 days, 4 days, 6 days, 8 days, 10 days, 15 days, 30 days, two months, 3 months, 4 months, 6 months, 1 year, 2 years, 3 years, 4 years, or more. In various aspects, the methods treat by way increasing the survival of the subject.

[00203] The antigen binding proteins of the present disclosure also may be used to detect DLK1 in a sample or diagnose a DLK1-positive cancer. Therefore, the present disclosure provides methods of detecting DLK1 in a sample. In various embodiments, the method comprises contacting the sample with an antigen-binding protein, a conjugate, or a fusion protein, as described herein, and assaying for an immunocomplex comprising the antigen-binding protein, conjugate or fusion protein bound to DLK1. The present disclosure also provides methods of diagnosing a DLK1 -positive cancer in a subject. In various embodiments, the method comprises contacting a biological sample comprising cells or tissue obtained from the subject with an antigen-binding protein, a conjugate, or a fusion protein, as described herein, and assaying for an immunocomplex comprising the antigen-binding protein, conjugate or fusion protein bound to DLK1.

[00204] *Subjects*

[00205] In some embodiments of the present disclosure, the subject is a mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits, mammals from the order Carnivora, including Felines (cats) and Canines (dogs), mammals from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). In some aspects, the mammals are of the order Primates,

Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). In some aspects, the mammal is a human.

[00206] *Kits*

[00207] In some embodiments, the antigen-binding proteins of the present disclosure are provided in a kit. In various aspects, the kit comprises the antigen-binding protein(s) as a unit dose. For purposes herein "unit dose" refers to a discrete amount dispersed in a suitable carrier. In various aspects, the unit dose is the amount sufficient to provide a subject with a desired effect, e.g., inhibition of tumor growth, reduction of tumor size, treatment of cancer. Accordingly, provided herein are kits comprising an antigen-binding protein of the present disclosure optionally provided in unit doses. In various aspects, the kit comprises several unit doses, e.g., a week or month supply of unit doses, optionally, each of which is individually packaged or otherwise separated from other unit doses. In some embodiments, the components of the kit/unit dose are packaged with instructions for administration to a patient. In some embodiments, the kit comprises one or more devices for administration to a patient, e.g., a needle and syringe, and the like. In some aspects, the antigen-binding protein of the present disclosure, a pharmaceutically acceptable salt thereof, a conjugate comprising the antigen-binding protein, or a multimer or dimer comprising the antigen-binding protein, is pre-packaged in a ready to use form, e.g., a syringe, an intravenous bag, etc. In some aspects, the kit further comprises other therapeutic or diagnostic agents or pharmaceutically acceptable carriers (e.g., solvents, buffers, diluents, etc.), including any of those described herein. In particular aspects, the kit comprises an antigen-binding protein of the present disclosure, along with an agent, e.g., a therapeutic agent, used in chemotherapy or radiation therapy.

[00208] *Various embodiments*

[00209] In various embodiments of the present disclosure, the antigen-binding protein binds to a human DLK1 protein (SEQ ID NO: 19 or 20).

[00210] In some embodiments, the antigen-binding protein of the present disclosure comprises a Fc polypeptide. In some embodiments, the antigen-binding protein of the present disclosure comprises a Fc polypeptide comprising an afucosylated glycan.

[00211] In various aspects, the antigen-binding protein of the present disclosure is an antibody, e.g., a monoclonal antibody. In various instances, the antigen-binding protein is an IgG. In various aspects, the antigen-binding protein inhibits at least about 50% colony

growth in a soft agar 3D proliferation assays or inhibits tumor growth in xenograft mice injected with human cancer cells. In various aspects, the antigen-binding protein inhibits tumor growth of in xenograft mice injected with ovarian cancer cells, melanoma cancer cells, bladder cancer cells, or endometrial cancer cells. In various instances, the antigen-binding protein inhibits at least 50% tumor growth in xenograft mice injected with ovarian cancer cells, bladder cancer cells, or endometrial cancer cells.

[00212] The present disclosure provides a bispecific antigen-binding protein that binds DLK1 and a second antigen, wherein the antigen-binding protein that binds DLK1 is any one of the antigen-binding protein described herein. In some embodiments, the bispecific antigen-binding protein comprises: (a) a heavy chain variable region amino acid sequence set forth in Table 1 and/or Fig. 19-Fig. 30, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (b) a light chain variable region amino acid sequence set forth in Table 1 and/or Fig. 19-Fig. 30, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; or (c) both (a) and (b). In some embodiments, the variant sequence has at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity. In some embodiments, the bispecific antigen-binding protein comprises a Fc polypeptide. In some embodiments, the bispecific antigen-binding protein comprises a Fc polypeptide comprising an afucosylated glycan.

[00213] In various aspects, a bispecific antigen-binding protein binds DLK1 and a second antigen. In some embodiments, a bispecific antigen-binding protein comprises an antigen-binding fragment of an antibody specific for the second antigen. In various embodiments, the second antigen is a cell surface protein expressed by a T cell, optionally a component of the T-cell receptor (TCR), for example CD3. In some embodiments, the second antigen is CD3. In some embodiments, the second antigen is CD3E.

[00214] In various embodiments, the second antigen is a costimulatory molecule which assists in T-cell activation, e.g., CD40 or 4-1BB (CD137). In various embodiments, the second antigen is an Fc receptor, optionally, a Fc gamma receptor, Fc-alpha receptor, or Fc-epsilon receptor. In some embodiments, the Fc receptor is CD64 (Fc-gamma RI), CD32 (Fc-gamma RIIA), CD16A (Fc-gamma RIIIA), CD16b (Fc-gamma RIIIb), FcεRI, CD23

(Fc-epsilon RII), CD89 (Fc-epsilon RI), Fcα/μR, or FcRn. In some embodiments, the Fc receptor is CD16A.

[00215] In various embodiments, the second antigen is an immune checkpoint molecule, e.g., a protein involved in the immune checkpoint pathway, optionally, A2AR, B7-H3, B7-H4, BTLA, CTLA4, IDO, KIR, LAG3, NOX2, PD-1, TIM3, VISTA, or SIGLEC7. In some embodiments, the immune checkpoint molecule is PD-1, LAG3, TIM3, or CTLA4. In various embodiments, the bispecific antigen-binding protein comprises an scFv, a Fab, or a F(ab)2' of any of the presently disclosed DLK1 antibodies.

[00216] In various embodiments, the bispecific antigen-binding protein comprises an antigen-binding protein comprising a sequence set forth in Table 1 and/or Fig. 19-Fig. 30, or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity. In some embodiments, the variant sequence has at least or about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%sequence identity. In various embodiments, the bispecific antigen-binding protein comprises a structure of a nanobody, a diabody, a BiTE®, DART, TandAb, CrossMab, or HSAbody. The present disclosure provides a conjugate comprising an antigen-binding protein or a bispecific antigen-binding protein described herein and a heterologous moiety. In some embodiments, the antigen-binding protein comprises the amino acid sequence set forth in Table 1 and/or Fig. 19-Fig. 30. In some embodiments, the conjugate comprises a cytotoxic agent or a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is an anti-mitotic agent which inhibits cell division by blocking tubulin polymerization. In some embodiments, the anti-mitotic agent is an auristatin. In some embodiments, the auristatin is MMAE.

[00217] In various embodiments, the conjugate of the present disclosure is conjugated to the antigen-binding protein via a cleavable linker. In some embodiments, the cleavable linker is VC-PAB.

[00218] In some embodiments, the conjugate comprises an antigen-binding protein that is an antibodythe antibody is a monoclonal antibody, optionally wherein the monoclonal antibody is an IgG antibody. In some embodiments, the antibody is a human antibody, humanized antibody, or a chimeric antibody.

[00219] In various embodiments, the conjugate of the present disclosure has an average number of units of the agent conjugated per antigen-binding protein in a range of 1 to 8, preferably wherein the average number of units of the agent conjugated per antigen-binding protein is in a range of 3-8. In some embodiments, the conjugate is a heterogeneous conjugate. In other embodiments, the conjugate is a homogeneous conjugate. In some embodiments, the conjugate comprises a heterologous moiety or an agent, wherein the agent is conjugated at a specific site of the antigen-binding protein. In some embodiments, the specific site is an unpaired cysteine residue.

[00220] The present disclosure also provides a fusion protein comprising an antigen-binding protein or a bispecific antigen-binding protein described herein. The present disclosure further provides a nucleic acid comprising a nucleotide sequence encoding an antigen-binding protein, a bispecific antigen-binding protein, a conjugate, or a fusion protein, of the present disclosure. The present disclosure provides a vector comprising the nucleic acid comprising a nucleotide sequence encoding an antigen binding protein, a conjugate, or a fusion protein, of the present disclosure. The present disclosure additionally provides a host cell comprising the nucleic acid or the vector of the present disclosure.

[00221] The present disclosure provides a method of producing an antigen-binding protein or a bispecific antigen-binding protein that binds to a DLK1 protein, comprising (i) culturing the host cell of the present disclosure in a cell culture medium, wherein the host cell comprises a nucleic acid comprising a nucleotide sequence encoding an antigen binding protein or a bispecific antigen-binding protein described herein, and (ii) harvesting the antigen-binding protein or a bispecific antigen-binding protein from the cell culture medium. Also, provided is a method of producing a fusion protein comprising an antigen-binding protein or a bispecific antigen-binding protein that binds to a DLK1 protein, comprising (i) culturing the host cell of the present disclosure in a cell culture medium, wherein the host cell comprises a nucleic acid comprising a nucleotide sequence encoding a fusion protein of the present disclosure, and (ii) harvesting the fusion protein from the cell culture medium.

[00222] The present disclosure furthermore provides a method of producing a pharmaceutical composition comprising combining an antigen-binding protein, a bispecific antigen-binding protein, a conjugate, a fusion protein, a nucleic acid, a vector, a host cell, of the present disclosure, or a combination thereof, and a pharmaceutically acceptable carrier,

diluent or excipient. Also provided are pharmaceutical compositions comprising antigen-binding protein, a bispecific antigen-binding protein, a conjugate, a fusion protein, a nucleic acid, a vector, a host cell, of the present disclosure, or a combination thereof, and a pharmaceutically acceptable carrier, diluent or excipient.

[00223] Provided herein is a method of treating a subject with a DLK1-expressing cancer comprising administering to the subject a pharmaceutical composition described herein in an amount effective to treat the cancer. Also provided is a method of inhibiting tumor growth in a subject, comprising administering to the subject a pharmaceutical composition described herein in an amount effective to inhibit tumor growth. The present disclosure provides a method of reducing tumor size in a subject, comprising administering to the subject a pharmaceutical composition described herein in an amount effective to reduce tumor size. Further provided is a method of preventing the recurrence of cancer in a subject, comprising administering to the subject a pharmaceutical composition described herein in an amount effective to prevent the recurrence of cancer.

[00224] The present disclosure provides a method of detecting DLK1 (CLD18.2) in a sample, comprising contacting the sample with an antigen-binding protein, a bispecific antigen-binding protein, a conjugate, or a fusion protein, of the present disclosure, and assaying for an immunocomplex comprising the antigen-binding protein, conjugate or fusion protein bound to CLD18.2. Also provided herein is a method of diagnosing a DLK1-positive cancer in a subject, comprising contacting a biological sample comprising cells or tissue obtained from the subject with an antigen-binding protein, a bispecific antigen-binding protein, a conjugate, or a fusion protein, of the present disclosure, and assaying for an immunocomplex comprising the antigen-binding protein, conjugate or fusion protein bound to DLK1.

[00225] The present disclosure also provides a method of treating cancer in a subject diagnosed to be a low over-expresser of DLK1. In various embodiments, the method comprises administering to the subject a presently disclosed pharmaceutical composition in an amount effective to prevent the recurrence of cancer. In some aspects, the administering induces apoptosis in tumor cells, optionally, the administering induces apoptosis in cells expressing DLK1. In various aspects, the subject has a tumor and the tumor is semi-quantitatively categorized into one of four groups: high expressers, moderate expressers, low expressers, and non-expressers.

[00226] *Exemplary Embodiments*

1. An antigen-binding protein comprising:
    a. CDRs 1-3 derived from a heavy chain variable region comprising the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAASGFSISDYYMAWVRQAPG KGLEWVANINYDGTNTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCVRSYYYYGMEYWGQGTTVTVSS (SEQ ID NO: 45) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity; and/or
    b. CDRs 1-3 derived from a light chain variable region comprising the amino acid sequence: DIQMTQSPSSLSASVGDRVTITCRASHDVSTAVAWYQQKPGK APKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQHYRIPLTFGQGTKLEIK (SEQ ID NO: 46) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity.
2. An antigen-binding protein comprising:
    a. CDRs 1-3 derived from a heavy chain variable region comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVSGFSLSIYSVHWVRQPPGK GLEWIGLIWGGGSTDYNPSLKSRVTISKDTSKNQVSLKLSSVT AADTAVYYCARKEGNYLWFAYWGQGTLVTVSS (SEQ ID NO: 47) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity; and/or
    b. CDRs 1-3 derived from a light chain variable region comprising the amino acid sequence: DIVMTQSPDSLAVSLGERVTMNCKSSQSLLQSSNQKNYLAWY QQKPGQPPKLLVYFASTRESGVPDRFSGSGSGTDFTLTISSVQA EDVAVYYCQQHYSIPLTFGQGTKLEIK (SEQ ID NO: 48) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity.

3. An antigen-binding protein comprising:
    a. CDRs 1-3 derived from a heavy chain variable region comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVSWVRQPPGK GLEWIGVIWGDGSTSYNPSLKSRVTISKDTSKNQVSLKLSSVT AADTAVYYCAKPDGPLGQGTLVTVSS (SEQ ID NO: 49) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity; and/or
    b. CDRs 1-3 derived from a light chain variable region comprising the amino acid sequence: DIVMTQSPLSLPVTPGEPASISCRSSQSLVHINGNTYLHWYLQK PGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYCSQTTHVPWTFGQGTKLEIK (SEQ ID NO: 50) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity.
4. An antigen-binding protein comprising:
    a. a heavy chain CDR1 comprising the amino acid sequence of: GFSISDYY (SEQ ID NO: 1) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
    b. a heavy chain CDR2 comprising the amino acid sequence of:: INYDGTNT (SEQ ID NO: 2) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
    c. a heavy chain CDR3 comprising the amino acid sequence of: VRSYYYYGMEY (SEQ ID NO: 3) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
    d. a light chain CDR1 comprising the amino acid sequence of: HDVSTA (SEQ ID NO: 4) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

e. a light chain CDR2 comprising the amino acid sequence of: SAS (SEQ ID NO: 5) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

f. a light chain CDR3 comprising the amino acid sequence of: QQHYRIPLT (SEQ ID NO: 6) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; or

g. a combination of any two or moreof (a)-(f).

5. An antigen-binding protein comprising:

a. a heavy chain CDR1 comprising the amino acid sequence of: GFSLSIYS (SEQ ID NO: 7) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

b. a heavy chain CDR2 amino acid sequence of: IWGGGST (SEQ ID NO: 8) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

c. a heavy chain CDR3 comprising the amino acid sequence of: ARKEGNYLWFAY (SEQ ID NO: 9) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

d. a light chain CDR1 comprising the amino acid sequence of: QSLLQSSNQKNY (SEQ ID NO: 10) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

e. a light chain CDR2 comprising the amino acid sequence of: FAS (SEQ ID NO: 11) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

f. a light chain CDR3 amino acid sequence of: QQHYSIPLT (SEQ ID NO: 12) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence

identity; or

g. a combination of any two or moreof (a)-(f).

6. An antigen-binding protein comprising:
   a. a heavy chain CDR1 amino acid sequence of: GFSLTSYG (SEQ ID NO: 13) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
   b. a heavy chain CDR2 comprising the amino acid sequence of: IWGDGST (SEQ ID NO: 14) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
   c. a heavy chain CDR3 comprising the amino acid sequence of: AKPDGP (SEQ ID NO: 15) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
   d. a light chain CDR1 comprising the amino acid sequence of: QSLVHINGNTY (SEQ ID NO: 16) a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
   e. a light chain CDR2 amino acid sequence of: KVS (SEQ ID NO: 17) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
   f. a light chain CDR3 comprising the amino acid sequence of: SQTTHVPWT (SEQ ID NO: 18) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; or
   g. a combination of any two or moreof (a)-(f).

7. The antigen-binding protein of any one of 1-7, wherein the variant sequence has at least about 80%, at least about 85%, at least about 90% sequence identity, or at least or about 95% sequence identity.

8. The antigen-binding protein of 4, additionally comprising:
   a. a heavy chain FR1 comprising the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO: 21) or a

variant sequence thereof which differs by only one or two amino acids or which has at least or about 70%, 80%, 85%, 90% or 95% sequence identity;

b. a heavy chain FR2 comprising the amino acid sequence: MAWVRQAPGKGLEWVAN (SEQ ID NO: 22) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70%, 80%, 85%, 90% or 95% sequence identity;

c. a heavy chain FR3 comprising the amino acid sequence: YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC (SEQ ID NO: 23) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

d. a heavy chain FR4 comprising the amino acid sequence: WGQGTTVTVSS (SEQ ID NO: 24) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

e. a light chain FR1 comprising the amino acid sequence: DIQMTQSPSSLSASVGDRVTITCRAS (SEQ ID NO: 25) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

f. a light chain FR2 comprising the amino acid sequence: VAWYQQKPGKAPKLLIY (SEQ ID NO: 26) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

g. a light chain FR3 comprising the amino acid sequence: YRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO: 27) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

h. a light chain FR4 comprising the amino acid sequence:

FGQGTKLEIK (SEQ ID NO: 28) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 785%, 90% or 95% sequence identity; or

i. a combination of any two or moreof (a)-(h).

9. The antigen-binding protein of 5, additionally comprising:

a. a heavy chain FR1 comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVS (SEQ ID NO: 29) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

b. a heavy chain FR2 comprising the amino acid sequence: VHWVRQPPGKGLEWIGL (SEQ ID NO: 30) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

c. a heavy chain FR3 comprising the amino acid sequence: DYNPSLKSRVTISKDTSKNQVSLKLSSVTAADTAVYYC (SEQ ID NO: 31) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

d. a heavy chain FR4 comprising the amino acid sequence: WGQGTLVTVSS (SEQ ID NO: 32) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

e. a light chain FR1 comprising the amino acid sequence: DIVMTQSPDSLAVSLGERVTMNCKSS (SEQ ID NO: 33) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

f. a light chain FR2 comprising the amino acid sequence: LAWYQQKPGQPPKLLVY (SEQ ID NO: 34) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

g. a light chain FR3 comprising the amino acid sequence:

TRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC (SEQ ID NO: 35) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

h. a light chain FR4 comprising the amino acid sequence of: FGQGTKLEIK (SEQ ID NO: 36) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity; or

i. a combination of any two or moreof (a)-(h).

10. The antigen-binding protein of 6, additionally comprising:

a. a heavy chain FR1 comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVS (SEQ ID NO: 37) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

b. a heavy chain FR2 comprising the amino acid sequence of: VSWVRQPPGKGLEWIGV (SEQ ID NO: 38) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

c. a heavy chain FR3 comprising the amino acid sequence: SYNPSLKSRVTISKDTSKNQVSLKLSSVTAADTAVYYC (SEQ ID NO: 39) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

d. a heavy chain FR4 comprising the amino acid sequence: LGQGTLVTVSS (SEQ ID NO: 40) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

e. a light chain FR1 comprising the amino acid sequence: DIVMTQSPLSLPVTPGEPASISCRSS (SEQ ID NO: 41) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence

identity;

f. a light chain FR2 comprising the amino acid sequence: LHWYLQKPGQSPQLLIY (SEQ ID NO: 42) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

g. a light chain FR3 comprising the amino acid sequence: NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC (SEQ ID NO: 43) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

h. a light chain FR4 amino acid sequence of: FGQGTKLEIK (SEQ ID NO: 44) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity; or

i. a combination of any two or moreof (a)-(h).

11. The antigen-binding protein of any one of 1, 4, 7, and 8 comprising:

a. a heavy chain variable region comprising the amino acid sequence of: EVQLVESGGGLVQPGGSLRLSCAASGFSISDYYMAWVRQAPG KGLEWVANINYDGTNTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCVRSYYYYGMEYWGQGTTVTVSS (SEQ ID NO: 45) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity; and/or

b. a light chain variable region comprising the amino acid sequence: DIQMTQSPSSLSASVGDRVTITCRASHDVSTAVAWYQQKPGK APKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQHYRIPLTFGQGTKLEIK (SEQ ID NO: 46) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity.

12. The antigen-binding protein of any one of 2, 5, 7, and 9 comprising:

a. a heavy chain variable region comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVSGFSLSIYSVHWVRQPPGK

GLEWIGLIWGGGSTDYNPSLKSRVTISKDTSKNQVSLKLSSVT AADTAVYYCARKEGNYLWFAYWGQGTLVTVSS (SEQ ID NO: 47) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity; and/or

b. a light chain variable region comprising the amino acid sequence: DIVMTQSPDSLAVSLGERVTMNCKSSQSLLQSSNQKNYLAWY QQKPGQPPKLLVYFASTRESGVPDRFSGSGSGTDFTLTISSVQA EDVAVYYCQQHYSIPLTFGQGTKLEIK (SEQ ID NO: 48) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity.

13. An antigen-binding protein of any one of 3, 6, 7, and 10 comprising:

a. a heavy chain variable region comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVSWVRQPPGK GLEWIGVIWGDGSTSYNPSLKSRVTISKDTSKNQVSLKLSSVT AADTAVYYCAKPDGPLGQGTLVTVSS (SEQ ID NO: 49) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity; and/or

b. a light chain variable region comprising the amino acid sequence: DIVMTQSPLSLPVTPGEPASISCRSSQSLVHINGNTYLHWYLQK PGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYCSQTTHVPWTFGQGTKLEIK (SEQ ID NO: 50) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity.

14. An antigen-binding protein that specifically binds to human Delta Like Non-Canonical Notch Ligand 1 (DLK1) comprising:

a. an antibody heavy chain comprising the amino acid sequence of: QVQLQESGPGLVKPSETLSLTCTVSGFSLSIYSVHWVRQPPGKGL EWIGLIWGGGSTDYNPSLKSRVTISKDTSKNQVSLKLSSVTAADT AVYYCARKEGNYLWFAYWGQGTLVTVSSASTKGPSVFPLAPSS

KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 51); and

b. an antibody light chain comprising the amino acid sequence of:
DIVMTQSPDSLAVSLGERVTMNCKSSQSLLQSSNQKNYLAWYQ
QKPGQPPKLLVYFASTRESGVPDRFSGSGSGTDFTLTISSVQAED
VAVYYCQQHYSIPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO: 52).

15. The antigen-binding protein of any one of the previous embodiments, wherein:
    a. the antigen-binding protein binds to a human DLK1 protein isoform-1 (UniProtKB ID: P80370-1) having an amino acid sequence according to SEQ ID NO: 19;
    b. the antigen-binding protein binds to a human DLK1 protein isoform-2 (UniProtKB ID: P80370-2) having an amino acid sequence according to SEQ ID NO: 69;
    c. the antigen-binding protein binds an extracellular domain of human DLK1 with a dissociation constant (K_D) of about less than 10 nM, 5 nM, 2.5 nM, 1 nM, 0.5 nM, 0.25 nM, 100 pM, 50 pM, 25 pM, 10 pM or 5 pM;
    d. the antigen-binding protein does not bind the mouse DLK1 protein (UniProtKB ID: Q09163) having an amino acid sequence according to SEQ ID NO: 20, or the antigen-binding protein binds the mouse DLK1 protein with at least 100-fold lower affinity than the human DLK1 protein; or
    e. a combination thereof.

16. The antigen-binding protein of any one of the previous embodiments, wherein the antigen-binding protein binds human DLK1 isoform 1 (UniProt ID: P80370-1) and isoform 2 (UniProt ID: P80370-2).
17. The antigen-binding protein of 16, wherein the antigen-binding protein binds human DLK1 isoform 1 (UniProt ID: P80370-1) and isoform 2 (UniProt ID: P80370-2) with a KD:
    a. less than about 6 nM,
    b. about 5 nM,
    c. about 2 nM, or
    d. about 1 nM.
18. The antigen-binding protein of any one of the previous embodiments, wherein the antigen-binding protein binds:
    a. an epitope within the EGF-like 2 and EGF-like 3 domains of the human DLK1 isoform 1 protein (UniProt ID: P80370-1); and/or
    b. a polypeptide comprising the amino acid residues 55-113 of the human DLK1 isoform 1 protein (UniProt ID: P80370-1).
19. The antigen-binding protein of any one of previous embodiments, wherein the antigen-binding protein binds the human DLK1 protein and induces (a) an antibody-dependent cell-mediated cytotoxicity (ADCC) response, and/or (b) a complement-dependent cytotoxicity (CDC) response in DLK1-positive cells.
20. The antigen-binding protein of any one of the previous embodiments, which is an antibody or antigen-binding antibody fragment.
21. The antigen-binding protein of 20, wherein the antibody is a monoclonal antibody.
22. The antigen-binding protein of 20, wherein the antibody is a chimeric antibody, a human antibody, or a humanized antibody.
23. The antigen-binding protein of any one of 20-22, wherein the antibody is an IgG.
24. The antigen-binding protein of 23, wherein the IgG is selected from IgG1, IgG2, IgG3 and IgG4.
25. The antigen-binding protein of 23 or 24, wherein the IgG is IgG1.
26. The antigen-binding protein of 20, wherein the antigen-binding antibody fragment

is selected from the group consisting of scFv, $F(ab')_2$, Fab, Fab' and Fv.

27. The antigen-binding protein of 20, wherein the antigen-binding antibody fragment is a single chain variable fragment (scFv).
28. The antigen-binding protein of 27, wherein the scFv comprises:
    a. the variable light (V_L) and variable heavy (V_H) chains of any of the antigen-binding protein of any one of previous embodiments;
    b. the amino acid sequence set forth in SEQ ID NO: 45 and SEQ ID NO: 46;
    c. the amino acid sequence set forth in SEQ ID NO: 47 and SEQ ID NO: 48;
    d. the amino acid sequence set forth in SEQ ID NO: 49 and SEQ ID NO: 50; or
    e. the amino acid sequence set forth in SEQ ID NOs: 57, 58, or 59, or an antigen-binding portion thereof.
29. The antigen-binding protein of 27, wherein the scFv comprises the amino acid sequence set forth in SEQ ID NO: 58 or an antibody-binding portion thereof.
30. The antigen-binding protein of any one of the previous embodiments, wherein the antigen-binding protein is a bispecific antigen-binding protein or a bispecific T cell engager (BiTE).
31. The antigen-binding protein of 30, wherein the bispecific antigen-binding protein or the BiTE comprises the amino acid sequence set forth in
    a. SEQ ID NO: 45 and SEQ ID NO: 46;
    b. SEQ ID NO: 47 and SEQ ID NO: 48; or
    c. SEQ ID NO: 49 and SEQ ID NO: 50.
32. The antigen-binding protein of 30 or 31, wherein the bispecific antigen-binding protein or the BiTE comprises the scFv of 28 or 29.
33. The antigen-binding protein of any one of 30-32, wherein the bispecific antigen-binding protein or the BiTE binds DLK1 and a T cell surface marker.
34. The antigen-binding protein of 33, wherein the T cell surface marker is the CD3 protein.
35. The antigen-binding protein of any one of 30-34, wherein the bispecific antigen-binding protein or the BiTE comprises the amino acid sequence set forth in SEQ ID NOs: 60, 61, or 62 or the antigen-binding portion thereof.
36. The antigen-binding protein of any one of 30-35, wherein the bispecific antigen-

binding protein or the BiTE comprises the amino acid sequence set forth in SEQ ID NO: 60 or the antigen-binding portion thereof.

37. The antigen-binding protein of any one of 30-35, wherein the bispecific antigen-binding protein or the BiTE comprises the amino acid sequence set forth in SEQ ID NO: 61 or the antigen-binding portion thereof.
38. The antigen-binding protein of any one of 30-35, wherein the bispecific antigen-binding protein or the BiTE elicits T cell activation in the presence of a DLK-positive cell.
39. The antigen-binding protein of any one of the previous embodiments, which inhibits tumor growth in a xenograft mouse injected with human cancer cells.
40. The antigen-binding protein of any one of the previous embodiments, comprising a Fc polypeptide comprising an afucosylated glycan.
41. A conjugate comprising an antigen-binding protein of any one of the previous embodiments or those described herein.
42. The conjugate of 41 comprising a detectable marker, a cytotoxic agent, or a chemotherapeutic agent.
43. The conjugate of 42, wherein the chemotherapeutic agent is an anti-mitotic agent which inhibits cell division by blocking tubulin polymerization.
44. The conjugate of 43, wherein the anti-mitotic agent is an auristatin.
45. The conjugate of 44, wherein the auristatin is monomethyl auristatin E or MMAE.
46. The conjugate of any one of 42-45, wherein the agent or the marker is conjugated to the antigen-binding protein via a cleavable linker or a non-cleavable linker.
47. The conjugate of 46, wherein the cleavable linker is VC-PAB.
48. The conjugate of any one of 41-47, wherein the antigen-binding protein is an antibody.
49. The conjugate of 48, wherein the antibody is a monoclonal antibody.
50. The conjugate of 49, wherein the antibody is a human antibody, a humanized antibody, or a chimeric antibody.
51. The conjugate of 50, wherein the antibody is an IgG antibody, optionally wherein the IgG is IgG1, IgG2, IgG3, or IgG4.
52. The conjugate of any one of 41-52, wherein an average number of units of

the agent conjugated per antigen-binding protein is in a range of 1 to 8, preferably wherein the average number of units of the agent conjugated per antigen-binding protein is (a) in a range of 3-8, or (b) 4.

53. The conjugate of any one of 41-52, wherein the conjugate is a heterogeneous conjugate.
54. The conjugate of any one of 41-52, wherein the conjugate is a homogeneous conjugate.
55. The conjugate of any one of 42-54, wherein the agent is conjugated at a specific site of the antigen-binding protein.
56. The conjugate of 55, wherein the specific site is an unpaired cysteine residue.
57. The conjugate of any one of 41-56, wherein the conjugate comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 45 and SEQ ID NO: 46 conjugated to VC-PAB-MMAE.
58. The conjugate of any one of 41-56, wherein the conjugate comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 47 and SEQ ID NO: 48 conjugated to VC-PAB-MMAE.
59. The conjugate of any one of 41-56, wherein the conjugate comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 49 and SEQ ID NO: 50 conjugated to VC-PAB-MMAE.
60. The conjugate of any one of 41-56, wherein the conjugate comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 51 and SEQ ID NO: 52 conjugated to VC-PAB-MMAE.
61. A fusion protein comprising an antigen-binding protein of any one of the previous embodiments.
62. A nucleic acid comprising a nucleotide sequence encoding an antigen binding protein of any one of 1-40, a conjugate of any one of 41-60, or a fusion protein of 61.
63. The nucleic acid of 62, wherein the nucleic acid is a cDNA.
64. A vector (e.g., expression vector) comprising the nucleic acid of 62 or 63.
65. The vector of 64, additionally comprising an internal ribosome entry site (IRES).
66. A host cell comprising the nucleic acid of 62 or 63, or the vector of 64 or 65.
67. The host cell of 66, wherein the host cell is a bacterial cell.

68. The host cell of 66, wherein the host cell is a eukaryotic cell.
69. The host cell of 68, wherein the eukaryotic cell is a mammalian cell.
70. The host cell of 69, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.
71. A method of producing an antigen-binding protein that binds to a Delta Like Non-Canonical Notch Ligand 1 (DLK1) protein, comprising (i) culturing the host cell of any one of 66-70 in a cell culture medium, and (ii) harvesting the antigen-binding protein from the cell culture medium.
72. A method of producing a fusion protein comprising an antigen-binding protein that binds to a Delta Like Non-Canonical Notch Ligand 1 (DLK1) protein, comprising (i) culturing the host cell of any one of 66-70 in a cell culture medium, and (ii) harvesting the fusion protein from the cell culture medium.
73. A method of producing a pharmaceutical composition, the method comprising combining (a) an antigen-binding protein of any one of 1-40, a conjugate of any one of 41-60, a fusion protein of 61, a nucleic acid of 62 or 63, a vector of 64 or 65, a host cell of any one of 66-70, or any combination thereof; and (b) a pharmaceutically acceptable carrier, diluent and/or excipient.
74. A pharmaceutical composition comprising an antigen-binding protein of any one of 1-40, a conjugate of any one of 41-60, a fusion protein of 61, a nucleic acid of 62 or 63, a vector of 64 or 65, a host cell of any one of 66-70, or any combination thereof; and (b) a pharmaceutically acceptable carrier, diluent and/or excipient.
75. A method of treating a subject with a DLK1-expressing cancer comprising administering to the subject a pharmaceutical composition of 74 to treat the cancer.
76. A method of inhibiting tumor growth in a subject, comprising administering to the subject a pharmaceutical composition of 74 to inhibit tumor growth.
77. A method of reducing tumor size in a subject, comprising administering to the subject a pharmaceutical composition of 74 in to reduce tumor size.
78. A method of preventing the recurrence of cancer in a subject, comprising

administering to the subject a pharmaceutical composition of 74 to prevent the recurrence of cancer.

79. A method of treating cancer in a subject diagnosed to be a low over-expresser of DLK1, comprising administering to the subject a pharmaceutical composition of 74 to prevent the recurrence of cancer.
80. The method of any one of 75-79 wherein the administering induces apoptosis in tumor cells.
81. The method of any one of 75-79 wherein the administering induces apoptosis in cells expressing Delta Like Non-Canonical Notch Ligand 1 (DLK1).
82. A method of detecting Delta Like Non-Canonical Notch Ligand 1 (DLK1) in a sample, comprising contacting the sample with an antigen-binding protein of any one of 1-40, a conjugate of any one of 41-60, or a fusion protein of 61, and assaying for an immunocomplex comprising the antigen-binding protein, conjugate or fusion protein bound toDLK1.
83. A method of diagnosing a Delta Like Non-Canonical Notch Ligand 1 (DLK1)-positive cancer in a subject, comprising contacting a biological sample comprising cells or tissue obtained from the subject with an antigen-binding protein of any one of 1-40, a conjugate of any one of 41-60, or a fusion protein of 61, and assaying for an immunocomplex comprising the antigen-binding protein, conjugate or fusion protein bound toDLK1.
84. The method of 83, further comprising treating the subject diagnosed to have DLK1-positive cancer by administering to the subject an antigen-binding protein of any one of 1-40, a conjugate of any one of 41-60, or a fusion protein of 61.
85. A method of activating a T cell to target a DLK1-expressing cancer cell in a subject, the method comprising administering to the subject a bispecific T cell engager (BiTE) of any one of 30-38.
86. A method of inducing an antibody-dependent cell-mediated cytotoxicity (ADCC) response against a DLK1-expressing cancer cell in a subject, the method comprising administering to the subject an antigen-binding protein that binds DLK1, wherein the antigen-binding protein comprises an Fc effector function; and the VH region and VL region of the antigen-binding protein of any

one of the previous embodiments or those described herein.

87. The method of any one of the previous embodiments, wherein the subject is a mammal, optionally a dog, a cat, a mouse, or a human.

88. A kit comprising (a) an antigen-binding protein of any one of 1-40, a conjugate of any one of 41-60, a fusion protein of 61, a nucleic acid of 62 or 63, a vector of 64 or 65, a host cell of any one of 66-70, or any combination thereof; and (b) an instruction for use.

[00227] The following examples are given merely to illustrate the present disclosure and not in any way to limit its scope.

EXAMPLES

EXAMPLE 1

[00228] This example describes the production of DLK1 specific antibodies.

[00229] Mice were immunized with 3T3 cells overexpressing a full length, epitope-tagged human DLK1 protein using a mammalian expression vector encoding a human DLK1-myc-DDK fusion protein. The amino acid sequence of the human DLK1 protein has UniProtKB Accession Number P80370-1 and an amino acid sequence of:
MTATEALLRVLLLLLAFGHSTYGAECFPACNPQNGFCEDDNVCRCQPGWQGPL
CDQCVTSPGCLHGLCGEPGQCICTDGWDGELCDRDVRACSSAPCANNRTCVSL
DDGLYECSCAPGYSGKDCQKKDGPCVINGSPCQHGGTCVDDEGRASHASCLCP
PGFSGNFCEIVANSCTPNPCENDGVCTDIGGDFRCRCPAGFIDKTCSRPVTNCAS
SPCQNGGTCLQHTQVSYECLCKPEFTGLTCVKKRALSPQQVTRLPSGYGLAYR
LTPGVHELPVQQPEHRILKVSMKELNKKTPLLTEGQAICFTILGVLTSLVVLGTV
GIVFLNKCETWVSNLRYNHMLRKKKNLLLQYNSGEDLAVNIIFPEKIDMTTFSK
EAGDEEI (SEQ ID NO: 19).

[00230] Splenocytes were harvested from the immunized mice and fused with myeloma lines by BTX Electrofusion (BTX, Holliston, MA) to generate hydridomas. Primary hybridoma cultures were generated and cultured in 384-well plates. The ability of the antibodies to bind peptides and/or human cancer cells that expressed DLK1 was assessed by ELISA assays and/or flow cytometry. The potential positive antibodies were re-arrayed into 96 well plates further screened by flow cytometry against endogenous and artificial cell line models.

[00231] Hybridomas positive for producing antibodies that bind human DLK1 protein were identified, and nucleic acid sequences encoding immunoglobulin light and heavy chain directed against human DLK1 protein isolated. These nucleic acid sequences were used to produce DLK1 antibodies formatted as full-length IgG antibodies (e.g., human IgG1) using ExpiCHO™ expression. The heavy and light chain variable regions of the antibodies were cloned into an antibody expression vector which was engineered in the lab based on a pcDNA™3.4-TOPO® vector (Catalog Number: A14697, ThermoFisher Scientific, USA). Transfection of the antibody expression vector into CHO cells, according to protocol provided in the kit (ExpiCHO™ Expression System, Catalog Number: A29133, ThermoFisher Scientific, USA)), resulted in production of a bicistronic mRNA in which an

IRES drives the expression of the second immunoglobulin chain. The produced antibodies were purified using protein A/G resins. Cell surface binding of the antibodies to DLK1 was determined by FACS in which DLK1 antibodies were directly conjugated with Alexa Fluor® 647 NHS Ester (Succinimidyl Ester), Cat# A20106 (ThermoFisher Scientific) following the manufacturer's protocol.

[00232] DLK1-expressing cells were used in FACS assays to determine the DLK1 antibody's ability to bind to DLK1 on the surface of cells. HEK293-T cells engineered to express human, monkey, mouse or rat DLK1 fused to a fluorescent protein (mGFP) were used as artificial models of DLK1 expression. COR-L279 (a human small cell lung carcinoma cell line), JR (a human rhabdomyosarcoma cell line), and H524 (a human small cell lung carcinoma cell line) were used as endogenous models of DLK1 expression, while SNU-C1 (a human colorectal cancer cell line), LS513 (a human colorectal cancer cell line), and M202 (a human melanoma cell line) were used as endogenous models lacking DLK1 expression.

[00233] For each type of cell tested and for each mAb, cells were detached from the surface of the culture flasks by versene (instead of trypsin) in order to protect the cell surface proteins. The detached cells were then incubated with Alexa Fluor®647-labeled DLK1 mAbs for 30 min in the dark on ice at a pre-determined concentration. The DLK1 mAbs were directly labeled with Alexa Fluor® 647 NHS Ester (Succinimidyl Ester). Alternatively, unlabeled DLK1 antibodies may be detected using a fluorescently labeled secondary antibody. After washing, the cells were read by a BD Accuri™ Flow Cytometer C6 to detect antibody-antigen protein binding in channel FL4H.

EXAMPLE 2

[00234] This example demonstrates the humanization of antibodies of the present disclosures.

[00235] Antibodies were selected for humanization analysis. The heavy chain variable (VH) and light chain variable (VL) sequences of mouse monoclonal anti-DLK1 antibodies were compared to a library of known human germline sequences from human V_H genes and human $V_L$kappa genes (IMGT® the international ImMunoGeneTics information system® www.imgt.org; founder and director: Marie-Paule Lefranc, Montpellier, France).; the databases used were IMGT human V_H genes (F+ORF, 273 germline sequences) and IMGT

human VLkappa genes (F+ORF, 74 germline sequences). The acceptor human germline was chosen from those closest in sequence to the parental antibody.

[00236] Alteration of human germline framework (i.e., non-CDR residues in VH and VL; abbreviated as FR) positions to corresponding parental murine sequence might be required to optimize binding of the humanized antibody. The sequences for versions of humanized antibodies are provided in Table 1 and Fig 19-Fig. 33.

[00237] Humanized antibodies, DLK1-547-h2, DLK1-548-h4, DLK1-557-h3, DLK1-557-f2, DLK1-559-h4, DLK1-559-f2, DLK1-561-f1, DLK1-562-h10 and DLK1-565-f2; (note that "DLK1-" may be replaced with "04-0", "mab-" or "DLK1-mab-" which refers to the same humanized antibody, for example, DLK1-547-h2 is the same as 04-0547-h2, mab-547-h2 and DLK1-mab-547-h2, respectively; further, expression plasmid DNAs may be designated by a lowercase letter "p" preceding the antibody name, such as p04-0547-h2 refers to an expression plasmid encoding anti-DLK1 antibody 04-0547-h2), were constructed and expressed as essentially described in Example 1. FACS assays were carried out to determine relative antigen binding strengths of the humanized antibodies (at either 1 μg or 0.2 μg) for binding to DLK1 overexpressed in HEK297T cell line. Corresponding parental antibodies (antibodies prior to humanization) were used as controls and may be designated with "chim".

[00238] Based on the *in vitro* antigen binding data, three humanized antibodies (04-547-h2 (also designated as DLK1-547-h2, 04-0547-h2), 04-561-F1 (also designated as DLK1-561-F1, 04-0561-F1) and 04-562-h10 (also designated as DLK1-562-h10, 04-0562-h10)) were selected for further testing and development. The antibodies were derived from mouse monoclonal antibody DLK1-547, DLK1-561, and DLK1-562.

[00239] *In vivo* binding studies of the humanized versions of monoclonal anti-DLK1 antibodies, DLK1-547-h2, DLK1-548-h4, DLK1-557-h3, DLK1-557-f2, DLK1-559-h4, DLK1-559-f2, DLK1-561-f1, DLK1-562-h10 and DLK1-565-f2, as well as original mouse monoclonal anti-DLK1 antibodies, DLK1-547, DLK1-548, DLK1-557 and DLK1-559, and as antibody-drug conjugates (ADC; antibody-MMAE conjugate) were carried out in xenograft mice injected with DLK1-positive human liver cancer cell line HepG2, DLK1-positive small cell lung carcinoma cell lines COR-L279 (also referred to as CORL279) and NCI-H524 (also referred to as H524), DLK1-positive human rhabdomyosarcoma cell line JR-1 (also referred to as JR), DLK1-negative colorectal cancer cell lines SNU-C1 (also

referred to as SNUC1) and LS513, and DLK1-negative human melanoma cell line M202. Briefly, xenograft models of different human cancer cell lines were established in six-week-old CD-1 athymic nude mice (Charles River Laboratories). After tumors reached an average size of 150 to 450 mm^3, mice were randomized into treatment groups. Humanized antibodies were diluted in sterile saline to a working concentration of 1 mg/mL for intravenous tail vein (IV) injection. Tumor xenografts were measured with calipers three times a week, and tumor volume in mm^3 was determined by multiplying height × width × length. Mice were treated for 2-7 weeks. At the end of study, animals were euthanized and tumor tissue was excised and divided to be stored as snap-frozen or formalin fixed paraffin embedded (FFPE) tissue for biomarker analysis.

[00240] The results of the xenograft assays are shown in Figs. 1-5.

[00241] Fig. 1 shows tumor growth inhibitory properties of four different mouse monoclonal antibodies (DLK1-547, DLK1-548, DLK1-557 and DLK1-559) directed against DLK1 protein in mice bearing xenograft of DLK1-positive HepG2 human hepatocellular cancer cells. Fig. 1A shows HEPG2 (DLK1+) cell line xenografts treated with non-targeting IgG control antibody or mouse monoclonal antibodies directed against DLK1. All mice were treated by IV tail vein injection at 10 mg antibody/kg once per week for 4 repeat doses. 6 mice per arm. Line represents mean tumor volume ± SEM. Fig. 1B shows a bar chart representing the mean change in tumor volume (± SEM) in each treatment group over the 23 days of treatment. All work was carried out under an Institutional Animal Care and Use Committee approved protocol.

[00242] Fig. 2 shows efficacy of a panel of humanized DLK1 mAbs inhibiting growth of DLK1-positive CORL279 human small cell lung cancer (SCLC) cell line xenografts without adversing affecting body weight. Fig. 2A shows CORL279 (DLK1+) cell line xenografts treated with non-targeting IgG control antibody or 9 different humanized antibodies directed against DLK1. All mice were treated by IV tail vein injection at 10 mg antibody/kg once per week for 3 repeat doses. 6 mice per arm. Line represents mean tumor volume ± SEM. Fig. 2B is a bar chart representing the mean change in tumor volume (± SEM) in each treatment group over the 21 days of treatment. Fig. 2C is a graph showing changes in percent body weight.

[00243] Fig. 3 shows selective efficacy of a DLK1-ADC (humanized mAb) in human cancer cell line xenografts. Fig. 3A-C show result of CORL279, JR and H524 (all DLK1+)

cell line xenografts treated with non-targeting IgG control antibody, DLK1-561-f1 (humanized-mAb). Mice were treated with control antibody at 10 mg/kg or the ADC at 5 mg/kg, all by IV tail vein injection 3 weekly repeat doses. Note dramatic reduction in tumor volume (mm^3), indicating great efficacy of DLK1-ADCs on DLK1-positive tumors. In contrast, similar treated of DLK1-negative tumors did not lead to dramatic reduction in tumor volume. Fig. 3D-F show SNUC1, LS513 and M202 (all DLK1-) cell line xenografts treated with control or DLK1-ADC as described above. 6-8 mice per arm. Lines represents mean tumor volume ± SEM.

[00244] Fig. 4 shows efficacy of humanized DLK1-ADCs in inhibiting growth and reducing tumor volume of DLK1-positive CORL279 human SCLC cell line xenografts. Fig. 5A shows result from treating CORL279 (DLK1+) cell line xenografts with non-targeting IgG control antibody or 3 different DLK1-ADCs generated from humanized DLK1-mAbs. Mice were treated with control antibody or ADCs by IV tail vein injection at 5 mg/kg once per week for 3 repeat doses. 7 mice per arm. Line represents mean tumor volume ± SEM. Fig. 4B is a bar chart representing the mean change in tumor volume (± SEM) in each treatment group over the 13 days of study (last day of control arm). Fig. 4C is a graph showing changes in percent body weight from start of treatment. Body weight initially drops with the ADCs but then recovered for the humanized DLK-ADC by day 23. Due to significant tumor growth in animals treated with non-targeting IgG-ADC control antibody, the animals were sacrificed on day 13 when average size of the xenograft is about 1200 mm^3.

[00245] Fig. 5 shows that the humanized DLK1-ADCs do not have anti-tumor activity in DLK1-negative M202 human melanoma cell line xenografts. Fig. 5A shows result from treating M202 ($DLK1^-$) cell line xenografts with non-targeting IgG control antibody or 3 different DLK1-ADCs generated from humanized DLK1-mAbs. Mice were treated with control antibody or ADCs by IV tail vein injection at 5 mg/kg once per week for 3 repeat doses. 8 mice per arm. Line represents mean tumor volume ± SEM. Fig. 5B is a bar chart representing the mean change in tumor volume (± SEM) in each treatment group over the 31 days of study (last day of control arm).

[00246] DKL1 antibody drug conjugates (ADCs) presented herein have been conjugated to a cytotoxic agent MMAE via a cleavable linker VC-PAB (thus comprising VC-PAB-MMAE). The average number of MMAE per antibody is 4, as measured by HIC and/or MS.

ADCs are heterogenous conjugates with ~90% of MMAEs conjugated to the interchain disulfides between the heavy chain and light chain of the IgG (reacted with the thiol of the cysteines from the reduced interchain disulfides). ADCs have been prepared at Wuxi Bio with their DAR4 technology.

[00247] Figs. 6, 8 and 9 provide a summary of biochemical, biophysical and cell biological properties of three lead humanized anti-DLK1 antibodies, 04-0561-F1, 04-0562-h10 and 04-0547-h2. These three antibodies bind strongly to DLK1-positive COR-L279 human small cell lung carcinoma cells than DLK1-negative M202 human melanoma cells. Antibody binding affinity for human DLK1-overexpressed on surface of HEK293T cells or DLK1-positive COR-L279 cells shows a dissociation constant (KD) of about 0.5 nM to 4 nM for 04-0561-F1 and 04-0562-h10 antibodies with a slightly higher KD of about 5 nM to 9 nM for 04-0547-h2 antibody. On recombinant fusion protein comprising human DLK1 extracellular domain (ECD) and monomeric immunoglobulin Fc domain (DLK1 ECD-mFc), the measured KDs are in the range of 2 pM to 20 pM for the two former antibodies and around 300 pM for 04-0547-h2 antibody. Similarly, EC50 determined using human DLK1 ECD-mFc fusion protein in an ELISA format showed that 04-0561-f1 and 04-0562-h10 antibodies have similar binding affinities at about 0.5 nM for the recombinant protein, while 04-0547-h2 antibody has a lower binding affinity, approximately 20-fold less. Thus, among the top three humanized anti-DLK1 antibodies, 04-0561-F1 and 04-0562-h10 antibodies have a lower KD and EC50 values than 04-0547-h2 antibody.

[00248] Size exclusion chromatography reveals that all three purified antibodies are primarily monomers with little antibody aggregation or fragmentation (Fig. 6).

