ANTI-TRANSFERRIN RECEPTOR ANTIBODIES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/570,683, filed on March 27, 2024, the entire contents of which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] The delivery of drugs to the central nervous system has been a challenge in the treatment of neurological diseases in part due to the difficulties in penetrating the blood brain barrier.

SUMMARY

[0003] This disclosure relates to single chain antibody agents that bind to a transferrin receptor (TfR1) and methods of making and using the same, e.g., for delivering payloads to brain tissue.

[0004] Provided herein is a single chain antibody agent polypeptide that binds to a transferrin receptor 1 (TfR1) or a variant or a fragment thereof.

[0005] In some embodiments, a single chain antibody agent comprises one or more complementarity determining regions (CDRs). In some embodiments, a single chain antibody agent comprises a CDR1, a CDR2 and a CDR3.

[0006] In some embodiments, a single chain antibody agent is or comprises a single domain antibody (e.g., a VHH).

[0007] Also provided herein is a polynucleotide encoding a single chain antibody agent polypeptide disclosed herein.

[0008] Further disclosed herein are vectors comprising a polynucleotide encoding a single chain antibody agent polypeptide disclosed herein and cells comprising a single chain antibody agent polypeptide disclosed herein.

[0009] In some embodiments, a cell is contacted with a vector encoding the single chain antibody agent polypeptide. In some embodiments, a cell is contacted with the single chain

antibody agent polypeptide. In some embodiments, a cell is an in vivo cell. In some embodiments, a cell is an ex vivo cell. In some embodiments, a cell expresses hTfR1.

[0010] This disclosure also provides a composition comprising a single chain antibody agent polypeptide disclosed herein.

[0011] In some embodiments of a composition disclosed herein, a single chain antibody agent polypeptide or a polynucleotide encoding a single chain antibody agent polypeptide is encapsulated in a delivery vehicle.

[0012] In some embodiments of a composition disclosed herein, a single chain antibody agent polypeptide or a polynucleotide encoding a single chain antibody agent polypeptide is conjugated to a delivery vehicle.

[0013] In some embodiments, a delivery vehicle is a liposome.

[0014] In some embodiments, a delivery vehicle is an LNP.

[0015] In some embodiments of a composition disclosed herein, a single chain antibody agent polypeptide or a polynucleotide encoding a single chain antibody agent polypeptide is encapsulated in an LNP.

[0016] In some embodiments of a composition disclosed herein, a single chain antibody agent polypeptide is conjugated to an LNP. In some embodiments, a single chain antibody agent polypeptide conjugated to an LNP is displayed on the surface of the LNP.

[0017] In some embodiments, a composition is a pharmaceutical composition. In some embodiments, a pharmaceutical composition comprises one or more excipients. In some embodiments, a pharmaceutical composition is formulated for intramuscular, intradermal, or intravenous delivery.

[0018] Also provided herein is a method of delivering a pharmaceutical composition disclosed herein, to a cell, tissue or subject.

[0019] In some embodiments, delivering comprises administering a pharmaceutical composition to a cell, tissue or subject.

[0020] In some embodiments, a method is a treatment method.

[0021] In some embodiments, a subject is a human.

[0022] In some embodiments, administering a pharmaceutical composition alleviates one or more symptoms of a disease or condition and/or treats a disease or condition.

[0023] In some embodiments of any of the single chain antibody agent polypeptides, polynucleotides, compositions or methods disclosed herein, a single chain antibody agent polypeptide comprises a sequence provided in **Table 1.**

[0024] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR1 sequence provided in Table 1; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR1 sequence provided in **Table 1;** (ii) a CDR2 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR2 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR2 sequence provided in **Table 1**; and/or (iii) a CDR3 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR3 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR3 sequence provided in **Table 1.**

[0025] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 3; or a sequence having

1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 3; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0026] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0027] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 1, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0028] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 158, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0029] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 159, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0030] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 160, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0031] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of

SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

[0032] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 8.

[0033] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 5, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0034] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 122, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0035] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 135, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0036] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 136, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0037] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 10; or a sequence with at least 85%, at least 86%, at least 87%, at least

88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 11; and/or (iii) a CDR3 sequence of SEQ ID NO: 12; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 12; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 12.

[0038] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; and (iii) a CDR3 sequence of SEQ ID NO: 12.

[0039] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 9, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0040] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 14; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 14; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or (iii) a CDR3 sequence of SEQ ID NO: 16; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 16; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 16.

[0041] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 16.

[0042] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 13, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0043] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 18; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 18; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 19; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 19; and/or (iii) a CDR3 sequence of SEQ ID NO: 20; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 20; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 20.

[0044] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; and (iii) a CDR3 sequence of SEQ ID NO: 20.

[0045] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 17, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0046] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 22; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 22; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 23; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 23; and/or (iii) a CDR3 sequence of SEQ ID NO: 24; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 24; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 24.

[0047] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; and (iii) a CDR3 sequence of SEQ ID NO: 24.

[0048] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 21, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0049] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 26; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 26; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 27; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 27; and/or (iii) a CDR3 sequence of SEQ ID NO: 28; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 28; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 28.

[0050] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; and (iii) a CDR3 sequence of SEQ ID NO: 28.

[0051] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 25, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0052] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 30; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 30; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 31; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 31; and/or (iii) a CDR3 sequence of SEQ ID NO: 32; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 32; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 32.

[0053] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; and (iii) a CDR3 sequence of SEQ ID NO: 32.

[0054] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 29, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0055] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 34; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 34; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 35; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 35; and/or (iii) a CDR3 sequence of SEQ ID NO: 36; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 36; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 36.

[0056] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; and (iii) a CDR3 sequence of SEQ ID NO: 36.

[0057] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 33, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0058] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 38; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 38; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 39; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 39; and/or (iii) a CDR3 sequence of SEQ ID NO: 40; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 40; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 40.

[0059] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; and (iii) a CDR3 sequence of SEQ ID NO: 40.

[0060] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 37, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0061] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 42; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 42; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 42; (ii) a CDR2 sequence of SEQ ID NO: 43; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 43; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 43; and/or (iii) a CDR3 sequence of SEQ ID NO: 44; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 44; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 44.

[0062] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 42; (ii) a CDR2 sequence of SEQ ID NO: 43; and (iii) a CDR3 sequence of SEQ ID NO: 44.

[0063] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 41, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0064] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 46; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 46; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 47; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 47; and/or (iii) a CDR3 sequence of SEQ ID NO: 48; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 48; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 48.

[0065] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; and (iii) a CDR3 sequence of SEQ ID NO: 48.

[0066] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 45, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0067] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 50; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 50; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 50; (ii) a CDR2 sequence of

SEQ ID NO: 51; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 51; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 51; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0068] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 50; (ii) a CDR2 sequence of SEQ ID NO: 51; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0069] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 49, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0070] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 117; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 117; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0071] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0072] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 116, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0073] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0074] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0075] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 118, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0076] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least

88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0077] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0078] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 120, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0079] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0080] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0081] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 121, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0082] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 124; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 124; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

[0083] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 8.

[0084] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 123, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0085] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 127; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 127; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 128; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 128; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 128.

[0086] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 128.

[0087] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 126, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0088] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 131; or a sequence with at least 85%, at least 86%, at least 87%, at

least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 131; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 131.

[0089] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 131.

[0090] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 129, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0091] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 133; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 133; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 133; and/or (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

[0092] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 133; and (iii) a CDR3 sequence of SEQ ID NO: 134.

[0093] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 132, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0094] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO:134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

[0095] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 134.

[0096] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 137, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0097] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least

95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 139; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 139; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 139.

[0098] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 139.

[0099] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 138, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0100] In some embodiments of any of the single chain antibody agent polypeptides, polynucleotides, compositions or methods disclosed herein, a single chain antibody agent binds to a human TfR1 (hTfR1).

[0101] In some embodiments of any of the single chain antibody agent polypeptides, polynucleotides, compositions or methods disclosed herein, a single chain antibody agent binds to a cyno TfR.

[0102] In some embodiments of any of the single chain antibody agent polypeptides, polynucleotides, compositions or methods disclosed herein, a single chain antibody agent binds to hTfR1 with an affinity (K_D) of about 10nM to about 1000nM when fused to an Fc domain.

[0103] In some embodiments of any of the single chain antibody agent polypeptides, polynucleotides, compositions or methods disclosed herein, a single chain antibody agent binds to hTfR1 with an affinity (K_D) of about 10nM to about 1000nM when fused to a C terminus of an Fc domain.

[0104] In some embodiments of any of the single chain antibody agent polypeptides, polynucleotides, compositions or methods disclosed herein, a single chain antibody agent binds

to hTfR1 with an affinity (K_D) of about 10nM to about 1000nM when fused to a N terminus of an Fc domain.

[0105] In some embodiments of any of the single chain antibody agent polypeptides, polynucleotides, compositions or methods disclosed herein, a single chain antibody agent binds to an epitope on an ectodomain of hTfR1. In some embodiments, a single chain antibody agent binds to hTfR1 at a site other than a transferrin binding site.

[0106] In some embodiments, a single chain antibody agent binds to hTfR1 in the presence of transferrin.

[0107] In some embodiments, a single chain antibody agent binds to hTfR1 in the absence of transferrin.

[0108] In some embodiments of any of the single chain antibody agent polypeptides, polynucleotides, compositions or methods disclosed herein, a single chain antibody agent is characterized in that when administered to a cell, tissue or subject, the single chain antibody agent polypeptide does not compete with transferrin for binding to hTfR1.

[0109] In some embodiments of any of the single chain antibody agent polypeptides, polynucleotides, compositions or methods disclosed herein, a single chain antibody agent further comprises at least one heavy chain constant region (CH).

[0110] In some embodiments, a single chain antibody agent comprises a CH2 domain and a CH3 domain, e.g., an Fc domain. In some embodiments, a Fc domain is an Fc domain of an immunoglobulin (Ig) isotype.

[0111] In some embodiments, an Ig isotype is IgA, IgD, IgG, IgM, or IgE. In some embodiments, an Ig isotype is IgG, e.g., a human IgG. In some embodiments, an IgG is or comprises IgG1, lgG2, lgG3, or lgG4.

[0112] In some embodiments, a Fc domain comprises a modification. In some embodiments, a modification modulates one or more properties of the antibody agent.

[0113] In some embodiments, a single chain antibody agent polypeptide further comprises a half-life extender. In some embodiments, a half-life extender is or comprises

albumin, e.g., human serum albumin. In some embodiments, a half-life extender comprises a modification that increases binding to neonatal Fc receptor (FcRn).

[0114] In some embodiments of any of the single chain antibody agent polypeptides, polynucleotides, compositions or methods disclosed herein, a single chain antibody agent is fused to one or more polypeptides.

[0115] In some embodiments, one or more polypeptides is an antibody agent or a fragment thereof. In some embodiments, the antibody agent binds to an antigen other than hTfR1.

[0116] In some embodiments, a single chain antibody agent polypeptide is conjugated to a polynucleotide. In some embodiments, a polynucleotide is an antisense oligo (ASO), an inhibitory RNA (e.g., siRNA), a microRNA, a locked nucleic acid (LNA), a peptide nucleic acid (PNA), or an aptamer.

[0117] In some embodiments, a single chain antibody agent polypeptide is conjugated to a small molecule, e.g., ADC.

[0118] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0119] **FIGS. 1A-1D** show binding profiles of exemplary VHHs disclosed herein to human TfR1 (**FIG. 1A**), cyno TfR (**FIG. 1B**) or mouse TfR (**FIG. 1C**). **FIG. 1D** is a schematic of the monovalent VHH-Fcs used for generating the data in **FIGS. 1A-1C**.

[0120] **FIG. 2** is a series of graphs showing binding affinities for VHH Clones A, B, and D assessed by surface plasmon resonance (SPR).

[0121] **FIG. 3** is a graph showing transferrin binding to hTfR1 in CHO cells expressing hTfR1 incubated with both transferrin and VHH Clones A-E.

[0122] **FIG. 4** is a series of graphs showing binding affinities of VHH Clones A-E to hTfR1 ectodomain (ECD) in the absence or presence of transferrin. Binding was assessed by SPR.

[0123] **FIG. 5** shows transcytosis of VHH Clones A, B, D and E as assessed by accumulation of VHHs in a transwell assay with MDCK II cells expressing human or mouse TfR1.

[0124] **FIGS. 6A-6B** are graphs showing the concentration of VHH Fc in the serum and brain lysate of mice administered a control antibody or Clones A, C, D and E formatted as monovalent VHH-Fc.

[0125] **FIGS. 7A-7B** show schematics depicting cryo-EM structures of human TfR1 in complex with Clone A VHH and human holo transferrin. **FIG. 7A** shows a schematic depicting a cryo-EM density map of human TfR1 in complex with Clone A VHH and transferrin at 2.4 Å resolution. **FIG. 7B** shows a schematic depicting an atomic model of a human TfR1/Clone A VHH/transferrin complex.

[0126] **FIGS. 8A-8B** show schematics depicting a cryo-EM structure of human TfR1 in complex with Clone B VHH and human holo transferrin. **FIG. 8A** shows a schematic depicting a cryo-EM density map of transferrin receptor in complex with Clone B VHH and transferrin at 2.7 Å resolution. **FIG. 8B** shows a schematic depicting an atomic model of TfR1/Clone B VHH/transferrin complex.

[0127] **FIG. 9** shows a schematic depicting an atomic model of a Clone A VHH-human TfR1 ectodomain (ECD) complex, highlighting amino acid residues divergent between cyno and human TfR1 ECD (R325, G724) as well as the Clone A VHH residues in close contact (D53, Q99, V101) with human TfR1.

[0128] **FIGS. 10A-10C** are graphs showing binding affinities of VHH Clones A, F, G, H, I, J, K, L, and M formatted as monovalent VHH-Fc fusions to human TfR1 (**FIG. 10A**), cyno

TfR1 (**FIG. 10B**) or mouse TfR1 (**FIG. 10C**). Exemplary clones tested were formatted as VHH-Fc fusions, where VHH was fused on the N-terminus of Fc.

[0129] **FIGS. 11A-11C** are graphs showing binding affinities of VHH Clone A and certain affinity variants of VHH Clone A (clones Q, R, S and T) to human TfR1 (**FIG. 11A**), cyno TfR1 (**FIG. 11B**) or mouse TfR1 (**FIG. 11C**). Exemplary affinity variants of VHH Clone A, Q, R, S and T tested were formatted as monovalent VHH-Fc fusions (VHH fused on the C-terminus of Fc).

[0130] **FIGS. 12A-12B** show a schematic depicting an atomic model of a Clone B VHH-human TfR1 extracellular domain (ECD) complex, highlighting stabilizing mutations (**FIG. 12A**) and non-stabilizing mutations (**FIG.12B**), with the dotted lines representing intended disulfide bonds. As depicted in **FIG.12A**, stabilizing mutations comprise pairs of two cysteine residues including A33C/T101C, A50C/Y107C, and N59C/Y107C. As depicted in **FIG.12B**, non-stabilizing mutations comprise pairs of two cysteine residues including A98C/Y32C, A33C/G108C and A50C/G35C.

[0131] **FIGS. 13A-13C** are thermal stability plots of exemplary VHHs. **FIG. 13A** is a thermal stability plot of VHH Clone B. **FIGS. 13B** and **13C** are thermal stability plots for two VHH Clone B variants (Clone Y and Clone BB) with increased melting temperature (as indicated by the presence of a Tm2 peak).

[0132] **FIGS. 14A-14C** are graphs showing binding affinities of VHH Clone B and exemplary variants of VHH Clone B (clones Y, Z, AA, BB and CC) formatted as monovalent VHH-Fc fusions to human TfR1 (VHH fused on the N-terminus of Fc) (**FIG. 14A**), cyno TfR1 (**FIG. 14B**) or mouse TfR1 (**FIG. 14C**).

DEFINITIONS

[0133] Unless otherwise defined herein, technical and scientific terms used in the present description have the meanings that are commonly understood by those of ordinary skill in the art. For purposes of interpreting this specification, the following description of terms will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In

the event that any description of a term set forth conflicts with any document incorporated herein by reference, the description of the term set forth below shall control.

[0134] ***Antibody agent*:** As used herein, the term "antibody agent" refers to an agent that specifically binds to a particular antigen. In some embodiments, the term encompasses any polypeptide or polypeptide complex that includes immunoglobulin structural elements sufficient to confer specific binding. In some embodiments, an antibody agent is a single chain antibody agent. Other exemplary antibody agents include, but are not limited to monoclonal antibodies or polyclonal antibodies. In some embodiments, an antibody agent may include one or more constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, an antibody agent may include one or more sequence elements are humanized, primatized, chimeric, etc, as is known in the art. In many embodiments, the term "antibody agent" is used to refer to one or more of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, embodiments, an antibody agent utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgA, IgG, IgE or IgM antibodies; bi- or multi-specific antibodies (e.g., Zybodies®, etc); antibody fragments such as Fab fragments, Fab' fragments, F(ab')2 fragments, Fd' fragments, Fd fragments, and isolated CDRs or sets thereof; single chain Fvs; polypeptide-Fc fusions; single domain antibodies (e.g., shark single domain antibodies such as IgNAR or fragments thereof); cameloid antibodies; masked antibodies (e.g., Probodies®); Small Modular ImmunoPharmaceuticals ("SMIPs™"); single chain or Tandem diabodies (TandAb®); VHHs; Anticalins®; Nanobodies® minibodies; BiTE®s; ankyrin repeat proteins or DARPINs®; Avimers®; DARTs; TCR-like antibodies;, Adnectins®; Affilins®; Trans-bodies®; Affibodies®; TrimerX®; MicroProteins; Fynomers®, Centyrins®; and KALBITOR®s. In many embodiments, an antibody agent is or comprises a polypeptide whose amino acid sequence includes one or more structural elements recognized by those skilled in the art as a complementarity determining region (CDR). In some embodiments an antibody agent is or comprises a polypeptide comprising at least one CDR (e.g., at least one heavy chain CDR and/or at least one light chain CDR). In some embodiments, an antibody agent is or comprises a polypeptide comprising structural elements recognized by those skilled in the art as an immunoglobulin variable domain. In some embodiments, an antibody agent is a polypeptide

protein having a binding domain which is homologous or largely homologous to an immunoglobulin-binding domain.

[0135] ***Chimeric antibody:*** The term "chimeric antibody" refers to an antibody in which a portion of the heavy and/or light chain is derived from a first source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0136] ***Conservative amino acid substitution.*** The phrase "conservative amino acid substitution" as used herein refers to a substitution in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been generally defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is considered to be a conservative substitution. Generally, conservative substitutions in the sequences of polypeptides and/or antibodies do not abrogate the binding of the polypeptide or antibody to the target binding site. Methods of identifying nucleotide and amino acid conservative substitutions that do not eliminate binding are well-known in the art.

[0137] ***Conjugate.*** The term "conjugate" as used herein refers to a combination in which two substances are linked by a covalent bond (e.g., an antibody of the disclosure joined to a therapeutic agent). In the conjugate, the two substances may be directly connected or may be connected via a linker. In some embodiments, one of the two substances is an antibody agent of the disclosure, and the other is a physiologically active substance, e.g., a polynucleotide, or a small molecule.. The linker may be a cleavable linker or a non-cleavable linker.

[0138] ***Effective amount.*** The term "effective amount" or "therapeutically effective amount" as used herein refers to an amount of an antibody agent of the disclosure that is required to reach the tissue of interest, or to an amount of a conjugate, a fusion protein or polypeptide, or a complex comprising an antibody agent of the disclosure and a therapeutic agent that is

sufficient to reduce and/or ameliorate the severity and/or duration of (i) a disease, disorder or condition in a subject, and/or (ii) a symptom in a subject. The term also encompasses an amount of a conjugate necessary for the (i) reduction or amelioration of the advancement or progression of a given disease, disorder, or condition, (ii) reduction or amelioration of the recurrence, development, or onset of a given disease, disorder, or condition, and/or (iii) the improvement or enhancement of the prophylactic or therapeutic effect(s) of another agent or therapy (e.g., an agent other than the conjugates provided herein).

[0139] ***Epitope:*** The terms "epitope" and "antigenic determinant" are used interchangeably herein and refer to a portion of an antigen or target capable of being recognized and bound by a particular an antibody agent. When the antigen or target is a polypeptide, epitopes can be formed both from contiguous amino acids and/or noncontiguous amino acids juxtaposed by tertiary folding of the protein. Epitopes formed from contiguous amino acids (also referred to as linear epitopes) are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding (also referred to as conformational epitopes) are typically lost upon protein denaturing. An epitope typically includes at least 3 amino acids in a unique spatial conformation. Epitopes can be predicted using software bioinformatic tools available on the internet. X-ray crystallography or electron microscopy (e.g., cryo-electron microscopy) may be used to characterize an epitope on a target protein by analyzing the amino acid residue interactions of an antigen/antibody complex.

[0140] ***Humanized antibody:*** The term "humanized antibody" as used herein refers to an antibody that comprises a human heavy chain variable region and a light chain variable region wherein the native CDR amino acid residues are replaced by residues from corresponding CDRs from a nonhuman antibody (e.g., mouse, rat, rabbit, or nonhuman primate), wherein the nonhuman antibody has the desired specificity, affinity, and/or activity. In some embodiments, one or more framework region amino acid residues of the human heavy chain or light chain variable regions are replaced by corresponding residues from nonhuman antibody. Furthermore, humanized antibodies can comprise amino acid residues that are not found in the human antibody or in the nonhuman antibody. In some embodiments, these modifications are made to further refine and/or optimize antibody characteristics. In some embodiments, the humanized

antibody comprises at least a portion of an immunoglobulin constant region (e.g., CH1, hinge, CH2, CH3, Fc), typically that of a human immunoglobulin.

[0141] ***Isolated.*** The term "isolated" as used herein refers to a polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition that is in a form not found in nature. An "isolated" antibody is substantially free of material from the cellular source from which it is derived. In some embodiments, isolated polypeptides, soluble proteins, antibodies, polynucleotides, vectors, cells, or compositions are those that have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition that is isolated is substantially pure. A polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition can be isolated from a natural source (e.g., tissue) or from a source such as an engineered cell line.

[0142] ***Percent identity.*** The terms "identical" or percent "identity" in the context of two or more polynucleotides or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity may be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that may be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variants thereof. In some embodiments, two nucleic acids or polypeptides of the disclosure are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, 96%, 97%, 98%, 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the sequences that is at least about 10, at least about 20, at least about 20-40, at least about 40-60 nucleotides or amino acid residues, at least about 60-80 nucleotides or amino acid residues in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 nucleotides or amino acid residues, such as at least about

80-100 nucleotides or amino acid residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, for example, (i) the coding region of a nucleotide sequence or (ii) an amino acid sequence.

