Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3231574 A1 2023/04/06

(21) 3 231 574

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) **Date de dépôt PCT/PCT Filing Date:** 2022/09/28
(87) **Date publication PCT/PCT Publication Date:** 2023/04/06
(85) **Entrée phase nationale/National Entry:** 2024/03/12
(86) **N° demande PCT/PCT Application No.:** CA 2022/051437
(87) **N° publication PCT/PCT Publication No.:** 2023/049999
(30) **Priorités/Priorities:** 2021/09/29 (US63/249,728); 2022/04/01 (US63/326,572)

(51) **Cl.Int./Int.Cl.** ***C07K 16/30*** (2006.01), ***C07K 16/28*** (2006.01)

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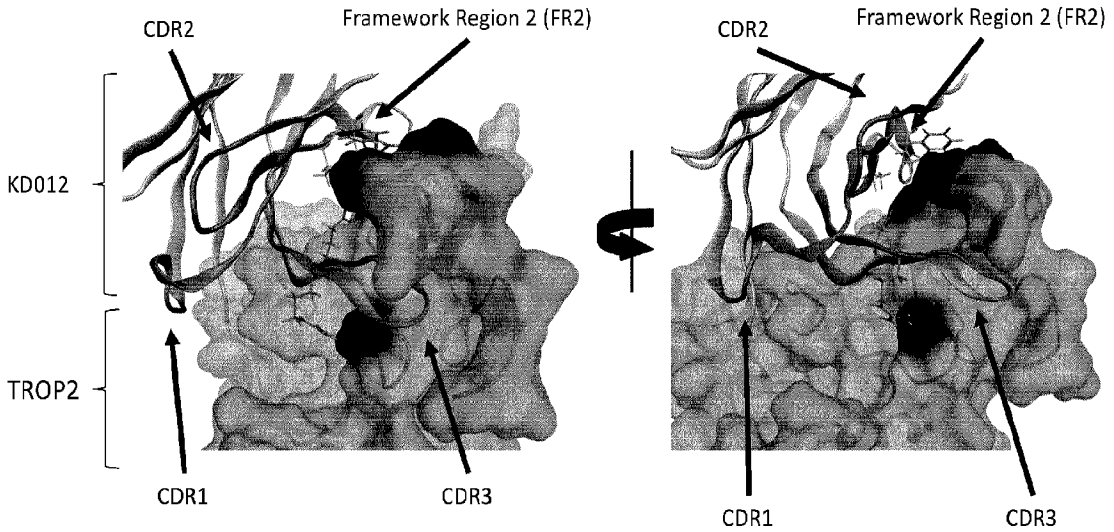
(54) **Titre : AGENTS DE LIAISON CIBLANT DES CELLULES TUMORALES EXPRIMANT TROP2**
(54) **Title: BINDING AGENTS TARGETING TROP2-EXPRESSING TUMOR CELLS**

Figure 20C



(57) **Abrégé/Abstract:**
The present disclosure generally relates to binding agents, such as antibodies and antigen binding fragments thereof, that are capable of binding to trophoblast cell surface antigen-2 (TROP2). Also disclosed herein are binding agents that are capable of targeting TROP2-expressing tumor cells and their use for the treatment of cancer. Single domain antibodies that specifically binds to amino acid residues of the extracellular domain of TROP2 are provided.

Date Submitted: 2024/03/12

CA App. No.: 3231574

Abstract:

The present disclosure generally relates to binding agents, such as antibodies and antigen binding fragments thereof, that are capable of binding to trophoblast cell surface antigen-2 (TROP2). Also disclosed herein are binding agents that are capable of targeting TROP2-expressing tumor cells and their use for the treatment of cancer. Single domain antibodies that specifically binds to amino acid residues of the extracellular domain of TROP2 are provided.

TITLE: BINDING AGENTS TARGETING TROP2-EXPRESSING TUMOR CELLS

TECHNICAL FIELD

The present disclosure generally relates to binding agents, such as antibodies and antigen binding fragments thereof, that are capable of binding to trophoblast cell surface antigen-2 (TROP2). Also disclosed herein are binding agents that are capable of targeting TROP2-expressing tumor cells and their use for the treatment of cancer. Single domain antibodies that specifically binds to amino acid residues of the extracellular domain of TROP2 are provided.

