Abstract:

Title: LRP-8 BINDING PROTEINS

(57) Abstract: The disclosure provides binding proteins that specifically bind to LRP-8 and optionally cross the blood brain barrier (BBB), localize to the brain and/or localize to the spinal cord.
LRP-8 BINDING PROTEINS

This application claims priority to U.S. Provisional Application Serial No. 62/090,878, filed December 11, 2014, which is hereby incorporated by reference in its entirety.

FIELD

The present disclosure provides LRP-8 binding proteins, including those that can participate in endocytosis, transcytosis, and/or crossing the blood brain barrier (BBB).

BACKGROUND

Low density lipoprotein receptor-related protein 8 (LRP-8) (also called ApoER2) is a cell surface receptor and is a member of the LDL receptor family. LRP-8 is abundant in the brain and placenta. LRP-8 ligands include reelin, ApoE, selenoprotein P and endoplasmic reticulum resident receptor-associated protein (RAP). LRP-8 plays a role in endocytosis, transcytosis and signal transduction, in particular in embryonic neuronal migration and postnatal long-term potentiation. LRP-8 isoform 1 consists of 963 amino acids and is separated into a ligand binding domain of eight ligand binding regions (Accession No: Q14114.4). While all LDL receptor family proteins consist of a cytoplasmic tail, LRP-8 is unique in that it contains a proline-rich 59 amino acid insert encoded by the alternatively spliced exon 19. This insert allows for protein interactions that are unable to occur with other LDL receptors and also diminishes the function of LRP-8 in lipoprotein endocytosis (Myant (2010) Proc. Biol. Sci. 277(1680): 345-51).

Through interactions with one of its ligands, reelin, LRP-8 also plays a critical role in the migration of neurons during development. Another LDL family member, VLDLR, also interacts with reelin, and together these two receptors modulate brain development and function. LRP-8 also functions as a receptor for the cholesterol transport protein apolipoprotein E. Decreased expression of LRP-8 is thus associated with certain neurological diseases. For instance, studies show that manipulation of LRP-8 can lead to Alzheimer's disease. A decrease in LRP8 expression is observed in patients with Alzheimer's disease. LRP-8 synthesis can lead to increased gamma secretase activity, a protease which cleaves LRP-8 as well as amyloid precursor protein (APP) into amyloid β (Aβ), resulting in degrading products that control the expression
of a tau protein, which ultimately leads to Alzheimer's disease (Carter (2007) Neurochem Int. 50(1): 12-38). LRP-8 activity has also been linked to antiphospholipid syndrome and major depressive disorder (MDD).

The blood-brain barrier (BBB) is a highly selective permeability barrier formed by brain endothelial cells that separates circulating blood from the brain extracellular fluid. It acts to effectively protect the brain from many common bacterial infections. While the BBB allows for the passage of water, some gases, and selective molecules, the BBB severely limits the penetration of large molecule drugs into the brain. Antibodies are generally too large to cross the BBB, and only certain antibiotics are able to cross. In some cases, a drug must be administered directly into the cerebrospinal fluid. However, drugs delivered directly to the cerebrospinal fluid often do not effectively penetrate into the brain tissue itself.

Several mechanisms have been developed for drug targeting in the brain that involve going "through" or "behind" the BBB. One of the strategies devised to overcome this obstacle includes utilizing transcytosis trafficking pathways of endogenous receptors expressed at the brain capillary endothelium. Recombinant proteins, such as monoclonal antibodies, have been designed against these receptors to enable receptor-mediated drug delivery. Recent studies suggest that antibodies with low affinity to BBB receptors, such as the transferrin receptor (TfR), offer the potential to substantially increase BBB transport and CNS retention of associated therapeutics. Moieties/molecules compared with high-affinity antibodies. These low affinity antibodies maximize brain uptake while minimizing reverse transcytosis back to the blood and also maximize the extent of accumulation after therapeutic dosing (Atwal et al. (2011) Sci. Transl. Med. 3: 84ra43; Yu et al. (2011) Sci. Transl. Med. 3(84): 84ra44).

Yet, the safety of administering such antibodies and conjugates is not well known.

Other BBB receptors used for binding an antibody to mediate transport across the BBB include the insulin receptor, insulin-like growth factor receptor (IGF receptor), LRP-8, low density lipoprotein receptor-related protein 1 (LRP1), glucose transporter 1 (Glut1) and heparin-binding epidermal growth factor-like growth factor (HB-EGF).

Antibodies and binding proteins that target some of these receptors and transport therapeutic agents across the BBB have been proposed.

Moreover, engineered proteins, such as antibodies, fragments, and multispecific binding proteins capable of binding two or more antigens, are known in the art. Such
multispecific binding proteins can be generated using cell fusion, chemical conjugation, or recombinant DNA techniques. There are a variety of multispecific binding protein structures known in the art and many structures and methods have distinct advantages or disadvantages.

Bispecific antibodies have been produced, for instance, using quadroma technology. Bispecific antibodies can also be produced by chemical conjugation of two different mAbs. Other approaches include coupling of two parental antibodies with a hetero-bifunctional crosslinker, production of tandem single-chain Fv molecules, diabodies, bispecific diabodies, single-chain diabodies, and di-diabodies. In addition, a multivalent antibody construct comprising two Fab repeats in the heavy chain of an IgG and capable of binding four antigen molecules has been described (see PCT Publication No. WO 01/77342 and Miller et al. (2003) J. Immunol. 170(9):4854-61).

US Patent No. 7,612,181 (incorporated herein by reference in its entirety) provides a novel family of binding proteins capable of binding two or more antigens with high affinity, which are called dual variable domain binding proteins (DVD-Ig binding protein) or dual variable domain immunoglobulins (DVD-Ig). DVD-Ig molecules are binding proteins that may be used to bind two distinct epitopes on the same molecule or two different molecules simultaneously. DVD-Ig molecules are unique binding proteins comprised of two variable domains fused to N-terminal constant regions. The variable domains may be directly fused to one another or connected via synthetic peptide linkers of assorted length and amino acid composition. DVD-Ig binding proteins may be engineered with intact and functional Fc domains, or otherwise modified constant domains, allowing them to mediate appropriate effector functions and exhibit other desired properties. The DVD-Ig format, due to its flexibility of choice of variable domain pair, orientation of two antigen-binding domains, and the length of the linker that joins them, may provide novel therapeutic modalities.

There remains a need for constructs, including multispecific constructs, exhibiting better targeting, efficiency, and/or efficacy in binding to LRP-8, and/or improved transport and delivery of therapeutic agents across the blood brain barrier. Improved targeting of LRP-8 may lead to improvements in, e.g., preventing, diagnosing, and/or treating disorders such as brain disorders, neurological diseases, and/or brain cancers. Also, while a variety of structures have been provided in the art,
with various advantages and disadvantages, new variable domain sequences can further improve the properties of binding proteins targeting LRP-8, or their cognate receptors.

**SUMMARY**

This disclosure provides proteins that bind LRP-8. Binding proteins of the disclosure include but are not limited to antibodies, antigen binding portions thereof, and multivalent and/or multispecific binding proteins such as dual variable domain immunoglobulin (DVD-Ig) binding proteins that can bind LRP-8. The disclosure also provides methods and compositions for targeting an LRP-8 binding protein to the brain and/or spinal cord and/or across the blood brain barrier (BBB), as well as pharmaceutically relevant animal models.

In one aspect, the disclosure provides a binding protein that specifically binds to human LRP-8. In certain aspects, the disclosure provides a binding protein that specifically binds to human and/or cynomolgus LRP-8. In certain aspects, the binding proteins bind LRP-8 expressed on brain vascular endothelium of a subject and facilitate uptake of a composition into the brain of the subject.

In certain embodiments, the binding protein also specifically binds to mouse and/or rat LRP-8. In certain aspects the binding protein undergoes endocytosis into HEK293 cells expressing LRP-8 at a rate between 1.5 and 2.5 times the endocytosis of control IgG into HEK293 cells expressing LRP-8. In other embodiments, the binding protein undergoes transcytosis across a Caco-2 cell monolayer expressing LRP-8 at a rate between 1.5 and 2.0 times the transcytosis of control IgG across a Caco-2 cell monolayer.

In certain embodiments, the binding protein specifically binds to residues 33-622 of human LRP-8 isoform 3. In certain embodiments, the binding protein specifically binds to one or both of an amino acid sequence comprising the sequence of CR1 (SEQ ID NO:2) and an amino acid sequence comprising the sequence of CR2 (SEQ ID NO:3).

In certain embodiments, disclosed herein are binding proteins comprising first and second polypeptide chains forming a binding domain for LRP-8, wherein each polypeptide chain comprises 1, 2, or 3 CDRs from, or at least 80% homology to, a VH or VL sequence listed in any one of Tables 2-7. In some embodiments, a binding protein comprises a first polypeptide chain comprising three CDRs from a VH sequence...
listed in any one of Tables 2-7, and a second polypeptide chain comprising three CDRs from the corresponding VL sequence listed in any one of Tables 2-7. In some embodiments, a binding protein comprises a first polypeptide chain comprising a VH sequence listed in any one of Tables 2-7, and a second polypeptide chain comprising the corresponding VL sequence listed in any one of Tables 2-7.

In certain embodiments, the LRP-8 binding protein comprises six CDRs: CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, as defined below:

CDR-H1: RFTFSNX1GMS, wherein X1 is F or Y;
CDR-H2: TISSGGRYTYYPDX2VKG, wherein X2 is S or H;
CDR-H3: DYLYAMDY;
CDR-L1: RSSQSLVYSX3X4NTYLH, wherein X3 is N, T, R, W or P, and wherein X4 is G, E, L or K;
CDR-L2: KVSNRFS; and
CDR-L3: SQSTHVPLT.

In certain embodiments, the LRP-8 binding protein comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises the sequence of

EVQLVESGGGLVKPGGLSKLCAASRFTFTSNFGMSWVRQTPDKRLEWVATISS
GGRTYYPDX1VKGRFTISRDNAKNTLYLQMSSLRSEDATAMYCARDLYAM

or

EVQLVESGGGLVKPGGLSKLCAASRFTFTSNYGMSWVRQTPDKRLEWVATIS
SGGRTYYPDX1VKGRFTISRDNAKNTLYLQMSSLRSEDATAMYCARDLYAM

wherein X1 is S or H, and

wherein the light chain variable domain comprises the sequence of

DVVMQTQTPLSLPVLSDLQASISCRSSLVYXS2X3NTYLYHLWQLKPQG
SPKVLMYKVSNRFSGVSDRFSGSGSHTDLKISRVEAEDLVYFCSSQSTHVPL
TFGAGTKLELK,

wherein X2 is N, T, R, W or P and X3 is G, E, L or K.

In certain embodiments, the LRP-8 binding protein comprises six CDRs: CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, as defined below:

CDR-H1: GFTVSDYYMA;
CDR-H2: SISYEGSSTYYGDSVKG;
In certain embodiments, the LRP-8 binding protein comprises a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises the sequence of
EVQLVESGGGLVQPGSRSLKLSCAASGFTVSDYMYMAWVRQAPKKGLLEWASIS
YEGSSTYYGDSVKGRFTISRDNAKSILYLQMNSLRSEDTATYYCARPLRYYGY
NYRFAYWGQGTTLVTSS and wherein the light chain variable domain comprises the sequence of
DIQMSQSPPLSASVGDRVTLSCKASQNIHKNLDDWQQKHEAPKLLIYYTDN
LQTGIPSFGSGSGTDYTLTISSLQPEDVATYYCYQYNQPTFGAGTKLELQ,
wherein the bold portions are CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and
CDR-L3, respectively.

In another aspect, the disclosure provides dual variable domain (DVD-Ig) binding proteins that specifically bind to LRP-8. In certain aspects, the DVD-Ig binding proteins bind LRP-8 expressed on brain vascular endothelium of a subject and facilitate uptake of a composition into the brain of the subject.

In an embodiment, the LRP-8 binding protein comprises a heavy chain polypeptide, wherein the polypeptide comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain; VD2 is a second heavy chain variable domain; C is a heavy chain constant domain; X1 is a linker with the proviso that it is not CH1; X2 is an Fc region; and n is independently 0 or 1. In some embodiments, the VD1 and/or VD2 comprises three CDRs: CDR-H1, CDR-H2, CDR-H3, as defined below:
CDR-H1: RFTFSNX1GMS, wherein X1 is F or Y or GFTVSDYMA; CDR-H2:
TISSGGRYTTYPDX2VKG, wherein X2 is S or H or SISYEGSTTYGDSVKG; and
CDR-H3: DYLYAMDY or PLRYYGNYRFAY.

In some embodiments, VD1 and/or VD2 on the heavy chain polypeptide comprises an amino acid sequence of
EVQLVESGGGLVKPGSQLKLSCAASRFTSFNFGMSWVRQTPDKRLEWVATISS
GGRYTTYPDXIVKGRFTISRDNAKNTLYLQMSSLRSEDAMYYCARDYLYAM
DYWGQGTSVTSS, or
EVQLVESGGDLVKPGGSLKLSRAFSNYSGLMPSWVRQTPSKRLEWVATIS
SGGRTYLPDPXIVKGRFTISRDNAKNTLYLQMSSLRSEDTAMYCARDYLYA
MDYWGQGTSVTSS,

wherein \( X_1 \) is R or S; or

EVQLVESGGGLVQPGSRLKLCAASGFTVSDY YMAWVRQAPKKGLEYV AVASIS
YEGSSTYYGDSDKGFTISRDNAKSILYLQMSSLRSEDTATYYCARPLRLYGY
NYRFAYWGQGLTVTSS, wherein the CDRs are bolded.

In some embodiments, \( \text{VD1} \) and/or \( \text{VD2} \) on the heavy chain polypeptide
comprises an amino acid sequence of

EVQLVESGGLVQPGSRLKLCAASGFTVSDY YMAWVRQAPKKGLEYV AVASIS
YEGSSTYYGDSDKGFTISRDNAKSILYLQMSSLRSEDTATYYCARPLRLYGY
NYRFAYWGQGLTVTSS,

or

EVQLVESGGGLVQPGSRLKLCAASGFTVSDY YMAWVRQAPKKGLEYV AVASIS
YEGSSTYYGDSDKGFTISRDNAKSILYLQMSSLRSEDTATYYCARPLRLYGY
NYRFAYWGQGLTVTSS, wherein the CDRs are bolded.

In some embodiments, the \( \text{LRP-8} \) binding protein comprises a light chain
polypeptide, wherein the polypeptide comprises \( \text{VD1}-(\text{X}1)n-\text{VD2}-\text{C} \), where \( \text{VD1} \) is a
first light chain variable domain; \( \text{VD2} \) is a second light chain variable domain; \( \text{C} \) is a
light chain constant domain; \( \text{X}1 \) is a linker with the proviso that it is not CL; and \( n \) is 0
or 1. In some embodiments, the polypeptide chain does not comprise an Fc region. In
some embodiments, the \( \text{VD1} \) and/or \( \text{VD2} \) comprises three CDRs: CDR-L1, CDR-L2,
CDR-L3, as defined below: CDR-L1: RSSQSLVYXSX3X4NYTLH, wherein \( X3 \) is N, T,
R, W or P, and wherein \( X4 \) is G, E, L or K or KASQNIHNL; CDR-L2: KVSNRFSP or
YTDNLQ; and CDR-L3: SQSTHVPIL or YQYNSGPT.

In another embodiment, \( \text{VD1} \) and/or \( \text{VD2} \) on the heavy chain polypeptide
comprises an amino acid sequence of

DVVM TQTPLSLPVSLGDQASISCRSSQ SLVYSX2X3NTY HLWYQLQKPGPSKVL
MYKVSNNRFSGVSDRFSGSSTDFTLKISRVEAEDLGFFCSQSTHVPLTFGA
GTKLELK,

wherein \( X2 \) is N, T, R, W or P and \( X3 \) is G, E, L or K; or

DIQMSQSPPVLSASVGDRVSLCKASQNIHNLWYQQKHGEAPKLIYYTDL
LQTGISRFSGSGTDTYTLTSSLDPEDVATYYCYQYNPSPTFGAGTKLELQ,

wherein the CDRs are bolded.
In another embodiment, VD1 and/or VD2 on the light chain polypeptide comprises an amino acid sequence of

DVVMTQTPLSLPVSLGDQASICRSSLVYSNGNTLYLHWYLMKPGQSPKVLM
YKVSNRFSGVSDRFGGSGGDFTLISKVAEADLGYYFCQSTHVPLTFGAGT
KLELK, wherein the CDRs are bolded.

In an embodiment, the binding proteins disclosed herein comprise a heavy chain polypeptide as discussed above and a light chain polypeptide as discussed above.

In certain embodiments, the LRP-8 binding protein comprises first and second polypeptide chain, wherein each polypeptide chain comprises VD1-(X1)n-VD2-(X2)n, wherein VD1 is a first variable domain; VD2 is a second variable domain; C is a constant domain; X1 is a linker; X2 is an Fc region on the first polypeptide chain and X2 is absent on the second polypeptide chain (i.e., n is 0 for (X2)n on the second chain); n is independently 0 or 1 on the first and second chains; wherein the VD1 domains on the first and second polypeptide chains form a first functional target binding site; and wherein the VD2 domains on the first and second polypeptide chains form a second functional target binding site. In some embodiments, the antigen target for the binding site formed by the VD1 and/or VD2 domains on the first and second polypeptide chains is LRP-8. In some embodiments, the VD1 and/or VD2 domains on the first and second polypeptide chains comprise the CDRs and/or variable domains from clone ML199.1 1H1.5B2. In some embodiments, the VD1 and/or VD2 domains on the first and second polypeptide chains comprise the CDRs and/or variable domains from clone BGK-2C8.8C. In some embodiments, the VD1 and/or VD2 domains on the first and second polypeptide chains comprise the CDRs and/or variable domains from clone ML201-8F3.3D7. In some embodiments, the VD1 and/or VD2 domains on the first and second polypeptide chains comprise the CDRs and/or variable domains from clone BGK.9D10-2. In some embodiments, the VD1 and/or VD2 domains on the first and second polypeptide chains comprise the CDRs and/or variable domains from clone CL-105967. In some embodiments, the VD1 and/or VD2 domains on the first polypeptide chain comprise a CDR-H1 of RFTFSNX1GMS, wherein X1 is F or Y or GFTVSDYYYY; a CDR-H2 of TISGGGRYYYPDX2VKG, wherein X2 is S or H or SISYEFSSTYYGDSVKG; and a CDR-H3 of DYLYAMDY or PLRYYGNYRFAY; and the VD1 and/or VD2 domains on the second polypeptide chain comprise a CDR-L1 of RSSQSLVYSX3X4NTYLY, wherein X3 is N, T, R, W or P, and wherein X4 is G, E,
L or K or KASQNIHKNLD; a CDR-L2 of KVSNRFS or YTDNLQT; and a CDR-L3 of SQSTHVPLT or YQYNSGPT.

In certain embodiments, the LRP-8 binding protein comprises heavy chain polypeptide and a light chain polypeptide, wherein the heavy chain polypeptide comprises VDI-(X1)n-VD2-C-(X2)n, wherein VDI is a first heavy chain variable domain; VD2 is a second heavy chain variable domain; C is a heavy chain constant domain; X1 is a linker with the proviso that it is not CHI; X2 is an Fc region; n is independently 0 or 1; and wherein the light chain polypeptide comprises VDI-(X1)n-VD2-C, wherein VDI is a first light chain variable domain; VD2 is a second light chain variable domain; C is a light chain constant domain; X1 is a linker with a proviso that it is not CL; X2 is an Fc region; n is 0 or 1; and wherein the light chain polypeptide does not comprise an Fc region. In some embodiments, the VDI domains on the heavy and light chain polypeptides form a first functional target binding site; and wherein the VD2 domains on the heavy and light chain polypeptides form a second functional target binding site. In some embodiments, the antigen target for the binding site formed by the VDI and/or VD2 domains is LRP-8. In some embodiments, the VDI and/or VD2 domains on the heavy and light chain polypeptides comprise the CDRs and/or variable domains from clone ML199.1 H1.5B2. In some embodiments, the VDI and/or VD2 domains on the heavy and light chain polypeptides comprise the CDRs and/or variable domains from clone BGK-2C8.8C. In some embodiments, the VDI and/or VD2 domains on the heavy and light chain polypeptides comprise the CDRs and/or variable domains from clone ML201-8F3.3D7. In some embodiments, the VDI and/or VD2 domains on the heavy and light chain polypeptides comprise the CDRs and/or variable domains from clone BGK.9D10-2. In some embodiments, the VDI and/or VD2 domains on the heavy and light chain polypeptides comprise the CDRs and/or variable domains from clone CL-105967. In some embodiments, the VDI and/or VD2 domains on the heavy chain polypeptide comprise a CDR-H1 of RFTFSNXIGMS, wherein X1 is F or Y or GFTVSDYYMA; a CDR-H2 of TISSGGRTYYYPD2VKG, wherein X2 is S or H or SISYEGSSTYYGDSDKVG; and a CDR-H3 of DLYAYMDY or PLRYGYNRFAY; and the VDI and/or VD2 domains on the light chain polypeptide comprise a CDR-L1 of RSSQSLVYXS3X4NTYLH, wherein X3 is N, T, R, W or P, and wherein X4 is G, E, L or K or KASQNIHKNLD; a CDR-L2 of KVSNRFS or YTDNLQT; and a CDR-L3 of SQSTHVPLT or YQYNSGPT.
In certain embodiments, the LRP-8 binding protein can also bind a brain antigen which benefits from transport to the brain via binding of the LRP-8 binding domain to that antigen.

In certain embodiments, the binding protein comprises two first polypeptide chains and two second polypeptide chains. In another embodiment, the Fc region comprises a variant sequence Fc region. In another embodiment, the Fc region comprises an Fc region selected from the group consisting of IgGl, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD.

In another aspect, the disclosure provides a binding protein conjugate comprising a binding protein as described herein, the binding protein conjugate further comprising an immunoadhesion molecule, an imaging agent, a therapeutic agent, or a cytotoxic agent.

In one embodiment, the imaging agent is a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, or biotin. In certain embodiments, the imaging agent is \( ^{3} \text{H}, ^{14} \text{C}, ^{35} \text{S}, ^{90} \text{Y}, ^{99} \text{Tc}, ^{111} \text{In}, ^{125} \text{I}, ^{131} \text{I}, ^{177} \text{Lu}, ^{166} \text{Ho}, \) or \( ^{153} \text{Sm}. \)

In one aspect, the disclosure provides an isolated nucleic acid encoding the amino acid sequence of a polypeptide chain or both polypeptide chains of a binding protein described herein. In another embodiment, a vector or vectors (e.g., pcDNA, pTT, pTT3, pEFBOS, pBV, pJV, pcDNA3.1 TOPO, pEF6 TOPO, pHybE, pBOS or pBJ) is provided encoding the isolated nucleic acid sequence or sequences that encode a binding protein disclosed herein.

In another aspect, a host cell is transformed with the vector(s) disclosed herein. In certain embodiments, the host cell is a prokaryotic cell. In an embodiment, the host cell is E.Coli. In some embodiments, the host cell is a eukaryotic cell. In certain embodiments, the eukaryotic cell is selected from the group consisting of a protist cell, animal cell, plant cell, and fungal cell. In yet another embodiment, the host cell is a mammalian cell including, but not limited to, CHO, COS, NSO, SP2, PER.C6, or a fungal cell such as Saccharomyces cerevisiae, or an insect cell such as Sf9.

In another aspect, the disclosure provides a method of producing a binding protein, comprising culturing a host cell described herein in culture medium under conditions sufficient to produce the binding protein.
In another aspect, the disclosure provides a binding protein produced by a method of culturing a host cell described herein in culture medium under conditions sufficient to produce the binding protein.

In another aspect, the disclosure provides a pharmaceutical composition comprising a binding protein described herein, and a pharmaceutically acceptable carrier.

In one embodiment, the pharmaceutical composition includes at least one additional agent. In certain embodiments, the additional agent is an an imaging agent, a cytotoxic agent, an angiogenesis inhibitor, a kinase inhibitor, a co-stimulation molecule blocker, an adhesion molecule blocker, an anti-cytokine antibody or functional fragment thereof, a detectable label or reporter, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, a cytokine antagonist, budenoside, epidermal growth factor, a corticosteroid, cyclosporin, sulfasalazine, an aminosalicylate, 6-mercaptopurine, azathioprine, metronidazole, a lipoxygenase inhibitor, mesalamine, olsalazine, balsalazide, an antioxidant, a thromboxane inhibitor, a growth factor, an elastase inhibitor, a pyridinyl-imidazole compound, an antibody, antagonist or agonist of TNF, LT, IL-1, IL-1R, IL-2, IL-4, IL-6, IL-6R, IL-7, IL-8, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-18, IL-23, TGF-β, EMAP-II, GM-CSF, FGF, PDGF, CD2, CD3, CD4, CD8, CD-19, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or a ligand thereof, methotrexate, FK506, rapamycin, mycophenolate mofetil, leflunomide, ibuprofen, prednisolone, a phosphodiesterase inhibitor, an adenosine agonist, an antithrombotic agent, a complement inhibitor, an adrenergic agent, IRAK, NIK, IKK, p38, a MAP kinase inhibitor, an IL-1β converting enzyme inhibitor, a TNFa-converting enzyme inhibitor, a T-cell signaling inhibitor, a metalloproteinase inhibitor, an angiotensin converting enzyme inhibitor, a soluble cytokine receptor, a soluble p55 TNF receptor, a soluble p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R or combinations thereof.
In one aspect, the disclosure provides an LRP-8 binding protein or composition as described herein for use in therapy. In certain embodiments, the disclosure provides a binding protein for use in treating a subject for a disease or a disorder. In some embodiments, the binding protein may be used for treatment by administering to the subject the binding protein such that treatment is achieved. In an embodiment, the LRP-8 binding proteins of the disclosure are used to deliver a drug or other therapeutic agent to the brain. In certain embodiments, the LRP-8 binding protein binds a target in the brain that is associated with a disease.

In various embodiments, the binding proteins disclosed herein are used in the treatment of brain disorders, e.g., an autoimmune or inflammatory disease of the brain, an infectious disorder of the brain, a neurological disorder, a neurodegenerative disorder, a brain cancer, or a brain metastasis. In certain embodiments, the disorder is Huntington's chorea, Parkinson's disease, Alzheimer's disease, dementia, acute or chronic spinal cord injury, multiple sclerosis, stroke, mental disorders, depression, schizophrenia, acute pain or chronic pain.

In another embodiment, the binding protein is administered or suitable for administration to a subject by a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavity, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraoesael, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathecal, intrathoracic, intruterine, intravesical, bolus, epidural, vaginal, rectal, buccal, sublingual, intranasal, or transdermal route.

In another aspect, the disclosure provides an isolated polypeptide, comprising an amino acid sequence of human LRP-8 CR1 peptide (SEQ ID NO:2) or human LRP-8 CR2 peptide (SEQ ID NO:3). In some embodiments, the peptide is a cyclic peptide. In some aspects, the disclosure also provides a method of generating an LRP-8 binding protein by immunizing a mammal with an isolated polypeptide described herein.

In another aspect, the disclosure provides methods of determining the presence of LRP-8 or fragment thereof in a test sample by an immunoassay, comprising contacting the sample with the LRP-8 binding protein described herein.
BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A shows a high resolution structure of CR7 from LRP as presented previously (Simonovic et al. (2001) Biochemistry 40(50): 15127-34, PDB:1J8E). This structure shows a loop/turn motif that is stabilized by a disulfide linkage (circled).

FIG. 1B shows a multiple sequence alignment of two complement-like repeat regions in LRP-8: CR1 and CR2.

FIG. 2A shows a multiple sequence alignment of mouse, human, and cynomolgus LRP-8 isoform 3 sequences.

FIG. 2B shows a multiple sequence alignment of human and cynomolgus LRP-8 isoform 1 sequences.

FIG. 3A shows FACS binding analysis of LRP-8 expressing HEK293 cells (ordinate) to ML199 antibodies from mice immunized using CR1 and CR1. The antibodies clones analyzed were 11H1, 20E8, 12C2, 12F6 and 12G11 (abscissa). An HA-Tag antibody was used as a control.

FIG. 3B shows FACS binding analysis of HEK293 cells that do not express LRP-8 (ordinate) to ML199 antibodies. The antibodies clones analyzed were 11H1, 20E8, 12C2, 12F6 and 12G11 (abscissa). An HA-Tag antibody was used as a control.

FIG. 4A shows FACS binding analysis of LRP-8 expressing HEK293 cells (ordinate) to ML199-1 H1 antibody and ML201 antibodies (abscissa). An HA-Tag antibody was used as a control.

FIG. 4B shows FACS binding of HEK293 cells that do not express LRP-8 (ordinate) to ML199-1 H1 antibody and ML201 antibodies (abscissa). An HA-Tag antibody was used as a control.

FIG. 5 shows immunohistochemistry (IHC) data from a mouse in vivo pharmacokinetics (PK) study using an anti-LRP-8 antibody, ML199.11H1.5B2 mu/hu IgG1m/k. The data show enhanced uptake into the brain 24 hours after 30 mg/kg (mpK) intravenous (IV) dosing. Positive IHC staining is observed in parenchyma and neuronal cells.

FIG. 6 shows IHC data from a mouse in vivo pharmacokinetics PK study using an anti-LRP-8 antibody, ML199.1 lh1.5B2 mu/hu IgG1l shows positive IHC staining of vasculature two hours after 40 mpK IV dosing of the anti-LRP-8 antibody.
FIG. 7 shows a set of photographs from a mouse in vivo staining study using anti-LRP-8 antibodies, ML199.1 1H1.5B2 and 8F3.3D7, and an anti-TfR antibody as a positive control (20 mpk or 40 mpk IV dosing). A human IgG antibody was used as a negative control. Staining data show that the anti-LRP-8 antibodies had enhanced uptake into brain 24 hours after dosing. The photographs show positive IHC staining of parenchyma and neuronal cells.

FIG. 8 shows a set of photographs from a mouse in vivo staining study using anti-LRP-8 antibodies ML199.1 1H1.5B2 and an anti-TfR antibody as a positive control (50 mpk IV dose). The staining data show enhanced uptake of anti-LRP-8 antibody, ML199.1H1.5B2, into brain at 24 hours after IV dosing. A human IgG antibody was used as a control. The photographs (cerebellum/purkinje cells: first row; pons/medulla: second row; cortex: third row) show positive staining of parenchyma and neurons.

FIG. 9 shows a set of photographs from a mouse in vivo staining study of the spinal cord using anti-LRP-8 antibodies ML.199.1 1H1.5B2 and ab58216 (45 mpk or 50 mpk IV dose). The data show enhanced uptake of anti-LRP-8 antibody, ML199.1H1.5B2 into the spinal cord 24 hours after IV administration. The photographs show positive IHC staining of parenchyma and neurons.

FIG. 10 shows binding of anti-LRP-8 parental antibody ML199.1 1H1.5B2 and variants to mLRRP-8 expressing HEK293 cells in an MSD-ECL assay.

FIG. 11A shows flow cytometry relative cell count (ordinate) as compared with FL2-height (PI staining) for hLRP-8 and mLRRP-8 using antibodies BGK-5D10-E4, BGK-6E3-F4 and BGK-2C8-E6-D3.

FIG. 11B shows binding data (folder over isotype control) for anti-LRP-8 antibodies described herein.

FIG. 12A shows FACS data for LRP-8-transfected HEK293 cells versus wild type HEK293 cells (ordinate) as a function of antibody concentration (abscissa) for antibodies BGK-2C8.8c and ML199-1 1H1.5B2 SN. A positive control antibody and an anti-mlgG negative control antibody were also analyzed.

FIG. 12B shows the EC50 (nM) binding data for the antibodies of Figure 12A.

FIG. 12C shows binding of LRP-8 antibody BGK-2C8.8c to cells overexpressing human LRP-8 and cyno LRP-8 in a MSD-ECL assay.
FIG. 13A shows binding of LRP-8 antibodies ML199.1 1H1.5B2, BGK-2C8.8C, and BGT-9D10-2 to cells overexpressing mouse LPR-8 variant 1 in a MSD-ECL assay.

FIG. 13B shows binding of LRP-8 antibodies ML199.1 1H1.5B2, BGK-2C8.8C, and BGT-9D10-2 to cells overexpressing human LRP-8 variant 3 in a MSD-ECL assay.

FIG. 13C shows binding of LRP-8 antibodies ML199.1 1H1.5B2, BGK-2C8.8C, and BGT-9D10-2 to cells overexpressing cyno LRP-8 variant 1 in a MSD-ECL assay.

FIG. 14 shows a set of photographs from a mouse *in vivo* staining study using anti-LRP-8 antibody 11H1.5B2 and an hlgG antibody as a positive control.

FIG. 15 shows a set of photographs from a mouse spinal cord *in vivo* staining study using anti-LRP-8 antibodies 11H1.5B2, BGK.2C8.8C, BGK.9D10-2, and an hlgG antibody as a positive control.

FIG. 16A is a schematic showing a procedure for an epitope binning assay using anti-LRP-8 antibodies.

FIG. 16B shows a representative epitope binning assay using anti-LRP-8 antibodies.

FIG. 17A shows binding of anti-LRP-8 ML199.1 1H1.5B2 antibody to CR1 and CR2 peptides.

FIG. 17B shows the sequence alignment between CR1 and CR2 peptides and indicates alanine mutants introduced in variants of CR1 peptide (CR1.1, CR1.2, CR1.3, and CR1.4).

FIG. 17C shows binding of anti-LRP-8 ML199.1 1H1.5B2 antibody to CR1, CR1.1, CR1.2, CR1.3, and CR1.4 peptides.

FIG. 18A shows one view of a crystal structure for anti-LRP-8. 11H1.5B2 antibody in complex with the CR1 peptide, determined at 1.72 A resolution.

FIG. 18B shows another view of a crystal structure of anti-LRP-8. 11H1.5B2 antibody in complex with the CR1 peptide, determined at 1.72 A resolution.

FIG. 19 shows the results of a pharmacokinetic study with IV dose-ranging of chimeric anti-LRP-8 ML199.1 1H1.5B2 antibody in CD1 mice.

