



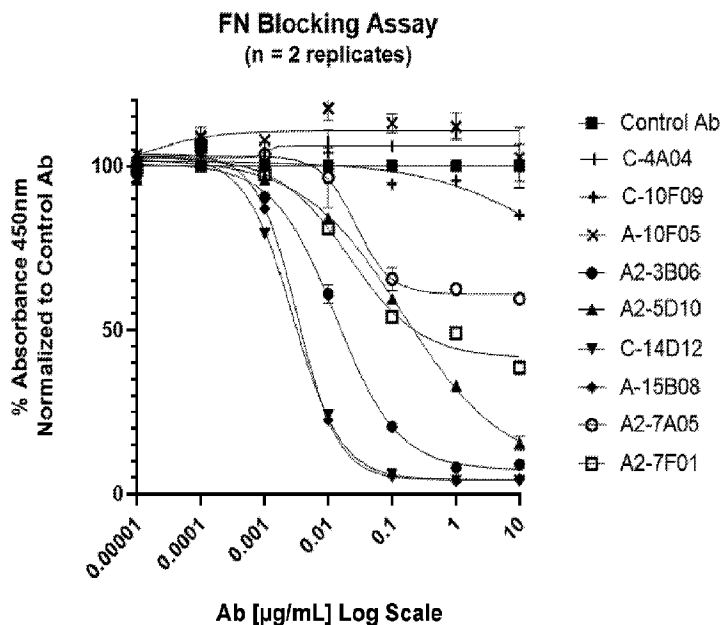
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(54) **Titre : AGENTS DE LIAISON A L'INTEGRINE ALPHA 5 BETA 1 ET LEURS UTILISATIONS**
(54) **Title: ALPHA 5 BETA 1 INTEGRIN BINDING AGENTS AND USES THEREOF**



(57) **Abrégé/Abstract:**

The present disclosure provides $\alpha 5 \beta 1$ integrin binding agents (e.g., antibodies, including multispecific antibodies, such as bispecific antibodies) and uses thereof.

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Abstract:

The present disclosure provides 51 integrin binding agents (e.g., antibodies, including multispecific antibodies, such as bispecific antibodies) and uses thereof.

ALPHA 5 BETA 1 INTEGRIN BINDING AGENTS AND USES THEREOF**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of U.S. Provisional Application No. 63/187,371, filed May 11, 2021, the disclosure of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] This application incorporates by reference a Sequence Listing submitted with this application as a text file entitled “14700-001-228_SEQ_LISTING.txt,” created on May 8, 2022, and of 142,533 bytes in size.

FIELD

[0003] The present disclosure relates generally to binding agents, such as antibodies, that bind to alpha 5 beta 1 ($\alpha 5 \beta 1$) integrin, including human $\alpha 5 \beta 1$ integrin, and methods of their use.

BACKGROUND

[0004] Integrins are transmembrane proteins that bind extracellular matrix (ECM) components and regulate cell adhesion, migration and activation. Each integrin is composed of an α and a β transmembrane integrin subunit. There are 18 α integrin subunits and 8 β integrin subunits in the human genome and they combine to generate 23 unique heterodimeric integrins. These heterodimers modulate cell behavior through mechanisms known as “inside-out” and “outside-in” signaling. In the former, intracellular proteins bind the integrin cytoplasmic domain and stabilizes a conformation that binds extracellular ligands with high affinity. Then, through “outside-in” signaling the ligand-bound integrin stimulates intracellular signaling cascades that modulate cell behaviors.

[0005] The $\alpha 5 \beta 1$ integrin is known as the fibronectin (FN) receptor because of its high affinity for the FN in the extracellular matrix (ECM). This binding is mediated by the ligand-binding site at the interface between the α and β subunits in the

headpiece of $\alpha 5\beta 1$ and an arginine-glycine-aspartic acid (RGD) peptide motif in the Type III repeats of FN. The $\alpha 5\beta 1$ integrin binds additional RGD-containing proteins like osteopontin and fibrillin along with proteins that lack RGD motifs including CD40L, IL-1b and the TNF- α converting enzyme ADAM-17. Consistent with the tissue distribution of its ligands, $\alpha 5\beta 1$ is expressed by a variety of cell-types including endothelial cells, mast cells and macrophage lineages in peripheral tissues and the central nervous system (CNS) (e.g., microglia and perivascular macrophages).

[0006] The association of $\alpha 5\beta 1$ integrin with tumor angiogenesis is well-established. In addition, $\alpha 5\beta 1$ has been demonstrated to be present on tumor cells. Antibodies that bind $\alpha 5\beta 1$ have been shown not only to inhibit angiogenesis but also facilitate killing of $\alpha 5\beta 1$ expressing tumor cells. The association of $\alpha 5\beta 1$ integrin with neuroinflammatory diseases including multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) has also been demonstrated. Antibodies that bind to $\alpha 5\beta 1$ have been shown to ameliorate symptoms in the experimental autoimmune encephalitis (EAE) model of MS and the SOD1^{G93A} transgenic model of ALS. Although expression of $\alpha 5\beta 1$ would appear to give it the potential to be a target in anti-angiogenesis and cancer therapies as well as in neuroinflammatory disease therapies, clinical success with antibodies targeting $\alpha 5$ integrin has not yet been achieved.

[0007] Accordingly, there remains a need in the art for agents that can target $\alpha 5\beta 1$ integrin to treat, prevent, or alleviate $\alpha 5$ -mediated diseases, disorders, or conditions, including those involving cells expressing $\alpha 5\beta 1$, such as tumor cells and macrophages.

SUMMARY

[0008] The present disclosure provides $\alpha 5\beta 1$ integrin binding agents, including human $\alpha 5\beta 1$ integrin binding agents. Such agents include antibodies that bind to $\alpha 5\beta 1$ integrin, for example, monospecific or multispecific (e.g., bispecific) antibodies that bind to $\alpha 5\beta 1$ integrin. Such antibodies, in some embodiments, compete for the

binding of human $\alpha 5 \beta 1$ integrin with an antibody having a heavy chain variable region and a light chain variable region as described herein (e.g., Tables 1-6).

[0009] The present disclosure also provides compositions comprising an $\alpha 5 \beta 1$ integrin binding agent. Such compositions, in some embodiments, include antibodies that bind to $\alpha 5 \beta 1$ integrin, for example, monospecific or multispecific (e.g., bispecific) antibodies that bind to $\alpha 5 \beta 1$ integrin. Such compositions, in some embodiments, include antibodies that compete for the binding of human $\alpha 5 \beta 1$ integrin with an antibody having a heavy chain variable region and a light chain variable region described herein (e.g., Tables 1-6).

[0010] The present disclosure also provides methods of treating, preventing, or alleviating an $\alpha 5 \beta 1$ integrin-mediated disease, disorder, or condition, including methods of treating, preventing, or alleviating one or more symptoms of the disease, disorder, or condition with an $\alpha 5 \beta 1$ integrin binding agent or a composition comprising the agent, including an $\alpha 5 \beta 1$ integrin binding agent or composition comprising the agent. Such compositions include antibodies that bind to $\alpha 5 \beta 1$ integrin, for example, monospecific or multispecific (e.g., bispecific) antibodies that bind to $\alpha 5 \beta 1$ integrin.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] **FIG. 1** illustrates exemplary results from fibronectin (FN) inhibition assays, further described in Example 5.

[0012] **FIGs. 2A-2B** show sequence alignments of heavy chain variable regions and light chain variable regions of (i) A-15B08, A2-3B06, A2-5D10, C-14D12 and (ii) A2-7A05, A2-7F01, including exemplary consensus sequences for VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3. Boundaries of CDRs are indicated according to Kabat, AbM, Chothia, Contact, and IMGT (see *also*, e.g., Tables 1-6).

[0013] **FIGs. 2C-2D** show sequence alignments of heavy chain variable regions and light chain variable regions of A-15B08-T62A and exemplary human engineered

variable region A-15B08_Low and A-15B08_Low+Mod. Boundaries of CDRs are indicated according to Kabat, AbM, Chothia, Contact, and IMGT.

[0014] FIGs. 2E-2F show sequence alignments of heavy chain variable regions and light chain variable regions of A-7A05 and exemplary human engineered variable region A-7A05_Low and A-7A05_Low+Mod. Boundaries of CDRs are indicated according to Kabat, AbM, Chothia, Contact, and IMGT.

[0015] FIGs. 2G-2H show sequence alignments of heavy chain variable regions and light chain variable regions of C-14D12 and exemplary human engineered C-14D12_Low and C-14D12_Low+Mod. Boundaries of CDRs are indicated according to Kabat, AbM, Chothia, Contact, and IMGT.

[0016] FIG. 3 shows a sequence alignment of exemplary Fc sequences, including variant Fc sequences.

[0017] FIGs. 4A and 4B show exemplary results of antibodies provided herein that disrupt the $\alpha 5\beta 1$ -FN integrin-ligand complex, further described in Example 7.

[0018] FIG. 5 shows exemplary results of $\alpha 5\beta 1$ integrin FN blocking assays, further described in Example 8.

[0019] FIG. 6 shows exemplary results of the potency of chimeras as compared to hybridomas, further described in Example 8.

[0020] FIG. 7 shows exemplary SDS-PAGE results of various IgG4 chimeric antibodies, further described in Example 8.

[0021] FIG. 8 shows exemplary results of conformation assays, further described in Example 9.

[0022] FIG. 9 shows exemplary results of cellular adhesion assays, further described in Example 10.

DETAILED DESCRIPTION

[0023] The present disclosure provides $\alpha 5\beta 1$ integrin binding agents. Such agents include antibodies (e.g., monospecific or multispecific, including bispecific) that bind to $\alpha 5\beta 1$ integrin, including antibodies that bind to human $\alpha 5\beta 1$ integrin. Such binding agents are useful in compositions and in methods of treating,

preventing, or alleviating an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition, including one or more symptoms of the disease, disorder, or condition. An $\alpha 5\beta 1$ integrin-mediated disease, disorder, and conditions include a cancer, an angiogenesis-mediated disease (e.g., a disease with abnormal angiogenesis), and an inflammatory disease (e.g., a neuroinflammatory disease). In addition, $\alpha 5\beta 1$ integrin binding agents described herein, such as $\alpha 5\beta 1$ integrin binding antibodies (e.g., monospecific or multispecific antibodies, including bispecific antibodies), are useful to (i) inhibit $\alpha 5\beta 1$ integrin binding to fibronectin, (ii) inhibit angiogenesis, and/or (iii) treat or alleviate one or more symptoms of (i) a cancer, (ii) an angiogenesis-mediated disease, disorder, or condition, or (iii) an inflammatory disease, disorder, or condition. An $\alpha 5\beta 1$ integrin binding agent as described herein, such as an $\alpha 5\beta 1$ integrin binding antibody (e.g., monospecific or multispecific, including bispecific), is useful in compositions and in methods of treatment of an $\alpha 5\beta 1$ -mediated disease, disorder, or condition.

[0024] The term “ $\alpha 5$ integrin,” “CD49e,” or “ $\alpha 5$ integrin polypeptide” and similar terms refers to a polypeptide (“polypeptide” and “protein” are used interchangeably herein) or any native $\alpha 5$ integrin from any vertebrate source, including mammals such as primates (e.g., humans, cynomolgus monkey (cyno)), dogs, and rodents (e.g., mice and rats), unless otherwise indicated. $\alpha 5$ integrin, also known in the art as Integrin alpha-5, ITGA5 protein, CD49e antigen, Glycoprotein Ic (GPIc), VLA5A, FNRA, and fibronectin receptor subunit alpha, has multiple domains, including beta-propeller (e.g., with seven 60 amino acids FG-GAP repeats), thigh, genu, calf1, calf2, transmembrane, and intracellular domains as well as four calcium binding sites. The term $\alpha 5$ integrin encompasses “full-length,” unprocessed $\alpha 5$ integrin, as well as any form of $\alpha 5$ integrin or any fragment thereof that results from processing in the cell. The term $\alpha 5$ integrin also encompasses naturally occurring variants of $\alpha 5$ integrin, such as SNP variants, splice variants and allelic variants. An $\alpha 5$ integrin in association with $\beta 1$ as a heterodimer is known in the art to interact with a number of ligands (e.g., fibronectin) and this interaction leads to protein conformational

changes and signal transduction, leading to changes in cellular activity, such as cell adhesion, proliferation, apoptosis, migration, and phagocytosis. The full-length amino acid sequence of human $\alpha 5$ integrin is provided below (exemplary signal sequence = italic text; exemplary extracellular domain = underline text):

*MGSRTPE*SPLHAVQLRWGPRRRPPLLPLLLLLPPPPRVGGFNLD*AEAPAVLSGPP*
GSFFGFSVEFYRPGTDGVSVLVGAPKANTSQPGVLQGGAVYLCPWGASPTQCTPI
EFDSKGSRLLESSLSSEGE*EEPVEYKSLQWFGATVRAHGSSILACAPLYSWRTEKE*
PLSDPVGTCYLSTDNFRILEYAPCRSDFSWAAGQGYCQGGFSAEFTKTGRVVLG
GPGSYFWQGGQILSATQE*QIAESYYPEYLINLVQGGQLQTRQASSIYDDSYLGYSVAV*
GEFSGDDT*EDFVAGV*PKGNLT*YGYVTILNGSDIRSLYNFSGEQMASYFGYAVAATD*
VNGDGLDDLLVGAPLLMDRTPDGRPQEVGRVYVYLQHPAGIEPTPTLTLTGHDEF
GRFGSSLTPLGDLDDQDGYNDVAIGAPFGGETQQGVVFVFPGGPGGLGSKPSQVL
QPLWAASHTPDFFGSALRGGRDLDGNGYPDLIVGSFGVDKAVVYRGRPIVSASAS
LTIFPAMFNPEERSCSLEGNPVACINLSFCLNASGKHVADSIGFTVELQLDWQKQK
GGVRRALFLASRQATLTQTLLIQNGAREDCREMKIYLRNESEFRDKLSPIHIALNFSL
DPQAPVDSHGLRPALHYQSKSRIEDKAQILLDCGEDNICVPDLQLEVFGEQNHVYL
GDKNALNLTFHAQNVGEGGAYEAELRVTAPPEAEYSGLVRHPGNFSSLSCDYFAV
NQSRLLVCDLGNPMKAGASLWGGLRFTVPHLRDTKKTIQFDFQILSKNLNNSQSDV
VSFRLSVEAQAQVTLNGVSKPEAVLFPVSDWHPRDQPQKEEDLGPVHHVYELIN
QGPSSISQGVLELSCPQALEGQQLLYVTRVTGLNCTTNHPINPKGLELDPEGSLHH
QKREAPSRSSASSGPQILKCPEAECFRLRCELGPLHQQESQSLQLHFRVWAKTF
LQREHQPFSLQCEAVYKALKMPYRILPRQLPQKERQVATAVQWTKAEGSYGVPLW
IIILAILFGLLLLGLLIYILYKLGFFKRSLPYGTAMEKAQLKPPATSDA (SEQ ID
 NO:111).

[0025] The full-length amino acid sequence of mouse $\alpha 5$ integrin is provided below (exemplary signal sequence = italic text; exemplary extracellular domain = underline text):

*MGSWTPRSPRSPLHAVLLRWGPRRLPPLLPLLLLLWPPPLQVGGFNLD*AEAPAVL
SGPPGSLFGFSVEFYRPGRDGVSVLVGAPKANTSQPGVLQGGAVYVCPWGTSP
QCTTIQFDSKGSRILESSLYSAKG*EEPVEYKSLQWFGATVRAHGSSILACAPLYSW*
RTEKD*PN*D*PVGTCYLSTENFRILEYAPCRSDFGSAAGQGYCQGGFSAEFTKTG*
RVVLG*G*P*GSYFWQGGQILSATQE*QISESYYPEYLINPVQGGQLQTRQASSVYDDSYLG*
YSVAVGEFSGDDT*EDFVAGV*PKGNLT*YGYVTVLNGSDIHSLYNVS*GEQMASYFGY*

AVAATDTNGDGLDDLLVGAPLLMERTADGRPQEVGRVYIYLQRPAGIDPTPTLTLT
GQDEF SRFGSSLTPLGDLDDQDGYNDVAIGAPFGGEAQGGVVFIFPGGPGGLSTKP
SQVLQPLWAAGRTPDFFGSALRGGRDLDDGNGYPDLIVGSFGVDKALVYRGRPIISA
SASLTIFPSMFNPEERSCSLEGNPVSCINLSFCLNASGKHVPNSIGFEVELQLDWQK
QKGGVRRALFLT SKQATLTQTLLIQNGAREDCREMKIYLRNESEFRDKLSPIHIALNF
SLDPKAPMDSHGLRPVLHYQSKSRIEDKAQILLDCGEDNICVPDLQLDVYGEKKHV
YLGDKNALNLTFHAQNLGEGGAYEAELRV TAPLEAEYSGLVRHPGNFSSLS CDYFA
VNQRSQLVCDLGNPMKAGTSLWGGLRFTVPHLQDTKKT IQFDFQILSKNLNNSQS
NVVSFPLSVEAQAQVSLNGVSKPEAVIFPVSDWNPQDQPQKEEDLGPVHHVYELI
NQGPSSISQGVLELSCPQALEGQQLLYVT KVTGLSNCTSNYTPNSQGLELDPETSP
HHLQKREAPGRSSTASGTQVLKCPEAKCFRLRCEFGPLHRQESRSLQLHFRVWAK
TFLQREYQPFSLQCEAVYEAL KMPYQILPRQLPQKKLQVATAVQWTKAEGSNGVP
 LWIILAILFGLLLLGLLIYVLYKLGFFKRSLPYGTAMEKAQLKPPATSDA (SEQ ID
 NO:112).

[0026] Other related $\alpha 5$ integrin polypeptides that are also encompassed by the term $\alpha 5$ integrin include fragments, derivatives (e.g., substitution, deletion, truncations, and insertion variants), fusion polypeptides, and interspecies homologs that retain $\alpha 5$ integrin activity and/or are sufficient to generate an anti- $\alpha 5$ integrin immune response. As those skilled in the art will appreciate, an $\alpha 5$ integrin binding agent (e.g., an antibody) described herein can bind to an $\alpha 5$ integrin polypeptide, an $\alpha 5$ integrin polypeptide fragment, an $\alpha 5$ integrin antigen, and/or an $\alpha 5$ integrin epitope. An epitope may be part of a larger $\alpha 5$ integrin antigen, which may be part of a larger $\alpha 5$ integrin polypeptide fragment, which, in turn, may be part of a larger $\alpha 5$ integrin polypeptide. An $\alpha 5$ integrin may exist in a native or denatured form. An $\alpha 5$ integrin polypeptide described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods. An $\alpha 5$ integrin polypeptide may comprise a polypeptide having the same amino acid sequence as a corresponding $\alpha 5$ integrin polypeptide derived from nature. Orthologs to the $\alpha 5$ integrin polypeptide are also well known in the

[0027] The term “ $\beta 1$ integrin,” “CD29,” or “ $\beta 1$ integrin polypeptide” and similar terms refers to a polypeptide (“polypeptide” and “protein” are used interchangeably

herein) or any native $\beta 1$ integrin from any vertebrate source, including mammals such as primates (e.g., humans, cynomolgus monkey (cyno), dogs, and rodents (e.g., mice and rats), unless otherwise indicated. $\beta 1$ integrin, also known in the art as Integrin beta-1, ITGB1 protein, CD29 antigen, fibronectin receptor subunit beta, and Glycoprotein IIa, has multiple domains, including $\beta 1$ headpiece, hybrid, and plexin-semaphoring-integrin (PSI) domains, four integrin-epidermal growth factor domains (I-EGF1, I-EGF2, I-EGF3, I-EGF4), β -tail, transmembrane, and intracellular domains. The term $\beta 1$ integrin encompasses “full-length,” unprocessed $\beta 1$ integrin, as well as any form of $\beta 1$ integrin or any fragment thereof that results from processing in the cell. The term $\beta 1$ integrin also encompasses naturally occurring variants of $\beta 1$ integrin, such as SNP variants, splice variants and allelic variants. A $\beta 1$ integrin in association with $\alpha 5$ as a heterodimer is known in the art to interact with a number of ligands (e.g., fibronectin) and this interaction leads to protein conformational changes and signal transduction, leading to changes in cellular activity, such as cell adhesion, proliferation, apoptosis, migration, and phagocytosis. The full-length amino acid sequence of human $\beta 1$ integrin is provided below (exemplary signal sequence = italic text; exemplary extracellular domain = underline text):

*MNLQPIFWIGLISSVCCVFAQT*DENRCLKANAKSCGECIQAGPNCGWCTNSTFLQE
GMPTSARCDDLEALKKKGCPPDDIENPRGSKDIKKNKVTNRSKGTAELKLPEDIT
QIQPQQQLVLRRLRSGEPQTFTLKFKRAEDYPIDLYYLMDSL SYMKDDLENVKSLGTDL
MNEMRRITSDFRIGFGSFVEKTVMPYISTTPAKLRNPCTSEQNCTSPFSYKNVLSLT
NKGEVFNELVGKQRISGNLDSPEGGFDAIMQVAVCGSLIGWRNVTRLLVFSTDAGF
HFAGDGKLGGIVLPNDGQCHLENNMYTMSHYDYDPSIAHLVQKLENNIQTIFAVTE
EFQPVYKELKNLIPKSAVGTL SANSSNVIQLIIDAYNSLSSEVILENGKLSEGV TISYK
SYCKNGVNGTG ENGRKCSNISIGDEVQFEISITSNKCPKKDSDFS KIRPLGFT EEEV
VILQYICECECQSEGIPE SPKCHEGNGTFECGACRCNEGRVGRHCECSTDEVNSE
DMDAYCRKENSSEICSNNGECVCGQCVC RKRDNTNEIYSGKFCECDNFNCDRSN
GLICGGNGVCKCRVCECNPNYTG SACDCSLDTSTCEASNGQICNGRGICECGVCK
CTDPKFQGGTCEMCQTCLGVCAEHKECVQCRAFNKG EKDTCTQECSYFNITKVE
SRDKLPQPVPDPVSHCKEKDVDDCWFYFTYSVNGNNEVMVHVVENPECPTGPD

IPIVAGVVAGIVLIGLALLLIWKLLMIIHDRREFAKFEKEKMNAKWDTGENPIYKSAVT
TVVNPKEYEGK (SEQ ID NO:113).

[0028] The full-length amino acid sequence of mouse $\beta 1$ integrin is provided below (exemplary signal sequence = italic text; exemplary extracellular domain = underline text):

*MNLQLVSWIGLISLICSVFGQ*TDKNRCLKANAKSCGECIQAGPNCGWCTNTTFLQE
GMPTSARCDDLEALKKKKGCQPSDIENPRGSQTIKKNKNVTNRSKGMAEKLRPEDIT
QIQPQQLLLKLRSGEPQKFTLKFRAEDYPIDLYYLMDSLYSMKDDLENVKS LGTDL
MNEMRRITSDFRIGFGSFVEKTVMPYISTTPAKLRNPCTSEQNCTSPFSYKNVLSLT
DRGEFFNELVGQQRISGNLDSPEGGFDAIMQVAVCGSLIGWRNVTRLLVFSTDAGF
HFAGDGKLG GIVLPNDGQCHLENNVYTMSHYYDYPSIAHLVQKLSENNIQTIFAVTE
EFQPVYKELKNLIPKSAVGTLSGNSSNVIQLIIDAYNSLSSEVILENSKLDPGVTINYK
SYCKNGVNGTGENGRKCSNISIGDEVQFEISITANKCPNKESETIKIKPLGFTEEEVEV
VLQFICKCNCQSHGIPASPKCHEGNGTFECGACRCNEGRVGRHCECSTDEVNSE
DMDAYCRKENSSEICSNNGECVCGQCVCRKRDNTNEIYSGKFCECDNFNCDRSN
GLICGGNGVCRRCRVCECYPNYTGSACDCSLDTGPCLASNGQICNGRGICECGACK
CTDPKFQGPTCETCQTCLGVCAEHKECVQCRAFNKGEKKDTC AQEC SHFNLT KV
ESREKLQPVPVQVDPVTHCKEKDIDDCWFYFTYSVNGNNEAIVHV VETPDCPTGPDII
PIVAGVVAGIVLIGLALLLIWKLLMIIHDRREFAKFEKEKMNAKWDTGENPIYKSAVTT
VVNPKEYEGK (SEQ ID NO:114).

[0029] The term “ $\alpha 4$ integrin,” “CD49d,” or “ $\alpha 4$ integrin polypeptide” and similar terms refers to a polypeptide (“polypeptide” and “protein” are used interchangeably herein) or any native $\alpha 4$ integrin from any vertebrate source, including mammals such as primates (e.g., humans, cynomolgus monkey (cyno)), dogs, and rodents (e.g., mice and rats), unless otherwise indicated. An $\alpha 4$ integrin, also known in the art as integrin alpha-4, ITGA4 protein, CD49d, VLA-4 subunit alpha, has multiple domains, including beta-propeller (e.g., with seven 60 amino acids FG-GAP repeats), thigh, genu, calf1, calf2, transmembrane, and intracellular domains, and also has three calcium binding sites. The term $\alpha 4$ integrin encompasses “full-length,” unprocessed $\alpha 4$ integrin, as well as any form of $\alpha 4$ integrin or any fragment thereof that results from processing in the cell. The term $\alpha 4$ integrin also encompasses

naturally occurring variants of $\alpha 4$ integrin, such as SNP variants, splice variants and allelic variants. A $\alpha 4$ integrin in association with $\beta 1$ as a heterodimer is known in the art to interact with a number of ligands (e.g., VCAM1, fibronectin) and this interaction leads to protein conformational changes and signal transduction, leading to changes in cellular activity, such as cell adhesion, proliferation, migration, and phagocytosis.

The full-length amino acid sequence of human $\alpha 4$ integrin is provided below

(exemplary signal sequence = italic text; exemplary extracellular domain = underline text):

*MAWEARREPGPRRAAVRETVMLLLCLGVPTGRPY*NVDTESALLYQGPHNTLFGYS
VVLHSHGANRWLLVGAPTANWLANASVINPGAIRCRIGKNPGQTCEQLQLGSPN
GEPCGKTCLEERDNQWLGVTLRQPGENGSIIVTCGHRWKNIFYIKNENKLPTGGC
YGVPPDLRTELSKRIAPCYQDYVKKFGENFASCQAGISSFYTKDLIVMGAPGSSYW
TGSLFVYNITTNKYKAFLDKQNQVKFGSYLGYSVGAGHFRSQHTTEVVGGAPQHE
QIGKAYIFSIDEKELNILHEMKGKLGSYFGASVCAVDLADGFSDLLVGAPMQSTIR
EEGRVFVYINSGSGAVMNAMEINLVGSDKYAARFGESIVNLGDIDNDGFEDVAIGA
PQEDDLQGAIIYNGRADGISSTFSQRIEGLQISKSLSMFGQSIGQIDADNNGYVD
VAVGAFRSDSAVLLRTRPVVVDASLSHPESVNRTKFDCVENGWPSVCIDLTLCFS
YKGKEVPGYIVLFYNMSLDVNRKAESPFRFYFSSNGTSDVITGSIQVSSREANCRT
HQAFMRKDVRDILTPIQIEAAYHLGPHVISKRSTEEFPPLQPILQQKKEKDIMKKTINF
ARFCAHENC SADLQVSAKIGFLKPHENKTYLAVGSMKTLMLNVSLFNAGDDAYETT
LHVKLVPGLYFIKILEEEKQINCEVTDNSGVVQLDCSIGYIYVDHLRSRIDISFLLDVSS
LSRAEEDLSITVHATCENEEEMDNLKHSRVTVAIPLKYEKLVHGFVNPTSFVYGS
NDENEPETCMVEKMNLTFHVINTGNSMAPNVSVEIMVPNSFSPQTDKLFNILDVQT
TTGECHFENYQRVCALEQQKSAMQTLKGIVRFLSKTDKRLLYCIKADPHCLNFLCN
FGKMESGKEASVHIQLEGRPSILEMDETSALKFEIRATGFPEPNPRVIELNKDENVA
HVLLEGLHHQRPKRYFTIVIISSSLLLGLIVLLLISYVMWKAGFFKRQYKSILQEENRR
DSWSYINSKSNDD (SEQ ID NO:115).

[0030] The term “fibronectin” or “FN” and similar terms refers to a polypeptide (“polypeptide” and “protein” are used interchangeably herein) or any native fibronectin from any vertebrate source, including mammals such as primates (e.g., humans, cynomolgus monkey (cyno)), dogs, and rodents (e.g., mice and rats), unless otherwise indicated. Fibronectin exists as a dimer or multimer linked through

disulfide bonds and has a multimodular structure composed predominantly of three different repeats termed FN-I, FN-II, and FN-III. In dimeric form, each of the two fibronectin subunits consists of 12 FN-I, 2 FN-II, and 15 to 17 FN-III modules, respectively. The term fibronectin also encompasses naturally occurring variants of fibronectin, such as SNP variants, splice variants and allelic variants. Fibronectin is an essential component of the extracellular matrix and has multiple protein-binding domains, including domains for fibrin-binding, collagen-binding, fibulin-1-binding, heparin-binding and syndecan-binding. Fibronectin is known in the art to interact (*e.g.*, via RGD) with integrins and is a ligand for $\alpha 5\beta 1$ integrin, $\alpha 8\beta 1$ integrin and $\alpha v\beta 3$ integrin. The full-length amino acid sequence of human fibronectin is provided below (exemplary signal sequence = italic text):

*MLRGP*GPGLLLLAVQCLGTAVPSTGASKSKRQAQQMVQPQSPVAVSQSKPGCYD
 NGKHYQINQQWERTYLGNALVCTCYGGSRGFNCESKPEAEETCFDKYTGNTRYV
 GDTYERPKDSMIWDCTCIGAGRGRISCTIANRCHEGGQSYKIGDTWRRPHETGGY
 MLECVCLGNGKGEWTCKPIAEKCFDHAAGTSYVVGETWEKPYQGWMMVDCTCL
 GEGSGRITCTSRNRCNDQDTRTSYRIGDTWSKKDNRGNLLQCICTGNRGEWKC
 ERHTSVQTTSSGSGPFTDVRAAVYQPQPHPQPPPYGHCVTDSGVVYSVGMQWLK
 TQGNKQMLCTCLGNGVSCQETAQTQYGGNSNGEPCVLPFTYNGRTFYSCCTEG
 RQDGHLCWSTTSNYEQDQKYSFCTDHTVLVQTRGGNSNGALCHFPFLYNNHNYT
 DCTSEGRDNDNMKWCGTTQNYDADQKFGFCPMAAHEEICTTNEGVMYRIGDQWD
 KQHDMGHMMRCTCVGNRGEWTCIAYSQLRDQCIVDDITYNVNDTFHKRHEEGH
 MLNCTCFGQGGRGRWKCDPVDQCQDSETGTFYQIGDSWEKYVHGVRYQCICYGR
 GIGEWHCQPLQTYPSSSGPVEVFITETPSQPNSHPIQWNAPQPISHISKYILRWPK
 NSVGRWKEATIPGHLNSYTIKGLKPGVVYEGQLISIQQYGHQEVTRFDFTTTSTSTP
 VTSNTVTGETTPFSPLVATSESVTEITASSFVVSWSASDTVSGFRVEYELSEEGDE
 PQYLDLPSTATSVNIPDLLPGRKYIVNVYQISEDGEQSLILSTSQTAPDAPPDTTVD
 QVDDTSIVVRWSRPQAPITGYRIVYSPSVEGSSTELNLPETANSVTLSDLQPGVQY
 NITIYAVEENQESTPVVIQQETTGTTPRSDTVSPRDLQFVEVTDVKVTIMWTPPESA
 VTGYRVDVIPVNLPGEHGQRLPISRNTFAEVTGLSPGVTTYFKVFAVSHGRESKPL
 TAQQTTKLDAPTNLQFVNEDSTVLVRWTPPRAQITGYRLTVGLTRRGQPRQYNV
 GPSVSKYPLRNLQPASEYTVSLVAIKGNQESPKATGVFTTLQPGSSIPPYNTEVTET
 TIVITWTPAPRIGFKLGVRPSQGGEAPREVTSDSGSIVVSGLTGVEYVYTIQVLRD

GQERDAPIVNKVVTPPLSPPTNLHLEANPDTGVLTVSWERSTTPDITGYRITTTPTNG
 QQGNSLEEVVHADQSSCTFDNLSPGLEYNVSVYTVKDDKESVPISDTIPEVPQLTD
 LSFVDITDSSIGLRWTPLNSSTIIGYRITVVAAGEGIPIFEDFVDSSVGYYTVTGLEPGI
 DYDISVITLINGGESAPTTLTQQTAVPPPTDLRFTNIGPDTMRVTWAPPPSIDLTNFL
 VRYSVPVKNEDVAELSPSDNAVVLTNLLPGTEYVVS SVSSVYEQHESTPLRGRQK
 TGLDSPTGIDFSDITANSFTVHWIAPRATITGYRIRHHPEHFSGRPREDRVPHSRNSI
 TLTNLTPGTEYVVSIVALNGREESPLLIGQQSTVSDVPRDLEVVAATPTSLLISWDAP
 AVTVRYRITYGETGGNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSP
 ASSKPISINYRTEIDKPSQMQVTDVQDNSISVKWLPSSSPVTGYRVTTTPKNGPGPT
 KTKTAGPDQTEMTIEGLQPTVEYVVS VYAQNPSGESQPLVQTAVTNIDRPKGLAFT
 DVDVDSIKIAWESPQQQVSRVRYTYSSPEDGIHELFPAPDGEEDTAELQGLRPGSE
 YTVSVVALHDDMESQPLIGTQSTAIPAPTDLKFQVTPPTSLSAQWTPPNVQLTGYR
 VRVTPKEKTGPMKEINLAPDSSSVVVSGLMVATKYEVSVYALKDTLTSRPAQGVVT
 TLENVSPRRRARVTDATETTITISWRTKTETITGFQVDAVPANGQTPIQRTIKPDVRS
 YTITGLQPGTDYKIYLYTLNDNARSSPVVIDASTAIDAPSNLRFLATTPNSLLVSWQP
 PRARITGYIIKYEKPGSPPREVVPRPRPGVTEATITGLEPGTEYTIYVIALKNNQKSEP
 LIGRKKTDQLVTLPHPNLHGPEILDVPSTVQKTPFVTHPGYDTGNGIQLPGTSG
 QQPSVGQQMIFEEHGFRRTPPTTATPIRHRPRPYPPNVGEEIQIGHIPREDVDYHL
 YPHGPGLNPNASTGQEALSQTTISWAPFQDTSEYIISCHPVGTDEEPLQFRVPGTS
 TSATLTGLTRGATYNVIVEALKDQQRHKVREEVTVGNSVNEGLNQPTDDSCFDPY
 TVSHYAVGDEWERMSESGFKLLCQCLGFGSGHFRCDSSRWCHDNGVNKYIGEK
 WDRQGENGQMMSCTCLGNGKGFEKCDPHEATCYDDGKTYHVGEQWQKEYLGAI
 CSCTCFGGQRGWRCDNCRRPGGEPSPGTTGQSYNQYSQRYHQRTNTNVNCPI
 ECFMPLDVQADREDSRE (SEQ ID NO:116).

[0031] As used herein, the term “binding agent” or a grammatical equivalent thereof refers to a molecule (*e.g.*, an antibody) with one or more antigen binding sites that binds an antigen. In some embodiments, an $\alpha 5 \beta 1$ integrin binding agent as described herein is an antibody, antibody fragment, or other peptide-based molecule that binds to $\alpha 5 \beta 1$ integrin, such as human $\alpha 5 \beta 1$ integrin.

[0032] The term “antibody,” “immunoglobulin,” or “Ig” is used interchangeably herein, and is used in the broadest sense and specifically covers, for example polyclonal antibodies, monoclonal antibodies (including agonist, antagonist,

neutralizing antibodies, full length monoclonal antibodies), antibody compositions with polypepitopic or monoepitopic specificity, recombinantly produced antibodies, monospecific antibodies, multispecific antibodies (including bispecific antibodies), synthetic antibodies, chimeric antibodies, humanized antibodies, or human versions of antibodies having full length heavy and/or light chains. The present disclosure also includes antibody fragments (and/or polypeptides that comprise antibody fragments) that retain $\alpha 5$ integrin binding characteristics. Non-limiting examples of antibody fragments include antigen-binding regions and/or effector regions of the antibody, *e.g.*, Fab, Fab', F(ab')₂, Fv, scFv, (scFv)₂, single chain antibody molecule, dual variable region antibody, single variable region antibody, linear antibody, V region, a multispecific antibody formed from antibody fragments, F(ab)₂, Fd, Fc, diabody, di-diabody, disulfide-linked Fvs (dsFv), single-domain antibody (*e.g.*, VHH, nanobody) or other fragments (*e.g.*, fragments consisting of the variable regions of the heavy and light chains that are non-covalently coupled),. In general terms, a variable region domain may be any suitable arrangement of immunoglobulin heavy (VH) and/or light (VL) variable regions. For example, the present disclosure also includes tetrameric antibodies comprising two heavy chain and two light chain molecules, an antibody light chain monomer, and an antibody heavy chain monomer. Thus, for example, the variable region domain may be dimeric and contain VH-VH, VH-VL, or VL-VL dimers that bind $\alpha 5$ integrin. If desired, the VH and VL chains may be covalently coupled either directly or through a linker to form a single chain Fv (scFv). For ease of reference, scFv proteins are referred to herein as included in the category "antibody fragments." Another form of an antibody fragment is a peptide comprising one or more complementarity determining regions (CDRs) of an antibody. CDRs (also termed "minimal recognition units" or "hypervariable region") can be obtained by constructing polynucleotides that encode the CDR of interest. Such polynucleotides are prepared, for example, by using the polymerase chain reaction to synthesize the variable region using mRNA of antibody-producing cells as a template (see, for example, Larrick et al., *Methods: A Companion to Methods in*

Enzymology, 2:106 (1991); Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in Monoclonal Antibodies Production, Engineering and Clinical Application, Ritter et al. (eds.), page 166, Cambridge University Press (1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in Monoclonal Antibodies: Principles and Applications, Birch et al., (eds.), page 137, Wiley-Liss, Inc. (1995)). Antibody fragments may be incorporated into single domain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, variable domains of new antigen receptors (v-NAR), and bis-single chain Fv regions (see, e.g., Hollinger and Hudson, Nature Biotechnology, 23(9):1126-1136, 2005). The binding agent, in some embodiments, contains a light chain and/or a heavy chain constant region, such as one or more constant regions, including one or more IgG1, IgG2, IgG3 and/or IgG4 constant regions. In some embodiments, antibodies can include epitope-binding fragments of any of the above. The antibodies described herein can be of any class (e.g., IgG, IgE, IgM, IgD, and IgA) or any subclass (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) of immunoglobulin molecule. Antibodies may be $\alpha 5\beta 1$ binding antibodies, including antagonistic antibodies or agonistic antibodies.

[0033] The term "monospecific" when used in reference to a binding agent (e.g., an antibody) as used herein denotes a binding agent that has one or more binding sites each of which bind to the same epitope of the same antigen.

[0034] The term "bispecific" when used in reference to a binding agent (e.g., an antibody) means that the binding agent is able to specifically bind to at least two distinct antigenic determinants, for example two binding sites each formed by a pair of an antibody heavy chain variable domain (VH) and an antibody light chain variable domain (VL) binding to different antigens or to different epitopes on the same antigen. Such a bispecific binding agent (e.g., an antibody) may have a 1+1 format. Other bispecific binding agent (e.g., an antibody) formats may be 2+1 or 1+2 formats (comprising two binding sites for a first antigen or epitope and one binding site for a second antigen or epitope) or 2+2 formats (comprising two binding sites for a first

antigen or epitope and two binding sites for a second antigen or epitope). When a bispecific binding agent (e.g., an antibody) comprises two antigen binding sites, each may bind to a different antigenic determinant. Such a bispecific binding agent (e.g., an antibody) may bind to two different epitopes on the same antigen (e.g., epitopes on $\alpha 5 \beta 1$ integrin) or on different antigens (e.g., an epitope on $\alpha 5 \beta 1$ integrin and an epitope on $\alpha \nu \beta 1$ integrin).

[0035] The terms “identical” or percent “identity” in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that can be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variants thereof. In some embodiments, two nucleic acids or polypeptides are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, 96%, 97%, 98%, 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the amino acid sequences that is at least about 10 residues, at least about 20 residues, at least about 40-60 residues, at least about 60-80 residues in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 residues, such as at least about 80-100 residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a target protein or an antibody. In some embodiments, identity exists over a region of the nucleotide sequences that is at

least about 10 bases, at least about 20 bases, at least about 40-60 bases, at least about 60-80 bases in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 bases, such as at least about 80-1000 bases or more, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as a nucleotide sequence encoding a protein of interest.

[0036] A “conservative amino acid substitution” is one in which one amino acid residue is replaced with another amino acid residue having a side chain with similar chemical characteristics. Families of amino acid residues having similar side chains have been generally defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. Generally, conservative substitutions in the sequences of the polypeptides, soluble proteins, and/or antibodies of the disclosure do not abrogate the binding of the polypeptide, soluble protein, or antibody containing the amino acid sequence, to the target binding site. Methods of identifying amino acid conservative substitutions which do not eliminate binding are well-known in the art.

[0037] The terms “polypeptide” refers to polymers of amino acids of any length. The polymer can be linear or branched, it can comprise modified amino acids, and it can include (*e.g.*, be interrupted by) non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as linkage to or conjugation with (directly or indirectly) a moiety such as a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an

amino acid (including, for example, unnatural amino acids), as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure can be based upon antibodies or other members of the immunoglobulin superfamily, in some embodiments, the polypeptides can occur as single chains.

[0038] As used herein, an “antigen” is a moiety or molecule that contains an epitope to which a binding agent (e.g., an antibody) can bind. As such, an antigen can be bound by an antibody. In some embodiments, the antigen, to which a binding agent (e.g., an antibody) described herein binds, is $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin), or a fragment thereof.

[0039] As used herein, an “epitope” is a term in the art and refers to a localized region of an antigen to which an antibody can bind. An epitope can be a linear epitope or a conformational, non-linear, or discontinuous, epitope. In the case of a polypeptide antigen, for example, an epitope can be contiguous amino acids of the polypeptide (a “linear” epitope) or an epitope can comprise amino acids from two or more non-contiguous regions of the polypeptide (a “conformational,” “non-linear” or “discontinuous” epitope), e.g., human $\alpha 5\beta 1$ integrin. It will be appreciated by one of skill in the art that, in general, a linear epitope may or may not be dependent on secondary, tertiary, or quaternary structure. For example, in some embodiments, an antibody binds to a group of amino acids regardless of whether they are folded in a natural three dimensional protein structure. In other embodiments, an antibody requires amino acid residues making up the epitope to exhibit a particular conformation (e.g., bend, twist, turn or fold) in order to recognize and bind the epitope.

[0040] An antibody binds “an epitope” or “essentially the same epitope” or “the same epitope” as a reference antibody, when the two antibodies recognize identical, overlapping or adjacent epitopes in a three-dimensional space. The most widely used and rapid methods for determining whether two antibodies bind to identical, overlapping or adjacent epitopes in a three-dimensional space are competition

assays, which can be configured in a number of different formats, for example, using either labeled antigen or labeled antibody. In some assays, the antigen is immobilized on a 96-well plate, or expressed on a cell surface, and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured using radioactive, fluorescent or enzyme labels.

[0041] “Epitope binning” is the process of grouping antibodies based on the epitopes they recognize. More particularly, epitope binning comprises methods and systems for discriminating the epitope recognition properties of different antibodies, for example, using competition assays. Such assays can be combined with computational processes for clustering antibodies based on their epitope recognition properties and identifying antibodies having distinct binding specificities.

