[0006] All references cited herein, including patent applications, patent publications, and Genbank Accession numbers are herein incorporated by reference, as if each individual reference were specifically and individually indicated to be incorporated by reference in its entirety.

SUMMARY

[0006a] In a first aspect, the present disclosure provides a bifunctional protein construct comprising a first binding moiety and a second binding moiety, wherein the first binding moiety specifically binds to a muscle-specific molecule, and wherein the second binding moiety specifically binds to a Notch receptor and activates the Notch receptor.

[0006b] In a second aspect, the present disclosure provides one or more isolated nucleic acids encoding the bifunctional protein construct of the disclosure, or one or more vectors comprising the one or more isolated nucleic acids, optionally wherein the one or more vectors is a viral vector.

[0006c] In a third aspect, the present disclosure provides a host cell expressing the bifunctional protein construct of the disclosure, comprising the one or more isolated nucleic acids of the diclosure, or comprising the one or more vectors of the disclosure.

[0006d] In a fourth aspect, the present disclosure provides a pharmaceutical composition comprising: i) the bifunctional protein construct of the disclosure, the one or more isolated nucleic acids of the disclosure, or the one or more vectors of the disclosure; and ii) a pharmaceutically acceptable excipient.

[0006e] In a fifth aspect, the present disclosure provides a method of producing a bifunctional protein construct, comprising:

(a) culturing a host cell comprising the one or more isolated nucleic acids of the disclosure or the one or more vectors of the disclosure, or the host cell of the disclosure, under a condition suitable for the expression of the bifunctional protein construct; and

(b) recovering the expressed bifunctional protein construct from the cultured host cell.

[0006f] In a sixth aspect, the present disclosure provides a use of the bifunctional protein construct of the disclosure or the pharmaceutical composition of the disclosure in the manufacture of a medicament for treating a muscle-related disease in an individual, optionally wherein:

(a) the muscle-related disease is selected from the group consisting of Pompe disease, centronuclear myopathy, fibrodysplasia ossificans progressive (FOP), Friedreich's ataxia (FRDA), familial hypertrophic cardiomyopathy, Laing distal myopathy, myofibrillar myopathy, and muscular dystrophy, optionally wherein the muscle disease is muscular dystrophy, further optionally wherein the muscular dystrophy comprises one or more of: Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), limb-girdle muscular dystrophy (LGMD), congenital muscular dystrophy (CMD), facioscapulohumeral muscular dystrophy (FSHD), myotonic dystrophy (DM), oculopharyngeal muscular dystrophy (OPMD), distal muscular dystrophy (DD), congenital myopathy, Charcot-Marie-Tooth (CMT) disorder, and Emery-Dreifuss muscular dystrophy (EDMD); and/or

(b) the bifunctional protein construct or the pharmaceutical composition is to be administered intravenously, subcutaneously, or intramuscularly.

[0006g] In a seventh aspect, the present disclosure provides an engineered DLL4 extracellular domain (ECD), wherein

(a) the engineered DLL4 ECD comprises a mutation selected from the group consisting of T52N and T135N, and wherein the amino acid position is in reference to a reference DLL4 ECD comprising the amino acid sequence of SEQ ID NO: 126, optionally wherein the engineered DLL4 ECD further comprises a mutation at one or more amino acid positions selected from the group consisting of G2, E14, R66, P80, F81, H168, Q220, N231, and N260, further optionally wherein the further mutation is selected from the group consisting of G2S, E14H, R66S, R66T, P80L, F81L, H168Y, Q220H, N231D, and N260D; and/or

(b) the engineered DLL4 ECD comprises a mutation selected from the group consisting of:

(i) T52N and T135N;

(ii) T52N, R66S, and T135N;

(iii) E14H, T52N, R66T, P80L, T135N, and N231D;

(iv) T52N, R66T, P80L, T135N, Q220H, and N260D;

(v) G2S, T52N, F81L, T135N, and H168Y;

(vi) G2S, T52N, F81L, R66S, T135N, and H168Y;

(vii) G2S, E14H, T52N, F81L, R66T, P80L, T135N, H168Y, and N231D; and

(viii) G2S, T52N, R66T, P80L, F81L, T135N, H168Y, Q220H, and N260D,

optionally wherein the engineered DLL4 ECD comprises an amino acid sequence selected from the group consisting of any one of SEQ ID NOs: 261-264.

[0006h] In an eighth aspect, the present disclosure provides a protein construct comprising the engineered DLL4 ECD of the disclosure, optionally wherein the protein construct further comprises a binding moiety that specifically binds to a muscle-specific molecule.

[0007] The present application in one example provides a bifunctional protein construct comprising a first binding moiety and a second binding moiety, wherein the first binding moiety specifically binds to a muscle-specific molecule, and wherein the second binding moiety specifically binds to a Notch receptor and activates the Notch receptor.

[0008] In some examples according to the bifunctional protein construct described above, the muscle-specific molecule is a target antigen on the sarcolemma, between the sarcolemma and the basal lamina, or in the basal lamina. In some examples, the target antigen is selected from the group consisting of laminin, agrin, nidogen, perlecan, and M-cadherin (CDH15). In some examples, the target antigen is a component of the dystrophin-associated glycoprotein complex (DGC). In some examples, the target antigen is selected from the group consisting of α-dystroglycan (α-DG), β-DG, laminin-211, perlecan, collagen, α-sarcoglycan, β-sarcoglycan, γ-sarcoglycan, δ-sarcoglycan, ε-sarcoglycan, ζ-sarcoglycan, biglycan, sarcospan, and matriglycan on α-DG. In some examples, the target antigen is a laminin subunit alpha-2 (LAMA2), CDH15, α-DG, or matriglycan on α-DG.

[0009] In some examples according to any of the bifunctional protein constructs described above, the first binding moiety comprises an antibody moiety specifically binding to the muscle-specific molecule. In some examples, the antibody moiety is selected from the group consisting of a full-length antibody, a Fab, a Fab', a $F(ab')_2$, an scFv, and an sdAb.

[0010] In some examples according to any of the bifunctional protein constructs described above, the antibody moiety specifically binds to LAMA2 (anti-LAMA2 antibody moiety). In some examples, the anti-LAMA2 antibody moiety comprises: (i) an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 3, an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 5, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 6; (ii) an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 8, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 9, an LC-CDR1

comprising the amino acid sequence of SEQ ID NO: 10, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 12; (iii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 54, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (iv) comprises an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 54, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (v) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 54, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (vi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 54, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 69; (vii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 54, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (viii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 54, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (ix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 54, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; (x) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 54, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73; (xi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 55, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 65; (xii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 55, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (xiii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 55, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (xiv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 55, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (xv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 55, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL

comprising the amino acid sequence of SEQ ID NO: 69; (xvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 55, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (xvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 55, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (xviii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 55, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; (xix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 55, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73; (xx) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 56, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 65; (xxi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 56, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (xxii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 56, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (xxiii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 56, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (xxiv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 56, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 69; (xxv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 56, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (xxvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 56, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (xxvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 56, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; (xxviii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 56, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73; (xxix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid

sequence of SEQ ID NO: 57, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 65; (xxx) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 57, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (xxxi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 57, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (xxxii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 57, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (xxxiii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 57, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 69; (xxxiv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 57, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (xxxv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 57, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (xxxvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 57, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; (xxxvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 57, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73; (xxxviii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 58, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 65; (xxxix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 58, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (xl) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 58, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (xli) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 58, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (xlii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 58, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID

NO: 69; (xliii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 58, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (xliv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 58, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (xlv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 58, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; (xlvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 58, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73; (xlvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 59, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 65; (xlviii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 59, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (xlix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 59, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (l) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 59, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (li) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 59, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 69; (lii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 59, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (liii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 59, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (liv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 59, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; (lv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 59, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73; (lvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 60, and an LC-

CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 65; (lvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 60, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (lviii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 60, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (lix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 60, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (lx) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 60, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 69; (lxi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 60, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (lxii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 60, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (lxiii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 60, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; (lxiv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 60, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73; (lxv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 61, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 65; (lxvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 61, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (lxvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 61, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (lxviii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 61, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (lxix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 61, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 69; (lxx) an HC-CDR1, HC-CDR2, and

HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 61, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (lxxi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 61, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (lxxii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 61, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; (lxxiii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 61, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73; (lxxiv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 62, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 65; (lxxv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 62, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (lxxvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 62, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (lxxvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 62, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (lxxviii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 62, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 69; (lxxix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 62, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (lxxx) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 62, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (lxxxi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 62, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; (lxxxii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 62, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73; (lxxxiii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 63, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL

comprising the amino acid sequence of SEQ ID NO: 65; (lxxxiv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 63, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (lxxxv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 63, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (lxxxvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 63, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (lxxxvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 63, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 69; (lxxxviii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 63, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (lxxxix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 63, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (xc) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 63, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; (xci) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 63, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73; (xcii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 64, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 65; (xciii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 64, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 66; (xciv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 64, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 67; (xcv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 64, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 68; (xcvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 64, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 69; (xcvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid

sequence of SEQ ID NO: 64, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 70; (xcviii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 64, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 71; (xcix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 64, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 72; or (c) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 64, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 73. In some examples, the anti-LAMA2 antibody moiety comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 3, an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 5, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 6. In some examples, the anti-LAMA2 antibody moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 49, and a VL comprising the amino acid sequence of SEQ ID NO: 50.

