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(71) Applicant: **ITI HEALTH, INC.** [US/US]; 1076 Autumn Hill Court, Crozet, VA 22932 (US).

(72) Inventors: **KELLY, Kimberly, A.**; 1076 Autumn Hill Court, Crozet, VA 22932 (US). **DIMASTROMATTEO, Julien**; 1076 Autumn Hill Court, Crozet, VA 22932 (US).

(74) Agent: **MACDONALD, Kevin**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

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[Continued on next page]

(54) Title: PLECTIN-1 BINDING ANTIBODIES AND USES THEREOF

(57) Abstract: Aspects of the disclosure provide compositions and methods for treating cancer characterized by surface expression of plectin-1. In some embodiments, the disclosure provides anti-plectin-1 antibodies. In some embodiments, the anti-plectin-1 antibodies are conjugated to a targeted moiety (e.g., a therapeutic moiety or a detectable label).

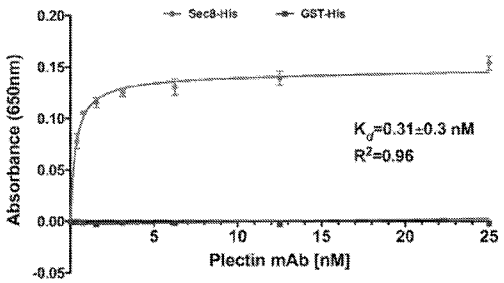

FIG. 3A

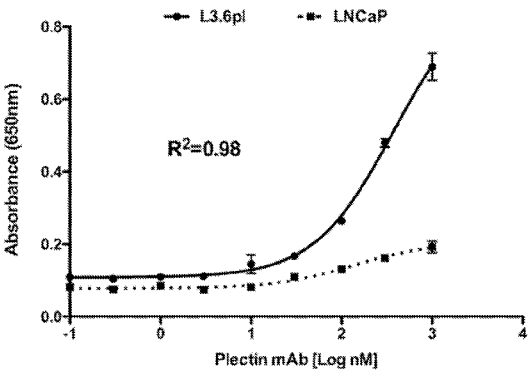

FIG. 3B

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LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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PLECTIN-1 BINDING ANTIBODIES AND USES THEREOF

RELATED APPLICATIONS

This Application claims the benefit of the filing date under 35 U.S.C. § 119(e) of U.S. provisional Application serial number 62/320,117, filed April 8, 2016, entitled "PLECTIN-1 BINDING ANTIBODIES AND USES THEREOF", the entire contents of which are incorporated herein by reference.

BACKGROUND

Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer death in the United States showing a rapid clinical course leading to death. Once diagnosed, PDAC has a median survival of 6 months and a 5-year survival rate of only 3 percent (Li et al., Lancet 363:1049-1057 (2004)).

As chemotherapy and radiotherapy have only modest benefits, and surgery is only possible in 20% of patients, early detection that allows surgical resection offers the best hope for longer survival (Yeo et al., Ann Surg 222:580-588 (1995); discussion 588-592). Indeed, the detection of PDAC or high-grade precursors in high-risk patient groups (e.g., hereditary cancer syndromes, chronic pancreatitis, and new-onset diabetes) represents a critical unmet need in the cancer diagnostic portfolio (Brentnall et al., Ann. Intern. Med. 131:247-255 (1999); Canto et al., Clin. Gastroenterol. Hepatol. 2:606-621 (2004)).

Serum CA-19-9 is the clinically used biomarker; however, it lacks the sensitivity needed to detect early-stage PDAC (Goggins, J. Clin. Oncol. 23:4524-4531 (2005)). In addition, cross-sectional abdominal imaging has proven to be unreliable to detect early-stage PDAC in high-risk patients (Pelaez-Luna et al., Am J Gastroenterol 102:2157-2163 (2007)).

Thus a high priority in this field of medicine is the identification of biomarkers for the development of binding ligands as diagnostics, such as imaging probes for detecting pre-neoplastic/early invasive lesions and for use in treatments.

SUMMARY

Aspects of the present disclosure relate to a recognition that successful development of clinically useful antibody-based agents, such as antibody drug conjugates (ADCs), is influenced by the specificity and selectivity of the agent for its target. Plectin-1 is a useful biomarker for a variety of cancers, including ovarian, esophageal, and head and neck

squamous cells carcinomas, as well as pancreatic ductal adenocarcinoma. In contrast with antibody targets, such as CD30, which is targeted by Brentuximab vedotin, and Her2, which is targeted by Ado-trastuzumab Emtansine, plectin-1 is a particularly useful target because it is present on the cell surface exclusively in certain cancer cells (*e.g.*, pancreatic ductal adenocarcinoma cells, ovarian cancer cells, *etc.*), thus giving exquisite specificity and selectivity. Accordingly, in some embodiments, the disclosure relates to antibodies and antigen binding fragments that bind specifically to plectin-1 on the surface of cancer cells, and methods of use thereof. In some embodiments, binding of an anti-plectin-1 antibody as described by the disclosure to a plectin-1 expressing cell induces death (*e.g.*, triggers apoptosis) of the cell.

In some aspects, the disclosure provides an antibody or antigen binding fragment that specifically binds an amino acid sequence having at least 85% identity to SEQ ID NO: 92. In some aspects, the disclosure provides an antibody or antigen binding fragment that specifically binds an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97% at least 98% or at least 99% identity to SEQ ID NO: 92. In some embodiments, the antibody specifically binds an amino acid sequence set forth as: SEQ ID NO: 92.

In some aspects, the disclosure provides an antibody, or antigen binding fragment, that specifically binds to cell-surface exposed plectin-1 antigen and that comprises six complementarity determining regions (CDRs): CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, wherein CDRH1 comprises a sequence as set forth in SEQ ID NO: 18, CDRH2 comprises a sequence as set forth in SEQ ID NO: 20, CDRH3 comprises a sequence as set forth in SEQ ID NO: 22, CDRL1 comprises a sequence as set forth in SEQ ID NO: 40, CDRL2 comprises a sequence as set forth in SEQ ID NO: 42, and CDRL3 comprises a sequence as set forth in SEQ ID NO: 44; or wherein CDRH1 comprises a sequence as set forth in SEQ ID NO: 62, CDRH2 comprises a sequence as set forth in SEQ ID NO: 64, CDRH3 comprises a sequence as set forth in SEQ ID NO: 66, CDRL1 comprises a sequence as set forth in SEQ ID NO: 84, CDRL2 comprises a sequence as set forth in SEQ ID NO: 86, and CDRL3 comprises a sequence as set forth in SEQ ID NO: 88.

In some embodiments, CDRH1 comprises a sequence as set forth in SEQ ID NO: 18, CDRH2 comprises a sequence as set forth in SEQ ID NO: 20, CDRH3 comprises a sequence as set forth in SEQ ID NO: 22, CDRL1 comprises a sequence as set forth in SEQ ID NO: 40,

CDRL2 comprises a sequence as set forth in SEQ ID NO: 42, and CDRL3 comprises a sequence as set forth in SEQ ID NO: 44.

In some aspects, the disclosure provides an antibody or antigen binding fragment that specifically binds to cell-surface exposed plectin-1, wherein the antibody or antigen binding fragment comprises variable heavy chain region comprising a complementarity determining region 3 (CDRH3) having a sequence set forth as: SEQ ID NO: 22 or SEQ ID NO: 66. In some embodiments, the antibody further comprises a light chain variable region comprising a complementarity determining region 3 (CDRL3) having a sequence set forth as: SEQ ID NO: 44 or SEQ ID NO: 88.

In some embodiments, the antibody, or antigen binding fragment comprises the heavy chain variable domain sequence of SEQ ID NO: 24. In some embodiments, the antibody, or antigen binding fragment comprises the light chain variable domain sequence of SEQ ID NO: 46. In some embodiments, the antibody, or antigen binding fragment comprises the heavy chain variable domain sequence of SEQ ID NO: 24 and the light chain variable domain sequence of SEQ ID NO: 46.

In some embodiments, the antibody, or antigen binding fragment CDRH1 comprises a sequence as set forth in SEQ ID NO: 62, CDRH2 comprises a sequence as set forth in SEQ ID NO: 64, CDRH3 comprises a sequence as set forth in SEQ ID NO: 66, CDRL1 comprises a sequence as set forth in SEQ ID NO: 84, CDRL2 comprises a sequence as set forth in SEQ ID NO: 86, and CDRL3 comprises a sequence as set forth in SEQ ID NO: 88.

In some embodiments, the antibody, or antigen binding fragment comprises the heavy chain variable domain sequence of SEQ ID NO: 68. In some embodiments, the antibody, or antigen binding fragment comprises the light chain variable domain sequence of SEQ ID NO: 90. In some embodiments, the antibody, or antigen binding fragment comprises the heavy chain variable domain sequence of SEQ ID NO: 68 and the light chain variable domain sequence of SEQ ID NO: 90.

In some aspects, the disclosure provides an antibody or antigen binding fragment that specifically binds to a cell-surface exposed plectin-1, wherein the antibody or antigen binding fragment comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO: 24 or SEQ ID NO: 68. In some embodiments, the antibody or antigen binding fragment further comprises a light chain variable region having a sequence set forth as: SEQ ID NO: 46 or SEQ ID NO: 90.

In some embodiments, the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO 24 and a light chain variable region having a sequence set forth as: SEQ ID NO: 46.

In some embodiments, the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO 68 and a light chain variable region having a sequence set forth as: SEQ ID NO: 90.

In some aspects, the disclosure provides an antibody that comprises a heavy chain variable region having a sequence set that shares at least 85% identity with SEQ ID NO: 15 and a light chain variable region that shares at least 85% identity with SEQ ID NO: 37. In some aspects, the disclosure provides an antibody that comprises a heavy chain variable region having a sequence set that shares at least 90% (*e.g.*, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identity with SEQ ID NO: 15 and a light chain variable region that shares at least 90% (*e.g.*, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identity with SEQ ID NO: 37.

In some aspects, the disclosure provides an antibody that comprises a heavy chain variable region having a sequence set that shares at least 85% identity with SEQ ID NO: 59 and a light chain variable region that shares at least 85% identity with SEQ ID NO: 81. In some aspects, the disclosure provides an antibody that comprises a heavy chain variable region having a sequence set that shares at least 90% (*e.g.*, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identity with SEQ ID NO: 59 and a light chain variable region that shares at least 90% (*e.g.*, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identity with SEQ ID NO: 81.

In some embodiments, an antibody, or antigen binding fragment described by the disclosure comprises a heavy chain constant domain having a sequence as set forth in SEQ ID NO: 15 or SEQ ID NO: 59.

In some embodiments, an antibody, or antigen binding fragment as described by the disclosure comprises a heavy chain constant domain selected from the group consisting of IgG, IgG1, IgG2, IgG2A, IgG2B, IgG2C, IgG3, IgG4, IgA1, IgA2, IgD, IgM, and IgE constant domains.

In some embodiments, an antibody, or antigen binding fragment described by the disclosure is a monoclonal antibody, a humanized antibody, a diabody, a chimeric antibody, a Fab fragment, a F(ab')2 fragment, an affibody, or an Fv fragment.

In some embodiments, the disclosure relates to antibody-drug conjugates targeted against plectin-1. In some embodiments, an antibody described by the disclosure (*e.g.*, an anti-plectin-1 antibody) is coupled to a targeted agent. In some embodiments, the targeted agent is a detectable moiety. In some embodiments, the detectable moiety is selected from the group consisting of a radioactive isotope, a magnetic compound, an x-ray absorber, a chemical compound, a biological tag, and a fluorescent molecule.

In some embodiments, the targeted agent is a therapeutic agent. In some embodiments, the therapeutic agent is a cytotoxic moiety or an immunomodulatory moiety.

In some embodiments, the antibody is coupled to the targeted agent via a linker. In some embodiments, the linker is a flexible amino acid sequence. In some embodiments, the linker is a photolinker.

In some embodiments, the targeted agent comprises a physiologically inert nanoparticle. In some embodiments, the nanoparticle is magnetic, fluorescent, or radioactive. In some embodiments, the targeted agent comprises a fluorochrome.

In some aspects, the disclosure provides an antibody, or antigen binding fragment, that competes or cross-competes for binding to an amino acid sequence set forth as: SEQ ID NO: 92 with an antibody, or antigen binding fragment as described by the disclosure (*e.g.*, an anti-plectin-1 antibody). In some embodiments, the antibody or antigen binding fragment competes or cross-competes with an equilibrium dissociation constant, Kd, of less than 10^{-6} M between the antibody or antigen binding fragment, and its antigen.

In some aspects, the disclosure provides a composition comprising an antibody as described by the disclosure (*e.g.*, an anti-plectin-1 antibody), optionally further comprising a pharmaceutically acceptable excipient.

In some aspects, the disclosure provides an isolated nucleic acid encoding a protein comprising three complementarity determining regions (CDRs): CDRH1, CDRH2, and CDRH3, wherein CDRH3 comprises a sequence as set forth in SEQ ID NO: 22. In some embodiments, CDRH1 comprises a sequence as set forth in SEQ ID NO: 18. In some embodiments, CDRH2 comprises a sequence as set forth in SEQ ID NO: 20.

In some aspects, the disclosure provides an isolated nucleic acid encoding a protein comprising three complementarity determining regions (CDRs): CDRL1, CDRL2, and CDRL3, wherein CDRL3 comprises a sequence as set forth in SEQ ID NO: 44. In some

embodiments, CDRL1 comprises a sequence as set forth in SEQ ID NO: 40. In some embodiments, CDRL2 comprises a sequence as set forth in SEQ ID NO: 42.

In some aspects, the disclosure provides an isolated nucleic acid encoding a protein comprising three complementarity determining regions (CDRs): CDRH1, CDRH2, and CDRH3, wherein CDRH3 comprises a sequence as set forth in SEQ ID NO: 66. In some embodiments, CDRH1 comprises a sequence as set forth in SEQ ID NO: 62. In some embodiments, CDRH2 comprises a sequence as set forth in SEQ ID NO: 64.

In some aspects, the disclosure provides an isolated nucleic acid encoding a protein comprising three complementarity determining regions (CDRs): CDRL1, CDRL2, and CDRL3, wherein CDRL3 comprises a sequence as set forth in SEQ ID NO: 88. In some embodiments, CDRL1 comprises a sequence as set forth in SEQ ID NO: 84. In some embodiments, CDRL2 comprises a sequence as set forth in SEQ ID NO: 86.

In some aspects, the disclosure provides an isolated nucleic acid comprising a sequence as set forth in a sequence selected from the group consisting of SEQ ID NO: 15, 24, 37, 46, 59, 68, 81, or 90.

In some aspects, the disclosure provides an isolated cell (*e.g.*, a host cell) comprising a nucleic acid as described by the disclosure. In some embodiments, the isolated cell is a bacterial cell, a yeast cell, a mammalian cell, or an insect cell. In some embodiments, the cell is a hybridoma cell.

In some aspects, the disclosure provides a method for targeting an agent to a cancer cell in a subject, the method comprising administering to the subject an antibody or composition as described by the disclosure (*e.g.*, an anti-plectin-1 antibody or a composition comprising an anti-plectin-1 antibody), coupled to a targeted agent, wherein the antibody binds to plectin-1 on the surface of the cancer cell in the subject.

In some aspects, the disclosure provides a method for treating cancer, the method comprising administering to a subject having cancer an effective amount an antibody or composition as described by the disclosure (*e.g.*, an anti-plectin-1 antibody or a composition comprising an anti-plectin-1 antibody).

In some aspects, the disclosure provides a method for detecting a cancer cell, the method comprising administering to a subject having cancer an effective amount of the method comprising administering to the subject an antibody or composition as described by

the disclosure (*e.g.*, an anti-plectin-1 antibody or a composition comprising an anti-plectin-1 antibody).

In some embodiments of methods described by the disclosure, the antibody or composition is administered at a dose in a range of 1 ng/kg and 100 mg/kg.

In some embodiments of methods described by the disclosure, the cancer cell is an ovarian cancer cell, esophageal cancer cell, head and neck squamous cell carcinoma cancer cell, or pancreatic cancer cell. In some embodiments, the cancer cell is a pancreatic ductal adenocarcinoma cell. In some embodiments of methods described by the disclosure, the subject is a mammal, optionally a human.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 shows the *in vitro* validation of different clones on YapC- or HPDE-coated plates.

FIG. 2 shows further *in vitro* validation of the cell lines using a cell killing assay.

FIGs. 3A-3B show PAb1 binding specificity on recombinant human C-terminal portion of Plectin-1 protein (FIG. 3A, Sec8-His) and plectin-1-positive L3.6pl cancer cells (FIG. 3B).

FIGs. 4A-4G show internalization of PAb1 in L3.6pl plectin-1-positive cancer cells. FIGs. 4A-4B show representative confocal images of L3.6pl after staining of PAb1 (FIG. 4A) and IgG ctrl (FIG. 4B) merged with endosomal marker Lamp-1. Staining of Lamp-1 (FIG. 4C), PAb1 (FIG. 4D) and co-localization of LAMP-1 and PAb1 (FIG. 4E) are shown. FIG. 4F shows data indicating that a significant portion of PAb1 merged with Lamp-1 whereas IgG control did not. FIG 4G shows quantification of internalized ^{125}I- PAb1 radioactivity after incubation at 37°C, 4°C or in combination with excess of cold PAb1 in L3.6pl plectin-1-positive cells and LNCap plectin-1-negative cells; Comp. refers to competition assay.

FIGs. 5A-5D show induction of cancer cell death by apoptosis after treatment with PAb1. FIG. 5A shows fluorescent minus one (FMO) flow cytometry data of L3.6pl cells. FIG. 5B shows L3.6pl AnnexinV positive cells after 72h control IgG treatment. FIG. 5C shows L3.6pl AnnexinV positive cells after 72h PAb1 treatment. FIG. 5D shows L3.6pl cancer cells experienced significantly more apoptosis after PAb1 treatment compared to control IgG (D).*,$p<0.05$.

FIGs. 6A-6D show effects of PAb1 treatment on tubulin anisotropy of YapC cancer

cells. FIG. 6A shows confocal microscopy images of YapC after tubulin staining without treatment. FIG. 6B shows confocal microscopy images of YapC after tubulin staining 10 min. post monomethyl auristatin E (MMAE) treatment. FIG. 6C shows confocal microscopy images of YapC after tubulin staining 24h post PAb1 treatment. FIG. 6D shows a decrease of anisotropy in cells treated with PAb1 compared to non-treated controls.

FIGs. 7A-7C show co-localization of PAb1 with tubulin in YapC cancer cells. FIG. 7A shows confocal microscopy images of YapC cells after tubulin staining. FIG. 7B shows confocal microscopy images of YapC cells after PAb1 staining. FIG. 7C shows co-localization (arrows) of tubulin staining and PAb1 staining.

FIGs. 8A-8E show *in vivo* PAb1 dose-escalating treatment of immunocompromised mice bearing a subcutaneous YapC tumor. FIG. 8A shows that after 11 days of treatment, tumor volume is significantly lower in mice administered 3mg/kg PAb1 than control IgG mice. 1mg/kg PAb1 treatment group elicited a significant reduction of tumor volume at day 14. The two higher doses of PAb1 showed a significantly lower tumor volume compared to 0.3mg/kg group. *, $p<0.05$, IgG vs 3mg/kg PAb1; #, $p<0.05$, IgG vs 1mg/kg PAb1; °, $p<0.05$, 0.3 vs 3mg/kg PAb1; •, $p<0.05$, 0.3 vs 1mg/kg PAb1. FIG. 8B shows the average body weight of the animal of each group. Note that the animal did not lose weight during the entire duration of the treatment.

DETAILED DESCRIPTION

Antibodies that Bind Plectin-1

The present disclosure provides antibodies and antigen binding fragments that bind to plectin-1 on the surface of cancer cells. The monoclonal antibodies of the disclosure may be murine, humanized or chimeric or in other forms. A detailed description of the antibodies of the disclosure as well as methods for the production and identification of the antibodies of the disclosure is provided herein.

Plectin-1 is a high molecular weight protein (500 kDa) that links intermediate filaments to microtubules and microfilaments, in addition to anchoring the cytoskeleton the plasma and nuclear membranes (reviewed in Sonnenberg, et al., Exp Cell Res 313:2189-2203 (2007)).

Generally, plectin-1 levels are low in normal pancreatic ductal cells but its expression is upregulated in cells having certain cancers (*e.g.*, precursor pancreatic intraepithelial

neoplasis (PanINs), pancreatic ductal adenocarcinoma cells (PDACs), ovarian cancer cells, *etc.*). Plectin-1 exhibits distinct cytoplasm and nuclear localization in normal fibroblasts, whereas an aberrant expression on the cell membrane is observed in cells having certain cancers (*e.g.*, PDACs). Altered subcellular localization of plectin-1 has also been observed in an autoimmune condition, paraneoplastic pemphigus, and in the associated lymphoproliferative neoplasm, Castleman's disease (Aho *et al.*, J Invest Dermatol 113:422-423 (1999)). Plectin-1 also has important roles in signal transduction. Thus, plectin-1 in cells having certain cancers (*e.g.*, precursor pancreatic intraepithelial neoplasis (PanINs), pancreatic ductal adenocarcinoma cells (PDACs), ovarian cancer cells, *etc.*) may have an impact on signaling pathways that regulate cell migration, polarity and energy metabolism related to carcinogenesis. Accordingly, in some embodiments, the disclosure provides antibodies and antigen binding fragments that bind to plectin-1 on the surface of cancer cells.