BACKGROUND

Trophoblast cell surface antigen-2 (TROP2) is a membrane-bound protein expressed at low levels in normal adult tissues. TROP2 has pleiotropic functions including inducing cell signaling and formation of cell-cell junctions by mediating interactions with extracellular and intracellular proteins. TROP2 has an extracellular epidermal growth factor (EGF)-like domain, followed by a 23 amino acids transmembrane domain and the cytoplasmic tail. The EGF-like domain contains a cysteine-rich domain, a thyroglobulin type-1 domain, and a cysteine-poor domain. In situations of TROP2 deficiency, its role in normal tissues appears to be compensated by expression of EpCAM, which is highly related to TROP2. TROP2 is highly expressed in a wide range of late-stage epithelial cancers including breast cancer and pancreatic cancer (Stepan *et al.*, J. Histochem and Cytochem, 2011, the entire content of which is incorporated herein by reference). Overexpression of TROP2 was shown to confer oncogenic behavior in several *in vitro* and *in vivo* models. TROP2 upregulation in solid cancers is associated with increased tumor aggressiveness, metastasis, and an overall decreased survival in large groups of difficult-to-treat cancers, making it an attractive target for cancer therapy.

TROP2 positive cancers have been successfully targeted clinically by antibody-drug conjugates (ADC) but not by naked functional antibodies. ADC's have associated drug toxicity in healthy tissues, off-target effects, and payload delivery issues. Given ADC dose-limiting side effects, narrow therapeutic windows, and efficacy limitations, there is a pressing need to improve anti-TROP2 therapeutics for patients who are poorly served by current therapies, if at all.

Camelids and cartilaginous fishes naturally produce antibodies composed of functional homodimeric heavy chain only antibodies (HCAbs) (Hamers-Casterman et al., 1993; Muyldermans and Smider, 2016). The heavy chains of HCAbs lack the first constant domain (CH1) and differs from classical antibodies by only a few amino acids substitutions normally involved in light chain pairing (Muyldermans et al., 1994; Vu et al., 1997). These substitutions (Val37Phe/Tyr, Gly44Glu, Leu45Arg, and Trp47Gly) are present in framework region 2 (FR2). The antigen-binding fragment of HCAbs is referred to as, VHH or nanobody®. VHHs have a molecular weight of around 15 kDa which makes them amenable to applications that require enhanced tissue penetration or rapid clearance, such as radioisotope-based imaging. However, for therapeutic applications, the VHH half-life usually needs to be increased so as to minimize renal clearance and optimize therapeutic efficacy (De Vlieger et al., Antibodies 8(1), 1-22, 2019). Although methods to increase VHH half-life such as PEGylation, N-glycosylation, HSA or other carrier protein fusions have been exploited, such constructs can introduce immunogenicity or have limited success.

VHHs have been exploited as building blocks to make bispecific and multispecific antibodies. In some studies, bivalent constructs have been shown to be have increased avidity or affinity compared to the monovalent form (Conrath et al., 2001; Coppieters et al., 2006; Hmila et al., 2008; Simmons et al., 2006 and Hultberg et al., 2011, Jähnichen et al. (2010), Fridy et al., 2014).

Novel TROP2 binding agents including antibodies and antigen binding fragments thereof such as single domain antibodies and antigen binding fragments thereof are disclosed herein.

SUMMARY

The present disclosure therefore generally relates to a binding agent that is capable, amongst other things, of binding trophoblast cell surface antigen-2 (TROP2). Binding agents of the present disclosure specifically bind to TROP2.

Although, the binding agents may have activity against tumor cells when unconjugated, the present disclosure also encompasses a conjugated form of such binding agents. Binding agents described herein may, in some instances, have no or low activity against tumor cells in a naked form and may thus benefit from being conjugated with a therapeutic moiety.

The present disclosure therefore provides in some aspects unconjugated binding agents. In other aspects, the present disclosure provides conjugated binding agents.