[0011] In some examples according to any of the bifunctional protein constructs described above, the anti-LAMA2 antibody moiety is an anti-LAMA2 scFv. In some examples, the anti-LAMA2 scFv comprises the amino acid sequence of SEQ ID NO: 51. In some examples, the anti-LAMA2 antibody moiety is an anti-LAMA2 Fab. In some examples, the anti-LAMA2 Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 52, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 53.

[0012] In some examples according to any of the bifunctional protein constructs described above, the anti-LAMA2 antibody moiety comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 8, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 9, an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 10, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 12.

[0013] In some examples according to any of the bifunctional protein constructs described above, the anti-LAMA2 antibody moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 54, and a VL comprising the amino acid sequence of SEQ ID

NO: 65. In some examples, the anti-LAMA2 antibody moiety comprises: (i) a VH comprising the amino acid sequence of SEQ ID NO: 54, and a VL comprising the amino acid sequence of SEQ ID NO: 65; (ii) a VH comprising the amino acid sequence of SEQ ID NO: 54, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (iii) a VH comprising the amino acid sequence of SEQ ID NO: 54, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (iv) a VH comprising the amino acid sequence of SEQ ID NO: 54, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (v) a VH comprising the amino acid sequence of SEQ ID NO: 54, and a VL comprising the amino acid sequence of SEQ ID NO: 69; (vi) a VH comprising the amino acid sequence of SEQ ID NO: 54, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (vii) a VH comprising the amino acid sequence of SEQ ID NO: 54, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (viii) a VH comprising the amino acid sequence of SEQ ID NO: 54, and a VL comprising the amino acid sequence of SEQ ID NO: 72; (ix) a VH comprising the amino acid sequence of SEQ ID NO: 54, and a VL comprising the amino acid sequence of SEQ ID NO: 73; (x) a VH comprising the amino acid sequence of SEQ ID NO: 55, and a VL comprising the amino acid sequence of SEQ ID NO: 65; (xi) a VH comprising the amino acid sequence of SEQ ID NO: 55, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (xii) a VH comprising the amino acid sequence of SEQ ID NO: 55, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (xiii) a VH comprising the amino acid sequence of SEQ ID NO: 55, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (xiv) a VH comprising the amino acid sequence of SEQ ID NO: 55, and a VL comprising the amino acid sequence of SEQ ID NO: 69; (xv) a VH comprising the amino acid sequence of SEQ ID NO: 55, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (xvi) a VH comprising the amino acid sequence of SEQ ID NO: 55, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (xvii) a VH comprising the amino acid sequence of SEQ ID NO: 55, and a VL comprising the amino acid sequence of SEQ ID NO: 72; (xviii) a VH comprising the amino acid sequence of SEQ ID NO: 55, and a VL comprising the amino acid sequence of SEQ ID NO: 73; (xix) a VH comprising the amino acid sequence of SEQ ID NO: 56, and a VL comprising the amino acid sequence of SEQ ID NO: 65; (xx) a VH comprising the amino acid sequence of SEQ ID NO: 56, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (xxi) a VH comprising the amino acid sequence of SEQ ID NO: 56, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (xxii) a VH comprising the amino acid sequence of SEQ ID NO: 56, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (xxiii) a VH comprising the amino acid sequence of SEQ ID NO: 56, and a VL

comprising the amino acid sequence of SEQ ID NO: 69; (xxiv) a VH comprising the amino acid sequence of SEQ ID NO: 56, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (xxv) a VH comprising the amino acid sequence of SEQ ID NO: 56, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (xxvi) a VH comprising the amino acid sequence of SEQ ID NO: 56, and a VL comprising the amino acid sequence of SEQ ID NO: 72; (xxvii) a VH comprising the amino acid sequence of SEQ ID NO: 56, and a VL comprising the amino acid sequence of SEQ ID NO: 73; (xxviii) a VH comprising the amino acid sequence of SEQ ID NO: 57, and a VL comprising the amino acid sequence of SEQ ID NO: 65; (xxix) a VH comprising the amino acid sequence of SEQ ID NO: 57, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (xxx) a VH comprising the amino acid sequence of SEQ ID NO: 57, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (xxxi) a VH comprising the amino acid sequence of SEQ ID NO: 57, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (xxxii) a VH comprising the amino acid sequence of SEQ ID NO: 57, and a VL comprising the amino acid sequence of SEQ ID NO: 69; (xxxiii) a VH comprising the amino acid sequence of SEQ ID NO: 57, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (xxxiv) a VH comprising the amino acid sequence of SEQ ID NO: 57, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (xxxv) a VH comprising the amino acid sequence of SEQ ID NO: 57, and a VL comprising the amino acid sequence of SEQ ID NO: 72; (xxxvi) a VH comprising the amino acid sequence of SEQ ID NO: 57, and a VL comprising the amino acid sequence of SEQ ID NO: 73; (xxxvii) a VH comprising the amino acid sequence of SEQ ID NO: 58, and a VL comprising the amino acid sequence of SEQ ID NO: 65; (xxxviii) a VH comprising the amino acid sequence of SEQ ID NO: 58, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (xxxix) a VH comprising the amino acid sequence of SEQ ID NO: 58, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (xl) a VH comprising the amino acid sequence of SEQ ID NO: 58, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (xli) a VH comprising the amino acid sequence of SEQ ID NO: 58, and a VL comprising the amino acid sequence of SEQ ID NO: 69; (xlii) a VH comprising the amino acid sequence of SEQ ID NO: 58, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (xliii) a VH comprising the amino acid sequence of SEQ ID NO: 58, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (xliv) a VH comprising the amino acid sequence of SEQ ID NO: 58, and a VL comprising the amino acid sequence of SEQ ID NO: 72; (xlv) a VH comprising the amino acid sequence of SEQ ID NO: 58, and a VL comprising the amino acid sequence of SEQ ID NO: 73; (xlvi) a VH comprising the amino

acid sequence of SEQ ID NO: 59, and a VL comprising the amino acid sequence of SEQ ID NO: 65; (xlvii) a VH comprising the amino acid sequence of SEQ ID NO: 59, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (xlviii) a VH comprising the amino acid sequence of SEQ ID NO: 59, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (xlix) a VH comprising the amino acid sequence of SEQ ID NO: 59, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (l) a VH comprising the amino acid sequence of SEQ ID NO: 59, and a VL comprising the amino acid sequence of SEQ ID NO: 69; (li) a VH comprising the amino acid sequence of SEQ ID NO: 59, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (lii) a VH comprising the amino acid sequence of SEQ ID NO: 59, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (liii) a VH comprising the amino acid sequence of SEQ ID NO: 59, and a VL comprising the amino acid sequence of SEQ ID NO: 72; (liv) a VH comprising the amino acid sequence of SEQ ID NO: 59, and a VL comprising the amino acid sequence of SEQ ID NO: 73; (lv) a VH comprising the amino acid sequence of SEQ ID NO: 60, and a VL comprising the amino acid sequence of SEQ ID NO: 65; (lvi) a VH comprising the amino acid sequence of SEQ ID NO: 60, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (lvii) a VH comprising the amino acid sequence of SEQ ID NO: 60, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (lviii) a VH comprising the amino acid sequence of SEQ ID NO: 60, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (lix) a VH comprising the amino acid sequence of SEQ ID NO: 60, and a VL comprising the amino acid sequence of SEQ ID NO: 69; (lx) a VH comprising the amino acid sequence of SEQ ID NO: 60, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (lxi) a VH comprising the amino acid sequence of SEQ ID NO: 60, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (lxii) a VH comprising the amino acid sequence of SEQ ID NO: 60, and a VL comprising the amino acid sequence of SEQ ID NO: 72; (lxiii) a VH comprising the amino acid sequence of SEQ ID NO: 60, and a VL comprising the amino acid sequence of SEQ ID NO: 73; (lxiv) a VH comprising the amino acid sequence of SEQ ID NO: 61, and a VL comprising the amino acid sequence of SEQ ID NO: 65; (lxv) a VH comprising the amino acid sequence of SEQ ID NO: 61, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (lxvi) a VH comprising the amino acid sequence of SEQ ID NO: 61, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (lxvii) a VH comprising the amino acid sequence of SEQ ID NO: 61, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (lxviii) a VH comprising the amino acid sequence of SEQ ID NO: 61, and a VL comprising the amino acid sequence of SEQ ID NO: 69; (lxix) a VH comprising the amino

acid sequence of SEQ ID NO: 61, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (lxx) a VH comprising the amino acid sequence of SEQ ID NO: 61, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (lxxi) a VH comprising the amino acid sequence of SEQ ID NO: 61, and a VL comprising the amino acid sequence of SEQ ID NO: 72; (lxxii) a VH comprising the amino acid sequence of SEQ ID NO: 61, and a VL comprising the amino acid sequence of SEQ ID NO: 73; (lxxiii) a VH comprising the amino acid sequence of SEQ ID NO: 62, and a VL comprising the amino acid sequence of SEQ ID NO: 65; (lxxiv) a VH comprising the amino acid sequence of SEQ ID NO: 62, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (lxxv) a VH comprising the amino acid sequence of SEQ ID NO: 62, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (lxxvi) a VH comprising the amino acid sequence of SEQ ID NO: 62, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (lxxvii) a VH comprising the amino acid sequence of SEQ ID NO: 62, and a VL comprising the amino acid sequence of SEQ ID NO: 69; (lxxviii) a VH comprising the amino acid sequence of SEQ ID NO: 62, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (lxxix) a VH comprising the amino acid sequence of SEQ ID NO: 62, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (lxxx) a VH comprising the amino acid sequence of SEQ ID NO: 62, and a VL comprising the amino acid sequence of SEQ ID NO: 72; (lxxxi) a VH comprising the amino acid sequence of SEQ ID NO: 62, and a VL comprising the amino acid sequence of SEQ ID NO: 73; (lxxxii) a VH comprising the amino acid sequence of SEQ ID NO: 63, and a VL comprising the amino acid sequence of SEQ ID NO: 65; (lxxxiii) a VH comprising the amino acid sequence of SEQ ID NO: 63, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (lxxxiv) a VH comprising the amino acid sequence of SEQ ID NO: 63, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (lxxxv) a VH comprising the amino acid sequence of SEQ ID NO: 63, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (lxxxvi) a VH comprising the amino acid sequence of SEQ ID NO: 63, and a VL comprising the amino acid sequence of SEQ ID NO: 69; (lxxxvii) a VH comprising the amino acid sequence of SEQ ID NO: 63, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (lxxxviii) a VH comprising the amino acid sequence of SEQ ID NO: 63, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (lxxxix) a VH comprising the amino acid sequence of SEQ ID NO: 63, and a VL comprising the amino acid sequence of SEQ ID NO: 72; (xc) a VH comprising the amino acid sequence of SEQ ID NO: 63, and a VL comprising the amino acid sequence of SEQ ID NO: 73; (xci) a VH comprising the amino acid sequence of SEQ ID NO: 64, and a VL comprising the amino acid sequence of