Fig. 8 shows long term storage (5 weeks) and temperature stress (RT or 37°C) did not cause significant antibody aggregation and degradation (by SEC) at the concentrations of 10, 20, 50, or 75 mg/ml. Specifically, after 5 weeks of storage at 37°C, for 04-0561-F1, less than 3.5% of degradation (for all four concentrations) and ~4% of aggregation (only in 75 mg/ml) were observed; whereas, for 04-562-h10, less than 8% of degradation was observed. When analyzed by non-reducing SDS-PAGE, long term storage (5 weeks) and temperature stress (RT or 37°C) did not have significant effects on the antibody integrity at the concentrations of 10, 20, 50, or 75 mg/ml. However, some antibody degradations were detected after 5 weeks storage at 37°C at all concentrations (10, 20, 50, and 75 mg/ml); 04-0561-F1 had less degradation level compared with 04-0562-h10.

[00249] The effects of different temperatures and 7-day storage at 10 mg/mL on the stability of the humanized anti-DLK1 antibody, 04-0547-h2, is shown in Fig. 9, where 100% of the purified antibody remained as a monomer with no detectable aggregate or fragments, which was also verified by SDS-PAGE analysis after 1 week of storage at -80°C, 4°C, RT or 37°C.

[00250] When examined for cross reactivity to human and non-human DLK1 proteins, all three humanized anti-DLK1 antibodies were demonstrated to bind to human and monkey DLK1-overexpressed on HEK293T cells but varies in their abilities to bind rodent DLK1 proteins (Fig. 6). In particular, 04-0547-h2 antibody bound mouse and rat DLK1 proteins, whereas, 04-0562-h10 antibody less so for mouse than rat DLK1 protein and 04-0561-F1 antibody appeared not to bind either of the rodent DLK1 proteins. In terms of cross-species reactivity, 04-0547-h2 antibody bound all four DLK1 proteins, while 04-0562-h10 preferred binding to human, monkey and rat DLK1 proteins over mouse DLK1 protein. Best humanized anti-DLK1 antibody with the least cross reactivity is 04-0561-F1 which bound human and monkey DLK1 proteins but not mouse or rat DLK1 protein. Additional cross reactivity data are provided in Fig. 11.

[00251] Similarity in the amino acid sequences of human, monkey, mouse and rat DLK1 proteins are shown in a sequence alignment in Fig. 10. Human DLK1 protein sequence is provided above and may be obtained using UniProtKB Accession Number P80370-1. The mouse DLK1 protein may be obtained using UniProtKB Accession Number Q09163 and has an amino acid sequence of:

MIATGALLRVLLLLLAFGHSTYGAECDPPCDPQYGFCEADNVCRCHVGWEGPL
CDKCVTAPGCVNGVCKEPWQCICKDGWDGKFCEIDVRACTSTPCANNGTCVD
LEKGQYECSCTPGFSGKDCQHKAGPCVINGSPCQHGGACVDDEGQASHASCLC
PPGFSGNFCEIVAATNSCTPNPCENDGVCTDIGGDFRCRCPAGFVDKTCSRPVSN
CASGPCQNGGTCLQHTQVSFECLCKPPFMGPTCAKKRGASPVQVTHLPSGYGL
TYRLTPGVHELPVQQPEQHILKVSMKELNKSTPLLTEGQAICFTILGVLTSLVVL
GTVAIVFLNKCETWVSNLRYNHTFRKKKNLLLQYNSGEELAVNIIFPEKIDMTT
FNKEAGDEEI (SEQ ID NO: 20).

[00252] Antibody internalization rate determined on native DLK1-positive COR-L279 cells showed a 50% internalization time of about 10 mins with internalization complete in about 1 hr (Fig. 6). Fig. 12 shows internalization data for chimeric anti-DLK1 antibodies,

04-0557m and 04-0559m, wherein the light chain and heavy chain variable regions of human IgG1 were replaced with mouse anti-DLK1 monoclonal antibody light chain and heavy chain variable regions, respectively, in $DLK1^+$ COR-L279 cells. Internalization of benchmark antibodies, KO_DLK1-hIgG1 and Li-DLK1-hIgG1, are also shown. Uptake of humanized anti-DLK1 antibodies, 04-0547-h2, 04-0548-h4, 04-0557-h3 and 04-557-F2, in HEPG2 cells is shown in Fig. 13 and COR-L279 cells in Fig. 14. Further, internalization data in COR-L279 cells are provided in Figs. 15 and 16 for the humanized anti-DLK1 antibodies, 04-0559-h4, 04-559-F2 (also referred to as 04-0559-F2), 04-0561-F1, 04-0562-h10, and 04-565-F2 (also referred to as 04-0565-F2), as well as for benchmark KO_DLK1-hIgG1 antibody and non-targeting hIgG1 control and no antibody body. Appearance of COR-L279 cells at 48 hrs after incubating with AF-647 labeled anti-DLK1 antibodies are shown in Fig. 17 for 04-0547-h2, 04-0548-h4, 04-0557-h3, 04-557-F2, 04-0559-h4, 04-559-F2, 04-0567-F1, 04-0562-h10, and 04-565-F2 as well as for benchmark KO_DLK1-hIgG1 antibody and negative controls using non-targeting hIgG1 or no antibody. Similarly, internalization of the AF-647 labeled MMAE-conjugated lead antibodies (04-0547-h2-MMAE-AF647, 04-0561-F1-MMAE-AF647 and 04-0562-h10-MMAE-AF647) along with lead antibody, 04-0561-F1, labeled with AF-647 without MMAE as a conjugate (04-0561-F1-AF647) is shown in Fig. 7. Internalization of the ADCs were complete within about 1.5 hrs in COR-L279 cells (see also Fig. 18).

[00253] The amino acid sequence of the framework (FR) and complementarity-determining regions (CDRs) for the heavy and light chain varible regions as well as amino acid sequence of the entire heavy and light chain variable regions (V_H and V_L) for the humanized DLK1 antibodies (04-0547-h2, 04-0561-F1, 04-0562-h10, 04-0548-h4, 04-0557-F2, 04-0557-h3, 04-0559-F2, 04-0559-h4, 04-0565-F2) are provided in Figs. 19-29 with Kabat and AbM denoted sequences in Figures 19-27 and IMGT-denoted sequences in Figures 28-29. Note the presence of "RTV" tripeptide in the light chain variable region framework 4 (LFR4) for Kabat and AbM-denoted sequences using the algorithm in abYsis (Swindells MB, Porter CT, Couch M, Hurst J, Abhinandan KR, Nielsen JH, Macindoe G, Hetherington J, Martin AC. abYsis: Integrated Antibody Sequence and Structure-Management, Analysis, and Prediction. J Mol Biol. 2017 Feb 3;429(3):356-364; World Wide Web at abysis.org). For the light chain variable region identified in Figs. 19-27, this light chain variable region is joined to a kappa light chain constant region which comprises

the sequence:

AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC.

This sequence corresponds to sequence of the kappa light chain constant region as given in UniProt ID: P01834, except for the presence of three amino acids "RTV" at the amino-terminal end in UniProt ID: P01834. For the light chain variable region sequence provided in Figs. 28 and 29, the entire kappa light chain may be obtained by appending to the C-terminal end of the variable light chain region, the amino acid sequence as provide in UniProt ID: P01834, namely:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC.

[00254] Amino acid sequence for the constant region of human IgG1 and IgG2, used in humanizing anti-DLK1 antibody is provided in Fig. 30.

[00255] DNA sequences encoding the humanized DLK1 antibody light and heavy chain variable regions are provided in Fig. 31 and the DNA sequences encoding the human light and heavy chain constant regions are provided in Fig. 32. Joining the sequences of the DNA encoding the light or heavy chain variable region of Fig. 31 followed by DNA sequence of the light or heavy chain constant region of Fig. 32 produces a DNA sequence encoding a full length light or heavy chain, respectively.

[00256] Heavy and light chain variable region coding sequences of the humanized anti-DLK1 antibody (04-0561-F1) and the mouse monoclonal anti-DLK1 antibody (04-0561-m) are provided in Fig. 33.

[00257] Analysis of the cross-reactivity data for one of the three lead anti-DLK1 antibodies showed that 04-0561-F1 (also referred to as DLK-561-F1) binds human and monkey but not mouse or rat DLK1 protein (Fig. 6). While all members of the DLK1 family members are related to each other, human and mouse DLK1 proteins shared about 85% amino acid sequence identity (see Fig. 10). The DLK protein is a membrane-bound protein comprising a signal peptide which is cleaved off the mature protein, six EGF-like repeats clustered at the N-terminal half of the extracellular domain, a helical transmembrane domain and a cytoplasmic domain (see Fig. 36A). To map the location of the DLK1 antibody binding epitope in the long form of human DLK1 protein (long form or isoform 1) which is absent in the long form of mouse DLK1 protein, Applicants aligned the

amino acid sequences of the two proteins and substituted a contiguous stretch of the amino acid sequence in the human DLK1 protein with a corresponding stretch from the mouse DLK1 protein (see Fig. 35 for differences in amino acids between the long form of human and mouse DLK1 proteins as well as the locations of regions 1-8 in aqua color (top two pairs of arrows). Dividing the extracellular domain (amino acid residues 24-303) of the human DLK1 protein into 4 regions (region 1-4) and replacing each region with a corresponding region from the mouse DLK1 protein, narrowed the location of the epitope in the human DLK1 protein to regions 1 and 2 or from amino acids 24-169. DLK1-561-F1 conjugated with AF-647 fluorophore (DLK1-561-F1-AF647) failed to bind HEK293T cells expressing the mGFP protein fusion to the long form of chimeric human DLK1 protein in which region 1 (834 HEK293T DLK1HM1-mGFP) or region 2 (835 HEK293T DLK1HM2-mGFP) has been replaced with corresponding region from the long form of mouse DLK1 protein (see Fig. 34), as analyzed by flow cytometry. Binding of DLK1-561-F1-AF647 to these two HEK293T cells, 834 and 835, was greatly reduced compared with the binding to HEK293T cells expressing the parental long form of human DLK1 protein as a fusion to mGFP protein (HEK293T DLK1 mGFP G06 (long form) or HEK293T cells expressing fusion proteins in which region 3 or 4 was used to swap out human DLK1 protein sequences with the mouse DLK1 protein sequences (836 HEK293T DLK1HM3-mGFP and 837 HEK293T DLK1HM4-mGFP).

[00258] To further define the location of the epitope in the long form of human DLK1 protein, regions 1 and 2 were divided further into smaller regions 5-8 and similar swapping experiments were performed. Swapping out region 5 or 8 in Fig. 35 did not affect binding of the DLK1-561-F1-AF647 antibody to the HEK293T cells expression mGFP protein fusion to the long form of chimeric human DLK1 with the mouse region 5 (850 HEK293T DLK1HM5-mGFP) or region 8 (849 HEK293T DLK1HM8-mGFP) sequences, indicating that the epitope for the DLK1-561-F1 antibody binding does not reside in either region 5 or 8. In contrast, swapping out region 6 or 7 of the long form of the chimeric human DLK1 protein with mouse sequences resulted in the loss of DLK1-561-F1-AF647 antibody to HEK293T cells (see 851 HEK293T DLK1 HM6-GFP and 848 HEK293T DLK1 HM7-mGFP in Fig. 34). Thus, the binding site for DLK1-561-F1 antibody resides in region 6 and 7 of the long form of the human DLK1 protein.

[00259] Analysis of the GFP fluorescence shows that the loss of DLK1-561-F1-AF647 antibody binding to 834, 835, 848 and 851 cells was not a result of the lack of expression of the chimeric fusion protein. Negative control experiments using a non-targeting hIgG1 antibody conjugated to AF647 fluorophore (hIgG1-AF647) and no antibody treatment (no ab) showed dependence of AF647 fluorescence on the presence of the DLK1-binding activity.

[00260] Based on the epitope mapping experiments of Figs. 34 and 35, the epitope for the humanized DLK1 antibody, DLK1-561-F1 (also referred to as 04-0561-F1), resides within about amino acid residues 56-113 of the long form of human DLK1 protein (isoform 1), which forms EGF-like 2 and EGF-like 3 structural domains (see Fig. 36A).

[00261] In addition to the long form (or full length form) of the DLK1 protein with 383 amino acids (isoform 1; also called DLK1-FL; see UniProt ID: P80370-1), expression of the human DLK1 gene can also give rise to an alternatively splice mRNA producing a short form of the DLK1 protein (isoform 2; also called DLK1-MB; see UniProt ID: P80370-2) with 310 amino acids, lacking 73 amino acids from the amino acid position 229-301 of the isoform 1. The shorter DLK1-MB lacks a juxtamembrane region with a cleavage site for a disintegrin and metalloprotease, ADAM17, which is also known as a TNFα converting enzyme (TACE). The large bolt identified the ADAM17-mediated juxtamembrane cleavage site for the DLK1-FL but missing in DLK1-MB in Fig. 36B. Smaller bolts identify the other protease cleavage site. The short form, DLK1-MB, is expressed more in cancer than the long (full length) form, DLK1-FL. The DLK1 isoform-2 (UniProt ID: P80370-2) comprises the following sequence:
MTATEALLRVLLLLLAFGHSTYGAECFPACNPQNGFCEDDNVCRCQPGWQGPLCD
QCVTSPGCLHGLCGEPGQCICTDGWDGELCDRDVRACSSAPCANNRTCVSLDDGL
YECSCAPGYSGKDCQKKDGPCVINGSPCQHGGTCVDDEGRASHASCLCPPGFSGN
FCEIVANSCTPNPCENDGVCTDIGGDFRCRCPAGFIDKTCSRPVTNCASSPCQNGGT
CLQHTQGQAICFTILGVLTSLVVLGTVGIVFLNKCETWVSNLRYNHMLRKKKNLLL
QYNSGEDLAVNIIFPEKIDMTTFSKEAGDEEI (SEQ ID NO: 69).

DLK1 isoform	Other name used herein
DLK1 isoform 1 (UniProt ID: P80370-1)	DLK1-FL
DLK1 isoform 2 (UniProt ID: P80370-2)	DLK1-MB

[00262] As amino acid number 56-113 of DLK1-FL protein is present in the human DLK1-MB protein, the ability of the lead anti-DLK1 antibody, DLK1-561-F1, to also bind the shorter DLK1-MB protein was analyzed in a flow cytometry assay (Fig. 37). Binding of a benchmark anti-DLK1 antibody, LegoChem 18A5, was also analyzed, as well as a negative control non-targeting human IgG1 antibody (hIgG1). The antibodies were either directly labeled with Alexa Fluor® 647 (ThermoFisher Scientific catalog #: A20006) or were indirectly labeled with AF-647 conjugated anti-His tag secondary antibody (BioLegend catalog #: 652513) to detect $(His)_6$ epitope at the C-terminus of the unlabeled primary antibody. Parental HEK 293T cells (HEK293T parental) or HEK293T cells expressing the mGFP protein fusion to DLK1-FL (HEK293T DLK1 mGFP G06 (long form)) or DLK1-MB (832-HEK293T DLK1 Iso2-mGFP mass pop (short form)) were incubated with either the DLK1-561-F1 antibody (561-F1-AF647 or 561-F1), LegoChem 18A5 (LegoChem 18A5-AF647 or LegoChem 18A5), or hIgG1 (hIgG1-AF647 or hIgG1). In the case of an unlabeled primary antibody, an additional incubation with AF647-labeled anti-His tag secondary antibody was performed. After washing, cells were analyzed for AF647 fluorescence (FL4-H channel) by flow cytometry on an iQue platform (Sartorius). As expected, DLK1-561-F1 binds both isoforms of human DLK1 protein, DLK-FL and DLK-MB. However, unlike DLK1-561-F1 anti-DLK1 antibody, LegoChem A185 benchmark anti-DLK1 antibody only binds the long form of DLK1 protein (isoform 1 or DLK1-FL). Its binding to the short form of human DLK1 protein is comparable to the binding observed with negative control non-targeting human IgG1 antibody (hIgG1). Thus, when compared to a benchmark anti-DLK1 antibody, LegoChem 18A5, Applicants' lead antibody, DLK-561-F1, bound both isoforms of human DLK1, whereas LegoChem 18A5 failed to bind or bind weakly to human DLK1-MB, an isoform of human DLK1 which is often expressed in human cancers.

[00263] To determine the EC50 of the interaction between the two isoforms of human DLK1 protein; and DLK1-561-F1 or the ChemLego 18A antibody, ELISA assay was performed using plates coated with either the DLK1 ECD-mFc long form or the DLK1 ECD-mFc short form (1μg/50μl PBS/well; 4°C overnight; Blocking: 5%BSA/PBS, 200μl/well, 37°C for 2hrs). Plates were incubated with DLK1 Abs serially diluted 2-fold starting from 400 ng/ml in 5% BSA/PBS at RT for 1.5 hrs. Mouse anti-human IgG Fc secondary antibody conjugated to HRP (Novus catalog number: NBP1-78623H) at 1:3000

dilution was applied to each well at 50 µl/well and further incubated at RT for 1hr. The ELISA was developed first with Thermo Scientific Pierce 1-Step™ Ultra TMB-ELISA substrate solution and then with addition of sulfuric acid stop solution. The ELISA plate was read on a plate reader at 450 nm. Fig. 38 shows absorption at 450 nm (OD450nm) as a function of anti-DLK1 antibody concentration. DLK1-561-F1 antibody showed a dose response curve with higher concentrations of the antibody producing greater absorption at 450 nm in the wells coated with either the long or short form of the human DLK1 protein (i.e., DLK1-FL or DLK1-MB, respectively) with an EC50 value of about 0.81 to 0.87 nM. In contrast, LegoChem 18A5 antibody showed a shallower dose response curve with an EC50 value of about 17.9 nM for binding to the long form of the human DLK1 protein (i.e., DLK1-FL or isoform 1). Binding of LegoChem 18A5 antibody to human DLK1-MB, the shorter form or isoform 2 of human DLK1 was not observed in the ELISA. Thus, DLK1-561-F1 antibody binds both isoforms of the human DLK1 protein, and its binding affinity to the long form of human DLK1 protein (DLK1-FL or isoform 1) is significantly higher (about over 20-fold) than that of LegoChem 18A5 antibody for human DLK1-FL. Further, while DLK1-561-F1 binds the shorter form of human DLK1 protein (DLK1-MB or isoform 2) with similar affinity to the long form of human DLK1 protein (DLK1-FL or isoform 1), LegoChem 18A5 failed to bind the shorter human DLK1-MB or isoform 2 of human DLK1 protein.

[00264] Antibody-dependent cellular cytotoxicity (ADCC) function of the lead anti-DLK1 antibody, DLK1-561-F1 (also called 04-0561-F1 and 561-F1), was assessed on the native cells positive for DLK1 expression (COR-L279) or negative for DLK1 expression (M202), as well as the HEK293 cells engineered to overexpress the short form of the human DLK1 protein (DLK1-MB or isoform 2). These native or engineered cells were incubated over a range of antibody concentrations for the 561-F1 anti-DLK1 antibody or human IgG1 non-targeting antibody control for 18-20 hrs, followed by co-incubation with Jurkat-Lucia™ NFAT-CD16 effector cells (InvivoGen, San Diego, USA) for 6 hrs. NFAT activation, indicative of the induced ADCC response, was assessed by determining Lucia luciferase activity in the supernatant. As shown in Fig. 39, the DLK1-561-F1 antibody induced significant ADCC activity on native DLK1$^+$ COR-L279 cells with an EC_{50} value of about 2.16 nM (Fig. 39A) and HEK293 cells expressing the short form of the human DLK1 protein with an EC_{50} value of about 101 ng/ml (Fig. 39B). The robust ADCC activity

observed with the DLK-561-F1 antibody is dependent on the expression of DLK1 protein, as DLK1-negative M202 cells produced minimal ADCC response with the maximal response similar to the ADCC response observed with the non-targeting hIgG1 control antibody (Fig. 39A). Unlike the DLK1-561-F1 antibody, LegoChem 18A5 benchmark antibody failed to produce an ADCC response on the target HEK293 cells engineered to express the short form of the human DLK1 protein (DLK1-MB or isoform 2) (Fig. 39B). Thus, DLK1-561-F1 antibody induces ADCC in native and overexpressed models of DLK1 expression.

[00265] Light and heavy chain variable regions of three lead humanized antibodies, 04-0547-h2, 04-0561-F1 and 04-0562-h10, were used to produce single chain variable fragments (scFvs). In addition, each of the anti-DLK1 scFv was used to produce a bispecific antibody comprising both an anti-DLK1 scFv and anti-CD3 scFv, so as to produce an anti-DLK1-CD3 bispecific T-cell engager (BiTE). Amino acid sequences for the three scFvs, 04-0547-h2scfv, 04-0561-F1scFv and 04-0562-h10scFv, along with the DNA sequence encoding these scFvs are provided in Table 1 (SEQ ID NOs: 57-59 and 63-65). Table 1 also provides the amino acid sequence and DNA sequence for the three respective BiTEs, 04-0547-h2Bs, 04-0561-F1Bs and 04-0562-h10Bs (SEQ ID NOs: 60-62 and 66-68). Each scFv and BiTE additionally comprises a $(His)_6$ epitope tag at the C-terminus.

[00266] DLK1-dependent binding of the scFvs and BiTEs was assessed by flow cytometry on an iQue platform (Sartorius). Briefly, each scFv or BiTE antibody was incubated with either DLK1+ COR-L279 cells or DLK1- M202 cells, followed by incubation with a secondary antibody, AF-647 labeled anti-His tag antibody. AF-647 fluorescence was captured in FL4-H channel and its average was reported in the upper table shown in Fig. 40. Background fluorescence and fluorescence due to binding cells by the secondary antibody in the absence of a primary antibody are reported in the lower table. Robust AF-647 fluorescence was observed for the $DLK1^+$ COR-L279 cells incubated with DLK1 scFv and BiTE antibodies, which was significantly above that observed for the DLK^- M202 cells, or for the COR-L279 cells incubated only with the AF-647-labeled secondary antibody. Thus, DLK1 scFvs and BiTEs selectively bound to the DLK1 positive COR-L279 cells but not to the DLK1 negative M202 cells.

[00267] T-cell activation activity of the anti-DLK1 BiTEs were assayed using Jurkat cells and a NFAT-RE reporter construct. T-cell activation was measured using Promega's T Cell Activation Bioassay kit (NFAT-RE J1621). Multiple cell lines (Raji, JR, CORL-279) were seeded at a density of 25,000 - 40,000 cells/well in white 96 well plate wells and incubated at 37°C overnight, after which thaw-and-use Jurkat T cells included in the assay kit were added to the seeded cells (1:1 cell ratio) and treated with DLK1 bispecific antibodies. Treated plates were incubated in 37°C for 6 hours, after which Bio-Glo reagent (included in kit) was added and immediately read on the Varioskan LUX plate reader. The units were RLU. As shown in Figure 41, cell lines overexpressing DKL1 (JR and COR-L279) induced activation of T cells when treated with either the 04-0547-h2Bs or 04-0561-F1Bs BiTEs, whereas there was decreased levels of activation in the DLK1-negative Raji cell line. The 04-0562-h10Bs BiTE did not induce T-cell activation. Each BiTE additionally comprises a $(His)_6$ epitope tag at the C-terminus.

[00268] All references, including publications, patent application s, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[00269] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the disclosure (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted.

[00270] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range and each endpoint, unless otherwise indicated herein, and each separate value and endpoint is incorporated into the specification as if it were individually recited herein. As used herein, the term "about" when used before a numerical designation, e.g., temperature, time, amount, concentration, and such other, including a range, indicates approximations which may vary by (+) or (-) 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%,

[00271] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and

all examples, or various language (e.g., "such as") provided herein, is intended merely to better illuminate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure.

[00272] Preferred embodiments of this disclosure are described herein, including the best mode known to the inventors for carrying out the disclosure. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the disclosure to be practiced otherwise than as specifically described herein. Accordingly, this disclosure includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the disclosure unless otherwise indicated herein or otherwise clearly contradicted by context.

Table 1

Seq ID NO.:	Sequence	description
01	GFSISDYY	{04-0547-h2} heavy chain CDR1
02	INYDGTNT	{04-0547-h2} heavy chain CDR2
03	VRSYYYYGMEY	{04-0547-h2} heavy chain CDR3
04	HDVSTA	{04-0547-h2} light chain CDR1
05	SAS	{04-0547-h2} light chain CDR2
06	QQHYRIPLT	{04-0547-h2} light chain CDR3
07	GFSLSIYS	{04-0561-F1} heavy chain CDR1
08	IWGGGST	{04-0561-F1} heavy chain CDR2
09	ARKEGNYLWFAY	{04-0561-F1} heavy chain CDR3
10	QSLLQSSNQKNY	{04-0561-F1} light chain CDR1
11	FAS	{04-0561-F1} light chain CDR2
12	QQHYSIPLT	{04-0561-F1} light chain CDR3
13	GFSLTSYG	{04-0562-h10} heavy chain CDR1
14	IWGDGST	{04-0562-h10} heavy chain CDR2
15	AKPDGP	{04-0562-h10} heavy chain CDR3
16	QSLVHINGNTY	{04-0562-h10} light chain CDR1
17	KVS	{04-0562-h10} light chain CDR2
18	SQTTHVPWT	{04-0562-h10} light chain CDR3
19	MTATEALLRVLLLLLAFGHSTYGA ECFPACNPQNGFCEDDNVCRCQPG WQGPLCDQCVTSPGCLHGLCGEPG QCICTDGWDGELCDRDVRACSSAP CANNRTCVSLDDGLYECSCAPGYS GKDCQKKDGPCVINGSPCQHGGTC VDDEGRASHASCLCPPGFSGNFCEI	human Delta Like Non-Canonical Notch Ligand 1 (DLK1) protein having UniProtKB Accession Number P80370-1, corresponding to the full length protein or isoform 1 (DLK1-FL)

	VANSCTPNPCENDGVCTDIGGDFR CRCPAGFIDKTCSRPVTNCASSPCQ NGGTCLQHTQVSYECLCKPEFTGL TCVKKRALSPQQVTRLPSGYGLAY RLTPGVHELPVQQPEHRILKVSMK ELNKKTPLLTEGQAICFTILGVLTSL VVLGTVGIVFLNKCETWVSNLRYN HMLRKKKNLLLQYNSGEDLAVNII FPEKIDMTTFSKEAGDEEI	
20	MIATGALLRVLLLLLAFGHSTYGA ECDPPCDPQYGFCEADNVCRCHVG WEGPLCDKCVTAPGCVNGVCKEP WQCICKDGWDGKFCEIDVRACTST PCANNGTCVDLEKGQYECSCTPGF SGKDCQHKAGPCVINGSPCQHGGA CVDDEGQASHASCLCPPGFSGNFC EIVAATNSCTPNPCENDGVCTDIGG DFRCRCPAGFVDKTCSRPVSNCAS GPCQNGGTCLQHTQVSFECLCKPP FMGPTCAKKRGASPVQVTHLPSGY GLTYRLTPGVHELPVQQPEQHILK VSMKELNKSTPLLTEGQAICFTILG VLTSLVVLGTVAIVFLNKCETWVS NLRYNHTFRKKKNLLLQYNSGEEL AVNIIFPEKIDMTTFNKEAGDEEI	mouse DLK1 protein having UniProtKB Accession Number Q09163
21	EVQLVESGGGLVQPGGSLRLSCAA S	{04-0547-h2} a heavy chain FR1
22	MAWVRQAPGKGLEWVAN	{04-0547-h2} a heavy chain FR2
23	YYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYC	{04-0547-h2} a heavy chain FR3
24	WGQGTTVTVSS	{04-0547-h2} a heavy chain FR4
25	DIQMTQSPSSLSASVGDRVTITCRA S	{04-0547-h2} a light chain FR1
26	VAWYQQKPGKAPKLLIY	{04-0547-h2} a light chain FR2
27	YRYTGVPSRFSGSGSGTDFTLTISSL QPEDFATYYC	{04-0547-h2} a light chain FR3
28	FGQGTKLEIK	{04-0547-h2} a light chain FR4
29	QVQLQESGPGLVKPSETLSLTCTVS	{04-0561-F1} a heavy chain FR1
30	VHWVRQPPGKGLEWIGL	{04-0561-F1} a heavy chain FR2
31	DYNPSLKSRVTISKDTSKNQVSLKL SSVTAADTAVYYC	{04-0561-F1} a heavy chain FR3
32	WGQGTLVTVSS	{04-0561-F1} a heavy chain FR4
33	DIVMTQSPDSLAVSLGERVTMNCK SS	{04-0561-F1} a light chain FR1
34	LAWYQQKPGQPPKLLVY	{04-0561-F1} a light chain FR2
35	TRESGVPDRFSGSGSGTDFTLTISSV QAEDVAVYYC	{04-0561-F1} a light chain FR3
36	FGQGTKLEIK	{04-0561-F1} a light chain FR4
37	QVQLQESGPGLVKPSETLSLTCTVS	{04-0562-h10} a heavy chain FR1
38	VSWVRQPPGKGLEWIGV	{04-0562-h10} a heavy chain FR2
39	SYNPSLKSRVTISKDTSKNQVSLKL SSVTAADTAVYYC	{04-0562-h10} a heavy chain FR3

40	LGQGTLVTVSS	{04-0562-h10} a heavy chain FR4
41	DIVMTQSPLSLPVTPGEPASISCRSS	{04-0562-h10} a light chain FR1
42	LHWYLQKPGQSPQLLIY	{04-0562-h10} a light chain FR2
43	NRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYYC	{04-0562-h10} a light chain FR3
44	FGQGTKLEIK	{04-0562-h10} a light chain FR4
45	EVQLVESGGGLVQPGGSLRLSCAA SGFSISDYYMAWVRQAPGKGLEW VANINYDGTNTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVY YCVRSYYYYGMEYWGQGTTVTVS S	{04-0547-h2} heavy chain variable region
46	DIQMTQSPSSLSASVGDRVTITCRA SHDVSTAVAWYQQKPGKAPKLLIY SASYRYTGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQHYRIPLTFG QGTKLEIK	{04-0547-h2} light chain variable region
47	QVQLQESGPGLVKPSETLSLTCTVS GFSLSIYSVHWVRQPPGKGLEWIG LIWGGGSTDYNPSLKSRVTISKDTS KNQVSLKLSSVTAADTAVYYCAR KEGNYLWFAYWGQGTLVTVSS	{04-0561-F1} heavy chain variable region
48	DIVMTQSPDSLAVSLGERVTMNCK SSQSLLQSSNQKNYLAWYQQKPG QPPKLLVYFASTRESGVPDRFSGSG SGTDFTLTISSVQAEDVAVYYCQQ HYSIPLTFGQGTKLEIK	{04-0561-F1} light chain variable region
49	QVQLQESGPGLVKPSETLSLTCTVS GFSLTSYGVSWVRQPPGKGLEWIG VIWGDGSTSYNPSLKSRVTISKDTS KNQVSLKLSSVTAADTAVYYCAKP DGPLGQGTLVTVSS	{04-0562-h10} heavy chain variable region
50	DIVMTQSPLSLPVTPGEPASISCRSS QSLVHINGNTYLHWYLQKPGQSPQ LLIYKVSNRFSGVPDRFSGSGSGTD FTLKISRVEAEDVGVYYCSQTTHV PWTFGQGTKLEIK	{04-0562-h10} light chain variable region
51	QVQLQESGPGLVKPSETLSLTCTVSG FSLSIYSVHWVRQPPGKGLEWIGLIW GGGSTDYNPSLKSRVTISKDTSKNQV SLKLSSVTAADTAVYYCARKEGNYL WFAYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSRDE	{04-0561-F1} heavy chain Note: the sequence shows the sequence of the polypeptide that is secreted. The signal peptide that gets cleaved off before secretion comprises the additional sequence of: MGWSCIILFLVATATGVHS, or alternatively, MEFGLSWVFLVALFRGVQC, at the N-terminal end.

	LTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	
52	DIVMTQSPDSLAVSLGERVTMNCKS SQSLLQSSNQKNYLAWYQQKPGQPP KLLVYFASTRESGVPDRFSGSGSGTD FTLTISSVQAEDVAVYYCQQHYSIPL TFGQGTKLEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC	{04-0561-F1} light chain Note: the sequence shows the sequence of the polypeptide that is secreted. The signal peptide that gets cleaved off before secretion comprises the additional sequence of: METDTLLLWVLLLWVPGSTG, or alternatively, MDMRVPAQLLGLLLLWLSGARC, at the N-terminal end.
53	CAGGTTCAGCTTCAAGAAAGTGG CCCAGGACTCGTCAAACCGTCTG AAACTCTCTCCTTGACCTGCACCG TCTCCGGCTTCTCTTTGAGTATAT ACTCGGTGCACTGGGTCAGGCAG CCACCTGGCAAGGGACTGGAGTG GATCGGCCTGATCTGGGGTGGTG GATCTACTGACTACAATCCTTCCC TCAAGAGCAGGGTTACCATTTCG AAGGACACATCTAAGAATCAGGT GTCCCTGAAGCTCTCTTCTGTCAC GGCGGCAGACACAGCTGTGTACT ATTGTGCCCGCAAGGAAGGCAAT TATTTGTGGTTCGCCTACTGGGGC CAAGGCACCCTGGTGACAGTGTC CAGTGCTAGCACCAAGGGCCCAT CGGTCTTCCCCCTGGCACCCTCCT CCAAGAGCACCTCTGGGGGCACA GCGGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGA CGGTGTCGTGGAACTCAGGCGCC CTGACCAGCGGCGTGCACACCTT CCCGGCCGTCCTACAGTCCTCAG GACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTT GGGCACCCAGACCTACATCTGCA ACGTGAATCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTTGA GCCCAAATCTTGTGACAAAACTC ACACATGCCCACCGTGCCCAGCA CCTGAACTCCTGGGGGGACCGTC AGTCTTCCTCTTCCCCCCAAAACC CAAGGACACCCTCATGATCTCCC GGACCCCTGAGGTCACATGCGTG GTGGTGGACGTGAGCCACGAAGA CCCTGAGGTCAAGTTCAACTGGT ACGTGGACGGCGTGGAGGTGCAT AATGCCAAGACAAAGCCGCGGGA	Nucleic acid encoding {04-0561-F1} heavy chain Note: this nucleic acid sequence does not comprise the sequence encoding the signal peptide. However, when a signal sequence is included, one embodiment of the nucleic acid begins at the 5' end with the sequence, ATGGGATGGTCATGTATCATCCTT TTTCTGGTAGCAACTGCAACTGG AGTACATAGC, which encodes a signal peptide with an amino acid sequence, MGWSCIILFLVATATGVHS. This signal peptide at the amino terminus is cleave off from the nascent protein to produce a mature immunoglobulin heavy chain.

	GGAGCAGTACAACAGCACGTACC GTGTGGTCAGCGTCCTCACCGTCC TGCACCAGGACTGGCTGAATGGC AAGGAGTACAAGTGCAAGGTCTC CAACAAAGCCCTCCCAGCCCCCA TCGAGAAAACCATCTCCAAAGCC AAAGGGCAGCCCCGAGAACCACA GGTGTACACCCTGCCCCCATCCC GGGACGAGCTGACCAAGAACCAG GTCAGCCTGACCTGCCTGGTCAA AGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGG CAGCCGGAGAACAACTACAAGAC CACGCCTCCCGTGCTGGACTCCG ACGGCTCCTTCTTCCTCTACAGCA AGCTCACCGTGGACAAGAGCAGG TGGCAGCAGGGGAACGTCTTCTC ATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACGCAGAAG AGCCTCTCCCTGTCTCCGGGCAA A	
54	GATATTGTGATGACACAGTCGCC AGATTCCCTGGCCGTGTCTCTCGG CGAACGGGTCACCATGAACTGCA AGAGCAGTCAGTCGCTGCTTCAA TCGTCAAACCAAAAGAACTACCT GGCTTGGTATCAGCAAAAGCCTG GTCAACCCCCAAAATTGCTGGTTT ACTTCGCAAGCACTAGAGAAAGC GGCGTGCCCGATCGATTTAGCGG TTCAGGATCTGGAACCGACTTCA CACTCACAATAAGCAGCGTACAA GCGGAGGACGTTGCCGTGTACTA TTGCCAACAACACTACTCCATCCC TCTGACCTTCGGCCAAGGCACAA AGCTGGAGATCAAACGTACGGTG GCTGCACCATCTGTCTTCATCTTC CCGCCATCTGATGAGCAGTTGAA ATCTGGAACTGCCTCTGTTGTGTG CCTGCTGAATAACTTCTATCCCAG AGAGGCCAAAGTACAGTGGAAG GTGGATAACGCCCTCCAATCGGG TAACTCCCAGGAGAGTGTCACAG AGCAGGACAGCAAGGACAGCAC CTACAGCCTCAGCAGCACCCTGA CGCTGAGCAAAGCAGACTACGAG AAACACAAAGTCTACGCCTGCGA AGTCACCCATCAGGGCCTGAGCT CGCCCGTCACAAAGAGCTTCAAC AGGGGAGAGTGT	Nucleic acid encoding {04-0561-F1} light chain Note: this nucleic acid sequence does not comprise the sequence encoding the signal peptide. However, when a signal sequence is included, one embodiment of the nucleic acid begins at the 5' end with the sequence, ATGGAGACAGACACACTCCTGCT ATGGGTACTGCTGCTCTGGGTTCC AGGCTCCACCGGC, which encodes a signal peptide with an amino acid sequence, METDTLLLWVLLLWVPGSTG. This signal peptide at the amino terminus is cleave off from the nascent protein to produce a mature immunoglobulin light chain.
55	CAAGTTCAATTGCAAGAAAGCGG CCCAGGGTTGGTTAAACCTTCCG AAACTTTGTCCCTTACTTGTACGG	Antibody 04-0561-F1 heavy chain DNA sequence

	TTTCTGGATTTTCACTCAGTATAT ATTCTGTACATTGGGTAAGACAA CCACCAGGTAAAGGGCTCGAATG GATTGGACTTATTTGGGGTGGCG GGAGTACAGATTATAATCCTAGT TTGAAATCAAGAGTTACCATATC TAAAGATACATCTAAGAATCAAG TTTCCTTGAAGCTCTCATCCGTCA CTGCAGCGGATACAGCTGTCTAT TATTGTGCTCGTAAAGAAGGGAA TTATTTGTGGTTTGCTTATTGGGG ACAAGGGACTCTTGTCACAGTTA GTTCTGCTAGCACCAAGGGCCCA TCGGTCTTCCCCCTGGCACCCTCC TCCAAGAGCACCTCTGGGGGCAC AGCGGCCCTGGGCTGCCTGGTCA AGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGC CCTGACCAGCGGCGTGCACACCT TCCCGGCTGTCCTACAGTCCTCAG GACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTT GGGCACCCAGACCTACATCTGCA ACGTGAATCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAAGTTGA GCCCAAATCTTGTGACAAAACTC ACACATGCCCACCGTGCCCAGCA CCTGAACTCCTGGGGGGACCGTC AGTCTTCCTCTTCCCCCCAAAACC CAAGGACACCCTCATGATCTCCC GGACCCCTGAGGTCACATGCGTG GTGGTGGACGTGAGCCACGAAGA CCCTGAGGTCAAGTTCAACTGGT ACGTGGACGGCGTGGAGGTGCAT AATGCCAAGACAAAGCCGCGGGA GGAGCAGTACAACAGCACGTACC GGGTGGTCAGCGTCCTCACCGTC CTGCACCAGGACTGGCTGAATGG CAAGGAGTACAAGTGCAAGGTCT CCAACAAAGCCCTCCCAGCCCCC ATCGAGAAAACCATCTCCAAAGC CAAAGGGCAGCCCCGAGAACCAC AGGTGTACACCCTGCCCCCATCC CGGGATGAGCTGACCAAGAACCA GGTCAGCCTGACCTGCCTGGTCA AAGGCTTCTATCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGG GCAGCCGGAGAACAACTACAAGA CCACGCCTCCCGTGCTGGACTCC GACGGCTCCTTCTTCCTCTACAGC AAGCTCACCGTGGACAAGAGCAG GTGGCAGCAGGGGAACGTCTTCT CATGCTCCGTGATGCATGAGGCT	Note: this nucleic acid sequence does not comprise the sequence encoding the signal peptide. However, when a signal sequence is included, one embodiment of the nucleic acid begins at the 5' end with the sequence, ATGGAGTTTGGGCTGAGCTGGG TTTTCCTCGTTGCTCTTTTTAGA GGTGTCCAGTGT, which encodes a signal peptide with an amino acid sequence, MEFGLSWVFLVALFRGVQC. This signal peptide at the amino terminus is cleave off from the nascent protein to produce a mature immunoglobulin heavy chain.

	CTGCACAACCACTACACGCAGAA GAGCCTCTCCCTGTCTCCGGGTAA ATGA	
56	GACATCGTGATGACCCAGAGCCC CGACAGCCTGGCCGTGAGCCTGG GCGAGAGGGTGACCATGAACTGC AAGAGCAGCCAGAGCCTGCTGCA GAGCAGCAACCAGAAGAACTACC TGGCCTGGTACCAGCAGAAGCCC GGCCAGCCCCCCAAGCTGCTGGT GTACTTCGCCAGCACCAGGGAGA GCGGCGTGCCCGACAGGTTCAGC GGCAGCGGCAGCGGCACCGACTT CACCCTGACCATCAGCAGCGTGC AGGCCGAGGACGTGGCCGTGTAC TACTGCCAGCAGCACTACAGCAT CCCCCTGACCTTCGGCCAGGGCA CCAAGCTGGAGATCAAGCGTACG GTGGCGGCGCCATCTGTCTTCATC TTCCCGCCATCTGATGAGCAGTTG AAATCTGGAACTGCCTCTGTTGTG TGCCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGA AGGTGGATAACGCCCTCCAATCG GGTAACTCCCAGGAGAGTGTCAC AGAGCAGGACAGCAAGGACAGC ACCTACAGCCTCAGCAGCACCCT GACGCTGAGCAAAGCAGACTACG AGAAACACAAAGTCTACGCCTGC GAAGTCACCCATCAGGGCCTGAG CTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTTAG	Antibody 04-0561-F1 light chain DNA sequence Note: this nucleic acid sequence does not comprise the sequence encoding the signal peptide. However, when a signal sequence is included, one embodiment of the nucleic acid begins at the 5' end with the sequence, ATGGACATGAGGGTCCCTGCTC AGCTCCTGGGGCTCCTGCTGCT CTGGCTCTCAGGTGCCAGATGT, which encodes a signal peptide with an amino acid sequence, MDMRVPAQLLGLLLLWLSGARC. This signal peptide at the amino terminus is cleave off from the nascent protein to produce a mature immunoglobulin light chain.
57	DIQMTQSPSSLSASVGDRVTITCRA SHDVSTAVAWYQQKPGKAPKLLIY SASYRYTGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQHYRIPLTFG QGTKLEIKGGGGSGGGGSGGGGSE VQLVESGGGLVQPGGSLRLSCAAS GFSISDYYMAWVRQAPGKGLEWV ANINYDGTNTYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYY CVRSYYYYGMEYWGQGTTVTVSS	04-0547-h2scfv, single chain Fv of 04-0547-h2 DLK1 antibody. The scFv may optionally further comprise at its C-terminus a $(His)_6$-epitope tag.
58	DIVMTQSPDSLAVSLGERVTMNCK SSQSLLQSSNQKNYLAWYQQKPG QPPKLLVYFASTRESGVPDRFSGSG SGTDFTLTISSVQAEDVAVYYCQQ HYSIPLTFGQGTKLEIKGGGGSGGG GSGGGGSQVQLQESGPGLVKPSET LSLTCTVSGFSLSIYSVHWVRQPPG KGLEWIGLIWGGGSTDYNPSLKSR VTISKDTSKNQVSLKLSSVTAADTA VYYCARKEGNYLWFAYWGQGTL VTVSS	04-0561-F1scfv, single chain Fv of 04-0561-F1 DLK1 antibody. The scFv may optionally further comprise at its C-terminus a $(His)_6$-epitope tag.

59	DIVMTQSPLSLPVTPGEPASISCRSS QSLVHINGNTYLHWYLQKPGQSPQ LLIYKVSNRFSGVPDRFSGSGSGTD FTLKISRVEAEDVGVYYCSQTTHV PWTFGQGTKLEIKGGGGSGGGGSG GGGSQVQLQESGPGLVKPSETLSLT CTVSGFSLTSYGVSWVRQPPGKGL EWIGVIWGDGSTSYNPSLKSRVTIS KDTSKNQVSLKLSSVTAADTAVYY CAKPDGPLGQGTLVTVSS	04-0562-h10scfv, single chain Fv of 04-0562-h10 DLK1 antibody. The scFv may optionally further comprise at its C-terminus a $(His)_6$-epitope tag.
60	DIQMTQSPSSLSASVGDRVTITCRA SHDVSTAVAWYQQKPGKAPKLLIY SASYRYTGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQHYRIPLTFG QGTKLEIKGGGGSGGGGSGGGGSE VQLVESGGGLVQPGGSLRLSCAAS GFSISDYYMAWVRQAPGKGLEWV ANINYDGTNTYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYY CVRSYYYYGMEYWGQGTTVTVSS GGGGSDIKLQQSGAELARPGASVK MSCKTSGYTFTRYTMHWVKQRPG QGLEWIGYINPSRGYTNYNQKFKD KATLTTDKSSSTAYMQLSSLTSEDS AVYYCARYYDDHYCLDYWGQGT TLTVSSVEGGSGGSGGSGGSGGVD DIQLTQSPAIMSASPGEKVTMTCRA SSSVSYMNWYQQKSGTSPKRWIY DTSKVASGVPYRFSGSGSGTSYSLT ISSMEAEDAATYYCQQWSSNPLTF GAGTKLELK	04-0547-h2Bs, anti-DLK1-CD3 bispecific T-cell engager (BiTE) comprising 04-0547-h2scfv. and anti-CD3 scFv The bispecific antigen-binding protein or the BiTE may optionally further comprise a $(His)_6$-epitope tag at its C-terminus.
61	DIVMTQSPDSLAVSLGERVTMNCK SSQSLLQSSNQKNYLAWYQQKPG QPPKLLVYFASTRESGVPDRFSGSG SGTDFTLTISSVQAEDVAVYYCQQ HYSIPLTFGQGTKLEIKGGGGSGGG GSGGGGSQVQLQESGPGLVKPSET LSLTCTVSGFSLSIYSVHWVRQPPG KGLEWIGLIWGGGSTDYNPSLKSR VTISKDTSKNQVSLKLSSVTAADTA VYYCARKEGNYLWFAYWGQGTL VTVSSGGGGSDIKLQQSGAELARP GASVKMSCKTSGYTFTRYTMHWV KQRPGQGLEWIGYINPSRGYTNYN QKFKDKATLTTDKSSSTAYMQLSS LTSEDSAVYYCARYYDDHYCLDY WGQGTTLTVSSVEGGSGGSGGSGG SGGVDDIQLTQSPAIMSASPGEKVT MTCRASSSVSYMNWYQQKSGTSP KRWIYDTSKVASGVPYRFSGSGSG TSYSLTISSMEAEDAATYYCQQWS SNPLTFGAGTKLELK	04-0561-F1Bs, anti-DLK1-CD3 bispecific T-cell engager (BiTE) comprising 04-0561-F1scfv and anti-CD3 scFv. The bispecific antigen-binding protein or the BiTE may optionally further comprise a $(His)_6$-epitope tag at its C-terminus.