[0042] As used herein, the terms “specifically binds,” “specifically recognizes,” “immunospecifically binds,” “selectively binds,” “immunospecifically recognizes” and “immunospecific” are analogous terms in the context of antibodies and refer to molecules that bind to an antigen (*e.g.*, epitope) as such binding is understood by one skilled in the art. In some embodiments, “specifically binds” means, for instance that a polypeptide or molecule interacts more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to the epitope, protein, or target molecule than with alternative substances, including related and unrelated proteins. For example, a molecule that specifically binds to an antigen may bind to other peptides or polypeptides, generally with lower affinity as determined by, *e.g.*, immunoassays, Biacore™, KinExA 3000 instrument (Sapidyne Instruments, Boise, ID), or other assays known in the art. In some embodiments, an antibody or antigen binding domain binds to or specifically binds to an antigen when it binds to an antigen with higher affinity than to any cross-reactive antigen as determined using experimental techniques, such as radioimmunoassays (RIA) and enzyme linked immunosorbent assays (ELISAs). Typically a specific or selective reaction will be at least twice background signal or noise and may be more than 10 times background. See, *e.g.*, Fundamental Immunology 332-36 (Paul ed., 2d ed.

1989) for a discussion regarding binding specificity. In some embodiments, the extent of binding of an antibody or antigen binding domain to a “non-target” protein is less than about 10% of the binding of the antibody or antigen binding domain to its particular target antigen, for example, as determined by fluorescence activated cell sorting (FACS) analysis or RIA. In some embodiments, molecules that specifically bind to an antigen bind to the antigen with a K_a that is at least 2 logs, 2.5 logs, 3 logs, 4 logs or greater than the K_a when the molecules bind to another antigen. In some embodiments, molecules that specifically bind to an antigen do not cross react with other proteins. In some embodiments, molecules that specifically bind to an antigen do not cross react with other non- $\alpha 5\beta 1$ integrin proteins. In some embodiments “specifically binds” means, for instance, that a polypeptide or molecule binds a protein or target with a K_D of about 0.1mM or less, but more usually less than about 1 μ M. In some embodiments, “specifically binds” means that a polypeptide or molecule binds a target with a K_D of at least about 0.1 μ M or less, at least about 0.01 μ M or less, or at least about 1nM or less. Because of the sequence identity between homologous proteins in different species, specific binding can include a polypeptide or molecule that recognizes a protein or target in more than one species. Likewise, because of homology within certain regions of polypeptide sequences of different proteins, specific binding can include a polypeptide or molecule that recognizes more than one protein or target. It is understood that, in some embodiments, a polypeptide or molecule that specifically binds a first target may or may not specifically bind a second target. As such, “specific binding” does not necessarily require (although it can include) exclusive binding, *e.g.*, binding to a single target. Thus, a polypeptide or molecule can, in some embodiments, specifically bind more than one target. In some embodiments, multiple targets can be bound by the same antigen-binding site on the polypeptide or molecule. For example, an antibody can, in certain instances, comprise two identical antigen-binding sites, each of which specifically binds the same epitope on two or more proteins. In some alternative embodiments, an antibody can be bispecific and

comprise at least two antigen-binding sites with differing specificities. Generally, but not necessarily, reference to “binding” means “specific binding”.

[0043] “Binding affinity” generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., a binding protein such as an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a binding molecule X for its binding partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present disclosure. In one embodiment, the “ K_D ” or “ K_D value” may be measured by biolayer interferometry (BLI) using, for example, the OctetQK384 system (ForteBio, Menlo Park, CA). Alternatively, the K_D may be also be measured in a radiolabeled antigen binding assay (RIA), for example, performed with the Fab version of an antibody of interest and its antigen (Chen, *et al.*, (1999) J. Mol Biol 293:865-881) or using surface plasmon resonance (SPR) assays by Biacore, using, for example, a BIAcore™-2000 or a BIAcore™-3000 (Biacore, Inc., Piscataway, NJ). An “on-rate” or “rate of association” or “association rate” or “ k_{on} ,” as well as an “off-rate” or “rate of dissociation” or “dissociation rate” or “ k_{off} ,” may can also be determined with the same SPR or BLI techniques described above using, for example, the OctetQK384 system (ForteBio, Menlo Park, CA) or a BIAcore™-2000 or a BIAcore™-3000 (Biacore, Inc., Piscataway, NJ), respectively.

[0044] The term “compete” when used in the context of $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies) means binding agents that compete for the same epitope or binding site on a target, which includes competition between such binding agents as

determined by an assay in which the binding agent under study prevents or inhibits the specific binding of a reference molecule (*e.g.*, a reference ligand, or reference antigen binding protein, such as a reference antibody) to a common antigen (*e.g.*, $\alpha 5\beta 1$ integrin). Numerous types of competitive binding assays can be used to determine if a test binding agent competes with a reference molecule for binding to $\alpha 5\beta 1$ integrin (*e.g.*, human $\alpha 5\beta 1$ integrin). Examples of assays that can be employed include solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (*see, e.g.*, Stahli et al., (1983) *Methods in Enzymology* 9:242-253); solid phase direct biotin-avidin EIA (*see, e.g.*, Kirkland et al., (1986) *J. Immunol.* 137:3614-3619) solid phase direct labeled assay, solid phase direct labeled sandwich assay (*see, e.g.*, Harlow and Lane, (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Press); solid phase direct label RIA using 1-125 label (*see, e.g.*, Morel et al., (1988) *Molec. Immunol.* 25:7-15); solid phase direct biotin-avidin EIA (*see, e.g.*, Cheung, et al., (1990) *Virology* 176:546-552); and direct labeled RIA (Moldenhauer et al., (1990) *Scand. J. Immunol.* 32:77-82). Typically, such an assay involves the use of a purified antigen (*e.g.*, $\alpha 5\beta 1$ integrin, such as human $\alpha 5\beta 1$ integrin) bound to a solid surface or cells bearing either of an unlabelled test antigen binding protein (*e.g.*, test $\alpha 5\beta 1$ integrin antibody) or a labeled reference antigen binding protein (*e.g.*, reference $\alpha 5\beta 1$ integrin antibody). Competitive inhibition may be measured by determining the amount of label bound to the solid surface or cells in the presence of the test antigen binding protein. Usually the test antigen binding protein is present in excess. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and/or antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference for antibodies steric hindrance to occur (*e.g.*, similar epitope or overlapping epitope). Additional details regarding methods for determining competitive binding are described herein, as shown in Example 6. Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a

common antigen by at least 20%, for example, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% or 75%. In some instance, binding is inhibited by at least 80%, 85%, 90%, 95%, 96% or 97%, 98%, 99% or more.

[0045] As used herein, the term “constant region” or “constant domain” is a well-known antibody term of art and refers to an antibody portion, *e.g.*, for example, a carboxyl terminal portion of a light and/or heavy chain which is not directly involved in binding of an antibody to antigen but which can exhibit various effector functions, such as interaction with the Fc receptor. The term include the portion of an immunoglobulin molecule having a generally more conserved amino acid sequence relative to an immunoglobulin variable domain.

[0046] Antibody “effector functions” refer to those biological activities attributable to the Fc region (*e.g.*, a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor); and B cell activation.

[0047] The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including, for example, native sequence Fc regions, recombinant Fc regions, and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is often defined to stretch from an amino acid residue at position Cys226 (according to the EU numbering system), or from Pro230 (according to the EU numbering system), to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. An exemplary Fc region sequence is provided below (CH2 domain = bold text; CH3 domain = underline text):

CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSP
GK (SEQ ID NO:133).

[0048] A “functional Fc region” possesses an “effector function” of a native sequence Fc region. Exemplary “effector functions” include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding region or binding domain (*e.g.*, an antibody variable region or domain) and can be assessed using various assays as disclosed.

[0049] A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature, and not manipulated, modified, and/or changed (*e.g.*, isolated, purified, selected, including or combining with other sequences such as variable region sequences) by a human. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof. Exemplary IgG1 and IgG4 Fc sequences are shown in **FIG. 3**.

[0050] A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, (*e.g.*, substituting, addition, or deletion) preferably one or more amino acid substitution(s). In some embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, for example, from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in

a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region described herein can possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, or at least about 90% homology therewith, for example, at least about 95% homology therewith. The variant Fc region herein described herein may have a loss of effector function (e.g., silent Fc). An exemplary variant Fc region ("silent Fc") sequence is provided below (CH2 domain = bold text with amino acid changes underlined; CH3 domain = underline text):

CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP

GK (SEQ ID NO:134). Exemplary variant Fc sequences are shown in **FIG. 3**, including the following variants (according to the EU numbering system): N297A/Q (N297A or N297Q), LALA (L234A, L235A), LALAPS (L234A, L235A, P331S), LALAPG (L234A, L235A, P329G), and TM (L234F, L235E, P331S).

[0051] As used herein, the term "heavy chain" when used in reference to an antibody refers to a polypeptide chain of about 50-70 kDa, wherein the amino-terminal portion includes a variable region of about 120 to 130 or more amino acids, and a carboxy-terminal portion includes one or more constant regions. The "heavy chain" can refer to any distinct types, e.g., for example, alpha (α), delta (δ), epsilon (ϵ), gamma (γ) and mu (μ), based on the amino acid sequence of the constant domain, which give rise to IgA, IgD, IgE, IgG and IgM classes of antibodies, respectively, including subclasses of IgG, e.g., IgG1, IgG2, IgG3 and IgG4.

[0052] As used herein, the term "light chain" when used in reference to an antibody can refer to a polypeptide chain of about 25 kDa, wherein the amino-terminal portion includes a variable region of about 100 to about 110 or more amino acids, and a carboxy-terminal portion includes a constant region. The approximate length of a light chain is 211 to 217 amino acids. There are two distinct types, e.g.,

kappa (κ) of lambda (λ) based on the amino acid sequence of the constant domains. Light chain amino acid sequences are well known in the art.

[0053] The terms “antigen binding fragment,” “antigen binding domain,” “antigen binding region,” and similar terms refer to that portion of an antibody, which comprises the amino acid residues that interact with an antigen and confer on the binding fragment, domain, or region its specificity and affinity for the antigen (*e.g.*, the CDRs). “Antigen binding fragment” as used herein include “antibody fragment,” which comprise a portion of an antibody including one or more CDRs, such as the antigen binding or variable region of the antibody.

[0054] Antibodies described herein include, but are not limited to, synthetic antibodies, monoclonal antibodies, recombinantly produced antibodies, multispecific antibodies (*e.g.*, including bispecific antibodies), human antibodies, humanized antibodies, chimeric antibodies, intrabodies, single-chain Fvs (scFv) (*e.g.*, including monospecific, bispecific, etc.), camelized antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

[0055] In some embodiments, antibodies described herein include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, including molecules that contain one or more antigen binding sites that bind to an $\alpha 5\beta 1$ integrin antigen.

[0056] Antibodies can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA or IgY), any class, (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2), or any subclass (*e.g.*, IgG2a or IgG2b) of immunoglobulin molecule. In some embodiments, antibodies described herein are IgG antibodies (*e.g.*, human IgG), or a class (*e.g.*, human IgG1, IgG2, IgG3 or IgG4) or subclass thereof.

[0057] In some embodiments, an antibody is a 4-chain antibody unit comprising two heavy (H) chain / light (L) chain pairs, wherein the amino acid sequences of the H chains are identical and the amino acid sequences of the L chains are identical. In some embodiments, the H and L chains comprise constant regions, for example,

human constant regions. In some embodiments, the L chain constant region of such antibodies is a kappa or lambda light chain constant region, for example, a human kappa or lambda light chain constant region. In some embodiments, the H chain constant region of such antibodies comprise a gamma heavy chain constant region, for example, a human gamma heavy chain constant region. In some embodiments, such antibodies comprise IgG constant regions, for example, human IgG constant regions (e.g., IgG1, IgG2, IgG3, and/or IgG4 constant regions).

[0058] An antibody or fragment thereof may preferentially bind to $\alpha 5\beta 1$ integrin, such as human $\alpha 5\beta 1$ integrin, meaning that the antibody or fragment thereof binds $\alpha 5\beta 1$ integrin with greater affinity than it binds to an unrelated control protein and/or binds human $\alpha 5\beta 1$ integrin with greater affinity than it binds to an unrelated control protein. For example, the antibody or fragment thereof may specifically recognize and bind $\alpha 5\beta 1$ integrin or a portion thereof. "Specific binding" indicates that the antibody or fragment thereof binds to $\alpha 5\beta 1$ integrin with an affinity that is at least 5, 10, 15, 20, 25, 50, 100, 250, 500, 1000, or 10,000 times greater than the affinity for an unrelated control protein (e.g., hen egg white lysozyme). In some embodiments, the antibody or fragment thereof may bind $\alpha 5\beta 1$ integrin substantially exclusively (e.g., is able to distinguish $\alpha 5\beta 1$ integrin from other known polypeptides, for example, by virtue of measurable differences in binding affinity). In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) may react with $\alpha 5\beta 1$ integrin sequences other than human $\alpha 5\beta 1$ integrin sequences (e.g., cynomolgous $\alpha 5\beta 1$ integrin sequences).

[0059] The term "variable region" or "variable domain" refers to a portion of the light or heavy chains of an antibody that is generally located at the amino-terminal of the light or heavy chain and has a length of about 120 to 130 amino acids in the heavy chain and about 100 to 110 amino acids in the light chain, and are used in the binding and specificity of each particular antibody for its particular antigen. The variable region of the heavy chain may be referred to as "VH." The variable region of the light chain may be referred to as "VL." The term "variable" refers to the fact that

certain segments of the variable regions differ extensively in sequence among antibodies. The V region mediates antigen binding and defines specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the approximately 110-amino acid span of the variable regions. Instead, the V regions consist of less variable (*e.g.*, relatively invariant) stretches called framework regions (FRs) of about 15-30 amino acids separated by shorter regions of greater variability (*e.g.*, extreme variability) called “hypervariable regions” or alternatively called “complementarity determining regions.” The variable regions of heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4), largely adopting a β sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see, *e.g.*, Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991)). The constant regions are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). The variable regions differ extensively in sequence between different antibodies. The variability in sequence is concentrated in the CDRs while the less variable portions in the variable region are referred to as framework regions (FR). The CDRs of the light and heavy chains are primarily responsible for the interaction of the antibody with antigen. In specific embodiments, the variable region is a human variable region.

[0060] The term “hypervariable region,” “HVR,” “HV,” “complementarity determining region,” or “CDR” when used herein refers to the regions of an antibody variable region that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). A number of hypervariable region

delineations are in use and are encompassed herein. The Kabat CDRs are based on sequence variability and are the most commonly used (see, e.g., Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (see, e.g., Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software (see, e.g., Martin, in Antibody Engineering, Vol. 2, Chapter 3, Springer Verlag). The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these hypervariable regions or CDRs are noted below.

[0061] A universal numbering system has been developed and widely adopted, ImMunoGeneTics (IMGT) Information System® (Lefranc *et al.*, Dev. Comp. Immunol. 27(1):55-77 (2003)). IMGT is an integrated information system specializing in immunoglobulins (IG), T cell receptors (TR) and major histocompatibility complex (MHC) of human and other vertebrates. Herein, the CDRs are referred to in terms of both the amino acid sequence and the location within the light or heavy chain. As the "location" of the CDRs within the structure of the immunoglobulin variable domain is conserved between species and present in structures called loops, by using numbering systems that align variable domain sequences according to structural features, CDR and framework residues and are readily identified. This information can be used in grafting and replacement of CDR residues from immunoglobulins of one species into an acceptor framework from, typically, a human antibody. An additional numbering system (AHon) has been developed by Honegger and

Plückthun, *J. Mol. Biol.* 309: 657-670 (2001). Correspondence between the numbering system, including, for example, the Kabat numbering and the IMGT unique numbering system, is well known to one skilled in the art (see, e.g., Kabat, *supra*; Chothia and Lesk, *supra*; Martin, *supra*; Lefranc *et al.*, *supra*) and is also illustrated below. An Exemplary system, shown herein, combines Kabat and Chothia.

	Exemplary	IMGT	Kabat	AbM	Chothia	Contact
V_H CDR1	26-35	27-38	31-35	26-35	26-32	30-35
V_H CDR2	50-65	56-65	50-65	50-58	52a/53-55	47-58
V_H CDR3	95-102	105-117	95-102	95-102	96-101	93-101
V_L CDR1	24-34	27-38	24-34	24-34	26-32	30-36
V_L CDR2	50-56	56-65	50-56	50-56	50-52	46-55
V_L CDR3	89-97	105-117	89-97	89-97	91-96	89-96

[0062] Hypervariable regions may comprise “extended hypervariable regions” as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 or 26-35A (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. As used herein, the terms “hypervariable region,” “HVR,” “HV,” “complementarity determining region,” or “CDR” are used interchangeably.

[0063] The term “vector” refers to a substance that is used to carry or include a nucleic acid sequences, including for example, in order to introduce a nucleic acid sequence into a host cell. Vectors applicable for use include, for example, expression vectors, plasmids, phage vectors, viral vectors, episomes and artificial chromosomes, which can include selection sequences or markers operable for stable integration into a host cell’s chromosome. Additionally, the vectors can include one or more selectable marker genes and appropriate expression control sequences. Selectable marker genes that can be included, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Expression control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like which are well known in the art. When two or more nucleic acid molecules are to be co-expressed (e.g., both an antibody heavy and light chain

or an antibody VH and VL) both nucleic acid molecules can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding nucleic acids can be operationally linked to one common expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter. The introduction of nucleic acid molecules into a host cell can be confirmed using methods well known in the art. Such methods include, for example, nucleic acid analysis such as Northern blots or polymerase chain reaction (PCR) amplification of mRNA, or immunoblotting for expression of gene products, or other suitable analytical methods to test the expression of an introduced nucleic acid sequence or its corresponding gene product. It is understood by those skilled in the art that the nucleic acid molecules are expressed in a sufficient amount to produce a desired product (e.g., an $\alpha 5\beta 1$ integrin binding agent as described herein), and it is further understood that expression levels can be optimized to obtain sufficient expression using methods well known in the art.

[0064] An “ $\alpha 5\beta 1$ integrin-mediated disease” and “ $\alpha 5\beta 1$ integrin-mediated disorder” and “ $\alpha 5\beta 1$ integrin-mediated condition” are used interchangeably and refer to any disease, disorder or condition that is completely or partially caused by or is the result of $\alpha 5\beta 1$ integrin or the interaction of $\alpha 5\beta 1$ integrin with fibronectin and/or alternatively any disease, disorder, or condition in which it is desirable to inhibit the *in vivo* effects of the interaction of $\alpha 5\beta 1$ integrin with fibronectin. An $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition includes a cancer, an angiogenesis-mediated disease (e.g., a disease with abnormal angiogenesis), and an inflammatory disease (e.g., a neuroinflammatory disease, including MS and ALS). In some embodiments, an $\alpha 5\beta 1$ integrin-mediated disease includes a disease, disorder or condition that is a cancer that is characterized by or associated with tumor cells that express or overexpress an $\alpha 5\beta 1$ integrin. In some embodiments, an $\alpha 5\beta 1$ integrin-mediated disease includes a disease, disorder or condition that is characterized by or associated with abnormally increased angiogenic activity of cells (e.g., tumor

cells). In some embodiments, an $\alpha 5\beta 1$ integrin-mediated disease is a disease, disorder or condition that is specifically associated with abnormal angiogenesis (e.g., an ocular disease such as diabetic retinopathy or age-induced macular degeneration). In some embodiments, an $\alpha 5\beta 1$ integrin-mediated disease includes a disease, disorder or condition that is an inflammatory disease that is characterized by or associated with an inflammatory immune response (e.g., an inflammatory autoimmune disease such as multiple sclerosis). In some embodiments, an $\alpha 5\beta 1$ integrin-mediated disease includes a disease, disorder or condition that is a neuroinflammatory disease that is characterized by or associated with neurodegeneration (e.g., MS or ALS).

[0065] An “effective amount” is generally an amount sufficient to reduce the severity and/or frequency of one or more symptoms, eliminate the one or more symptoms and/or underlying cause, prevent the occurrence of one or more symptoms and/or their underlying cause, and/or improve or remediate the damage that results from or is associated with a disease, disorder, or condition. In some embodiments, the effective amount is a therapeutically effective amount or a prophylactically effective amount.

[0066] The term “therapeutically effective amount” as used herein refers to the amount of an agent (e.g., an antibody described herein or any other agent described herein) that is sufficient to reduce and/or ameliorate the severity and/or duration of a given disease, disorder or condition, and/or a symptom related thereto. A therapeutically effective amount of an agent, including a therapeutic agent, can be an amount necessary for (i) reduction or amelioration of the advancement or progression of a given disease, disorder, or condition, (ii) reduction or amelioration of the recurrence, development or onset of a given disease, disorder or conditions, and/or (iii) to improve or enhance the prophylactic or therapeutic effect of another therapy (e.g., a therapy other than the administration of an $\alpha 5\beta 1$ integrin binding agent such as an antibody described herein). A “therapeutically effective amount” of a substance/molecule/agent of the present disclosure (e.g., an $\alpha 5\beta 1$ integrin

antibody) may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule/agent, to elicit a desired response in the individual. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of the substance/molecule/agent are outweighed by the therapeutically beneficial effects. In some embodiments, the term “therapeutically effective amount” refers to an amount of an antibody or other agent (e.g., or drug) effective to “treat” a disease, disorder, or condition, in a subject or mammal.

[0067] A “prophylactically effective amount” is an amount of a pharmaceutical composition that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of a disease, disorder or condition, or reducing the likelihood of the onset (or reoccurrence) of a disease, disorder, or condition or associated symptom(s). The full therapeutic or prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically or prophylactically effective amount may be administered in one or more administrations.

[0068] The term “pharmaceutically acceptable” as used herein means being approved by a regulatory agency of the Federal or a state government, or listed in the U.S. Pharmacopeia, European Pharmacopeia or other generally recognized Pharmacopeia for use in animals, and more particularly in humans.

[0069] “Carriers” as used herein include carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the carrier is an aqueous pH buffered solution. Examples of carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (e.g., less than about 10 amino acid residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans;

chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™. The term “carrier” can also refer to a diluent, adjuvant (e.g., Freund’s adjuvant (complete or incomplete)), excipient, or vehicle with which the therapeutic is administered. Such carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a exemplary carrier when a composition (e.g., a pharmaceutical composition) is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable excipients (e.g., pharmaceutical excipients) include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. Compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral compositions, including formulations, can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable carriers are described in Remington’s Pharmaceutical Sciences (1990) Mack Publishing Co., Easton, PA. Compositions, including pharmaceutical compounds, may contain a prophylactically or therapeutically effective amount of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), for example, in isolated or purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject (e.g., patient). The formulation should suit the mode of administration.

[0070] In some embodiments, the present disclosure provides $\alpha 5\beta 1$ integrin binding agents that can be used herein as therapeutic agents. Such agents include antibodies (e.g., monospecific or multispecific, including bispecific) that bind to $\alpha 5\beta 1$

integrin. Exemplary antibodies include polyclonal, monoclonal, humanized, human, bispecific, and heteroconjugate antibodies, as well as variants thereof having increased or decreased affinity or other properties.

[0071] In some embodiments, described herein are $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies) that bind to $\alpha 5\beta 1$ integrin, including an $\alpha 5\beta 1$ integrin polypeptide, an $\alpha 5\beta 1$ integrin polypeptide fragment, an $\alpha 5\beta 1$ integrin peptide or an $\alpha 5\beta 1$ integrin epitope. In some embodiments, the $\alpha 5\beta 1$ integrin binding agents are human, humanized, or chimeric antibodies (e.g., comprising human constant regions) that bind $\alpha 5\beta 1$ integrin, including an $\alpha 5$ integrin polypeptide, an $\alpha 5$ integrin polypeptide fragment, an $\alpha 5$ integrin peptide or an $\alpha 5$ integrin epitope. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), such as a human $\alpha 5\beta 1$ integrin binding agent, can bind to $\alpha 5\beta 1$ integrin expressed on the surface of a mammalian (e.g., human) cell, including an $\alpha 5\beta 1$ integrin expressing tumor cell. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) binds an $\alpha 5\beta 1$ integrin extracellular epitope exposed on a cell such as a tumor cell (e.g., an $\alpha 5\beta 1$ integrin epitope). In some embodiments, described herein is an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) that binds to $\alpha 5\beta 1$ integrin, such as human $\alpha 5\beta 1$ integrin or portions thereof. In some embodiments, $\alpha 5\beta 1$ integrin is a human $\alpha 5\beta 1$ integrin. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent is a human $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody that binds to human $\alpha 5\beta 1$ integrin). An exemplary amino acid sequence of human $\alpha 5$ integrin and of human $\beta 1$ integrin is described herein.

[0072] In some embodiments, the $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies) described herein compete for the binding to $\alpha 5\beta 1$ integrin, such as human $\alpha 5\beta 1$ integrin, with an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) that comprises a VH region, VL region, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 of any one of the antibodies described herein, such as an amino acid sequence of a VH region, VL region, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 as set forth in Tables 1-6. Accordingly, in some

embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) described herein competes for the binding to $\alpha 5\beta 1$ integrin, such as human $\alpha 5\beta 1$ integrin, with an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) that comprises one, two, and/or three VH CDRs and/or one, two, and/or three VL CDRs from: (a) the antibody designated A-15B08; (b) the antibody designated A2-3B06; (c) the antibody designated A2-5D10; (d) the antibody designated A2-7A05; (e) the antibody designated A2-7F01; or (f) the antibody designated C-14D12, as shown in Tables 1-6. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) described herein competes for the binding to $\alpha 5\beta 1$ integrin, such as human $\alpha 5\beta 1$ integrin, with an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) that comprises one, two, and/or three VH CDRs and one, two, and/or three VL CDRs from: a) the antibody designated A-15B08; (b) the antibody designated A2-3B06; (c) the antibody designated A2-5D10; (d) the antibody designated A2-7A05; (e) the antibody designated A2-7F01; or (f) the antibody designated C-14D12, as shown in Tables 1-6. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) described herein competes for the binding to $\alpha 5\beta 1$ integrin, such as human $\alpha 5\beta 1$ integrin, with an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) that comprises a VH region and VL region from: a) the antibody designated A-15B08; (b) the antibody designated A2-3B06; (c) the antibody designated A2-5D10, (d) the antibody designated A2-7A05, (e) the antibody designated A2-7F01, or (f) the antibody designated C-14D12, as shown in Tables 1-6. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) described herein competes for the binding to $\alpha 5\beta 1$ integrin, such as human $\alpha 5\beta 1$ integrin, with an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) that comprises: (a) a VH region comprising the amino acid sequence of SEQ ID NO:25 or humanized variant thereof and a VL region comprising the amino acid sequence of SEQ ID NO:26 or humanized variant thereof; (b) a VH region comprising the amino acid sequence of SEQ ID NO:42 or humanized variant thereof and a VL region comprising the amino acid sequence of SEQ ID NO:43 or humanized variant thereof; (c) a VH region comprising the amino acid sequence of SEQ ID NO:51 or humanized

variant thereof and a VL region comprising the amino acid sequence of SEQ ID NO:52 or humanized variant thereof; (d) a VH region comprising the amino acid sequence of SEQ ID NO:77 or humanized variant thereof and a VL region comprising the amino acid sequence of SEQ ID NO:78 or humanized variant thereof; (e) a VH region comprising the amino acid sequence of SEQ ID NO:91 or humanized variant thereof and a VL region comprising the amino acid sequence of SEQ ID NO:92 or humanized variant thereof, or (f) a VH region comprising the amino acid sequence of SEQ ID NO:109 or humanized variant thereof and a VL region comprising the amino acid sequence of SEQ ID NO:110 or humanized variant thereof.

[0073] In some embodiments, the $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies) described herein comprise a VH region, VL region, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 of any one of the antibodies described herein, such as an amino acid sequence of a VH region, VL region, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 as set forth in Tables 1-6.

Accordingly, in some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) described herein comprises one, two, and/or three heavy chain CDRs and/or one, two, and/or three light chain CDRs from: (a) the antibody designated A-15B08; (b) the antibody designated A2-3B06; (c) the antibody designated A2-5D10; (d) the antibody designated A2-7A05; (e) the antibody designated A2-7F01; or (f) the antibody designated C-14D12, as shown in Tables 1-6. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) described herein comprises one, two, and/or three heavy chain CDRs and one, two, and/or three light chain CDRs from: (a) the antibody designated A-15B08; (b) the antibody designated A2-3B06; (c) the antibody designated A2-5D10; (d) the antibody designated A2-7A05; (e) the antibody designated A2-7F01; or (f) the antibody designated C-14D12, as shown in Tables 1-6.

[0074] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) comprises a VH region, which comprises VH CDR1, VH CDR2, and/or VH CDR3,

and a VL region, which comprises VL CDR1, VL CDR2, and/or VL CDR3, of any one of the binding agents described herein (see, e.g., Table 1, Table 2, Table 3, Table 4, Table 5, Table 6). Accordingly, in some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) described herein comprises one, two, and/or three heavy chain CDRs (e.g., VH CDR1, VH CDR2, and/or VH CDR3) and/or one, two, and/or three light chain CDRs (e.g., VL CDR1, VL CDR2, and/or VL CDR3) from Table 1. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent described herein comprises one, two, and/or three heavy chain CDRs (e.g., VH CDR1, VH CDR2, and/or VH CDR3) and/or one, two, and/or three light chain CDRs (e.g., VL CDR1, VL CDR2, and/or VL CDR3) from Table 2. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) described herein comprises one, two, and/or three heavy chain CDRs (e.g., VH CDR1, VH CDR2, and/or VH CDR3) and/or one, two, and/or three light chain CDRs (e.g., VL CDR1, VL CDR2, and/or VL CDR3) from Table 3. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent described herein comprises one, two, and/or three heavy chain CDRs (e.g., VH CDR1, VH CDR2, and/or VH CDR3) and/or one, two, and/or three light chain CDRs (e.g., VL CDR1, VL CDR2, and/or VL CDR3) from Table 4. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent described herein comprises one, two, and/or three heavy chain CDRs (e.g., VH CDR1, VH CDR2, and/or VH CDR3) and/or one, two, and/or three light chain CDRs (e.g., VL CDR1, VL CDR2, and/or VL CDR3) from Table 5. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent described herein comprises one, two, and/or three heavy chain CDRs (e.g., VH CDR1, VH CDR2, and/or VH CDR3) and/or one, two, and/or three light chain CDRs (e.g., VL CDR1, VL CDR2, and/or VL CDR3) from Table 6. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) described herein is multispecific (e.g., bispecific) and comprises a first binding domain that comprises one, two, and/or three heavy chain CDRs (e.g., VH CDR1, VH CDR2, and/or VH CDR3) and/or one, two, and/or three light chain CDRs (e.g., VL CDR1, VL CDR2, and/or VL CDR3) from Table 1, Table 2, Table 3, Table 4, Table 5, or Table 6, and a second binding domain that comprises one, two, and/or

three heavy chain CDRs (e.g., VH CDR1, VH CDR2, and/or VH CDR3) and/or one, two, and/or three light chain CDRs (e.g., VL CDR1, VL CDR2, and/or VL CDR3) from a binding agent that binds to a second target antigen that is not $\alpha 5\beta 1$ integrin (e.g., $\alpha \nu \beta 3$ integrin, $\alpha 4\beta 1$ integrin, $\alpha 4\beta 7$ integrin, TREM2, TNF α , IL-6, IL-1 β , CSF1, CSF-1R, C1Q, CD40L, FGFR, IL-12, and Type I interferons).

[0075] The antibody designated A-15B08 comprises CDR sequences according to Kabat and/or Chothia, AbM, Contact, or IMGT as shown in Table 1 and in some embodiments can comprise a VH sequence that is SEQ ID NO:25 or a humanized variant thereof and a VL sequence that is SEQ ID NO:26 or a humanized variant thereof.

[0076] The antibody designated A2-3B06 comprises CDR sequences according to Kabat and/or Chothia, AbM, Contact, or IMGT as shown in Table 2 and in some embodiments can comprise a VH sequence that is SEQ ID NO:42 or a humanized variant thereof and a VL sequence that is SEQ ID NO:43 or a humanized variant thereof.

[0077] The antibody designated A2-5D10 comprises CDR sequences according to Kabat and/or Chothia, AbM, Contact, or IMGT as shown in Table 3 and in some embodiments can comprise a VH sequence that is SEQ ID NO:51 or a humanized variant thereof and a VL sequence that is SEQ ID NO:52 or a humanized variant thereof.

[0078] The antibody designated A2-7A05 comprises CDR sequences according to Kabat and/or Chothia, AbM, Contact, or IMGT as shown in Table 4 and in some embodiments can comprise a VH sequence that is SEQ ID NO:77 or a humanized variant thereof and a VL sequence that is SEQ ID NO:78 or a humanized variant thereof.

[0079] The antibody designated A2-7F01 comprises CDR sequences according to Kabat and/or Chothia, AbM, Contact, or IMGT as shown in Table 5 and in some embodiments can comprise a VH sequence that is SEQ ID NO:91 or a humanized

variant thereof and a VL sequence that is SEQ ID NO:92 or a humanized variant thereof.

[0080] The antibody designated C-14D12 comprises CDR sequences according to Kabat and/or Chothia, AbM, Contact, or IMGT as shown in Table 6 and in some embodiments can comprise a VH sequence that is SEQ ID NO:109 or a humanized variant thereof and a VL sequence that is SEQ ID NO:110 or a humanized variant thereof.

Table 1: Antibody A-15B08

		Exemplary	IMGT	Kabat	Chothia	Contact	AbM
VH CDR Seq.	VH CDR1	GFSLTSYGVH (SEQ ID NO:1)	GFSLTSYG (SEQ ID NO:7)	SYGVH (SEQ ID NO:12)	GFSLTSY (SEQ ID NO:13)	TSYGVH (SEQ ID NO:18)	GFSLTSYGVH (SEQ ID NO:1)
	VH CDR2	VIWSDGSTTYS TLKS (SEQ ID NO:2)	IWSDGST (SEQ ID NO:8)	VIWSDGSTTYS TLKS (SEQ ID NO:2)	SDG (SEQ ID NO:14)	WLVIWSDGSTT (SEQ ID NO:19)	VIWSDGSTT (SEQ ID NO:24)
	VH CDR3	HYDYDGDWFAY (SEQ ID NO:3)	ARHYDYDGDWF AY (SEQ ID NO:9)	HYDYDGDWFAY (SEQ ID NO:3)	YDYDGDWFA (SEQ ID NO:15)	ARHYDYDGDWF A (SEQ ID NO:20)	HYDYDGDWFAY (SEQ ID NO:3)
VL CDR Seq.	VL CDR1	TASSRVSSNSLH (SEQ ID NO:4)	SRVSSNS (SEQ ID NO:10)	TASSRVSSNSLH (SEQ ID NO:4)	SSRVSSNS (SEQ ID NO:16)	SSNSLHWY (SEQ ID NO:21)	TASSRVSSNSLH (SEQ ID NO:4)
	VL CDR2	STSNLAS (SEQ ID NO:5)	STS (SEQ ID NO:11)	STSNLAS (SEQ ID NO:5)	STS (SEQ ID NO:11)	LWYSTSNLA (SEQ ID NO:22)	STSNLAS (SEQ ID NO:5)
	VL CDR3	HQYLRSPT (SEQ ID NO:6)	HQYLRSPT (SEQ ID NO:6)	HQYLRSPT (SEQ ID NO:6)	YLRSP (SEQ ID NO:17)	HQYLRSPT (SEQ ID NO:23)	HQYLRSPT (SEQ ID NO:6)
VH Sequence: QVQLKESGPGGLVAPSQLSITCTISGFSLTSYGVHWRQPPGKGLEWLVIWSDGSTTYNSTLKSRLSISKDNSKSQVFFKMNSLQTDDTAMY YCARHYDYDGDWFAYWGQGLTVSA (SEQ ID NO:25)							
VL Sequence: QIVLTQSPAIMSASLGERVTMTCTASSRVSSNSLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGGTYSYLTSSMEAEADAATYYCHQYL RSPPTFGGKLEIK (SEQ ID NO:26)							

NST in VH CDR2 is alternatively NSA or X₁ SX₂, wherein X₁ is N, Q, S, or A and/or X₂ is A or T

Table 2: Antibody A2-3B06

		Exemplary	IMGT	Kabat	Chothia	Contact	AbM
VH CDR Seq.	VH CDR1	GFSLTTYGVH (SEQ ID NO:27)	GFSLTYG (SEQ ID NO:31)	TYGVH (SEQ ID NO:34)	GFSLTTY (SEQ ID NO:35)	TTYGVH (SEQ ID NO:38)	GFSLTTYGVH (SEQ ID NO:27)
	VH CDR2	VIWSDGSTTYS ALKS (SEQ ID NO:28)	IWSDGST (SEQ ID NO:8)	VIWSDGSTTYS ALKS (SEQ ID NO:28)	SDG (SEQ ID NO:14)	WLVIWSDGSTT (SEQ ID NO:19)	VIWSDGSTT (SEQ ID NO:24)
	VH CDR3	HGGLLRDAMD Y (SEQ ID NO:29)	VRHGGLLRDA MDY (SEQ ID NO:32)	HGGLLRDAMD Y (SEQ ID NO:29)	GGLLRDAMD (SEQ ID NO:36)	VRHGGLLRDA MD (SEQ ID NO:39)	HGGLLRDAMD Y (SEQ ID NO:29)
VL CDR Seq.	VL CDR1	TASSSVSSNSFH (SEQ ID NO:30)	SSVSSNS (SEQ ID NO:33)	TASSSVSSNSFH (SEQ ID NO:30)	SSSVSSNS (SEQ ID NO:37)	SSNSFWY (SEQ ID NO:40)	TASSSVSSNSFH (SEQ ID NO:30)
	VL CDR2	STSNLAS (SEQ ID NO:5)	STS (SEQ ID NO:11)	STSNLAS (SEQ ID NO:5)	STS (SEQ ID NO:11)	LMLYSTSNLA (SEQ ID NO:41)	STSNLAS (SEQ ID NO:5)
	VL CDR3	HQYLRSPT (SEQ ID NO:6)	HQYLRSPT (SEQ ID NO:6)	HQYLRSPT (SEQ ID NO:6)	YLRSP (SEQ ID NO:17)	HQYLRSPT (SEQ ID NO:23)	HQYLRSPT (SEQ ID NO:6)
VH Sequence: QVQLKESGPGLVAPSQLSITCTISGFSLTTYGVHWRQPPGKGLEWLVIIWSDGSTTYSALKSRNLNITKDNSKQVFLKMNSLQTDITAMY YCVRHGGLLRDAMDYWGQGTSTVSS (SEQ ID NO:42)							
VL Sequence: QIVLTQSPAIMSASLGERVTMTCTASSSVSSNSFWYQQKPGSPKLVLYSTSNLASGVPARFSGSGGTSYSLTISSMEAEADAATYYCHQY LRSPPTFGGGTKLEIK (SEQ ID NO:43)							

Table 3: Antibody A2-5D10

		Exemplary	IMGT	Kabat	Chothia	Contact	AbM
VH CDR Seq.	VH CDR1	GFSLTSGVH (SEQ ID NO:1)	GFSLTSG (SEQ ID NO:7)	SYGVH (SEQ ID NO:12)	GFSLTSG (SEQ ID NO:13)	TSYGVH (SEQ ID NO:18)	GFSLTSGVH (SEQ ID NO:1)
	VH CDR2	VIWSDGSTTNS TLKS (SEQ ID NO:2)	IWSDGST (SEQ ID NO:8)	VIWSDGSTTNS TLKS (SEQ ID NO:2)	SDG (SEQ ID NO:14)	WLVIWSDGSTT (SEQ ID NO:19)	VIWSDGSTT (SEQ ID NO:24)
	VH CDR3	HYDYGDFWFA (SEQ ID NO:3)	ARHYDYDGDWF AY (SEQ ID NO:9)	HYDYGDFWFA (SEQ ID NO:3)	YDYGDFWFA (SEQ ID NO:15)	ARHYDYDGDWF A (SEQ ID NO:20)	HYDYGDFWFA (SEQ ID NO:3)
VL CDR Seq.	VL CDR1	TASSSVSSRCLH (SEQ ID NO:44)	SSVSSRC (SEQ ID NO:46)	TASSSVSSRCLH (SEQ ID NO:44)	SSSVSSRC (SEQ ID NO:47)	SSRCLHWY (SEQ ID NO:49)	TASSSVSSRCLH (SEQ ID NO:44)
	VL CDR2	STSNLAS (SEQ ID NO:5)	STS (SEQ ID NO:11)	STSNLAS (SEQ ID NO:5)	STS (SEQ ID NO:11)	LWYSTSNLA (SEQ ID NO:22)	STSNLAS (SEQ ID NO:5)
	VL CDR3	HQYYRSPPT (SEQ ID NO:45)	HQYYRSPPT (SEQ ID NO:45)	HQYYRSPPT (SEQ ID NO:45)	YYRSPPT (SEQ ID NO:48)	HQYYRSPPT (SEQ ID NO:50)	HQYYRSPPT (SEQ ID NO:45)
VH Sequence: QVQLKESGPGGLVAPSQLSITCTISGFSLTSGVHWRQPPGKGLEWLVIIWSDGSTTNS TLKSRLSISKDNSKQVFLKMNSLQTDDTAMY YCARHYDYDGDWFAYWGQGLTVSA (SEQ ID NO:51)							
VL Sequence: QILLTQSPAIMSASLGERVTMTCTASSSVSSRCLHWYQQKPGSSPKLWIYSTSNLASGVPARFRGSGTSYSLTISSMEAEADAATYYCHQYY RSPPTFGGKLEIK (SEQ ID NO:52)							

NST in VH CDR2 is alternatively NSA or X₁ SX₂, wherein X₁ is N, Q, S, or A and/or X₂ is A or T. C in VL CDR1 is alternatively S, A, or V.