SEQ ID NO: 65; (xcii) a VH comprising the amino acid sequence of SEQ ID NO: 64, and a VL comprising the amino acid sequence of SEQ ID NO: 66; (xciii) a VH comprising the amino acid sequence of SEQ ID NO: 64, and a VL comprising the amino acid sequence of SEQ ID NO: 67; (xciv) a VH comprising the amino acid sequence of SEQ ID NO: 64, and a VL comprising the amino acid sequence of SEQ ID NO: 68; (xcv) a VH comprising the amino acid sequence of SEQ ID NO: 64, and a VL comprising the amino acid sequence of SEQ ID NO: 69; (xcvi) a VH comprising the amino acid sequence of SEQ ID NO: 64, and a VL comprising the amino acid sequence of SEQ ID NO: 70; (xcvii) a VH comprising the amino acid sequence of SEQ ID NO: 64, and a VL comprising the amino acid sequence of SEQ ID NO: 71; (xcviii) a VH comprising the amino acid sequence of SEQ ID NO: 64, and a VL comprising the amino acid sequence of SEQ ID NO: 72; or (xcix) a VH comprising the amino acid sequence of SEQ ID NO: 64, and a VL comprising the amino acid sequence of SEQ ID NO: 73.

[0014] In some examples according to any of the bifunctional protein constructs described above, the anti-LAMA2 antibody moiety is an anti-LAMA2 scFv. In some examples, the anti-LAMA2 scFv comprises the amino acid sequence of SEQ ID NO:145.

[0015] In some examples according to any of the bifunctional protein constructs described above, the anti-LAMA2 antibody moiety is an anti-LAMA2 Fab. In some examples, the anti-LAMA2 Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 165, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 166.

[0016] In some examples according to any of the bifunctional protein constructs described above, the antibody moiety specifically binds to matriglycan on α-DG (anti-matriglycan antibody moiety). In some examples, the anti-matriglycan antibody moiety comprises: (i) an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 74, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 76, an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 77, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 78, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 79; (ii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 90, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 95; (iii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 90, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 96; (iv) an HC-CDR1, HC-CDR2,

and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 90, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 97; (v) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 90, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 98; (vi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 91, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 95; (vii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 91, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 96; (viii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 91, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 97; (ix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 91, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 98; (x) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 92, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 95; (xi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 92, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 96; (xii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 92, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 97; (xiii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 92, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 98; (xiv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 93, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 95; (xv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 93, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 96; (xvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 93, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 97; or (xvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 93, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL

comprising the amino acid sequence of SEQ ID NO: 98. In some examples, the anti-matriglycan antibody moiety comprises a VH comprising the amino acid sequence of any one of SEQ ID NOs: 89-93, and a VL comprising the amino acid sequence of any one of SEQ ID NOs: 94-98.

[0017] In some examples according to any of the bifunctional protein constructs described above, the anti-matriglycan antibody moiety comprises: (i) a VH comprising the amino acid sequence of SEQ ID NO: 89, and a VL comprising the amino acid sequence of SEQ ID NO: 94; (ii) a VH comprising the amino acid sequence of SEQ ID NO: 90, and a VL comprising the amino acid sequence of SEQ ID NO: 95; (iii) a VH comprising the amino acid sequence of SEQ ID NO: 90, and a VL comprising the amino acid sequence of SEQ ID NO: 96; (iv) a VH comprising the amino acid sequence of SEQ ID NO: 90, and a VL comprising the amino acid sequence of SEQ ID NO: 97; (v) a VH comprising the amino acid sequence of SEQ ID NO: 90, and a VL comprising the amino acid sequence of SEQ ID NO: 98; (vi) a VH comprising the amino acid sequence of SEQ ID NO: 91, and a VL comprising the amino acid sequence of SEQ ID NO: 95; (vii) a VH comprising the amino acid sequence of SEQ ID NO: 91, and a VL comprising the amino acid sequence of SEQ ID NO: 96; (viii) a VH comprising the amino acid sequence of SEQ ID NO: 91, and a VL comprising the amino acid sequence of SEQ ID NO: 97; (ix) a VH comprising the amino acid sequence of SEQ ID NO: 91, and a VL comprising the amino acid sequence of SEQ ID NO: 98; (x) a VH comprising the amino acid sequence of SEQ ID NO: 92, and a VL comprising the amino acid sequence of SEQ ID NO: 95; (xi) a VH comprising the amino acid sequence of SEQ ID NO: 92, and a VL comprising the amino acid sequence of SEQ ID NO: 96; (xii) a VH comprising the amino acid sequence of SEQ ID NO: 92, and a VL comprising the amino acid sequence of SEQ ID NO: 97; (xiii) a VH comprising the amino acid sequence of SEQ ID NO: 92, and a VL comprising the amino acid sequence of SEQ ID NO: 98; (xiv) a VH comprising the amino acid sequence of SEQ ID NO: 93, and a VL comprising the amino acid sequence of SEQ ID NO: 95; (xv) a VH comprising the amino acid sequence of SEQ ID NO: 93, and a VL comprising the amino acid sequence of SEQ ID NO: 96; (xxvi) a VH comprising the amino acid sequence of SEQ ID NO: 93, and a VL comprising the amino acid sequence of SEQ ID NO: 97; or (xxvii) a VH comprising the amino acid sequence of SEQ ID NO: 93, and a VL comprising the amino acid sequence of SEQ ID NO: 98.

[0018] In some examples according to any of the bifunctional protein constructs described above, the anti-matriglycan antibody moiety is an anti-matriglycan scFv. In some

examples, the anti-matriglycan scFv comprises the amino acid sequence of SEQ ID NO: 99.

[0019] In some examples according to any of the bifunctional protein constructs described above, the anti-matriglycan antibody moiety is an anti-matriglycan Fab. In some examples, the anti-matriglycan Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 100, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 101.

[0020] In some examples according to any of the bifunctional protein constructs described above, the antibody moiety specifically binds to CDH15 (anti-CDH15 antibody moiety). In some examples, the anti-CDH15 antibody moiety comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 102, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 103, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 104, an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 105, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 106, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 107.

[0021] In some examples according to any of the bifunctional protein constructs described above, the anti-CDH15 antibody moiety comprises a VH comprising the amino acid sequence of SEQ ID NO: 108, and a VL comprising the amino acid sequence of SEQ ID NO: 109.

[0022] In some examples according to any of the bifunctional protein constructs described above, the anti-CDH15 antibody moiety is an anti-CDH15 scFv. In some examples, the anti-CDH15 scFv comprises the amino acid sequence of SEQ ID NO: 110.

[0023] In some examples according to any of the bifunctional protein constructs described above, the anti-CDH15 antibody moiety is an anti-CDH15 Fab. In some examples, the anti-CDH15 Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 111, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 112.

[0024] In some examples according to any of the bifunctional protein constructs described above, the first binding moiety comprises a non-antibody moiety specifically binding to the muscle-specific molecule. In some examples, the non-antibody moiety comprises a protein domain selected from the group consisting of a laminin G-like domain (LG domain) of laminin, an LG domain of agrin, an LG domain of nidogen, an LG domain of perlecan, the laminin coiled-coil binding domain of agrin, and the laminin γ binding domain of nidogen. In some examples, wherein the non-antibody moiety comprises an LG

domain of laminin. In some examples, the non-antibody moiety comprises an LG domain of laminin subunit α-1 (LAMA1), LAMA2, laminin subunit α-3 (LAMA3), laminin subunit α-4 (LAMA4), or laminin subunit α-5 (LAMA5). In some examples, the non-antibody moiety comprises the amino acid sequence of any of SEQ ID NOs: 113-117.

[0025] In some examples according to any of the bifunctional protein constructs described above, the non-antibody moiety comprises an LG domain of LAMA2. In some examples, the non-antibody moiety comprises LG4-5 domains of LAMA2 (LAMA2 LG4-5). In some examples, the LAMA2 LG4-5 comprises the amino acid sequence of SEQ ID NO: 118 or 119.

[0026] In some examples according to any of the bifunctional protein constructs described above, the non-antibody moiety comprises an LG domain of perlecan. In some examples, the non-antibody moiety comprises the amino acid sequence of SEQ ID NO: 120.