62	DIVMTQSPLSLPVTPGEPASISCRSS QSLVHINGNTYLHWYLQKPGQSPQ LLIYKVSNRFSGVPDRFSGSGSGTD FTLKISRVEAEDVGVYYCSQTTHV PWTFGQGTKLEIKGGGGSGGGGSG GGGSQVQLQESGPGLVKPSETLSLT CTVSGFSLTSYGVSWVRQPPGKGL EWIGVIWGDGSTSYNPSLKSRVTIS KDTSKNQVSLKLSSVTAADTAVYY CAKPDGPLGQGTLVTVSSGGGGSD IKLQQSGAELARPGASVKMSCKTS GYTFTRYTMHWVKQRPGQGLEWI GYINPSRGYTNYNQKFKDKATLTT DKSSSTAYMQLSSLTSEDSAVYYC ARYYDDHYCLDYWGQGTTLTVSS VEGGSGGSGGSGGSGGVDDIQLTQ SPAIMSASPGEKVTMTCRASSSVSY MNWYQQKSGTSPKRWIYDTSKVA SGVPYRFSGSGSGTSYSLTISSMEA EDAATYYCQQWSSNPLTFGAGTKL ELK	04-0562-h10Bs, anti- DLK1-CD3 bispecific T-cell engager (BiTE) comprising 04-0562-h10scfv and anti-CD3 scFv The bispecific antigen-binding protein or the BiTE may optionally further comprise a $(His)_6$-epitope tag at its C-terminus.
63	GACATCCAGATGACACAGAGCCC TAGCAGCCTGTCTGCCAGCGTGG GAGACAGAGTGACCATCACCTGT AGAGCCAGCCACGATGTGTCTAC AGCCGTGGCCTGGTATCAGCAGA AGCCTGGAAAGGCCCCTAAGCTG CTGATCTACAGCGCCAGCTACAG ATACACCGGCGTGCCCAGCAGAT TTTCTGGCAGCGGCTCTGGCACC GACTTCACCCTGACCATATCTAGC CTGCAGCCTGAGGACTTCGCCAC CTACTACTGCCAGCAGCACTACA GAATCCCTCTGACCTTTGGCCAG GGCACCAAGCTGGAAATCAAGGG TGGTGGTGGTTCTGGAGGAGGAG GATCTGGAGGGGGGGGTCCGAG GTGCAGCTGGTTGAATCTGGCGG AGGACTGGTTCAGCCTGGCGGAT CTCTGAGACTGTCTTGTGCCGCCA GCGGCTTCAGCATCAGCGACTAC TATATGGCCTGGGTCCGACAGGC CCCTGGCAAAGGACTTGAGTGGG TCGCCAACATCAACTACGACGGC ACCAACACCTACTACGCCGACAG CGTGAAGGGCAGATTCACCATCA GCCGGGACAACAGCAAGAACACC CTGTACCTGCAGATGAACAGCCT GAGAGCCGAGGACACCGCCGTGT ACTATTGTGTGCGGAGCTACTACT ATTACGGCATGGAATACTGGGGC CAGGGCACCACCGTGACAGTCTC TTCT	DNA encoding 04-0547-h2scfv

64	GACATCGTGATGACCCAGAGCCC CGACAGCCTGGCCGTGAGCCTGG GCGAGAGGGTGACCATGAACTGC AAGAGCAGCCAGAGCCTGCTGCA GAGCAGCAACCAGAAGAACTACC TGGCCTGGTACCAGCAGAAGCCC GGCCAGCCCCCCAAGCTGCTGGT GTACTTCGCCAGCACCAGGGAGA GCGGCGTGCCCGACAGGTTCAGC GGCAGCGGCAGCGGCACCGACTT CACCCTGACCATCAGCAGCGTGC AGGCCGAGGACGTGGCCGTGTAC TACTGCCAGCAGCACTACAGCAT CCCCCTGACCTTCGGCCAGGGCA CCAAGCTGGAGATCAAGGGTGGT GGTGGTTCTGGAGGAGGAGGATC TGGAGGGGGGGGGTCCCAAGTTC AATTGCAAGAAAGCGGCCCAGGG TTGGTTAAACCTTCCGAAACTTTG TCCCTTACTTGTACGGTTTCTGGA TTTTCACTCAGTATATATTCTGTA CATTGGGTAAGACAACCACCAGG TAAAGGGCTCGAATGGATTGGAC TTATTTGGGGTGGCGGGAGTACA GATTATAATCCTAGTTTGAAATCA AGAGTTACCATATCTAAAGATAC ATCTAAGAATCAAGTTTCCTTGA AGCTCTCATCCGTCACTGCAGCG GATACAGCTGTCTATTATTGTGCT CGTAAAGAAGGGAATTATTTGTG GTTTGCTTATTGGGGACAAGGGA CTCTTGTCACAGTTAGTTCT	DNA encoding 04-0561-F1scfv
65	GACATCGTGATGACACAGAGCCC TCTGAGCCTGCCTGTGACACCTG GCGAACCTGCCAGCATCAGCTGT AGAAGCAGCCAGAGCCTGGTGCA CATCAACGGCAACACCTACCTGC ACTGGTATCTGCAGAAGCCCGGC CAGTCTCCTCAGCTGCTGATCTAC AAGGTGTCCAACCGGTTCAGCGG CGTGCCCGATAGATTTTCTGGCA GCGGCTCTGGCACCGACTTCACC CTGAAGATCTCCAGAGTGGAAGC CGAGGACGTGGGCGTGTACTACT GTAGCCAGACCACACACGTGCCC TGGACATTTGGACAGGGCACCAA GCTGGAAATCAAGGGTGGTGGTG GTTCTGGAGGAGGAGGATCTGGA GGGGGGGGGTCCCAGGTTCAGCT GCAAGAGTCTGGCCCTGGCCTGG TCAAGCCTAGCGAAACACTGAGC CTGACCTGTACCGTGTCCGGCTTT AGCCTGACAAGCTACGGCGTGTC	DNA encoding 04-0562-h10scfv

	CTGGGTCCGACAGCCTCCTGGAA AAGGCCTGGAATGGATCGGAGTG ATCTGGGGCGACGGCAGCACCAG CTATAACCCTAGCCTGAAGTCCA GAGTGACCATCAGCAAGGACACC AGCAAGAACCAGGTGTCCCTGAA GCTGAGCAGCGTGACAGCCGCTG ATACCGCCGTGTACTACTGCGCC AAACCTGATGGACCTCTCGGCCA GGGAACACTGGTCACAGTCTCTT CT	
66	GACATCCAGATGACACAGAGCCC TAGCAGCCTGTCTGCCAGCGTGG GAGACAGAGTGACCATCACCTGT AGAGCCAGCCACGATGTGTCTAC AGCCGTGGCCTGGTATCAGCAGA AGCCTGGAAAGGCCCCTAAGCTG CTGATCTACAGCGCCAGCTACAG ATACACCGGCGTGCCCAGCAGAT TTTCTGGCAGCGGCTCTGGCACC GACTTCACCCTGACCATATCTAGC CTGCAGCCTGAGGACTTCGCCAC CTACTACTGCCAGCAGCACTACA GAATCCCTCTGACCTTTGGCCAG GGCACCAAGCTGGAAATCAAGGG TGGTGGTGGTTCTGGAGGAGGAG GATCTGGAGGGGGGGGGTCCGAG GTGCAGCTGGTTGAATCTGGCGG AGGACTGGTTCAGCCTGGCGGAT CTCTGAGACTGTCTTGTGCCGCCA GCGGCTTCAGCATCAGCGACTAC TATATGGCCTGGGTCCGACAGGC CCCTGGCAAAGGACTTGAGTGGG TCGCCAACATCAACTACGACGGC ACCAACACCTACTACGCCGACAG CGTGAAGGGCAGATTCACCATCA GCCGGGACAACAGCAAGAACACC CTGTACCTGCAGATGAACAGCCT GAGAGCCGAGGACACCGCCGTGT ACTATTGTGTGCGGAGCTACTACT ATTACGGCATGGAATACTGGGGC CAGGGCACCACCGTGACAGTCTC TTCTGGCGGCGGCGGCAGCGACA TCAAGCTGCAGCAGAGCGGCGCC GAGCTGGCCAGGCCCGGCGCCAG CGTGAAGATGAGCTGCAAGACCA GCGGCTACACCTTCACCAGGTAC ACCATGCACTGGGTGAAGCAGAG GCCCGGCCAGGGCCTGGAGTGGA TCGGCTACATCAACCCCAGCAGG GGCTACACCAACTACAACCAGAA GTTCAAGGACAAGGCCACCCTGA CCACCGACAAGAGCAGCAGCACC	DNA encoding 04-0547-h2Bs or an anti- DLK1-CD3 bispecific T-cell engager (BiTE)

	GCCTACATGCAGCTGAGCAGCCT GACCAGCGAGGACAGCGCCGTGT ACTACTGCGCCAGGTACTACGAC GACCACTACTGCCTGGACTACTG GGGCCAGGGCACCACCCTGACCG TGAGCAGCGTGGAGGGCGGCAGC GGCGGCAGCGGCGGCAGCGGCG GCAGCGGCGGCGTGGACGACATC CAGCTGACCCAGAGCCCCGCCAT CATGAGCGCCAGCCCCGGCGAGA AGGTGACCATGACCTGCAGGGCC AGCAGCAGCGTGAGCTACATGAA CTGGTACCAGCAGAAGAGCGGCA CCAGCCCCAAGAGGTGGATCTAC GACACCAGCAAGGTGGCCAGCGG CGTGCCCTACAGGTTCAGCGGCA GCGGCAGCGGCACCAGCTACAGC CTGACCATCAGCAGCATGGAGGC CGAGGACGCCGCCACCTACTACT GCCAGCAGTGGAGCAGCAACCCC CTGACCTTCGGCGCCGGCACCAA GCTGGAGCTGAAG	
67	GACATCGTGATGACCCAGAGCCCC GACAGCCTGGCCGTGAGCCTGGGC GAGAGGGTGACCATGAACTGCAAG AGCAGCCAGAGCCTGCTGCAGAGC AGCAACCAGAAGAACTACCTGGCC TGGTACCAGCAGAAGCCCGGCCAG CCCCCCAAGCTGCTGGTGTACTTCG CCAGCACCAGGGAGAGCGGCGTGC CCGACAGGTTCAGCGGCAGCGGCA GCGGCACCGACTTCACCCTGACCAT CAGCAGCGTGCAGGCCGAGGACGT GGCCGTGTACTACTGCCAGCAGCA CTACAGCATCCCCCTGACCTTCGGC CAGGGCACCAAGCTGGAGATCAAG GGTGGTGGTGGTTCTGGAGGAGGA GGATCTGGAGGGGGGGGGTCCCAA GTTCAATTGCAAGAAAGCGGCCCA GGGTTGGTTAAACCTTCCGAAACTT TGTCCCTTACTTGTACGGTTTCTGG ATTTTCACTCAGTATATATTCTGTA CATTGGGTAAGACAACCACCAGGT AAAGGGCTCGAATGGATTGGACTT ATTTGGGGTGGCGGGAGTACAGAT TATAATCCTAGTTTGAAATCAAGAG TTACCATATCTAAAGATACATCTAA GAATCAAGTTTCCTTGAAGCTCTCA TCCGTCACTGCAGCGGATACAGCT GTCTATTATTGTGCTCGTAAAGAAG GGAATTATTTGTGGTTTGCTTATTG GGGACAAGGGACTCTTGTCACAGT TAGTTCTGGCGGCGGCGGCAGCGA	DNA encoding 04-0561-F1Bs or an anti- DLK1-CD3 bispecific T-cell engager (BiTE)

	CATCAAGCTGCAGCAGAGCGGCGC CGAGCTGGCCAGGCCCGGCGCCAG CGTGAAGATGAGCTGCAAGACCAG CGGCTACACCTTCACCAGGTACACC ATGCACTGGGTGAAGCAGAGGCCC GGCCAGGGCCTGGAGTGGATCGGC TACATCAACCCCAGCAGGGGCTAC ACCAACTACAACCAGAAGTTCAAG GACAAGGCCACCCTGACCACCGAC AAGAGCAGCAGCACCGCCTACATG CAGCTGAGCAGCCTGACCAGCGAG GACAGCGCCGTGTACTACTGCGCC AGGTACTACGACGACCACTACTGC CTGGACTACTGGGGCCAGGGCACC ACCCTGACCGTGAGCAGCGTGGAG GGCGGCAGCGGCGGCAGCGGCGGC AGCGGCGGCAGCGGCGGCGTGGAC GACATCCAGCTGACCCAGAGCCCC GCCATCATGAGCGCCAGCCCCGGC GAGAAGGTGACCATGACCTGCAGG GCCAGCAGCAGCGTGAGCTACATG AACTGGTACCAGCAGAAGAGCGGC ACCAGCCCCAAGAGGTGGATCTAC GACACCAGCAAGGTGGCCAGCGGC GTGCCCTACAGGTTCAGCGGCAGC GGCAGCGGCACCAGCTACAGCCTG ACCATCAGCAGCATGGAGGCCGAG GACGCCGCCACCTACTACTGCCAG CAGTGGAGCAGCAACCCCCTGACC TTCGGCGCCGGCACCAAGCTGGAG CTGAAG	
68	GACATCGTGATGACACAGAGCCCT CTGAGCCTGCCTGTGACACCTGGCG AACCTGCCAGCATCAGCTGTAGAA GCAGCCAGAGCCTGGTGCACATCA ACGGCAACACCTACCTGCACTGGT ATCTGCAGAAGCCCGGCCAGTCTC CTCAGCTGCTGATCTACAAGGTGTC CAACCGGTTCAGCGGCGTGCCCGA TAGATTTTCTGGCAGCGGCTCTGGC ACCGACTTCACCCTGAAGATCTCCA GAGTGGAAGCCGAGGACGTGGGCG TGTACTACTGTAGCCAGACCACAC ACGTGCCCTGGACATTTGGACAGG GCACCAAGCTGGAAATCAAGGGTG GTGGTGGTTCTGGAGGAGGAGGAT CTGGAGGGGGGGGGTCCCAGGTTC AGCTGCAAGAGTCTGGCCCTGGCC TGGTCAAGCCTAGCGAAACACTGA GCCTGACCTGTACCGTGTCCGGCTT TAGCCTGACAAGCTACGGCGTGTC CTGGGTCCGACAGCCTCCTGGAAA AGGCCTGGAATGGATCGGAGTGAT	DNA encoding 04-0562-h10Bs or an anti- DLK1-CD3 bispecific T-cell engager (BiTE)

	CTGGGGCGACGGCAGCACCAGCTA TAACCCTAGCCTGAAGTCCAGAGT GACCATCAGCAAGGACACCAGCAA GAACCAGGTGTCCCTGAAGCTGAG CAGCGTGACAGCCGCTGATACCGC CGTGTACTACTGCGCCAAACCTGAT GGACCTCTCGGCCAGGGAACACTG GTCACAGTCTCTTCTGGCGGCGGCG GCAGCGACATCAAGCTGCAGCAGA GCGGCGCCGAGCTGGCCAGGCCCG GCGCCAGCGTGAAGATGAGCTGCA AGACCAGCGGCTACACCTTCACCA GGTACACCATGCACTGGGTGAAGC AGAGGCCCGGCCAGGGCCTGGAGT GGATCGGCTACATCAACCCCAGCA GGGGCTACACCAACTACAACCAGA AGTTCAAGGACAAGGCCACCCTGA CCACCGACAAGAGCAGCAGCACCG CCTACATGCAGCTGAGCAGCCTGA CCAGCGAGGACAGCGCCGTGTACT ACTGCGCCAGGTACTACGACGACC ACTACTGCCTGGACTACTGGGGCC AGGGCACCACCCTGACCGTGAGCA GCGTGGAGGGCGGCAGCGGCGGCA GCGGCGGCAGCGGCGGCAGCGGCG GCGTGGACGACATCCAGCTGACCC AGAGCCCCGCCATCATGAGCGCCA GCCCCGGCGAGAAGGTGACCATGA CCTGCAGGGCCAGCAGCAGCGTGA GCTACATGAACTGGTACCAGCAGA AGAGCGGCACCAGCCCCAAGAGGT GGATCTACGACACCAGCAAGGTGG CCAGCGGCGTGCCCTACAGGTTCA GCGGCAGCGGCAGCGGCACCAGCT ACAGCCTGACCATCAGCAGCATGG AGGCCGAGGACGCCGCCACCTACT ACTGCCAGCAGTGGAGCAGCAACC CCCTGACCTTCGGCGCCGGCACCA AGCTGGAGCTGAAG	
69	MTATEALLRVLLLLLAFGHSTYGAE CFPACNPQNGFCEDDNVCRCQPGWQ GPLCDQCVTSPGCLHGLCGEPGQCIC TDGWDGELCDRDVRACSSAPCANN RTCVSLDDGLYECSCAPGYSGKDCQ KKDGPCVINGSPCQHGGTCVDDEGR ASHASCLCPPGFSGNFCEIVANSCTP NPCENDGVCTDIGGDFRCRCPAGFID KTCSRPVTNCASSPCQNGGTCLQHT QGQAICFTILGVLTSLVVLGTVGIVFL NKCETWVSNLRYNHMLRKKKNLLL QYNSGEDLAVNIIFPEKIDMTTFSKEA GDEEI	Human DLK1 isoform 2 (UniProtKB Accession Number P80370-2) amino acid sequence

* The nucleic acid molecules comprising the sequences set forth in SEQ ID NOs: 53 or 55, while different at the nucleotide level, both encode the mature (secreted) heavy chain of 04-0561-F1. Similarly, the nucleic acid molecules comprising the sequences set forth in SEQ ID NOs: 54 and 56, while different, both encode the mature (secreted) light chain of 04-0561-F1. Missing from each coding sequence are the start codon (ATG) and sequence for signal peptide (which present in the nascently translated protein gets cleaved off from the mature protein). SEQ ID NOs 53 and 54 were codon-optimized. Accordingly, both SEQ ID NOs: 53 and 55 encode the identical heavy chain of 04-0561-F1; and both SEQ ID NOs: 54 and 56 encode the identical light chain of 04-0561-F1.

* The scFvs and bispecific antibodies (e.g., BiTEs) used herein further comprises a C-terminal $(His)_6$ epitope tag, which facilitated purification and detection.

* It is well understood in the art that a newly synthesized polypeptide destined for the secretory pathway (e.g., a protein that is secreted or inserted into a membrane) comprises a signal peptide. Thus, any one of the polypeptides presented herein may further comprise a signal peptide, variations of amino acid sequences of which are well known in the art.

Table 2: Alternative DLK1 antibody names and expression vectors for production of DLK1 antibodies

Antibody	**Antibody name (m: mouse VHVL)**	**Antibody expression vector name**
chimeric anti-DLK1 antibody*	04-0547m (also called 04-547m, 04-0547-m, 04-547-m, or DLK1-547)	p04-0547m (also called p04-547m, p04-0547-m or p04-547-m)
chimeric anti-DLK1 antibody*	04-0548m (also called 04-548m, 04-0548-m, 04-548-m or DLK1-548)	p04-0548m (also called p04-548m, p04-0548-m or p04-548-m)
chimeric anti-DLK1 antibody*	04-0557m (also called 04-557m, 04-0557-m, 04-557-m or DLK1-557)	p04-0557m (also called p04-557m, p04-0557-m or p04-557-m)
chimeric anti-DLK1 antibody*	04-0559m (also called 04-559m, 04-0559-m, 04-	p04-0559m (also called p04-559m, p04-0559-m or p04-559-m)

	559-m or DLK1-559)	
chimeric anti-DLK1 antibody*	04-0561-m (also called 04-561m, 04-0561-m, 04-561-m or DLK1-561)	p04-0561-m (also called p04-561m, p04-0561-m, p04-561-m)
humanized anti-DLK1 antibody (IgG1)	04-0547-h2 (also called 04-547-h2, 547-h2, mab-547-h2, DLK1-mab-547-h2 or DLK1-547-h2)	p04-0547-h2 (also called p04-547-h2, p562-h2, pmab-547-h2, pDLK1-mab-547-h2 or pDLK1-547-h2)
humanized anti-DLK1 antibody (IgG1)	04-0548-h4 (also called 04-548-h4, 548-h4, mab-548-h4, DLK1-mab-548-h4 or DLK1-548-h4)	p04-0548-h4 (also called p04-548-h4, p548-h4, pmab-548-h4, pDLK1-mab-548-h4 or pDLK1-548-h4)
humanized anti-DLK1 antibody (IgG1)	04-0557-F2 (also called 04-557-F2, 557-F2, mab-557-F2, DLK1-mab-557-F2 or DLK1-557-F2)	p04-0557-F2 ((also called p04-557-F2, p557-F2, pmab-557-F2, pDLK1-mab-557-F2 or pDLK1-557-F2)
humanized anti-DLK1 antibody (IgG1)	04-0557-h3 (also called 04-557-h3, 557-h3, mab-557-h3, DLK1-mab-557-h3 or DLK1-557-h3)	p04-0557-h3 (also called p04-557-h3, p557-h3, pmab-557-h3, pDLK1-mab-557-h3 or pDLK1-557-h3)
humanized anti-DLK1 antibody (IgG1)	04-0559-F2 (also called 04-559-F2, 559-F2, mab-559-F2, DLK1-mab-559-F2, DLK1-559-F2 or DLK1-559-f2)	p04-0559-F2 (also called p04-559-F2, p559-F2, pmab-559-F2, pDLK1-mab-559-F2, pDLK1-559-F2, or pDLK1-559-f2)
humanized anti-DLK1 antibody (IgG1)	04-0559-h4 (also called 04-559-h4, 559-h4, mab-559-h4, DLK1-mab-	p04-0559-h4 (also called p04-559-h4, p559-h4, pmab-559-h4, pDLK1-mab-559-h4 or pDLK1-559-h4)

	559-h4 or DLK1-559-h4)	
humanized anti-DLK1 antibody (IgG1)	04-0561-F1 (also called 04-561-F1, 561-F1, mab-561-F1, DLK1-mab-561-F1, DLK1-561-F1 or DLK1-561-f1)	p04-0561-F1 (also called p04-561-F1, p561-F1, pmab-561-F1, pDLK1-mab-561-F1, pDLK1-561-F1 or pDLK1-561-f1)
humanized anti-DLK1 antibody (IgG1)	04-0562-h10 (also called 04-562-h10, 562-h10, mab-562-h10, DLK1-mab-562-h10 or DLK1-562-h10)	p04-0562-h10 (also called p04-562-h10, p562-h10, pmab-562-h10, pDLK1-mab-562-h10 or pDLK1-562-h10)
humanized anti-DLK1 antibody (IgG1)	04-0565-F2 (also called 04-565-F2, 565-F2, mab-565-F2, DLK1-mab-565-F2, DLK1-565-F2 or DLK1-565-f2)	p04-0565-F2 (also called p04-565-F2, p565-F2, pmab-565-F2, pDLK1-mab-565-F2, pDLK1-565-F2 or pDLK1-565-f2))
	LegoChem 18A5 (also called 18A5)	pLegoChem_18A5
	Li-DLK1-hIgG1	pLi-DLK1-hIgG1
	Ko_DLK1-hIgG1 (also called KO_DLK1-hIgG1)	pKo_DLK1-hIgG1 (also called pKO_DLK1-hIgG1)
non-targeting human IgG1 negative control antibody	hIgG1 (also called hIgG, Hu-IgG1)	phIgG1 (also called phIgG or pHu-IgG1)
scFv comprising light and heavy chain variable regions of 04-0547-h2 antibody	04-0547-h2scFv (also called DLK1-547-h2scfv)	p04-0547-h2scFv (also called pDLK1-547-h2scfv)
scFv comprising light and heavy chain variable regions of 04-0561-F1 antibody	04-0561-F1scFv (also called DLK1-561-F1scfv)	p04-0561-F1scFv (also called pDLK1-561-F1scfv)

scFv comprising light and heavy chain variable regions of 04-0562-h10 antibody	04-0562-h10scFv (also called DLK1-562-h10scfv)	p04-0562-h10scFv (also called pDLK1-562-h10scfv)
bispecific T-cell engager (BiTE) Fv comprising 04-0547-h2scFv antigen-binding portion and scFv that binds CD3 on surface of T cells	04-0547-h2Bs (also called DLK1-547-h2Bs)	p04-0547-h2Bs (also called pDLK1-547-h2Bs)
bispecific T-cell engager (BiTE) Fv comprising 04-0561-F1scFv antigen-binding portion and scFv that binds CD3 on surface of T cells	04-0561-F1Bs (also called DLK1-561-F1Bs)	p04-0561-F1Bs (also called pDLK1-561-F1Bs)
bispecific T-cell engager (BiTE) Fv comprising 04-0562-h10scFv antigen-binding portion and scFv that binds CD3 on surface of T cells	04-0562-h10Bs (also called DLK1-562-h10Bs)	p04-0562-h10Bs (also called pDLK1-562-h10Bs)

* chimeric anti-DLK1 antibody comprises mouse light and heavy chain variable regions and human IgG1 constant region, where human IgG1 light and heavy chain variable regions were replaced with the variable regions of mouse anti-DLK1 antibody.

Table 3: DLK1 antibody conjugates

Antibody Conjugate@	**Description**
04-0547-h2-MMAE (also called DLK1-ADC-547-h2)	04-0547-h2 antibody conjugated to MMAE
04-0561-F1-MMAE (also called DLK1-ADC-561-f1)	04-0561-F1 antibody conjugated to MMAE
04-0562-h10-MMAE (also called DLK1-ADC-562-h10)	04-0562-h10 antibody conjugated to MMAE
04-0547-h2-MMAE-AF647	04-0547-h2 antibody conjugated to both MMAE and Alexa Fluor® 647

04-0561-F1-MMAE-AF647	04-0561-F1 antibody conjugated to both MMAE and Alexa Fluor® 647
04-0562-h10-MMAE-AF647	04-0562-h10 antibody conjugated to both MMAE and Alexa Fluor® 647
04-0561-F1-AF647 (also called 561-F1-AF647, DLK1-561-F1-AF647)	04-0561-F1 conjugated to Alexa Fluor® 647
LegoChem 18A5-AF647	LegoChem 18A5 antibody conjugated to Alexa Fluor® 647
hIgG1-AF647 (negative control antibody conjugate)	Human IgG1 antibody conjugated to Alexa Fluor® 647

@MMAE conjugation is performed at a free thiol group of a cysteine residue using MC-VC-PAB-MMAE.

WHAT IS CLAIMED IS:

1. An antigen-binding protein comprising:
    a. CDRs 1-3 derived from a heavy chain variable region comprising the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAASGFSISDYYMAWVRQAPG KGLEWVANINYDGTNTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCVRSYYYYGMEYWGQGTTVTVSS (SEQ ID NO: 45) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity; and/or
    b. CDRs 1-3 derived from a light chain variable region comprising the amino acid sequence: DIQMTQSPSSLSASVGDRVTITCRASHDVSTAVAWYQQKPGK APKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQHYRIPLTFGQGTKLEIK (SEQ ID NO: 46) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity.

2. An antigen-binding protein comprising:
    a. CDRs 1-3 derived from a heavy chain variable region comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVSGFSLSIYSVHWVRQPPGK GLEWIGLIWGGGSTDYNPSLKSRVTISKDTSKNQVSLKLSSVT AADTAVYYCARKEGNYLWFAYWGQGTLVTVSS (SEQ ID NO: 47) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity; and/or
    b. CDRs 1-3 derived from a light chain variable region comprising the amino acid sequence: DIVMTQSPDSLAVSLGERVTMNCKSSQSLLQSSNQKNYLAWY QQKPGQPPKLLVYFASTRESGVPDRFSGSGSGTDFTLTISSVQA EDVAVYYCQQHYSIPLTFGQGTKLEIK (SEQ ID NO: 48) or a

variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity.

3. An antigen-binding protein comprising:
    a. CDRs 1-3 derived from a heavy chain variable region comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVSWVRQPPGK GLEWIGVIWGDGSTSYNPSLKSRVTISKDTSKNQVSLKLSSVT AADTAVYYCAKPDGPLGQGTLVTVSS (SEQ ID NO: 49) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity; and/or
    b. CDRs 1-3 derived from a light chain variable region comprising the amino acid sequence: DIVMTQSPLSLPVTPGEPASISCRSSQSLVHINGNTYLHWYLQK PGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYCSQTTHVPWTFGQGTKLEIK (SEQ ID NO: 50) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 70% sequence identity.

4. An antigen-binding protein comprising:
    a. a heavy chain CDR1 comprising the amino acid sequence of: GFSISDYY (SEQ ID NO: 1) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
    b. a heavy chain CDR2 comprising the amino acid sequence of:: INYDGTNT (SEQ ID NO: 2) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
    c. a heavy chain CDR3 comprising the amino acid sequence of: VRSYYYYGMEY (SEQ ID NO: 3) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

d. a light chain CDR1 comprising the amino acid sequence of: HDVSTA (SEQ ID NO: 4) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
e. a light chain CDR2 comprising the amino acid sequence of: SAS (SEQ ID NO: 5) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
f. a light chain CDR3 comprising the amino acid sequence of: QQHYRIPLT (SEQ ID NO: 6) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; or
g. a combination of any two or moreof (a)-(f).

5. An antigen-binding protein comprising:
a. a heavy chain CDR1 comprising the amino acid sequence of: GFSLSIYS (SEQ ID NO: 7) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
b. a heavy chain CDR2 amino acid sequence of: IWGGGST (SEQ ID NO: 8) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
c. a heavy chain CDR3 comprising the amino acid sequence of: ARKEGNYLWFAY (SEQ ID NO: 9) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
d. a light chain CDR1 comprising the amino acid sequence of: QSLLQSSNQKNY (SEQ ID NO: 10) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;
e. a light chain CDR2 comprising the amino acid sequence of: FAS (SEQ ID NO: 11) or a variant sequence thereof which differs by

only one or two amino acids or which has at least or about 70% sequence identity;

f. a light chain CDR3 amino acid sequence of: QQHYSIPLT (SEQ ID NO: 12) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; or

g. a combination of any two or moreof (a)-(f).

6. An antigen-binding protein comprising:

a. a heavy chain CDR1 amino acid sequence of: GFSLTSYG (SEQ ID NO: 13) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

b. a heavy chain CDR2 comprising the amino acid sequence of: IWGDGST (SEQ ID NO: 14) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

c. a heavy chain CDR3 comprising the amino acid sequence of: AKPDGP (SEQ ID NO: 15) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

d. a light chain CDR1 comprising the amino acid sequence of: QSLVHINGNTY (SEQ ID NO: 16) a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

e. a light chain CDR2 amino acid sequence of: KVS (SEQ ID NO: 17) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity;

f. a light chain CDR3 comprising the amino acid sequence of: SQTTHVPWT (SEQ ID NO: 18) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; or

g. a combination of any two or moreof (a)-(f).

7. The antigen-binding protein of any one of claims 1-7, wherein the variant sequence has at least about 80%, at least about 85%, at least about 90% sequence identity, or at least or about 95% sequence identity.

8. The antigen-binding protein of claim 4, additionally comprising:
    a. a heavy chain FR1 comprising the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO: 21) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70%, 80%, 85%, 90% or 95% sequence identity;
    b. a heavy chain FR2 comprising the amino acid sequence: MAWVRQAPGKGLEWVAN (SEQ ID NO: 22) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70%, 80%, 85%, 90% or 95% sequence identity;
    c. a heavy chain FR3 comprising the amino acid sequence: YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC (SEQ ID NO: 23) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;
    d. a heavy chain FR4 comprising the amino acid sequence: WGQGTTVTVSS (SEQ ID NO: 24) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;
    e. a light chain FR1 comprising the amino acid sequence: DIQMTQSPSSLSASVGDRVTITCRAS (SEQ ID NO: 25) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;
    f. a light chain FR2 comprising the amino acid sequence: VAWYQQKPGKAPKLLIY (SEQ ID NO: 26) or a variant

sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

g. a light chain FR3 comprising the amino acid sequence: YRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO: 27) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

h. a light chain FR4 comprising the amino acid sequence: FGQGTKLEIK (SEQ ID NO: 28) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 785%, 90% or 95% sequence identity; or

i. a combination of any two or moreof (a)-(h).

9. The antigen-binding protein of claim 5, additionally comprising:

a. a heavy chain FR1 comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVS (SEQ ID NO: 29) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

b. a heavy chain FR2 comprising the amino acid sequence: VHWVRQPPGKGLEWIGL (SEQ ID NO: 30) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

c. a heavy chain FR3 comprising the amino acid sequence: DYNPSLKSRVTISKDTSKNQVSLKLSSVTAADTAVYYC (SEQ ID NO: 31) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

d. a heavy chain FR4 comprising the amino acid sequence: WGQGTLVTVSS (SEQ ID NO: 32) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

e. a light chain FR1 comprising the amino acid sequence: DIVMTQSPDSLAVSLGERVTMNCKSS (SEQ ID NO: 33) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;
f. a light chain FR2 comprising the amino acid sequence: LAWYQQKPGQPPKLLVY (SEQ ID NO: 34) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;
g. a light chain FR3 comprising the amino acid sequence: TRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC (SEQ ID NO: 35) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;
h. a light chain FR4 comprising the amino acid sequence of: FGQGTKLEIK (SEQ ID NO: 36) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity; or
i. a combination of any two or moreof (a)-(h).

10. The antigen-binding protein of claim 6, additionally comprising:
a. a heavy chain FR1 comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVS (SEQ ID NO: 37) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;
b. a heavy chain FR2 comprising the amino acid sequence of: VSWVRQPPGKGLEWIGV (SEQ ID NO: 38) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;
c. a heavy chain FR3 comprising the amino acid sequence: SYNPSLKSRVTISKDTSKNQVSLKLSSVTAADTAVYYC (SEQ ID NO: 39) or a variant sequence thereof which differs by only one or

two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

d. a heavy chain FR4 comprising the amino acid sequence: LGQGTLVTVSS (SEQ ID NO: 40) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

e. a light chain FR1 comprising the amino acid sequence: DIVMTQSPLSLPVTPGEPASISCRSS (SEQ ID NO: 41) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

f. a light chain FR2 comprising the amino acid sequence: LHWYLQKPGQSPQLLIY (SEQ ID NO: 42) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

g. a light chain FR3 comprising the amino acid sequence: NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC (SEQ ID NO: 43) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity;

h. a light chain FR4 amino acid sequence of: FGQGTKLEIK (SEQ ID NO: 44) or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 85%, 90% or 95% sequence identity; or

i. a combination of any two or moreof (a)-(h).

11. The antigen-binding protein of any one of claims 1, 4, 7, and 8 comprising:

a. a heavy chain variable region comprising the amino acid sequence of: EVQLVESGGGLVQPGGSLRLSCAASGFSISDYYMAWVRQAPG KGLEWVANINYDGTNTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCVRSYYYYGMEYWGQGTTVTVSS (SEQ ID

NO: 45) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity; and/or

b. a light chain variable region comprising the amino acid sequence: DIQMTQSPSSLSASVGDRVTITCRASHDVSTAVAWYQQKPGK APKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQHYRIPLTFGQGTKLEIK (SEQ ID NO: 46) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity.

12. The antigen-binding protein of any one of claims 2, 5, 7, and 9 comprising:

a. a heavy chain variable region comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVSGFSLSIYSVHWVRQPPGK GLEWIGLIWGGGSTDYNPSLKSRVTISKDTSKNQVSLKLSSVT AADTAVYYCARKEGNYLWFAYWGQGTLVTVSS (SEQ ID NO: 47) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity; and/or

b. a light chain variable region comprising the amino acid sequence: DIVMTQSPDSLAVSLGERVTMNCKSSQSLLQSSNQKNYLAWY QQKPGQPPKLLVYFASTRESGVPDRFSGSGSGTDFTLTISSVQA EDVAVYYCQQHYSIPLTFGQGTKLEIK (SEQ ID NO: 48) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity.

13. An antigen-binding protein of any one of claims 3, 6, 7, and 10 comprising:

a. a heavy chain variable region comprising the amino acid sequence: QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVSWVRQPPGK GLEWIGVIWGDGSTSYNPSLKSRVTISKDTSKNQVSLKLSSVT

AADTAVYYCAKPDGPLGQGTLVTVSS (SEQ ID NO: 49) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity; and/or

b. a light chain variable region comprising the amino acid sequence: DIVMTQSPLSLPVTPGEPASISCRSSQSLVHINGNTYLHWYLQK PGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYCSQTTHVPWTFGQGTKLEIK (SEQ ID NO: 50) or a variant sequence thereof which differs by only 1-5 amino acids or which has at least or about 85%, 90%, 95%, 98% or 99% sequence identity.

14. An antigen-binding protein that specifically binds to human Delta Like Non-Canonical Notch Ligand 1 (DLK1) comprising:

a. an antibody heavy chain comprising the amino acid sequence of: QVQLQESGPGLVKPSETLSLTCTVSGFSLSIYSVHWVRQPPGKGL EWIGLIWGGGSTDYNPSLKSRVTISKDTSKNQVSLKLSSVTAADT AVYYCARKEGNYLWFAYWGQGTLVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO: 51); and

b. an antibody light chain comprising the amino acid sequence of: DIVMTQSPDSLAVSLGERVTMNCKSSQSLLQSSNQKNYLAWYQ QKPGQPPKLLVYFASTRESGVPDRFSGSGSGTDFTLTISSVQAED VAVYYCQQHYSIPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 52).

15. The antigen-binding protein of any one of the previous claims, wherein:
    a. the antigen-binding protein binds to a human DLK1 protein isoform-1 (UniProtKB ID: P80370-1) having an amino acid sequence according to SEQ ID NO: 19;
    b. the antigen-binding protein binds to a human DLK1 protein isoform-2 (UniProtKB ID: P80370-2) having an amino acid sequence according to SEQ ID NO: 69;
    c. the antigen-binding protein binds an extracellular domain of human DLK1 with a dissociation constant (K_D) of about less than 10 nM, 5 nM, 2.5 nM, 1 nM, 0.5 nM, 0.25 nM, 100 pM, 50 pM, 25 pM, 10 pM or 5 pM;
    d. the antigen-binding protein does not bind the mouse DLK1 protein (UniProtKB ID: Q09163) having an amino acid sequence according to SEQ ID NO: 20, or the antigen-binding protein binds the mouse DLK1 protein with at least 100-fold lower affinity than the human DLK1 protein; or
    e. a combination thereof.

16. The antigen-binding protein of any one of the previous claims, wherein the antigen-binding protein binds human DLK1 isoform 1 (UniProt ID: P80370-1) and isoform 2 (UniProt ID: P80370-2).

17. The antigen-binding protein of claim 16, wherein the antigen-binding protein binds human DLK1 isoform 1 (UniProt ID: P80370-1) and isoform 2 (UniProt ID: P80370-2) with a KD:
    a. less than about 6 nM,
    b. about 5 nM,
    c. about 2 nM, or
    d. about 1 nM.

18. The antigen-binding protein of any one of the previous claims, wherein the

antigen-binding protein binds:

a. an epitope within the EGF-like 2 and EGF-like 3 domains of the human DLK1 isoform 1 protein (UniProt ID: P80370-1); and/or
b. a polypeptide comprising the amino acid residues 55-113 of the human DLK1 isoform 1 protein (UniProt ID: P80370-1).

19. The antigen-binding protein of any one of previous claims, wherein the antigen-binding protein binds the human DLK1 protein and induces (a) an antibody-dependent cell-mediated cytotoxicity (ADCC) response, and/or (b) a complement-dependent cytotoxicity (CDC) response in DLK1-positive cells.

20. The antigen-binding protein of any one of the previous claims, which is an antibody or antigen-binding antibody fragment.

21. The antigen-binding protein of claim 20, wherein the antibody is a monoclonal antibody.

22. The antigen-binding protein of claim 20, wherein the antibody is a chimeric antibody, a human antibody, or a humanized antibody.

23. The antigen-binding protein of any one of claims 20-22, wherein the antibody is an IgG.

24. The antigen-binding protein of claim 23, wherein the IgG is selected from IgG1, IgG2, IgG3 and IgG4.

25. The antigen-binding protein of claim 23 or 24, wherein the IgG is IgG1.

26. The antigen-binding protein of claim 20, wherein the antigen-binding antibody fragment is selected from the group consisting of scFv, $F(ab')_2$, Fab, Fab' and Fv.

27. The antigen-binding protein of claim 20, wherein the antigen-binding antibody

fragment is a single chain variable fragment (scFv).

28. The antigen-binding protein of claim 27, wherein the scFv comprises:
    a. the variable light (V_L) and variable heavy (V_H) chains of any of the antigen-binding protein of any one of previous claims;
    b. the amino acid sequence set forth in SEQ ID NO: 45 and SEQ ID NO: 46;
    c. the amino acid sequence set forth in SEQ ID NO: 47 and SEQ ID NO: 48;
    d. the amino acid sequence set forth in SEQ ID NO: 49 and SEQ ID NO: 50; or
    e. the amino acid sequence set forth in SEQ ID NOs: 57, 58, or 59, or an antigen-binding portion thereof.

29. The antigen-binding protein of claim 27, wherein the scFv comprises the amino acid sequence set forth in SEQ ID NO: 58 or an antibody-binding portion thereof.

30. The antigen-binding protein of any one of the previous claims, wherein the antigen-binding protein is a bispecific antigen-binding protein or a bispecific T cell engager (BiTE).

31. The antigen-binding protein of claim 30, wherein the bispecific antigen-binding protein or the BiTE comprises the amino acid sequence set forth in
    a. SEQ ID NO: 45 and SEQ ID NO: 46;
    b. SEQ ID NO: 47 and SEQ ID NO: 48; or
    c. SEQ ID NO: 49 and SEQ ID NO: 50.

32. The antigen-binding protein of claim 30 or 31, wherein the bispecific antigen-binding protein or the BiTE comprises the scFv of claim 28 or 29.

33. The antigen-binding protein of any one of claims 30-32, wherein the bispecific antigen-binding protein or the BiTE binds DLK1 and a T cell surface marker.

34. The antigen-binding protein of claim 33, wherein the T cell surface marker is the CD3 protein.

35. The antigen-binding protein of any one of claims 30-34, wherein the bispecific antigen-binding protein or the BiTE comprises the amino acid sequence set forth in SEQ ID NOs: 60, 61, or 62 or the antigen-binding portion thereof.

36. The antigen-binding protein of any one of claims 30-35, wherein the bispecific antigen-binding protein or the BiTE comprises the amino acid sequence set forth in SEQ ID NO: 60 or the antigen-binding portion thereof.

37. The antigen-binding protein of any one of claims 30-35, wherein the bispecific antigen-binding protein or the BiTE comprises the amino acid sequence set forth in SEQ ID NO: 61 or the antigen-binding portion thereof.

38. The antigen-binding protein of any one of claims 30-35, wherein the bispecific antigen-binding protein or the BiTE elicits T cell activation in the presence of a DLK-positive cell.

39. The antigen-binding protein of any one of the previous claims, which inhibits tumor growth in a xenograft mouse injected with human cancer cells.

40. The antigen-binding protein of any one of the previous claims, comprising a Fc polypeptide comprising an afucosylated glycan.

41. A conjugate comprising an antigen-binding protein of any one of the previous claims or those described herein.

42. The conjugate of claim 41 comprising a detectable marker, a cytotoxic agent, or a chemotherapeutic agent.

43. The conjugate of claim 42, wherein the chemotherapeutic agent is an anti-

mitotic agent which inhibits cell division by blocking tubulin polymerization.

44. The conjugate of claim 43, wherein the anti-mitotic agent is an auristatin.

45. The conjugate of claim 44, wherein the auristatin is monomethyl auristatin E or MMAE.

46. The conjugate of any one of claims 42-45, wherein the agent or the marker is conjugated to the antigen-binding protein via a cleavable linker or a non-cleavable linker.

47. The conjugate of claim 46, wherein the cleavable linker is VC-PAB.

48. The conjugate of any one of claims 41-47, wherein the antigen-binding protein is an antibody.

49. The conjugate of claim 48, wherein the antibody is a monoclonal antibody.

50. The conjugate of claim 49, wherein the antibody is a human antibody, a humanized antibody, or a chimeric antibody.

51. The conjugate of claim 50, wherein the antibody is an IgG antibody, optionally wherein the IgG is IgG1, IgG2, IgG3, or IgG4.

52. The conjugate of any one of claims 41-52, wherein an average number of units of the agent conjugated per antigen-binding protein is in a range of 1 to 8, preferably wherein the average number of units of the agent conjugated per antigen-binding protein is (a) in a range of 3-8, or (b) 4.

53. The conjugate of any one of claims 41-52, wherein the conjugate is a

heterogeneous conjugate.

54. The conjugate of any one of claims 41-52, wherein the conjugate is a homogeneous conjugate.

55. The conjugate of any one of claims 42-54, wherein the agent is conjugated at a specific site of the antigen-binding protein.

56. The conjugate of claim 55, wherein the specific site is an unpaired cysteine residue.

57. The conjugate of any one of claims 41-56, wherein the conjugate comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 45 and SEQ ID NO: 46 conjugated to VC-PAB-MMAE.

58. The conjugate of any one of claims 41-56, wherein the conjugate comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 47 and SEQ ID NO: 48 conjugated to VC-PAB-MMAE.

59. The conjugate of any one of claims 41-56, wherein the conjugate comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 49 and SEQ ID NO: 50 conjugated to VC-PAB-MMAE.

60. The conjugate of any one of claims 41-56, wherein the conjugate comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 51 and SEQ ID NO: 52 conjugated to VC-PAB-MMAE.

61. A fusion protein comprising an antigen-binding protein of any one of the previous claims.

62. A nucleic acid comprising a nucleotide sequence encoding an antigen binding protein of any one of claims 1-40, a conjugate of any one of claims

41-60, or a fusion protein of claim 61.

63. The nucleic acid of claim 62, wherein the nucleic acid is a cDNA.

64. A vector (e.g., expression vector) comprising the nucleic acid of claim 62 or 63.

65. The vector of claim 64, additionally comprising an internal ribosome entry site (IRES).

66. A host cell comprising the nucleic acid of claim 62 or 63, or the vector of claim 64 or 65.

67. The host cell of claim 66, wherein the host cell is a bacterial cell.

68. The host cell of claim 66, wherein the host cell is a eukaryotic cell.

69. The host cell of claim 68, wherein the eukaryotic cell is a mammalian cell.

70. The host cell of claim 69, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

71. A method of producing an antigen-binding protein that binds to a Delta Like Non-Canonical Notch Ligand 1 (DLK1) protein, comprising (i) culturing the host cell of any one of claims 66-70 in a cell culture medium, and (ii) harvesting the antigen-binding protein from the cell culture medium.

72. A method of producing a fusion protein comprising an antigen-binding protein that binds to a Delta Like Non-Canonical Notch Ligand 1 (DLK1) protein, comprising (i) culturing the host cell of any one of claims 66-70 in a cell culture medium, and (ii) harvesting the fusion protein from the cell culture medium.

73. A method of producing a pharmaceutical composition, the method comprising combining (a) an antigen-binding protein of any one of claims 1-40, a conjugate of any one of claims 41-60, a fusion protein of claim 61, a nucleic acid of claim 62 or 63, a vector of claim 64 or 65, a host cell of any one of claims 66-70, or any combination thereof; and (b) a pharmaceutically acceptable carrier, diluent and/or excipient.

74. A pharmaceutical composition comprising an antigen-binding protein of any one of claims 1-40, a conjugate of any one of claims 41-60, a fusion protein of claim 61, a nucleic acid of claim 62 or 63, a vector of claim 64 or 65, a host cell of any one of claims 66-70, or any combination thereof; and (b) a pharmaceutically acceptable carrier, diluent and/or excipient.

75. A method of treating a subject with a DLK1-expressing cancer comprising administering to the subject a pharmaceutical composition of claim 74 to treat the cancer.

76. A method of inhibiting tumor growth in a subject, comprising administering to the subject a pharmaceutical composition of claim 74 to inhibit tumor growth.

77. A method of reducing tumor size in a subject, comprising administering to the subject a pharmaceutical composition of claim 74 in to reduce tumor size.

78. A method of preventing the recurrence of cancer in a subject, comprising administering to the subject a pharmaceutical composition of claim 74 to prevent the recurrence of cancer.

79. A method of treating cancer in a subject diagnosed to be a low over-expresser of DLK1, comprising administering to the subject a pharmaceutical composition of claim 74 to prevent the recurrence of cancer.

80. The method of any one of claims 75-79 wherein the administering induces apoptosis in tumor cells.

81. The method of any one of claims 75-79 wherein the administering induces apoptosis in cells expressing Delta Like Non-Canonical Notch Ligand 1 (DLK1).

82. A method of detecting Delta Like Non-Canonical Notch Ligand 1 (DLK1) in a sample, comprising contacting the sample with an antigen-binding protein of any one of claims 1-40, a conjugate of any one of claims 41-60, or a fusion protein of claim 61, and assaying for an immunocomplex comprising the antigen-binding protein, conjugate or fusion protein bound toDLK1.

83. A method of diagnosing a Delta Like Non-Canonical Notch Ligand 1 (DLK1)-positive cancer in a subject, comprising contacting a biological sample comprising cells or tissue obtained from the subject with an antigen-binding protein of any one of claims 1-40, a conjugate of any one of claims 41-60, or a fusion protein of claim 61, and assaying for an immunocomplex comprising the antigen-binding protein, conjugate or fusion protein bound to DLK1.

84. The method of claim 83, further comprising treating the subject diagnosed to have DLK1-positive cancer by administering to the subject an antigen-binding protein of any one of claims 1-40, a conjugate of any one of claims 41-60, or a fusion protein of claim 61.

85. A method of activating a T cell to target a DLK1-expressing cancer cell in a subject, the method comprising administering to the subject a bispecific T cell engager (BiTE) of any one of claims 30-38.

86. A method of inducing an antibody-dependent cell-mediated cytotoxicity (ADCC) response against a DLK1-expressing cancer cell in a subject, the method comprising administering to the subject an antigen-binding protein that binds DLK1, wherein the antigen-binding protein comprises an Fc effector function; and the VH region and VL region of the antigen-binding protein of any one of the previous claims or those described herein.

87. The method of any one of the previous claims, wherein the subject is a mammal, optionally a dog, a cat, a mouse, or a human.

88. A kit comprising (a) an antigen-binding protein of any one of claims 1-40, a conjugate of any one of claims 41-60, a fusion protein of claim 61, a nucleic acid of claim 62 or 63, a vector of claim 64 or 65, a host cell of any one of claims 66-70, or any combination thereof; and (b) an instruction for use.