Antibodies or antigen binding fragments thereof such as single domain antibodies (sdAbs) or antigen binding fragments that specifically bind to at least one epitope comprising amino acid residues of the extracellular domain (ECD) of TROP2 are provided. The single domain antibodies (sdAbs) of the present disclosure have unique binding features and activity.

For example, the complementarity determining region 3 (CDR3) of the sdAbs forms a loop fitting into a groove within the cysteine rich domain (CRD) of the extracellular domain (ECD) of TROP2. Amino acid residues of the CDR3 and of the framework region 2 (FR2) interact with amino acid residues of TROP2 cysteine rich domain and together form a clip around the CRD as a bi-paratopic antibody. These unique properties confer high affinity and a slow dissociation rate to the sdAbs disclosed herein. Surprisingly, amino acid residues of the complementarity determining region 1 (CDR1) and of the complementarity determining region 2 (CDR2) do not appear to interact with amino acid residues of the CRD.

Moreover, the anti-TROP2 single domain antibodies of the present disclosure possess anti-tumor activity without drug conjugation.

For example, the anti-TROP2 single domain antibodies of the present disclosure are capable of inducing antibody-dependent cellular cytotoxicity (ADCC) and/or antibody-dependent cellular phagocytosis (ADCP). Moreover, the anti-TROP2 single domain antibodies of the present disclosure are also capable of internalization making them amenable to deliver drug payloads into the tumor microenvironment or into tumor cells.

The single domain antibodies of the present disclosure also interfere/block TROP2 function.

Exemplary embodiments of binding agents of the present disclosure therefore include single domain antibodies or antigen binding fragments thereof that specifically binds to at least one epitope comprising amino acids of the cysteine rich domain of TROP2. In some embodiment, the single domain antibody or antigen binding fragment thereof comprises FR2 amino acid residues and CDR3 amino acid residues that interact with amino acid residues of the CRD of

TROP2. The single domain antibody or antigen binding fragment thereof may have an affinity of $\leq 10^{-6}$ M for human TROP2.

The single domain antibodies of the present disclosure may be used to treat disorders or diseases associated with expression or overexpression of TROP2, such as cancer.

The binding function of sdAbs is conferred by a single polypeptide chain namely, the heavy chain variable region. This unique property provides formatting flexibility. For example, multiple sdAbs or sdAb antigen binding domains may be fused into a single polypeptide chain conferring multivalence and/or multispecificity. In addition, multiple polypeptide chains may be assembled to increase the diversity (multi-paratopic binding) or avidity of interactions with TROP2. Moreover, the sdAbs antigen binding domains may be fused to any type of polypeptide chain including for example and without limitation, fusion with a heavy chain and/or light chain of a native antibody or antigen binding fragment thereof, with protein scaffold, with immune cell modulating agent and the like.

As such, the present disclosure not only relates to the single domain antibodies disclosed herein but also more generally to binding agents that comprise one or more antigen binding domains of the single domain antibodies disclosed herein. Accordingly, in an embodiment, the binding agent may comprise an antigen binding domain of a single domain antibody disclosed herein.

In addition, the present disclosure relates to binding agents that compete with the single domain antibodies of the present disclosure. For example, single domain antibodies that compete with one of more single domain antibodies disclosed herein for the binding of at least one epitope comprising amino acid residues of the extracellular domain (ECD) of TROP2 are provided.

In some embodiments, the binding agent of the present disclosure binds to human TROP2. In other embodiments, the binding agent of the present disclosure binds to cynomolgus or Rhesus TROP2. Accordingly, the binding agent of the present disclosure may be used in the treatment of human diseases or disorders. Moreover, the pre-clinical development of the binding agents of the present disclosure may be carried out in cynomolgus or Rhesus monkeys and other related non-human primates.

In accordance with the present disclosure, the binding agent disclosed herein is capable of targeting tumor cells expressing TROP2.

In accordance with the present disclosure, the binding agent disclosed herein is capable of causing regression of tumors expressing TROP2.

In accordance with the present disclosure, the binding agent disclosed herein is also capable of causing growth inhibition of tumors expressing TROP2.

In other embodiments, the binding agent is capable of causing inhibition of tumor growth via antibody-dependent cellular cytotoxicity (ADCC).

In other embodiments, the binding agent is capable of causing inhibition of tumor growth via antibody-dependent cellular phagocytosis (ADCP).