[0027] In some examples according to any of the bifunctional protein constructs described above, the non-antibody moiety comprises an LG domain of agrin. In some examples, the non-antibody moiety comprises the amino acid sequence of SEQ ID NO: 121.

[0028] In some examples according to any of the bifunctional protein constructs described above, the non-antibody moiety comprises a laminin coiled-coil binding domain of agrin. In some examples, the laminin coiled-coil binding domain of agrin comprises the amino acid sequence of SEQ ID NO: 122.

[0029] In some examples according to any of the bifunctional protein constructs described above, the non-antibody moiety comprises a laminin γ binding domain of nidogen. In some examples, the laminin γ binding domain of nidogen comprises the amino acid sequence of SEQ ID NO: 123 or 124.

[0030] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety comprises an extracellular domain (ECD) or a variant thereof of a Notch ligand selected from the group consisting of Delta-like 1 (DLL1), DLL3, DLL4, Jagged1 (Jag1), and Jag2.

[0031] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety comprises a DLL4 ECD or a variant thereof. In some examples, the second binding moiety comprises a DLL4 ECD, and wherein the DLL4 ECD comprises the amino acid sequence of SEQ ID NO: 125. In some examples, wherein the second binding moiety comprises a variant DLL4 ECD, and wherein the variant

DLL4 ECD comprises MNNL domain, DSL domain, and EGF1-5 domains of DLL4, or a sequence having at least about 90% sequence identity to the sequence of MNNL domain, DSL domain, and EGF1-5 domains of DLL4. In some examples, the variant DLL4 ECD comprises the amino acid sequence of any of SEQ ID NOs: 126-129 and 260-267.

[0032] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety comprises a DLL1 ECD or a variant thereof. In some examples, the second binding moiety comprises a DLL1 ECD, and wherein the DLL1 ECD comprises the amino acid sequence of SEQ ID NO: 130.

[0033] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety comprises a DLL3 ECD or a variant thereof. In some examples, the second binding moiety comprises a DLL3 ECD, and wherein the DLL3 ECD comprises the amino acid sequence of SEQ ID NO: 131.

[0034] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety comprises a Jag1 ECD or a variant thereof. In some examples, the second binding moiety comprises a Jag1 ECD, and wherein the Jag1 ECD comprises the amino acid sequence of SEQ ID NO: 132. In some examples, wherein the second binding moiety comprises a variant Jag1 ECD, and wherein the variant Jag1 ECD comprises MNNL domain, DSL domain, and EGF1-6 domains of Jag1, or a sequence having at least about 90% sequence identity to the sequence of MNNL domain, DSL domain, and EGF1-6 domains of Jag1. In some examples, the variant Jag1 ECD comprises the amino acid sequence of SEQ ID NO: 133 or 134.

[0035] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety comprises a Jag2 ECD or a variant thereof. In some examples, the second binding moiety comprises a Jag2 ECD, and wherein the Jag2 ECD comprises the amino acid sequence of SEQ ID NO: 135.

[0036] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety is an anti-Notch antibody moiety that activates the Notch receptor. In some examples, the anti-Notch agonist antibody moiety is selected from the group consisting of a full-length antibody, a Fab, a Fab', a $F(ab')_2$, an scFv, and an sdAb.

[0037] In some examples according to any of the bifunctional protein constructs described above, the first binding moiety is fused to the second binding moiety via an optional linker. In some examples, the first binding moiety is fused to the N-terminus of the second binding moiety. In some examples, the first binding moiety is fused to the C-

terminus of the second binding moiety. In some examples, the bifunctional protein construct comprises (i) the second binding moiety comprising an ECD or a variant thereof of a Notch ligand selected from the group consisting of DLL1, DLL3, DLL4, Jag1, and Jag2; and (ii) the first binding moiety comprising: (a) an antibody moiety, wherein the antibody moiety is an scFv, a Fab, or an sdAb specifically binding to a muscle-specific molecule; or (b) a non-antibody moiety, wherein the non-antibody moiety comprises a protein domain selected from the group consisting of an LG domain of laminin, an LG domain of agrin, an LG domain of nidogen, and an LG domain of perlecan. In some examples, the first binding moiety comprises two or more non-antibody moieties connected in tandem.

[0038] In some examples according to any of the bifunctional protein constructs described above, the first binding moiety comprises an LAMA2 LG4-5 comprising the amino acid sequence of SEQ ID NO: 118 or 119. In some examples, the second binding moiety comprises a variant DLL4 ECD, and wherein the variant DLL4 ECD comprises the amino acid sequence of any of SEQ ID NOs: 126-129 and 260-267. In some examples, the bifunctional protein construct comprises the amino acid sequence of any of SEQ ID NOs: 136-139. In some examples, the second binding moiety comprises a DLL1 ECD, and wherein the DLL1 ECD comprises the amino acid sequence of SEQ ID NO: 130. In some examples, the bifunctional protein construct comprises the amino acid sequence of SEQ ID NO: 140. In some examples, the second binding moiety comprises a DLL3 ECD, and wherein the DLL3 ECD comprises the amino acid sequence of SEQ ID NO: 131. In some examples, the bifunctional protein construct comprises the amino acid sequence of SEQ ID NO: 141. In some examples, the second binding moiety comprises a variant Jag1 ECD, and wherein the variant Jag1 ECD comprises the amino acid sequence of SEQ ID NO: 133 or 134. In some examples, the bifunctional protein construct comprises the amino acid sequence of SEQ ID NO: 142 or 143. In some examples, the second binding moiety comprises a Jag2 ECD, and wherein the Jag2 ECD comprises the amino acid sequence of SEQ ID NO: 135. In some examples, the bifunctional protein construct comprises the amino acid sequence of SEQ ID NO: 144.

[0039] In some examples according to any of the bifunctional protein constructs described above, the first binding moiety comprises an anti-LAMA2 scFv. In some examples, the anti-LAMA2 scFv comprises the amino acid sequence of SEQ ID NO: 145. In some examples, the bifunctional protein construct comprises the amino acid sequence of any of SEQ ID NOs: 146-154.

[0040] In some examples according to any of the bifunctional protein constructs described above, the first binding moiety comprises an anti-matriglycan scFv. In some examples, the anti-matriglycan scFv comprises the amino acid sequence of SEQ ID NO: 155. In some examples, the bifunctional protein construct comprises the amino acid sequence of any of SEQ ID NOs: 156-164.

[0041] In some examples according to any of the bifunctional protein constructs described above, the first binding moiety comprises a Fab specifically binding to a muscle-specific molecule. In some examples, the second binding moiety is fused to the N-terminus of the VL of the Fab via an optional linker.

[0042] In some examples according to any of the bifunctional protein constructs described above, the first binding moiety comprises an anti-LAMA2 Fab. In some examples, the anti-LAMA2 Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 165, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 166. In some examples, the bifunctional protein construct comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 165, and a fusion polypeptide comprising the second binding moiety and the second polypeptide of the anti-LAMA2 Fab, and wherein the fusion polypeptide comprises the amino acid sequence of any of SEQ ID NOs: 167-175.

[0043] In some examples according to any of the bifunctional protein constructs described above, the first binding moiety comprises an anti-matriglycan Fab. In some examples, the anti-matriglycan Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 176, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 177. In some examples, the bifunctional protein construct comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 176, and a fusion polypeptide comprising the second binding moiety and the second polypeptide of the anti-matriglycan Fab, and wherein the fusion polypeptide comprises the amino acid sequence of any of SEQ ID NOs: 178-186.

[0044] In some examples according to any of the bifunctional protein constructs described above, the first binding moiety is fused to the second binding moiety via a carrier protein. In some examples, the carrier protein is selected from the group consisting of an human serum albumin (HSA), an anti-HSA antibody moiety, and a subunit of an Fc domain. In some examples, the first binding moiety is fused to the N-terminus of the carrier protein via an optional first linker, and the second binding moiety is fused to the C-terminus of the carrier protein via an optional second linker. In some examples, the first binding

moiety is fused to the C-terminus of the carrier protein via an optional first linker, and the second binding moiety is fused to the N-terminus of the carrier protein via an optional second linker. In some examples, the bifunctional protein construct comprises (i) the second binding moiety comprising an ECD or a variant thereof of a Notch ligand selected from the group consisting of DLL1, DLL3, DLL4, Jag1, and Jag2; and (ii) the first binding moiety comprising: (a) an antibody moiety, wherein the antibody moiety is an scFv, a Fab, or an sdAb specifically binding to a muscle-specific molecule; or (b) a non-antibody moiety, wherein the non-antibody moiety comprises a protein domain selected from the group consisting of an LG domain of laminin, an LG domain of agrin, an LG domain of nidogen, and an LG domain of perlecan. In some examples, the first binding moiety comprises two or more non-antibody moieties connected in tandem.

[0045] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct further comprises an Fc domain comprising a first subunit and a second subunit. In some examples, the Fc domain is derived from human IgG1, human IgG2, or human IgG4. In some examples, the Fc domain is derived from human IgG1 comprising the amino acid sequence of SEQ ID NO: 187. In some examples, each subunit of the Fc domain comprises a mutation that reduces or abolishes the effector function. In some examples, each subunit of the Fc domain comprises an L234A/L235A mutation (EU numbering).