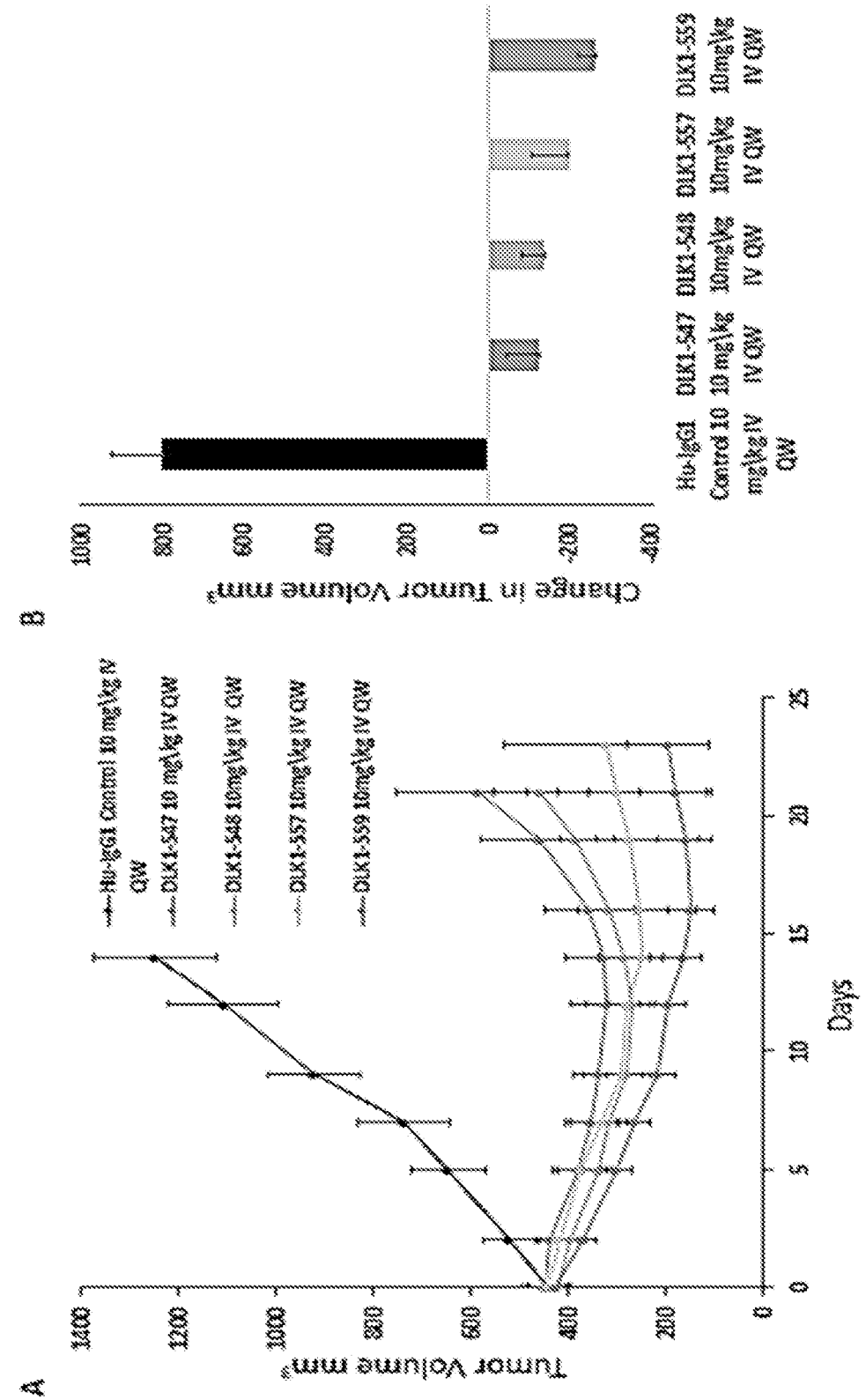

A
Tumor Volume mm³
Days
Hu-IgG1 Control 10 mg\kg IV QW
DLX1-547 10 mg\kg IV QW
DLX1-548 10mg\kg IV QW
DLX1-557 10mg\kg IV QW
DLX1-559 10mg\kg IV QW
B
Change in Tumor Volume mm³
Hu-IgG1 Control 10 mg\kg IV QW
DLX1-547 10 mg\kg IV QW
DLX1-548 10mg\kg IV QW
DLX1-557 10mg\kg IV QW
DLX1-559 10mg\kg IV QW

Fig. 1

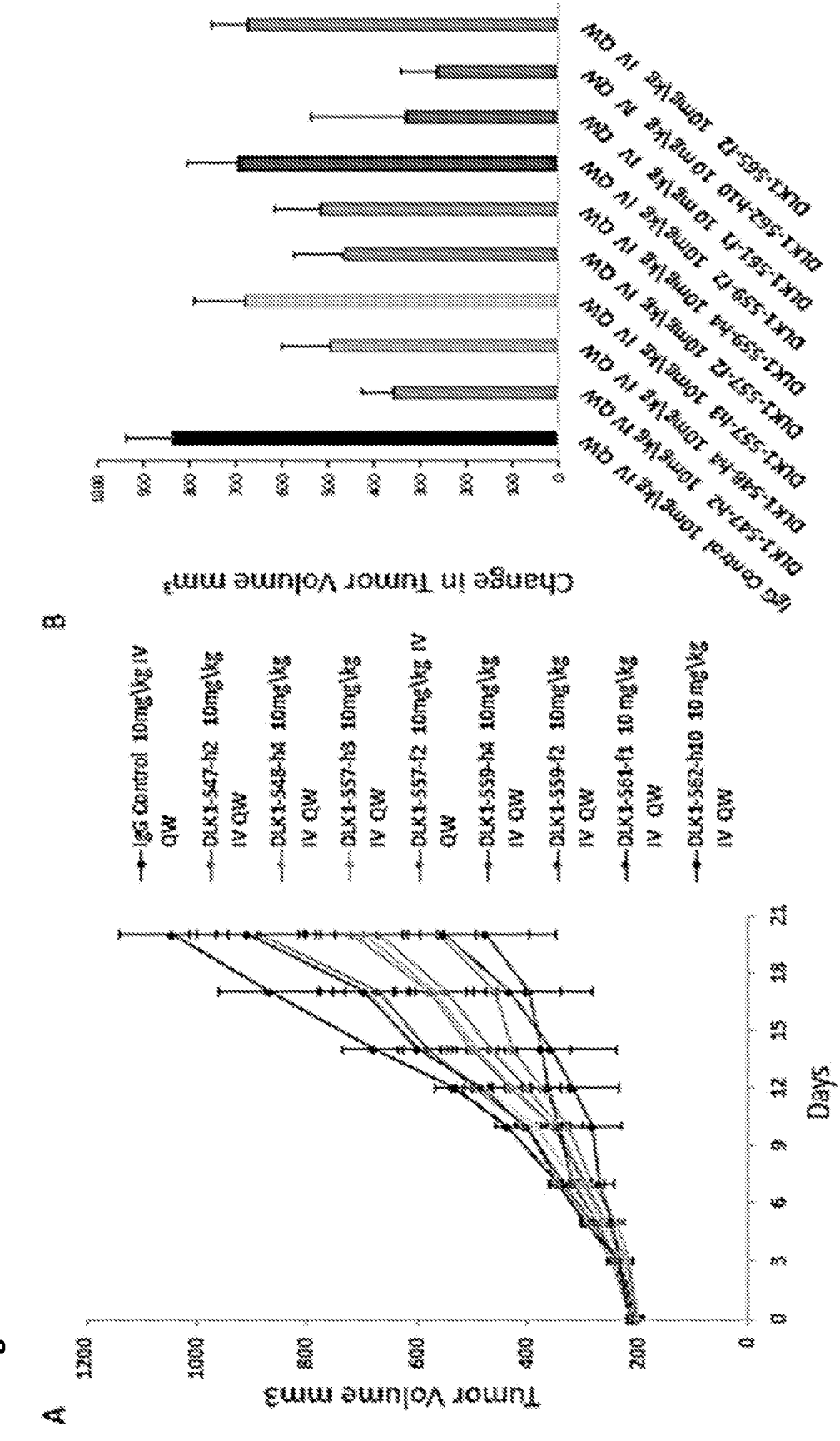
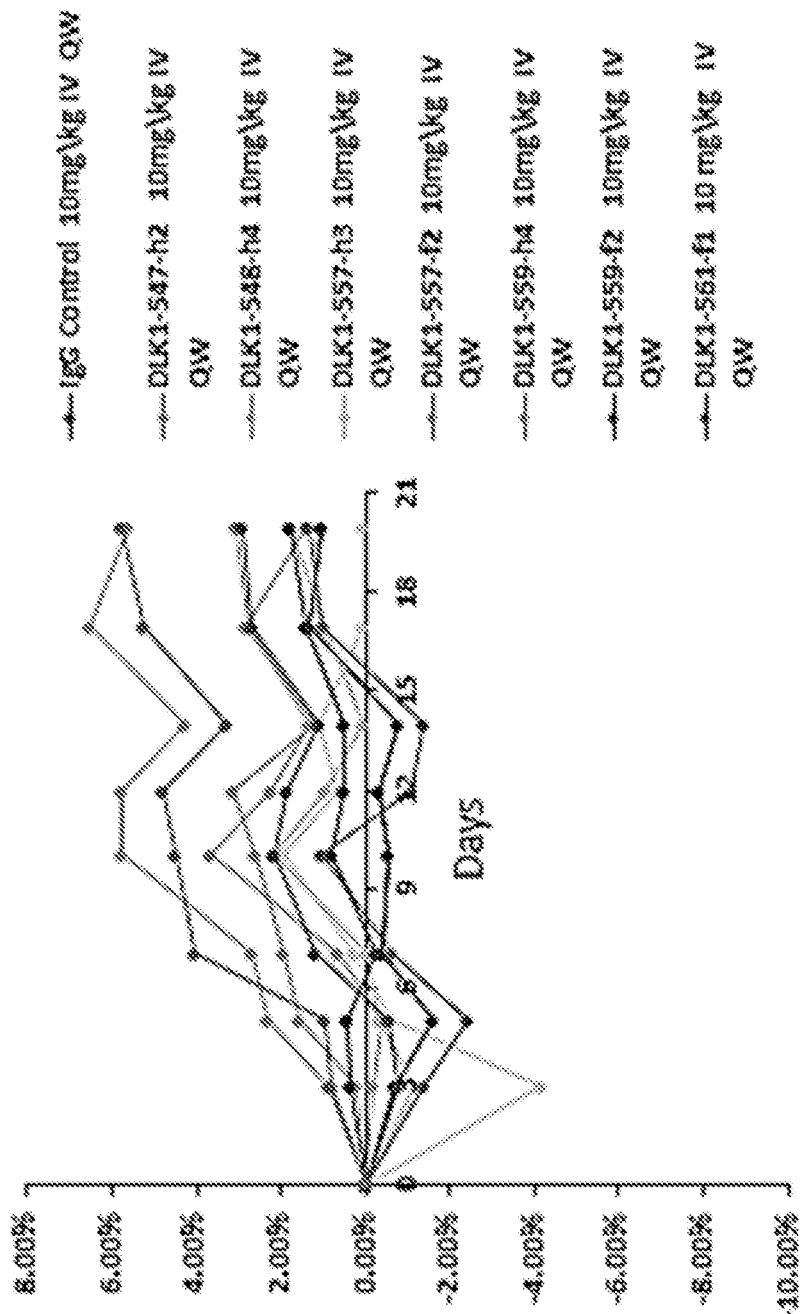

A
Tumor Volume mm3
Days
IgG Control 10mg\kg IV QW
QW
DLK1-547-h2 10mg\kg IV QW
IV QW
DLK1-548-h4 10mg\kg IV QW
IV QW
DLK1-559-h3 10mg\kg IV QW
IV QW
DLK1-557-h2 10mg\kg IV QW
QW
DLK1-559-h4 10mg\kg IV QW
IV QW
DLK1-559-h2 10mg\kg IV QW
IV QW
DLK1-561-h1 10 mg\kg IV QW
IV QW
DLK1-562-h10 10 mg\kg IV QW
B
Change in Tumor Volume mm3
IgG Control 10mg\kg IV QW
DLK1-547-h2 10mg\kg IV QW
DLK1-548-h4 10mg\kg IV QW
DLK1-557-h2 10mg\kg IV QW
DLK1-559-h4 10mg\kg IV QW
DLK1-559-h2 10mg\kg IV QW
DLK1-561-h1 10 mg\kg IV QW
DLK1-562-h10 10 mg\kg IV QW
DLK1-565-h2 10mg\kg IV QW

Fig. 2


% Body Weight
8.00%
6.00%
4.00%
2.00%
0.00%
-2.00%
-4.00%
-6.00%
-8.00%
-10.00%
0
3
6
9
12
15
18
21
Days
IgG Control 10mg\kg IV QW
DLK1-547-h2 10mg\kg IV QW
DLK1-548-h4 10mg\kg IV QW
DLK1-557-h3 10mg\kg IV QW
DLK1-557-f2 10mg\kg IV QW
DLK1-559-h4 10mg\kg IV QW
DLK1-559-f2 10mg\kg IV QW
DLK1-561-f1 10 mg\kg IV QW

C

Fig. 2, Cont.

Fig. 3

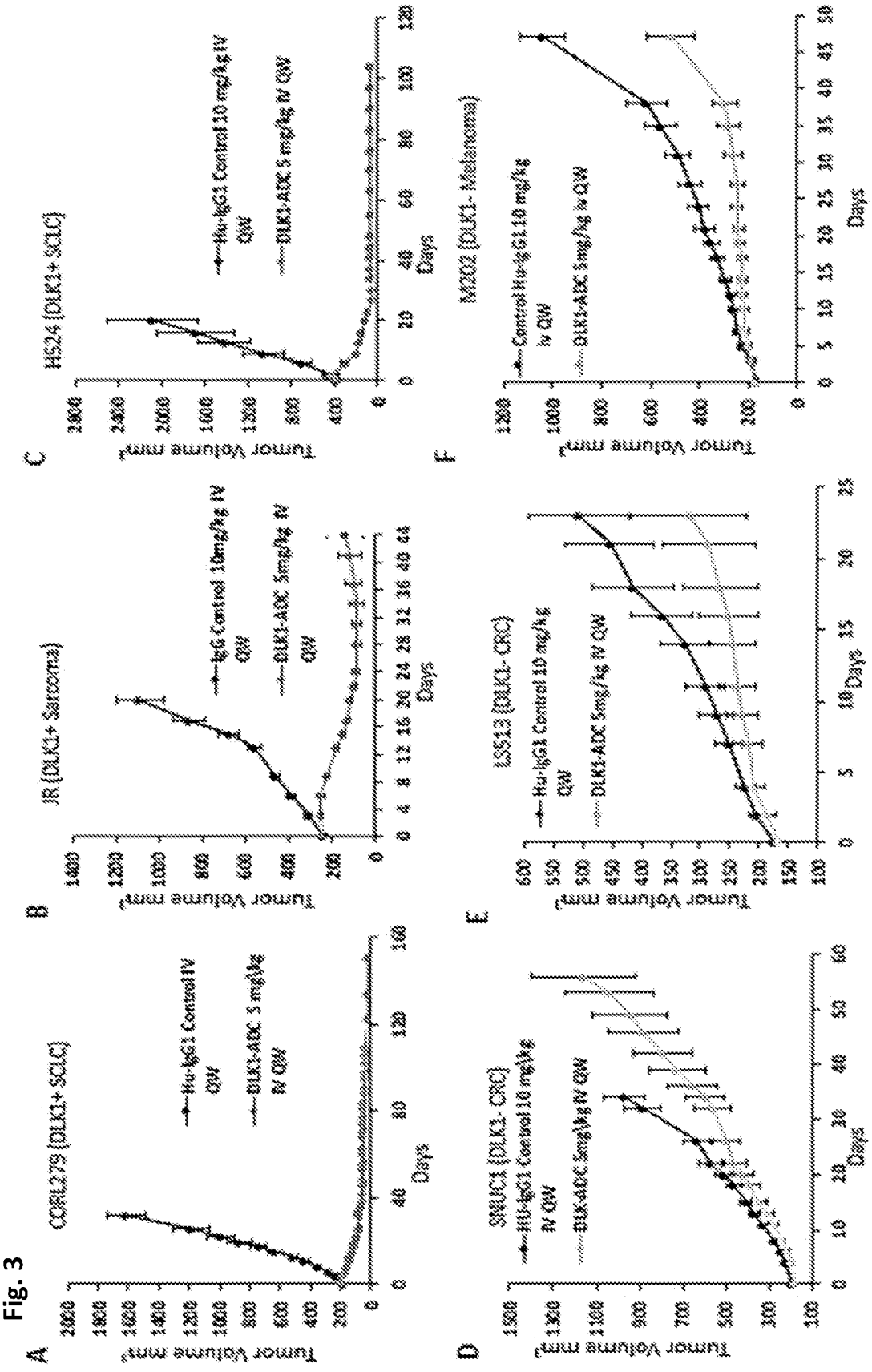

A
CORL279 (DLK1+ SCLC)
Tumor Volume mm³
Days
Hu-IgG1 Control IV QW
DLK1-ADC 5 mg/kg IV QW
B
JR (DLK1+ Sarcoma)
Tumor Volume mm³
Days
IgG Control 10mg/kg IV QW
DLK1-ADC 5mg/kg IV QW
C
HS24 (DLK1+ SCLC)
Tumor Volume mm³
Days
Hu-IgG1 Control 10 mg/kg IV QW
DLK1-ADC 5 mg/kg IV QW
D
SNUC1 (DLK1- CRC)
Tumor Volume mm³
Days
HU-IgG1 Control 10 mg/kg IV QW
DLK1-ADC 5mg/kg IV QW
E
LS513 (DLK1- CRC)
Tumor Volume mm³
Days
Hu-IgG1 Control 10 mg/kg QW
DLK1-ADC 5mg/kg IV QW
F
M202 (DLK1- Melanoma)
Tumor Volume mm³
Days
Control Hu-IgG1 10 mg/kg Iv QW
DLK1-ADC 5mg/kg Iv QW

FIG. 4A

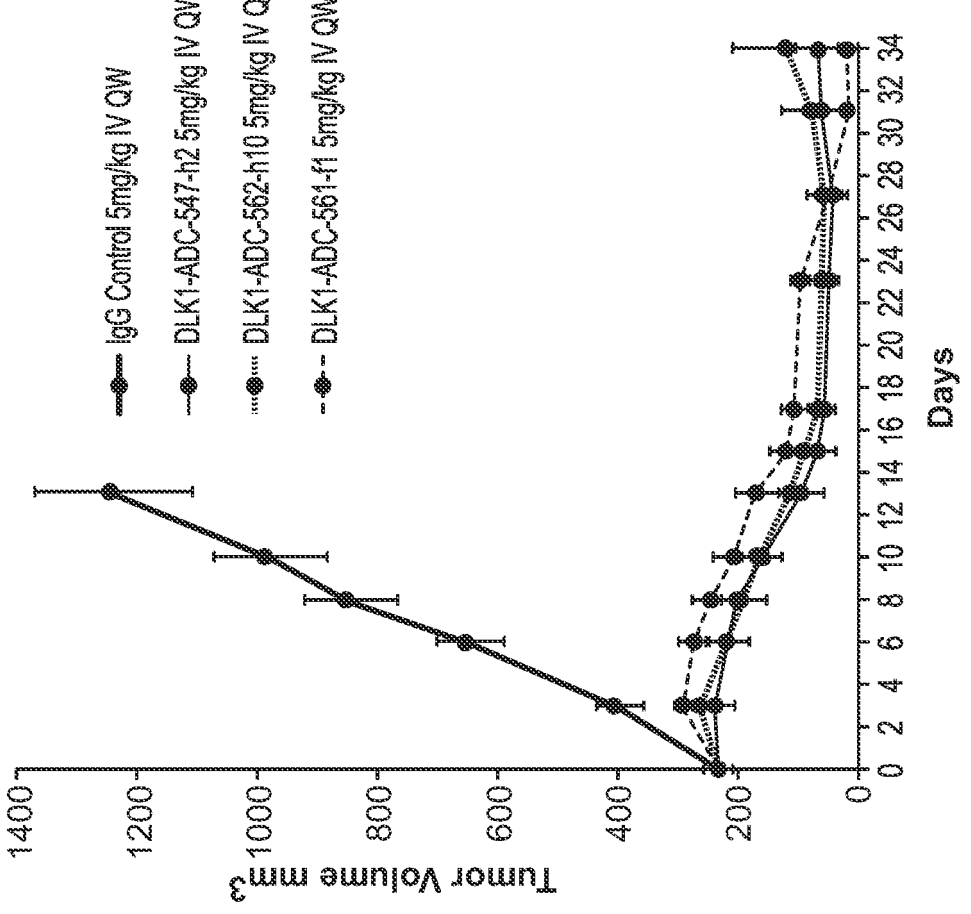
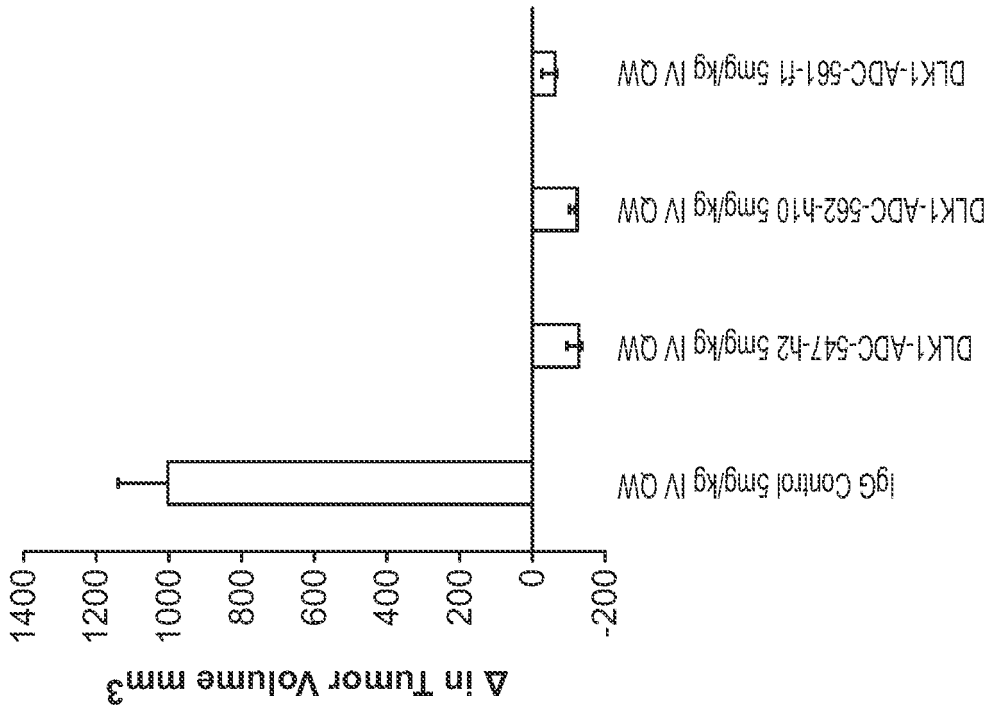

Tumor Volume mm3
1400
1200
1000
800
600
400
200
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Days
IgG Control 5mg/kg IV QW
DLK1-ADC-547-h2 5mg/kg IV QW
DLK1-ADC-562-h10 5mg/kg IV QW
DLK1-ADC-561-f1 5mg/kg IV QW

FIG. 4B


Δ in Tumor Volume mm3
1400
1200
1000
800
600
400
200
0
-200
IgG Control 5mg/kg IV QW
DLK1-ADC-547-h2 5mg/kg IV QW
DLK1-ADC-562-h10 5mg/kg IV QW
DLK1-ADC-561-f1 5mg/kg IV QW

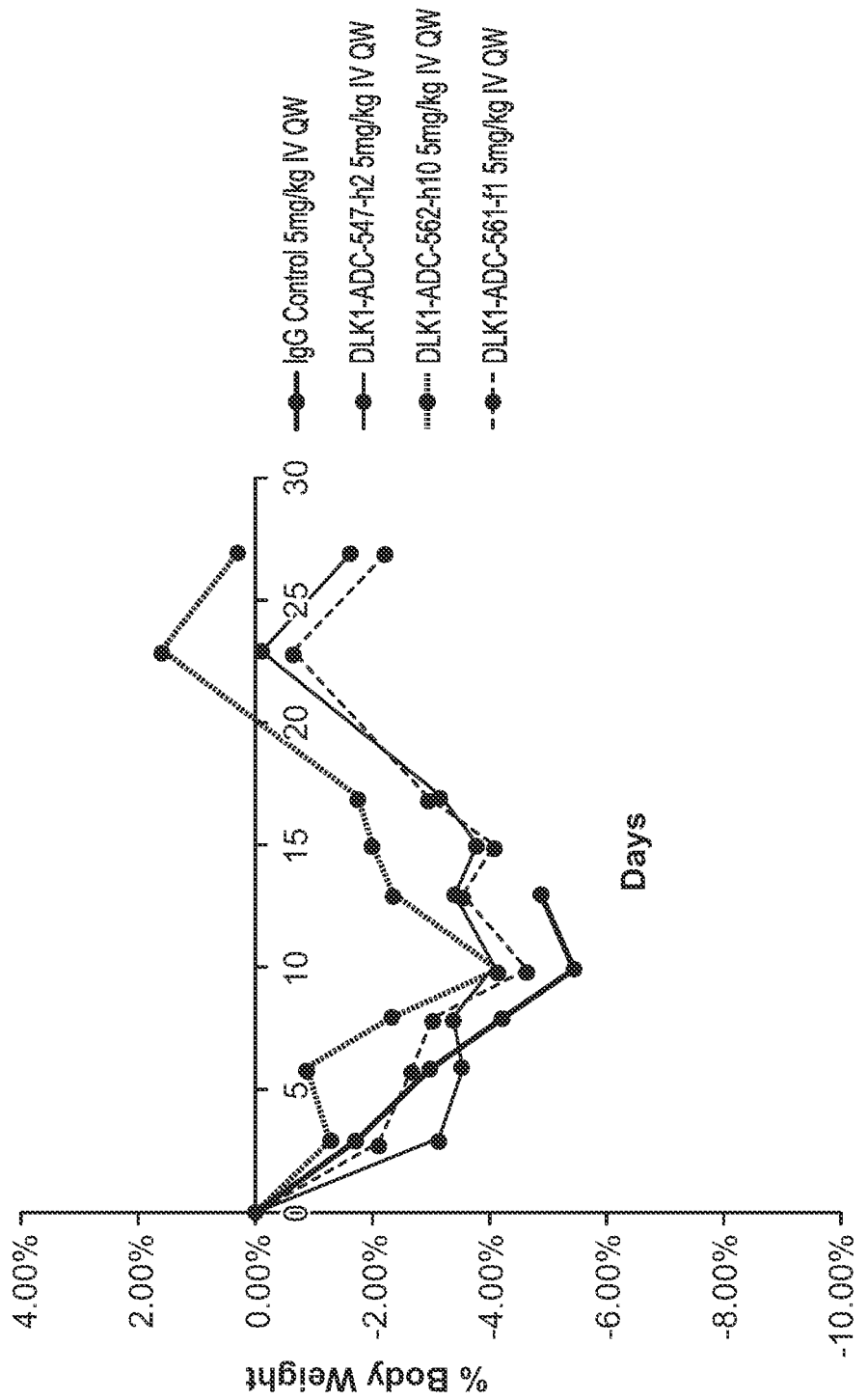

IgG Control 5mg/kg IV QW
DLK1-ADC-547-h2 5mg/kg IV QW
DLK1-ADC-562-h10 5mg/kg IV QW
DLK1-ADC-561-f1 5mg/kg IV QW
% Body Weight
4.00%
2.00%
0.00%
-2.00%
-4.00%
-6.00%
-8.00%
-10.00%
0
5
10
15
20
25
30
Days

FIG. 4C

FIG. 5A

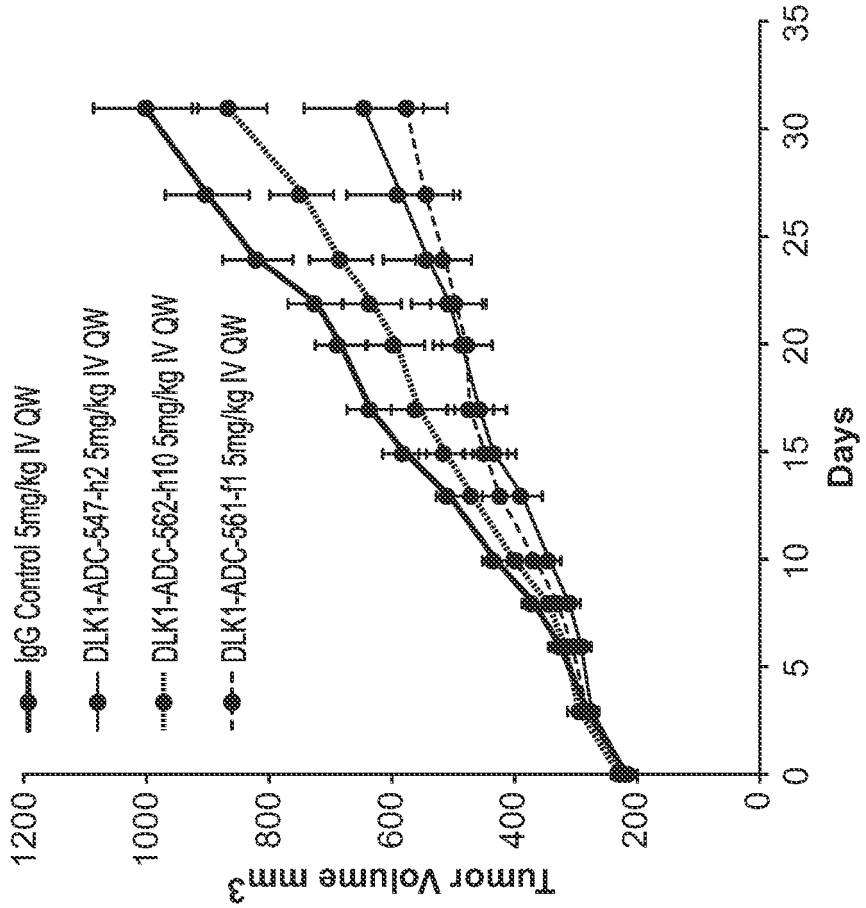

IgG Control 5mg/kg IV QW
DLK1-ADC-547-h2 5mg/kg IV QW
DLK1-ADC-562-h10 5mg/kg IV QW
DLK1-ADC-561-f1 5mg/kg IV QW
Tumor Volume mm3
Days
0
200
400
600
800
1000
1200
0
5
10
15
20
25
30
35

FIG. 5B

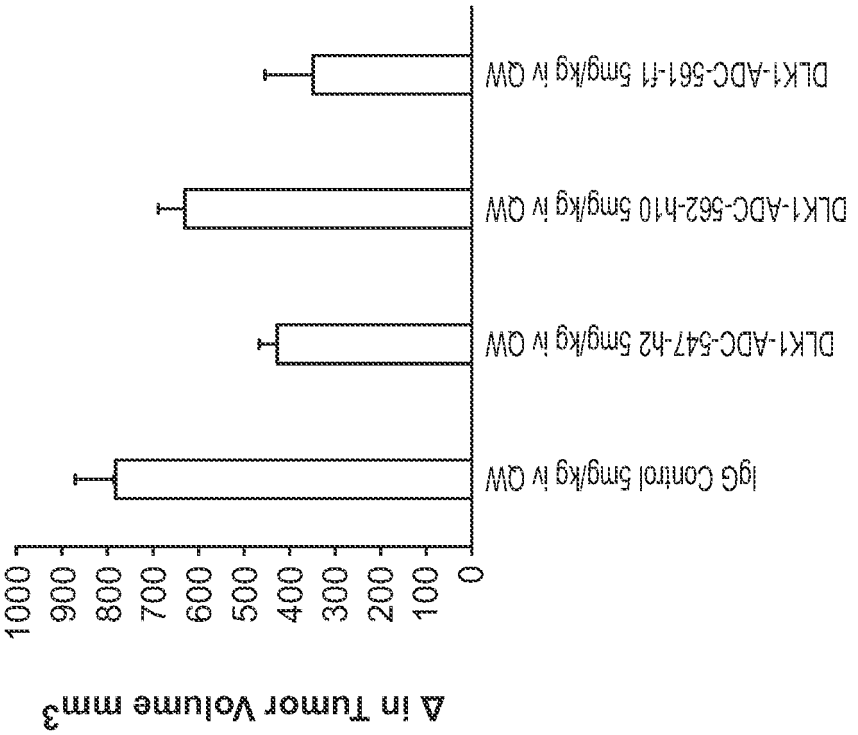

Δ in Tumor Volume mm3
0
100
200
300
400
500
600
700
800
900
1000
IgG Control 5mg/kg iv QW
DLK1-ADC-547-h2 5mg/kg iv QW
DLK1-ADC-562-h10 5mg/kg iv QW
DLK1-ADC-561-f1 5mg/kg iv QW

FIG. 6

Humanized DLK1 Abs		04-0561-F1	04-0562-h10	04-0547-h2
Antibody Binding Activity by Flow Cytometry (20µg/ml)	CORL-279 (SCLC, DLK+)	337,998	222,123	186,310.0
	M202 (Melanoma, DLK+)	7,039	6,878	5,208.0
ADC Binding Activity by Flow Cytometry (20µg/ml)	CORL-279 (SCLC, DLK+)	314,568	311,529	225,123.5
	M202 (Melanoma, DLK+)	5,110	4,512	4,385.0
Antibody Binding Affinity - K D by Kin ExA (nM)	HEK293 DLK1-mGFP G05 Cells	1.11	1.92	5.34
	CORL-279 cells(adherent)	0.49	3.55	8.20
Antibody Binding Kinetics by Octet384 using Recombinant Protein DLK1 ECD-mFc	KD (pM)	18.63	3.76	297.80
	Ka (1/Ms)	4.82E+04	2.14E+05	1.55E+04
	Kdis (1/s)	8.98E-04	8.06E-04	4.62 E-03
EC50 (nM) by EL ISA	Recombinant DLK1 ECD-mFc	0.684	0.514	10.319
Cross Reactivity to Three Tox Models by Flow (20µg/ml)	HEK293T Human DLK1mGFP G05	33,564.27	42,292.55	35,585.27
	HEK293T Monkey DLK1mGFP mass	36,870.05	48,292.38	34,062.05
	HEK293T Mouse DLK1mGFP mass	755.89	11,257.42	47,071.18
	HEK293T Rat DLK1mGFP mass pop	715.92	32,662.19	33.757.18
Cross Reactivity to Three Tox Models - KD by KinExA (nM)	HEK293T Human DLK1mGFP G05	1.11	1.92	5.34
	HEK293T Monkey DLK1mGFP mass	2.19	4.69	9.89
	HEK293T Mouse DLK1mGFP mass	not defined	200.04	13.01
	HEK293T Rat DLK1mGFP mass pop	not defined	4.43	5.96

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FIG. 6 CONT.

Humanized DLK1 Abs		04-0561-F1	04-0562-h10	04-0547-h2
Antibody Aggregation by SEC using AKTA Pure 25	Aggregates (% of Area)	1.77	2.71	0.99
	Monomers (% of Area)	97.00	97.29	99.01
	Fragements (% of Area)	1.23	0.00	0.00
Ab Hydrophobicity-HIC Retesion Time (min)		30.11	30.78	37.11
Antibody Tm in PBS (^{o}C)		69.02	69.35	68.46
Antibody Tagg in PBS (^{o}C)		93.47	90.20	88.72
Antibody Titer in ExpiCHO-S (~mg purified Ab/ml Medium)		0.433	0.572	0.676
Antibody Internalization Rate (Native Positive Cell Line COR-L279)	Started time	~0min		
	Internalization 50% time	~10mins		
	Finished time	~1 hour		

Fig. 7

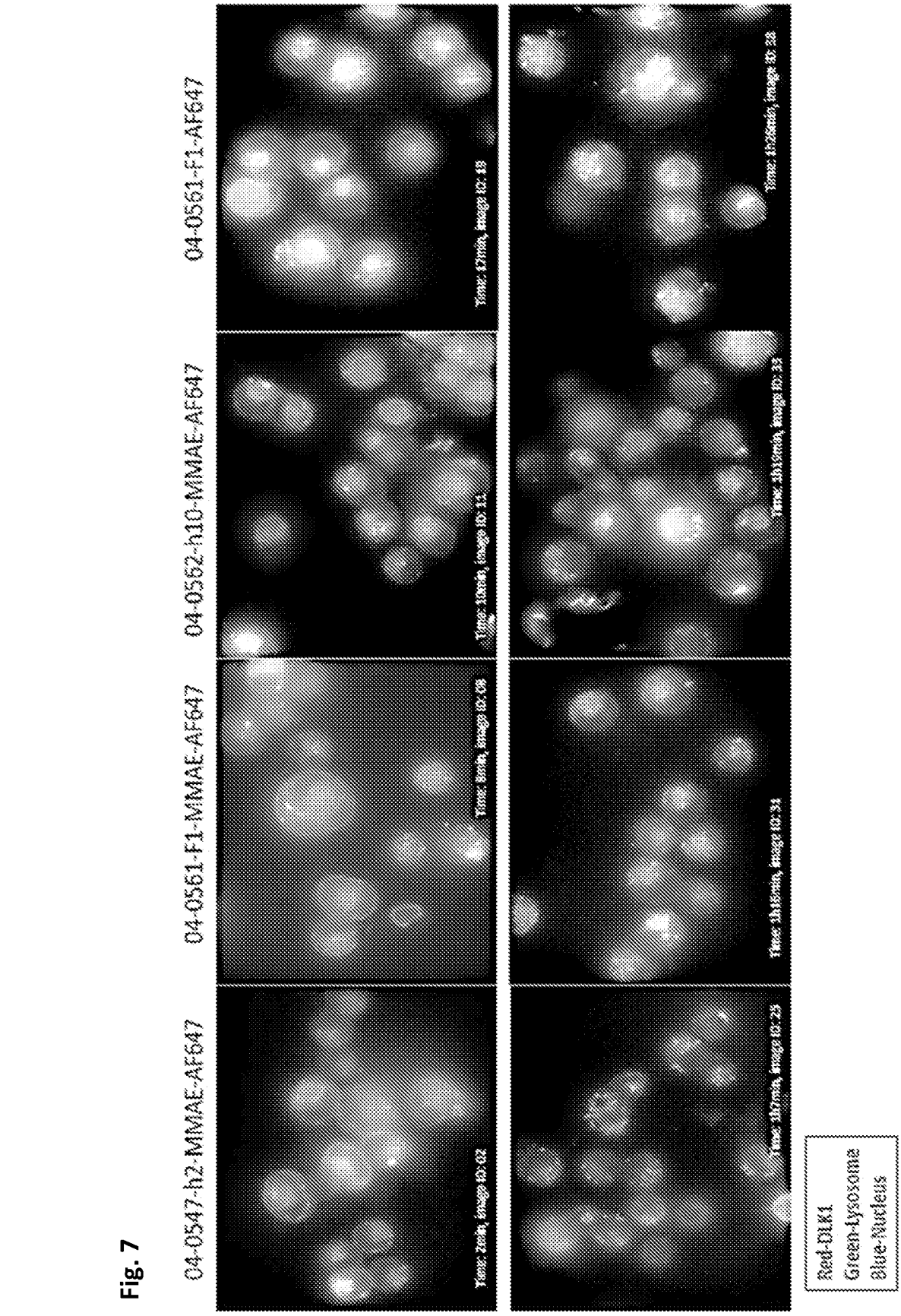
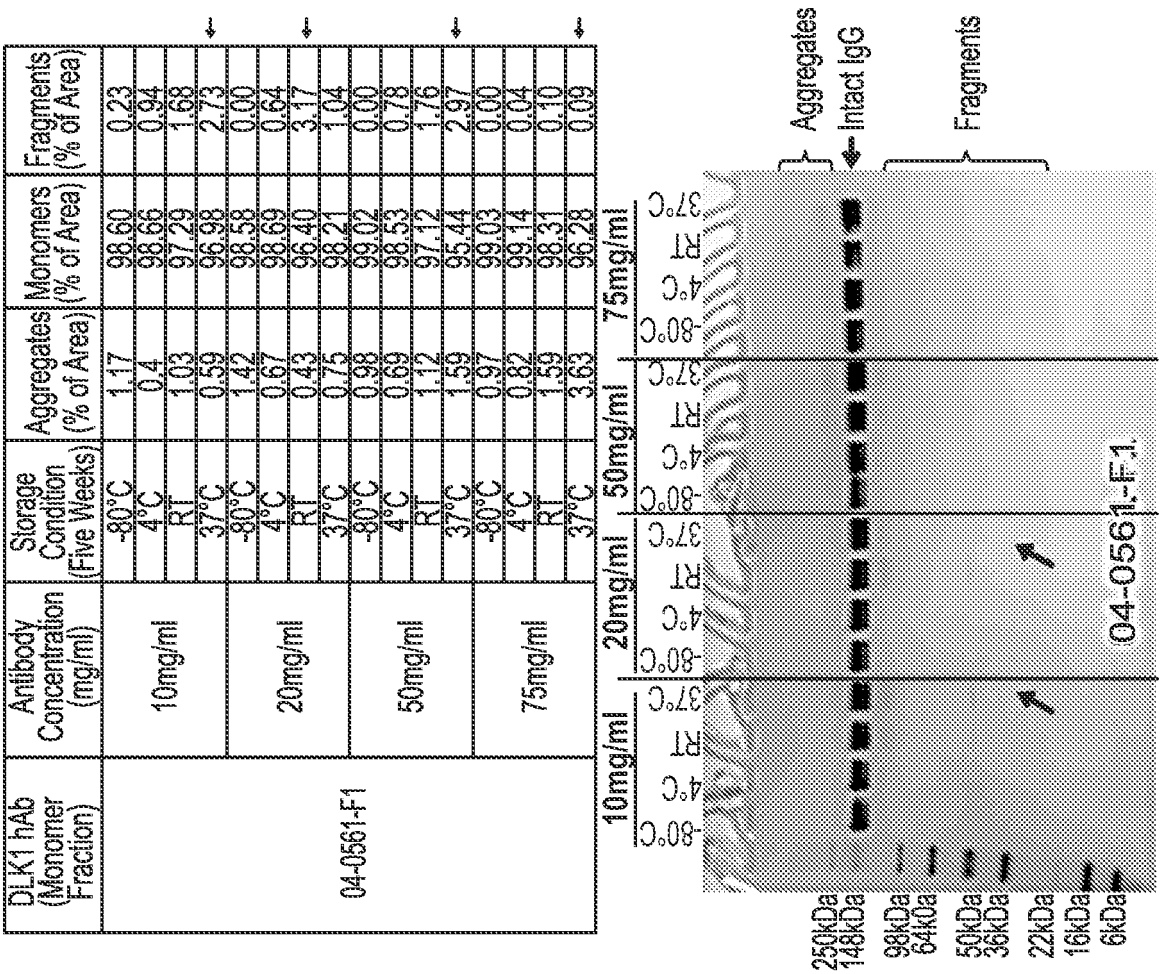

04-0547-h2-MMAE-AF647
04-0561-F1-MMAE-AF647
04-0562-h10-MMAE-AF647
04-0561-F1-AF647
Red-DLK1
Green-Lysosome
Blue-Nucleus

FIG. 8

DLK1 hAb (Monomer Fraction)	Antibody Concentration (mg/ml)	Storage Condition (Five Weeks)	Aggregates (% of Area)	Monomers (% of Area)	Fragments (% of Area)
04-0561-F1	10mg/ml	-80°C	1.17	98.60	0.23
		4°C	0.4	98.66	0.94
		RT	1.03	97.29	1.68
		37°C	0.59	96.98	2.73
	20mg/ml	-80°C	1.42	98.58	0.00
		4°C	0.67	98.69	0.64
		RT	0.43	96.40	3.17
		37°C	0.75	98.21	1.04
	50mg/ml	-80°C	0.98	99.02	0.00
		4°C	0.69	98.53	0.78
		RT	1.12	97.12	1.76
		37°C	1.59	95.44	2.97
	75mg/ml	-80°C	0.97	99.03	0.00
		4°C	0.82	99.14	0.04
		RT	1.59	98.31	0.10
		37°C	3.63	96.28	0.09



DLK1 hAb (Monomer Fraction)	Antibody Concentration (mg/ml)	Storage Condition (Five Weeks)	Aggregates (% of Area)	Monomers (% of Area)	Fragments (% of Area)
04-0562-h10	10mg/ml	-80°C	0.00	99.93	0.07
		4°C	0.00	99.70	0.30
		RT	0.20	99.12	0.68
		37°C	0.18	95.12	4.71
	20mg/ml	-80°C	0.11	98.16	1.73
		4°C	0.10	96.92	2.98
		RT	0.14	98.82	1.04
		37°C	0.15	91.86	7.99
	50mg/ml	-80°C	0.31	99.48	0.00
		4°C	0.31	98.48	1.21
		RT	0.42	98.32	1.26
		37°C	0.28	91.83	7.89
	75mg/ml	-80°C	0.47	99.47	0.06
		4°C	0.16	99.84	0.00
		RT	0.45	99.55	0.00
		37°C	1.06	93.43	5.52

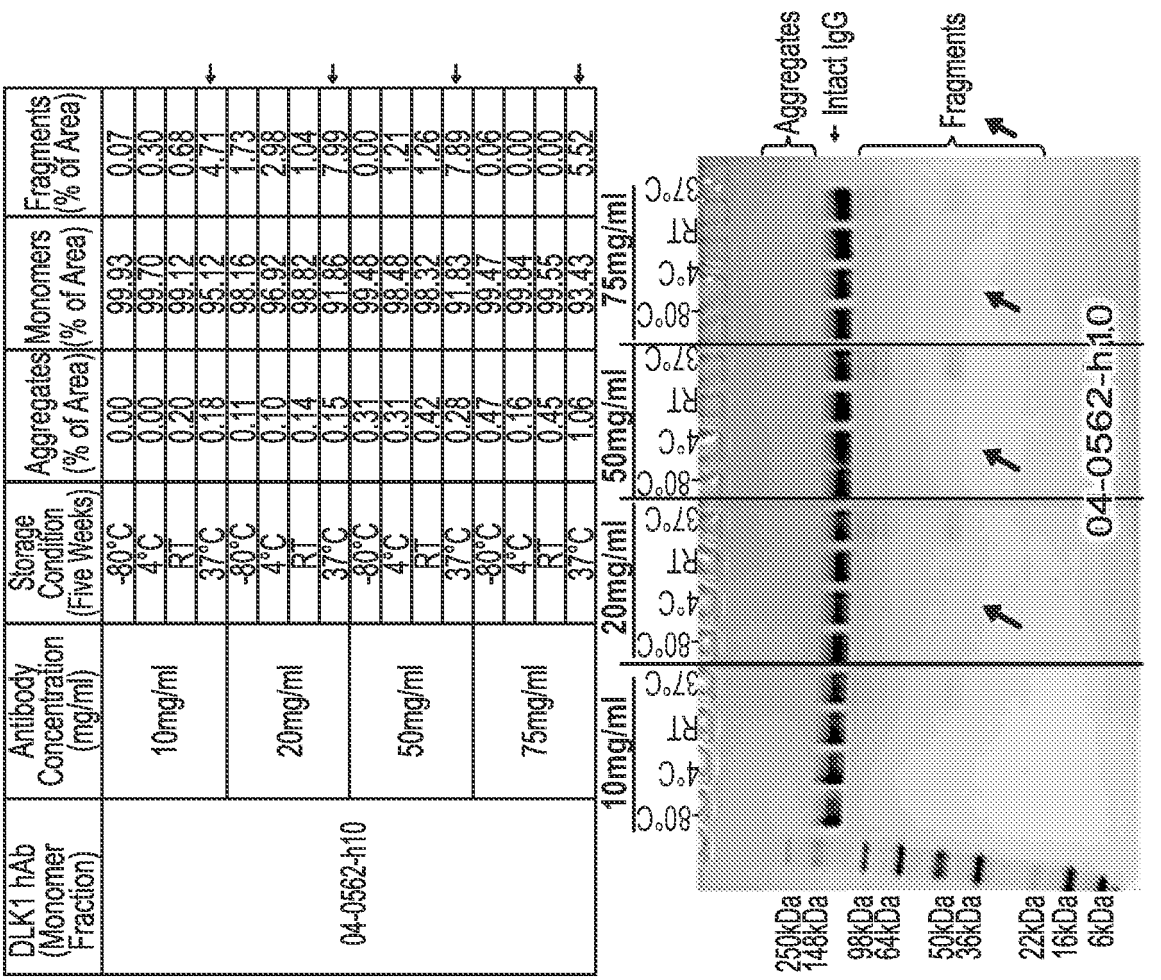
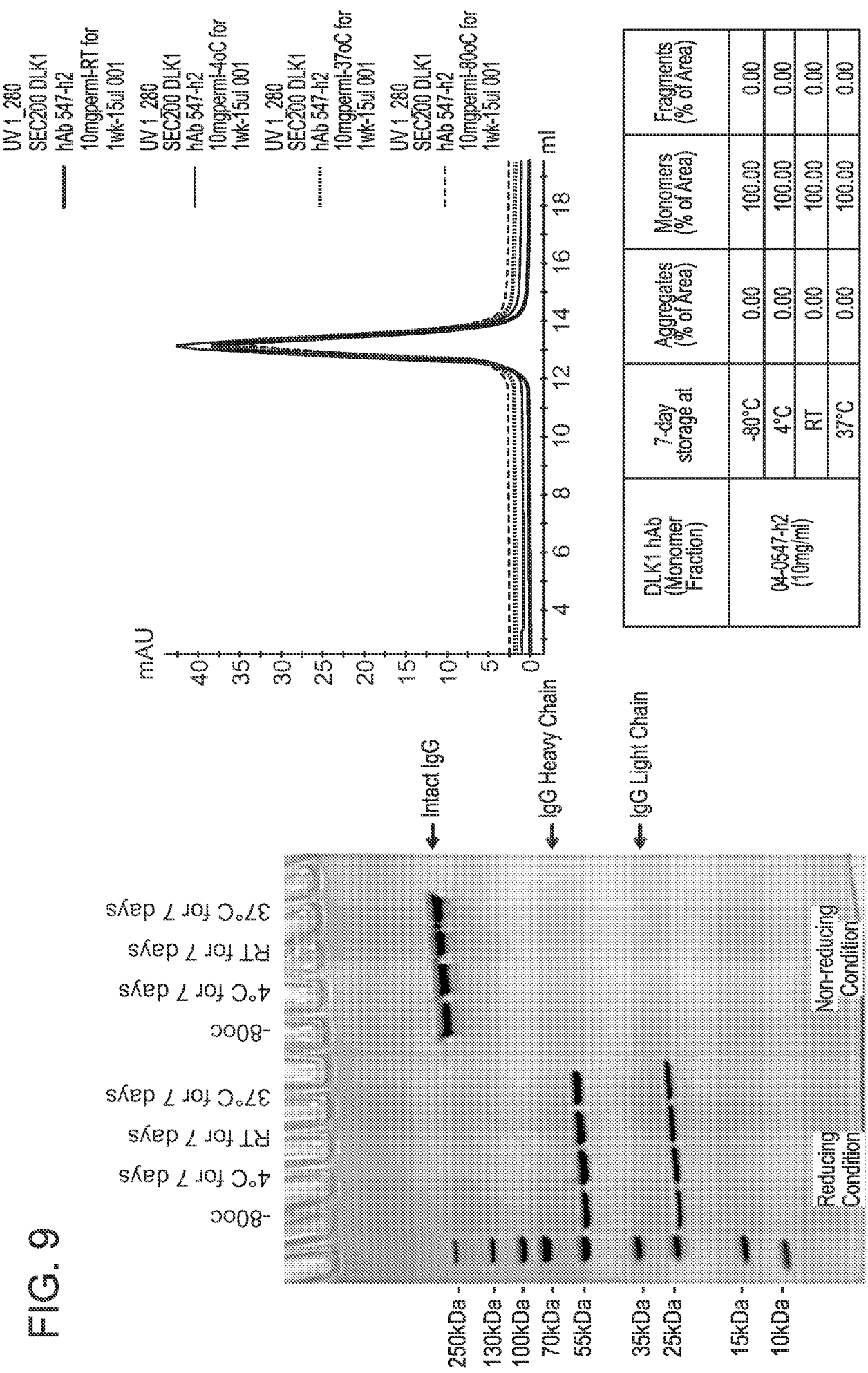