[0143] ***Pharmaceutically acceptable.*** The term "pharmaceutically acceptable" as used herein refers to a substance approved or approvable by a regulatory agency or listed in the U.S. Pharmacopeia, European Pharmacopeia, or other generally recognized pharmacopeia for use in animals, including humans.

[0144] ***Pharmaceutically acceptable excipient.*** The terms "pharmaceutically acceptable excipient, carrier, or adjuvant" as used herein refer to an excipient, carrier, or adjuvant that can be administered to a subject, together with at least one antibody agent of the disclosure, and that is generally safe, non-toxic, and has no effect on the pharmacological activity of the therapeutic agent. In general, those of skill in the art and the U.S. FDA consider a pharmaceutically acceptable excipient, carrier, or adjuvant to be an inactive ingredient of any formulation.

[0145] ***Polypeptide.*** The terms "polypeptide" and "peptide" and "protein" are used interchangeably herein and refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid, including but not limited to, unnatural amino acids, as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure may be based upon antibodies, the term "polypeptide" encompasses polypeptides as a single chain and polypeptides of two or more associated chains.

[0146] ***Polynucleotide.*** The terms "polynucleotide" and "nucleic acid" and "nucleic acid molecule" are used interchangeably herein and refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase.

[0147] ***Single chain antibody agent:*** The term "single chain antibody agent" as used herein refers to a polypeptide that comprises at least one variable domain that includes at least two complementarity determining regions (CDRs) and structural elements recognized by those skilled in the art as an immunoglobulin variable domain. In some embodiments, a single chain antibody agent includes three CDRs. In some embodiments, a single chain antibody agent comprises a polypeptide whose amino acid sequence includes at least one CDR (e.g., at least one heavy chain CDR and/or at least one light chain CDR) that is substantially identical to one found in a reference antibody. In some embodiments an included CDR is substantially identical to a reference CDR in that it is either identical in sequence or contains between 1-5 amino acid substitutions as compared with the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that it shows at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or 100% sequence identity with the reference CDR. In some embodiments, a single chain antibody agent is a single domain antibody. In some embodiments, a single domain antibody is a camelid single domain antibody derived from a camelid heavy-chain antibody (HcAb). In some embodiments, a camelid single domain antibody is also referred to as a VHH. In some embodiments, a single domain antibody is a shark single domain antibody derived from a shark heavy-chain antibody (HcAb). In some embodiments, a shark single domain antibody is also referred to as a VNAR. In some embodiments, a single chain antibody agent is a single chain fragment variable (scFv) which is a single protein chain in which the V_H and V_L regions pair to form monovalent molecules see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). In some embodiments, a single chain antibody agent may include one or more sequence elements that are humanized, primatized, chimeric, etc, as is known in the art. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (e.g., attachment of a glycan, a payload [e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc], or other pendant group [e.g., poly-ethylene glycol, etc.]).

[0148] ***Substantially pure.*** The term "substantially pure" as used herein refers to material that is at least 50% pure (i.e., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[0149] ***Specifically binds.*** The term "specifically binds" as used herein refers to an antibody agent that interacts more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to a particular antigen, epitope, protein, or target molecule than with alternative substances. An antibody agent that specifically binds an antigen can be identified, for example, by immunoassays, ELISAs, surface plasmon resonance (SPR), or other techniques known to those of skill in the art. In some embodiments, an antibody that specifically binds an antigen (e.g., human TfR1) can bind related antigens (e.g., cyno TfR1). An antibody agent that specifically binds an antigen can bind the target antigen at a higher affinity than its affinity for a different antigen. The different antigen can be a related antigen. In some embodiments, an antibody agent that specifically binds an antigen can bind the target antigen with an affinity that is at least 20 times greater, at least 30 times greater, at least 40 times greater, at least 50 times greater, at least 60 times greater, at least 70 times greater, at least 80 times greater, at least 90 times greater, or at least 100 times greater, than its affinity for a different antigen. In some embodiments, an antibody agent that specifically binds a particular antigen binds a different antigen at such a low affinity that binding cannot be detected using an assay described herein or otherwise known in the art. In some embodiments, affinity is measured using SPR technology in a Biacore system as described herein or as known to those of skill in the art.

[0150] ***Therapeutic effect.*** The term "therapeutic effect" as used herein refers to the effect and/or ability of an agent, e.g., an antibody agent, a conjugate, a fusion protein or polypeptide, or a complex comprising the antibody of the disclosure to reduce and/or ameliorate the severity and/or duration of (i) a disease, disorder, or condition in a subject, and/or (ii) a symptom in a subject. The term also encompasses the ability of an agent, e.g., a conjugate, to (i) reduce or ameliorate the advancement or progression of a given disease, disorder, or condition, (ii) reduce or ameliorate the recurrence, development, or onset of a given disease, disorder, or condition, and/or (iii) to improve or enhance the prophylactic or therapeutic effect(s) of another agent or therapy (e.g., an agent other than the conjugates provided herein).

[0151] *Vector:* The term "vector" as used herein means a construct that is capable of delivering, and usually expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid, or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, and DNA or RNA expression vectors encapsulated in liposomes.

DETAILED DESCRIPTION

[0152] The present disclosure provides antibodies that specifically bind transferrin receptor 1 (TfR1). Related polypeptides, polynucleotides, vectors, cells, compositions and conjugates comprising the antibodies, methods of making the antibodies, and methods of delivering the compositions and conjugates are also provided. The disclosure also provides methods of using the novel anti-TfR1 antibodies.

TfR1 and Anti-TfR1 Antibodies

[0153] Transferrin receptor, also known as CD71, is a transmembrane glycoprotein expressed in various sites of the human body at differing levels, whose function is to mediate cellular uptake of iron from a plasma glycoprotein, transferrin. Iron uptake from transferrin involves the binding of transferrin to the transferrin receptor, internalization of transferrin within an endocytic vesicle by receptor-mediated endocytosis and the release of iron from the protein by a decrease in endosomal pH. Ponka P, Lok CN. Int J Biochem Cell Biol. 1999 Oct;31(10):1111-37 and Xiaopeng Mo, in Brain Targeted Drug Delivery System, 2019. Apotransferrin (i.e., non-iron conjugate) binds to TfR when bound to two Fe 3+ ions to form holotransferrin (i.e., iron conjugate). The complex of TfR and holotransferrin is translocated into the cell by receptor-mediated endocytosis. CD71 and transferrin dissociate in an endosomal environment, and transferrin moves into the cell while CD71 is recycled to the cell membrane. Thus, transferrin is thought to translocate into cells by proper binding to TfR and proper dissociation. The transferrin receptor system has been exploited for delivery of anticancer drugs and proteins, therapeutic genes into malignant cells, and to deliver other therapeutic agents across the blood brain barrier to the brain.

[0154] In humans and cynomolgus monkeys, two transferrin receptors, TfR1 and TfR2 have been characterized. TfR1 is a high affinity ubiquitously expressed receptor while expression of TfR2 is restricted to certain cell types and is unaffected by intracellular iron concentrations. TfR2 binds to transferrin with a 25-30 fold lower affinity than TfR1. The antibodies of the present disclosure bind to TfR1.

[0155] The sequences for human TfR1, cyno TfR1, and mouse TfR1 are as follows:

[0156] Human TfR1 (UniProt No. P02786.2; SEQ ID NO: 53)

[0157] MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLAVDEEENAD
NNTKANVTKPKRCSGSICYGTIAVIVFFLIGFMIGYLGYCKGVEPKTECERLAGTESPVRE
EPGEDFPAARRLYWDDLKRKLSEKLDSTDFTGTIKLLNENSYVPREAGSQKDENLALYV
ENQFREFKLSKVWRDQHFVKIQVKDSAQNSVIIVDKNGRLVYLVENPGGYVAYSKAATV
TGKLVHANFGTKKDFEDLYTPVNGSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKF
PIVNAELSFFGHAHLGTGDPYTPGFPSFNHTQFPPSRSSGLPNIPVQTISRAAAEKLFGNM
EGDCPSDWKTDSTCRMVTSESKNVKLTVSNVLKEIKILNIFGVIKGFVEPDHYVVVGAQ
RDAWGPGAAKSGVGTALLLKLAQMFSDMVLKDGFQPSRSIIFASWSAGDFGSVGATEW
LEGYLSSLHLKAFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQNVKHPVTGQFLYQDS
NWASKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELIERIPELNKVA
RAAAEVAGQFVIKLTHDVELNLDYERYNSQLLSFVRDLNQYRADIKEMGLSLQWLYSA
RGDFFRATSRLTTDFGNAEKTDRFVMKKLNDRVMRVEYHFLSPYVSPKESPFRHVFWGS
GSHTLPALLENLKLRKQNNGAFNETLFRNQLALATWTIQGAANALSGDVWDIDNEF

[0158] Cyno TfR1 (UniProt No. G8F602; SEQ ID NO: 54)

[0159] MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLAVDDEENA
DNNTKANGTKPKRCGGNICYGTIAVIIFFLIGFMIGYLGYCKGVEPKTECERLAGTESPAR
EEPEEDFPAAPRLYWDDLKRKLSEKLDTTDFTSTIKLLNENLYVPREAGSQKDENLALYIE
NQFREFKLSKVWRDQHFVKIQVKDSAQNSVIIVDKNGGLVYLVENPGGYVAYSKAATVT
GKLVHANFGTKKDFEDLDSPVNGSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPI
VKADLSFFGHAHLGTGDPYTPGFPSFNHTQFPPSQSSGLPNIPVQTISRAAAEKLFGNME
GDCPSDWKTDSTCKMVTSENKSVKLTVSNVLKETKILNIFGVIKGFVEPDHYVVVGAQR
DAWGPGAAKSSVGTALLLKLAQMFSDMVLKDGFQPSRSIIFASWSAGDFGSVGATEWLE

GYLSSLHLKAFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQDVKHPVTGRSLYQDSN
WASKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELVERIPELNKVA
RAAAEVAGQFVIKLTHDTELNLDYERYNSQLLLFLRDLNQYRADVKEMGLSLQWLYSA
RGDFFRATSRLTTDFRNAEKRDKFVMKKLNDRVMRVEYYFLSPYVSPKESPFRHVFWGS
GSHTLSALLESLKLRRQNNSAFNETLFRNQLALATWTIQGAANALSGDVWDIDNEF

[0160] Mouse TfR1 (Genbank No. NP_035768.1; SEQ ID NO: 55)

[0161] MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLAADEEENA
DNNMKASVRKPKRFNGRLCFAAIALVIFFLIGFMSGYLGYCKRVEQKEECVKLAETEET
DKSETMETEDVPTSSRLYWADLKTLLSEKLNSIEFADTIKQLSQNTYTPREAGSQKDESL
AYYIENQFHEFKFSKVWRDEHYVKIQVKSSIGQNMVTIVQSNGNLDPVESPEGYVAFSKP
TEVSGKLVHANFGTKKDFEELSYSVNGSLVIVRAGEITFAEKVANAQSFNAIGVLIYMDK
NKFPVVEADLALFGHAHLGTGDPYTPGFPSFNHTQFPPSQSSGLPNIPVQTISRAAAEKLF
GKMEGSCPARWNIDSSCKLELSQNQNVKLIVKNVLKERRILNIFGVIKGYEEPDRYVVVG
AQRDALGAGVAAKSSVGTGLLLKLAQVFSDMISKDGFRPSRSIIFASWTAGDFGAVGATE
WLEGYLSSLHLKAFTYINLDKVVLGTSNFKVSASPLLYTLMGKIMQDVKHPVDGKSLYR
DSNWISKVEKLSFDNAAYPFLAYSGIPAVSFCFCEDADYPYLGTRLDTYEALTQKVPQLN
QMVRTAAEVAGQLIIKLTHDVELNLDYEMYNSKLLSFMKDLNQFKTDIRDMGLSLQWL
YSARGDYFRATSRLTTDFHNAEKTNRFVMREINDRIMKVEYHFLSPYVSPRESPFRHIFW
GSGSHTLSALVENLKLRQKNITAFNETLFRNQLALATWTIQGVANALSGDIWNIDNEF

[0162] The present disclosure provides single chain antibody agents that bind TfR1. In some embodiments, single chain antibody agents provided herein are derived from camelid heavy chain antibodies (HcAb). In some embodiments, a single chain antibody agent disclosed herein is or comprises a heavy chain variable domain (VHH) from a camelid heavy chain antibody (HcAb), or a fragment thereof.

[0163] In some embodiments, a single chain antibody agent is isolated. In some embodiments, a single chain antibody agent is substantially pure.

[0164] In some embodiments, a single chain antibody agent is a recombinant antibody.

[0165] In some embodiments, a single chain antibody agent is a chimeric antibody.

[0166] In some embodiments, a single chain antibody agent is a humanized antibody. Various methods for generating humanized antibodies are known in the art. In some embodiments, a humanized antibody comprises one or more amino acid residues that have been introduced into its sequence from a source that is non-human. In some embodiments, humanization is performed by substituting one or more non-human CDR sequences for the corresponding CDR sequences of a human antibody.

[0167] The choice of which human heavy chain variable region and/or light chain variable region are used for generating humanized antibodies can be made based on a variety of factors and by a variety of methods known in the art. In some embodiments, the "best-fit" method is used where the sequence of the variable region of a non-human (*e.g.*, rodent) antibody is screened against the entire library of known human variable region sequences. The human sequence that is most similar to that of the non-human (*e.g.*, rodent) sequence is selected as the human variable region framework for the humanized antibody. In some embodiments, a particular variable region framework derived from a consensus sequence of all human antibodies of a particular subgroup of light or heavy chains is selected as the variable region framework. In some embodiments, the variable region framework sequence is derived from the consensus sequences of the most abundant human subclasses. In some embodiments, human germline genes are used as the source of the variable region framework sequences.

[0168] Other methods for humanization include, but are not limited to, (i) a method called "superhumanization" that is described as the direct transfer of CDRs to a human germline framework, (ii) a method termed Human String Content (HSC) that is based on a metric of "antibody humanness", (iii) methods based on generation of large libraries of humanized variants (including phage, ribosomal, and yeast display libraries), and (iv) methods based on framework region shuffling.

[0169] In some embodiments, a single chain antibody agent is fused, e.g., linked, to one or more additional antibody agents or fragments thereof forming a multispecific antibody agent.

[0170] In some embodiment, a multispecific antibody is a bispecific antibody. Bispecific antibodies are capable of recognizing and binding at least two different antigens or epitopes. The different epitopes can either be within the same molecule (*e.g.*, two epitopes on TfR1) or on

different molecules (*e.g.*, one epitope on TfR1 and one epitope on a different target). In some embodiments, a bispecific antibody has enhanced potency as compared to an individual antibody or to a combination of more than one antibody. In some embodiments, a bispecific antibody has reduced toxicity as compared to an individual antibody or to a combination of more than one antibody. It is known to those of skill in the art that any therapeutic agent may have unique pharmacokinetics (PK) (*e.g.*, circulating half-life). In some embodiments, a bispecific antibody has the ability to synchronize the PK of two active binding agents wherein the two individual binding agents have different PK profiles. In some embodiments, a bispecific antibody has the ability to concentrate the actions of two agents in a common area (*e.g.*, tissue) in a subject. In some embodiments, a bispecific antibody has the ability to concentrate the actions of two agents to a common target (*e.g.*, a specific cell type). In some embodiments, a bispecific antibody has the ability to target the actions of two agents to more than one biological pathway or function. In some embodiments, a bispecific antibody has the ability to target two different cells and bring them closer together.

[0171] In some embodiments, a bispecific antibody has decreased toxicity and/or side effects. In some embodiments, a bispecific antibody has decreased toxicity and/or side effects as compared to a mixture of the two individual antibodies or the antibodies as single agents. In some embodiments, a bispecific antibody has an increased therapeutic index. In some embodiments, a bispecific antibody has an increased therapeutic index as compared to a mixture of the two individual antibodies or the antibodies as single agents.

[0172] Several techniques for making bispecific antibodies are known by those skilled in the art. In some embodiments, the bispecific antibodies comprise heavy chain constant regions with modifications in the amino acids that are part of the interface between the two heavy chains. These modifications are made to enhance heterodimer formation and generally reduce or eliminate homodimer formation. In some embodiments, the bispecific antibodies are generated using a knobs-into-holes (KIH) strategy. See., e.g., Ridgway et al. Protein Eng. 1996;9(7):617-21 and Klein et al. MAbs. 2012;4(6):653-663.

[0173] In some embodiments, the bispecific antibodies comprise an scFv that covalently links the VH and VL and removes CH1 and CL. In some embodiments, the bispecific antibodies comprise an scFab or Fcab (see, e.g., Wozniak-Knopp et al. PEDS 2010;23(4):289-97), single-

domain antibodies (e.g., with VHHs from camelid species or sharks), or Duet Mabs (see, e.g., Mazor et al. Mabs 2015;7(2):377-89). Bispecific antibodies can be intact antibodies or antibody fragments comprising antigen-binding sites.

[0174] In some embodiments, a bispecific or multispecific antibody disclosed here comprises on one arm a single chain antibody agent disclosed herein (e.g., a VHH) and on another or additional arm one or more antibody agents, e.g., a full antibody, a Fab, a scFv, a VHH, etc.

[0175] Anti-TfR1 antibodies with more than two specificities are contemplated in this disclosure. In some embodiments, trispecific or tetraspecific antibodies are generated. Anti-TfR1 antibodies with more than two valencies are contemplated. In some embodiments, trivalent or tetravalent antibodies are generated.

[0176] CDRs of an antibody are defined by those skilled in the art using a variety of methods/systems. These systems and/or definitions have been developed and refined over a number of years and include Kabat, Chothia, IMGT, AbM, and Contact. The Kabat definition is based on sequence variability and is commonly used. The Chothia definition is based on the location of the structural loop regions. The IMGT system is based on sequence variability and location within the structure of the variable domain. The AbM definition is a compromise between Kabat and Chothia. The Contact definition is based on analyses of the available antibody crystal structures. An Exemplary system is a combination of Kabat and Chothia. Software programs (*e.g.*, abYsis) are available and known to those of skill in the art for analysis of antibody sequence and determination of CDRs.

[0177] In some embodiments, a single chain antibody agent disclosed herein comprises a single chain antibody agent sequence provided in **Table 1**.

Table 1: Sequence for exemplary single chain antibody agents

Clone	Feature	SEQ ID NO	Sequence
A	VHH	1	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGDAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSVWGQGTLVTVSS
	CDR1	2	GSIPPISVMY

Clone	Feature	SEQ ID NO	Sequence
	CDR2	3	WVGDAGNTAYADSVRG
	CDR3	4	KSDQSV
B	VHH	5	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYAMGWFR QAPGKEREFVAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEETFGITWYGSHEEDFRS WGQGTLVTVSS
	CDR1	6	GRPFSNYAMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	8	AAEETFGITWYGSHEEDFRS
C	VHH	9	DVQLQESGGGLVQPGGSLRLSCVASGSITPASVMYWYRQ APGKEREFVAWVSSHENTAYADSVRGRFTISRDDAKNTA YLQMNSLKPEDTGVYYCKSDQSPWGQGTQVTVSS
	CDR1	10	GSITPASVMY
	CDR2	11	WVSSHENTAYADSVRG
	CDR3	12	KSDQSP
D	VHH	13	DVQLQESGGGPVQPGGSLRLSCVASGSITPINVMYWYRQ APGKGREFVAWVGSAGNTAYADSVRGRFTVSRDDAKNT AYLQMNSLKSEDTGVYFCRTDQSPWGQGTQVTVSS
	CDR1	14	GSITPINVMY
	CDR2	15	WVGSAGNTAYADSVRG
	CDR3	16	RTDQSP
E	VHH	17	DVQLQESGGGPVQPGGSLRLSCVASGSITPINVMYWYRQ APGKGREFVAWVGSHGNTAYADSVRGRFTVSRDNVGNT AYLQMDSLKSEDTGVYYCKTDQSAWGQGTQVTVSS
	CDR1	18	GSITPINVMY
	CDR2	19	WVGSHGNTAYADSVRG
	CDR3	20	KTDQSA
F	VHH	21	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGsAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSVWGQGTLVTVSS
	CDR1	22	GSIPPISVMY
	CDR2	23	WVGSAGNTAYADSVRG
	CDR3	24	KSDQSV
G	VHH	25	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGDgGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSVWGQGTLVTVSS
	CDR1	26	GSIPPISVMY
	CDR2	27	WVGDGGNTAYADSVRG
	CDR3	28	KSDQSV

Clone	Feature	SEQ ID NO	Sequence
H	VHH	29	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGDAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSSWGQGTLVTVSS
	CDR1	30	GSIPPISVMY
	CDR2	31	WVGDAGNTAYADSVRG
	CDR3	32	KSDQSS
I	VHH	33	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGDAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSAWGQGTLVTVSS
	CDR1	34	GSIPPISVMY
	CDR2	35	WVGDAGNTAYADSVRG
	CDR3	36	KSDQSA
J	VHH	37	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGDAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSPWGQGTLVTVSS
	CDR1	38	GSIPPISVMY
	CDR2	39	WVGDAGNTAYADSVRG
	CDR3	40	KSDQSP
K	VHH	41	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGSAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSAWGQGTLVTVSS
	CDR1	42	GSIPPISVMY
	CDR2	43	WVGSAGNTAYADSVRG
	CDR3	44	KSDQSA
L	VHH	45	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGDAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDSSVWGQGTLVTVSS
	CDR1	46	GSIPPISVMY
	CDR2	47	WVGDAGNTAYADSVRG
	CDR3	48	KSDSSV
M	VHH	49	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGDAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDGSVWGQGTLVTVSS
	CDR1	50	GSIPPISVMY
	CDR2	51	WVGDAGNTAYADSVRG
	CDR3	52	KSDGSV
N	VHH	158	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGDAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSVWGCGTLVTVSS
	CDR1	2	GSIPPISVMY
	CDR2	3	WVGDAGNTAYADSVRG