In some embodiments, antibodies, also known as immunoglobulins, are tetrameric glycosylated proteins composed of two light (L) chains of approximately 25 kDa each and two heavy (H) chains of approximately 50 kDa each. Two types of light chain, termed lambda and kappa, may be found in antibodies. Depending on the amino acid sequence of the constant domain of heavy chains, immunoglobulins can be assigned to five major classes: A, D, E, G, and M, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. Each light chain typically includes an N-terminal variable (V) domain (V_L) and a constant (C) domain (C_L). Each heavy chain typically includes an N-terminal V domain (V_H), three or four C domains (C_H1-3), and a hinge region. The C_H domain most proximal to V_H is designated as C_H1. The V_H and V_L domains consist of four regions of relatively conserved sequences called framework regions (FR1, FR2, FR3, and FR4), which form a scaffold for three regions of hypervariable sequences (complementarity determining regions, CDRs). The CDRs contain most of the residues responsible for specific interactions of the antibody with the antigen. CDRs are referred to as CDR1, CDR2, and CDR3. Accordingly, CDR constituents on the heavy chain are referred to as CDRH1, CDRH2, and CDRH3, while CDR constituents on the light chain are referred to as CDRL1, CDRL2, and CDRL3. The CDRs typically refer to the Kabat CDRs, as described in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services (1991), eds. Kabat *et al.* Another standard for characterizing the antigen binding site is to refer to the hypervariable loops as described by Chothia. See, *e.g.*,

Chothia, D. *et al.* (1992) J. Mol. Biol. 227:799-817; and Tomlinson *et al.* (1995) EMBO J. 14:4628-4638. Still another standard is the AbM definition used by Oxford Molecular's AbM antibody modeling software. See, generally, *e.g.*, Protein Sequence and Structure Analysis of Antibody Variable Domains. In: Antibody Engineering Lab Manual (Ed.: Duebel, S, and Kontermann, R., Springer-Verlag, Heidelberg). Embodiments described with respect to Kabat CDRs can alternatively be implemented using similar described relationships with respect to Chothia hypervariable loops or to the AbM-defined loops, or combinations of any of these methods.

In some embodiments, anti-plectin-1 antibodies of the present disclosure and the nucleic acid molecules of the present disclosure that encode the antibodies include the CDR amino acid and nucleic acid sequences shown in Table 1 below.

Table 1.

Antibody	**CDRH1**	**CDRH2**	**CDRH3**	**CDRL1**	**CDRL2**	**CDRL3**
Pab2						
Amino acid:	(SEQ ID NO: 18)	(SEQ ID NO: 20)	(SEQ ID NO: 22)	(SEQ ID NO: 40)	(SEQ ID NO: 42)	(SEQ ID NO: 44)
Nuc. Acid:	(SEQ ID NO: 7)	(SEQ ID NO: 9)	(SEQ ID NO: 11)	(SEQ ID NO: 29)	(SEQ ID NO: 31)	(SEQ ID NO: 33)
Pab1						
Amino acid:	(SEQ ID NO: 62)	(SEQ ID NO: 64)	(SEQ ID NO: 66)	(SEQ ID NO: 84)	(SEQ ID NO: 86)	(SEQ ID NO: 88)
Nuc. Acid:	(SEQ ID NO: 51)	(SEQ ID NO: 53)	(SEQ ID NO: 55)	(SEQ ID NO: 73)	(SEQ ID NO: 75)	(SEQ ID NO: 77)

In some embodiments, anti-plectin-1 binding agents (*e.g.*, anti-plectin-1 antibodies) of the disclosure include any antibody or antigen binding fragment that includes a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, or CDRL3, or combinations thereof, as provided for any one of the antibodies shown in Table 1. In some embodiments, anti- plectin-1 binding agents include the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 of any one of the antibodies shown in Table 1. The disclosure also includes any nucleic acid sequence that encodes a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, or CDRL3 as provided for any one of the antibodies shown in Table 1. Antibody heavy and light chain CDR3 domains may play a particularly important role in the binding specificity/affinity of an antibody for an antigen. Accordingly, the anti-plectin-1 antibodies of the disclosure, or the nucleic acid molecules thereof, may include at least the heavy and/or light chain CDR3s of antibodies as shown in Table 1 or as set forth by SEQ ID NOs: 15, 22, 24, 37, 44, 46, 59, 66, 68, 81, 88 or 90.

The complete amino acid and nucleic acid sequences for the heavy chain variable region and light chain variable region of the antibodies listed in Table 2.

Table 2.

Antibody	Heavy Chain Variable Region	Light Chain Variable Region
PAb2		
Amino acid:	SEQ ID NO: 24	SEQ ID NO: 46
Nuc. Acid:	SEQ ID NO: 13	SEQ ID NO: 35
PAb1		
Amino acid:	SEQ ID NO: 68	SEQ ID NO: 90
Nuc. Acid:	SEQ ID NO: 57	SEQ ID NO: 79

In some embodiments, anti-plectin antibodies of the disclosure include any antibody that includes a heavy chain variable domain or a light chain variable domain or both as shown in Table 1, or as described in the sequence listing of this disclosure (*e.g.*, SEQ ID NOs: 15, 24, 37, 46, 59, 68, 81, or 90). The disclosure also includes any nucleic acid molecule encoding an antibody that includes a heavy chain variable domain or a light chain variable domain nucleic acid sequence, or both, as shown in Table 1 or as described in the sequence listing of this disclosure (*e.g.*, SEQ ID NOs: 4, 13, 26, 35, 48, 57, 70, or 79).

Anti-plectin-1 antibodies of this disclosure may optionally comprise antibody constant regions or parts thereof. For example, a V_L domain may be attached at its C-terminal end to a light chain constant domain like Cκ or Cλ. Similarly, a V_H domain or portion thereof may be attached to all or part of a heavy chain like IgA, IgD, IgE, IgG, and IgM, and any isotype subclass. Antibodies may include suitable constant regions (see, for example, Kabat et al., Sequences of Proteins of Immunological Interest, No. 91-3242, National Institutes of Health Publications, Bethesda, Md. (1991)). Therefore, antibodies within the scope of this may disclosure include V_H and V_L domains, or an antigen binding portion thereof, combined with constant regions known in the art. In some embodiments, anti-plectin-1 antibodies of the disclosure comprise a heavy chain constant region comprising a sequence represented by SEQ ID NOs: 4, 14, 26, 36, 48, 58, 70, or 80.

In certain embodiments, the V_H and/or V_L domains may be reverted to germline sequence, *e.g.*, the FR of these domains are mutated using conventional molecular biology techniques to match those produced by the germline cells. In other embodiments, the FR sequences remain diverged from the consensus germline sequences.

In some embodiments, anti-plectin-1 antibodies or antigen binding fragments may or may not include the framework region of the antibodies, for example as set forth in SEQ ID NOs: 6, 8, 10, 12, 17, 19, 21, 23, 28, 30, 32, 34, 39, 41, 43, 45, 50, 52, 54, 56, 61, 63, 65, 67, 72, 74, 76, 78, 83, or 85. In some embodiments, anti-plectin-1 antibodies are murine antibodies. In some embodiments, anti-plectin-1 antibodies are chimeric or humanized antibodies.

It should be appreciated that, in some embodiments, the disclosure contemplates variants (*e.g.*, homologs) of amino acid and nucleic acid sequences for the heavy chain variable region and light chain variable region of the antibodies. "Homology" refers to the percent identity between two polynucleotides or two polypeptide moieties. The term "substantial homology", when referring to a nucleic acid, or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in about 90 to 100% of the aligned sequences. For example, in some embodiments, nucleic acid sequences sharing substantial homology are at least 90%, at least 91%, at least 92% at least 93%, at least 94%, at least 95%, at least 96% at least 97%, at least 98% at least 99% sequence identity. When referring to a polypeptide, or fragment thereof, the term "substantial homology" indicates that, when optimally aligned with appropriate gaps, insertions or deletions with another polypeptide, there is nucleotide sequence identity in about 90 to 100% of the aligned sequences. The term "highly conserved" means at least 80% identity, preferably at least 90% identity, and more preferably, over 97% identity. For example, in some embodiments, highly conserved proteins share at least 85%, at least 90%, at least 91%, at least 92% at least 93%, at least 94%, at least 95%, at least 96% at least 97%, at least 98% at least 99% identity. In some cases, highly conserved may refer to 100% identity. Identity is readily determined by one of skill in the art by, for example, the use of algorithms and computer programs known by those of skill in the art.

In some embodiments, an anti-plectin-1 antibodies of the disclosure can bind to plectin-1 with high affinity, *e.g.*, with a Kd less than 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M or lower. For example, anti-plectin-1 antibodies or antigen binding fragments thereof can bind to plectin-1with an affinity between 5 pM and 500 nM, *e.g.*, between 50 pM and 100 nM, *e.g.*, between 500 pM and 50 nM. The disclosure also includes antibodies or antigen binding fragments that compete with any of the antibodies described herein for binding to plectin-1 and that have an affinity of 50 nM or lower (*e.g.*, 20 nM or lower, 10 nM or lower, 500 pM or lower, 50 pM or lower, or 5 pM or lower). The affinity and binding kinetics of the anti-plectin-1antibody can be tested using any method known in the art including but not limited to biosensor technology (*e.g.*, OCTET or BIACORE).

As used herein, the term "antibody" generally refers to an immunoglobulin. All derivatives thereof which maintain or possess specific binding ability are also provided herein. An antibody preparation may be monoclonal or polyclonal.

As used herein, the term "antibody fragment" or "antigen binding fragment" refers to any derivative of an antibody which is less than full-length. Generally, an antigen binding fragment retains at least a significant portion of the full-length antibody's specific binding ability. Examples of antigen binding fragments include, but are not limited to, Fab, Fab', F(ab')2, scFv, Fv, dsFv diabody, affibodies, and Fd fragments. Antigen binding fragments may be produced by any appropriate means. For instance, an antigen binding fragment may be enzymatically or chemically produced by fragmentation of an intact antibody or it may be recombinantly produced from a gene encoding the partial antibody sequence. Alternatively, an antigen binding fragment may be wholly or partially synthetically produced. An antigen binding fragment may optionally be a single chain antibody fragment. Alternatively, a fragment may comprise multiple chains which are linked together, for instance, by disulfide linkages. An antigen binding fragment may also optionally be a multimolecular complex. A functional antigen binding fragment will typically comprise at least about 50 amino acids and more typically will comprise at least about 200 amino acids.

Single-chain Fvs (scFvs) are recombinant antigen binding fragments consisting of only the variable light chain (VL) and variable heavy chain (VH) covalently connected to one another by a polypeptide linker. Either VL or VH may be the NH2-terminal domain. The polypeptide linker may be of variable length and composition so long as the two variable domains are bridged without serious steric interference. Typically, the linkers are comprised

primarily of stretches of glycine and serine residues with some glutamic acid or lysine residues interspersed for solubility.

Diabodies are dimeric scFvs. The components of diabodies typically have shorter peptide linkers than most scFvs, and they show a preference for associating as dimers.

A Fv fragment is an antigen binding fragment which consists of one VH and one VL domain held together by noncovalent interactions. The term dsFv is used herein to refer to an Fv with an engineered intermolecular disulfide bond to stabilize the VH-VL pair.

A F(ab')2 fragment is an antigen binding fragment essentially equivalent to that obtained from immunoglobulins (typically IgG) by digestion with an enzyme pepsin at pH 4.0-4.5. The fragment may be recombinantly produced.

A Fab fragment is an antigen binding fragment essentially equivalent to that obtained by reduction of the disulfide bridge or bridges joining the two heavy chain pieces in the F(ab')2 fragment. The Fab' fragment may be recombinantly produced.

A Fab fragment is an antigen binding fragment essentially equivalent to that obtained by digestion of immunoglobulins (typically IgG) with the enzyme papain. The Fab fragment may be recombinantly produced. The heavy chain segment of the Fab fragment is the Fd piece.

An affibody is a small protein comprising a three-helix bundle that functions as an antigen binding molecule (*e.g.*, an antibody mimetic). Generally, affibodies are approximately 58 amino acids in length and have a molar mass of approximately 6 kDa. Affibody molecules with unique binding properties are acquired by randomization of 13 amino acids located in two alpha-helices involved in the binding activity of the parent protein domain. Specific affibody molecules binding a desired target protein can be isolated from pools (libraries) containing billions of different variants, using methods such as phage display.

Production of Antibodies that Bind Plectin-1

Numerous methods may be used for obtaining antibodies, or antigen binding fragments thereof, of the disclosure. For example, antibodies can be produced using recombinant DNA methods. Monoclonal antibodies may also be produced by generation of hybridomas (see *e.g.*, Kohler and Milstein (1975) Nature, 256: 495-499) in accordance with known methods. Hybridomas formed in this manner are then screened using standard

methods, such as enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (*e.g.*, OCTET or BIACORE) analysis, to identify one or more hybridomas that produce an antibody that specifically binds with a specified antigen. Any form of the specified antigen may be used as the immunogen, *e.g.*, recombinant antigen, naturally occurring forms, any variants or fragments thereof, as well as antigenic peptide thereof (*e.g.*, any of the epitopes described herein as a linear epitope or within a scaffold as a conformational epitope). One exemplary method of making antibodies includes screening protein expression libraries that express antibodies or fragments thereof (*e.g.*, scFv), *e.g.*, phage or ribosome display libraries. Phage display is described, for example, in Ladner et al., U.S. Pat. No. 5,223,409; Smith (1985) Science 228:1315-1317; Clackson et al. (1991) Nature, 352: 624-628; Marks et al. (1991) J. Mol. Biol., 222: 581-597WO92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; and WO 90/02809.

In addition to the use of display libraries, the specified antigen (*e.g.*, plectin-1) can be used to immunize a non-human animal, *e.g.*, a rodent, *e.g.*, a mouse, hamster, or rat. In one embodiment, the non-human animal is a mouse.

In another embodiment, a monoclonal antibody is obtained from the non-human animal, and then modified, *e.g.*, made chimeric, using recombinant DNA techniques known in the art. A variety of approaches for making chimeric antibodies have been described. See *e.g.*, Morrison *et al.*, Proc. Natl. Acad. Sci. U.S.A. 81:6851, 1985; Takeda *et al.*, Nature 314:452, 1985, Cabilly *et al.*, U.S. Pat. No. 4,816,567; Boss *et al.*, U.S. Pat. No. 4,816,397; Tanaguchi *et al.*, European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom Patent GB 2177096B.

Antibodies can also be humanized by methods known in the art. For example, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; and Oxford Molecular, Palo Alto, Calif.). Fully humanized antibodies, such as those expressed in transgenic animals are within the scope of the invention (see, *e.g.*, Green *et al.* (1994) Nature Genetics 7, 13; and U.S. Patent Nos. 5,545,806 and 5,569,825).

For additional antibody production techniques, see Antibodies: A Laboratory Manual, Second Edition. Edited by Edward A. Greenfield, Dana-Farber Cancer Institute, ©2014. The present disclosure is not necessarily limited to any particular source, method of production, or other special characteristics of an antibody.

Some aspects of the present invention relate to isolated cells (*e.g.*, host cells) transformed with a polynucleotide or vector. Host cells may be a prokaryotic or eukaryotic cell. The polynucleotide or vector which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained extrachromosomally. The host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell. In some embodiments, fungal cells are, for example, those of the genus Saccharomyces, in particular those of the species *S. cerevisiae*. The term "prokaryotic" includes all bacteria which can be transformed or transfected with a DNA or RNA molecules for the expression of an antibody or the corresponding immunoglobulin chains. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. The term "eukaryotic" includes yeast, higher plants, insects and vertebrate cells, *e.g.*, mammalian cells, such as NSO and CHO cells. Depending upon the host employed in a recombinant production procedure, the antibodies or immunoglobulin chains encoded by the polynucleotide may be glycosylated or may be non-glycosylated. Antibodies or the corresponding immunoglobulin chains may also include an initial methionine amino acid residue.

In some embodiments, once a vector has been incorporated into an appropriate host, the host may be maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the immunoglobulin light chains, heavy chains, light/heavy chain dimers or intact antibodies, antigen binding fragments or other immunoglobulin forms may follow; see, Beychok, Cells of Immunoglobulin Synthesis, Academic Press, N.Y., (1979). Thus, polynucleotides or vectors are introduced into the cells which in turn produce the antibody or antigen binding fragments. Furthermore, transgenic animals, preferably mammals, comprising the aforementioned host cells may be used for the large scale production of the antibody or antibody fragments.

The transformed host cells can be grown in fermenters and cultured according to techniques known in the art to achieve optimal cell growth. Once expressed, the whole antibodies, their dimers, individual light and heavy chains, other immunoglobulin forms, or antigen binding fragments, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like; see, Scopes, "Protein Purification", Springer Verlag, N.Y. (1982). The antibody or antigen binding fragments can then be isolated from the growth

medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the, *e.g.*, microbially expressed antibodies or antigen binding fragments may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies directed, *e.g.*, against the constant region of the antibody.

Aspects of the disclosure relate to a hybridoma, which provides an indefinitely prolonged source of monoclonal antibodies. As used herein, "hybridoma cell" refers to an immortalized cell derived from the fusion of B lymphoblasts with a myeloma fusion partner. For preparing monoclonal antibody-producing cells (*e.g.*, hybridoma cells), an individual animal whose antibody titer has been confirmed (*e.g.*, a mouse) is selected, and 2 days to 5 days after the final immunization, its spleen or lymph node is harvested and antibody-producing cells contained therein are fused with myeloma cells to prepare the desired monoclonal antibody producer hybridoma. Measurement of the antibody titer in antiserum can be carried out, for example, by reacting the labeled protein, as described hereinafter and antiserum and then measuring the activity of the labeling agent bound to the antibody. The cell fusion can be carried out according to known methods, for example, the method described by Kochler and Milstein (Nature 256:495 (1975)). As a fusion promoter, for example, polyethylene glycol (PEG) or Sendai virus (HVJ), preferably PEG is used.

Examples of myeloma cells include NS-1, P3U1, SP2/0, AP-1 and the like. The proportion of the number of antibody producer cells (spleen cells) and the number of myeloma cells to be used is preferably about 1:1 to about 20:1. PEG (preferably PEG 1000-PEG 6000) is preferably added in concentration of about 10% to about 80%. Cell fusion can be carried out efficiently by incubating a mixture of both cells at about 20ºC to about 40 ºC, preferably about 30 ºC to about 37 ºC for about 1 minute to 10 minutes.

Various methods may be used for screening for a hybridoma producing the antibody (*e.g.*, against a tumor antigen or autoantibody of the present invention). For example, where a supernatant of the hybridoma is added to a solid phase (*e.g.*, microplate) to which antibody is adsorbed directly or together with a carrier and then an anti-immunoglobulin antibody (if mouse cells are used in cell fusion, anti-mouse immunoglobulin antibody is used) or Protein A labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the protein bound to the solid phase. Alternately, a supernatant of the hybridoma is added to a solid phase to which an anti-immunoglobulin antibody or Protein A

is adsorbed and then the protein labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the protein bound to the solid phase.

Selection of the monoclonal antibody can be carried out according to any known method or its modification. Normally, a medium for animal cells to which HAT (hypoxanthine, aminopterin, thymidine) are added is employed. Any selection and growth medium can be employed as long as the hybridoma can grow. For example, RPMI 1640 medium containing 1% to 20%, preferably 10% to 20% fetal bovine serum, GIT medium containing 1% to 10% fetal bovine serum, a serum free medium for cultivation of a hybridoma (SFM-101, Nissui Seiyaku) and the like can be used. Normally, the cultivation is carried out at 20 ºC to 40 ºC, preferably 37 ºC for about 5 days to 3 weeks, preferably 1 week to 2 weeks under about 5% CO_2 gas. The antibody titer of the supernatant of a hybridoma culture can be measured according to the same manner as described above with respect to the antibody titer of the anti-protein in the antiserum.

As an alternative to obtaining immunoglobulins directly from the culture of hybridomas, immortalized hybridoma cells can be used as a source of rearranged heavy chain and light chain loci for subsequent expression and/or genetic manipulation. Rearranged antibody genes can be reverse transcribed from appropriate mRNAs to produce cDNA. If desired, the heavy chain constant region can be exchanged for that of a different isotype or eliminated altogether. The variable regions can be linked to encode single chain Fv regions. Multiple Fv regions can be linked to confer binding ability to more than one target or chimeric heavy and light chain combinations can be employed. Any appropriate method may be used for cloning of antibody variable regions and generation of recombinant antibodies.