FIG. 9



DLK1 hAb (Monomer Fraction)	7-day storage at	Aggregates (% of Area)	Monomers (% of Area)	Fragments (% of Area)
04-0547-h2 (10mg/ml)	-80°C	0.00	100.00	0.00
	4°C	0.00	100.00	0.00
	RT	0.00	100.00	0.00
	37°C	0.00	100.00	0.00

FIG. 10

```
sp|P80370|DLK1_HUMAN            MTATEALLRVLLLLLAFGHSTYGAECFPACNPQNGFCEDDNVCRCQPGWQGPLCDQCVTS   60
tr|A0A2K5TMQ6|A0A2K5TMQ6_MACFA  MTATEALLRVLLLLLAFGHSTHGAECFPACNPQNGFCEDDNVCRCQPGWQGPLCDQCVTS   60
sp|Q09163|DLK1_MOUSE            MTATGALLRVLLLLLAFGHSTYGAECDPPCDPQYGFCEADNVCRCHVGWEGPLCDKCVTA   60
sp|O70534|DLK1_RAT              MTATGALLRVLLLLLAFGHSTYGAECDPACDPQHGFCEADNVCRCEPGWEGPLCEKCVTS   60
                                * ** ***************:**** * *:** **** ******. **:****::***:

sp|P80370|DLK1_HUMAN            PGCLHGLCGEPGQCICTDGWDGELCDRDVRACSSAPCANNRTCVSLDDGLYECSCAPGYS   120
tr|A0A2K5TMQ6|A0A2K5TMQ6_MACFA  PGCLHGLCEEPWQCICTDGWDGKLCDRDVRACSSTPCANNGTCVSLDDGLYECSCAPGYS   120
sp|Q09163|DLK1_MOUSE            PGCVNGVCKEPWQCICKDGWDGKFCEIDVRACTSTPCANNGTCVDLEKGQYECSCAPGFS   120
sp|O70534|DLK1_RAT              PGCVNGLCEEPWQCVCKEGWDGKFCEIDIRACTSTPCANNGTCVDLEKGQYECSCTPGFS   120
                                ***::*:* ** **:*.:****::*: *:***:*:***** *** .*:.* *****:**:*

sp|P80370|DLK1_HUMAN            GKDCQKKDGPCVINGSPCQHGGTCVDDEGRASHASCLCPPGFSGNFCEIVA--NSCTPNP   178
tr|A0A2K5TMQ6|A0A2K5TMQ6_MACFA  GKDCQKKDGPCVINGSPCQHGGTCVDDEGRASHASCLCPPGFSGNFCEIVA--NSCTPNP   178
sp|Q09163|DLK1_MOUSE            GKDCQHKAGPCVINGSPCQHGGACVDDEGQASHASCLCPPGFSGNFCEIVAATNSCTPNP   180
sp|O70534|DLK1_RAT              GKDCQHKAGPCVINGSPCQHGGACVDDEGRASHASCLCPPGFSGNFCEIV--TNSCTPNP   178
                                *****:* *************:******:*******************   *******

sp|P80370|DLK1_HUMAN            CENDGVCTDIGGDFRCRCPAGFIDKTCSRPVTNCASSPCQNGGTCLQHTQVSYECLCKPE   238
tr|A0A2K5TMQ6|A0A2K5TMQ6_MACFA  CENDGVCTDIGGDFRCRCPAGFIDKTCSRPVTNCASGPCQNGGTCLQHTQVSYECLCKPE   238
sp|Q09163|DLK1_MOUSE            CENDGVCTDIGGDFRCRCPAGFVDKTCSRPVSNCASGPCQNGGTCLQHTQVSFECLCKPE   240
sp|O70534|DLK1_RAT              CENDGVCTDIGGDFRCRCPAGFVDKTCSRPVSNCASGPCLNGGTCLQHTQVSFECLCKPP   238
                                *********************:********:****.** ***********:******
```

FIG. 10 CONT.

```
sp|P80370|DLK1_HUMAN              FTGLTCVKKRALSPQQVTRLPSGYGLAYRLTPGVHELPVQQPEHRILKVSMKELNKKTPL  298
tr|A0A2K5TMQ6|A0A2K5TMQ6_MACFA    FTGLTCVKKRALSPQQVTHLPSGYGLTYRLTPGVHELPVPQPEHRVLKVSMKELNKNTPL  298
sp|Q09163|DLK1_MOUSE              FMGPTCAKKRGASPVQVTHLPSGYGLTYRLTPGVHELPVQQPEQHILKVSMKELNKSTPL  300
sp|O70534|DLK1_RAT                FMGPTCAKKRGTSPVQVTHLPSGYGLTYRLTPGVHELPVQQPEHHILKVSMKELNKSAPL  298
                                  * * **.***. ** ***:*******:************ ***:::********** .:**

sp|P80370|DLK1_HUMAN              LTEGQAICFTILGVLTSLVVLGTVGIVFLNKCETWVSNLRYNHMLRKKKNLLLQYNSGED  358
tr|A0A2K5TMQ6|A0A2K5TMQ6_MACFA    LTEGQAICFTILGVLTSLVVLGTVGIVFLNKCETWVSNLRYNRMLRKKKNLLLQYNSGED  358
sp|Q09163|DLK1_MOUSE              LTEGQAICFTILGVLTSLVVLGTVGIVFLNKCETWVSNLRYNHTFRKKKNLLLQYNSGEE  360
sp|O70534|DLK1_RAT                LTEGQAICFTILGVLTSLVVLGTVAIVFLNKCEAWVSNLRYNHMLRKKKNLLLQYNSGEE  358
                                  ***********************.********:********: :**************:

sp|P80370|DLK1_HUMAN              LAVNIIFPEKIDMTTFSKEAGDEEI    383
tr|A0A2K5TMQ6|A0A2K5TMQ6_MACFA    LAVNIIFPEKIDMTTFSKEAGGEEI    383
sp|Q09163|DLK1_MOUSE              LAVNIIFPEKIDMTTFNKEAGDEEI    385
sp|O70534|DLK1_RAT                LAVNIIFPEKIDMTTFNKEAGDEDI    383
                                  ***************.****.*:*
```

ECD

DLK1	Uniprot #	NCBI Accession#	Length (aa)	Identity
Homo sapiens	P80370-1	NP_003827	383	100.00%
Crab-eating macaque	A0A2K5TMQ6		383	96.34%
Mus musculus	Q09163	NP_034182	385	84.94%
Rattus norvegicus	O70534	NP_446196	383	85.12%

FIG. 11

FL4-H (DLK1)	DLK1 hAb 04-561-F1		DLK1 hAb 04-562-h10		DLK1 hAb 04-547-h2		2nd Ab only
	1µg/reaction	0.2µg/reaction	1µg/reaction	0.2µg/reaction	1µg/reaction	0.2µg/reaction	
HEK293T Human DLK1mGFP Clone G06	33,564.27	30,016.52	42,492.55	33,739.18	35,585.27	16,069.86	632.14
HEK293T Monkey DLK1mGFP mass pop	36,870.05	28,916.76	48,292.38	36,723.74	34,062.05	12,381.48	773.98
HEK293T Mouse DLK1mGFP mass pop	756.89	748.81	11,257.42	3,735.64	47,071.18	26,034.03	718.47
HEK293T Rat DLK1mGFP mass pop	716.92	680.78	32,662.19	26,919.31	33,757.18	16,907.12	656.76
	Reacts with monkey, not mouse nor rate		Reacts with monkey, mouse (weak), and rate		Reacts with monkey, mouse and rate		

FL1-H (mGFP)	DLK1 hAb 04-561-F1		DLK1 hAb 04-562-h10		DLK1 hAb 04-547-h2		2nd Ab only
	1µg/reaction	0.2µg/reaction	1µg/reaction	0.2µg/reaction	1µg/reaction	0.2µg/reaction	
HEK293T Human DLK1mGFP Clone G06	151,727.77	166,659.31	162,433.89	164,124.17	161,265.94	148,745.34	167,161.71
HEK293T Monkey DLK1mGFP mass pop	317,813.40	328,481.83	320,881.67	320,533.12	331,073.73	314,336.37	324,924.52
HEK293T Mouse DLK1mGFP mass pop	315,599.99	307,902.81	298,054.95	311,362.23	329,231.36	312,063.14	313,500.04
HEK293T Rat DLK1mGFP mass pop	278,276.76	275,398.27	241,876.83	264,243.65	275,952.69	271,814.55	271,393.11

FIG. 11 CONT.

Cell Model		DLK1 hAbs	KD			Expression Level		Equilibrium Curves	Detached by
			KD (nM)	Error%	95% Confidence Interval (CI)	Expression Level	95% Confidence Interval (CI)		
Human	HEK293T DLK1mGFP Human Clone G06	04-0561-F1	1.11	3.83	0.455nM - 2.19nM	1.024E+05	6.504E+04 - 1.476E+05	500pM, 20nM	Versene
		04-0562-h10	1.92	3.33	1.03nM - 3.34nM	1.184E+05	8.297E+04 - 1.616E+05		
		04-0547-h2	5.34	5.90	not defined	8.000E+06	not defined	400pM, 50nM	
Monkey	HEK293T DLK1mGFP Monkey mass pop	04-0561-F1	2.19	2.87	1.33nM - 3.45nM	1.310E+06	9.237E+05 - 1.758E+06	400pM, 50nM	Versene
		04-0562-h10	4.69	2.28	3.41nM - 6.36nM	1.310E+06	9.919E+05 - 1.676E+06		
		04-0547-h2	9.89	3.92	5.15nM - 17.83nM	1.141E+06	5.720E+05 - 1.909E+06		
Mouse	HEK293T DLK1mGFP Mouse mass pop	04-0561-F1	no defined		not defined	not defined	not defined	400pM, 50nM	Versene
		04-0562-h10	200.04	3.19	not defined	1.358E+06	not defined		
		04-0547-h2	13.01	3.08	4.21nM - 26.32nM	5.490E+05	1.029E+05 - 1.177E+06		
Rat	HEK293T DLK1mGFP Rat mass pop	04-0561-F1	not defined		not defined	not defined		400pM, 50nM	Versene
		04-0562-h10	4.43	2.90	1.39nM - 9.51nM	5.211E+05	1.432E+05 - 9.601E+05		
		04-0547-h2	5.96	3.17	1.82nM - 13.53nM	5.686E+05	1.432E+05 - 1.091E+06		

Fig. 12

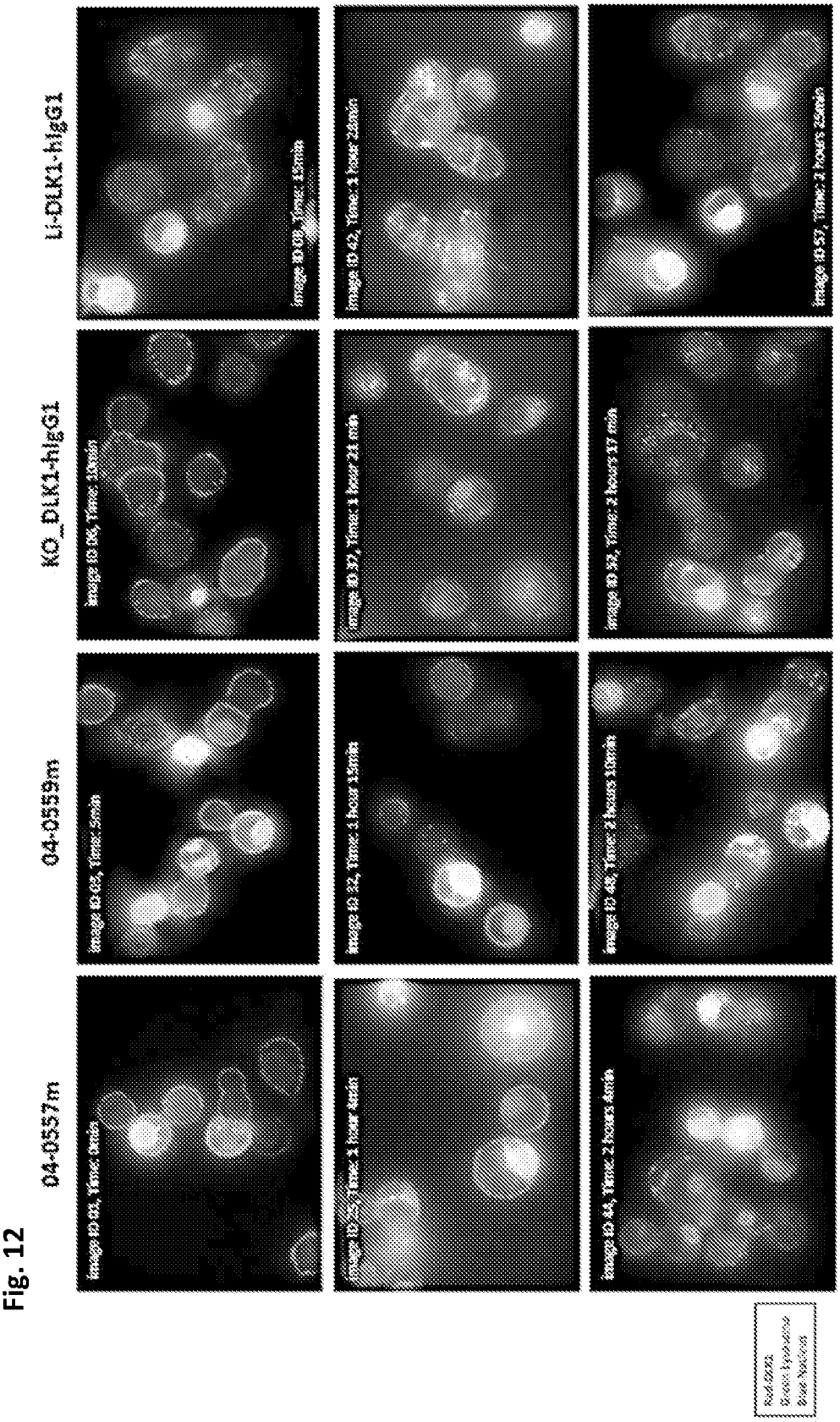

04-0557m
04-0559m
KO_DLK1-hIgG1
Li-DLK1-hIgG1
Image ID 01, Time: 0min
Image ID 05, Time: 5min
Image ID 06, Time: 15min
Image ID 02, Time: 1 hour 25min
Image ID 37, Time: 1 hour 21 min
Image ID 32, Time: 2 hours 17 min
Image ID 53, Time: 2 hours 25min
Image ID 54, Time: 2 hours 4min
Image ID 48, Time: 2 hours 10min

Fig. 13

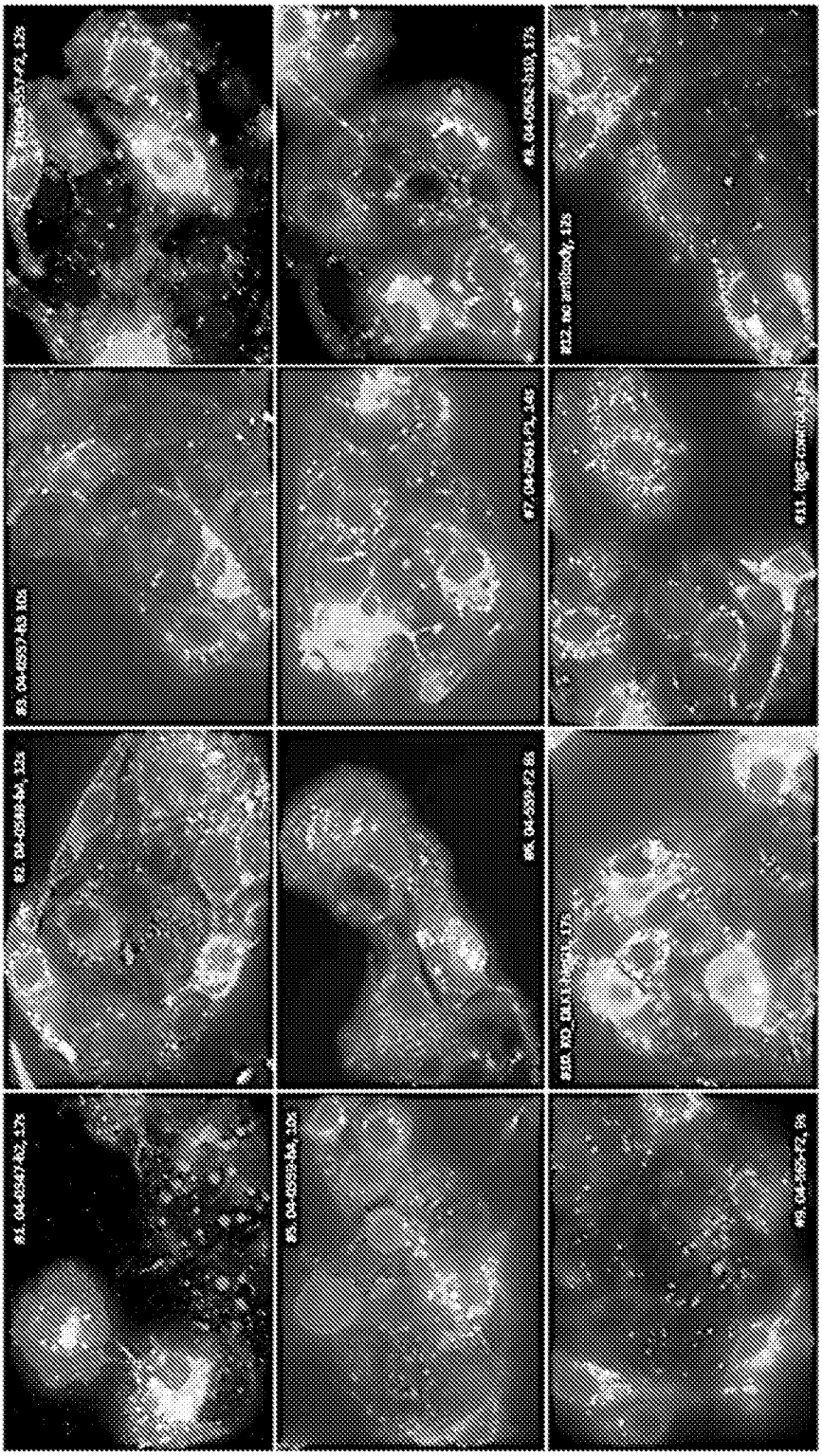


Fig. 14

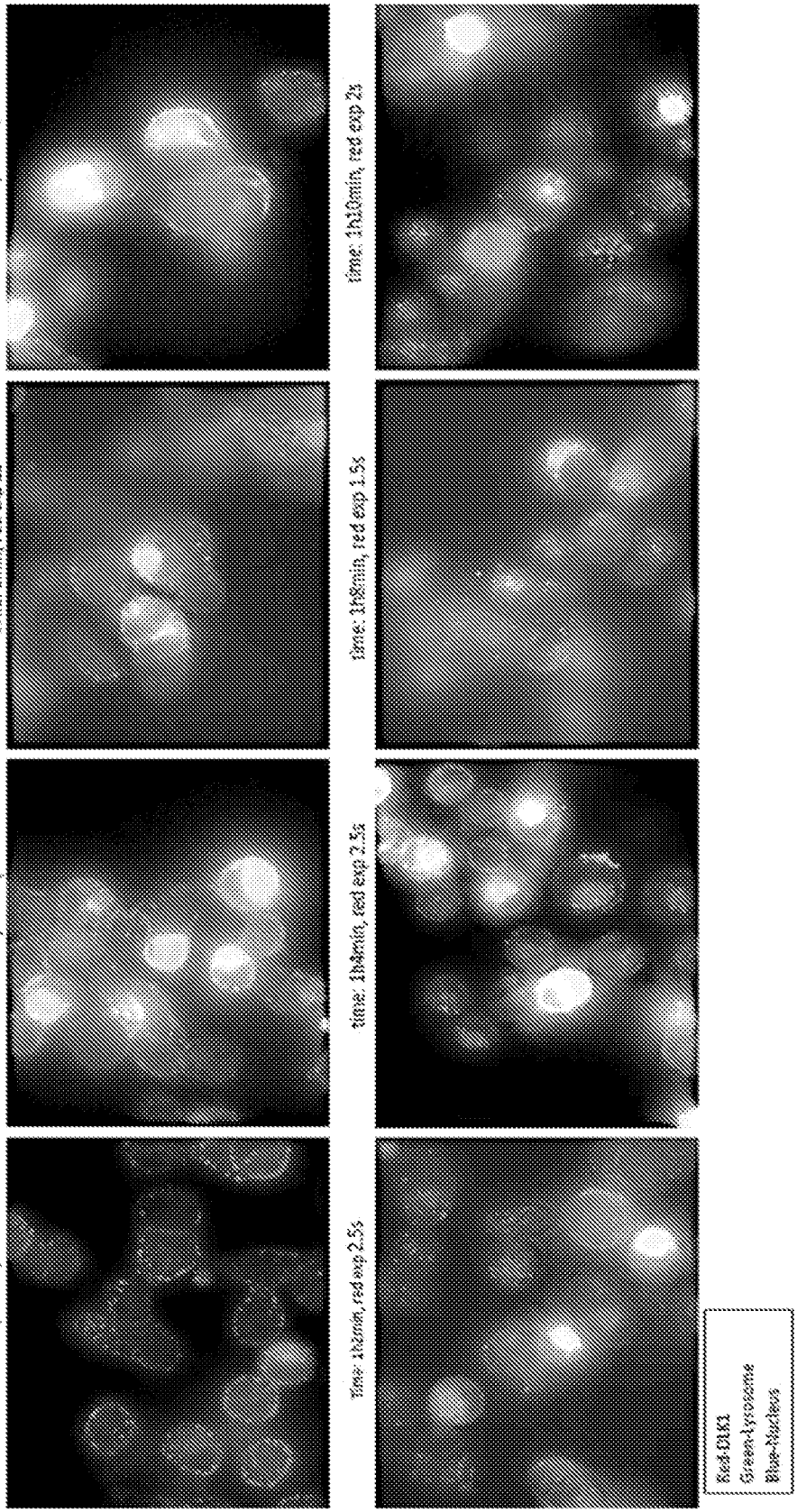

#1: 04-0547-h2
Time: 1min, red exp 1.2s
#2: 04-0548-h4
Time: 2min, red exp 1s
#3: 04-0557-h3
time: 5min, red exp 1s
#4: 04-557-r2
time: 13min, red exp 2s
time: 1hr4min, red exp 2.5s
time: 1hr8min, red exp 2.5s
time: 1hr8min, red exp 1.5s
time: 1hr10min, red exp 2s
Red-CD63
Green-lysosome
Blue-Nucleus

Fig. 15

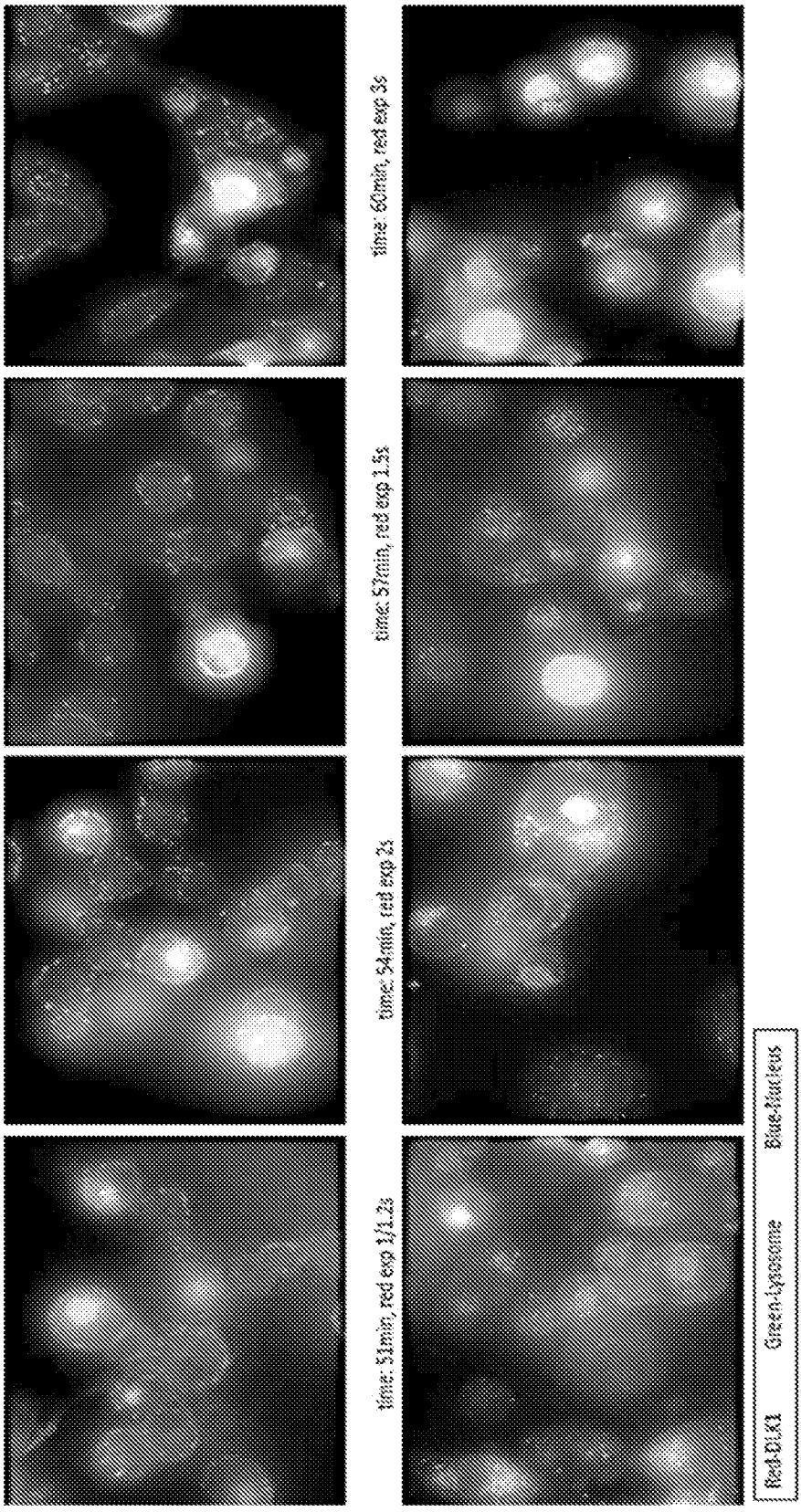

#5: 04-0559-b4
time: 3min, red exp 1/1.5s
#6: 04-559-f2
time: 5min, red exp 1s
#7: 04-0561-f1
time: 8min, red exp 1.2s
#8: 04-0562-h10
time: 11min, red exp 1s
time: 31min, red exp 1/1.2s
time: 54min, red exp 2s
time: 57min, red exp 1.5s
time: 60min, red exp 3s
Red-DLK1
Green-Lysosome
Blue-Nucleus

Fig. 16

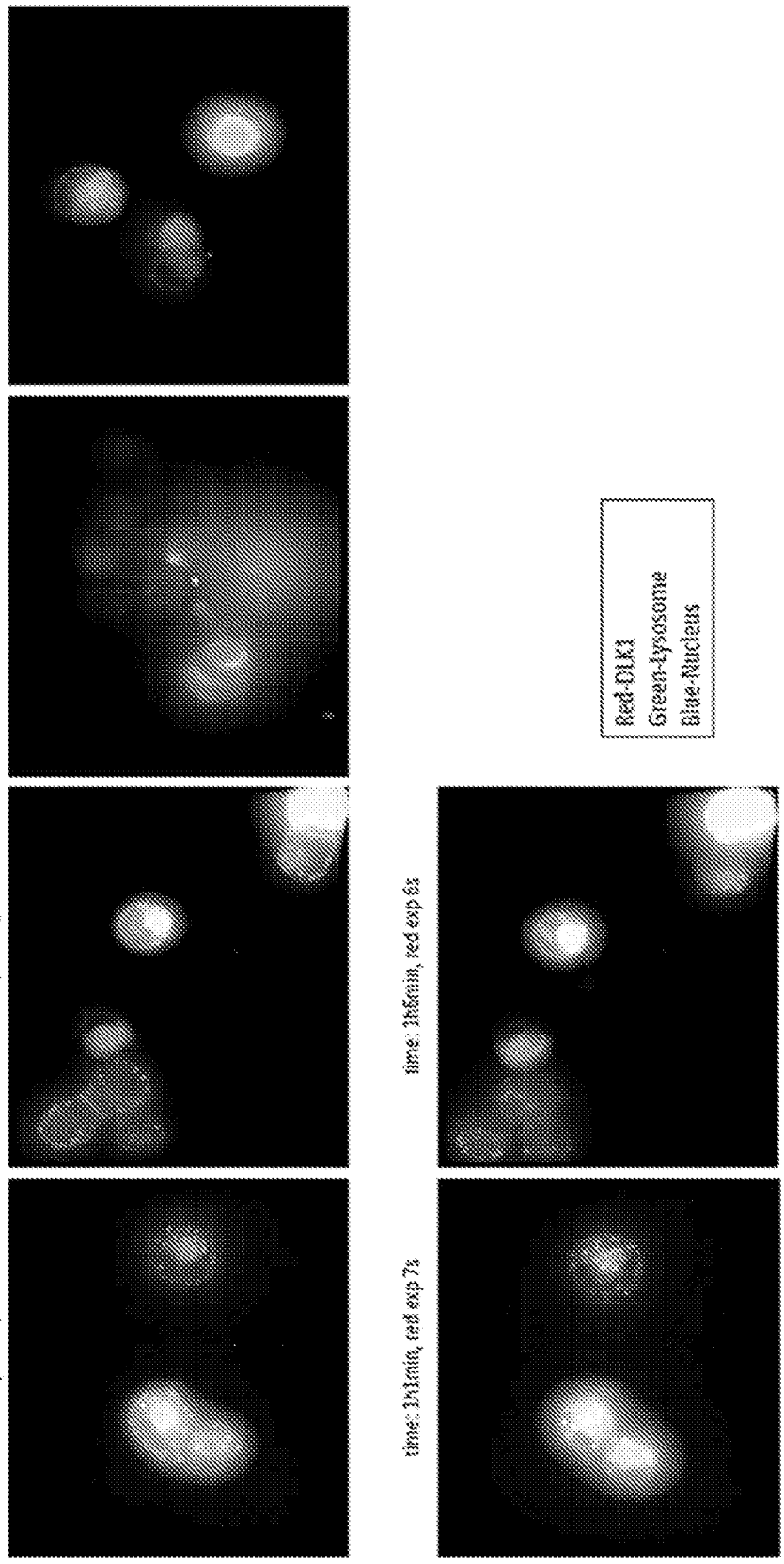

#3: 04-585-f2
time: 0min, red exp 6s
#10: KO_DLK1-hIgG1
time: 11min, red exp 5s
#11: hIgG1 control
time: 2min, red exp 5s
no any Antibody
time: 25min, red exp 5s
time: 1h1min, red exp 7s
time: 1h8min, red exp 6s
Red-DLK1
Green-Lysosome
Blue-Nucleus

Fig. 17

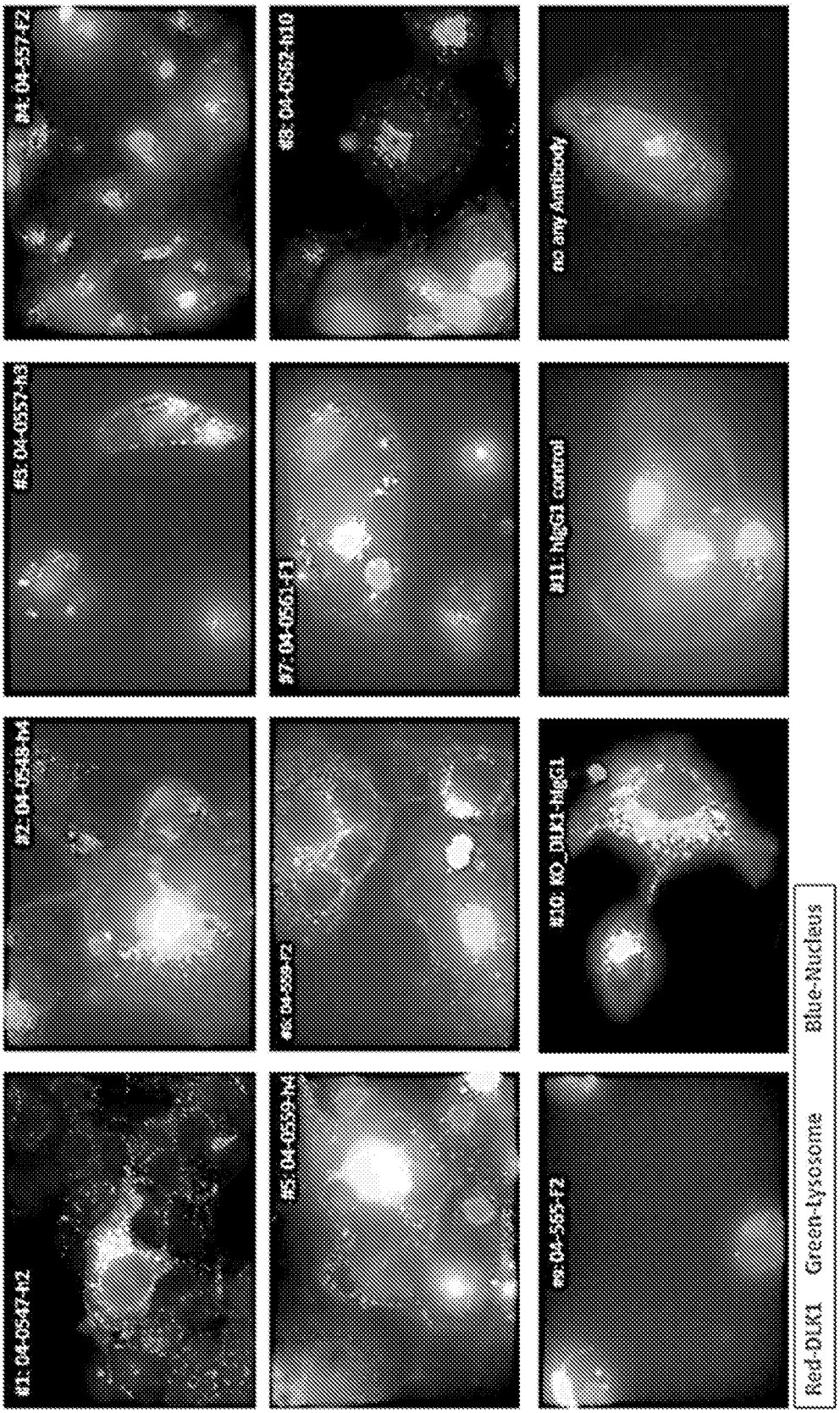

#1: 04-0547-h2
#2: 04-0548-h4
#3: 04-0557-h3
#4: 04-557-f2
#5: 04-0559-h4
#6: 04-558-f2
#7: 04-0561-f1
#8: 04-0562-h10
#9: 04-565-f2
#10: KO_DLK1-Mgl1
#11: Mgl1 control
no any Antibody
Red-DLK1 Green-Lysosome Blue-Nucleus

Fig. 18

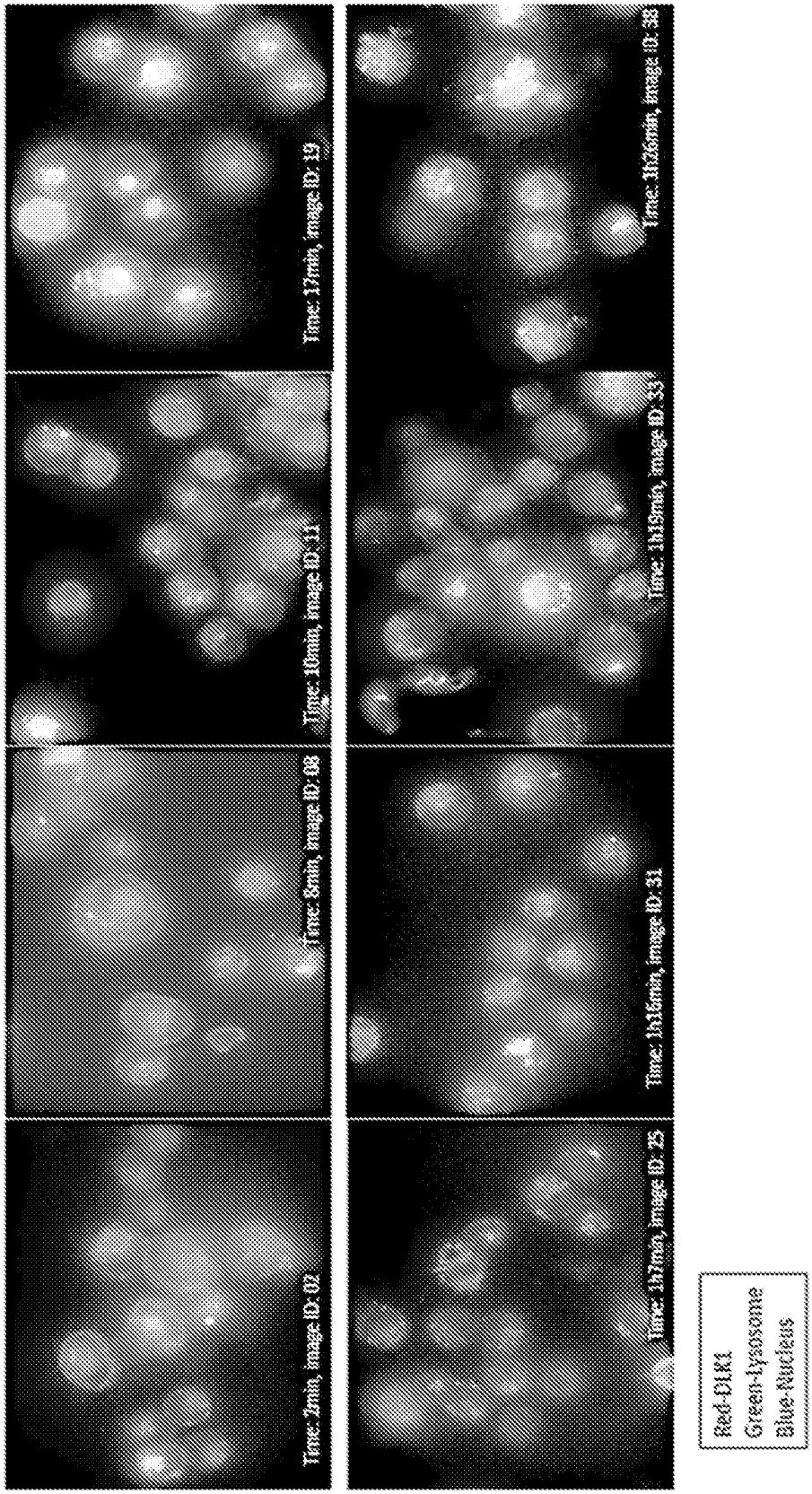

04-0547-h2-MMAE-AF647
04-0561-f1-MMAE-AF647
04-0562-h10-MMAE-AF647
04-0561-f1-AF647
Time: 2min, image ID: 02
Time: 8min, image ID: 08
Time: 10min, image ID: 11
Time: 17min, image ID: 19
Time: 1h7min, image ID: 25
Time: 1h16min, image ID: 31
Time: 1h19min, image ID: 33
Time: 1h26min, image ID: 38
Red-DLK1
Green-Lysosome
Blue-Nucleus

FIG. 19

Humanized DLK1 antibody of 04-0547-h2

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0547-h2_VH	Kabat	Sequence Fragment	EVQLVESGGGLVQPGGSLRLSCAASGFSIS	DYYMA	WVRQAPGKGLEWVA	NINYDGTNTYYADSVKG	RFTISRDNSKNTLYQMNSLRAEDTAVYYCVR
04-0547-h2_VH	Kabat	Residues	1 - 30	31 - 35	36 - 49	50 - 66	67 - 98
04-0547-h2_VH	Kabat	Length	30	5	14	17	32

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0547-h2_VL	Kabat	Sequence Fragment	DIQMTQSPSSLSASVGDRVTITC	RASHDVSTAVA	WYQQKPGKAPKLLIY	SASYRYT	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
04-0547-h2_VL	Kabat	Residues	1 - 23	24 - 34	35 - 49	50 - 56	57 - 88
04-0547-h2_VL	Kabat	Length	23	11	15	7	32

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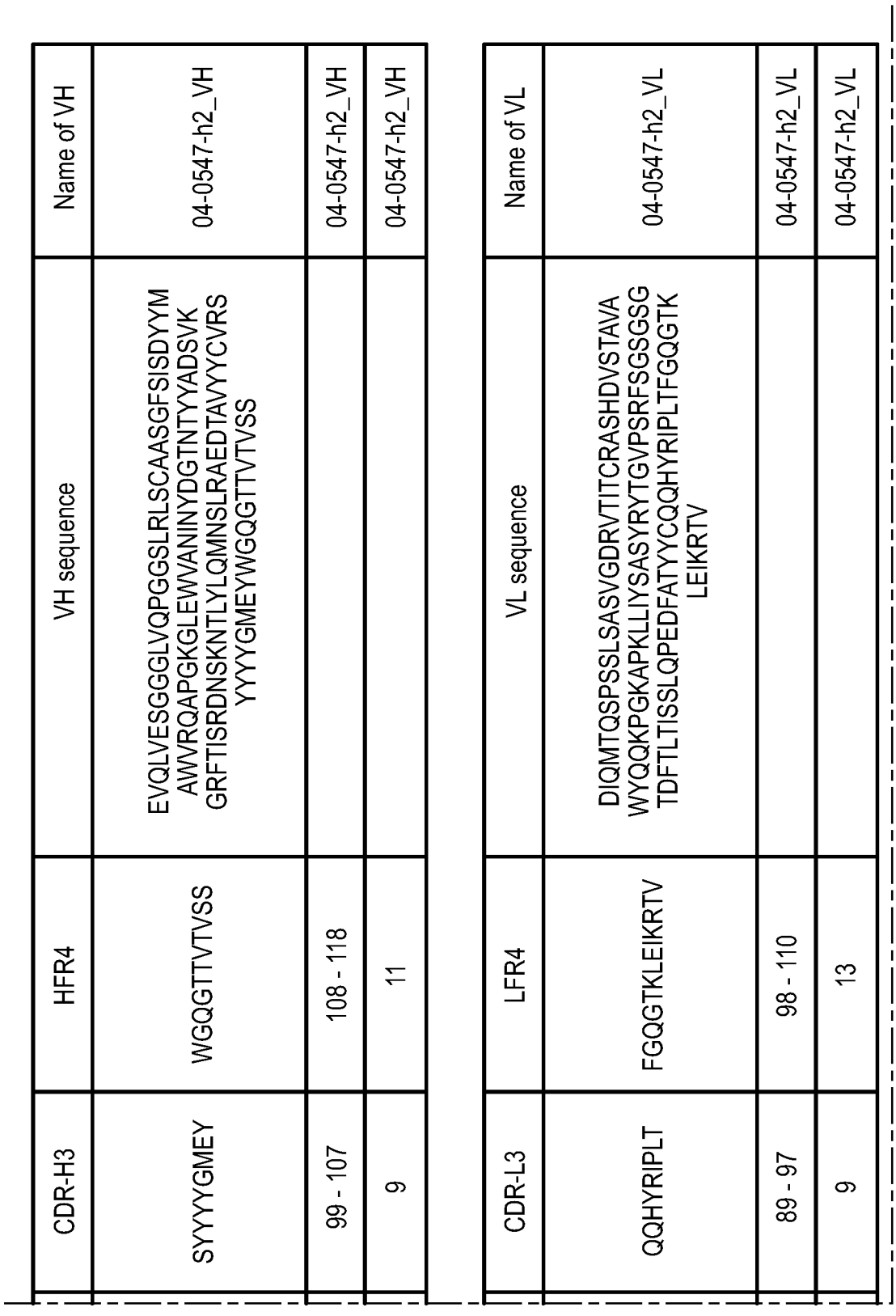
FIG. 19 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SYYYYGMEY	WGQGTTVTVSS	EVQLVESGGGLVQPGGSLRLSCAASGFSISDYYM AWVRQAPGKGLEWVANINYDGTNTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVRS YYYYGMEYWGQGTTVTVSS	04-0547-h2_VH
99 - 107	108 - 118		04-0547-h2_VH
9	11		04-0547-h2_VH

CDR-L3	LFR4	VL sequence	Name of VL
QQHYRIPLT	FGQGTKLEIKRTV	DIQMTQSPSSLSASVGDRVTITCRASHDVSTAVA WYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQHYRIPLTFGQGTK LEIKRTV	04-0547-h2_VL
89 - 97	98 - 110		04-0547-h2_VL
9	13		04-0547-h2_VL

FIG. 19 CONT.