Clone	Feature	SEQ ID NO	Sequence
	CDR3	4	KSDQSV
O	VHH	159	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGDAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSVWGCGTLVTVPP
	CDR1	2	GSIPPISVMY
	CDR2	3	WVGDAGNTAYADSVRG
	CDR3	4	KSDQSV
P	VHH	160	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGDAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSVWGQGTLVTVPP
	CDR1	2	GSIPPISVMY
	CDR2	3	WVGDAGNTAYADSVRG
	CDR3	4	KSDQSV
Q	VHH	116	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGSGGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSVWGQGTLVTVSS
	CDR1	2	GSIPPISVMY
	CDR2	117	WVGSGGNTAYADSVRG
	CDR3	4	KSDQSV
R	VHH	118	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGAAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDQSVWGQGTLVTVSS
	CDR1	2	GSIPPISVMY
	CDR2	119	WVGAAGNTAYADSVRG
	CDR3	4	KSDQSV
S	VHH	120	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGSAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDGSVWGQGTLVTVSS
	CDR1	2	GSIPPISVMY
	CDR2	15	WVGSAGNTAYADSVRG
	CDR3	52	KSDGSV
T	VHH	121	QVQLVESGGGLVQPGGSLTLSCVASGSIPPISVMYWYRQA PGKEREFVAWVGAAGNTAYADSVRGRFTISRDNAKNTAY LQMNSLKPEDTGVYYCKSDGSVWGQGTLVTVSS
	CDR1	2	GSIPPISVMY
	CDR2	119	WVGAAGNTAYADSVRG
	CDR3	52	KSDGSV
U	VHH	122	QVQLVESGGGLVQAGGSLRLSCANSGRPFSNYAMGWFR QAPGKEREFVAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEETFGITWYGSHEEDFRS WGQGTLVTVSS

Clone	Feature	SEQ ID NO	Sequence
	CDR1	6	GRPFSNYAMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	8	AAEETFGITWYGSHEEDFRS
V	VHH	123	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYAMCWFR QAPGKEREFVACISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEETFGITWYGSHEEDFRS WGQGTLVTVSS
	CDR1	124	GRPFSNYAMC
	CDR2	125	CISRTGGSSNYANSVKG
	CDR3	8	AAEETFGITWYGSHEEDFRS
W	VHH	126	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNCAMGWFR QAPGKEREFVAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCACEETFGITWYGSHEEDFRS WGQGTLVTVSS
	CDR1	127	GRPFSNCAMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	128	ACEETFGITWYGSHEEDFRS
X	VHH	129	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYCMGWFR QAPGKEREFVAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEETFGITWYCSHEEDFRS WGQGTLVTVSS
	CDR1	130	GRPFSNYCMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	131	AAEETFGITWYCSHEEDFRS
Y	VHH	132	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYAMGWFR QAPGKEREFVAAISRTGGSSCYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEETFGITWCGSHEEDFRS WGQGTLVTVSS
	CDR1	6	GRPFSNYAMG
	CDR2	133	AISRTGGSSCYANSVKG
	CDR3	134	AAEETFGITWCGSHEEDFRS
Z	VHH	135	QVQLVESGGGLVQAGGSLRLSCLNSGRPFSNYAMGWFR QAPGKEREFVAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEETFGITWYGSHEEDFRS WGQGTLVTVSS
	CDR1	6	GRPFSNYAMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	8	AAEETFGITWYGSHEEDFRS
AA	VHH	136	QVQLVESGGGLVQAGGSMRLSCANSGRPFSNYAMGWFR QAPGKEREFVAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEETFGITWYGSHEEDFRS WGQGTLVTVSS

Clone	Feature	SEQ ID NO	Sequence
	CDR1	6	GRPFSNYAMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	8	AAEETFGITWYGSHEEDFRS
BB	VHH	137	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYAMGWFR QAPGKEREFVACISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEETFGITWCGSHEEDFRS WGQGTLVTVSS
	CDR1	6	GRPFSNYAMG
	CDR2	125	CISRTGGSSNYANSVKG
	CDR3	134	AAEETFGITWCGSHEEDFRS
CC	VHH	138	QVQLVESGGGLVQAGGSMRLSCLNSGRPFSNYCMGWFR QAPGKEREFVAAISRTGGSSNYANSVKGRFTITKSDAKTT VYLQMNSLKPEDTAVYYCAAEECFGITWYGSHEEDFRS WGQGTLVTVSS
	CDR1	130	GRPFSNYCMG
	CDR2	7	AISRTGGSSNYANSVKG
	CDR3	139	AAEECFGITWYGSHEEDFRS

[0178] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR1 sequence provided in Table 1; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR1 sequence provided in **Table 1;** (ii) a CDR2 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR2 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR2 sequence provided in **Table 1**; and/or (iii) a CDR3 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR3 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR3 sequence provided in **Table 1.**

[0179] In some embodiments, a single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 3; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 3; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0180] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0181] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 1, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0182] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 158, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0183] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 159, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0184] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 160, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0185] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

[0186] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 8.

[0187] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 5, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0188] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 122, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0189] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 135, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0190] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 136, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0191] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 10; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 11; and/or (iii) a CDR3 sequence of SEQ ID NO: 12; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 12; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 12.

[0192] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; and (iii) a CDR3 sequence of SEQ ID NO: 12.

[0193] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 9, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0194] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 14; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 14; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or (iii) a CDR3 sequence of SEQ ID NO: 16; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 16; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 16.

[0195] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 16.

[0196] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 13, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0197] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 18; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 18; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 19; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 19; and/or (iii) a CDR3 sequence of SEQ ID NO: 20; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 20; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 20.

[0198] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; and (iii) a CDR3 sequence of SEQ ID NO: 20.

[0199] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 17, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0200] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 22; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 22; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 23; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 23; and/or (iii) a CDR3 sequence of SEQ ID NO: 24; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 24; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 24.

[0201] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; and (iii) a CDR3 sequence of SEQ ID NO: 24.

[0202] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 21, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0203] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 26; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 26; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 27; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 27; and/or (iii) a CDR3 sequence of SEQ ID NO: 28; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 28; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 28.

[0204] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; and (iii) a CDR3 sequence of SEQ ID NO: 28.

[0205] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 25, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0206] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 30; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 30; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 31; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 31; and/or (iii) a CDR3 sequence of SEQ ID NO: 32; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 32; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 32.

[0207] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; and (iii) a CDR3 sequence of SEQ ID NO: 32.

[0208] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 29, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0209] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 34; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 34; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 35; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 35; and/or (iii) a CDR3 sequence of SEQ ID NO: 36; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 36; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 36.

[0210] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; and (iii) a CDR3 sequence of SEQ ID NO: 36.

[0211] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 33, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0212] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 38; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 38; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 39; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 39; and/or (iii) a CDR3 sequence of SEQ ID NO: 40; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 40; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 40.

[0213] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; and (iii) a CDR3 sequence of SEQ ID NO: 40.

[0214] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 37, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0215] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 42; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 42; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 42; (ii) a CDR2 sequence of

SEQ ID NO: 43; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 43; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 43; and/or (iii) a CDR3 sequence of SEQ ID NO: 44; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 44; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 44.

[0216] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 42; (ii) a CDR2 sequence of SEQ ID NO: 43; and (iii) a CDR3 sequence of SEQ ID NO: 44.

[0217] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 41, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0218] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 46; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 46; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 47; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 47; and/or (iii) a CDR3 sequence of SEQ ID NO: 48; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 48; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 48.

[0219] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; and (iii) a CDR3 sequence of SEQ ID NO: 48.

[0220] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 45, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0221] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 50; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 50; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 50; (ii) a CDR2 sequence of SEQ ID NO: 51; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 51; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 51; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0222] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 50; (ii) a CDR2 sequence of SEQ ID NO: 51; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0223] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 49, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0224] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least

88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 117; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 117; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0225] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0226] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 116, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0227] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0228] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0229] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 118, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0230] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0231] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0232] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 120, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0233] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0234] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0235] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 121, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0236] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 124; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 124; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at

least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

[0237] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 8.

[0238] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 123, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0239] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 127; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 127; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 128; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 128; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 128.

[0240] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 128.

[0241] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 126, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0242] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 131; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 131; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 131.

[0243] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 131.

[0244] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 129, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0245] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 133; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 133; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 133; and/or (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

[0246] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6, (ii) a CDR2 sequence of SEQ ID NO: 133; and (iii) a CDR3 sequence of SEQ ID NO: 134.

[0247] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 132, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0248] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

[0249] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 6, (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 134.

[0250] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 137, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0251] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 139; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 139; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 139.

[0252] In some embodiments, a single chain antibody agent comprises (i) a CDR1 sequence of SEQ ID NO: 130, (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 139.

[0253] In some embodiments, a single chain antibody agent comprises the sequence of SEQ ID NO: 138, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0254] In some embodiments, a single chain antibody agent disclosed herein binds to human TfR1 (hTfR1). In some embodiments, a single chain antibody agent binds to hTfR1 with an affinity (K_D) of about 10nM to about 1000nM, e.g., when fused to an Fc domain. In some embodiments, a single chain antibody agent is fused to a C terminus of an Fc domain. In some embodiments, a single chain antibody agent is fused to a N terminus of an Fc domain.

[0255] In some embodiments, binding affinity of a single chain antibody agent to hTfR1 is about 10nM to about 1000nM, about 20nm to about 1000nM, about 30 to about 1000nM, about 40 to about 1000nM, about 50 to about 1000nM, about 60 to about 1000nM, about 70 to about 1000nM, about 80 to about 1000nM, about 90 to about 1000nM, about 100 to about 1000nM, about 200 to about 1000nM, about 300 to about 1000nM, about 400 to about 1000nM, about 500 to about 1000nM, about 600 to about 1000nM, about 700 to about 1000nM, about 800 to about 1000nM, about 900 to about 1000nM, about 10nM to about 900 nM, about 10nM to about 800 nM, about 10nM to about 700 nM, about 10nM to about 600 nM, about 10nM to about 500 nM, about 10nM to about 400 nM, about 10nM to about 300 nM, about 10nM to about 200 nM, about 10nM to about 100 nM, about 10nM to about 90 nM, about 10nM to about 80 nM, about 10nM to about 70 nM, about 10nM to about 60 nM, about 10nM to about 50 nM, about 10nM to about 40 nM, about 10nM to about 30 nM, about 10nM to about 20 nM, e.g., when fused to an Fc domain. In some embodiments, binding affinity of a single chain antibody agent to hTfR1 is about 10nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 200 nM, about 300 nM, about 400 nM, about 500 nM, about 600 nM, about 700 nM, about 800 nM, about 900 nM, about 1000 nM, e.g., when fused to an Fc domain.

[0256] In some embodiments, binding affinity of a single chain antibody agent to hTfR1 is about 10nM to about 1000nM, about 20nm to about 1000nM, about 30 to about 1000nM, about 40 to about 1000nM, about 50 to about 1000nM, about 60 to about 1000nM, about 70 to about 1000nM, about 80 to about 1000nM, about 90 to about 1000nM, about 100 to about 1000nM, about 200 to about 1000nM, about 300 to about 1000nM, about 400 to about 1000nM, about 500 to about 1000nM, about 600 to about 1000nM, about 700 to about 1000nM, about 800 to about 1000nM, about 900 to about 1000nM, about 10nM to about 900 nM, about 10nM to about 800 nM, about 10nM to about 700 nM, about 10nM to about 600 nM, about 10nM to about 500 nM, about 10nM to about 400 nM, about 10nM to about 300 nM, about 10nM to about 200 nM, about 10nM to about 100 nM, about 10nM to about 90 nM, about 10nM to about 80 nM, about 10nM to about 70 nM, about 10nM to about 60 nM, about 10nM to about 50 nM, about 10nM to about 40 nM, about 10nM to about 30 nM, about 10nM to about 20 nM, e.g., when fused to a C terminus of an Fc domain. In some embodiments, binding affinity of a single chain

antibody agent to hTfR1 is about 10nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 200 nM, about 300 nM, about 400 nM, about 500 nM, about 600 nM, about 700 nM, about 800 nM, about 900 nM, about 1000 nM, e.g., when fused to a C terminus of an Fc domain.

[0257] In some embodiments, binding affinity of a single chain antibody agent to hTfR1 is about 10nM to about 1000nM, about 20nm to about 1000nM, about 30 to about 1000nM, about 40 to about 1000nM, about 50 to about 1000nM, about 60 to about 1000nM, about 70 to about 1000nM, about 80 to about 1000nM, about 90 to about 1000nM, about 100 to about 1000nM, about 200 to about 1000nM, about 300 to about 1000nM, about 400 to about 1000nM, about 500 to about 1000nM, about 600 to about 1000nM, about 700 to about 1000nM, about 800 to about 1000nM, about 900 to about 1000nM, about 10nM to about 900 nM, about 10nM to about 800 nM, about 10nM to about 700 nM, about 10nM to about 600 nM, about 10nM to about 500 nM, about 10nM to about 400 nM, about 10nM to about 300 nM, about 10nM to about 200 nM, about 10nM to about 100 nM, about 10nM to about 90 nM, about 10nM to about 80 nM, about 10nM to about 70 nM, about 10nM to about 60 nM, about 10nM to about 50 nM, about 10nM to about 40 nM, about 10nM to about 30 nM, about 10nM to about 20 nM, e.g., when fused to a N terminus of an Fc domain. In some embodiments, binding affinity of a single chain antibody agent to hTfR1 is about 10nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 200 nM, about 300 nM, about 400 nM, about 500 nM, about 600 nM, about 700 nM, about 800 nM, about 900 nM, about 1000 nM, e.g., when fused to a N terminus of an Fc domain.

[0258] In some embodiments, a single chain antibody agent disclosed herein is fused to an Fc domain, e.g., as described herein. In some embodiments, a single chain antibody agent is fused to a C terminus of an Fc domain. In some embodiments, a single chain antibody agent is fused to a N terminus of an Fc domain.

[0259] In some embodiments, a single chain antibody agent disclosed herein binds to cyno TfR1.

[0260] In some embodiments, a single chain antibody agent disclosed herein binds to mouse TfR1.

[0261] In some embodiments, a single chain antibody agent disclosed herein binds to an epitope on an ectodomain of hTfR1. In some embodiments, an epitope is a linear epitope. In some embodiments, an epitope is a conformational epitope.

[0262] In some embodiments, a single chain antibody agent disclosed herein binds to a portion of an apical domain of hTfR1. In some embodiments, a single chain antibody agent disclosed herein binds to a portion of a helical domain of hTfR1. In some embodiments, a single chain antibody agent disclosed herein binds to a portion of an apical domain of hTfR1 and to a portion of a helical domain of hTfR1.

[0263] In some embodiments, a single chain antibody agent disclosed herein binds to hTfR1 at a site other than a transferrin binding site. In some embodiments, a single chain antibody agent disclosed herein binds to hTfR1 in the presence of transferrin. In some embodiments, a single chain antibody agent disclosed herein binds to hTfR1 in the absence of transferrin. In some embodiments, a single chain antibody agent disclosed herein binds to hTfR1 with similar affinity in the presence or in the absence of transferrin.

[0264] In some embodiments, a single chain antibody agent disclosed herein is characterized in that when administered to a cell, tissue or subject, the single chain antibody agent polypeptide does not compete with transferrin for binding to hTfR1.

Exemplary TfR1 epitopes

[0265] TfR1 binding single domain antibody agents described herein can bind to one or more epitopes on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1). In some embodiments, a TfR1 binding single domain antibody agent described herein binds to one or more epitopes on human TfR1. In some embodiments, a TfR1 binding single domain antibody agent described herein binds to one or more epitopes on cyno TfR1.

[0266] In some embodiments, an epitope on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) bound by a single domain antibody agent described herein is a linear epitope.

[0267] In some embodiments, an epitope on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) bound by a single domain antibody agent described herein is a conformational epitope.

[0268] In some embodiments, an epitope on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) bound by a single domain antibody agent described herein is a discontinuous epitope.

[0269] In some embodiments, an epitope on TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) bound by a single domain antibody agent described herein comprises one or more domains of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1). In some embodiments, one or more domains of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) is or comprises an extracellular domain of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) or a fragment thereof. In some embodiments, one or more domains of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1) is or comprises an apical domain, a helical domain, a protease-like domain of TfR1 (e.g., human TfR1, cyno TfR1, and/or mouse TfR1), and/or fragments of one or more of the foregoing domains.

[0270] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises an extracellular domain of human TfR1 or a fragment thereof. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises: (1) an apical domain of human TfR1or a fragment thereof, (2) a helical domain of human TfR1or a fragment thereof, or (3) an apical domain of human TfR1or a fragment thereof, and a helical domain of human TfR1or a fragment thereof.

[0271] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises: (1) one or more residues K189, Y309, F321, P322, P323, S324, R325, L329, or L381 from an apical domain of human TfR1, and/or (2) one or more residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729 or R732 from a helical domain of human TfR1.

[0272] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, Y309, F321, P322, P323, S324, R325, L329, or L381 from an apical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, R325, L329, or L381 from an apical domain of human TfR1.

[0273] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, R732 from a helical domain of human TfR1.

[0274] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, R325, L329, or L381 from an apical domain of human TfR1, and one or more residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, Y309, F321, P322, P323, S324, R325, L329, or L381 from an apical domain of human TfR1, and all of residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, R325, L329, or L381 from an apical domain of human TfR1, and all of residues E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, or R732 from a helical domain of human TfR1.

[0275] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises: (1) one or more residues K189, P323, S324, R325, L381, or E383 from an apical domain of human TfR1, and/or (2) one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1.

[0276] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, R325, L381, or E383 from an apical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, L381, and E383 from an apical domain of human TfR1.

[0277] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1.

[0278] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, L381, and E383 from an apical domain of human TfR1 and one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, R325, L381, or E383 from an apical domain of human TfR1 and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, L381, and E383 from an apical domain of human TfR1, and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1.

[0279] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises: (1) one or more residues K189, P323, S324, R325, or L381 from an apical domain of human TfR1, and/or (2) one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1.

[0280] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, R325, or L381 from an apical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, and L381 from an apical domain of human TfR1.

[0281] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an

epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1.

[0282] In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, and L381 from an apical domain of human TfR1 and one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, R325, or L381 from an apical domain of human TfR1 and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1. In some embodiments, an epitope on human TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, R325, and L381 from an apical domain of human TfR1 and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of human TfR1.

[0283] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises an extracellular domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises an apical domain, a helical domain, or an apical domain and a helical domain of cyno TfR1.

[0284] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises: (1) one or more residues K189, Y309, F321, P322, P323, S324, Q325, L329, or L381 from an apical domain of cyno TfR1, and/or (2) one or more residues E634, M635, G636, R719, N722, N723, S724, A725, F726, N727, E728, T729, or R732 from a helical domain of cyno TfR1.

[0285] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, Y309, F321, P322, P323, S324, Q325, L329, or L381 from an apical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of

residues K189, Y309, F321, P322, P323, S324, Q325, L329, and L381 from an apical domain of cyno TfR1.

[0286] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues E634, M635, G636, R719, N722, N723, S724, A725, F726, N727, E728, T729, or R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues E634, M635, G636, R719, N722, N723, S724, A725, F726, N727, E728, T729, and R732 from a helical domain of cyno TfR1.

[0287] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, Q325, L329, and L381 from an apical domain of cyno TfR1, and one or more residues E634, M635, G636, R719, N722, N723, S724, A725, F726, N727, E728, T729, or R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, Y309, F321, P322, P323, S324, Q325, L329, or L381 from an apical domain of cyno TfR1, and all of residues E634, M635, G636, R719, N722, N723, S724, A725, F726, N727, E728, T729, and R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, Y309, F321, P322, P323, S324, Q325, L329, and L381 from an apical domain of cyno TfR1, and all of residues E634, M635, G636, R719, N722, N723, S724, A725, F726, N727, E728, T729, and R732 from a helical domain of cyno TfR1.

[0288] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises: (1) one or more residues K189, P323, S324, Q325, L381, or E383 from an apical domain of cyno TfR1, and/or (2) one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of cyno TfR1.

[0289] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, Q325, L381, or E383 from an apical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1

bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, Q325, L381, and E383 from an apical domain of cyno TfR1.

[0290] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of cyno TfR1.

[0291] In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, Q325, L381, and E383 from an apical domain of cyno TfR1 and one or more residues E634, M635, R719, N723, N727, E728, T729, or R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises one or more residues K189, P323, S324, Q325, L381, or E383 from an apical domain of cyno TfR1 and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of cyno TfR1. In some embodiments, an epitope on cyno TfR1 bound by a single domain antibody agent described herein comprises all of residues K189, P323, S324, Q325, L381, and E383 from an apical domain of cyno TfR1, and all of residues E634, M635, R719, N723, N727, E728, T729, and R732 from a helical domain of cyno TfR1.

Constant Regions of Anti-TfR1 Antibodies

[0292] In some embodiments, a single chain antibody agent disclosed herein (e.g., a VHH) comprises a heavy chain constant region. A heavy chain constant region includes any one or all of the following constant heavy chain (CH) domains: CH1, hinge, CH2, and/or CH3 domain(s) or any combination thereof.

[0293] In some embodiments, the CH domain is from an IgG1 molecule or an IgG4 molecule. In some embodiments, the CH domain is from an IgG2 molecule, an IgG3 molecule, or an IgG1 molecule.

[0294] In some embodiments, a single chain antibody agent disclosed herein (e.g., a VHH) comprises a CH2 and a CH3 domain, e.g., an Fc domain. In some embodiments, an Fc domain is a wildtype Fc domain. In some embodiments, an Fc domain is a mutated Fc domain, e.g., having one or more modifications that alters binding and/or activity of an Fc domain.

[0295] As an example, a single chain antibody agent disclosed herein (e.g., a VHH) can be fused to any one of the following constant heavy chain (CH) constructs as shown in **Table 8** below. In some embodiments, the hinge region is any hinge region known in the art. In some embodiments, the hinge region is naturally occurring, e.g., from a naturally occurring IgG1, IgG2, IgG3, or IgG4 molecule. In other embodiments, the hinge region contains modification(s) relative to a naturally occurring hinge.

[0296] In some embodiments, an anti-TfR1 antibody of the disclosure is one in which at least one or more of the constant regions has been modified or deleted. In some embodiments, an antibody may comprise one or more modifications to the heavy chain constant domain (CH1, CH2 or CH3) and/or to the light chain constant region (CL). In some embodiments, the heavy chain constant region of the modified antibody comprises at least one human constant region. In some embodiments, the heavy chain constant region of the modified antibody comprises more than one human constant region. In some embodiments, modifications to the constant region comprise additions, deletions, or substitutions of one or more amino acids in one or more regions. In some embodiments, a single chain antibody agent disclosed herein is directly fused to a hinge region. In some embodiments, a single chain antibody agent disclosed herein comprises a VHH fused to an Fc.

[0297] In some embodiments, an anti-TfR1 antibody of the disclosure contains a linker (e.g., a linker as shown in **Table 8** below). In some embodiments, a linker is positioned between the Fc region and the Fab region of an anti-TfR1 antibody of the disclosure.