FIG. 20 shows the results of a pharmacokinetic study with chimeric anti-LRP-8.M199.1 1H1.5B2 antibody in a 4 day period after a single dose administration.
mg/kg). The serum PK of anti-LRP-8 was comparable to control IgG and significant brain and spinal cord uptake was shown at 24 hours compared to control IgG.

**FIG. 21A** shows the reelin count in cynoLRP8-293 cells.

**FIG. 21B** shows the results of cell-based competition assay using cynoLRP8-293 cells and a reelin HU-flag.

**FIG. 22A** and **FIG. 22B** show heavy chain and light chain sequence alignment of all hLRP8 antibodies.

**DETAILED DESCRIPTION**

This disclosure provides low density Lipoprotein Receptor-Related Protein 8 (LRP-8) binding proteins, including, but not limited to, anti-LRP-8 antibodies or antigen-binding portions thereof that bind LRP-8, and multivalent, multispecific binding proteins such as dual variable domain immunoglobulin (DVD-Ig) binding proteins that bind LRP-8. Various aspects of the disclosure relate to LRP-8 antibodies, multispecific molecules, antibody fragments, and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such LRP-8 binding proteins. Methods of using the LRP-8 binding proteins of the disclosure to cross the BBB, mediate localization to the brain and/or mediate localization to the spinal cord are also encompassed by the disclosure. The disclosure also encompasses any binding protein or antibody capable of binding to the same epitope bound by any of the antibodies and binding proteins disclosed herein, as well as any binding protein or antibody capable of competing for binding to LRP-8 with a binding protein described herein. In certain aspects of the disclosure, an LRP-8 binding protein has one or more of the following properties: 1) binds to an extracellular domain of LRP-8; 2) binds to LRP-8 with minimal interruption of natural ligand binding; 3) internalizes as part of a binding protein-receptor complex; 4) transcytoses to the albuminal side of endothelial cells; 5) increases binding protein uptake to targeted tissue; 6) comprises a DVD-Ig; and/or 7) is cross-reactive with LRP-8 in a non-human mammal (e.g., one or any combination of cynomolgus monkey, rat or mouse). In some embodiments, a cross-reactive binding protein is one that exhibits at least about 50% of the binding affinity to a second species as observed for a first species (e.g., at least about 50% of the affinity to mouse LRP-8 as observed for human LRP-8).

According to certain embodiments, an LRP-8 binding protein binds to LRP-8 (e.g., human LRP-8 and/or cyno LRP-8). In certain embodiments, an LRP-8 binding
protein binds to an epitope between about amino acids 1 and 120 of human LRP-8 (SEQ ID NO: 1). In certain embodiments, an LRP-8 binding protein binds to one or both of human LRP-8 CR1 peptide (SEQ ID NO:2) or human LRP-8 CR2 peptide (SEQ ID NO:3). In other embodiments, an LRP-8 binding protein binds to one or both of conjugated human LRP-8 CR1 peptide (SEQ ID NO:5) or conjugated human LRP-8 CR2 peptide (SEQ ID NO:6). In other embodiments, an LRP-8 binding protein binds to a CR1 / CR2 consensus sequence (SEQ ID NO:4). In some embodiments, an LRP-8 binding protein binds to one or more of CR1, CR1.1, CR1.2, CR1.3, CR1.4, and CR2 (sequences provided in Table 1). In some embodiments, an LRP-8 binding protein binds to CR1, CR1.2, CR1.3, and CR2, but does not bind to CR1.1 and/or CR1.4 (sequences provided in Table 1). In certain embodiments, an LRP-8 binding protein binds to an epitope in LRP-8 comprising or consisting of amino acid residues 47-57 and 60 of human LRP-8 (SEQ ID NO:1). In certain embodiments, an LRP-8 binding protein binds to an epitope in LRP-8 comprising or consisting of amino acid residues 47-66 of human LRP-8 (SEQ ID NO:1), or comprising or consisting of four or more, or five or more, amino acid residues from within that amino acid stretch of human LRP-8. In certain embodiments, an LRP-8 binding protein binds to a conformational epitope, consisting of or comprising discontinues residues of human LRP-8 (SEQ ID NO: 1). In certain embodiments, an LRP-8 binding protein binds to an epitope in LRP-8 comprising one or more amino acid residues selected from 52(F), 56(C), and 56(N), or all three amino acid residues of human LRP-8 (SEQ ID NO: 1). In certain embodiments, an LRP-8 binding protein binds to an epitope in LRP-8 comprising one or more amino acid residues selected from 47(C), 52(F), 54(C), 56(N), 59(C), 60(1), 64(W), and 66(C) of human LRP-8 (SEQ ID NO: 1). In various embodiments, the epitope is a conformational epitope and comprises portions of human LRP-8 (SEQ ID NO: 1) contacted by the antibody in addition to those listed above. In various embodiments, the binding protein comprises clone ML199.1 1H1.5B2, or the CDR and/or variable domains from that clone. In some embodiments, the binding protein exhibits cross-reactivity with LRP-8 in a non-human mammal (e.g., one or any combination of cynomolgus monkey, rat or mouse).

In various embodiments, an epitope is determined by obtaining an X-ray crystal structure of an antibody: antigen complex and determining which residues on LRP-8 are within a specified distance of residues on the antibody of interest, wherein the specified
distance is, 5 Å or less, e.g., 5Å, 4Å, 3Å, 2Å, 1Å or any distance in between. In some embodiments, the epitope is defined as a stretch of 8 or more contiguous amino acid residues along the LRP-8 sequence in which at least 50%, 70% or 85% of the residues are within the specified distance of the antibody or binding protein in the X-ray crystal structure.

In various embodiments, the binding proteins disclosed herein are capable of binding LRP-8 (e.g., human, cyno, and/or mouse LRP-8). In various embodiments, the binding proteins disclosed herein are capable of binding LRP-8 without disrupting one or more biological function of LRP-8. The biological functions of LRP-8 include, for example, the recognition of a natural ligand such as reelin, ApoE, selenoprotein P and RAP, and the activation of downstream signaling pathways including PI3K pathway (see e.g., Herz et al. (2009) Curr. Opin. Lipidol. 20(3): 190-196; and Kurokawa et al. (2014) J. Biol. Chem. 289(13): 9195-9207). In some embodiments, binding to LRP-8 without disrupting one or more biological function allows for targeting of binding proteins to the brain/nervous system and crossing the blood-brain barrier (e.g., for delivery of a second agent, multispecific binding protein, or conjugate) without disrupting the natural functions of LRP-8 functions. In some embodiments, the binding protein comprises clone ML199.11H1.5B2, or the CDR and/or variable domains from that clone. In various embodiments, a binding protein comprising an LRP-8 binding domain disclosed herein is able to cross the blood-brain barrier in either a monospecific or multispecific format, or both. In various embodiments, the binding proteins disclosed herein exhibit an effective affinity, epitope, charge, and/or valency to enhance uptake across the blood-brain barrier.

In various embodiments, the binding protein is capable of binding LRP-8, and comprises clone ML199.11H1.5B2. In various embodiments, the binding protein is capable of binding LRP-8, and comprises the CDR and/or variable domain amino acid sequences of clone ML199.11H1.5B2. In various embodiments, the binding protein is capable of binding LRP-8, and comprises clone 11H1. In various embodiments, the binding protein is capable of binding LRP-8, and comprises the CDR and/or variable domain amino acid sequences of clone 11H1. In various embodiments, the binding protein is capable of binding LRP-8, and comprises clone BGK-2C8.8C. In various embodiments, the binding protein is capable of binding LRP-8, and comprises the CDR and/or variable domain amino acid sequences of clone BGK-2C8.8C. In various
In various embodiments, the binding protein is capable of binding LRP-8, and comprises clone ML201-8F3.3D7. In various embodiments, the binding protein is capable of binding LRP-8, and comprises the CDR and/or variable domain amino acid sequences of clone ML201-8F3.3D7. In various embodiments, the binding protein is capable of binding LRP-8, and comprises clone BGK.9D10-2. In various embodiments, the binding protein is capable of binding LRP-8, and comprises the CDR and/or variable domain amino acid sequences of clone BGK.9D10-2. In an embodiment, the binding protein is capable of binding LRP-8, and comprises CL-105967.

In some embodiments, the binding protein exhibits cross-reactivity with LRP-8 in a non-human mammal (e.g., one or any combination of cynomolgus monkey, rat or mouse).

Table 1 shows amino acid sequences of LRP-8 proteins, including various complement-like repeat regions of LRP-8 ("CR," also known as ligand binding repeats) and variant CR regions, as well as conserved regions across species.

### Table 1. LRP-8 Protein Amino Acid Sequences

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<thead>
<tr>
<th>Protein</th>
<th>Sequence Identifier</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human LRP-8</td>
<td>SEQ ID NO:1</td>
<td>MGLPEPGPLRLALLLLLLLLLLLLQQLQHLA</td>
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<td>DMRCYRDADESMGSTMVTRAVIGIIVP</td>
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<td>VYKRTTEEEEDDELHIGRTAQIGHVYPARV</td>
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**cynoLRP-8**

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| LLEMQLQHLAAAAADPLLGGQGPAKECEKDQFQCRNERCIPSVWRCDEDDDCLDLHSDEDDCPKCTCSDSFTDNCNGHICHERWKCDGEECPDGSDESEATCTLTGCHGNEFQCGDGTCVLAIKRCNQEQDCPDGSDEAGCLQVPTFTLGNNRRPRGRLNECLHNNGGCCHISTDLKGFECTCPAGFQQLLDQKTCGDIDECKPDADCSIQICVNYGKFKECYPYEMDLTTKNCKAAAGKSPSLIFTNRHVRRVRLDKVRNYSLPRLMLKNVKNVALDMEXTRNRYWCDLSYRKYSAAYMNDKASKPEQEVILDEQLEHSPGALVADWDVKHIYWTDSNGKTSISVATVGGGRCRTLFSRNLSEPFRAIAVDPQLGFGMWHDGQNQAKIEKSGLGVDKLHQCGLSSIDFGNQKRMILLSSTDIFLSHPFGIAVFEDKFVFWTDLNEAEIFSANRNLGTEISLAENLNNHPHDIVFHKLQPFRAADACKLSCKLSVQPNGGCEYLCIPAPQISSHPKYSTCACPDTMWLPMKRCRYRCDGPEACGPRERFCGRDGGAICPERWVCDRQDFCDSRSDAEACLGRPQGATSAPAAACATQAQFACRSSECVHLGWRCDGDRCKDSDKDEACPLGTLGCHNELFQCGDGTCVLAIKRCNQEQDCPDGSDEAGCLQGLNCHLNNGGCSCHIDTDLKIGFECTCPAGFQLLDQKTCGDIDECKPDADCSQICVNNGYKFKECYPYEMDLTTKNCKAAAGKSPSLIFTNRHVRRVRLDKVRNYSLPRLMLKNVKNVALDMEXTRNRYWCDLSYRKYSAAYMNDKASKPEQEVILDEQLEHSPGALVADWDVKHIYWTDSNGKTSISVATVGGGRCRTLFSRNLSEPFRAIAVDPQLGFGMWHDGQNQAKIEKSGLGVDKLHQCGLSSIDFGNQKRMILLSSTDIFLSHPFGIAVFEDKFVFWTDLNEAEIFSANRNLGTEISLAENLNNHPHDIVFHKLQPFRAADACKLSCKLSVQPNGGCEYLCIPAPQISSHPKYSTCACPDTMWLPMKRCRYRCDGPEACGPRERFCGRDGGAICPERWVCDRQDFCDSRSDAEACLGRPQGATSAPAAACATQAQFACRSSECVHLGWRCDGDRCKDSDKDEACPLGTLGCHNELFQCGDGTCVLAIKRCNQEQDCPDGSDEAGCLQGLNCHLNNGGCSCHIDTDLKIGFECTCPAGFQLLDQKTCGDIDECKPDADCSQICVNNGYKFKECYPYEMDLTTKNCKAAAGKSPSLIFTNRHVRRVRLDKVRNYSLPRLMLKNVKNVALDMEXTRNRYWCDLSYRKYSAAYMNDKASKPEQEVILDEQLEHSPGALVADWDVKHIYWTDSNGKTSISVATVGGGRCRTLFSRNLSEPFRAIAVDPQLGFGMWHDGQNQAKIEKSGLGVDKLHQCGLSSIDFGNQKRMILLSSTDIFLSHPFGIAVFEDKFVFWTDLNEAEIFSANRNLGTEISLAENLNNHPHDIVFHKLQPFRAADACKLSCKLSVQPNGGCEYLCIPAPQISSHPKYSTCACPDTMWLPMKRCRYRCPQSTSTTLPSSTRTGFPATGAPGTIVHRSTDQHSTETPNLAAAVPSSVSPPRAPSILSTLSPATSNSHGYGNEDSKMGSTVTAAVIGIIPVIVIALCMSGYLWRWKRKNTKSMFNPDVPYRTKTEDDEDELHIGRTAQIGHVYPARVALSEDGLP
*Predicted cyno LRP-8 isoform 1 signal peptide is underline

In certain embodiments, this disclosure provides binding proteins that specifically bind LRP-8. In some embodiments, a binding protein, including a DVD-Ig binding protein, antibody, or fragment thereof, is capable of binding LRP-8 and has a heavy or light chain comprising at least about 80%, 90%, 95%, 99%, or 100% homology to CDRs 1-3 or to the full variable domains of any of the sequences in Tables 2-7 or 18. As used herein, the term percent (%) homology defines the percentage of residues in an amino acid sequence that are identical to a reference sequence after aligning the sequences and introducing gaps and other spacing, e.g., using the BLAST alignment software.

In certain embodiments, the binding proteins disclosed herein have at least about 80%, homology to the VH and VL domains of antibodies or binding proteins that specifically bind LRP-8, e.g., binding proteins or antibodies LRP-8 comprising CDRs and/or variable domains selected from those identified in Tables 2-7. Each VH and VL domain of a binding protein contains three CDR domains: CDR-H1, CDR-H2, CDR-H3, and CDR-L1, CDR-L2, and CDR-L3. According to certain embodiments, a binding protein described herein can contain 1, 2, or 3 CDRs having at least 80% homology to the CDRs in a VH or VL sequence listed in any one of Tables 2-7. In some embodiments, a binding protein described herein can comprise 1, 2, 3, 4, 5, or 6 CDRs having at least 80% homology to the CDRs in a VH domain and its paired VL domain disclosed in any one of Tables 2-7. In some embodiments, the binding proteins disclosed herein have sequences that have at least 80% homology to a VH domain and its paired VL domain disclosed in any one of Tables 2-7.

In certain embodiments, a binding protein disclosed herein can compete for binding with an antibody or binding protein that specifically bind LRP-8. In certain embodiments, a binding protein disclosed herein can compete for binding with an antibody or binding protein comprising CDRs and/or variable domains selected from those identified in Tables 2-7. In certain embodiments, a binding protein disclosed herein can compete for binding with clone ML199.11H1.5B2. In certain embodiments, a binding protein disclosed herein can compete for binding with clone 11H1. In certain embodiments, a binding protein disclosed herein can compete for binding with clone BGK-2C8.8C. In certain embodiments, a binding protein disclosed herein can compete for binding with clone ML201-8F3.3D7. In certain embodiments, a binding protein
disclosed herein can compete for binding with clone BGK.9D10-2. In certain embodiments, a binding protein disclosed herein can compete for binding with clone CL-105967.

In certain embodiments, competitive binding can be evaluated using routine cross-blocking assays, such as the assay described in ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1st edition 1988, 2nd edition 2014). In some embodiments, competitive binding is identified when a test antibody or binding protein reduces binding of a reference antibody or binding protein (e.g., a binding protein comprising CDRs and/or variable domains selected from those identified in Tables 2-7) to LRP-8 by at least about 50% in the cross-blocking assay (e.g., 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.5%, or more, or any percentage in between), and/or vice versa. In some embodiments, competitive binding can be due to shared or similar (e.g., partially overlapping) epitopes, or due to steric hindrance where antibodies or binding proteins bind at nearby epitopes. See, e.g., Tzartos, Methods in Molecular Biology, vol. 66, Epitope Mapping Protocols, pages 55-66, Humana Press Inc. (1998) ("only marked mutual crosscompetition should be taken as unequivocal evidence of overlapping epitopes, since weak or one-way inhibition may simply reflect a decrease in affinity owing to steric or allosteric effects. Therefore, we completely ignored cases of weak inhibition (<25%) and essentially only considered inhibition of >50%"). In some embodiments, competitive binding can be used to sort groups of binding proteins that share similar epitopes, e.g., those that compete for binding can be "binned" as a group of binding proteins that have overlapping or nearby epitopes, while those that do not compete are placed in a separate group of binding proteins that do not have overlapping or nearby epitopes.

In various embodiments, the VH and VL domains disclosed herein are 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical (i.e., they share the specified percent homology) to one or more of those shown in Tables 2-7. According to certain embodiments, an LRP-8 binding protein can have one or more heavy chain variable domains and/or one or more light chain variable domains as set forth in any one of Tables 2-7. In certain embodiments, an LRP-8 binding protein can have CDR sequences selected from one or more of the heavy chain variable domains and/or one or more light chain variable domains as set forth in any one of Tables 2-7. In some embodiments, a binding protein disclosed herein comprises heavy chain CDR
sequences and/or a heavy chain variable domain and its paired light chain CDR sequences and/or a paired light chain variable domain selected from Tables 2-7.

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<th>SEQ ID NO:</th>
<th>Clone</th>
<th>Heavy Chain Variable Domain (CDRs in bold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL-33867 (ML201-2B4.2B1.2H10)</td>
<td>DVKLAVSGGLVKPGSRSLCALASGFTLSRYAMSRVQTPKRELEWVAY &lt;br&gt; IRNGYDIYIADTVKGRFTISRDNNLYLQMSLKLSEDATMYCRTRE &lt;br&gt; SYNFNFPQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>CL-33866 (ML201-8F3.3D7)</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVAT &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>CL-33865 (ML199-11H1.5B2)</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVAT &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>hML199-11H1-5B2.VH.1</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVAT &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>hML199-11H1-5B2.VH.1a</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVST &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.15</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVST &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.16</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVST &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.17</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVST &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.18</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVST &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.19</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVST &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.20</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVST &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.21</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVST &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY &lt;br&gt; LLNYFYQQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>ab58216</td>
<td>EVHLQGSGYFVNLKASGVKICSASGYYGFYNVKGSHGSLQVWR &lt;br&gt; INFYSGDTFFQNKPKGKATLTVQDSNNTLERSLITSEDSAYFIVFACED &lt;br&gt; IGRAWYQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>BGK-2C8.E6.D3</td>
<td>QYTKLVEGSLQPQSQLSLCTSFSGSLNLGVGVRQGQSGLGEEW &lt;br&gt; ANLIIWDDGDFNPSLKLQELTVQDSNNTLERSLITSEDSAYFIVFACED &lt;br&gt; IGRAWYQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>BGK-5D10-E4</td>
<td>QVQLEKTVQGDPVQPTQLTSLCTVQSFSLNYPVQTVVRQGQSGLGEEW &lt;br&gt; ANLIIWDDGDFNPSLKLQELTVQDSNNTLERSLITSEDSAYFIVFACED &lt;br&gt; IGRAWYQQQTTLTVSS</td>
<td></td>
</tr>
<tr>
<td>BGK-6E3-F4</td>
<td>EVQLVESGGGLVKPGSRLCALASGFTLSRYAMSRVQTPKRELEWVAT &lt;br&gt; ISSLGRTYDPSVKGRTISRDNNLYLQMSLKLSEDATMYCARDY</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. LRP-8 Binding Protein Heavy Chain Variable Domain Amino Acid Sequences (CDRs In Bold)
In certain embodiments, an LRP-8 binding protein can comprise CDR sequences selected from one or more of the following heavy chain CDR sequences in Table 3. In some embodiments, an LRP-8 binding protein can include a set of heavy chain CDR sequences (e.g., an HCDR1, HCDR2, and HCDR3) selected from any of the sets shown below. In some embodiments, a binding protein can comprise a heavy chain CDR set selected from Table 3 and any light chain CDR set selected from Table 6. In some embodiments, a binding protein can comprise a heavy chain CDR set selected from Table 3 and any light chain CDR set in Table 6 (e.g., the heavy chain CDR set from clone ML199-1H1.5B2 paired with the light chain CDR set from clone ML199-11H1.5B2).

Table 3. LRP-8 Binding Protein Heavy Chain CDR Amino Acid Sequences

<table>
<thead>
<tr>
<th>Clone</th>
<th>HCDR1</th>
<th>HCDR2</th>
<th>HCDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL-33867 (ML201-2B4.2B1.2H10)</td>
<td>GFTLSRYAMS (SEQ ID NO: XX)</td>
<td>YIRNGGDYYADTVKG (SEQ ID NO: XX)</td>
<td>EGSYYNFDY (SEQ ID NO: XX)</td>
</tr>
<tr>
<td>CL-33866 (ML201-8F3.3D7)</td>
<td>RFTFSNFGMS (SEQ ID NO: XX)</td>
<td>TISSGGRYTYPPSVKG (SEQ ID NO: XX)</td>
<td>DYLYAMDY (SEQ ID NO: XX)</td>
</tr>
</tbody>
</table>
In certain embodiments, an LRP-8 binding protein can comprise a heavy chain variable domain sequence selected from any of the following sequences in Table 4. In some embodiments, a binding protein can comprise a heavy chain variable domain.
sequence selected from Table 4 and any light chain variable domain sequence selected from Table 7. In some embodiments, a binding protein can comprise a heavy chain variable domain sequence selected from Table 4 and its paired light chain variable domain sequence in Table 6 (e.g., the heavy chain variable domain from clone ML199-11H1.5B2 paired with the light chain variable domain from clone ML199-1 1H1.5B2).

Table 4. LRP-8 Binding Protein Heavy Chain Variable Domain Amin Acid Sequences (CDRs in bold, gaps introduced to align frameworks; also depicted in Figure 22A)

<table>
<thead>
<tr>
<th>SEQ ID NO:</th>
<th>Clone</th>
<th>Heavy Chain Variable Domain (CDRs in bold, gaps introduced to align frameworks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL-33867 (ML201-2B4 2B1 2H10)</td>
<td>DVKLVASGGGLVKPGSRLSCAASGFTLSRY --AMSVWQFPEKRELEWVIYRNGDYIYADTVKGRFTISRDANNTLQLQMS LKSEDTAMYCTREGS-----YNYFDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>CL-33866 (ML201-8F3 3D7)</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQTFDRKRELEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRSEDTAMYCAR-DY-----LYAMDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>CL-33865 (ML199-11H1.5B2)</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQTFDRKRELEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRSEDTAMYCAR-DY-----LYAMDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>lhML199-11H1-5B2.VH.1</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQAFKQGKLEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRAEDTAVYYCAR-DY-----LYAMDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>lhML199-11H1-5B2.VH.1a</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQAFKQGKLEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRAEDTAVYYCAR-DY-----LYAMDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.15</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQAPFKQGKLEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRAEDTAVYYCAR-DY-----LYAMDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.16</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQAPFKQGKLEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRAEDTAVYYCAR-DY-----LYAMDYWQQGTTTVSS</td>
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<tr>
<td>ML199.11H1.5B2.1A.17</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQAPFKQGKLEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRAEDTAVYYCAR-DY-----LYAMDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.18</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQAPFKQGKLEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRAEDTAVYYCAR-DY-----LYAMDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.19</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQAPFKQGKLEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRAEDTAVYYCAR-DY-----LYAMDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.20</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQAPFKQGKLEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRAEDTAVYYCAR-DY-----LYAMDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.21</td>
<td>EVQLVESGGGLVKPGSRLSCAASRFTSNF --GMSVVRQAPFKQGKLEWVAISSGRTYYPDSVKGRFTISRDANNTLQLQMS LRAEDTAVYYCAR-DY-----LYAMDYWQQGTTTVSS</td>
<td></td>
</tr>
<tr>
<td>ab58216</td>
<td>EVHLQFGVEFVKPGSRLSCKASGYIITSGY --FLNVQVQSHVSKLEWVGRINYSGDFFNNQKFGKATLTVDKSNTAHMLRSLTSEDSAYCARED-----GRFAWQQGTTTVSS</td>
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</tr>
</tbody>
</table>
5 Table 5. LRP-8 Binding Protein Light Chain Variable Domain Sequences (CDRs In Bold)

<table>
<thead>
<tr>
<th>SEQ ID NO:</th>
<th>Clone</th>
<th>Light Chain Variable Domain (CDRs in bold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL-33867 (ML201-2B4.2B1.2H10)</td>
<td><strong>DVLM</strong>&lt;br&gt;<strong>MTQ</strong>&lt;br&gt;<strong>FTLSLPVLGQGSI</strong>&lt;br&gt;<strong>CSSQIVS</strong>&lt;br&gt;<strong>HNS</strong>&lt;br&gt;<strong>NT</strong>&lt;br&gt;<strong>LEW</strong>&lt;br&gt;<strong>YI</strong>&lt;br&gt;<strong>QPGQSP</strong>&lt;br&gt;<strong>NF</strong>&lt;br&gt;<strong>LL</strong>&lt;br&gt;<strong>I</strong>&lt;br&gt;<strong>K</strong>&lt;br&gt;<strong>VSNRFS</strong>&lt;br&gt;<strong>GPD</strong>&lt;br&gt;<strong>R</strong>&lt;br&gt;<strong>F</strong>&lt;br&gt;<strong>G</strong>&lt;br&gt;<strong>IS</strong>&lt;br&gt;<strong>G</strong>&lt;br&gt;<strong>T</strong>&lt;br&gt;<strong>K</strong>&lt;br&gt;<strong>E</strong>&lt;br&gt;<strong>L</strong>&lt;br&gt;<strong>I</strong>&lt;br&gt;<strong>Q</strong>&lt;br&gt;<strong>K</strong>&lt;br&gt;<strong>Q</strong>&lt;br&gt;<strong>S</strong>&lt;br&gt;<strong>H</strong>&lt;br&gt;<strong>V</strong>&lt;br&gt;<strong>P</strong>&lt;br&gt;<strong>N</strong>&lt;br&gt;<strong>F</strong>&lt;br&gt;<strong>G</strong>&lt;br&gt;<strong>G</strong>&lt;br&gt;</td>
<td></td>
</tr>
<tr>
<td>CL-33866 (ML201-8F3.3D7)</td>
<td><strong>DVMT</strong>&lt;br&gt;<strong>MQ</strong>&lt;br&gt;<strong>TL</strong>&lt;br&gt;<strong>FTL</strong>&lt;br&gt;<strong>VSDLGQGSI</strong>&lt;br&gt;<strong>CSSQIVS</strong>&lt;br&gt;<strong>HNS</strong>&lt;br&gt;<strong>NT</strong>&lt;br&gt;<strong>LEW</strong>&lt;br&gt;<strong>YI</strong>&lt;br&gt;<strong>QPGQSP</strong>&lt;br&gt;<strong>NF</strong>&lt;br&gt;<strong>LL</strong>&lt;br&gt;<strong>I</strong>&lt;br&gt;<strong>K</strong>&lt;br&gt;<strong>VSNRFS</strong>&lt;br&gt;<strong>GPD</strong>&lt;br&gt;<strong>R</strong>&lt;br&gt;<strong>F</strong>&lt;br&gt;<strong>G</strong>&lt;br&gt;<strong>IS</strong>&lt;br&gt;<strong>G</strong>&lt;br&gt;<strong>T</strong>&lt;br&gt;<strong>K</strong>&lt;br&gt;<strong>E</strong>&lt;br&gt;<strong>L</strong>&lt;br&gt;<strong>I</strong>&lt;br&gt;<strong>Q</strong>&lt;br&gt;<strong>K</strong>&lt;br&gt;<strong>Q</strong>&lt;br&gt;<strong>S</strong>&lt;br&gt;<strong>H</strong>&lt;br&gt;<strong>V</strong>&lt;br&gt;<strong>P</strong>&lt;br&gt;<strong>N</strong>&lt;br&gt;<strong>F</strong>&lt;br&gt;<strong>G</strong>&lt;br&gt;<strong>G</strong>&lt;br&gt;</td>
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</tr>
</tbody>
</table>

According to certain embodiments, an LRP-8 binding protein can comprise one or more light chain variable domains as set forth in Table 5.
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<th>Document Content</th>
</tr>
</thead>
<tbody>
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<td>LTGFAGTKLEL K</td>
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<td>CL-33865 (ML199-1) H1.5B2</td>
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</tr>
<tr>
<td>hML199-HHI-5B2VL. 1a</td>
</tr>
<tr>
<td>hML199-HHI-5B2VL. 1b</td>
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<td>ML199. 11H1.5B2. 1A. 16</td>
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</tr>
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</tr>
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<td>ML199. 11H1.5B2. 1A.20</td>
</tr>
<tr>
<td>ML199. 11H1.5B2. 1A.21</td>
</tr>
<tr>
<td>ab582 16</td>
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<tr>
<td>BGK-2C8.E6.D3</td>
</tr>
<tr>
<td>BGK-2C8.8C</td>
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<td>CL-105959 (BGK-7F7)</td>
</tr>
<tr>
<td>CL-105960 (BGK-7F7)</td>
</tr>
</tbody>
</table>
According to certain embodiments, an LRP-8 binding protein can comprise one or more sequences selected from or homologous to the following light chain CDR sequences in Table 6.

### Table 6. LRP-8 Binding Protein Light Chain CDR Sequences

<table>
<thead>
<tr>
<th>Clone</th>
<th>LCDR1</th>
<th>LCDR2</th>
<th>LCDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL-33867 (ML201-2B4.2B1.2H10)</td>
<td>RSSQIVHSNGNTYLE (SEQ ID NO: XX)</td>
<td>KVSNRFS (SEQ ID NO: XX)</td>
<td>FQGSHVPLT (SEQ ID NO: XX)</td>
</tr>
<tr>
<td>CL-33866 (ML201-8F3.3D7)</td>
<td>RSSQLVYSNGNTYLH (SEQ ID NO: XX)</td>
<td>KVSNRFS (SEQ ID NO: XX)</td>
<td>SQSTHVPLT (SEQ ID NO: XX)</td>
</tr>
<tr>
<td>CL-33865 (ML199-11H1.5B2)</td>
<td>RSSQLVYSNGNTYLH (SEQ ID NO: XX)</td>
<td>KVSNRFS (SEQ ID NO: XX)</td>
<td>SQSTHVPLT (SEQ ID NO: XX)</td>
</tr>
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<td>hML199-11H1-5B2VL.1</td>
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<td>KVSNRFS (SEQ ID NO: XX)</td>
<td>SQSTHVPLT (SEQ ID NO: XX)</td>
</tr>
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<td>KVSNRFS (SEQ ID NO: XX)</td>
<td>SQSTHVPLT (SEQ ID NO: XX)</td>
</tr>
<tr>
<td>hML199-11H1-5B2VL.1.b</td>
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<td>SQSTHVPLT (SEQ ID NO: XX)</td>
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<td>KVSNRFS (SEQ ID NO: XX)</td>
<td>SQSTHVPLT (SEQ ID NO: XX)</td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.16</td>
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<td>KVSNRFS (SEQ ID NO: XX)</td>
<td>SQSTHVPLT (SEQ ID NO: XX)</td>
</tr>
<tr>
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<td>RSSQLVYSNLTNYLH (SEQ ID NO: XX)</td>
<td>KVSNRFS (SEQ ID NO: XX)</td>
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</tr>
<tr>
<td>ML199.11H1.5B2.1A.18</td>
<td>RSSQSLVYSNKNTYLH</td>
<td>KVSNRFS</td>
<td>SQSTHVPLT</td>
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<tr>
<td>ML199.11H1.5B2.1A.19</td>
<td>RSSQSLVYSRGNTYLH</td>
<td>KVSNRFS</td>
<td>SQSTHVPLT</td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.20</td>
<td>RSSQSLVYSWGNTYLH</td>
<td>KVSNRFS</td>
<td>SQSTHVPLT</td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.21</td>
<td>RSSQSLVYSPGNTYLH</td>
<td>KVSNRFS</td>
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<td>ab58216</td>
<td>RSSQTVIHSNGNTYLE</td>
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<td>RASEGVSJNYMH</td>
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<td>BGK-5D10-E4</td>
<td>LASEDIYNNLNA</td>
<td>FTSNLQD</td>
<td>LQDSEYPLT</td>
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<td>BGK-6E3-F4</td>
<td>KSSQSLSSGKQNYLA</td>
<td>LASTRES</td>
<td>QOHYDTPLT</td>
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<td>BGK-2C8.8C</td>
<td>KASQNIHKNLD</td>
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<td>YANSLED</td>
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<td>CL-105959 (BGK-7A11)</td>
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<td>RDDKRPD</td>
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<tr>
<td>CL-105960 (BGK-7F7)</td>
<td>KRSTANIGSNYVN</td>
<td>RDDRKP</td>
<td>QSYSSGINI</td>
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<td>FTSTLES</td>
<td>LHYNPLPPW</td>
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<td>CL-105969 (BGK-6B5-2)</td>
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<td>RDDKRPD</td>
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<td>CL-134994</td>
<td>RASQSVGSCYLA</td>
<td>GASSRAT</td>
<td>QHYVRSPIT</td>
</tr>
<tr>
<td>CL-135325</td>
<td>RASQSIGSILN</td>
<td>AASSLQS</td>
<td>QQSYIPPL</td>
</tr>
<tr>
<td>CL-135359</td>
<td>RASQGICTYLN</td>
<td>AASSLQS</td>
<td>QSYNPPPLT</td>
</tr>
</tbody>
</table>
According to certain embodiments, an LRP-8 binding protein can comprise one or more light chain variable domains as set forth in Table 7.