Table 4: Antibody A2-7A05

		Exemplary	IMGT	Kabat	Chothia	Contact	AbM
VH CDR Seq.	VH CDR1	GYFTIYWIN (SEQ ID NO:53)	GYFTIYW (SEQ ID NO:59)	IYWIN (SEQ ID NO:64)	GYFTIY (SEQ ID NO:65)	TIYWIN (SEQ ID NO:70)	GYFTIYWIN (SEQ ID NO:53)
	VH CDR2	KIYPGSISTDYNE KFKS (SEQ ID NO:54)	IYPGSIST (SEQ ID NO:60)	KIYPGSISTDYNE KFKS (SEQ ID NO:54)	PGSI (SEQ ID NO:66)	WIGKIYPGSISTD (SEQ ID NO:71)	KIYPGSISTD (SEQ ID NO:76)
	VH CDR3	TGTGGLAY (SEQ ID NO:55)	AITGTGGLAY (SEQ ID NO:61)	TGTGGLAY (SEQ ID NO:55)	GTGGLA (SEQ ID NO:67)	AITGTGGLA (SEQ ID NO:72)	TGTGGLAY (SEQ ID NO:55)
VL CDR Seq.	VL CDR1	RASSSVNYMY (SEQ ID NO:56)	SSVNY (SEQ ID NO:62)	RASSSVNYMY (SEQ ID NO:56)	SSSVNY (SEQ ID NO:68)	NYMYWY (SEQ ID NO:73)	RASSSVNYMY (SEQ ID NO:56)
	VL CDR2	FTSSLAP (SEQ ID NO:57)	FTS (SEQ ID NO:63)	FTSSLAP (SEQ ID NO:57)	FTS (SEQ ID NO:63)	LWYFTSSLA (SEQ ID NO:74)	FTSSLAP (SEQ ID NO:57)
	VL CDR3	QQFTTSPFT (SEQ ID NO:58)	QQFTTSPFT (SEQ ID NO:58)	QQFTTSPFT (SEQ ID NO:58)	FTTSPF (SEQ ID NO:69)	QQFTTSPF (SEQ ID NO:75)	QQFTTSPFT (SEQ ID NO:58)
VH Sequence: QVQLQQPGAELVKPGASVKLSCKASGYFTIYWINWVKRPGQGLEWIGKIYPGSISTDYNEKFKSKATLTVDTSSTAYMQLSSLTSDDSAV YYCAITGTGGLAYWGQGLTVTSA (SEQ ID NO:77)							
VL Sequence: ENVLTQSPAIMSASLGEKVTMTCRASSSVNYMYWYQQKSDASPKLWYFTSSLAPGVPGRFSGSGSGNSYSLTISTMEGEDAAITYCQQFTT SPFTFGSGTKLEIK (SEQ ID NO:78)							

Table 5: Antibody A2-7F01

		Exemplary	IMGT	Kabat	Chothia	Contact	AbM
VH CDR Seq.	VH CDR1	GYTFTIYWIN (SEQ ID NO:53)	GYTFTIYW (SEQ ID NO:59)	IYWIN (SEQ ID NO:64)	GYTFTIY (SEQ ID NO:65)	TIYWIN (SEQ ID NO:70)	GYTFTIYWIN (SEQ ID NO:53)
	VH CDR2	NIYPGSSSTNYN EKFKT (SEQ ID NO:79)	IYPGSSST (SEQ ID NO:82)	NIYPGSSSTNYN EKFKT (SEQ ID NO:79)	PGSS (SEQ ID NO:84)	WIGNIYPGSSST N (SEQ ID NO:87)	NIYPGSSSTN (SEQ ID NO:90)
	VH CDR3	TGTGGFAY (SEQ ID NO:80)	AITGTGGFAY (SEQ ID NO:83)	TGTGGFAY (SEQ ID NO:80)	GTGGFA (SEQ ID NO:85)	AITGTGGFA (SEQ ID NO:88)	TGTGGFAY (SEQ ID NO:80)
VL CDR Seq.	VL CDR1	RASSSVNYMY (SEQ ID NO:56)	SSVNY (SEQ ID NO:62)	RASSSVNYMY (SEQ ID NO:56)	SSSVNY (SEQ ID NO:68)	NYMYWY (SEQ ID NO:73)	RASSSVNYMY (SEQ ID NO:56)
	VL CDR2	FTSSLAP (SEQ ID NO:57)	FTS (SEQ ID NO:63)	FTSSLAP (SEQ ID NO:57)	FTS (SEQ ID NO:63)	LWYFTSSLA (SEQ ID NO:74)	FTSSLAP (SEQ ID NO:57)
	VL CDR3	QQLTGSPFT (SEQ ID NO:81)	QQLTGSPFT (SEQ ID NO:81)	QQLTGSPFT (SEQ ID NO:81)	LTGSPF (SEQ ID NO:86)	QQLTGSPF (SEQ ID NO:89)	QQLTGSPFT (SEQ ID NO:81)
VH Sequence: QVQLQQPGAELVKPGASVKLSCKASGYTFTIYWINWVKQRPQGQLEWIGNIYPGSSSTNYNEKFKTKATLTVDTSSTAYMQLSSLTSDDSAV YYCAITGTGGFAYWGQGLTVTSA (SEQ ID NO:91)							
VL Sequence: ENVLTQSPAIMSASLGEKVTMSCRASSSVNYMYWYQQKSDASPKLWYFTSSLAPGVPTRFSGSGSGNSFSLTISSMEGEDAATYYCQQLTG SPFTFGSGTRLEIK (SEQ ID NO:92)							

Table 6: Antibody C-14D12

		Exemplary	IMGT	Kabat	Chothia	Contact	AbM
VH CDR Seq.	VH CDR1	GFSLTDYGVH (SEQ ID NO:93)	GFSLTDYG (SEQ ID NO:97)	DYGVH (SEQ ID NO:100)	GFSLTDY (SEQ ID NO:101)	TDYGVH (SEQ ID NO:105)	GFSLTDYGVH (SEQ ID NO:93)
	VH CDR2	VIWSDGSTTYS ALKS (SEQ ID NO:28)	IWSDGST (SEQ ID NO:8)	VIWSDGSTTYS ALKS (SEQ ID NO:28)	SDG (SEQ ID NO:14)	WLVIWSDGSTT (SEQ ID NO:19)	VIWSDGSTT (SEQ ID NO:24)
	VH CDR3	HAPSFIRYGSRY DALDY (SEQ ID NO:94)	ARHAPSFIRYGS RYDALDY (SEQ ID NO:98)	HAPSFIRYGSRY DALDY (SEQ ID NO:94)	APSFIRYGSRYD ALD (SEQ ID NO:102)	ARHAPSFIRYGS RYDALD (SEQ ID NO:106)	HAPSFIRYGSRY DALDY (SEQ ID NO:94)
VL CDR Seq.	VL CDR1	TASSSVTSSFLH (SEQ ID NO:95)	SSVTSSF (SEQ ID NO:99)	TASSSVTSSFLH (SEQ ID NO:95)	SSSVTSSF (SEQ ID NO:103)	TSSFLHWY (SEQ ID NO:107)	TASSSVTSSFLH (SEQ ID NO:95)
	VL CDR2	STSNLAS (SEQ ID NO:5)	STS (SEQ ID NO:11)	STSNLAS (SEQ ID NO:5)	STS (SEQ ID NO:11)	LWYSTSNLA (SEQ ID NO:22)	STSNLAS (SEQ ID NO:5)
	VL CDR3	HQYHRSPPT (SEQ ID NO:96)	HQYHRSPPT (SEQ ID NO:96)	HQYHRSPPT (SEQ ID NO:96)	YHRSPPT (SEQ ID NO:104)	HQYHRSPPT (SEQ ID NO:108)	HQYHRSPPT (SEQ ID NO:96)
VH Sequence: QVQLKESGPDVLVAPSQLSITCTVSGFSLTDYGVHWRQPPGKLEWLVIWSDGSTTYSALKSRLSITKDTSKRQVFLKMNSLQTDTTAM YYCARHAPSFIRYGSRYDALDYWGQGSTVTSS (SEQ ID NO:109)							
VL Sequence: QIVLTQSPAIMSASLGERVTLTCTASSSVTSSFLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSTYSLTSSMEAEADAATYYCHQYH RSPPTFGGGTKLEIK (SEQ ID NO:110)							

[0081] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise a VH region or VH domain. In other embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise a VL region or VL domain. In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein have a combination of (i) a VH domain or VH region; and/or (ii) a VL domain or VL region.

[0082] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise heavy chain having a combination of (i) a VH domain comprising CDRs according to Kabat and/or Chothia, AbM, Contact, or IMGT described in any one of Tables 1-6; and (ii) one or more heavy chain constant domains (e.g., CH1, Hinge, CH2, and CH3). An exemplary IgG heavy chain comprises any VH domain as described herein and the following CH1, Hinge, CH2, and CH3 amino acid sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
VLDSGDGSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSPGK (SEQ
ID NO:117). Another exemplary IgG heavy chain comprises any VH domain with
CDRs as described herein and the following CH1, Hinge, CH2, and CH3 amino acid
sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV

HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP
PVLDSDSGFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO:118). Exemplary Fc sequences are shown in **FIG. 3**.

[0083] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise a light chain having a combination of (i) a VL domain comprising CDRs described in any one of Tables 1-6; and (ii) a light chain constant domain (CL). An exemplary light chain (e.g., for pairing with an IgG heavy chain) comprises any VL domain with CDRs according to Kabat and/or Chothia, AbM, Contact, or IMGT as described herein and the following CL amino acid sequence:

RTVAAPSVFIFPPSDSQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES
VTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ
ID NO:119).

[0084] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise (a) a heavy chain having a combination of (i) a VH domain with CDRs according to Kabat and/or Chothia, AbM, Contact, or IMGT described in any one of Tables 1-6, and (ii) one or more heavy chain constant domains (e.g., CH1, Hinge, CH2, and CH3); and (b) a light chain having a combination of (i) a VL domain with CDRs according to Kabat and/or Chothia, AbM, Contact, or IMGT described in any one of Tables 1-6, and (ii) a light chain constant domain (CL).

[0085] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including a human $\alpha 5\beta 1$ integrin binding agent, described herein comprises one or more CDRs, including six CDRs, for example, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 identified in Table 1. In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including a human $\alpha 5\beta 1$ integrin binding agent, described herein comprises one or more, including six CDRs, for example, VH CDR1, VH

CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 identified in Table 2. In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including a human $\alpha 5\beta 1$ integrin binding agent, described herein comprises one or more, including six CDRs, for example, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 identified in Table 3. In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including a human $\alpha 5\beta 1$ integrin binding agent, described herein comprises one or more, including six CDRs, for example, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 identified in Table 4. In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including a human $\alpha 5\beta 1$ integrin binding agent, described herein comprises one or more, including six CDRs, for example, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 identified in Table 5. In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including a human $\alpha 5\beta 1$ integrin binding agent, described herein comprises one or more, including six CDRs, for example, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 identified in Table 6. In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including a human $\alpha 5\beta 1$ integrin binding agent, described herein comprises one or more, including six CDRs, for example, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 identified in Tables 1, 2, 3, 4, 5 and/or 6.

[0086] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 1. In other embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including

three CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 1. In yet other embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 1 and one or more CDRs, including three VL CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 1.

[0087] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 2. In other embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 2. In yet other embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 2 and one or more CDRs, including three VL CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 2.

[0088] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM,

Contact, or IMGT listed in Table 3. In other embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 3. In yet other embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 3 and one or more CDRs, including three VL CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 3.

[0089] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 4. In other embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 4. In yet other embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 4 and one or more CDRs, including three VL CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 4.

[0090] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (*e.g.*, antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 5. In other embodiments, $\alpha 5\beta 1$ integrin binding agents (*e.g.*, antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 5. In yet other embodiments, $\alpha 5\beta 1$ integrin binding agents (*e.g.*, antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 5 and one or more CDRs, including three VL CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 5.

[0091] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (*e.g.*, antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 6. In other embodiments, $\alpha 5\beta 1$ integrin binding agents (*e.g.*, antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 6. In yet other embodiments, $\alpha 5\beta 1$ integrin binding agents (*e.g.*, antibodies, such as bispecific antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein comprise one or more CDRs, including three VH CDRs, for example, VH CDR1, VH CDR2, and/or VH CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table

6 and one or more CDRs, including three VL CDRs, for example, VL CDR1, VL CDR2, and/or VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT listed in Table 6.

[0092] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody, such as a bispecific antibody) described herein comprises one or more complementarity determining regions (CDRs) comprising an amino acid sequence selected from a group consisting of SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody, such as a bispecific antibody) described herein comprises two or more complementarity determining regions (CDRs) comprising an amino acid sequence selected from a group consisting of SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody, such as a bispecific antibody) described herein comprises three or more complementarity determining regions (CDRs) comprising an amino acid sequence selected from a group consisting of SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody, such as a bispecific antibody) described herein comprises four or more complementarity determining regions (CDRs) comprising an amino acid sequence selected from a group consisting of SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody, such as a bispecific antibody) described herein comprises five or more complementarity determining regions (CDRs) comprising an amino acid sequence selected from a group consisting of SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody, such as a bispecific antibody) described herein comprises six or more complementarity determining regions (CDRs) comprising an amino acid sequence selected from a group consisting of SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108.

[0093] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprise one or more (*e.g.*, one, two or three) VH CDRs listed in Tables 1-6. In other embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises one or more (*e.g.*, one, two or three) VL CDRs listed in Tables 1-6. In yet other embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises one or more (*e.g.*, one, two or three) VH CDRs listed in Tables 1-6 and one or more VL CDRs listed in Tables 1-6. Accordingly, in some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises a VH CDR1 having the amino acid sequence of any one of SEQ ID NOS:1, 7, 12, 13, 18, 27, 31, 34, 35, 38, 53, 59, 64, 65, 70, 93, 97, 100, 101, and 105. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises a VH CDR2 having the amino acid sequence of any one of SEQ ID NOS:2, 8, 14, 19, 24, 28, 54, 60, 66, 71, 76, 79, 82, 84, 87, and 90. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises a VH CDR3 having the amino acid sequence of any one of SEQ ID NOS:3, 9, 15, 20, 29, 32, 36, 39, 55, 61, 67, 72, 80, 83, 85, 88, 94, 98, 102, and 106. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises a VH CDR1 and/or a VH CDR2 and/or a VH CDR3 independently selected from a VH CDR1, VH CDR2, VH CDR3 as set forth in any one of the amino acid sequences as set forth in Table 1-6. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises a VL CDR1 having the amino acid sequence of any one of SEQ ID NOS:4, 10, 16, 21, 30, 33, 37, 40, 44, 46, 47, 49, 56, 62, 68, 73, 95, 99, 103, and 107. In another embodiment, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises a VL CDR2 having the amino acid sequence of any one of SEQ ID NOS:5, 11, 22, 41, 57, 63, and 74. In some embodiments, an $\alpha 5\beta 1$

integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises a VL CDR3 having the amino acid sequence of any one of SEQ ID NOS:6, 17, 23, 45, 48, 50, 58, 69, 75, 81, 86, 89, 96, 104, and 108. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises a VL CDR1 and/or a VL CDR2 and/or a VL CDR3 independently selected from a VL CDR1, VL CDR2, VL CDR3 as set forth in any one of the amino acid sequences as set forth in Tables 1-6.

[0094] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises a heavy chain variable (VH) region comprising: (1) a VH CDR1 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:1, 27, 53, or 93, (ii) SEQ ID NO:7, 31, 59, or 97, (iii) SEQ ID NO:12, 34, 64, or 100, (iv) SEQ ID NO:13, 35, 65, or 101, and (v) SEQ ID NO:18, 38, 70, or 105; (2) a VH CDR2 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:2, 28, 54, or 79, (ii) SEQ ID NO:8, 60, or 82, (iii) SEQ ID NO:14, 66, or 84 (iv) SEQ ID NO:19, 71, or 87, and (v) SEQ ID NO:24, 76; or 90, and (3) a VH CDR3 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:3, 29, 55, 80, or 94, (ii) SEQ ID NO:9, 32, 61, 83, or 98, (iii) SEQ ID NO:15, 36, 67, 85, or 102, and (iv) SEQ ID NO:20, 39, 72, 88, or 106; and/or a light chain variable (VL) region comprising: (1) a VL CDR1 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:4, 30, 44, 56, or 95, (ii) SEQ ID NO:10, 33, 46, 62, or 99, (iii) SEQ ID NO:16, 37, 47, 68, or 103, and (iv) SEQ ID NO:21, 40, 49, 73, or 107; (2) a VL CDR2 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:5 or 57, (ii) SEQ ID NO:11 or 63, and (iii) SEQ ID NO:22, 41, or 74; and (3) a VL CDR3 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:6, 45, 58, 81, or 96, (ii) SEQ ID NO:17, 48, 69, 86, or 104, and (iii) SEQ ID NO:23, 50, 75, 89, or 108.

[0095] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises a heavy chain variable

(VH) region comprising: (1) a VH CDR1 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:1, 27, 53, or 93, (ii) SEQ ID NO:7, 31, 59, or 97, (iii) SEQ ID NO:12, 34, 64, or 100, (iv) SEQ ID NO:13, 35, 65, or 101, and (v) SEQ ID NO:18, 38, 70, or 105; (2) a VH CDR2 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:2, 28, 54, or 79, (ii) SEQ ID NO:8, 60, or 82, (iii) SEQ ID NO:14, 66, or 84 (iv) SEQ ID NO:19, 71, or 87, and (v) SEQ ID NO:24, 76; or 90, and (3) a VH CDR3 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:3, 29, 55, 80, or 94, (ii) SEQ ID NO:9, 32, 61, 83, or 98, (iii) SEQ ID NO:15, 36, 67, 85, or 102, and (iv) SEQ ID NO:20, 39, 72, 88, or 106.

[0096] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody, such as a bispecific antibody) described herein comprises a light chain variable (VL) region comprising: (1) a VL CDR1 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:4, 30, 44, 56, or 95, (ii) SEQ ID NO:10, 33, 46, 62, or 99, (iii) SEQ ID NO:16, 37, 47, 68, or 103, and (iv) SEQ ID NO:21, 40, 49, 73, or 107; (2) a VL CDR2 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:5 or 57, (ii) SEQ ID NO:11 or 63, and (iii) SEQ ID NO:22, 41, or 74; and (3) a VL CDR3 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:6, 45, 58, 81, or 96, (ii) SEQ ID NO:17, 48, 69, 86, or 104, and (iii) SEQ ID NO:23, 50, 75, 89, or 108.

[0097] In some embodiments, described herein is an antibody or fragment thereof that binds to $\alpha 5\beta 1$ integrin, wherein the antibody or fragment thereof comprises: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:1, 27, 53, or 93, (ii) SEQ ID NO:7, 31, 59, or 97, (iii) SEQ ID NO:12, 34, 64, or 100, (iv) SEQ ID NO:13, 35, 65, or 101, and (v) SEQ ID NO:18, 38, 70, or 105; (2) a VH CDR2 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:2, 28, 54, or 79, (ii) SEQ ID NO:8, 60, or 82, (iii) SEQ ID NO:14, 66, or 84 (iv) SEQ ID NO:19, 71, or 87, and (v) SEQ ID NO:24, 76; or 90, and (3) a VH CDR3 having an

amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:3, 29, 55, 80, or 94, (ii) SEQ ID NO:9, 32, 61, 83, or 98, (iii) SEQ ID NO:15, 36, 67, 85, or 102, and (iv) SEQ ID NO:20, 39, 72, 88, or 106; and/or a light chain variable (VL) region comprising: (1) a VL CDR1 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:4, 30, 44, 56, or 95, (ii) SEQ ID NO:10, 33, 46, 62, or 99, (iii) SEQ ID NO:16, 37, 47, 68, or 103, and (iv) SEQ ID NO:21, 40, 49, 73, or 107; (2) a VL CDR2 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:5, or 57, (ii) SEQ ID NO:11, or 63, and (iii) SEQ ID NO:22, 41, or 74; and (3) a VL CDR3 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:6, 45, 58, 81, or 96, (ii) SEQ ID NO:17, 48, 69, 86, or 104, and (iii) SEQ ID NO:23, 50, 75, 89, or 108.

[0098] In some embodiments, described herein is an antibody or fragment thereof that binds to $\alpha 5 \beta 1$ integrin, wherein the antibody or fragment thereof comprises a heavy chain variable (VH) region comprising: (1) a VH CDR1 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:1, 27, 53, or 93, (ii) SEQ ID NO:7, 31, 59, or 97, (iii) SEQ ID NO:12, 34, 64, or 100, (iv) SEQ ID NO:13, 35, 65, or 101, and (v) SEQ ID NO:18, 38, 70, or 105; (2) a VH CDR2 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:2, 28, 54, or 79, (ii) SEQ ID NO:8, 60, or 82, (iii) SEQ ID NO:14, 66, or 84 (iv) SEQ ID NO:19, 71, or 87, and (v) SEQ ID NO:24, 76; or 90, and (3) a VH CDR3 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:3, 29, 55, 80, or 94, (ii) SEQ ID NO:9, 32, 61, 83, or 98, (iii) SEQ ID NO:15, 36, 67, 85, or 102, and (iv) SEQ ID NO:20, 39, 72, 88, or 106.

[0099] In some embodiments, described herein is an antibody or fragment thereof that binds to $\alpha 5 \beta 1$ integrin, wherein the antibody or fragment thereof comprises a light chain variable (VL) region comprising: (1) a VL CDR1 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:4, 30, 44, 56, or 95, (ii) SEQ ID NO:10, 33, 46, 62, or 99, (iii) SEQ ID NO:16, 37, 47, 68, or 103, and (iv) SEQ ID NO:21, 40, 49, 73, or 107; (2) a VL CDR2 having an amino acid

sequence of selected from the group consisting of: (i) SEQ ID NO:5, or 57, (ii) SEQ ID NO:11, or 63, and (iii) SEQ ID NO:22, 41, or 74; and (3) a VL CDR3 having an amino acid sequence of selected from the group consisting of: (i) SEQ ID NO:6, 45, 58, 81, or 96, (ii) SEQ ID NO:17, 48, 69, 86, or 104, and (iii) SEQ ID NO:23, 50, 75, 89, or 108.

[00100] In some embodiments, described herein is an antibody or fragment thereof that binds to $\alpha 5\beta 1$ integrin comprising all three heavy chain complementarity determining regions (CDRs) and/or all three light chain CDRs from: (i) the antibody designated A-15B08 that comprises a VH sequence that is SEQ ID NO:25 or humanized variant thereof and a VL sequence that is SEQ ID NO:26 or humanized variant thereof; (ii) the antibody designated A2-3B06 that comprises a VH sequence that is SEQ ID NO:42 or humanized variant thereof and a VL sequence that is SEQ ID NO:43 or humanized variant thereof; (iii) the antibody designated A2-5D10 that comprises a VH sequence that is SEQ ID NO:51 or humanized variant thereof and a VL sequence that is SEQ ID NO:52 or humanized variant thereof; (iv) the antibody designated A2-7A05 that comprises a VH sequence that is SEQ ID NO:77 or humanized variant thereof and a VL sequence that is SEQ ID NO:78 or humanized variant thereof; (v) the antibody designated A2-7F01 that comprises a VH sequence that is SEQ ID NO:91 or humanized variant thereof and a VL sequence that is SEQ ID NO:92 or humanized variant thereof; or (vi) the antibody designated C-14D12 that comprises a VH sequence that is SEQ ID NO:109 or humanized variant thereof and a VL sequence that is SEQ ID NO:110 or humanized variant thereof. In some embodiments, the antibody or fragment thereof comprises all three heavy chain CDRs and/or all three light chain CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) from the antibody designated A-15B08. In some embodiments, antibody or fragment thereof comprises all three heavy chain CDRs and/or all three light chain CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) from the antibody designated A2-3B06. In some embodiments, the antibody or fragment thereof comprises all three heavy chain CDRs and/or all three light chain CDRs

(according to Kabat and/or Chothia, AbM, Contact, or IMGT) from the antibody designated A2-5D10. In some embodiments, the antibody or fragment thereof comprises all three heavy chain CDRs and/or all three light chain CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) from the antibody designated A2-7A05. In some embodiments, antibody or fragment thereof comprises all three heavy chain CDRs and/or all three light chain CDRs from the antibody (according to Kabat and/or Chothia, AbM, Contact, or IMGT) designated A2-7F01. In some embodiments, the antibody or fragment thereof comprises all three heavy chain CDRs and/or all three light chain CDRs from the antibody (according to Kabat and/or Chothia, AbM, Contact, or IMGT) designated C-14D12. In some embodiments, the antibody or fragment thereof competes for the binding with an antibody or fragment thereof that comprises: (i) all three heavy chain CDRs and/or all three light chain CDRs from the antibody designated A-15B08 (see, e.g., Table 1), (ii) all three heavy chain CDRs and/or all three light chain CDRs from the antibody designated A2-3B06 (see, e.g., Table 2), (iii) all three heavy chain CDRs and/or all three light chain CDRs from the antibody designated A2-5D10 (see, e.g., Table 3), or (iv) all three heavy chain CDRs and/or all three light chain CDRs from the antibody designated C-14D12 (see, e.g., Table 6). In some embodiments, the antibody or fragment thereof competes for the binding with an antibody or fragment thereof that comprises: (i) all three heavy chain CDRs and/or all three light chain CDRs from the antibody designated A2-7A05 (see, e.g., Table 4), or (ii) all three heavy chain CDRs and/or all three light chain CDRs from the antibody designated A2-7F01 (see, e.g., Table 5).

[00101] In some embodiments, described herein is an antibody or fragment thereof that binds to $\alpha 5 \beta 1$ integrin, wherein the antibody comprises: (a) a heavy chain variable (VH) region comprising a VH CDR1, a VH CDR2, and a VH CDR3 amino acid sequence as set forth in Tables 1-6; and/or (b) a light chain variable (VL) region comprising a VL CDR1, a VL CDR2, and a VL CDR3 amino acid sequence as set forth in Tables 1-6. In some embodiments, the antibody comprises a heavy chain variable (VH) region comprising a VH CDR1, a VH CDR2, and a VH CDR3 amino

acid sequence as set forth in Tables 1-6. In some embodiments, the antibody comprises a light chain variable (VL) region comprising a VL CDR1, a VL CDR2, and a VL CDR3 amino acid sequence as set forth in Tables 1-6.

[00102] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:1, 7, 12, 13, and 18; (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 8, 14, 19, and 24; and (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:3, 9, 15, and 20; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:4, 10, 16, and 21; (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 22; and (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:6, 17, and 23.

[00103] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:1; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:2; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:3; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:4; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:6.

[00104] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:7; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:8; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:9; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:10; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:11; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:6.

[00105] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:12; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:2; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:3; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:4; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:6.

[00106] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:13; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:14; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:15; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:16; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:11; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:17.

[00107] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:18; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:19; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:21; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:22; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:23.

[00108] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:1; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:24; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:3; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the

amino acid sequence of SEQ ID NO:4; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:6.

[00109] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:27, 31, 34, 35, and 38; (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:8, 14, 19, 24, and 28; and (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:29, 32, 36, and 39; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:30, 33, 37, and 40; (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 41 and (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:6, 17, and 23.

[00110] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:27; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:28; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:29; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:30; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:6.

[00111] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:31; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:8; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:32; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:33; (2) a VL CDR2 having the amino acid

sequence of SEQ ID NO:11; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:6.

[00112] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:34; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:28; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:29; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:30; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:6.

[00113] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:35 (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:14; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:36; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:37; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:11; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:17.

[00114] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:38; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:19; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:39; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:40; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:41; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:23.

[00115] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:27; (2) a VH CDR2 having the amino acid sequence of

SEQ ID NO:24; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:29; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:30; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:6

[00116] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:1, 7, 12, 13, and 18; (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 8, 14, 19, and 24; and (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:3, 9, 15, and 20; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:44, 46, 47, and 49; (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 22; and (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:45, 48, and 50.

[00117] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:1; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:2; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:3; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:44; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:45.

[00118] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:7; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:8; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:9; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino

acid sequence of SEQ ID NO:46; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:11; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:45.

[00119] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:12; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:2; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:3; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:44; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:45.

[00120] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:13; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:14; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:15; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:47; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:11; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:48.

[00121] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:18; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:19; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:49; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:22; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:50.

[00122] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid

sequence of SEQ ID NO:1; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:24; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:3; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:44; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:45.

[00123] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:53, 59, 64, 65, and 70; (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:54, 60, 66, 71, and 76; and (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:55, 61, 67, and 72; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:56, 62, 68, and 73; (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:57, 63, and 74 and (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:58, 69, and 75.

[00124] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:53; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:54; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:55; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:56; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:57; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:58.

[00125] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:59; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:60; and (3) a VH CDR3 having the amino acid sequence of SEQ ID

NO:61; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:62; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:63; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:58.

[00126] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:64; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:54; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:55; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:56; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:57; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:58.

[00127] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:65; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:66; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:67; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:68; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:63; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:69.

[00128] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:70; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:71; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:72; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:73; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:74; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:75.

[00129] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:53; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:76; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:55; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:56; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:57; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:58.

[00130] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 53, 59, 64, 65, and 70; (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:79, 82, 84, 87, and 90; and (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:80, 83, 85, and 88; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:56, 62, 68, and 73; (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:57, 63, and 74 and (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 86, and 89.

[00131] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:53; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:79; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:80; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:56; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:57; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:81

[00132] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid

sequence of SEQ ID NO:59; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:82; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:83; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:62; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:63; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:81.

[00133] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:64; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:79; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:80; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:56; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:57; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:81.

[00134] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:65; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:84; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:85; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:68; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:63; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:86.

[00135] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:70; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:87; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:88; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:73; (2) a VL CDR2 having the amino acid

sequence of SEQ ID NO:74; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:89.

[00136] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:53; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:90; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:80; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:56; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:57; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:81.

[00137] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:93, 97, 100, 101, and 105; (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:8, 14, 19, 24, and 28; and (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:94, 98, 102, and 106; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:95, 99, 103, and 107; (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 22 and (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:96, 104, and 108.

[00138] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:93; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:28; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:94; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:95; (2) a VL CDR2 having the amino acid

sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:96.

[00139] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:97; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:8; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:98; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:99; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:11; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:96.

[00140] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:100; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:28; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:94; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:95; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:96.

[00141] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:101; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:14; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:102; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:103; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:11; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:104.

[00142] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid

sequence of SEQ ID NO:105; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:19; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:106; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:107; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:22; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:108.

[00143] In some embodiments, described herein is an antibody comprising: (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:93; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:24; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:94; and (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:95; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:96.

[00144] In some embodiments, described herein is an antibody comprising a VH region and/or VL region described herein, which further comprises human framework sequences. In some embodiment, the VH region and/or VL region further comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and/or a framework 4 (FR4) sequence.

[00145] In some embodiments, the antibody described herein is a monoclonal antibody. In some embodiments, the monoclonal antibody is a humanized, human or chimeric antibody. In some embodiments, the antibody described herein is a Fab, Fab', F(ab')₂, Fv, scFv, (scFv)₂, single chain antibody molecule, dual variable region antibody, single variable region antibody, linear antibody, V region, or a multispecific antibody formed from antibody fragments.

[00146] In some embodiments, the CDRs disclosed herein include consensus sequences derived from groups of related antibodies (see, *e.g.*, Tables 1-6). As described herein, a "consensus sequence" refers to amino acid sequences having conserved amino acids common among a number of sequences and variable amino

acids that vary within a given amino acid sequence. The exemplary CDR consensus sequences provided include CDRs corresponding to CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and/or CDRL3. Exemplary consensus sequences of CDRs of $\alpha 5\beta 1$ integrin binding agents (*e.g.*, antibodies, such as bispecific antibodies) are shown in **FIGs. 2A and 2B**. In some embodiments, the CDRs disclosed herein include exemplary consensus sequences derived from groups of related antibodies (see, *e.g.*, Tables 1-6 including, for example, a first exemplary group from Tables 1, 2, 3, and 6 and a second exemplary group from Tables 4 and 5). Exemplary consensus sequences of CDRs of $\alpha 5\beta 1$ integrin binding agents (*e.g.*, antibodies, such as bispecific antibodies) are shown in **FIGs. 2A and 2B**. Accordingly, in some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody, such as a bispecific antibody) described herein comprises (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence GFSLT_{X1}YGVH (SEQ ID NO:120), wherein X₁ is a naturally occurring amino acid (*e.g.*, S, T, or D); (2) a VH CDR2 having the amino acid sequence VIWSDGSTTYX₁SX₂LKS (SEQ ID NO:121), wherein X₁ and/or X₂ are each (or any) independently a naturally occurring amino acid (*e.g.*, X₁ is N, Q, S, or A and/or X₂ is A or T); and (3) a VH CDR3 having the amino acid of H X₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀Y (SEQ ID NO:122) or HX₁X₂X₃X₄X₅X₆X₇X₈X₉RYDALX₁₀Y (SEQ ID NO:123), wherein X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, and/or X₁₀ are each (or any) independently a naturally occurring amino acid (*e.g.*, X₁ is Y, G, or A, X₂ is D, G, or P, X₃ is Y, L, or S, X₄ is D, L, or F, X₅ is G, R, or I, X₆ is D or R, X₇ is W, D, or Y, X₈ is F, A, or G, X₉ is absent, M, or S, and/or X₁₀ is D or A; and/or (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence TASSX₁VX₂SX₃X₄X₅H (SEQ ID NO:124), wherein X₁, X₂, X₃, X₄, and/or X₅ are each (or any) independently a naturally occurring amino acid (*e.g.*, X₁ is R or S, X₂ is S or T, X₃ is N, R, or S, X₄ is S, F, C, A, or V, and/or X₅ is L or F); (2) a VL CDR2 having the amino acid sequence STSNLAS (SEQ ID NO:125); and (3) a VL CDR3 having the amino acid sequence HQYX₁RSPPT (SEQ ID NO:126), wherein X₁ is a naturally occurring amino acid (*e.g.*, L, Y, or

H). Accordingly, in some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody, such as a bispecific antibody) described herein comprises (a) a heavy chain variable (VH) region comprising: (1) a VH CDR1 having the amino acid sequence GYTFTIYWIN (SEQ ID NO:127); (2) a VH CDR2 having the amino acid sequence of X_1 IYPGSX₂STX₃YNEKFKX₄ (SEQ ID NO:128), wherein X_1 , X_2 , X_3 , and/or X_4 are each (or any) a naturally occurring amino acid (e.g., X_1 is K or N, X_2 is I or S, X_3 is D or N, and/or X_4 is S or T); and (3) a VH CDR3 having the amino acid of TGTGGX₁AY (SEQ ID NO:129), wherein X_1 is a naturally occurring amino acid (e.g., X_1 is absent, L, or F); and/or (b) a light chain variable (VL) region comprising: (1) a VL CDR1 having the amino acid sequence RASSSVNYMY (SEQ ID NO:130); (2) a VL CDR2 having the amino acid sequence FTSSLAP (SEQ ID NO:131); and (3) a VL CDR3 having the amino acid sequence QQX₁TX₂SPFT (SEQ ID NO:132), wherein X_1 and/or X_2 are each (or any) a naturally occurring amino acid (e.g., X_1 is F or L and/or X_2 is T or G).

[00147] In some embodiments, described herein is a binding agent (e.g., an antibody) that binds to essentially the same epitope as an antibody or fragment thereof of any one of the antibodies described herein. In some embodiments, described herein is a binding agent (e.g., an antibody) that competes for binding to human $\alpha 5\beta 1$ integrin with an antibody or fragment thereof of any one described herein. In some embodiments, the binding agent is an antibody or fragment thereof.

[00148] In certain aspects, the CDRs of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, can be determined according to the Kabat system (Kabat et al. (1971) Ann. NY Acad. Sci. 190:382-391 and, Kabat et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

[00149] In certain aspects, the CDRs of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, can be determined according to the Chothia system, which will be referred to herein as the "Chothia

CDRs” (see, *e.g.*, Chothia and Lesk, 1987, J. Mol. Biol., 196:901-917; Al-Lazikani et al., 1997, J. Mol. Biol., 273:927-948; Chothia et al., 1992, J. Mol. Biol., 227:799-817; Tramontano A et al., 1990, J. Mol. Biol. 215(1):175-82; and U.S. Patent No. 7,709,226).

[00150] In certain aspects, the CDRs of an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, can be determined according to the ImMunoGeneTics (IMGT) system, which will be referred to herein as the “IMGT CDRs”, for example, as described in Lefranc, M.-P., 1999, The Immunologist, 7:132-136 and Lefranc, M.-P. et al., 1999, Nucleic Acids Res., 27:209-212.

[00151] In certain aspects, the CDRs of an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, can be determined according to the AbM system, which will be referred to herein as the “AbM CDRs,” for example as described in MacCallum et al., 1996, J. Mol. Biol., 262:732-745. See also, *e.g.*, Martin, A., “Protein Sequence and Structure Analysis of Antibody Variable Domains,” in Antibody Engineering, Kontermann and Dübél, eds., Chapter 31, pp. 422-439, Springer-Verlag, Berlin (2001).

[00152] In certain aspects, the CDRs of an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, can be determined according to the Contact system, which will be referred to herein as the “Contact CDRs” (see, *e.g.*, MacCallum RM et al., 1996, J Mol Biol 5: 732-745). The Contact CDRs are based on an analysis of the available complex crystal structures.

[00153] In some embodiments, the position of one or more CDRs along the VH (*e.g.*, CDR1, CDR2, or CDR3) and/or VL (*e.g.*, CDR1, CDR2, or CDR3) region of an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, described herein (see, *e.g.*, CDRs according to Kabat and/or Chothia, AbM, Contact, or IMGT in Tables 1-6) may vary by one, two, three, four, five, or six amino acid positions (*e.g.*, one or more amino acid modifications) so long as binding to $\alpha 5\beta 1$ integrin (*e.g.*, human $\alpha 5\beta 1$ integrin) is maintained (*e.g.*, substantially

maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). For example, in some embodiments, the position defining a CDR (according to Kabat and/or Chothia, AbM, Contact, or IMGT) of any of Tables 1, 2, 3, 4, 5, or 6 may vary by shifting the N-terminal and/or C-terminal boundary of the CDR by one, two, three, four, five, or six amino acids, relative to the current CDR position, so long as binding to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In other embodiments, the length of one or more CDRs along the VH (e.g., CDR1, CDR2, or CDR3) and/or VL (e.g., CDR1, CDR2, or CDR3) region of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, described herein (see, e.g., CDRs according to Kabat and/or Chothia, AbM, Contact, or IMGT in Tables 1-6) may vary (e.g., be shorter or longer) by one, two, three, four, five, or more amino acids, so long as binding to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). For example, in some embodiments, a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be one, two, three, four, five or more amino acids shorter than one or more of the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) described by SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108, so long as binding to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In other embodiments, a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be one, two, three, four, five or more amino acids longer than one or more of the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) described by SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108, so long as binding to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In other embodiments, the amino terminus of a VH and/or VL CDR1,

CDR2, and/or CDR3 described herein may be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) described by SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108, so long as binding to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In other embodiments, the carboxy terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) described by SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108, so long as binding to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In other embodiments, the amino terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) described by SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108, so long as binding to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In some embodiments, the carboxy terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) described by SEQ ID NOS:1-24, 27-41, 44-50, 53-76, 79-90 and 93-108, so long as binding to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). Any method known in the art can be used to ascertain whether binding to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained, for example, the binding assays and conditions described in the "Examples" section described herein. For

example, Example 2 described herein describes an assay for measuring binding to $\alpha 5 \beta 1$ integrin (e.g., human $\alpha 5 \beta 1$ integrin).

[00154] In other embodiments, the $\alpha 5 \beta 1$ integrin binding agents (e.g., antibodies), including human $\alpha 5 \beta 1$ integrin binding agents, presented herein that bind to $\alpha 5 \beta 1$ integrin, comprise conservative sequence modifications (e.g., modifications of one or more amino acids in one or more CDRs as described above). With respect to polypeptides that are $\alpha 5 \beta 1$ integrin binding agents (e.g., antibodies), such as human $\alpha 5 \beta 1$ integrin binding agents, conservative sequence modifications include conservative amino acid substitutions that include ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, in some embodiments, a predicted nonessential amino acid residue in an $\alpha 5 \beta 1$ integrin binding agent is replaced with another amino acid residue from the same side chain family. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen binding are well-known in the art (see, e.g., Brummell et al., *Biochem.* 32:1180-1187 (1993); Kobayashi et al. *Protein Eng.* 12(10):879-884 (1999); and Burks et al. *Proc. Natl. Acad. Sci. USA* 94:412-417 (1997)). In some embodiments, the nucleotide and amino acid sequence modifications refer to at most 1, 2, 3, 4, 5, or 6 amino acid substitutions to the CDRs described in Table 1, Table 2, Table 3, Table 4, Table 5, or Table 6. Thus, for example, each such CDR may contain up to 5 conservative amino acid substitutions, for example up to (not more than) 4 conservative amino acid substitutions, for example up to (not more than) 3 conservative amino acid

substitutions, for example up to (not more than) 2 conservative amino acid substitutions, or no more than 1 conservative amino acid substitution.

[00155] The present disclosure provides variants of the antibodies described herein (see, e.g., Table 1, Table 3). The antibody designated as A-15B08-T62A described in Example 8 below is such an exemplary antibody variant. A-15B08-T62A was generated by replacing the Threonine residue at position 62 in the CDRH2 of antibody A-15B08 with an Alanine to remove a putative N-glycosylation site. The VH, VL, and CDR sequences according to various numbering schemes of A-15B08-T62A are shown in **FIGs. 2C** and **2D**. More specifically, the antibody designated as A-15B08-T62A comprises a VH comprising the amino acid sequence of SEQ ID NO:135 and a VL comprising the amino acid sequence of SEQ ID NO:26. The 6 CDR sequences of A-15B08-T62A according to Kabat, AbM, Chothia, Contact, and IMGT are shown in **FIGs. 2C** and **2D**.

[00156] In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:135. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:135; and a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In some embodiments, the CDRs are according to Chothia numbering. In some embodiments, the CDRs are according to Contact numbering. In some embodiments, the CDRs are according to IMGT. In some embodiments, the CDRs are according to a combination of two or more numbering schemes selected from Kabat, AbM, Chothia, Contact, and IMGT.

[00157] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:135 and a VL comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:26, and the binding of the antibody or fragment thereof to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%).

[00158] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence of SEQ ID NO:135 and a VL comprising an amino acid sequence of SEQ ID NO:26.

[00159] If desired, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, is linked or conjugated (directly or indirectly) to a moiety with effector function, such as cytotoxic activity (e.g., a chemotherapeutic moiety or a radioisotope) or immune recruitment activity, to form an antibody-drug conjugate (ADC). Moieties that are linked or conjugated (directly or indirectly) include drugs that are cytotoxic or non-cytotoxic. Alternatively or in addition, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, is optionally linked or conjugated (directly or indirectly) to a moiety that facilitates isolation from a mixture (e.g., a tag) or a moiety with reporter activity (e.g., a detection label or reporter protein). It will be appreciated that the features of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, described herein extend also to a polypeptide comprising an $\alpha 5\beta 1$ integrin binding agent fragment.

[00160] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein are conjugated or recombinantly linked (directly or indirectly) to a therapeutic agent (e.g., a cytotoxic agent) or to a diagnostic or detectable agent (e.g., a labeled agent, including a labeled antibody). The conjugated or recombinantly linked antibodies can be useful,

for example, for diagnosing, treating and/or preventing $\alpha 5\beta 1$ integrin-mediated diseases, disorders, and conditions, including a cancer (e.g., a cancer associated with or characterized by tumor cells that express or overexpress $\alpha 5\beta 1$ integrin), an angiogenesis-mediated disease (e.g., a disease associated with or characterized by abnormal angiogenesis), and an inflammatory disease (e.g., a neuroinflammatory disease, including MS and ALS).

[00161] Such diagnosis and/or detection, including with a diagnostic agent and/or a detectable agent, can be accomplished, for example, by coupling an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) to detectable substances (e.g., a labeled agent, including a labeled antibody) including, for example: enzymes, including, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, including, but not limited to, streptavidin/biotin or avidin/biotin; fluorescent materials, including, but not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, or phycoerythrin; luminescent materials, including, but not limited to, luminol; bioluminescent materials, including, but not limited to, luciferase, luciferin, or aequorin; chemiluminescent material, including, but not limited to, an acridinium based compound or a HALOTAG; radioactive materials, including, but not limited to, iodine (^{131}I , ^{125}I , ^{123}I , and ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{115}In , ^{113}In , ^{112}In , and ^{111}In), technetium (^{99}Tc), thallium (^{201}Tl), gallium (^{68}Ga and ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{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 , ^{113}Sn , or ^{117}Sn ; positron emitting metals using various positron emission tomographies; and non-radioactive paramagnetic metal ions.