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0547-h2_VH	AbM	Sequence Fragment	EVQLVESGGGLVQPGGSLRLSCAAS	GFSISDYYMA	WVRQAPGKGLEWVA	NINYDGTNTY	YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVR
04-0547-h2_VH	AbM	Residues	1 - 25	26 - 35	36 - 49	50 - 59	60 - 98
04-0547-h2_VH	AbM	Length	25	10	14	10	39

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0547-h2_VL	AbM	Sequence Fragment	DIQMTQSPSSLSASVGDRVTITC	RASHDVSTAVA	WYQQKPGKAPKLLIY	SASYRYT	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
04-0547-h2_VL	AbM	Residues	1 - 23	24 - 34	35 - 49	50 - 56	57 - 88
04-0547-h2_VL	AbM	Length	23	11	15	7	32

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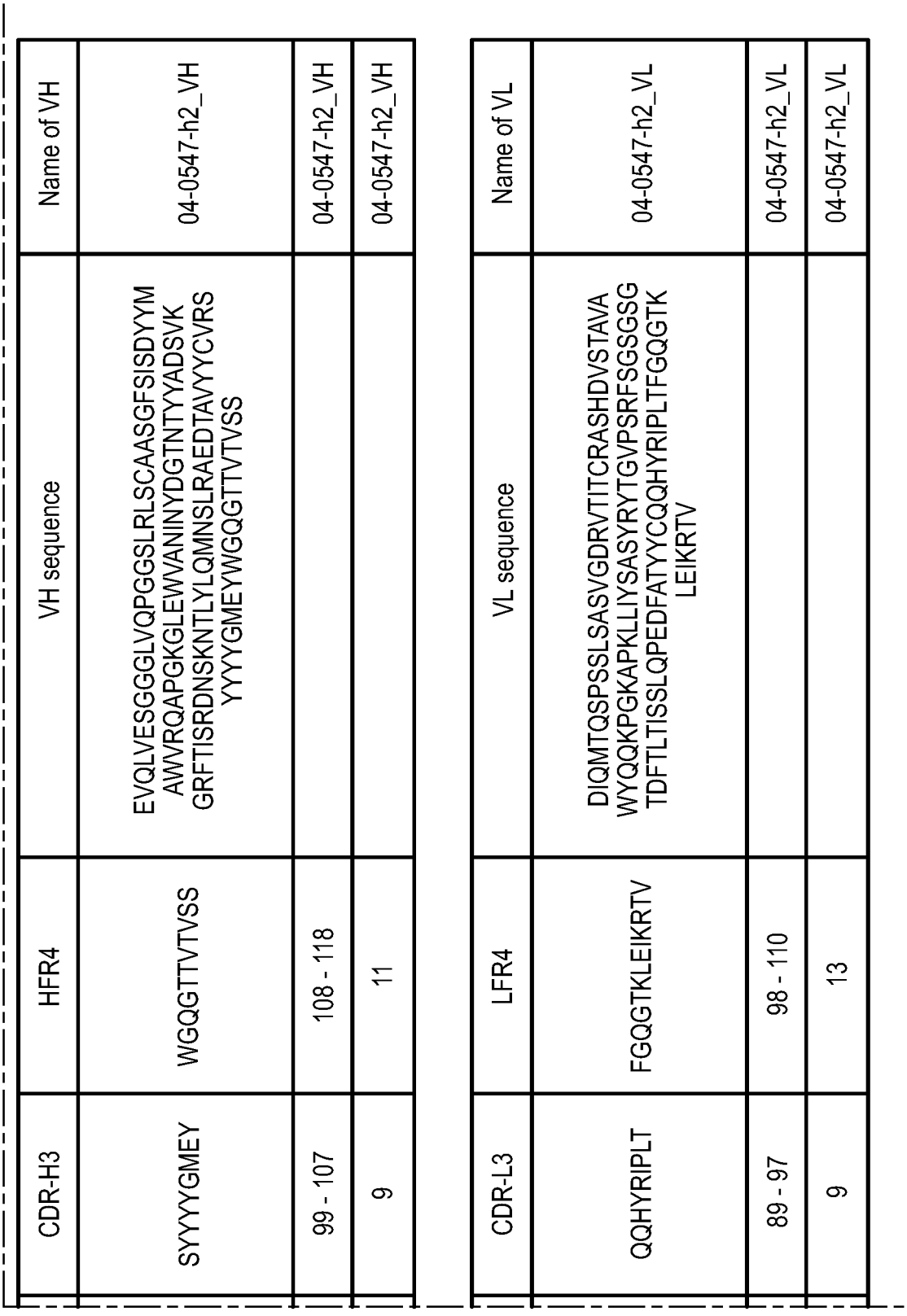
FIG. 19 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SYYYYGMEY	WGQGTTVTVSS	EVQLVESGGGLVQPGGSLRLSCAASGFSISDYYM AWVRQAPGKGLEWVANINYDGTNTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVRS YYYYGMEYWGQGTTVTVSS	04-0547-h2_VH
99 - 107	108 - 118		04-0547-h2_VH
9	11		04-0547-h2_VH

CDR-L3	LFR4	VL sequence	Name of VL
QQHYRIPLT	FGQGTKLEIKRTV	DIQMTQSPSSLSASVGDRVTITCRASHDVSTAVA WYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQHYRIPLTFGQGTK LEIKRTV	04-0547-h2_VL
89 - 97	98 - 110		04-0547-h2_VL
9	13		04-0547-h2_VL

FIG. 20

Humanized DLK1 antibody of 04-0561-F1

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0561-F1_VH	Kabat	Sequence Fragment	QVQLQESGPGLVKPSETLSLTCTVSGFSLS	IYSVH	WVRQPPGKGLEWIG	LIWGGGSTDYNPSLKS	RVTISKDTSKNQVSLKLSSVTAADTAVYYCAR
04-0561-F1_VH	Kabat	Residues	1 - 30	31 - 35	36 - 49	50 - 65	66 - 97
04-0561-F1_VH	Kabat	Length	30	5	14	16	32

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0561-F1_VL	Kabat	Sequence Fragment	DIVMTQSPDSLAVSLGERVTMNC	KSSQSLLQSSNQKNYLA	WYQQKPGQPPKLLVY	FASTRES	GVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC
04-0561-F1_VL	Kabat	Residues	1 - 23	24 - 40	41 - 55	56 - 62	63 - 94
04-0561-F1_VL	Kabat	Length	23	17	15	7	32

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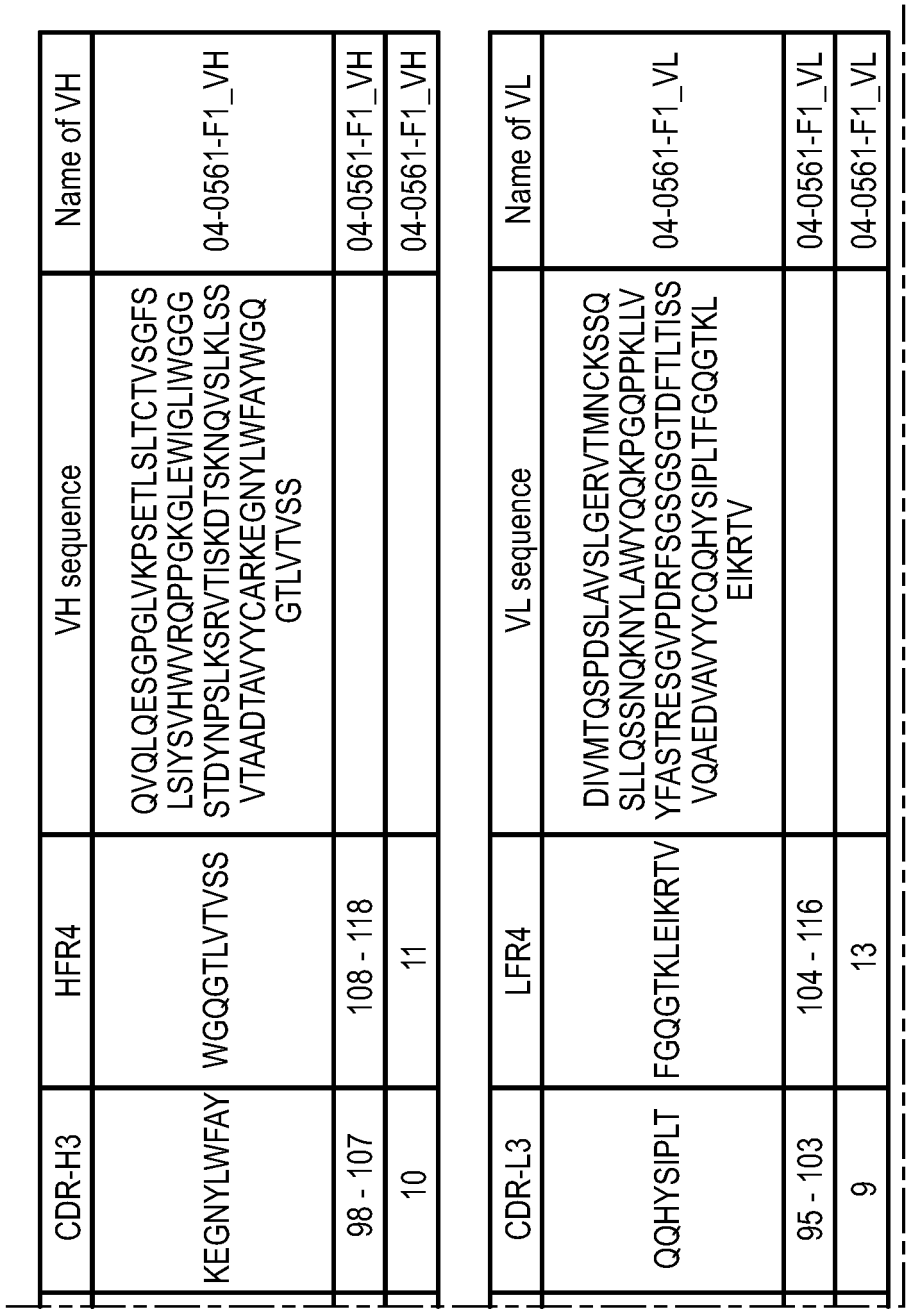
FIG. 20 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
KEGNYLWFAY	WGQGTLVTVSS	QVQLQESGPGLVKPSETLSLTCTVSGFS LSIYSVHWVRQPPGKGLEWIGLIWGGG STDYNPSLKSRVTISKDTSKNQVSLKLSS VTAADTAVYYCARKEGNYLWFAYWGQ GTLVTVSS	04-0561-F1_VH
98 - 107	108 - 118		04-0561-F1_VH
10	11		04-0561-F1_VH

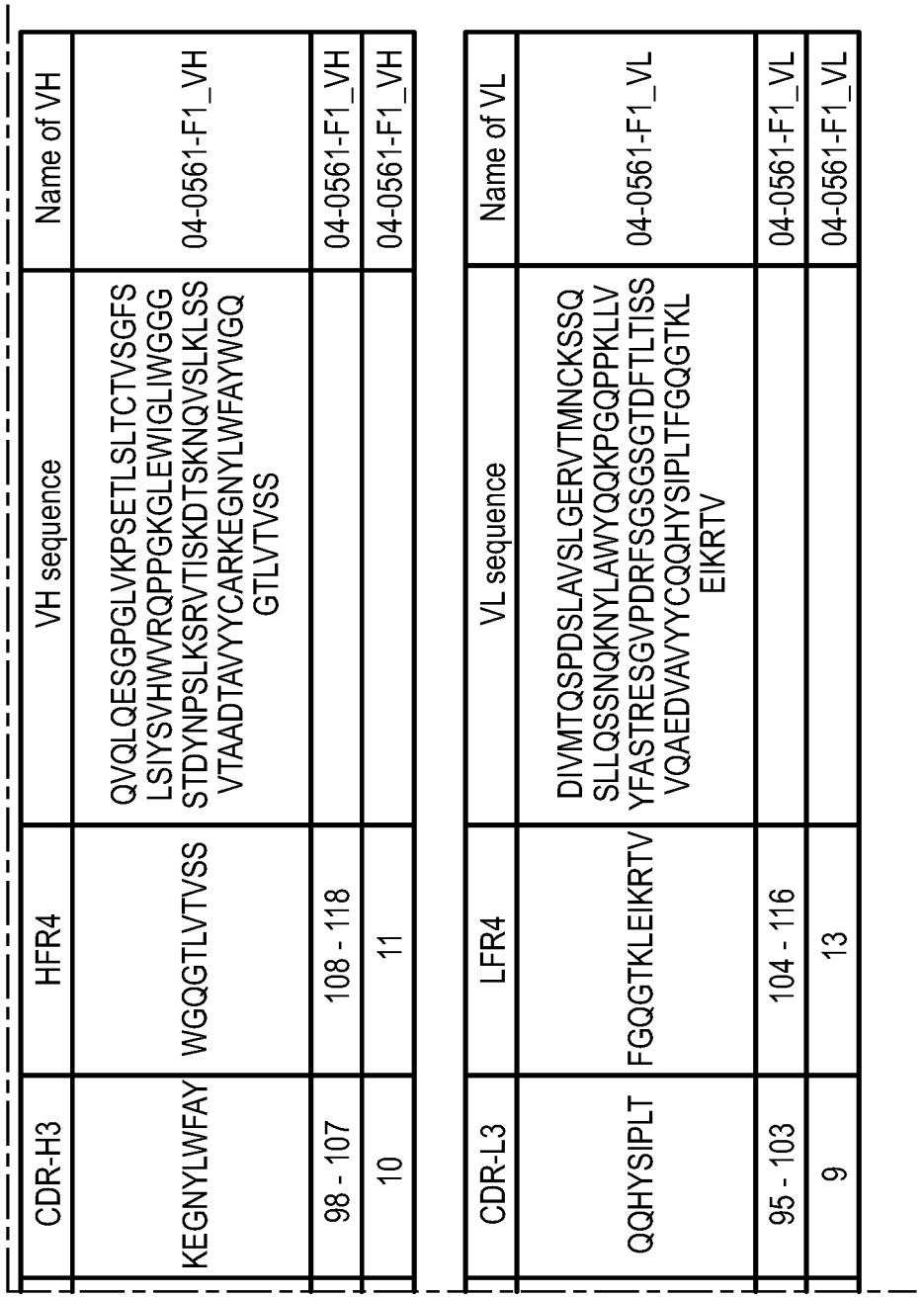
CDR-L3	LFR4	VL sequence	Name of VL
QQHYSIPLT	FGQGTKLEIKRTV	DIVMTQSPDSLAVSLGERVTMNCKSSQ SLLQSSNQKNYLAWYQQKPGQPPKLLV YFASTRESGVPDRFSGSGSGTDFTLTISS VQAEDVAVYYCQQHYSIPLTFGQGTKL EIKRTV	04-0561-F1_VL
95 - 103	104 - 116		04-0561-F1_VL
9	13		04-0561-F1_VL

FIG. 20 CONT.

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0561-F1_VH	AbM	Sequence Fragment	QVQLQESGPGLVKPSETLSLTCTVS	GFSLSIYSVH	WVRQPPGKGLEWIG	LIWGGGSTD	YNPSLKSRVTISKDTSKNQVSLKLSSVTAADTAVYYCAR
04-0561-F1_VH	AbM	Residues	1 - 25	26 - 35	36 - 49	50 - 58	59 - 97
04-0561-F1_VH	AbM	Length	25	10	14	9	39

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0561-F1_VL	AbM	Sequence Fragment	DIVMTQSPDSLAVSLGERVTMNC	KSSQSLLQSSNQKNYLA	WYQQKPGQPPKLLVY	FASTRES	GVPDRFSGSGSGTDFTLTISSVQAEDVAVYYC
04-0561-F1_VL	AbM	Residues	1 - 23	24 - 40	41 - 55	56 - 62	63 - 94
04-0561-F1_VL	AbM	Length	23	17	15	7	32

FIG. 20 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
KEGNYLWFAY	WGQGTLVTVSS	QVQLQESGPGLVKPSETLSLTCTVSGFS LSIYSVHWVRQPPGKGLEWIGLIWGGG STDYNPSLKSRVTISKDTSKNQVSLKLSS VTAADTAVYYCARKEGNYLWFAYWGQ GTLVTVSS	04-0561-F1_VH
98 - 107	108 - 118		04-0561-F1_VH
10	11		04-0561-F1_VH

CDR-L3	LFR4	VL sequence	Name of VL
QQHYSIPLT	FGQGTKLEIKRTV	DIVMTQSPDSLAVSLGERVTMNCKSSQ SLLQSSNQKNYLAWYQQKPGQPPKLLV YFASTRESGVPDRFSGSGSGTDFTLTISS VQAEDVAVYYCQQHYSIPLTFGQGTKL EIKRTV	04-0561-F1_VL
95 - 103	104 - 116		04-0561-F1_VL
9	13		04-0561-F1_VL

FIG. 21

Humanized DLK1 antibody of 04-0562-h10

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0562-h10_VH	Kabat	Sequence Fragment	QVQLQESGPGLVKPSETLSLTCTVSGFSLT	SYGVS	WVRQPPGKGLEWIG	VIWGDGSTSYNPSLKS	RVTISKDTSKNQVSLKLSSVTAADTAVYYCAK
04-0562-h10_VH	Kabat	Residues	1 - 30	31 - 35	36 - 49	50 - 65	66 - 97
04-0562-h10_VH	Kabat	Length	30	5	14	16	32

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0562-h10_VL	Kabat	Sequence Fragment	DIVMTQSPLSLPVTPGEPASISC	RSSQSLVHINGNTYLH	WYLQKPGQSPQLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
04-0562-h10_VL	Kabat	Residues	1 - 23	24 - 39	**40 - 54**	55 - 61	62 - 93
04-0562-h10_VL	Kabat	Length	23	16	15	7	32

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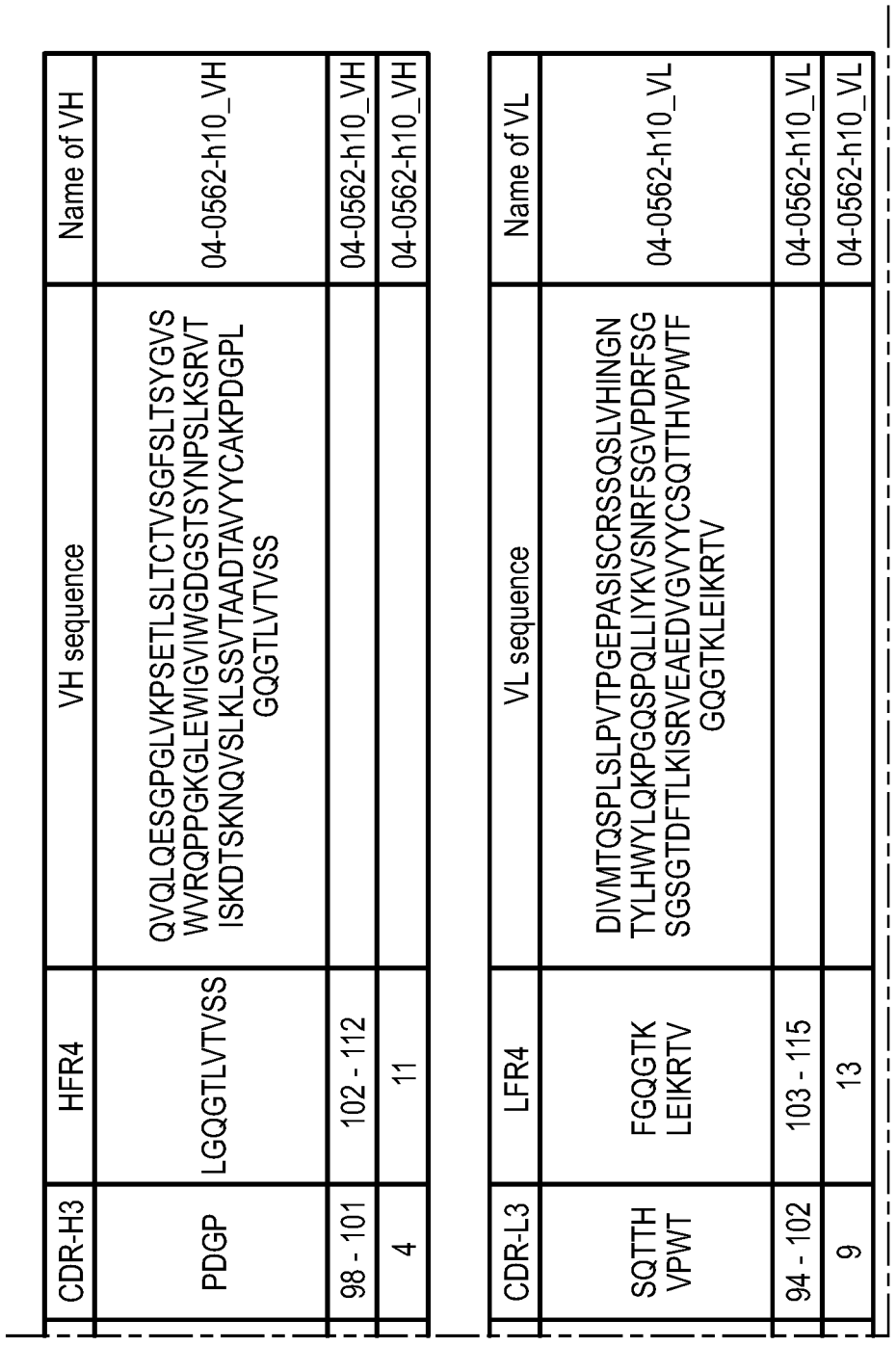
FIG. 21 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
PDGP	LGQGTLVTVSS	QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVS WVRQPPGKGLEWIGVIWGDGSTSYNPSLKSRVT ISKDTSKNQVSLKLSSVTAADTAVYYCAKPDGPL GQGTLVTVSS	04-0562-h10_VH
98 - 101	102 - 112		04-0562-h10_VH
4	11		04-0562-h10_VH

CDR-L3	LFR4	VL sequence	Name of VL
SQTTH VPWT	FGQGTK LEIKRTV	DIVMTQSPLSLPVTPGEPASISCRSSQSLVHINGN TYLHWYLQKPGQSPQLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISRVEAEDVGVYYCSQTTHVPWTF GQGTKLEIKRTV	04-0562-h10_VL
94 - 102	103 - 115		04-0562-h10_VL
9	13		04-0562-h10_VL

FIG. 21 CONT.

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0562-h10_VH	AbM	Sequence Fragment	QVQLQESGPGLVKP SETLSLTCTVS	GFSLT SYGVS	WVRQPPGK GLEWIG	VIWGDGS TS	YNPSLKSRVTISKDTSKNQ VSLKLSSVTAADTAVYYCAK
04-0562-h10_VH	AbM	Residues	1 - 30	26 - 35	36 - 49	50 - 58	59 - 97
04-0562-h10_VH	AbM	Length	25	10	14	9	39

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0562-h10_VL	AbM	Sequence Fragment	DIVMTQSPLSLPVTP GEPASISC	RSSQS LVHING NTYLH	WYLQKPGQ SPQLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKI SRVEAEDVGVYYC
04-0562-h10_VL	AbM	Residues	1 - 23	24 - 39	**40 - 54**	55 - 61	62 - 93
04-0562-h10_VL	AbM	Length	23	16	15	7	32

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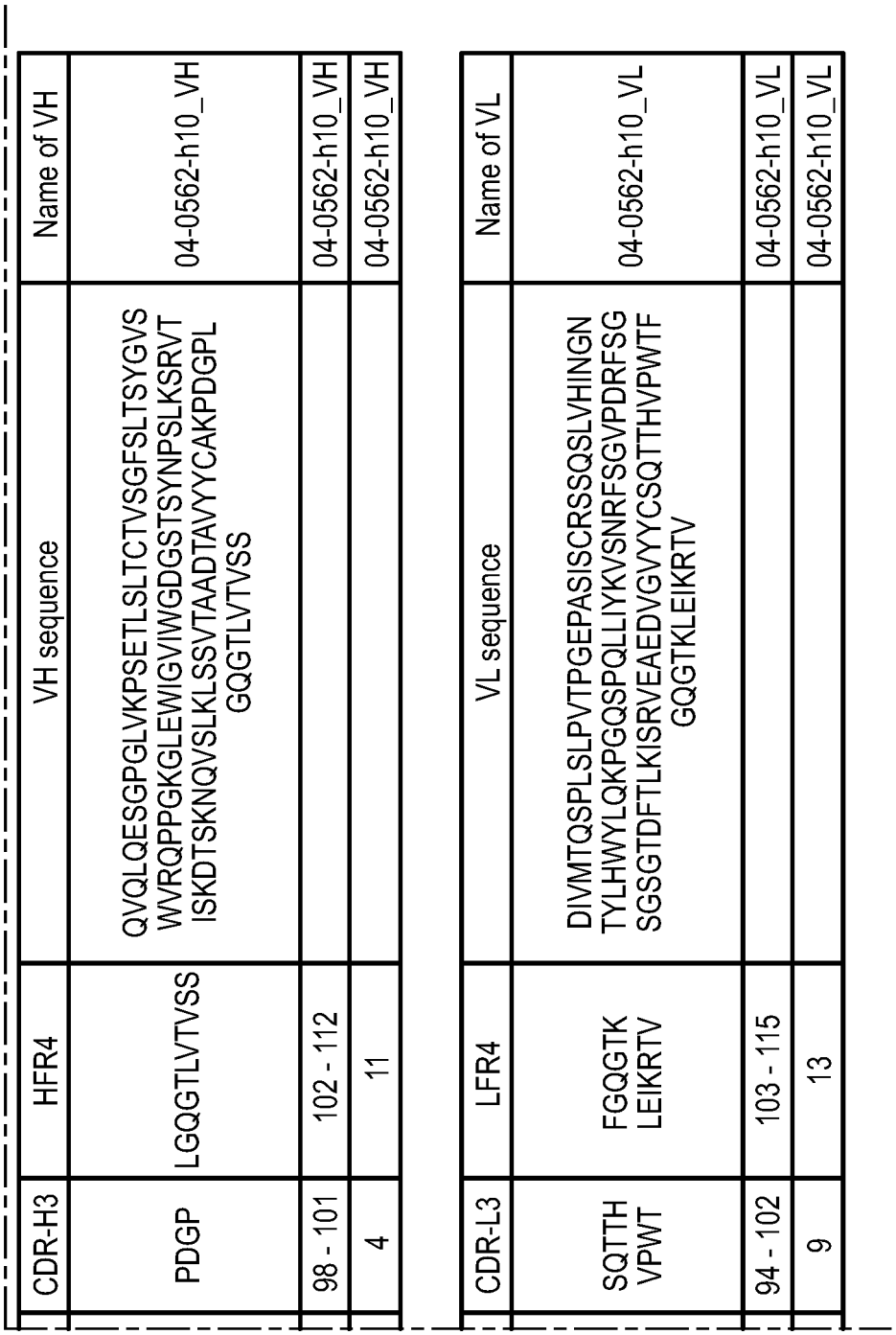
FIG. 21 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
PDGP	LGQGTLVTVSS	QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVS WVRQPPGKGLEWIGVIWGDGSTSYNPSLKSRVT ISKDTSKNQVSLKLSSVTAADTAVYYCAKPDGPL GQGTLVTVSS	04-0562-h10_VH
98 - 101	102 - 112		04-0562-h10_VH
4	11		04-0562-h10_VH

CDR-L3	LFR4	VL sequence	Name of VL
SQTTH VPWT	FGQGTK LEIKRTV	DIVMTQSPLSLPVTPGEPASISCRSSQSLVHINGN TYLHWYLQKPGQSPQLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISRVEAEDVGVYYCSQTTHVPWTF GQGTKLEIKRTV	04-0562-h10_VL
94 - 102	103 - 115		04-0562-h10_VL
9	13		04-0562-h10_VL

FIG. 22

Humanized DLK1 antibody of 04-0548-h4

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0548-h4_VH	Kabat	Sequence Fragment	EVQFVESGGGLVQPG GSLRLSCAASGFTFS	NFWMN	WVRQAPGK GLEWIA	QIRLKSDN YGTHYAD SVKG	RFTISRDNSKNTVYLQ MNSLRAEDTGVYYCTE
04-0548-h4_VH	Kabat	Residues	1 - 30	31 - 35	36 - 49	50 - 68	69 - 100
04-0548-h4_VH	Kabat	Length	30	5	14	19	32

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0548-h4_VL	Kabat	Sequence Fragment	DIQMTQSPSSLSAS VGDRVTITC	RASQD VSAAVA	WYQQKPG KAPKVLIY	SASNRYT	GVPSRFSGSGSGTDF TFTISSVQPEDFATYYC
04-0548-h4_VL	Kabat	Residues	1 - 23	24 - 34	**35 - 49**	50 - 56	57 - 88
04-0548-h4_VL	Kabat	Length	23	11	15	7	32

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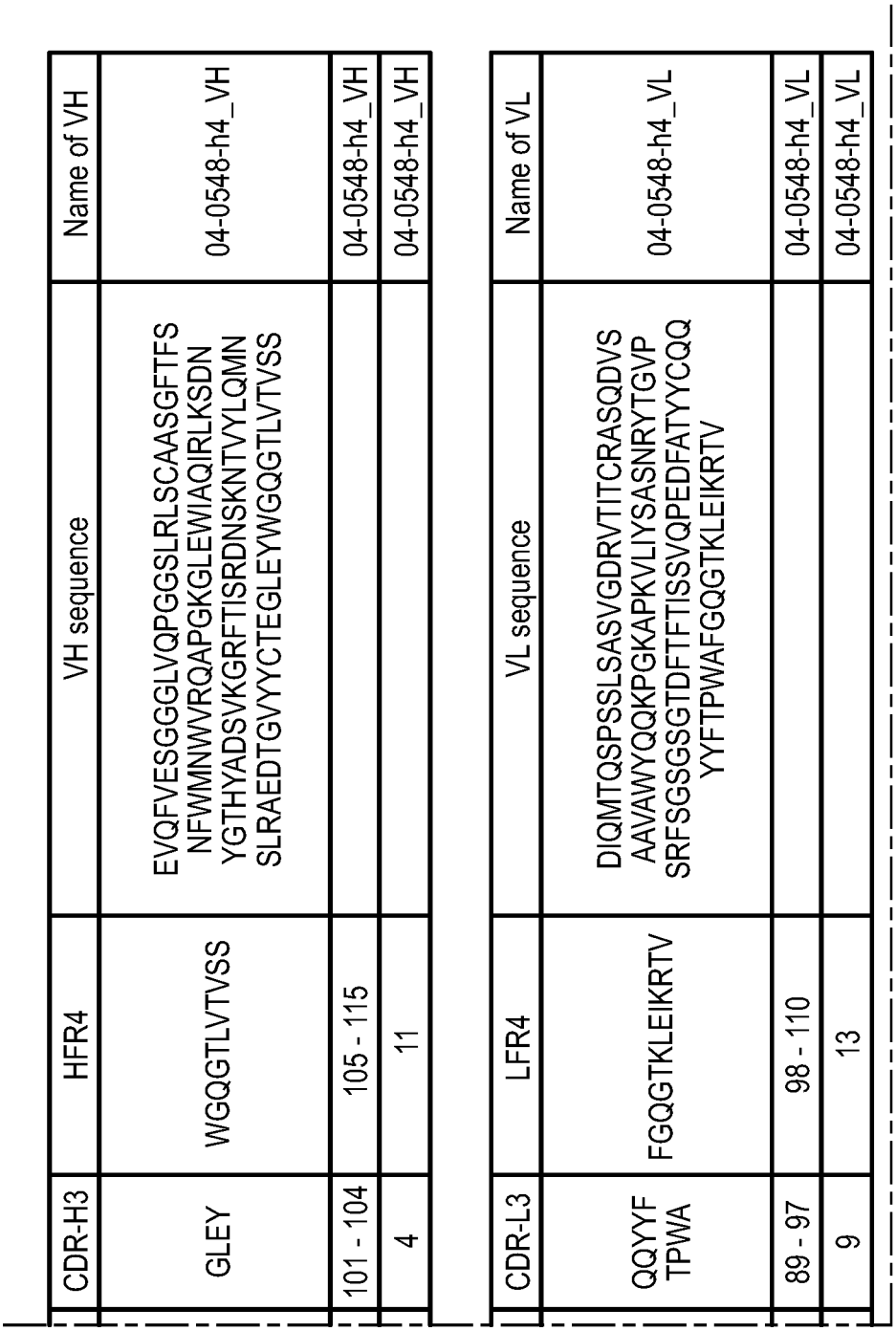
FIG. 22 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
GLEY	WGQGTLVTVSS	EVQFVESGGGLVQPGGSLRLSCAASGFTFS NFWMNWVRQAPGKGLEWIAQIRLKSDN YGTHYADSVKGRFTISRDNSKNTVYLQMN SLRAEDTGVYYCTEGLEYWGQGTLVTVSS	04-0548-h4_VH
101 - 104	105 - 115		04-0548-h4_VH
4	11		04-0548-h4_VH

CDR-L3	LFR4	VL sequence	Name of VL
QQYYF TPWA	FGQGTKLEIKRTV	DIQMTQSPSSLSASVGDRVTITCRASQDVS AAVAWYQQKPGKAPKVLIYSASNRYTGVP SRFSGSGSGTDFTFTISSVQPEDFATYYCQQ YYFTPWAFGQGTKLEIKRTV	04-0548-h4_VL
89 - 97	98 - 110		04-0548-h4_VL
9	13		04-0548-h4_VL

FIG. 22 CONT.

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0548-h4_VH	AbM	Sequence Fragment	EVQFVESGGGLVQ PGGSLRLSCAAS	GFTFSN FWMN	WVRQAPG KGLEWIA	QIRLKSDN YGTH	YADSVKGRFTISRDNS KNTVYLQMNSLRAE DTGVYYCTE
04-0548-h4_VH	AbM	Residues	1 - 25	26 - 35	36 - 49	50 - 61	62 - 100
04-0548-h4_VH	AbM	Length	25	10	14	12	39

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0548-h4_VL	AbM	Sequence Fragment	DIQMTQSPSSLSAS VGDRVTITC	RASQD VSAAVA	WYQQKPG KAPKVLIY	SASNRYT	GVPSRFSGSGSGTDF TFTISSVQPEDFATYYC
04-0548-h4_VL	AbM	Residues	1 - 23	24 - 34	**35 - 49**	50 - 56	57 - 88
04-0548-h4_VL	AbM	Length	23	11	15	7	32

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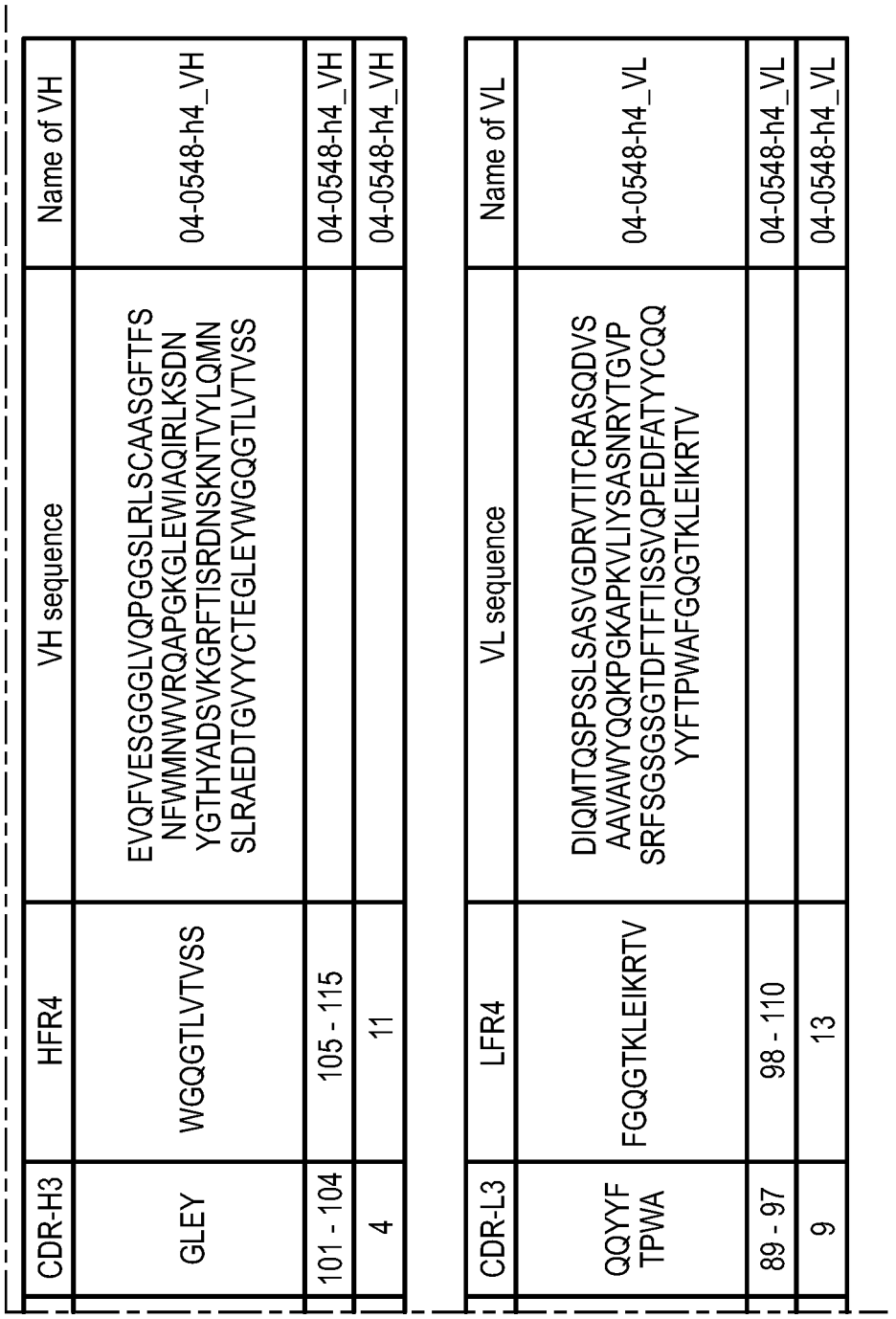
FIG. 22 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
GLEY	WGQGTLVTVSS	EVQFVESGGGLVQPGGSLRLSCAASGFTFS NFWMNWVRQAPGKGLEWIAQIRLKSDN YGTHYADSVKGRFTISRDNSKNTVYLQMN SLRAEDTGVYYCTEGLEYWGQGTLVTVSS	04-0548-h4_VH
101 - 104	105 - 115		04-0548-h4_VH
4	11		04-0548-h4_VH

CDR-L3	LFR4	VL sequence	Name of VL
QQYYF TPWA	FGQGTKLEIKRTV	DIQMTQSPSSLSASVGDRVTITCRASQDVS AAVAWYQQKPGKAPKVLIYSASNRYTGVP SRFSGSGSGTDFTFTISSVQPEDFATYYCQQ YYFTPWAFGQGTKLEIKRTV	04-0548-h4_VL
89 - 97	98 - 110		04-0548-h4_VL
9	13		04-0548-h4_VL

FIG. 23

Humanized DLK1 antibody of 04-0557-F2

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0557-F2_VH	Kabat	Sequence Fragment	QVQLVQSGAEVKKP GSSVKVSCKSSGYSFS	GYFLN	WMRQAPGQ GLEWMG	RIHPYQG DILYAQK FQG	RVTITADKSTSTAYMELSS LRSEDTAVYYCGR
04-0557-F2_VH	Kabat	Residues	1 - 30	31 - 35	36 - 49	50 - 66	67 - 98
04-0557-F2_VH	Kabat	Length	30	5	14	17	32

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0557-F2_VL	Kabat	Sequence Fragment	DIQMTQSPSSLSAS VGDRVTITC	RASQD IITTVA	WYQQKPG KAPKLLIY	SASYRYP	GVPSRFSGSGSGTDF TLTISSVQPEDFATYYC
04-0557-F2_VL	Kabat	Residues	1 - 23	24 - 34	**35 - 49**	50 - 56	57 - 88
04-0557-F2_VL	Kabat	Length	23	11	15	7	32

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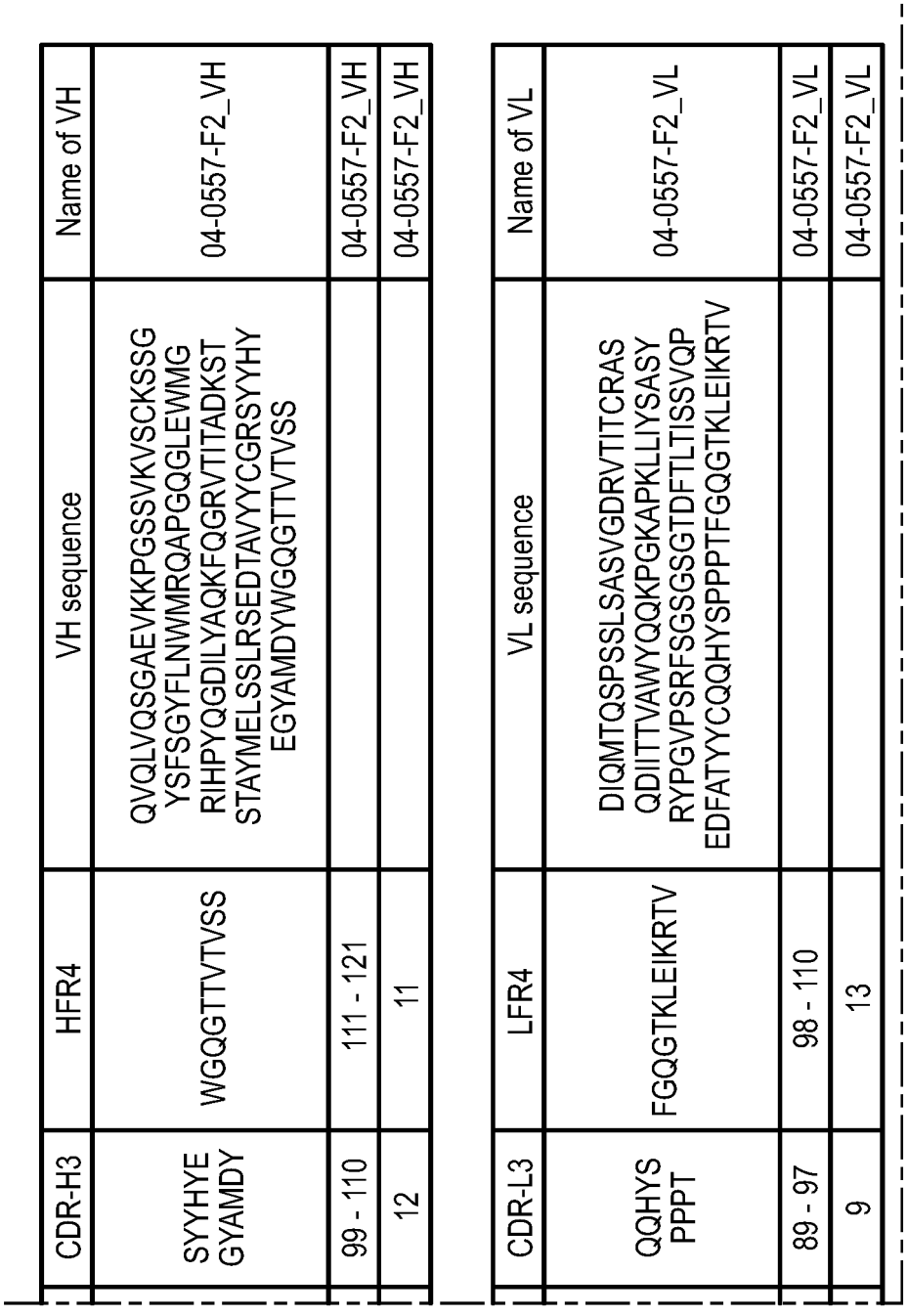
FIG. 23 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SYYHYE GYAMDY	WGQGTTVTVSS	QVQLVQSGAEVKKPGSSVKVSCKSSG YSFSGYFLNWMRQAPGQGLEWMG RIHPYQGDILYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCGRSYYHY EGYAMDYWGQGTTVTVSS	04-0557-F2_VH
99 - 110	111 - 121		04-0557-F2_VH
12	11		04-0557-F2_VH

CDR-L3	LFR4	VL sequence	Name of VL
QQHYS PPPT	FGQGTKLEIKRTV	DIQMTQSPSSLSASVGDRVTITCRAS QDIITTVAWYQQKPGKAPKLLIYSASY RYPGVPSRFSGSGSGTDFTLTISSVQP EDFATYYCQQHYSPPPTFGQGTKLEIKRTV	04-0557-F2_VL
89 - 97	98 - 110		04-0557-F2_VL
9	13		04-0557-F2_VL

FIG. 23 CONT.

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0557-F2_VH	AbM	Sequence Fragment	QVQLVQSGAEVKKP GSSVKVSCKSS	GYSFS GYFLN	WMRQAPG QGLEWMG	RIHPYQG DIL	YAQKFQGRVTITADKSTSTA YMELSSLRSEDTAVYYCGR
04-0557-F2_VH	AbM	Residues	1 - 25	26 - 35	36 - 49	50 - 59	60 - 98
04-0557-F2_VH	AbM	Length	25	10	14	10	39

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0557-F2_VL	AbM	Sequence Fragment	DIQMTQSPSSLSAS VGDRVTITC	RASQD IITTVA	WYQQKPG KAPKLLIY	SASYRYP	GVPSRFSGSGSGTDF TLTISSVQPEDFATYYC
04-0557-F2_VL	AbM	Residues	1 - 23	24 - 34	**35 - 49**	50 - 56	57 - 88
04-0557-F2_VL	AbM	Length	23	11	15	7	32

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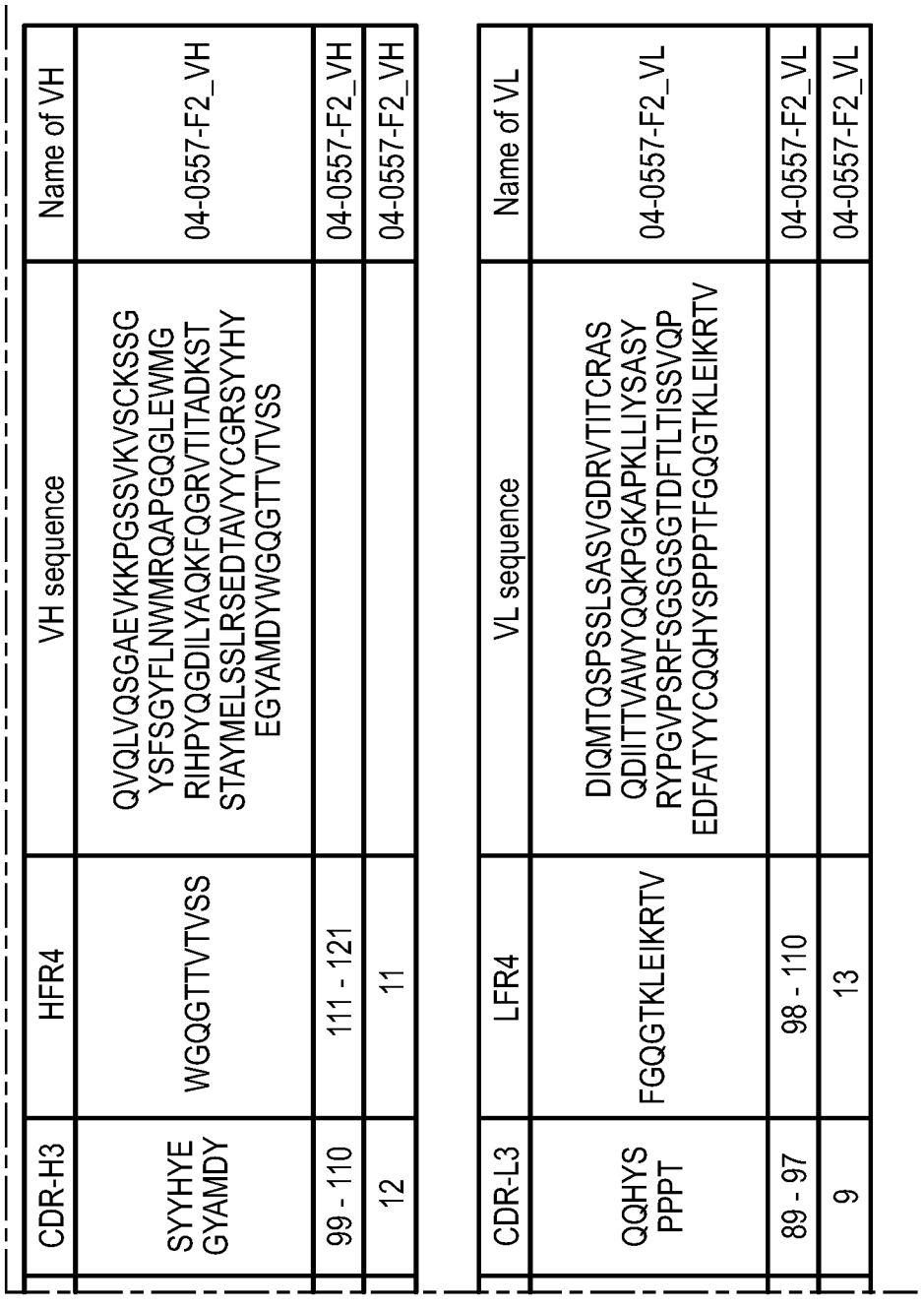
FIG. 23 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SYYHYE GYAMDY	WGQGTTVTVSS	QVQLVQSGAEVKKPGSSVKVSCKSSG YSFSGYFLNWMRQAPGQGLEWMG RIHPYQGDILYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCGRSYYHY EGYAMDYWGQGTTVTVSS	04-0557-F2_VH
99 - 110	111 - 121		04-0557-F2_VH
12	11		04-0557-F2_VH

CDR-L3	LFR4	VL sequence	Name of VL
QQHYS PPPT	FGQGTKLEIKRTV	DIQMTQSPSSLSASVGDRVTITCRAS QDIITTVAWYQQKPGKAPKLLIYSASY RYPGVPSRFSGSGSGTDFTLTISSVQP EDFATYYCQQHYSPPPTFGQGTKLEIKRTV	04-0557-F2_VL
89 - 97	98 - 110		04-0557-F2_VL
9	13		04-0557-F2_VL

FIG. 24

Humanized DLK1 antibody of 04-0557-h3

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0557-h3_VH	Kabat	Sequence Fragment	QVQLVQSGAEVKKP GSSVKVSCKSSGYSFS	GYFLN	WMRQAPGQ GLEWMG	RIHPYNG DILYAQK FQG	RVTITADKSTSTAYMELSS LRSEDTAVYYCGR
04-0557-h3_VH	Kabat	Residues	1 - 30	31 - 35	36 - 49	50 - 66	67 - 98
04-0557-h3_VH	Kabat	Length	30	5	14	17	32

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0557-h3_VL	Kabat	Sequence Fragment	DIQMTQSPSSLSAS VGDRVTITC	RASQD IITTVA	WYQQKPG KAPKLLIY	SASYRYP	GVPSRFSGSGSGTDF TLTISSVQPEDFATYYC
04-0557-h3_VL	Kabat	Residues	1 - 23	24 - 34	**35 - 49**	50 - 56	57 - 88
04-0557-h3_VL	Kabat	Length	23	11	15	7	32