### Table 7. LRP-8 Binding Protein Light Chain Variable Domain Amino Acid Sequences (CDRs in bold, gaps introduced to align frameworks; also depicted in Figure 22B)

<table>
<thead>
<tr>
<th>SEQ ID NO:</th>
<th>Clone</th>
<th>Light Chain Variable Domain (CDRs in bold, gaps introduced to align frameworks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL-33867  (ML201-2B4.2B1.2H10)</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSLVSNGLKYSLYQLKFGQPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>CL-33866  (ML201-8F3.3D7)</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSLVSNGLKYSLYQLKFGQPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>CL-33865  (ML199-11H1.5B2)</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSLVSNGLKYSLYQLKFGQPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>hML199-11H1-5B2VL.1</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSLVSNGLKYSLYQLKFGQPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>hML199-11H1-5B2VL.1a</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSLVSNGLKYSLYQLKFGQPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>hML199-11H1-5B2VL.1b</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSLVSNGLKYSLYQLKFGQPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.15</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSVSPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.16</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSVSPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.17</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSVSPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
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<tr>
<td>ML199.11H1.5B2.1A.18</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSVSPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
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<tr>
<td>ML199.11H1.5B2.1A.19</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSVSPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.20</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSVSPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>ML199.11H1.5B2.1A.21</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSVSPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
<tr>
<td>ab58216</td>
<td>DVLMTQFSLPVSLGDQASIRCSSQSVSPQNLIIKFKSNRIFSQSVFPRFSQSG--&lt;br&gt;SGTDFTLSKRMVEADLGYYFCQGSHVP-LTFGGGKLEIK</td>
<td></td>
</tr>
</tbody>
</table>
In some embodiments, the LRP-8 binding protein can include sequences that are at least about 80%, 90%, 95%, 99%, or 100% homologous to sequences in Tables 2-7.

In certain embodiments, the binding proteins described herein contain two or more sequences identical to or with homology to two or more VH sequences or fragments thereof. In certain embodiments, each of the VH sequences specifically binds to the same proteins. In this situation, the two VH sequences can bind to the same or different epitopes on the same protein. In other embodiments, the binding proteins described herein contain two or more sequences identical to or homologous with two or more VL sequences or fragments thereof. In certain embodiments, each of the VL sequences specifically binds to the same proteins. In this situation, the two VL sequences can bind to the same or different epitopes on the same protein. In certain embodiments, each of the VL sequences specifically binds to different proteins.
For example, the binding protein may be a bispecific or multispecific construct. The bispecific or multispecific construct may be monovalent or bivalent. Various bispecific or multispecific constructs are known in the art (see e.g., Spiess et al. (2015) Mol. Immunol. 67; 95-106). The bispecific or multispecific constructs include, but are not limited to, an asymmetric bispecific antibody, an asymmetric bispecific IgG4, a CrossMab binding protein, a DAF (dual action Fab antibody; two-in-one), a DAF (dual action Fab antibody; four-in-one), a DutMab, a DT-IgG, a knobs-in-holes binding protein, a Charge pair binding protein, a Fab-arm exchange binding protein, a SEEDbody, a Triomab (Triomab quadroma bispecific or removable bispecific), a LUZ-Y, a Fcab, a κλ-body, an iMab (innovative multimer), and an Orthogonal Fab. In some embodiments, the bispecific or multispecific construct is a DVD-Ig binding protein, an IgG(H)-scFv, an scFv-(H)IgG, an IgG(L)-scFv, an scFv-(L)IgG, an IgG(L, H)-Fv, an IgG(H)-V, a V(H)-IgG, an IgG(L)-V, a V(L)-IgG, a KIH IgG-scFab, a 2scFv-IgG, an IgG-2scFv, an scFv4-Ig, a Zybody, or a DVI-IgG (four-in-one). The bispecific or multispecific construct also can be a nanobody (or VHH), a bispecific tandem nanobody, a bispecific trivalent tandem nanobody, a nanobody-HSA, a BiTE (bispecific T-cell engager) binding protein, a Diabody, a DART (dual affinity retargeting) binding protein, a TandAb (tetravalent bispecific tandem antibody), an scDiabody, an scDiabody-CH3, a Diabody-CH3, a Triple Body, a Miniantibody, a Minibody, a TriBi minibody, an scFv-CH3 KIH, a Fab-scFv, an scFv-CH-CL-scFv, a F(ab')2, a F(ab')2 scFv2, an scFv-KIH, a Fab-scFv-Fc, a Tetraivalent HCAb, an scDiabody-Fc, a Diabody-Fc, a Tandem scFv-Fc, a Fabsc, a bsFc-1/2, a CODV-Ig (cross-over dual variable immunoglobulin), a biclonics antibody or an Intrabody. The bispecific or multispecific constructs also include, for example, a Dock and Lock binding protein, an ImmTAC, an HSAbody, an scDiabody-HSA, a Tandem scFv-Toxin, an IgG-IgG binding protein, a Cov-X-Body, and an scFvl-PEG-scFv2. In some embodiments, the bispecific or multispecific construct is a DVD-Ig binding protein, a CrossMab binding protein, a diabody, a tandem single-chain Fv molecule, a bispecific diabody, a single-chain diabody molecule, or a di-diabody. In some embodiments, the binding protein is a DVD-Ig binding protein. See, e.g., US Patent No. 7,612,181 (incorporated herein by reference in its entirety). The bispecific or multispecific construct may comprise one or more binding sites for LRP-8. The bispecific or multispecific construct may comprise binding sites only for LRP-8, or may comprise additional binding sites for other antigen
targets. The bispecific or multispecific construct may comprise binding sites for more than one epitope on LRP-8, e.g., using different CDR sets or variable domains from Tables 2-7 to form binding sites targeting different epitopes on LRP-8.

The binding proteins described herein can also be larger protein structures including three or more VH and/or VL domains, for example a triple variable domain immunoglobulin (TVD-Ig) binding protein.

The blood-brain-barrier (BBB) is layer of tightly packed endothelial cells that make up the walls of brain capillaries and prevent substances in the blood from diffusing freely into the brain. The instant disclosure improves upon the art by providing, in certain embodiments, binding proteins capable of binding a BBB antigen (LRP-8) and transcytosing into mouse and monkey brains, thereby facilitating critical preclinical testing, and would be expected to also exhibit similar activity in human brain. In certain aspects, the disclosure provides high molecular weight multivalent binding proteins (e.g., a DVD-Ig or other bispecific binding proteins) comprising at least one binding domain targeting an LRP-8 antigen combined with one or more second binding domains directed against a therapeutically relevant target. Unlike other binding proteins, the binding proteins of the disclosure may have one or more binding domains (e.g., one, two, or three binding domains) that are unoccupied upon BBB uptake such that they remain available for binding to the therapeutically relevant target molecule present in the brain. Additionally or alternatively, one or more of the binding domains may be pre-loaded with a therapeutic agent (e.g., an endogenous or exogenous therapeutic protein) to facilitate delivery of the agent to the brain. Accordingly, the binding proteins of the disclosure are well-suited for the treatment of brain and CNS diseases including, but not limited to, Alzheimer's disease (AD), Parkinson's disease (PD) or multiple sclerosis (MS). In some embodiments, the binding proteins are used to treat brain and CNS diseases including, but not limited to, Alzheimer's disease (AD), Parkinson's disease (PD) or multiple sclerosis (MS).

Also disclosed herein are methods for treating a disease or condition (e.g., a neurological and/or brain condition) in a human subject. Such methods comprise administering to an individual (human or other mammal) one or more binding proteins that bind LRP-8 or a portion thereof (e.g., CR1 and CR2), and another target.

Dual variable domain ("DVD-Ig") binding proteins of the disclosure comprise two or more antigen binding sites and may be tetravalent or multivalent binding
proteins. DVDs may be monospecific, *i.e.*, capable of binding one antigen, or multispecific, *i.e.*, capable of binding two or more antigens. A DVD-Ig binding protein comprising two heavy chain DVD polypeptides and two light chain DVD polypeptides is referred to as a "DVD immunoglobulin" or "DVD-Ig". Each half of a DVD-Ig comprises a heavy chain DVD polypeptide and a light chain DVD polypeptide, and two or more antigen binding sites. Each binding site comprises a heavy chain variable domain and a light chain variable domain with a total of six CDRs involved in antigen binding per antigen binding site. A description of the design, expression, and characterization of DVD-Ig molecules is provided in U.S. Patent No. 7,612,181 and Wu et al. (2007) *Nature Biotechnol.* 25: 1290-1297. An example of such DVD-Ig molecules comprises a heavy chain that comprises the structural formula VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CHI, X2 is an Fc region, and n is 0 or 1, but preferably 1; and a light chain that comprises the structural formula VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CHI, and X2 does not comprise an Fc region; and n is 0 or 1, but preferably 1. Such a DVD-Ig may comprise two such heavy chains and two such light chains, wherein each chain comprises variable domains linked in tandem without an intervening constant region between the variable domains, wherein a heavy chain and a light chain associate to form tandem functional antigen binding sites, and a pair of heavy and light chains may associate with another pair of heavy and light chains to form a tetrameric binding protein with four functional antigen binding sites. In another example, a DVD-Ig molecule may comprise heavy and light chains that each comprise three variable domains (VD1, VD2, VD3) linked in tandem without an intervening constant region between variable domains, wherein a pair of heavy and light chains may associate to form three antigen binding sites, and wherein a pair of heavy and light chains may associate with another pair of heavy and light chains to form a tetrameric binding protein with six antigen binding sites.

For example, the DVD-Ig binding protein can bind to an amino acid comprising the sequence of SEQ ID NO: 2 or a portion thereof. In various embodiments, the DVD-Ig binding protein binds to an amino acid comprising the sequence of SEQ ID NO: 3 or
a portion thereof. In various embodiments the DVD-Ig binding proteins binds an amino acid comprising both of the sequences of SEQ ID NO:2 and SEQ ID NO:3. A DVD-Ig binding protein may bind one or more epitopes of LRP-8, such as the CR1 region of SEQ ID NO:2. In an embodiment, a DVD-Ig binding protein may bind an epitope of LRP-8 and an epitope of a second target antigen other than an LRP-8 polypeptide.

Also disclosed herein are methods for reducing one or more symptoms of a condition associated with the brain (e.g., a condition affecting neuronal or vascular tissues) in an individual comprising administering to the individual a binding protein that binds LRP-8 or a portion thereof and also another target. In some embodiments, the binding protein is a DVD-Ig binding protein including a variable heavy chain and/or a variable light chain comprising an amino acid sequence selected from the sequences described herein, whereby one or more symptoms of the condition is reduced.

In various embodiments, a binding protein comprising an LRP-8 binding domain as described herein can be linked to a second binding domain or a second active agent (e.g., a cytotoxic agent) via a linker sequence. The linker sequence may be a single amino acid or a linker polypeptide comprising two or more amino acid residues joined by peptide bonds. In an embodiment, a linker sequence may comprise or consist of a sequence selected from the group consisting of GGGGSG (SEQ ID NO:11), GGSGG (SEQ ID NO:12), GGGSGGGGGS (SEQ ID NO:13), GGSGGGGSG (SEQ ID NO:14), GGGGGGGSG (SEQ ID NO:15), GGGGGGGGGSSGGG (SEQ ID NO:16), GGGGGGGGGGSSGGG (SEQ ID NO:17), GGGGGGGGGGGGGS (SEQ ID NO:18), ASTKGP (SEQ ID NO:19), ASTKGPSVFPLAP (SEQ ID NO:20), TVAAP (SEQ ID NO:21), RTVAAP (SEQ ID NO:22), TVAAPSVFIFPP (SEQ ID NO:23), RTVAAPSVFIFPP (SEQ ID NO:24), AKTTPKLEEGFSEAR (SEQ ID NO:25), AKTTPKLEEGFSEARV (SEQ ID NO:26), AKTTPKLGG (SEQ ID NO:27), SAKTTPKLGG (SEQ ID NO:28), SAKTTP (SEQ ID NO:29), RADAAP (SEQ ID NO:30), RADAAPTVS (SEQ ID NO:31), RADAAAAGGPGS (SEQ ID NO:32), RADAAAAGGGGSGGGGSGGGGS (SEQ ID NO:33), SAKTTPKLGGFSEARV (SEQ ID NO:34), ADAAP (SEQ ID NO:35), ADAAPTVSIFPP (SEQ ID NO:36), QPKAAP (SEQ ID NO:37), QPKAAPSVTLFPP (SEQ ID NO:38), AKTTP (SEQ ID NO:39), AKTTPSVTPLAP (SEQ ID NO:40), AKTTAP (SEQ ID NO:41), AKTAPSYYPLAP (SEQ ID NO:42),
GENKVEYAPALMALS (SEQ ID NO:43), GPAKELTPLKEAKVS (SEQ ID NO:44), and GHEAAAVMQVQYPAS (SEQ ID NO:45).

The choice of linker sequences may be based on crystal structure analysis of several Fab molecules. There is a natural flexible linkage between the variable domain and the CH1/CL constant domain in Fab or antibody molecular structure. This natural linkage comprises approximately 10-12 amino acid residues, contributed by 4-6 residues from C-terminus of V domain and 4-6 residues from the N-terminus of CL/CH1 domain. DVD-Igs described herein can be generated using N-terminal 5-6 amino acid residues, or 11-12 amino acid residues, of CL or CH1 as linker in light chain and heavy chain of DVD-Ig, respectively. The N-terminal residues of CL or CH1 domains, particularly the first 5-6 amino acid residues, adopt a loop conformation without strong secondary structures, and therefore can act as flexible linkers between the two variable domains. The N-terminal residues of CL or CH1 domains are natural extension of the variable domains, as they are part of the Ig sequences, and therefore minimize to a large extent any immunogenicity potentially arising from the linkers and junctions.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of such terms should be clear. However, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. In this application, the use of the term "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including," as well as other forms, such as "includes" and "included," is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise. All ranges shall be interpreted to include the endpoints of those ranges unless stated otherwise.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, pathology, and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art unless stated otherwise. The methods and
techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturers' specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

That the present disclosure may be more readily understood, select terms are defined below.

The term "polypeptide" means any polymeric chain of amino acids. The terms "peptide" and "protein" are used interchangeably with the term polypeptide and also refer to a polymeric chain of amino acids. The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomelic or polymeric. The term "polypeptide" encompasses fragments and variants (including fragments of variants) thereof, unless otherwise contradicted by context. For an antigenic polypeptide, a fragment of polypeptide optionally contains at least one contiguous or nonlinear epitope of polypeptide. The precise boundaries of the at least one epitope fragment can be confirmed using ordinary skill in the art. The fragment comprises at least about 5 contiguous amino acids, such as at least about 10 contiguous amino acids, at least about 15 contiguous amino acids, or at least about 20 contiguous amino acids.

The term "isolated protein" or "isolated polypeptide" means a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally associated components that accompany it in its native state; is substantially free of other proteins from the same species; is expressed by a cell from a different species; or does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may
also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

The term "recovering" means the process of rendering a chemical species such as a polypeptide substantially free of naturally associated components by isolation, e.g., using protein purification techniques well known in the art.

The term "biological activity" means all inherent biological properties of a protein, e.g., for LRP-8, mediating one or any combination of endocytosis, transcytosis, signal transduction, brain localization, spinal cord localization, placental localization, testes localization and/or travel across the blood brain barrier (BBB), for example.

The terms "specific binding" or "specifically binding" in reference to the interaction of an antibody, a binding protein, or a peptide with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody. In certain embodiments, a binding protein that specifically binds to an antigen binds to that antigen with a $K_D$ greater than $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-9}$, $10^{-10}$, $10^{-11}$, $10^{-12}$, $10^{-13}$ or $10^{-14}$ M.

In other embodiments, a binding protein that specifically binds to an antigen binds to that antigen with a $K_D$ of between $10^{-6}$ and $10^{-7}$, $10^{-6}$ and $10^{-8}$, $10^{-6}$ and $10^{-9}$, $10^{-6}$ and $10^{-10}$, $10^{-6}$ and $10^{-11}$, $10^{-6}$ and $10^{-12}$, $10^{-6}$ and $10^{-13}$, $10^{-6}$ and $10^{-14}$, $10^{-9}$ and $10^{-10}$, $10^{-9}$ and $10^{-11}$, $10^{-9}$ and $10^{-12}$, $10^{-9}$ and $10^{-13}$ or $10^{-9}$ and $10^{-14}$ M.

The term "antibody" broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art. Nonlimiting embodiments of which are discussed below.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable domain (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains: CH1, CH2, and CH3. Each light chain is comprised of a light chain variable domain (abbreviated herein as VL) and
a light chain constant region. The light chain constant region is comprised of one
domain, CL. The VH and VL regions can be further subdivided into regions of
hypervariability, termed complementarity determining regions (CDR), interspersed with
regions that are more conserved, termed framework regions (FR). Each VH and VL is
composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-
terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.
Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY),
class (e.g., IgG 1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

The term "Fc region" is used to define the C-terminal region of an
immunoglobulin heavy chain, which may be generated by papain digestion of an intact
antibody. The Fc region may be a native sequence Fc region or a variant Fc region.
The Fc region of an immunoglobulin generally comprises two constant domains, a CH2
domain, and a CH3 domain, and optionally comprises a CH4 domain. Replacements of
amino acid residues in the Fc portion to alter antibody effector function are known in
the art (U.S. Pat. Nos. 5,648,260 and 5,624,821). The Fc portion of an antibody
mediates several important effector functions, for example, cytokine induction, ADCC,
phagocytosis, complement dependent cytotoxicity (CDC), and half-life/clearance rate of
antibody and antigen-antibody complexes. In some cases these effector functions are
deletious, depending on the therapeutic objectives. Certain human IgG isotypes,
particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcyRs and
complement C3, respectively. Neonatal Fc receptors (FcRn) are the critical
components determining the circulating half-life of antibodies. In still another
embodiment at least one amino acid residue is replaced in the constant region of the
antibody, for example the Fc region of the antibody, such that effector functions of the
antibody are altered. The term "antigen-binding portion" of an antibody refers to one or
more fragments of an antibody that retain the ability to specifically bind to an antigen
(e.g., hLRP-8). Antigen-binding functions of an antibody can be performed by
fragments of a full-length antibody. Such antibody fragment embodiments may also be
incorporated in bispecific, dual specific, or multi-specific formats such as a DVD-Ig
format; specifically binding to two or more different antigens (e.g., h LRP-8 and a
different antigen molecule). Examples of binding fragments encompassed within the
term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent
fragment consisting of the VL, VH, CL, and CHI domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CHI domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al. (1989) Nature, 341: 544-546; PCT Publication No. WO 90/05144), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see, for example, Bird et al. (1988) Science 242: 423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85: 5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see, for example, Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448; Poljak (1994) Structure 2: 1121-1123; Kontermann and Dubel eds., Antibody Engineering, Springer-Verlag, N.Y. (2001), p. 790 (ISBN 3-540-41354-5). In addition single chain antibodies also include "linear antibodies" comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al. (1995) Protein Eng. 8(10): 1057-1062; and U.S. Patent No. 5,641,870).

An immunoglobulin constant (C) domain refers to a heavy (CH) or light (CL) chain constant domain. Murine and human IgG heavy chain and light chain constant domain amino acid sequences are known in the art.

The term "LRP-8 binding protein construct" (or "binding protein construct") refers to a polypeptide comprising one or more of the antigen binding portions of the disclosure linked to a linker or an immunoglobulin constant domain. A "linker polypeptide" comprises two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Such linker polypeptides are well
known in the art (see e.g., Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448; Poljak (1994) Structure 2: 1121-1123). An immunoglobulin constant domain refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences are known in the art and several are represented in Table 8. In various embodiments, the binding proteins and antibodies disclosed herein can comprise any of the constant domains listed in Table 8.

Table 8. Sequence of Human IgG Heavy Chain Constant Domain and Light Chain Constant Domain

<table>
<thead>
<tr>
<th>Protein</th>
<th>Sequence Identifier</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ig gamma-1 constant region</td>
<td>SEQ ID NO:</td>
<td>ASTKGSVFVFLAPSSSKTSTGTAALGCLVK</td>
</tr>
<tr>
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Still further, an LRP-8 binding protein, antibody, or antigen-binding portion thereof, may be part of a larger immunoadhesion molecule, formed by covalent or noncovalent association of the antibody antigen-binding portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov et al. (1995) Human Antibod. Hybridomas 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov et al. (1994) Mol. Immunol. 31:1047-1058). Antibody portions, such as Fab and F(ab’)2 fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antigen-binding portions thereof, and immunoadhesion molecules can be obtained using standard recombinant DNA techniques. An LRP-8 binding protein, such as an antigen-binding portion of an antibody may also be part of a DVD-Ig.

An "isolated antibody" is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds LRP-8 is substantially free of antibodies that specifically bind antigens other than LRP-8). An isolated antibody that specifically binds LRP-8 may, however, have cross-reactivity to other antigens, such as LRP-8 molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The term "monoclonal antibody" or "mAb" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on
the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method.

The term "human antibody" includes antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the disclosure may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. However, the term "human antibody" does not include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody" includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes, or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

The term "chimeric antibody" refers to antibodies that comprise heavy and light chain variable domain sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable domains linked to human constant regions.

The term "CDR-grafted antibody" refers to antibodies that comprise heavy and light chain variable domain sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable
domains in which one or more of the murine CDRs (e.g., CDR3) has been replaced with human CDR sequences.

The term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable domains of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable domains. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable domain capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda, M.D. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable domain of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia et al. (1987) J. Mol. Biol. 196: 901-917; and Chothia et al. (1989) Nature 342: 877-883) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2, and L3 or H1, H2, and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan et al. ((1995) FASEB J. 9:133-139) and MacCallum et al. ((1996) J. Mol. Biol. 262(5):732-745). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding.

The methods used herein may utilize CDRs defined according to any of these systems, although exemplary embodiments use Kabat or Chothia defined CDRs.

The terms "Kabat numbering", "Kabat definitions", and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy and light chain variable domains of an antibody, or an antigen binding portion thereof (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). For the heavy chain variable domain, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable domain, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

The growth and analysis of extensive public databases of amino acid sequences of variable heavy and light regions over the past twenty years have led to the understanding of the typical boundaries between framework regions (FR) and CDR sequences within variable region sequences and enabled persons skilled in this art to accurately determine the CDRs according to Kabat numbering, Chothia numbering, or other systems. See, e.g., Martin, "Protein Sequence and Structure Analysis of Antibody Variable Domains," Chapter 31, In Antibody Engineering, (Kontermann and Dubel, eds.) (Springer-Verlag, Berlin, 2001), especially pages 432-433. A useful method of determining the amino acid sequences of Kabat CDRs within the amino acid sequences of variable heavy (VH) and variable light (VL) regions is provided below:

To identify a CDR-L1 amino acid sequence: Starts approximately 24 amino acid residues from the amino terminus of the VL region; Residue before the CDR-L1 sequence is always cysteine (C); Residue after the CDR-L1 sequence is always a tryptophan (W) residue, typically Trp-Tyr-Gln (W-Y-Q), but also Trp-Leu-Gln (W-L-Q), Trp-Phe-Gln (W-F-Q), and Trp-Tyr-Leu (W-Y-L); Length is typically 10 to 17 amino acid residues.

To identify a CDR-L2 amino acid sequence: Starts always 16 residues after the end of CDR-L1; Residues before the CDR-L2 sequence are generally Ile-Tyr (I-Y), but also Val-Tyr (V-Y), Ile-Lys (I-K), and Ile-Phe (I-F); Length is always 7 amino acid residues.

To identify a CDR-L3 amino acid sequence: Starts always 33 amino acids after the end of CDR-L2; Residue before the CDR-L3 amino acid sequence is always a cysteine (C); Residues after the CDR-L3 sequence are always Phe-Gly-X-Gly (F-G-X-G) (SEQ ID NO:76), where X is any amino acid; Length is typically 7 to 11 amino acid residues.
To identify a CDR-H1 amino acid sequence: Starts approximately 31 amino acid residues from amino terminus of VH region and always 9 residues after a cysteine (C); Residues before the CDR-H1 sequence are always Cys-X-X-X-X-X-X-X (SEQ ID NO: XX), where X is any amino acid; Residue after CDR-H1 sequence is always a Trp (W), typically Trp-Val (W-V), but also Trp-Ile (W-I), and Trp-Ala (W-A); Length is typically 5 to 7 amino acid residues.

To identify a CDR-H2 amino acid sequence: Starts always 15 amino acid residues after the end of CDR-H1; Residues before CDR-H2 sequence are typically Leu-Glu-Trp-Ile-Gly (L-E-W-I-G) (SEQ ID NO: XX), but other variations also;

Residues after CDR-H2 sequence are Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala (K/R-L/I/V/F/T/A-T/S/I/A); Length is typically 16 to 19 amino acid residues.

To identify a CDR-H3 amino acid sequence: Starts always 33 amino acid residues after the end of CDR-H2 and always 3 after a cysteine (C) Residues before the CDR-H3 sequence are always Cys-X-X (C-X-X), where X is any amino acid, typically Cys-Ala-Arg (C-A-R); Residues after the CDR-H3 sequence are always Trp-Gly-X-Gly (W-G-X-G) (SEQ ID NO:78), where X is any amino acid; Length is typically 3 to 25 amino acid residues.

As used herein, the term "canonical" residue refers to a residue in a CDR or framework that defines a particular canonical CDR structure as defined by Chothia et al. ((1987) J. Mol. Biol. 196: 901-917); and Chothia et al. ((1992) J. Mol. Biol. 227: 799-817), both are incorporated herein by reference). According to Chothia et al., critical portions of the CDRs of many antibodies have nearly identical peptide backbone confirmations despite great diversity at the level of amino acid sequence. Each canonical structure specifies primarily a set of peptide backbone torsion angles for a contiguous segment of amino acid residues forming a loop.

An "affinity matured" antibody is an antibody with one or more alterations in one or more CDRs thereof which result in an improvement in the affinity of the antibody for a target antigen, compared to a parent antibody which does not possess the alteration(s). Exemplary affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. A variety of procedures for producing affinity matured antibodies are known in the art. For example, Marks et al. (1992) BioTechnology 10: 779-783 describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by...

The term "multivalent binding protein" denotes a binding protein comprising two or more antigen binding sites. A multivalent binding protein may be engineered to have three or more antigen binding sites, and is generally not a naturally occurring antibody. The term "multispecific binding protein" refers to a binding protein capable of binding two or more related or unrelated targets.

In some embodiments, the binding protein is a single chain dual variable domain immunoglobulin protein. The terms "single chain dual variable domain immunoglobulin protein" or "scDVD-Ig protein" or scFvDVD-Ig protein" refer to the antigen binding fragment of a DVD molecule that is analogous to an antibody single chain Fv fragment. scDVD-Ig proteins are described in U.S.S.N. 61/746,659; 14/141,498; and 14/141,500, incorporated herein by reference in their entireties. In an embodiment, the variable domains of a scDVD-Ig protein are antibody variable domains. In an embodiment, the variable domains are non-immunoglobulin variable domains (e.g., receptor).

In some embodiments, the binding protein is a DVD-Fab. The terms "DVD-Fab" or fDVD-Ig protein" refer to the antigen binding fragment of a DVD-Ig molecule that is analogous to an antibody Fab fragment. fDVD-Ig proteins are described in U.S.S.N. 61/746,663; 14/141,498; and 14/141,501, incorporated herein by reference in their entireties.

In some embodiments, the binding protein is a receptor DVD-Ig protein. The terms "receptor DVD-Ig protein" constructs, or "rDVD-Ig protein" refer to DVD-Ig constructs comprising at least one receptor-like binding domain. rDVD-Ig proteins are described in U.S.S.N. 61/746,616; and 14/141,499, incorporated herein by reference in their entireties.

The term "receptor domain" (RD), or receptor binding domain refers to the portion of a cell surface receptor, cytoplasmic receptor, nuclear receptor, or soluble receptor that functions to bind one or more receptor ligands or signaling molecules (e.g.,
toxins, hormones, neurotransmitters, cytokines, growth factors, or cell recognition molecules).

The terms multi-specific and multivalent IgG-like molecules or "pDVD-Ig" proteins are capable of binding two or more proteins (e.g., antigens). pDVD-Ig proteins are described in U.S.S.N. 14/141,502, incorporated herein by reference in its entirety. In certain embodiments, pDVD-Ig™ proteins are disclosed which are generated by specifically modifying and adapting several concepts. These concepts include but are not limited to: (1) forming Fc heterodimer using CH3 "knobs-into-holes" design, (2) reducing light chain missing pairing by using CH1/CL cross-over, and (3) pairing two separate half IgG molecules at protein production stage using "reduction then oxidation" approach.

In certain embodiments, a binding protein disclosed herein is a "half-DVD-Ig" comprised of one DVD-Ig heavy chain and one DVD-Ig light chain. The half-DVD-Ig™ protein preferably does not promote cross-linking observed with naturally occurring antibodies which can result in antigen clustering and undesirable activities. See U.S. Patent Publication No. 20120201746 which is incorporated by reference herein in its entirety.

In some embodiments, the binding protein is a pDVD-Ig protein. In one embodiment, a pDVD-Ig construct may be created by combining two halves of different DVD-Ig molecules, or a half DVD-Ig protein and half IgG molecule.

In some embodiments, the binding protein is an mDVD-Ig protein. As used herein "monobody DVD-Ig protein" or "mDVD-Ig protein" refers to a class of binding molecules wherein one binding arm has been rendered non-functional. mDVD-Ig proteins are described in U.S.S.N. 14/141,503, incorporated herein by reference in its entirety.

The Fc regions of the two polypeptide chains that have a formula of VDH-(X1)n-C-(X2)n may each contain a mutation, wherein the mutations on the two Fc regions enhance heterodimerization of the two polypeptide chains. In one aspect, knobs-into-holes mutations may be introduced into these Fc regions to achieve heterodimerization of the Fc regions. See Atwell et al. (1997) J. Mol. Biol. 270:26-35.

In some embodiments, the binding protein is a cross-over DVD-Ig protein. As used herein "cross-over DVD-Ig" protein or "coDVD-Ig" protein refers to a DVD-Ig protein wherein the cross-over of variable domains is used to resolve the issue of
affinity loss in the inner antigen-binding domains of some DVD-Ig molecules. coDVD-
Ig proteins are described in U.S. N. 14/141,504, incorporated herein by reference in its
entirety.

In certain embodiments, a binding protein that binds to LRP-8 (e.g., one or any
combination of human, cynomolgus, mouse and rat LRP-8) is provided as part of a
bispecific antibody. The term "bispecific antibody", as used herein, refers to full-length
antibodies that are generated by quadroma technology (see Milstein et al. (1983) Nature
305: 537-540), by chemical conjugation of two different monoclonal antibodies (see
Staerz et al. (1985) Nature 314: 628-634), or by knob-into-hole or similar approaches
which introduces mutations in the Fc region (see Holliger et al. (1993) Proc. Natl. Acad.
Sci. USA 90(14): 6444-6448), resulting in multiple different immunoglobulin species of
which only one is the functional bispecific antibody. By molecular function, a
bispecific antibody binds one antigen (or epitope) on one of its two binding arms (one
pair of HC/LC), and binds a different antigen (or epitope) on its second arm (a different
pair of HC/LC). By this definition, a bispecific antibody has two distinct antigen
binding arms (in both specificity and CDR sequences), and is monovalent for each
antigen it binds.

The term "dual-specific antibody", as used herein, refers to full-length antibodies
that can bind two different antigens (or epitopes) in each of its two binding arms (a pair
of HC/LC) (see PCT Publication No. WO 02/02773). Accordingly a dual-specific
binding protein has two identical antigen binding arms, with identical specificity and
identical CDR sequences, and is bivalent for each antigen to which it binds.

A "functional antigen binding site" of a binding protein is one that is capable of
binding a target antigen. The antigen binding affinity of the antigen binding site is not
necessarily as strong as the parent antibody from which the antigen binding site is
derived, but the ability to bind antigen must be measurable using any one of a variety of
methods known for evaluating antibody binding to an antigen. Moreover, the antigen
binding affinity of each of the antigen binding sites of a multivalent antibody herein
need not be quantitatively the same.

As used herein, the terms "donor" and "donor antibody" refer to an antibody
providing one or more CDRs. In an exemplary embodiment, the donor antibody is an
antibody from a species different from the antibody from which the framework regions
are obtained or derived. In the context of a humanized antibody, the term "donor antibody" refers to a non-human antibody providing one or more CDRs.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable domain minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable domain of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub-regions constituting a framework region.

As used herein, the terms "acceptor" and "acceptor antibody" refer to the antibody providing or nucleic acid sequence encoding at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% of the amino acid sequences of one or more of the framework regions. In some embodiments, the term "acceptor" refers to the antibody amino acid providing or nucleic acid sequence encoding the constant region(s). In yet another embodiment, the term "acceptor" refers to the antibody amino acid providing or nucleic acid sequence encoding one or more of the framework regions and the constant region(s). In a specific embodiment, the term "acceptor" refers to a human antibody amino acid or nucleic acid sequence that provides or encodes at least 80%, preferably, at least 85%, at least 90%, at least 95%, at least 98%, or 100% of the amino acid sequences of one or more of the framework regions. In accordance with this embodiment, an acceptor may contain at least 1, at least 2, at least 3, least 4, at least 5, or at least 10 amino acid residues that does (do) not occur at one or more specific positions of a human antibody. An acceptor framework region and/or acceptor constant region(s) may be, e.g., derived or obtained from a germline antibody gene, a mature antibody gene, a functional antibody (e.g., antibodies well known in the art, antibodies in development, or antibodies commercially available).
Human heavy chain and light chain acceptor sequences are known in the art. In one embodiment of the disclosure the human heavy chain and light chain acceptor sequences are selected from the sequences listed from V-base (http://vbase.mrc-cpe.cam.ac.uk/) or from EVIGT®, the international ImMunoGeneTics Information System® (http://imgt.cines.fr/textes/IMGTrepertoire/LocusGenes/). In another embodiment of the disclosure the human heavy chain and light chain acceptor sequences are selected from the sequences described in Table 3 and Table 4 of U.S. Patent Publication No. 2011/0280800, incorporated by reference herein in their entireties.

As used herein, the term "germline antibody gene" or "gene fragment" refers to an immunoglobulin sequence encoded by non-lymphoid cells that have not undergone the maturation process that leads to genetic rearrangement and mutation for expression of a particular immunoglobulin. (See, e.g., Shapiro et al. (2002) Crit. Rev. Immunol. 22(3): 183-200; Marchalonis et al. (2001) Adv. Exp. Med. Biol. 484:13-30). One of the advantages provided by various embodiments of the present disclosure stems from the recognition that germline antibody genes are more likely than mature antibody genes to conserve essential amino acid sequence structures characteristic of individuals in the species, hence less likely to be recognized as from a foreign source when used therapeutically in that species.