[00162] Labeled agents (e.g., a labeled antibody) which specifically bind to an $\alpha 5\beta 1$ integrin can be used for diagnostic purposes to detect, diagnose, or monitor an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition. Described herein are

methods for the detection of an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition comprising: (a) assaying the expression of an $\alpha 5\beta 1$ integrin in cells or a tissue sample of a subject using one or more $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies) as described herein that specifically bind to the $\alpha 5\beta 1$ integrin; and (b) comparing the level of the $\alpha 5\beta 1$ integrin with a control level, (e.g., levels in normal tissue samples such as from a patient not having an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition) or from the same patient before disease onset), whereby an increase in the assayed level of $\alpha 5\beta 1$ integrin compared to the control level of the $\alpha 5\beta 1$ integrin is indicative of an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition. Also described herein is a diagnostic assay for diagnosing an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition comprising: (a) assaying for the level of an $\alpha 5\beta 1$ integrin in cells or a tissue sample of an individual using one or more $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies) as described herein that specifically bind to an $\alpha 5\beta 1$ integrin; and (b) comparing the level of the $\alpha 5\beta 1$ integrin with a control level (e.g., levels in normal tissue samples), whereby an increase in the assayed $\alpha 5\beta 1$ integrin level compared to the control level of the $\alpha 5\beta 1$ integrin is indicative of an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition. In certain embodiments, described herein is a method of treating an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition in a subject, comprising: (a) assaying for the level of an $\alpha 5\beta 1$ integrin in cells or a tissue sample of the subject using one or more $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies) as described herein that specifically bind to an $\alpha 5\beta 1$ integrin; and (b) comparing the level of the $\alpha 5\beta 1$ integrin with a control level (e.g., levels in normal tissue samples), whereby an increase in the assayed $\alpha 5\beta 1$ integrin level compared to the control level of the $\alpha 5\beta 1$ integrin is indicative of an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition. In some embodiments, the method further comprises (c) administering an effective amount of an $\alpha 5\beta 1$ integrin binding agent (e.g., antibody) herein to the subject identified as having the $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition. A more definitive diagnosis of an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition may allow health

professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition.

[00163] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein are components in kits. In some embodiments, kits comprise an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) or a composition (e.g., a pharmaceutical composition) comprising the $\alpha 5\beta 1$ integrin binding agent (e.g., the antibody), packaged into suitable packaging material. A kit optionally includes a label or packaging insert including a description of the components or instructions for use *in vitro*, *in vivo*, or *ex vivo*, of the components therein.

[00164] Also described herein are $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies) that are recombinantly linked or conjugated (covalent or non-covalent conjugations, directly or indirectly) to a heterologous protein or polypeptide (or fragment thereof, for example, to a polypeptide (e.g., of about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90, or about 100 amino acids) to generate fusion proteins, as well as uses thereof. In particular, described herein are fusion proteins comprising an antigen-binding fragment of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, described herein (e.g., comprising CDR1, CDR2, and/or CDR3 of VH and/or VL) and a heterologous protein, polypeptide, or peptide. In some embodiments, the heterologous protein, polypeptide, or peptide that an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) is linked to is useful for targeting the $\alpha 5\beta 1$ integrin binding agent to a particular cell (e.g., an $\alpha 5\beta 1$ integrin-expressing cell, including a tumor cell).

[00165] Moreover, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies), including human $\alpha 5\beta 1$ integrin binding agents, described herein can be linked (directly or indirectly) to marker or “tag” sequences, such as a peptide, to facilitate purification. In some embodiments, the marker or tag amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (see, e.g., QIAGEN, Inc.), among others,

many of which are commercially available. For example, as described in Gentz *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86:821-24, hexa-histidine provides for convenient purification of a fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin (“HA”) tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984, Cell 37:767-78), and the “FLAG” tag.

[00166] Methods for linking or conjugating (directly or indirectly) moieties (including polypeptides) to antibodies are well known in the art, any one of which can be used to make an antibody-drug conjugate or fusion protein described herein.

[00167] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) described herein is a fusion protein. The term “fusion protein” as used herein refers to a polypeptide that comprises an amino acid sequence of a binding agent (*e.g.*, an antibody) and an amino acid sequence of a heterologous polypeptide or protein (*e.g.*, a polypeptide or protein not normally a part of the antibody (*e.g.*, a non- $\alpha 5\beta 1$ integrin binding antibody). In some embodiments, the fusion protein retains the biological activity of an $\alpha 5\beta 1$ integrin binding agent. In some embodiments, the fusion protein comprises an $\alpha 5\beta 1$ integrin antibody VH region, VL region, VH CDR (one, two or three VH CDRs), and/or VL CDR (one, two or three VL CDRs), wherein the fusion protein binds to an $\alpha 5\beta 1$ integrin epitope, an $\alpha 5\beta 1$ integrin fragment and/or an $\alpha 5\beta 1$ integrin polypeptide.

[00168] Fusion proteins may be generated, for example, through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as “DNA shuffling”). DNA shuffling may be employed to alter the activities of $\alpha 5\beta 1$ integrin binding agents (*e.g.*, antibodies), including human $\alpha 5\beta 1$ integrin binding agents, as described herein, including, for example, $\alpha 5\beta 1$ integrin binding agents with higher affinities and lower dissociation rates. In some embodiments, $\alpha 5\beta 1$ integrin binding agents, including human $\alpha 5\beta 1$ integrin binding agents, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion, or other methods prior to recombination. A polynucleotide

encoding an $\alpha 5\beta 1$ integrin binding agent described herein may be recombined with one or more components, motifs, sections, parts, domains, fragments, *etc.* of one or more heterologous molecules.

[00169] An $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody), including a human $\alpha 5\beta 1$ integrin binding agents, described herein may also be attached to solid supports, which are useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride, or polypropylene.

[00170] An $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, described herein can also be linked or conjugated (directly or indirectly) to a second antibody to form an antibody heteroconjugate.

[00171] The linker may be a “cleavable moiety” facilitating release of the linked or conjugated agent in a cell, but non-cleavable linkers are also contemplated herein. Linkers for use in conjugates (*e.g.*, antibody-drug conjugates) of the present disclosure include, without limitation, acid labile linkers (*e.g.*, hydrazone linkers), disulfide-containing linkers, peptidase-sensitive linkers (*e.g.*, peptide linkers comprising amino acids, for example, valine and/or citrulline such as citrulline-valine or phenylalanine-lysine), photolabile linkers, dimethyl linkers, thioether linkers, or hydrophilic linkers designed to evade multidrug transporter-mediated resistance.

[00172] Conjugates of an antibody and agent, including wherein the agent is a drug for the preparation of ADC, may be made using a variety of bifunctional protein coupling agents such as BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate). The present disclosure further contemplates that conjugates of antibodies and agents, including wherein the agent is a drug for the preparation of ADC, may be prepared using any suitable methods as disclosed in the art (see, *e.g.*, Bioconjugate Techniques (Hermanson ed., 2d ed. 2008)).

[00173] Conventional conjugation strategies for antibodies and agents, including wherein the agent is a drug for the preparation of ADC, have been based on random conjugation chemistries involving the ϵ -amino group of Lys residues or the thiol group of Cys residues, which results in heterogeneous conjugates. Recently developed techniques allow site-specific conjugation to antibodies, resulting in homogeneous loading and avoiding conjugate subpopulations with altered antigen-binding or pharmacokinetics. These include engineering of “thiomabs” comprising cysteine substitutions at positions on the heavy and light chains that provide reactive thiol groups and do not disrupt immunoglobulin folding and assembly or alter antigen. In another method, selenocysteine is cotranslationally inserted into an antibody sequence by recoding the stop codon UGA from termination to selenocysteine insertion, allowing site specific covalent conjugation at the nucleophilic selenol group of selenocysteine in the presence of the other natural amino acids.

[00174] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, described herein is conjugated to a cytotoxic agent. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, disclosed herein can be optionally conjugated with one or more cytotoxic agent(s) disclosed herein or known in the art in order to generate an ADC. In some embodiments, the cytotoxic agent is a chemotherapeutic agent including, but not limited to, methotrexate, adriamycin, doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents. In some embodiments, the cytotoxic agent is an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof, including, but not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolacca americana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcin, crotin, Sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin,

enomycin, and the tricothecenes. In some embodiments, the cytotoxic agent is a radioisotope to produce a radioconjugate or a radioconjugated agent. A variety of radionuclides are available for the production of radioconjugated agents including, but not limited to, ^{90}Y , ^{125}I , ^{131}I , ^{123}I , ^{111}In , ^{131}In , ^{105}Rh , ^{153}Sm , ^{67}Cu , ^{67}Ga , ^{166}Ho , ^{177}Lu , ^{186}Re , ^{188}Re , and ^{212}Bi . Conjugates of a polypeptide or molecule and one or more small molecule toxins, such as a calicheamicin, maytansinoids, a trichothene, and CC1065, and the derivatives of these toxins that have toxin activity, can also be used. Conjugates of a polypeptide or molecule and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene).

[00175] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, described herein is conjugated to a drug such as a signal transduction modulator, a pro-apoptotic agent, a mitotic inhibitor, an anti-tumor antibiotic, an immunomodulating agent, a nucleic acid for gene therapy, an alkylating agent, an anti-angiogenic agent, an anti-metabolite, a boron-containing agent, a chemoprotective agent, a hormone agent, an anti-hormone agent, a corticosteroid, a photoactive therapeutic agent, an oligonucleotide, a radionuclide agent, a radiosensitizer, a topoisomerase inhibitor, and a tyrosine kinase inhibitor. In some embodiments, the mitotic inhibitor is a dolastatin, an auristatin, a maytansinoid, and a plant alkaloid. In some embodiments, the drug is a dolastatin, an auristatin, a maytansinoid, and a plant alkaloid. An example of an auristatin is monomethylaurisatin F (MMAF) or monomethylauristatin E (MMAE). Examples of maytansinoids include, but are not limited to, DM1, DM2, DM3, and

DM4. In some embodiments, the anti-tumor antibiotic is selected from the group consisting of an actinomycine, an anthracycline, a calicheamicin, and a duocarmycin. In some embodiments, the actinomycine is a pyrrolobenzodiazepine (PBD).

[00176] An $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, as described herein may be monospecific, bispecific, trispecific or of greater multispecificity. Such agents may include antibodies. Multispecific antibodies, such as bispecific antibodies, are monoclonal antibodies that have binding specificities for at least two different targets (*e.g.*, $\alpha 5\beta 1$ integrin and $\alpha \nu$ integrin) or two different epitopes on the same target (*e.g.*, a bispecific antibody directed to $\alpha 5\beta 1$ integrin with a first binding domain for a first epitope of an $\alpha 5\beta 1$ integrin, and a second binding domain for a second epitope of $\alpha 5\beta 1$ integrin). In some embodiments, the multispecific (*e.g.*, bispecific) antibodies can be constructed based on the sequences of the antibodies described herein, for example, the CDR sequences in Table 1, Table 2, Table 3, Table 4, Table 5, and/or Table 6. In some embodiments, the multispecific antibodies described herein are bispecific antibodies. In some embodiments, bispecific antibodies are mouse, chimeric, human or humanized antibodies. In some embodiments, one of the binding specificities of the multispecific antibody is for $\alpha 5\beta 1$ integrin and the other is for any other target (*e.g.*, $\alpha \nu \beta 3$ integrin). In some embodiments, a multispecific (*e.g.*, bispecific) antibody can comprise more than one target (*e.g.*, antigen) binding domain, in which different binding domains are specific for different targets (*e.g.*, a first binding domain that binds to $\alpha 5\beta 1$ integrin and a second binding domain that binds another target (*e.g.*, $\alpha \nu \beta 3$ integrin). In some embodiments, multispecific (*e.g.*, bispecific) antibody molecules can bind than one (*e.g.*, two or more) epitopes on the same target (*e.g.*, $\alpha 5\beta 1$ integrin).

[00177] Methods for making multispecific antibodies are known in the art, such as, by co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (see, *e.g.*, Milstein and Cuello, 1983, *Nature* 305:537-40). For further details of generating multispecific antibodies (*e.g.*,

bispecific antibodies), see, for example, Bispecific Antibodies (Kontermann ed., 2011).

[00178] Exemplary structures of multispecific antibodies are known in the art and are further described in Weidle *et al.*, 2013, Cancer Genomics & Proteomics 10: 1-18; Brinkman *et al.*, 2017, MABS, 9:2, 182-212; Godar *et al.*, 2018, Expert Opinion on Therapeutic Patents, 28:3, 251-276; and Spiess *et al.*, 2015, Mol. Immunol. 67 95-106.

[00179] For example, bispecific antibody molecules can be classified into different structural groups: (i) bispecific immunoglobulin G (BslgG); (ii) IgG appended with an additional antigen-binding moiety; (iii) bispecific antibody fragments; (iv) bispecific fusion proteins; and (v) bispecific antibody conjugates. As a non-limiting example, BslgG formats can include crossMab, DAF (two-in-one), DAF (four-in-one), DutaMab, DT-IgG, knobs-in-holes common LC, knobs-in-holes assembly, charge pair, Fab-arm exchange, SEEDbody, triomab, LUZ-Y, Fcab, $\kappa\lambda$ -body, orthogonal Fab.

[00180] In some embodiments, BslgG comprises heavy chains that are engineered for heterodimerization. For example, heavy chains can be engineered for heterodimerization using a “knobs-into-holes” strategy, a SEED platform, a common heavy chain (*e.g.*, in $\kappa\lambda$ -bodies), and use of heterodimeric Fc regions. Strategies are known in the art to avoid heavy chain pairing of homodimers in BslgG, including knobs-into-holes, duobody, azymetric, charge pair, HA-TF, SEEDbody, and differential protein A affinity.

[00181] Another bispecific antibody format is IgG appended with an additional antigen-binding moiety. For example, monospecific IgG can be engineered to have bispecificity by appending an additional antigen-binding unit onto the monospecific IgG, *for example*, at the N- or C- terminus of either the heavy or light chain. Exemplary additional antigen-binding units include single domain antibodies (*e.g.*, variable heavy chain or variable light chain), engineered protein scaffolds, and paired antibody variable domains (*e.g.*, single chain variable fragments or variable

fragments). Non-limiting examples of appended IgG formats include dual variable domain IgG (DVD-Ig), IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, zybod, and DVI-IgG (four- in-one). See Spiess et al. Mol. Immunol. 67(2015):95-106. In some embodiments, an exemplary antibody format is a B-Body format for monospecific or multispecific (e.g., bispecific antibodies) as described in, for example, International Patent Application Publication No. WO 2018/075692 and US Patent Application Publication No. 2018/0118811.

[00182] Bispecific antibody fragments (BsAb) are a format of bispecific antibody molecules that lack some or all of the antibody constant domains. For example, some BsAb lack an Fc region. In some embodiments, bispecific antibody fragments include heavy and light chain regions that are connected by a peptide linker that permits efficient expression of the BsAb in a single host cell. Non-limiting examples of bispecific antibody fragments include, but are not limited to, nanobody, nanobody-HAS, BiTE, Diabody, DART, TandAb, scDiabody, scDiabody-CH3, Diabody-CH3, triple body, miniantibody, minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv, scFv-CH-CL-scFv, F(ab')₂, F(ab')₂-scFv₂, scFv-KIH, Fab-scFv-Fc, tetravalent HCAb, scDiabody-Fc, Diabody-Fc, tandem scFv-Fc, and intrabody.

[00183] Bispecific fusion proteins include antibody fragments linked to other proteins. For example bispecific fusion proteins can be linked to other proteins to add additional specificity and/or functionality. In some embodiments, the dock-and-lock (DNL) method can be used to generate bispecific antibody molecules with higher valency. For example, bispecific antibody fusions to albumin binding proteins or human serum albumin can be extend the serum half-life of antibody fragments. In some embodiments, chemical conjugation, for example, chemical conjugation of antibodies and/or antibody fragments, can be used to create BsAb molecules. An exemplary bispecific antibody conjugate includes the CovX-body format, in which a low molecular weight drug is conjugated site-specifically to a single reactive lysine in

each Fab arm or an antibody or fragment thereof. In some embodiments, the conjugation improves the serum half-life.

[00184] Methods of production of multispecific antibodies, including bispecific antibodies, are known in the art. For example, multispecific antibodies, including bispecific antibodies, can be produced by separate expression of the component antibodies in different host cells and subsequent purification/assembly or by expression of the component antibodies in a single host cell. Purification of multispecific (e.g., bispecific) antibody molecules can be performed by various methods known in the art, including affinity chromatography.

[00185] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies), including human $\alpha 5\beta 1$ integrin binding agents, disclosed herein can be provided in any antibody format disclosed herein or known in the art. As a non-limiting example, in some embodiments, the $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies), including human $\alpha 5\beta 1$ integrin binding agents, can be selected from Fabs-in-tandem-Ig (FIT-Ig); DVD-Ig; hybrid hybridoma (quadroma or tetradoma); anticalin platform (Pieris); diabodies; single chain diabodies; tandem single chain Fv fragments; TandAbs, Trispecific Abs (Affimed); Darts dual affinity retargeting (Macrogenics); Bispecific Xmabs (Xencor); Bispecific T cell engagers (BiTE; Amgen; 55kDa); Triplebodies; Tribody = Fab-scFv Fusion Protein multifunctional recombinant antibody derivatives (CreativeBiolabs); Duobody platform (Genmab); dock and lock platform; knobs-into-holes (KIH) platform; humanized bispecific IgG antibody (REGN1979) (Regeneron); Mab2 bispecific antibodies (F-Star); DVD-Ig = dual variable domain immunoglobulin (AbbVie); kappa-lambda bodies; TBTI = tetravalent bispecific tandem Ig; and CrossMab (Roche).

[00186] In some embodiments, a multispecific (e.g., bispecific) antibody disclosed herein comprises an $\alpha 5\beta 1$ integrin binding domain and one or more additional binding domains that bind to one or more targets that are not $\alpha 5\beta 1$ integrin (e.g., αv integrin). In some embodiments, a multispecific (e.g., bispecific) antibody disclosed herein comprises an $\alpha 5\beta 1$ integrin binding domain that comprises the CDRs

(according to Kabat and/or Chothia, AbM, Contact, or IMGT) of the VH and/or VL amino acid sequences of Table 1. In some embodiments, a multispecific (e.g., bispecific) antibody disclosed herein comprises an $\alpha 5\beta 1$ integrin binding domain that comprises the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) of the VH and/or VL amino acid sequences of Table 2. In some embodiments, a multispecific (e.g., bispecific) antibody disclosed herein comprises an $\alpha 5\beta 1$ integrin binding domain that comprises the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) of the VH and/or VL amino acid sequences of Table 3. In some embodiments, a multispecific (e.g., bispecific) antibody disclosed herein comprises an $\alpha 5\beta 1$ integrin binding domain that comprises the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) of the VH and/or VL amino acid sequences of Table 4. In some embodiments, a multispecific (e.g., bispecific) antibody disclosed herein comprises an $\alpha 5\beta 1$ integrin binding domain that comprises the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) of the VH and/or VL amino acid sequences of Table 5. In some embodiments, a multispecific (e.g., bispecific) antibody disclosed herein comprises an $\alpha 5\beta 1$ integrin binding domain that comprises the CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) of the VH and/or VL amino acid sequences of Table 6.

[00187] In some embodiments, described herein is a multispecific (e.g., bispecific) antibody comprising a binding domain which binds to $\alpha 5\beta 1$ integrin that comprises VH and VL CDRs (e.g., VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT) as set forth in Table 1. In some embodiments, described herein is a multispecific (e.g., bispecific) antibody comprising a binding domain which binds to $\alpha 5\beta 1$ integrin that comprises VH and VL CDRs (e.g., VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT) as set forth in Table 2. In some embodiments, described herein is a multispecific (e.g., bispecific) antibody comprising a binding domain which binds to $\alpha 5\beta 1$ integrin that comprises VH and VL CDRs (e.g., VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and

VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT) as set forth in Table 3. In some embodiments, described herein is a multispecific (e.g., bispecific) antibody comprising a binding domain which binds to $\alpha 5\beta 1$ integrin that comprises VH and VL CDRs (e.g., VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT) as set forth in Table 4. In some embodiments, described herein is a multispecific (e.g., bispecific) antibody comprising a binding domain which binds to $\alpha 5\beta 1$ integrin that comprises VH and VL CDRs (e.g., VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT) as set forth in Table 5. In some embodiments, described herein is a multispecific (e.g., bispecific) antibody comprising a binding domain which binds to $\alpha 5\beta 1$ integrin that comprises VH and VL CDRs (e.g., VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 according to Kabat and/or Chothia, AbM, Contact, or IMGT) as set forth in Table 6.

[00188] Antibodies that bind $\alpha 5\beta 1$ integrin may be obtained by any suitable method, such as (but not limited to) immunization with whole tumor cells comprising $\alpha 5\beta 1$ integrin and collection of antibodies, recombinant techniques, or screening libraries of antibodies or antibody fragments using $\alpha 5\beta 1$ integrin extracellular domain epitopes. Monoclonal antibodies may be generated using a variety of known techniques (see, e.g., Coligan et al. (eds.), *Current Protocols in Immunology*, 1:2.5.12.6.7 (John Wiley & Sons 1991); *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.) (1980); *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press (1988); and Picksley et al., "Production of monoclonal antibodies against proteins expressed in *E. coli*," in *DNA Cloning 2: Expression Systems*, 2nd Edition, Glover et al. (eds.), page 93 (Oxford University Press 1995)). For example, an exemplary technique for generating monoclonal antibodies comprises immunizing an animal with a human $\alpha 5\beta 1$ integrin antigen and

generating a hybridoma from spleen cells taken from the animal. A hybridoma may produce a monoclonal antibody or antibody fragment that binds $\alpha 5\beta 1$ integrin.

[00189] In some embodiments, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries, including as described herein. In some embodiments, antibody phage libraries can be generated using the techniques described in, for example, *Antibody Phage Display: Methods and Protocols*, P.M. O'Brien and R. Aitken, eds, Humana Press, Totawa N.J., 2002. In some embodiments, antibody clones can be selected by screening phage libraries. Phage libraries can contain phage that display various fragments of antibody variable region (Fv) fused to phage coat protein (e.g., Fab, scFv). Such phage libraries are screened for antibodies against the desired antigen. Clones expressing Fv fragments (e.g., Fab, scFv) capable of binding to the desired antigen are adsorbed to the antigen and thus separated from the non-binding clones in the library. The binding clones are then eluted from the antigen, and can be further enriched by additional cycles of antigen adsorption/elution.

[00190] Variable domains can be displayed functionally on phage, either as single-chain Fv (scFv) fragments, in which VH and VL are covalently linked through a short, flexible peptide, or as Fab fragments, in which they are each fused to a constant domain and interact non-covalently, as described, for example, in Winter *et al.*, *Ann. Rev. Immunol.*, 12: 433-455 (1994).

[00191] Repertoires of VH and VL genes can be separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be searched for antigen-binding clones as described, for example, in Winter *et al.*, *supra*. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned to provide a single source of human antibodies to a wide range of non-self and also self antigens without any immunization as described, for example, by Griffiths *et al.*, *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning the unrearranged V-gene segments from

stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro* as described, for example, by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992).

[00192] Screening of the libraries can be accomplished by various techniques known in the art. For example, $\alpha 5\beta 1$ integrin (*e.g.*, an $\alpha 5\beta 1$ integrin polypeptide, fragment or epitope) can be used to coat the wells of adsorption plates, expressed on host cells affixed to adsorption plates or used in cell sorting, or conjugated to biotin for capture with streptavidin-coated beads, or used in any other method for panning display libraries. The selection of antibodies with slow dissociation kinetics (*e.g.*, good binding affinities) can be promoted by use of long washes and monovalent phage display as described in Bass *et al.*, *Proteins*, 8: 309-314 (1990) and in WO 92/09690, and a low coating density of antigen as described in Marks *et al.*, *Biotechnol.*, 10: 779-783 (1992).

[00193] An $\alpha 5\beta 1$ integrin binding agent (*e.g.*, antibody) can be obtained by designing a suitable antigen screening procedure to select for the phage clone of interest followed by construction of a full length $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) clone using VH and/or VL sequences (*e.g.*, the Fv sequences), or various CDR sequences from VH and VL sequences, from the phage clone of interest and suitable constant region (*e.g.*, Fc) sequences described in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3.

[00194] Likewise, human antibodies that bind $\alpha 5\beta 1$ integrin may be generated by any of a number of techniques including, but not limited to, Epstein Barr Virus (EBV) transformation of human peripheral blood cells (*e.g.*, containing B lymphocytes), *in vitro* immunization of human B cells, fusion of spleen cells from immunized transgenic mice carrying inserted human immunoglobulin genes, isolation from human immunoglobulin V region phage libraries, or other procedures as known in the art and based on the disclosure herein. Methods for obtaining human antibodies from transgenic animals are further described, for example, in Bruggemann *et al.*,

Curr. Opin. Biotechnol., 8: 455-58, 1997; Jakobovits et al., *Ann. N. Y. Acad. Sci.*, 764: 525-35, 1995; Green et al., *Nature Genet.*, 7: 13-21, 1994; Lonberg et al., *Nature*, 368: 856-859, 1994; Taylor et al., *Int. Immun.* 6: 579-591, 1994; and U.S. Patent No. 5,877,397.

[00195] For example, human antibodies that bind $\alpha 5 \beta 1$ integrin may be obtained from transgenic animals that have been engineered to produce specific human antibodies in response to antigenic challenge. For example, International Patent Publication No. WO 98/24893 discloses transgenic animals having a human Ig locus, wherein the animals do not produce functional endogenous immunoglobulins due to the inactivation of endogenous heavy and light chain loci. Transgenic non-primate mammalian hosts capable of mounting an immune response to an immunogen, wherein the antibodies have primate constant and/or variable regions, and wherein the endogenous immunoglobulin encoding loci are substituted or inactivated, also have been described. For example, International Patent Publication No. WO 96/30498 discloses the use of the Cre/Lox system to modify the immunoglobulin locus in a mammal, such as to replace all or a portion of the constant or variable region to form a modified antibody molecule. For example, International Patent Publication No. WO 94/02602 discloses non-human mammalian hosts having inactivated endogenous Ig loci and functional human Ig loci. For example, U.S. Patent No. 5,939,598 discloses methods of making transgenic mice in which the mice lack endogenous heavy chains, and express an exogenous immunoglobulin locus comprising one or more xenogeneic constant regions. Using a transgenic animal, such as a transgenic animal described herein, an immune response can be produced to a selected antigenic molecule, and antibody producing cells can be removed from the animal and used to produce hybridomas that secrete human-derived monoclonal antibodies. Immunization protocols, adjuvants, and the like are known in the art, and are used in immunization of, for example, a transgenic mouse as described, for example, in International Patent Publication No. WO 96/33735.

The monoclonal antibodies can be tested for the ability to inhibit or neutralize the biological activity or physiological effect of the corresponding protein.

[00196] The present disclosure provides humanized antibodies that bind $\alpha 5\beta 1$ integrin, including human $\alpha 5\beta 1$ integrin. Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Humanized antibodies that bind $\alpha 5\beta 1$ integrin may be produced using techniques known to those skilled in the art (e.g., Zhang et al., *Molecular Immunology*, 42(12): 1445-1451, 2005; Hwang et al., *Methods*, 36(1): 35-42, 2005; Dall’Acqua et al., *Methods*, 36(1): 43-60, 2005; Clark, *Immunology Today*, 21(8): 397-402, 2000, and U.S. Patent Nos. 6,180,370; 6,054,927; 5,869,619; 5,861,155; 5,712,120; and 4,816,567.

[00197] In some cases, the humanized antibodies are constructed by CDR grafting, in which the amino acid sequences of the six complementarity determining regions (CDRs) of the parent non-human antibody (e.g., rodent) are grafted onto a human antibody framework. For example, Padlan *et al.* (*FASEB J.* 9:133-139, 1995) determined that only about one third of the residues in the CDRs actually contact the antigen, and termed these the “specificity determining residues,” or SDRs. In the technique of SDR grafting, only the SDR residues are grafted onto the human antibody framework (see, e.g., Kashmiri *et al.*, *Methods* 36: 25-34, 2005).

[00198] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies, can be important to reduce antigenicity. For example, according to the so-called “best-fit” method, the sequence of the variable domain of a non-human (e.g., rodent) antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent may be selected as the human framework for the humanized antibody (see, e.g., Sims *et al.* (1993) *J. Immunol.* 151:2296; Chothia *et al.* (1987) *J. Mol. Biol.* 196:901. Another method uses a particular framework derived from the

consensus sequences of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (see, *e.g.*, Carter *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; Presta *et al.* (1993) *J. Immunol.*, 151:2623. In some cases, the framework is derived from the consensus sequences of the most abundant human subclasses, V_L6 subgroup I (V_L6I) and V_H subgroup III (V_HIII). In another method, human germline genes are used at the source of the framework regions.

[00199] In an alternative paradigm based on comparison of CDRs, called Superhumanization, FR homology is irrelevant. The method consists of comparison of the non-human sequence with the functional human germline gene repertoire. Those genes encoding the same or closely related canonical structures to the murine sequences are then selected. Next, within the genes sharing the canonical structures with the non-human antibody, those with highest homology within the CDRs are chosen as FR donors. Finally, the non-human CDRs are grafted onto these FRs (see, *e.g.*, Tan *et al.*, *J. Immunol.* 169: 1119-1125, 2002).

[00200] It is further generally desirable that antibodies be humanized with retention of their affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. These include, for example, WAM (Whitelegg and Rees, *Protein Eng.* 13: 819-824, 2000), Modeller (Sali and Blundell, *J. Mol. Biol.* 234: 779-815, 1993), and Swiss PDB Viewer (Guex and Peitsch, *Electrophoresis* 18: 2714-2713, 1997). Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, for example, the analysis of residues that influence the ability of the candidate

immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[00201] Another method for antibody humanization is based on a metric of antibody humanness termed Human String Content (HSC). This method compares the mouse sequence with the repertoire of human germline genes and the differences are scored as HSC. The target sequence is then humanized by maximizing its HSC rather than using a global identity measure to generate multiple diverse humanized variants. (see, *e.g.*, Lazar *et al.*, *Mol. Immunol.* 44: 1986-1998, 2007).

[00202] In addition to the methods described above, empirical methods may be used to generate and select humanized antibodies. These methods include those that are based upon the generation of large libraries of humanized variants and selection of the best clones using enrichment technologies or high throughput screening techniques. Antibody variants may be isolated from phage, ribosome and yeast display libraries as well as by bacterial colony screening (see, *e.g.*, Hoogenboom, *Nat. Biotechnol.* 23: 1105-1116, 2005; Dufner *et al.*, *Trends Biotechnol.* 24: 523-529, 2006; Feldhaus *et al.*, *Nat. Biotechnol.* 21: 163-70, 2003; Schlapschky *et al.*, *Protein Eng. Des. Sel.* 17: 847-60, 2004).

[00203] In the FR library approach, a collection of residue variants are introduced at specific positions in the FR followed by selection of the library to select the FR that best supports the grafted CDR. The residues to be substituted may include some or all of the "Vernier" residues identified as potentially contributing to CDR structure (see, *e.g.*, Foote and Winter, *J. Mol. Biol.* 224: 487-499, 1992), or from the more limited set of target residues identified by Baca *et al.* (*J. Biol. Chem.* 272: 10678-10684, 1997).

[00204] In FR shuffling, whole FRs are combined with the non-human CDRs instead of creating combinatorial libraries of selected residue variants (see, e.g., Dall'Acqua *et al.*, *Methods* 36: 43-60, 2005). The libraries may be screened for binding in a two-step selection process, first humanizing VL, followed by VH. Alternatively, a one-step FR shuffling process may be used. Such a process has been shown to be more efficient than the two-step screening, as the resulting antibodies exhibited improved biochemical and physico-chemical properties including enhanced expression, increased affinity and thermal stability (see, e.g., Damschroder *et al.*, *Mol. Immunol.* 44: 3049-60, 2007).

[00205] The "humaneering" method is based on experimental identification of essential minimum specificity determinants (MSDs) and is based on sequential replacement of non-human fragments into libraries of human FRs and assessment of binding. It begins with regions of the CDR3 of non-human VH and VL chains and progressively replaces other regions of the non-human antibody into the human FRs, including the CDR1 and CDR2 of both VH and VL. This methodology typically results in epitope retention and identification of antibodies from multiple sub-classes with distinct human V-segment CDRs. Humaneering allows for isolation of antibodies that are 91-96 % homologous to human germline gene antibodies. (see, e.g., Alfenito, Cambridge Healthtech Institute's Third Annual PEGS, The Protein Engineering Summit, 2007).

[00206] The "human engineering" method involves altering an non-human antibody or antibody fragment, such as a mouse or chimeric antibody or antibody fragment, by making specific changes to the amino acid sequence of the antibody so as to produce a modified antibody with reduced immunogenicity in a human that nonetheless retains the desirable binding properties of the original non-human antibodies. Generally, the technique involves classifying amino acid residues of a non-human (e.g., mouse) antibody as "low risk", "moderate risk", or "high risk" residues. The classification is performed using a global risk/reward calculation that evaluates the predicted benefits of making particular substitution (e.g., for

immunogenicity in humans) against the risk that the substitution will affect the resulting antibody's folding and/or are substituted with human residues. The particular human amino acid residue to be substituted at a given position (e.g., low or moderate risk) of a non-human (e.g., mouse) antibody sequence can be selected by aligning an amino acid sequence from the non-human antibody's variable regions with the corresponding region of a specific or consensus human antibody sequence. The amino acid residues at low ("Low") and/or moderate ("Mod") risk positions in the non-human sequence can be substituted for the corresponding residues in the human antibody sequence according to the alignment. Techniques for making human engineered proteins are described in greater detail in Studnicka *et al.*, Protein Engineering, 7: 805-814 (1994), U.S. Patents 5,766,886, 5,770,196, 5,821,123, and 5,869,619, and PCT Application Publication WO 93/11794.

[00207] Exemplary humanized antibodies generated based on the above-referenced human engineering method are provided herein, including humanized antibodies designated as A-15B08_Low, A-15B08_Low+Mod, A2-7A05_Low, A2-7A05_Low+Mod, C-14D12_Low and C-14D12_Low+Mod. The VH, VL, and CDR sequences according to various numbering schemes (e.g., Kabat, AbM, Chothia, Contact, and IMGT) of these humanized antibodies are shown in **FIGs. 2C, 2D, 2E, 2F, 2G, and 2H**.

[00208] More specifically, the antibody designated as A-15B08_Low comprises a VH comprising the amino acid sequence of SEQ ID NO:136 and a VL comprising the amino acid sequence of SEQ ID NO:137. The 6 CDR sequences of A-15B08_Low according to Kabat, AbM, Chothia, Contact, and IMGT are shown in **FIGs. 2C and 2D**.

[00209] The antibody designated as A-15B08_Low+Mod comprises a VH comprising the amino acid sequence of SEQ ID NO:138 and a VL comprising the amino acid sequence of SEQ ID NO:139. The 6 CDR sequences of A-15B08_Low+Mod according to Kabat, AbM, Chothia, Contact, and IMGT are shown in **FIGs. 2C and 2D**.

[00210] The antibody designated as A2-7A05_Low comprises a VH comprising the amino acid sequence of SEQ ID NO:140 and a VL comprising the amino acid sequence of SEQ ID NO:141. The 6 CDR sequences of A2-7A05_Low according to Kabat, AbM, Chothia, Contact, and IMGT are shown in **FIGs. 2E** and **2F**.

[00211] The antibody designated as A2-7A05_Low+Mod comprises a VH comprising the amino acid sequence of SEQ ID NO:142 and a VL comprising the amino acid sequence of SEQ ID NO:143. The 6 CDR sequences of A2-7A05_Low+Mod according to Kabat, AbM, Chothia, Contact, and IMGT are shown in **FIGs. 2E** and **2F**.

[00212] The antibody designated as C-14D12_Low comprises a VH comprising the amino acid sequence of SEQ ID NO:144 and a VL comprising the amino acid sequence of SEQ ID NO:145. The 6 CDR sequences of C-14D12_Low according to Kabat, AbM, Chothia, Contact, and IMGT are shown in **FIGs. 2G** and **2H**.

[00213] The antibody designated as C-14D12_Low+Mod comprises a VH comprising the amino acid sequence of SEQ ID NO:146 and a VL comprising the amino acid sequence of SEQ ID NO:147. The 6 CDR sequences of C-14D12_Low+Mod according to Kabat, AbM, Chothia, Contact, and IMGT are shown in **FIGs. 2G** and **2H**.

[00214] In some embodiments, provided herein is an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody or fragment thereof that binds $\alpha 5\beta 1$ integrin, e.g., human $\alpha 5\beta 1$ integrin) comprising one or more CDR sequence(s) from A-15B08_Low, A-15B08_Low+Mod, A2-7A05_Low, A2-7A05_Low+Mod, C-14D12_Low and C-14D12_Low+Mod, as shown in **FIGs. 2C, 2D, 2E, 2F, 2G, and 2H**.

[00215] In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:136. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:137. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent

provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:136; and a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:137. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In some embodiments, the CDRs are according to Chothia numbering. In some embodiments, the CDRs are according to Contact numbering. In some embodiments, the CDRs are according to IMGT. In some embodiments, the CDRs are according to a combination of two or more numbering schemes selected from Kabat, AbM, Chothia, Contact, and IMGT.

[00216] In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:138. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:139. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:138; and a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:139. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In some embodiments, the CDRs are according to Chothia numbering. In some embodiments, the CDRs are according to Contact numbering. In some embodiments, the CDRs are according to IMGT. In some embodiments, the CDRs are according to a combination of two or more numbering schemes selected from Kabat, AbM, Chothia, Contact, and IMGT.

[00217] In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:140. In some embodiments,

the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:141. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:140; and a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:141. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In some embodiments, the CDRs are according to Chothia numbering. In some embodiments, the CDRs are according to Contact numbering. In some embodiments, the CDRs are according to IMGT. In some embodiments, the CDRs are according to a combination of two or more numbering schemes selected from Kabat, AbM, Chothia, Contact, and IMGT.

[00218] In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:142. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:143. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:142; and a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:143. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In some embodiments, the CDRs are according to Chothia numbering. In some embodiments, the CDRs are according to Contact numbering. In some embodiments, the CDRs are according to IMGT. In some embodiments, the CDRs are according to a combination of two or more numbering schemes selected from Kabat, AbM, Chothia, Contact, and IMGT.

[00219] In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:144. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:145. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:144; and a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:145. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In some embodiments, the CDRs are according to Chothia numbering. In some embodiments, the CDRs are according to Contact numbering. In some embodiments, the CDRs are according to IMGT. In some embodiments, the CDRs are according to a combination of two or more numbering schemes selected from Kabat, AbM, Chothia, Contact, and IMGT.

[00220] In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:146. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:147. In some embodiments, the $\alpha 5\beta 1$ integrin binding agent provided herein comprises a VH comprising a VH CDR1, VH CDR2, and VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:146; and a VL comprising a VL CDR1, VL CDR2, and VL CDR3 as set forth in the VL comprising the amino acid sequence of SEQ ID NO:147. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In some embodiments, the CDRs are according to Chothia numbering. In some embodiments, the CDRs are according to Contact

numbering. In some embodiments, the CDRs are according to IMGT. In some embodiments, the CDRs are according to a combination of two or more numbering schemes selected from Kabat, AbM, Chothia, Contact, and IMGT.

[00221] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:136 and a VL comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:137, and the binding of the antibody or fragment thereof to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%).

[00222] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:138 and a VL comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:139, and the binding of the antibody or fragment thereof to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%).

[00223] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:140 and a VL comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:141, and the binding of the antibody or fragment thereof to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%).

[00224] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence having at least 75%, 80%,

85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:142 and a VL comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:143, and the binding of the antibody or fragment thereof to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%).

[00225] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:144 and a VL comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:145, and the binding of the antibody or fragment thereof to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%).

[00226] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:146 and a VL comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:147, and the binding of the antibody or fragment thereof to $\alpha 5\beta 1$ integrin (e.g., human $\alpha 5\beta 1$ integrin) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%).

[00227] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence of SEQ ID NO:136 and a VL comprising an amino acid sequence of SEQ ID NO:137.

[00228] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence of SEQ ID NO:138 and a VL comprising an amino acid sequence of SEQ ID NO:139.

[00229] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence of SEQ ID NO:140 and a VL comprising an amino acid sequence of SEQ ID NO:141.

[00230] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence of SEQ ID NO:142 and a VL comprising an amino acid sequence of SEQ ID NO:143.

[00231] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence of SEQ ID NO:144 and a VL comprising an amino acid sequence of SEQ ID NO:145.

[00232] In some embodiments, the antibody or fragment thereof provided herein comprises a VH comprising an amino acid sequence of SEQ ID NO:146 and a VL comprising an amino acid sequence of SEQ ID NO:147.

[00233] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent described herein comprises a non-antibody protein scaffold. Non-limiting examples of such a non-antibody protein scaffold include a fibronectin scaffold, an anticalin, an adnectin, an affibody, a DARPIn, a fynomer, an affitin, an affilin, an avimer, a cysteine-rich knottin peptide, or an engineered Kunitz-type inhibitor. Methods for generating such non-antibody protein scaffolds are well known in the art, any one of which can be used to generate an $\alpha 5\beta 1$ integrin binding agent comprising a non-antibody protein scaffold (see, e.g., Simeon and Chen, *Protein Cell*, 9(1):3-14 (2018); Yang et al., *Annu Rev Anal Chem (Palo Alto Calif)*. 10(1):293-320 (2017)).

[00234] Further provided are the materials for generating $\alpha 5\beta 1$ integrin binding agents, including human $\alpha 5\beta 1$ integrin binding agents, and fragments thereof. For example, an isolated cell (e.g., a hybridoma, a transformed or transfected cell) may produce an $\alpha 5\beta 1$ integrin binding agent (e.g., antibody or antibody fragment). In this regard, a cell (e.g., an isolated cell) may produce an antibody or fragment thereof comprising a VH and a VL as shown in Table 1, 2, 3, 4, 5, or 6 for A-15B08, A2-3B06, A2-5D10, A2-7A05, A2-7F01, or C-14D12AB1, respectively, or as shown in **FIGs. 2C-2H** for A-15B08-T62A, A-15B08_Low, A-15B08_Low+Mod, A2-7A05_Low,

A2-7A05_Low+Mod, C-14D12_Low and C-14D12_Low+Mod.. In some embodiments, polynucleotides described herein may comprise one or more nucleic acid sequences encoding an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, antibody or antibody fragment). In some embodiments, the polynucleotide is an isolated and/or recombinant polynucleotide. In various aspects, the isolated polynucleotide comprises a nucleotide sequence that encodes an antibody heavy chain variable region (V_H) and/or an antibody light chain variable region (V_L), wherein the V_H and the V_L comprise CDRs (according to Kabat and/or Chothia, AbM, Contact, or IMGT) identical to CDRs as shown in Table 1, CDRs as shown in Table 2, CDRs as shown in Table 3, CDRs as shown in Table 4, CDRs as shown in Table 5, CDRs as shown in Table 6, or CDRs as shown in **FIGs. 2C-2H**.

[00235] In some embodiments, one or more vectors (*e.g.*, expression vectors) may comprise one or more polynucleotides for expression of the one or more polynucleotides in a suitable host cell. Such vectors are useful, for example, for amplifying the polynucleotides in host cells to create useful quantities thereof, and for expressing binding agents, such as antibodies or antibody fragments, using recombinant techniques.