In yet other embodiments, the binding agent may be used to deliver a payload to tumor cells expressing TROP2. Accordingly, in some embodiments, the binding agent may be used as an antibody drug conjugate (ADC).

In some embodiments, the binding agent is capable of causing direct inhibition of tumor growth. For example, the activity of the binding agent may be independent of ADCC, ADCP or ADC function.

The present disclosure relates to a binding agent that comprises one or more antigen binding domains, wherein at least one of the one or more antigen binding domains comprises an antigen binding fragment thereof that is capable of binding to TROP2.

As such, in accordance with the present disclosure, the antigen binding fragment is from a single domain antibody (heavy chain only antibody) disclosed herein.

Also, in accordance with the present disclosure, the antigen binding fragment comprises a single domain antibody variable region.

Also, in accordance with the present disclosure, the antigen binding fragment comprises the CDRs of a single domain antibody.

Therefore, the present disclosure relates to a binding agent that comprises one or more antigen binding domains, wherein at least one of the one or more antigen binding domains

comprises an antigen binding domain of a single domain antibody that is capable of binding to TROP2.

The present disclosure also particularly relates to a binding agent that comprises one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to human TROP2.

The binding agent of the present disclosure is essentially polypeptidic. In some embodiments, the binding agent of the present disclosure is essentially composed of one, two or more than two polypeptide chains. However, the binding agent may comprise non-polypeptidic moieties such as for example, small molecules, sugars, radioactive labels and the like. In some embodiments, the binding agent is composed of one heavy chain. In some embodiments, the binding agent is composed of two heavy chains. In other embodiments, the binding agent is composed of more than two heavy chains.

In some embodiments, the binding agent of the present disclosure may be capable of binding to the extracellular domain (ECD) of TROP2 (as represented by amino acids 27 to 274 of TROP2 (e.g., SEQ ID NO:39 or SEQ ID NO:40), see Goldenberg *et al.*, 2018, Ikeda, M. *et al.*, 2015, the entire contents of which are incorporated herein by reference). In some embodiments, the binding agent of the present disclosure binds to the extracellular domain of human TROP2.

In some embodiments, the binding agent of the present disclosure may be capable of binding to an epitope comprising amino acids of the extracellular domain of TROP2. In some embodiments, the binding agent of the present disclosure binds to an epitope comprising amino acids of the extracellular domain of human TROP2.

In some embodiments, the binding agent of the present disclosure may be capable of binding to an epitope including amino acid residues comprised within amino acid sequence 27-274 of TROP2. In some embodiments, the binding agent of the present disclosure binds to an epitope including amino acid residues comprised within amino acid sequence 27-274 of human TROP2.

In some embodiments, the binding agent of the present disclosure may be capable of binding to the cysteine-rich domain (CRD) of TROP2 (as represented by amino acids 27 to 73 of

TROP2 (e.g., SEQ ID NO:39 or SEQ ID NO:40)). In some embodiments, the binding agent of the present disclosure binds to the cysteine-rich domain of human TROP2.

In some embodiments, the binding agent of the present disclosure may be capable of binding to an epitope comprising amino acids of the cysteine-rich domain of TROP2. In some embodiments, the binding agent of the present disclosure binds to an epitope comprising amino acids of the cysteine-rich domain of human TROP2.

In some embodiments, the binding agent of the present disclosure may be capable of binding to the thyroglobulin type-1 domain of TROP2 (as represented by amino acids 74 to 146 of TROP2 (SEQ ID NO:39 or SEQ ID NO:40)).

In some embodiments, the binding agent of the present disclosure binds to the thyroglobulin type-1 domain of human TROP2.

In some embodiments, the binding agent of the present disclosure may be capable of binding to an epitope comprising amino acids of the thyroglobulin type-1 domain of TROP2. In some embodiments, the binding agent of the present disclosure binds to an epitope comprising amino acids of the thyroglobulin type-1 domain of human TROP2.

In an exemplary embodiment, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-274 or a fragment thereof.

In another exemplary embodiment, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-146 or a fragment thereof.

In another exemplary embodiment, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-73 or a fragment thereof.