As used herein, the term "key" residues refer to certain residues within the variable domain that have more impact on the binding specificity and/or affinity of an antibody, in particular a humanized antibody. A key residue includes, but is not limited to, one or more of the following: a residue that is adjacent to a CDR, a potential glycosylation site (can be either N- or O-glycosylation site), a rare residue, a residue capable of interacting with the antigen, a residue capable of interacting with a CDR, a canonical residue, a contact residue between heavy chain variable domain and light chain variable domain, a residue within the Vernier zone, and a residue in the region that overlaps between the Chothia definition of a variable heavy chain CDR and the Kabat definition of the first heavy chain framework.

The term "humanized antibody" refers to antibodies that comprise heavy and light chain variable domain sequences from a non-human species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", i.e., more similar to human germline variable sequences. One type of
humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences. Also "humanized antibody" is an antibody or a variant, derivative, analog or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab')2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In an embodiment, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

A humanized antibody may be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype including without limitation IgGl, IgG2, IgG3, and IgG4. The humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using techniques well known in the art.

The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In an exemplary
embodiment, such mutations, however, will not be extensive. Usually, at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (see, e.g., Winnaker, From Genes to Clones (Verlaggesellschaft, Weinheim, Germany 1987)). In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

With respect to constructing DVD-Ig or other binding protein molecules, a "linker" is used to denote a single amino acid or a polypeptide ("linker polypeptide") comprising two or more amino acid residues joined by peptide bonds and used to link one or more antigen binding portions. Such linker polypeptides are well known in the art (see, e.g., Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448; Poljak (1994) Structure 2: 1121-1123).

As used herein, "Vernier" zone refers to a subset of framework residues that may adjust CDR structure and fine-tune the fit to antigen as described by Foote et al. (1992) J. Mol. Biol., 224: 487-499, which is incorporated herein by reference. Vernier zone residues form a layer underlying the CDRs and may impact on the structure of CDRs and the affinity of the antibody.

As used herein, the term "neutralizing" refers to neutralization of the biological activity of an antigen (e.g., LRP-8 or another antigen) when a binding protein specifically binds the antigen. A neutralizing binding protein described herein can bind to LRP-8 or another antigen resulting in the inhibition of a biological activity of the LRP-8 or other antigen. The neutralizing binding protein can bind LRP-8 or another antigen and reduce a biologically activity of the LRP-8 or other antigen by at least about 20%, 40%, 60%, 80%, 85%, or more. Inhibition of a biological activity of LRP-8 or other antigen by a neutralizing binding protein can be assessed by measuring one or more indicators of LRP-8 or other antigen biological activity well known in the art; for example, inhibition of endocytosis and/or transcytosis.
The term "activity" includes activities such as the binding specificity/affinity of a binding protein for an antigen, for example, a binding protein that specifically binds to an LRP-8 antigen and/or the neutralizing potency of an LRP-8 binding protein.

The term "epitope" includes any polypeptide determinant capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by a binding protein.

An epitope may be determined by obtaining an X-ray crystal structure of an antibody:antigen complex and determining which residues on the antigen (LRP-8) are within a specified distance of residues on the antibody of interest, wherein the specified distance is, 5 Å or less, e.g., 5Å, 4Å, 3Å, 2Å, 1Å or any distance in between. In some embodiments, the epitope is defined as a stretch of 8 or more contiguous amino acid residues along the LRP-8 sequence in which at least 50%, 70% or 85% of the residues are within the specified distance of the antibody or binding protein in the X-ray crystal structure.

In certain embodiments, a binding protein is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. Binding proteins that bind to the same or similar epitopes will likely cross-compete (one prevents the binding or modulating effect of the other). Cross-competition, however, can occur even without epitope overlap, e.g., if epitopes are adjacent in three-dimensional space and/or due to steric hindrance.


The term "K_\text{on}" (also "Kon", "kon"), as used herein, is intended to refer to the on rate constant for association of a binding protein (e.g., a DVD-Ig) to an antigen to form an association complex, e.g., binding protein/antigen complex, as is known in the art.
The "$K_o$" also is known by the terms "association rate constant", or "$ka$", as used interchangeably herein. This value indicates the binding rate of a binding protein to its target antigen or the rate of complex formation between an antibody and antigen as is shown by the equation below:

$$\text{Binding protein ("Ab") + Antigen ("Ag")} \rightarrow \text{Ab-Ag}.\$$

The term "$K_{off}$" (also "Koff, "koff), as used herein, is intended to refer to the off rate constant for dissociation, or "dissociation rate constant", of a binding protein (e.g., an DVD-Ig) from an association complex (e.g., a binding protein/antigen complex) as is known in the art. This value indicates the dissociation rate of an antibody from its target antigen or separation of Ab-Ag complex over time into free binding protein and antigen as shown by the equation below:

$$\text{Ab+Ag^}\text{Ab-Ag}.\$$

The term "$K_D$" (also "$K_a$"), as used herein, is intended to refer to the "equilibrium dissociation constant", and refers to the value obtained in a titration measurement at equilibrium, or by dividing the dissociation rate constant (Koff) by the association rate constant (Kon). The association rate constant (Kon), the dissociation rate constant (Koff), and the equilibrium dissociation constant (K) are used to represent the binding affinity of a binding protein to an antigen. Methods for determining association and dissociation rate constants are well known in the art. Using fluorescence-based techniques offers high sensitivity and the ability to examine samples in physiological buffers at equilibrium. Other experimental approaches and instruments such as a BIAcore® (biomolecular interaction analysis) assay can be used (e.g., instrument available from BIAcore International AB, a GE Healthcare company, Uppsala, Sweden). Additionally, a KinExA® (Kinetic Exclusion Assay) assay, available from Sapidyne Instruments (Boise, Id.) can also be used.

The terms "label" and "detectable label" mean a moiety attached to a specific binding partner, such as a binding protein or an analyte, e.g., to render the reaction between members of a specific binding pair, such as a binding protein and an analyte, detectable. The specific binding partner, e.g., binding protein or analyte, so labeled is referred to as "detectably labeled." Thus, the term "labeled binding protein" as used herein, refers to a protein with a label incorporated that provides for the identification of the binding protein. In an embodiment, the label is a detectable marker that can produce a signal that is detectable by visual or instrumental means, e.g., incorporation of a
radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin or streptavidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., $^3$H, $^{14}$C, $^{35}$S, $^{90}$Y, $^{99}$Tc, $^{111}$In, $^{125}$I, $^{131}$I, $^{177}$Lu, $^{166}$Ho, or $^{153}$Sm), chromogens, fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), and magnetic agents (e.g., gadolinium chelates). Representative examples of labels commonly employed for immunoassays include moieties that produce light, e.g., acridinium compounds, and moieties that produce fluorescence, e.g., fluorescein. Other labels are described herein. In this regard, the moiety itself may not be detectably labeled but may become detectable upon reaction with yet another moiety. Use of the term "detectably labeled" is intended to encompass the latter type of detectable labeling.

The term "binding protein conjugate" refers to a binding protein described herein chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. Preferably the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. When employed in the context of an immunoassay, a binding protein conjugate may be a detectably labeled antibody, which is used as the detection antibody.

The terms "crystal" and "crystallized" as used herein, refer to a binding protein (e.g., a DVD-Ig), or antigen binding portion thereof, that exists in the form of a crystal. Crystals are one form of the solid state of matter that is distinct from other forms such as the amorphous solid state or the liquid crystalline state. Crystals are composed of regular, repeating, three-dimensional arrays of atoms, ions, molecules (e.g., proteins
such as DVD-Igs, or molecular assemblies (e.g., antigen/binding protein complexes). These three-dimensional arrays are arranged according to specific mathematical relationships that are well-understood in the field. The fundamental unit, or building block, that is repeated in a crystal is called the asymmetric unit. Repetition of the asymmetric unit in an arrangement that conforms to a given, well-defined crystallographic symmetry provides the "unit cell" of the crystal. Repetition of the unit cell by regular translations in all three dimensions provides the crystal. See Giege et al., Chapter 1, In Crystallization of Nucleic Acids and Proteins, a Practical Approach, 2nd ed., Ducruix and Giege (eds.), Oxford University Press, N. Y. (1999) p. 1-16.

The term "polynucleotide" means a polymeric form of two or more nucleotides, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "isolated polynucleotide" shall mean a polynucleotide (e.g., of genomic, cDNA, or synthetic origin, or some combination thereof) that, by virtue of its origin, the "isolated polynucleotide" is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence.

The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form
of vector. However, the disclosure is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. "Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. The term "expression control sequence" as used herein refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

The term "recombinant host cell" (or simply "host cell"), is intended to refer to a cell into which exogenous DNA has been introduced. In an embodiment, the host cell comprises two or more (e.g., multiple) nucleic acids encoding antibodies, such as the host cells described in U.S. Pat. No. 7,262,028, for example. Such terms are intended to refer not only to the particular subject cell, but also to the progeny of such a cell.

Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. In an embodiment, host cells include prokaryotic and eukaryotic cells selected from any
of the Kingdoms of life. In another embodiment, eukaryotic cells include protist, fungal, plant and animal cells. In another embodiment, host cells include but are not limited to the prokaryotic cell line *Escherichia coli*; mammalian cell lines CHO, HEK 293, COS, NSO, SP2 and PER.C6; the insect cell line Sf9; and the fungal cell *Saccharomyces cerevisiae*.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1989).

The terms "regulate" and "modulate" are used interchangeably, and, as used herein, refer to a change or an alteration in the activity of a molecule of interest (e.g., the biological activity of LRP-8 or another antigen). Modulation may be an increase or a decrease in the magnitude of a certain activity or function of the molecule of interest. Exemplary activities and functions of a molecule include, but are not limited to, binding characteristics, enzymatic activity, cell receptor activation, and signal transduction.

Correspondingly, the term "modulator," as used herein, is a compound capable of changing or altering an activity or function of a molecule of interest (e.g., the biological activity of LRP-8 or another antigen). For example, a modulator may cause an increase or decrease in the magnitude of a certain activity or function of a molecule compared to the magnitude of the activity or function observed in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of at least one activity or function of a molecule. Exemplary inhibitors include, but are not limited to, proteins, peptides, antibodies, peptibodies, carbohydrates or small organic molecules. Peptibodies are described, e.g., in PCT Publication No. WO 01/83525.

The term "agonist," as used herein, refers to a modulator that, when contacted with a molecule of interest, causes an increase in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed
in the absence of the agonist. Particular agonists of interest may include, but are not limited to, LRP-8 polypeptides, nucleic acids, carbohydrates, or any other molecule that binds to LRP-8.

The terms "antagonist" and "inhibitor," as used herein, refer to a modulator that, when contacted with a molecule of interest causes a decrease in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the antagonist. Particular antagonists of interest include those that block or modulate the biological or immunological activity of human LRP-8. Antagonists and inhibitors of human LRP-8 may include, but are not limited to, proteins, nucleic acids, carbohydrates, or any other molecules, which bind to human LRP-8.

As used herein, the term "effective amount" refers to the amount of a therapy that is sufficient to reduce or ameliorate the severity and/or duration of a disorder or one or more symptoms thereof; prevent the advancement of a disorder; cause regression of a disorder; prevent the recurrence, development, onset, or progression of one or more symptoms associated with a disorder; detect a disorder; or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (e.g., prophylactic or therapeutic agent).

"Patient" and "subject" may be used interchangeably herein to refer to an animal, such as a mammal, including a primate (for example, a human, a monkey (e.g., a cynomolgus monkey), and a chimpanzee), a non-primate (for example, a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse or a whale), a bird (e.g., a duck or a goose), and a fish (e.g. zebrafish or a shark). Preferably, a patient or subject is a human, such as a human being treated or assessed for a disease, disorder or condition, a human at risk for a disease, disorder or condition, a human having a disease, disorder or condition, and/or human being treated for a disease, disorder or condition.

The term "sample," as used herein, is used in its broadest sense. A "biological sample," as used herein, includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things include, but are not limited to, humans, non-human primates, mice, rats, monkeys, dogs, rabbits and other animals. Such substances include, but are not limited to, blood (e.g., whole blood), plasma,
serum, urine, amniotic fluid, synovial fluid, endothelial cells, leukocytes, monocytes, other cells, organs, tissues, bone marrow, lymph nodes and spleen.

"Control" refers to a composition known to not contain analyte ("negative control") or to contain analyte ("positive control"). A positive control can comprise a known concentration of analyte. "Control," "positive control," and "calibrator" may be used interchangeably herein to refer to a composition comprising a known concentration of analyte. A "positive control" can be used to establish assay performance characteristics and is a useful indicator of the integrity of reagents (e.g., analytes).

"Risk" refers to the possibility or probability of a particular event occurring either presently or at some point in the future. "Risk stratification" refers to an array of known clinical risk factors that allows physicians to classify patients into a low, moderate, high or highest risk of developing a particular disease, disorder or condition.

"Specific" and "specificity" in the context of an interaction between members of a specific binding pair (e.g., an antigen (or fragment thereof) and a binding protein (or antigenically reactive fragment thereof)) refer to the selective reactivity of the interaction. The phrase "specifically binds to" and analogous phrases refer to the ability of binding proteins to bind specifically to analyte (or a fragment thereof) and not bind specifically to other entities.

"Specific binding partner" is a member of a specific binding pair. A specific binding pair comprises two different molecules, which specifically bind to each other through chemical or physical means. Therefore, in addition to antigen and binding protein specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin (or streptavidin), carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, and antibodies, including monoclonal and polyclonal antibodies as well as complexes, fragments, and variants (including fragments of variants) thereof, whether isolated or recombinantly produced.

"Variant" as used herein means a polypeptide that differs from a given polypeptide (e.g., binding proteins or LRP-8 polypeptide) in amino acid sequence by the addition (e.g., insertion), deletion, or conservative substitution of amino acids, but that
retains one or more biological activity of the given polypeptide (e.g., a variant LRP-8 may compete with wild-type LRP-8 for binding to an anti-LRP-8 binding protein). A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity and degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydrophobic index of amino acids, as understood in the art (see, e.g., Kyte et al. (1982) J. Mol. Biol. 157: 105-132).

The hydrophobic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydrophobicity indexes can be substituted and still retain protein function. In one aspect, amino acids having hydrophobic indexes of ±2 are substituted. The hydrophilicity of amino acids also can be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity (see, e.g., U.S. Patent No. 4,554,101). Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. In one aspect, substitutions are performed with amino acids having hydrophilicity values within ±2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties. "Variant" also can be used to describe a polypeptide or fragment thereof that has been differentially processed, such as by proteolysis, phosphorylation, or other post-translational modification, yet retains its biological activity or antigen reactivity, e.g., the ability to bind to LRP-8. Use of "variant" herein is intended to encompass fragments of a variant unless otherwise contradicted by context.

Alternatively or additionally, a "variant" is to be understood as a polynucleotide or protein which differs in comparison to the polynucleotide or protein from which it is derived by one or more changes in its length or sequence. The polypeptide or
polynucleotide from which a protein or nucleic acid variant is derived is also known as
the parent polypeptide or polynucleotide. The term "variant" comprises "fragments" or
"derivatives" of the parent molecule. Typically, "fragments" are smaller in length or
size than the parent molecule, whilst "derivatives" exhibit one or more differences in
their sequence in comparison to the parent molecule. Also encompassed are modified
molecules such as but not limited to post-translationally modified proteins (e.g.
glycosylated, biotinylated, phosphorylated, ubiquitinated, palmitoylated, or
proteolytically cleaved proteins) and modified nucleic acids such as methylated DNA.
Also mixtures of different molecules such as but not limited to RNA-DNA hybrids, are
encompassed by the term "variant". Typically, a variant is constructed artificially,
preferably by gene-technological means whilst the parent polypeptide or polynucleotide
is a wild-type protein or polynucleotide. However, also naturally occurring variants are
to be understood to be encompassed by the term "variant" as used herein. Further, the
variants usable in the present disclosure may also be derived from homologs, orthologs,
or paralogs of the parent molecule or from artificially constructed variant, provided that
the variant exhibits at least one biological activity of the parent molecule, i.e. is
functionally active.

Alternatively or additionally, a "variant" as used herein, can be characterized by
a certain degree of sequence identity to the parent polypeptide or parent polynucleotide
from which it is derived. More precisely, a protein variant in the context of the present
disclosure exhibits at least 80% sequence identity to its parent polypeptide. A
polynucleotide variant in the context of the present disclosure exhibits at least 80%
sequence identity to its parent polynucleotide. The term "at least 80% sequence
identity" is used throughout the specification with regard to polypeptide and
polynucleotide sequence comparisons. This expression preferably refers to a sequence
identity of at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least
85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at
least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least
98%, or at least 99% to the respective reference polypeptide or to the respective
reference polynucleotide.

The similarity of nucleotide and amino acid sequences, i.e. the percentage of
sequence identity, can be determined via sequence alignments. Such alignments can be
carried out with several art-known algorithms, preferably with the mathematical
algorithm of Karlin and Altschul (Karlin et al. (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5877), with hmmalign (HMMER package, http://hmmmer.wustl.edu/) or with the CLUSTAL algorithm (Thompson et al. (1994) Nucleic Acids Res. 22:4673-80) available e.g. on http://www.ebi.ac.uk/Tools/clustalw/ or on http://www.ebi.ac.uk/Tools/clustalw2/index.html or on http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html. Preferred parameters used are the default parameters as they are set on http://www.ebi.ac.uk/Tools/clustalw/ or http://www.ebi.ac.uk/Tools/clustalw2/index.html. The grade of sequence identity (sequence matching) may be calculated using e.g. BLAST, BLAT or BlastZ (or BlastX).

A similar algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) J. Mol. Biol. 215: 403-410. BLAST polynucleotide searches are performed with the BLASTN program, score = 100, word length = 12, to obtain polynucleotide sequences that are homologous to those nucleic acids which encode mir-146a. BLAST protein searches are performed with the BLASTP program, score = 50, word length = 3, to obtain amino acid sequences homologous to mir-146a. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used. Sequence matching analysis may be supplemented by established homology mapping techniques like Shuffle-LAGAN (Brudno et al. (2003) Bioinformatics, 19 Suppl 1: 154-162) or Markov random fields. When percentages of sequence identity are referred to in the present application, these percentages are calculated in relation to the full length of the longer sequence, if not specifically indicated otherwise.

1. Anti LRP-8 DVD-Ig Binding Proteins

In various embodiments, provided herein are DVD-Ig binding proteins that bind one or more epitopes of LRP-8 and/or another antigen other than an LRP-8 polypeptide. An exemplary embodiment of such DVD-Ig molecules comprises a heavy chain that comprises the structural formula VDl-(X1)n-VD2-C-(X2)n, wherein VDl is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker, X2 is an Fc region on the first polypeptide chain and X2 does not comprise an Fc region on the second polypeptide chain; n is independently 0 or 1 on the first and second chains; and a light chain that comprises the
structural formula VD1-(XI)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, XI is a linker, and X2 is an Fc region on the first polypeptide chain and X2 does not comprise an Fc region on the second polypeptide chain; n is independently 0 or 1 on the first and second chains. Such a DVD-Ig may comprise two such heavy chains and two such light chains, wherein each chain comprises variable domains linked in tandem without an intervening constant region between the variable domains, wherein a heavy chain and a light chain associate to form two tandem antigen binding sites, and a pair of heavy and light chains may associate with another pair of heavy and light chains to form a tetrameric binding protein with four antigen binding sites. In another embodiment, a DVD-Ig molecule may comprise heavy and light chains that each comprise three variable domains, e.g., VD1, VD2, VD3, linked in tandem without an intervening constant region between variable domains, wherein a pair of heavy and light chains may associate to form three antigen binding sites, and wherein a pair of heavy and light chains may associate with another pair of heavy and light chains to form a tetrameric binding protein with six antigen binding sites.

Each variable domain (VD) in a DVD-Ig may be obtained from one or more "parent" monoclonal antibodies that bind one or more desired antigens or epitopes, such as LRP-8 antigens or epitopes. General methods of making DVD-Ig and properties associated with DVD-Igs are described in U.S. Patent No. 8,841,417, incorporated by reference herein in its entirety. Specific methods used with the DVD-Ig specifically presented herein are provided below.

II. Use of LRP-8 Binding Proteins in Various Diseases

In some embodiments, LRP-8 binding proteins of the disclosure are useful as therapeutic molecules to treat various diseases, particularly diseases in which crossing the BBB would be advantageous for treatment, e.g., in disorders of the brain and/or spinal cord. Such LRP-8 binding proteins may bind one or more targets involved in a specific disease. Examples of such targets in various diseases are described below. In some embodiments, a binding protein disclosed herein is administered to a patient in need of treatment. In some embodiments, the patient is one who would benefit from altered LRP-8 levels and/or function. In some embodiments, administration of a binding protein does not alter normal LRP-8 biologic function, but binding of the
administered protein to LRP-8 allows for transport across the blood-brain barrier. In some embodiments, the binding protein comprises a second therapeutic agent (e.g., an agent acting on the nervous system) that is delivered across the blood-brain barrier by binding of the protein to LRP-8. In some embodiments, the binding protein is administered to a human patient. In some embodiments, the binding protein is administered to a non-human patient. In some embodiments, the binding protein exhibits cross-reactivity with LRP-8 in a non-human mammal (e.g., one or any combination of cynomolgus monkey, rat or mouse), allowing for binding and transport across the blood-brain barrier in any of these species. In certain embodiments, the binding protein comprises clone ML199.1 1H1.5B2, or the CDR and/or variable domains from that clone.

Altered expression of LRP-8 is associated with certain neurological diseases. Thus, in one aspect, an LRP-8 binding protein is used for treating a neurological disease or disorder. Neurological diseases include, but are not limited to a brain disorder, an autoimmune or inflammatory disease of the brain, an infectious disorder of the brain, a neurological disorder, a neurodegenerative disorder, a brain cancer, a brain metastasis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, multiple sclerosis, stroke, mental disorders, depression, schizophrenia, acute pain, and chronic pain.

In various embodiments, the LRP-8 binding protein comprises a second binding domain or therapeutic agent targeting a brain antigen and which benefits from transport to the brain via binding of the LRP-8 binding domain to that antigen.

In an embodiment, a disorder that may be treated by administering to a subject an LRP-8 binding protein described herein includes, but is not limited to, diabetes; uveitis; neuropathic pain; osteoarthritic pain; inflammatory pain; rheumatoid arthritis; osteoarthritis; juvenile chronic arthritis; septic arthritis; Lyme arthritis; psoriatic arthritis; reactive arthritis; spondyloarthropathy; systemic lupus erythematosus (SLE); Crohn's disease; ulcerative colitis; inflammatory bowel disease; autoimmune diabetes; insulin dependent diabetes mellitus; thyroiditis; asthma; allergic diseases; psoriasis; dermatitis; scleroderma; graft versus host disease; organ transplant rejection; acute immune disease associated with organ transplantation; chronic immune disease associated with organ transplantation; sarcoidosis; atherosclerosis; disseminated intravascular coagulation (DIC); Kawasaki's disease; Grave's disease; nephrotic syndrome; chronic fatigue syndrome; Wegener's granulomatosis; Henoch-Schoenlein
purpurea; microscopic vasculitis of the kidneys; chronic active hepatitis; autoimmune uveitis; septic shock; toxic shock syndrome; sepsis syndrome; cachexia; infectious diseases; parasitic diseases; acute transverse myelitis; Huntington's chorea; Parkinson's disease; Alzheimer's disease; stroke; primary biliary cirrhosis; hemolytic anemia; malignancies; heart failure; myocardial infarction; Addison's disease; sporadic polyglandular deficiency type I; polyglandular deficiency type II (Schmidt's syndrome); acute respiratory distress syndrome (ARDS); alopecia; alopecia areata; seronegative arthropathy; arthropathy; Reiter's disease; psoriatic arthropathy; ulcerative colitic arthropathy; enteropathic synovitis; chlamydia; Yersinia and Salmonella associated arthropathy; spondyloarthritis; atheromatous disease/arteriosclerosis; atopic allergy; autoimmune bullous disease; pemphigus vulgaris; pemphigus foliaceus; pemphigoid; linear IgA disease; autoimmune haemolytic anemia; Coombs positive haemolytic anemia; acquired pernicious anemia; juvenile pernicious anemia; myalgic encephalitis/Royal Free disease; chronic mucocutaneous candidiasis; giant cell arteritis (GCA); primary sclerosing hepatitis; cryptogenic autoimmune hepatitis; acquired immunodeficiency syndrome (AIDS); acquired immunodeficiency related diseases; hepatitis B; hepatitis C; common varied immunodeficiency (common variable hypogammaglobulinemia); dilated cardiomyopathy; female infertility; ovarian failure; premature ovarian failure; fibrotic lung disease; cryptogenic fibrosing alveolitis; post-inflammatory interstitial lung disease; interstitial pneumonitis; connective tissue disease associated interstitial lung disease; mixed connective tissue disease associated lung disease; systemic sclerosis associated interstitial lung disease; rheumatoid arthritis associated interstitial lung disease; systemic lupus erythematosus associated lung disease; dermatomyositis/polymyositis associated lung disease; Sjögren's disease associated lung disease; ankylosing spondylitis associated lung disease; vasculitic diffuse lung disease; haemosiderosis associated lung disease; drug-induced interstitial lung disease; fibrosis; radiation fibrosis; bronchiolitis obliterans; chronic eosinophilic pneumonia; lymphocytic infiltrative lung disease; postinfectious interstitial lung disease; gouty arthritis; autoimmune hepatitis; type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis); type-2 autoimmune hepatitis (anti-LKM antibody hepatitis); autoimmune mediated hypoglycemia; type B insulin resistance with acanthosis nigricans; hypoparathyroidism; osteoarthritis; primary sclerosing cholangitis; psoriasis type 1; psoriasis type 2; idiopathic leucopenia; autoimmune neutropaenia;
renal disease NOS; glomerulonephritides; microscopic vasculitis of the kidneys; Lyme disease; discoid lupus erythematosus; idiopathic male infertility; nitric oxide-associated male infertility; sperm autoimmunity; multiple sclerosis (all subtypes, including primary progressive, secondary progressive, relapsing remitting); sympathetic ophthalmia;
pulmonary hypertension secondary to connective tissue disease; Goodpasture's syndrome; pulmonary manifestation of polyarteritis nodosa; acute rheumatic fever; rheumatoid spondylitis; Still's disease; systemic sclerosis; Sjogren's syndrome; Takayasu's disease/arteritis; autoimmune thrombocytopenia (AITP); idiopathic thrombocytopenia; autoimmune thyroid disease; hyperthyroidism; goitrous autoimmune hypothyroidism (Hashimoto's disease); atrophic autoimmune hypothyroidism; primary myxoedema; phacogenic uveitis; primary vasculitis; vitiligo; acute liver disease; chronic liver disease; alcoholic cirrhosis; alcohol-induced liver injury; cholestasis; hypercholesterolemia; idiosyncratic liver disease; drug-induced hepatitis; non-alcoholic steatohepatitis; allergy; group B Streptococci (GBS) infection; mental disorders (e.g., depression and schizophrenia); Th2 Type and Th1 Type mediated diseases; acute and chronic pain (different forms of pain); cancer (such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate, and rectal cancer); hematopoietic malignancies; leukemia; lymphoma; abetalipoproteinemia; acrocyanosis; acute and chronic parasitic or infectious processes; acute leukemia; acute lymphoblastic leukemia (ALL); T-cell ALL; FAB ALL; acute myeloid leukemia (AML); acute or chronic bacterial infection; acute pancreatitis; acute renal failure; adenocarcinomas; atrial ectopic beats; AIDS dementia complex; alcohol-induced hepatitis; allergic conjunctivitis; allergic contact dermatitis; allergic rhinitis; allograft rejection; alpha-1-antitrypsin deficiency; amyotrophic lateral sclerosis; anemia; angina pectoris; anterior horn cell degeneration; anti-CD3 therapy; antiphospholipid syndrome; anti-receptor hypersensitivity reactions; aortic and peripheral aneurysms; aortic dissection; arterial hypertension; arteriosclerosis; arteriovenous fistula; ataxia; atrial fibrillation (sustained or paroxysmal); atrial flutter; atrioventricular block; B cell lymphoma; bone graft rejection; bone marrow transplant (BMT) rejection; bundle branch block; Burkitt's lymphoma; burns; cardiac arrhythmias; cardiac stun syndrome; cardiac tumors; cardiomyopathy; cardiopulmonary bypass inflammation response; cartilage transplant rejection; cerebellar cortical degenerations; cerebellar disorders; chaotic or multifocal atrial tachycardia; chemotherapy associated disorders; chronic myelocytic leukemia (CML); chronic alcoholism; chronic
inflammatory pathologies; chronic lymphocytic leukemia (CLL); chronic obstructive pulmonary disease (COPD); chronic salicylate intoxication; colorectal carcinoma; congestive heart failure; conjunctivitis; contact dermatitis; cor pulmonale; coronary artery disease; Creutzfeldt-Jakob disease; culture negative sepsis; cystic fibrosis; cytokine therapy associated disorders; dementia pugilistica; demyelinating diseases; dengue hemorrhagic fever; dermatitis; dermatologic conditions; diabetes mellitus; diabetic arteriosclerotic disease; diffuse Lewy body disease; dilated congestive cardiomyopathy; disorders of the basal ganglia; Down's syndrome in middle age; drug-induced movement disorders induced by drugs which block CNS dopamine receptors; drug sensitivity; eczema; encephalomyelitis; endocarditis; endocrinopathy; epiglottitis; Epstein-Barr virus infection; erythromelalgia; extrapyramidal and cerebellar disorders; familial hemophagocytic lymphohistiocytosis; fetal thymus implant rejection; Friedreich's ataxia; functional peripheral arterial disorders; fungal sepsis; gas gangrene; gastric ulcer; glomerular nephritis; graft rejection of any organ or tissue; gram negative sepsis; gram positive sepsis; granulomas due to intracellular organisms; hairy cell leukemia; Hallervorden-Spatz disease; Hashimoto's thyroiditis; hay fever; heart transplant rejection; hemochromatosis; hemodialysis; hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura; hemorrhage; hepatitis A; His bundle arrhythmias; HIV infection/HIV neuropathy; Hodgkin's disease; hyperkinetic movement disorders; hypersensitivity reactions; hypersensitivity pneumonitis; hypertension; hypokinetic movement disorders; hypothalamic-pituitary-adrenal axis evaluation; idiopathic Addison's disease; idiopathic pulmonary fibrosis (IPF); antibody mediated cytotoxicity; asthenia; infantile spinal muscular atrophy; inflammation of the aorta; influenza a; ionizing radiation exposure; iridocyclitis/uveitis/optic neuritis; ischemia-reperfusion injury; ischemic stroke; juvenile rheumatoid arthritis; juvenile spinal muscular atrophy; Kaposi's sarcoma; kidney transplant rejection; legionella; leishmaniasis; leprosy; lesions of the corticospinal system; lipedema; liver transplant rejection; lymphedema; malaria; malignant lymphoma; malignant histiocytosis; malignant melanoma; meningitis; meningococccemia; metabolic syndrome migraine headache; idiopathic migraine headache; mitochondrial multisystem disorder; mixed connective tissue disease; monoclonal gammopathy; multiple myeloma; multiple systems degenerations (Menzel; Dejerine-Thomas; Shy-Drager; and Machado-Joseph); myasthenia gravis; mycobacterium avium intracellulare; mycobacterium tuberculosis;
myelodysplasia syndrome; myocardial infarction; myocardial ischemic disorders; nasopharyngeal carcinoma; neonatal chronic lung disease; nephritis; nephrosis; neurodegenerative diseases; neurogenic I muscular atrophies; neutropenic fever; non-Hodgkin's lymphoma; occlusion of the abdominal aorta and its branches; occlusive arterial disorders; OKT3® therapy; orchitis/epididymitis; orchitis/vasectomy reversal procedures; organomegaly; osteoporosis; pancreas transplant rejection; pancreatic carcinoma; paraneoplastic syndrome/hypercalcemia of malignancy; parathyroid transplant rejection; pelvic inflammatory disease; perennial rhinitis; pericardial disease; peripheral atherosclerotic disease; peripheral vascular disorders; peritonitis; pernicious anemia; Pneumocystis carinii pneumonia; pneumonia; POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome); post perfusion syndrome; post pump syndrome; post-MI cardiomyopathy syndrome; preeclampsia; progressive supranuclear palsy; primary pulmonary hypertension; radiation therapy; Raynaud's phenomenon; Raynaud's disease; Refsum's disease; regular narrow QRS tachycardia; renovascular hypertension; reperfusion injury; restrictive cardiomyopathy; sarcomas; senile chorea; senile dementia of Lewy body type; seronegative arthropathies; shock; sickle cell anemia; skin allograft rejection; skin changes syndrome; small bowel transplant rejection; solid tumors; specific arrhythmias; spinal ataxia; spinocerebellar degenerations; streptococcal myositis; structural lesions of the cerebellum; subacute sclerosing panencephalitis; syncope; syphilis of the cardiovascular system; systemic anaphylaxis; systemic inflammatory response syndrome; systemic onset juvenile rheumatoid arthritis; telangiectasia; thromboangitis obliterans; thrombocytopenia; toxicity; transplants; trauma/hemorrhage; type III hypersensitivity reactions; type IV hypersensitivity; unstable angina; uremia; urosepsis; urticaria; valvular heart diseases; varicose veins; vasculitis; venous diseases; venous thrombosis; ventricular fibrillation; viral and fungal infections; viral encephalitis/aseptic meningitis; viral-associated hemophagocytic syndrome; Wernicke-Korsakoff syndrome; Wilson's disease; xenograft rejection of any organ or tissue; acute coronary syndromes; acute idiopathic polynu-ritis; acute inflammatory demyelinating polyradiculoneuropathy; acute ischemia; adult Still's disease; alopecia areata; anaphylaxis; anti-phospholipid antibody syndrome; aplastic anemia; arteriosclerosis; atopic eczema; atopic dermatitis; autoimmune dermatitis; autoimmune disorder associated with Streptococcus infection; autoimmune enteropathy;
autoimmune hearing loss; autoimmune lymphoproliferative syndrome (ALPS); autoimmune myocarditis; autoimmune premature ovarian failure; blepharitis; bronchiectasis; bullous pemphigoid; cardiovascular disease; catastrophic antiphospholipid syndrome; celiac disease; cervical spondylosis; chronic ischemia; cicatricial pemphigoid; clinically isolated syndrome (CIS) with risk for multiple sclerosis; conjunctivitis; childhood onset psychiatric disorder; dacryocystitis; dermatomyositis; diabetic retinopathy; disk herniation; disk prolapse; drug induced immune hemolytic anemia; endocarditis; endometriosis; endophthalmitis; episcleritis; erythema multiforme; erythema multiforme major; gestational pemphigoid; Guillain-Barre syndrome (GBS); hay fever; Hughes syndrome; idiopathic Parkinson's disease; idiopathic interstitial pneumonia; IgE-mediated allergy; immune hemolytic anemia; inclusion body myositis; infectious ocular inflammatory disease; inflammatory demyelinating disease; inflammatory heart disease; inflammatory kidney disease; iritis; keratitis; keratoconjunctivitis sicca; Kussmaul disease or Kussmaul-Meier disease; Landry's paralysis; Langerhan's cell histiocytosis; livedo reticularis; macular degeneration; microscopic polyangiitis; Morbus Bechterev; motor neuron disorders; mucous membrane pemphigoid; multiple organ failure; myasthenia gravis; myelodysplastic syndrome; myocarditis; nerve root disorders; neuropathy; non-A non-B hepatitis; optic neuritis; osteolysis; pauciarticular JRA; peripheral artery occlusive disease (PAOD); peripheral vascular disease (PVD); peripheral artery; disease (PAD); phlebitis; polyarteritis nodosa (or periarteritis nodosa); polychondritis; polymyalgia rheumatica; poliomyelitis; polyarticular JRA; polyendocrine deficiency syndrome; polymyositis; polymyalgia rheumatica (PMR); post-pump syndrome; primary Parkinsonism; secondary Parkinsonism; prostatitis; pure red cell aplasia; primary adrenal insufficiency; recurrent neuromyelitis optica; restenosis; rheumatic heart disease; SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis); secondary amyloidosis; shock lung; scleritis; sciatica; secondary adrenal insufficiency; silicone associated connective tissue disease; Sneddon-Wilkinson dermatosis; spondylitis ankylosans; Stevens-Johnson syndrome (SJS); systemic inflammatory response syndrome; temporal arteritis; toxoplasmonic retinitis; toxic epidermal necrolysis; transverse myelitis; TRAPS (tumor necrosis factor receptor type 1 (TNFR)-associated periodic syndrome); type B insulin resistance with acanthosis nigricans; type I allergic reaction; type II diabetes; urticaria; usual interstitial pneumonia (UIP); vernal conjunctivitis; viral retinitis; Vogt-Koyanagi-
Harada syndrome (VKH syndrome); wet macular degeneration; wound healing; or *Yersinia* and *Salmonella* associated arthropathy.