[0046] In some examples according to any of the bifunctional protein constructs described above, either the first subunit of the Fc domain or the second subunit of the Fc domain comprises an H435R/Y436F mutation (EU numbering). In some examples, the Fc domain comprises knob-into-hole mutations, and wherein: i) the first subunit of the Fc domain comprises a knob mutation, and the second subunit of the Fc domain comprises a hole mutation; or ii) the second subunit of the Fc domain comprises a knob mutation, and the first subunit of the Fc domain comprises a hole mutation. In some examples, the knob mutation is T366W (EU numbering), and the hole mutation is T366S/L368A/Y407V (EU numbering). In some examples, the Fc domain comprises charged-pair mutations, and wherein: i) an amino acid residue in the first subunit of the Fc domain is replaced with a positively charged residue, and an amino acid residue in the second subunit of the Fc domain is replaced with a negatively charged residue; or ii) an amino acid residue in the first subunit of the Fc domain is replaced with a negatively charged residue, and an amino acid residue in the second subunit of the Fc domain is replaced with a positively charged residue. In some examples, the Fc domain comprises i) an amino acid residue at D399 (EU numbering) in

the first subunit of the Fc domain is replaced with a positively charged residue, and an amino acid residue at K409 (EU numbering) in the second subunit of the Fc domain is replaced with a negatively charged residue; or ii) an amino acid residue at K409 (EU numbering) in the first subunit of the Fc domain is replaced with a negatively charged residue, and an amino acid residue at D399 (EU numbering) in the second subunit of the Fc domain is replaced with a positively charged residue.

[0047] In some examples according to any of the bifunctional protein constructs described above, each subunit of the Fc domain comprises a mutation that reduces half-life. In some examples, each subunit of the Fc domain comprises an H435A mutation (EU numbering). In some examples, the Fc domain is derived from human IgG1, and wherein each subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 188.

[0048] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct comprises: i) a first unit of a second binding moiety specifically binding to a first Notch receptor, and ii) a second unit of a second binding moiety specifically binding to a second Notch receptor, and wherein the first unit of the second binding moiety and the second unit of the second binding moiety each independently comprises an ECD or a variant thereof of a Notch ligand selected from the group consisting of DLL1, DLL3, DLL4, Jag1, and Jag2. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a DLL4 ECD, and wherein the DLL4 ECD comprises the amino acid sequence of any one of SEQ ID NO: 125-129. In some examples, the first unit of the second binding moiety and the second unit of the second binding moiety are the same. In some examples, the first unit of the second binding moiety and the second unit of the second binding moiety are different. In some examples, the bifunctional protein construct comprises: i) a first unit of a first binding moiety specifically binding to a first muscle-specific molecule, and ii) a second unit of a first binding moiety specifically binding to a second muscle-specific molecule. In some examples, the first unit of the first binding moiety and the second unit of the first binding moiety are the same. In some examples, the first unit of the first binding moiety and the second unit of the first binding moiety are different. In some examples, the first unit of the first binding moiety comprises a first antibody moiety specifically binding to the first muscle-specific molecule, the second unit of the first binding moiety comprises a second antibody moiety specifically binding to the second muscle-specific molecule, and wherein the first antibody moiety and the second antibody moiety are each independently selected from the group consisting of a Fab, an scFv, and an sdAb.

[0049] In some examples according to any of the bifunctional protein constructs described above, the first antibody moiety is a first Fab (Fab1), and the second antibody moiety is a second Fab (Fab2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': a first unit of a second binding moiety specifically binding to a first Notch receptor – optional linker – the first subunit of the Fc domain – optional linker – VH1-(H1-CH1); ii) a second polypeptide comprising from N' to C': a second unit of a second binding moiety specifically binding to a second Notch receptor – optional linker – the second subunit of the Fc domain – optional linker – VH2-(H2-CH1); iii) a third polypeptide comprising from N' to C': VL1-(L1-CL); and iv) a fourth polypeptide comprising from N' to C': VL2-(L2-CL); and wherein VL1-(L1-CL) and VH1-(H1-CH1) form Fab1, and VH2-(H2-CH1) and VL2-(L2-CL) form Fab2. In some examples, the first antibody moiety is a first Fab (Fab1), and the second antibody moiety is a second Fab (Fab2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': a first unit of a second binding moiety specifically binding to a first Notch receptor – optional linker – the first subunit of the Fc domain – optional linker – VL1-(L1-CL); ii) a second polypeptide comprising from N' to C': a second unit of a second binding moiety specifically binding to a second Notch receptor – optional linker – the second subunit of the Fc domain – optional linker – VL2-(L2-CL); iii) a third polypeptide comprising from N' to C': VH1-(H1-CH1); and iv) a fourth polypeptide comprising from N' to C': VH2-(H2-CH1); and wherein VL1-(L1-CL) and VH1-(H1-CH1) form Fab1, and VH2-(H2-CH1) and VL2-(L2-CL) form Fab2.

[0050] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct comprises Fab1 and Fab2 both specifically bind to LAMA2. In some examples, the anti-LAMA2 Fab1 and/or anti-LAMA2 Fab2 comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 165, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 166. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a variant DLL4 ECD, and wherein the variant DLL4 ECD comprises the amino acid sequence of any of SEQ ID NOs: 126-129 and 260-267. In some examples, wherein the third polypeptide and the fourth polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 165, and the first polypeptide and the second polypeptide of the bifunctional protein construct each comprises the amino acid sequence of any of SEQ ID NOs: 189-192. In some examples, the first unit of the second binding moiety and/or the second unit of the second

binding moiety comprises a DLL1 ECD, and wherein the DLL1 ECD comprises the amino acid sequence of SEQ ID NO: 130. In some examples, the third polypeptide and the fourth polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 165, and the first polypeptide and the second polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 193. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a DLL3 ECD, and wherein the DLL3 ECD comprises the amino acid sequence of SEQ ID NO: 131. In some examples, the third polypeptide and the fourth polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 165, and the first polypeptide and the second polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 194. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a variant Jag1 ECD, and wherein the variant Jag1 ECD comprises the amino acid sequence of SEQ ID NO: 133 or 134. In some examples, the third polypeptide and the fourth polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 165, and the first polypeptide and the second polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 195 or 196. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a Jag2 ECD, and wherein the Jag2 ECD comprises the amino acid sequence of SEQ ID NO: 135. In some examples, the third polypeptide and the fourth polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 165, and the first polypeptide and the second polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 197.

[0051] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct comprises Fab1 and Fab2 both specifically bind to matriglycan. In some examples, the anti-matriglycan Fab1 and/or anti-matriglycan Fab2 comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 176, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 177. In some examples, the third polypeptide and the fourth polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 176, and wherein: (i) the first polypeptide and the second polypeptide of the bifunctional protein construct each comprises the amino acid sequence of any of SEQ ID NOs: 198-201; (ii) the first polypeptide and the second polypeptide of the bifunctional protein construct each

comprises the amino acid sequence of SEQ ID NO: 202; (iii) the first polypeptide and the second polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 203; (iv) the first polypeptide and the second polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 204 or 205; or (v) the first polypeptide and the second polypeptide of the bifunctional protein construct each comprises the amino acid sequence of SEQ ID NO: 206.

[0052] In some examples according to any of the bifunctional protein constructs described above, the first antibody moiety is a first sdAb (sdAb1), and the second antibody moiety is a second sdAb (sdAb2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': a first unit of a second binding moiety specifically binding to a first Notch receptor – optional linker – the first subunit of the Fc domain – optional linker – sdAb1; and ii) a second polypeptide comprising from N' to C': a second unit of a second binding moiety specifically binding to a second Notch receptor – optional linker – the second subunit of the Fc domain – optional linker – sdAb2. In some examples, the first antibody moiety is a first scFv (scFv1), and the second antibody moiety is a second scFv (scFv2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': a first unit of a second binding moiety specifically binding to a first Notch receptor – optional linker – the first subunit of the Fc domain – optional linker – scFv1; and ii) a second polypeptide comprising from N' to C': a second unit of a second binding moiety specifically binding to a second Notch receptor – optional linker – the second subunit of the Fc domain – optional linker – scFv2.

[0053] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct comprises scFv1 and scFv2 both specifically bind to LAMA2. In some examples, anti-LAMA2 scFv1 and/or anti-LAMA2 scFv2 comprises the amino acid sequence of SEQ ID NO: 145 or 268. In some examples, the linker comprises an amino acid sequence of any one of SEQ ID NOs: 207-222. In some examples, the first subunit of the Fc domain and the second subunit of the Fc domain each comprises the amino acid sequence of SEQ ID NO: 188. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a variant DLL4 ECD, and wherein the variant DLL4 ECD comprises the amino acid sequence of any of SEQ ID NOs: 126-129 and 260-267. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising the amino acid sequence of any of SEQ ID NOs: 223-226. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding

moiety comprises a DLL1 ECD, and wherein the DLL1 ECD comprises the amino acid sequence of SEQ ID NO: 130. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising the amino acid sequence of SEQ ID NO: 227. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a DLL3 ECD, and wherein the DLL3 ECD comprises the amino acid sequence of SEQ ID NO: 131. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising the amino acid sequence of SEQ ID NO: 228. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a variant Jag1 ECD, and wherein the variant Jag1 ECD comprises the amino acid sequence of SEQ ID NO: 133 or 134. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising the amino acid sequence of SEQ ID NO: 229 or 230. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a Jag2 ECD, and wherein the Jag2 ECD comprises the amino acid sequence of SEQ ID NO: 135. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising the amino acid sequence of SEQ ID NO: 231.