In accordance with the present disclosure the fragment may comprise at least 10 amino acid residues of human TROP2.

For example, the binding agent, or antigen binding domain(s) of the binding agent may bind to a fragment of at least 10 amino acid residues of a human TROP2 amino acid sequence consisting of amino acid residues 27-146.

In accordance with the present disclosure the fragment may comprise between 10 to 110 amino acid residues, including for example between 10 to 100 amino acid residues, between 10 to 90 amino acid residues, between 10 to 80 amino acid residues, between 10 to 70 amino acid residues, between 10 to 60 amino acid residues, between 10 to 50 amino acid residues, between 10 to 40 amino acid residues, between 10 to 30 amino acid residues, between 10 to 29 amino acid residues, between 10 to 28 amino acid residues, between 10 to 27 amino acid residues, between 10 to 26 amino acid residues, between 10 to 25 amino acid residues, between 10 to 24 amino acid residues, between 10 to 23 amino acid residues, between 10 to 22 amino acid residues, between 10 to 21 amino acid residues, between 10 to 20 amino acid residues, between 10 to 19 amino acid residues, between 10 to 18 amino acid residues, between 10 to 17 amino acid residues, between 10 to 16 amino acid residues, between 10 to 15 amino acid residues, between 10 to 14 amino acid residues, between 10 to 13 amino acid residues, between 10 to 12 amino acid residues between 10 to 11 amino acid residues of a human TROP2 amino acid sequence consisting of amino acid residues 27-146.

In another example, the binding agent, or antigen binding domain(s) of the binding agent may bind to a fragment of at least 10 amino acid residues of a human TROP2 amino acid sequence consisting of amino acid residues 27-73.

In accordance with the present disclosure the fragment may comprise of between 10 to 45 amino acid residues, including for example between 10 to 40 amino acid residues, between 10 to 35 amino acid residues, between 10 to 30 amino acid residues, between 10 to 29 amino acid residues, between 10 to 28 amino acid residues, between 10 to 27 amino acid residues, between 10 to 26 amino acid residues, between 10 to 25 amino acid residues, between 10 to 24 amino acid residues, between 10 to 23 amino acid residues, between 10 to 22 amino acid residues, between 10 to 21 amino acid residues, between 10 to 20 amino acid residues, between 10 to 19 amino acid residues, between 10 to 18 amino acid residues, between 10 to 17 amino acid residues, between 10 to 16 amino acid residues, between 10 to 15 amino acid residues, between 10 to 14 amino acid residues, between 10 to 13 amino acid residues, between 10 to 12 amino acid residues between 10

to 11 amino acid residues of a human TROP2 amino acid sequence consisting of amino acid residues 27-73.

In some instances, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-56 of human TROP2 or a fragment thereof.

In other instances, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-66 of human TROP2 or a fragment thereof.

In yet other instances, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 47-56 of human TROP2 or a fragment thereof.

In other instances, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 60-66 of human TROP2 or a fragment thereof.

In yet other instances, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 47-66 of human TROP2 or a fragment thereof.

For example, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-56, 27-66, 28-66, 29-66, 30-66, 31-66, 32-66, 33-66, 34-66, 35-66, 36-66, 37-66, 38-66, 39-66, 40-66, 41-66, 42-66, 43-66, 44-66, 45-66, 46-66 or 47-66.

For example, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to an epitope consisting of amino acid residues 27-56, 27-66, 28-66, 29-66, 30-66, 31-66, 32-66, 33-66, 34-66, 35-66, 36-66, 37-66, 38-66, 39-66, 40-66, 41-66, 42-66, 43-66, 44-66, 45-66, 46-66 or 47-66 of TROP2.

In some embodiments, the binding agent of the present disclosure may not be capable of binding to the cysteine-poor domain (CPD) of TROP2 (as represented by amino acids 147 to 274 of TROP2). In some embodiments, the binding agent of the present disclosure does not bind to the cysteine-poor domain (CPD) of human TROP2. In some embodiments, the binding agent of the

present disclosure may not be capable of significantly binding to the cysteine-poor domain (CPD) of TROP2. In some embodiments, the binding agent of the present disclosure does not significantly bind to the cysteine-poor domain (CPD) of human TROP2.