[00236] In some embodiments, one or more vectors are expression vectors wherein one or more polynucleotides encoding antibody sequences are operatively linked to one or more polynucleotides comprising expression control sequences. Autonomously replicating recombinant expression constructs such as plasmid and viral DNA vectors incorporating one or more polynucleotides encoding antibody sequences that bind $\alpha 5\beta 1$ integrin are specifically contemplated. Expression control DNA sequences include promoters, enhancers, and operators, and are generally selected based on the expression systems in which the expression construct (*e.g.*, expression vector) is to be utilized. Promoter and enhancer sequences are generally selected for the ability to increase gene expression, while operator sequences are generally selected for the ability to regulate gene expression. Expression constructs (*e.g.*, expression vectors) may also include sequences encoding one or more

selectable markers that permit identification of host cells bearing the construct. Expression constructs (e.g., expression vectors) may also include sequences that facilitate, and preferably promote, homologous recombination in a host cell. In some embodiments, expression constructs (e.g., expression vectors) can also include sequences necessary for replication in a host cell.

[00237] Exemplary expression control sequences include promoter/enhancer sequences, including, for example, cytomegalovirus promoter/enhancer (Lehner et al., *J. Clin. Microbiol.*, 29: 2494-2502, 1991; Boshart et al., *Cell*, 41: 521-530, 1985); Rous sarcoma virus promoter (Davis et al., *Hum. Gene Ther.*, 4: 151, 1993); Tie promoter (Korhonen et al., *Blood*, 86(5): 1828-1835, 1995); simian virus 40 promoter; DRA (downregulated in adenoma; Alrefai et al., *Am. J. Physiol. Gastrointest. Liver Physiol.*, 293: G923-G934, 2007); MCT1 (monocarboxylate transporter 1; Cuff et al., *Am. J. Physiol. Gastrointest. Liver Physiol.*, G977-G979, 2005); and Math1 (mouse atonal homolog 1; Shroyer et al., *Gastroenterology*, 132: 2477-2478, 2007), for expression in mammalian cells, the promoter being operatively linked upstream (e.g., 5') of a polypeptide coding sequence. In another variation, the promoter is an epithelial-specific promoter or endothelial-specific promoter. Polynucleotides may also optionally include a suitable polyadenylation sequence (e.g., the SV40 or human growth hormone gene polyadenylation sequence) operably linked downstream (e.g., 3') of the polypeptide coding sequence.

[00238] If desired, the one or more polynucleotides also optionally comprise nucleotide sequences encoding secretory signal peptides fused in frame with the polypeptide sequences. The secretory signal peptides direct secretion of the antibody polypeptides by the cells that express the one or more polynucleotides, and are cleaved by the cell from the secreted polypeptides. The one or more polynucleotides may further optionally comprise sequences whose only intended function is to facilitate large scale production of the vector. One can manufacture and administer polynucleotides for gene therapy using procedures that have been described in the literature for a variety of transgenes. See, e.g., Isner et al.,

Circulation, 91: 2687-2692, 1995; and Isner et al., *Human Gene Therapy*, 7: 989-1011, 1996.

[00239] In some embodiments, polynucleotides may further comprise additional sequences to facilitate uptake by host cells and expression of the antibody or fragment thereof (and/or any other peptide). In some embodiments, a “naked” transgene encoding an antibody or fragment thereof described herein (e.g., a transgene without a viral, liposomal, or other vector to facilitate transfection) is employed.

[00240] Any suitable vectors may be used to introduce one or more polynucleotides that encode an antibody or fragment thereof into the host. Exemplary vectors that have been described include replication deficient retroviral vectors, including but not limited to lentivirus vectors (see, e.g., Kim et al., *J. Virol.*, 72(1): 811-816, 1998; Kingsman & Johnson, *Scrip Magazine*, October, 1998, pp. 43-46); parvoviral vectors, such as adeno-associated viral (AAV) vectors (U.S. Patent Nos. 5,474,935; 5,139,941; 5,622,856; 5,658,776; 5,773,289; 5,789,390; 5,834,441; 5,863,541; 5,851,521; 5,252,479; Gnatenko et al., *J. Invest. Med.*, 45: 87-98, 1997); adenoviral (AV) vectors (see, e.g., U.S. Patent Nos. 5,792,453; 5,824,544; 5,707,618; 5,693,509; 5,670,488; 5,585,362; Quantin et al., *Proc. Natl. Acad. Sci. USA*, 89: 2581-2584, 1992; Stratford Perricaudet et al., *J. Clin. Invest.*, 90: 626-630, 1992; and Rosenfeld et al., *Cell*, 68: 143-155, 1992); an adenoviral adeno-associated viral chimeric (U.S. Patent No. 5,856,152) or a vaccinia viral or a herpesviral vector (U.S. Patent Nos. 5,879,934; 5,849,571; 5,830,727; 5,661,033; 5,328,688); Lipofectin mediated gene transfer (BRL); liposomal vectors (U.S. Patent No. 5,631,237); and combinations thereof. Any of these expression vectors can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., *Molecular Cloning, a Laboratory Manual*, 2d edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New

York, N.Y. (1994). Optionally, viral vectors are rendered replication-deficient by, for example, deleting or disrupting select genes required for viral replication.

[00241] Other non-viral delivery mechanisms contemplated include calcium phosphate precipitation (Graham and Van Der Eb, *Virology*, 52: 456-467, 1973; Chen and Okayama, *Mol. Cell Biol.*, 7: 2745-2752, 1987; Rippe et al., *Mol. Cell Biol.*, 10: 689-695, 1990) DEAE-dextran (Gopal, *Mol. Cell Biol.*, 5: 1188-1190, 1985), electroporation (Tur-Kaspa et al., *Mol. Cell Biol.*, 6: 716-718, 1986; Potter et al., *Proc. Nat. Acad. Sci. USA*, 81: 7161-7165, 1984), direct microinjection (Harland and Weintraub, *J. Cell Biol.*, 101: 1094-1099, 1985, DNA-loaded liposomes (Nicolau and Sene, *Biochim. Biophys. Acta*, 721: 185-190, 1982; Fraley et al., *Proc. Natl. Acad. Sci. USA*, 76: 3348-3352, 1979; Felgner, *Sci Am.*, 276(6): 102-6, 1997; Felgner, *Hum Gene Ther.*, 7(15): 1791-3, 1996), cell sonication (Fechheimer et al., *Proc. Natl. Acad. Sci. USA*, 84: 8463-8467, 1987), gene bombardment using high velocity microprojectiles (Yang et al., *Proc. Natl. Acad. Sci USA*, 87: 9568-9572, 1990), and receptor-mediated transfection (Wu and Wu, *J. Biol. Chem.*, 262: 4429-4432, 1987; Wu and Wu, *Biochemistry*, 27: 887-892, 1988; Wu and Wu, *Adv. Drug Delivery Rev.*, 12: 159-167, 1993).

[00242] An expression vector (or an antibody or fragment thereof described herein) may be entrapped in a liposome. See, e.g., Ghosh and Bachhawat, In: *Liver diseases, targeted diagnosis and therapy using specific receptors and ligands*, Wu G, Wu C ed., New York: Marcel Dekker, pp. 87-104 (1991); Radler et al., *Science*, 275(5301): 810-814, 1997). Also contemplated are various commercial approaches involving "lipofection" technology. In some embodiments, the liposome may be complexed with a hemagglutinating virus (HVJ). This has been shown to facilitate fusion with the cell membrane and promote cell entry of liposome-encapsulated DNA (see, e.g., Kaneda et al., *Science*, 243: 375-378, 1989). In some embodiments, the liposome is complexed or employed in conjunction with nuclear nonhistone chromosomal proteins (HMG-1) (see, e.g., Kato et al., *J. Biol. Chem.*, 266: 3361-3364, 1991). In some embodiments, the liposomes are complexed or employed in

conjunction with both HVJ and HMG-1. Such expression constructs have been successfully employed in transfer and expression of nucleic acid *in vitro* and *in vivo*. In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, is included in the liposome to target the liposome to cells (such as tumor cells) expressing $\alpha 5\beta 1$ integrin on their surface.

[00243] A cell may comprise one or more polynucleotides or one or more vectors, for example, the cell is transformed or transfected with one or more polynucleotides encoding an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, or the one or more vectors comprising the one or more polynucleotides. In some embodiments, cells express and produce an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, containing one or more, including six CDRs having at least 75% identity (e.g., 75%, 80%, 85%, 90%, 95%, 100%) to the CDRs of A-15B08 (see, e.g., Table 1). In some embodiments, the cell expresses and produces an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, containing the V_H and the V_L comprising CDRs identical to those of A2-3B06 (see, e.g., Table 2). In some embodiments, the cell expresses and produces an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, containing the V_H and the V_L comprising CDRs identical to those of A2-5D10 (see, e.g., Table 3). In some embodiments, the cell expresses and produces an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, containing the V_H and the V_L comprising CDRs identical to those of A2-7A05 (see, e.g., Table 4). In some embodiments, the cell expresses and produces an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, containing the V_H and the V_L comprising CDRs identical to those of A2-7F01 (see, e.g., Table 5). In some embodiments, the cell expresses and produces an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, containing the V_H and the V_L comprising CDRs identical to those of C-14D12 (see, e.g., Table 6). The cells may be prokaryotic cells, such as *Escherichia coli* (see, e.g., Pluckthun et al.,

Methods Enzymol., 178: 497-515, 1989), or eukaryotic cells, such as an animal cell (e.g., a myeloma cell, Chinese Hamster Ovary (CHO) cell, or hybridoma cell), yeast (e.g., *Saccharomyces cerevisiae*), or a plant cell (e.g., a tobacco, corn, soybean, or rice cell). Use of mammalian host cells may provide for translational modifications (e.g., glycosylation, truncation, lipidation, and phosphorylation) that may be desirable to confer optimal biological activity on recombinant expression products. Similarly, polypeptides (e.g., $\alpha 5\beta 1$ integrin binding agents such as antibodies), including human $\alpha 5\beta 1$ integrin binding agents) may be glycosylated or non-glycosylated and/or have been covalently modified to include one or more water soluble polymer attachments such as polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol.

[00244] Methods for introducing DNA or RNA into host cells are well known and include transformation, transfection, electroporation, nuclear injection, or fusion with carriers such as liposomes, micelles, ghost cells, and protoplasts. Such host cells are useful for amplifying polynucleotides and also for expressing polypeptides encoded by the polynucleotides. In this regard, a process for the production of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) may comprise culturing a host cell and isolating the $\alpha 5\beta 1$ integrin binding agent. Transferring a naked DNA expression construct into cells can be accomplished using particle bombardment, which depends on the ability to accelerate DNA coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (see, e.g., Klein et al., *Nature*, 327: 70-73, 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (see, e.g., Yang et al., *Proc. Natl. Acad. Sci USA*, 87: 9568-9572, 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads. A host cell may be isolated and/or purified. A host cell also may be a cell transformed *in vitro* to cause transient or permanent expression of the polypeptide *in vivo*. A host cell may also be an isolated cell transformed *ex vivo* and introduced

post-transformation, for example, to produce the polypeptide *in vivo* for therapeutic purposes. The definition of host cell explicitly excludes a transgenic human being.

[00245] A variety of methods for producing antibodies from polynucleotides are generally well-known. For example, basic molecular biology procedures are described by Maniatis et al., *Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, New York, 1989 (see also Maniatis et al, 3rd ed., Cold Spring Harbor Laboratory, New York, 2001). Additionally, numerous publications describe techniques suitable for the preparation of antibodies by manipulation of DNA, creation of expression vectors, and transformation and culture of appropriate cells (see, e.g., Mountain and Adair, Chapter 1 in *Biotechnology and Genetic Engineering Reviews*, Tombs ed., Intercept, Andover, UK, 1992); and *Current Protocols in Molecular Biology*, Ausubel ed., Wiley Interscience, New York, 1999).

[00246] An $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, is produced using any suitable method, for example, isolated from an immunized animal, recombinantly or synthetically generated, or genetically-engineered, including as described above. Antibody fragments derived from an antibody are obtained by, for example, proteolytic hydrolysis of an antibody. For example, papain or pepsin digestion of whole antibodies yields a 5S fragment termed $F(ab')_2$ or two monovalent Fab fragments and an Fc fragment, respectively. $F(ab)_2$ can be further cleaved using a thiol reducing agent to produce 3.5S Fab monovalent fragments. Methods of generating antibody fragments are further described in, for example, Edelman et al., *Methods in Enzymology*, 1: 422 Academic Press (1967); Nisonoff et al., *Arch. Biochem. Biophys.*, 89: 230-244, 1960; Porter, *Biochem. J.*, 73: 119-127, 1959; U.S. Patent No. 4,331,647; and by Andrews, S.M. and Titus, J.A. in *Current Protocols in Immunology* (Coligan et al., eds), John Wiley & Sons, New York (2003), pages 2.8.1 2.8.10 and 2.10A.1 2.10A.5.

[00247] An $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding agent, can be genetically engineered. For example, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including a human $\alpha 5\beta 1$ integrin binding

agent, comprises, for example, a variable region or variable domain generated by recombinant DNA engineering techniques. In this regard, a variable region is optionally modified by insertions, deletions, or changes in the amino acid sequence of the antibody to produce an antibody of interest, including as described above. Polynucleotides encoding complementarity determining regions (CDRs) of interest, including CDRs as listed in Tables 1-6, are prepared, for example, by using polymerase chain reaction to synthesize variable regions using mRNA of antibody producing cells as a template (see, e.g., Courtenay Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter et al. (eds.), page 166 (Cambridge University Press 1995); Ward et al., "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch et al., (eds.), page 137 (Wiley Liss, Inc. 1995); and Larrick et al., *Methods: A Companion to Methods in Enzymology*, 2: 106-110, 1991). Current antibody manipulation techniques allow construction of engineered variable region domains containing at least one CDR and, optionally, one or more framework amino acids from a first antibody and the remainder of the variable region domain from a second antibody. Such techniques are used, for example, to humanize an antibody or to improve its affinity for a binding target.

[00248] "Humanized antibodies" are antibodies in which CDRs of heavy and light variable chains of non-human immunoglobulins are transferred into a human variable domain. Constant regions need not be present, but if they are, they optionally are substantially identical to human immunoglobulin constant regions, for example, at least about 85-90%, about 95%, 96%, 97%, 98%, 99% or more identical, in some embodiments. Hence, in some instances, all parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of natural human immunoglobulin sequences. For example, humanized antibodies are human immunoglobulins (e.g., host antibody) in which hypervariable region residues of the host antibody are replaced by hypervariable region residues from a non-

human species (donor antibody) such as mouse, rat, rabbit, or a non-human primate having the desired specificity, affinity, and capacity.

[00249] In some embodiments, $\alpha 5\beta 1$ integrin binding agents (*e.g.*, antibodies) described herein are useful in compositions and in methods of treating, preventing, or alleviating an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition, including one or more symptoms of the disease, disorder, or condition. In some embodiments, the subject is diagnosed with an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition. The $\alpha 5\beta 1$ integrin-mediated diseases, disorders, and conditions include, but are not limited to, a cancer (*e.g.*, a cancer associated with or characterized by tumor cells that express or overexpress $\alpha 5\beta 1$ integrin), an angiogenesis-mediated disease (*e.g.*, a disease associated with or characterized by abnormal angiogenesis), and an inflammatory disease (*e.g.*, a neuroinflammatory disease, including MS and ALS).

[00250] In some embodiments, described herein is a method for treating a cancer or a tumor in a subject comprising administering to the subject an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) described herein or fragment thereof or a pharmaceutical composition comprising the binding agent (*e.g.*, antibody) described herein. In some embodiments, the subject is diagnosed with a cancer.

[00251] In some embodiments, described herein is a method for alleviating one or more symptoms associated with a cancer or a tumor in a subject comprising administering to the subject an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) described herein or fragment thereof or a pharmaceutical composition comprising the binding agent (*e.g.*, antibody) described herein.

[00252] In some embodiments, described herein is a method (i) for treating an angiogenesis-mediated disease, disorder, or condition or (ii) for inhibiting angiogenesis in a subject (*e.g.*, with a tumor) comprising administering to the subject an $\alpha 5\beta 1$ integrin binding agent (*e.g.*, an antibody) described herein or fragment thereof or a pharmaceutical composition comprising the binding agent (*e.g.*,

antibody) described herein. In some embodiments, the subject is diagnosed with an angiogenesis-mediated disease, disorder, or condition.

[00253] In some embodiments, described herein is a method for alleviating one or more symptoms associated with an angiogenesis-mediated disease, disorder, or condition in a subject comprising administering to the subject an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) described herein or fragment thereof or a pharmaceutical composition comprising the binding agent (e.g., antibody) described herein.

[00254] In some embodiments, described herein is a method for treating an inflammatory disease, disorder, or condition, including a neuroinflammatory disease, disorder, or condition (e.g., MS, ALS), in a subject comprising administering to the subject an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) described herein or fragment thereof or a pharmaceutical composition comprising the binding agent (e.g., antibody) described herein. In some embodiments, the subject is diagnosed with an inflammatory disease, disorder, or condition, including a neuroinflammatory disease, disorder, or condition (e.g., MS, ALS).

[00255] In some embodiments, described herein is a method for alleviating one or more symptoms associated with an inflammatory disease, including a neuroinflammatory disease, disorder, or condition (e.g., MS, ALS), in a subject comprising administering to the subject an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) described herein or fragment thereof or a pharmaceutical composition comprising the binding agent (e.g., antibody) described herein.

[00256] The subject of a method described above can be administered one or more therapeutic agents described herein in combination with an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) described herein or fragment thereof or a pharmaceutical composition comprising the binding agent (e.g., antibody) described herein.

[00257] In some embodiments, the antibody is a human antibody, including, but not limited to, an antibody having variable regions in which both the framework and

CDR regions are derived from human germline immunoglobulin sequences as described, for example, in Kabat et al. (1991) *Sequences of proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242. If the antibody contains a constant region, the constant region also preferably is derived from human germline immunoglobulin sequences. Human antibodies may comprise amino acid residues not encoded by human germline immunoglobulin sequences, for example, to enhance the activity of the antibody, but do not comprise CDRs derived from other species (e.g., a mouse CDR placed within a human variable framework region).

[00258] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) binds to and kills tumor cells in cell culture. Such cell culture may include tumor cells expressing or overexpressing $\alpha 5\beta 1$ integrin. Tumor cells include, but are not limited to, breast cancer cells, bladder cancer cells, melanoma cells, prostate cancer cells, mesothelioma cells, lung cancer cells, testicular cancer cells, thyroid cancer cells, squamous cell carcinoma cells, glioblastoma cells, neuroblastoma cells, uterine cancer cells, colorectal cancer cells, stomach cancer cells, bladder cancer cells, and pancreatic cancer cells.

[00259] In some embodiments, described herein is a method of inhibiting abnormal angiogenesis in a subject (e.g., with a tumor). For example, the method comprises administering an amount of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), such as a human $\alpha 5\beta 1$ integrin binding agent described herein, effective to inhibit the abnormal angiogenesis. In some embodiments, the method includes administering an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), including an $\alpha 5\beta 1$ integrin binding agent, that competes for binding with antibody A-15B08, antibody A2-3B06, antibody A2-5D10, antibody A2-7A05, antibody A2-7F01, and/or antibody C-14D12AB1 (see, e.g., CDRs and VH/VL of Tables 1, 2, 3, 4, 5 and/or 6), to human $\alpha 5\beta 1$ integrin and/or binds the region of an $\alpha 5\beta 1$ integrin recognized by antibody A-15B08, antibody A2-3B06, antibody A2-5D10, antibody A2-7A05, antibody A2-7F01, and/or antibody C-14D12 (see, e.g., CDRs and VH/VL of Tables 1, 2, 3, 4, 5, and/or 6),

resulting in inhibition of abnormal angiogenesis. In some embodiments, one or more binding agents (e.g., antibodies), polynucleotides, vectors, and/or cells as described above can be used in methods of inhibiting abnormal angiogenesis *in vivo* (e.g., in a method of treating cancer in a subject).

[00260] A method of modulating (e.g., inhibiting, reducing, preventing) tumor growth in a subject also is provided. For example, the method comprises administering to the subject a composition comprising an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) in an amount effective to modulate tumor growth in the subject. “Tumor” refers to any neoplastic cell growth or proliferation, whether malignant or benign, and to all pre-cancerous and cancerous cells and tissues. The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated or abnormal cell growth and includes all malignant neoplasms including, but not limited to: carcinoma, lymphoma, blastoma, sarcoma, and leukemia. Examples of cancers include, but are not limited to: breast cancer (including metastatic breast cancer), cervical cancer, colon cancer, colorectal cancer (including metastatic colorectal cancer), lung cancer (including non-small cell lung cancer), fibrosarcoma, non-Hodgkins lymphoma (NHL), chronic lymphocytic leukemia, bladder cancer, pancreatic cancer, renal cell cancer, spleen cancer, prostate cancer including hormone refractory prostate cancer, liver cancer, head and neck cancer, stomach cancer, bladder cancer, melanoma, ovarian cancer, mesothelioma, soft tissue cancer, gastrointestinal stromal tumor, glioblastoma multiforme and multiple myeloma. Also provided is a method of treating, preventing, or ameliorating a cancer by administering an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) such as a human $\alpha 5\beta 1$ integrin binding agent, to a subject in need thereof, alone or in combination with another agent. Also provided is a method of treating, preventing, or ameliorating one or more symptoms of a cancer by administering an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) such as a human $\alpha 5\beta 1$ integrin binding agent, to a subject in need thereof, alone or in combination with another agent.

[00261] “Inhibiting” abnormal angiogenesis does not require a 100% inhibition.

Any inhibition that reduces tumor growth and/or metastasis is contemplated.

Similarly, “modulating” tumor growth refers to reducing the size of the tumor, slowing tumor growth, or inhibiting an increase in the size of an existing tumor. Complete abolition of a tumor is not required; any decrease in tumor size or slowing of tumor growth constitutes a beneficial biological effect in a subject. In this regard, tumor cell removal may be enhanced by, for example, at least about 5%, at least about 10% or at least about 20% compared to levels of removal observed in the absence of the method (e.g., in a biologically-matched control subject or specimen that is not exposed to the agent of the method). The effect is detected by, for example, a reduction in tumor size or tumor metastasis, a decrease or maintenance of the levels of tumor markers, or reduction or maintenance of a tumor cell population. In some embodiments, removal of tumor cells is enhanced by, for example, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more (about 100%) compared to the removal of tumor cells in the absence of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) of the method.

[00262] A particular administration regimen of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) for a particular subject will depend, in part, upon the agent used, the amount of agent administered, the route of administration, and the cause and extent of any side effects. The amount of agent (e.g., an antibody) administered to a subject (e.g., a mammal, such as a human) should be sufficient to effect the desired response over a reasonable time frame. According, in some embodiments, the amount of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) or pharmaceutical composition described herein administered to a subject is an effective amount. In some embodiments, the amount of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) or pharmaceutical composition described herein administered to a subject is a therapeutically effective amount. In some aspects, the method comprises administering, for example, from about 0.1 $\mu\text{g/kg}$ to up to about 100 mg/kg or more.

Some conditions or disease states require prolonged treatment, which may or may not entail administering doses of $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies), including human $\alpha 5\beta 1$ integrin binding agents (e.g., antibodies), over multiple administrations.

[00263] Suitable routes of administering a composition comprising an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), such as a human $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), are well known in the art. Although more than one route can be used to administer an agent (e.g., an antibody), a particular route can provide a more immediate and more effective reaction than another route. Depending on the circumstances, a composition comprising an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) such as a human $\alpha 5\beta 1$ integrin binding agent is applied or instilled into body cavities, absorbed through the skin or mucous membranes, ingested, inhaled, and/or introduced into circulation. For example, it may be desirable to deliver a composition comprising an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), such as a human $\alpha 5\beta 1$ integrin binding agent, through injection by intravenous, subcutaneous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, intralesional, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, urethral, vaginal, or rectal means, by sustained release systems, or by implantation devices. If desired, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), such as a human $\alpha 5\beta 1$ integrin binding agent, is administered regionally via intraarterial or intravenous administration feeding the region of interest, for example, via the hepatic artery for delivery to the liver. Alternatively, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), such as a human $\alpha 5\beta 1$ integrin binding agent, is administered locally via implantation of a membrane, sponge, or another appropriate material on to which the binding agent has been absorbed or encapsulated. Where an implantation device is used, the device is, one aspect, implanted into any suitable tissue or organ, and delivery of an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), such as a human $\alpha 5\beta 1$ integrin binding agent, is, for

example, via diffusion, timed-release bolus, or continuous administration. In other aspects, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) is administered directly to exposed tissue during tumor resection or other surgical procedures.

[00264] The present disclosure provides a composition, such as pharmaceutical composition, comprising an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) such as a human $\alpha 5\beta 1$ integrin binding agent and a carrier (e.g., a pharmaceutically acceptable carrier). The particular carrier employed may depend on chemico-physical considerations, such as solubility and lack of reactivity with the binding agent or co-therapy, and by the route of administration. Pharmaceutically acceptable carriers are well-known in the art, examples of which are described herein. Illustrative pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Injectable formulations are further described in, for example, *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Co., Philadelphia. Pa., Banker and Chalmers. eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)). A pharmaceutical composition comprising an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) such as a human $\alpha 5\beta 1$ integrin binding agent is, in one aspect, placed within containers, along with packaging material that provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions include a tangible expression describing the reagent concentration, as well as, in some embodiments, relative amounts of excipient ingredients or diluents (e.g., water, saline or PBS) that may be necessary to reconstitute the pharmaceutical composition.

[00265] In some aspects, a method described herein further comprises administering one or more additional agents, including therapeutic agents, which may be present in a composition or may be administered with an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody), such as a human $\alpha 5\beta 1$ integrin binding agent, or provided in a separate composition using the same or a different route of

administration. The one or more additional agents, including therapeutic agents, may be administered (e.g., for combination therapy) together or separately (e.g., simultaneously, alternatively, sequentially) with an $\alpha 5\beta 1$ integrin binding agent (e.g., antibody). Such additional therapeutic agents include, but are not limited to, therapeutic antibodies, immunotherapies and immunotherapeutic agents, cytotoxic agents, chemotherapeutic agents, and inhibitors.

[00266] Therapeutic antibodies that can be used with an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) as described herein (e.g., for combination therapy) include, but are not limited to, an $\alpha \nu \beta 3$ binding antibody (e.g., etaracizumab), an $\alpha 4\beta 1$ binding antibody (e.g., natalizumab), an $\alpha 4\beta 7$ binding antibody (e.g., vedolizumab), a TREM2 binding antibody (e.g., AL002), a TNF α binding antibody (e.g., adalimumab), CSF1 binding antibody (e.g., MCS110), CSF-1R binding antibody (e.g., AMG820), C1Q binding antibody (ANX005), CD40L binding antibody (e.g., ruplizumab), an FGFR antibody (e.g., bemarituzumab), IL-1 β binding antibody (e.g., canakinumab, gevokizumab), IL-6 binding antibody (e.g., tocilizumab), IL-12 binding antibody (e.g., ustekinumab), and an antibody that binds type I interferons (IFN) (e.g., sifalimumab).

[00267] Immunotherapies and immunotherapeutic agents that can be used with an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) as described herein (e.g., for combination therapy) include, but are not limited to, cytokines, interleukins, tumor necrosis factors, and combinations thereof. In some embodiments, the immunotherapy includes an immunotherapeutic agent that modulates immune responses, for example, a checkpoint inhibitor or a checkpoint agonist. In some embodiments, the immunotherapeutic agent is an antibody modulator that targets PD-1, PD-L1, PD-L2, CEACAM (e.g., CEACAM-I, -3 and/or -5), CTLA-4, TIM-3, LAG-3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, TGF beta, OX40, 41BB, LIGHT, CD40, GITR, TGF-beta, TIM-3, SIRP-alpha, VSIG8, BTLA, SIGLEC7, SIGLEC9, ICOS, B7H3, B7H4, FAS, and/or BTNL2 among others known in the art. In some embodiments, the immunotherapeutic agent is an agent that increases natural killer (NK) cell activity. In some embodiments, the immunotherapeutic agent is an agent

that inhibits suppression of an immune response. In some embodiments, the immunotherapeutic agent is an agent that inhibits suppressor cells or suppressor cell activity. In some embodiments, the immunotherapeutic agent is an agent or therapy that inhibits Treg activity. In some embodiments, the immunotherapeutic agent is an agent that inhibits the activity of inhibitory immune checkpoint receptors.

[00268] In some embodiments, the immunotherapeutic agent includes a T cell modulator chosen from an agonist or an activator of a costimulatory molecule. In one embodiment, the agonist of the costimulatory molecule is chosen from an agonist (e.g., an agonistic antibody or antigen-binding fragment thereof, or a soluble fusion) of GITR, OX40, ICOS, SLAM (e.g., SLAMF7), HVEM, LIGHT, CD2, CD27, CD28, CDS, ICAM-I, LFA-I (CD1 Ia/CD18), ICOS (CD278), 4-1BB (CD137), CD30, CD40, BAFFR, CD7, NKG2C, NKp80, CD160, B7-H3, or CD83 ligand. In other embodiments, the effector cell combination includes a bispecific T cell engager (e.g., a bispecific antibody molecule that binds to CD3 and a tumor antigen (e.g., EGFR, PSCA, PSMA, EpCAM, HER2 among others).

[00269] Cytotoxic agents that can be used with an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) as described herein (e.g., for combination therapy) include a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Exemplary cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., I^{131} , I^{125} , Y^{90} , and Re^{186}); chemotherapeutic agents; and toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof.

[00270] Chemotherapeutic agents that can be used with an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) as described herein (e.g., for combination therapy) include chemical compounds useful in the treatment of cancer. Examples of chemotherapeutic agents include, but are not limited to: alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide,

triethylenethiophosphoramidate and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including a the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gamma11 and calicheamicin omega11 (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994))); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminoglutethimide,

mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone mitoxantrone mopidanmol nitraerine, pentostatin; phenamet pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (e.g., T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumburg, Ill.), and TAXOTERE® doxetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); inhibitors of PKC-alpha, Raf, H-Ras and EGFR (e.g., erlotinib (Tarceva™)) that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene,

droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON• toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC- α , Raf and H-Ras; ribozymes such as a VEGF expression inhibitor (e.g., ANGIOZYME® ribozyme) and a HER2 expression inhibitor; vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; Vinorelbine and Etoposides, and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[00271] Inhibitors that can be used with an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) as described herein (e.g., for combination therapy) include, but are not limited to, kinase inhibitors such as FAK inhibitors (e.g., GSK2256098), MEK inhibitors (e.g., cobimetinib, rametinib, binimetinib, selumetinib), tyrosine kinase inhibitors (e.g., cabozantinib); EGFR inhibitors (e.g., erlotinib); Janus kinase (JAK)1-selective inhibitors (e.g., baricitinib, tofacitinib, upadacitinib), CSF-1R inhibitors (e.g., BLZ945); C-kit inhibitors (e.g., masitinib); and FGFR inhibitors (e.g., erdafitinib).

[00272] In some embodiments, an $\alpha 5\beta 1$ integrin binding agent (e.g., an antibody) as disclosed herein can be used in combination with inhibitors of PD-1 or inhibitors of PD-L1, e.g., an anti-PD-1 monoclonal antibody or an anti-PD-L1 monoclonal antibody, for example, nivolumab (Opdivo), pembrolizumab (Keytruda, MK-3475), atezolizumab, or avelumab.

[00273] In some embodiments, an $\alpha 5 \beta 1$ integrin binding agent (e.g., an antibody) as disclosed herein can be used in combination with CTLA-4 inhibitors, e.g., an anti-CTLA-4 antibody, for example, ipilimumab (Yervoy), or with antibodies to cytokines, or with bispecific antibodies that bind to PD-L1 and CTLA-4 or PD-1 and CTLA-4, or with other anti-cancer agents.

[00274] The additional agent may be a pharmaceutically acceptable salt, ester, amide, hydrate, and/or prodrug of any of the therapeutic agents described above or other agents.

[00275] The additional therapeutic agent may be a pharmaceutically acceptable salt, ester, amide, hydrate, and/or prodrug of any of the therapeutic agents described above or other agents.

[00276] It is understood that modifications which do not substantially affect the activity of the various embodiments described herein are also provided within the definition of the subject matter described herein. Accordingly, the following examples are intended to illustrate but not limit the present disclosure.

EXAMPLES

Example 1: Antibody Generation and Initial Screening for $\alpha 5$ Integrin Binding

[00277] NZBW and CD-1 mice, four of each, were injected with 100 μ g purified recombinant human $\alpha 5 \beta 1$ integrin heterodimer (rh- $\alpha 5 \beta 1$; Acro Biosystems, Newark, DE; cat. no. IT1-H52W5). Four weeks later cells from spleens and draining lymph nodes were fused to create hybridomas. Approximately 4000 hybridomas supernatants (2,500 from NZBW strain and 1,500 from CD-1 strain) were screened by FLOW cytometry for positivity on K562 cells (ATCC® CCL-243™; Manassas, VA 20110) that had been activated with 10ng/mL PMA (Phorbol 12-myristate 13-acetate; Sigma-Aldrich, St. Louis, MO, cat. no. 5.00582) for 24 hours, cryopreserved and thawed just prior to use. In short, hybridoma supernatants were incubated with the activated K562 cells for 20 minutes, washed, then incubated with a fluorescent conjugated detecting antibody for 20 minutes, washed, resuspended in 7-Aminoactinomycin D, and Mean Fluorescence Intensity (MFI) measured on a Guava

cytometer (Luminex Corporation, Austin, TX 78727). As shown in Table 7, 249 positive clones were selected for further screening based on hybridoma supernatants that had MFI's that were significantly higher ($>1.5x$) than hybridoma media only (MFI of 125 on the K562 cells).

[00278] Table 7

Antibody	MFI
A-2B04	1685
A-2E09	1629
A-2H03	373
A-3A08	1185
A-3B08	1621
A-3B12	1601
A-3C02	2081
A-3E08	895
A-3F10	2027
A-3G09	2241
A-3H05	2084
A-4D08	2192
A-4F09	2367
A-4G02	1977
A-4H03	406
A-5C03	593
A-5D12	1642
A-5F02	1839
A-5G04	3474
A-5H05	4210
A-5H09	1797
A-6A05	2607
A-6A12	2251
A-6B09	366
A-6C10	2267
A-6D10	1030
A-6E04	4763
A-6E11	1662
A-6H03	345
A-7A10	685
A-7B03	1882
A-7C07	2355
A-7C09	2384
A-7D04	1277

Antibody	MFI
A-7D06	742
A-7F05	3921
A-7F06	2057
A-7F09	2598
A-7G10	1509
A-7H02	2208
A-7H08	1924
A-8C06	2433
A-8C07	2656
A-8E08	2444
A-8G02	2294
A-9A08	2647
A-9B08	2662
A-9C03	2912
A-9E05	2419
A-9E06	2286
A-9F09	1829
A-9F10	348
A-10B02	630
A-10B04	1793
A-10F05	4140
A-10F08	2076
A-10G02	2136
A-11A10	2500
A-11B02	2288
A-11C01	2622
A-11C08	2862
A-11D06	921
A-11E10	3009
A-11F06	5239
A-11G01	1606
A-11G03	1390
A-11G06	4472
A-11H02	3070

Antibody	MFI
A-11H07	1890
A-12A11	3056
A-12B06	2950
A-12F09	368
A-12H10	3929
A-13A04	676
A-13A10	2684
A-13F10	3296
A-13H08	2638
A-14B11	3367
A-14C09	4703
A-14F03	553
A-14G05	3726
A-14G08	4207
A-15B08	1388
A-15C03	1973
A-15D10	2342
A-15E11	1850
A-15H02	4046
A-15H03	3577
A-15H11	6572
A2-10A11	2348
A2-10B05	1630
A2-10B12	2791
A2-10C10	2279
A2-10C11	2513
A2-10E01	2400
A2-10G01	2466
A2-10G06	2464
A2-1A05	1452
A2-1C04	1778
A2-1E01	1787
A2-1F09	1560
A2-1G10	1654

Antibody	MFI
A2-1H04	1805
A2-1H07	1748
A2-1H09	1879
A2-2A06	1470
A2-2A07	2354
A2-2A11	1823
A2-2B01	1927
A2-2C11	1474
A2-2D03	1868
A2-2D05	1781
A2-2E06	1317
A2-2F05	1715
A2-3B01	1873
A2-3B03	1920
A2-3B06	2136
A2-3B11	1575
A2-3D02	1243
A2-3E04	1758
A2-3E11	1265
A2-3G01	2069
A2-3G03	2230
A2-3G10	1910
A2-3H06	2138
A2-4B05	1885
A2-4B07	1695
A2-4D11	2005
A2-4E06	1169
A2-4E10	1799
A2-4G01	2256
A2-4H01	1908
A2-5A07	1528
A2-5B03	1928
A2-5B04	1102
A2-5B09	4143
A2-5C04	1982
A2-5D07	2055
A2-5D10	1890
A2-5F01	729
A2-5F02	2352
A2-5F10	1946
A2-5G04	2039

Antibody	MFI
A2-5H05	1565
A2-5H11	2268
A2-6A07	969
A2-6A08	1548
A2-6A10	1289
A2-6C05	4281
A2-6C08	2375
A2-6C10	2121
A2-6E02	4173
A2-6F01	2481
A2-6G03	2200
A2-6H11	2272
A2-7A04	2248
A2-7A05	5503
A2-7E10	1950
A2-7F01	6255
A2-8B01	2300
A2-8B07	1102
A2-8C07	2594
A2-8D08	1896
A2-8D12	2779
A2-8E07	888
A2-8E09	2424
A2-8G08	2665
A2-8H06	2547
A2-8H11	4366
A2-9A03	990
A2-9A11	3395
A2-9B05	2169
A2-9C07	1972
A2-9C11	1096
A2-9D05	4685
A2-9D06	2028
A2-9F05	1987
A2-9F09	2290
A2-9G08	4093
A2-9G11	2327
A2-9H10	2471
C-1G03	1561
C-1H02	803
C-2F04	1243

Antibody	MFI
C-2F10	687
C-2G03	2634
C-2H02	525
C-2H03	4654
C-3C11	415
C-3D02	4681
C-3F08	448
C-3H10	399
C-4A04	3047
C-4C02	388
C-4G03	519
C-4H08	471
C-5C04	447
C-5E04	2407
C-5F01	399
C-5G01	500
C-5G02	5121
C-5G03	371
C-5H03	2640
C-6B10	658
C-6C12	386
C-6F08	472
C-6G03	391
C-6H03	1719
C-6H07	418
C-7B08	6727
C-7B11	5609
C-8A09	714
C-8F09	475
C-8F11	4472
C-8G11	2693
C-8H03	2537
C-8H07	449
C-8H11	7289
C-9A06	334
C-9B01	3623
C-9C11	936
C-9E11	594
C-9G02	1717
C-9H03	1984
C-10A12	408

Antibody	MFI
C-10F09	670
C-11C09	573
C-11E01	5272
C-11H03	1595
C-12A04	3479
C-12H05	1107
C-12H09	6609
C-13A11	355

Antibody	MFI
C-13B02	629
C-13D04	7126
C-13E09	4379
C-13F01	5012
C-13H01	764
C-14D12	2015
C-14H03	1124
C-14H10	360

Antibody	MFI
C-15B04	354
C-15C02	6294
C-15C08	546
C-15E04	489
C-15F08	3987
C-15F10	365
C-15F11	5822
C-15H03	1177

Example 2: Screening for $\alpha 5$ Binding Specificity

[00279] The 249 positive hybridoma supernatants selected as described in Example 1 were screened for reactivity to rh- $\alpha 5\beta 1$ in a plate-based ELISA.

Immulon4 HBX ELISA 96-well plates (Thermo Fisher Scientific, Waltham, MA, cat. no. 3855) were coated with rh- $\alpha 5\beta 1$ (R&D Systems, Minneapolis, MN 55413, cat. no. 3230-A5), at 1 μ g/mL in PBS supplemented with 0.5 mM $MgCl_2$, $MnCl_2$ and $CaCl_2$ and incubated overnight at 4°C. Plates were washed 3 times with Wash Buffer (1x Tris Buffered Saline containing 0.05% Tween20), blocked with 2% BSA in 1xTBS (made from 10X Thermo Scientific Blocker BSA (10X) in TBS; Thermo Fisher Scientific, Waltham, MA, cat. no. 37520) for 2 hours at room temperature (RT) then incubated with hybridoma supernatants diluted 1:10 with Standard Diluent (2% BSA, 1xTBS, 0.05% Tween20). Following a 1 hour incubation at RT, wells were washed 3 times, incubated with biotinylated goat anti-mouse secondary antibody at 1:8000 dilution (Invitrogen, Carlsbad, CA, cat. no. 62-6540) for 1 hour, washed 3 times, then incubated for 30 minutes with poly-HRP Streptavidin (Thermo Fisher Scientific, Waltham, MA, cat. no. N200), washed 4 times, incubated with TMB (Thermo Fisher Scientific, Waltham, MA, cat. no. N301) for 5-10 minutes, followed by addition of ELISA Stop Solution (Invitrogen, Carlsbad, CA, cat. no. SS04). Absorbance 450nm was measured. Results are shown in Tables 8A and 8B.