[0054] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct comprises scFv1 and scFv2 both specifically bind to matriglycan. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising: (i) the amino acid sequence of any one of SEQ ID NOs: 232-235; (ii) the amino acid sequence of SEQ ID NO: 236; (iii) the amino acid sequence of SEQ ID NO: 237; (iv) the amino acid sequence of SEQ ID NO: 238 or 239; or (v) the amino acid sequence of SEQ ID NO: 240.

[0055] In some examples according to any of the bifunctional protein constructs described above, the first antibody moiety is a first Fab (Fab1), and the second antibody moiety is a second Fab (Fab2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': VH1-(H1-CH1) – optional linker – the first subunit of the Fc domain – optional linker – a first unit of a second binding moiety specifically binding to a first Notch receptor; ii) a second polypeptide comprising from N' to C': VH2-(H2-CH1) – optional linker – the second subunit of the Fc domain – optional linker – a second unit of a second binding moiety specifically binding to a second Notch receptor; iii) a third polypeptide comprising from N' to C': VL1-(L1-CL); and iv) a fourth polypeptide

comprising from N' to C': VL2-(L2-CL); and wherein VL1-(L1-CL) and VH1-(H1-CH1) form Fab1, and VH2-(H2-CH1) and VL2-(L2-CL) form Fab2.

[0056] In some examples according to any of the bifunctional protein constructs described above, the first antibody moiety is a first sdAb (sdAb1), and the second antibody moiety is a second sdAb (sdAb2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': sdAb1 – optional linker – the first subunit of the Fc domain – optional linker – a first unit of a second binding moiety specifically binding to a first Notch receptor; and ii) a second polypeptide comprising from N' to C': sdAb2– optional linker – the second subunit of the Fc domain – optional linker – a second unit of a second binding moiety specifically binding to a second Notch receptor.

[0057] In some examples according to any of the bifunctional protein constructs described above, the first antibody moiety is a first scFv (scFv1), and the second antibody moiety is a second scFv (scFv2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': scFv1– optional linker – the first subunit of the Fc domain – optional linker – a first unit of a second binding moiety specifically binding to a first Notch receptor; and ii) a second polypeptide comprising from N' to C': scFv2– optional linker – the second subunit of the Fc domain – optional linker – a second unit of a second binding moiety specifically binding to a second Notch receptor.

[0058] In some examples according to any of the bifunctional protein constructs described above, the first antibody moiety is a first Fab (Fab1), and the second antibody moiety is a second Fab (Fab2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': VH1-(H1-CH1) – optional linker – the first subunit of the Fc domain; ii) a second polypeptide comprising from N' to C': VH2-(H2-CH1) – optional linker – the second subunit of the Fc domain; iii) a third polypeptide comprising from N' to C': VL1-(L1-CL) – optional linker – a first unit of a second binding moiety specifically binding to a first Notch receptor; and iv) a fourth polypeptide comprising from N' to C': VL2-(L2-CL) – optional linker – a second unit of a second binding moiety specifically binding to a second Notch receptor; and wherein VL1-(L1-CL) and VH1-(H1-CH1) form Fab1, and VH2-(H2-CH1) and VL2-(L2-CL) form Fab2.

[0059] In some examples according to any of the bifunctional protein constructs described above, the first antibody moiety is a first Fab (Fab1), and the second antibody moiety is a second Fab (Fab2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': VH1-(H1-CH1) – optional linker – the first subunit of the Fc domain; ii) a second polypeptide comprising from N' to C': VH2-(H2-CH1) – optional

linker – the second subunit of the Fc domain; iii) a third polypeptide comprising from N' to C': a first unit of a second binding moiety specifically binding to a first Notch receptor – optional linker – VL1-(L1-CL); and iv) a fourth polypeptide comprising from N' to C': a second unit of a second binding moiety specifically binding to a second Notch receptor – optional linker – VL2-(L2-CL); and wherein VL1-(L1-CL) and VH1-(H1-CH1) form Fab1, and VH2-(H2-CH1) and VL2-(L2-CL) form Fab2.

[0060] In some examples according to any of the bifunctional protein constructs described above, the first antibody moiety is a first Fab (Fab1), and the second antibody moiety is a second Fab (Fab2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': a first unit of a second binding moiety specifically binding to a first Notch receptor – optional linker – VH1-(H1-CH1) – optional linker – the first subunit of the Fc domain; ii) a second polypeptide comprising from N' to C': a second unit of a second binding moiety specifically binding to a second Notch receptor – optional linker – VH2-(H2-CH1) – optional linker – the second subunit of the Fc domain; iii) a third polypeptide comprising from N' to C': VL1-(L1-CL); and iv) a fourth polypeptide comprising from N' to C': VL2-(L2-CL); and wherein VL1-(L1-CL) and VH1-(H1-CH1) form Fab1, and VH2-(H2-CH1) and VL2-(L2-CL) form Fab2.

[0061] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct further comprises a third unit of a second binding moiety specifically binding to a third Notch receptor, and a fourth unit of a second binding moiety specifically binding to a fourth Notch receptor, and wherein the third unit of the second binding moiety and the fourth unit of the second binding moiety each independently comprises an ECD or a variant thereof of a Notch ligand selected from the group consisting of DLL1, DLL3, DLL4, Jag1, and Jag2. In some examples, the third unit of the second binding moiety and the fourth unit of the second binding moiety each independently comprises an ECD or a variant thereof of DLL4. In some examples, the third unit of the second binding moiety and/or the fourth unit of the second binding moiety comprises a DLL4 ECD, and wherein the DLL4 ECD comprises the amino acid sequence of SEQ ID NO: 125. In some examples, the third unit of the second binding moiety and/or the fourth unit of the second binding moiety comprises a variant DLL4 ECD, and wherein the variant DLL4 ECD comprises MNNL domain, DSL domain, and EGF1-5 domains of DLL4. In some examples, the variant DLL4 ECD comprises the amino acid sequence of any one of SEQ ID NO: 126-129 and 260-267. In some examples, all four units of the second

binding moiety are the same. In some examples, at least one of the four units of the second binding moiety is different from the others.

[0062] In some examples according to any of the bifunctional protein constructs described above, the first antibody moiety is a first Fab (Fab1), and the second antibody moiety is a second Fab (Fab2); wherein the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': a first unit of a second binding moiety specifically binding to a first Notch receptor – optional linker – VH1-(H1-CH1) – optional linker – the first subunit of the Fc domain; ii) a second polypeptide comprising from N' to C': a second unit of a second binding moiety specifically binding to a second Notch receptor – optional linker – VH2-(H2-CH1) – optional linker – the second subunit of the Fc domain; iii) a third polypeptide comprising from N' to C': a third unit of a second binding moiety specifically binding to a third Notch receptor – optional linker – VL1-(L1-CL); and iv) a fourth polypeptide comprising from N' to C': a fourth unit of a second binding moiety specifically binding to a fourth Notch receptor – optional linker – VL2-(L2-CL); and wherein VL1-(L1-CL) and VH1-(H1-CH1) form Fab1, and VH2-(H2-CH1) and VL2-(L2-CL) form Fab2.

[0063] In some examples according to any of the bifunctional protein constructs described above, the first unit of the first binding moiety comprises a first non-antibody moiety specifically binding to the first muscle-specific molecule, the second unit of the first binding moiety comprises a second non-antibody moiety specifically binding to the second muscle-specific molecule, and wherein the first non-antibody moiety and the second non-antibody moiety are each independently selected from the group consisting of an LG domain of laminin, an LG domain of agrin, an LG domain of nidogen, and an LG domain of perlecan. In some examples, the first unit of the first binding moiety comprises two or more first non-antibody moieties connected in tandem, and the second unit of the first binding moiety comprises two or more second non-antibody moieties connected in tandem. In some examples, the first non-antibody moiety and/or the second non-antibody moiety comprises LAMA2 LG4-5 comprising the amino acid sequence of SEQ ID NO: 118 or 119.

[0064] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': the first unit of the first binding moiety – optional linker – the first subunit of the Fc domain – optional linker – a first unit of a second binding moiety specifically binding to a first Notch receptor; and ii) a second polypeptide comprising from N' to C': the second unit of the first binding moiety – optional linker – the second subunit of

the Fc domain – optional linker – a second unit of a second binding moiety specifically binding to a second Notch receptor.

[0065] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct comprises: i) a first polypeptide comprising from N' to C': a first unit of a second binding moiety specifically binding to a first Notch receptor – optional linker – the first subunit of the Fc domain – optional linker – the first unit of the first binding moiety; and ii) a second polypeptide comprising from N' to C': a second unit of a second binding moiety specifically binding to a second Notch receptor – optional linker – the second subunit of the Fc domain – optional linker – the second unit of the first binding moiety.

[0066] In some examples according to any of the bifunctional protein constructs described above, the first unit of the first binding moiety comprises two LAMA2 LG4-5 connected in tandem, and the second unit of the first binding moiety comprises two LAMA2 LG4-5 connected in tandem. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a variant DLL4 ECD, and wherein the variant DLL4 ECD comprises the amino acid sequence of any of SEQ ID NOs: 126-129 and 260-267. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising the amino acid sequence of any one of SEQ ID NOs: 241-244. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a DLL1 ECD, and wherein the DLL1 ECD comprises the amino acid sequence of SEQ ID NO: 130. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising the amino acid sequence of SEQ ID NO: 245. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a DLL3 ECD, and wherein the DLL3 ECD comprises the amino acid sequence of SEQ ID NO: 131. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising the amino acid sequence of SEQ ID NO: 246. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a variant Jag1 ECD, and wherein the variant Jag1 ECD comprises the amino acid sequence of SEQ ID NO: 133 or 134. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising the amino acid sequence of SEQ ID NO: 247 or 248. In some examples, the first unit of the second binding moiety and/or the second unit of the second binding moiety comprises a Jag2 ECD, and wherein the Jag2 ECD comprises

the amino acid sequence of SEQ ID NO: 135. In some examples, the bifunctional protein construct comprises a first polypeptide and a second polypeptide each comprising the amino acid sequence of SEQ ID NO: 249.