Also disclosed herein are methods of treating pain in an individual (human or other mammal) comprising the step of administering to the individual a protein that binds LRP-8 in combination with a protein that binds a non-LRP-8 antigen. In an embodiment, the binding proteins are administered in combination, for example, in a mixture, by successive administration, or by concurrent administration. In another aspect, a method of treating pain in an individual comprises the step of administering to the individual a multispecific protein that comprises at least one antigen binding site that binds LRP-8 and at least one antigen binding site that binds a non-LRP-8 antigen.

### III. Pharmaceutical Compositions

The disclosure also provides pharmaceutical compositions comprising one or more of the LRP-8 binding proteins LRP-8 of the disclosure and a pharmaceutically acceptable carrier. The pharmaceutical compositions comprising binding proteins of the disclosure are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating of a disorder or one or more symptoms thereof, and/or in research. In a specific embodiment, a composition comprises one or more antibodies of the disclosure. In another embodiment, the pharmaceutical composition comprises one or more antibodies of the disclosure and one or more prophylactic or therapeutic agents other than antibodies of the disclosure for treating a disorder in which LRP-8 activity is detrimental or in which crossing the BBB, localizing to the brain and/or localizing to the spinal cord is advantageous. In an embodiment, the prophylactic or therapeutic agents are known to be useful for or having been or currently being used in the prevention, treatment, management, or amelioration of a disorder or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier, diluent or excipient.

The LRP-8 binding proteins of the disclosure can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises a binding protein of the disclosure and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are
physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the binding protein.

Various delivery systems are known and can be used to administer one or more antibodies of the disclosure or the combination of one or more antibodies of the disclosure and a prophylactic agent or therapeutic agent useful for preventing, managing, treating, or ameliorating a disorder or one or more symptoms thereof, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the binding protein, receptor-mediated endocytosis (see, e.g., Wu et al. (1987) J. Biol. Chem. 262: 4429-4432), construction of a nucleic acid as part of a retroviral or other vector. Methods of administering a prophylactic or therapeutic agent of the disclosure include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, intratumoral administration, and mucosal administration (e.g., intranasal and oral routes). In addition, pulmonary administration can be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., U.S. Patent Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913 and 5,290,540; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entireties. In one embodiment, a binding protein of the disclosure, combination therapy, or a composition of the disclosure is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.). In a specific embodiment, prophylactic or therapeutic agents of the disclosure are administered intramuscularly, intravenously, intratumorally, orally, intranasally, pulmonary, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal, and intestinal mucosa,
etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

In an embodiment, specific binding of antibody-coupled carbon nanotubes (CNTs) to tumor cells in vitro, followed by their highly specific ablation with near-infrared (NIR) light can be used to target tumor cells. For example, biotinylated polar lipids can be used to prepare stable, biocompatible, noncytotoxic CNT dispersions that are then attached to one or two different neutralite avidin-derivatized DVD-Igs directed against one or more tumor antigens (e.g., CD22) (Chakravarty et al. (2008) Proc. Natl. Acad. Sci. USA 105:8697-8702).

In a specific embodiment, it may be desirable to administer the prophylactic or therapeutic agents of the disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, the implant being of a porous or non-porous material, including membranes and matrices, such as sialastic membranes, polymers, fibrous matrices (e.g., Tissuel®), or collagen matrices. In one embodiment, an effective amount of one or more antibodies of the disclosure antagonists is administered locally to the affected area to a subject to prevent, treat, manage, and/or ameliorate a disorder or a symptom thereof. In another embodiment, an effective amount of one or more antibodies of the disclosure is administered locally to the affected area in combination with an effective amount of one or more therapies (e.g., one or more prophylactic or therapeutic agents) other than a binding protein of the disclosure of a subject to prevent, treat, manage, and/or ameliorate a disorder or one or more symptoms thereof.

al. (1989) J. Neurosurg. 71: 105-112; U.S. Patent Nos. 5,679,377; 5,916,597; 5,912,015; 5,989,463; 5,128,326; PCT Publication Nos. WO 99/15154; and WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In an exemplary embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, p. 115-138 (1984)).


In a specific embodiment, where the composition of the disclosure is a nucleic acid encoding a prophylactic or therapeutic agent, the nucleic acid can be administered in vivo to promote expression of its encoded prophylactic or therapeutic agent, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic®, DuPont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al. (1991) Proc. Natl. Acad. Sci. USA 88: 1864-1868). Alternatively, a nucleic acid can be
introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

A pharmaceutical composition of the disclosure is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral, intranasal (*e.g.*, inhalation), transdermal (*e.g.*, topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic, such as lignocaine, to ease pain at the site of the injection.

If the compositions of the disclosure are to be administered topically, the compositions can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, *e.g.*, Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity preferably greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (*e.g.*, preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (*e.g.*, a gaseous propellant, such as FREON®) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art.

If the method of the disclosure comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the
present disclosure can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

If the method of the disclosure comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gelcaps, solutions, suspensions, and the like. Tablets or capsules can be prepared by conventional means with pharmacologically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulfate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmacologically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

The method of the disclosure may comprise pulmonary administration, e.g., by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, e.g., U.S. Patent Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; and 5,290,540; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein.
by reference their entireties. In a specific embodiment, a binding protein of the
disclosure, combination therapy, and/or composition of the disclosure is administered
using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc.,
Cambridge, Mass.).

The method of the disclosure may comprise administration of a composition
formulated for parenteral administration by injection (e.g., by bolus injection or
continuous infusion). Formulations for injection may be presented in unit dosage form
(e.g., in ampoules or in multi-dose containers) with an added preservative. The
compositions may take such forms as suspensions, solutions or emulsions in oily or
aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing
and/or dispersing agents. Alternatively, the active ingredient may be in powder form
for constitution with a suitable vehicle (e.g., sterile pyrogen-free water) before use.

The methods of the disclosure may additionally comprise of administration of
compositions formulated as depot preparations. Such long acting formulations may be
administered by implantation (e.g., subcutaneously or intramuscularly) or by
intramuscular injection. Thus, for example, the compositions may be formulated with
suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil)
or ion exchange resins, or as sparingly soluble derivatives (e.g., as a sparingly soluble
salt).

The methods of the disclosure encompass administration of compositions
formulated as neutral or salt forms. Pharmaceutically acceptable salts include those
formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic,
tartaric acids, etc., and those formed with cations such as those derived from sodium,
potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-
ethylamino ethanol, histidine, procaine, etc.

Generally, the ingredients of compositions are supplied either separately or
mixed together in unit dosage form, for example, as a dry lyophilized powder or water
free concentrate in a hermetically sealed container such as an ampoule or sachet
indicating the quantity of active agent. Where the mode of administration is infusion,
composition can be dispensed with an infusion bottle containing sterile pharmaceutical
grade water or saline. Where the mode of administration is by injection, an ampoule of
sterile water for injection or saline can be provided so that the ingredients may be mixed
prior to administration.
In particular, the disclosure also provides that one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the disclosure is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. In one embodiment, one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the disclosure is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. Preferably, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the disclosure is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, at least 100 mg or at least 200 mg/mL. The lyophilized prophylactic or therapeutic agents or pharmaceutical compositions of the disclosure should be stored at between 2°C and 8°C in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions of the disclosure should be administered within 1 week, preferably within 5 days, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the disclosure is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. Preferably, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, more preferably at least 0.5 mg/ml, at least 1 mg/ml, at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml, at least 100 mg/ml or at least 200 mg/mL. The liquid form should be stored at between 2°C and 8°C in its original container.

The binding protein of the disclosure can be incorporated into a pharmaceutical composition suitable for parenteral administration. Preferably, the binding protein will be prepared as an injectable solution containing 0.1-250 mg/ml binding protein. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampoule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10 mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or
potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10%, mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05%> polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants. The pharmaceutical composition comprising a binding protein of the disclosure prepared as an injectable solution for parenteral administration, can further comprise an agent useful as an adjuvant, such as those used to increase the absorption, or dispersion of a therapeutic protein (e.g., DVD-Ig). A particularly useful adjuvant is hyaluronidase (such as Hylenex® recombinant human hyaluronidase). Addition of hyaluronidase in the injectable solution improves human bioavailability following parenteral administration, particularly subcutaneous administration. It also allows for greater injection site volumes (i.e., greater than 1 ml) with less pain and discomfort, and minimum incidence of injection site reactions (see, PCT Publication No. WO 2004/078140 and US Publication No. 2006/104968).

The compositions provided in this disclosure may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In an exemplary embodiment, the binding protein is administered by intravenous infusion or injection. In another preferred embodiment, the binding protein is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to
high drug concentration. Sterile injectable solutions can be prepared by incorporating
the active compound (i.e., binding protein) in the required amount in an appropriate
solvent with one or a combination of ingredients enumerated above, as required,
followed by filtered sterilization. Generally, dispersions are prepared by incorporating
the active compound into a sterile vehicle that contains a basic dispersion medium and
the required other ingredients from those enumerated above. In the case of sterile,
lyophilized powders for the preparation of sterile injectable solutions, the preferred
methods of preparation are vacuum drying and spray-drying that yields a powder of the
active ingredient plus any additional desired ingredient from a previously sterile-filtered
solution thereof. The proper fluidity of a solution can be maintained, for example, by
the use of a coating such as lecithin, by the maintenance of the required particle size in
the case of dispersion and by the use of surfactants. Prolonged absorption of injectable
compositions can be brought about by including, in the composition, an agent that
delays absorption, for example, monostearate salts and gelatin.

The binding proteins of the present disclosure can be administered by a variety
of methods known in the art, although for many therapeutic applications, the preferred
route/mode of administration is subcutaneous injection, intravenous injection or
infusion. As will be appreciated by the skilled artisan, the route and/or mode of
administration will vary depending upon the desired results. In certain embodiments,
the active compound may be prepared with a carrier that will protect the compound
against rapid release, such as a controlled release formulation, including implants,
transdermal patches, and microencapsulated delivery systems. Biodegradable,
biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides,
polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the
preparation of such formulations are patented or generally known to those skilled in the
art. See, e.g., Robinson, J.R. (ed.) Sustained and Controlled Release Drug Delivery

In certain embodiments, a binding protein of the disclosure may be orally
administered, for example, with an inert diluent or an assimilable edible carrier. The
compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell
gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet.
For oral therapeutic administration, the compounds may be incorporated with excipients
and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs,
suspensions, syrups, wafers, and the like. To administer a compound of the disclosure by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, a binding protein of the disclosure is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders in which LRP-8 activity is detrimental. For example, an anti-human LRP-8 binding protein of the disclosure may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (e.g., antibodies that bind other cytokines or that bind cell surface molecules).

Furthermore, one or more antibodies of the disclosure may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

In certain embodiments, a binding protein to LRP-8 or fragment thereof is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in U.S. Patent No. 6,660,843, which is hereby incorporated by reference for any purpose.

In a specific embodiment, nucleic acid sequences comprising nucleotide sequences encoding a binding protein of the disclosure or another prophylactic or therapeutic agent of the disclosure are administered to treat, prevent, manage, or ameliorate a disorder or one or more symptoms thereof by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the disclosure, the nucleic acids produce their encoded binding protein or prophylactic or therapeutic agent of the disclosure that mediates a prophylactic or therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present disclosure. Detailed descriptions of various methods of gene therapy are disclosed in US Publication No. 2005/0042664, which is incorporated herein by reference.

A binding protein of the disclosure also can be administered with one or more additional agents useful in the treatment of various diseases, or conjugated to one or
more such agent. Binding proteins of the disclosure, can be used alone or in combination to treat such diseases. It should be understood that the binding proteins of the disclosure can be used alone or in combination with an additional agent, e.g., a therapeutic agent, the additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the binding protein of the present disclosure. The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition, e.g., an agent that affects the viscosity of the composition.

It should further be understood that the combinations which are to be included within this disclosure are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations, which are part of this disclosure, can be the antibodies of the present disclosure and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

Preferred combinations are non-steroidal anti-inflammatory drug(s) also referred to as NSAIDS which include drugs like ibuprofen. Other preferred combinations are corticosteroids including prednisolone; the well-known side-effects of steroid use can be reduced or even eliminated by tapering the steroid dose required when treating patients in combination with the anti-LRP-8 binding proteins of this disclosure. Non-limiting examples of therapeutic agents for rheumatoid arthritis with which a binding protein can be combined include, but are not limited to, the following: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-3, JL-4, JL-5, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, IL-21, interferons, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the disclosure, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, CTLA or their ligands including CD 154 (gp39 or CD40L).

Preferred combinations of therapeutic agents may interfere at different points in the autoimmune and subsequent inflammatory cascade; preferred examples include
TNF antagonists like chimeric, humanized or human TNF antibodies, D2E7, (PCT Publication No. WO 97/29131), CA2 (Remicade™), CDP 571, and soluble p55 or p75 TNF receptors, derivatives, thereof, (p75TNFRlgG (Enbrel™) or p55TNFRlgG (Lenercept), and also TNFa converting enzyme (TACE) inhibitors; similarly IL-1 inhibitors (Interleukin-1 -converting enzyme inhibitors, IL-IRA etc.) may be effective for the same reason. Other preferred combinations include Interleukin 11. Yet another preferred combination are other key players of the autoimmune response which may act parallel to, dependent on or in concert with LRP-8 function. Yet another preferred combination are non-depleting anti-CD4 inhibitors. Yet other preferred combinations include antagonists of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2) including antibodies, soluble receptors or antagonistic ligands.

The binding proteins of the disclosure may also be combined with agents, such as methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquine/hydroxychloroquine, pencillamine, aurothiolumate (intramuscular and oral), azathioprine, colchicine, corticosteroids (oral, inhaled and local injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signaling by proinflammatory cytokines such as TNF-a or IL-1 (e.g., IRAK, NIK, IKK, p38, or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TNFa converting enzyme (TACE) inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulphasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g., soluble p55 or p75 TNF receptors and the derivatives p75TNFRlgG (Enbrel™) and p55TNFRlgG (Lenercept)), sIL-1RI, sIL-1RII, sIL-6R), anti-inflammatory cytokines (e.g., IL-4, IL-10, IL-11, IL-13 and TGFβ), celecoxib, folic acid, hydroxychloroquine sulfate, rofecoxib, etanercept, infliximab, naproxen, valdecoxib, sulfasalazine, methylprednisolone, meloxicam, methylprednisolone acetate, gold sodium thiomolate, aspirin, triamcinolone acetonide, propoxyphene napsylate/apap, folate, nabumetone, diclofenac, piroxicam, etodolac, diclofenac sodium, oxaprozin, oxycodone HC1, hydrocodone bitartrate/apap, diclofenac.
sodium/misoprostol, fentanyl, anakinra, human recombinant, tramadol HC1, salsalate, sulindac, cyanocobalamin/fa/pyridoxine, acetaminophen, alendronate sodium, prednisolone, morphine sulfate, lidocaine hydrochloride, indomethacin, glucosamine sulffrondroitin, amitriptyline HC1, sulfadiazine, oxycodone HC1/acetaminophen, olopatadine HC1, misoprostol, naproxen sodium, omeprazole, cyclophosphamide, rituximab, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-18, anti-IL15, BIRB-796, SCIO-469, VX-702, AMG-548, VX-740, Roflumilast, IC-485, CDC-801, and Mesopram. Preferred combinations include methotrexate or leflunomide in moderate or severe rheumatoid arthritis cases, cyclosporine.

Non-limiting additional agents which can also be used in combination with a binding protein to treat rheumatoid arthritis (RA) include, but are not limited to, the following: non-steroidal anti-inflammatory drug(s) (NSAIDs); cytokine suppressive anti-inflammatory drug(s) (CSAIDs); CDP-571/BAY-10-3356 (humanized anti-TNFa antibody; Celltech/Bayer); cA2/infliximab (chimeric anti-TNFa antibody; Centocor); 75 kdTNFR-IgG/etanercept (75 kD TNF receptor-IgG fusion protein; Immunex; see e.g., Moreland et al., Abstract No. 813 (1994) Arthritis Rheum., 37:5295; Baumgartner et al., (1996) J. Invest. Med., 44(3):235A; 55 kdTNF-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); IDEC-CE9.1/SB 210396 (non-depleting primatized anti-CD4 antibody; IDEC/SmithKline; see e.g., Kaine et al., Abstract No. 195 (1995) Arthritis Rheum., 38:5185); DAB 486-IL-2 and/or DAB 389-IL-2 (IL-2 fusion proteins; Seragen; see e.g., Sewell et al., (1993) Arthritis Rheum., 36(9):1223-1233); Anti-Tac (humanized anti-IL-2Ra; Protein Design Labs/Roche); IL-4 (anti-inflammatory cytokine; DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10, anti-inflammatory cytokine; DNAX/Schering); IL-4; IL-10 and/or IL-4 agonists (e.g., agonist antibodies); IL-IRA (IL-1 receptor antagonist; Synergy/Amgem); anakinra (Kinaret®/Amgen); TNF-bp/s-TNF (soluble TNF binding protein; see e.g., Evans et al., Abstract No. 1540 (1996) Arthritis Rheum., 39(9) (supplement): 5284); Kapadia et al., (1995) Amer. J. Physiol. Heart and Circulatory Physiology, 268: H517-H525); RP73401 (phosphodiesterase Type IV inhibitor; see e.g., Chikanza et al., Abstract No. 1527 (1996) Arthritis Rheum., 39(9) (supplement): 5282); MK-966 (COX-2 Inhibitor; see e.g., Erich et al., Abstract Nos. 328 and 329 (1996) Arthritis Rheum., 39(9)(supplement):S81); Iloprost (see e.g., Scholz P., Abstract No. 336 (1996) Arthritis Rheum., 39(9) (supplement) :S82); methotrexate; thalidomide (see e.g., Lee et al.,
Abstract No. 1524 (1996) Arthritis Rheum., 39(9)(supplement):5282) and thalidomide-related drugs (e.g., Celgen); leflunomide (anti-inflammatory and cytokine inhibitor; see e.g., Finnegan et al., Abstract No. 627 (1996) Arthritis Rheum., 39(9)(supplement):S131); Thoss et al., (1996) Inflamm. Res., 45:103-107); tranexamic acid (inhibitor of plasminogen activation; see e.g., Ronday et al., Abstract No. 1541 (1996) Arthritis Rheum., 39(9)(supplement):5284); T-614 (cytokine inhibitor; see e.g., Hara et al., Abstract No. 1526 (1996) Arthritis Rheum., 39(9)(supplement):5282); prostaglandin E1 (see e.g., Moriuchi et al., Abstract No. 1528 (1996) Arthritis Rheum., 39(9)(supplement):5282); Tenidap (non-steroidal anti-inflammatory drug; see e.g., Guttadauria, M., Abstract No. 1516 (1996) Arthritis Rheum., 39(9)(supplement):5280); Naproxen (non-steroidal anti-inflammatory drug; see e.g., Fiebich et al., (1996) Neuro. Report, 7:1209-1213); Meloxicam (non-steroidal anti-inflammatory drug); Ibuprofen (non-steroidal anti-inflammatory drug); Piroxicam (non-steroidal anti-inflammatory drug); Diclofenac (non-steroidal anti-inflammatory drug); Indomethacin (non-steroidal anti-inflammatory drug); Sulfasalazine (see e.g., Farr et al., Abstract No. 1519 (1996) Arthritis Rheum., 39(9)(supplement):S281); Azathioprine (see e.g., Hickey et al., Abstract No. 1521 (1996) Arthritis Rheum., 39(9)(supplement):S281); ICE inhibitor (inhibitor of the enzyme interleukin-1β converting enzyme); zap-70 and/or lck inhibitor (inhibitor of the tyrosine kinase zap-70 or lck); VEGF inhibitor and/or VEGF-R inhibitor (inhibitors of vascular endothelial cell growth factor or vascular endothelial cell growth factor receptor; inhibitors of angiogenesis); corticosteroid anti-inflammatory drugs (e.g., SB203580); TNF-convertase inhibitors; anti-IL-12 antibodies; anti-IL-18 antibodies; interleukin-1 (see e.g., Keith Jr. et al., Abstract No. 1613 (1996) Arthritis Rheum., 39(9)(supplement):S296); interleukin-1 (see e.g., Bessis et al., Abstract No. 1681 (1996) Arthritis Rheum., 39(9)(supplement):5308); interleukin-17 inhibitors (see e.g., Lotz et al. Abstract No. 559 (1996) Arthritis Rheum., 39(9)(supplement):5120); gold; penicillamine; chloroquine; chlorambucil; hydroxychloroquine; cyclosporine; cyclophosphamide; total lymphoid irradiation; anti-thymocyte globulin; anti-CD4 antibodies; CD5-toxins; orally-administered peptides and collagen; lobenzarit disodium; Cytokine Regulating Agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); ICAM-1 antisense phosphorothioate oligo-deoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); prednisone; orgotein; glycosaminoglycan polysulphate; minocycline; anti-IL2R
antibodies; marine and botanical lipids (fish and plant seed fatty acids; see e.g., DeLuca et al., (1995) Rheum. Dis. Clin. North Am., 21:759-777); auranofin; phenylbutazone; meclofenamic acid; flufenamic acid; intravenous immune globulin; zileuton; azaribine; mycophenolic acid (RS-61443); tacrolimus (FK-506); sirolimus (rapamycin); amiprilose (therafectin); cladribine (2-chlorodeoxyadenosine); methotrexate; bcl-2 inhibitors (see Bruncko et al., (2007) J. Med. Chem., 50(4):641-662); antivirals and immune modulating agents.

In one embodiment, the binding protein described herein is administered in combination with one of the following agents for the treatment of rheumatoid arthritis (RA): small molecule inhibitor of KDR, small molecule inhibitor of Tie-2; methotrexate; prednisone; celecoxib; folic acid; hydroxychloroquine sulfate; rofecoxib; etanercept; infliximab; leflunomide; naproxen; valdecoxib; sulfasalazine; methylprednisolone; ibuprofen; meloxicam; methylprednisolone acetate; gold sodium thiомalate; aspirin; azathioprine; triamcinolone acetonide; propoxyphene napsylate/apap; folate; nabumetone; diclofenac; piroxicam; etodolac; diclofenac sodium; oxaprozin; oxycodone HC1; hydrocodone bitartrate/apap; diclofenac sodium/misoprostol; fentanyl; anakinra, human recombinant; tramadol HC1; salsalate; sulindac; cyanocobalamin/fa/pyridoxine; aceterminophen; alendronate sodium; prednisolone; morphine sulfate; lidocaine hydrochloride; indomethacin; glucosamine sulfate/chondroitin; cyclosporine; amitriptyline HC1; sulfadiazine; oxycodone HC1 /acetaminophen; olopataidine HC1; misoprostol; naproxen sodium; omeprazole; mycophenolate mofetil; cyclophosphamide; rituximab; IL-1 TRAP; MRA; CTLA4-IG; IL-18 BP; IL-12/23; anti-IL 18; anti-IL 15; BIRB-796; SCIO-469; VX-702; AMG-548; VX-740; Roflumilast; IC-485; CDC-801; and mesopram.

Non-limiting examples of therapeutic agents for inflammatory bowel disease with which a binding protein of the disclosure can be combined include the following: budesonide; epidermal growth factor; corticosteroids; cyclosporin, sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL- Iβ mAbs; anti-IL-6 mAbs; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-17, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the
disclosure, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands. The binding proteins of the disclosure may also be combined with agents, such as methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signaling by proinflammatory cytokines such as TNFa or IL-1 (e.g., IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TNFa converting enzyme inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g., soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R) and anti-inflammatory cytokines (e.g., IL-4, IL-10, IL-1β, IL-13 and TGFp) and bcl-2 inhibitors.

Non-limiting examples of therapeutic agents for multiple sclerosis (MS) with which binding proteins of the disclosure can be combined include the following: corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; tizanidine; interferon-pia (AVONEX; Biogen); interferon-pib (BETASERON; Chiron/Berlex); interferon a-n3 (Interferon Sciences/Fujimoto), interferon-a (Alfa Wassermann/J&J), interferon β1 A-IF (Serono/Inhale Therapeutics), Peginterferon a 2b (Enzon/Schering-Plough), Copolymer 1 (Cop-1; COPAXONE; Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; clabribine; antibodies to or antagonists of other human cytokines or growth factors and their receptors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-23, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Binding proteins of the disclosure can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD19, CD20, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. Binding proteins of the disclosure, may also be combined with agents, such as methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signaling by proinflammatory cytokines such as TNFa or IL-1 (e.g., IRAK, NIK, IKK, p38 or...
MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TACE inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g., soluble p55 or p75 TNF receptors, sIL-1RI, sIL-IRII, sIL-6R), anti-inflammatory cytokines (e.g., IL-4, IL-10, IL-13 and TGFP) and bcl-2 inhibitors.

Examples of therapeutic agents for multiple sclerosis with which binding proteins of the disclosure can be combined include interferon-β, for example, IFNpia and IFNpib; Copaxone; corticosteroids; caspase inhibitors, for example inhibitors of caspase-1; IL-1 inhibitors; TNF inhibitors; and antibodies to CD40 ligand and CD80.

The binding proteins of the disclosure, may also be combined with agents, such as alemtuzumab, dronabinol, Unimed, daclizumab, mitoxantrone, xaliproden hydrochloride, fampridine, glatiramer acetate, natalizumab, sinnabidol, a-immunokine NNS03, ABR-215062, AnergiX.MS, chemokine receptor antagonists, BBR-2778, calagualine, CPI-1189, LEM (liposome encapsulated mitoxantrone), THC.CBD (cannabinoid agonist) MBP-8298, mesopram (PDE4 inhibitor), MNA-715, anti-IL-6 receptor antibody, neurovax, pirfenidone allotrap 1258 (RDP-1258), sTNF-R1, talampanel, teriflunomide, TGF-beta2, tiplimotide, VLA-4 antagonists (for example, TR-14035, VLA4 Ultrahaler, Antegran-ELAN/Biogen), interferon gamma antagonists, IL-4 agonists.

Non-limiting examples of therapeutic agents for angina with which binding proteins of the disclosure can be combined include the following: aspirin, nitroglycerin, isosorbide mononitrate, metoprolol succinate, atenolol, metoprolol tartrate, amlodipine besylate, diltiazem hydrochloride, isosorbide dinitrate, clopidogrel bisulfate, nifedipine, atorvastatin calcium, potassium chloride, furosemide, simvastatin, verapamil HC1, digoxin, propranolol hydrochloride, carvedilol, lisinopril, spironolactone, hydrochlorothiazide, enalapril maleate, nadolol, ramipril, enoxaparin sodium, heparin sodium, valsartan, sotalol hydrochloride, fenofibrate, ezetimibe, bumetanide, losartan potassium, lisinopril/hydrochlorothiazide, felodipine, captopril, bisoprolol fumarate.

Non-limiting examples of therapeutic agents for ankylosing spondylitis with which binding proteins of the disclosure can be combined include the following: ibuprofen, diclofenac and misoprostol, naproxen, meloxicam, indomethacin, diclofenac,
celecoxib, rofecoxib, sulfasalazine, methotrexate, azathioprine, minocyclin, prednisone, etanercept, infliximab.

Non-limiting examples of therapeutic agents for asthma with which binding proteins of the disclosure can be combined include the following: albuterol, salmeterol/fluticasone, montelukast sodium, fluticasone propionate, budesonide, prednisone, salmeterol xinafoate, levalbuterol HCl, albuterol sulfate/ipratropium, prednisolone sodium phosphate, triamcinolone acetonide, beclomethasone dipropionate, ipratropium bromide, azithromycin, pirbuterol acetate, prednisolone, theophylline anhydrous, methylprednisolone sodium succinate, clarithromycin, zafirlukast, formoterol fumarate, influenza virus vaccine, methylprednisolone, amoxicillin trihydrate, flunisolide, allergy injection, cromolyn sodium, fexofenadine hydrochloride, flunisolide/menthol, amoxicillin/clavulanate, levofoxacin, inhaler assist device, guaifenesin, dexamethasone sodium phosphate, moxifloxacin HCl, doxycycline hyclate, guaifenesin/d-methorphan, p-ephedrine/code/chlorphenir, gatifloxacin, cetirizine hydrochloride, mometasone furoate, salmeterol xinafoate, benzonatate, cephalexin, pe/hydrocodone/chlorphenir, cetirizine HCl/pseudoephed, phenylephrine/code/promethazine, codeine/promethazine, cefprozil, dexamethasone, guaifenesin/pseudoephedrine, chlorpheniramine/hydrocodone, nedocromil sodium, terbutaline sulfate, epinephrine, methylprednisolone, metaproterenol sulfate.

Non-limiting examples of therapeutic agents for COPD with which binding proteins of the disclosure can be combined include the following: albuterol sulfate/ipratropium, ipratropium bromide, salmeterol/fluticasone, albuterol, salmeterol xinafoate, fluticasone propionate, prednisone, theophylline anhydrous, methylprednisolone sodium succinate, montelukast sodium, budesonide, formoterol fumarate, triamcinolone acetonide, levofoxacin, guaifenesin, azithromycin, beclomethasone dipropionate, levalbuterol HCl, flunisolide, ceftriaxone sodium, amoxicillin trihydrate, gatifloxacin, zafirlukast, amoxicillin/clavulanate, flunisolide/menthol, chlorpheniramine/hydrocodone, metaproterenol sulfate, methylprednisolone, mometasone furoate, p-ephedrine/code/chlorphenir, pirbuterol acetate, p-ephedrine/loratadine, terbutaline sulfate, tiotropium bromide, (R,R)-formoterol, TgAAT, Cilomilast, Roflumilast.