[00280] Table 8A

Antibody	Absorbance 450nm			Selected Human $\alpha 5$ Specific
	Human $\alpha 5\beta 1$	Mouse $\alpha 5\beta 1$	Human $\alpha 4\beta 1$	
A-2B04	0.1084	0.2165	0.0974	No
A-2E09	0.1163	0.1417	0.1034	No
A-2H03	0.3487	0.2382	0.2448	No
A-3A08	0.3521	0.6708	0.0769	Yes
A-3B08	0.3792	0.0911	0.0767	No
A-3B12	0.441	0.0652	0.075	No
A-3C02	0.082	0.0632	0.0808	No
A-3E08	4	0.0852	2.5769	No
A-3F10	0.0903	0.0734	0.0775	No
A-3G09	0.1338	0.0879	0.1013	No
A-3H05	0.1382	0.1028	0.1045	No
A-4D08	0.1095	0.0848	0.0844	No
A-4F09	0.1227	0.0945	0.0914	No
A-4G02	0.6869	0.0959	0.0801	Yes
A-4H03	0.2637	0.1811	0.2028	No
A-5C03	0.0629	0.0539	0.0601	No
A-5D12	0.075	0.068	0.0797	No
A-5F02	0.13	0.1027	0.0994	No
A-5G04	0.172	0.0667	0.0757	No
A-5H05	0.3463	0.1424	0.1587	No
A-5H09	0.0818	0.0646	0.0789	No
A-6A05	0.6434	0.109	0.1038	No
A-6A12	0.1472	0.1202	0.1103	No
A-6B09	0.8353	0.5175	0.5583	No
A-6C10	0.4028	0.0969	0.0858	No
A-6D10	0.306	0.0791	0.0859	No
A-6E04	0.7985	0.3352	0.3234	No
A-6E11	1.0874	0.1883	0.1816	Yes
A-6H03	0.1106	0.0935	0.0885	No
A-7A10	0.1075	0.0747	0.0864	No
A-7B03	0.2604	0.1575	0.1568	No
A-7C07	0.106	0.0836	0.0867	No
A-7C09	0.8062	0.1092	0.1198	No
A-7D04	3.9383	0.0676	2.5841	No
A-7D06	0.613	0.0755	0.0848	Yes
A-7F05	0.344	0.0634	0.0835	No
A-7F06	0.1247	0.0868	0.0943	No
A-7F09	0.3208	0.0922	0.1038	No
A-7G10	0.2368	0.0704	0.0757	No
A-7H02	0.0873	0.0679	0.0711	No

Antibody	Absorbance 450nm			Selected Human $\alpha 5$ Specific
	Human $\alpha 5\beta 1$	Mouse $\alpha 5\beta 1$	Human $\alpha 4\beta 1$	
A-7H08	0.0707	0.0795	0.0632	No
A-8C06	0.1241	0.0827	0.088	No
A-8C07	0.3763	0.085	0.079	No
A-8E08	0.1166	0.089	0.088	No
A-8G02	0.1299	0.0926	0.0946	No
A-9A08	0.0898	0.0718	0.0753	No
A-9B08	0.7456	0.0999	0.0944	No
A-9C03	0.129	0.0859	0.1017	No
A-9E05	0.1206	0.0853	0.0754	No
A-9E06	0.0691	0.0608	0.0623	No
A-9F09	0.0579	0.0642	0.0637	No
A-9F10	0.0649	0.0536	0.0652	No
A-10B02	0.5028	0.2765	0.2848	No
A-10B04	0.5087	0.1134	0.1122	No
A-10F05	0.6922	0.2621	0.26	Yes
A-10F08	0.0761	0.0939	0.0792	No
A-10G02	0.1068	0.0863	0.0809	No
A-11A10	0.1154	0.0935	0.0858	No
A-11B02	0.0846	0.0693	0.1524	No
A-11C01	0.0851	0.0712	0.0685	No
A-11C08	0.3658	0.096	0.097	No
A-11D06	0.6792	0.1354	0.1377	Yes
A-11E10	0.1307	0.0863	0.1018	No
A-11F06	0.2314	0.0799	0.0905	No
A-11G01	0.5191	0.0766	0.0843	Yes
A-11G03	4	0.1105	2.644	No
A-11G06	0.7794	0.0659	0.0643	No
A-11H02	1.1794	0.2536	0.2508	No
A-11H07	0.5646	0.2788	1.6954	No
A-12A11	0.1206	0.0915	0.0882	No
A-12B06	0.1374	0.1035	0.1058	No
A-12F09	0.1329	0.0682	0.0745	No
A-12H10	0.725	0.0814	0.0972	Yes
A-13A04	0.2271	0.0961	1.5607	No
A-13A10	1.0457	0.1158	0.1057	Yes
A-13F10	0.1028	0.0805	0.083	No
A-13H08	0.0712	0.0706	0.0676	No
A-14B11	0.0855	0.0827	0.0857	No
A-14C09	0.2857	0.0665	0.0683	No
A-14F03	0.065	0.0696	0.0721	No
A-14G05	0.1379	0.0944	0.1161	No
A-14G08	1.1672	0.1101	0.1082	Yes

Antibody	Absorbance 450nm			Selected Human $\alpha 5$ Specific
	Human $\alpha 5\beta 1$	Mouse $\alpha 5\beta 1$	Human $\alpha 4\beta 1$	
A-15B08	0.8241	0.0924	0.0855	Yes
A-15C03	0.4782	0.0709	0.0793	Yes
A-15D10	4	0.0647	2.5858	No
A-15E11	0.384	0.0594	0.0946	No
A-15H02	0.952	0.1077	0.1149	No
A-15H03	1.4724	0.2658	0.2527	No
A-15H11	1.2506	0.0799	0.081	Yes
C-1G03	0.0681	0.0479	0.073	No
C-1H02	0.2343	0.1417	0.1873	No
C-2F04	0.288	0.089	0.0937	No
C-2F10	0.1306	1.2717	1.4726	No
C-2G03	0.1596	0.0794	0.1668	No
C-2H02	0.0769	0.0525	0.0666	No
C-2H03	2.7936	1.9471	2.5027	No
C-3C11	2.8486	0.0836	2.4763	No
C-3D02	0.5177	0.3261	0.3997	No
C-3F08	2.495	0.0598	2.7046	No
C-3H10	1.587	0.0594	2.1689	No
C-4A04	1.8716	0.1389	0.121	Yes
C-4C02	2.8954	0.1083	2.3639	No
C-4G03	0.2835	0.2123	0.2072	No
C-4H08	2.9081	0.0939	2.7376	No
C-5C04	3.168	0.2312	2.6409	No
C-5E04	0.1665	0.0835	0.0941	No
C-5F01	1.5882	0.4283	0.4783	No
C-5G01	2.7418	0.0865	2.2995	No
C-5G02	0.653	0.3808	0.3941	No
C-5G03	0.7942	0.573	0.5658	No
C-5H03	0.068	0.059	0.1312	No
C-6B10	3.1915	0.1824	2.3102	No
C-6C12	2.8865	0.1219	2.187	No
C-6F08	3.1197	0.1017	2.6153	No
C-6G03	0.0633	0.0507	0.0727	No
C-6H03	0.0604	0.0559	0.0791	No
C-6H07	1.224	0.0743	0.0942	No
C-7B08	0.3579	0.2375	0.2159	Yes
C-7B11	0.5058	0.3476	0.34	No
C-8A09	0.772	0.0618	0.0857	No
C-8F09	2.6852	0.1054	2.4293	No
C-8F11	0.2259	0.0648	0.087	No
C-8G11	0.8047	0.0872	0.1645	Yes
C-8H03	0.0823	0.0798	0.1158	No

Antibody	Absorbance 450nm			Selected Human $\alpha 5$ Specific
	Human $\alpha 5\beta 1$	Mouse $\alpha 5\beta 1$	Human $\alpha 4\beta 1$	
C-8H07	0.9156	0.0772	0.0882	No
C-8H11	1.0302	0.4727	0.4897	Yes
C-9A06	0.0912	2.3936	2.6409	No
C-9B01	0.1404	0.1146	0.1017	No
C-9C11	0.1389	0.1578	0.1478	No
C-9E11	2.823	0.1228	2.3282	No
C-9G02	0.1484	0.1135	0.077	No
C-9H03	0.0769	0.0947	0.073	No
C-10A12	2.5345	0.1411	2.3139	No
C-10F09	1.9713	0.1087	0.0803	Yes
C-11C09	0.9653	0.1033	0.0883	No
C-11E01	0.2311	0.1651	0.146	No
C-11H03	0.072	0.0922	0.0613	No
C-12A04	0.1	0.1018	0.1352	No
C-12H05	2.9323	0.1314	2.3868	No
C-12H09	0.3533	0.2619	0.2516	No
C-13A11	1.0918	0.1344	0.1019	No
C-13B02	2.701	0.1381	2.2382	No
C-13D04	0.6535	0.5035	0.4125	No
C-13E09	0.2133	0.14	0.1134	No
C-13F01	0.4665	0.3354	0.3691	No
C-13H01	0.3363	0.1236	2.119	No
C-14D12	0.702	0.1699	0.1707	Yes
C-14H03	0.0653	0.0973	0.0713	No
C-14H10	2.0178	0.1617	0.1417	No
C-15B04	2.4029	0.1089	2.4022	No
C-15C02	1.0536	0.7504	0.8032	Yes
C-15C08	3.0525	0.1459	2.3658	No
C-15E04	2.8959	0.1376	2.3174	No
C-15F08	0.0688	0.0969	0.0939	No
C-15F10	0.1581	0.1178	0.0787	No
C-15F11	0.068	0.0947	0.071	No
C-15H03	0.0822	0.1094	0.0916	No

[00281] Table 8B

Antibody	Absorbance 450nm			Selected Human $\alpha 5$ Specific
	Human $\alpha 5\beta 1$	Mouse $\alpha 5\beta 1$	Human $\alpha 4\beta 1$	
A2-1A05	0.3128	0.0652	0.0645	No
A2-1C04	0.2881	0.0563	0.0605	No
A2-1E01	0.3006	0.0575	0.0632	No

Antibody	Absorbance 450nm			Selected Human $\alpha 5$ Specific
	Human $\alpha 5\beta 1$	Mouse $\alpha 5\beta 1$	Human $\alpha 4\beta 1$	
A2-1F09	0.2492	0.0493	0.0575	No
A2-1G10	0.2958	0.0525	0.0598	No
A2-1H04	0.3068	0.055	0.0591	No
A2-1H07	0.326	0.0518	0.0563	No
A2-1H09	0.3069	0.0514	0.0575	No
A2-2A06	0.3083	0.0522	0.057	No
A2-2A07	2.0978	0.0499	0.0592	No
A2-2A11	0.2576	0.0575	0.0612	No
A2-2B01	0.0954	0.0511	0.0563	No
A2-2C11	0.2295	0.055	0.0594	No
A2-2D03	0.269	0.0588	0.0651	No
A2-2D05	0.2356	0.0527	0.0619	No
A2-2E06	0.3011	0.0551	0.0656	No
A2-2F05	0.2787	0.0533	0.0615	No
A2-3B01	0.3051	0.0581	0.0615	No
A2-3B03	0.3221	0.0534	0.0598	No
A2-3B06	2.0288	0.049	0.0558	Yes
A2-3B11	0.2829	0.0551	0.0613	No
A2-3D02	0.2303	0.0529	0.0594	No
A2-3E04	0.239	0.0522	0.0576	No
A2-3E11	0.2059	0.0531	0.0566	No
A2-3G01	0.2653	0.0573	0.0649	No
A2-3G03	0.2784	0.0566	0.0648	No
A2-3G10	0.291	0.0533	0.0646	No
A2-3H06	0.2919	0.0551	0.0613	No
A2-4B05	0.3177	0.0546	0.0665	No
A2-4B07	0.3282	0.0601	0.0631	No
A2-4D11	0.2957	0.0545	0.0628	No
A2-4E06	2.0963	0.05	0.0563	No
A2-4E10	0.2842	0.0538	0.0636	No
A2-4G01	2.0015	0.091	0.0852	No
A2-4H01	0.1636	0.0521	0.0601	No
A2-5A07	1.5964	0.1552	0.123	No
A2-5B03	0.2409	0.0563	0.0639	No
A2-5B04	0.1644	0.0517	0.0621	No
A2-5B09	1.8497	0.0612	0.0676	No
A2-5C04	0.326	0.0568	0.0635	No
A2-5D07	0.3061	0.0551	0.062	No
A2-5D10	2.3107	0.0647	0.0683	Yes
A2-5F01	0.5058	0.0505	0.057	No
A2-5F02	0.3518	0.0583	0.063	No
A2-5F10	0.2895	0.0538	0.06	No

Antibody	Absorbance 450nm			Selected Human $\alpha 5$ Specific
	Human $\alpha 5\beta 1$	Mouse $\alpha 5\beta 1$	Human $\alpha 4\beta 1$	
A2-5G04	0.3432	0.059	0.0654	No
A2-5H05	1.4644	0.06	0.0642	No
A2-5H11	0.1404	0.0581	0.0636	No
A2-6A07	0.1111	0.0538	0.0601	No
A2-6A08	0.3056	0.06	0.0664	No
A2-6A10	0.1633	0.0526	0.0614	No
A2-6C05	1.8893	0.0863	0.0825	No
A2-6C08	0.3231	0.0576	0.0636	No
A2-6C10	2.0145	0.0551	0.0609	Yes
A2-6E02	1.7497	0.0535	0.0592	No
A2-6F01	0.3692	0.0566	0.064	No
A2-6G03	0.3429	0.066	0.0678	No
A2-6H11	0.3617	0.0549	0.0609	No
A2-7A04	0.3179	0.0549	0.0595	No
A2-7A05	2.0454	0.0682	0.0713	Yes
A2-7E10	0.2291	0.0634	0.0666	No
A2-7F01	2.0314	0.0563	0.0617	Yes
A2-8B01	0.3258	0.0659	0.07	No
A2-8B07	1.9484	0.0649	2.2718	No
A2-8C07	0.2728	0.0545	0.0613	No
A2-8D08	0.2894	0.0611	0.067	No
A2-8D12	0.2973	0.0549	0.0631	No
A2-8E07	0.3154	0.0588	0.0631	No
A2-8E09	0.2372	0.0524	0.06	No
A2-8G08	0.305	0.0568	0.0628	No
A2-8H06	0.2819	0.0567	0.0628	No
A2-8H11	1.4458	0.0515	0.0595	Yes
A2-9A03	0.0962	0.0577	0.0613	No
A2-9A11	1.9121	0.0619	0.0608	Yes
A2-9B05	0.2211	0.0566	0.0672	No
A2-9C07	0.2783	0.0577	0.0656	No
A2-9C11	2.3712	0.0638	0.0679	No
A2-9D05	2.2156	0.0758	0.0686	No
A2-9D06	0.3405	0.0605	0.0632	No
A2-9F05	0.2276	0.0571	0.0594	No
A2-9F09	1.8866	0.0758	0.0705	No
A2-9G08	1.9022	0.0508	0.0586	No
A2-9G11	0.2839	0.0546	0.0624	No
A2-9H10	0.1615	0.0537	0.0682	No
A2-10A11	0.1696	0.0619	0.0648	No
A2-10B05	0.13	0.0596	0.0641	No
A2-10B12	0.232	0.059	0.0654	No

Antibody	Absorbance 450nm			Selected Human $\alpha 5$ Specific
	Human $\alpha 5\beta 1$	Mouse $\alpha 5\beta 1$	Human $\alpha 4\beta 1$	
A2-10C10	0.2857	0.0606	0.0656	No
A2-10C11	0.2625	0.0568	0.0618	No
A2-10E01	0.2733	0.0562	0.0618	No
A2-10G01	0.2942	0.0634	0.0637	No
A2-10G06	0.3191	0.0576	0.064	Yes

[00282] In parallel the 249 hybridomas were screened for specificity to the human $\alpha 5$ subunit by testing for reactivity to rh- $\alpha 4\beta 1$ (R&D Systems, Minneapolis, MN 55413; cat. no. 3230-A5 & 5668-A4). Results are shown in Tables 8A and 8B. Any hybridoma that reacted to both rh- $\alpha 4\beta 1$ and rh- $\alpha 5\beta 1$ is likely to be $\beta 1$ subunit specific and not considered $\alpha 5$ specific. Using the protocol described above, 29 hybridoma supernatants reacted strongly (>1.0 Absorbance 450nm) with rh- $\alpha 4\beta 1$ and were not considered to be $\alpha 5$ specific.

[00283] In addition, hybridomas were tested for cross-reactivity to recombinant mouse $\alpha 5\beta 1$ (rm- $\alpha 5\beta 1$; R&D Systems, Minneapolis, MN 55413; cat. no. 7728-A5) using the same protocol as above except that the plates were coated with rm- $\alpha 5\beta 1$. Results are shown in Tables 8A and 8B. Three hybridomas exhibited strong binding to rm- $\alpha 5\beta 1$ (>1.0 Absorbance 450nm), but also had strong reactivity to rh- $\alpha 4\beta 1$.

[00284] Of the 220 hybridomas that were shown to have specific binding to the $\alpha 5$ subunit, 28 were selected for further characterization and represented a range of anti- $\alpha 5$ binding antibodies having Absorbance 450nm readings in the range of 0.32 to 2.3 without cross-reactivity to rh- $\alpha 4\beta 1$. None had detectable cross-reactivity to rm- $\alpha 5\beta 1$ in this 1:10 dilution single point assay.

Example 3: Selection of Antibodies with High $\alpha 5$ Binding Affinity

[00285] Kinetic analysis was performed on the 28 hybridoma supernatants selected as described in Example 2 using ForteBio Octet BMIA instrument (ForteBio, Fremont, CA) to calculate the equilibrium dissociation constants ($K_D = k_{off}/k_{on}$) of the antibody clones binding to 20nM rh- $\alpha 5\beta 1$ (Acro Biosystems, Newark, DE; cat. no. IT1-H52W5). Anti-Mouse IgG Fc biosensors were used to load each clone

supernatant. Assay steps were as follows: Sensor Check (30s) --> Load Ab (700s) --> Quench (480s) --> Baseline(480s) --> Ab Assoc. (600s) --> Dissoc. (600s). The kinetics data are shown in Table 9. Twenty of the hybridomas had a K_D less than 10 nanomolar (nM), ranging from 0.4 to 7.8 nM, and were selected for further characterization.

[00286] Table 9

Antibody	K_D	k_{on}	k_{off}	Selected
A-3A08	5.08E-09	2.44E+05	1.24E-03	Yes
A-4G02	1.23E-09	3.21E+05	3.96E-04	Yes
A-6E11	3.22E-07	1.80E+05	5.80E-02	No
A-7D06	4.75E-09	2.70E+05	1.28E-03	Yes
A-10F05	7.84E-09	3.87E+05	3.03E-03	Yes
A-11D06	3.29E-08	9.56E+05	3.14E-02	No
A-11G01	5.03E-09	2.61E+05	1.32E-03	Yes
A-12H10	3.83E-08	1.21E+06	4.64E-02	No
A-13A10	4.19E-08	1.74E+06	7.27E-02	No
A-14G08	8.45E-10	2.34E+05	1.98E-04	Yes
A-15B08	1.31E-09	3.28E+05	4.31E-04	Yes
A-15C03	4.67E-09	2.76E+05	1.29E-03	Yes
A-15H11	9.01E-10	2.15E+05	1.93E-04	Yes
A2-10G06	5.85E-07	2.91E+05	1.70E-01	No
A2-3B06	4.74E-09	2.89E+05	1.37E-03	Yes
A2-5D10	2.32E-09	6.93E+05	1.61E-03	Yes
A2-6C10	3.50E-09	2.60E+05	9.11E-04	Yes
A2-7A05	8.00E-10	2.22E+05	1.78E-04	Yes
A2-7F01	1.30E-09	2.42E+05	3.14E-04	Yes
A2-8H11	4.81E-09	2.89E+05	1.39E-03	Yes
A2-9A11	5.02E-09	2.77E+05	1.39E-03	Yes
C-4A04	2.44E-09	1.12E+05	2.74E-04	Yes
C-7B08	4.90E-08	4.01E+05	1.97E-02	No
C-8G11	4.42E-10	2.71E+05	1.20E-04	Yes
C-8H11	1.07E-07	2.01E+05	2.14E-02	No
C-10F09	2.40E-09	3.00E+05	7.20E-04	Yes
C-14D12	3.79E-10	2.63E+05	9.94E-05	Yes
C-15C02	7.31E-07	3.34E+04	2.44E-02	No

Example 4: Variable Region Sequencing to Identify Unique Antibody Sequences

[00287] DNA sequencing of the heavy and light chain variable regions of the 20 clones selected as described in Example 3 was performed. DNA was isolated from hybridoma cell pellets and sequenced using the Sanger method. Sequence alignments revealed 9 unique sequence heavy and light chain pairs, assigned group numbers 1 thru 9. One antibody clone was selected to represent each unique sequence group as shown in Table 10.

[00288] Table 10

Antibody	Sequence Group	Variable Region Sequence	Selected Unique mAbs
A-3A08	8	H + L	-
A-4G02	3	H + L	-
A-7D06	8	H + L	-
A-10F05	9	H + L	+
A-11G01	8	H + L	-
A-14G08	2	H + L	-
A-15B08	3	H + L	+
A-15C03	8	H	-
A-15H11	2	H + L	-
A2-3B06	8	H + L	+
A2-5D10	5	H + L	+
A2-6C10	8	H + L	-
A2-7A05	2	H + L	+
A2-7F01	4	H + L	+
A2-8H11	8	H + L	-
A2-9A11	8	H + L	-
C-4A04	7	H + L	+
C-8G11	1	H + L	-
C-10F09	6	H + L	+
C-14D12	1	H + L	+

Example 5: Selection of Antibodies that Inhibit Fibronectin Binding to $\alpha 5\beta 1$

[00289] To determine whether the 9 unique hybridoma clones selected as described in Example 4 were able to inhibit the binding of $\alpha 5\beta 1$ integrin to fibronectin (FN), the antibodies were first purified from hybridoma supernatants by Protein A

chromatography, protein concentrations measured by BCA assay (Pierce™ BCA Protein Assay Kit; Thermo Fisher Scientific, Waltham, MA, cat. no. 23225) and then tested in a quantitative FN inhibition assay in an ELISA format.

[00290] Immulon4 HBX ELISA 96-well plates were coated with FN by incubation overnight at 4°C with 2.5µg/mL human FN (R&D Systems, Minneapolis, MN 55413, cat. no. 1918-FN) in 1xPBS (0.01M phosphate buffer and 0.154M NaCl, pH 7.4). Plates were then washed 3 times with Wash Buffer (1x Tris Buffered Saline containing 0.05% Tween20), blocked with 2% BSA in 1xTBS for 2 hours at room temperature (RT). Antibodies were diluted in Standard Diluent (2% BSA, 1xTBS, 0.05% Tween20) containing 0.1 µg/mL rh-α5β1-6xHis tagged protein (Acro Biosystems, Newark, DE, cat. no. IT1-H52W5), to generate an 11 point 1:3 antibody dilution series ranging from 10,000 ng/mL to 0.17 ng/mL. Isotype control antibody (Control Ab; Ms IgG2a EMD Millipore Corp, Billerica, MA, cat. no. PP102) was used to normalize data across different assay runs. For the assays, 100uL of the antibody dilution series / His-tagged-α5β1 mixture was added to the wells after the blocking solution was removed and wells were washed 3 times. Following 1 hour at RT, the wells were washed 3 times, incubated with biotinylated Anti-6xHis-Tag Ab (Invitrogen, Carlsbad, CA, cat. no. MAI-21315-BTIN) at 1:1000 in Standard Diluent for 1 hour, washed 3 times, incubated for 30 minutes with poly-HRP Streptavidin (Thermo Fisher Scientific, Waltham, MA, cat. no. N200), washed 4 times, incubated with TMB substrate (Thermo Fisher Scientific, Waltham, MA, cat. no. N301) for 2-5 minutes, followed by addition of ELISA Stop Solution (Invitrogen, Carlsbad, CA, cat. no. SS04). Absorbance 450nm was measured. Data points were normalized to the isotype Control Ab values at each concentration and reported as % Absorbance 450nm normalized to Control Ab. Non-linear regression analysis was used to fit curves (4-parameter) to the data using GraphPad Prism version 9.0.2 (GraphPad Software, LLC, San Diego, CA). Results are shown in **FIG. 1** and Table 11.

[00291] Table 11

Antibody	IC50	% Max Inhibition
A-10F05	N/A	N/A
A-15B08	0.0033	96
A2-3B06	0.0130	93
A2-5D10	0.1475	93
A2-7A05	0.0271	41
A2-7F01	0.0215	60
C-4A04	N/A	N/A
C-10F09	N/A	N/A
C-14D12	0.0028	96
Control Ab	N/A	0

[00292] Three classes of antibodies were identified as defined by their ability to strongly inhibit the binding of $\alpha 5\beta 1$ to FN ($\geq 93\%$ maximal inhibition), partially inhibit binding to FN ($\leq 60\%$ maximal inhibition) or not inhibit FN-binding (Table 11). The 6 antibodies that strongly or partially inhibited the binding of $\alpha 5\beta 1$ to FN were selected for further characterization.

Example 6: Competition Binding Studies to Identify Epitope Binding Groups

[00293] Epitope analysis via competition binding studies was performed using Biolayer Interferometry (BLI) on the 6 antibodies selected as described in Example 5. The anti- $\alpha 5\beta 1$ FN blocking antibodies A2-7A05, C-14D12 and A-15B08 were immobilized on sensors and incubated with each of the other antibodies that had been preincubated with rh- $\alpha 5\beta 1$, to determine whether this antibody-antigen association prevents or allows binding to the antibody on the sensor. The magnitude of observed binding (response) was compared to binding of antigen alone under the same conditions. If the overall response was greater than 120% of antigen binding alone, then the antibodies can pair with each other. If response was less than 80% of response of antigen binding alone, then the antibodies block each other. The protocol was as follows: preincubate the panel of antibodies with antigen (1 hr). Equilibrate the sensor (30 seconds (s)) --> Load lead Ab on the sensor (700s) ----> Quench (480s) ----> Read baseline (480s) --> measure preincubated Ab+Ag

association with loaded sensor (600s). The results are shown in Tables 12A and 12B.

[00294] For example, rh- $\alpha 5\beta 1$ complexed with A2-7A05 or A2-7F01 was able to bind C-14D12 and A-15B08 on the sensor. rh- $\alpha 5\beta 1$ complexed with A-15B08, A2-3B06, A2-5D10 or C-14D12 was able to bind to A2-7A05 on the sensor. A2-3B06 and C-14D12 when complexed with $\alpha 5\beta 1$ were not able to bind A-15B08 on the sensor and A-15B08 and A2-3B06 complexed with rh- $\alpha 5\beta 1$ were not able to bind C-14D12 on the sensor. The results in Tables 12A and 12B show that two epitope groups are represented by these 6 antibodies. A2-7A05 and A2-7F01 represent one group and A-15B08, A2-3B06, A2-5D10 and C-14D12 represent the second group.

[00295] Table 12A

Immobilized mAb	Secondary mAb*	Response (nm)	% Response	Compete
A-15B08	C-14D12	-0.15	-72.85%	+
A-15B08	A-15B08	-0.15	-73.81%	+
A-15B08	A2-3B06	0.10	50.05%	+
A-15B08	no mAb	0.21	100.00%	Control
A-15B08	A2-5D10	0.22	106.82%	+
A-15B08	A2-7F01	0.29	142.59%	-
A-15B08	A2-7A05	0.31	150.63%	-
A2-7A05	A2-7A05	-0.17	-451.35%	+
A2-7A05	A2-7F01	-0.17	-472.70%	+
A2-7A05	no mAb	0.04	100.00%	Control
A2-7A05	A2-3B06	0.07	198.65%	-
A2-7A05	C-14D12	0.11	309.19%	-
A2-7A05	A2-5D10	0.17	448.38%	-
A2-7A05	A-15B08	0.25	666.22%	-
C-14D12	C-14D12	-0.11	-50.49%	+
C-14D12	A-15B08	-0.09	-38.40%	+
C-14D12	A2-3B06	0.12	53.28%	+
C-14D12	no mAb	0.22	100.00%	Control
C-14D12	A2-5D10	0.24	106.12%	+
C-14D12	A2-7F01	0.30	133.09%	-
C-14D12	A2-7A05	0.31	139.39%	-

* Pre-Mixed with 20nM rh- $\alpha 5\beta 1$

[00296] Table 12B

		On Sensor		
		A2-7A05	A-15B08	C-14D12
Pre-incubated with $\alpha 5\beta 1$	A2-7A05	+	-	-
	A2-7F01	+	-	-
	C-14D12	-	+	+
	A-15B08	-	+	+
	A2-3B06	-	+	+
	A2-5D10	-	+	+

Example 7: Antibody Binding Disrupts the $\alpha 5\beta 1$ -FN Integrin-Ligand Complex

[00297] Surface Plasmon Resonance (SPR) and a cell-based assay were used to test the effect of the antibodies on the dissociation of rh- $\alpha 5\beta 1$ protein bound to human FN protein. The antibodies tested are representatives of the two groups of antibodies identified in Example 6 that define two different epitope binding groups and have distinct ligand blocking properties. They are A-15B08, a strong blocker of FN binding and A2-7A05, a partial blocker of FN binding. In addition, the small molecule antagonist cyclic RGD (cRGD; Creative-Peptides, Shirley, New York, 11967, cat. no. CP22175) was tested as a comparator that inhibits $\alpha 5\beta 1$ integrin binding to FN by competing at the ligand binding pocket.

[00298] Description of the SPR method used: FN protein dissolved in water was manually printed onto the bare gold-coated (thickness 47nm) PlexArray Nanocapture Sensor Chip (Plexera Bioscience, Seattle, WA) at 40% humidity. The chip was incubated in 80% humidity at 4°C for overnight and rinsed with 10x PBST (0.1M phosphate buffer, 1.54M NaCl, pH7.4, 0.5% Tween20) for 10 minutes (min), 1x PBST (0.01M phosphate buffer pH7.4, 0.154M NaCl, 0.05% Tween20) for 10 min, and deionized water twice for 10 min. The chip was then blocked with 5% (w/v) non-fat milk in water overnight, and washed with 10x PBST for 10 min, 1x PBST for 10 min, and deionized water twice for 10 min before being dried under a stream of nitrogen prior to use. SPR measurements were performed using PlexArray HT (Plexera Bioscience, Seattle, WA), a high-throughput surface plasmon resonance imaging (SPRi) platform. Collimated light (660 nm) passes through the coupling prism,

reflects off the SPR-active gold surface, and is received by the CCD camera. Buffers and samples were injected by a non-pulsatile piston pump into the 30 μ L flow cell that was mounted on the coupling prim. Each SPR measurement cycle contained four steps: washing with 1xPBS running buffer at a constant rate of 2 μ L/second (s) to obtain a stable baseline, injection of 400nM rh- α 5 β 1 for binding to FN at 5 μ L/s for 300s (to reach equilibrium), followed by injection of running buffer alone at 2 μ L/s for 50s to allow for dissociation of rh- α 5 β 1, and lastly injection of 1 μ M antibody at 2 μ L/s for 250s. All the measurements were performed at 25°C. SPR binding responses (a.u.) were recorded and plotted over time.

[00299] Both antibodies positively impacted dissociation of rh- α 5 β 1 protein bound to FN but to different degrees and cRGD did not. **FIG. 4A** shows the overlays of SPR responses, with and without the addition of the antibodies or cRGD. The injection of either antibody resulted in a transient increase in SPR response indicating the formation of a ternary complex of FN, rh- α 5 β 1 protein and the antibody. Strikingly, A-15B08 antibody, previously shown to be a strong blocker of FN binding by ELISA, resulted in a rapid dissociation of the FN- α 5 β 1 complex as detected by the SPR response declining nearly to baseline during the 250s that antibody is injected and SPR response is measured. This property of A-15B08 to rapidly dissociate the complex contrasts with published results that show a much slower rate of dissociation by other anti- α 5 β 1 antibodies under similar conditions (Mould *et al.*, J Bio Chem., 30; 291 (40): 20993-21007 (2016)). The A2-7A05 antibody, that was shown by ELISA to block α 5 β 1 binding to FN only partially, did not cause such a rapid decrease in SPR response, although did lead to partial dissociation at a slower rate, comparable to the previously published results by Mould *et al.* (2016) for a number of other antibodies. In contrast, injection of the small molecule antagonist cRGD did not have any discernable effect on the dissociation rate of the complex. Both antibodies had similar binding affinities to rh- α 5 β 1 protein (K_D 1.31E-09 and 0.8E-09 for A-15B08 and A2-7A05, respectively, see also Table 9) suggesting that the difference in rate of dissociation induced by these

antibodies is not a specific function of binding affinity, but instead an intrinsic property of the binding interaction. As previously shown by Mould *et al.* (2016), ligand site competitive antagonists such as cRGD are not effective at dissociation of ligand-integrin complexes as are allosteric antagonist antibodies suggesting that an allosteric mechanism is involved in the dissociation activity seen with the antibodies tested here.

[00300] The A-15B08 and A2-7A05 antibodies and cRGD were also tested for their ability to induce dissociation of cellular $\alpha 5 \beta 1$ integrin from FN. U87MG cells (HTB-14™, ATCC, Manassas, VA) which were originally derived from a Glioblastoma tumor, are known to express $\alpha 5 \beta 1$ integrin, and adhere to FN coated plates. After an overnight incubation on FN coated plates, the U87MG cells formed a loosely packed monolayer and have extended spindle shapes. If the cells are induced to detach from the plates, by trypsin for example, the attachment points are released, and the cells become round. This type of change in morphology was used to assess whether the antibodies and the small molecule inhibitor cRGD can induce U87MG cells to dissociate from FN coated plates.

[00301] The following method was used to assess cellular detachment activity: A 96-well cell culture plate (Thermo Fisher Scientific, Waltham, MA, cat. no. 165306) was coated with FN (Human Fibronectin, R&D Systems, Minneapolis, MN 55413, cat. no. 1918-FN) by incubation of 50uL per well 32µg/mL FN in 1xPBS for 1 hour at 37°C, then washed 2x with EMEM media (ATCC, Manassas, VA, cat. no. 30-2003) supplemented with 10% Fetal Bovine Serum (FBS; ATCC, Manassas, VA, cat. no. 30-2021). U87MG cells were plated at 20,000 cells per well in EMEM media + 10% FBS overnight to allow for maximum adherence. The next day the media was replaced with fresh media that included a 1:5 dilution series of antibodies A-15B08, A2-7A05 or isotype control IgG4 at concentrations of 2.0, 0.4, 0.08 and 0.016µg/mL or cRGD at 20, 4, 0.8 and 0.16µM. All tests were run in duplicate. Cell morphology was assessed, and images were captured using a 10X objective of an ECHO Rebel light microscope (ECHO, San Diego, CA).

[00302] The results show that antibody A-15B08, representative of the strong FN blocking antibodies, caused significant cell rounding due to detachment of cellular contacts at concentrations as low as 0.4µg/mL of the antibody, which was seen at both 1 hour and 3 hours, the latter being the time at which images were captured (**FIG. 4B**). The partial antagonist A2-7A05 also exhibited cellular detachment at the 0.4µg/mL antibody concentration but at a much lower level and was only obvious at 3 hours. The weaker ability of this antibody to detach adherent cells is consistent with it being only a partial inhibitor. IgG4 isotype control and cRGD treatments did not show significant cell detachment at any of the concentrations or time points assayed (**FIG. 4B**). The extent at which the two antibodies dissociated cells from FN coated wells is consistent with the SPR dissociation data generated with purified proteins (**FIG. 4A**), where the strong antagonist antibody caused rapid dissociation of the integrin-FN complex and the partial antagonist only weakly induced dissociation.

Example 8: Generation of Human IgG4 Chimeras and Removal of N-Glycosylation Site in CDRH2 Does Not Significantly Impact the Antibodies FN Blocking Activity

[00303] Antibody expression plasmids were constructed as human IgG4 chimeras with variable domains from antibodies A-15B08, C-14D12 and A2-7A05. In addition, the Threonine residue at position 62 in the CDRH2 of antibody A-15B08 was changed to an Alanine to remove a putative N-glycosylation site and designated as IgG4 clone A-15B08-T62A. The VH and VL sequences as well as 6 CDR sequences (according to various numbering schemes) of A-15B08-T62A are shown in **FIGs. 2C** and **2D**. Correctness of the sequences was verified with Sanger sequencing and plasmid concentrations determined by measuring the absorption at a wavelength of 260nm. The expression clones were transfected into suspension-adapted CHO K1 cells and grown in an animal-component free, serum-free medium. Supernatants were harvested by centrifugation and subsequent filtration (0.2µm filter). The antibody was purified using MabSelect™ SuRe™ (Cytiva, Marlborough, MA). Purity was determined by analytical size exclusion chromatography with an Agilent AdvanceBio

SEC column (300A 2.7 μ m 7.8 x 300mm; Agilent Technologies, Inc., Santa Clara, CA) using PBS as running buffer at 0.8mL/min. Yields were determined by Absorbance 280nm. All 4 chimeric antibodies, A-15B08, A-15B08-T62A, C-14D12 and A2-7A05, expressed at high levels with yields of 55, 53, 43 and 24 mg from 250mL culture, respectively.

[00304] Freeze-thaw stability was tested on a small aliquot of each chimera antibody by overnight storage at -80°C followed by thaw on ice, repeated three times. All 4 chimeric antibodies were stable to 3x freeze-thaws cycles as judged by their ability to inhibit the binding of $\alpha 5\beta 1$ integrin to fibronectin (FN), compared to the antibodies that were only stored at 4°C. The ELISA method used was as described in Example 2. IC50s were calculated by non-linear regression analysis curve-fitting (4-parameter) of the ELISA data (**FIG. 5**) using GraphPad Prism version 9.0.2 (GraphPad Software, LLC, San Diego, CA). The resulting IC50s indicate that 3x Freeze-Thaw (F/T) only very minimally effected potency of the antibodies in the FN-blocking assay (Table 13). In addition, the potency of the IgG4 chimeras was compared to the potency of the mouse hybridomas that they were derived from using the same FN-blocking ELISA and IC50 determination method. The results show that the chimeras had slightly higher potency than the hybridomas in all cases by approximately 2-fold (see **FIG. 6** & Table 14) except in the case of A2-7A05 where the difference was less (IC50 0.115 vs. 0.130).

Table 13

Antibody	IC50	
	4°C	3x F/T
A-15B08	0.015	0.017
A-15B08-T62A	0.018	0.020
C-14D12	0.021	0.023
A2-7A05	0.121	0.119

Table 14

Antibody	IC50	
	Hybridoma	Chimera
A-15B08	0.040	0.020
A-15B08-T62A	N/A	0.021
C-14D12	0.045	0.021
A2-7A05	0.130	0.115

[00305] The glycosylation state of A-15B08 was determined by comparing the mobility of its heavy chain in SDS-PAGE to that of A-15B08-T62A which contains a T to A mutation at position 62 of the heavy chain variable domain in the putative N-glycosylation site NST. 2µg of each antibody was separated by SDS-PAGE using a Bolt™ 4-12.5% Tris-Bis Plus (Invitrogen, Carlsbad, CA, cat. no. NW04120BOX) mini protein gel run using MOPS buffer (Invitrogen, Carlsbad, CA, cat. no. B0001). The mobility of the antibody heavy chain A-15B08-T62A with the mutated N-glycosylation site migrated similarly to the other 2 antibody heavy chains from C-14D12 and A2-7A05 that did not contain a putative N-glycosylation site. Notably, the heavy chain of A-15B08 migrated slower than the other 3 antibodies providing evidence that it was indeed glycosylated at this site (**FIG. 7**). Based on this data it is expected that antibody A2-5D10 (not mutated nor tested by SDS-PAGE) would also be glycosylated at the analogous NST sequence in its CDRH2.

Example 9: Antibody Binding to α5β1 Integrin Shifts Its Conformation to an Inactive Form

[00306] The ability of the anti-α5 antibodies to modulate the conformation of the integrin from an active to an inactive conformation was assayed using an antibody 12G10 (mouse anti-human integrin beta1/CD29 antibody, Novus Biologicals, Littleton, CO, cat. no. NB100-63255) that preferentially binds the β1-chain when the integrin is in an active or open conformation. A Nunc MaxiSorp Flat-Bottom 96-well plate (Invitrogen, Waltham, MA, cat. no. 44-2404-21) was coated with 12G10 antibody at 2µg/mL in 0.2M carbonate-bicarbonate buffer, pH9.4 (Thermo Scientific,

Rockford, IL, cat. No. 28382) by incubation overnight at 4°C. Plates were then washed 3 times with Wash Buffer (1x Tris Buffered Saline containing 0.05% Tween20), then blocked with 2% BSA in 1xTBS for 2 hours at room temperature (RT). The human IgG4 chimeric versions of the antibodies were diluted in Standard Diluent (2% BSA, 1xTBS, 0.05% Tween20) containing 0.05 µg/mL rh-α5β1-6xHis tagged protein (Acro Biosystems, Newark, DE, cat. no. IT1-H52W5) and 0.5mM MnCl₂ (TEKnova, Hollister, CA, cat. no. M0350) to generate a 7 point 1:5 antibody dilution series ranging from 10,000 ng/mL to 0.64 ng/mL. In addition to human IgG4 chimeric antibodies A-15B08, C-14D12 and A2-7A05, three other antibodies tested included IgG4 isotype control (human IgG4, Kappa, anti-fluorescein Ab00102-13.0, Absolute Antibody, Wilton, UK), anti-integrin alpha-5 clone SNAKA51 (MilliporeSigma, St. Louis, MO, cat. no. MABT201) known to induce integrin alpha-5 into an active conformation, and finally the 12G10 antibody itself which can directly compete with the 12G10 bound to the MaxiSorp plate. For the assays, 100µL of the antibody dilution series, His-tagged-α5β1 mixture was added to the wells after the blocking solution was removed and wells were washed 3 times. After 1 hour at RT, the wells were washed 3 times, incubated with biotinylated Anti-6xHis-Tag Ab (Invitrogen, Carlsbad, CA, cat. no. MAI-21315-BTIN) at 1:1000 in Standard Diluent for 1 hour, washed 3 times, incubated for 30 minutes with poly-HRP Streptavidin (Thermo Fisher Scientific, Waltham, MA, cat. no. N200), washed 3 times, incubated with TMB substrate (Thermo Fisher Scientific, Waltham, MA, cat. no. N301) for 2-5 minutes, followed by addition of ELISA Stop Solution (Invitrogen, Carlsbad, CA, cat. no. SS04). Absorbance 450nm was measured. Data points were normalized to the absorbance of the well containing no antibody for each dilution series and reported as Percent Binding. Non-linear regression analysis was used to fit curves (3-parameter) to the data using GraphPad Prism version 9.0.2 (GraphPad Software, LLC, San Diego, CA). The results are presented in **FIG. 8**.

[00307] The results show that two antibodies, A-15B08 and C-14D12, that were previously shown to strongly inhibit the binding of α5β1 to FN, reduced α5β1 integrin

binding to 12G10 by approximately 50%, while the antibody A2-7A05, that partially inhibited binding to FN, did not reduce $\alpha 5\beta 1$ integrin binding to 12G10. The IgG4 isotype control similarly did not reduce $\alpha 5\beta 1$ integrin binding to 12G10. The SNAKA51 antibody which is known to shift $\alpha 5\beta 1$ integrin to an active conformation increased $\alpha 5\beta 1$ integrin binding to 12G10. Without being bound by any theory, the antibodies that strongly inhibit $\alpha 5\beta 1$ integrin binding to its primary ligand FN may do so, in part, by shifting the conformation of $\alpha 5\beta 1$ integrin into an inactive conformational state.

Example 10: Antibody Inhibition of Cellular Adhesion to Fibronectin

[00308] Cell surface $\alpha 5\beta 1$ integrin receptors interact with FN to promote cellular adhesion. To test whether the human IgG4 chimeric anti- $\alpha 5\beta 1$ antibodies inhibit adhesion, a plate-based cell adhesion assay was developed for use with the U87MG cell line (HTB-14™, ATCC, Manassas, VA) which is derived from a Glioblastoma tumor and is known to express $\alpha 5\beta 1$ integrin. U87MG cells were grown to 80% confluence in EMEM media (ATCC, Manassas, VA, cat. no. 30-2003) supplemented with 10% Fetal Bovine Serum (FBS; ATCC, Manassas, VA, cat. no. 30-2021) in a 5% CO₂, 37°C incubator. Cells were dissociated from flasks with 0.05% Trypsin, 0.02% EDTA (Lifeline Cell Technology, Frederick, MD, cat. no. CM0017), washed once with Dulbecco's 1x PBS (DPBS) Modified which contains calcium and magnesium (HyClone, Logan, UT, cat. no. SH30028-02) and resuspended in EMEM media without supplementation at a cellular concentration of 2,000,000 cells per mL. Cells were rested in the incubator for 30 minutes before mixing with a six point 1:3 dilution series of the antibodies with final concentrations ranging from 3 to 0.01 $\mu\text{g/mL}$ in a 96-well polypropylene round-bottom plate. 100,000 rested cells were pre-mixed with each dilution of antibody to a final volume of 100 μL and then transferred to a Human FN coated 96-well plate (R&D Systems, Minneapolis, MN, cat. no. CWP001). The cell-antibody mixtures were incubated for 1 hour at 37°C, 5% CO₂. Non-adhered cells were removed from the wells by inversion of the plate on an absorbent pad, then washed 2x with 200 μL each well with 1x DPBS without Calcium

and Magnesium (EMD Millipore Corp, Billerica, MA, cat. no. TMS-012-A). 100 μ L 1x DPBS containing Hoechst 33342 dye (Thermo Fisher Scientific, Waltham, MA, cat. no. 62249) at the recommended dilution (1:2000) was added to each well to fluorescently stain nuclei to facilitate cell counting. Cells that remained adhered to the wells were counted using image analysis software provided by the ImageXpress Pico Automated Cell Imaging System (Molecular Devices, San Jose, CA) which is based on counting fluorescently stained nuclei, in this case using a DAPI filter to detect the Hoechst nuclear staining. Non-linear regression analysis was used to fit curves (4-parameter) to the data using GraphPad Prism version 9.0.2 (GraphPad Software, LLC, San Diego, CA).