[0067] In some examples according to any of the bifunctional protein constructs described above, i) the first binding moiety is fused to the N-terminus of the first subunit of the Fc domain via an optional first linker, and the second binding moiety is fused to the N-terminus of the second subunit of the Fc domain via an optional second linker; or ii) the first binding moiety is fused to the N-terminus of the second subunit of the Fc domain via an optional first linker, and the second binding moiety is fused to the N-terminus of the first subunit of the Fc domain via an optional second linker. In some examples, the first binding moiety is a Fab specifically binding to the muscle-specific molecule, and wherein the C-terminus of the CH1 of the Fab is fused to the N-terminus of the first subunit of the Fc domain or the second subunit of the Fc domain via the optional first linker. In some examples, the second binding moiety comprises an ECD or a variant thereof of a Notch ligand selected from the group consisting of DLL1, DLL3, DLL4, Jag1, and Jag2.

[0068] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct comprises: i) a first unit of a second binding moiety specifically binding to a first Notch receptor, and ii) a second unit of a second binding moiety specifically binding to a second Notch receptor; wherein the first unit of the second binding moiety is fused to the N-terminus of the first subunit of the Fc domain via an optional first linker, and the second unit of the second binding moiety is fused to the N-terminus of the second subunit of the Fc domain via an optional second linker; and wherein the first binding moiety is fused to the C-terminus of either the first subunit of the Fc domain or the second subunit of the Fc domain via an optional third linker. In some examples, the bifunctional protein construct comprises: i) a first unit of a first binding moiety specifically binding to a first muscle-specific molecule, and ii) a second unit of a first binding moiety specifically binding to a second muscle-specific molecule; and wherein the first unit of the first binding moiety is fused to the C-terminus of the first subunit of the Fc domain via an optional third linker, and the second unit of the first binding moiety is fused to the C-terminus of the second subunit of the Fc domain via an optional fourth linker.

[0069] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety is fused to the N-terminus of the first subunit of the Fc domain via an optional linker; wherein the bifunctional protein construct comprises one or more units of the first binding moiety specifically binding to one or more muscle-

specific molecules; and wherein each of the one or more units of the first binding moiety is independently fused via an optional linker to one of: i) the C-terminus of the first subunit of the Fc domain; ii) the N-terminus of the second subunit of the Fc domain; and iii) the C-terminus of the second subunit of the Fc domain.

[0070] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety is fused to the N-terminus of the second subunit of the Fc domain via an optional linker; wherein the bifunctional protein construct comprises one or more units of the first binding moiety specifically binding to one or more muscle-specific molecules; and wherein each of the one or more units of the first binding moiety is independently fused via an optional linker to one of: i) the N-terminus of the first subunit of the Fc domain; ii) the C-terminus of the first subunit of the Fc domain; and iii) the C-terminus of the second subunit of the Fc domain.

[0071] In some examples according to any of the bifunctional protein constructs described above, the bifunctional protein construct comprises: i) a first unit of a second binding moiety specifically binding to a first Notch receptor, and ii) a second unit of a second binding moiety specifically binding to a second Notch receptor; wherein the first unit of the second binding moiety is fused to the C-terminus of the first subunit of the Fc domain via an optional first linker, and the second unit of the second binding moiety is fused to the C-terminus of the second subunit of the Fc domain via an optional second linker; and wherein the first binding moiety is fused to the N-terminus of either the first subunit of the Fc domain or the second subunit of the Fc domain via an optional third linker. In some examples, the bifunctional protein construct comprises: i) a first unit of a first binding moiety specifically binding to a first muscle-specific molecule, and ii) a second unit of a first binding moiety specifically binding to a second muscle-specific molecule; and wherein the first unit of the first binding moiety is fused to the N-terminus of the first subunit of the Fc domain via an optional third linker, and the second unit of the first binding moiety is fused to the N-terminus of the second subunit of the Fc domain via an optional fourth linker.

[0072] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety is fused to the C-terminus of the first subunit of the Fc domain via an optional linker; wherein the bifunctional protein construct comprises one or more units of the first binding moiety specifically binding to one or more muscle-specific molecules; and wherein each of the one or more units of the first binding moiety is independently fused via an optional linker to one of: i) the N-terminus of the first subunit of

the Fc domain; ii) the N-terminus of the second subunit of the Fc domain; and iii) the C-terminus of the second subunit of the Fc domain.

[0073] In some examples according to any of the bifunctional protein constructs described above, the second binding moiety is fused to the C-terminus of the second subunit of the Fc domain via an optional linker; wherein the bifunctional protein construct comprises one or more units of the first binding moiety specifically binding to one or more muscle-specific molecules; and wherein each of the one or more units of the first binding moiety is independently fused via an optional linker to one of: i) the N-terminus of the first subunit of the Fc domain; ii) the C-terminus of the first subunit of the Fc domain; and iii) the N-terminus of the second subunit of the Fc domain.

[0074] In some examples according to any of the bifunctional protein constructs described above, i) the one or more units of the second binding moiety each independently comprises an ECD or a variant thereof of a Notch ligand selected from the group consisting of DLL1, DLL3, DLL4, Jag1, and Jag2; and ii) the one or more units of the first binding moiety each independently comprises an antibody moiety specifically binding to a muscle-specific molecule, and wherein the antibody moiety is independently selected from the group consisting of an scFv, a Fab, and an sdAb. In some examples, i) the two units of the second binding moiety are the same; and/or ii) the two or more units of the first binding moiety are the same. In some examples, i) the two units of the second binding moiety are different; and/or ii) at least one of the two or more units of the first binding moiety is different from the other(s). In some examples, the Fc domain comprises knob-into-hole mutations and/or charged-pair mutations. In some examples, (i) the first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 348, and the second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 255; (ii) the first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 255, and the second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 348; (iii) the first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 256, and the second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 257; (iv) the first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 257, and the second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 256; (v) the first subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 258, and the second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 259; or (vi) the first subunit of the Fc domain comprises the amino acid

sequence of SEQ ID NO: 259, and the second subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 258.

[0075] In another example, provided herein is one or more isolated nucleic acids encoding any of the bifunctional protein construct described above.

[0076] In another example, provided herein is one or more vectors comprising one or more isolated nucleic acids described above. In some examples, the one or more vectors is a viral vector. In some examples, the viral vector is an adeno-associated virus (AAV) vector or a lentiviral vector.

[0077] In another example, provided herein is a host cell expressing any of the bifunctional protein constructs described above, the one or more isolated nucleic acids described above, or the one or more vectors described above. In some examples, the host cell is a Chinese hamster ovary (CHO) cell or an HEK293 cell.

[0078] Also provided are pharmaceutical compositions comprising: i) any of the bifunctional protein constructs described above, the one or more isolated nucleic acids described above, or the one or more vectors described above; and ii) a pharmaceutically acceptable excipient.

[0079] In another example, provided herein is a method of producing a bifunctional protein construct, comprising: i) culturing a host cell comprising the one or more isolated nucleic acids described above or the one or more vectors described above, or the host cell described above, under a condition suitable for the expression of the bifunctional protein construct; and ii) recovering the expressed bifunctional protein construct from the cultured host cell. In some examples, the method further comprises introducing the one or more isolated nucleic acids or the one or more vectors into the host cell.

[0080] In another example, provided herein is a method of treating a muscle-related disease in an individual, comprising administering to the individual an effective amount of any of the bifunctional protein constructs described above or the pharmaceutical composition described above. In some examples, the muscle-related disease is selected from the group consisting of Pompe disease, centronuclear myopathy, fibrodysplasia ossificans progressive (FOP), Friedreich's ataxia (FRDA), familial hypertrophic cardiomyopathy, Laing distal myopathy, myofibrillar myopathy, and muscular dystrophy. In some examples, the muscle disease is muscular dystrophy. In some examples, the muscular dystrophy comprises one or more of: Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), limb-girdle muscular dystrophy (LGMD), congenital muscular dystrophy (CMD), facioscapulohumeral muscular dystrophy (FSHD), myotonic dystrophy (DM),

oculopharyngeal muscular dystrophy (OPMD), distal muscular dystrophy (DD), congenital myopathy, Charcot-Marie-Tooth (CMT) disorder, and Emery-Dreifuss muscular dystrophy (EDMD). In some examples, the bifunctional protein construct or the pharmaceutical composition is administered intravenously, subcutaneously, or intramuscularly. In some examples, the individual is a human.