Non-limiting examples of therapeutic agents for HCV with which binding proteins of the disclosure can be combined include the following: Interferon-alpha-2a,
Interferon-alpha-2b, Interferon-alpha conl, Interferon-alpha-nl, PEGylated interferon-alpha-2a, PEGylated interferon-alpha-2b, ribavirin, Peginterferon alfa-2b+ribavirin, Ursodeoxycholic Acid, Glycyrrhizic Acid, Thymalfasin, Maxamine, VX-497 and any compounds that are used to treat HCV through intervention with the following targets:

- HCV polymerase
- HCV protease
- HCV helicase
- HCV IRES (internal ribosome entry site)

Non-limiting examples of therapeutic agents for idiopathic pulmonary fibrosis with which binding proteins of the disclosure can be combined include the following:

- prednisone
- azathioprine
- albuterol
- colchicine
- albuterol sulfate
- digoxin
- interferon
- methylprednisolone sod succ
- lorazepam
- furosemide
- lisinopril
- nitroglycerin
- spironolactone
- cyclophosphamide
- ipratropium bromide
- actinomycin d
- alteplase
- fluticasone propionate
- levofloxacin

Non-limiting examples of therapeutic agents for myocardial infarction with which binding proteins of the disclosure can be combined include the following:

- aspirin
- nitroglycerin
- metoprolol tartrate
- enoxaparin sodium
- heparin sodium
- clopidogrel bisulfate
- carvedilol
- atenolol
- morphine sulfate
- metoprolol succinate
- warfarin sodium
- lisinopril
- isosorbide mononitrate
- digoxin
- furosemide
- simvastatin
- ramipril
- tenecteplase
- enalapril maleate
- torsemide
- retavase
- losartan potassium
- quinapril HCl/mag carb
- bumetanide
- alteplase
- enalaprilat
- amiodarone hydrochloride
- tirofiban HCl m-hydrate
- diltiazem hydrochloride
- captopril
- irbesartan
- valsartan
- propranolol hydrochloride
- fosinopril sodium
- lidocaine hydrochloride
- eptifibatide
- cefazolin sodium
- atropine sulfate
- aminocaproic acid
- spironolactone
- interferon
- sotalol hydrochloride
- potassium chloride
- docusate sodium
- dobutamine HCl
- alprazolam
- pravastatin sodium
- atorvastatin calcium
- midazolam hydrochloride
- meperidine hydrochloride
- isosorbide dinitrate
- epinephrine
- dopamine hydrochloride
- bivalirudin
- rosvastatin
- ezetimibe/simvastatin
- avasimibe
- cariperide

Non-limiting examples of therapeutic agents for psoriasis with which binding proteins of the disclosure can be combined include the following:

- small molecule inhibitor of KDR
- small molecule inhibitor of Tie-2
- calcipotriene
- clobetasol propionate
- triamcinolone acetonide
- halobetasol propionate
- tazarotene
- methotrexate
- fluocinonide
- betamethasone diprop augmented
- fluocinolone acetonide
- acitretin
- tar
shampoo, betamethasone valerate, mometasone furoate, ketoconazole,
pramoxine/fluocinolone, hydrocortisone valerate, flurandrenolide, urea, betamethasone,
clobetasol propionate/emoll, fluticasone propionate, azithromycin, hydrocortisone,
moisturizing formula, folic acid, desonide, pimecrolimus, coal tar, diflorasone diacetate,
etanercept folate, lactic acid, methoxsalen, hc/bismuth subgal/znox/resor,
methylprednisolone acetate, prednisone, sunscreen, halcinonide, salicylic acid,
anthralin, clocortolone pivalate, coal extract, coal tar/salicylic acid, coal tar/salicylic acid/sulfur, desoximetasone, diazepam, emollient, fluocinonide/emollient, mineral oil/castor oil/na lact, mineral oil/peanut oil, petroleum/isopropyl myristate, psoralen,
salicylic acid, soap/tribromsalan, thimerosal/boric acid, celecoxib, infliximab,
cyclosporine, alefacect, efalizumab, tacrolimus, pimecrolimus, PUVA, UVB,
sulfasalazine.

Non-limiting examples of therapeutic agents for psoriatic arthritis with which
binding proteins of the disclosure can be combined include the following: methotrexate,
etanercept, rofecoxib, celecoxib, folic acid, sulfasalazine, naproxen, leflunomide,
methylprednisolone acetate, indomethacin, hydroxychloroquine sulfate, prednisone,
sulindac, betamethasone diprop augmented, infliximab, methotrexate, folate,
triamcinolone acetonide, diclofenac, dimethylsulfoxide, piroxicam, diclofenac sodium,
ketoprofen, meloxicam, methylprednisolone, nabumetone, tolmetin sodium,
calcipotriene, cyclosporine, diclofenac sodium/misoprostol, fluocinonide, glucosamine sulfate, gold sodium thiomalate, hydrocodone bitartrate/apap, ibuprofen, risedronate sodium, sulfadiazine, thioguanine, valdecoxib, alefacect, efalizumab and bcl-2 inhibitors.

Non-limiting examples of therapeutic agents for restenosis with which binding
proteins of the disclosure can be combined include the following: sirolimus, paclitaxel,
everolimus, tacrolimus, Zotarolimus, aceterminophen.

Non-limiting examples of therapeutic agents for sciatica with which binding
proteins of the disclosure can be combined include the following: hydrocodone bitartrate/apap, rofecoxib, cyclobenzaprine HCl, methylprednisolone, naproxen,
ibuprofen, oxycodeone HCl /aceterminophen, celecoxib, valdecoxib, methylprednisolone acetate, prednisone, codeine phosphate/apap, tramadol HCl /aceterminophen,
metaxalone, meloxicam, methocarbamol, lidocaine hydrochloride, diclofenac sodium,
gabapentin, dexamethasone, carisoprodol, ketorolac tromethamine, indomethacin,
acetaminophen, diazepam, nabumetone, oxycodone HCl, tizanidine HCl, diclofenac sodium/misoprostol, propoxyphene napsylate/apap, asa/oxycod/oxycodone ter, ibuprofen/hydrocodone bit, tramadol HCl, etodolac, propoxyphene HCl, amitriptyline HCl, carisoprodol/codeine phos/asa, morphine sulfate, multivitamins, naproxen sodium, orphenadrine citrate, temazepam.

Examples of therapeutic agents for SLE (lupus) with which binding proteins of the disclosure can be combined include the following: NSAIDS, for example, diclofenac, naproxen, ibuprofen, piroxicam, indomethacin; COX2 inhibitors, for example, Celecoxib, rofecoxib, valdecoxib; anti-malarials, for example, hydroxychloroquine; Steroids, for example, prednisone, prednisolone, budesonide, dexamethasone; cytotoxics, for example, azathioprine, cyclophosphamide, mycophenolate mofetil, methotrexate; inhibitors of PDE4 or purine synthesis inhibitor, for example Cellcept. Binding proteins of the disclosure, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid, olsalazine, Imuran and agents which interfere with synthesis, production or action of proinflammatory cytokines such as IL-1, for example, caspase inhibitors like IL-1β converting enzyme inhibitors and IL-1ra. Binding proteins of the disclosure may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors; or molecules that target T cell activation molecules, for example, CTLA-4-IgG or anti-B7 family antibodies, anti-PD-1 family antibodies.

Binding proteins of the disclosure, can be combined with IL-1 1 or anti-cytokine antibodies, for example, fonotolizumab (anti-IFNγ antibody), or anti-receptor receptor antibodies, for example, anti-IL-6 receptor antibody and antibodies to B-cell surface molecules. Antibodies of the disclosure or antigen binding portion thereof may also be used with LJP 394 (abetimus), agents that deplete or inactivate B-cells, for example, Rituximab (anti-CD20 antibody), lymphostat-B (anti-BlyS antibody), TNF antagonists, for example, anti-TNF antibodies, Adalimumab (PCT Publication No. WO 97/29131; HUMIRA®), CA2 (REMCIDADE®), CDP 571, TNFR-Ig constructs, (p75TNFRIgG (ENBREL®) and p55TNFRIgG (LENERCEPT®)) and bcl-2 inhibitors, because bcl-2 overexpression in transgenic mice has been demonstrated to cause a lupus like phenotype (see Marquina R. et al., (2004) J. Immunol., 172(1 1):7177-7185), therefore inhibition is expected to have therapeutic effects.

The pharmaceutical compositions of the disclosure may include a "therapeutically effective amount" or a "prophylactically effective amount" of a binding
protein of the disclosure. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the binding protein may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the binding protein to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the binding protein, are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.
IV. Diagnostics

The disclosure herein also provides diagnostic applications. LRP-8 binding proteins of the disclosure may be employed in any of a variety of formats to detect LRP-8 in vivo, in vitro, or ex vivo (e.g., in cells or tissues that have been obtained from a living subject, subjected to a procedure, then returned to the subject). LRP-8 multispecific binding proteins of the disclosure offer the further advantage of being capable of binding to an epitope of LRP-8 as well as other antigens or epitopes in various diagnostic and detection assay formats.

In an aspect, the disclosure provides methods of determining the presence of at least one antigen or fragment thereof in a test sample by an immunoassay comprising the LRP-8 binding protein described herein. In another embodiment, the method further comprises: (i) contacting the test sample with the at least one LRP-8 binding protein, wherein the binding protein binds to an epitope on LRP-8 or fragment thereof so as to form a first complex; (ii) contacting the first complex with the at least one detectable label, wherein the detectable label binds to an epitope of the LRP-8 binding protein or an epitope on the antigen or fragment thereof that is not bound by the LRP-8 binding protein to form a second complex; and (iii) detecting the presence of LRP-8 or fragment thereof in the test sample based on the signal generated by the detectable label in the second complex, wherein the presence of LRP-8 or fragment thereof is identified or indicated by analyzing the signal generated by the detectable label.

In another embodiment, the method further comprises: (i) contacting the test sample with the at least one LRP-8 binding protein, wherein the LRP-8 binding protein binds to an epitope on LRP-8 the antigen or fragment thereof so as to form a first complex; (ii) contacting the first complex with the at least one detectable label, wherein the detectable label competes with LRP-8 or fragment thereof for binding to the LRP-8 binding protein so as to form a second complex; and (iii) detecting the presence of LRP-8 or fragment thereof in the test sample based on the signal generated by the detectable label in the second complex, wherein the presence of LRP-8 or fragment thereof is measured by analyzing the signal generated by the detectable label.

In one embodiment, the test sample is from a patient and the method further comprises diagnosing, prognosticating, or assessing the efficiency of therapeutic/prophylactic treatment of the patient, and optionally wherein if the method
further comprises assessing the efficacy of therapeutic/prophylactic treatment of the patient, the method optionally further comprises modifying the therapeutic/prophylactic treatment of the patient as needed to improve efficacy. In another embodiment, the method is adapted for use in an automated system or a semi-automated system. In another embodiment, the method determines the presence of more than one antigen in the sample.

In one aspect, the disclosure provides a method of determining the amount or concentration of LRP-8 or fragment thereof in a test sample by an immunoassay, wherein the immunoassay (a) employs at least one agent and at least one detectable label and (b) comprises comparing a signal generated by the detectable label with a control or a calibrator comprising LRP-8 or fragment thereof, wherein the calibrator is optionally part of a series of calibrators in which each calibrator differs from the other calibrators in the series by the concentration of LRP-8 or fragment thereof, and wherein the at least one agent comprises a LRP-8 binding protein described herein.

In one embodiment, the method comprises: (i) contacting the test sample with the at least one LRP-8 binding protein, wherein the LRP-8 binding protein binds to an epitope on LRP-8 or fragment thereof so as to form a first complex; (ii) contacting the first complex with the at least one detectable label, wherein the detectable label binds to an epitope on LRP-8 or fragment thereof that is not bound by the LRP-8 binding protein to form a second complex; and (iii) determining the amount or concentration of the antigen or fragment thereof in the test sample based on the signal generated by the detectable label in the second complex, wherein the amount or concentration of the antigen or fragment thereof is identified by analyzing the signal generated by the detectable label.

V. Kits

A kit for assaying a test sample for the presence, amount, or concentration of an analyte (or a fragment thereof) in a test sample is also provided. The kit comprises at least one component for assaying the test sample for LRP-8 (or fragments thereof) and instructions for assaying the test sample for the analyte (or a fragment thereof). The at least one component for assaying the test sample for the analyte (or a fragment thereof) can include a composition comprising an anti-LRP-8 multispecific binding protein, such
as a DVD-Ig (or a fragment, a variant, or a fragment of a variant thereof), as described herein and which is optionally immobilized on a solid phase.

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the disclosure described herein are obvious and may be made using suitable equivalents without departing from the scope of the disclosure or the embodiments disclosed herein.

Having now described the binding proteins and methods of making and using them of the disclosure in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the disclosure.
EXEMPLARY

The following examples are intended to be illustrative and in no way limit the scope of the disclosure.

5 Example 1. Materials and Methods

Example 1.1. Cynomolgus LRP-8 De Novo Cloning

Example 1.1.1. Cynomolgus LRP-8 (isoform 1) De Novo Cloning

Five cynomolgus cDNAs from the NGS database that has 13 tissues from a 99F donor and limited data from 4 donors were set as a workstation. The predicted cynomolgus LRP8 (isoform 1) was obtained. The cDNA sequences were translated in silico and the protein sequences of all tissues were aligned. The brain sequences were from different animals (234B, 481B, 568B, 571B, and 99F-HIPO). The protein sequence of cynomolgus LRP-8 isoform 1 is shown in Table 1.

Example 1.1.2. Cynomolgus LRP-8 (isoform 3) De Novo Cloning

Eight cynomolgus cDNAs were purchased from a commercial source (BioChain Institute, CA). The eight cDNAs were derived from eight different tissues from five different donors (Table 9). The taxon ID and subspecies of these tissues are unknown.

Table 9. List of Commercial Sources of Cynomolgus cDNA from Different Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>BioChain Cat #</th>
<th>Lot #</th>
<th>Donor #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>C1534035-Cy</td>
<td>B412038</td>
<td>S12822N</td>
</tr>
<tr>
<td>Kidney</td>
<td>C1534142-Cy</td>
<td>B509183</td>
<td>S12495N</td>
</tr>
<tr>
<td>Testis</td>
<td>C1534260-Cy</td>
<td>B312050</td>
<td>S12805N</td>
</tr>
<tr>
<td>Lung</td>
<td>C1534152-Cy</td>
<td>B509138</td>
<td>S12805N</td>
</tr>
<tr>
<td>Heart</td>
<td>C1534122-Cy</td>
<td>B406122</td>
<td>S12821N</td>
</tr>
<tr>
<td>Spleen</td>
<td>C1534246-Cy</td>
<td>B606234</td>
<td>S12823N</td>
</tr>
<tr>
<td>Liver</td>
<td>C1534149-Cy</td>
<td>B603082</td>
<td>S12495N</td>
</tr>
<tr>
<td>Small intestine</td>
<td>C1534226-Cy</td>
<td>B402223</td>
<td>S12495N</td>
</tr>
</tbody>
</table>

Primer sets located in the 5’ and 3’ UTRs were designed based on the human LRP-8 sequence (Accession # NP_059992). The cDNAs from all tissues were used as templates and standard PCR analyses were performed. Two out of eight tissues (brain and testis) produced the expected PCR products. The PCR products from those tissues
were cloned into a TA cloning vector (Invitrogen, CA), and multiple subclones (approximately 25) were sequenced. The cDNA sequences were translated in silico and the amino acid sequences of all tissues were aligned. The testis and brain sequences were from different animals (Table 9). The cynomolgus (Macaca fascicularis, crab-eating macaque) amino acid sequences from all animals were identical to each other. The Macaca fascicularis amino acid sequences from brain and testis were aligned with the Homo sapiens (human) sequence.

Example 1.2. LRP-8 Stable Cell Line Generation

HEK293H and 3T12 cells were cultured in T25 culture flasks and incubated at 37°C, 5% CO₂, and cells were passaged every four to five days. On the day before transfection, cells were diluted to 2x10⁵ cells in 2ml/well in a 6 well plate at 99% cell viability. The Homo sapiens LRP-8 (isoform3) (Accession # NP_059992) amino acid sequence was identified from GenBank. The Mus musculus (mouse) LRP-8 (isoform2) (Accession # NP_001074395) amino acid sequence was identified from GenBank. Cynomolgus LRP-8 amino acid sequence was identified in house by de novo cloning according to Example 1.1. The LRP-8 cDNAs were each cloned into a pCMV vector.

HEK293H and 3T12 cells were pre-incubated in six wells plate (2 ml/well in Opti-MEM) at 37°C/5% CO₂. A mixture of 2.5 µg plasmid DNA and 10 µl Lipofectamine2000 (Invitrogen) in 500 µl Opti-MEM was incubated at room temperature (RT) for 20 minutes, and then added to the cells. The cells were incubated at 37°C/5% CO₂ for 4 hours. The cells were incubated in culture medium at 37°C/5% CO₂ overnight. On the day after transfection, 2 ml of selection media with 0.5mg/ml G-418 (final concentration) was added to each well. Growth media were changed on the transfected cells every 4-5 days. Stable clonal cell lines were generated by serial dilution of the parental cells and subsequent expansion of isolated single cell colonies. For characterization of cell lines by FACS, the 3T12 transfected cells were dissociated using PBS-based Cell Dissociation Buffer (Invitrogen). HEK293 transfected cells were dissociated using growth medium, washed and re-suspended in cold PBS (pH 7.2)/2% FBS (FACS Buffer) to 1x10⁶ cells/ml, incubated one hour at 4°C with the primary antibodies, and analyzed using an Accuri C6 flow cytometer.
Example 1.3. Endocytosis Assay

HEK293 cells overexpressing human LRP-8 were collected and 0.3x10^6 cells were plated into each well. Cells were then blocked with 5% normal goat serum on ice for 1 hour. After three washes with PBS, cells were re-suspended in 50 µl primary antibody 3 µg/ml in 5% normal goat serum for one hour on ice. Unbound primary antibody was removed by three washes with PBS. Cells were then re-suspended in 50 µl PBS/2% FBS and incubated at 31°C the indicated times. Cells were placed on ice after incubation and washed three times with PBS. External antibody signal was blocked with unconjugated secondary antibody at 20 µg/ml on ice for one hour. After three washes with PBS, dead cells were stained for thirty minutes with eFlour660 (eBioscience) on ice. After three washes with PBS, cells were fixed and made permeable with BD Fix/Perm solution as described by the manufacturer. The internalized antibody signal was detected using a FITC-conjugated secondary antibody at 2 µg/ml. Unbound secondary antibody was removed by washing the cells three times with PBS. Cells were re-suspended in PBS, and each plate was read using an EnVision (without eFlour660 staining) or FACS Accuri (with eFlour660 staining) flow cytometer.

Example 1.4. Transcytosis Assay

Human epithelial colorectal adenocarcinoma Caco-2 cells (ECACC) were cultured and maintained as recommended by the manufacturer. Cells (2.5 x 10^4) suspended in 200 µl culture medium were plated on the top side of a transwell filter (Corning #3470). A one milliliter (ml) of culture medium was added to the bottom side of the transwell. Cells were then cultured at 37°C, 5% CO₂ for 21 days before the assay. 50% of the medium was refreshed every three to four days and transepithelial electrical resistance (TEER) was measured with EVOM and ENDOFIM (World Precision Instruments). Antibodies were prepared as a 10x solution in PBS. A volume (20 µl) of a test sample was added to the top side of the transwell. After an indicated time, 100 µl of each sample was collected from the bottom side of the transwell. At the end of the transcytosis assay, the TEER of each transwell was measured to ensure the integrity of the monolayer.

Antibody concentrations in the samples were determined using an Electrochemiluminescence-Meso Scale Discovery (ECL-MSD) assay. The MSD plate (MSD Cat# L15XB-3 / L11XB-3) was coated with an F(ab')2 fragment of Fc fragment-
specific capture antibody overnight at 4°C. The plate was blocked with 3% MSD blocking buffer (MSD Cat#R93AA-01) for one hour at 25°C, and then washed with 1x Tris-Buffered Saline and Tween 20 (TTBS) wash buffer. Standards and samples were diluted in 1% MSD assay buffer or 0.1% serum containing 1% MSD assay buffer. Each antibody was used as an internal standard to quantitate respective antibody concentrations. Each assay plate was incubated for two hours at 25°C and bound antibody was detected using goat anti-human/mouse/rat Sulfo-TAG (MSD). Plate values were read/calculated using an MSD SECTOR Imager 6000 system. Each concentration was determined from the standard curve with a five-parameter nonlinear regression program using Excel Fit software (N=3 for each test article).

**Example 1.5. Cell-Based FACS Assay**

LRP-8 stable cells and parental cells were collected and incubated in FACS buffer (1xPBS / 2% FCS) and an aliquot was removed for control wells. Parental cells were labeled with CFSE (5(6)-Carboxyfluorescein N-hydroxysuccinimidyl ester). 5x10^4 cells/well of equal numbers of CFSE labeled parental cells and un-labeled LRP-8 stable cells were mixed and centrifuged for ten minutes at 1200 rpm. After adding FACS buffer, cells were spun at 2000 rpm for three minutes and then incubated for 15 minutes at 4°C with mAb from 30 μg/ml, with a 3-fold dilution across the plate. After three washes with FACS buffer, cells were incubated for 15 minutes at 4°C with 50 μl of secondary Thermo-scientific APC antibody diluted 1:500. Unbound secondary antibodies were removed by three washes with FACS buffer, and the cells were re-suspended in 50 μl of FACS buffer and analysed using a BD FACSCanto system (BD Biosciences, CA).

**Example 1.6. Antibody Affinity Measurements (Cell-Based MSD Assay)**

A cell-based ECL-MSD binding assay was used to determine antibody affinity. FIEK293 cells overexpressing human, monkey or mouse LRP-8 were added onto MSD 96-well plate (MSD Cat# L15XB-3 / L11XB-3) and incubated at 37°C for one hour. Cells were blocked using 15% FBS (Hyclone, Thermo Scientific Cat# SH300700.03) at RT for 30 minutes with mild agitation. Plates were washed with DPBS three times and Abs or DVD-Ig binding proteins were added to the well. After incubation at RT for one hour, plates were washed with DPBS and goat anti-human or
anti-mouse Sulfo TAG (MSD Cat#R32AJ-l) was added. Plates were incubated at RT for one hour, washed with DPBS and immersed in MSD read buffer (MSD Cat#R92TD-2) before reading on a MSD SECTOR Imager 6000. EC50 values were obtained using Xlfit4 software system.

Example 1.7. Measuring Antibody Concentration in Mouse Brain, Spinal Cord, and Serum

Wild type C57B1/6N or Balb/c mice 6-8 weeks were injected intravenously or intraperitoneally with antibodies. After the indicated time, mice were perfused using DPBS with Heparin (1000 units/L) at a rate of 2 ml/minute for ten minutes. The brain of each murine subject was extracted, vertically divided into equal halves and the half brains were homogenized using Bullet Blender Blue (NextAdvance BBX24B) and zirconium beads (NextAdvance ZROB05/ZROB10) in 1% NP-40 (Thermo Scientific Cat#28324) in PBS containing protease inhibitors (Roche Diagnostics Ref#06538304001). Homogenized brain samples were rotated at 4°C for one hour before spinning at 14,000 rpm for 20 minutes. Supernatant material was isolated for brain antibody measurement. The spinal cord was collected in some instances for further analysis. An incision was made post axis and prior to hips. The spinal cord was cut and placed in a cryogenic test tube and snap frozen. A second segment of spinal cord (0.06-0.08g) was cut and placed flat on an Iftc cassette and fixed in 4% PFA. Homogenization and antibody detection methods were the same as those used for brain tissue.

Whole blood was collected from tail nick or cardiac puncture (terminal).

Whole blood from tail nick was diluted 1:5 in assay buffer with EDTA and was snap frozen. Whole blood from cardiac puncture was collected in serum separator microcontainer tubes (BD Diagnostics, Ref#365956), allowed to clot for 30 minutes, and spun down at 13k rpm at RT for 8 minutes. Supernatant was isolated for antibody measurement in serum.

Antibody concentrations were measured with an ECL-MSD assay. MSD plates (MSD Cat# L15XB-3 / LI1XB-3) were coated with an F(ab')2 fragment of donkey anti-human IgG, Fc fragment-specific polyclonal antibody (Jackson ImmunoResearch Code# 709-006-098), or donkey anti-mouse IgG (Jackson ImmunoResearch Code# )
overnight at 4°C. Plates were blocked with 3% MSD blocking buffer (MSD Cat#R93AA-01) for 1 hour at 25°C. Plates were washed with 1x TTBS wash buffer. Standards and samples diluted in 1% MSD assay buffer or 0.1% serum containing 1% MSD assay buffer were added. Each antibody was used as an internal standard to quantify respective antibody concentrations. Plates were incubated for two hours at 25°C and bound antibody was detected with goat anti-human Sulfo-TAG (MSD Cat#R32AJ-l). Plates were read on an MSD SECTOR Imager 6000 system. Antibody concentrations were determined from the standard curve with a five-parameter nonlinear regression program using Excel Fit software. Each group contained three animals unless otherwise indicated. Data were expressed as means +/- SD.

**Example 1.8. Immunohistochemistry and Evaluation**

Antibody treated mice were perfused and divided into equal halves as described above, and half brains were fixed in 4% paraformaldehyde for 6 hours. Following fixative, tissues were processed through a graded series RUSH protocol (Leica TP 1050 Tissue Processor) of alcohol to xylene and then embedded in paraffin (Leica EG1 150H). 5 µM brain sections were cut with a microtome (Microm, HM355S). Sections were de-paraffinized and rehydrated to water and placed into Tris with tween-20 buffer (Teknova Cat#T5155). Staining was performed on a Dako autostainer links 48 system. Briefly, the sections were blocked with 3% hydrogen peroxide plus methanol for 30 minutes, washed with wash buffer then incubated for 8 minutes with protease I (Ventana Ref#760-2018). Sections were blocked with a streptavidin and biotin blocking kit (Vector Laboratories Cat#SP-2002) for 8 minutes each, followed by Dako protein block for 30 minutes. The sections were incubated for 1 hour at RT with a biotinylated donkey anti-human IgG (H+L) F(ab’)(Jackson ImmunoResearch Code#709-066-149) at 15 µg/ml followed by an R.T.U Vectastain Universal ABC Kit PK-7100 (Vector Labs, UK) for 30 minutes at RT. The sections were then reacted with diaminobenzidine (DAB) chromogen (Dako Ref#K3468) for 3 minutes to form a brown precipitate, washed with water, counterstained with Gill Modified Hematoxylin (EMD Harleco Ref#65065) for 30 seconds and bluing reagent (Richard-Allan Scientific Ref#7301), dehydrated and mounted for microscopic observation. Five sections from different regions of the brain were stained. Representative images from
the cerebellum and cortex sections were captured. All settings (filters and light levels) for each image were kept constant throughout the experiment. Staining intensity of vasculature, parenchyma and neurons were visually scored under a microscope using 0 to 4 scale, where 0 is no staining; 1 is light staining at small portion of tissue; 2 is light staining at most tissue, 3 is moderate staining at most tissue; 4 is strong staining at most tissue. The data was evaluated in a blind manner. The average score of each group with at least three animals was reported.

Example 1.9. Aldevron cDNA Immunization and Antibody Generation

Human LRP-8 Extracellular domain (ECD) cDNA was subcloned into an Aldevron proprietary immunization vector. Genetic immunization introduces the cDNA encoding the target sequence into the skin of rat, the target protein was expressed, and an immune response was generated. The screening system developed using the GENOVAC Antibody Technology at Aldevron Freiburg is based on screening vectors expressing the target protein that are transiently transfected into mammalian cells. In this case, hLRP-8 and mLRP-8 were transiently expressed for screening. Bleeds and hybridoma supernatants were screened using flow cytometry.

Example 1.10. Epitope Binning

Example 1.10.1 Cell-Based Anti-LRP-8 Competition Assay

An anti-LRP-8 competition assay was based on a cell-based Electrochemiluminescence-Meso Scale Discovery Assay (ECL-MSD) binding assay. HEK293 cells overexpressing mouse or cynomolgus monkey LRP-8 were added onto MSD 96-well plate (MSD Cat# L15XB-3 / L11XB-3) and incubated at 37°C for 1 hour. Cells were blocked using 15% FBS (Hyclone, Thermo Scientific Cat# SH300700.03) at RT for 30 minutes with mild agitation and plates were washed with DPBS 3 times. Competing LRP-8 antibodies with human Fc and mouse Fc domains were used in two competition combinations:

Example 1.10.2 Competition 1 (Competitor Ab with Mouse Fc)

A 1:1 mixture of fixed concentrated anti-LRP-8 human Fc Ab and a titer of competitor LRP8 mouse Fc Ab were added onto the plate and incubated at room temp
for 1 hour. After washing, anti-human SULFO-TAG Ab was added on the plate and incubated for 1 hour.

**Example 1.10.3 Competition 2 (Competitor Ab with Human Fc)**

A 1:1 mixture of fixed concentrated anti-LRP-8 mouse Fc Ab and a titer of competitor LRP-8 human Fc Ab were added onto the plate and incubated at room temp for 1 hour. After washing, an anti-mouse SULFO-TAG Ab was added to the plate and incubated for 1 hour. Plates were washed with DPBS and immersed in MSD read buffer T surfactant free (MSD Cat# R92TD-2) before reading on an MSD SECTOR Imager 6000. Data were obtained and analyzed using a GraphPad Prism 6 software package (GraphPad Software, Inc., La Jolla, CA).

**Example 1.11. Crystallographic Study of anti-LRP-8 ML199.11H1.5B2 Fab in Complex with CR1 Peptide**

Fab fragment of LRP-8 11H1.5B2 was prepared by papain cleavage of the parent antibody, anti-LRP-8 ML199.1 11H1.5B2 [mu/hu IgGl/k] LALA chimeric antibody. Papain was activated with 50mM cysteine in PBS, pH 7.4 buffer. Anti-LRP-8 ML199. 11H1.5B2 chimeric antibody in PBS, pH 7.4 buffer was mixed with papain at 1:100 weight ratio of papain to the antibody and incubated for 1 hr at 37° C. The reaction was quenched with 5 mM iodoacetamide. The mixture was purified on 5 ml Mab SelectSure resin (GE Healthcare) where the Fab fragment was collected as flow through. The flow through was concentrated using an Ultrafree-15 Biomax 10 kDa molecular weight cut-off (MWCO) centrifugal device (Millipore). The concentrated mixture was purified on 2.6 cm x 60 cm Sephacryl 200 HiPrep column (GE Healthcare) pre-equilibrated in 50 mM HEPES, 50 mM NaCl, pH 7.5 buffer.

CR1 peptide was dissolved with the protein buffer from the last purification step (50 mM NaCl, 50 mM HEPES, pH 7.5) to a final concentration of 100 mM. The peptide was added to the LPR-8 Fab sample (29.4 mg/ml) to a final molar ratio (peptide over protein) of 8:1. Sitting drop vapor diffusion method was used by mixing equal volume of LRP-8-peptide complex and the crystallization reagent of 25% PEG 4000, 0.2 M Ammonium Sulfate, 0.1 M Sodium acetate/HCl, pH 4.6. Thin stacking plate crystals were initially found with 2-3 days and continued to grow to their full size within one week. Single plate crystals were separated and flash frozen into liquid
nitrogen using 20% propylene glycol plus the crystallization solution as the cryo-
protectant. Diffraction data were collected at a temperature of 100 K using beamline
XALOC (BL13) at ALBA synchrotron, Spain.

Diffraction data for the complex crystal structure were processed using the
5 program autoPROC from Global Phasing Ltd. The Fab fragment and CR1 peptide
complex dataset was processed in the space group P21 with the following unit cell
dimensions: a=41.3 b=79.8 c=67.1, β=95.5. A maximum likelihood molecular
replacement solution was determined using the program PHASER using an Fab search
model reported previously (Protein Data Bank entry 1VPO). Coordinates for 1Fab
molecule were found based on the molecular replacement solution. Preliminary
refinement of the resulting solution was conducted using REFMAC and the program
BUSTER. The model for CR1 peptide and edits to the Fab scaffold were built
manually using the program COOT and examination of 2Fo-Fc and Fo-Fc electron-
density maps. Refinement concluded with the addition of water molecules using
BUSTER. Final refinement statistics reported Rfree/Rwork values of 0.23/0.20.

The contacts between the Fab fragment and CR1 peptide involve both critical
hydrogen bond and hydrophobic interactions which stabilize the interface. A list of
molecular contacts (measuring within a 4.0 Å range) were generated using the program
NCONT in the CCP4 suite of programs. The contacts were measured between the
peptide and the corresponding light and heavy chains of the Fab fragment.

Example 1.12. Binding Studies of Mutant Peptides to Delineate Residues
Implicated In Binding

Example 1.12.1 Mutagenesis of CR1 and CR2 Peptides

Cyclic peptides CR1 and CR2, based on loop sequences from LRP-8, were
designed and used as tools for antibody generation and binding studies for the generated
antibodies.

3A7Q GSGPAKECEKDQFCRNRCIPSVWRCDEDDCLDHSDEDDCPK
CR1 ------------ CEKDQ FQRN ERCIPS VWRC [eye (1,13)]
CR2 ------------ CADSD FTSDN GHCIHERWKC [eye (1=13)]

These peptides were shown by Biacore studies to bind several subsequently
generated antibodies, including anti-LRP-8 ML199-1 1H1.5B2 antibody. Based on the
aligned sequence of CR1 and CR2 peptide, the sequence FxSxN appeared to be common in both binding peptides, and therefore likely important for the binding epitope. Note that the serine in the CR1 and CR2 peptide sequences was already changed from the parent LRP-8 protein sequence, removing an unpaired cysteine. Modified forms of cyclic peptide CR1, containing the following changes were synthesized, and examined for binding to chimeric anti-LRP-8 ML199-1 1H1.5B2 antibody ([hu IgGl/k] LALA) by both BIACORE and direct ELISA assays.

CR1 [eye (1, 13)] H2N- CEKDQFQSRNERCIPSVWR (Koa) -amide
CR1.1 [eye (1, 13)] H2N- CEKDQAQSRNERCIPSVWR (Koa) -amide
CR1.2 [eye (1, 13)] H2N- CEKDQFQARNERCIPSVWR (Koa) -amide
CR1.3 [eye (1, 13)] H2N- CEKDQFQARERCIPSVWR (Koa) -amide
CR1.4 [eye (1, 13)] H2N- CEKDQAQARAERC IPSVWR (Koa) -amide

In both assays, peptides CR1.2 and CR1.3 bound with affinity similar that of the unmodified CR1, whereas peptides CR1.1 & CR1.4, both containing the F->A substitution, did not bind. This indicated that the phenylalanine in the sequence -FQSRN- is required for antibody binding, and thus a key residue in the epitope.

**Example 1.12.2 CR1/CR2 Peptide anti-LRP-8 Binding ELISA**

High binding MSD plates (MSD Cat# L15XB-3 / LI1XB-3) were coated with 1 μg/ml of CR1 or CR2 peptide overnight at 4°C. The next day, the plate was blocked with 3% MSD blocking buffer (MSD Cat# R93AA-01) for 1 hour at RT. The plates were washed with TTBS buffer (20mM Tris; 0.5% Tween, 150mM sodium chloride; pH 7.5) three times and a titer of anti-LRP-8 human Fc Ab was added. After incubating for 1 hour at RT, plate was washed and anti-human SULFO-TAG Ab was added to the plate and incubated for 1 hour. The plates were washed and immersed in MSD read buffer T with surfactant (MSD Cat# R92TC-1) before reading on an MSD SECTOR Imager 6000. Data were obtained and analyzed using a GraphPad Prism 6 software package (GraphPad Software, Inc., La Jolla, CA).
Example 2. Generation of anti-LRP-8 Antibodies Based on LRP-8 Peptides

Example 2.1. Design of LRP-8 Peptides as Immunogen

LRP-8 contains complement-like repeat regions (CR) in its sequence and these were used as antigenic peptides. A high resolution structure of CR7 from LRP was solved to 1.8 Å resolution as presented previously (Simonovic et al. (2001) Biochem. 40(50): 15127-34). This structure shows a loop/turn motif that is stabilized by a disulfide linkage (Figure 1A). When examining two complement-like repeat regions in LRP-8 (CR1 and CR2), this loop/motif was predicted by sequence as compared to the protein x-ray structure of LRP (Protein Data Bank ID: 1J8E). This sequence is outlined in Figure IB. Due to an internal Cys in the sequence, this residue was mutated to serine (Ser; shown in Figure IB in bold) to increase peptide stability and improve peptide aggregation. The peptide was cyclized to mimic the beta turn as displayed by the structure 1J8E. The resulting designed peptides are shown below (KLH - keyhole limpet hemocyanin).