Accordingly, the binding agent, or antigen binding domain(s) or of the binding agent does not significantly bind to or is not capable of binding to a polypeptide that comprises a human TROP2 amino acid sequence consisting of amino acid residues 147-274 or a fragment thereof.

In an exemplary embodiment, the binding agent, or antigen binding domain(s) of the binding agent may not be capable of binding to one or more polypeptide selected from polypeptides comprising a human TROP2 amino acid sequence comprising deletion of at least 10 amino acid residues within amino acid residues 27-73.

In accordance with the present disclosure, the binding agent, or antigen binding domain(s) of the binding agent may not be capable of binding to one or more polypeptide selected from polypeptides comprising a human TROP2 amino acid sequence comprising deletion of amino acid residues 27-36, 27-44, 27-56, 27-66, 47-56, 47-66, 60-66 and/or 27-73.

Alternatively, the binding agent, or antigen binding domain(s) of the binding agent may have a reduced binding to one or more polypeptide selected from polypeptides comprising a human TROP2 amino acid sequence comprising deletion of amino acid residues 27-36, 27-44, 27-56, 27-66, 47-56, 47-66, 60-66 and/or 27-73 compared to a human TROP2 polypeptide that does not have such deletion including for example, the full human TROP2 polypeptide (SEQ ID NO:39).

For example, the binding agent, or antigen binding domain(s) of the binding agent may not be capable of binding to one or more polypeptide selected from polypeptides comprising a human TROP2 amino acid sequence comprising deletion of amino acid residues 28-66, 29-66, 30-66, 31-66, 32-66, 33-66, 34-66, 35-66, 36-66, 37-66, 38-66, 39-66, 40-66, 41-66, 42-66, 43-66, 44-66, 45-66, 46-66 or 47-66.

In yet another exemplary embodiment, the binding agent, or antigen binding domain(s) of the binding agent may not be capable of binding to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 149 or have a reduced binding to such polypeptide compared to a human TROP2 polypeptide that does not have such deletion including for example, the full human TROP2 polypeptide (SEQ ID NO:39).

In an embodiment, the binding agent binds to human TROP2 protein (e.g., SEQ ID NO:39) or to a TROP2 homologue or TROP2 variant having a sequence at least 80% identical to the human TROP2 protein thereof or to a portion of human TROP2 protein (e.g., an extracellular domain of human TROP2: SEQ ID NO:164), of the homologue or variant thereof.

In some embodiment, the TROP2 homologue is a TROP2 sequence or portion thereof of a primate.

In some embodiment, the binding agent, or antigen binding domain(s) of the binding agent of the present disclosure binds to an epitope that is common to human TROP2 and to cynomolgus TROP2.

In some embodiment, the binding agent, or antigen binding domain(s) of the binding agent of the present disclosure binds to an epitope that is similar in human TROP2 and to cynomolgus TROP2.

In some embodiment, the binding agent, or antigen binding domain(s) of the binding agent of the present disclosure is capable of binding human TROP2 and cynomolgus TROP2.

In some embodiment, the binding agent, or antigen binding domain(s) of the binding agent of the present disclosure preferentially binds to human TROP2 over mouse TROP2 or has a better affinity for human TROP2 over mouse TROP2.

In some embodiment, the binding agent, or antigen binding domain(s) of the binding agent of the present disclosure preferentially binds to human TROP2 over rat TROP2 or has a better affinity for human TROP2 over rat TROP2.

In some embodiment, the binding agent, or antigen binding domain(s) of the binding agent of the present disclosure binds to amino acid residues that are common to the extracellular domain of human TROP2 and to the extracellular domain of cynomolgus TROP2.

In some embodiment, the binding agent, or antigen binding domain(s) of the binding agent of the present disclosure binds to amino acid residues that are not present in the extracellular domain of mouse TROP2.

In some embodiment, the binding agent, or antigen binding domain(s) of the binding agent of the present disclosure binds to amino acid residues that are not present in the extracellular domain of rat TROP2.

In some embodiment, the binding agent, or antigen binding domain(s) of the binding agent of the present binds to amino acid residues that are present in the extracellular domain of human TROP2, cynomolgus TROP2, mouse TROP2 and rat TROP2.