Table 8: Constant Region, Hinge Sequences, and Linker Sequences

Domain	**Sequence**
IgG1 or IgG4 CH1	
IgG1 CH1 (no hinge) (SEQ ID NO: 58)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKV

IgG1 CH1 (with hinge underlined) (SEQ ID NO: 59)	ASTKGPSVFPLAPSSKSTSGGTAALG**C**LVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYI**C**NVN**HKPSNTKVDK**KVEP**KSC**DKTHT**C**PP
IgG1 CH1 (no hinge) (SEQ ID NO: 60)	ASTKGPSVFPLAPSSKSTSGGTAALG**C**LVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYI**C**NVN**HKPSNTKVDKR**V
IgG1 CH1 (with hinge underlined) (SEQ ID NO: 61)	ASTKGPSVFPLAPSSKSTSGGTAALG**C**LVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYI**C**NVN**HKPSNTKVDKR**VESKYGPP**C**P
IgG1 CH1 (no hinge) (SEQ ID NO: 62)	ASTKGPSVFPLAPSSKSTSGGTAALG**C**LVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYI**C**NVN**HKPSNTKVDK**KV
IgG1 CH1 (with hinge underlined) (SEQ ID NO: 63)	ASTKGPSVFPLAPSSKSTSGGTAALG**C**LVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYI**C**NVN**HKPSNTKVDK**KVEP**KSC**
IgG4 CH1 (no hinge) (SEQ ID NO: 64)	ASTKGPSVFPLAP**C**SRSTSESTAALG**C**LVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT KTYT**C**NVD**HKPSNTKVDK**RV
IgG4 CH1 (with hinge underlined) (SEQ ID NO: 65)	ASTKGPSVFPLAP**C**SRSTSESTAALG**C**LVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT KTYT**C**NVD**HKPSNTKVDK**RVES
Fc hIgG1.agly DelPG (SEQ ID NO: 66)	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQ STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
Fc hIgG1.agly DelG (SEQ ID NO: 67)	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQ STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
Fc hIgG1.agly (SEQ ID NO: 68)	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQ STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY

	PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
HingeFc hIgG1.agly (SEQ ID NO: 69)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
HingeFc hIgG1.agly(KnobS) (SEQ ID NO: 70)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP G
Fc hIgG1.agly(KnobS) (SEQ ID NO: 71)	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQ STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
HingeFc hIgG1.agly(HoleS) (SEQ ID NO: 72)	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQ STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
Hinge region	
IgG1 hinge A (SEQ ID NO: 73)	EPKSCDKTHTCPPCPAPELLGGP
IgG1 hinge B (SEQ ID NO: 74)	EP**K**S**C**DKTHT**C**PP
IgG1 hinge C (SEQ ID NO: 75)	EP**K**S**C**
IgG4 hinge D (SEQ ID NO: 76)	ESKYGPPCPPCPAPEFLGGP
IgG4 hinge E (SEQ ID NO: 77)	ESKYGPP**C**P
IgG4 hinge F	ES

IgG1 hinge G (SEQ ID NO: 78)	EPKS
IgG1 hinge H (SEQ ID NO: 79)	EPKSCD
IgG1 hinge I (SEQ ID NO: 80)	EPKSCDK
IgG1 hinge J (SEQ ID NO: 81)	EPKSCDKT
IgG1 hinge K (SEQ ID NO: 82)	EPKSCDKTH
IgG1 hinge L (SEQ ID NO: 83)	EPKSCDKTHT
IgG1 hinge M (SEQ ID NO: 84)	EPKSCDKTHTC
IgG1 hinge N (SEQ ID NO: 85)	EPKSCDKTHTCP
IgG1 hinge O (SEQ ID NO: 86)	EPKSCDKTHTCPPC
IgG1 hinge P (SEQ ID NO: 87)	EPKSCDKTHTCPPCP
IgG1 hinge Q (SEQ ID NO: 88)	EPKSCDKTHTCPPCPA
IgG1 hinge R (SEQ ID NO: 89)	EPKSCDKTHTCPPCPAP
IgG1 hinge S (SEQ ID NO: 90)	EPKSCDKTHTCPPCPAPE
IgG1 hinge T (SEQ ID NO: 91)	EPKSCDKTHTCPPCPAPEL
IgG1 hinge U (SEQ ID NO: 92)	EPKSCDKTHTCPPCPAPELL

IgG1 hinge V (SEQ ID NO: 93)	EPKSCDKTHTCPPCPAPELLG
IgG1 hinge W (SEQ ID NO: 94)	EPKSCDKTHTCPPCPAPELLGG
IgG4 hinge X	ESK
IgG4 hinge Y (SEQ ID NO: 95)	ESKY
IgG4 hinge Z (SEQ ID NO: 96)	ESKYG
IgG4 hinge ZA (SEQ ID NO: 97)	ESKYGP
IgG4 hinge ZB (SEQ ID NO: 98)	ESKYGPP
IgG4 hinge ZC (SEQ ID NO: 99)	ESKYGPPC
IgG4 hinge ZD (SEQ ID NO: 100)	ESKYGPPCPP
IgG4 hinge ZE (SEQ ID NO: 101)	ESKYGPPCPPC
IgG4 hinge ZF (SEQ ID NO: 102)	ESKYGPPCPPCP
IgG4 hinge ZG (SEQ ID NO: 103)	ESKYGPPCPPCPA
IgG4 hinge ZH (SEQ ID NO: 104)	ESKYGPPCPPCPAP
IgG4 hinge ZI (SEQ ID NO: 105)	ESKYGPPCPPCPAPE
IgG4 hinge ZJ (SEQ ID NO: 106)	ESKYGPPCPPCPAPEF

IgG4 hinge ZK (SEQ ID NO: 107)	ESKYGPPCPPCPAPEFL
IgG4 hinge ZL (SEQ ID NO: 108)	ESKYGPPCPPCPAPEFLG
IgG4 hinge ZM (SEQ ID NO: 109)	ESKYGPPCPPCPAPEFLGG
IgG4 hinge ZN (SEQ ID NO: 110)	ESKYGPPCPPCPAPEFLGGP
Linker	
LinkerS	S
LinkerSG2	SGG
LinkgerSG4 (SEQ ID NO: 111)	SGGGG
LinkerSG4SG4 (SEQ ID NO: 112)	SGGGGSGGGG
LinkerSG4SG4SG4 (SEQ ID NO: 113)	SGGGGSGGGGSGGGG
LinkerG3SG5 (SEQ ID NO: 114)	GGGSGGGGG
LinkerG4SG4SG4S (SEQ ID NO: 115)	GGGGSGGGGSGGGGS
LinkerTG	TG

[0298] Other exemplary constant regions, e.g., hinge regions, that can be combined with the antibody variable regions described herein include but are not limited to the hinge regions described in Peters SJ, et al. J Biol Chem. 2012 Jul 13;287(29):24525-33; and Heads JT, et al. Protein Sci. 2012 Sep;21(9):1315-22; incorporated herein by reference in their entirety.

[0299] The present disclosure further embraces additional variants and equivalents that are substantially homologous to the recombinant, monoclonal, chimeric, humanized, and human

antibodies, or antibody fragments thereof, described herein. In some embodiments, it is desirable to modulate the binding affinity of the antibody. In some embodiments, it is desirable to modulate biological properties of the antibody, including but not limited to, specificity, thermostability, expression level, effector function(s), glycosylation, immunogenicity, and/or solubility. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of an antibody, such as changing the number or position of glycosylation sites or altering membrane anchoring characteristics.

[0300] Effector function of antibodies can be modulated by amino acid mutations and/or domain substitutions (e.g., including but not limited to those described in Dumet et al. MABS 2019; 11(8):1341-50). Additional characteristics such as pharmacokinetics (e.g., Dall'acqua et al J of Immunology 2002;169 (9) 5171-80), glycosylation, immunogenicity, solubility, and stability can be engineered by modification of Fc by mutations or substitutions. In addition, novel antigen specificity can be engineered into constant domains to create new paratopes (e.g., Wozniak-Knopp et al. PEDS 2010;23(4):289-97).

[0301] Variations may be a substitution, deletion, or insertion of one or more nucleotides encoding the antibody or polypeptide that results in a change in the amino acid sequence as compared with the native antibody or polypeptide sequence. In some embodiments, amino acid substitutions are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, e.g., conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. In some embodiments, the substitution, deletion, or insertion includes less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the parent molecule. In some embodiments, variations in the amino acid sequence that are biologically useful and/or relevant may be determined by systematically making insertions, deletions, or substitutions in the sequence and testing the resulting variant proteins for activity as compared to the parent protein.

[0302] In some embodiments, variants may include addition of amino acid residues at the amino- and/or carboxyl-terminal end of the antibody or polypeptide. The length of additional

amino acids residues may range from one residue to a hundred or more residues. In some embodiments, a variant comprises an N-terminal methionyl residue. In some embodiments, the variant comprises an additional polypeptide/protein (e.g., Fc region) to create a fusion protein. In some embodiments, a variant is engineered to be detectable and may comprise a detectable label and/or protein (e.g., a fluorescent tag or an enzyme).

[0303] In some embodiments, a cysteine residue not involved in maintaining the proper conformation of an antibody is substituted or deleted to modulate the antibody's characteristics, for example, to improve oxidative stability and/or prevent aberrant disulfide crosslinking. Conversely, in some embodiments, one or more cysteine residues are added to create disulfide bond(s) to improve stability.

[0304] In some embodiments, an antibody of the present disclosure comprises variant hinge regions incapable of forming disulfide linkages between identical heavy chains (e.g., reduce homodimer formation). In some embodiments, the antibodies comprise heavy chains with changes in amino acids that result in altered electrostatic interactions. In some embodiments, the antibodies comprise heavy chains with changes in amino acids that result in altered hydrophobic/hydrophilic interactions.

[0305] In some embodiments, an antibody of the present disclosure is "deimmunized". The deimmunization of antibodies generally consists of introducing specific amino acid mutations (e.g., substitutions, deletions, additions) that result in removal of predicted T-cell epitopes without significantly reducing the binding affinity or other desired characteristics of the antibody.

[0306] The variant antibodies or polypeptides described herein may be generated using methods known in the art, including but not limited to, site-directed mutagenesis, alanine scanning mutagenesis, and PCR mutagenesis.

[0307] In some embodiments an anti-TfR1 antibody described herein is chemically modified. In some embodiments, the anti-TfR1 antibody has been chemically modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, and/or linkage to a cellular ligand or other protein. Any of numerous chemical modifications may be carried out by known techniques.

[0308] Generally speaking, antigen-antibody interactions are non-covalent and reversible, formed by a combination of hydrogen bonds, hydrophobic interactions, electrostatic and van der Waals forces. When describing the strength of an antigen-antibody complex, the terms affinity and/or avidity are often used. The binding of an antibody to its antigen is a reversible process, and the affinity of the binding is typically reported as an equilibrium dissociation constant (K_D). K_D is the ratio of an antibody dissociation rate (koff, also referred to herein as k_d) (how quickly it dissociates from its antigen) to the antibody association rate (kon) (how quickly it binds to its antigen). In some embodiments, K_D values are determined by measuring the kon and koff rates of a specific antibody/antigen interaction and then using a ratio of these values to calculate the K_D value. In some embodiments, K_D values are used to evaluate and rank the strength of individual antibody/antigen interactions. The lower the K_D of an antibody, the higher the affinity of the antibody for its target. In some embodiments, affinity is measured using SPR technology in a Biacore system. Avidity gives a measure of the overall strength of an antibody-antigen complex. It is dependent on three major parameters: (i) affinity of the antibody for the target, (ii) valency of both the antibody and antigen, and (iii) structural arrangement of the parts that interact.

Methods of Making Anti-TfR1 Antibodies

[0309] The anti-TfR1 antibodies described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthesis methods to constructing a DNA sequence encoding polypeptide sequences and expressing those sequences in a suitable host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide functional variants thereof. In some embodiments, a DNA sequence encoding a polypeptide of interest is constructed by chemical synthesis using an oligonucleotide synthesizer. Oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize a polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to

construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5′ or 3′ overhangs for complementary assembly.

[0310] Once assembled (by synthesis, site-directed mutagenesis, or another method), a polynucleotide sequence encoding a particular polypeptide of interest can be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction enzyme mapping, and/or expression of a biologically active polypeptide in a suitable host. As is well-known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[0311] In some embodiments, a recombinant expression vector is used to amplify and express DNA encoding an antibody against human TfR1. For example, a recombinant expression vector can be a replicable DNA construct that includes synthetic or cDNA-derived DNA fragments encoding a polypeptide chain of an anti-TfR1 antibody operatively linked to suitable transcriptional and/or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence that is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Regulatory elements can include an operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants can also be included. DNA regions are "operatively linked" when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor that participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. In some embodiments,

structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. In some embodiments, in situations where recombinant protein is expressed without a leader or transport sequence, a polypeptide may include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

[0312] The choice of an expression control sequence and an expression vector generally depends upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus, and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including pCR1, pBR322, pMB9 and their derivatives, and wider host range plasmids, such as M13 and other filamentous single-stranded DNA phages.

[0313] In some embodiments, an anti-TfR1 antibody of the present disclosure is expressed from one or more vectors. In some embodiments, a heavy chain polypeptide is expressed by a vector. Thus, the present disclosure provides vectors encoding an anti-TfR1 antibody (e.g., a single chain antibody agent, e.g., a VHH) described herein. In one embodiment, the vector encodes a heavy chain polypeptide of an anti-TfR1 antibody described herein. In one embodiment, the vector encodes a single chain anti-TfR1 antibody described herein, e.g., a VHH.

[0314] Suitable host cells for expression of an anti-TfR1 antibody or a TfR1 protein or fragment thereof to use as an antigen or immunogen include prokaryotes, yeast cells, insect cells, or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram-negative or gram-positive organisms, for example *E. coli* or *Bacillus*. Higher eukaryotic cells include established cell lines of mammalian origin as described herein. Cell-free translation systems may also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts, as well as methods of protein production, including antibody production are well-known in the art.

[0315] Various mammalian culture systems may be used to express recombinant polypeptides. Expression of recombinant proteins in mammalian cells may be desirable because these proteins are generally correctly folded, appropriately modified, and biologically functional.

Examples of suitable mammalian host cell lines include, but are not limited to, COS-7 (monkey kidney-derived), L-929 (murine fibroblast-derived), C127 (murine mammary tumor-derived), 3T3 (murine fibroblast-derived), CHO (Chinese hamster ovary-derived), HeLa (human cervical cancer-derived), BHK (hamster kidney fibroblast-derived), HEK-293 (human embryonic kidney-derived) cell lines and variants thereof. Mammalian expression vectors can comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5′ or 3′ flanking non-transcribed sequences, and 5′ or 3′ non-translated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

[0316] Expression of recombinant proteins in insect cell culture systems (*e.g.*, baculovirus) also offers a robust method for producing correctly folded and biologically functional proteins. Baculovirus systems for production of heterologous proteins in insect cells are well-known to those of skill in the art.

[0317] Thus, the present disclosure provides cells comprising the anti-TfR1 antibody described herein. The present disclosure also provides cells comprising one or more polynucleotides encoding an anti-TfR1 antibody described herein or one or more vectors encoding anti-TfR1 antibody described herein. In one embodiment, the cell comprises a polynucleotide encoding an anti-TfR1 antibody described herein. In one embodiment, the cell comprises a polynucleotide encoding a heavy chain of an anti-TfR1 antibody described herein. In one embodiment, the cell comprises a vector encoding a an anti-TfR1 antibody described herein. In one embodiment, the cell comprises a vector encoding a heavy chain of an anti-TfR1 antibody described herein. In some embodiments, the cells produce the anti-TfR1 antibodies described herein. In some embodiments, the cells produce an antibody. In some embodiments, the cells produce an antibody that binds human TfR1. In some embodiments, the cells produce an antibody that binds cyno TfR1. In some embodiments, the cells produce an antibody that binds human TfR1 and cyno TfR1. In some embodiments, the cell is a prokaryotic cell (*e.g.*, *E. coli*). In some embodiments, the cell is a eukaryotic cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a hybridoma cell.

[0318] Proteins produced by a host cell can be purified according to any suitable method. Standard methods include chromatography (*e.g.*, ion exchange, affinity, and sizing column

chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexa-histidine maltose binding domain, influenza coat sequence, and glutathione-S-transferase can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Affinity chromatography used for purifying immunoglobulins include, but are not limited to, Protein A, Protein G, and Protein L chromatography. Isolated proteins can be physically characterized using techniques known to those of skill in the art, including but not limited to, proteolysis, size exclusion chromatography (SEC), mass spectrometry (MS), nuclear magnetic resonance (NMR), isoelectric focusing (IEF), high performance liquid chromatography (HPLC), and x-ray crystallography. The purity of isolated proteins can be determined using techniques known to those of skill in the art, including but not limited to, SDS-PAGE, SEC, capillary gel electrophoresis, IEF, and capillary isoelectric focusing (cIEF).

[0319] In some embodiments, supernatants from expression systems that secrete recombinant protein into culture media are first concentrated using a commercially available protein concentration filter, for example, an Amicon® or Millipore Pellicon® ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. In some embodiments, an anion exchange resin is employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose, or other types commonly employed in protein purification. In some embodiments, a cation exchange step is employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In some embodiments, a hydroxyapatite media is employed, including but not limited to, ceramic hydroxyapatite (CHT). In some embodiments, one or more reverse-phase HPLC steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, are employed to further purify a recombinant protein. In some embodiments, hydrophobic interaction chromatography (HIC) is used to separate recombinant proteins based on their hydrophobicity. HIC is a useful separation technique for purifying proteins while maintaining biological activity due to the use of conditions and matrices that operate under less denaturing conditions than some other techniques. Some or all of the foregoing purification steps, in various combinations, can be employed to provide a homogeneous recombinant protein.

Polynucleotides

[0320] In some embodiments, the disclosure encompasses polynucleotides comprising polynucleotides that encode a polypeptide (*e.g.*, an anti-TfR1 single chain antibody agent) described herein. The term "polynucleotides that encode a polypeptide" encompasses a polynucleotide that includes only coding sequences for the polypeptide as well as a polynucleotide that includes additional coding and/or non-coding sequences. The polynucleotides of the disclosure can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA; and can be double-stranded or single-stranded, and if single stranded can be the coding strand or non-coding (anti-sense) strand. In some embodiments, a polynucleotide comprises a nucleotide sequence encoding a single chain anti-TfR1 antibody agent, e.g., a VHH, as described herein.

[0321] In some embodiments, a polynucleotide comprises a nucleotide sequence encoding a polypeptide comprising a VHH amino acid sequence depicted in **Table 1.** In some embodiments, a polynucleotide comprises a nucleotide sequence encoding a polypeptide comprising a VHH amino acid sequence having at least 90% identity to a VHH amino acid sequence depicted in **Table 1.**

[0322] In some embodiments, a polynucleotide disclosed herein is a polynucleotide variant. In some embodiments, a polynucleotide variant can contain alterations in the coding regions, non-coding regions, or both. In some embodiments, a polynucleotide variant contains alterations that produce silent substitutions, additions, or deletions, but does not alter the properties or activities of the encoded polypeptide. In some embodiments, a polynucleotide variant comprises silent substitutions that results in no change to the amino acid sequence of the polypeptide (due to the degeneracy of the genetic code). In some embodiments, a polynucleotide variant comprises one or more mutated codons comprising one or more (e.g., 1, 2, or 3) substitutions to the codon that change the amino acid encoded by that codon. Methods for introducing one or more substitutions into a codon are known in the art, such as, e.g., PCR mutagenesis and site-directed mutagenesis. Polynucleotide variants can be produced for a variety of reasons, for example, to optimize codon expression for a particular host (*e.g.*, change codons

in the human mRNA to those preferred by a bacterial host such as *E. coli*). In some embodiments, a polynucleotide variant comprises at least one silent mutation in a non-coding or a coding region of the sequence.

[0323] In some embodiments, a polynucleotide variant is produced to modulate or alter expression (or expression levels) of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to increase expression of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to decrease expression of the encoded polypeptide. In some embodiments, a polynucleotide variant has increased expression of the encoded polypeptide as compared to a parental polynucleotide sequence. In some embodiments, a polynucleotide variant has decreased expression of the encoded polypeptide as compared to a parental polynucleotide sequence.

[0324] In some embodiments, a polynucleotide comprises the coding sequence for a polypeptide (*e.g.*, a single chain antibody agent) fused in the same reading frame to a polynucleotide that aids in expression and/or secretion of a polypeptide from a host cell (*e.g.*, a leader sequence that functions as a secretory sequence for controlling transport of a polypeptide). The polypeptide can have the leader sequence cleaved by the host cell to form a "mature" form of the polypeptide.

[0325] In some embodiments, a polynucleotide comprises the coding sequence for a polypeptide (*e.g.*, an antibody) fused in the same reading frame to a marker or tag sequence. For example, in some embodiments, a marker sequence is a hexa-histidine tag (HIS-tag) that allows for efficient purification of the polypeptide fused to the marker. In some embodiments, a marker sequence is a hemagglutinin (HA) tag derived from the influenza hemagglutinin protein when a mammalian host (*e.g.*, COS-7 cells) is used. In some embodiments, the marker sequence is a FLAG™ tag. In some embodiments, a marker is used in conjunction with other markers or tags.

[0326] In some embodiments, a polynucleotide is isolated. In some embodiments, a polynucleotide is substantially pure.

Vectors and Cells

[0327] Vectors and cells comprising each and every one of the polynucleotides described herein are also provided. In some embodiments, an expression vector comprises a polynucleotide molecule encoding an anti-TfR1 single chain antibody agent described herein. In some embodiments, an expression vector comprises a polynucleotide molecule encoding a polypeptide that is part of a an anti-TfR1 single chain antibody agent described herein. In some embodiments, an expression vector comprises a polynucleotide molecule encoding a VHH polypeptide of an anti-TfR1 single chain antibody agent described herein.

[0328] In some embodiments, a host cell comprises an expression vector comprising a polynucleotide molecule encoding an anti-TfR1 single chain antibody agent described herein. In some embodiments, a host cell comprises an expression vector comprising a polynucleotide molecule encoding a polypeptide that is part of an anti-TfR1 single chain antibody agent described herein. In some embodiments, a host cell comprises a polynucleotide molecule encoding an anti-TfR1 single chain antibody agent described herein. In some embodiments, a host cell comprises an expression vector comprising a polynucleotide molecule encoding a VHH polypeptide of an anti-TfR1 single chain antibody agent described herein.