In some embodiments, an appropriate nucleic acid that encodes variable regions of a heavy and/or light chain is obtained and inserted into an expression vectors which can be transfected into standard recombinant host cells. A variety of such host cells may be used. In some embodiments, mammalian host cells may be advantageous for efficient processing and production. Typical mammalian cell lines useful for this purpose include CHO cells, 293 cells, or NSO cells. The production of the antibody or antigen binding fragment may be undertaken by culturing a modified recombinant host under culture conditions appropriate for the growth of the host cells and the expression of the coding sequences. The antibodies or antigen binding fragments may be recovered by isolating them from the culture. The

expression systems may be designed to include signal peptides so that the resulting antibodies are secreted into the medium; however, intracellular production is also possible.

The disclosure also includes a polynucleotide encoding at least a variable region of an immunoglobulin chain of the antibodies described herein. In some embodiments, the variable region encoded by the polynucleotide comprises at least one complementarity determining region (CDR) of the VH and/or VL of the variable region of the antibody produced by any one of the above described hybridomas.

Polynucleotides encoding antibody or antigen binding fragments may be, *e.g.*, DNA, cDNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric nucleic acid molecule comprising any of those polynucleotides either alone or in combination. In some embodiments, a polynucleotide is part of a vector. Such vectors may comprise further genes such as marker genes which allow for the selection of the vector in a suitable host cell and under suitable conditions.

In some embodiments, a polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells. Expression of the polynucleotide comprises transcription of the polynucleotide into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known to those skilled in the art. They may include regulatory sequences that facilitate initiation of transcription and optionally poly-A signals that facilitate termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally associated or heterologous promoter regions. Possible regulatory elements permitting expression in prokaryotic host cells include, *e.g.*, the PL, Lac, Trp or Tac promoter in *E. coli*, and examples of regulatory elements permitting expression in eukaryotic host cells are the AOX1 or GAL1 promoter in yeast or the CMV-promoter, SV40-promoter, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells.

Beside elements which are responsible for the initiation of transcription such regulatory elements may also include transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system employed, leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the polynucleotide and are well known in the art. The leader sequence(s) is (are)

assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a portion thereof, into, for example, the extracellular medium. Optionally, a heterologous polynucleotide sequence can be used that encode a fusion protein including a C- or N-terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product.

In some embodiments, polynucleotides encoding at least the variable domain of the light and/or heavy chain may encode the variable domains of both immunoglobulin chains or only one. Likewise, a polynucleotides may be under the control of the same promoter or may be separately controlled for expression. Furthermore, some aspects relate to vectors, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that comprise a polynucleotide encoding a variable domain of an immunoglobulin chain of an antibody or antigen binding fragment; optionally in combination with a polynucleotide that encodes the variable domain of the other immunoglobulin chain of the antibody.

In some embodiments, expression control sequences are provided as eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector into targeted cell population (*e.g.*, to engineer a cell to express an antibody or antigen binding fragment). A variety of appropriate methods can be used to construct recombinant viral vectors. In some embodiments, polynucleotides and vectors can be reconstituted into liposomes for delivery to target cells. The vectors containing the polynucleotides (*e.g.*, the heavy and/or light variable domain(s) of the immunoglobulin chains encoding sequences and expression control sequences) can be transferred into the host cell by suitable methods, which vary depending on the type of cellular host.

Modifications

Some aspects of the disclosure relate to antibody-drug conjugates targeted against plectin-1. As used herein, "antibody drug conjugate" refers to molecules comprising an antibody, or antigen binding fragment thereof, linked to a targeted molecule (*e.g.*, a biologically active molecule, such as a therapeutic molecule, and/or a detectable label).

Accordingly, in some embodiments, antibodies or antigen binding fragments of the disclosure may be modified with a detectable label, including, but not limited to, an enzyme, prosthetic group, fluorescent material, luminescent material, bioluminescent material, radioactive material, positron emitting metal, nonradioactive paramagnetic metal ion, and affinity label for detection and isolation of plectin-1. The detectable substance may be coupled or conjugated either directly to the polypeptides of the disclosure or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. Non-limiting examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, glucose oxidase, or acetylcholinesterase; non-limiting examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; non-limiting examples of suitable fluorescent materials include biotin, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, or phycoerythrin; an example of a luminescent material includes luminol; non-limiting examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include a radioactive metal ion, *e.g.*, alpha-emitters or other radioisotopes such as, for example, iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115}mIn, ^{113}mIn, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99}mTc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{86}R, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, and tin (^{113}Sn, ^{117}Sn). The detectable substance may be coupled or conjugated either directly to the anti-plectin-1 antibodies of the disclosure or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. Anti-plectin-1 antibodies conjugated to a detectable substance may be used for diagnostic assays as described herein.

In some embodiments, antibodies or antigen binding fragments of the disclosure may be modified with a therapeutic moiety (*e.g.*, therapeutic agent). As used herein, the term "therapeutic agent" refers to chemicals or drugs or proteins that are able to inhibit cell function, inhibit cell replication or kill mammalian cells, preferably human cells. Examples of therapeutic agents include but are not limited to cytotoxic moieties, radioisotopes, molecules of plant, fungal, or bacterial origin (*e.g.*, plant-derived toxins (*e.g.*, secondary metabolites), glycosides, antimicrobial compounds (*e.g.*, streptomycin, penicillin, *etc.*),

biological proteins (*e.g.*, protein toxins), particles (*e.g.*, recombinant viral particles, *e.g.*, via a viral coat protein), or mixtures thereof. The therapeutic agent can be an intracellularly active drug or other agent, such as short-range radiation emitters, including, for example, short-range, high-energy alpha-emitters (*e.g.*, ^{131}I).

In some embodiments, the therapeutic agent is an immunomodulatory moiety (*e.g.*, immunomodulatory agent). As used herein, "immunomodulatory agent" refers to a compound or molecule that increases or decreases the immune response of a subject in response to the agent. For example, an immunomodulatory agent may enhance the immune response of a subject to a tumor, *e.g.*, increase the level of inflammatory cytokines such as interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNF-α). Examples of immunomodulatory agents that increase the immune response of a subject include granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod, cellular membrane fractions from bacteria, certain interleukins and cytokines (*e.g.*, IL-1β, IL-6, and TNF-α), and immune checkpoint inhibitors (*e.g.*, PD-1 inhibitors, PD1-L inhibitors, *etc.*). In some embodiments, an immunomodulatory agent may decrease the immune response of a subject (*e.g.*, mediate or achieve immunosuppression). Examples of immunosuppressive immunomodulators include but are not limited to immunosuppressive drugs (*e.g.*, glucococorticoids, cytostatics, anti-inflammatory monoclonal antibodies (*e.g.*, anti-IL-2 receptor antibodies), and drugs targeting immunophilins (*e.g.*, ciclosporin, sirolimus, *etc.*).

In some embodiments, the antibody is coupled to the targeted agent via a linker. As used herein, the term "linker" refers to a molecule or sequence, such as an amino acid sequence, that attaches, as in a bridge, one molecule or sequence to another molecule or sequence. "Linked," "conjugated," or "coupled" means attached or bound by covalent bonds, or non-covalent bonds, or other bonds, such as van der Waals forces. Antibodies described by the disclosure can be linked to the targeted agent (*e.g.*, therapeutic moiety or detectable moiety) directly, *e.g.*, as a fusion protein with protein or peptide detectable moieties (with or without an optional linking sequence, *e.g.*, a flexible linker sequence) or via a chemical coupling moiety. A number of such coupling moieties are known in the art, e.g., a peptide linker or a chemical linker, *e.g.*, as described in International Patent Application Publication No. WO 2009/036092. In some embodiments, the linker is a flexible amino acid sequence. Examples of flexible amino acid sequences include glycine and serine rich linkers, which comprise a stretch of two or more glycine residues, (*e.g.*, GGGS; SEQ ID NO: 93). In some

embodiments, the linker is a photolinker. Examples of photolinkers include ketyl-reactive benzophenone (BP), anthraquinone (AQ), nitrene-reactive nitrophenyl azide (NPA), and carbene-reactive phenyl-(trifluoromethyl)diazirine (PTD).

In some embodiments, the targeted agent comprises a physiologically inert nanoparticle. Examples of nanoparticles developed and used for imaging cancer cells, include magnetic nanoparticles and their magnetofluorescent analogues (see, *e.g.*, Weissleder *et al.*, Nat. Biotechnol., 19:316-317 (2001); McCarthy *et al.*, Nanomedicine, 2:153-167 (2007); Hogemann *et al.*, Bioconjug. Chem., 11:941-946 (2000), and Josephson *et al.*, Bioconjug. Chem., 10:186-191 (1999)) which are contemplated for use with isolated peptide ligands and phage displayed peptides. Multimodal nanoparticles are known that incorporate both magnetic and fluorescent molecules within the same molecule and are used for fluorescent microscopy (which detects the fluorescent part of this very small particle) and MRI (which detects its magnetic portion). In some embodiments, the nanoparticle is magnetic, fluorescent, or radioactive. In some embodiments, the targeted agent comprises a fluorochrome.

Pharmaceutical Compositions

In some aspects, the disclosure relates to pharmaceutical compositions comprising anti-plectin-1 antibodies. In some embodiments, the composition comprises an anti-plectin-1 antibody and a pharmaceutically acceptable carrier. As used herein the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions. Pharmaceutical compositions can be prepared as described below. The active ingredients may be admixed or compounded with any conventional, pharmaceutically acceptable carrier or excipient. The compositions may be sterile.

Typically, pharmaceutical compositions are formulated for delivering an effective amount of an agent (*e.g.*, an anti-plectin-1 antibody or antibody drug conjugate comprising an anti-plectin-1 antibody and a targeted agent). In general, an "effective amount" of an active

agent refers to an amount sufficient to elicit the desired biological response (*e.g.*, killing of a cancerous cell or suppression of tumor growth). An effective amount of an agent may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of the compound, the disease being treated (*e.g.*, certain cancers characterized by surface expression of plectin-1), the mode of administration, and the patient.

A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known in the art. See, for example, REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Ed. (1990).

It will be understood by those skilled in the art that any mode of administration, vehicle or carrier conventionally employed and which is inert with respect to the active agent may be utilized for preparing and administering the pharmaceutical compositions of the present disclosure. Illustrative of such methods, vehicles and carriers are those described, for example, in Remington's Pharmaceutical Sciences, 4th ed. (1970), the disclosure of which is incorporated herein by reference. Those skilled in the art, having been exposed to the principles of the disclosure, will experience no difficulty in determining suitable and appropriate vehicles, excipients and carriers or in compounding the active ingredients therewith to form the pharmaceutical compositions of the disclosure.

An effective amount, also referred to as a therapeutically effective amount, of a compound (for example, an anti-plectin-1 antibody or antibody drug conjugate comprising an anti-plectin-1 antibody and a targeted agent) is an amount sufficient to ameliorate at least one adverse effect associated with cancer (*e.g.*, tumor growth, metastasis). The therapeutically effective amount to be included in pharmaceutical compositions depends, in each case, upon several factors, *e.g.*, the type, size and condition of the patient to be treated, the intended mode of administration, the capacity of the patient to incorporate the intended dosage form, *etc.* Generally, an amount of active agent is included in each dosage form to provide from about 0.1 to about 250 mg/kg, and preferably from about 0.1 to about 100 mg/kg. One of ordinary skill in the art would be able to determine empirically an appropriate therapeutically effective amount.

Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body

weight, severity of adverse side-effects and selected mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to treat the particular subject. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular therapeutic agent being administered, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular nucleic acid and/or other therapeutic agent without necessitating undue experimentation.

In some cases, compounds of the disclosure are prepared in a colloidal dispersion system. Colloidal dispersion systems include lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. In some embodiments, a colloidal system of the disclosure is a liposome. Liposomes are artificial membrane vessels which are useful as a delivery vector *in vivo* or *in vitro*. It has been shown that large unilamellar vesicles (LUVs), which range in size from 0.2 - 4.0 μm can encapsulate large macromolecules.

Liposomes may be targeted to a particular tissue by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein. Ligands which may be useful for targeting a liposome to, for example, an smooth muscle cell include, but are not limited to: intact or fragments of molecules which interact with smooth muscle cell specific receptors and molecules, such as antibodies, which interact with the cell surface markers of cancer cells. Such ligands may easily be identified by binding assays well known to those of skill in the art. In still other embodiments, the liposome may be targeted to a tissue by coupling it to an antibody known in the art.

Compounds described by the disclosure may be administered alone (*e.g.*, in saline or buffer) or using any delivery vehicle known in the art. For instance the following delivery vehicles have been described: cochleates; Emulsomes; ISCOMs; liposomes; live bacterial vectors (*e.g.*, *Salmonella*, *Escherichia coli*, *Bacillus Calmette-Guérin*, *Shigella*, *Lactobacillus*); live viral vectors (*e.g.*, Vaccinia, adenovirus, Herpes simplex); microspheres; nucleic acid vaccines; polymers (*e.g.,* carboxymethylcellulose, chitosan); polymer rings; proteosomes; sodium fluoride; transgenic plants; virosomes; and, virus-like particles.

The formulations of the disclosure are administered in pharmaceutically acceptable solutions, which may routinely contain pharmaceutically acceptable concentrations of salt,

buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients.

The term pharmaceutically-acceptable carrier means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a human or other vertebrate animal. The term carrier denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the compounds of the present disclosure, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

In addition to the formulations described herein, the compounds may also be formulated as a depot preparation. Such long-acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries

such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, see Langer R (1990) Science 249:1527-1533, which is incorporated herein by reference.

The compounds may be administered *per se* (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Suitable buffering agents include: acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the compounds into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the compounds into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product. Liquid dose units are vials or ampoules. Solid dose units are tablets, capsules and suppositories.

Treatment Methods

Aspects of the disclosure relate to the discovery of antibodies that specifically bind to plectin-1 on the surface of certain cancer cells. In some embodiments, binding of an anti-plectin-1 antibody as described by the disclosure to certain cancer cells induces death (*e.g.*, triggers apoptosis) of the cells. Without wishing to be bound by any particular theory, antibodies described by the disclosure are useful, in some embodiments, for treating cancer characterized by surface expression of plectin-1. As used herein, "treating cancer" refers to

decreasing the number of cancer cells in a patient, slowing the growth of cancer cells in a patient, reducing the metastasis of cancer cells in a patient and includes any type of response for either relieving cancer symptoms or increasing the life-span of a patient.

Examples of cancers characterized by surface expression of plectin-1 include but are not limited to ovarian cancer cell, esophageal cancer cell, head and neck squamous cell carcinoma cancer cell, or pancreatic cancer cell (*e.g.*, pancreatic ductal adenocarcinoma (PDAC)). However, it should be appreciated that other cancers (such as lung cancer, bladder cancer, breast cancer, esophageal cancer, mouth cancer, tongue cancer, gum cancer, skin cancer (e.g., melanoma, basal cell carcinoma, Kaposi's sarcoma, etc.), muscle cancer, heart cancer, liver cancer, bronchial cancer, cartilage cancer, bone cancer, stomach cancer, prostate cancer, testicular cancer, cervical cancer, endometrial cancer, uterine cancer, colon cancer, colorectal, gastric cancer, kidney cancer, bladder cancer, lymphoma cancer, spleen cancer, thymus cancer, thyroid cancer, brain cancer, neuronal cancer, mesothelioma, gall bladder cancer, ocular cancer (*e.g.*, cancer of the cornea, cancer of uvea, cancer of the choroids, cancer of the macula, vitreous humor cancer, *etc.*), joint cancer (such as synovium cancer), glioblastoma, white blood cell cancer (*e.g.*, lymphoma, leukemia, etc.), hereditary non-polyposis cancer (HNPC), colitis-associated cancer, *etc.* Cancers are further exemplified by sarcomas (such as osteosarcoma and Kaposi's sarcoma) may be treated using anti-plectin-1 antibodies described by the disclosure.

In some aspects, the disclosure provides a method for treating cancer, the method comprising administering to a subject having cancer an effective amount an antibody or composition as described by the disclosure (*e.g.*, an anti-plectin-1 antibody or a composition comprising an anti-plectin-1 antibody). In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

Generally, antibodies and pharmaceutical compositions of the disclosure preferably contain a pharmaceutically acceptable carrier or excipient suitable for rendering the compound or mixture administrable orally as a tablet, capsule or pill, or parenterally, intravenously, intradermally, intramuscularly or subcutaneously, or transdermally.

The pharmaceutical compositions containing an anti-plectin-1 antibody and/or other compounds can be administered by any suitable route for administering medications. A variety of administration routes are available. The particular mode selected will depend, of course, upon the particular agent or agents selected, the particular condition being treated,

and the dosage required for therapeutic efficacy. The methods of this disclosure, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces therapeutic effect without causing clinically unacceptable adverse effects. Various modes of administration are discussed herein. For use in therapy, an effective amount of the anti-plectin-1 antibody and/or other therapeutic agent can be administered to a subject by any mode that delivers the agent to the desired surface, *e.g.*, mucosal, systemic.

Administering the pharmaceutical composition of the present disclosure may be accomplished by any means known to the skilled artisan. Routes of administration include but are not limited to oral, parenteral, intravenous, intramuscular, intraperitoneal, intranasal, sublingual, intratracheal, inhalation, subcutaneous, ocular, vaginal, and rectal. Systemic routes include oral and parenteral. Several types of devices are regularly used for administration by inhalation. These types of devices include metered dose inhalers (MDI), breath-actuated MDI, dry powder inhaler (DPI), spacer/holding chambers in combination with MDI, and nebulizers.

For oral administration, the compounds can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the disclosure to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers for neutralizing internal acid conditions or may be administered without any carriers.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol

or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present disclosure may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.,* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active compounds may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the compounds, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-, di-, and tri-glycerides; hydrogel release systems; silastic systems; peptide-based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which an agent of the disclosure is contained in a form within a matrix such as those described in U.S. Pat. Nos. 4,452,775, 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Pat. Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

The anti-plectin-1 antibodies and compositions described by the disclosure can be administered to a subject (*e.g.*, a subject having cancer) on multiple occasions. In some embodiments, the number of occasions in which an antibody or composition of the disclosure is delivered to a subject is in a range of 2 to 10 times (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times). In some embodiments, a heterologous nucleic acid is delivered to a subject more than 10 times.

In some embodiments, a dose of an antibody or composition of the disclosure is administered to a subject no more than once per calendar day (*e.g.*, a 24-hour period). In some embodiments, a dose of an antibody or composition of the disclosure is administered to

a subject no more than once per 2, 3, 4, 5, 6, or 7 calendar days. In some embodiments, a dose of an antibody or composition of the disclosure is administered to a subject no more than once per calendar week (*e.g.*, 7 calendar days). In some embodiments, a dose of an antibody or composition of the disclosure is administered to a subject no more than bi-weekly (*e.g.*, once in a two calendar week period). In some embodiments, a dose of an antibody or composition of the disclosure is administered to a subject no more than once per calendar month (*e.g.*, once in 30 calendar days). In some embodiments, a dose of an antibody or composition of the disclosure is administered to a subject no more than once per six calendar months. In some embodiments, a dose of an antibody or composition of the disclosure is administered to a subject no more than once per calendar year (*e.g.*, 365 days or 366 days in a leap year).

Immunoassays

In some embodiments, the disclosure relates to a method for detecting a plectin-1 on the surface of cells, *e.g.*, cancer cells, *in situ* or *in vitro*. In some embodiments, the disclosure relates to a method for detecting plectin-1 on the surface of cells in a sample obtained from a subject. The sample may be obtained from a subject, for example, by extracting a tumor or portion thereof from a subject. In some embodiments, cells may be isolated from the tumor. However, in some embodiments, cells may be examined in the context of an isolated tumor.

In some embodiments, a method for detecting a plectin-1 *in situ* involve delivering to a subject a plectin-1 antibody or antigen binding fragment conjugated to a label (*e.g.*, a radioactive label) under conditions in which the antibody or antigen binding fragment is able to form binding complexes with an accessible epitope of plectin-1 on cells, *e.g.*, cancer cells, in the subject; and detecting the label in the subject (*e.g.*, using autoradiography or other nuclear medicines detection techniques, including single photon emission computed tomography (SPECT), positron emission tomography (PET) and scintigraphy).

In some embodiments, a method for detecting a plectin-1 in a tumor sample obtained from a subject involve (a) contacting the sample with the antibody or antigen binding fragment under conditions suitable for binding of the antibody or antigen binding fragment to the antigen, if the antigen is present in the sample, thereby forming binding complexes; and (b) determining the level of the antibody or antigen binding fragment bound to the antigen

(*e.g.*, determining the level of the binding complexes), *e.g.*, at the surface of a cell of the tumor.