**CR1:**
[Cyc (1,13)] H2N-CEKDQFQSRNRCIPSVWR(KAoa)-amide
10mgs, >90% Purity (5mgs uncoupled, 5mgs to KLH via Aoa)

**CR2:**
[Cyc (1,13)] H2N -CADSDFTSDNGHCHERWK(KAoa)-amide
10mgs, >90% Purity (5mgs uncoupled, 5mgs to KLH via Aoa)

Sequence analysis shows that CR1 and CR2 peptides share sequence similarities (Figure IB), and are identical in the analyzed cynomolgus and human samples shown in Figure 2A. Figure 2B shows the alignment between human and cyno LRP-8 isoform 3.

Example 2.2. Antibody Generation and Screening

KLH-conjugated CR1 and CR2 peptides were synthesized by New England Peptide (Gardner, MA). Equal amounts of CR1 and CR2 (50 µg) were mixed for immunization and were injected in mice subcutaneously every three weeks for four times before the mouse spleens were harvested. Lymphocytes were isolated and fused with NSO cells with a well-established protocol. Hybridoma supernatant (SN) was used for cell-based FACS with hLRP-8-HEK293 stable cells and HEK293 parental cells. Supernatants (SN) that bind to hLRP-8-HEK293 stable cells, but not to HEK293
parental cells were selected. All positive SNs were tested against the 3T12-LRP-8 cells to further confirm binding. Two fusions (ML199 and ML201) were generated and about six positively binding hybridomas were selected. Three monoclonal antibodies (ML201-2B4.2B1.2H10; ML201-8F3.3D7; and ML199-1H1.5B2) were subcloned.

**Example 2.3. Anti-LRP-8 and Mu/Hu Chimeric Antibody Generation**

Anti-LRP-8 antibodies were produced at AbbVie Bioresearch Center. The antibody variable domain DNAs were codon optimized and cloned into expression vectors to produce mouse or human IgG proteins. The antibody constructs were expressed in HEK293 cells and purified according to established methods. Expression yield was measured with a Nanodrop spectrophotometer. Percentile of monomer was determined by size exclusion chromatography (SEC). Heavy chain and light chain variable regions of three monoclonal antibodies (ML201-2B4.2B1.2H10; ML201-8F3.3D7; and ML199-1H1.5B2) are summarized in Tables 2 and 5.

**Example 2.4. Murine anti-LRP-8 Antibody Analysis**

The ML199 and ML201 antibodies were analyzed for binding to LRP-8 transfected HEK293 cells and control HEK293 cells (Figures 3 and 4). Data showed that the ML199-1H1, ML201-8F3, and ML201-2B4 clones effectively bound human, mouse, and cyno LRP-8 (Tables 10-12).

**Table 10. Binding Data For anti-LRP-8 M199-11H1.5B2 Antibody**

<table>
<thead>
<tr>
<th>Clone</th>
<th>huLRP-8 cell based EC50 (nM)</th>
<th>Reactivity</th>
<th>Endocytosis (fold-over Ig)</th>
<th>Transcytosis (folder-over Ig)</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-LRP-8,11H1.5B2</td>
<td>0.4</td>
<td>hu, cyno, mu</td>
<td>1.7</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Table 11. ECL-MSD Binding with 10 µg/ml Abs (Data Re-Expressed as Fold over mlgG Control)**

<table>
<thead>
<tr>
<th></th>
<th>human_LRP-8 HEK293</th>
<th>cyno-LRP-8 HEK293</th>
<th>mouse-LRP-8 HEK293</th>
<th>HEK293 parental</th>
</tr>
</thead>
<tbody>
<tr>
<td>ab58216</td>
<td>17.3*</td>
<td>1.2</td>
<td>19.2*</td>
<td>0.9</td>
</tr>
<tr>
<td>mFc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ML199-</td>
<td>15.1*</td>
<td>7.0**</td>
<td>23.1*</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Table 12. Endocytosis and Transcytosis Data (Data Expressed as Fold over IgG Control)

<table>
<thead>
<tr>
<th></th>
<th>Endocytosis hLRP-8-HEK293 (FACS-based)</th>
<th>Transcytosis Caco-2 hLRP-8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ab58216 mFc</td>
<td>2.6***</td>
</tr>
<tr>
<td></td>
<td>ML199-11H1-5B2 muFc</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>ML201-8F3-3D7 muFc</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>ML201-2B4.2B1.2H10 muFc</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*More than 10 fold; ** more than 5-10 fold; *** more than 2-5 fold

**Example 2.5. Chimeric anti-LRP-8 Antibody Analysis**

Chimeric anti-LRP-8 antibody (ML199.1 1H1.5B2 mu/hu IgGlm/k) was intravenously administered to subjects and analyzed for IHC staining. Mouse *in vivo* PK study data using an intravenous dose (30 mpk) of anti-LRP-8, ML199. 11H1.5B2 mu/hu IgGlm/k antibody showed enhanced uptake into brain in 24 hours (Figure 5). Clear positive IHC staining was parenchyma and neuronal cells. Anti-LRP-8, ML199. 1H1.5B2 mu/hu IgGlm/k at an intravenous dose of 40 mpk also produced positive IHC staining of vasculature in two hours (Figure 6). As shown in Figure 7, anti-LRP-8 antibodies (i.e., anti-LRP-8 ML199.1 1H1.5B2 and anti-LRP-8 8F3.3D7 40mpk) and control Tfr antibody had enhanced uptake into brain in 24 hours. Positive IHC staining of parenchyma and neuronal cells was observed. Tables 13 and 14 show anti-LRP-8 PK study IHC score and data for antibody concentration detected in brain, spinal cord, and serum samples (e.g., homogenates). V, P, and N in Tables 13 and 14 refer to vasculature, parenchyma, and neuron, respectively.
### Table 13. MSD Data for LRP-8 Antibodies

<table>
<thead>
<tr>
<th>ANIMAL #</th>
<th>Test Article</th>
<th>Treatment (Single/IV)</th>
<th>MSD-brain, spinal cord and serum</th>
<th>IHC-brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Conc, nM</td>
<td>% Ab/serum</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>2hr/40mpk</td>
<td>10.91</td>
<td>2.08</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3.98</td>
<td>1.31</td>
<td>4443.69</td>
</tr>
<tr>
<td>3</td>
<td>anti-LRP-8 (ML199.1H1.5 B2) hFc Lot2194993</td>
<td>1.76</td>
<td>2.13</td>
<td>4720.77</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>4hr/40mpk</td>
<td>3.89</td>
<td>0.98</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>10.93</td>
<td>1.37</td>
<td>3261.31</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>4hr/40mpk</td>
<td>2.20</td>
<td>1.65</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>4hr/40mpk</td>
<td>12.52</td>
<td>5.82</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>5.56</td>
<td>2.68</td>
<td>2005.72</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>24hr/40mpk</td>
<td>5.15</td>
<td>4.30</td>
</tr>
<tr>
<td>10</td>
<td>anti-LRP-8 (ML201.8F3.3 D7) hFc LOT2195691</td>
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<td>4.30</td>
<td>3719.01</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>24hr/40mpk</td>
<td>8.11</td>
<td>3630.11</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>5.23</td>
<td>3721.20</td>
<td>0.14</td>
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<tr>
<td>34</td>
<td>IgG1/K mut (234,235) Lot2140396</td>
<td>1.43</td>
<td>0.94</td>
<td>848.40</td>
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<td>35</td>
<td></td>
<td>0.77</td>
<td>0.28</td>
<td>1121.89</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>0.61</td>
<td>0.30</td>
<td>853.10</td>
</tr>
<tr>
<td>37</td>
<td>(TfR) hFc Lot1892291</td>
<td>9.5</td>
<td>1085.95</td>
<td>0.87</td>
</tr>
<tr>
<td>38</td>
<td></td>
<td>7.23</td>
<td>1114.89</td>
<td>0.65</td>
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<tr>
<td>39</td>
<td></td>
<td>6.09</td>
<td>915.12</td>
<td>0.67</td>
</tr>
</tbody>
</table>

LLP for anti-LRP-8 (ML199.1 H1.5B2): Brain/Spine = 4.12ng/mL & Serum = 0.02ng/mL
LLP for anti-LRP-8 (ML201.8F3.3D7): Brain/Spine = 4.12ng/mL & Serum = 0.05ng/mL
LLP for IgG: Brain/Spine = 0.46ng/mL & Serum = 0.05ng/mL
LLP for (TfR): Brain/Spine = 0.46ng/mL & Serum = 0.05ng/mL
## Table 14. Anti-LRP-8 PK Study IHC Score and Concentration in Brain Homogenates and Serum

<table>
<thead>
<tr>
<th>SN</th>
<th>14106</th>
<th>IHC Brain</th>
<th>IHC Spinal Cord</th>
<th>MSD-Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/N</td>
<td>Number</td>
<td>Cat &amp; Dose</td>
<td>Male Animal</td>
<td>Score (0-4)</td>
</tr>
<tr>
<td>Animal #</td>
<td>Group</td>
<td>Timepoint/R OUT E/Dose</td>
<td></td>
<td>V</td>
</tr>
<tr>
<td>1-3</td>
<td>anti-LRP-8 (ML199.11H1.5B2) hFc Lot2194993</td>
<td>28hr/IV/50mpk (Multiple)</td>
<td>Balb/C</td>
<td>3</td>
</tr>
<tr>
<td>4-6</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>7-9</td>
<td></td>
<td>C57Bl/6N</td>
<td>4</td>
<td>1</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>10-12</td>
<td>anti-LRP-8 (ab58216) hFc Lot2196879</td>
<td>28hr/IV/45mpk (Multiple)</td>
<td>Balb/C</td>
<td>3</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>3</td>
</tr>
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<td></td>
<td>C57Bl/6N</td>
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<td>0</td>
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<td></td>
<td></td>
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<td>3</td>
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<td>16-18</td>
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<td>C57Bl/6N</td>
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<td>0.5</td>
</tr>
<tr>
<td>25-27</td>
<td>higG1/K mut (234,235) Lot2140396</td>
<td>24hr/IV/50mpk (Single)</td>
<td>Balb/C</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
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</tr>
<tr>
<td>28-30</td>
<td></td>
<td>C57Bl/6N</td>
<td>4</td>
<td>0.5</td>
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<td></td>
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</tr>
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<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>31-33</td>
<td></td>
<td>SJL</td>
<td>2</td>
<td>0</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>34-36</td>
<td>(TTR) Lot 1892324</td>
<td>24hr/IV/50mpk (Single)</td>
<td>C57Bl/6N</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>
Additional data show enhanced uptake of anti-LRP-8.ML199.1 1H1.5B2 antibody into brain at 24 hours with positive staining of parenchyma and neurons (Figure 8). Enhanced uptake into the spinal cord was observed at 24 hours for LRP-8 antibody intravenously administered 24 hours earlier. Positive IHC staining of parenchyma and neurons were observed (Figure 9). Data showed that the administered LRP-8 effectively crossed the blood-brain-barrier.

**Example 2.6. Anti-LRP-8, ML199-11H1-5B2 Humanization**

Variable domain sequences for anti-LRP-8 antibody, ML199-1 1H1-5B2 were obtained by cDNA cloning using well-established methods. Variable region residues were annotated according to the Kabat numbering system. The canonical structures of the CDRs were determined according to Huang et al. (2005) Methods 36:35-42. Canonical structure was assigned: ML199-1 1H1-5B2 VH: 1-3. A search of vh.l-3.fasta for VH acceptor human framework found that the FR4 region sequence of hIGHJ6*01 has the highest similarity to that of ML199-1 1H1-5B2 VH sequence. IGHJ6*01 was used as the acceptor sequence. All other hJH FR4 sequences are also possible acceptor sequences. By calculating the structural important VH position, IGHV3-21*01 was chosen as a human acceptor framework sequence. For light chain humanization, using the same method, IGKV2-28*01 was chosen as a likely human acceptor framework sequence. IGKV2-30*01 may also be used as an acceptor for humanization in other embodiments. Variable domain sequences of humanized variants of anti-LRP-8 antibody ML199-1 1H1-5B2 are shown in Tables 2 and 5. Humanized variants of anti-LRP-8 antibody ML199-1 1H1-5B2 either showed lower binding to LRP-8 and/or yielded lower expression in HEK 293 cells (Table 15).

**Table 15. Binding Affinity and Production Yield of Humanized Variant Antibodies**

<table>
<thead>
<tr>
<th>Clone</th>
<th>Light Chain</th>
<th>Heavy Chain</th>
<th>mLRP8 cell binding EC50 (nM)</th>
<th>Yield (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML199-11H1.5B2VH.1/ML.1</td>
<td>VL.1</td>
<td>VH.1</td>
<td>22.45</td>
<td>2.84</td>
</tr>
<tr>
<td>ML199-11H1.5B2VH.1/ML.1a</td>
<td>VL.1a</td>
<td>VH.1</td>
<td>13.44</td>
<td>0.10</td>
</tr>
<tr>
<td>ML199-11H1.5B2VH.1/ML.1b</td>
<td>VL.1b</td>
<td>VH.1</td>
<td>7.92</td>
<td>0.74</td>
</tr>
</tbody>
</table>
Example 2.7. Anti-LRP-8, ML199-11H1-5B2VH/VLla Liability Free Variants

Protein liability motifs were found in HCDR2 and LCDR1 of humanized anti-LRP-8 antibody, ML199-11H1-5B2VH/VLla. Firstly, 59 variants using the combination VHl/VLla as template and introducing mutations in each liability on HCDR2 and LCDR1 were made as shown below:

\[
\begin{array}{|c|c|c|}
\hline
\text{hML199-HH1-5B2VH.l} & \text{VL.l} & \text{VH.la} \\
\hline
\text{hML199-HH1-5B2VH.la} & \text{VL.la} & \text{VH.la} \\
\hline
\end{array}
\]

\[
\begin{array}{|c|c|c|}
\hline
\text{ML199-11H1.5B2VH.la/VL.l} & 6.83 & 6.01 \\
\hline
\text{ML199-11H1.5B2VH.la/VL.lb} & 41.84 & 1.57 \\
\hline
\text{ML199-11H1.5B2VH.lb/VL.la} & 19.65 & 3.14 \\
\hline
\text{ML199-11H1.5B2} & \text{chimeric VL} & \text{chimeric VH} \\
\hline
\end{array}
\]

where, \(x\) = Any amino acid but: M, C, N, D or Q.
\[
\begin{array}{|c|c|c|}
\hline
\text{ML199-1} & \text{VL.l} & \text{VH.la} \\
\hline
\text{ML199-11H1-5B2VH/VLla} & 6.83 & 6.01 \\
\hline
\text{ML199-11H1-5B2VH.la/VL.la} & 41.84 & 1.57 \\
\hline
\text{ML199-11H1-5B2VH.lb/VL.la} & 19.65 & 3.14 \\
\hline
\text{ML199-11H1.5B2} & \text{chimeric VL} & \text{chimeric VH} \\
\hline
\end{array}
\]

\(z\) = Any amino acid but: M, C, G, S or N. No P in VHl.

Heavy chain and light chain variable domains of the liability free variants of anti-LRP-8 ML199-11H1-5B2VH 1/VLla antibody are shown in Tables 2 and 5.

Supernatants from each variant were tested for expression and binding to LRP-8. Selected liability free variants were purified (Table 16) and their affinity was determined by cell-based ECL-MSD binding assay (Figure 10). Removal of liability sequences yielded variants with either lower binding affinity to LRP-8 and/or yielded lower expression in HEK 293 cells.
Table 16. Structural Information and Production Yield of hML199-HH1.5B2 VH/VL\textit{la} Variants

<table>
<thead>
<tr>
<th>Protein name</th>
<th>VH mutation</th>
<th>VL mutation</th>
<th>Yield (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL-73742 hCg1 LALA/k (ML199.11H1.5B2 1A.15)</td>
<td>S62H</td>
<td>G29E</td>
<td>0.64</td>
</tr>
<tr>
<td>CL-73743 hCg1 LALA/k (ML199.11H1.5B2 1A.16)</td>
<td>S62H</td>
<td>N28T</td>
<td>0.68</td>
</tr>
<tr>
<td>CL-73744 hCg1 LALA/k (ML199.11H1.5B2 1A.17)</td>
<td>S62H</td>
<td>G29L</td>
<td>0.54</td>
</tr>
<tr>
<td>CL-73745 hCg1 LALA/k (ML199.11H1.5B2 1A.18)</td>
<td>S62H</td>
<td>G29K</td>
<td>0.46</td>
</tr>
<tr>
<td>CL-73746 hCg1 LALA/k (ML199.11H1.5B2 1A.19)</td>
<td>S62H</td>
<td>N28R</td>
<td>1.30</td>
</tr>
<tr>
<td>CL-73747 hCg1 LALA/k (ML199.11H1.5B2 1A.20)</td>
<td>S62H</td>
<td>N28W</td>
<td>0.68</td>
</tr>
<tr>
<td>CL-73748 hCg1 LALA/k (ML199.11H1.5B2 1A.21)</td>
<td>S62H</td>
<td>N28P</td>
<td>5.92</td>
</tr>
</tbody>
</table>

Example 3. Generation of Anti-LRP-8 Antibodies Based on GENOVAC\textsuperscript{TM} Technology

Example 3.1. Aldevron cDNA Immunization and Antibody Generation

Additional anti-LRP-8 antibodies were generated by using genetic immunization with GENOVAC\textsuperscript{TM} antibody technology by Aldevron Freiburg as described above. In this Example, FL_hLRP-8 cDNA was used and 108 positive binders were collected and analyzed using mouse cross reactivity assays and endocytosis assays. Twenty five candidates were selected for scale up using cyno cross-reactivity assays, endocytosis assays, and transcytosis assays (Table 17). Data in Table 17 shows effective binding of the antibodies to LRP-8 (see also Figure 11).
Table 17. Cell-Based MSD Binding (Fold Over IgG Control) and FACS Data for the LRP-8 Antibodies Developed Using GENOVACTM Antibody Technology

<table>
<thead>
<tr>
<th>clone name</th>
<th>human LRP8 HEK293</th>
<th>cyno-LRP8-HEK293</th>
<th>mouse-LRP8-HEK293</th>
<th>HEK293 parental</th>
<th>Endocytosis hLRP8-293</th>
<th>Transcytosis Caco-2_hLRP8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1to5 1to25 1to125</td>
<td>1to5 1to25 1to125</td>
<td>1to5 1to25 1to125</td>
<td>1to5 1to25 1to125</td>
<td>1to5 1to25 1to125</td>
<td>fold over-IgG fold over-IgG</td>
</tr>
<tr>
<td>BGK-2C8</td>
<td>5.3 4.9 4.0</td>
<td>15.3 15.0 11.2</td>
<td>9.2 8.5 3.2</td>
<td>0.4 0.3 0.2</td>
<td>5.76</td>
<td>2.29</td>
</tr>
<tr>
<td>BKG-5D10</td>
<td>28.9 27.2 22.9</td>
<td>27.6 28.9 29.2</td>
<td>0.8 1.4 1.6</td>
<td>0.3 0.2 0.2</td>
<td>6.33</td>
<td>2.33</td>
</tr>
<tr>
<td>BGK-6B5</td>
<td>5.3 3.8 3.1</td>
<td>5.6 5.8 5.2</td>
<td>0.3 0.6 0.8</td>
<td>0.3 0.2 0.2</td>
<td>2.34</td>
<td>1.08</td>
</tr>
<tr>
<td>BGK-6E3</td>
<td>8.7 7.3 6.7</td>
<td>3.1 2.2 1.8</td>
<td>0.9 1.0 0.9</td>
<td>1.1 0.5 0.3</td>
<td>4.76</td>
<td>3.06</td>
</tr>
<tr>
<td>BGK-7F7</td>
<td>5.0 5.2 5.0</td>
<td>2.0 1.9 1.8</td>
<td>3.5 6.0 5.3</td>
<td>0.4 0.3 0.2</td>
<td>5.18</td>
<td>2.17</td>
</tr>
<tr>
<td>BGK-9D10</td>
<td>7.9 6.7 6.0</td>
<td>2.4 1.7 1.6</td>
<td>1.7 1.4 1.1</td>
<td>0.8 0.4 0.2</td>
<td>5.09</td>
<td>0.96</td>
</tr>
</tbody>
</table>
Example 3.2. Anti-LRP-8 and Mu/Hu Chimeric Antibody Generation and Murine Anti-LRP-8 Antibody Analysis

Anti-LRP-8 antibody variable domain DNAs were codon optimized and cloned into expression vectors to produce mouse or human IgG proteins. The antibody constructs were expressed in HEK293 cells and purified according to established methods. Expression yield was measured with a Nanodrop spectrophotometer. Percentage of monomer was determined by size exclusion chromatography (SEC).

Nine (9) monoclonal antibodies were obtained (BGK-2C8.E6.D3; BGK-5D10-E4; BGK-6E3-F4; BGK-2C8.8C; BGK-2H4; BGK-7A1 1; BGK-7F7; BGK-9D10-2; BGK-6B5-2). Tables 2 and 5 show heavy chain and light chain variable region sequences for the 10 monoclonal LRP-8 antibodies that were constructed using this method.

Using a cell-based MSD assay, anti-LRP-8 BGK-2C8.8C antibody reacted with both human LRP-8 (hLRP-8) and cyno LRP-8 (cLRP-8) (Figure 12).

Example 4. In Vitro and In Vivo Analysis of Anti-LRP-8 Antibodies

Example 4.1. In Vitro Cell-Based Assay for Cross-reactivity

Anti-LRP-8 antibodies generated in Examples 2 and 3 were further analyzed for cross-reactivity in a cell-based binding assay using cynomolgus LRP-8, mouse LRP-8, and human LRP-8. Figure 13 shows representative data obtained from four monoclonal anti-LRP-8 antibodies, CL-105967 (BGK-9D10-2), BGK-2C8.8C, and ML199-11H1.5B2, demonstrating that all of those antibodies were capable of cross-reacting with LRP-8 from mouse, cynomolgus, and human. Table 18 summarizes the results obtained from the anti-LRP-8 antibodies in a cell-based binding assay. Most of the tested antibodies showed the ability to cross-react with cynomolgus LRP-8. Table 18 also shows the percentage of monomer of each antibody tested in this assay as determined by size exclusion chromatography (SEC).
Table 18. Binding Data and Percentage of Monomer of Chimeric anti-LRP-8 Antibodies

<table>
<thead>
<tr>
<th>Clone</th>
<th>SEC% Monomer</th>
<th>Binding hLRP8 EC50 (nM)</th>
<th>Binding cynoLRP8 EC50 (nM)</th>
<th>Binding mLRP8 EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML199-11H1.5B2</td>
<td>100</td>
<td>0.22</td>
<td>0.21</td>
<td>0.32</td>
</tr>
<tr>
<td>ML201-8F3.3D7</td>
<td>100</td>
<td>0.16</td>
<td>NT**</td>
<td>NT</td>
</tr>
<tr>
<td>BGK-2C8.8C</td>
<td>100</td>
<td>2.62</td>
<td>0.82</td>
<td>21.98</td>
</tr>
<tr>
<td>BGK-9D10-2</td>
<td>100</td>
<td>2.29</td>
<td>0.64</td>
<td>0.43</td>
</tr>
<tr>
<td>BGK-7F7</td>
<td>100</td>
<td>0.11</td>
<td>0.35</td>
<td>3.92</td>
</tr>
<tr>
<td>BGK-6B5-2</td>
<td>100</td>
<td>0.15</td>
<td>0.51</td>
<td>41.8</td>
</tr>
<tr>
<td>BGK-7A11</td>
<td>98</td>
<td>0.12</td>
<td>0.53</td>
<td>27.63</td>
</tr>
<tr>
<td>BGK-2H4</td>
<td>100</td>
<td>0.21</td>
<td>1.08</td>
<td>NB*</td>
</tr>
<tr>
<td>BGK.2C8.E6.D3 [r/m IgG2a/K]</td>
<td>99</td>
<td>0.1</td>
<td>0.31</td>
<td>NB</td>
</tr>
<tr>
<td>BGK.5D10.E4 [r/m IgG2a/K]</td>
<td>95</td>
<td>0.24</td>
<td>0.77</td>
<td>NB</td>
</tr>
<tr>
<td>BGK.6E3.F4 [r/m IgG2a/K]</td>
<td>100</td>
<td>0.11</td>
<td>0.25</td>
<td>NB</td>
</tr>
<tr>
<td>hIgGl/K mut control</td>
<td>-</td>
<td>NB</td>
<td>NB</td>
<td>NB</td>
</tr>
</tbody>
</table>

*NB: No binding observed  
**NT: No test data available

5 Example 4.2. Anti-LRP-8 PK Study IHC score and Concentration in Brain Homogenates and Serum

Anti-LRP-8 antibodies were administered to mice through an intravenous or intraperitoneal route in order to quantitatively measure their capacities to cross the blood brain barrier in the subjects. As can be seen in Figure 14, with a single intravenous injection or three consecutive intravenous (IV) injections of anti-LRP-8 ML199.11H1.5B2 antibody (50mpk x 1 or 50mpk x 3), clear vasculature IHC staining in all brain regions as well as neuronal staining in pons, medulla, spinal cord, and cortex was observed. With anti-LRP-8 ML199.11H1.5B2 antibody administration, enhanced parenchyma staining in all brain regions was observed as compared with control IgG administration. Figure 15 shows the results obtained from anti-LRP-8 antibodies, ML199.11H1.5B2, BGK-2C8.8C, and BGK-9D10-2
from IHC staining in spinal cord.

Table 19 shows anti-LRP-8 PK study IHC assessment and mouse in vivo PK study data including antibody concentration detected in brain, spinal cord, and serum samples (e.g., homogenates). Table 19 also summarizes the initial assessment of stability of the tested antibodies based on serum exposure, with 4 antibodies (ML199.1 1H1.5B2, ML201-8F3.3D7, BGK-2C8.8C, and BGK-9D 10-2) showing enhanced brain uptake in mice.

**Table 19. Anti-LRP-8 PK Study IHC Assessment and Concentration in Brain Homogenates and Serum**

<table>
<thead>
<tr>
<th>Clone Name</th>
<th>Serum exposure (24h in vivo)</th>
<th>Mouse brain uptake</th>
<th>IV/IP Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IHC</td>
<td>Brain, nM</td>
</tr>
<tr>
<td>ML199-11H1.5B2</td>
<td>Good</td>
<td>Yes</td>
<td>7.9 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>Yes</td>
<td>6.9 ± 2.5</td>
</tr>
<tr>
<td>ML201-8F3.3D7</td>
<td>Good</td>
<td>Yes</td>
<td>8.7 ± 3.8</td>
</tr>
<tr>
<td>BGK-2C8.8C</td>
<td>Good</td>
<td>Yes</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>BGK-9D10-2</td>
<td>reduced</td>
<td>Yes</td>
<td>8.1 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>Yes</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>BGK-7F7</td>
<td>low</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>BGK-6B5-2</td>
<td>low</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>BGK-7A11</td>
<td>low</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>BGK-2H4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BGK.2C8.E6.D3</td>
<td>[r/m IgG2a/K]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BGK.5D10.E4</td>
<td>[r/m IgG2a/K]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BGK.6E3.F4</td>
<td>[r/m IgG2a/K]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>hlgG1/K mut control</td>
<td>Good</td>
<td>No</td>
<td>3.05 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>No</td>
<td>4.8 ± 4.0</td>
</tr>
</tbody>
</table>

**Example 4.3 Antibodies Generated by a Yeast Display Method**

Additional anti-LRP-8 antibodies were generated by a yeast-display method. Heavy chain and light chain variable domains of the additional antibodies are shown in Tables 2 and 5. Those antibodies were also tested in a cell-based binding assay and
shown to bind cyno LRP-8 as well as mouse LRP-8 (Table 20).

Table 20. Binding of Anti-LRP-8 Antibodies to Cyno and Mouse LRP8

<table>
<thead>
<tr>
<th>Clone</th>
<th>ELISA using CR1 Peptide (OD450)</th>
<th>cyno LRP8 FACS (ratio +/−)</th>
<th>mouse LRP8 FACS (ratio +/−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML199.11H1.5B2</td>
<td>4.00</td>
<td>141.2</td>
<td>282.6</td>
</tr>
<tr>
<td>CL-134994</td>
<td>0.37</td>
<td>19.4</td>
<td>10.0</td>
</tr>
<tr>
<td>CL-135325</td>
<td>0.17</td>
<td>16.5</td>
<td>21.2</td>
</tr>
<tr>
<td>CL-135359</td>
<td>0.10</td>
<td>12.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Negative IgG control</td>
<td>0.08</td>
<td>0.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Example 4.4. Epitope Binning

Anti-LRP-8 antibodies were tested in a label-free cell-based competition assay in order to determine which antibodies are capable of binding to LRP-8 simultaneously. If antibodies were not able to bind LRP-8 simultaneously (therefore possibly competing for the same or substantially similar epitope), those antibodies were assigned to the same "epitope bin." If antibodies were capable of binding to LRP-8 simultaneously and therefore did not compete for binding to LRP-8, those antibodies were assigned to a different "epitope bin."

Figure 16A shows a schematic assay procedure. Figure 16B shows representative data obtained from the competition assay using cyno LRP-8-HEK 293 stable cells. In this representative experiment, 30 µg/ml of anti-LRP-8 BGK.6E3.F4 antibody was incubated with varying amounts of antibodies including chimeric anti-LRP-8 antibodies, BGK.2C8.E6D3, BGK.7F7, BGK.6B5-2, BGK.7A1 1, or a negative control, hlgG. As a positive control, chimeric anti-LRP-8 BGK.6E3.F4 antibody was used to demonstrate the degree of self-competition.

As shown in Figure 16B, the competition between anti-LRP-8 BGK.6E3.F4 antibody and the subset of antibodies including BGK.7F7, BGK.6B5-2, BGK.7A1 1 was observed, and therefore those antibodies were assigned to the same epitope bin. There was no competition observed between anti-LRP-8 BGK.6E3.F4 and BGK.2C8.E6D3 antibodies, and therefore these two antibodies were assigned to a different epitope bin. Table 21 summarizes the exemplary epitope binning assignments based on the cyno LRP-8-HEK293 cell-based competition assay.
(antibodies capable of binding human and cyno LRP-8 are listed in bold; antibodies capable of binding human, mouse, and cyno LRP-8 are underlined; and antibodies that demonstrated brain penetration in mice are listed in italic).

Table 21. Epitope Binning

<table>
<thead>
<tr>
<th>Bin 1</th>
<th>Bin 2</th>
<th>Bin 3</th>
<th>Bin 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML199-11H1.5B2</td>
<td>BGK.2C8.8C</td>
<td>BGK.6E3.F4</td>
<td>BGK.9D10.2</td>
</tr>
<tr>
<td>ML201-8F3.3D7</td>
<td>BGK.7E7</td>
<td>BGK.2H4</td>
<td></td>
</tr>
<tr>
<td>CL-135325</td>
<td>BGK.6B5.2</td>
<td>BGK.5D10.E4</td>
<td></td>
</tr>
<tr>
<td>CL-135359</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antibodies with significant sequence homology to the CDR sequences of anti-LRP-8-ML 199-1 1H1.5B2 antibody would be expected to bind to the same or substantially similar epitope of anti-LRP-8-ML 199-1 1H1.5B2 antibody, and therefore compete for binding to LRP-8 in a competition assay. Those competing antibodies would exhibit desirable biological properties of anti-LRP-8-ML 199-1 1H1.5B2 antibody when evaluated in *in vitro* and/or *in vivo* assays described herein and known in the art.

**Example 4.5. Mutagenesis Analysis**

In order to determine the key residues involved in the interaction between anti-LRP-8 antibodies and LRP-8 protein, a mutagenesis analysis was performed based on CR1 peptide that was used as an immunogen for generating the ML199 and ML201 antibodies. Anti-LRP-8 ML199-1 1H1.5B2 antibody was capable of binding CR1 and CR2 peptides with similar affinity. Affinity of Anti-LRP-8 ML199-1 1H1.5B2 to CR1 and CR2 is 1.5 nM and 2.1 nM respectively (Figure 17A). This, indicatets that the antibody may recognize residues shared by the peptides. The sequence alignment between CR1 and CR2 peptides shows that residues including F6, S8, and N10 are conserved between CR1 and CR2 (Figure 17B). Based on the alignment, variants of CR1 peptide were generated by mutating the conserved residues to alanine (CR1.1, CR1.2, CR1.3, and CR1.4; see also Table 1).

As shown in Figure 17C, in an ELISA binding assay, anti-LRP-8 ML199-
1H1.5B2 antibody was not able to bind CR1 variants of which F6 was mutated to alanine (CR1.1 and CR1.4) while the mutation of other residues did not affect the interaction between the antibody and the peptide (CR1.2, and CR1.3). The similar result was also obtained based on a BIACORE kinetic binding assay. These experiments demonstrate that F6 of CR1 peptide is a part of the key binding site of anti-LRP-8 ML199-1 1H1.5B2 antibody.