[00309] Results are shown in **FIG. 9**. The average number of U87MG cells that adhered to the FN coated wells in the absence of antibody was 37,022 with a standard deviation (stdev) 2,364 (n=16). Cell counts from wells without FN coating on average had 628 cells with a stdev of 143 (n=12). The highest concentration of antibodies tested at 3 μ g/mL inhibited adhesion ranging from 78% to 86% for the antibodies that are strong blockers of FN binding, A-15B08, A-15B08-T62A and C-14D12, and 25% for antibody A2-7A05 that was shown to be a partial blocker of FN binding (Table 15). The data from this cellular adhesion blocking assay demonstrated the inhibitory effects of the present antibodies on the binding of $\alpha 5\beta 1$ with Fn and on cellular adhesion.

Table 15

Antibody	Cell Counts (Curve Bottom)	Cell Counts (Curve Top)	% Inhibition	IC50
A-15B08 IgG4 Chimera	4783	33768	86	0.12
A-15B08-T62A IgG4 Chimera	6318	35447	82	0.15
C-14D12 IgG4 Chimera	8044	37180	78	0.14
A2-7A05 IgG4 Chimera	28819	38202	25	0.27
IgG4 Isotype	31993	49996	N/A	N/A
No mAb	34148	40905	N/A	N/A

EMBODIMENTS

1. An antibody or fragment thereof that competes for binding to $\alpha 5\beta 1$ integrin with an antibody comprising:

(A)(i) a heavy chain variable region having an amino acid sequence of SEQ ID NO:25 and a light chain variable region having an amino acid sequence of SEQ ID NO:26; (ii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:42 and a light chain variable region having an amino acid sequence of SEQ ID NO:43; (iii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:51 and a light chain variable region having an amino acid sequence of SEQ ID NO:52; (iv) a heavy chain variable region having an amino acid sequence of SEQ ID NO:109 and a light chain variable region having an amino acid sequence of SEQ ID NO:110; (v) a heavy chain variable region having an amino acid sequence of SEQ ID NO:135 and a light chain variable region having an amino acid sequence of SEQ ID NO:26; (vi) a heavy chain variable region having an amino acid sequence of SEQ ID NO:136 and a light chain variable region having an amino acid sequence of SEQ ID NO:137; (vii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:138 and a light chain variable region having an amino acid sequence of SEQ ID NO:139; (viii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:144 and a light chain variable region having an amino acid sequence of SEQ ID NO:145; (ix) a heavy chain variable region having an amino acid sequence of SEQ ID NO:146 and a light chain variable region having an amino acid sequence of SEQ ID NO:147; or

(B)(i) a heavy chain variable region having an amino acid sequence of SEQ ID NO:77 and a light chain variable region having an amino acid sequence of SEQ ID NO:78; (ii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:91 and a light chain variable region having an amino acid sequence of SEQ ID NO:92; (iii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:140 and a light chain variable region having an amino acid sequence of SEQ ID NO:141; and/or (iv) a heavy chain variable region having an amino acid sequence of SEQ ID NO:142 and a light chain variable region having an amino acid sequence of SEQ ID NO:143.

2. An antibody or fragment thereof that binds to $\alpha 5 \beta 1$ integrin, wherein the antibody or fragment thereof comprises:

(a) a heavy chain variable (V_H) region comprising:

(1) a V_H CDR1 having an amino acid sequence selected from the group consisting of:

- (i) SEQ ID NO:1, 27, 53, or 93,
- (ii) SEQ ID NO:7, 31, 59, or 97,
- (iii) SEQ ID NO:12, 34, 64, or 100,
- (iv) SEQ ID NO:13, 35, 65, or 101, and
- (v) SEQ ID NO:18, 38, 70, or 105;

(2) a V_H CDR2 having an amino acid sequence selected from the group consisting of:

- (i) SEQ ID NO:2, 28, 54, or 79,
- (ii) SEQ ID NO:8, 60, or 82,
- (iii) SEQ ID NO:14, 66, or 84,
- (iv) SEQ ID NO:19, 71, or 87, and
- (v) SEQ ID NO:24, 76, or 90; and

(3) a V_H CDR3 having an amino acid sequence selected from the group consisting of:

- (i) SEQ ID NO:3, 29, 55, 80, or 94,
- (ii) SEQ ID NO:9, 32, 61, 83, or 98,
- (iii) SEQ ID NO:15, 36, 67, 85, or 102, and
- (iv) SEQ ID NO:20, 39, 72, 88, or 106;

and

(b) a light chain variable (V_L) region comprising:

(1) a V_L CDR1 having an amino acid sequence selected from the group consisting of:

- (i) SEQ ID NO:4, 30, 44, 56, or 95,
- (ii) SEQ ID NO:10, 33, 46, 62, or 99,

- (iii) SEQ ID NO:16, 37, 47, 68, or 103, and
- (iv) SEQ ID NO:21, 40, 49, 73, or 107;
- (2) a V_L CDR2 having an amino acid sequence selected from the group consisting of:
 - (i) SEQ ID NO:5 or 57,
 - (ii) SEQ ID NO:11 or 63, and
 - (iii) SEQ ID NO:22, 41, or 74; and
- (3) a V_L CDR3 having an amino acid sequence selected from the group consisting of:
 - (i) SEQ ID NO:6, 45, 58, 81, or 96;
 - (ii) SEQ ID NO:17, 48, 69, 86, or 104, and
 - (iii) SEQ ID NO:23, 50, 75, 89, or 108.

3. An antibody or fragment thereof that binds to $\alpha 5 \beta 1$ integrin, wherein the antibody or fragment thereof comprises a heavy chain variable (V_H) region comprising:

- (1) a V_H CDR1 having an amino acid sequence selected from the group consisting of:
 - (i) SEQ ID NO:1, 27, 53, or 93,
 - (ii) SEQ ID NO:7, 31, 59, or 97,
 - (iii) SEQ ID NO:12, 34, 64, or 100,
 - (iv) SEQ ID NO:13, 35, 65, or 101, and
 - (v) SEQ ID NO:18, 38, 70, or 105;
- (2) a V_H CDR2 having an amino acid sequence selected from the group consisting of:
 - (i) SEQ ID NO:2, 28, 54, or 79,
 - (ii) SEQ ID NO:8, 60, or 82,
 - (iii) SEQ ID NO:14, 66, or 84,
 - (iv) SEQ ID NO:19, 71, or 87, and
 - (v) SEQ ID NO:24, 76, or 90; and

(3) a V_H CDR3 having an amino acid sequence selected from the group consisting of:

- (i) SEQ ID NO:3, 29, 55, 80, or 94,
- (ii) SEQ ID NO:9, 32, 61, 83, or 98,
- (iii) SEQ ID NO:15, 36, 67, 85, or 102, and
- (iv) SEQ ID NO:20, 39, 72, 88, or 106.

4. An antibody or fragment thereof that binds to $\alpha 5\beta 1$ integrin, wherein the antibody or fragment thereof comprises a light chain variable (V_L) region comprising:

(1) a V_L CDR1 having an amino acid sequence selected from the group consisting of:

- (i) SEQ ID NO:4, 30, 44, 56, or 95,
- (ii) SEQ ID NO:10, 33, 46, 62, or 99,
- (iii) SEQ ID NO:16, 37, 47, 68, or 103, and
- (iv) SEQ ID NO:21, 40, 49, 73, or 107;

(2) a V_L CDR2 having an amino acid sequence selected from the group consisting of:

- (i) SEQ ID NO:5 or 57,
- (ii) SEQ ID NO:11 or 63, and
- (iii) SEQ ID NO:22, 41, or 74; and

(3) a V_L CDR3 having an amino acid sequence selected from the group consisting of:

- (i) SEQ ID NO:6, 45, 58, 81, or 96,
- (ii) SEQ ID NO:17, 48, 69, 86, or 104, and
- (iii) SEQ ID NO:23, 50, 75, 89, or 108.

5. An antibody or fragment thereof that binds to $\alpha 5\beta 1$ integrin comprising all three heavy chain complementarity determining regions (CDRs) or all three light chain CDRs from:

the antibody designated A-15B08 that comprises a V_H sequence that is SEQ ID NO:25 and a V_L sequence that is SEQ ID NO:26;

the antibody designated A-15B08-T62A that comprises a VH sequence that is SEQ ID NO:135 and a VL sequence that is SEQ ID NO:26;

the antibody designated A-15B08_Low that comprises a VH sequence that is SEQ ID NO:136 and a VL sequence that is SEQ ID NO:137;

the antibody designated A-15B08_Low+Mod that comprises a VH sequence that is SEQ ID NO:138 and a VL sequence that is SEQ ID NO:139; the antibody designated A2-3B06 that comprises a VH sequence that is SEQ ID NO:42 and a VL sequence that is SEQ ID NO:43;

the antibody designated A2-5D10 that comprises a VH sequence that is SEQ ID NO:51 and a VL sequence that is SEQ ID NO:52;

the antibody designated A2-7A05 that comprises a VH sequence that is SEQ ID NO:77 and a VL sequence that is SEQ ID NO:78;

the antibody designated A2-7A05_Low that comprises a VH sequence that is SEQ ID NO:140 and a VL sequence that is SEQ ID NO:141;

the antibody designated A2-7A05_Low+Mod that comprises a VH sequence that is SEQ ID NO:142 and a VL sequence that is SEQ ID NO:143;

the antibody designated A2-7F01 that comprises a VH sequence that is SEQ ID NO:91 and a VL sequence that is SEQ ID NO:92;

the antibody designated C-14D12 that comprises a VH sequence that is SEQ ID NO:109 and a VL sequence that is SEQ ID NO:110;

the antibody designated C-14D12_Low that comprises a VH sequence that is SEQ ID NO:144 and a VL sequence that is SEQ ID NO:145;
or

the antibody designated C-14D12_Low+Mod that comprises a VH sequence that is SEQ ID NO:146 and a VL sequence that is SEQ ID NO:147.

6. The antibody or fragment thereof of embodiment 5, wherein the antibody or fragment thereof comprises all three heavy chain CDRs and all three light chain CDRs from the antibody designated A-15B08.

7. The antibody or fragment thereof of embodiment 5, wherein the antibody or fragment thereof comprises all three heavy chain CDRs and all three light chain CDRs from the antibody designated A2-3B06.

8. The antibody or fragment thereof of embodiment 5, wherein the antibody or fragment thereof comprises all three heavy chain CDRs and all three light chain CDRs from the antibody designated A2-5D10.

9. The antibody or fragment thereof of embodiment 5, wherein the antibody or fragment thereof comprises all three heavy chain CDRs and all three light chain CDRs from the antibody designated A2-7A05.

10. The antibody or fragment thereof of embodiment 5, wherein the antibody or fragment thereof comprises all three heavy chain CDRs and all three light chain CDRs from the antibody designated A2-7F01.

11. The antibody or fragment thereof of embodiment 5, wherein the antibody or fragment thereof comprises all three heavy chain CDRs and all three light chain CDRs from the antibody designated C-14D12.

12. An antibody or fragment thereof that binds to $\alpha 5\beta 1$ integrin, wherein the antibody comprises:

(a) a heavy chain variable (V_H) region comprising a V_H CDR1, a V_H CDR2, and a V_H CDR3 amino acid sequence as set forth in Tables 1-6;
or

(b) a light chain variable (V_L) region comprising a V_L CDR1, a V_L CDR2, and a V_L CDR3 amino acid sequence as set forth in Tables 1-6.

13. The antibody or fragment thereof of embodiment 12, wherein the antibody comprises:

(a) a heavy chain variable (V_H) region comprising a V_H CDR1, a V_H CDR2, and a V_H CDR3 amino acid sequence as set forth in Tables 1-6;
and

(b) a light chain variable (V_L) region comprising a V_L CDR1, a V_L CDR2, and a V_L CDR3 amino acid sequence as set forth in Tables 1-6.

14. The antibody or fragment thereof of embodiment 12, wherein the antibody comprises a heavy chain variable (V_H) region comprising a V_H CDR1, a V_H CDR2, and a V_H CDR3 amino acid sequence as set forth in Tables 1-6.

15. The antibody or fragment thereof of embodiment 12, wherein the antibody comprises a light chain variable (V_L) region comprising a V_L CDR1, a V_L CDR2, and a V_L CDR3 amino acid sequence as set forth in Tables 1-6.

16. The antibody or fragment thereof of embodiment 12, wherein the antibody comprises:

(a) a heavy chain variable (V_H) region comprising:

- (1) a V_H CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:1, 7, 12, 13, and 18;
- (2) a V_H CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 8, 14, 19, and 24; and
- (3) a V_H CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:3, 9, 15, and 20;

and

(b) a light chain variable (V_L) region comprising:

- (1) a V_L CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:4, 10, 16, and 21;
- (2) a V_L CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 22; and
- (3) a V_L CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:6, 17, and 23.

17. The antibody or fragment thereof of embodiment 16, wherein the antibody comprises:

(a) a heavy chain variable (V_H) region comprising:

- (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:1;

(2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:2; and

(3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:3;

and

(b) a light chain variable (V_L) region comprising:

(1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:4;

(2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and

(3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:6.

18. The antibody or fragment thereof of embodiment 16, wherein the antibody comprises:

(a) a heavy chain variable (V_H) region comprising:

(1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:7;

(2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:8; and

(3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:9;

and

(b) a light chain variable (V_L) region comprising:

(1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:10;

(2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:11; and

(3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:6.

19. The antibody or fragment thereof of embodiment 16, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:12;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:2; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:3;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:4;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:6.

20. The antibody or fragment thereof of embodiment 16, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:13;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:14; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:15;

and

- (b) a light chain variable (V_L) region comprising:

- (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:16;
- (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:11; and
- (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:17.

21. The antibody or fragment thereof of embodiment 16, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:18;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:19; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:20;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:21;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:22; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:23.

22. The antibody or fragment thereof of embodiment 16, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:1;

- (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:24; and
- (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:3;
- and
- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:4;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:6.

23. The antibody or fragment thereof of embodiment 12, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:27, 31, 34, 35, and 38;
 - (2) a V_H CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:8, 14, 19, 24, and 28; and
 - (3) a V_H CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:29, 32, 36, and 39;
- and
- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:30, 33, 37, and 40;
 - (2) a V_L CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 41 and
 - (3) a V_L CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:6, 17, and 23.

24. The antibody or fragment thereof of embodiment 23, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:27;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:28; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:29;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:30;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:6.

25. The antibody or fragment thereof of embodiment 23, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:31;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:8; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:32;

and

- (b) a light chain variable (V_L) region comprising:

- (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:33;
- (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:11; and
- (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:6.

26. The antibody or fragment thereof of embodiment 23, wherein the antibody comprises:

(a) a heavy chain variable (V_H) region comprising:

- (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:34;
- (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:28; and
- (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:29;

and

(b) a light chain variable (V_L) region comprising:

- (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:30;
- (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and
- (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:6.

27. The antibody or fragment thereof of embodiment 23, wherein the antibody comprises:

(a) a heavy chain variable (V_H) region comprising:

- (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:35;

- (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:14; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:36;
- and
- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:37;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:11; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:17.

28. The antibody or fragment thereof of embodiment 23, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:38;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:19; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:39;
- and
- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:40;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:41; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:23.

29. The antibody or fragment thereof of embodiment 23, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:27;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:24; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:29;and
- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:30;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:6.

30. The antibody or fragment thereof of embodiment 12, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:1, 7, 12, 13, and 18;
 - (2) a V_H CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 8, 14, 19, and 24; and
 - (3) a V_H CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:3, 9, 15, and 20;and
- (b) a light chain variable (V_L) region comprising:

- (1) a V_L CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:44, 46, 47, and 49;
- (2) a V_L CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 22; and
- (3) a V_L CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:45, 48, and 50.

31. The antibody or fragment thereof of embodiment 30, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:1;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:2; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:3;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:44;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:45.

32. The antibody or fragment thereof of embodiment 30, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:7;

- (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:8; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:9;
- and
- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:46;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:11; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:45.

33. The antibody or fragment thereof of embodiment 30, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:12;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:2; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:3;
- and
- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:44;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:45.

34. The antibody or fragment thereof of embodiment 30, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:13;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:14; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:15;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:47;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:11; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:48.

35. The antibody or fragment thereof of embodiment 30, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:18;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:19; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:20;

and

- (b) a light chain variable (V_L) region comprising:

- (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:49;
- (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:22; and
- (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:50.

36. The antibody or fragment thereof of embodiment 30, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:1;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:24; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:3;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:44;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:45.

37. The antibody or fragment thereof of embodiment 12, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:53, 59, 64, 65, and 70;

- (2) a V_H CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:54, 60, 66, 71, and 76; and
- (3) a V_H CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:55, 61, 67, and 72;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:56, 62, 68, and 73;
 - (2) a V_L CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:57, 63, and 74; and
 - (3) a V_L CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:58, 69, and 75.

38. The antibody or fragment thereof of embodiment 37, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:53;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:54; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:55;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:56;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:57; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:58.

39. The antibody or fragment thereof of embodiment 37, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:59;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:60; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:61;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:62;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:63; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:58.

40. The antibody or fragment thereof of embodiment 37, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:64;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:54; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:55;

and

- (b) a light chain variable (V_L) region comprising:

- (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:56;
- (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:57; and
- (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:58.

41. The antibody or fragment thereof of embodiment 37, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:65;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:66; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:67;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:68;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:63; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:69.

42. The antibody or fragment thereof of embodiment 37, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:70;

(2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:71; and

(3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:72;

and

(b) a light chain variable (V_L) region comprising:

(1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:73;

(2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:74; and

(3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:75.

43. The antibody or fragment thereof of embodiment 37, wherein the antibody comprises:

(a) a heavy chain variable (V_H) region comprising:

(1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:53;

(2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:76; and

(3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:55;

and

(b) a light chain variable (V_L) region comprising:

(1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:56;

(2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:57; and

(3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:58.

44. The antibody or fragment thereof of embodiment 12, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:53, 59, 64, 65, and 70;
 - (2) a V_H CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:79, 82, 84, 87, and 90; and
 - (3) a V_H CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:80, 83, 85, and 88;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:56, 62, 68, and 73;
 - (2) a V_L CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:57, 63, and 74; and
 - (3) a V_L CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 86, and 89.

45. The antibody or fragment thereof of embodiment 44, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:53;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:79; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:80;

and

- (b) a light chain variable (V_L) region comprising:

- (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:56;
- (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:57; and
- (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:81.

46. The antibody or fragment thereof of embodiment 44, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:59;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:82; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:83;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:62;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:63; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:81.

47. The antibody or fragment thereof of embodiment 44, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:64;

(2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:79; and

(3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:80;

and

(b) a light chain variable (V_L) region comprising:

(1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:56;

(2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:57; and

(3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:81.

48. The antibody or fragment thereof of embodiment 44, wherein the antibody comprises:

(a) a heavy chain variable (V_H) region comprising:

(1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:65;

(2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:84; and

(3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:85;

and

(b) a light chain variable (V_L) region comprising:

(1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:68;

(2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:63; and

(3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:86.

49. The antibody or fragment thereof of embodiment 44, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:70;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:87; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:88;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:73;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:74; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:89.

50. The antibody or fragment thereof of embodiment 44, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:53;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:90; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:80;

and

- (b) a light chain variable (V_L) region comprising:

- (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:56;
- (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:57; and
- (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:81.

51. The antibody or fragment thereof of embodiment 12, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:93, 97, 100, 101, and 105;
 - (2) a V_H CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:8, 14, 19, 24, and 28; and
 - (3) a V_H CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:94, 98, 102, and 106;
- and
- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:95, 99, 103, and 107;
 - (2) a V_L CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 22; and
 - (3) a V_L CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:96, 104, and 108.

52. The antibody or fragment thereof of embodiment 51, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:93;

(2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:28; and

(3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:94;

and

(b) a light chain variable (V_L) region comprising:

(1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:95;

(2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5 and

(3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:96.

53. The antibody or fragment thereof of embodiment 51, wherein the antibody comprises:

(a) a heavy chain variable (V_H) region comprising:

(1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:97;

(2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:8; and

(3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:98;

and

(b) a light chain variable (V_L) region comprising:

(1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:99;

(2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:11; and

(3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:96.

54. The antibody or fragment thereof of embodiment 51, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:100;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:28; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:94;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:95;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:96.

55. The antibody or fragment thereof of embodiment 51, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:101;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:14; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:102;

and

- (b) a light chain variable (V_L) region comprising:

- (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:103;
- (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:11; and
- (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:104.

56. The antibody or fragment thereof of embodiment 51, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:105;
 - (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:19; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:106;

and

- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:107;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:22; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:108.

57. The antibody or fragment thereof of embodiment 51, wherein the antibody comprises:

- (a) a heavy chain variable (V_H) region comprising:
 - (1) a V_H CDR1 having the amino acid sequence of SEQ ID NO:93;

- (2) a V_H CDR2 having the amino acid sequence of SEQ ID NO:24; and
 - (3) a V_H CDR3 having the amino acid sequence of SEQ ID NO:94;
- and
- (b) a light chain variable (V_L) region comprising:
 - (1) a V_L CDR1 having the amino acid sequence of SEQ ID NO:95;
 - (2) a V_L CDR2 having the amino acid sequence of SEQ ID NO:5; and
 - (3) a V_L CDR3 having the amino acid sequence of SEQ ID NO:96.

58. The antibody or fragment thereof of any one of embodiments 12-57, wherein the V_H region or V_L region further comprises human framework sequences.

59. The antibody or fragment thereof of embodiment 58, wherein the V_H region and V_L region further comprises human framework sequences.

60. The antibody or fragment thereof of any one of embodiments 12-57, wherein the V_H region or V_L region further comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and/or a framework 4 (FR4) sequence.

61. The antibody or fragment thereof of embodiment 60, wherein the V_H region and V_L region further comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and a framework 4 (FR4) sequence.

62. The antibody or fragment thereof of any one of embodiments 1-61, wherein the antibody is a monoclonal antibody.

63. The antibody or fragment thereof of embodiment 62, wherein the monoclonal antibody is a humanized, human or chimeric antibody.

64. The antibody or fragment thereof of any one of embodiments 1-63, which is a Fab, Fab', F(ab')₂, Fv, scFv, (scFv)₂, single chain antibody molecule, dual variable

region antibody, single variable region antibody, linear antibody, V region, or a multispecific antibody formed from antibody fragments.

65. The antibody or fragment thereof of any one of embodiments 1-64, which is conjugated or recombinantly fused to a diagnostic agent, detectable agent or therapeutic agent.

66. The antibody or fragment thereof of embodiment 65, wherein the therapeutic agent is a chemotherapeutic agent, cytotoxin, or drug.

67. A binding agent that binds to essentially the same epitope as an antibody or fragment thereof of any one of embodiments 1-66.

68. The binding agent of embodiment 67, which is an antibody or fragment thereof.

69. The binding agent of embodiment 67, which comprises a non-antibody protein scaffold.

70. The binding agent of embodiment 69, wherein the non-antibody protein scaffold comprises a fibronectin scaffold, an anticalin, an adnectin, an affibody, a DARPIn, a fynomer, an affitin, an affilin, an avimer, a cysteine-rich knottin peptide, or an engineered Kunitz-type inhibitor.

71. A binding agent that competes for binding to human $\alpha 5 \beta 1$ integrin with an antibody or fragment thereof of any one of embodiments 1-66.

72. The binding agent of embodiment 71, wherein the binding agent is an antibody or fragment thereof.

73. One or more vectors comprising one or more polynucleotides encoding the antibody or fragment thereof of any one of embodiments 1-66.

74. A pharmaceutical composition that comprises the antibody or fragment thereof of any one of embodiments 1-63, and a pharmaceutically acceptable carrier.

75. A method for treating an $\alpha 5 \beta 1$ integrin-mediated disease, disorder or condition in a subject comprising administering to the subject the antibody or fragment thereof of any one of embodiments 1-66 or the pharmaceutical composition of embodiment 74.

76. A method for alleviating one or more symptoms associated with an $\alpha 5\beta 1$ integrin-mediated disease, disorder, or condition in a subject comprising administering to the subject the antibody or fragment thereof of any one of embodiments 1-66 or the pharmaceutical composition of embodiment 74.

77. A method for treating a cancer or a tumor in a subject comprising administering to the subject the antibody or fragment thereof of any one of embodiments 1-66 or the pharmaceutical composition of embodiment 74.

78. A method for alleviating one or more symptoms associated with a cancer or a tumor in a subject comprising administering to the subject the antibody or fragment thereof of any one of embodiments 1-66 or the pharmaceutical composition of embodiment 74.

79. A method for treating an angiogenesis-mediated disease, disorder, or condition in a subject comprising administering to the subject the antibody or fragment thereof of any one of embodiments 1-66 or the pharmaceutical composition of embodiment 74.

80. A method for alleviating one or more symptoms associated with an angiogenesis-mediated disease, disorder, or condition in a subject comprising administering to the subject the antibody or fragment thereof of any one of embodiments 1-66 or the pharmaceutical composition of embodiment 74.

81. A method for treating an inflammatory disease, disorder, or condition in a subject comprising administering to the subject the antibody or fragment thereof of any one of embodiments 1-66 or the pharmaceutical composition of embodiment 74.

82. A method for alleviating one or more symptoms associated with an inflammatory disease, disorder or condition in a subject comprising administering to the subject the antibody or fragment thereof of any one of embodiments 1-66 or the pharmaceutical composition of embodiment 74.

83. The method of any one of embodiments 75-82, wherein the subject is administered one or more therapeutic agents in combination with the antibody or fragment thereof or the pharmaceutical composition.

[00310] Throughout this application various publications, patents, patent applications and other documents have been referenced. The disclosures of these publications, patents, patent applications and other documents in their entireties are hereby incorporated by reference in this application for all purposes, including in order to more fully describe the state of the art to which this the subject matter disclosed herein pertains. Although the disclosed subject matter has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the disclosed subject matter. Many variations will become apparent to those skilled in the art upon review of this specification.

WHAT IS CLAIMED IS:

1. An antibody or fragment thereof that competes for binding to $\alpha 5\beta 1$ integrin with an antibody comprising:

(A)(i) a heavy chain variable region having an amino acid sequence of SEQ ID NO:25 and a light chain variable region having an amino acid sequence of SEQ ID NO:26; (ii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:42 and a light chain variable region having an amino acid sequence of SEQ ID NO:43; (iii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:51 and a light chain variable region having an amino acid sequence of SEQ ID NO:52; (iv) a heavy chain variable region having an amino acid sequence of SEQ ID NO:109 and a light chain variable region having an amino acid sequence of SEQ ID NO:110; (v) a heavy chain variable region having an amino acid sequence of SEQ ID NO:135 and a light chain variable region having an amino acid sequence of SEQ ID NO:26; (vi) a heavy chain variable region having an amino acid sequence of SEQ ID NO:136 and a light chain variable region having an amino acid sequence of SEQ ID NO:137; (vii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:138 and a light chain variable region having an amino acid sequence of SEQ ID NO:139; (viii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:144 and a light chain variable region having an amino acid sequence of SEQ ID NO:145; (ix) a heavy chain variable region having an amino acid sequence of SEQ ID NO:146 and a light chain variable region having an amino acid sequence of SEQ ID NO:147; or

(B)(i) a heavy chain variable region having an amino acid sequence of SEQ ID NO:77 and a light chain variable region having an amino acid sequence of SEQ ID NO:78; (ii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:91 and a light chain variable region having an amino acid sequence of SEQ ID NO:92; (iii) a heavy chain variable region having an amino acid sequence of SEQ ID NO:140 and a light chain variable region having an amino acid sequence of SEQ ID NO:141; and/or (iv) a heavy chain variable region having an amino acid sequence of SEQ ID NO:142 and a light chain variable region having an amino acid sequence of SEQ ID NO:143.

2. The antibody or fragment thereof of claim 1 that binds to $\alpha 5 \beta 1$ integrin and that comprises: (i) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:25, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:26; (ii) a VH CDR1, a VH CDR2, a VH CDR3 as set forth in SEQ ID NO:42, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:43; (iii) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:51, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:52; (iv) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:77, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:78; (v) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:91, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:92; (vi) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:109, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:110; (vii) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:135, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:26; (viii) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:136, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:137; (ix) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:138, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:139; (x) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:140, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:141; (xi) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:142, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:143; (xii) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:144, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:145; or (xiii) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in SEQ ID NO:146, and/or a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in SEQ ID NO:147.

3. The antibody or fragment thereof of claim 1 that binds to $\alpha 5 \beta 1$ integrin and that comprises:

(a) a heavy chain variable (VH) region comprising a VH CDR1, a VH CDR2, and a VH CDR3 amino acid sequence as set forth in Tables 1-6;

or

- (b) a light chain variable (VL) region comprising a VL CDR1, a VL CDR2, and a VL CDR3 amino acid sequence as set forth in Tables 1-6.
4. The antibody or fragment thereof of claim 3, that comprises:
- (a) a heavy chain variable (VH) region comprising a VH CDR1, a VH CDR2, and a VH CDR3 amino acid sequence as set forth in Tables 1-6;
- and
- (b) a light chain variable (VL) region comprising a VL CDR1, a VL CDR2, and a VL CDR3 amino acid sequence as set forth in Tables 1-6.
5. The antibody or fragment thereof of claim 3, that comprises a heavy chain variable (VH) region comprising a VH CDR1, a VH CDR2, and a VH CDR3 amino acid sequence as set forth in Tables 1-6.
6. The antibody or fragment thereof of claim 3, that comprises a light chain variable (VL) region comprising a VL CDR1, a VL CDR2, and a VL CDR3 amino acid sequence as set forth in Tables 1-6.
7. The antibody or fragment thereof of claim 1 that binds to $\alpha 5\beta 1$ integrin and that comprises:
- (a) a heavy chain variable (VH) region comprising:
- (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:1, 7, 12, 13, and 18;
- (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 8, 14, 19, and 24;
- and
- (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:3, 9, 15, and 20;
- and
- (b) a light chain variable (VL) region comprising:
- (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:4, 10, 16, and 21;

- (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 22; and
- (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:6, 17, and 23.

8. The antibody or fragment thereof of claim 1 that binds to $\alpha 5\beta 1$ integrin and that comprises:

- (a) a heavy chain variable (VH) region comprising:
 - (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:27, 31, 34, 35, and 38;
 - (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:8, 14, 19, 24, and 28; and
 - (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:29, 32, 36, and 39;
- and
- (b) a light chain variable (VL) region comprising:
 - (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:30, 33, 37, and 40;
 - (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 41 and
 - (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:6, 17, and 23.

9. The antibody or fragment thereof of claim 1 that binds to $\alpha 5\beta 1$ integrin and that comprises:

- (a) a heavy chain variable (VH) region comprising:
 - (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:1, 7, 12, 13, and 18;
 - (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 8, 14, 19, and 24; and

- (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:3, 9, 15, and 20;
- and
- (b) a light chain variable (VL) region comprising:
 - (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:44, 46, 47, and 49;
 - (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 22; and
 - (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:45, 48, and 50.

10. The antibody or fragment thereof of claim 1 that binds to $\alpha 5\beta 1$ integrin and that comprises:

- (a) a heavy chain variable (VH) region comprising:
 - (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:53, 59, 64, 65, and 70;
 - (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:54, 60, 66, 71, and 76; and
 - (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:55, 61, 67, and 72;
- and
- (b) a light chain variable (VL) region comprising:
 - (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:56, 62, 68, and 73;
 - (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:57, 63, and 74; and
 - (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:58, 69, and 75.

11. The antibody or fragment thereof of claim 1 that binds to $\alpha 5\beta 1$ integrin and that comprises:

- (a) a heavy chain variable (VH) region comprising:

- (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:53, 59, 64, 65, and 70;
 - (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:79, 82, 84, 87, and 90; and
 - (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:80, 83, 85, and 88;
- and
- (b) a light chain variable (VL) region comprising:
 - (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:56, 62, 68, and 73;
 - (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:57, 63, and 74; and
 - (3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 86, and 89.

12. The antibody or fragment thereof of claim 1 that binds to $\alpha 5 \beta 1$ integrin and that comprises:

- (a) a heavy chain variable (VH) region comprising:
 - (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:93, 97, 100, 101, and 105;
 - (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:8, 14, 19, 24, and 28; and
 - (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:94, 98, 102, and 106;
- and
- (b) a light chain variable (VL) region comprising:
 - (1) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:95, 99, 103, and 107;
 - (2) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 11, and 22; and

(3) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:96, 104, and 108.

13. The antibody or fragment thereof of any one of claims 3-12, wherein the VH region or VL region further comprises human framework sequences.

14. The antibody or fragment thereof of claim 13, wherein the VH region and VL region further comprises human framework sequences.

15. The antibody or fragment thereof of any one of claims 3-12, wherein the VH region or VL region further comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and/or a framework 4 (FR4) sequence.

16. The antibody or fragment thereof of claim 15, wherein the VH region and VL region further comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and a framework 4 (FR4) sequence.

17. The antibody or fragment thereof of any one of claims 1-16, wherein the antibody is a monoclonal antibody.

18. The antibody or fragment thereof of claim 17, wherein the monoclonal antibody is a humanized, human or chimeric antibody.

19. The antibody or fragment thereof of any one of claims 1-18, which is a Fab, Fab', F(ab')₂, Fv, scFv, (scFv)₂, single chain antibody molecule, dual variable region antibody, single variable region antibody, linear antibody, V region, or a multispecific antibody formed from antibody fragments.

20. The antibody or fragment thereof of any one of claims 1-19, which is conjugated or recombinantly fused to a diagnostic agent, detectable agent or therapeutic agent.

21. The antibody or fragment thereof of claim 20, wherein the therapeutic agent is a chemotherapeutic agent, cytotoxin, or drug.

22. A binding agent that binds to essentially the same epitope as an antibody or fragment thereof of any one of claims 1-21.

23. The binding agent of claim 22, which is an antibody or fragment thereof.

24. The binding agent of claim 22, which comprises a non-antibody protein scaffold.

25. The binding agent of claim 24, wherein the non-antibody protein scaffold comprises a fibronectin scaffold, an anticalin, an adnectin, an affibody, a DARPin, a fynomer, an affitin, an affilin, an avimer, a cysteine-rich knottin peptide, or an engineered Kunitz-type inhibitor.

26. A binding agent that competes for binding to human $\alpha 5$ integrin with an antibody or fragment thereof of any one of claims 1-21.

27. The binding agent of claim 26, wherein the binding agent is an antibody or fragment thereof.

28. One or more vectors comprising one or more polynucleotides encoding the antibody or fragment thereof of any one of claims 1-21.

29. A host cell comprising one or more vectors of claim 28.

30. A composition that comprises the antibody or fragment thereof of any one of claims 1-21, and a pharmaceutically acceptable carrier.

31. A method for treating an $\alpha 5 \beta 1$ integrin-mediated disease, disorder or condition in a subject comprising administering to the subject the antibody or fragment thereof of any one of claims 1-21 or the composition of claim 30.

32. A method for alleviating one or more symptoms associated with an $\alpha 5 \beta 1$ integrin-mediated disease, disorder or condition in a subject comprising

administering to the subject the antibody or fragment thereof of any one of claims 1-21 or the composition of claim 30.

33. A method for treating a cancer or a tumor in a subject comprising administering to the subject the antibody or fragment thereof of any one of claims 1-21 or the composition of claim 30.

34. A method for alleviating one or more symptoms associated with a cancer or a tumor in a subject comprising administering to the subject the antibody or fragment thereof of any one of claims 1-21 or the pharmaceutical composition of claim 30.

35. A method for treating an angiogenesis-mediated disease, disorder, or condition in a subject comprising administering to the subject the antibody or fragment thereof of any one of claims 1-21 or the composition of claim 30.

36. A method for alleviating one or more symptoms associated with a cancer or a tumor in a subject comprising administering to the subject the antibody or fragment thereof of any one of claims 1-21 or the composition of claim 30.

37. A method for treating an inflammatory disease, disorder, or condition in a subject comprising administering to the subject the antibody or fragment thereof of any one of claims 1-21 or the pharmaceutical composition of claim 30.

38. A method for alleviating one or more symptoms associated with an inflammatory disease, disorder, or condition in a subject comprising administering to the subject the antibody or fragment thereof of any one of claims 1-21 or the composition of claim 30.

39. The method of any one of claims 31-38, wherein the subject is administered one or more therapeutic agents in combination with the antibody or fragment thereof or the pharmaceutical composition.

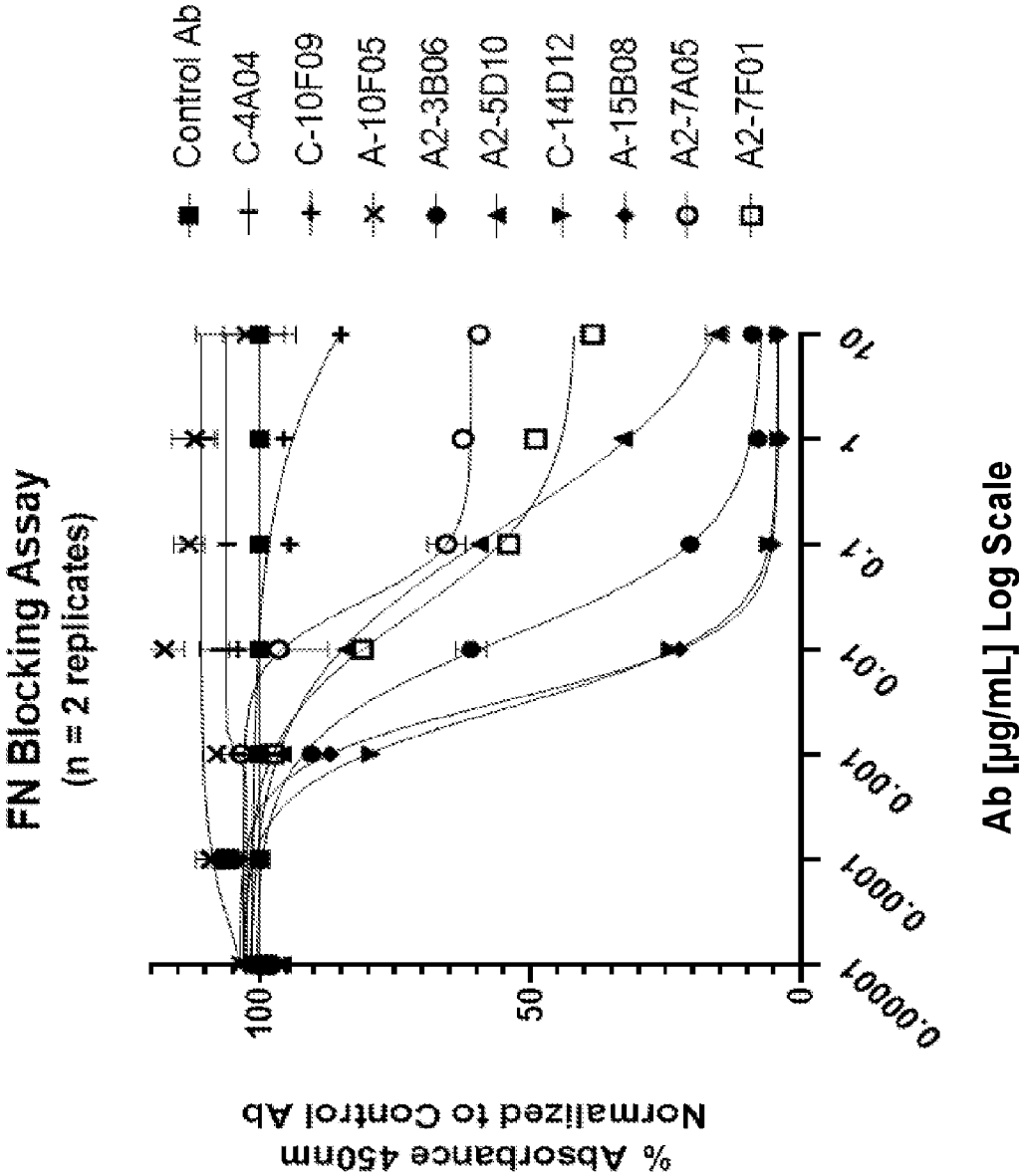


FIG. 1

VH Domain									
Kabat	1	10	22	31--35	40	50--a-----60----	65		
AbM	1	10	22	26-----ab---35	40	50--a-----58	65		
Chothia	1	10	22	26-----ab32	40	a-55	65		
Contact	1	10	22	30ab---35	40	47-----a-----58	65		
IMGT	1		23	27-----38	41	56-----65	74		
A-15B08	QVQLKESGPGLVAPSQSLITCTIS GFSLT--SYGVH WVRQPPGKGLEWLVIW-SDGSTTYNSTLKS								
A2-3B06	QVQLKESGPGLVAPSQSLITCTIS GFSLT--TYGVH WVRQPPGKGLEWLVIW-SDGSTTYNSALKS								
A2-5D10	QVQLKESGPGLVAPSQSLITCTIS GFSLT--SYGVH WVRQPPGKGLEWLVIW-SDGSTTYNSTLKS								
C-14D12	QVQLKESGPDLVAPSQSLITCTVS GFSLT--DYGVIWVRQPPGKGLEWLVIW-SDGSTTYNSALKS								
consensus	GFSLT--xYGVH (SEQ ID NO:120)								
A2-7A05	QVQLQQPGAELVKPGASVKLSCKAS GYTFT--IYWIN WVKQRPQGQGLEWIGKIYPGSISTDYNEKFKS								
A2-7F01	QVQLQQPGAELVKPGASVKLSCKAS GYTFT--IYWIN WVKQRPQGQGLEWIGNIYPGSSSTNYNEKFKT								
consensus	GYTFT--IYWIN xIYPGSxSTxYNEKFKx (SEQ ID NO:128)								

FIG. 2A

Kabat	70	80	abc	90	95	110	
AbM	70	80	abc	90	95	110	
Chothia	70	80	abc	90	96	110	
Contact	70	80	abc	90	93	110	
IMGT	75	89		105	117		
A-15B08	RLSISKDNSKSQVFFKMNSLQTTDDTAMYYCAR	HYDYDGDWF	-----AY	WGQGTLLVTVSA	(SEQ ID NO:25)		
A2-3B06	RLNITKDNSKSQVFLKMNSLQTTDDTAMYYCVR	HGGLLRDAM	-----DY	WGQGTSLTVSS	(SEQ ID NO:42)		
A2-5D10	RLSISKDNSKSQVFLKMNSLQTTDDTAMYYCAR	HYDYDGDWF	-----AY	WGQGTLLVTVSA	(SEQ ID NO:51)		
C-14D12	RLSITKDTSKRQVFLKMNSLQTTDDTAMYYCAR	HAPSFIRYGSRYDALDY		WGQGTSLTVSS	(SEQ ID NO:109)		
consensus	HxxxxxxxxxRYDALxY (SEQ ID NO:123)						
A2-7A05	KATLTVDTSSTAYMQLSSLTSDDSAVYYCAI	TGTGGL	-----AY	WGQGTLLVTVSA	(SEQ ID NO:77)		
A2-7F01	KATLTVDTSSTAYMQLSSLTSDDSAVYYCAI	TGTGGF	-----AY	WGQGTLLVTVSA	(SEQ ID NO:91)		
consensus	TGTGGx-----AY (SEQ ID NO:129)						

FIG. 2A (cont.)