Analysis of Physical/Chemical Properties of Anti-TfR1 Antibodies

[0329] Anti-TfR1 single chain antibody agents of the present disclosure may be analyzed for their physical/chemical properties and/or biological activities by various methods known in the art. In some embodiments, an anti-TfR1 single chain antibody agent is tested for its ability to bind TfR1 (e.g., human TfR1 and/or cyno TfR1). Binding assays include, but are not limited to, SPR (e.g., Biacore), ELISA, and flow cytometry. In some embodiments, an anti-TfR1 single chain antibody agent is tested for its ability to inhibit, reduce, or block binding of transferrin to its TfR1 receptor. In some embodiments, an anti-TfR1 single chain antibody agent is tested for its ability to inhibit, reduce, or block TfR1 activity. In some embodiments, an anti-TfR1 single chain antibody agent is tested for its ability to internalize with TFR1 and induce increased internalization of TfR1. In addition, antibodies may be evaluated for solubility, stability, thermostability, viscosity, expression levels, expression quality, and/or purification efficiency.

[0330] In some embodiments, assays are provided for identifying an anti-TfR1 single chain antibody agent that affects TfR1 activity. In some embodiments, SPR, ELISA, or FACS assays are used to assess the ability of an anti-TfR1 antibody to block binding of TfR1 to Tf. In some embodiments, cytotoxicity assays are used to assess the ability of an anti- single chain antibody agent to affect natural killer (NK) cell activity. In some embodiments, proliferation assays are used to assess the ability of an anti-TfR1 antibody to affect T-cell activity.

[0331] In some embodiments, an anti-TfR1 single chain antibody agent described herein is an antagonist of human TfR1.

[0332] In some embodiments, an anti-TfR1 single chain antibody agent described herein is not an antagonist of human TfR1.

Anti-TfR1 Antibody Conjugates and Complexes

[0333] The present disclosure also provides conjugates comprising an anti-TfR1 single chain antibody agent described herein conjugated to a second molecule. In some embodiments, the second molecule comprises any agent, e.g., therapeutic agent, described herein.

[0334] Conjugates comprising an anti-TfR1 single chain antibody agent described herein may be made using any suitable method known in the art. In some embodiments, the components of the conjugate are linked by covalent interactions. In some embodiments, conjugates are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyidithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene).

[0335] In some embodiments, an anti-TfR1 single chain antibody agent described herein is conjugated to a detectable substance or molecule that allows the agent to be used for diagnosis and/or detection. A detectable substance can include, but is not limited to, enzymes, such as

horseradish peroxidase, alkaline phosphatase, beta-galactosidase, and acetylcholinesterase; prosthetic groups, such as biotin and flavine(s); fluorescent materials, such as, umbelliferone, fluorescein, fluorescein isothiocyanate (FITC), rhodamine, tetramethylrhodamine isothiocyanate (TRITC), dichlorotriazinylamine fluorescein, dansyl chloride, cyanine (Cy3), and phycoerythrin; bioluminescent materials, such as luciferase; radioactive materials, such as 212Bi, 14C, 57Co, 51Cr, 67Cu, 18F, 68Ga, 67Ga, 153Gd, 159Gd, 68Ge, 3H, 166Ho, 131I, 125I, 123I, 121I, 115In, 113In, 112In, 111In, 140La, 177Lu, 54Mn, 99Mo, 32P, 103Pd, 149Pm, 142Pr, 186Re, 188Re, 105Rh, 97Ru, 35S, 47Sc, 75Se, 153Sm, 113Sn, 117Sn, 85Sr, 99mTc, 201Ti, 133Xe, 90Y, 69Yb, 175Yb, 65Zn; positron emitting metals; and magnetic metal ions.

[0336] An anti-TfR1 single chain antibody agent described herein can also be conjugated to a second antibody to form an antibody heteroconjugate.

[0337] In some embodiments, the anti-TfR1 single chain antibody agent of the present disclosure can be conjugated to a molecule or drug, such as a nucleic acid, e.g., an antisense oligonucleotide, a short interfering RNA (siRNA), an RNA such as messenger RNA (mRNA), microRNA (miRNA), guide RNA (gRNA), a phosphoroamidate morpholino oligomer or an aptamer, etc. In some embodiments, the anti-TfR1 single chain antibody agent is conjugated to a particle, e.g., lipid particle or nanoparticle, which can contain a therapeutic agent such as one described herein. In some embodiments, the anti-TfR1 single chain antibody agent is conjugated to a viral particle, e.g., a viral particle comprising a therapeutic nucleic acid and/or protein, e.g., a viral particle for gene therapy (e.g., an adeno-associated virus or a lentivirus). The anti-TfR1 single chain antibody agent may be linked to the drug by a linker. In some embodiments, the anti-TfR1 single chain antibody agent is conjugated to a small molecule such as a cytotoxic agent (e.g., maitansine). In some embodiments, the anti-TfR1 single chain antibody agent is conjugated to an anti-inflammatory agent (e.g., a glucocorticoid). In some embodiments, the anti-TfR1 single chain antibody agent is conjugated to a half-life extension moiety (e.g., polyethylene glycol). Methods of preparing antibody-nucleic acid conjugates, such as the conjugates contemplated in this disclosure are well-known in the art. See, e.g., US Patent Application Publication No. US20190240346, and US Patent Nos. US10881743 and US10550188, and International Patent Application Publication No. WO1991004753, the disclosures of which are incorporated by reference herein in their entirety.

[0338] In some embodiments, the conjugate is a fusion protein. Fusion proteins comprising an anti-TfR1 single chain antibody agent described herein can be made using any suitable method known in the art. Such a fusion protein can include a fusion of an anti-TfR1 single chain antibody agent of the disclosure (including bispecific, multispecific, or multivalent anti-TfR1 antibodies) with a therapeutic polypeptide or antibody. In one instance, an anti-TfR1 single chain antibody agent described herein is conjugated via a fusion protein to an anti-beta amyloid antibody (e.g., aducanumab). In one instance, an anti-TfR1 single chain antibody agent described herein is conjugated via a fusion protein to rituximab. In one instance, an anti-TfR1 single chain antibody agent described herein is conjugated via a fusion protein to an enzyme (e.g., iduronate 2-sulfatase, glucocerebrosidase, alpha-L-iduronidase, or sulfamidase). In one instance, avidin can be added to the C-terminus of the heavy chain to produce a fusion protein as described in Candelaria PV et al. Front Immunol. 2021;12:607692. The fusion protein may be further conjugated or complexed to a second molecule or drug, such as a biotinylated drug, as described in Daniels TR, et al. Biochim Biophys Acta. 2012;1820(3):291-317.

[0339] In some embodiments, complexes comprising an anti-TfR1 single chain antibody agent described herein can be made using any suitable method known in the art. In some embodiments, the components of the complex are linked by non-covalent interactions. Such compounds comprise an anti-TfR1 single chain antibody agent complexed with another agent, e.g., therapeutic agent, or complexed with a lipid or particle which comprises another therapeutic agent.

[0340] In some embodiments, a particle that is conjugated to or complexed with an anti-TfR1 single chain antibody agent described herein may comprise a liposome or lipid nanoparticle (LNP). Liposomes or LNPs are particles comprising a plurality of lipid molecules physically associated with each other by intermolecular forces.

[0341] In some embodiments, liposomes or LNPs can be used to encapsulate one or more single chain antibody agent polypeptides or polynucleotides encoding one or more single chain antibody agent polypeptides, for delivery.

[0342] In some embodiments, one or more single chain antibody agent polypeptides disclosed herein can be conjugated to LNPs such that one or more single chain antibody agent polypeptides are displayed on the surface of the LNPs.

[0343] LNPs contain cationic lipids and are useful for delivering polyanions such as nucleic acids. Other lipids that can be included in LNPs are neutral lipids (i.e., uncharged or zwitterionic lipids), anionic lipids, helper lipids that enhance transfection, and stealth lipids that increase the length of time for which nanoparticles can exist in vivo. Examples of suitable cationic lipids, neutral lipids, anionic lipids, helper lipids, and stealth lipids can be found in WO2016/010840 and WO201/173054, each of which is herein incorporated by reference in its entirety for all purposes. In some embodiments, a lipid nanoparticle can comprise a cationic lipid and one or more other components. In some embodiments, the other component can comprise a helper lipid such as cholesterol. In some embodiments, the other components can comprise a helper lipid such as cholesterol and a neutral lipid such as distearoylphosphatidylcholine (DSPC). In some embodiments, the other components can comprise a helper lipid such as cholesterol, an optional neutral lipid such as DSPC, and a stealth lipid such as S010, S024, S027, S031, or S033.

[0344] Liposomes are made from amphiphilic lipids which can form bilayers in an aqueous environment to encapsulate an aqueous core. A polypeptide or polynucleotide may be incorporated into the aqueous core. These lipids can have an anionic, cationic or zwitterionic hydrophilic head groups. Liposomes can be formed from a single lipid or from a mixture of lipids. A mixture may comprise (1) a mixture of anionic lipids; (2) a mixture of cationic lipids; (3) a mixture of zwitterionic lipids; (4) a mixture of anionic lipids and cationic lipids; (5) a mixture of anionic lipids and zwitterionic lipids; (6) a mixture of zwitterionic lipids and cationic lipids; or (7) a mixture of anionic lipids, cationic lipids and zwitterionic lipids. Similarly, a mixture may comprise both saturated and unsaturated lipids. Exemplary phospholipids include, but are not limited to, phosphatidylethanolamines, phosphatidylcholines, phosphatidylserines, and phosphatidylglycerols. Exemplary cationic lipids include, but are not limited to, 1,2-distearyloxy-N,N-dimethyl-3-aminopropane (DSDMA), dioleoyl trimethylammonium propane (DOTAP), 1,2- dioleyloxy-N,Ndimethyl-3-aminopropane (DODMA), 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane

(DLenDMA). Zwitterionic lipids include, but are not limited to, acyl zwitterionic lipids and ether zwitterionic lipids. Examples of useful zwitterionic lipids include dodecylphosphocholine, DPPC, and DOPC.

Tissue Targeting and Use of Anti-TfR1 Antibodies

[0345] In some embodiments, an anti-TfR1 single chain antibody agent described can be used to target brain tissue and transport an agent, e.g., a therapeutic agent across the blood brain barrier for the treatment of a neurological disorder. Exemplary neurological disorders include Alzheimer's disease, Parkinson's disease, frontotemporal dementia, ALS, Huntington's disease, multiple sclerosis, spinal muscular atrophy, muscular dystrophy, spinal cord injury, stroke, ophthalmological conditions, acute or chronic optic neuritis, psychiatric disorders, Tourette's disease brain injury, brain tumors, and epilepsy.

[0346] Exemplary therapeutic agents for the treatment of Alzheimer's disease include caprylic triglyceride, anti-tau antibody, anti-beta amyloid antibody, anti-DKK1 antibody, APOE antagonist antibody, donepezil, quinidine, a serotonin 6 receptor antagonist, a beta-secretase inhibitor, a RAGE antagonist, a BACE inhibitor, an amyloid beta-protein inhibitor, a phosphodiesterase 9A inhibitor, bisnorcymserine, bryostatin-1, an alpha-7 potentiator, a purinoceptor P2Y6 agonist, a tau protein aggregation/TDP-43 aggregation inhibitor, N3pG-Aß mAb, an mGlu2 agonist, quinazolinone, a mitochondrial protein stimulant, an amyloid precursor protein secretase inhibitor, a 5HT6 antagonist, R-phenserine, an amyloid beta/tau protein inhibitor, a MAO-B inhibitor, an Lp-PLA2 inhibitor, a 5-HT6 receptor antagonist, a BET protein inhibitor, an anti-protofibrillar AB mAb, nomethiazole, a histamine H3 receptor antagonist, a PPAR-delta/gamma agonist, abeotaxane, and a p38 mitogen-activated protein kinase inhibitor.

[0347] Exemplary therapeutic agents for the treatment of ALS include an anti-SOD1 antibody, anti-DR6 antibody, anti-DPR antibody, dexpramipexole, arimoclomal, GM6, ibudilast, a macrophage modulator, a NOGO-A inhibitor, and a troponin complex stimulant.

[0348] Exemplary therapeutic agents for the treatment of brain injury include apomorphine, a cytokine inhibitor/neuropeptide receptor modulator, and a progesterone receptor agonist.

[0349] Exemplary therapeutic agents for the treatment of brain tumors include an IDH1 inhibitor, doxorubicin, paclitaxel, an anti-EGFRvIII antibody-drug conjugate, bevacizumab, a FGF-R kinase inhibitor, a PI3K inhibitor, cabozantinib, iodine I 131 derlotuximab biotin, a PDGFR inhibitor, carboxyamidotriazole orotate, a non-neurotoxic derivative of penclomidine, golvatinib, dexanabinol, a TGF-beta 1 kinase inhibitor, afatinib, an IDO inhibitor, cabazitaxel, a Src kinase/pre-tubulin inhibitor, a SMO protein inhibitor, an endothelin A/B receptor antagonist, a proteasome inhibitor, a T-type calcium channel antagonist, a thapsigargin analogue, irinotecan, nivolumab, a CSF-1R inhibitor, pelareorep, an EGFR antagonist, an exportin-1 protein inhibitor/ nuclear protein inhibitor, a BIRC5 protein inhibitor, evofosfamide, abeotaxane, ENG protein inhibitor, trans-sodium crocetinate, an N7-alkylating agent, a targeted anti-angiogenic agent, and veliparib.

[0350] Exemplary therapeutic agents for the treatment of epilepsy include everolimus, eslicarbazepine acetate, alprazolam, brivaracetam, carbamazepine, cannabidiol, a 4-aminobutyrate transaminase inhibitor, perampanel, a GABA-A receptor agonist, synthetic huperzine, pregabalin, clobazam, diazepam, a $GABA_A$ synaptic and extra-synaptic receptor modulator, topiramate IV, lacosamide, and a serotonin receptor agonist.

[0351] Exemplary therapeutic agents for the treatment of genetic disorders (e.g., Friedrich's ataxia, late infantile neuronal ceroid, spinal and bulbar muscular atrophy, ataxia telangiectasia, pantothenate kinase-associated neurodegeneration, spinal muscular atrophy, familial amyloid polyneuropathy, Rett syndrome, Leigh syndrome, Wilson's disease) include a NF/E2 related factor 2 stimulant, interferon gamma-1b, rhTPP1 enzyme replacement therapy, vatiquinone, deferiprone, nusinersen, ISIS-TTRRX, a serotonin 1A receptor agonist, cytokine inhibitors/neuropeptide receptor modulator, an siRNA inhibitor targeting TTR, phosphopantothenate replacement, DcpS inhibitor, cysteamine bitartrate, indolepropionic acid, a transthyretin dissociation inhibitor, and bis-choline tetrathiomolybdate.

[0352] Exemplary therapeutic agents for the treatment of headache include an anti-CGRP mAb, a CGRP receptor antagonist mAb, sumatriptan, dextromethorphan/quinidine, onabotulinumtoxinA, a serotonin-1F receptor agonist, a nNOS inhibitor/5HT, dihydroergotamine, cyclobenzaprine, and aspirin/sumatriptan combination.

[0353] Exemplary therapeutic agents for the treatment of Huntington's disease include laquinimod, a PDE10 inhibitor, pridopidine, cysteamine bitartrate, and aVMAT2 inhibitor.

[0354] Exemplary therapeutic agents for the treatment of multiple sclerosis include natalizumab, monomethyl fumarate prodrug, anti-LINGO-1 antibody, a Nck protein modulator, a S1PR-1/5 receptor agonist, fingolimod, an anti-CD52 mAb, idebenone, a PPAR-gamma agonist/modulator, laquinimod, a tyrosine kinase inhibitor, an anti-CD19 mAb, ibudilast, guanabenz, an anti-CD20 mAb, interferon beta-1b, an IL-7 receptor inhibitor, a S1P1 receptor agonist, a myelin protein stimulant, estriol succinate, imilecleucel-T, an anti-VLA 2 mAb, a BAFF-R modulator, a CD100 antigen inhibitor, an anti-DR6 antibody, and an NF-kappa B inhibitor.

[0355] Exemplary therapeutic agents for the treatment of muscular dystrophy include a myostatin inhibitor, drisapersen, eteplirsen, halofuginone, idebenone, ISIS-DMPKR$_X$, a (steroid receptor agonist, a GAPDH inhibitor, a genetic transcription inhibitor, tadalafil, ataluren, and a glucocorticoid receptor agonist.

[0356] Exemplary therapeutic agents for the treatment of pain include a neublastin, P2X3 purinoreceptor antagonist, a SNARE protein antagonist, oxycodone-naltrexone core (abuse resistant), amitriptyline/ketamine, rintatolimod, a cannabinoid receptor CB2 agonist, a non-eryhropoietic peptide, a PPAR-gamma agonist, a glycogen phosphorylase inhibitor, a NMDA receptor antagonist, zoledronic acid, an early growth response protein 1 inhibitor, a (histamine-3 receptor antagonist, buprenorphine, a cytokine inhibitor, cebranopadol, celecoxib, an arachidonic acid analog, a synthetic capsaicin, a Nav1.7 sodium channel inhibitor, an opioid kappa receptor agonist, duloxetine, a nerve growth factor stimulant, dexmedetomidine, a voltage-gated sodium channel inhibitor, bupivacaine, an angiotensin type 2 receptor antagonist, a nerve growth factor inhibitor, a p38 inhibitor, rapastinel, levorphanol, a CGRP mAb, pregabalin, an mGlu2/3 receptor agonist, a CACNA2D1 protein modulator, a bone resorption factor inhibitor, neublastin, a mu-opioid analgesic, an nNOS inhibitor, O-desmethyltramadol, palmitoylethanolamide, a GABA A agonist, a TRPV-1 receptor agonist, nabiximols, a cyclo-oxygenase 2 inhibitor, a nerve growth factor modulator, cyclobenzaprine, flurbiprofen, a fatty acid amide hydrolase inhibitor, and ibuprofen/phosphatidylcholine.

[0357] Exemplary therapeutic agents for the treatment of Parkinson's disease include amantadine, apomorphine, an alpha7 nicotine acetylcholine receptor partial agonist, an anti-alpha-synuclein antibody, alpha-synuclein inhibitor, levodopa, a D1 potentiator, dipraglurant, a serotonin 1A/1B partial agonist, fipamezole, GM6, a retinoid X receptor agonist, istradefylline, rotigotine, pramipexole/rasagiline, R-phenserine, a serotonin 2A/6 receptor antagonist, an adenosine A2A receptor antagonist, safinamide, and a dopamine receptor agonist.

[0358] Exemplary therapeutic agents for the treatment of spasticity include baclofen, onabotulinumtoxinA, abobotulinumtoxinA, arbaclofen, nabiximols, and incobotulinumtoxinA.

[0359] Exemplary therapeutic agents for the treatment of spinal cord injury include an anti-Lingo-1 antibody, anti-NgR1 antibody, neublastin, a nervous system modulator, a Rho GTP-binding protein-inhibitor, and fibroblast growth factor receptor.

[0360] Exemplary therapeutic agents for the treatment of stroke include natalizumab, recombinant mutant form of human wild-type activated protein C, ticagrelor, dalfampridine, aspirin, nimodipine microparticles, GM6, a PARP inhibitor, a PDZ domain inhibitor, a beta amyloid inhibitor, dabigatran, and sodium nitrite.

[0361] Exemplary therapeutic agents for the treatment of Tourette's Syndrome include a histamine-3 receptor antagonist, a 4-aminobutyrate transaminase inhibitor, abobotulinumtoxinA, ecopipam, a VMAT2 inhibitor, acamprosate, and vigabatrin.

[0362] Other exemplary therapeutic agents for the treatment of other neurological disorders include a myostatin inhibitor, NF/E2 related factor 2 stimulant, anti-tau antibody, a myeloperoxidase inhibitor, a mitochondrial permeability transition pore inhibitor, belimumab, type II-B activin receptor modulator mAb, a C1 esterase inhibitor, ferric carboxymaltose, amifampridine, fingolimod, a monoamine oxidase B inhibitor, a neurotransmitter modulator, a dopamine receptor agonist, an anti-CD19 mAb, a VMAT2 inhibitor, a CD20 mAb, thymosin beta-4, an anti-IL-6 receptor mAb, eculizumab, an AMPA receptor modulator, a steroid hydroxylase inhibitor, pyridoxal phosphate, abeotaxane, aceneuramic acid, and sodium oxybate.

[0363] In some embodiments, the conjugate is a fusion polypeptide comprising an anti-TfR1 single chain antibody agent described herein and a whole antibody or antibody fragment (a therapeutic agent). In certain embodiments, the whole antibody or antibody fragment is an anti-

beta amyloid antibody, an anti-tau antibody, an anti-alpha synuclein antibody, an anti-TDP-43 antibody, an anti-LINGO-1 antibody, an anti-LINGO-2 antibody, an anti-LINGO-3 antibody, an anti-LINGO-4 antibody, an anti-TREM2 antibody, an anti-C9orf72 dipeptide repeat poly-GA antibody (i.e., antibody capable of binding a dipeptide repeat (DPR) of poly-glycine–alanine (GA) having at least 6 repeats $(GA)_6$ as translated from the chromosome 9 open reading frame 72 (C9orf72) gene, an anti-TWEAK antibody, or an anti-TWEAK-R antibody.

[0364] In some embodiments, the conjugate is a fusion polypeptide comprising an anti-TfR1 single chain antibody agent described herein and an antisense oligonucleotide (e.g., nusinersen).

Pharmaceutical Compositions

[0365] The present disclosure provides compositions comprising an anti-TfR1 single chain antibody agent described herein. The present disclosure also provides pharmaceutical compositions comprising an anti-TfR1 single chain antibody agent described herein and a pharmaceutically acceptable vehicle.

[0366] In some embodiments, a composition (e.g., a pharmaceutical composition) comprising a single chain antibody agent polypeptide or a polynucleotide encoding a single chain antibody agent polypeptide comprises a delivery vehicle. In some embodiments, a single chain antibody agent polypeptide or a polynucleotide encoding a single chain antibody agent polypeptide is encapsulated in a delivery vehicle. In some embodiments, a delivery vehicle is an LNP. In some embodiments, a delivery vehicle is a liposome.

[0367] Formulations are prepared for storage and/or use by combining an anti-TfR1 single chain antibody agent of the present disclosure with a pharmaceutically acceptable vehicle (e.g., a carrier or excipient). Those of skill in the art generally consider pharmaceutically acceptable carriers, excipients, and/or stabilizers to be inactive ingredients of a formulation or pharmaceutical composition.