As used herein a binding complex refers to a biomolecular complex of antibody or antigen binding fragments bound to antigen (*e.g.*, plectin-1 protein). Binding complexes may comprise antibodies or antigen binding fragments with a single specificity or two or more antibodies or antigen binding fragments with different specificities. In one embodiment, a binding complex comprises two or more antibodies recognizing different antigenic sites on the same antigen. In some instances, an antibody or antigen binding fragment may be bound to an antigen, having bound to it other biomolecules such as RNA, DNA, polysaccharides or proteins. In one embodiment, a binding complex comprises two or more antibodies recognizing different antigens. In some embodiments, an antibody or antigen binding fragment in a binding complex (*e.g.*, an immobilized antibody or antigen binding fragment bound to antigen), may itself by bound, as an antigen, to an antibody or antigen binding fragment (*e.g.*, a detectably labeled antibody or antigen binding fragment). Thus, binding complexes may, in some instances, comprise multiple antigens and multiple antibodies or antigen binding fragments. Antigens present in binding complexes may or may not be in their native in situ conformation. In some embodiments, a binding complex is formed between an antibody or antigen binding fragment and a purified protein antigen, or isolated proteins comprising antigen, in which the antigen is not in its native in situ conformation. In some embodiments, a binding complex is formed between an antibody or antigen binding fragment and a purified protein antigen, in which the antigen is not in its native in situ conformation and is immobilized on solid support (*e.g.*, a PVDF membrane). In some embodiments, a binding complex is formed with an antibody or antigen binding fragment and, for example, a cell surface protein that is present in situ in a native confirmation (*e.g.*, on the surface of a cell). Antibodies or antigen binding fragments in binding complexes may or may not be detectably labeled. In some embodiments, binding complexes comprise detectably labeled antibodies or antigen binding fragments and non-labeled antibodies or antigen binding fragments. In some embodiments, binding complexes comprise detectably labeled antigen. In some embodiments, antibodies or antigen binding fragments, in binding complexes, are immobilized to one or more solid supports. In some embodiments, antigens, in binding complexes, are immobilized to one or more solid supports. Exemplary solid supports are disclosed herein and will be apparent to one of ordinary skill in the art. The

foregoing examples of binding complexes are not intended to be limiting. Other examples of binding complexes will be apparent to one or ordinary skill in the art.

In any of the detection, diagnosis, and monitoring methods, the antibody, or antigen binding fragments, or antigen may be conjugated to a solid support surface, either directly or indirectly. Methods for conjugation to solid supports are standard and can be accomplished via covalent and non-covalent interactions. Non-limiting examples of conjugation methods include: adsorption, cross-linking, protein A/G - antibody interactions, and streptavidin-biotin interactions. Other methods of conjugation will be readily apparent to one of ordinary skill in the art.

In some aspects, the foregoing detection, diagnosis, and monitoring methods include comparing the level of the antibody or antigen binding fragment bound to the antigen (*e.g.*, binding complexes) to one or more reference standards. The reference standard may be, for example, the level of a corresponding plectin-1 in a subject that does or does not have preeclampsia. In one embodiment, the reference standard is the level of plectin-1 detected in a sample that does not contain plectin-1 (*e.g.*, a background level). Alternatively, a background level can be determined from a sample that contains a particular plectin-1, by contacting the sample with non-specific antibodies (*e.g.*, antibodies obtained from non-immune serum). Then again, the reference standard may be the level of plectin-1 detected in a sample that does contain plectin-1 (*e.g.*, a positive control). In some cases, the reference standard may be a series of levels associated with varying concentrations of plectin-1 in a sample and useful for quantifying the concentration of plectin-1 in the test sample. The foregoing examples of reference standards are not limiting and other suitable reference standard will be readily apparent to one of ordinary skill in the art.

Another embodiment relates to a diagnostic composition comprising any one of the above described antibodies, antigen binding fragments, polynucleotides, vectors or cells and optionally suitable means for detection. The antibodies or antigen binding fragments are, for example, suited for use in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. Examples of immunoassays which can utilize the antibody or antigen binding fragments are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the Enzyme Linked Immunoassay (ELISA), radioimmunoassay (RIA), the sandwich (immunometric assay), flow cytometry, the western blot assay, immunoprecipitation assays, immunohistochemistry,

immuno-microscopy, lateral flow immuno-chromatographic assays, and proteomics arrays. The antigens and antibodies or antigen binding fragments can be bound to many different solid supports (*e.g.*, carriers, membrane, columns, proteomics array, etc.). Examples of solid support materials include glass, polystyrene, polyvinyl chloride, polyvinylidene difluoride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, such as nitrocellulose, polyacrylamides, agaroses, and magnetite. The nature of the support can be either fixed or suspended in a solution (*e.g.*, beads).

By a further embodiment, antibodies and antigen binding fragments provided herein may also be used in a method for evaluating plectin-1 expression in a subject by obtaining a biological sample from the subject which may be a blood sample, any other appropriate body fluid sample (*e.g.*, lymph fluid), or a tissue sample (*e.g.*, pancreatic tissue, ovarian tissue, tissue from the head or neck of a subject, breast tissue, lung tissue, *etc.*). The procedure may comprise contacting the sample (*e.g.*, pancreatic tissue), or protein sample isolated therefrom, with an antibody, or antigen binding fragment, under conditions enabling the formation of binding complexes between antibody or antigen binding fragment and antigen. The level of such binding complexes may then be determined by methods known in the art.

In some embodiments, the biological sample is contacted with the antibody or antigen binding fragment under conditions suitable for binding of the antibody or antigen binding fragment to a plectin-1 protein, if the antigen is present in the sample, and formation of binding complexes consisting of antibody, or antigen binding fragment, bound to the antigen. This contacting step is typically performed in a reaction chamber, such as a tube, plate well, membrane bath, cell culture dish, microscope slide, and the like. In some embodiments, the antibody or antigen binding fragment is immobilized on a solid support. In some embodiments, the antigen is immobilized on a solid support. In some embodiments, the solid support is the surface of a the reaction chamber. In some embodiments, the solid support is of a polymeric membrane (*e.g.*, nitrocellulose strip, Polyvinylidene Difluoride (PVDF) membrane, *etc.*). Other appropriate solid supports may be used.

In some embodiments, the antibody and antigen binding fragment is immobilized on the solid support prior to contacting with the antigen. In other embodiments, immobilization of the antibody and antigen binding fragment is performed after formation of binding complexes. In still other embodiments, antigen is immobilized on a solid support prior to formation of binding complexes. In some embodiments, a detection reagent is added to the

reaction chamber to detect immobilized binding complexes. In some embodiments, the detection reagent comprises a detectably labeled secondary antibody directed against the antigen. In some embodiments, the primary antibody or antigen binding fragment is itself detectable labeled, and is thereby the detection reagent.

In one aspect, detection methods comprise the steps of immobilizing antibodies or antigen binding fragments to a solid support; applying a sample (*e.g.*, a biological sample or isolated protein sample) to the solid support under conditions that permit binding of antigen to the antibodies or antigen binding fragment, if present in the sample; removing the excess sample from the solid support; applying detectably labeled antibodies or antigen binding fragments under conditions that permit binding of the detectably labeled antibodies or antigen binding fragments to the antigen-bound immobilized antibodies or antigen binding fragments; washing the solid support and assaying for the presence of label on the solid support.

In some embodiments, the antigen is immobilized on the solid support, such as a PVDF membrane, prior to contacting with the antibody and antigen binding fragment in a reaction chamber (*e.g.*, a membrane bath). A detection reagent is added to the reaction chamber to detect immobilized binding complexes. In some embodiments, the detection reagent comprises a detectably labeled secondary antibody directed against the antigen. In some embodiments, the detection reagent comprises a detectably labeled secondary antibody directed against the primary antibody or antigen binding fragment. As disclosed herein, the detectable label may be, for example, a radioisotope, a fluorophore, a luminescent molecule, an enzyme, a biotin-moiety, an epitope tag, or a dye molecule. In some embodiments, the primary antibody or antigen binding fragment is itself detectable labeled, and is thereby the detection reagent. Suitable detectable labels are described herein, and will be readily apparent to one of ordinary skill in the art.

Accordingly, diagnostic kits, suitable for home or clinical use (point of care service), are provided that comprise (a) detectably labeled and/or non-labeled antibodies or antigen binding fragments, as antigen binding reagents (*e.g.*, plectin-1 binding reagents); (b) a detection reagent; and, optionally, (c) complete instructions for using the reagents to detect antigens in a sample. In some embodiments, the diagnostic kit includes the antibody, or antigen binding fragment, and/or plectin-1 immobilized on a solid support. Any of the solid supports described herein are suitable for incorporation in the diagnostic kits. In a preferred embodiment, the solid support is the surface of a reaction chamber of a plate well. Typically,

the plate well is in a multi-well plate having a number of wells selected from: 6, 12, 24, 96, 384, and 1536, but it is not so limited. In other embodiments, the diagnostic kits provide a detectably labeled antibody or antigen binding fragment. Diagnostic kits are not limited to these embodiments and other variations in kit composition will be readily apparent to one of ordinary skill in the art.

The present disclosure is further illustrated by the following Examples, which in no way should be construed as further limiting. The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference.

EXAMPLES

Expression and Purification of Human Plectin-1 in E. coli

Expression-ready constructs, both His-tagged vectors (15 mg/L, SEQ ID NO: 2) and GST-tagged vectors (30 mg/L, SEQ ID NO: 3), were used to generate monoclonal antibodies. To evaluate expression, the plasmids were transformed in strain B of *E. coli*. The trials were conducted in 4 mL test tubes, and the following variables were examined: temperature, expression time, and concentration of isopropyl β-D-1-thiogalactopyranoside (IPTG).

Cells were harvested by centrifugation. Cell pellets were lysed by sonication and target protein was obtained with one-step purification using a nickel column. Fractions were pooled and dialyzed against the storage buffer. Different storage buffers were used to determine which yielded the most stable protein with a concentration greater than 0.4 mg/mL. Proteins were analyzed by SDS-PAGE and Western blot using standard protocols to obtain molecular weight and purity measurements. The concentration of the protein was determined with a Bradford protein assay, using bovine serum albumin (BSA) as a standard.

Monoclonal Development of Anti-human Plectin-1 Protein

A specific panel of anti-human plectin-1 protein monoclonal antibodies which recognize the target protein (underlined in SEQ ID NO: 1; set forth in SEQ ID NO: 92) was developed.

First, five BALB/c mice were immunized with GenScript's MonoExpress immunization protocol and observed for two weeks.

Electrofusion was used to perform two fusions. The average fusion efficiency using this process is around 1 hybridoma/5000 B cells. The anticipated yield of hybridoma clones was $2x10^4$, and the fused cells were plated into 96-well plates. An ELISA was performed to screen the fusion proteins for positive clones. Supernatants from the positive clones were then further screened by ELISA against the target protein. 10*His-tagged protein was used as the counter screen. Selected clones were positive against the target protein and negative against the 10*His-tagged protein. The positive clones were expanded into 24-well plates coated with human recombinant Sec 8 (plectin-1 Section 8) and 2mL of supernatant for each clone was collected before the cells were frozen for storage. Table 3 shows the OD_{450nm} for cell lines grown on plates coated with human recombinant plectin-1 section 8.

Table 3: OD_{450} Results for Experimental Cell Lines

Cell Line	OD 450		Host Strain
	A	B	
PAb3	2.647	0.084	MOUSE
PAb2	2.428	0.082	
PAb1	2.323	0.117	
PAb6	2.484	0.093	
PAb7	2.400	0.109	
PAb8	2.257	0.085	
PAb9	2.616	0.113	
PAb4	2.484	0.118	
PAb10	2.326	0.132	
PAb11	2.418	0.110	
PC (antiserum 1:1k)	2.254	0.215	
PAb12	2.422	0.107	
PAb13	2.223	0.093	

PAb14	2.292	0.084	
PAb15	2.498	0.086	
PAb16	2.223	0.087	
PAb5	2.453	0.097	
PAb17	2.546	0.086	
PAb18	2.589	0.098	
PAb19	2.552	0.081	
PAb20	2.558	0.073	
NC (medium)	0.068	0.073	

Positive primary clones from the two fusions were sub-cloned by limiting dilution to ensure the sub-clones were derived from a single parental cell. The clones were grown for three generations. The sub-clones were further screened by ELISA. Based on the results of the ELISA, two stable sub-clonal cell lines of each primary clone were cryopreserved.

Clones PAb1 and PAb2, exhibited the highest specific plectin binding potential (FIG. 1). Clones PAb3, PAb4, and PAb5 also demonstrated ability to kill cancer cells (FIG. 2).

Roller bottles were used to produce the antibodies at a concentration of approximately 15 mg/L. The antibody proteins were further purified using protein A/G affinity column chromatography and dialyzed into PBS buffer for storage. For quality control, the antibodies underwent a purity test by SDS-PAGE, concentration determination by absorption at OD_{280nm}, and antigen reactivity by ELISA.

Selected antibodies were subjected to standard full length antibody sequencing. The antibodies underwent total RNA extraction, RT-PCR, and 5' RACE and 3' RACE PCR. The target PCR fragments of the variable and constant regions were gel-purified and cloned into sequencing vectors. At least five independent positive clones of each chain were sequenced in order to deduce the consensus sequence.

Monoclonal Antibody Sequencing of PAb1 and PAb2

PAb1 and PAb2 were sequenced using the following procedure. Total RNA was isolated from the hybridoma cells recovered by GenScript using TRIzol® reagent (Ambion,

Cat. No.: 15596-026) and the procedure from the technical manual of TRIzol® reagent. The total RNA was then analyzed by agarose gel electrophoresis. Isotype-specific anti-sense primers or universal primers were used to reverse transcribe the total RNA into cDNA with the PrimeScript™ 1st Strand cDNA Synthesis Kit using the manufacturer's protocol. The antibody fragments of V_H, V_L, C_H, and C_L were amplified. Amplified antibody fragments were then separately cloned into a standard cloning vector using standard molecular cloning procedures. Colony PCR was performed to identify clones with inserts of correct sizes. More than five single colonies with inserts of correct sizes were sequenced for each antibody fragment.

Ultimately, five single colonies with correct V_H, V_L, C_H, C_L insert sizes were sent for sequencing. The V_H, V_L, C_H, C_L genes of five different clones were found to be nearly identical. The PAb1 and PAb2 consensus sequences, listed in the Sequences section below, represent the sequences of the PAb1 and PAb2 antibodies.

In vitro assays using PAb1

In vitro binding assays were performed. FIG. 3A shows PAb1 binds specifically to a recombinant human C-terminal portion of plectin-1 protein. Data indicate that PAb1 binds selectively to recombinant human Sec8-His protein with high affinity (*e.g.*, a $K_d < 1$ nM). FIG. 3B shows PAb1 binding specificity on plectin-1 positive L3.6pl cancer cells; PAb1 did not bind to plectin-1 negative LNCaP cells.

FIGs. 4A-4G show internalization of PAb1 in L3.6pl plectin-1 positive cancer cells. Representative confocal microscopy images demonstrating staining of L3.6pl cells with PAb1 (FIG. 4A) or IgG control (FIG. 4B), merged with endosomal marker LAMP-1, are shown. Co-localization of PAb1 and LAMP-1 was observed (FIGs. 4C-4E). Quantification assays indicated that a significant portion of PAb1 merged with LAMP-1, whereas IgG control did not. Measurement of internalized ^{125}I- PAb1 radioactivity after incubation at 37 °C, 4 °C, or in combination with cold PAb1 in L3.6pl cells, indicated a decrease in radioactivity in both cell lines at 4 °C vs. 37 4 °C, while internalization activity decreased only in the L3.6pl cells during competition with cold PAb1 (Comp.), as shown in FIG. 4G.

FIGs. 5A-5D show data relating to induction of cancer cell death by apoptosis after treatment with PAb1. FIG. 5A shows a fluorescence minus one control experiment of L3.6pl

cells by flow cytometry. FIG. 5B shows L3.6pl Annexin V-positive cells 72 hours after treatment with IgG control. FIG. 5C shows L3.6pl Annexin V-positive cells 72 hours after treatment with PAb1. Data indicate PAb1-treated L3.6pl cells experienced significantly more apoptosis (as assessed by Annexin V) compared to control IgG-treated cells (FIG. 5D).

Survival of cancer cell types and healthy cell types was measured 72 hours after treatment with either PAb1 or IgG control. EC50s were calculated by logistical nonlinear regression and reported as the concentration of mAb (nM) that reduced cell viability by 50%. Data are shown in Table 4 (below).

Table 4

Cell name	Origin	Phenotype	Tissue type	Plectin-1mAb EC50 (nM)	Plectin-1 mAb Cell survival min. (%)	IgG EC50 (nM)	IgG Cell survival min. (%)
Keratinocyte	Human	Normal	Skin	500	80	no fit	90
HPDE	Human	Normal	Pancreas	300	80	330	64
RL14	Human	Normal	Heart	4020	55	345	82
HEK293T	Human	Normal	Kidney	324	32	65	95
L3.6pl	Human	Cancer	Pancreas	34	23	5049	9
YapC	Human	Cancer	Pancreas	43	19	225	38
OVCAR8	Human	Cancer	Ovary	63	16	no fit	100
SKOV3	Human	Cancer	Ovary	53	6	no fit	83

The effect of PAb1 treatment on tubulin anisotropy of YapC cancer cells was examined by confocal microscopy. FIG. 6A shows tubulin staining in YapC cells that were not treated with PAb1. FIG. 6B shows tubulin staining 10 minutes post monomethyl auristatin E (MMAE) treatment; MMAE blocks tubulin polymerization. FIG. 6C shows tubulin staining 24 hours post PAb1 treatment. Data indicate a decrease in anisotrophy in cells treated with PAb1 compared to untreated control cells (FIG. 6D).

Co-localization of PAb1 with tubulin in YapC cancer cells was also investigated. Representative confocal microscopy images of YapC after tubulin staining (FIG. 7A) and PAb1 staining (FIG. 7B) are shown. Data indicate that tubulin and PAb1 co-localize (FIG. 7C; arrows) on the surface of dying cells. Increased PAb1 staining was also observed on couple cells.

In vitro assays using PAb1

Immunocompromised mice bearing a subcutaneous YapC tumor were administered either PAb1 or IgG control. Data indicated that after 11 days, the mice treated with 3 mg/kg PAb1 had a significantly smaller tumor volume than mice treated with the IgG control (FIG. 8A). Data also indicated that 1 mg/kg PAb1 of mice elicited a significant reduction of tumor volume at day 14. Two higher doses (1 mg/kg and 3 mg/kg) of PAb1 resulted in a significantly lower tumor volume compared to the mice treated with 0.3 mg/kg PAb1 (FIG. 8A), indicating a dose-dependent effect. Data also indicated that animals treated with PAb1 did not lose weight during the entire duration of treatment (FIG. 8B). Photos of mice treated with 3 mg/kg PAb1 at Day 0, Day 14 and Day 25 are also shown (FIGs. 8C-8E).

SEQUENCES

>SEQ ID NO: 1 – Plectin (hemidesmosomal protein 1), *Homo sapiens;* target protein underlined

MVAGMLMPRDQLRAIYEVLFREGVMVAKKDRRPRSLHPHVPGVTNLQVMRAMASLRARG
LVRETFAWCHFYWYLTNEGIAHLRQYLHLPPEIVPASLQRVRRPVAMVMPARRTPHVQAVQ
GPLGSPPKRGPLPTEEQRVYRRKELEEVSPETPVVPATTQRTLARPGPEPAPATDERDRVQKK
TFTKWVNKHLIKAQRHISDLYEDLRDGHNLISLLEVLSGDSLPREKGRMRFHKLQNVQIALD
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>SEQ ID NO: 2 – pET-10NC-Plec C term: *His tag*-**EK cleavage site**-Human plectin 1 (section 8)-stop codon (344 amino acids; MW=36959.2; predicted pI=8.80)

MRS*HHHHHHHHHH*RSGTG**DDDDK**AMADIGSEFELRRQACGFRSRSSSVGSSSSYPIS
PAVSRTQLASWSDPTEETGPVAGILDTETLEKVSITEAMHRNLVDNITGQRLLEAQAC
TGGIIDPSTGERFPVTDAVNKGLVDKIMVDRINLAQKAFCGFEDPRTKTKMSAAQAL
KKGWLYYEAGQRFLEVQYLTGGLIEPDTPGRVPLDEALQRGTVDARTAQKLRDVG
AYSKYLTCPKTKLKISYKDALDRSMVEEGTGLRLLEAAAQSTKGYYSPYSVSGSGST
AGSRTGSRTGSRAGSRRGSFDATGSGFSMTFSSSSYSSSGYGRRYASGSSSLGGPESA
VA.

>SEQ ID NO: 3 – pGEX2t-Section 8: *GST tag*-**Thrombin cleavage site**-Human plectin 1 (section 8)-stop codon (540 amino acids; MW=59809.3; predicted pI=8.15)

*MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLT QSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMF EDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYI AWPLQGWQATFGGGDHPPK*SD**LVPRGS**EFELRRQACGFRSRSSSVGSSSSYPISPAVSRTQLA SWSDPTEETGPVAGILDTETLEKVSITEAMHRNLVDNITGQRLLEAQACTGGIIDPSTGERFPV TDAVNKGLVDKIMVDRINLAQKAFCGFEDPRTKTKMSAAQALKKGWLYYEAGQRFLEVQY LTGGLIEPDTPGRVPLDEALQRGTVDARTAQKLRDVGAYSKYLTCPKTKLKISYKDALDRSM VEEGTGLRLLEAAAQSTKGYYSPYSVSGSGSTAGSRTGSRTGSRAGSRRGSFDATGSGFSMT FSSSSYSSSGYGRRYASGSSSLGGPESAVA.