VL Domain									
Kabat	1	10	20	24-27-----34	40	50-----56			
AbM	1	10	20	24-----30a--34	40	50-----56			
Chothia	1	10	20	26-----32	40	50--			
Contact	1	10	20	30a-----36	40	46-----55			
IMGT	1		23	27-----38	41	56-65	69		
A-15B08	QIVLTQSPAIMSASLGERVTMTCTASSRVSSNSLHWYQQKPGSSPKLWIYSTSNLAS								
A2-3B06	QIVLTQSPAIMSASLGERVTMTCTASSSVSSNSFHWYQQKPGSSPKLWLYSTSNLAS								
A2-5D10	QILLTQSPAIMSASLGERVTMTCTASSSVSSRCLHWYQQKPGSSPKLWIYSTSNLAS								
C-14D12	QIVLTQSPAIMSASLGERVTLTCTASSSVTSSFLHWYQQKPGSSPKLWIYSTSNLAS								
consensus	TASSxVxSxxxH								STSNLAS
	(SEQ ID NO:124)								(SEQ ID NO:125)
A2-7A05	ENVLTQSPAIMSASLGEKVTMTCTRASSVN--YMYWYQQKSDASPKLWIYFTSSLAP								
A2-7F01	ENVLTQSPAIMSASLGEKVTMSCRASSVN--YMYWYQQKSDASPKLWIYFTSSLAP								
consensus	RASSVN--YMY								FTSSLAP
	(SEQ ID NO:130)								(SEQ ID NO:131)

FIG. 2B

Kabat	60	70	80	89-----97
AbM	60	70	80	89-----97
Chothia	60	70	80	91---96
Contact	60	70	80	89-----96
IMGT	70	89		105-----117
A-15B08	GVPARFSGSGTSYSLTISSM EAEDAATYYC			HQYLRSPPT FGGGTKLEIK (SEQ ID NO:26)
A2-3B06	GVPARFSGSGTSYSLTISSM EAEDAATYYC			HQYLRSPPT FGGGTKLEIK (SEQ ID NO:43)
A2-5D10	GVPARFRGSGTSYSLTISSM EAEDAATYYC			HQYRSPPT FGGGTKLEIK (SEQ ID NO:52)
C-14D12	GVPARFSGSGTSYSLTISSM EAEDAATYYC			HQVHRSPPT FGGGTKLEIK (SEQ ID NO:110)
consensus	HQYxRSPPT			(SEQ ID NO:126)
A2-7A05	GVPGRFSGSGNSYSLTISTMEGEDAATYYC			QQFTTSPPT FSGGTKLEIK (SEQ ID NO:78)
A2-7F01	GVPTRFSGSGNSFSLSLTISSM EGEDAATYYC			QQLTGSPPT FSGGTRLEIK (SEQ ID NO:92)
consensus	QQxTxSPPT			(SEQ ID NO:132)

FIG. 2B (cont.)

VH Domain									
Kabat	1	10	22	31--35	40	50-----60----	65		
AbM	1	10	22	26-----35	40	50-----58	65		
Chothia	1	10	22	26-----32	40	-55	65		
Contact	1	10	22	30-----35	40	47-----58	65		
IMGT	1		23	27-----38	41	56-----65	74		
A-15B08-T62A	QVQLKESGPGGLVAPSQSLSIITCTIS GFSLTSYGVH WVRQPPGKGLEWLV VIWSDGSTTYSALKS								
A-15B08_HC_Low	QVTLKESGPTLVKPTQTLLTITCTIS GFSLTSYGVH WVRQPPGKGLEWLV VIWSDGSTTYSALKS								
A-15B08_HC_Low+Mod	QVTLKESGPTLVKPTQTLLTITCTIS GFSLTSYGVH WVRQPPGKALEWLV VIWSDGSTTYSALKS								
Kabat	70	80	abc	90	95-----102	110			
AbM	70	80	abc	90	95-----102	110			
Chothia	70	80	abc	90	96-----101	110			
Contact	70	80	abc	90	93-----101	110			
IMGT	75	89		105-----117					
A-15B08-T62A	RLSISKDNSKSQVFFKMNSLQTDVTAMYYCAR HYDYDGDWFAY WGQGLTVTVSA (SEQ ID NO:135)								
A-15B08_HC_Low	RLTISKDNSKSQVFFMTNLDVTDTAMYYCAR HYDYDGDWFAY WGQGLTVTVSS (SEQ ID NO:136)								
A-15B08_HC_Low+Mod	RLTISKDTSKNQVFFMTNLDVTDTATYYCAR HYDYDGDWFAY WGQGLTVTVSS (SEQ ID NO:138)								

FIG. 2C

VL Domain									
Kabat	1	10	20	24-27-----34	40	50-----56			
AbM	1	10	20	24-----30a--34	40	50-----56			
Chothia	1	10	20	26-----32	40	50--			
Contact	1	10	20	30a-----36	40	46-----55			
IMGt	1		23	27-----38	41	56-65	69		
A-15B08-T62A*									
A-15B08_LC_Low									
A-15B08_LC_Low+Mod									
Kabat	60	70	80	89-----97					
AbM	60	70	80	89-----97					
Chothia	60	70	80	91---96					
Contact	60	70	80	89-----96					
IMGt	70		89	105-----117					
A-15B08-T62A*									
A-15B08_LC_Low									
A-15B08_LC_Low+Mod									

*VL domain sequence is the same between A-15B08 and A-15B08-T62A

FIG. 2D

VH Domain									
Kabat	1	10	22	31--35	40	50--a-----60----	65		
AbM	1	10	22	26-----35	40	50--a-----58	65		
Chothia	1	10	22	26-----32	40	a-55	65		
Contact	1	10	22	30-----35	40	47-----a-----58	65		
IMGT	1		23	27-----38	41	56-----65	74		
A2-7A05_HC	QVQLQQPGAELVKPGASVKLSCKAS GYTFTHYWIN WVKQRPQGLEWIG KIYPGSISTDYNEKFKS								
A2-7A05_HC_Low	QVQLVQPGAELVKPGASVKLSCKAS GYTFTHYWIN WVKQAPGGLEWIG KIYPGSISTDYNEKFKG								
A2-7A05_HC_Low+Mod	QVQLVQPGAELVKPGASVKLSCKAS GYTFTHYWIN WVRQAPGGLEWIG KIYPGSISTDYNNQKFQG								
Kabat	70	80	abc	90	95-----102	110			
AbM	70	80	abc	90	95-----102	110			
Chothia	70	80	abc	90	96--101	110			
Contact	70	80	abc	90	93-----101	110			
IMGT	75	89		105-----117					
A2-7A05_HC	KATLTVDTSSTAYMQLSSLTSDDSAVYYCAI TGTGGLAY WGQGTLLTVSA (SEQ ID NO:77)								
A2-7A05_HC_Low	KATLTVDTSSTAYMELSSLRSEDTAVYYCAI TGTGGLAY WGQGTLLTVSS (SEQ ID NO:140)								
A2-7A05_HC_Low+Mod	RATLTVDTSSTAYMELSSLRSEDTAVYYCAI TGTGGLAY WGQGTLLTVSS (SEQ ID NO:142)								

FIG. 2E

VL Domain									
Kabat	1	10	20	24-27----	34	40	50-----	56	
AbM	1	10	20	24-----	30-34	40	50-----	56	
Chothia	1	10	20	26----	32	40	50--		
Contact	1	10	20	30-----	36	40	46-----	55	
IMGT	1		23	27--	38	41	56-65	69	
A2-7A05_LC	ENVLTQSPALMSASLGKVTMTC RASSSVNYMY WYQKSDASPRLWIY FTSSLAP								
A2-7A05_LC_Low	ENVLTQSPGIMASLGERVTMSC RASSSVNYMY WYQKSGQSPRLWIY FTSSRAP								
A2-7A05_LC_Low+Mod	ENVLTQSPGILSASLGERVTMSC RASSSVNYMY WYQKSGQAPRLWIY FTSSRAP								
Kabat	60	70	80	89-----	97				
AbM	60	70	80	89-----	97				
Chothia	60	70	80	91----	96				
Contact	60	70	80	89-----	96				
IMGT	70	89		105-----	117				
A2-7A05_LC	GVPGRFSGSGSGNSYSLTISTMEGEDAATYYC QQFTTSPFT FGSGTKLEIK (SEQ ID NO:78)								
A2-7A05_LC_Low	GVPDRFSGSGSGTSYTLTISRMEGEDFATYYC QQFTTSPFT FGQGTKLEIK (SEQ ID NO:141)								
A2-7A05_LC_Low+Mod	GVPDRFSGSGSGTSYTLTISRMEGEDFATYYC QQFTTSPFT FGQGTKLEIK (SEQ ID NO:143)								

FIG. 2F

VH Domain									
Kabat	1	10	22	31--35	40	50-----60----	65		
AbM	1	10	22	26-----35	40	50-----58	65		
Chothia	1	10	22	26-----32	40	-55	65		
Contact	1	10	22	30-----35	40	47-----58	65		
IMGT	1		23	27-----38	41	56-----65	74		
C-14D12	QVQLKESGPDLLVAPSQSLSIITCTVS GFSLTDYGVH WVRQPPGKGLEWLV VIWSDGSTTYNSALKS								
C-14D12_HC_Low	QVTLKESGPTLVKPTQTLLTITCTVS GFSLTDYGVH WVRQPPGKGLEWLV VIWSDGSTTYNSALKS								
C-14D12_HC_Low+Mod	QVTLKESGPTLVKPTQTLLTITCTVS GFSLTDYGVH WVRQPPGKALEWLV VIWSDGSTTYNSSLKS								
Kabat	70	80	abc	90	95-----102	110			
AbM	70	80	abc	90	95-----102	110			
Chothia	70	80	abc	90	96-----101	110			
Contact	70	80	abc	90	93-----101	110			
IMGT	75	89		105-----117					10/21
C-14D12	RLSITKDTSKRQVFLKMNSLQTDTTAMYYCAR HAPSFIRYGSRYDALDY WGQGTSTTVSS (SEQ ID NO:109)								
C-14D12_HC_Low	RLTISKDTSKRQVLLTMTNLDTVDTAMYYCAR HAPSFIRYGSRYDALDY WGQGTLLVTVSS (SEQ ID NO:144)								
C-14D12_HC_Low+Mod	RLTISKDTSKNQVLLTMTNLDTVDTATYYCAR HAPSFIRYGSRYDALDY WGQGTLLVTVSS (SEQ ID NO:146)								

FIG. 2G

VL Domain									
Kabat	1	10	20	24-27-----34	40	50-----56			
AbM	1	10	20	24-----30a--34	40	50-----56			
Chothia	1	10	20	26-----32	40	50--			
Contact	1	10	20	30a-----36	40	46-----55			
IMGT	1		23	27-----38	41	56-65	69		
C-14D12	QIVLTQSPALMSASLGERVTLTC TASSSVTSSFLH WYQKPGSSPKLWIY STSNLAS								
C-14D12_LC_Low	EIVLTQSPGTMSASLGERVTLSC RASSSVTSSFLH WYQKPGQSPKLWIY STSNRAS								
C-14D12_LC_Low+Mod	EIVLTQSPGTLSASLGERVTLSC RASSSVTSSFLH WYQKPGQAPRLWIY STSNRAT								
Kabat	60	70	80	89-----97					
AbM	60	70	80	89-----97					
Chothia	60	70	80	91---96					
Contact	60	70	80	89-----96					
IMGT	70		89	105-----117					
C-14D12	GVPARFSGSGTSYSLTISSEAEADAATYYC HQYHRSPPT FGGGTKLEIK (SEQ ID NO:110)								
C-14D12_LC_Low	GVPDFSGSGGTDYTLTISRMEAEAFATYYC HQYHRSPPT FGQGTKLEIK (SEQ ID NO:145)								
C-14D12_LC_Low+Mod	GVPDFSGSGGTDYTLTISRMEAEAFATYYC HQYHRSPPT FGQGTKLEIK (SEQ ID NO:147)								

FIG. 2H

290	*	300	*	310	*	320	*	330	*	340	*
-----	---	-----	---	-----	---	-----	---	-----	---	-----	---

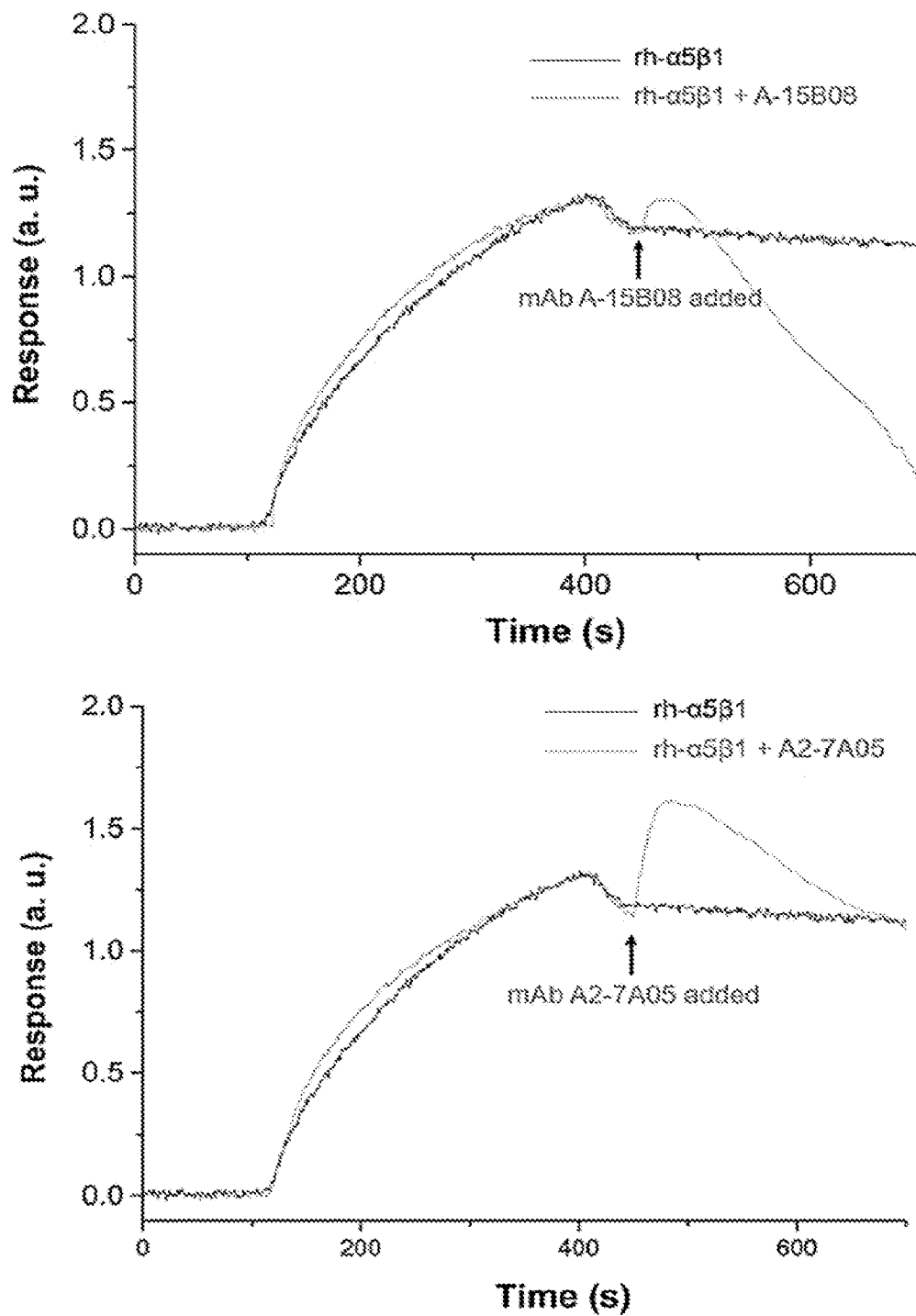
290	*	300	*	310	*	320	*	330	*	340	*
-----	---	-----	---	-----	---	-----	---	-----	---	-----	---

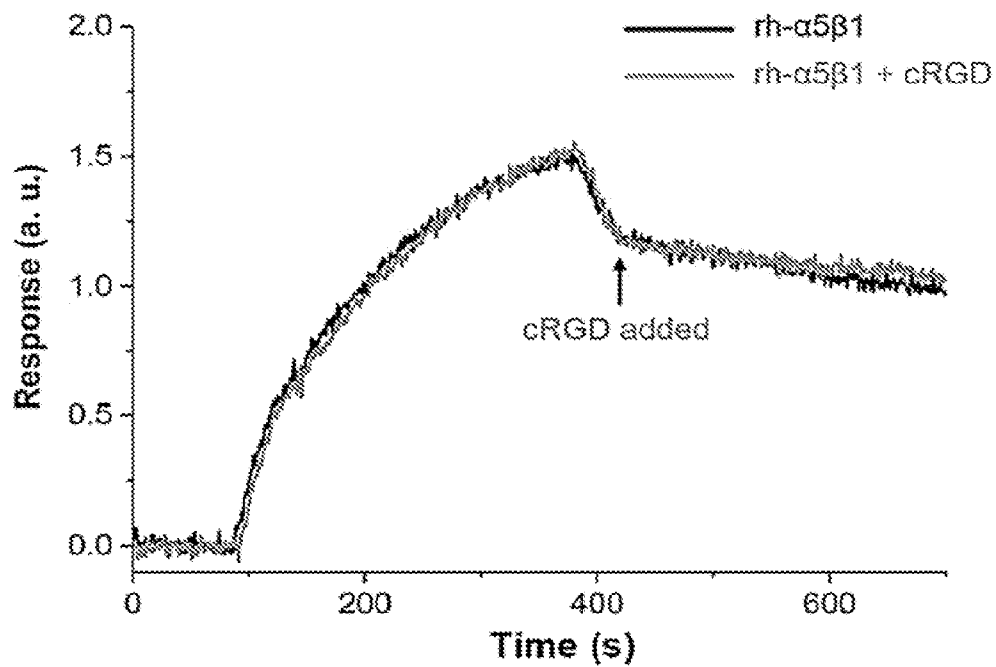
FIG. 3

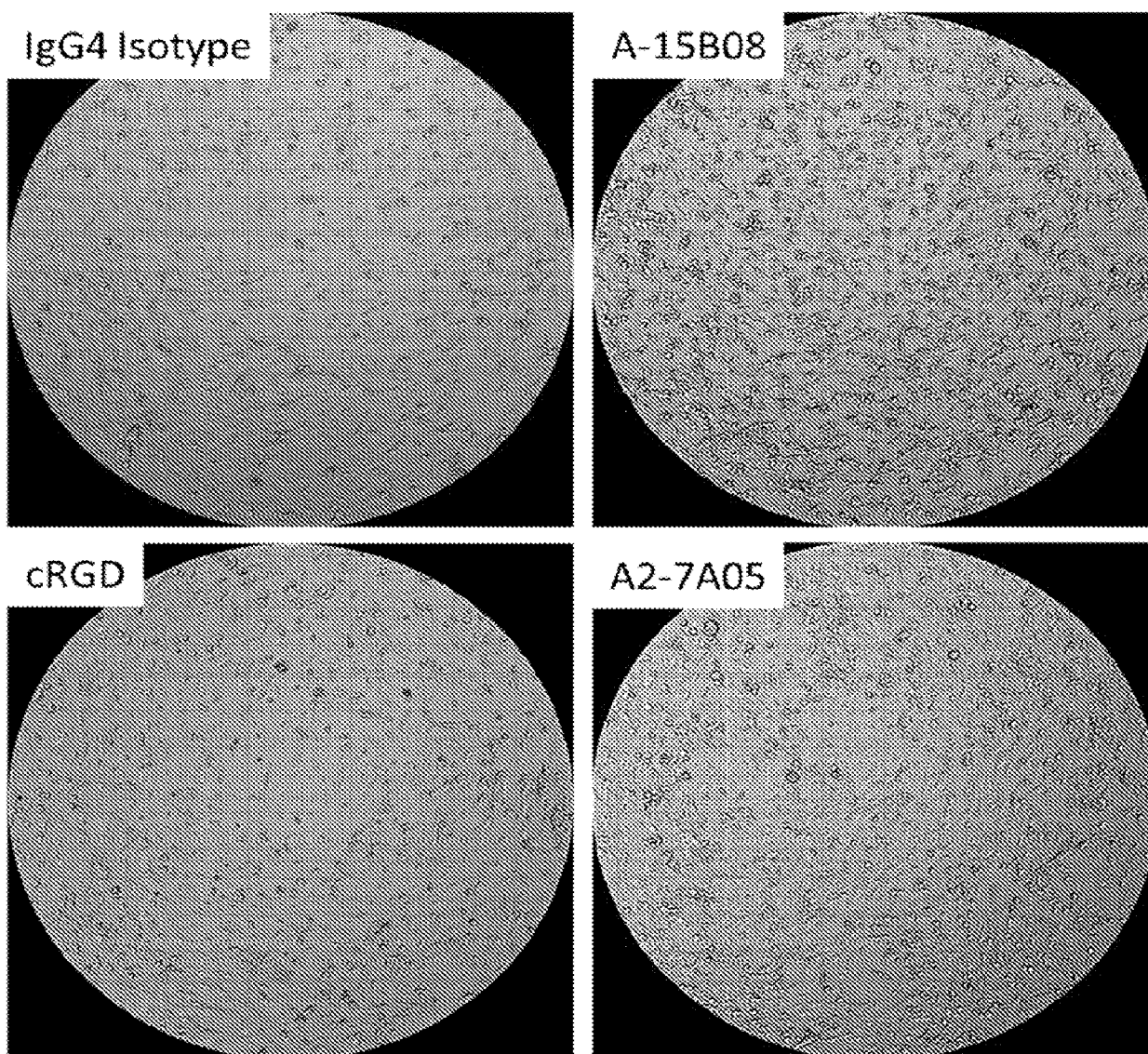
	350	360	370	380	390	400
	*	*	*	*	*	*
IgG1	PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFF					
IgG4	PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFF					
N297A/Q	PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFF					
LALA	PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFF					
LALAPS	PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFF					
LALAPG	PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFF					
TM	PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFF					
	410	420	430	440	447	
	*	*	*	*	*	
IgG1	LYSKLTVDKSRWQQQGNVVFSCVMHEALHNHYTQKSLSLSPGK	(SEQ ID NO:148)				
IgG4	LYSRLTVDKSRWQEGNVVFSCVMHEALHNHYTQKSLSLSPGK	(SEQ ID NO:149)				
N297A/Q	LYSKLTVDKSRWQQQGNVVFSCVMHEALHNHYTQKSLSLSPGK	(SEQ ID NO:150)				
LALA	LYSKLTVDKSRWQQQGNVVFSCVMHEALHNHYTQKSLSLSPGK	(SEQ ID NO:151)				
LALAPS	LYSKLTVDKSRWQQQGNVVFSCVMHEALHNHYTQKSLSLSPGK	(SEQ ID NO:152)				
LALAPG	LYSKLTVDKSRWQQQGNVVFSCVMHEALHNHYTQKSLSLSPGK	(SEQ ID NO:153)				
TM	LYSKLTVDKSRWQQQGNVVFSCVMHEALHNHYTQKSLSLSPGK	(SEQ ID NO:154)				

FIG. 3 (cont.)

14/21

**FIG. 4A**

**FIG. 4A (cont.)**

**FIG. 4B**

$\alpha 5\beta 1$ Integrin FN Blocking Assay
4°C vs. 3x Freeze/Thaw -80°C
(n = 2 replicates)

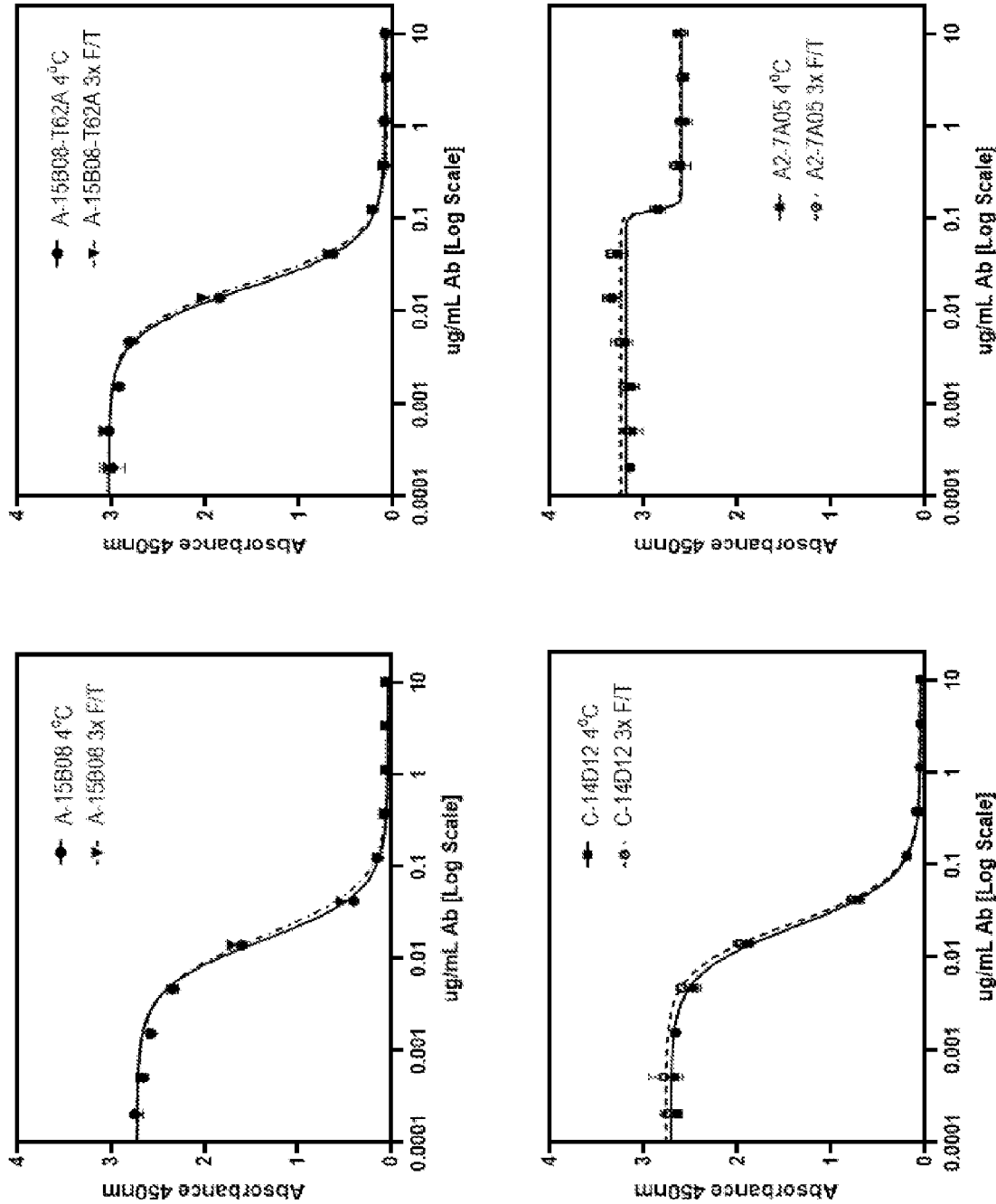
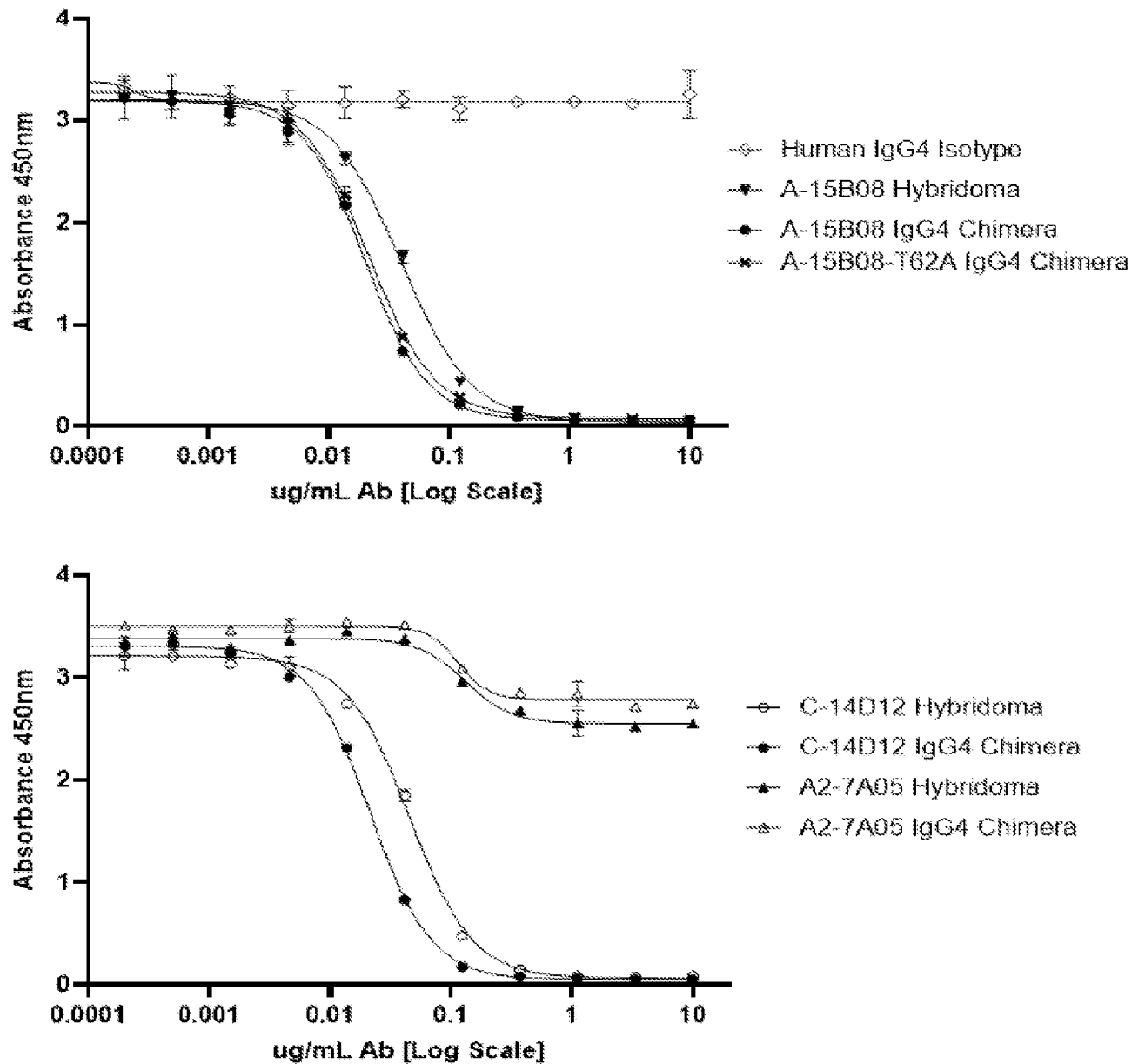


FIG. 5

Potency of Hybridomas vs. IgG4 Chimeras

**FIG. 6**

SDS-PAGE of IgG4 Chimeric Antibodies

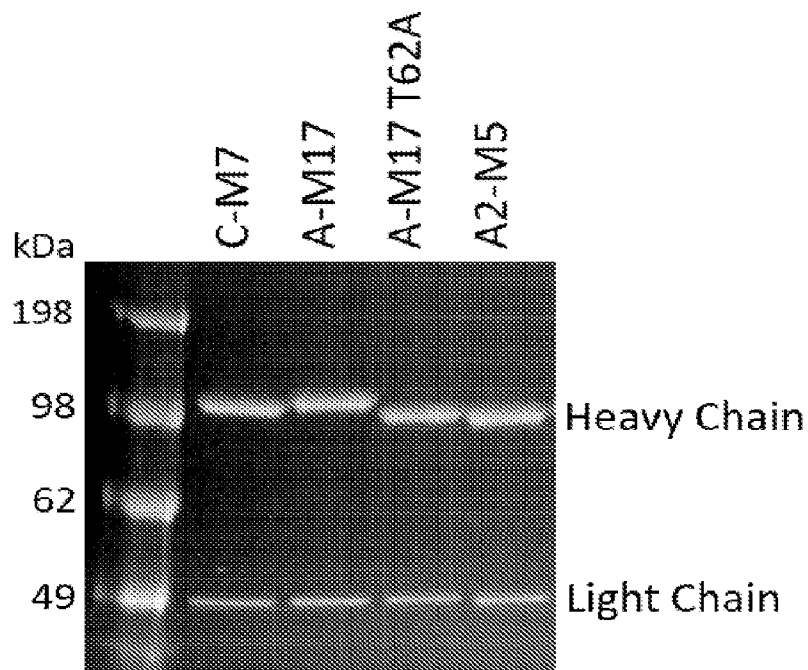


FIG. 7

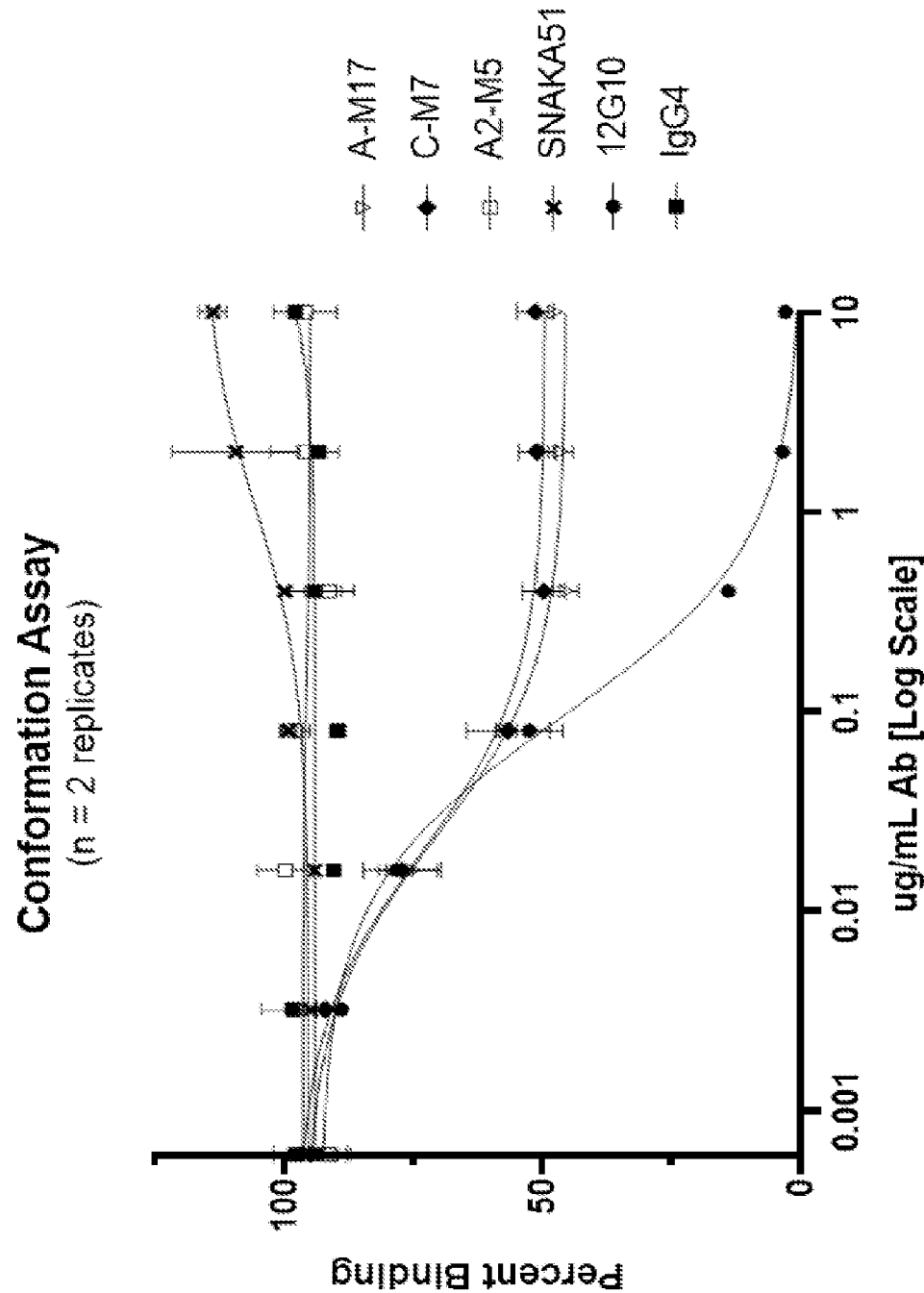


FIG. 8

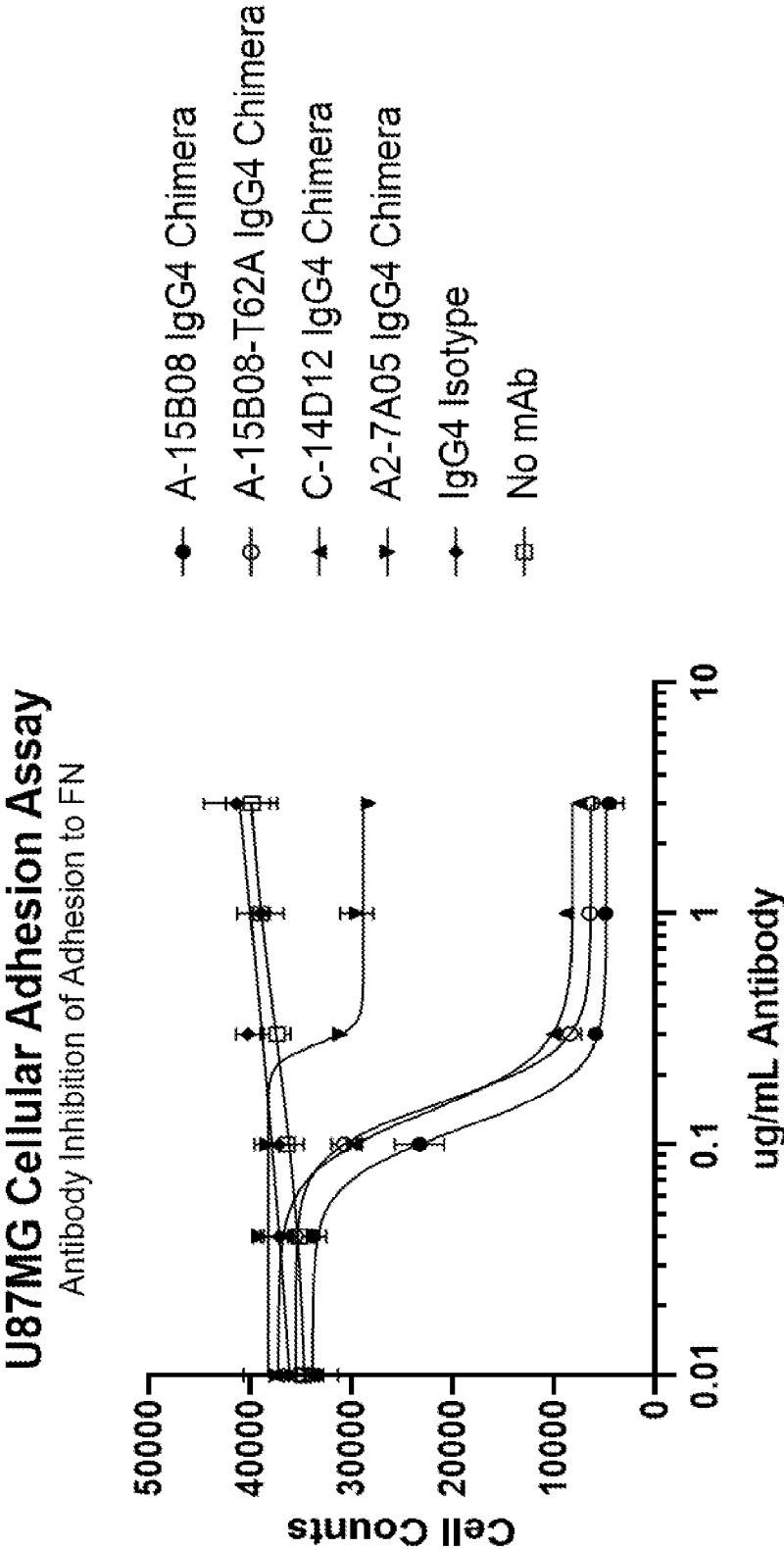


FIG. 9

FN Blocking Assay

(n = 2 replicates)

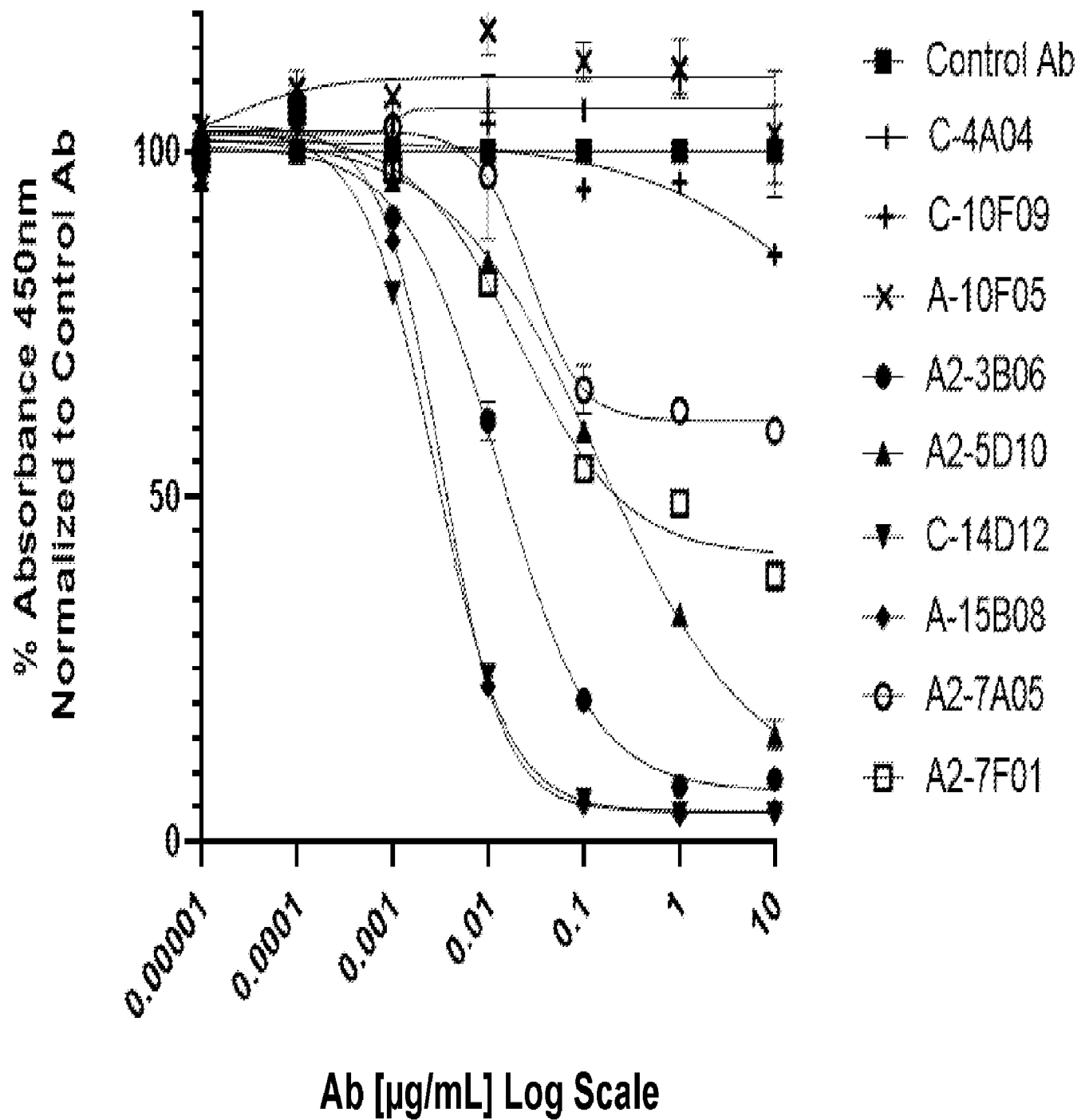


FIG. 1