[0368] Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride,

benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens, such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol; low molecular weight polypeptides (e.g., less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes such as Zn-protein complexes; and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG). (*Remington: The Science and Practice of Pharmacy, 22nd Edition*, 2012, Pharmaceutical Press, London.). In some embodiments, the formulation is in the form of an aqueous solution. In some embodiments, the formulation is lyophilized or in an alternative dried form.

[0369] The therapeutic formulation can be in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories. In solid compositions such as tablets the principal active ingredient is mixed with a pharmaceutical carrier. Conventional tableting ingredients include corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and diluents (e.g., water). These can be used to form a solid preformulation composition containing a homogeneous mixture of a compound of the present disclosure, or a non-toxic pharmaceutically acceptable salt thereof. The solid preformulation composition is then subdivided into unit dosage forms of a type described above. The tablets, pills, etc. of the formulation or composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner composition covered by an outer component. Furthermore, the two components can be separated by an enteric layer that serves to resist disintegration and permits the inner component to pass intact through the stomach or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials include a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

[0370] The binding agents of the present disclosure may be formulated in any suitable form for delivery to a target cell/tissue. In some embodiments, an anti-TfR1 single chain

antibody agent can be formulated as a liposome, microparticle, microcapsule, albumin microsphere, microemulsion, nano-particle, LNP, nanocapsule, or macroemulsion.

[0371] In some embodiments, a pharmaceutical formulation includes an anti-TfR1 single chain antibody agent of the present disclosure complexed with liposomes. Methods to produce liposomes are known to those of skill in the art. For example, some liposomes can be generated by reverse phase evaporation with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE).

[0372] In some embodiments, a pharmaceutical formulation includes an anti-TfR1 single chain antibody agent of the present disclosure complexed with LNPs.

[0373] In some embodiments, an anti-TfR1 antibody is formulated as a sustained-release preparation. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing an agent, where the matrices are in the form of shaped articles (e.g., films or microcapsules). Sustained-release matrices include but are not limited to polyesters, hydrogels such as poly(2-hydroxyethyl-methacrylate) or poly(vinyl alcohol), polylactides, copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(−)-3-hydroxybutyric acid.

[0374] The pharmaceutical compositions or formulations of the present disclosure can be administered in any number of ways for either local or systemic treatment. In some embodiments, administration is topical by epidermal or transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. In some embodiments, administration is pulmonary by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, and intranasal. In some embodiments, administration is oral. In some embodiments, administration is parenteral including intravenous, intraarterial, intratumoral, subcutaneous, intraperitoneal, intramuscular (e.g., injection or infusion), or intracranial (e.g., intrathecal or intraventricular). In some embodiments, administration is by intravenous injection or intravenous infusion. In some embodiments, administration is by intramuscular injection.

[0375] Various delivery systems are known and can be used to administer an anti-TfR1 single chain antibody agent described herein. In some embodiments, an anti-TfR1 antibody or a composition described herein is delivered in a controlled release or sustained release system. In some embodiments, a pump is used to achieve controlled or sustained release. In some embodiments, polymeric materials are used to achieve controlled or sustained release of the anti-TfR1 single chain antibody agent herein. Examples of polymers used in sustained release formulations include, but are not limited to, poly 2-hydroxy ethyl methacrylate, polymethyl methacrylate, polyacrylic acid, polyethylene-co-vinyl acetate, polymethacrylic acid, polyglycolides (PLG), polyanhydrides, poly N-vinyl pyrrolidone, polyvinyl alcohol (PVA), polyacrylamide, polyethylene glycol (PEG), polylactides (PLA), polylactide-co-glycolides (PLGA), and polyorthoesters. Any polymer used in a sustained release formulation should be inert, free of leachable impurities, stable on storage, sterile, and biodegradable.

[0376] Additional delivery systems can be used to administer an anti-TfR1 single chain antibody agent described herein including, but not limited to, injectable drug delivery devices and osmotic pumps. Injectable drug delivery devices include, for example, hand-held devices (e.g., autoinjectors) or wearable devices. Different types of osmotic pump systems may include single compartment systems, dual compartment systems, and multiple compartment systems.

Exemplary Embodiments

[0377] Exemplary embodiments as described below are also within the scope of the present disclosure:

[0378] Embodiment 1. A single chain antibody agent polypeptide that binds to a transferrin receptor 1 (TfR1) or a variant or a fragment thereof.

[0379] Embodiment 2. The single chain antibody agent polypeptide of embodiment 1, wherein the single chain antibody agent comprises one or more complementarity determining regions (CDRs).

[0380] Embodiment 3. The single chain antibody agent polypeptide of embodiment 2, wherein the single chain antibody agent comprises a CDR1, a CDR2 and a CDR3.

[0381] Embodiment 4. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent is or comprises a single domain antibody (e.g., a VHH).

[0382] Embodiment 5. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent polypeptide comprises a sequence provided in Table 1.

[0383] Embodiment 6. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent comprises: (i) a CDR1 sequence provided in Table 1; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR1 sequence provided in Table 1; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR1 sequence provided in Table 1; (ii) a CDR2 sequence provided in Table 1; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR2 sequence provided in Table 1; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR2 sequence provided in Table 1; and/or (iii) a CDR3 sequence provided in Table 1; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR3 sequence provided in Table 1; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR3 sequence provided in Table 1.

[0384] Embodiment 7. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at

least 98%, or at least 99% identity to SEQ ID NO: 3; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 3; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0385] Embodiment 8. The single chain antibody agent polypeptide of embodiment 7, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 3; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0386] Embodiment 9. The single chain antibody agent polypeptide of embodiment 7 or 8, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 1, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0387] Embodiment 10. The single chain antibody agent polypeptide of embodiment 7 or 8, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 158, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0388] Embodiment 11. The single chain antibody agent polypeptide of embodiment 7 or 8, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 159, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0389] Embodiment 12. The single chain antibody agent polypeptide of embodiment 7 or 8, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 160, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0390] Embodiment 13. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

[0391] Embodiment 14. The single chain antibody agent polypeptide of embodiment 13, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 8.

[0392] Embodiment 15. The single chain antibody agent polypeptide of embodiment 13 or 14, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 5, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0393] Embodiment 16. The single chain antibody agent polypeptide of embodiment 13 or 14, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 122, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0394] Embodiment 17. The single chain antibody agent polypeptide of embodiment 13 or 14, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 135, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0395] Embodiment 18. The single chain antibody agent polypeptide of embodiment 13 or 14, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 136, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0396] Embodiment 19. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 10; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 11; and/or (iii) a CDR3 sequence of SEQ ID NO: 12; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 12; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 12.

[0397] Embodiment 20. The single chain antibody agent polypeptide of embodiment 19, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 10; (ii) a CDR2 sequence of SEQ ID NO: 11; and (iii) a CDR3 sequence of SEQ ID NO: 12.

[0398] Embodiment 21. The single chain antibody agent polypeptide of embodiment 19 or 20, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 9, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0399] Embodiment 22. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 14; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 14; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or (iii) a CDR3 sequence of SEQ ID NO: 16; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 16; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 16.

[0400] Embodiment 23. The single chain antibody agent polypeptide of embodiment 22, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 14; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 16.

[0401] Embodiment 24. The single chain antibody agent polypeptide of embodiment 22 or 23, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 13, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0402] Embodiment 25. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 18; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 18; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 19; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 19; and/or (iii) a CDR3 sequence of SEQ ID NO: 20; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 20; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 20.

[0403] Embodiment 26. The single chain antibody agent polypeptide of embodiment 25, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 18; (ii) a CDR2 sequence of SEQ ID NO: 19; and (iii) a CDR3 sequence of SEQ ID NO: 20.

[0404] Embodiment 27. The single chain antibody agent polypeptide of embodiment 25 or 26, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 17, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0405] Embodiment 28. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 22; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 22; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at

least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 23; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 23; and/or (iii) a CDR3 sequence of SEQ ID NO: 24; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 24; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 24.

[0406] Embodiment 29. The single chain antibody agent polypeptide of embodiment 28, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 22; (ii) a CDR2 sequence of SEQ ID NO: 23; and (iii) a CDR3 sequence of SEQ ID NO: 24.

[0407] Embodiment 30. The single chain antibody agent polypeptide of embodiment 28 or 29, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 21, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0408] Embodiment 31. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 26; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 26; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 27; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 27; and/or (iii) a CDR3 sequence of SEQ ID NO: 28; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 28; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 28.

[0409] Embodiment 32. The single chain antibody agent polypeptide of embodiment 31, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 26; (ii) a CDR2 sequence of SEQ ID NO: 27; and (iii) a CDR3 sequence of SEQ ID NO: 28.

[0410] Embodiment 33. The single chain antibody agent polypeptide of embodiment 31 or 32, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 25, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0411] Embodiment 34. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 30; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 30; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 31; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 31; and/or (iii) a CDR3 sequence of SEQ ID NO: 32; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 32; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 32.

[0412] Embodiment 35. The single chain antibody agent polypeptide of embodiment 34, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 30; (ii) a CDR2 sequence of SEQ ID NO: 31; and (iii) a CDR3 sequence of SEQ ID NO: 32.

[0413] Embodiment 36. The single chain antibody agent polypeptide of embodiment 34 or 35, wherein the single chain antibody agent comprises the sequence of SEQ

ID NO: 29, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0414] Embodiment 37. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 34; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 34; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 35; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 35; and/or (iii) a CDR3 sequence of SEQ ID NO: 36; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 36; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 36.

[0415] Embodiment 38. The single chain antibody agent polypeptide of embodiment 37, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 34; (ii) a CDR2 sequence of SEQ ID NO: 35; and (iii) a CDR3 sequence of SEQ ID NO: 36.

[0416] Embodiment 39. The single chain antibody agent polypeptide of embodiment 37 or 38, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 33, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0417] Embodiment 40. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 38; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at

least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 38; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 39; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 39; and/or (iii) a CDR3 sequence of SEQ ID NO: 40; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 40; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 40.

[0418] Embodiment 41. The single chain antibody agent polypeptide of embodiment 40, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 38; (ii) a CDR2 sequence of SEQ ID NO: 39; and (iii) a CDR3 sequence of SEQ ID NO: 40.

[0419] Embodiment 42. The single chain antibody agent polypeptide of embodiment 40 or 41, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 37, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0420] Embodiment 43. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 42; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 42; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 42; (ii) a CDR2 sequence of SEQ ID NO: 43; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 43; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 43; and/or (iii) a CDR3 sequence of SEQ ID NO: 44;

or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 44; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 44.

[0421] Embodiment 44. The single chain antibody agent polypeptide of embodiment 43, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 42; (ii) a CDR2 sequence of SEQ ID NO: 43; and (iii) a CDR3 sequence of SEQ ID NO: 44.

[0422] Embodiment 45. The single chain antibody agent polypeptide of embodiment 43 or 44, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 41, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0423] Embodiment 46. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 46; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 46; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 47; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 47; and/or (iii) a CDR3 sequence of SEQ ID NO: 48; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 48; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 48.

[0424] Embodiment 47. The single chain antibody agent polypeptide of embodiment 46, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of

SEQ ID NO: 46; (ii) a CDR2 sequence of SEQ ID NO: 47; and (iii) a CDR3 sequence of SEQ ID NO: 48.

[0425] Embodiment 48. The single chain antibody agent polypeptide of embodiment 46 or 47, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 45, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0426] Embodiment 49. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 50; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 50; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 50; (ii) a CDR2 sequence of SEQ ID NO: 51; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 51; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 51; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0427] Embodiment 50. The single chain antibody agent polypeptide of embodiment 49, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 50; (ii) a CDR2 sequence of SEQ ID NO: 51; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0428] Embodiment 51. The single chain antibody agent polypeptide of embodiment 49 or 50, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 49, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least

89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0429] Embodiment 52. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 117; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 117; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0430] Embodiment 53. The single chain antibody agent polypeptide of embodiment 52, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 117; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0431] Embodiment 54. The single chain antibody agent polypeptide of embodiment 52 or 53, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 116, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0432] Embodiment 55. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or (iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

[0433] Embodiment 56. The single chain antibody agent polypeptide of embodiment 55, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 4.

[0434] Embodiment 57. The single chain antibody agent polypeptide of embodiment 55 or 56, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 118, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0435] Embodiment 58. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at

least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0436] Embodiment 59. The single chain antibody agent polypeptide of embodiment 58, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 15; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0437] Embodiment 60. The single chain antibody agent polypeptide of embodiment 59 or 59, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 120, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0438] Embodiment 61. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or (iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

[0439] Embodiment 62. The single chain antibody agent polypeptide of embodiment 61, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of

SEQ ID NO: 2; (ii) a CDR2 sequence of SEQ ID NO: 119; and (iii) a CDR3 sequence of SEQ ID NO: 52.

[0440] Embodiment 63. The single chain antibody agent polypeptide of embodiment 61 or 62, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 121, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0441] Embodiment 64. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 124; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 124; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or (iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

[0442] Embodiment 65. The single chain antibody agent polypeptide of embodiment 64, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 124; (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 8.

[0443] Embodiment 66. The single chain antibody agent polypeptide of embodiment 64 or 65, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 123, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least

89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0444] Embodiment 67. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 127; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 127; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 128; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 128; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 128.

[0445] Embodiment 68. The single chain antibody agent polypeptide of embodiment 67, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 127; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 128.

[0446] Embodiment 69. The single chain antibody agent polypeptide of embodiment 67 or 68, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 126, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0447] Embodiment 70. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 131; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 131; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 131.

[0448] Embodiment 71. The single chain antibody agent polypeptide of embodiment 70, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 131.

[0449] Embodiment 72. The single chain antibody agent polypeptide of embodiment 70 or 71, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 129, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0450] Embodiment 73. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 133; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 133; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 133; and/or (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

[0451] Embodiment 74. The single chain antibody agent polypeptide of embodiment 73, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 133; and (iii) a CDR3 sequence of SEQ ID NO: 134.

[0452] Embodiment 75. The single chain antibody agent polypeptide of embodiment 73 or 74, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 132, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0453] Embodiment 76. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or (iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO:134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

[0454] Embodiment 77. The single chain antibody agent polypeptide of embodiment 76, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of

SEQ ID NO: 6; (ii) a CDR2 sequence of SEQ ID NO: 125; and (iii) a CDR3 sequence of SEQ ID NO: 134.

[0455] Embodiment 78. The single chain antibody agent polypeptide of embodiment 76 or 77, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 137, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0456] Embodiment 79. The single chain antibody agent polypeptide of any one of embodiments 1-6, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or (iii) a CDR3 sequence of SEQ ID NO: 139; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 139; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 139.

[0457] Embodiment 80. The single chain antibody agent polypeptide of embodiment 79, wherein the single chain antibody agent comprises: (i) a CDR1 sequence of SEQ ID NO: 130; (ii) a CDR2 sequence of SEQ ID NO: 7; and (iii) a CDR3 sequence of SEQ ID NO: 139.

[0458] Embodiment 81. The single chain antibody agent polypeptide of embodiment 79 or 80, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 138, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least

89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

[0459] Embodiment 82. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent binds to a human TfR1 (hTfR1), or a cyno TfR.

[0460] Embodiment 83. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent binds to hTfR1 with an affinity (KD) of about 10nM to about 1000nM when fused to an Fc domain.

[0461] Embodiment 84. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent binds to an epitope on an ectodomain of hTfR1.

[0462] Embodiment 85. The single chain antibody agent polypeptide of embodiment 84, wherein the single chain antibody agent binds to hTfR1 at a site other than a transferrin binding site.

[0463] Embodiment 86. The single chain antibody agent polypeptide of embodiment 84 or 85, wherein the single chain antibody agent binds to hTfR1 in the presence of transferrin.

[0464] Embodiment 87. The single chain antibody agent polypeptide of embodiment 84 or 85, wherein the single chain antibody agent binds to hTfR1 in the absence of transferrin.

[0465] Embodiment 88. The single chain antibody agent polypeptide of any one of the preceding embodiments, characterized in that when administered to a cell, tissue or subject, the single chain antibody agent polypeptide does not compete with transferrin for binding to hTfR1.

[0466] Embodiment 89. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent polypeptide further comprises at least one heavy chain constant region (CH).

[0467] Embodiment 90. The single chain antibody agent polypeptide of embodiment 89, wherein the single chain antibody agent comprises a CH2 domain and a CH3 domain, e.g., an Fc domain.

[0468] Embodiment 91. The single chain antibody agent polypeptide of embodiment 90, wherein the Fc domain is an Fc domain of an immunoglobulin (Ig) isotype.

[0469] Embodiment 92. The single chain antibody agent polypeptide of embodiment 91, wherein the Ig isotype is IgA, IgD, IgG, IgM, or IgE.

[0470] Embodiment 93. The single chain antibody agent polypeptide of embodiment 92, wherein the Ig isotype is IgG, e.g., a human IgG.

[0471] Embodiment 94. The single chain antibody agent polypeptide of embodiment 93, wherein IgG is or comprises IgG1, lgG2, lgG3, or lgG4.

[0472] Embodiment 95. The single chain antibody agent polypeptide of any one of embodiments 90-94, wherein the Fc domain comprises a modification.

[0473] Embodiment 96. The single chain antibody agent polypeptide of embodiment 95, wherein the modification modulates one or more properties of the antibody agent.

[0474] Embodiment 97. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent polypeptide further comprises a half-life extender.

[0475] Embodiment 98. The single chain antibody agent polypeptide of embodiment 97, wherein the half-life extender is or comprises albumin, e.g., human serum albumin.

[0476] Embodiment 99. The single chain antibody agent polypeptide of embodiment 97, wherein the half-life extender comprises a modification that increases binding to neonatal Fc receptor (FcRn).

[0477] Embodiment 100. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent polypeptide is fused to one or more polypeptides.

[0478] Embodiment 101. The single chain antibody agent polypeptide of embodiment 100, wherein the one or more polypeptides is an antibody agent or a fragment thereof.

[0479] Embodiment 102. The single chain antibody agent polypeptide of embodiment 100 or 101, wherein the antibody agent binds to an antigen other than hTfR1.

[0480] Embodiment 103. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent polypeptide is conjugated to a polynucleotide.

[0481] Embodiment 104. The single chain antibody agent polypeptide of embodiment 103, wherein the polynucleotide is an antisense oligo (ASO), an inhibitory RNA (e.g., siRNA), a microRNA, a locked nucleic acid (LNA), a peptide nucleic acid (PNA), or an aptamer.

[0482] Embodiment 105. The single chain antibody agent polypeptide of any one of the preceding embodiments, wherein the single chain antibody agent polypeptide is conjugated to a small molecule, e.g., ADC.

[0483] Embodiment 106. A polynucleotide encoding the single chain antibody agent polypeptide of any one of embodiments 1-105.

[0484] Embodiment 107. A vector comprising a polynucleotide of embodiment 106.

[0485] Embodiment 108. A cell comprising the single chain antibody agent polypeptide of any one of embodiments 1-105.

[0486] Embodiment 109. The cell of embodiment 108, wherein the cell is contacted with a vector encoding the single chain antibody agent polypeptide.

[0487] Embodiment 110. The cell of embodiment 108, wherein the cell is contacted with the single chain antibody agent polypeptide.

[0488] Embodiment 111. The cell of embodiment 109 or 110, wherein the cell is an in vivo cell.

[0489] Embodiment 112. The cell of embodiment 109 or 110, wherein the cell is an ex vivo cell.

[0490] Embodiment 113. The cell of any one of embodiments 108-111, wherein the cell expresses hTfR1.

[0491] Embodiment 114. A composition comprising the single chain antibody agent polypeptide of any one of embodiments 1-105.

[0492] Embodiment 115. A composition comprising the polynucleotide of embodiment 106.

[0493] Embodiment 116. The composition of embodiment 114 or 115, wherein the single chain antibody agent polypeptide or the polynucleotide encoding the single chain antibody agent polypeptide is encapsulated in or conjugated to a delivery vehicle.

[0494] Embodiment 117. The composition of embodiment 116, wherein the delivery vehicle is a liposome, or an LNP.

[0495] Embodiment 118. The composition of embodiment 116 or 117, wherein the composition is a pharmaceutical composition.

[0496] Embodiment 119. The composition of embodiment 118, wherein the pharmaceutical composition comprises one or more excipients.

[0497] Embodiment 120. The composition of embodiment 118 or 119, wherein the pharmaceutical composition is formulated for intramuscular, intradermal, or intravenous delivery.

[0498] Embodiment 121. A method of delivering the pharmaceutical composition of any one of embodiments 118-120, to a cell, tissue or subject.

[0499] Embodiment 122. The method of embodiment 121, wherein delivering comprises administering the pharmaceutical composition to the cell, tissue or subject.

[0500] Embodiment 123. The method of embodiment 121 or 122, wherein the method is a treatment method.

[0501] Embodiment 124. The method of embodiment 123, wherein the subject is a human.

[0502] Embodiment 125. The method of any one of embodiments 122-124, wherein administering the pharmaceutical composition alleviates one or more symptoms of a disease or condition and/or treats a disease or condition.

EXAMPLES

[0503] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art can develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

Example 1: Identification of anti-TfR1 VHHs

[0504] This Example describes the identification and characterization of VHHs that bind to a transferrin receptor 1(TfR1).

[0505] *Generation of anti-TfR1 VHHs*: For VHH discovery, DNA, protein and cell line antigens were generated as follows: DNA comprised of plasmid vectors encoding full length human, murine or cyno TfR1 driven by a mammalian promoter. Protein antigens comprised of His-tagged TfR1 ectodomains with human, cyno, mouse or alpaca sequences. Cell line antigens comprised of alpaca skin stromal cells (ASSCs) engineered to stably express full-length human, cyno or mouse TfR1.

[0506] To generate single-domain antibodies against the TfR1 ectodomain (ECD), Alpacas were immunized (Capralogics, Inc.) using two immunization schemes. In scheme 1, two alpacas were immunized with 2 mg of murine TfR1 DNA via Dermojet intradermal injection on day 0. Four additional boosts on day 14, 28, 42 and 56 were performed with 2 mg of alternating murine, human or cyno TfR1 DNA in an identical manner. Three weeks following the DNA boosts, 50 million ASSC cells over-expressing human or mouse TfR1 were injected on days 77 and 91 via subcutaneous delivery with incomplete Freund's adjuvant administered in tandem. Following the DNA or ASSC cellular boosts, 300 mL whole blood collections were taken on days 63 and 98. In scheme 2, one alpaca was immunized with 1 mg of KLH conjugated His-tagged TfR1 ectodomain containing human, cyno, mouse or alpaca sequences on day 0, and again on day 7 via subcutaneous injection. Three additional injections were performed on days 17, 31 and 45 with 0.4 mg of protein. Two final injections consisting of 1 mg of protein were performed on days 45 and 52. All immunizations in scheme 2 were pre-mixed with GERBU

adjuvant prior to administration. A 300 mL whole blood collection was taken following the final boost on days 56 and 62.