Pab2 Sequences

Name	Sequence	SEQ ID NO:
Pab2 Heavy Chain	ATGAACTTCGGGCTCAGCTTGATTTTCCTTGCCCTCATTTTA AAAGGTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGG GAGACTTGGTGAAGCCTGGAGGGTCCCTGAAACTCTCCTGT GCAGCCTCTGGATTCACTTTCAGTAGGTATGGCATGTCTTG GGTTCGCCAGACTCCAGACAAGAGGCTGGAGTGGGTCGCA ACCATTAGTATTGGTGGTACTTACACCTACTATCCAGACAG TATGAAGGGGCGATTCACCATCTCCAGAGACAATGCCAAG AACACCCTGTACCTGCAAATGAGCAGTCTGAAGTCTGAGG ACACAGCCATGTATTACTGTGCAAGACGGGGGTATGGTAA CTACTCTTACTATGGTATGGACTACTGGGGTCAAGGAACCT CAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTC TATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCAT GGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGC CAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGT GTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACAC TCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCA GCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAG CACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGT TGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGT CTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATTA CTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGC AAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGA TGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAG CAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCAT CATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGC AGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAGAAAA CCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGT GTACACCATTCCACCTCCCAAGGAGCAGATGGCCAAGGAT AAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCCCTGA AGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCG	4

	GAGAACTACAAGAACACTCAGCCCATCATGGACACAGATG GCTCTTACTTCGTCTACAGCAAGCTCAATGTGCAGAAGAGC AACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACA TGAGGGCCTGCACAACCACCATACTGAGAAGAGCCTCTCC CACTCTCCTGGTAAATGA	
Pab2 Heavy Chain Leader	ATGAACTTCGGGCTCAGCTTGATTTTCCTTGCCCTCATTTTA AAAGGTGTCCAGTGT	5
Pab2 Heavy Chain FR1	GAGGTGCAGCTGGTGGAGTCTGGGGGAGACTTGGTGAAGC CTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTC ACTTTCAGT	6
Pab2 Heavy Chain CDR1	AGGTATGGCATGTCT	7
Pab2 Heavy Chain FR2	TGGGTTCGCCAGACTCCAGACAAGAGGCTGGAGTGGGTCG CA	8
Pab2 Heavy Chain CDR2	ACCATTAGTATTGGTGGTACTTACACCTACTATCCAGACAG TATGAAGGGG	9
Pab2 Heavy Chain FR 3	CGATTCACCATCTCCAGAGACAATGCCAAGAACACCCTGT ACCTGCAAATGAGCAGTCTGAAGTCTGAGGACACAGCCAT GTATTACTGTGCAAGA	10
Pab2 Heavy Chain CDR3	CGGGGGTATGGTAACTACTCTTACTATGGTATGGACTAC	11
Pab2 Heavy Chain FR 4	TGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA	12
Pab2 Heavy Chain Variable Region	GAGGTGCAGCTGGTGGAGTCTGGGGGAGACTTGGTGAAGC CTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTC ACTTTCAGTAGGTATGGCATGTCTTGGGTTCGCCAGACTCC AGACAAGAGGCTGGAGTGGGTCGCAACCATTAGTATTGGT GGTACTTACACCTACTATCCAGACAGTATGAAGGGGCGATT CACCATCTCCAGAGACAATGCCAAGAACACCCTGTACCTG CAAATGAGCAGTCTGAAGTCTGAGGACACAGCCATGTATT ACTGTGCAAGACGGGGGTATGGTAACTACTCTTACTATGGT ATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTC A	13
Pab2 Heavy Chain Constant Region	GCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGG ATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCC TGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGG AACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGC TGTCCTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGA CTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGC AACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGA AAATTGTGCCCAGGGATTGTGGTTGTAAGCCTTGCATATGT ACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAA GCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCA CGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGT CCAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAG CTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTT CCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACTGGC TCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGC TTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAG GCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCTCCC AAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCA	14

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Pab2 Heavy Chain	MNFGLSLIFLALILKGVQCEVQLVESGGDLVKPGGSLKLSCAA SGFTFSRYGMSWVRQTPDKRLEWVATISIGGTYTYYPDSMKG RFTISRDNAKNTLYLQMSSLKSEDTAMYYCARRGYGNYSYY GMDYWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLG CLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSD	15
Pab2 Heavy Chain Leader	MNFGLSLIFLALILKGVQC	16
Pab2 Heavy Chain FR1	EVQLVESGGDLVKPGGSLKLSCAASGFTFS	17
Pab2 Heavy Chain CDR1	RYGMS	18
Pab2 Heavy Chain FR2	WVRQTPDKRLEWVA	19
Pab2 Heavy Chain CDR2	TISIGGTYTYYPDSMKG	20
Pab2 Heavy Chain FR 3	RFTISRDNAKNTLYLQMSSLKSEDTAMYYCAR	21
Pab2 Heavy Chain CDR3	RGYGNYSYYGMDY	22
Pab2 Heavy Chain FR 4	WGQGTSVTVSS	23
Pab2 Heavy Chain Variable Region	EVQLVESGGDLVKPGGSLKLSCAASGFTFSRYGMSWVRQTPD KRLEWVATISIGGTYTYYPDSMKGRFTISRDNAKNTLYLQMS SLKSEDTAMYYCARRGYGNYSYYGMDYWGQGTSVTVSS	24
Pab2 Heavy Chain Constant Region	AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWN SGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVA HPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTI TLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQ FNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKT KGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQ WNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTF TCSVLHEGLHNHHTEKSLSHSPGK	25
Pab2 Light Chain	ATGAGGTTCTCTGCTCAGCTTCTGGGGCTGCTTGTGCTCTG GATCCCTGGATCCACTGCAGATATTGTGATGACGCAGGCTG CATTCTCCAATCCAGTCACTCTTGGAACATCAGCTTCCATC TCCTGCAGGTCTAGTAAGAGTCTCCTACATAGTAATGGCAT CACTTATTTGTATTGGTATCTGCAGAAGCCAGGCCAGTCTC CTCAGCTCCTGATTTATCAGATGTCCAACCTTGCCTCAGGA GTCCCAGACAGGTTCAGTAGCAGTGGGTCAGGAACTGATT TCACACTGAGAATCAGCAGAGTGGAGGCTGAGGATGTGGG TGTTTATTACTGTGCTCAAAATCTAGAACTTCCGCTCACGTT CGGTGCTGGGACCAAGCTGGAGCTGAAACGGGCTGATGCT GCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTT AACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACT TCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGG	26

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Pab2 Light Chain Leader	ATGAGGTTCTCTGCTCAGCTTCTGGGGCTGCTTGTGCTCTG GATCCCTGGATCCACTGCA	27
Pab2 Light Chain FR1	GATATTGTGATGACGCAGGCTGCATTCTCCAATCCAGTCAC TCTTGGAACATCAGCTTCCATCTCCTGC	28
Pab2 Light Chain CDR1	AGGTCTAGTAAGAGTCTCCTACATAGTAATGGCATCACTTA TTTGTAT	29
Pab2 Light Chain FR2	TGGTATCTGCAGAAGCCAGGCCAGTCTCCTCAGCTCCTGAT TTAT	30
Pab2 Light Chain CDR2	CAGATGTCCAACCTTGCCTCA	31
Pab2 Light Chain FR 3	GGAGTCCCAGACAGGTTCAGTAGCAGTGGGTCAGGAACTG ATTTCACACTGAGAATCAGCAGAGTGGAGGCTGAGGATGT GGGTGTTTATTACTGT	32
Pab2 Light Chain CDR3	GCTCAAAATCTAGAACTTCCGCTCACG	33
Pab2 Light Chain FR 4	TTCGGTGCTGGGACCAAGCTGGAGCTGAAA	34
Pab2 Light Chain Variable Region	GATATTGTGATGACGCAGGCTGCATTCTCCAATCCAGTCAC TCTTGGAACATCAGCTTCCATCTCCTGCAGGTC TAGTAAGAGTCTCCTACATAGTAATGGCATCACTTATTTGT ATTGGTATCTGCAGAAGCCAGGCCAGTCTCCTCAGCTCCTG ATTTATCAGATGTCCAACCTTGCCTCAGGAGTCCCAGACAG GTTCAGTAGCAGTGGGTCAGGAACTGATTTCACACTGAGA ATCAGCAGAGTGGAGGCTGAGGATGTGGGTGTTTATTACT GTGCTCAAAATCTAGAACTTCCGCTCACGTTCGGTGCTGGG ACCAAGCTGGAGCTGAAA	35
Pab2 Light Chain Constant Region	CTGTGCTCAAAATCTAGAACTTCCGCTCACGTTCGGTGCTG GGACCAAGCTGGAGCTGAAACGGGCTGATGCTGCACCAAC TGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTG GAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCC AAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAAC GACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAG CAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTG ACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTG AGGCCACTCACA AGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAAT GAGTGT	36
Pab2 Light Chain	MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISC RSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDR FSSSGSGTDFTLRISRVEAEDVGVYYCAQNLELPLTFGAGTKL ELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVK WKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYER HNSYTCEATHKTSTSPIVKSFNRNEC	37
Pab2 Light Chain Leader	MRFSAQLLGLLVLWIPGSTA	38

Pab2 Light Chain FR1	DIVMTQAAFSNPVTLGTSASISC	39
Pab2 Light Chain CDR1	RSSKSLLHSNGITYLY	40
Pab2 Light Chain FR2	WYLQKPGQSPQLLIY	41
Pab2 Light Chain CDR2	QMSNLAS	42
Pab2 Light Chain FR 3	GVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC	43
Pab2 Light Chain CDR3	AQNLELPLT	44
Pab2 Light Chain FR 4	FGAGTKLELK	45
Pab2 Light Chain Variable Region	DIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITYLYWYLQ KPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAED VGVYYCAQNLELPLTFGAGTKLELK	46
Pab2 Light Chain Constant Region	RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI DGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNS YTCEATHKTSTSPIVKSFNRNEC	47

Pab1 Sequences

Name	Sequence	SEQ ID NO:
Pab1 Heavy Chain	ATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGC CCAAAGTATCCAAGCACAGATCCAGTTGGTGCAGTCTGGA CCTGAGCTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCT GCAAGGCTTCTGGTTATACCTTCACAGACTATTCAATGCAC TGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGG GCTGGATAAACACTGAGACTGGTGAGCCAACATATGCAGA TGACTTCAAGGGACGGTTTGCCTTCTCTTTGGAAACCTCTG CCAGCACTGCCTATTTGCAGATCAACAACCTCAAAAATGA GGACACGGCTAC ATATTTCTGTGCCCCCGGAGGGTTTGCTTACTGGGGCCAAG GGACTCTGGTCACTGTCTCTGCAGCCAAAACAACACCCCCA TCAGTCTATCCACTGGCCCCTGGGTGTGGAGATACAACTGG TTCCTCCGTGACTCTGGGATGCCTGGTCAAGGGCTACTTCC CTGAGTCAGTGACTGTGACTTGGAACTCTGGATCCCTGTCC AGCAGTGTGCACACCTTCCCAGCTCTCCTGCAGTCTGGACT CTACACTATGAGCAGCTCAGTGACTGTCCCCTCCAGCACCT GGCCAAGTCAGACCGTCACCTGCAGCGTTGCTCACCCAGCC AGCAGCACCACGGTGGACAAAAAACTTGAGCCCAGCGGGC CCATTTCAACAATCAACCCCTGTCCTCCATGCAAGGAGTGT CACAAATGCCCAGCTCCTAACCTCGAGGGTGGACCATCCGT CTTCATCTTCCCTCCAAATATCAAGGATGTACTCATGATCT CCCTGACACCCAAGGTCACGTGTGTGGTGGTGGATGTGAG CGAGGATGACCCAGACGTCCAGATCAGCTGGTTTGTGAAC AACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAG	48

	AGGATTACAACAGTACTATCCGGGTGGTCAGCACCCTCCCC ATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAAAT GCAAGGTCAACAACAAAGACCTCCCATCACCCATCGAGAG AACCATCTCAAAAATTAAAGGGCTAGTCAGAGCTCCACAA GTATACATCTTGCCGCCACCAGCAGAGCAGTTGTCCAGGA AAGATGTCAGTCTCACTTGCCTGGTCGTGGGCTTCAACCCT GGAGACATCAGTGTGGAGTGGACCAGCAATGGGCATACAG AGGAGAACTACAAGGACACCGCACCAGTCCTGGACTCTGA CGGTTCTTACTTCATATATAGCAAGCTCAATATGAAAACAA GCAAGTGGGAGAAAACAGATTCCTTCTCATG CAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAG AAGACCATCTCCCGGTCTCCGGGTAAATGA	
Pab1 Heavy Chain Leader	ATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGC CCAAAGTATCCAAGCA	49
Pab1 Heavy Chain FR1	CAGATCCAGTTGGTGCAGTCTGGACCTGAGCTGAAGAAGC CTGGAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGTTAT ACCTTCACA	50
Pab1 Heavy Chain CDR1	GACTATTCAATGCAC	51
Pab1 Heavy Chain FR2	TGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGG GC	52
Pab1 Heavy Chain CDR2	TGGATAAACACTGAGACTGGTGAGCCAACATATGCAGATG ACTTCAAGGGA	53
Pab1 Heavy Chain FR 3	CGGTTTGCCTTCTCTTTGGAAACCTCTGCCAGCACTGCCTAT TTGCAGATCAACAACCTCAAAAATGAGGACACGGCTACAT ATTTCTGTGCCCCC	54
Pab1 Heavy Chain CDR3	GGAGGGTTTGCTTAC	55
Pab1 Heavy Chain FR 4	TGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA	56
Pab1 Heavy Chain Variable Region	CAGATCCAGTTGGTGCAGTCTGGACCTGAGCTGAAGAAGC CTGGAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGTTAT ACCTTCACAGACTATTCAATGCACTGGGTGAAGCAGGCTCC AGGAAAGGGTTTAAAGTGGATGGGCTGGATAAACACTGAG ACTGGTGAGCCAACATATGCAGATGACTTCAAGGGACGGT TTGCCTTCTCTTTGGAAACCTCTGCCAGCACTGCCTATTTGC AGATCAACAACCTCAAAAATGAGGACACGGCTACATATTT CTGTGCCCCCGGAGGGTTTGCTTACTGGGGCCAAGGGACTC TGGTCACTGTCTCTGCA	57
Pab1 Heavy Chain Constant Region	GCCAAAACAACACCCCCATCAGTCTATCCACTGGCCCCTGG GTGTGGAGATACAACTGGTTCCTCCGTGACTCTGGGATGCC TGGTCAAGGGCTACTTCCCTGAGTCAGTGACTGTGACTTGG AACTCTGGATCCCTGTCCAGCAGTGTGCACACCTTCCCAGC TCTCCTGCAGTCTGGACTCTACACTATGAGCAGCTCAGTGA CTGTCCCCTCCAGCACCTGGCCAAGTCAGACCGTCACCTGC AGCGTTGCTCACCCAGCCAGCAGCACCACGGTGGACAAAA AACTTGAGCCCAGCGGGCCCATTTCAACAATCAACCCCTGT CCTCCATGCAAGGAGTGTCACAAATGCCCAGCTCCTAACCT CGAGGGTGGACCATCCGTCTTCATCTTCCCTCCAAATATCA AGGATGTACTCATGATCTCCCTGACACCCAAGGTCACGTGT GTGGTGGTGGATGTGAGCGAGGATGACCCAGACGTCCAGA	58

	TCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCA GACACAAACCCATAGAGAGGATTACAACAGTACTATCCGG GTGGTCAGCACCCTCCCCATCCAGCACCAGGACTGGATGA GTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCT CCCATCACCCATCGAGAGAACCATCTCAAAAATTAAAGGG CTAGTCAGAGCTCCACAAGTATACATCTTGCCGCCACCAGC AGAGCAGTT GTCCAGGAAAGATGTCAGTCTCACTTGCCTGGTCGTGGGCT TCAACCCTGGAGACATCAGTGTGGAGTGGACCAGCAATGG GCATACAGAGGAGAACTACAAGGACACCGCACCAGTCCTG GACTCTGACGGTTCTTACTTCATATATAGCAAGCTCAATAT GAAAACAAGCAAGTGGGAGAAAACAGATTCCTTCTCATGC AACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGA AGACCATCTCCCGGTCTCCGGGTAAA	
Pab1 Heavy Chain	MAWVWTLLFLMAAAQSIQAQIQLVQSGPELKKPGETVKISCK ASGYTFTDYSMHWVKQAPGKGLKWMGWINTETGEPTYADD FKGRFAFSLETSASTAYLQINNLKNEDTATYFCAPGGFAYWG QGTLVTVSAAKTTPPSVYPLAPGCGDTTGSSVTLGCLVKGYF PESVTVTWNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSTWP SQTVTCSVAHPASSTTVDKKLEPSGPISTINPCPPCKECHKCPA PNLEGGPSVFIFPPNIKDVLMISLTPKVTCVVVDVSEDDPDVQI SWFVNNVEVHTAQTQTHREDYNSTIRVVSTLPIQHQDWMSG KEFKCKVNNKDLPSPIERTISKIKGLVRAPQVYILPPPAEQLSR KDVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPVLDSDG SYFIYSKLNMKTSKWEKTDSFSCNVRHEGLKNYYLKKTISRSP GK	59
Pab1 Heavy Chain Leader	MAWVWTLLFLMAAAQSIQA	60
Pab1 Heavy Chain FR1	QIQLVQSGPELKKPGETVKISCKASGYTFT	61
Pab1 Heavy Chain CDR1	DYSMH	62
Pab1 Heavy Chain FR2	WVKQAPGKGLKWMG	63
Pab1 Heavy Chain CDR2	WINTETGEPTYADDFKG	64
Pab1 Heavy Chain FR 3	RFAFSLETSASTAYLQINNLKNEDTATYFCAP	65
PAb1 Heavy Chain CDR3	GGFAY	66
PAb1 Heavy Chain FR 4	WGQGTLVTVSA	67
PAb1 Heavy Chain Variable Region	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPG KGLKWMGWINTETGEPTYADDFKGRFAFSLETSASTAYLQIN NLKNEDTATYFCAPGGFAYWGQGTLVTVSA	68
PAb1 Heavy Chain Constant Region	AKTTPPSVYPLAPGCGDTTGSSVTLGCLVKGYFPESVTVTWN SGSLSSSVHTFPALLQSGLYTMSSSVTVPSSTWPSQTVTCSVA HPASSTTVDKKLEPSGPISTINPCPPCKECHKCPAPNLEGGPSV FIFPPNIKDVLMISLTPKVTCVVVDVSEDDPDVQISWFVNNVE VHTAQTQTHREDYNSTIRVVSTLPIQHQDWMSGKEFKCKVN NKDLPSPIERTISKIKGLVRAPQVYILPPPAEQLSRKDVSLTCLV	69

	VGFNPGDISVEWTSNGHTEENYKDTAPVLDSDGSYFIYSKLN MKTSKWEKTDSFSCNVRHEGLKNYYLKKTISRSPGK	
PAb1 Light Chain	ATGAGGTGCCTAGCTGAGTTCCTGGGGCTGCTTGTGCTCTG GATCCCTGGAGCCATTGGGGATATTGTGATGACTCAGGCTG CACCCTCTGTACCTGTCACTCCTGGAGAGTCAGTATCCATC TCCTGCAGGTCTAGTAAGAGTCTCCTGCATAGTAATGGCAA CACTTACTTGTATTGGTTCCTGCAGAGGCCAGGCCAGTCTC CTCAGCTCCTGATATATCGGATGTCCAACCTTGCCTCAGGA GTCCCAGACAGGTTCAGTGGCAGTGGGTCAGGAACTGCTTT CACACTGAGAATCAGTAGAGTGGAGGCTGAGGATGTGGGT GTTTATTACTGTATGCAACATCTAGAATATCCGCTCACGTT CGGTGCTGGGACCAAGCTGGAGCTGAAACGGGCTGATGCT GCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTT AACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACT TCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGG CAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGAT CAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCC TCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTA TACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTG TCAAGAGCTTCAACAGGAATGAGTGTTAG	70
PAb1 Light Chain Leader	ATGAGGTGCCTAGCTGAGTTCCTGGGGCTGCTTGTGCTCTG GATCCCTGGAGCCATTGGG	71
PAb1 Light Chain FR1	GATATTGTGATGACTCAGGCTGCACCCTCTGTACCTGTCAC TCCTGGAGAGTCAGTATCCATCTCCTGC	72
PAb1 Light Chain CDR1	AGGTCTAGTAAGAGTCTCCTGCATAGTAATGGCAACACTTA CTTGTAT	73
PAb1 Light Chain FR2	TGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCTGAT ATAT	74
PAb1 Light Chain CDR2	CGGATGTCCAACCTTGCCTCA	75
PAb1 Light Chain FR 3	GGAGTCCCAGACAGGTTCAGTGGCAGTGGGTCAGGAACTG CTTTCACACTGAGAATCAGTAGAGTGGAGGCTGAGGATGT GGGTGTTTATTACTGT	76
PAb1 Light Chain CDR3	ATGCAACATCTAGAATATCCGCTCACG	77
PAb1 Light Chain FR 4	TTCGGTGCTGGGACCAAGCTGGAGCTGAAA	78
PAb1 Light Chain Variable Region	GATATTGTGATGACTCAGGCTGCACCCTCTGTACCTGTCAC TCCTGGAGAGTCAGTATCCATCTCCTGCAGGTCTAGTAAGA GTCTCCTGCATAGTAATGGCAACACTTACTTGTATTGGTTC CTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCTGATATATCG GATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGTG GCAGTGGGTCAGGAACTGCTTTCACACTGAGAATCAGTAG AGTGGAGGCTGAGGATGTGGGTGTTTATTACTGTATGCAAC ATCTAGAATATCCGCTCACGTTCGGTGCTGGGACCAAGCTG GAGCTGAAA	79
PAb1 Light Chain Constant Region	CGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATC CAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCT TCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGG AAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACA GTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCAT	80

	GAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGA CATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAA CTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT	
PAb1 Light Chain	MRCLAEFLGLLVLWIPGAIGDIVMTQAAPSVPVTPGESVSISC RSSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLASGVPD RFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPLTFGAGT KLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINV KWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYE RHNSYTCEATHKTSTSPIVKSFNRNEC	81
PAb1 Light Chain Leader	MRCLAEFLGLLVLWIPGAIG	82
PAb1 Light Chain FR1	DIVMTQAAPSVPVTPGESVSISC	83
PAb1 Light Chain CDR1	RSSKSLLHSNGNTYLY	84
PAb1 Light Chain FR2	WFLQRPGQSPQLLIY	85
PAb1 Light Chain CDR2	RMSNLAS	86
PAb1 Light Chain FR 3	GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC	87
PAb1 Light Chain CDR3	MQHLEYPLT	88
PAb1 Light Chain FR 4	FGAGTKLELKR	89
PAb1 Light Chain Variable Region	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQ RPGQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLRISRVEAED VGVYYCMQHLEYPLTFGAGTKLELKR	90
PAb1 Light Chain Constant Region	ADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKID GSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYT CEATHKTSTSPIVKSFNRNEC	91

>SEQ ID NO: 92 – Plectin (hemidesmosomal protein 1), *Homo sapiens;* target protein underlined

SSSDGVVKSMIIDRRSGRQYDIDDAIAKNLIDRSALDQYRAGTLSITEFADMLSGNAGGFRSR
SSSVGSSSSYPISPAVSRTQLASWSDPTEETGPVAGILDTETLEKVSITEAMHRNLVDNITGQR
LLEAQACTGGIIDPSTGERFPVTDAVNKGLVDKIMVDRINLAQKAFCGFEDPRTKTKMSAAQ
ALKKGWLYYEAGQRFLEVQYLTGGLIEPDTPGRVPLDEALQRGTVDARTAQKLRDVGAYSK
YLTCPKTKLKISYKDALDRSMVEEGTGLRLLEAAAQSTKGYYSPYSVSGSGSTAGSRTGSRT
GSRAGSRRGSFDATGSGFSMTFSSSSYSSSGYGRRYASGSSASLGGPESAVA

What is claimed is:

CLAIMS

1. An antibody or antigen binding fragment that specifically binds an amino acid sequence having at least 85% identity to SEQ ID NO: 92.

2. The antibody or antigen binding fragment of claim 1, wherein the antibody specifically binds an amino acid sequence set forth as: SEQ ID NO: 92.

3. An antibody or antigen binding fragment that specifically binds to a cell-surface exposed plectin-1, wherein the antibody or antigen binding fragment comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO: 24 or SEQ ID NO: 68.

4. The antibody or antigen binding fragment of claim 3 further comprising a light chain variable region having a sequence set forth as: SEQ ID NO: 46 or SEQ ID NO: 90.

5. The antibody of claim 3 or 4, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO 24 and a light chain variable region having a sequence set forth as: SEQ ID NO: 46.

6. The antibody of claim 3 or 4, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO 68 and a light chain variable region having a sequence set forth as: SEQ ID NO: 90.

7. The antibody of any one of claims 1 to 6, wherein the antibody specifically binds an amino acid sequence having at least 85% identity to SEQ ID NO: 92.

8. The antibody of any one of claims 1 to 7, wherein the antibody specifically binds an amino acid sequence set forth as SEQ ID NO: 92.

9. An antibody or antigen binding fragment that specifically binds to cell-surface exposed plectin-1, wherein the antibody or antigen binding fragment comprises variable

heavy chain region comprising a complementarity determining region 3 (CDRH3) having a sequence set forth as: SEQ ID NO: 22 or SEQ ID NO: 66.

10. The antibody or antigen binding fragment of claim 5 further comprising a light chain variable region comprising a complementarity determining region 3 (CDRL3) having a sequence set forth as: SEQ ID NO: 44 or SEQ ID NO: 88.

11. An antibody that comprises a heavy chain variable region having a sequence set that shares at least 85% identity with SEQ ID NO: 15 and a light chain variable region that shares at least 85% identity with SEQ ID NO: 37.

12. An antibody that comprises a heavy chain variable region having a sequence set that shares at least 85% identity with SEQ ID NO: 59 and a light chain variable region that shares at least 85% identity with SEQ ID NO: 81.

13. The antibody of any one of the preceding claims, wherein the antibody is coupled to a targeted agent.

14. The antibody of claim 13, wherein the targeted agent is a detectable moiety.

15. The antibody of claim 14, wherein the detectable moiety is selected from the group consisting of a radioactive isotope, a magnetic compound, an x-ray absorber, a chemical compound, a biological tag, and a fluorescent molecule.

16. The antibody of claim 13, wherein the targeted agent is a therapeutic agent.

17. The antibody of claim 16, wherein the therapeutic agent is a cytotoxic moiety or an immunomodulatory moiety.

18. The antibody of any one of claims 13 to 17, wherein the antibody is coupled to the targeted agent via a linker.

19. The antibody of claim 18, wherein the linker is a flexible amino acid sequence.

20. The antibody of claim 18, wherein the linker is a photolinker.

21. The antibody of any one of claims 13 to 20, wherein the targeted agent comprises a physiologically inert nanoparticle.

22. The antibody of claim 21, wherein the nanoparticle is magnetic, fluorescent, or radioactive.

23. The antibody of any one of claims 13 to 22, wherein the targeted agent comprises a fluorochrome.

24. An antibody, or antigen binding fragment, that specifically binds to cell-surface exposed plectin-1 antigen and that comprises six complementarity determining regions (CDRs): CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3,

wherein CDRH1 comprises a sequence as set forth in SEQ ID NO: 18, CDRH2 comprises a sequence as set forth in SEQ ID NO: 20, CDRH3 comprises a sequence as set forth in SEQ ID NO: 22, CDRL1 comprises a sequence as set forth in SEQ ID NO: 40, CDRL2 comprises a sequence as set forth in SEQ ID NO: 42, and CDRL3 comprises a sequence as set forth in SEQ ID NO: 44; or

wherein CDRH1 comprises a sequence as set forth in SEQ ID NO: 62, CDRH2 comprises a sequence as set forth in SEQ ID NO: 64, CDRH3 comprises a sequence as set forth in SEQ ID NO: 66, CDRL1 comprises a sequence as set forth in SEQ ID NO: 84, CDRL2 comprises a sequence as set forth in SEQ ID NO: 86, and CDRL3 comprises a sequence as set forth in SEQ ID NO: 88.

25. The antibody, or antigen binding fragment, of claim 24, wherein CDRH1 comprises a sequence as set forth in SEQ ID NO: 18, CDRH2 comprises a sequence as set forth in SEQ ID NO: 20, CDRH3 comprises a sequence as set forth in SEQ ID NO: 22,

CDRL1 comprises a sequence as set forth in SEQ ID NO: 40, CDRL2 comprises a sequence as set forth in SEQ ID NO: 42, and CDRL3 comprises a sequence as set forth in SEQ ID NO: 44.

26. The antibody, or antigen binding fragment, of claim 25, comprising the heavy chain variable domain sequence of SEQ ID NO: 24.

27. The antibody, or antigen binding fragment, of claim 25 or 26, comprising the light chain variable domain sequence of SEQ ID NO: 46.

28. The antibody, or antigen binding fragment, of any one of claims 25 to 27, comprising the heavy chain variable domain sequence of SEQ ID NO: 24 and the light chain variable domain sequence of SEQ ID NO: 46.

29. The antibody, or antigen binding fragment, of claim 24, wherein CDRH1 comprises a sequence as set forth in SEQ ID NO: 62, CDRH2 comprises a sequence as set forth in SEQ ID NO: 64, CDRH3 comprises a sequence as set forth in SEQ ID NO: 66, CDRL1 comprises a sequence as set forth in SEQ ID NO: 84, CDRL2 comprises a sequence as set forth in SEQ ID NO: 86, and CDRL3 comprises a sequence as set forth in SEQ ID NO: 88.

30. The antibody, or antigen binding fragment, of claim 29, comprising the heavy chain variable domain sequence of SEQ ID NO: 68.

31. The antibody, or antigen binding fragment, of claim 29 or 30, comprising the light chain variable domain sequence of SEQ ID NO: 90.

32. The antibody, or antigen binding fragment, of any one of claims 29 to 31, comprising the heavy chain variable domain sequence of SEQ ID NO: 68 and the light chain variable domain sequence of SEQ ID NO: 90.

33. The antibody, or antigen binding fragment, of any one of claims 1 to 32, wherein the antibody or antigen binding fragment, is a monoclonal antibody, a humanized antibody, a diabody, a chimeric antibody, a Fab fragment, a F(ab')2 fragment, affibody, or an Fv fragment.

34. The antibody, or antigen binding fragment, of any one of claims 1 to 33, wherein the antibody or antigen binding fragment, comprises a heavy chain constant domain having a sequence as set forth in SEQ ID NO: 15 or SEQ ID NO: 59.

35. The antibody, or antigen binding fragment, of any one of claims 1 to 34, wherein the antibody or antigen binding fragment comprises a heavy chain constant domain selected from the group consisting of IgG, IgG1, IgG2, IgG2A, IgG2B, IgG2C, IgG3, IgG4, IgA1, IgA2, IgD, IgM, and IgE constant domains.

36. The antibody, or antigen binding fragment, of any one of claims 1 to 35, wherein the antibody or antigen binding fragment is conjugated to an agent selected from the group consisting of a fluorescent agent, a luminescent agent, an enzymatic agent and a radioactive agent.

37. An antibody, or antigen binding fragment, that competes or cross-competes for binding to an amino acid sequence set forth as: SEQ ID NO: 92 with an antibody, or antigen binding fragment of any one of claims 1 to 36.

38. The antibody or antigen binding fragment of claim 37, wherein the antibody or antigen binding fragment competes or cross-competes with an equilibrium dissociation constant, Kd, of less than 10^{-6} M between the antibody or antigen binding fragment, and its antigen.

39. A composition comprising the antibody of any one of claims 1 to 38, optionally further comprising a pharmaceutically acceptable excipient.

40. An isolated nucleic acid encoding a protein comprising three complementarity determining regions (CDRs): CDRH1, CDRH2, and CDRH3, wherein CDRH3 comprises a sequence as set forth in SEQ ID NO: 22.

41. The isolated nucleic acid of claim 40, wherein CDRH1 comprises a sequence as set forth in SEQ ID NO: 18.

42. The isolated nucleic acid of claim 40 or 41, wherein CDRH2 comprises a sequence as set forth in SEQ ID NO: 20.

43. An isolated nucleic acid encoding a protein comprising three complementarity determining regions (CDRs): CDRL1, CDRL2, and CDRL3, wherein CDRL3 comprises a sequence as set forth in SEQ ID NO: 44.

44. The isolated nucleic acid of claim 43, wherein CDRL1 comprises a sequence as set forth in SEQ ID NO: 40.

45. The isolated nucleic acid of claim 43 or 44, wherein CDRL2 comprises a sequence as set forth in SEQ ID NO: 42.

46. An isolated nucleic acid encoding a protein comprising three complementarity determining regions (CDRs): CDRH1, CDRH2, and CDRH3, wherein CDRH3 comprises a sequence as set forth in SEQ ID NO: 66.

47. The isolated nucleic acid of claim 46, wherein CDRH1 comprises a sequence as set forth in SEQ ID NO: 62.

48. The isolated nucleic acid of claim 46 or 47, wherein CDRH2 comprises a sequence as set forth in SEQ ID NO: 64.

49. An isolated nucleic acid encoding a protein comprising three complementarity determining regions (CDRs): CDRL1, CDRL2, and CDRL3, wherein CDRL3 comprises a sequence as set forth in SEQ ID NO: 88.

50. The isolated nucleic acid of claim 49, wherein CDRL1 comprises a sequence as set forth in SEQ ID NO: 84.

51. The isolated nucleic acid of claim 49 or 50, wherein CDRL2 comprises a sequence as set forth in SEQ ID NO: 86.

52. An isolated nucleic acid comprising a sequence as set forth in a sequence selected from the group consisting of SEQ ID NO: 15, 24, 37, 46, 59, 68, 81, or 90.

53. An isolated cell comprising an isolated nucleic acid of any one of claims 40 to 52.

54. The isolated cell of claim 53, wherein the cell is a bacterial cell, a yeast cell, a mammalian cell, or an insect cell.

55. The isolated cell of claim 53 or 54, wherein the cell is a hybridoma cell.

56. A method for targeting an agent to a cancer cell in a subject, the method comprising administering to the subject a composition comprising an antibody as described in any one of claims 1 to 38, or the composition of claim 39, coupled to a targeted agent, wherein the antibody binds to plectin-1 on the surface of the cancer cell in the subject.

57. The method of claim 56, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO: 24 or SEQ ID NO: 68.

58. The method of claim 57 further comprising a light chain variable region having a sequence set forth as: SEQ ID NO: 46 or SEQ ID NO: 90.

59. The method of any one of claims 56 to 58, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO 24 and a light chain variable region having a sequence set forth as: SEQ ID NO: 46.

60. The method of any one of claims 56 to 58, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO 68 and a light chain variable region having a sequence set forth as: SEQ ID NO: 90.

61. The method of any one of claims 56 to 60, wherein the targeted agent is a detectable moiety.

62. The method of claim 61, wherein the detectable moiety is selected from the group consisting of a radioactive isotope, a magnetic compound, an x-ray absorber, a chemical compound, a biological tag, and a fluorescent molecule.

63. The method of any one of claims 56 to 60, wherein the targeted agent is a therapeutic agent.

64. The method of claim 63, wherein the therapeutic agent is a cytotoxic moiety or an immunomodulatory moiety.

65. The method of any one of claims 56 to 64, wherein the antibody is coupled to the targeted agent via a linker.

66. The method of claim 65, wherein the linker is a flexible amino acid sequence.

67. The method of claim 65, wherein the linker is a photolinker.

68. The method of any one of claims 56 to 67, wherein the targeted agent comprises a physiologically inert nanoparticle.

69. The method of claim 68, wherein the nanoparticle is magnetic, fluorescent, or radioactive.

70. The method of any one of claims 56 to 69, wherein the targeted agent comprises a fluorochrome.

71. The method of any one of claims 56 to 70, wherein the antibody or composition is administered at a dose in a range of 1 ng/kg and 100 mg/kg.

72. The method of any one of claims 56 to 71, wherein the cancer cell is an ovarian cancer cell, esophageal cancer cell, head and neck squamous cell carcinoma cancer cell, or pancreatic cancer cell.

73. The method of claim 72, wherein the cancer cell is a pancreatic ductal adenocarcinoma cell.

74. The method of any one of claims 56 to 73, wherein the subject is a mammal, optionally a human.

75. A method for treating cancer, the method comprising administering to a subject having cancer an effective amount of an antibody of any one of claims 1 to 38, or an effective amount of the composition of claim 39.

76. The method of claim 75, wherein the antibody is coupled to a targeted agent, and wherein the antibody binds to plectin-1 on the surface of the cancer cell in the subject.

77. The method of claim 75 or 76, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO: 24 or SEQ ID NO: 68.

78. The method of any one of claims 75 to 77, wherein the antibody further comprises a light chain variable region having a sequence set forth as: SEQ ID NO: 46 or SEQ ID NO: 90.

79. The method of claim 75 or 76, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO 24 and a light chain variable region having a sequence set forth as: SEQ ID NO: 46.

80. The method of any one of claims 75 to 77, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO 68 and a light chain variable region having a sequence set forth as: SEQ ID NO: 90.

81. The method of any one of claims 76 to 80, wherein the targeted agent is a detectable moiety.

82. The method of claim 81, wherein the detectable moiety is selected from the group consisting of a radioactive isotope, a magnetic compound, an x-ray absorber, a chemical compound, a biological tag, and a fluorescent molecule.

83. The method of any one of claims 76 to 82, wherein the targeted agent is a therapeutic agent.

84. The method of claim 83, wherein the therapeutic agent is a cytotoxic moiety or an immunomodulatory moiety.

85. The method of any one of claims 76 to 84, wherein the antibody is coupled to the targeted agent via a linker.

86. The method of claim 85, wherein the linker is a flexible amino acid sequence.

87. The method of claim 85, wherein the linker is a photolinker.

88. The method of any one of claims 76 to 87, wherein the targeted agent comprises a physiologically inert nanoparticle.

89. The method of claim 88, wherein the nanoparticle is magnetic, fluorescent, or radioactive.

90. The method of any one of claims 76 to 89, wherein the targeted agent comprises a fluorochrome.

91. The method of any one of claims 75 to 90, wherein the antibody or composition is administered at a dose in a range of 1 ng/kg and 100 mg/kg.

92. The method of any one of claims 75 to 91, wherein the cancer cell is an ovarian cancer cell, esophageal cancer cell, head and neck squamous cell carcinoma cancer cell, or pancreatic cancer cell.

93. The method of claim 92, wherein the cancer cell is a pancreatic ductal adenocarcinoma cell.

94. The method of any one of claims 75 to 93, wherein the subject is a mammal, optionally a human.

95. A method for detecting a cancer cell, the method comprising administering to a subject having cancer an effective amount of the antibody of any one of claims 1 to 38 or an effective amount of the composition of claim 39.

96. The method of claim 95, wherein the antibody is coupled to a targeted agent, and wherein the antibody binds to plectin-1 on the surface of the cancer cell in the subject.

97. The method of claim 95 or 96, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO: 24 or SEQ ID NO: 68.

98. The method of any one of claims 95 to 97, wherein the antibody further comprises a light chain variable region having a sequence set forth as: SEQ ID NO: 46 or SEQ ID NO: 90.

99. The method of claim 95 or 96, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO 24 and a light chain variable region having a sequence set forth as: SEQ ID NO: 46.

100. The method of any one of claims 95 to 97, wherein the antibody comprises a heavy chain variable region having a sequence set forth as: SEQ ID NO 68 and a light chain variable region having a sequence set forth as: SEQ ID NO: 90.

101. The method of any one of claims 96 to 100, wherein the targeted agent is a detectable moiety.

102. The method of claim 101, wherein the detectable moiety is selected from the group consisting of a radioactive isotope, a magnetic compound, an x-ray absorber, a chemical compound, a biological tag, and a fluorescent molecule.

103. The method of any one of claims 96 to 102, wherein the antibody is coupled to the targeted agent via a linker.

104. The method of claim 103, wherein the linker is a flexible amino acid sequence.

105. The method of claim 103, wherein the linker is a photolinker.

106. The method of any one of claims 96 to 105, wherein the targeted agent comprises a physiologically inert nanoparticle.

107. The method of claim 106, wherein the nanoparticle is magnetic, fluorescent, or radioactive.

108. The method of any one of claims 96 to 107, wherein the targeted agent comprises a fluorochrome.

109. The method of any one of claims 95 to 108, wherein the antibody or composition is administered at a dose in a range of 1 ng/kg and 100 mg/kg.

110. The method of any one of claims 95 to 109, wherein the cancer cell is an ovarian cancer cell, esophageal cancer cell, head and neck squamous cell carcinoma cancer cell, or pancreatic cancer cell.