[0081] In another example, the present inventions provides an antibody construct comprising an antibody moiety specifically recognizing matriglycan on α-DG (anti-matriglycan antibody moiety), wherein the anti-matriglycan antibody moiety comprises: (i) an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 74, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 76, an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 77, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 78, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 79; (ii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 90, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 95; (iii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 90, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 96; (iv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 90, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 97; (v) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 90, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 98; (vi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 91, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 95; (vii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 91, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 96; (viii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 91, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 97; (ix) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 91, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 98; (x) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 92, and an LC-

CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 95; (xi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 92, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 96; (xii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 92, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 97; (xiii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 92, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 98; (xiv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 93, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 95; (xv) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 93, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 96; (xvi) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 93, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 97; or (xvii) an HC-CDR1, HC-CDR2, and HC-CDR3 of a VH comprising the amino acid sequence of SEQ ID NO: 93, and an LC-CDR1, LC-CDR2, and LC-CDR3 of a VL comprising the amino acid sequence of SEQ ID NO: 98.

[0082] In some examples according to any of the antibody constructs described above, the anti-matriglycan antibody moiety comprises a VH comprising the amino acid sequence of any one of SEQ ID NOs: 89-93, and a VL comprising the amino acid sequence of any one of SEQ ID NOs: 94-98. In some examples, the anti-matriglycan antibody moiety comprises: (i) a VH comprising the amino acid sequence of SEQ ID NO: 89, and a VL comprising the amino acid sequence of SEQ ID NO: 94; (ii) a VH comprising the amino acid sequence of SEQ ID NO: 90, and a VL comprising the amino acid sequence of SEQ ID NO: 95; (iii) a VH comprising the amino acid sequence of SEQ ID NO: 90, and a VL comprising the amino acid sequence of SEQ ID NO: 96; (iv) a VH comprising the amino acid sequence of SEQ ID NO: 90, and a VL comprising the amino acid sequence of SEQ ID NO: 97; (v) a VH comprising the amino acid sequence of SEQ ID NO: 90, and a VL comprising the amino acid sequence of SEQ ID NO: 98; (vi) a VH comprising the amino acid sequence of SEQ ID NO: 91, and a VL comprising the amino acid sequence of SEQ ID NO: 95; (vii) a VH comprising the amino acid sequence of SEQ ID NO: 91, and a VL comprising the amino acid sequence of SEQ ID NO: 96; (viii) a VH comprising the amino acid sequence of SEQ ID

NO: 91, and a VL comprising the amino acid sequence of SEQ ID NO: 97; (ix) a VH comprising the amino acid sequence of SEQ ID NO: 91, and a VL comprising the amino acid sequence of SEQ ID NO: 98; (x) a VH comprising the amino acid sequence of SEQ ID NO: 92, and a VL comprising the amino acid sequence of SEQ ID NO: 95; (xi) a VH comprising the amino acid sequence of SEQ ID NO: 92, and a VL comprising the amino acid sequence of SEQ ID NO: 96; (xii) a VH comprising the amino acid sequence of SEQ ID NO: 92, and a VL comprising the amino acid sequence of SEQ ID NO: 97; (xiii) a VH comprising the amino acid sequence of SEQ ID NO: 92, and a VL comprising the amino acid sequence of SEQ ID NO: 98; (xiv) a VH comprising the amino acid sequence of SEQ ID NO: 93, and a VL comprising the amino acid sequence of SEQ ID NO: 95; (xv) a VH comprising the amino acid sequence of SEQ ID NO: 93, and a VL comprising the amino acid sequence of SEQ ID NO: 96; (xvi) a VH comprising the amino acid sequence of SEQ ID NO: 93, and a VL comprising the amino acid sequence of SEQ ID NO: 97; or (xvii) a VH comprising the amino acid sequence of SEQ ID NO: 93, and a VL comprising the amino acid sequence of SEQ ID NO: 98.

[0083] In some examples according to any of the antibody constructs described above, the anti-matriglycan antibody moiety is selected from the group consisting of a full-length antibody, a Fab, a Fab', a $F(ab')_2$, and an scFv. In some examples, the anti-matriglycan antibody moiety is an anti-matriglycan scFv. In some examples, the anti-matriglycan scFv comprises the amino acid sequence of SEQ ID NO: 99.

[0084] In some examples according to any of the antibody constructs described above, the anti-matriglycan antibody moiety is an anti-matriglycan Fab. In some examples, the anti-matriglycan Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 100, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 101.

[0085] In some examples according to any of the antibody constructs described above, the anti-matriglycan antibody moiety is an anti-matriglycan Fab. In some examples, the anti-matriglycan Fab comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 100, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 101.

[0086] In some examples according to any of the antibody constructs described above, the anti-matriglycan antibody moiety is an anti-matriglycan full-length antibody. In some examples, the anti-matriglycan full-length antibody comprises an Fc domain derived from human IgG1, human IgG2, or human IgG4. In some examples, the Fc

domain is derived from human IgG1 comprising the amino acid sequence of SEQ ID NO: 187. In some examples, each subunit of the Fc domain comprises an L234A/L235A mutation (EU numbering). In some examples, each subunit of the Fc domain comprises an H435A mutation (EU numbering). In some examples, each subunit of the Fc domain comprises an L234A/L235A/P329G mutation (EU numbering). In some examples, the Fc domain is derived from human IgG1, and wherein each subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 188. In some examples, the Fc domain is derived from human IgG1, and wherein each subunit of the Fc domain comprises the amino acid sequence of SEQ ID NO: 277.

[0087] In some examples according to any of the antibody constructs described above, the antibody construct further comprises a second binding moiety specifically binding to a second target molecule. In some examples, the second binding moiety specifically binds to a Notch receptor and activates the Notch receptor. In some examples, the second binding moiety comprises an ECD or a variant thereof of a Notch ligand selected from the group consisting of DLL1, DLL3, DLL4, Jag1, and Jag2.

[0088] In another example, the present invention provides an engineered DLL4 extracellular domain (ECD), wherein the engineered DLL4 ECD comprises a mutation selected from the group consisting of T52N and T135N, and wherein the amino acid position is in reference to a reference DLL4 ECD comprising the amino acid sequence of SEQ ID NO: 126. In some examples, the engineered DLL4 ECD further comprises a mutation at one or more amino acid positions selected from the group consisting of G2, E14, R66, P80, F81, H168, Q220, N231, and N260. In some examples, the further mutation is selected from the group consisting of G2S, E14H, R66S, R66T, P80L, F81L, H168Y, Q220H, N231D, and N260D. In some examples, the engineered DLL4 ECD comprises a mutation selected from the group consisting of: (i) T52N and T135N; (ii) T52N, R66S, and T135N; (iii) E14H, T52N, R66T, P80L, T135N, and N231D; (iv) T52N, R66T, P80L, T135N, Q220H, and N260D; (v) G2S, T52N, F81L, T135N, and H168Y; (vi) G2S, T52N, F81L, R66S, T135N, and H168Y; (vii) G2S, E14H, T52N, F81L, R66T, P80L, T135N, H168Y, and N231D; and (viii) G2S, T52N, R66T, P80L, F81L, T135N, H168Y, Q220H, and N260D. In some examples, the engineered DLL4 ECD comprises an amino acid sequence selected from the group consisting of any one of SEQ ID NOs: 261-264.

[0089] In another example according to any of the engineered DLL4 ECD described above, the present invention provides a protein construct comprising the engineered DLL4 ECD. In

some examples, the protein construct further comprises a binding moiety that specifically binds to a muscle-specific molecule.

[0090] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0091] FIGs. 1A-1M depict different configurations of bifunctional proteins comprising an anti-muscle target protein and a Notch ligand or agonist. FIG. 1A depicts a monovalent Notch ligand with an anti-muscle protein antibody (Ab) in “Knob-into-Hole” format. FIGs. 1B and 1C depict additional formats and configurations of bifunctional proteins, of which R1, R2, R3, and R4 can be an anti-muscle target protein or a Notch ligand/agonist. FIG. 1D depicts a bivalent fusion protein with Notch ligands fused to the N-terminus of the Fc and an anti-muscle protein antibody Fab fused to the C-terminus of the Fc. FIG. 1E depicts a bivalent fusion protein with Notch ligands fused to the N-terminus of the Fc and an anti-muscle protein antibody scFv fused to the C-terminus of the Fc. FIG. 1F depicts a bivalent fusion protein Notch ligands fused to the N-terminus of the Fc and an anti-muscle target protein antibody single chain Ab (VHH or nanobody) fused to the C-terminus of the Fc. FIG. 1G depicts a bivalent fusion protein with Notch ligands fused to the C-terminus of the Fc and an anti-muscle protein antibody Fab fused to the N-terminus of the Fc. FIG. 1H depicts a bivalent fusion protein with Notch ligands fused to the C-terminus of the Fc and an anti-muscle protein scFv fused to the N-terminus of the Fc. FIG. 1I depicts a bivalent fusion protein with Notch ligands fused to the C-terminus of Fc and an anti-muscle protein VHH/nanobody fused to the N-terminus of Fc. FIG. 1J depicts a bivalent fusion protein with Notch ligands fused to the C-terminus of the antibody light chains. FIG. 1K depicts a bivalent fusion protein with Notch ligands fused to the N-terminus of the antibody light chains. FIG. 1L depicts a bivalent fusion protein with Notch ligands fused to the N-terminus of the antibody heavy chains. FIG. 1M depicts a tetravalent fusion protein with Notch ligands fused to the N-terminus of the antibody light and heavy chains.

[0092] FIGs. 2A-2J depict different configurations of bifunctional proteins comprising a non-antibody moiety (e.g., an LG domain of laminin, an LG domain of agrin, an LG domain of nidogen, and an LG domain of perlecan) and a Notch ligand or agonist. FIG. 2A depicts a monovalent protein with a Notch ligand fused to the N-terminus of a laminin G-like domain (LG domain) monomer, or alternatively, a scFv, sdAb, or Fab to a muscle specific protein.