[0507] Circulating PBMCs were isolated from the whole blood collections from both immunization schemes by diluting with PBS at a 1:1 ratio and proceeding with a density gradient centrifugation method using Ficoll-Paque Plus (GE Healthcare, 17-1440-02). Isolated PBMCs were frozen at -80 in RNAlater (ThermoFisher, AM7021). The VHH libraries were generated from the PBMCs using methods described previously (Pardon, *Nature Protocols*, 2019, vol.9 issue 3). Total RNA was purified from PBMCs (Qiagen, 75142) and cDNA was generated via RT-PCR (ThermoFisher, 18091050). VHH repertoires were PCR amplified from total cDNA using a forward primer specific to the VHH leader sequence 5'-CTGGGTGGTCCTGGCTGC-3' (SEQ ID NO: 56) and reverse primers specific to the IgG2/3 hinge regions 5'-GGAGCTGGGGTCTTCGCTGTGGTGCG-3', 5'-TGGTTGTGGTTTTGGTGTCTTGGGTT-3' (SEQ ID NO: 57) (Maass et al., 2007). A second round of PCR was performed with primers specific to the VHH FW1 V-gene and FW4 J-gene regions found in the IMGT database, and the resulting amplicons were transformed into yeast via homologous recombination using published methods (Rappazzo, Science, 2021).

[0508] Yeast displayed VHH libraries were screened in accordance with the methods disclosed in US Patent Publications US20100056386A1 and US20090181855A1 as fusions to human IgG1 Fc. Iterative rounds of selection were performed against recombinant HIS tagged human and cyno TfR1, as well as human TfR1 pre-blocked with recombinant human holo-transferrin (Sigma; T0665). Colonies were subsequently sequenced to identify unique clones, using techniques known in the art. Following this campaign, antibodies were expressed from yeast and purified on protein A resin. Yeast-expressed antibodies were tested for binding to human TfR1 ECD recombinant protein using Bio-Layer Interferometry (BLI). Antibody binding was similarly performed against human TfR1 after pre-blocking of human TfR1 ECD with recombinant human holo-transferrin. BLI was performed on an Octet RED384 and Octet HTX instruments (ForteBio) according to standard procedures. Yeast-expressed antibodies were also tested for binding to CHO cells over-expressing full-length human, cyno and mouse TfR1. Cells were incubated with antibodies at a single 200 nM concentrations for 2 hours on ice, washed twice with isotonic buffer, then incubated with a fluorescently labeled anti-Fc secondary

antibody (Jackson; 109-546-008), fixed in 1% PFA and analyzed on a flow cytometer. Sequences of the resultant antibodies are provided in **Table 1** herein.

[0509] *Analysis of TfR1 binding:* To characterize antibody affinity against various species of TfR1, antibodies were generated recombinantly and tested for TfR1 binding via flow cytometry. Exemplary antibodies were generated as monovalent VHH-Fc fusions to human TfR1, with a VHH fused on the N-terminus of Fc or a VHH fused on the C-terminus of Fc. Exemplary antibody VHH reagents were generated in monovalent format fused to human IgG1(N297Q) Fc with knob mutation and paired with IgG1(N297Q) Fc with hole mutations (**FIG. 1D**). An N-terminal signal sequence preceded the antibody gene in all expression constructs to facilitate secretion extracellularly. Constructs were transiently transfected into Chinese Hamster Ovary-S (CHO-S) cells for expression and antibodies were purified from culture supernatant once cells were harvested. Proteins were purified by loading supernatant on HiTrap MabSelect SuRe columns (Cytiva), eluting with 25 mM sodium phosphate, 100 mM sodium chloride, pH 2.8, then neutralizing 1:60 (v:v) with 0.5 M sodium phosphate, pH 8.6. Presence of mis-paired homodimers was assessed by SDS-PAGE, and if >10% of total protein was mis-paired, antibodies were purified by MonoRab Anti-Camelid VHH affinity resin (GenScript), eluting with a stepwise pH gradient of 0.1 M glycine at pH 4.5, 4.0, and 3.5, then neutralizing with 1 M Tris-HCl, pH 8.0. Aggregate content was assessed, and if >5% of total protein, antibodies were further purified by size exclusion chromatography on a Superdex 75 column (Cytiva) in PBS.

[0510] Stable TfR1 expressing cell lines were generated where endogenous hamster TfR1 was knocked out of Chinese Hamster Ovary-S (CHO-S) cells by CRISPR-Cas9 manipulation and the engineered cells were transduced with plasmids encoding full length human, cyno, or mouse TfR1. Cell surface expression of TfR1 in all cell lines was verified by staining cells either with Alexa647-labeled human transferrin or appropriate antibodies against TfR1 followed by analysis by flow cytometry. Monovalent VHH-Fc fusions were incubated with stable cell lines at the indicated concentrations for 1-2 hours on ice, washed 3 times with isotonic buffer, incubated with PE-conjugated anti-hFc for 1 hour on ice, fixed with 1% paraformaldehyde for 15 min at RT, then analyzed by flow cytometry for detection of antibody signal by PE. Mean fluorescence intensity (MFI) of the PE fluorophore was calculated at each

concentration and EC50 values were determined by a 3-parameter logarithmic fit in the GraphPad Prism software. The binding profiles for VHH Clones A-E to human TfR1, cyno TfR1 or mouse TfR1 is shown in **FIGS. 1A-1C**.

[0511] For the set of VHH antibodies (**Table 2**), a range of cell surface affinities was measured for human TfR1 (EC50 ~ 6-180 nM) with cross-reactivity to cynomolgus and mouse TfR1 (EC50 ratio between species ~0.2-6).

Table 2: Cell surface affinities

	Flow Cytometry				
	huTfR1-CHO binding	**cyTfR1-CHO binding**	**muTfR1-CHO binding**	**Species cross-reactivity**	
VHH clone	**EC50 (nM)**	**EC50 (nM)**	**EC50 (nM)**	**Ratio cy/hu**	**Ratio mu/hu**
Clone A	27	97	150	3.6	5.6
Clone B	180	32	470	0.2	2.6
Clone C	6.6	9.2	8.7	1.4	1.3
Clone D	55	14	16	0.3	0.3
Clone E	14	13	20	0.9	1.4

[0512] This data demonstrates that all of the VHH clones bound to human TfR1, cyno TfR1 and mouse TfR1.

[0513] *Transferrin binding*: To further characterize antibody affinity against human and cyno TfR1, recombinant TfR1 ECD proteins were generated and tested for VHH-Fc binding via surface plasmon resonance (SPR). Recombinant human TfR1 ectodomain (hTfR1 ECD) (aa89-760) and cynomolgus macaque TfR1 ectodomain (cyTfR1 ECD) (aa89-760) with N-terminal 8xHis-Gly tags, were transiently expressed in CHO-S. TfR1 protein was purified using nickel Sepharose excel resin (Cytiva) eluting in 50 mM Tris, 400 mM NaCl, 10 mM MgCl2, 500 mM imidazole, pH 7.8, followed by size exclusion chromatography on a superdex 200 26/600 column equilibrated in PBS + 0.2 M NaCl. Eluate was aliquoted and stored at -80C with the addition of 10% glycerol to prevent protein aggregation upon freeze-thaw.

[0514] SPR binding analysis was performed on a Biacore 8K(+) instrument at room temperature in 10mM HEPES, 150mM NaCl, 3mM EDTA, 0.05%BSA, 0.005% P20, pH 7.4, using a CM5 chip with an anti-His capture kit (Cytiva). His-tagged human, cynomolgus monkey, or murine TfR1 ectodomain was captured by anti-His capture at 40-80pg/mm^2 on the sensor surface, then monovalent VHH-Fc fusion reagents were injected at concentrations ranging from 0.78 to 4000 nM. Binding sensorgrams were measured and analyzed with a 1:1 binding model using the Biacore Insight Evaluation software to produce monovalent affinity and kinetics parameters (equilibrium dissociation constant K_D, association rate constant k_a, and dissociation rate constant k_d). In cases where the 1:1 binding model did not fit the sensorgram data, a steady state analysis was performed to determine K_D.

[0515] All VHH clones bound to both human TfR1 and cyno TfR1. The binding kinetics and affinity parameters are provided in **Table 3** and exemplary binding curves are shown in **FIG. 2.**

Table 3: SPR binding analysis

	SPR (monovalent binding)						
	Human TfR1 ECD binding			Cyno TfR1 ECD Domain binding			Species cross-reactivity
Description	k_a ($M^{-1}s^{-1}$)	k_d (/s)	K_D (nM)	k_a ($M^{-1}s^{-1}$)	k_d (/s)	K_D (nM)	Ratio cy/hu
Clone A	3.5E+04	4.6E-03	133	3.4E+04	2.1E-02	616	4.6
Clone B			~1000			66	~0.07
Clone C	5.2E+04	3.4E-03	66	6.4E+04	5.9E-04	9.3	0.14
Clone D	3.2E+04	1.1E-02	330	1.3E+05	3.3E-03	25	0.07
Clone E	6.3E+04	1.0E-03	16	1.2E+05	4.9E-04	4.1	0.3

[0516] Monovalent affinity of the VHH antibodies for recombinant human TfR1 ECD ranged from K_D = 16 to ~1000 nM. Cross-reactivity between human and cynomolgus TfR1 protein was characterized by a K_D ratio that varied between 0.07 and 4.6 for different VHH antibodies.

[0517] *Transferrin blocking and displacement:* Competitive binding of the VHH antibodies with transferrin was tested by both flow cytometry and SPR. Displacement of cell

surface transferrin by VHH antibodies was characterized by incubating huTfR CHO with sub-saturating levels of AlexaFluor647-labeled human transferrin (3nM, Thermo T23366) together with VHH antibodies (at ~0.03-500 nM) or unlabeled holo human transferrin (500 nM). No transferrin displacement was observed for any of the VHH antibodies tested, as shown in **FIG. 3.** A quantification of transferrin binding is provided in **Table 4**.

[0518] Binding of VHH antibodies to human TfR1 ECD by SPR was compared to binding of antibody with holo human transferrin pre- saturated at 1000nM (T0665, Sigma). A slight weakening or strengthening of TfR1 binding affinity was observed in the presence of transferrin for some VHH antibodies, but the differences in K_D with and without transferrin were less than 2-fold (**FIG. 4;** and **Table 4**).

Table 4: Transferring blocking and displacement

	AlexaFluor647-Tf displacement	**hTfR1 ECD Affinity, K_D**	
Description	**% Tf bound (+500nM Ab)**	**Antibody alone**	**Antibody + 1uM Tf**
Clone A	92	85	114
Clone B	109	n.d.	n.d.
Clone C	96	67	53
Clone D	105	336	203
Clone E	104	14	17

[0519] Overall, the data provided herein demonstrates no direct competition between these VHH antibodies and transferrin for binding the transferrin receptor (TfR1).

[0520] *Transcytosis analysis.* To assess the cellular transport function of VHH antibodies, human or mouse TfR1 was expressed in Madin-Darby canine kidney II (MDCK II; ECACC 00062107), stably transduced with VSV-G pseudotyped lentiviral particles encoding a human TfR1 gene or mouse TfR1 gene under a human EF1a promoter with an Ires-Puromycin resistance cassette. Cells were selected for puromycin resistance to produce a MDCKII/huTfR or MDCKII/muTfR cell lines.

[0521] MDCKII/TfR cells were plated onto Corning 0.4 micron pore transwell inserts with 25,000 cells per insert in complete medium, 0.25ml in the insert and 1 ml in the bottom

chamber, and kept in an incubator at 37degC and 5% CO2. On day 4 the bottom media was fully replaced and half of the insert media was replaced with fresh media. On day 5, test article was added to the top well at 100 nM. On day 7, samples were collected from top and bottom chambers and analyzed for concentration of the test article. Quantitation of test article was performed using commonly available anti-human IgG reagents in a mesoscale discovery assay system, with results extrapolated from a titrated standard curve of the same test article.

[0522] Anti-TfR1 VHH antibodies showed varying levels of cellular transport in MDCKII/huTfR or MDCKII/muTfR cell lines, up to ~9x (Clone A) over a negative control antibody (**Table 5; FIG. 5**). Several VHH antibodies showed elevated transport in both human and mouse TfR cells, and one (Clone D) had >5-fold transport over control in both model systems.

Table 5: MDCKII Transcytosis

	MDCKII Transcytosis			
	MDCKII-huTfR		**MDCKII-muTfR**	
Description	**Accum. Mol. (fmol.)**	**Fold Over Control**	**Accum. Mol. (fmol.)**	**Fold Over Control**
Clone A	110	9	26	3
Clone B	11	1	14	1
Clone D	77	6	59	6
Clone E	65	5	26	3

[0523] *In vivo biodistribution.* To investigate the in vivo biodistribution of anti-TfR VHH antibodies, mice were administered a 20 mg/kg molar IgG equivalent IV dose of monovalent VHH-hFc antibodies. Four hours later, mice were anesthetized with ketamine/xylazine (100/10 mg/kg i.p.), blood samples were collected via cardiac puncture, and mice were perfused, through the left ventricle, with ice-cold PBS/Heparin (1ug/mL) at 2ml/min for 10 min to clear the vasculature of blood. Brains were then removed, and hemispheres flash frozen in liquid nitrogen. Serum was generated by allowing blood to clot for 15-30 minutes at room temperature, centrifugation at 2000g for 10min, freezing supernatants for further analysis.

[0524] Frozen brain hemispheres were homogenized in lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.25 % Na deoxycholate, 1 mM EDTA, 1 % NP40, complete Protease inhibitors) with zirconium oxide beads (ZROB05 and ZROB10) in a tissue homogenizer (NextAdvance Bullet Blender) for 10 min, then incubated at 4degC, rotating for 1 hour. Lysates were then cleared of debris by centrifugation at 12,000g for 20min.

[0525] VHH-Fc levels were analyzed in serum and brain lysate by MSD immunoassay. Samples were incubated on MSD plates (MSD, Cat# L15XB-3/L11XB-3) coated with anti-hIgG Fc capture reagent (Jackson ImmunoResearch, Cat# 709-006-098), detected with sulfo-tagged anti-hIgG (MSD, Cat# R32AJ-1), and quantified by interpolation on a standard curve generated for each test antibody.

[0526] Different anti-TfR VHH antibodies were detected at different levels in serum and brain of mice following IV administration (**Table 6; FIGS. 6A-6B**). The different VHHs distributed out of serum and into brain more than a non-binding negative control antibody. One VHH antibody (Clone A) had notably higher levels in serum and brain lysate relative to the other anti-TfR VHH antibodies. In some embodiments, this data suggests that VHH Clone A can be cleared from circulation more slowly and/or is also able to penetrate into brain.

Table 6: In vivo biodistribution

		In Vivo Biodistribution; 4hr		
		Serum	Brain Lysate	
ProtID	Description	hIgG conc. (nM)	hIgG conc. (nM)	Fold Over Control
9449	Clone A	2923	13.4	4.2
9458	Clone C	1810	5.0	1.6
9451	Clone D	1321	8.0	2.5
9448	Clone E	1375	5.6	1.7

[0527] *VHH structure in complex with TfR1.* To further characterize the binding of the VHHs against TfR1 the structure of the VHH bound to the TfR1/transferrin complex was determined using two sequence divergent VHHs: Clone A and Clone B.

[0528] Human TfR1-Tf binary complex formation: To form and enrich the human TfR1 ectodomain / holo-transferrin binary complex (hTfR1-Tf), 1 mg holo-transferrin (Sigma; T0665) was incubated with 0.5 mg human TfR1 ECD before size selection using a Superdex 200 column with 10 mM HEPES (pH 7.5) + 100mM NaCl as running buffer. Peaks corresponding to the binary complex were isolated and flash frozen. Presence of both human TfR1 ECD and holo-transferrin in the complex was verified using SDS-PAGE.

[0529] Grid preparation and data collection for cryo-electron microscopy studies: TfR1-Tf-VHH complex was prepared by adding 15 uLs of VHH monovalent Fc (2 mg/mL) to 60 uLs of hTfR1-Tf binary complex (0.4 mg/mL) immediately before electron microscopy grid preparation. 3.5 uLs of this complex was applied to glow discharged Quantifoil R1.2/1.3 grids, blotted for 5 seconds, then plunge frozen into liquid ethane using a Vitrobot Mark IV. Data collection was performed on a Glacios cryo-transmission electron microscope (Cryo-TEM) operating at 200 kV equipped with a K3 direct electron detector. ~6000 movies were collected at 36,000x magnification with a total electron dose of 43 e-/A$^{\circ 2}$ over 50 frames.

[0530] CryoEM data processing: Raw movies were motion corrected in RELION using MotionCor2 and CTF values were estimated using GCTF. Using an initial subset of 500 random micrographs, Topaz picking was performed using the general model. Particles were classified and good picks were used to train a Topaz model. The train model was then used to pick particles over the entire dataset. Pick particles were extracted and imported into cryoSPARC for 2D classification. A random subset of 100k particles was used in ab-initio to generate initial 3D references and then all particles were classified using heterogenous refinement. The best class was refined using homogeneous refinement and then particles were imported into RELION. Particles underwent iterative CTF refinements followed by Bayesian polishing. Particles were sorted using fixed angle 3D classification and best class was further refined to obtain best cryoEM density map at 2.4Å resolution.

[0531] Atomic model building and refinement: The crystal structure of hTfR1-Tf (PDB: 3S9N) and an AlphaFold model of VHH was docked into the cryoEM in Chimera then rigid body docking was performed in Phenix. Model was rebuilt manually using Coot followed by real space refinement in Phenix.

[0532] For Clone A, a cryo-EM structure was generated on the ternary complex of Clone A VHH, human TfR1 ECD, and holo human transferrin to a resolution of 2.4 Å. An atomic model of the complex was obtained by fitting a homology model of Clone A and available TfR1-Tf crystal structures into the cryo-EM density map followed by manual rebuilding (**FIGS. 7A-7B**). The model revealed that Clone A binds to an epitope spanning both peptide chains of the TfR1 homodimer in the helical and apical domains. There are 20 TfR1 residues and 23 Clone A residues that are within 4 Å of the binding partner. The predicted discontinuous epitope is formed by residues K189, Y309, F321, P322, P323, S324, R325, L329, and L381 from the apical domain and E634, M635, G636, R719, N722, N723, G724, A725, F726, N727, E728, T729, and R732 from the helical domain of human TfR1. The antigen-binding site of Clone A is formed by all three CDRs and is composed of P29, P30, S32, V33, Y35, W50, D53, A54, G55, N56, T57, D61, D98, Q99, S100, and V101 plus additional framework contacts at Y37, E44, R45, E46, F47, K96, and W102.

[0533] For Clone B, a cryo-EM structure was generated on the ternary complex of Clone B VHH, human TfR1 ECD, and holo human transferrin (Sigma T0665) to a resolution of 2.7 Å. An atomic model of the complex was obtained by rigid body fitting an IgFold prediction of Clone B and available TfR1-Tf crystal structures into the cryo-EM density map (**FIGS. 8A-8B**). The model revealed that Clone B binds to a similar epitope to that of Clone A, spanning both peptide chains of the TfR1 homodimer in the helical and apical domains. 14 TfR1 residues and 15 Clone B residues are within 4 Å of the binding partner. The discontinuous epitope is formed by residues K189, P323, S324, R325, L381, and E383 from the apical domain, and E634, M635, R719, N723, N727, E728, T729, and R732 from the helical domain of human TfR1. The antigen-binding site of Clone B is formed by all three CDRs and is composed of R27, P28, F29, S30, N31, R53, T54, G55, G56, F102, I104, and T105, plus additional framework contacts at D74, A75, and K76.

[0534] Since Clone A recognizes human and cynomolgus macaque homologs of TfR1, cyno and human sequence conversation in TfR1 epitope was examined (**FIG. 9**). The TfR1 epitope spanning both the apical and helical domains is highly conserved between human and cyno homologs, with only two amino acid residue substitutions at R325Q and G724S (hu>cyno TfR1), consistent with the observed cyno-human cross-reactivity of Clone A.

[0535] Based on the cryo-EM structure of Clone A-hTfR1, a cohort of Clone A mutants were designed with amino acid residue substitutions in Clone A paratope to modulate TfR1 affinity for optimal CNS-targeting, and to potentially minimize affinity gap to cyno and human TfR1. Three sites in Clone A were identified, i.e. D53, Q99, and V101, as key residues interacting with cyno-human divergent epitope regions, and introduced amino acid substitutions, D53S, Q99G, and V101S/A/P, that are predicted to strengthen hydrogen bonds and Van der Waals forces between VHH and cyno TfR1 (**Table 1**). Amino acid frequencies were referenced from available antibody variable regions in the NCBI Protein database and from observed sequence diversity from related antibodies in this discovery campaign to enhance the chance that the substituting amino acids are tolerated in CDRs.

[0536] *Clone A affinity modulation.* To characterize the effect of VHH Clone A mutagenesis on affinity for various species of TfR1, antibodies were generated recombinantly as monovalent VHH-Fc fusions, purified, and tested for TfR1 binding via flow cytometry and SPR, using methods described above.

[0537] Clone A variants showed a range of binding affinities to human TfR1 by both cell-based and protein-based binding assessment (**Tables 7** and **9; FIGS. 10A-10C** and **FIGS. 11A-11C**). The mutations introduced impacted human TfR1 binding over the range of EC_{50} = 8.8 – 93 nM compared to 30 nM for parental Clone A (N-terminus fusion), and EC_{50} = 103 – 1300 nM compared to 28 nM for parental Clone A mono-USD (C-terminus fusion) by flow cytometry, and K_D = 17 – 1030 nM and K_D = 470 – 11000 nM by SPR, respectively. Species cross-reactivity was improved for most mutants relative to the parent VHH. Specifically, while parent Clone A has ~5-fold weaker affinity to cynomolgus and mouse compared to human TfR1, the mutations A54G (Clone G), V101S (Clone H), or Q99G (Clone M) all bring the affinities to cynomolgus and mouse TfR1 within 3-fold of the affinity to human TfR1 by both flow cytometry and SPR (EC_{50} or K_D ratio = 0.3-3.0). Additionally, while parent Clone A mono-USD has ~13-fold weaker affinity to cynomolgus and mouse compared to human TfR1, the mutations D53S/A54G (Clone Q mono-USD), D53A (Clone R mono-USD), D53S/Q99G (Clone S mono-USD), or D53A/Q99G (Clone T mono-USD) all resulted in the affinities to cynomolgus and mouse TfR1 to be within 6-fold of the affinity to human TfR1 by flow cytometry, and within the same range of the affinity to human TfR1 by SPR (K_D ratio = 0.3-0.4). Without wishing to be bound by

theory, Clone A variants and Clone A mono-USD variants showed the ability to modulate affinity and lessen the gap in affinity between various species of TfR1.