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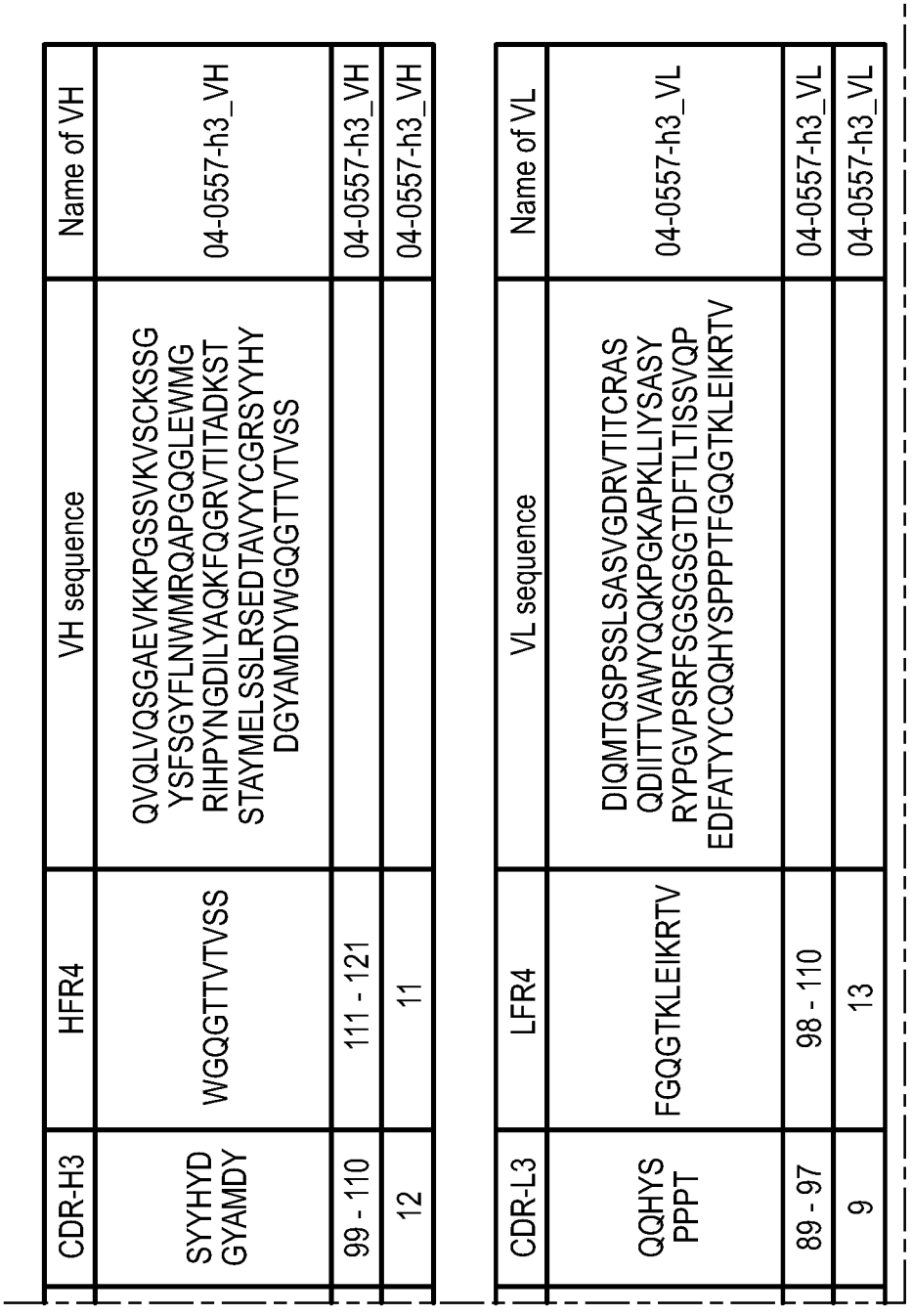
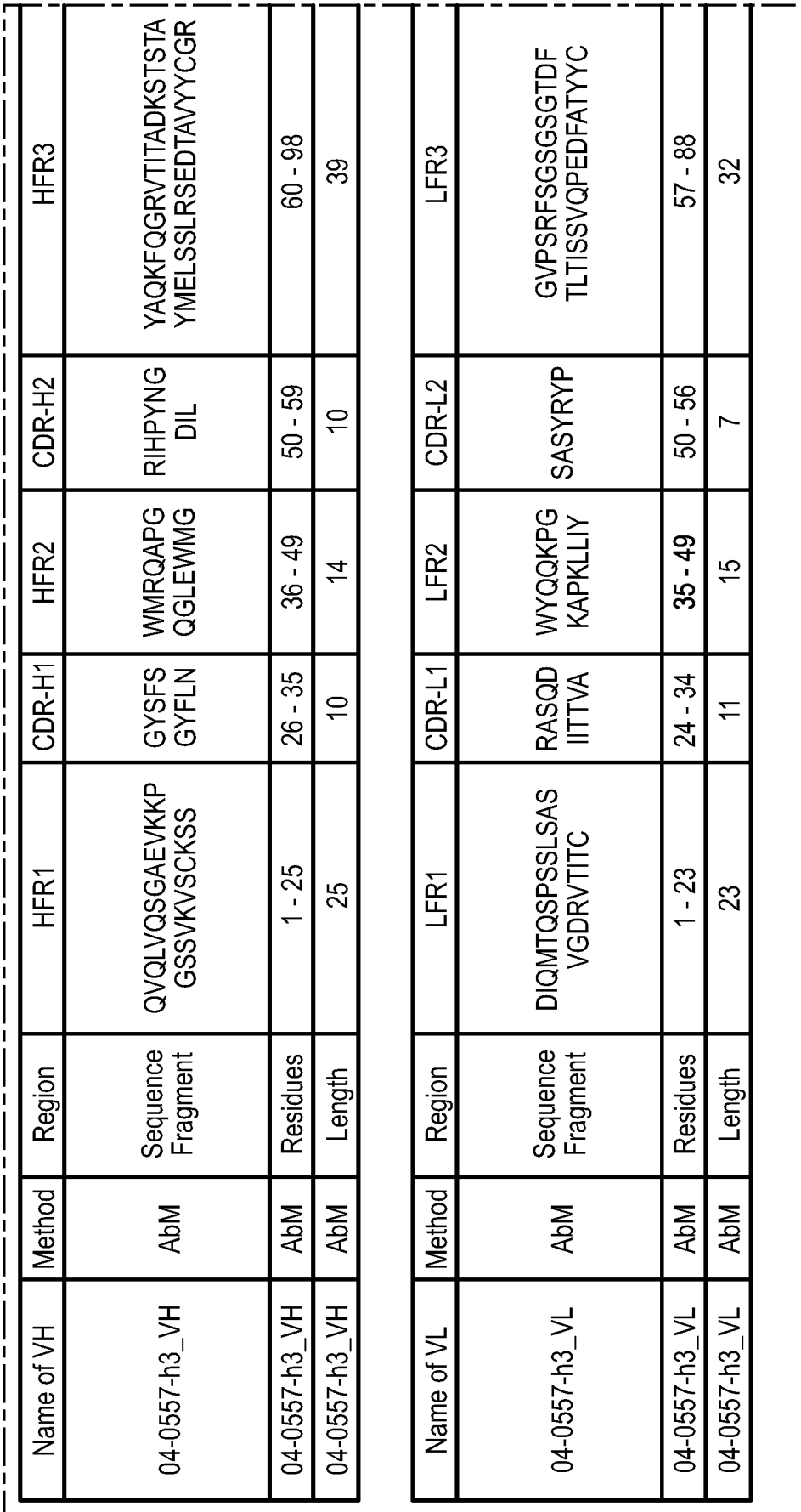
FIG. 24 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SYYHYD GYAMDY	WGQGTTVTVSS	QVQLVQSGAEVKKPGSSVKVSCKSSG YSFSGYFLNWMRQAPGQGLEWMG RIHPYNGDILYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCGRSYYHY DGYAMDYWGQGTTVTVSS	04-0557-h3_VH
99 - 110	111 - 121		04-0557-h3_VH
12	11		04-0557-h3_VH

CDR-L3	LFR4	VL sequence	Name of VL
QQHYS PPPT	FGQGTKLEIKRTV	DIQMTQSPSSLSASVGDRVTITCRAS QDIITTVAWYQQKPGKAPKLLIYSASY RYPGVPSRFSGSGSGTDFTLTISSVQP EDFATYYCQQHYSPPPTFGQGTKLEIKRTV	04-0557-h3_VL
89 - 97	98 - 110		04-0557-h3_VL
9	13		04-0557-h3_VL

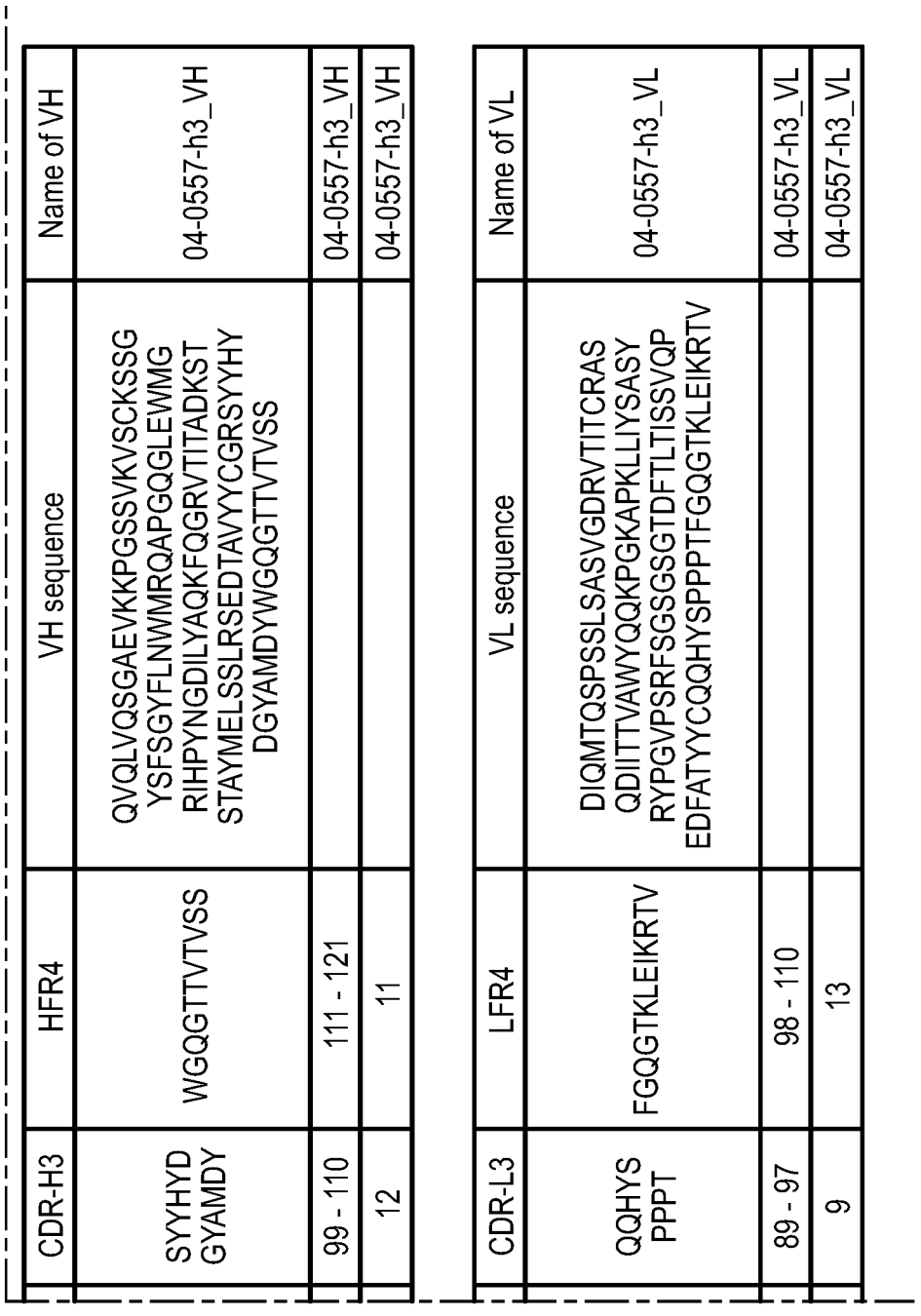
FIG. 24 CONT.



Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0557-h3_VH	AbM	Sequence Fragment	QVQLVQSGAEVKKP GSSVKVSCKSS	GYSFS GYFLN	WMRQAPG QGLEWMG	RIHPYNG DIL	YAQKFQGRVTITADKSTSTA YMELSSLRSEDTAVYYCGR
04-0557-h3_VH	AbM	Residues	1 - 25	26 - 35	36 - 49	50 - 59	60 - 98
04-0557-h3_VH	AbM	Length	25	10	14	10	39

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0557-h3_VL	AbM	Sequence Fragment	DIQMTQSPSSLSAS VGDRVTITC	RASQD IITTVA	WYQQKPG KAPKLLIY	SASYRYP	GVPSRFSGSGSGTDF TLTISSVQPEDFATYYC
04-0557-h3_VL	AbM	Residues	1 - 23	24 - 34	**35 - 49**	50 - 56	57 - 88
04-0557-h3_VL	AbM	Length	23	11	15	7	32

FIG. 24 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SYYHYD GYAMDY	WGQGTTVTVSS	QVQLVQSGAEVKKPGSSVKVSCKSSG YSFSGYFLNWMRQAPGQGLEWMG RIHPYNGDILYAQKFQGRVTITADKST STAYMELSSLRSEDTAVYYCGRSYYHY DGYAMDYWGQGTTVTVSS	04-0557-h3_VH
99 - 110	111 - 121		04-0557-h3_VH
12	11		04-0557-h3_VH

CDR-L3	LFR4	VL sequence	Name of VL
QQHYS PPPT	FGQGTKLEIKRTV	DIQMTQSPSSLSASVGDRVTITCRAS QDIITTVAWYQQKPGKAPKLLIYSASY RYPGVPSRFSGSGSGTDFTLTISSVQP EDFATYYCQQHYSPPPTFGQGTKLEIKRTV	04-0557-h3_VL
89 - 97	98 - 110		04-0557-h3_VL
9	13		04-0557-h3_VL

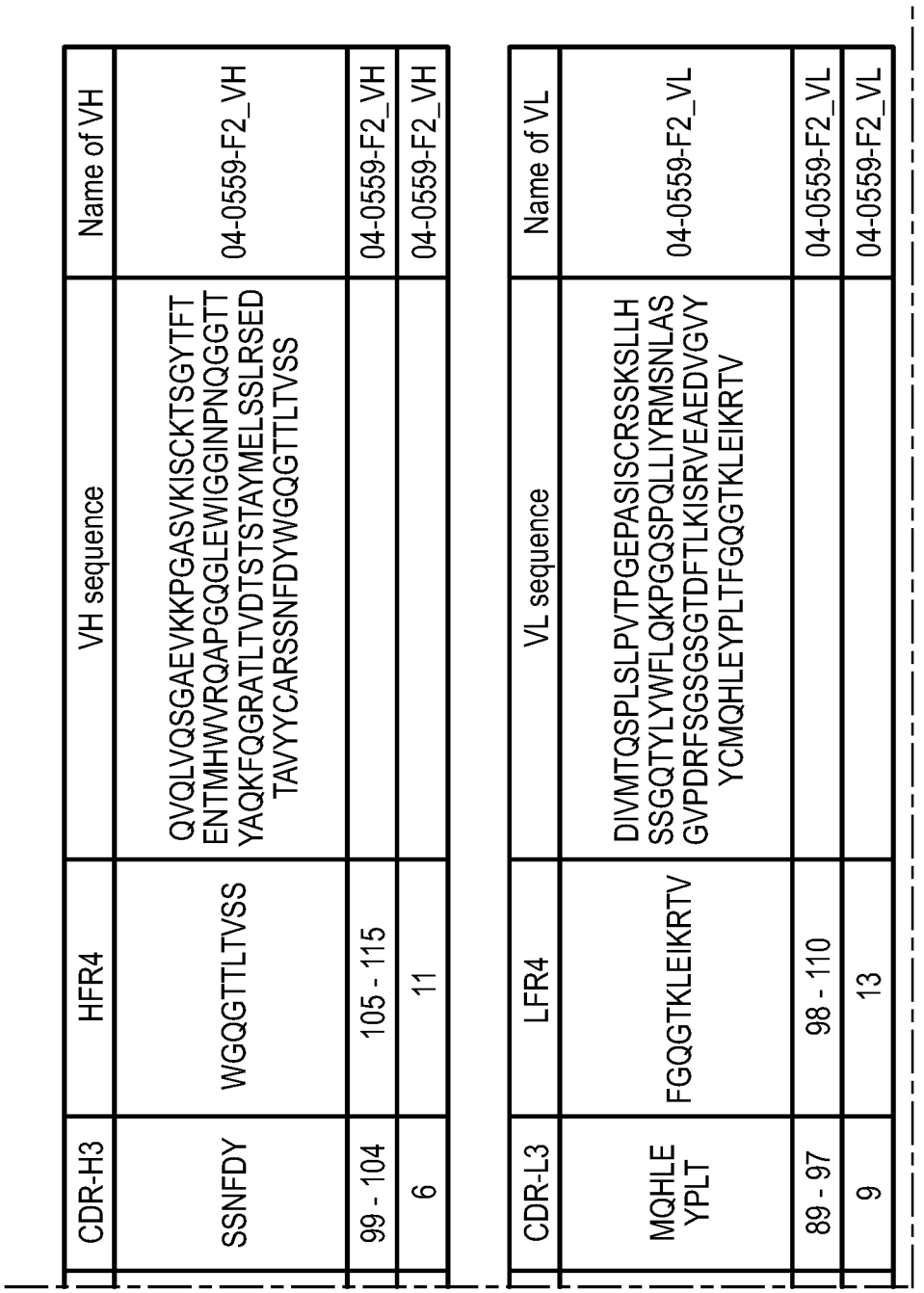
FIG. 25

Humanized DLK1 antibody of 04-0559-F2

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0559-F2_VH	Kabat	Sequence Fragment	QVQLVQSGAEVKKP GASVKISCKTSGYTFT	ENTMH	WVRQAPGQ GLEWIG	GINPNQG GTTYAQK FQG	RATLTVDTSTSTAYMELSS LRSEDTAVYYCAR
04-0559-F2_VH	Kabat	Residues	1 - 30	31 - 35	36 - 49	50 - 66	67 - 98
04-0559-F2_VH	Kabat	Length	30	5	14	17	32

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0559-F2_VL	Kabat	Sequence Fragment	DIVMTQSPLSLPVT PGEPASISC	RSSKSL LHSSG QTYLY	WFLQKPG QSPQLLIY	RMSNLAS	GVPDRFSGSGSGTDF TLKISRVEAEDVGVYYC
04-0559-F2_VL	Kabat	Residues	1 - 23	24 - 34	**35 - 49**	50 - 56	57 - 88
04-0559-F2_VL	Kabat	Length	23	11	15	7	32

FIG. 25 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SSNFDY	WGQGTTLTVSS	QVQLVQSGAEVKKPGASVKISCKTSGYTFT ENTMHWVRQAPGQGLEWIGGINPNQGGTT YAQKFQGRATLTVDTSTSTAYMELSSLRSED TAVYYCARSSNFDYWGQGTTLTVSS	04-0559-F2_VH
99 - 104	105 - 115		04-0559-F2_VH
6	11		04-0559-F2_VH

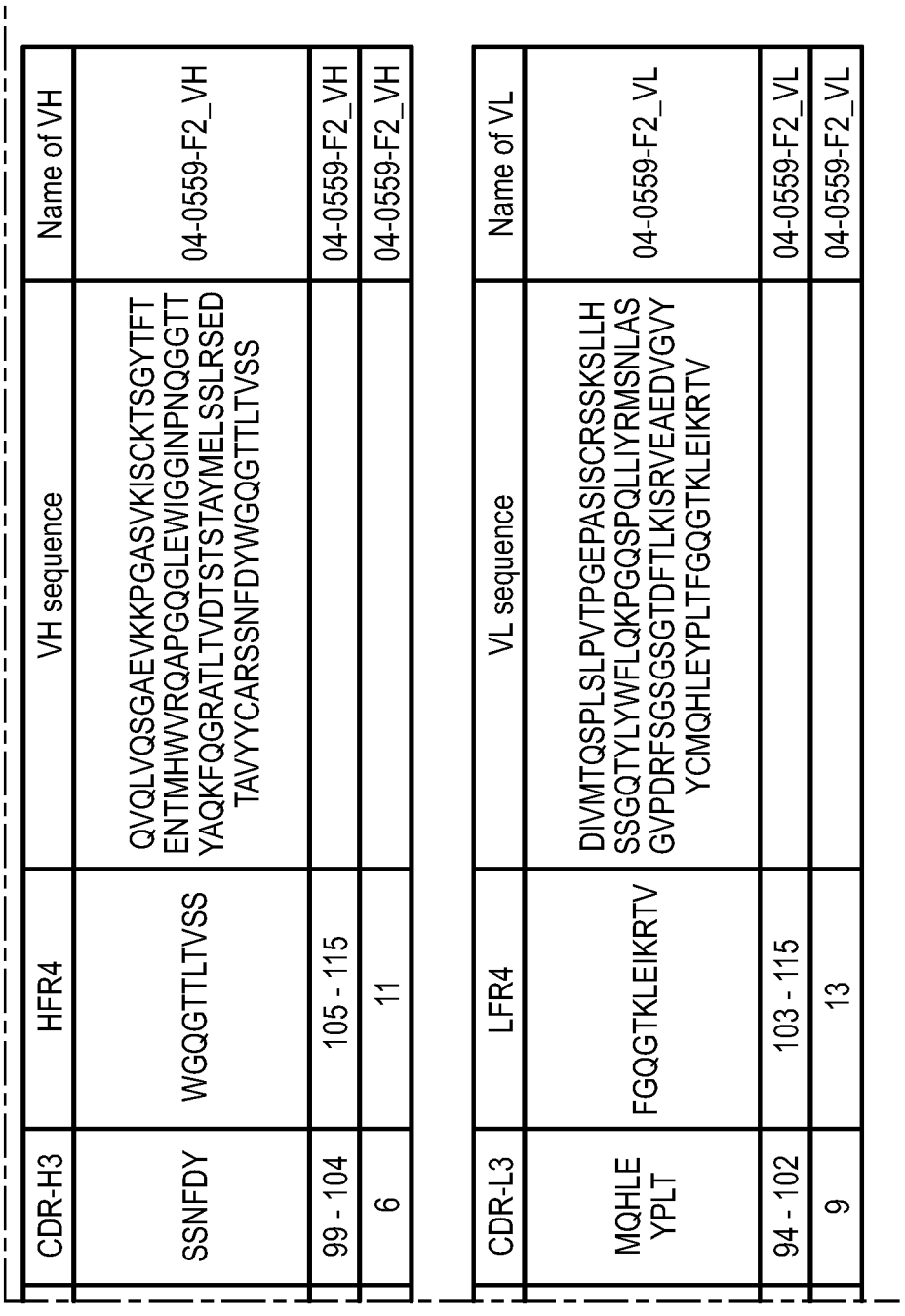
CDR-L3	LFR4	VL sequence	Name of VL
MQHLE YPLT	FGQGTKLEIKRTV	DIVMTQSPLSLPVTPGEPASISCRSSKSLLH SSGQTYLYWFLQKPGQSPQLLIYRMSNLAS GVPDRFSGSGSGTDFTLKISRVEAEDVGVY YCMQHLEYPLTFGQGTKLEIKRTV	04-0559-F2_VL
89 - 97	98 - 110		04-0559-F2_VL
9	13		04-0559-F2_VL

FIG. 25 CONT.

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0559-F2_VH	AbM	Sequence Fragment	QVQLVQSGAEVKKP GASVKISCKTS	GYTFT ENTMH	WVRQAPGQ GLEWIG	GINPNQ GGTT	YAQKFQGRATLTVDTS TSTAYMELSSLRSEDTA VYYCAR
04-0559-F2_VH	AbM	Residues	1 - 25	26 - 35	36 - 49	50 - 59	60 - 98
04-0559-F2_VH	AbM	Length	25	10	14	10	39

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0559-F2_VL	AbM	Sequence Fragment	DIVMTQSPLSLPVT PGEPASISC	RSSKSL LHSSG QTYLY	WFLQKPG QSPQLLIY	RMSNLAS	GVPDRFSGSGSGTDF TLKISRVEAEDVGVYYC
04-0559-F2_VL	AbM	Residues	1 - 23	24 - 39	**40 - 54**	55 - 61	62 - 93
04-0559-F2_VL	AbM	Length	23	16	15	7	32

FIG. 25 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SSNFDY	WGQGTTLTVSS	QVQLVQSGAEVKKPGASVKISCKTSGYTFT ENTMHWVRQAPGQGLEWIGGINPNQGGTT YAQKFQGRATLTVDTSTSTAYMELSSLRSED TAVYYCARSSNFDYWGQGTTLTVSS	04-0559-F2_VH
99 - 104	105 - 115		04-0559-F2_VH
6	11		04-0559-F2_VH

CDR-L3	LFR4	VL sequence	Name of VL
MQHLE YPLT	FGQGTKLEIKRTV	DIVMTQSPLSLPVTPGEPASISCRSSKSLLH SSGQTYLYWFLQKPGQSPQLLIYRMSNLAS GVPDRFSGSGSGTDFTLKISRVEAEDVGVY YCMQHLEYPLTFGQGTKLEIKRTV	04-0559-F2_VL
94 - 102	103 - 115		04-0559-F2_VL
9	13		04-0559-F2_VL

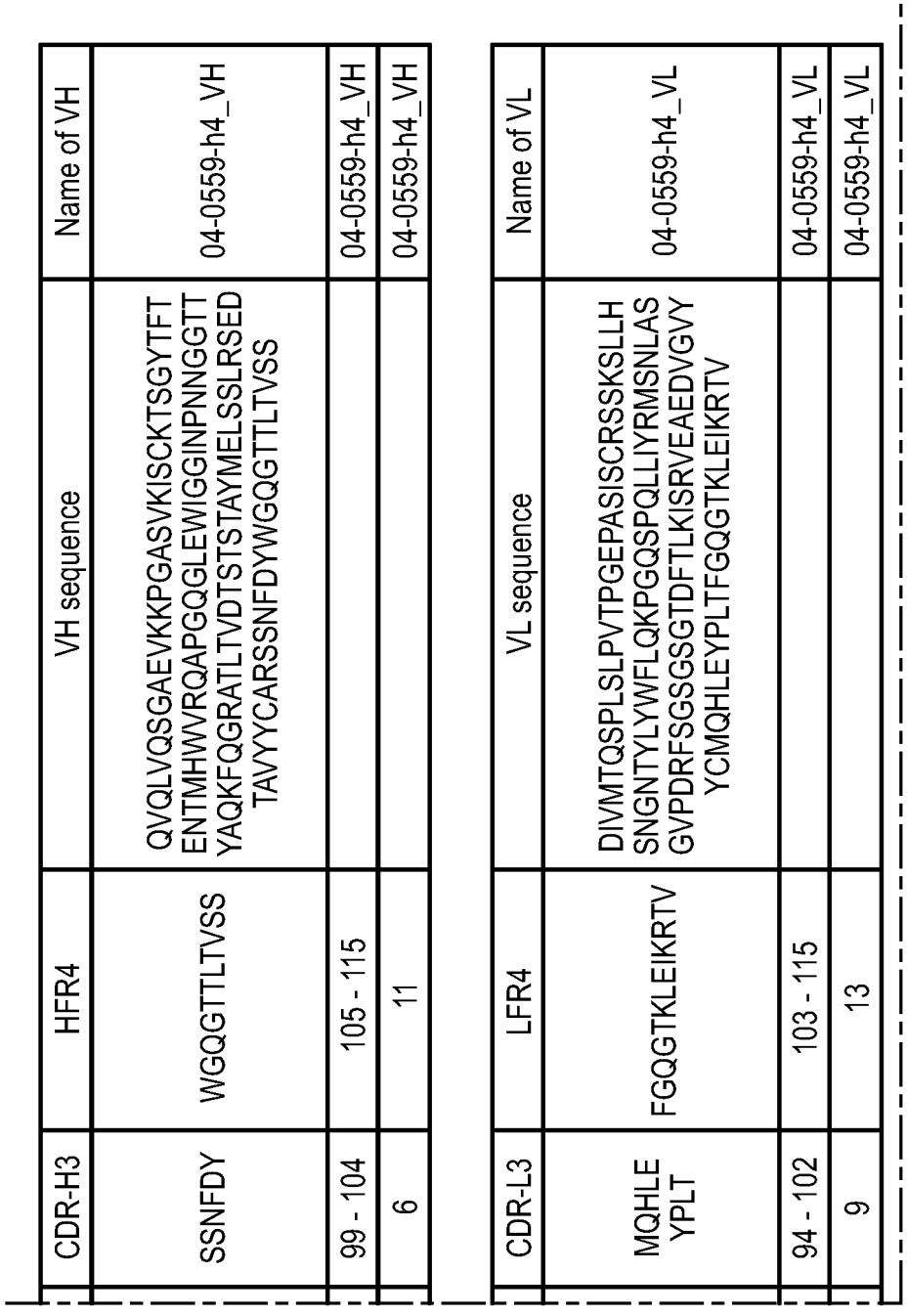
FIG. 26

Humanized DLK1 antibody of 04-0559-h4

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0559-h4_VH	Kabat	Sequence Fragment	QVQLVQSGAEVKKP GASVKISCKTSGYTFT	ENTMH	WVRQAPGQ GLEWIG	GINPNNG GTTYAQK FQG	RATLTVDTSTSTAYMELSS LRSEDTAVYYCAR
04-0559-h4_VH	Kabat	Residues	1 - 30	31 - 35	36 - 49	50 - 66	67 - 98
04-0559-h4_VH	Kabat	Length	30	5	14	17	32

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0559-h4_VL	Kabat	Sequence Fragment	DIVMTQSPLSLPVT PGEPASISC	RSSKSL LHSNG NTYLY	WFLQKPG QSPQLLIY	RMSNLAS	GVPDRFSGSGSGTDF TLKISRVEAEDVGVYYC
04-0559-h4_VL	Kabat	Residues	1 - 23	24 - 39	**40 - 54**	55 - 61	62 - 93
04-0559-h4_VL	Kabat	Length	23	16	15	7	32

FIG. 26 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SSNFDY	WGQGTTLTVSS	QVQLVQSGAEVKKPGASVKISCKTSGYTFT ENTMHWVRQAPGQGLEWIGGINPNNGGTT YAQKFQGRATLTVDTSTSTAYMELSSLRSED TAVYYCARSSNFDYWGQGTTLTVSS	04-0559-h4_VH
99 - 104	105 - 115		04-0559-h4_VH
6	11		04-0559-h4_VH

CDR-L3	LFR4	VL sequence	Name of VL
MQHLE YPLT	FGQGTKLEIKRTV	DIVMTQSPLSLPVTPGEPASISCRSSKSLLH SNGNTYLYWFLQKPGQSPQLLIYRMSNLAS GVPDRFSGSGSGTDFTLKISRVEAEDVGVY YCMQHLEYPLTFGQGTKLEIKRTV	04-0559-h4_VL
94 - 102	103 - 115		04-0559-h4_VL
9	13		04-0559-h4_VL

FIG. 26 CONT.

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0559-h4_VH	AbM	Sequence Fragment	QVQLVQSGAEVKKP GASVKISCKTS	GYTFT ENTMH	WVRQAPGQ GLEWIG	GINPNN GGTT	YAQKFQGRATLTVDTS TSTAYMELSSLRSEDTA VYYCAR
04-0559-h4_VH	AbM	Residues	1 - 25	26 - 35	36 - 49	50 - 59	60 - 98
04-0559-h4_VH	AbM	Length	25	10	14	10	39

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0559-h4_VL	AbM	Sequence Fragment	DIVMTQSPLSLPVT PGEPASISC	RSSKSL LHSNG NTYLY	WFLQKPG QSPQLLIY	RMSNLAS	GVPDRFSGSGSGTDF TLKISRVEAEDVGVYYC
04-0559-h4_VL	AbM	Residues	1 - 23	24 - 39	**40 - 54**	55 - 61	62 - 93
04-0559-h4_VL	AbM	Length	23	16	15	7	32

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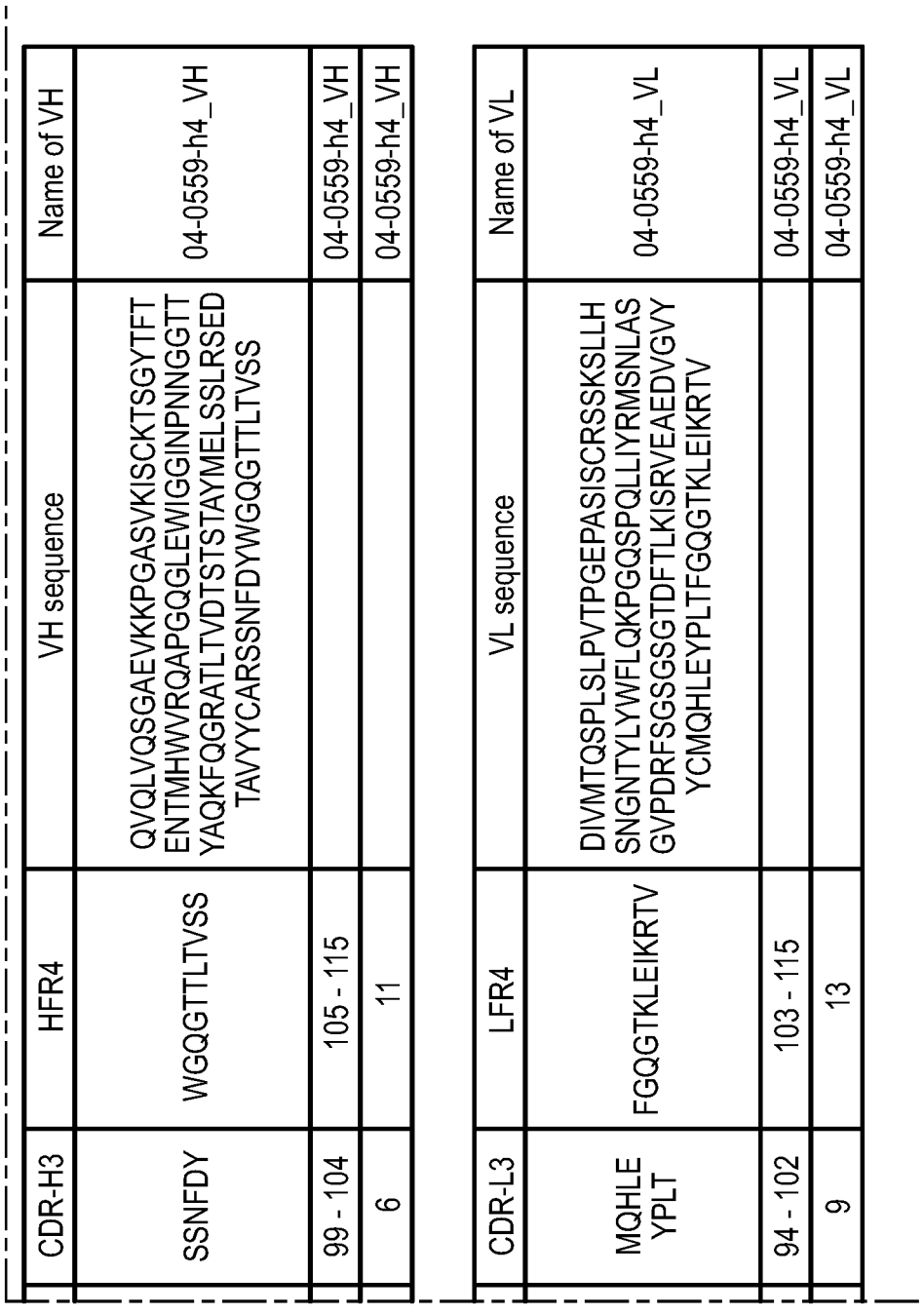
FIG. 26 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SSNFDY	WGQGTTLTVSS	QVQLVQSGAEVKKPGASVKISCKTSGYTFT ENTMHWVRQAPGQGLEWIGGINPNNGGTT YAQKFQGRATLTVDTSTSTAYMELSSLRSED TAVYYCARSSNFDYWGQGTTLTVSS	04-0559-h4_VH
99 - 104	105 - 115		04-0559-h4_VH
6	11		04-0559-h4_VH

CDR-L3	LFR4	VL sequence	Name of VL
MQHLE YPLT	FGQGTKLEIKRTV	DIVMTQSPLSLPVTPGEPASISCRSSKSLLH SNGNTYLYWFLQKPGQSPQLLIYRMSNLAS GVPDRFSGSGSGTDFTLKISRVEAEDVGVY YCMQHLEYPLTFGQGTKLEIKRTV	04-0559-h4_VL
94 - 102	103 - 115		04-0559-h4_VL
9	13		04-0559-h4_VL

FIG. 27

Humanized DLK1 antibody of 04-0565-F2

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0565-F2_VH	Kabat	Sequence Fragment	QVQLVQSGAEVKKPG ASVKVSCKASGFYIR	DTYIH	WVRQAPGQ GLEWMG	RIDPVYG HIKYAQK FQG	RVTMTADTSTSTAYMELSS LRSEDTAVYYCAR
04-0565-F2_VH	Kabat	Residues	1 - 30	31 - 35	36 - 49	50 - 66	67 - 98
04-0565-F2_VH	Kabat	Length	30	5	14	17	32

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0565-F2_VL	Kabat	Sequence Fragment	DIVMTQSPLSLPVT PGEPASISC	RSSESL LHSSG HTYLY	WFLQKPG QSPQLLIY	RMSNLAS	GVPDRFSGSGSGTDF TLKISRVEAEDVGVYYC
04-0565-F2_VL	Kabat	Residues	1 - 23	24 - 39	**40 - 54**	55 - 61	62 - 93
04-0565-F2_VL	Kabat	Length	23	16	15	7	32

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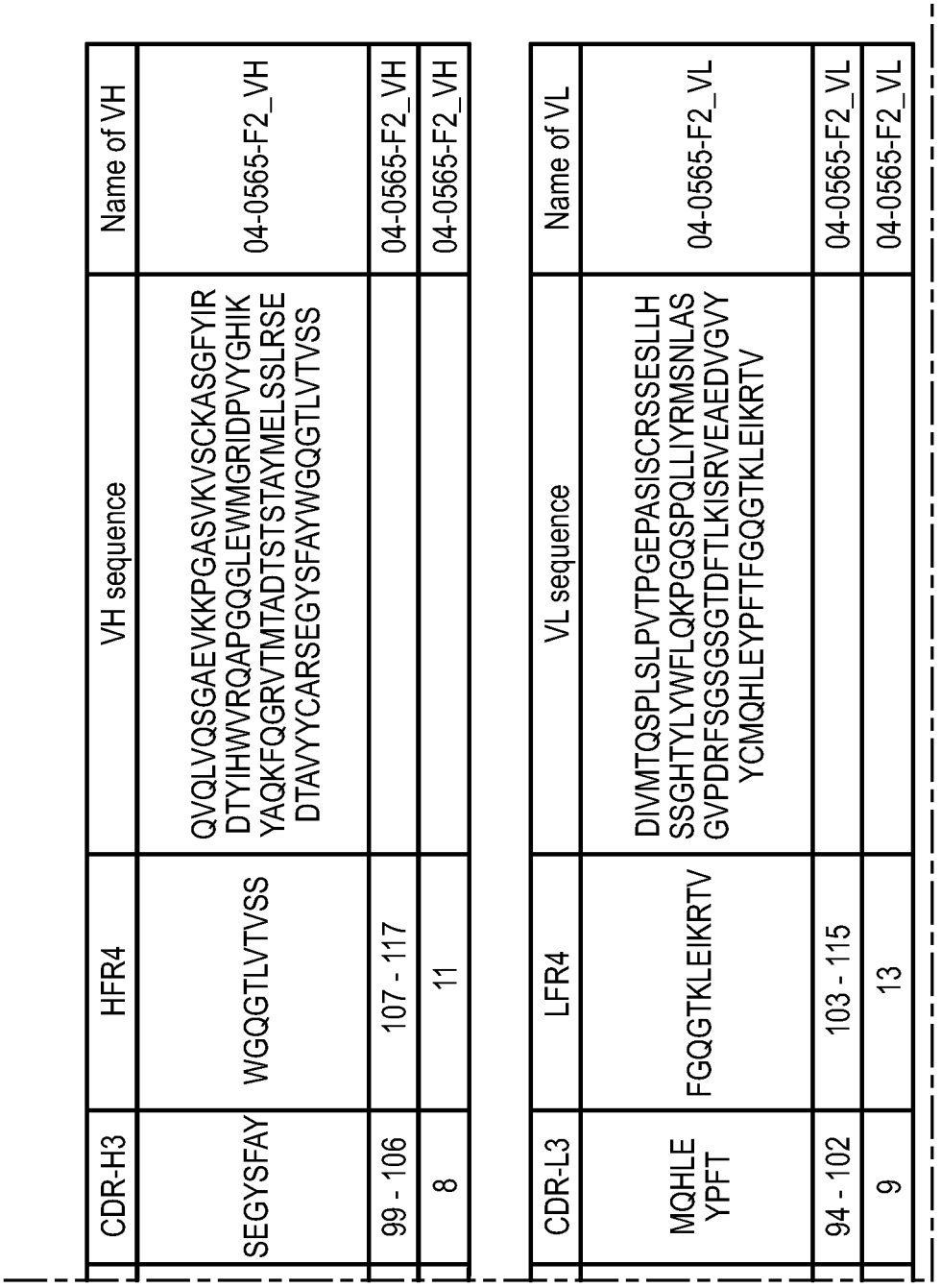
FIG. 27 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SEGYSFAY	WGQGTLVTVSS	QVQLVQSGAEVKKPGASVKVSCKASGFYIR DTYIHWVRQAPGQGLEWMGRIDPVYGHIK YAQKFQGRVTMTADTSTSTAYMELSSLRSE DTAVYYCARSEGYSFAYWGQGTLVTVSS	04-0565-F2_VH
99 - 106	107 - 117		04-0565-F2_VH
8	11		04-0565-F2_VH

CDR-L3	LFR4	VL sequence	Name of VL
MQHLE YPFT	FGQGTKLEIKRTV	DIVMTQSPLSLPVTPGEPASISCRSSESLLH SSGHTYLYWFLQKPGQSPQLLIYRMSNLAS GVPDRFSGSGSGTDFTLKISRVEAEDVGVY YCMQHLEYPFTFGQGTKLEIKRTV	04-0565-F2_VL
94 - 102	103 - 115		04-0565-F2_VL
9	13		04-0565-F2_VL

FIG. 27 CONT.

Name of VH	Method	Region	HFR1	CDR-H1	HFR2	CDR-H2	HFR3
04-0565-F2_VH	AbM	Sequence Fragment	QVQLVQSGAEVKKPG ASVKVSCKAS	GFYIR DTYIH	WVRQAPGQ GLEWMG	RIDPVYG HIK	YAQKFQGRVTMTADTSTST AYMELSSLRSEDTAVYYCAR
04-0565-F2_VH	AbM	Residues	1 - 25	26 - 35	36 - 49	50 - 59	60 - 98
04-0565-F2_VH	AbM	Length	25	10	14	10	39

Name of VL	Method	Region	LFR1	CDR-L1	LFR2	CDR-L2	LFR3
04-0565-F2_VL	AbM	Sequence Fragment	DIVMTQSPLSLPVT PGEPASISC	RSSESL LHSSG HTYLY	WFLQKPG QSPQLLIY	RMSNLAS	GVPDRFSGSGSGTDF TLKISRVEAEDVGVYYC
04-0565-F2_VL	AbM	Residues	1 - 23	24 - 39	**40 - 54**	55 - 61	62 - 93
04-0565-F2_VL	AbM	Length	23	16	15	7	32

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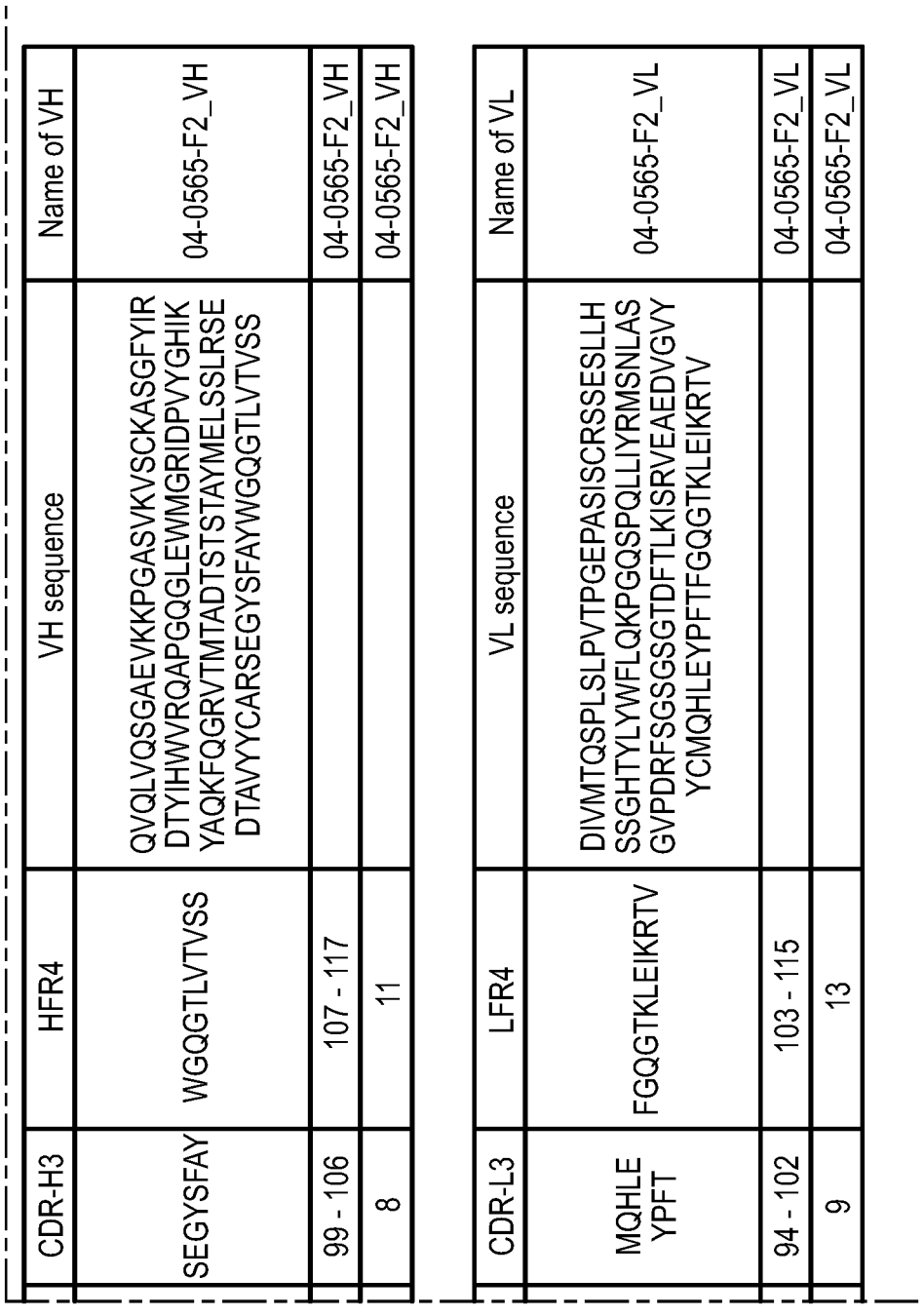
FIG. 27 CONT.



CDR-H3	HFR4	VH sequence	Name of VH
SEGYSFAY	WGQGTLVTVSS	QVQLVQSGAEVKKPGASVKVSCKASGFYIR DTYIHWVRQAPGQGLEWMGRIDPVYGHIK YAQKFQGRVTMTADTSTSTAYMELSSLRSE DTAVYYCARSEGYSFAYWGQGTLVTVSS	04-0565-F2_VH
99 - 106	107 - 117		04-0565-F2_VH
8	11		04-0565-F2_VH

CDR-L3	LFR4	VL sequence	Name of VL
MQHLE YPFT	FGQGTKLEIKRTV	DIVMTQSPLSLPVTPGEPASISCRSSESLLH SSGHTYLYWFLQKPGQSPQLLIYRMSNLAS GVPDRFSGSGSGTDFTLKISRVEAEDVGVY YCMQHLEYPFTFGQGTKLEIKRTV	04-0565-F2_VL
94 - 102	103 - 115		04-0565-F2_VL
9	13		04-0565-F2_VL

Name of antibody	Name of expression vector	Name of VH or VL	FR1-IMGT	CDR1-IMGT	FR2-IMGT	CDR2-IMGT	FR3-IMGT
04-0547-h2	p04-0547-h2	04-0547-h2_VH	EVQLVESG GGLVQPG GSLRLSC AAS	GFSIS DYY	MAWVR QAPGKG LEWVAN	INYDGTNT	YYADSVKGRFTIS RDNSKNTLYLQM NSLRAEDTAVYYC
		04-0547-h2_VL	DIQMTQS PSSLSASV GDRVTIT CRAS	HDVSTA	VAWYQ QKPGKA PKLLIY	SAS	YRYTGVPSRFSGS GSGTDFTLTISSLQ PEDFATYYC
04-0561-F1	p04-0561-F1	04-0561-F1_VH	QVQLQES GPGLVKPS ETLSLT CTVS	GFSLSIYS	VHWVR QPPGKG LEWIGL	IWGGGST	DYNPSLKSRVTISK DTSKNQVSLKLSS VTAADTAVYYC
		04-0561-F1_VL	DIVMTQS PDSLAVSL GERVTMN CKSS	QSLLQSS NQKNY	LAWYQQ KPGQPP KLLVY	FAS	TRESGVPDRFSGS GSGTDFTLTISSV QAEDVAVYYC
04-0562-h10	p04-0562-h10	04-0562-h10_VH	QVQLQE SGPGLVK PSETLS LTCTVS	GFSLT SYG	VSWVRQ PPGKGLE WIGV	IWGDGST	SYNPSLKSRVTISK DTSKNQVSLKLSS VTAADTAVYYC
		04-0562-h10_VL	DIVMTQS PLSLPVTP GEPASIS CRSS	QSLVHIN GNTY	LHWYLQ KPGQSP QLLIY	KVS	NRFSGVPDRFSGS GSGTDFTLKISRV EAEDVGVYYC

FIG. 28

CDR3-IMGT	FR4-IMGT	VH or VL sequence	Name of VH or VL	Project
VRSYYYY GMEY	WGQGTTVTVSS	EVQLVESGGGLVQPGGSLRLSCAASGFSISDYYMAWVRQAPGKGL EWVANINYDGTNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCVRSYYYYGMEYWGQGTTVTVSS	04-0547-h2_VH	DLK1
QQHYRIPLT	FGQGTKLEIK	DIQMTQSPSSLSASVGDRVTITCRASHDVSTAVAWYQQKPGKAPK LLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHYRI PLTFGQGTKLEIK	04-0547-h2_VL	DLK1
ARKEGNYL WFAY	WGQGTLVTVSS	QVQLQESGPGLVKPSETLSLTCTVSGFSLSIYSVHWVRQPPGKGLE WIGLIWGGGSTDYNPSLKSRVTISKDTSKNQVSLKLSSVTAADTAVY YCARKEGNYLWFAYWGQGTLVTVSS	04-0561-F1_VH	DLK1
QQHYSIPLT	FGQGTKLEIK	DIVMTQSPDSLAVSLGERVTMNCKSSQSLLQSSNQKNYLAWYQQK PGQPPKLLVYFASTRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVY YCQQHYSIPLTFGQGTKLEIK	04-0561-F1_VL	DLK1
AKPDGP	LGQGTLVTVSS	QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVSWVRQPPGKGLE WIGVIWGDGSTSYNPSLKSRVTISKDTSKNQVSLKLSSVTAADTAVY YCAKPDGPLGQGTLVTVSS	04-0562-h10_VH	DLK1
SQTTH VPWT	FGQGTKLEIK	DIVMTQSPLSLPVTPGEPASISCRSSQSLVHINGNTYLHWYLQKPGQ SPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQ TTHVPWTFGQGTKLEIK	04-0562-h10_VL	DLK1

FIG. 28 CONT.