111. The method of claim 110, wherein the cancer cell is a pancreatic ductal adenocarcinoma cell.

112. The method of any one of claims 95 to 111, wherein the subject is a mammal, optionally a human.

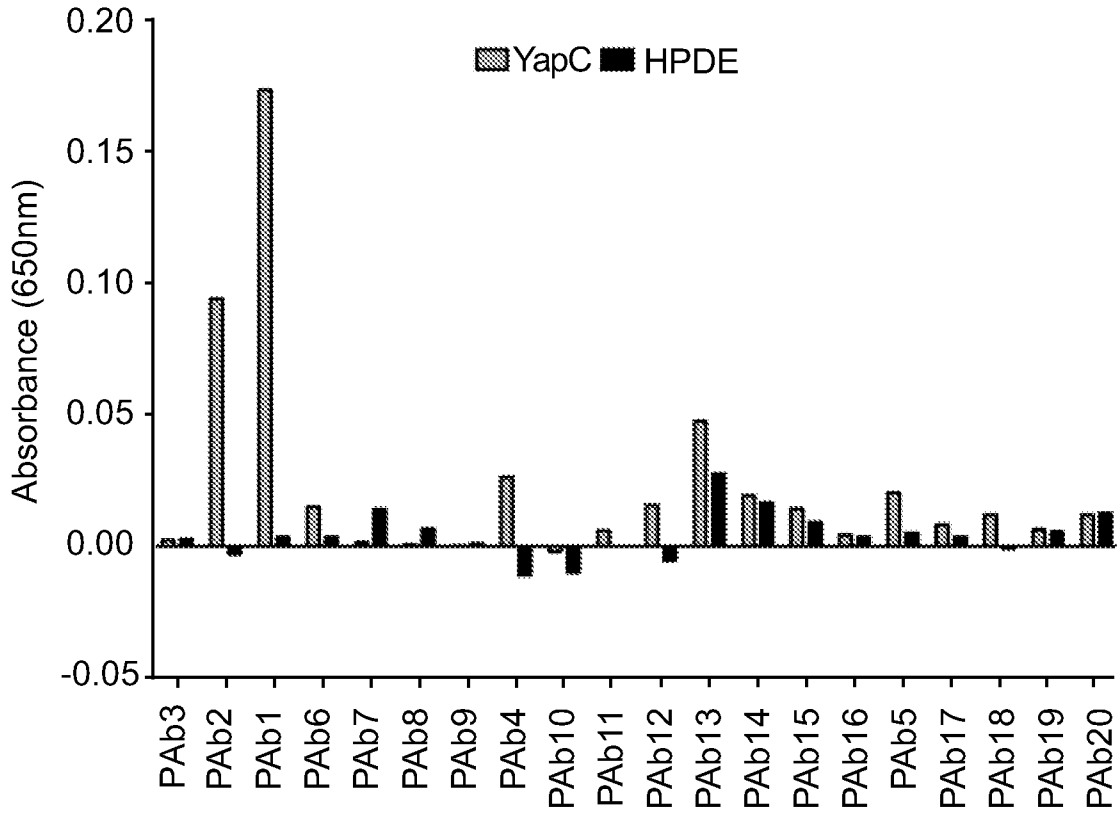
YapC
HPDE
Absorbance (650nm)
0.20
0.15
0.10
0.05
0.00
-0.05
PAb3
PAb2
PAb1
PAb6
PAb7
PAb8
PAb9
PAb4
PAb10
PAb11
PAb12
PAb13
PAb14
PAb15
PAb16
PAb5
PAb17
PAb18
PAb19
PAb20

FIG. 1

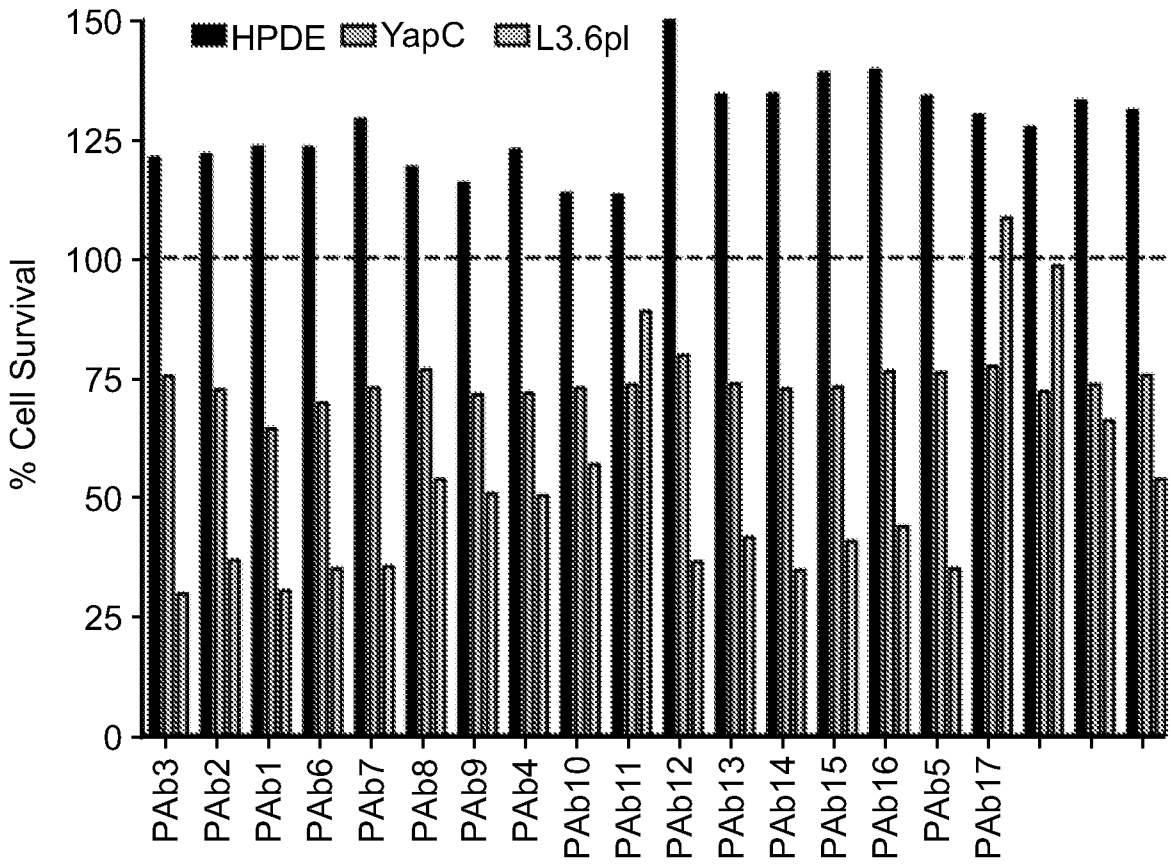
HPDE
YapC
L3.6pl
% Cell Survival
150
125
100
75
50
25
0
PAb3
PAb2
PAb1
PAb6
PAb7
PAb8
PAb9
PAb4
PAb10
PAb11
PAb12
PAb13
PAb14
PAb15
PAb16
PAb5
PAb17

FIG. 2

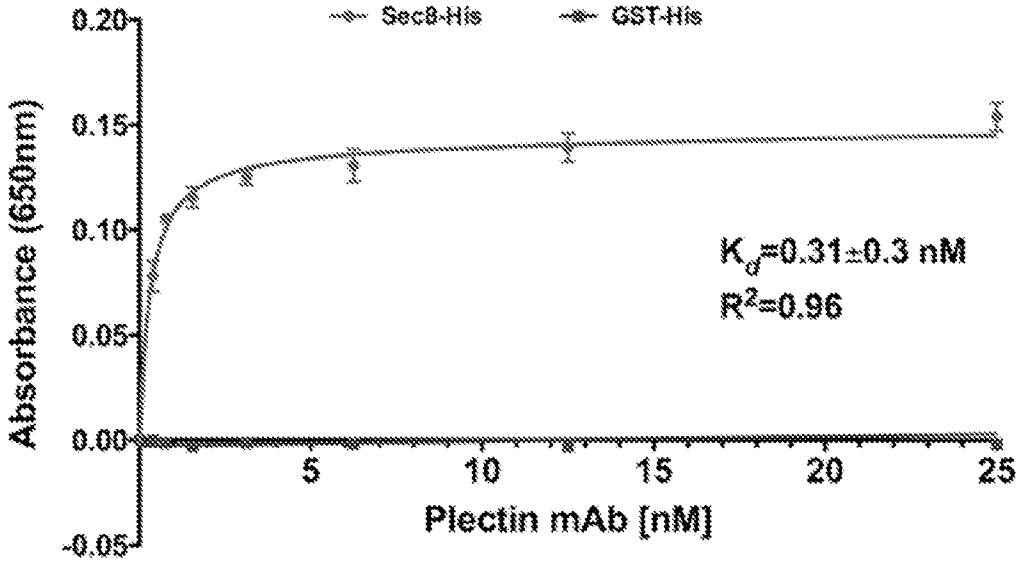
Sec8-His
GST-His
Absorbance (650nm)
0.20
0.15
0.10
0.05
0.00
-0.05
5
10
15
20
25
Plectin mAb [nM]
K_d=0.31±0.3 nM
R^2=0.96

FIG. 3A

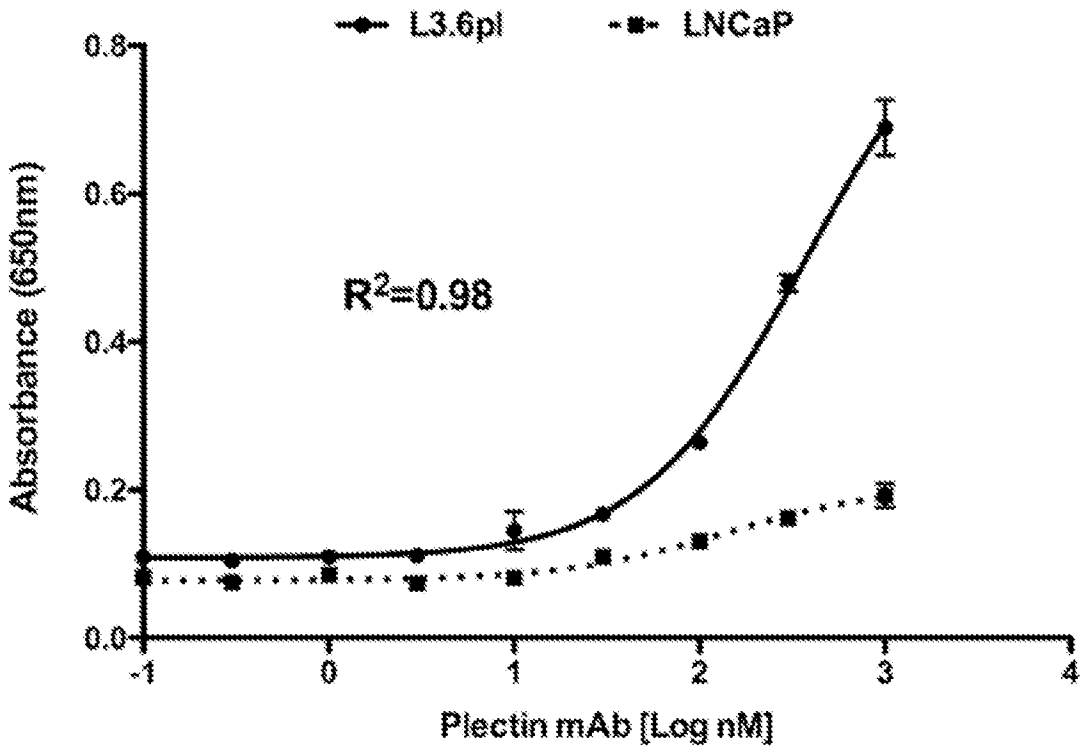
L3.6pl
LNCaP
Absorbance (650nm)
0.8
0.6
0.4
0.2
0.0
-1
0
1
2
3
4
Plectin mAb [Log nM]
R^2=0.98

FIG. 3B

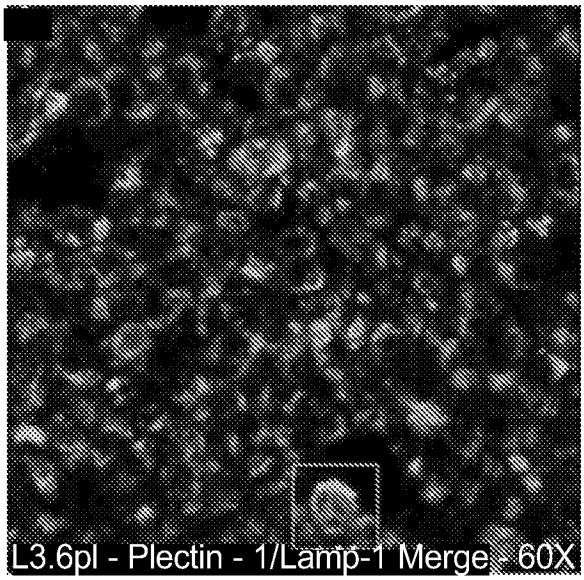

FIG. 4A

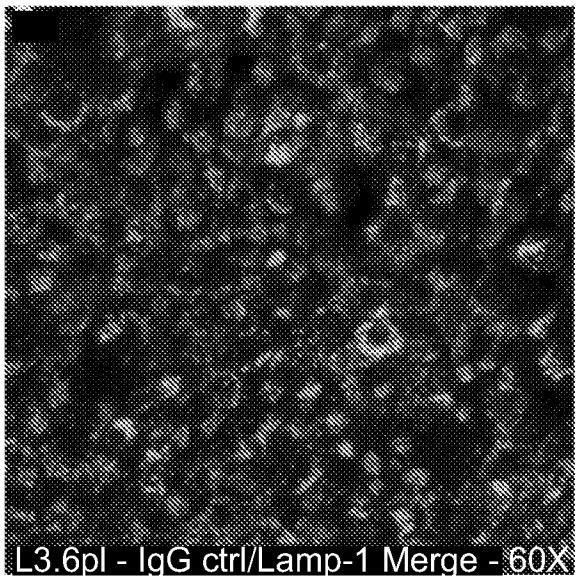

FIG. 4B

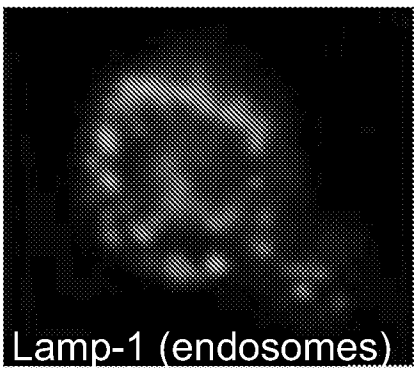

FIG. 4C

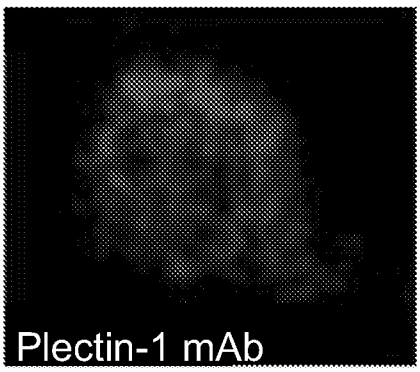

FIG. 4D

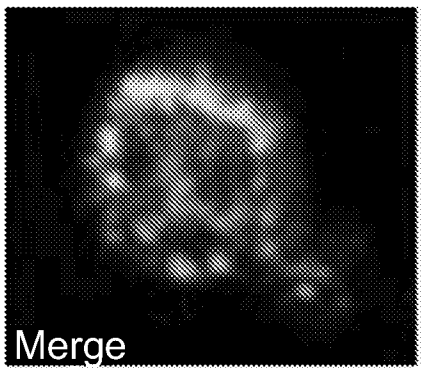

FIG. 4E

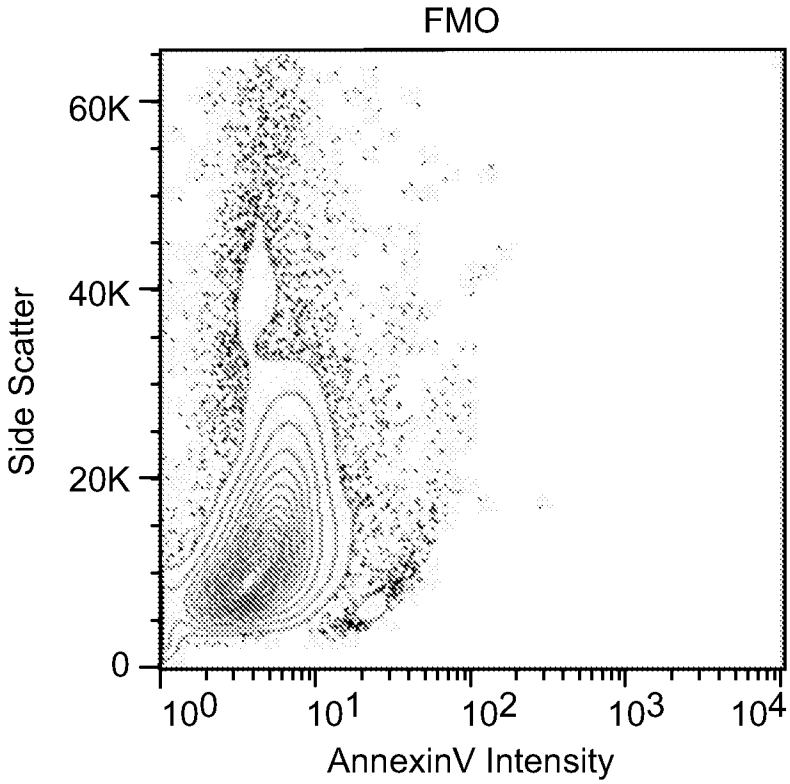
FMO
60K
40K
20K
0
Side Scatter
10^0
10^1
10^2
10^3
10^4
AnnexinV Intensity

FIG. 5A

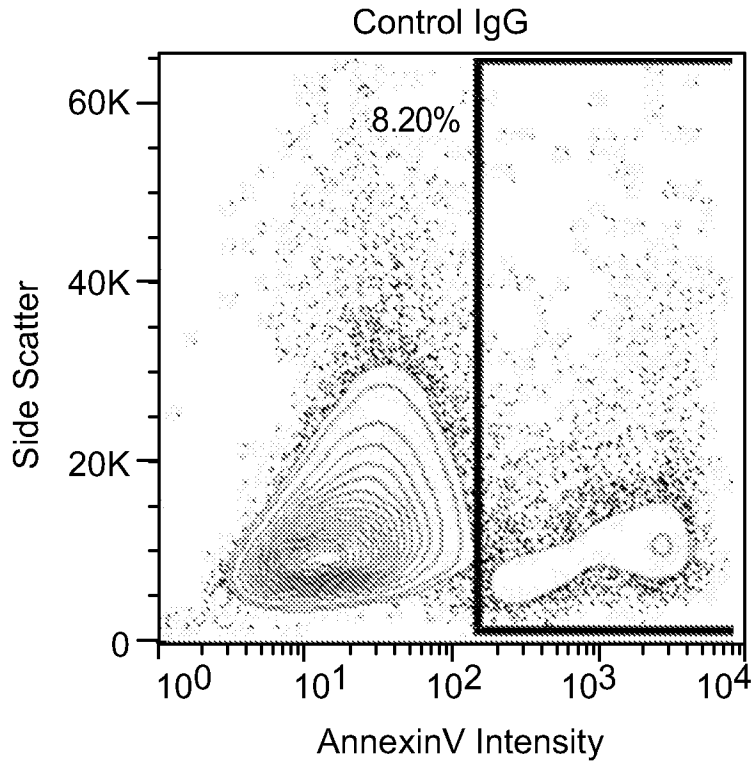
Control IgG
8.20%
60K
40K
20K
0
Side Scatter
10^0
10^1
10^2
10^3
10^4
AnnexinV Intensity

FIG. 5B

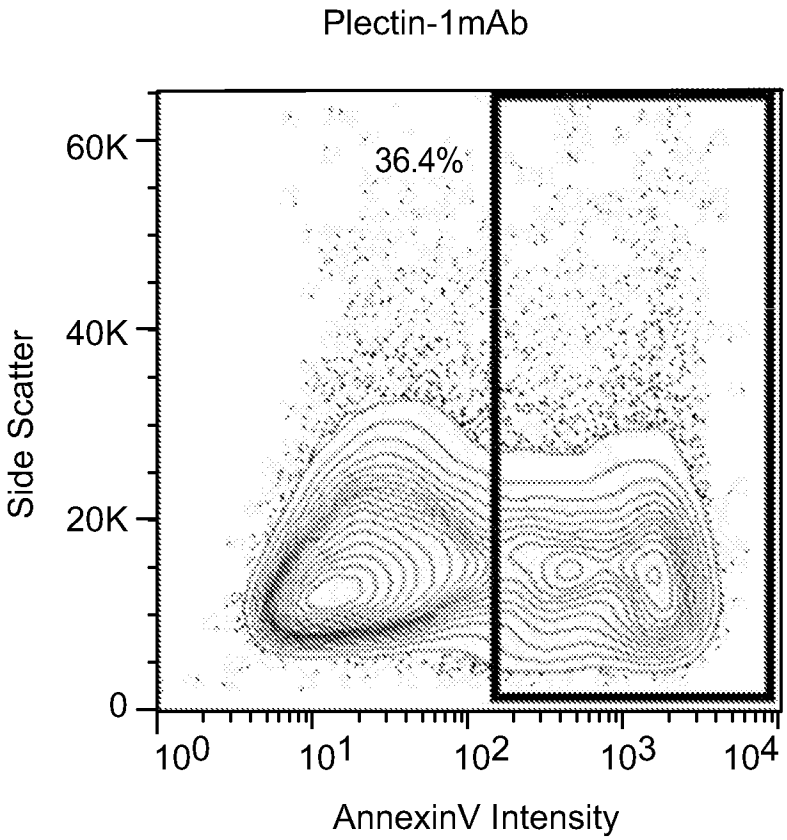
Plectin-1mAb
36.4%
60K
40K
20K
0
Side Scatter
10^0
10^1
10^2
10^3
10^4
AnnexinV Intensity