Table 7: Binding affinities of VHH Clone A variants

		Flow Cytometry					SPR (monovalent binding)						
		huTfR-CHO binding	cyTfR-CHO binding	muTfR-CHO binding	Species cross-reactivity		Human TfR ECD binding			Cyno TfR ECD Domain binding			Species cross-reactivity
VHH Clone	Mutation	EC_{50} (nM)	EC_{50} (nM)	EC_{50} (nM)	Ratio cy/hu	Ratio mu/hu	k_a ($M^{-1}s^{-1}$)	k_d (/s)	K_D (nM)	k_a ($M^{-1}s^{-1}$)	k_d (/s)	K_D (nM)	Ratio cy/hu
Clone A	parent	30	124	154	4.1	5.1	3.1E+04	3.5E-03	110	3.4E+04	1.8E-02	536	4.9
Clone F	D53S	93	48	280	0.5	3.0	1.8E+04	1.8E-02	1030	7.2E+04	1.5E-02	210	0.2
Clone G	A54G	46	29	115	0.6	2.5	3.8E+04	1.2E-02	330	4.8E+04	5.7E-03	120	0.4
Clone H	V101S	34	89	89	2.6	2.6	3.3E+04	5.4E-03	130	2.8E+04	1.0E-02	370	2.8
Clone I	V101A	22	43	69	2.0	3.1	4.0E+04	3.7E-03	91	3.5E+04	7.9E-03	230	2.5
Clone J	V101P	21	41	77	1.9	3.7	4.1E+04	3.6E-03	87	3.2E+04	7.8E-03	250	2.9
Clone K	D53S/V101A	81	21	85	0.3	1.1	2.1E+04	1.7E-02	830	8.1E+04	5.8E-03	72	0.1
Clone L	Q99S	8.8	30	88	3.4	10	6.4E+04	1.1E-03	17	4.7E+04	1.1E-02	240	14
Clone M	Q99G	60	180	166	3.0	2.7	4.2E+04	1.4E-02	330	4.7E+04	3.7E-02	790	2.4

Table 9: Binding affinities of VHH Clone A mono-USD variants

		Flow Cytometry					SPR (monovalent binding)						
		huTfR-CHO binding	cyTfR-CHO binding	muTfR-CHO binding	Species cross-reactivity		Human TfR ECD binding			Cyno TfR ECD Domain binding			Species cross-reactivity
VHH Clone	Mutation	EC_{50} (nM)	EC_{50} (nM)	EC_{50} (nM)	Ratio cy/hu	Ratio mu/hu	k_a ($M^{-1}s^{-1}$)	k_d (/s)	K_D (nM)	k_a ($M^{-1}s^{-1}$)	k_d (/s)	K_D (nM)	Ratio cy/hu
Clone A mono-USD	Parent mono-USD	28	340	370	12.0	13.1	1.20E+04	5.50E-03	470	1.40E+04	2.90E-02	2030	4.3
Clone Q mono-USD	D53S/A54G	103	183	590	1.8	5.8	8.00E+03	2.20E-02	2800	1.90E+04	1.90E-02	980	0.4
Clone R mono-USD	D53A	230	390	1300	1.7	5.7	6.60E+03	3.60E-02	5500	1.70E+04	2.30E-02	1330	0.2
Clone S mono-USD	D53S/Q99G	330	320	1200	1.0	3.6	8.10E+03	7.10E-02	8700	2.20E+04	5.00E-02	2300	0.3
Clone T mono-USD	D53A/Q99G	1300	800	~1300	0.6	1.0	steady state		11000	2.20E+04	6.20E-02	2800	0.3

[0538] Thus, mutagenesis aimed at improving epitope homology between species resulted in well matched affinities across mouse, cynomolgus, and human TfR1. The data provided herein supports the utility of exemplary anti-TfR1 VHH antibodies disclosed herein for delivering payloads to the brain.

[0539] Accordingly, the present Example demonstrates the identification and characterization of anti-TfR1 VHHs. The VHHs described herein can be conjugated to one or more payloads and used for delivering payloads to hTfR1 expressing cells and/or tissue such as muscle cells and/or tissue. Additionally, VHHs described herein can be fused to one or more other antibody agents to form multispecific molecules for targeting hTfR1 expressing cells and/or tissue. Taken together, the data provided herein supports the development of novel anti-hTfR1 VHHs for targeting hTfR1 expressing cells and/or tissue.

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Example 2: Exemplary VHH Clone B variants show enhanced stability

[0542] The present Example demonstrates that thermal stability of VHH Clone B can be modulated. To characterize the effect of mutagenesis on thermal stability of VHH Clone B, VHH Clone B variants were generated recombinantly as monovalent VHH-Fc fusions, purified, and tested by differential scanning calorimetry (DSC).

[0543] Methods: Design of VHH Clone B variants. Using the cryo-EM structure of Clone B-hTfR1, a cohort of Clone B mutations were designed with amino acid residue substitutions in Clone B outside of its paratope to modulate thermal stability. The following was observed: (i) G35C/A50C would bond CDR1 to CDR2 as in certain rabbit VH; (ii) Y32C/A98C or A33C/T101C or A33C/G108C would each bond CDR1 to CDR3 as in certain somatic VHH. Non limiting examples of rabbit VH disulfide bonds may include non-canonical disulfide bonds found in the VH domain for rabbit IgGs. Non limiting examples of somatic VHH disulfide bonds may include non-canonical disulfide bonds that commonly exist in VHHs across species like alpaca, llama, and shark. In these and other cases of disulfide bonds to CDR3 used in some VHH, the CDR3 position depends on the specific mature CDR3 sequence and conformation; and (iii) A50C/Y107C or N59C/Y107C would each bond CDR2 to CDR3, which is a novel disulfide design.

[0544] Results: Differential scanning calorimetry (DSC) was used to assess thermal stability of exemplary VHH Clone B variants. For VHH-Fcs analyzed with DSC, based on the thermal stability of the VHH-Fc, peaks corresponding to CH2, VHH and CH3 (in such order) could be observed. For example, in a VHH-Fc with a lower melting temperature, the CH2 and VHH peaks could unfold at a similar temperature and produce two peaks: a first peak with CH2 and VHH, and a second peak with CH3. As another example, increasing the melting temperature of the VHH in the VHH-Fc can produce three distinct peaks with a first CH2 peak, a second VHH peak, and a third CH3 peak.

[0545] VHH Clone B variants showed a range of thermal stabilities when assessed with DSC (**Table 10; FIGS. 12A-12B**). **FIG. 12A** highlights stabilizing mutations comprising pairs of cysteines of VHH Clone B, such as A33C/T101C, A50C/Y107C, and N59C/Y107C, and **FIG. 12B** highlights non-stabilizing mutations comprising pairs of cysteines of VHH Clone B, such as A98C/Y32C, A33C/G108C and A50C/G35C. The mutations introduced impacted thermal

stability over the range of Tm1/Tm2= 45.4 – 70.5 °C compared to 53.4 °C for parental VHH Clone B. As shown in **FIGS. 13A-13C,** mutations N59C/Y107C (Clone Y) and A50C/Y107C (Clone BB) increased melting temperature (as indicated by the presence of a Tm2 peak) compared to parental VHH Clone B.

[0546] For parental VHH Clone B two peaks are observed likely due to the unfolding of CH2 and VHH at similar temperatures (e.g., ~50-53°C) which combine to produce one broader peak, e.g., as compared to two peaks, one each forCH2 and VHH. For Clones Y and BB, as the melting temperature of the VHH increased (as shown in Table 10), the VHH peak shifted to a higher temperature and separated from the CH2 peak. Thus, all three peaks (e.g., CH2, VHH, and CH3) were able to be modeled and quantified. Clones Y, BB, and CC all resulted in increased melting temperature of the VHH, compared to parental VHH Clone B, indicating an increase in thermal stability.

Table 10: Thermal Stability of VHH Clone B variants

		DSC thermal stability			
VHH Clone	**Mutation**	TOnset (°C)	Tm_1 (°C)	Tm_2 (°C)	Tm_3 (°C)
Clone B	parent	45.3	53.4		80.0
Clone U	M18L/L23A	43.9	52.3		80.1
Clone V	G35C/A50C	39.1	49.4		79.9
Clone W	Y32C/A98C	34.4	45.4	51.0	79.9
Clone X	A33C/G108C	39.7	51.6		80.0
Clone Y	N59C/Y107C	45.0	54.4	65.1	80.4
Clone Z	M18L	44.4	53.2		79.9
Clone AA	L23A	43.2	51.4		79.8
Clone BB	A50C/Y107C	44.0	53.7	70.5	79.9
Clone CC	A33C/T101C	45.6	54.2	58.8	79.7

[0547] To characterize the effect of VHH Clone B stability mutagenesis on affinity for various species of TfR1, antibodies were generated recombinantly as monovalent VHH-Fc

fusions, purified, and tested for TfR1 binding via flow cytometry and SPR, using methods described above.

[0548] VHH Clone B variants showed a range of binding affinities to human TfR1 by both cell-based and protein-based binding assessment (**Table 11; FIGS. 14A-14C**). The mutations introduced impacted human TfR1 binding over the range of EC_{50} of 45 – 128 nM compared to 39 nM for parental Clone B by flow cytometry, and K_D of 903 – 1340 nM compared to 1000 nM for parental Clone B by SPR. Species cross-reactivity varied among the variants tested. Specifically, while parent Clone B had ~4-fold weaker affinity to cynomolgus and mouse TfR1 compared to human TfR1, most mutations maintained binding affinities to human and cynomolgus TfR1 relative to parental Clone B, with decreased binding affinity to mouse TfR1. Mutations A33C/T101C (Clone CC) (which had improved thermal stability) resulted in loss of binding to human and mouse TfR1, with decreased binding affinity to cynomolgus TfR1. Without wishing to be bound by any particular theory, this data suggests that altering certain Cysteine residues that may have a role in VHH folding and/or structure could increase thermal stability but also affect, e.g., negatively impact, binding to the antigen, e.g., TfR1.

[0549] Taken together this data demonstrates the ability to modulate the activity of single domain antibody agents that bind to TfR1, e.g., by mutagenesis. As shown herein, mutagenesis can be performed to increase thermal stability and/or increase binding affinity of a single domain antibody agent to TfR1, e.g., human TfR1, cyno TfR1 and/or mouse TfR1.

Table 11: Binding affinities of VHH Clone B variants

		Flow Cytometry					SPR (monovalent binding)	
		huTfR-CHO binding	cyTfR-CHO binding	muTfR-CHO binding	Species cross-reactivity		Human TfR ECD binding	Cyno TfR ECD binding
VHH Clone	Mutation	EC_{50} (nM)	EC_{50} (nM)	EC_{50} (nM)	Ratio hu/cy	Ratio mu/hu	K_D (nM)	K_D (nM)
Clone B	parent	39	9	143	4.2	3.6	1000	66
Clone U	M18L/L23A	n.d.	n.d.	n.d.	n.d.	n.d.	1130	43
Clone V	G35C/A50C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Clone W	Y32C/A98C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Clone X	A33C/G108C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Clone Y	N59C/Y107C	71	23	~1000	3.1	15	987	41
Clone Z	M18L	84	36	251	2.3	3.0	1340	40
Clone AA	L23A	128	19	208	6.7	1.6	1080	44
Clone BB	A50C/Y107C	45	15	406	3.0	9.0	903	32
Clone CC	A33C/T101C	no binding	300	no binding	n.d.	n.d.	no binding	469

CLAIMS

What is claimed is:

1. A single chain antibody agent polypeptide that binds to a transferrin receptor 1 (TfR1) or a variant or a fragment thereof.

2. The single chain antibody agent polypeptide of claim 1, wherein the single chain antibody agent comprises one or more complementarity determining regions (CDRs).

3. The single chain antibody agent polypeptide of claim 2, wherein the single chain antibody agent comprises a CDR1, a CDR2 and a CDR3.

4. The single chain antibody agent polypeptide of any one of the preceding claims, wherein the single chain antibody agent is or comprises a single domain antibody (e.g., a VHH).

5. The single chain antibody agent polypeptide of any one of the preceding claims, wherein the single chain antibody agent polypeptide comprises a sequence provided in **Table 1.**

6. The single chain antibody agent polypeptide of any one of the preceding claims, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR1 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR1 sequence provided in **Table 1**;

(ii) a CDR2 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR2 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR2 sequence provided in **Table 1**; and/or

(iii) a CDR3 sequence provided in **Table 1**; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a CDR3 sequence provided in **Table 1**; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to a CDR3 sequence provided in **Table 1**.

7. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

(ii) a CDR2 sequence of SEQ ID NO: 3; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 3; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 3; and/or

(iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

8. The single chain antibody agent polypeptide of claim 7, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 1, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

9. The single chain antibody agent polypeptide of claim 7, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 158, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at

least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

10. The single chain antibody agent polypeptide of claim 7, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 159, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

11. The single chain antibody agent polypeptide of claim 7, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 160, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

12. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6;

(ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or

(iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

13. The single chain antibody agent polypeptide of claim 12, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 5, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

14. The single chain antibody agent polypeptide of claim 12, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 122, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

15. The single chain antibody agent polypeptide of claim 12, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 135, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

16. The single chain antibody agent polypeptide of claim 12, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 136, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

17. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 10; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 10;

(ii) a CDR2 sequence of SEQ ID NO: 11; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 11; and/or

(iii) a CDR3 sequence of SEQ ID NO: 12; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 12; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 12.

18. The single chain antibody agent polypeptide of claim 17, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 9, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

19. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 14; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 14; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 14;

(ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or

(iii) a CDR3 sequence of SEQ ID NO: 16; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 16; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 16.

20. The single chain antibody agent polypeptide of claim 19, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 13, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

21. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 18; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 18; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 18;

(ii) a CDR2 sequence of SEQ ID NO: 19; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 19; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 19; and/or

(iii) a CDR3 sequence of SEQ ID NO: 20; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 20; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 20.

22. The single chain antibody agent polypeptide of claim 21, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 17, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

23. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 22; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at

least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 22; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 22;

(ii) a CDR2 sequence of SEQ ID NO: 23; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 23; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 23; and/or

(iii) a CDR3 sequence of SEQ ID NO: 24; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 24; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 24.

24. The single chain antibody agent polypeptide of claim 23, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 21, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

25. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 26; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 26; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 26;

(ii) a CDR2 sequence of SEQ ID NO: 27; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 27; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 27; and/or

(iii) a CDR3 sequence of SEQ ID NO: 28; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 28; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 28.

26. The single chain antibody agent polypeptide of claim 25, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 25, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

27. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 30; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 30; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 30;

(ii) a CDR2 sequence of SEQ ID NO: 31; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 31; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 31; and/or

(iii) a CDR3 sequence of SEQ ID NO: 32; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 32; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 32.

28. The single chain antibody agent polypeptide of claim 27, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 29, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

29. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 34; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 34; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 34;

(ii) a CDR2 sequence of SEQ ID NO: 35; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 35; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 35; and/or

(iii) a CDR3 sequence of SEQ ID NO: 36; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 36; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 36.

30. The single chain antibody agent polypeptide of claim 29, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 33, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

31. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 38; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 38; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 38;

(ii) a CDR2 sequence of SEQ ID NO: 39; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 39; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 39; and/or

(iii) a CDR3 sequence of SEQ ID NO: 40; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at

least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 40; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 40.

32. The single chain antibody agent polypeptide of claim 31, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 37, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

33. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 42; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 42; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 42;

(ii) a CDR2 sequence of SEQ ID NO: 43; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 43; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 43; and/or

(iii) a CDR3 sequence of SEQ ID NO: 44; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 44; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 44.

34. The single chain antibody agent polypeptide of claim 33, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 41, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

35. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 46; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 46; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 46;

(ii) a CDR2 sequence of SEQ ID NO: 47; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 47; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 47; and/or

(iii) a CDR3 sequence of SEQ ID NO: 48; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 48; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 48.

36. The single chain antibody agent polypeptide of claim 35, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 45, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

37. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 50; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 50; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 50;

(ii) a CDR2 sequence of SEQ ID NO: 51; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 51; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 51; and/or

(iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

38. The single chain antibody agent polypeptide of claim 37, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 49, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

39. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

(ii) a CDR2 sequence of SEQ ID NO: 117; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 117; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 117; and/or

(iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

40. The single chain antibody agent polypeptide of claim 39, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 116, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at

least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

41. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

(ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or

(iii) a CDR3 sequence of SEQ ID NO: 4; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 4.

42. The single chain antibody agent polypeptide of claim 41, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 118, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

43. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

(ii) a CDR2 sequence of SEQ ID NO: 15; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 15; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 15; and/or

(iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

44. The single chain antibody agent polypeptide of claim 43, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 120, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

45. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 2; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 2;

(ii) a CDR2 sequence of SEQ ID NO: 119; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 119; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 119; and/or

(iii) a CDR3 sequence of SEQ ID NO: 52; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 52; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 52.

46. The single chain antibody agent polypeptide of claim 45, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 121, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

47. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 124; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 124; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 124;

(ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or

(iii) a CDR3 sequence of SEQ ID NO: 8; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 8.

48. The single chain antibody agent polypeptide of claim 47, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 123, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

49. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 127; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 127; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 127;

(ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or

(iii) a CDR3 sequence of SEQ ID NO: 128; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 128; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 128.

50. The single chain antibody agent polypeptide of claim 49, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 126, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

51. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130;

(ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or

(iii) a CDR3 sequence of SEQ ID NO: 131; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at

least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 131; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 131.

52. The single chain antibody agent polypeptide of claim 51, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 129, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

53. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6;

(ii) a CDR2 sequence of SEQ ID NO: 133; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 133; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 133; and/or

(iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

54. The single chain antibody agent polypeptide of claim 53, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 132, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

55. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 6; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 6;

(ii) a CDR2 sequence of SEQ ID NO: 125; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 125; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 125; and/or

(iii) a CDR3 sequence of SEQ ID NO: 134; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO:134; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 134.

56. The single chain antibody agent polypeptide of claim 55, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 137, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

57. The single chain antibody agent polypeptide of any one of claims 1-6, wherein the single chain antibody agent comprises:

(i) a CDR1 sequence of SEQ ID NO: 130; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 130; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 130;

(ii) a CDR2 sequence of SEQ ID NO: 7; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at

least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 7; and/or

(iii) a CDR3 sequence of SEQ ID NO: 139; or a sequence with at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 139; or a sequence having 1, 2, 3, 4 or 5 substitutions relative to SEQ ID NO: 139.

58. The single chain antibody agent polypeptide of claim 57, wherein the single chain antibody agent comprises the sequence of SEQ ID NO: 138, or a sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto.

59. The single chain antibody agent polypeptide of any one of the preceding claims, wherein the single chain antibody agent binds to a human TfR1 (hTfR1), or a cyno TfR, optionally wherein the single chain antibody agent binds to hTfR1 with an affinity (K_D) of about 10nM to about 1000nM when fused to an Fc domain.

60. The single chain antibody agent polypeptide of any one of the preceding claims, wherein the single chain antibody agent binds to an epitope on an ectodomain of hTfR1.

61. The single chain antibody agent polypeptide of claim 60, wherein the single chain antibody agent binds to hTfR1 at a site other than a transferrin binding site.

62. The single chain antibody agent polypeptide of any one of the preceding claims, characterized in that when administered to a cell, tissue or subject, the single chain antibody agent polypeptide does not compete with transferrin for binding to hTfR1.

63. The single chain antibody agent polypeptide of any one of the preceding claims, wherein the single chain antibody agent polypeptide further comprises at least one heavy chain constant

region (CH), optionally wherein the single chain antibody agent comprises a CH2 domain and a CH3 domain, e.g., an Fc domain.

64. The single chain antibody agent polypeptide of claim 63, wherein the Fc domain is an Fc domain of an immunoglobulin (Ig) isotype.

65. The single chain antibody agent polypeptide of claim 63 or 64, wherein the Fc domain comprises a modification, optionally wherein the modification modulates one or more properties of the antibody agent.

66. The single chain antibody agent polypeptide of any one of the preceding claims, wherein the single chain antibody agent polypeptide further comprises a half-life extender.

67. The single chain antibody agent polypeptide of any one of the preceding claims, wherein the single chain antibody agent polypeptide is fused to one or more polypeptides,

optionally wherein the one or more polypeptides is an antibody agent or a fragment thereof that binds to an antigen other than hTfR1.

68. The single chain antibody agent polypeptide of any one of the preceding claims, wherein the single chain antibody agent polypeptide is conjugated to a polynucleotide, optionally wherein the polynucleotide is an antisense oligo (ASO), an inhibitory RNA (e.g., siRNA), a microRNA, a locked nucleic acid (LNA), a peptide nucleic acid (PNA), or an aptamer.

69. The single chain antibody agent polypeptide of any one of the preceding claims, wherein the single chain antibody agent polypeptide is conjugated to a small molecule, e.g., ADC.

70. A polynucleotide encoding the single chain antibody agent polypeptide of any one of claims 1-69.

71. A vector comprising a polynucleotide of claim 70.

72. A cell comprising the single chain antibody agent polypeptide of any one of claims 1-69.

73. The cell of claim 72, wherein the cell expresses hTfR1.

74. A composition comprising the single chain antibody agent polypeptide of any one of claims 1-69.

75. A composition comprising the polynucleotide of claim 70.

76. The composition of claim 74 or 75, wherein the single chain antibody agent polypeptide or the polynucleotide encoding the single chain antibody agent polypeptide is encapsulated in or conjugated to a delivery vehicle.

77. The composition of claim 76, wherein the delivery vehicle is a liposome, or an LNP.

78. The composition of claim 76 or 77, wherein the composition is a pharmaceutical composition optionally wherein the pharmaceutical composition comprises one or more excipients.

79. The composition of claim 78, wherein the pharmaceutical composition is formulated for intramuscular, intradermal, or intravenous delivery.

80. A method of delivering the pharmaceutical composition of claim 78 or 79, to a cell, tissue or subject.

81. The method of claim 80, wherein delivering comprises administering the pharmaceutical composition to the cell, tissue or subject.

82. The method of claim 80 or 81, wherein the method is a treatment method.

83. The method of claim 82, wherein the subject is a human.

84. The method of any one of claims 81-83, wherein administering the pharmaceutical composition alleviates one or more symptoms of a disease or condition and/or treats a disease or condition.