Name of antibodies	Name of expression vector	Name of VH & VL	FR1-IMGT	CDR1-IMGT	FR2-IMGT	CDR2-IMGT	FR3-IMGT
04-0548-h4	p04-0548-h4	04-0548-h4_VH	EVQFVES GGGLVQ PGGSLR LSCAAS	GFTFS NFW	MNWVRQ APGKGL EWIAQ	IRLKSD NYGT	HYADSVKGRFTISR DNSKNTVYLQMNS LRAEDTGVYYC
		04-0548-h4_VL	DIQMTQS PSSLSAS VGDRVT ITCRAS	QDVSAA	VAWYQQK PGKAPK VLIY	SAS	NRYTGVPSRFSGSG SGTDFTFTISSVQPE DFATYYC
04-0557-F2	p04-0557-F2	04-0557-F2_VH	QVQLVQS GAEVKK PGSSVK VSCKSS	GYSFS GYF	LNWMRQA PGQGLEW MGR	IHPYQGDI	LYAQKFQGRVTITA DKSTSTAYMELSSLR SEDTAVYYC
		04-0557-F2_VL	DIQMTQS PSSLSAS VGDRVT ITCRAS	QDIITT	VAWYQQK PGKAPK LLIY	SAS	YRYPGVPSRFSGSG SGTDFTLTISSVQPE DFATYYC
04-0559-F2	p04-0559-F2	04-0559-F2_VH	QVQLVQS GAEVKK PGASVK ISCKTS	GYTFT ENT	MHWVR QAPGQ GLEWIGG	INPNQGGT	TYAQKFQGRATLTV DTSTSTAYMELSSLR SEDTAVYYC
		04-0559-F2_VL	DIVMTQS PLSLPVT PGEPAS ISCRSS	KSLLHS SGQTY	LYWFLQK PGQSPQ LLIY	RMS	NLASGVPDRFSGSG SGTDFTLKISRVEAE DVGVYYC

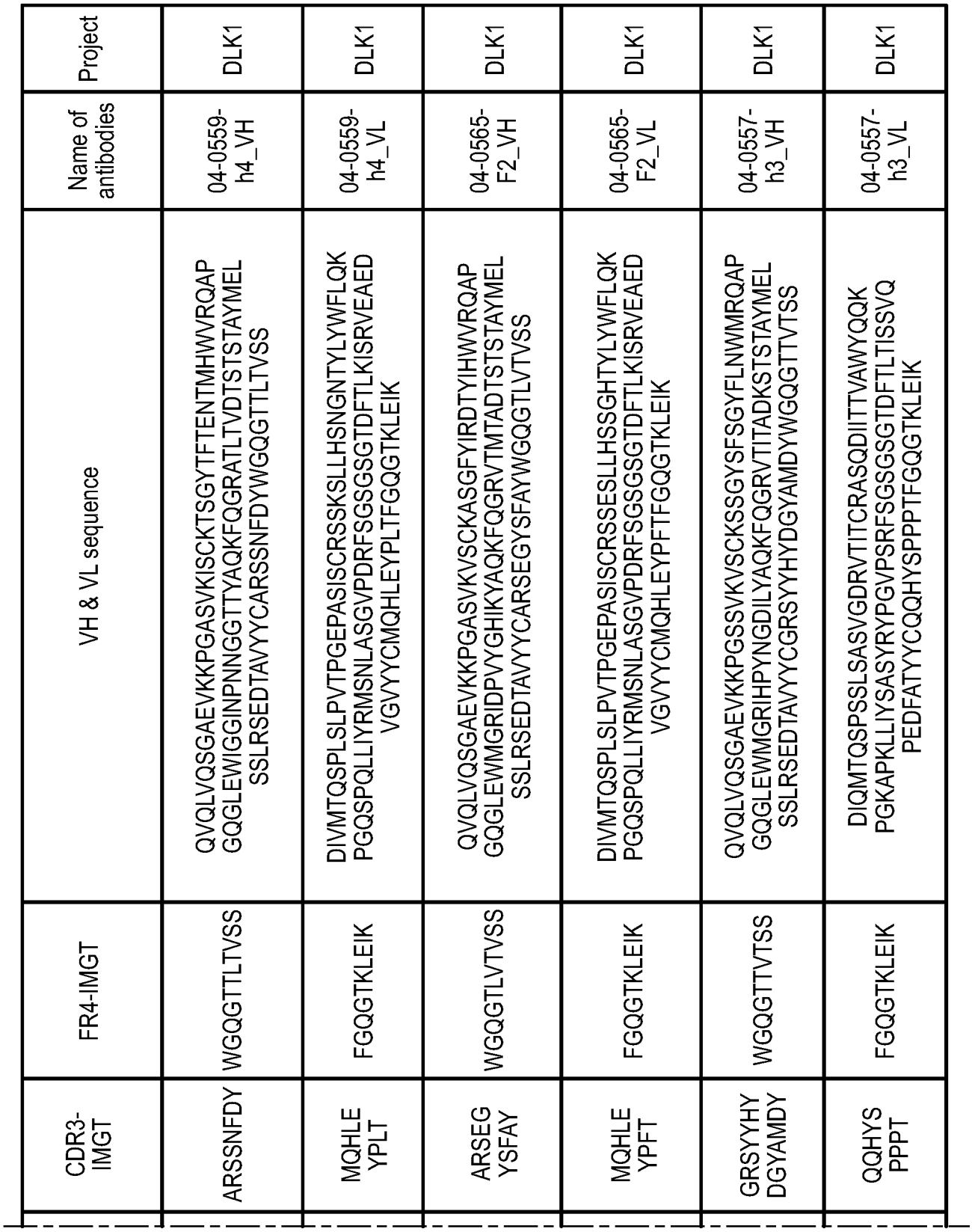
FIG. 29

CDR3-IMGT	FR4-IMGT	VH & VL sequence	Name of antibodies	Project
TEGLEY	WGQGTLVTVSS	EVQFVESGGGLVQPGGSLRLSCAASGFTFSNFWMNWVRQAP GKGLEWIAQIRLKSDNYGTHYADSVKGRFTISRDNSKNTVYLQM NSLRAEDTGVYYCTEGLEYWGQGTLVTVSS	04-0548-h4_VH	DLK1
QQYYF TPWA	FGQGTKLEIK	DIQMTQSPSSLSASVGDRVTITCRASQDVSAAVAWYQQK PGKAPKVLIYSASNRYTGVPSRFSGSGSGTDFTFTISSVQ PEDFATYYCQQYYFTPWAFGQGTKLEIK	04-0548-h4_VL	DLK1
GRSYYHY DGYAMDY	WGQGTTVTVSS	QVQLVQSGAEVKKPGSSVKVSCKSSGYSFSGYFLNWMRQAP GQGLEWMGRIHPYQGDILYAQKFQGRVTITADKSTSTAYMELS SLRSEDTAVYYCGRSYYHYDGYAMDYWGQGTTVTVSS	04-0557-F2_VH	DLK1
QQHYS PPPT	FGQGTKLEIK	DIQMTQSPSSLSASVGDRVTITCRASQDIITTVAWYQQK PGKAPKLLIYSASYRYPGVPSRFSGSGSGTDFTLTISSVQ PEDFATYYCQQHYSPPPTFGQGTKLEIK	04-0557-F2_VL	DLK1
ARSSNFDY	WGQGTTLTVSS	QVQLVQSGAEVKKPGASVKISCKTSGYTFTENTMHWVRQAP GQGLEWIGGINPNQGGTTYAQKFQGRATLTVDTSTSTAYMELS SLRSEDTAVYYCARSSNFDYWGQGTTLTVSS	04-0559-F2_VH	DLK1
MQHLE YPLT	FGQGTKLEIK	DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSSGQTYLYWFLQK PGQSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCMQHLEYPLTFGQGTKLEIK	04-0559-F2_VL	DLK1

FIG. 29
CONT.

FIG. 29
CONT.

Name of antibody	Name of expression vector	Name of VH & VL	FR1-IMGT	CDR1-IMGT	FR2-IMGT	CDR2-IMGT	FR3-IMGT
04-0559-h4	p04-0559-h4	04-0559-h4_VH	QVQLVQS GAEVKK PGASVK ISCKTS	GYTFT ENT	MHWVR QAPGQG LEWIGG	INPNNGGT	TYAQKFQGRATLTV DTSTSTAYMELSSLR SEDTAVYYC
		04-0559-h4_VL	DIVMTQS PLSLPVT PGEPAS ISCRSS	KSLLHS NGNTY	LYWFLQ KPGQS PQLLIY	RMS	NLASGVPDRFSGSG SGTDFTLKISRVEAE DVGVYYC
04-0565-F2	p04-0565-F2	04-0565-F2_VH	QVQLVQS GAEVKK PGASVK VSCKAS	GFYIR DTY	IHWVRQ APGQGL EWMGR	IDPVYGHI	KYAQKFQGRVTMTA DTSTSTAYMELSSLR SEDTAVYYC
		04-0565-F2_VL	DIVMTQS PLSLPVT PGEPAS ISCRSS	ESLLHS SGHTY	LYWFLQ KPGQS PQLLIY	RMS	NLASGVPDRFSGSG SGTDFTLKISRVEAE DVGVYYC
04-0557-h3	p04-0557-h3	04-0557-h3_VH	QVQLVQS GAEVKK PGSSVK VSCKSS	GYSFS GYF	LNWMRQ APGQGL EWMGR	IHPYNGDI	LYAQKFQGRVTITA DKSTSTAYMELSSLR SEDTAVYYC
		04-0557-h3_VL	DIQMTQS PSSLSAS VGDRVTI TCRAS	QDIITT	VAWYQQ KPGKAP KLLIY	SAS	YRYPGVPSRFSGSG SGTDFTLTISSVQPE DFATYYC



CDR3-IMGT	FR4-IMGT	VH & VL sequence	Name of antibodies	Project
ARSSNFDY	WGQGTTLTVSS	QVQLVQSGAEVKKPGASVKISCKTSGYTFTENTMHWVRQAP GQGLEWIGGINPNNGGTTYAQKFQGRATLTVDTSTSTAYMEL SSLRSEDTAVYYCARSSNFDYWGQGTTLTVSS	04-0559-h4_VH	DLK1
MQHLE YPLT	FGQGTKLEIK	DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGNTYLYWFLQK PGQSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCMQHLEYPLTFGQGTKLEIK	04-0559-h4_VL	DLK1
ARSEG YSFAY	WGQGTLVTVSS	QVQLVQSGAEVKKPGASVKVSCKASGFYIRDTYIHWVRQAP GQGLEWMGRIDPVYGHIKYAQKFQGRVTMTADTSTSTAYMEL SSLRSEDTAVYYCARSEGYSFAYWGQGTLVTVSS	04-0565-F2_VH	DLK1
MQHLE YPFT	FGQGTKLEIK	DIVMTQSPLSLPVTPGEPASISCRSSESLLHSSGHTYLYWFLQK PGQSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCMQHLEYPFTFGQGTKLEIK	04-0565-F2_VL	DLK1
GRSYYHY DGYAMDY	WGQGTTVTSS	QVQLVQSGAEVKKPGSSVKVSCKSSGYSFSGYFLNWMRQAP GQGLEWMGRIHPYNGDILYAQKFQGRVTITADKSTSTAYMEL SSLRSEDTAVYYCGRSYYHYDGYAMDYWGQGTTVTSS	04-0557-h3_VH	DLK1
QQHYS PPPT	FGQGTKLEIK	DIQMTQSPSSLSASVGDRVTITCRASQDIITTVAWYQQK PGKAPKLLIYSASYRYPGVPSRFSGSGSGTDFTLTISSVQ PEDFATYYCQQHYSPPPTFGQGTKLEIK	04-0557-h3_VL	DLK1

FIG. 29 CONT.

Fig. 30

Name	Description	Sequence
Human immunoglobulin kappa constant (IGKC)	Uniprot ID: P01834	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
Human immunoglobulin heavy constant gamma 1 (IGHG1)	Uniprot ID: P01857	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Immunoglobulin heavy constant gamma 2 (IGHG2)	Uniprot ID: P01859	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEY KCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQ PENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FIG. 31

Name	Description	Sequence
04-0565-F2_VL	DLK1 antibody light chain variable region coding sequence	GACATCGTGATGACCCAGAGCCCCCTGAGCCTGCCCGTGACCCCCGGCGAGCCCGCCAGCATCAGCTGCAGGAGCAGCGAGAGCCTGCTGCACAGCAACGGCGGCAACACCTACCTGTACTGGTTC CTGCAGAAGCCCGGCCAGAGCCCCCAGCTGCTGATCTACAAGATGAGCAACCTGGCCAGCGGCGTGCCCGACAGGTTCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGAAGATCAGCAG GGTGGAGGCCGAGGACGTGGGCGTGTACTACTGCATGCAGCACCTGGAGTACCCCTTCACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG
04-0565-F2_VH	DLK1 antibody heavy chain variable region codeing sequence	CAAGTACAACTTGTCCAGTCAGGAGCGGCAAGTTAAGAAGCCAGGGGCATCCGTCAAGTCTCCTGTAAAGCTTCTGGGTTTTATATTCGCCATACGTATATTCATTGGGTACGGCAAGCTCCAG GACAAGGGTTGGAATGGATGGGGCGGATTGATCCTGTCTATGGTCATATAAAATATGCGCAAAAGTTTCAAGGACGCGTCACAATGACTGCGGATACATCCACATCTACTGCGTATATGGAACT CTCTTCTCTCCGGTCAGAAGATACAGCTGTTTATTATTGTGCAAGAAGTGAAGGATATAGTTTGCTTATTGGGGACAAGGGACGGCTCGTTACAGTTTCCTCA
04-0562-h10_VL	DLK1 antibody light chain variable region coding sequence	GACATCGTGATGACACAGAGCCCTCTGAGCCTGCCTGTGACACCTGGCGAACCTGCCAGCATCAGCTGTAGAAGCAGCCACGAGCCTGGTGCACATCAACGGCAACACCTACCTGCACTGGTATC TGCAGAAGCCCGGCCAGTCTCCTCAGCTGCTGATCTACAAGGTGTCCAACCGGTTCAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACCCTGAAGATCTCCAGAGT GGAAGCCGAGGACGTGGGCGTGTACTACTGTAGCCAGAGCACCCACGTGCCCTGGACATTTGGACAGGGCACCAAGGTGGAAATCAAG
04-0562-h10_VH	DLK1 antibody heavy chain variable region codeing sequence	CAGGTTCAGCTGCAAGAGTCTGGCCCTGGGCCTGGTCAAGCCTAGCGAAACACTGAGCCTGACCTGTACCGTGTCCGGCTTTAGCCTGACAAGCTACGGGTGTGTCCTGGTCGACAGCCTCCTG GAAAAGGCCTGGAATGGATCGGAGTGATCTGGGGCGACGGCAGCACCAGCTATAACCCTAGCCTGAAGTCCAGAGTGACCATCAGCAAGGACACCAGCAAGAACCAGGTGTCCCTGAAGCTG AGCAGCGTGACAGCCGCTGATACCGCCGTGTACTACTGCGGCCAAGGGAACACTGGTCACAGTCTCTTCT
04-0561-F1_VL	DLK1 antibody light chain variable region coding sequence	GACATCGTGATGACCCAGAGCCCCCTGAGCCTGGCCGTGAGCCTGGGCGAGAGGGTGACCATGAACTGCAAGAGCAGCCAGAGCCTGCTGCAGAGCAGCAACCAGAAGAACTACCTGGCCTG GTACCAGCAGAAGCCCGGCCAGAGCCCCAAGCTGCTGGTGTACTACTGCCAGCAGCACTACAGCATCCCCCTGACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG

FIG. 31 CONT.

04-0561-F1_VH	DLK1 antibody heavy chain variable region codeing sequence	CAAGTTCAATTGCAAGAAAGCGGCCAGGGTTGGTTAAACCTTCGAAACTTTGTCCTTACTTGTACGGTTTCTGGATTTTCACTCAGTATATTATTCTGTACATTGGGTAAGACAACCAGCAGGT AAAGGGCTCGAATTGGATTGGACTTATTTGGGGTGGCGGGAGTACAGATTATAATCCTAGTTTGAAATCAAGATACCATATCTAAGATACATCAAGTTTCCTTGAAGCTCTCATC CGTCACTGCAGCGGATACAGCTGTCTATTATTGTGCTCGTAAAGAAGGGAATTATTTGTGGTTTGCTTATTGGGGACAAGGGACTCTTGTCACAGTTAGTTCT
04-0559-H4_VL	DLK1 antibody light chain variable region coding sequence	GACATCGTGATGACACAGAGCCCTCTGAGCCTGCCTGTGACACCTGGCGAACCTGCCAGCATCAGCTGCAGAAGCAGCAAGAGCCTGCTGCACAGCAACGGCAATACCTACCTGTACTGGTTCC TGCAGAAACCCGGCCAGTCTCCTCAGCTGCTGATCTACAGAATGAGCAACCTGGCCAGCGGCGTGCCCGATAGATTTTCTGGCTCTGGCAGCGGCACCGACTTCACCCTGAAGATCTCTAGAGT GGAAGCCGAGGACGTGGGCGTGTACTACTGTATGCAGCACCTGGAATACCCTCTGACCTTCGGCCAGGGCACCAAGCTGGAAATCAAG
04-0559-H4_VH	DLK1 antibody heavy chain variable region codeing sequence	CAGGTTCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCGGCTCCGTGAAGATCAGCTGCAAGACAAGCGGCTACACCTTCACCGAGAACACCATGCACTGGGTCCGACAGGCTCCA GGACAAGGCCTGGAATGGATCGGCGGCATCAACCCTAACAACGGCGGCACAACATACGCCCAGAAGTTCCAGGGCAGAGTCACCATGACCGTGGATACCAGCACAGACGACCGGCCTACATGGA ACTGAGCAGCCTGAGAAGCGAGGACACCGCCGTGTACTACTGTGCCAGCAAGCAGCAACTTCGACTACTGGGGCCAGGGCACAACCCTGACAGTCTCTTCT
04-0559-F2_VL	DLK1 antibody light chain variable region coding sequence	GACATCGTGATGACCCAGAGCCCCCTGAGCCTGCCCGTGACCCCCGGCGAGCCCGCCAGCATCAGCTGCAGGAGCAGCAAGAGCCTGCTGCACAGCAACGGCAACACCTACCTGTACTGGTTC CTGCAGAAGCCCGGCCAGAGCCCCCAGCTGCTGATCTACAGGATGAGCAACCTGGCCAGCGGCGTGCCCGACAGGTTCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGAAGATCAGCAG GGTGGAGGCCGAGGACGTGGGCGTGTACTACTGCATGCAGCACCTGGAGTACCCCCTGACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG
04-0559-F2_VH	DLK1 antibody heavy chain variable region codeing sequence	CAAGTCCAATTGGTCCAATCCGGAGCCGGAAGTAAACGAAACCGGGGCGCATCTGTCAAAATTAGTTGTAAAACTTCCGGATATACATTTACGGAAAATACAATGCATTGGTACGTCAAGCTCCAG GACAAGGACTCGAATGGATTGGTGGTATTAATCCTAATCAAGGTGGAACTACGTATGCTCAGAAATTTCAAGGAAGAGCTACTCTCACAGTCGATACATCCACATCCACGGCATATATTGGAATTG TCTTCTCTCCGCTCAGAAGATACAGCTGTATATTATTGTGATTATTGGGGACAAGGGACTACTCTCACAGTTTCAAGC

FIG. 31 CONT.

Name	Description	Sequence
04-0557-h3_VL	DLK1 antibody light chain variable region coding sequence	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGCCAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCAGCCAGGACATCATCACCACCGTG GCCTGGTATCAGCAGAAGCCTGGAAAGGCCCCTAAGCTGCTGATCTACAGCGCCAGCTACAGATACCCTGGCGTGCCCAGCAGATTTTCTGGCAGCGGC TCTGGCACCGACTTCACCCTGACAATCAGCAGCGTGCAGCCCGAGGATTTCGCCACCTACTACTGCCAGCAGCACTACAGCCCTCCACCTACATTTGGCCA GGGCACCAAGCTGGAAATCAAG
04-0557-h3_VH	DLK1 antibody heavy chain variable region coding sequence	CAGGTTCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCAGCAGCGTGAAGGTGTCCTGCAAGAGCAGCGGCTACAGCTTCTCCGGCTACTTC CTGAACTGGATGAGACAGGCCCCTGGACAGGGCCTCGAATGGATGGGCAAGAATCCATCCTTACAACGGCGACATCCTGTACGCCCAGAAATTCCAGGGC AGAGTGACCATCACCGCCGACAAGAGCACAAGCACCGCCTACATGGAACTGAGCAGCCTGAGAAGCGAGGACACCGCCGTGTACTACTGCGGCAGAAG CTACTACCACTACGACGGCTACGCCATGGACTATTGGGGCCAGGGAACCACCGTGACCGTTAGCTCT
04-0557-F2_VL	DLK1 antibody light chain variable region coding sequence	GATATTCAAATGACTCAATCCCCGTCATCCTTGTCTGCTAGTGTTGGAGATCGGCGTCACTATTACGTGTGGGGCATCCCAAGATATTATTACAACTGTTGCT TGGTATCAACAAAAGCCTGGTAAAGCTCCTAAATTGCTTATTTATAGTGCTTCCTATCGTTATCCAGGGGTTCCGTCCGGTTTTCTGGGTCCGGTTCTGGA ACAGATTTTACATTGACTATATCTCTGTCCAACCAGAAGATTTTGCTACTTATTATTGTCAACAACATTATTCCCCACCACCAACATTTGGACAAGGGACT AAACTCGAAATTAAA
04-0557-F2_VH	DLK1 antibody heavy chain variable region coding sequence	CAGGTGCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAGCCCGGCAGCGTGAAGGTGAGCTGCAAGAGCAGCGGCTACAGCTTCAGCGGCTACT TCCTGAACTGGATGAGGCAGGCCCCCGGCCAGGGCCTGGAGTGGATGGGCAGGATCCACCCCTACCAGGGCGACATCCTGTACGCCCAGAAGTTCCAG GGCAGGGTGACCATCACCGCCGACAAGAGCACCAGCACCGCCTACATGGAGCTGAGCAGCCTGAGGAGCGAGGACACCGCCGTGTACTACTGCGCCAG GAGCTACTACCACTACGAGGGCTACGCCATGGACTACTGGGGCCAGGGCACCACCGTGACCGTGAGCAGC

FIG. 31 CONT.

04-0548-h4_VL	DLK1 antibody light chain variable region coding sequence	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGCCAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCAGCCAGGATGTGTCTGCCGCCGTG GCTTGGTATCAGCAGAAGCCTGGAAAGGCCCCTAAGGTGCTGATCTACAGCGCCAGCAACAGATACACCGGCGTGCCCAGCAGATTTTCTGGCAGCGGC TCTGGCACCGACTTCACCTTTACAATCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACTACTTCACCCCTTGGCCCTTTGGCCA GGGCACCAAGCTGGAAATCAAG
04-0548-h4_Vh	DLK1 antibody heavy chain variable region coding sequence	GAGGTGCAGTTCGTGGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATCTCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAATTTCTGGA TGAACTGGGTCCGACAGGCCCCTGGCAAAGGCCTGGAATGGATCGCCCAGATCAGAACTGAAGTCCGACAACTACGGCACCCACTACGCCGACTCTGTGA AGGGCAGATTCACCATCAGCCGGGACAACAGCAAGAACACCGTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGGCGTGTACTACTGTACC GAAGGACTGGAATACTGGGGCCAGGGCACACTGGTCACAGTGTCATCT
04-0547-h2_VL	DLK1 antibody light chain variable region coding sequence	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGCCAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCAGCCAGGATGTGTCTACAGCCGTG GCCTGGTATCAGCAGAAGCCTGGAAAGGCCCCTAAGGTGCTGATCTACAGCGCCAGCTACAGATACACCGGCGTGCCCAGCAGATTTTCTGGCAGCGGC TCTGGCACCGACTTCACCCTGACCATATCTAGCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCAGCAGCACTACAGAATCCCTCTGACCTTTGGCCA GGGCACCAAGCTGGAAATCAAG
04-0547-h2_VH	DLK1 antibody heavy chain variable region coding sequence	GAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATCTCTGAGACTGTCTTGTGCCGCCAGCGGCTTCAGCATCAGGGACTACTAT ATGGCCTGGGTCCGACAGGCCCCTGGCAAAGGACTTGAGTGGGTCGCCAACATCAACTACGACGGCACCAACACCTACTACGCCGACAGCGTGAAGGG CAGATTCACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTATTGTGTGCGGAG CTACTACTATTACGGCATGGAATACTGGGGCCAGGGCACCACCGTGACAGTCTCTTCT

Fig. 32

Name	Description	Sequence
Human immunoglobulin kappa constant	for human IgG1 expression	CGTACGGTGGCGGCGCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTT GTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGG TAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCT GAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC ACAAAGAGCTTCAACAGGGGAGAGTGTTAG
Human immunoglobulin heavy constant gamma 1 (IGHG1)	for human IgG1 expression	GCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCC TGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGC CGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG CAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAG TCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGG ACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGA CAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAAC CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACC ACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Fig. 33

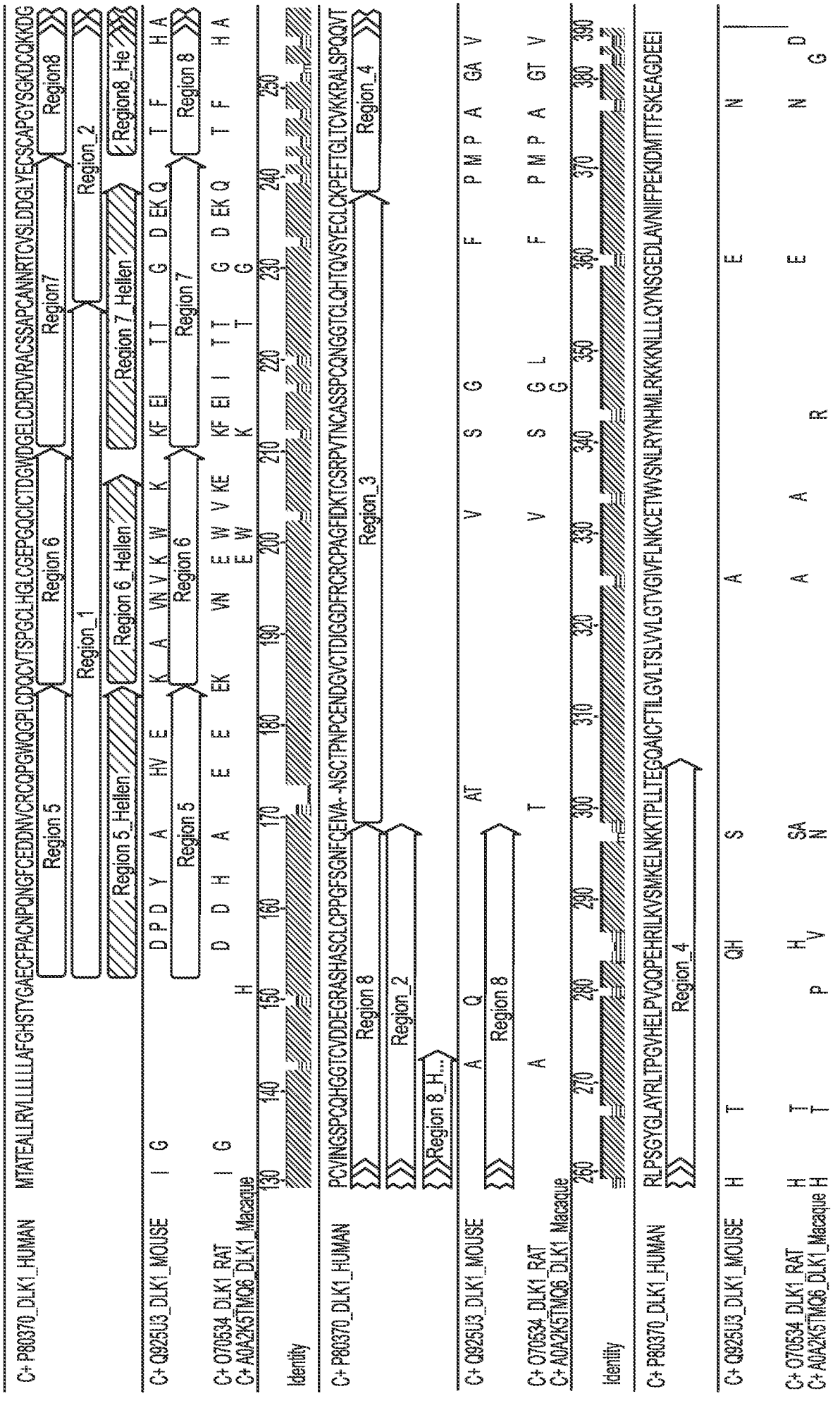
Name	Description	Sequence
04-0561-F1_VH	heavy chain variable region coding sequence of humanized anti-DLK1 antibody 04-0561-F1	CAAGTTCAATTGCAAGAAAGCGGCCCAGGGTTGGTTAAACCTTCCGAAACTTTGTCCCTTACTTGTACG GTTTCTGGATTTTCACTCAGTATATATTCTGTACATTGGGTAAGACAACCACCAGGTAAAGGGCTCGAAT GGATTGGACTTATTTGGGGTGGCGGGAGTACAGATTATAATCCTAGTTTGAAAATCAAGAGTTACCATATC TAAAGATACATCTAAGAATCAAGTTTCCTTGAAGCTCTCATCCGTCACTGCAGCGGATACAGCTGTCTATT ATTGTGCTCGTAAAGAAGGGAATTATTTGTGGTTTGCTTATTGGGGACAAGGGACTCTTGTCACAGTTA GTTCT
04-0561-F1_VL	light chain variable region coding sequence of humanized anti-DLK1 antibody 04-0561-F1	GACATCGTGATGACCCAGAGCCCCGACAGCCTGGCCGTGAGCCTGGGGCGAGAGGGTGACCATGAACT GCAAGAGCAGCCAGAGCCTGCTGCAGAGCAGCAACCAGAAGAACTACCTGGCCTGGTACCAGCAGAA GCCCGGCCAGCCCCCCAAGCTGCTGGTACTTCGCCAGCACCAGGGAGAGCGGCGTGCCCGACAGG TTCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCGTGCAGGCCGAGGACGTGG CCGTGTACTACTGCCAGCAGCACTACAGCATCCCCCCTGACCTTCGGCCAGGGCACCAAGCTGGAGATCA AG
04-0561-m_VH	Mouse VH variable region coding sequence of chimeric anti-DLK1 antibody 04-0561-m	CAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCACCCTCACAGAGCCTGTCCATCACATGCAC TGTCTCTGGGTTCTCATTATCCATATATAGTGTACACTGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAG TGGCTGGGATTGATATGGGGTGGTGGAAGCACAGACTATAATTCAGCTCTCAAATCCAGACTGAGCATC AGCAAGGACAACTCCAAGAGCCAAGTTTCTTAAAAATGAACAGTCTGCAAACTGATGACACAGCCAT GTACTACTGTGCCAGAAAGGATGGTAACTACCTCTGGTTTGCTACTGGGGCCAAGGGACTCTGGTCAC TGTCTCTGCA
04-0561-m_VL	mouse VL variable region coding sequence of chimeric anti-DLK1 antibody 04-0561-m	GACATTGTGATGACACAGTCTCCATCCTCCCTGGCTATGTCAGTAGGACAGAAGGTCACTATGAGCTGCA AGTCCAGTCAGAGCCTTTAAATAGTAGCAATCAAAAGAACTATTTGGCCTGGTACCAGCAGAAACCAG GACAGTCTCCTAAACTTCTGGTATATTTGCATCCACTAGGGAATCTGGGTCCCTGATCGCTTCATAGGC AGTGGATCTGGGACAGATTTCACTCTTACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGATTACTTC TGTCAGCAACATTATAGTATTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA

Fig. 34

Direct flow: FL4H/AF-647 signal	DLK1-561-F1-AF647	hIgG1-AF647	no ab
antibody concentration	20ug/ml	20ug/ml	0
HEK293T parental	6,563.00	4,432.00	1,282.00
HEK293T DLK1 mGFP G06 (long form)	436,889.50	9,870.00	1,649.00
834 HEK293T DLK1HM1-mGFP	18,999.00	8,757.00	1,385.00
835 HEK293T DLK1HM2-mGFP	21,274.00	8,513.00	1,251.00
836 HEK293T DLK1HM3-mGFP	567,238.50	8,769.00	1,657.50
837 HEK293T DLK1HM4-mGFP	501,850.50	8,483.50	1,516.00
850 HEK293T DLK1 HM5-mGFP	664,678.50	7,658.00	1,807.00
851 HEK293T DLK1 HM6-mGFP	19,194.50	6,461.00	1,992.00
848 HEK293T DLK1 HM7-mGFP	8,446.00	5,274.00	1,583.50
849 HEK293T DLK1 HM8-mGFP	660,063.00	4,357.00	1,653.50

Direct flow: GFP signal	DLK1-561-F1-AF647	hIgG1-AF647	no ab
antibody concentration	20ug/ml	20ug/ml	0
HEK293T parental	8,090.00	7,701.00	7,781.00
HEK293T DLK1 mGFP G06 (long form)	952,015.50	1,403,606.00	1,352,732.50
834 HEK293T DLK1HM1-mGFP	614,709.00	640,100.00	718,750.00
835 HEK293T DLK1HM2-mGFP	1,484,510.00	1,546,772.00	1,477,988.00
836 HEK293T DLK1HM3-mGFP	1,271,590.00	1,368,289.00	1,298,531.00
837 HEK293T DLK1HM4-mGFP	796,924.50	1,081,922.50	873,012.00
850 HEK293T DLK1 HM5-mGFP	2,200,934.00	2,352,481.00	1,943,135.00
851 HEK293T DLK1 HM6-mGFP	1,825,754.50	2,072,360.50	2,035,732.00
848 HEK293T DLK1 HM7-mGFP	4,743,904.50	5,309,634.00	4,856,410.50
849 HEK293T DLK1 HM8-mGFP	4,355,303.00	3,607,626.00	3,485,728.50

FIG. 35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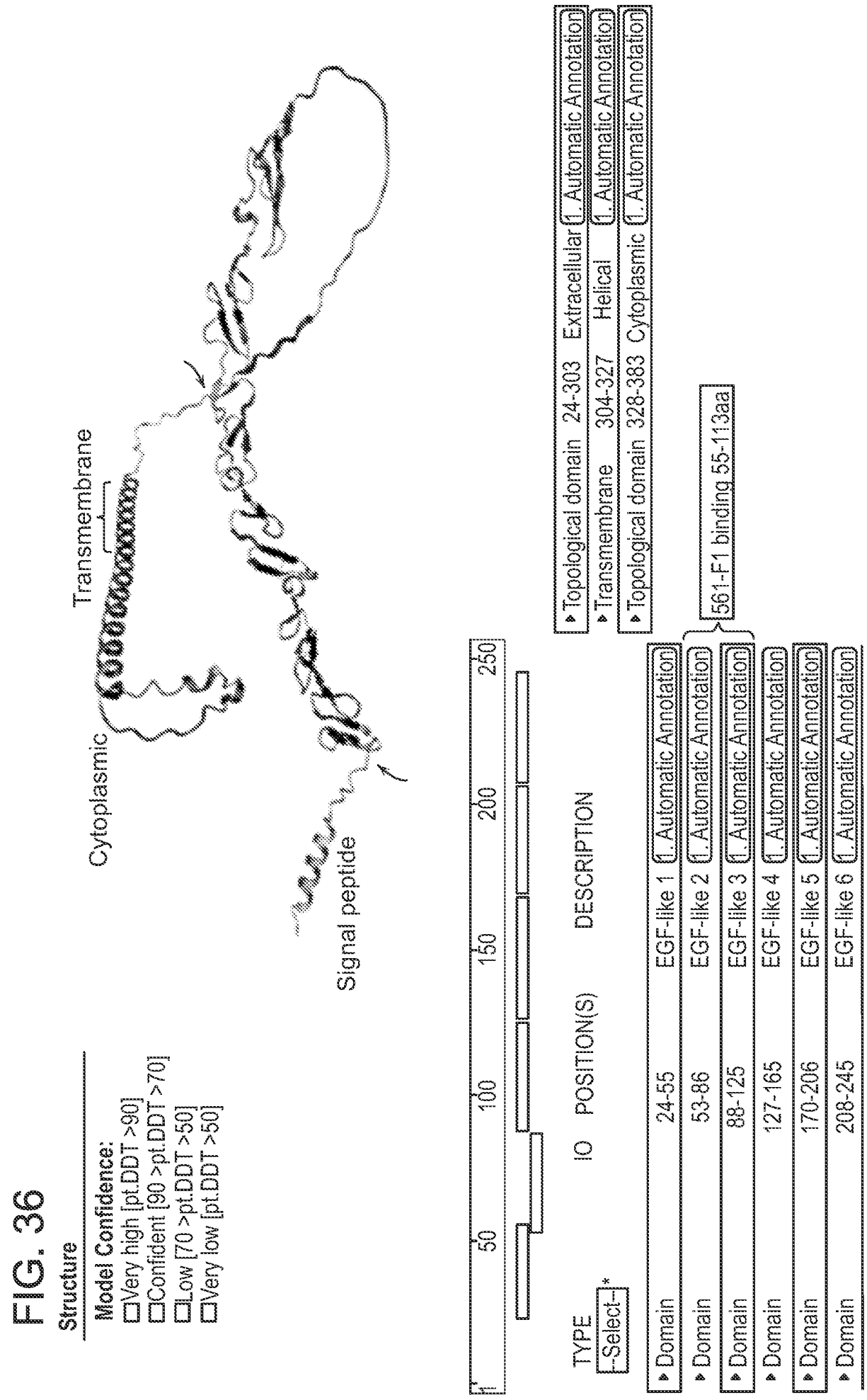
C+ Q925U3_DLK1_MOUSE
C+ O70534_DLK1_RAT
C+ A0A2K5TMQ6_DLK1_Macaque
Region 5
Region 6
Region_1
Region 7
Region_2
Region8
Region 5_Hellen
Region 6_Hellen
Region 7_Hellen
Region8_He
Region 8
Region_3
Region_4
Region 8_H...

FIG. 36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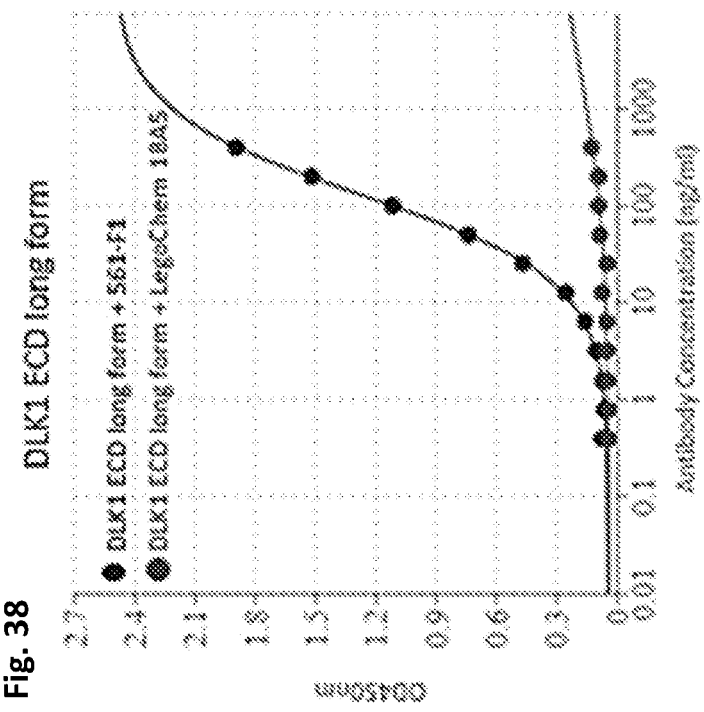
Confident [90 >pt.DDT >70]
Low [70 >pt.DDT >50]
Very low [pt.DDT >50]
Transmembrane
Cytoplasmic
Signal peptide
1
50
100
150
200
250
TYPE
-Select-
IO
POSITION(S)
DESCRIPTION
Topological domain 24-303 Extracellular 1. Automatic Annotation
Transmembrane 304-327 Helical 1. Automatic Annotation
Topological domain 328-383 Cytoplasmic 1. Automatic Annotation
561-F1 binding 55-113aa
Domain 24-55 EGF-like 1 1. Automatic Annotation
Domain 53-86 EGF-like 2 1. Automatic Annotation
Domain 88-125 EGF-like 3 1. Automatic Annotation
Domain 127-165 EGF-like 4 1. Automatic Annotation
Domain 170-206 EGF-like 5 1. Automatic Annotation
Domain 208-245 EGF-like 6 1. Automatic Annotation

Fig. 37

Direct flow: FL4H/AF-647 signal	561-F1-AF647	LegoChem 18A5-AF647	hIgG1-AF647
antibody concentration	20ug/ml	20ug/ml	20ug/ml
HEK293T parental	15,533.00	17,562.00	21,331.00
HEK293T DLK1 mGFP G06 (long form)	225,038.00	153,404.00	47,932.00
832-HEK293T DLK1 Iso2-mGFP mass pop (short form)	557,351.00	43,484.00	29,714.00

Indirect flow: FL4H/AF-647 signal	561-F1	LegoChem 18A5	hIgG1
1° antibody concentration	4ug/ml	4ug/ml	4ug/ml
HEK293T parental	1,727.50	2,827.00	3,130.00
HEK293T DLK1 mGFP G06 (long form)	32,920.00	20,415.00	4,116.00
832-HEK293T DLK1 Iso2-mGFP mass pop (short form)	220,664.00	3,348.00	2,895.00

Fig. 38



EC50 (nM)	DLK1-561-F1	LegoChem 18A5
Long form of DLK1 ECD-mFc (isoform 1)	0.870	17.887
Short form of DLK1 ECD-mFc (isoform 2)	0.810	No binding

FIG. 39A

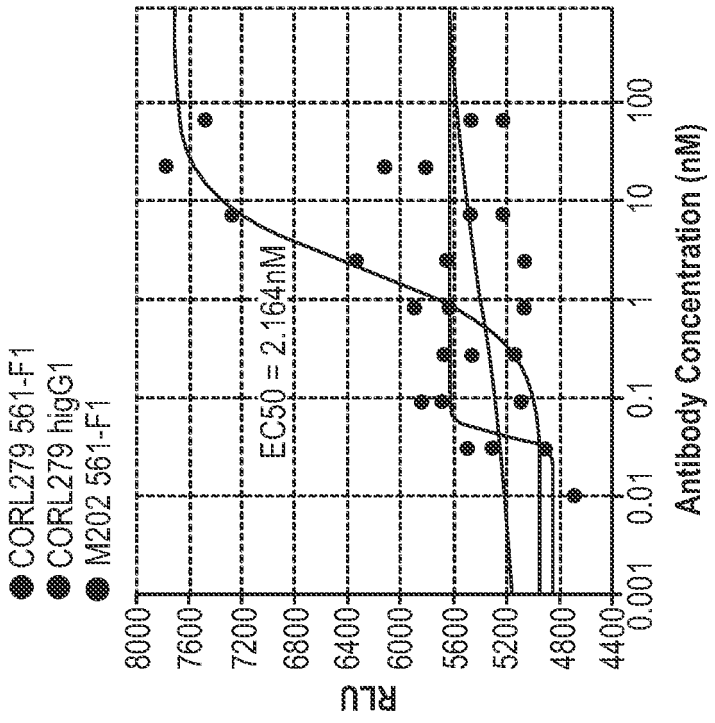


FIG. 39B

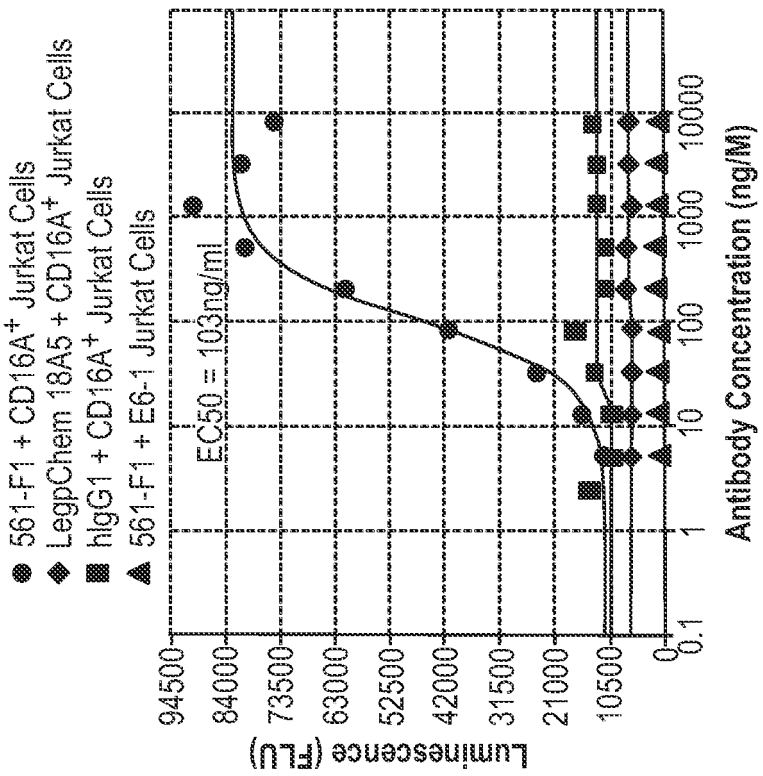
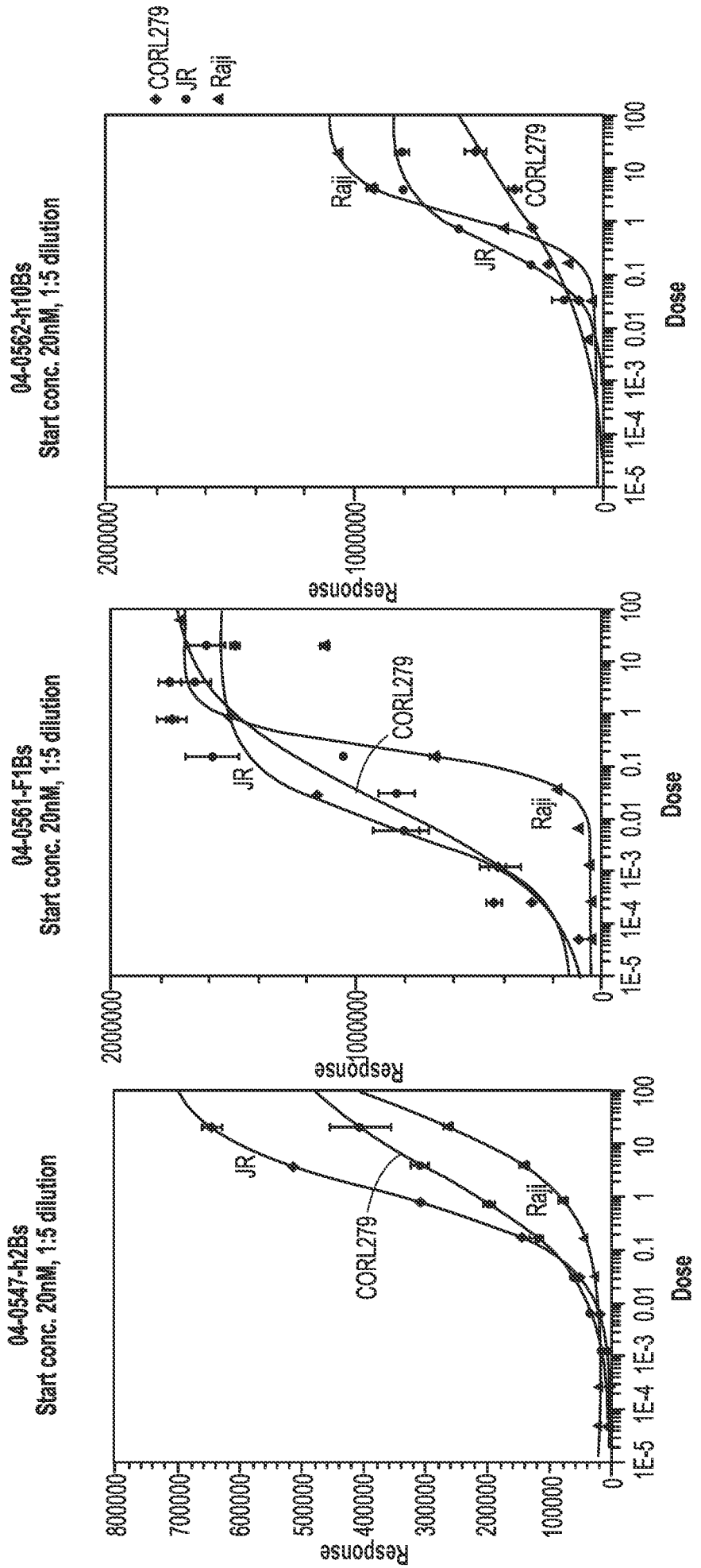


Fig. 40

Antibody		CORL279 (DLK1+)	M202 (DLK1-)
DLK1 scFv	04-0547-h2scFv	763,041.00	9,460.00
	04-0561-F1scFv	1,430,812.50	11,680.00
	04-0562-h10scFv	621,643.00	8,072.00
DLK1 BiTE	04-0547-h2Bs	424,744.00	15,031.00
	04-0561-F1Bs	1,158,268.00	124,757.00
	04-0562-h10Bs	900,449.00	15,794.00

Control	CORL279 (DLK1+)	M202 (DLK1-)
AF647-anti-His tag 2° Ab	63,034.00	16,139.00
no ab (cells only)	4,394.00	2,612.00

FIG. 41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Start conc. 20nM, 1:5 dilution
04-0561-F1Bs
Start conc. 20nM, 1:5 dilution
04-0562-h10Bs
Start conc. 20nM, 1:5 dilution
CORL279
JR
Raji
Response
Dose
800000
700000
600000
500000
400000
300000
200000
100000
2000000
1000000
0
1E-5
1E-4
1E-3
0.01
0.1
1
10
100