FIG. 5C

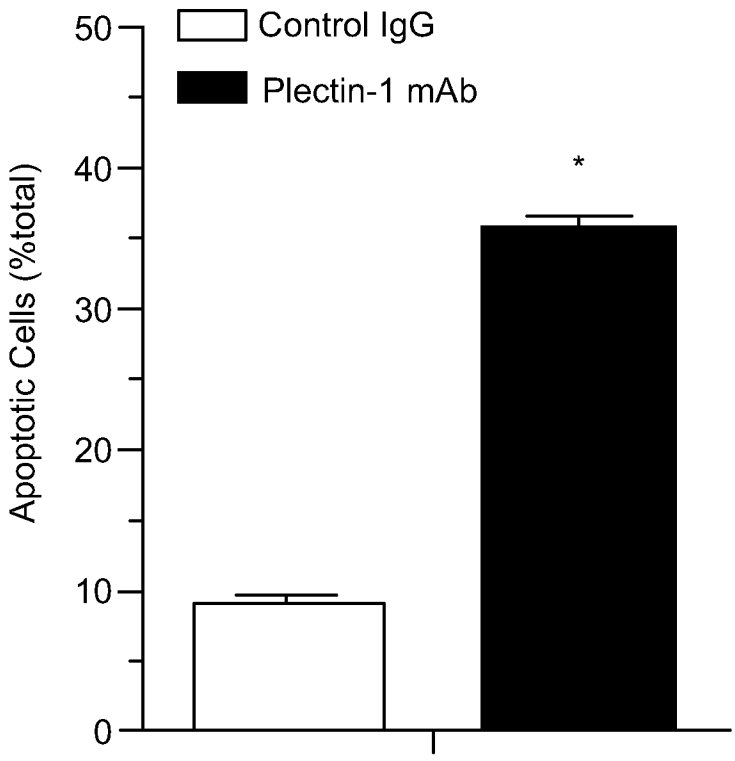
Control IgG
Plectin-1 mAb
*
50
40
30
20
10
0
Apoptotic Cells (%total)

FIG. 5D

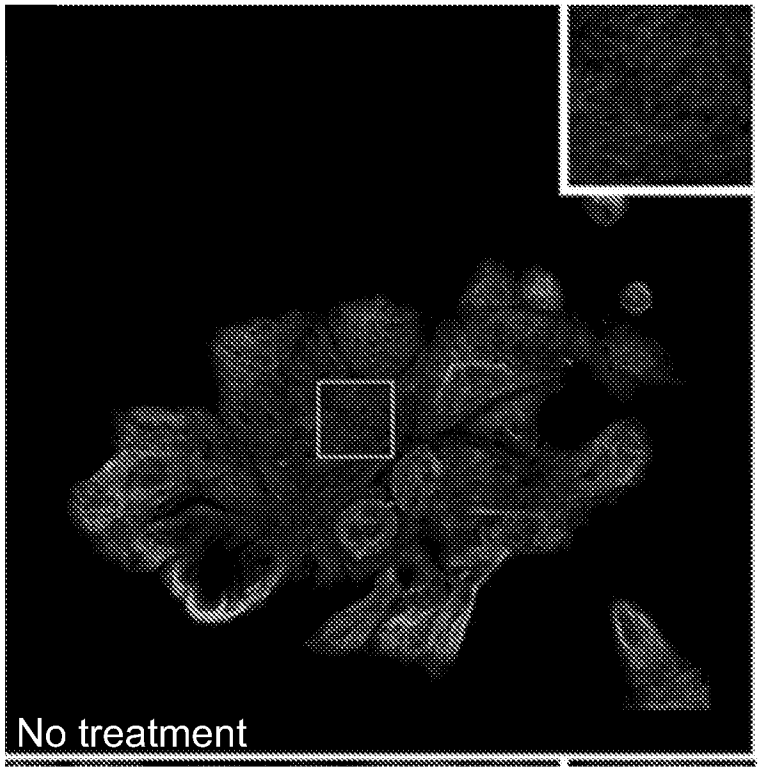

FIG. 6A

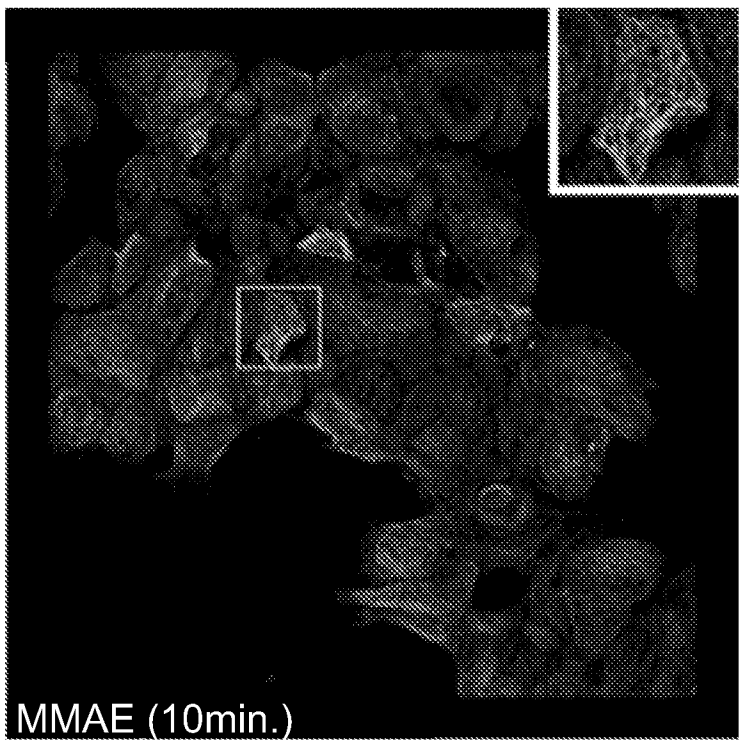

FIG. 6B

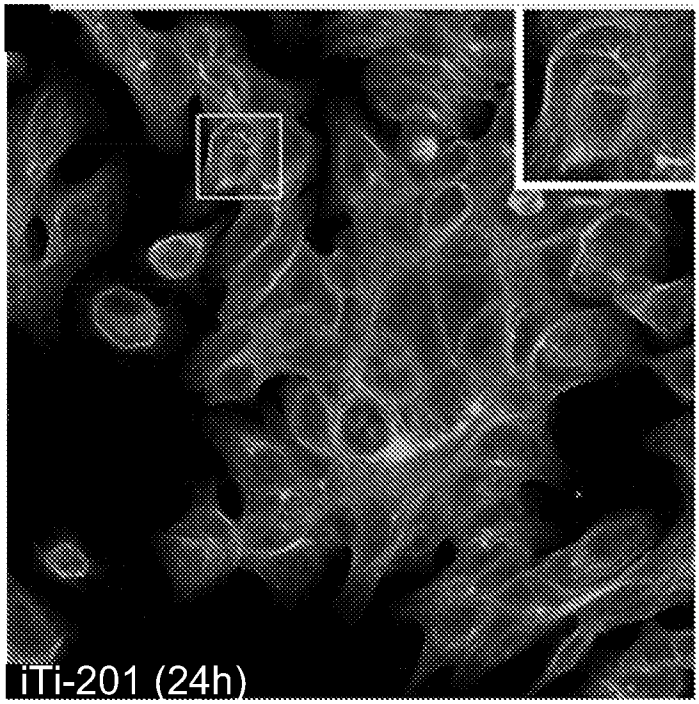
iTi-201 (24h)

FIG. 6C

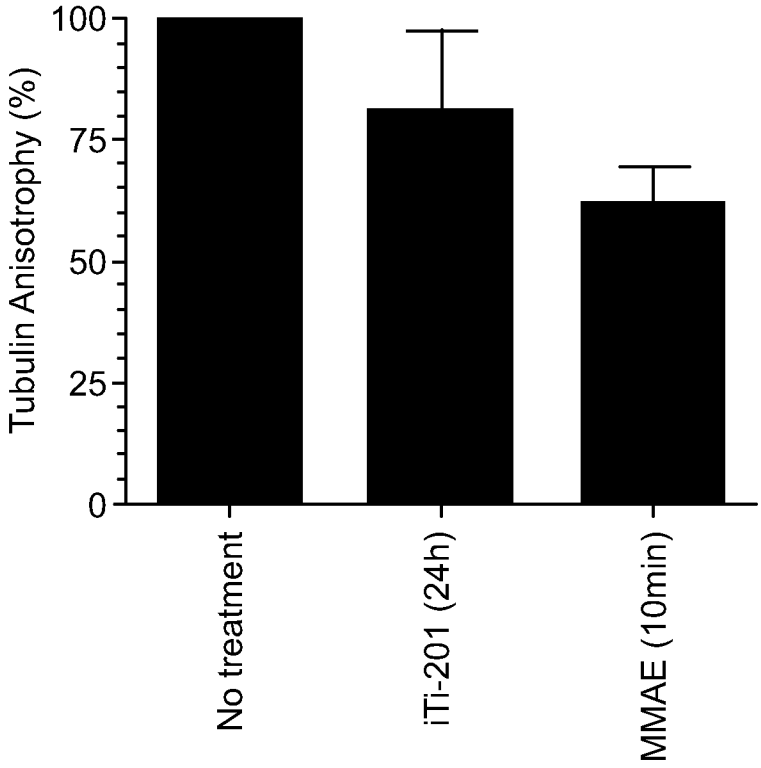
Tubulin Anisotrophy (%)
100
75
50
25
0
No treatment
iTi-201 (24h)
MMAE (10min)

FIG. 6D

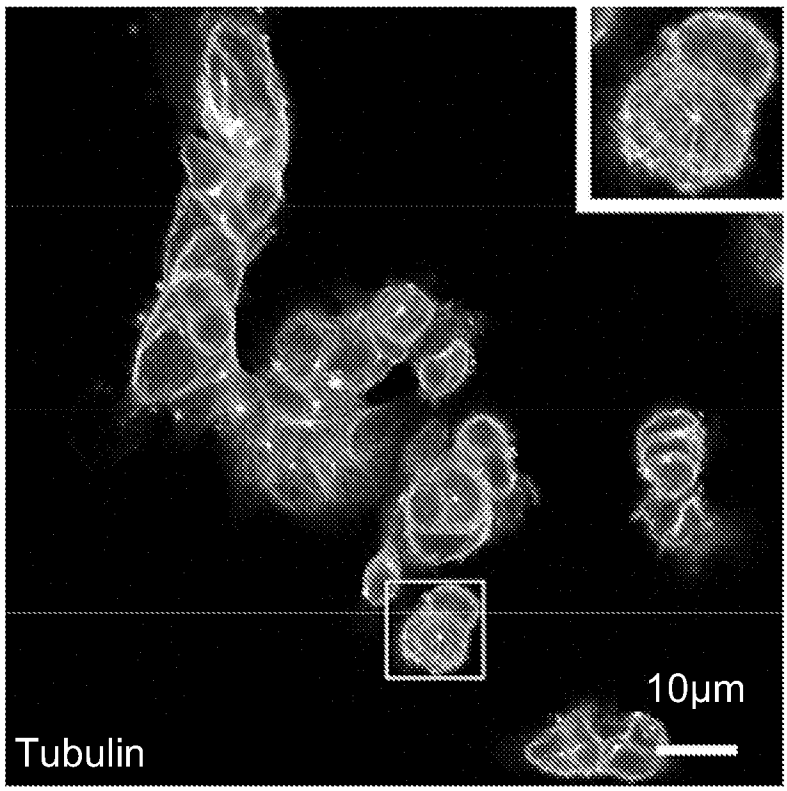

FIG. 7A

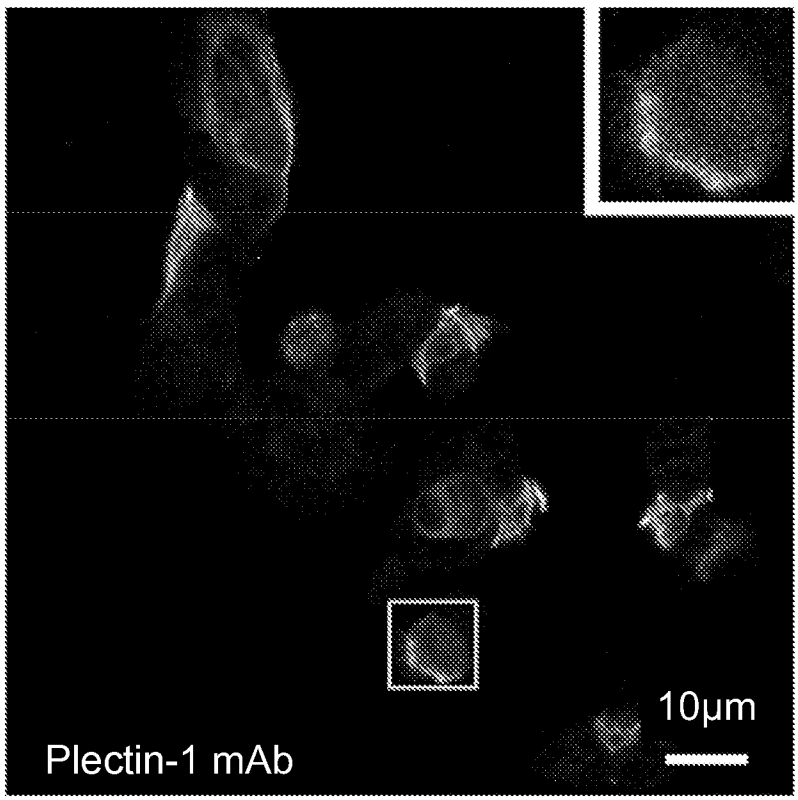

FIG. 7B

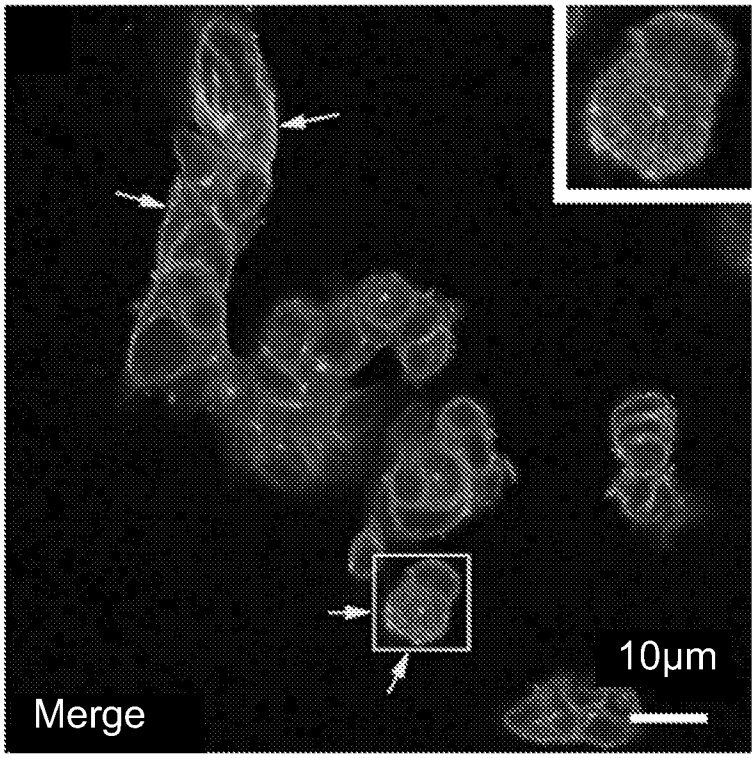
Merge
10μm

FIG. 7C

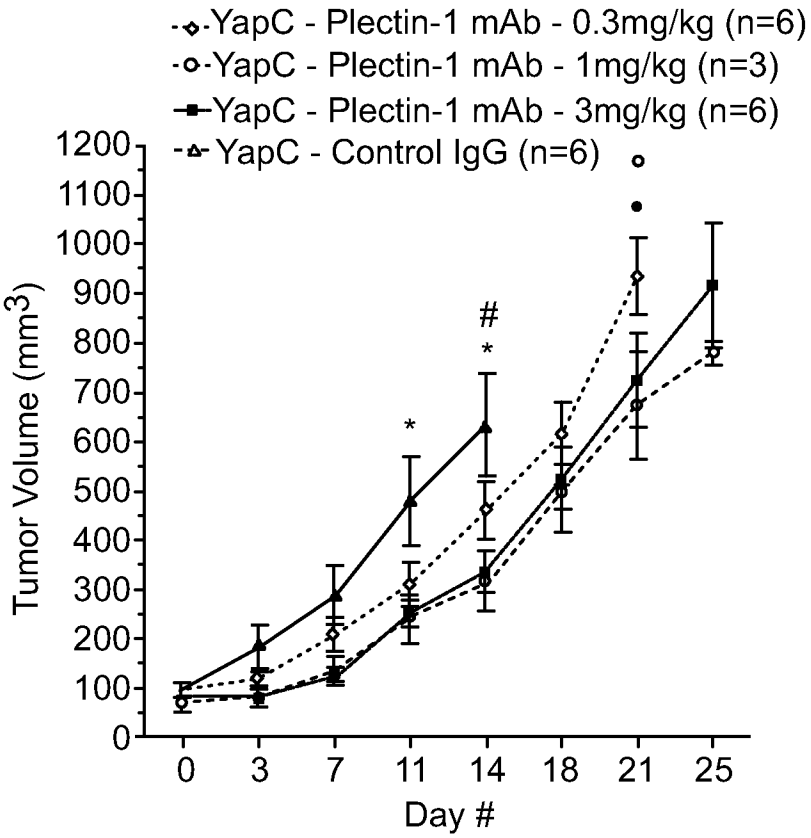
YapC - Plectin-1 mAb - 0.3mg/kg (n=6)
YapC - Plectin-1 mAb - 1mg/kg (n=3)
YapC - Plectin-1 mAb - 3mg/kg (n=6)
YapC - Control IgG (n=6)
Tumor Volume (mm3)
Day #
0
3
7
11
14
18
21
25
100
200
300
400
500
600
700
800
900
1000
1100
1200
*
*
#

FIG. 8A

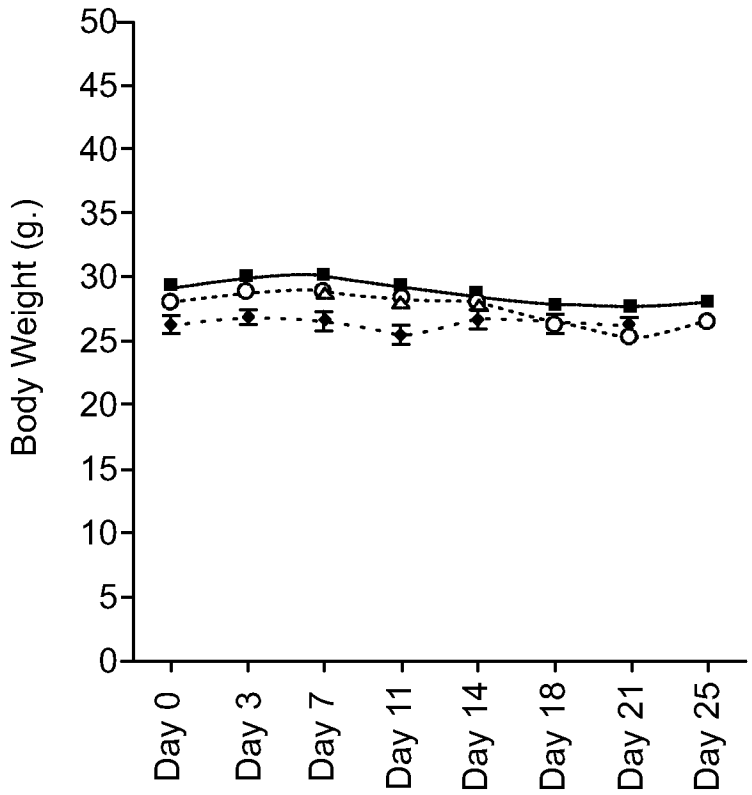

FIG. 8B

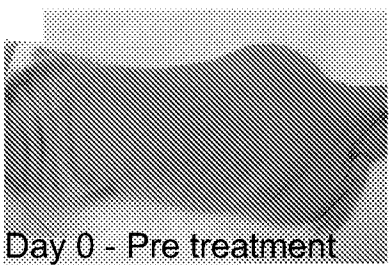

FIG. 8C

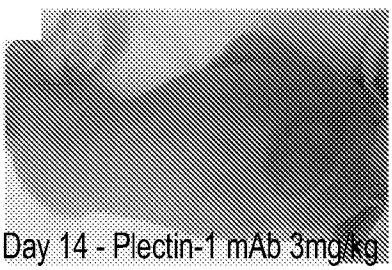

FIG. 8D

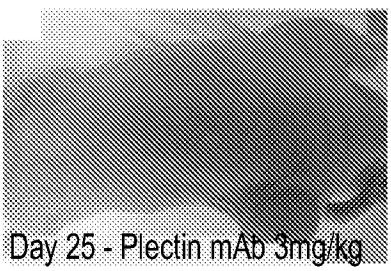

FIG. 8E