**Example 4.6. Crystal Structure of anti-LRP-8 Antibody with CR1 Peptide**

In order to determine the conformational epitope recognized by anti-LRP-8 ML199-1 1H1.5B2 antibody, the crystal structure of anti-LRP-8 ML199-1 1H1.5B2 antibody Fab fragment complexed with CR1 peptide was determined at 1.72 Å resolution. Figure 18A. The last 5 residues of the peptide are not seen in the crystal structure because those 5 residues were not resolved in electron density, indicating those residues do not make contact with the Fab fragment and are most likely flexible, thus not contributing to the epitope. The Fab fragment and the peptide were co-crystallized at an 8:1 molar ratio. The structure was determined by molecular replacement method (R_{f} = 0.23/0.20).

The detailed structural analysis confirms that anti-LRP-8 ML199-1 1H1.5B2 antibody makes significant contacts with CR1 peptide (Figure 18B). Using NCONT (ccp4 suite) program, 175 atomic contacts were identified within a 4.0 Å range. Based on the analysis, the region of CR1 peptide indicated in bold [CEKDQFQRNCFPSPWRC] was identified as a part of the comprehensive conformational epitope recognized by anti-LRP-8 ML199-1 1H1.5B2 antibody. Antibodies that bind to the same conformational epitope of anti-LRP-8 ML 199-1H1.5B2 antibody would be expected to possess similar biological properties of anti-LRP-8 ML 199-1 1H1.5B2 antibody.

**Example 4.7. Pharmacokinetic Analysis of Antibodies**

The pharmacokinetics of anti-LRP-8 antibody was evaluated in male CD-I mice. Groups of mice were dosed intravenously at a dose of 0.2, 1, or 5 mg/kg (5 mice per dose level). Serial blood samples were collected by tail vein puncture from 1 to 504 hours post-dose. Samples were diluted in assay buffer and analyzed using an anti-human IgG immunoassay to quantify the plasma concentration of anti-LRP-8.
Pharmacokinetic parameters were estimated by non-compartmental analysis using WinNonlin (Certara, Princeton, NJ).

Upon IV dosing, chimeric anti-LRP-8 ML199.1 1H1.5B2 antibody ([hu IgGl/k] LALA) exhibited typical bi-phasic pattern of distribution and elimination in CD-1 mice, with a volume of distribution of 85 to 135 mL/kg and clearance of 0.2 to 0.3 mL/h/kg (Figure 19). Terminal half-life of anti-LRP-8 antibody was approximately 312 to 335 hours. There was no indication of target-mediated disposition and no apparent change in elimination over time. PK was dose-linear across 0.2 to 5 mg/kg and exposure is approximately dose-proportional. Table 22 summarizes the pharmacokinetic parameters measured with chimeric anti-LRP-8 ML199.11H1.5B2 antibody ([hu IgGl/k] LALA) at various doses.

Table 22. Pharmacokinetic Parameters

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>N</th>
<th>AUC_{h1} (h*μg/ml)</th>
<th>AUC_{INF} (h*μg/ml)</th>
<th>Vss (mL/kg)</th>
<th>CL (mL/h/kg)</th>
<th>t_{1/2} (h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>IV</td>
<td>3</td>
<td>459±8</td>
<td>686±49</td>
<td>135±5.7</td>
<td>0.294±0.02</td>
<td>315</td>
</tr>
<tr>
<td>1</td>
<td>IV</td>
<td>4</td>
<td>3500±120</td>
<td>5110±240</td>
<td>84.6±18</td>
<td>0.197±0.01</td>
<td>312</td>
</tr>
<tr>
<td>5</td>
<td>IV</td>
<td>4</td>
<td>15300±900</td>
<td>23900±1140</td>
<td>106±11</td>
<td>0.211±0.01</td>
<td>335</td>
</tr>
</tbody>
</table>

*ti/2 reported as harmonic mean without variance

High dose (35 mg/kg) PK was examined by a single IV dosing in 8 week male C57B1/6 mice. Three mice were dosed for each group. Antibody concentration in serum was determined by ECL-MSD assay (Figure 20).

Example 4.8. Reelin Binding And ML199.11H1.5B2 Reelin Competition

Example 4.8.1 CR1/CR2 Peptide Ligand Binding Assay

High binding MSD plates (MSD Cat# L15XB-3 / L11XB-3) were coated with 1 μg/ml of CR1 or CR2 peptide overnight at 4°C. The next day, the plate was blocked with 3% MSD blocking buffer (MSD Cat# R93AA-01) for 1 hour at RT. The plates were washed with TTBS buffer (20mM Tris; 0.5% Tween, 150mM sodium chloride; pH 7.5) 3 times and the titer of reelin-HIS FLAG was added. After incubating for 1 hour at RT, the plate was washed and Anti-HIS (C-Term) Ab (Invitrogen Ref# 46-0693) was used to detect reelin. After incubating for 1 hour at RT, the plate was washed, and an anti-mouse SULFO-TAG Ab was added to the plate and incubated for 1 hour. The
plates were washed and immersed in MSD read buffer T with surfactant (MSD Cat# R92TC-1) before reading on an MSD SECTOR Imager 6000. Data were obtained and analyzed using a GraphPad Prism 6 software package (GraphPad Software, Inc., La Jolla, CA).

**Example 4.8.2 Cell-Based Reelin-Anti-LRP8 Competition Assay**

Anti-LRP8 competition assay was based on a cell-based Electrochemiluminescence-Meso Scale Discovery Assay (ECL-MSD) binding assay. HEK293 cells overexpressing cynomolgus monkey LRP-8 were added to the MSD 96-well plate (MSD Cat# L15XB-3/L11XB-3) and incubated at 37°C for 1 hour. Cells were blocked using 15% FBS (Hyclone, Thermo Scientific Cat# SH300700.03) at RT for 30 minutes with mild agitation, plates were washed with DPBS 3 times. Anti-LRP-8 antibody with a human Fc and reelin-HIS-FLAG were used in two competition combinations:

**Competition 1 (Competitor Anti-LRP8 Ab):** A 1:1 mixture of fixed concentrated reelin-HIS-FLAG and a titer of competitor LRP-8 were added to the plate and incubated at RT for 1 hour. After washing, reelin was detected with anti-HIS Ab for 1 hour followed by incubation with anti-mouse SULFO-TAG Ab for 1 hour.

**Competition 2 (Competitor Reelin):** A 1:1 mixture of fixed concentrated anti-LRP8 human Fc Ab and a titer of competitor reelin were added to the plate and incubated at RT for 1 hour. After washing, an anti-human SULFO-TAG Ab was added to the plate and incubated for 1 hour. The plates were washed with DPBS and immersed in MSD read buffer T surfactant free (MSD Cat# R92TD-2) before reading on an MSD SECTOR Imager 6000. Data were obtained and analyzed using a GraphPad Prism 6 software package (GraphPad Software, Inc., La Jolla, CA).

As shown in Figure 21A, reelin was capable of binding HEK293 cells overexpressing cynomolgus monkey LRP-8 in a dose-dependent manner. When the cells were incubated with 30 μg/ml of reelin and various amounts of either anti-LRP-8 ML199.1 1H1.5B2 antibody or human IgG as a negative control, neither anti-LRP-8 ML199.1 1H1.5B2 antibody nor human IgG blocked the interaction between reelin and cynomolgus LRP-8-HEK293 stable cells (Figure 21B).
**Incorporation by Reference**

**Equivalents**

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

1. A binding protein capable of binding LRP-8, comprising a heavy chain comprising a set of three heavy chain CDRs (CDR-H1, CDR-H2, and CDR-H3) selected from any set of heavy chain CDRs listed in Tables 2-4.

2. The binding protein of claim 1, wherein the binding protein further comprises a light chain comprising a set of three light chain CDRs (CDR-L1, CDR-L2, and CDR-L3) selected from any set of light chain CDRs listed in Tables 5-7.

3. A binding protein capable of binding LRP-8, comprising a heavy chain comprising a set of three heavy chain CDRs (CDR-H1, CDR-H2, and CDR-H3) and a light chain comprising a set of three light chain CDRs (CDR-L1, CDR-L2, and CDR-L3), wherein the heavy chain CDR set is selected from any set of heavy chain CDRs listed in Tables 2-4, and the light chain CDR set is the corresponding light chain CDR set from Tables 5-7.

4. A binding protein capable of binding LRP-8, comprising a heavy chain comprising a set of three heavy chain CDRs (CDR-H1, CDR-H2, and CDR-H3), wherein the CDRs are:
   (i) CDR-H1: RFTFSNXiGMS, wherein $X_1$ is F or Y, or GFTVSDYYMA;
   (ii) CDR-H2: TISSGGRTYYPDX, VKG, wherein $X_2$ is S or H, or SISYEGSSTYYGDSVKG; and
   (iii) CDR-H3: DYLYAMDY or PLRYYGYNYRFAY.

5. The binding protein of claim 4, wherein the heavy chain comprises a variable domain comprising an amino acid sequence of
   \[
   \text{EVQLVESGGGLVPRGS} \text{A} \text{S} \text{R} \text{F} \text{S} \text{N} \text{F} \text{G} \text{M} \text{S} \text{W} \text{V} \text{R} \text{Q} \text{P} \text{D} \text{K} \text{R} \text{L} \text{E} \text{W} \text{V} \text{A} \text{T} \text{I} \text{S} \text{S} \text{G} \text{G} \text{R} \text{Y} \text{T} \text{Y} \text{P} \text{D} x \text{I} \text{V} \text{K} \text{G} \text{R} \text{F} \text{T} \text{I} \text{S} \text{R} \text{D} \text{N} \text{A} \text{K} \text{N} \text{T} \text{L} \text{Y} \text{L} \text{Q} \text{M} \text{S} \text{S} \text{L} \text{R} \text{E} \text{D} \text{T} \text{A} \text{M} \text{Y} \text{Y} \text{C} \text{A} \text{R} \text{D} \text{Y} \text{L} \text{Y} \text{A} \text{D} \text{Y} \text{W} \text{G} \text{Q} \text{G} \text{T} \text{S} \text{V} \text{T} \text{V} \text{S}, \text{ or }
   \text{EVQLVESGGGLVPRGS} \text{A} \text{S} \text{R} \text{F} \text{S} \text{N} \text{F} \text{G} \text{M} \text{S} \text{W} \text{V} \text{R} \text{Q} \text{P} \text{D} \text{K} \text{R} \text{L} \text{E} \text{W} \text{V} \text{A} \text{T} \text{I} \text{S} \text{S} \text{G} \text{G} \text{R} \text{Y} \text{T} \text{Y} \text{P} \text{D} x \text{I} \text{V} \text{K} \text{G} \text{R} \text{F} \text{T} \text{I} \text{S} \text{R} \text{D} \text{N} \text{A} \text{K} \text{N} \text{T} \text{L} \text{Y} \text{L} \text{Q} \text{M} \text{S} \text{S} \text{L} \text{R} \text{E} \text{D} \text{T} \text{A} \text{M} \text{Y} \text{Y} \text{C} \text{A} \text{R} \text{D} \text{Y} \text{L} \text{Y} \text{A} \text{M} \text{D} \text{Y} \text{W} \text{G} \text{Q} \text{G} \text{T} \text{S} \text{V} \text{T} \text{V} \text{S},
   \]

130
wherein $X_1$ is R or S; or

EVQLVESGGGLVQPGSRSLKLSCAAASGFTVSDYFYMAYWRQAPKPGGLEWVASIS
YEIGSTYYGDSDKGRFTISREDNAKSLIQLMNSLRESDTAYCARPLRYGYNYRFAYWGQQTLNVTSS.

6. The binding protein of claim 4 or 5, further comprising a light chain comprising a set of three light chain CDRs (CDR-L1, CDR-L2, and CDR-L3), wherein the CDRs are:

   (i) CDR-L1: RSSQSLVVSX$_3$X$_4$NTYLH, wherein X$_3$ is N, T, R, W or P, and wherein X$_4$ is G, E, L or K, or KASQNIHKNLD;
   (ii) CDR-L2: KVSNRFS or YTDNLQT; and
   (iii) CDR-L3: SQSTHVPLT or YQYNSSGPT.

7. The binding protein of claim 6, wherein the light chain comprises a variable domain comprising an amino acid sequence of

   DVVMQTQTPLSLPVSLGDQASICRSSLQVYNSX$_2$X$_3$NTYHWLYQKPGQSPKVL
   MYKVSNRFSGVSDRFSGSGSTDFTLKISRVEAEDLVYFCQSQSTHVPLTFGA
   GTKLELK,

   wherein X$_2$ is N, T, R, W or P and $X_3$ is G, E, L or K; or
   DIQMSQSPPVLSASVGDRVTLSCKASQNIHKNLDWYQKHEAPKLILYTDN
   LQTGIPSRSFGSGSTDYTLTISSLQPEDVATYNYQNYNSGPTFGAGTKLELQ.

8. The binding protein of any one of claims 1-7, wherein the binding protein comprises a heavy chain CDR set of:

   (i) CDR-H1: RFTFSNYGMS;
   (ii) CDR-H2: TISSGGRYYPDSVKG; and
   (iii) CDR-H3: DYLYAMDY,

   and a light chain CDR set of:

   (i) CDR-L1: RSSQSLVYNSGNTYLH;
   (ii) CDR-L2: KVSNRFS; and
   (iii) CDR-L3: SQSTHVPLT.

9. The binding protein of claim 8, wherein the binding protein comprises a heavy chain variable domain of:
EVQLVESGGGLVKPGGLKLSCAASRGFTSFNYGMSWVRQTPDKRLEWVATISS
GGRYTYYPDVSKGRFTISRDNAKNTLYLQMSSLRSEDTAMYCARDYLYAMD
YWGGQGTSVTSS; and

a light chain variable domain of:
DVVMTQTPLSPLVSLGDQASICRSSQSLVYSNGNTLYHWYLQKPGQSPKVLM
YKVSNSRFSGVSDRFSGSSTFTKLISRVEAEGLVYFCQSTHVPLTFGAGTKLELK.

10. The binding protein of claim 1, wherein the binding protein comprises a heavy chain variable domain of:
EVQLVESGGGLVQPGRSLKLSCAASRGFTVSDDYMAWVRQAPKKGLEWVASIS
YEGSSTYYGDSVKGRFTISRDNASKSLYLQMNSLRSDETATYYCARPLRYYGY
NYRFAYWGGQTLVTSS, or the CDR sequence of that variable domain; and

a light chain variable domain of:
DIQMSQSSPVLASVGDRVTLSCKASQNIHKNLQDWYQKHEAPKLLLIIYTYDN
LQQTGIPSRSFGSGSTDVTTLTISSLQPEDVATYYCQYNPSGPTFGAGTKLELK,
or the CDR sequence of that variable domain.

11. The binding protein of claim 1, wherein the binding protein comprises a heavy chain variable domain of:
EVQLVESGGGLVQQGRSLKLSCAASRGFTSFNYYMAWVRQAPKKGLEWVATIT
TSGSRSFPDSDKGRFTISRDNASKSLYLQINSLKSEDATYYCARRGPGLYFD
HWGRGVMVTSS, or the CDR sequence of that variable domain; and

a light chain variable domain of:
DIQMTQSSPSLPSLGDKVTITCQASQNINIFIAWYQQPKGAPRLLIRFTSTL
ESDTPSRFSGGSGGRDYSFSISNVEEDFASYYCLHYDNLPPWTFGGGTKLELK,
or the CDR sequence of that variable domain.

12. The binding protein of claim 1, wherein the binding protein comprises a heavy chain variable domain of:
EVQLVESGGDLVKPGGLKLSCAASRGFTSFNSFGMSWVRQTPDKRLEWVATISS
GGRYTYYPDVSKGRFTISRDNAKNTLYLQMSSLRSEDTAMYCARDYLYAMD
DYWGQGTSVTSS, or the CDR sequence of that variable domain; and
a light chain variable domain of:
DVVMTQTPLSLPVLGDQASISCRSSQLVYSNGNTLYHWYLQKPGQSPKVLMYKVSNSRSGVDSRFGSGTDLKISRVEAEHLGVYFCQSTHVPLTFGAGTKLELK.

13. The binding protein of any one of claims 1-12, wherein the binding protein is an antibody.

14. The binding protein of claim 13, wherein the antibody is a monoclonal antibody.

15. The binding protein of any one of claims 1-12, wherein the binding protein is a multispecific binding protein.

16. The binding protein of claim 15, wherein the multispecific binding protein is a dual variable domain (DVD-Ig) binding protein.

17. The binding protein of claim 15 or 16, wherein the multispecific binding protein is also capable of binding a second antigen target in the brain or spinal cord other than LRP-8.

18. The binding protein of any one of claims 1-17, wherein the binding protein binds human LRP-8.

19. The binding protein of claim 18, wherein the binding protein is cross-reactive with at least one of cynomolgus monkey, rat, and mouse LRP-8.

20. The binding protein of claim 18, wherein the binding protein also binds cynomolgus monkey LRP-8.

21. The binding protein of any one of claims 1-20, wherein the binding protein binds to human LRP-8 with an EC50 of at most about 2.62 nM, or at most about 2.29 nM, or at most about 0.88 nM, or at most about 0.24 nM.
22. The binding protein of any one of claims 1-21, wherein the binding protein undergoes endocytosis by HEK293 cells expressing LRP-8 at a rate between 1.5 and 2.5 times the endocytosis of control IgG into HEK293 cells expressing LRP-8.

23. The binding protein of any one of claims 1-21, wherein the binding protein undergoes transcytosis across a Caco-2 cell monolayer expressing LRP-8 at a rate between 1.5 and 2.0 times the transcytosis of control IgG across a Caco-2 cell monolayer expressing LRP-8.

24. The binding protein of any one of claims 1-23, wherein the binding protein is able to cross the blood-brain barrier after binding to LRP-8.

25. The binding protein of any one of claims 1-24, further comprising a wild-type human lambda or kappa light chain constant region.

26. The binding protein of any one of claims 1-25, further comprising a wild-type human IgGl heavy chain constant region or a variant of a human IgGl heavy chain constant region.

27. The binding protein of claim 26, wherein the variant comprises a leucine (L) to alanine (A) mutation at amino acid positions 234 and 235 of human IgGl.

28. The binding protein of any one of claims 1-25, further comprising a heavy chain constant region selected from the group consisting of IgGl, IgG2, IgG3, IgG4, IgA, IgM, IgE, and IgD, or a variant thereof.

29. The binding protein of any one of claims 1-28, wherein the binding protein is a crystallized binding protein.

30. A binding protein that competes for binding to human LRP-8 with the binding protein of any one of claims 1-29.
31. A conjugate comprising the binding protein of any one of claims 1-30 and a second agent.

32. The conjugate of claim 31, wherein the second agent acts on the nervous system and is capable of being transported across the blood-brain barrier when the conjugate binds to LRP-8.

33. The conjugate of claim 31, wherein the second agent is an immunoadhesion molecule, an imaging agent, a therapeutic agent, or a cytotoxic agent.

34. The conjugate of claim 33, wherein the imaging agent is a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, or biotin.

35. An isolated nucleic acid or nucleic acids encoding the binding protein amino acid sequence of any one of claims 1-30.

36. A vector comprising the isolated nucleic acid or nucleic acids of claim 35.

37. A host cell comprising the vector of claim 36.

38. The host cell of claim 37, wherein the host cell is a prokaryotic cell, _Escherichia coli_, a eukaryotic cell, a protist cell, an animal cell, a plant cell, a fungal cell, a yeast cell, an Sf9 cell, a mammalian cell, an avian cell, an insect cell, a CHO cell or a COS cell.

39. A method of producing a binding protein, comprising culturing the host cell of claim 37 or 38 in culture medium under conditions sufficient to produce the binding protein.

40. A pharmaceutical composition comprising the binding protein of any one of claims 1-30 or the conjugate of claim 31, and a pharmaceutically acceptable carrier.
41. The pharmaceutical composition of claim 40, further comprising at least one additional agent.

42. The pharmaceutical composition according to claim 41, wherein the additional agent is an imaging agent, a cytotoxic agent, an angiogenesis inhibitor, a kinase inhibitor, a co-stimulation molecule blocker, an adhesion molecule blocker, an anti-cytokine antibody or functional fragment thereof, a detectable label or reporter, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

43. A method of treating a subject for a disease or a disorder by administering the binding protein of any one of claims 1-30 or the conjugate of claim 31 to the subject.

44. The method of claim 43, wherein the disorder is
   a. a brain disorder, an autoimmune or inflammatory disease of the brain, an infectious disorder of the brain, a neurological disorder, a neurodegenerative disorder, a brain cancer, or a brain metastasis, and/or
   b. Huntington's chorea, Parkinson's disease, Alzheimer's disease, dementia, acute or chronic spinal cord injury, multiple sclerosis, stroke, mental disorders, depression, schizophrenia, acute pain, or chronic pain.

45. The method of claim 43 or 44, wherein the administering to the subject is by a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteo, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intraretinal, intrarectal, intrarenal, intraretinal,
intraspinal, intrasynovial, intrathecal, intrathoracic, intrauterine, intravesical, bolus, epidural, vaginal, rectal, buccal, sublingual, intranasal, transdermal, or oral route.

46. Use of the binding protein of any one of claims 1-30 or the conjugate of claim 31 in the manufacture of a medicament for treating a subject for a disease or disorder.

47. The use of claim 46, wherein the disorder is
   a. a brain disorder, an autoimmune or inflammatory disease of the brain, an infectious disorder of the brain, a neurological disorder, a neurodegenerative disorder, a brain cancer, or a brain metastasis, and/or
   b. Huntington's chorea, Parkinson's disease, Alzheimer's disease, dementia, acute or chronic spinal cord injury, multiple sclerosis, stroke, mental disorders, depression, schizophrenia, acute pain, or chronic pain.

48. The binding protein of any one of claims 1-30 or the conjugate of claim 31 for use in treating a subject for a disease or a disorder.

49. The binding protein of claim 48, wherein the disorder is
   a. a brain disorder, an autoimmune or inflammatory disease of the brain, an infectious disorder of the brain, a neurological disorder, a neurodegenerative disorder, a brain cancer, or a brain metastasis, and/or
   b. Huntington's chorea, Parkinson's disease, Alzheimer's disease, dementia, acute or chronic spinal cord injury, multiple sclerosis, stroke, mental disorders, depression, schizophrenia, acute pain, or chronic pain.

50. A method of detecting the presence, amount, or concentration of LRP-8 or a fragment of LRP-8 in a test sample by an immunoassay, comprising contacting the test sample with at least one binding protein and at least one detectable label, and
wherein the at least one binding protein comprises the binding protein of any one of claims 1-30.

51. The method of claim 50, wherein the test sample is from a patient and the method further comprises diagnosing, prognosticating, or assessing the efficiency of therapeutic/prophylactic treatment of the patient, and wherein if the method further comprises assessing the efficacy of therapeutic/prophylactic treatment of the patient, the method optionally further comprises modifying the therapeutic/prophylactic treatment of the patient as needed to improve efficacy.

52. A kit for assaying a test sample for the presence, amount, or concentration of LRP-8 or a fragment of LRP-8 in the sample, the kit comprising (a) instructions for assaying the test sample for LRP-8 or a fragment of LRP-8, and (b) at least one binding protein comprising the binding protein of any one of claims 1-30.

53. The binding protein of any one of claims 1-3, wherein the variable domains that form a functional target site for LRP-8 comprise a heavy chain having at least about 80%, 90%, 95%, or 98% homology to any of those selected from Tables 2-4.

54. The binding protein of any one of claims 1-3, wherein the variable domains that form a functional target site for LRP-8 comprise a light chain having at least about 80%, 90%, 95%, or 98%, homology to any of those selected from Tables 5-7.

55. The binding protein of claim 1, wherein the binding protein comprises any one of the sequences selected from those listed in Tables 2-7, or any pair of heavy and light chain sequences listed in Tables 2-7.

56. A binding protein that binds to LRP-8 at an epitope comprising or consisting of amino acid residues 33-622 of human LRP-8 (SEQ ID NO:1).

57. A binding protein that binds to LRP-8 at an epitope comprising or consisting of amino acid residues 47-66; or amino acids residues 47-57; or amino acid residues 47-57 and 60; or amino acid residues 52-56; or amino acid residues 52(F), and 56(N); or
amino acid residues 47(C), 52(F), 56(N), 59(C), 60(1), 64(W), and 66(C) of human LRP-8 (SEQ ID NO:1).

58. The binding protein of claim 56 or 57, wherein the binding protein is a monoclonal antibody.

59. The binding protein of claim 56 or 57, wherein the binding protein is a multispecific binding protein.

60. The binding protein of claim 59, wherein the binding protein is a dual variable domain (DVD-Ig) binding protein.

61. A binding protein that competes for binding to human LRP-8 with the binding protein of any one of claims 53-60.

62. An LRP-8 binding protein, wherein the binding protein binds to human LRP-8 with an EC50 of at most about 2.62 nM, or at most about 2.29 nM, or at most about 0.88 nM, or at most about 0.24 nM.

63. The binding protein of claim 62, wherein the binding protein is a monoclonal antibody.

64. The binding protein of claim 62, wherein the binding protein is a multispecific binding protein.

65. The binding protein of claim 64, wherein the binding protein is a dual variable domain (DVD-Ig) binding protein.

66. An isolated polypeptide comprising an amino acid sequence of human LRP-8 CR1 peptide (SEQ ID NO:2) or human LRP-8 CR2 peptide (SEQ ID NO:3).

67. The polypeptide of claim 66, wherein the polypeptide is a cyclic peptide.
68. A method of generating an LRP-8 binding protein by immunizing a mammal with the polypeptide of claim 66 or 67.

69. The method of claim 68, wherein the mammal is a mouse.

70. The method of claim 68 or 69, wherein the method further comprises isolating the binding protein from a spleen of the mammal.

71. The method of any one of claims 68-70, wherein the method further comprises generating a hybridoma that is capable of producing the binding protein.

72. A polypeptide comprising the amino acid sequence of Cyno LRP-8 isoform 1.
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<td>DACELSVQPNGGCEYLCLPAPQISSHSPKTYTCACPDTMMLGDPMDKRCY</td>
<td>DACELSVQPNGGCEYLCLPAPQISSHSPKTYTCACPDTMMLGDPMDKRCY</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MOUSE</td>
<td>DACELSVQPNGGCEYLCLPAPQISSHSPKTYTCACPDTMMLGDPMDKRCY</td>
<td>DACELSVQPNGGCEYLCLPAPQISSHSPKTYTCACPDTMMLGDPMDKRCY</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CYNO</td>
<td>(721) 721</td>
<td>730</td>
<td>740</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>760</td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 2A**

SUBSTITUTE SHEET (RULE 26)
<table>
<thead>
<tr>
<th>CR1 Peptide</th>
<th>CR2 Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>KDQFQCRNRCIPSVWRCDEDDCLDHSDEDCPDKTCA</td>
<td>KDQFQCRNRCIPSVWRCDEDDCLDHSDEDCPDKTCA</td>
</tr>
<tr>
<td>EDPFQCRNRCIPLVWRCDEDDCSDNSDEDCPDKTCA</td>
<td>EDPFQCRNRCIPLVWRCDEDDCSDNSDEDCPDKTCA</td>
</tr>
<tr>
<td>KDQFQCRNRCIPSVWRCDEDDCLDHSDEDCPDKTCA</td>
<td>KDQFQCRNRCIPSVWRCDEDDCLDHSDEDCPDKTCA</td>
</tr>
<tr>
<td>KDQFQCRNRCIPSVWRCDEDDCLDHSDEDCPDKTCA</td>
<td>KDQFQCRNRCIPSVWRCDEDDCLDHSDEDCPDKTCA</td>
</tr>
</tbody>
</table>

**FIG. 2A**

CONTINUED

AT CR1 AND CR2, PEPTIDES IDENTICAL IN CYNO AND HUMAN LRP8
FIG. 2B-Continued

(401) huLRP8(NP_004622.2)[401]
cynoLRP8(XP_005543831)[398]
cynoLRP8(238B)[394]
cynoLRP8(481B)[377]
cynoLRP8(99F-Hipo)[368]
cynoLRP8(481B)G646A,C668A,T688G(377)
cynoLRP8(238B)p[388]
cynoLRP8(99F-Hipo)G740A,C1075T(370)

(501) huLRP8(NP_004622.2)[501]
cynoLRP8(XP_005543831)[498]
cynoLRP8(238B)[494]
cynoLRP8(481B)[477]
cynoLRP8(99F-Hipo)[468]
cynoLRP8(481B)G646A,C668A,T688G(477)
cynoLRP8(238B)p[488]
cynoLRP8(99F-Hipo)G740A,C1075T(470)

(601) huLRP8(NP_004622.2)[601]
cynoLRP8(XP_005543831)[598]
cynoLRP8(238B)[594]
cynoLRP8(481B)[577]
cynoLRP8(99F-Hipo)[568]
cynoLRP8(481B)G646A,C668A,T688G(577)
cynoLRP8(238B)p[588]
cynoLRP8(99F-Hipo)G740A,C1075T(570)

(701) huLRP8(NP_004622.2)[701]
cynoLRP8(XP_005543831)[698]
cynoLRP8(238B)[694]
cynoLRP8(481B)[677]
cynoLRP8(99F-Hipo)[669]
cynoLRP8(481B)G646A,C668A,T688G(677)
cynoLRP8(238B)p[688]
cynoLRP8(99F-Hipo)G740A,C1075T(670)
MOUSE IN VIVO PK STUDY WITH ANTI-LRP8, ML199.11H1.5B2 mu/hu IgG1m/k: ENHANCED UPTAKE INTO BRAIN IN 24 H: POSITIVE IHC STAINING OF PARENCHYMA AND NEURONAL CELLS

SN13226
30mpk IV 24hrs

ANTILRP8 11H1 PONS/MEDULLA

IHC: V=2.7 P=0.3 N=0.3

IgG CONTROL PONS/MEDULLA

ANTILRP8 11H1 CORTEX

IHC: V=2.7 P=0.1 N=0

IgG CONTROL CORTEX

→ VASCULATURE

→ NEURON

AVERAGE IHC SCORING: RANGE = 0-4
V: VASCULATURE
P: PARENCHYMA
N: NEURON

FIG. 5
FIG. 7

- VASCULATURE
- PARENCHYMA
- NEURON

**Anti-LRP8.8F3.3D7.40mpk**
- **Hc** (V = 3, P = 0.7, N = 0.2)
- **Brain (nM):** 7.7 ± 4.1
- **Serum (nM):** 229 ± 225.9

**Anti-LRP8.11H1.3B2.40mpk**
- **Hc** (V = 3, P = 0.3, N = 0.2)
- **Brain (nM):** 8.7 ± 3.8
- **Serum (nM):** 374 ± 80.6

**Anti-TR.20mpk**
- **Hc** (V = 3, P = 2.3, N = 1.7)
- **Brain (nM):** 17.6 ± 4.7
- **Serum (nM):** 0.39 ± 0.168

**hLgG CONTROL.20mpk**
- **Hc** (V = 1.7, P = 0, N = 0)
- **Brain (nM):** 0.9 ± 0.4
- **Serum (nM):** 940 ± 156.2

**hLgG CONTROL.50mpk**
- **Hc** (V = 1.7, P = 0, N = 0)
- **Brain (nM):** 4.1 ± 0.3
- **Serum (nM):** 1788 ± 229

**IV: 24 hours 40X images**
- **Pons Medulla Region**

- AVERAGE IHC SCORING: RANGE = 0.4

**SEPARATE STUDY**
- **hLgG CONTROL 50mpk**
FIG. 8
FIG. 11A

<table>
<thead>
<tr>
<th>Clone</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGK-5D10-E4</td>
<td>IgG2a/k</td>
</tr>
<tr>
<td>BGK-6E3-F4</td>
<td>IgG2a/k</td>
</tr>
<tr>
<td>BGK-2C8-E6-D3</td>
<td>IgG2b/k</td>
</tr>
</tbody>
</table>

**Graphs:**
- pB-LRP8-hum
- pB1-LRP8-mur
- Irrelevant Construct
  - 6G11 was lost in subcloning
  - BGK-5D10-E4 (HU, CYNO)
  - BGK-6E3-F4 (HU)
  - BGK-2C8-E6-D3 (HU, CYNO)

**FIG. 11B**

<table>
<thead>
<tr>
<th>SUP Before Subclone</th>
<th>MOUSE LRP8-HEK293</th>
<th>CYNO LRP8-HEK293</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fold Over Isotype</td>
<td>Fold Over Isotype</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Sup Clone Sup</td>
</tr>
<tr>
<td>T25 SUP BGK-2C8</td>
<td>53.19</td>
<td>131.97</td>
</tr>
<tr>
<td>T25 SUP BGK-6G11</td>
<td>24.56</td>
<td>5.14</td>
</tr>
<tr>
<td>T25 SUP BGK-7F7</td>
<td>27.42</td>
<td>21.64</td>
</tr>
</tbody>
</table>

**Tables:**
- Mouse LRP8-HEK293
- Cyto LRP8-HEK293

**Tables:**
- Fold Over Isotype Control
- Fold Over Isotype Sup Clone Sup
BGK-2C8.8C HECK293-mLRP8 BINDING FACS

FIG. 12A

FIG. 12B

BGK-2C8.8C ALSO REACTS WITH hLRP8 AND CYNOLRP8
(CELL-BASED MSD ASSAY)

FIG. 12C
<table>
<thead>
<tr>
<th></th>
<th>EC50 mM</th>
<th>EC50 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL-105967/10-2</td>
<td>0.26</td>
<td>51.21</td>
</tr>
<tr>
<td>BGK-2C8.8C</td>
<td>21.98</td>
<td>4396</td>
</tr>
<tr>
<td>11H1</td>
<td>0.27</td>
<td>41.04</td>
</tr>
<tr>
<td>hlgG</td>
<td>NB</td>
<td>NB</td>
</tr>
</tbody>
</table>

**FIG. 13A**

mlRPG assay, Anti-LRP8 Abs

**SIGNAL**

0.1

1

10

100

1000

10000

100000

20000

40000

60000

80000

100000

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FIG. 16A

LABEL-FREE CELL-BASED COMPETITION ASSAY

ANT-HUMAN Fc Sulf-TAG Ab
ANT-LRP8 hFc Ab
ANT-LRP8 MFC Ab
LRP8 EXPRESSING CELL
FIG. 22A

WH Alignment of ALL HLRP8 Antibodies (CDRs were highlighted in bold)

FIG. 22B

VL Alignment of ALL HLRP8 Antibodies (CDRs were highlighted in bold)