Accordingly, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to human TROP2 and to cynomolgus TROP2.

In exemplary embodiments, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a denatured form human TROP2.

In another exemplary embodiments, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a reduced form of human TROP2.

In yet other exemplary embodiments, the binding agent, or antigen binding domain(s) of the binding agent may be capable of binding to a deglycosylated form of human TROP2.

In accordance with an embodiment, the formation of an antigen binding domain may involve amino acid residues of a single polypeptide chain. For example, the amino acid residues forming the paratope may be located on a single polypeptide chain that is able to adopt a proper configuration for interacting with the epitope.

However, in accordance with another embodiment, the formation of an antigen binding domain may involve amino acid residues of two polypeptide chains. In some instances, a complete antigen binding domain may require the association of two polypeptide chains. For example, the amino acid residues forming the paratope may be located on two polypeptide chains that are able to associate and adopt a proper configuration for interacting with the epitope.

In accordance with the present disclosure, the binding agent, or antigen binding domain(s) of the binding agent comprises an amino acid sequence of one or more CDRs of a single domain antibody (of a heavy chain only antibody).

In an exemplary embodiment, the binding agent, or the antigen binding domain(s) may comprise the amino acid sequence of a CDR1, CDR2, CDR3, FR2 or combination thereof of a heavy chain only antibody.

In an exemplary embodiment, the binding agent, or the antigen binding domain(s) may comprise the amino acid sequence of the CDR3 of single domain antibody (of a heavy chain only antibody).

In another exemplary embodiment, the binding agent, or the antigen binding domain(s) may comprise the amino acid sequence of FR2 and CDR3 of a heavy chain only antibody. In a further exemplary embodiment, the FR2 and CDR3 amino acid sequence may be on same polypeptide chain. Alternatively, the FR2 and CDR3 amino acid sequence may be on separate polypeptide chains.

In another exemplary embodiment, the binding agent, or the antigen binding domain(s) may comprise the amino acid sequence of FR2 and CDR3 of a heavy chain only antibody and optionally the amino acid sequence of CDR1 and/or CDR2.

In accordance with the present disclosure, the order of CDR1, CDR2, CDR3, and/or FR2 amino acid sequence of the antigen binding domain may be the same as that of a single domain antibody. As such, the antigen binding domain comprises in a N- to C- terminal fashion CDR1, FR2, CDR2 and/or CDR3 amino acid sequences. In some instances, the antigen binding domain comprises in a N- to C- terminal fashion FR2 and CDR3 amino acid sequences. In other instances, the antigen binding domain, comprises in a N- to C- terminal fashion CDR1, FR2 and CDR3 amino acid sequences. In other instances, the antigen binding domain, comprises in a N- to C- terminal fashion CDR2, FR2 and CDR3 amino acid sequences. In other instances, the antigen binding domain, comprises in a N- to C- terminal fashion CDR1, CDR2, FR2 and CDR3 amino acid sequences. In accordance with the present disclosure, the antigen binding domain(s) may comprise an amino acid sequence of the variable region of a heavy chain only antibody.

In some embodiments, the antigen binding domain(s) may comprise an amino acid sequence of a camelid VH or VHH. In other embodiments, the antigen binding domain(s) may comprise an amino acid sequence of a mouse VH. In yet other embodiments, the antigen binding domain(s) may comprise an amino acid sequence of human VH. In a further embodiment, the

antigen binding domain(s) may comprise an amino acid sequence of humanized VH or VHH (e.g., a humanized camelid VH or VHH, or a humanized mouse VH).

The binding agent, or antigen binding domain(s) of the binding agent may come from various sources including from transgenic animals or non-transgenic animals, from screening of an antibody library etc. For example, antibodies may be obtained from hybridoma after immunization of rodents (mouse, rats etc.) or from larger mammals such as camelids. In some embodiments, the animal may be immunized with a TROP2 antigen.

In an embodiment, the amino acid sequence or nucleotide sequence of an antigen binding domain is obtained by immunization of a transgenic animal modified to express heavy chain only antibodies. In some instances, the sequence of the antigen binding domain is obtained from a transgenic mouse carrying modification at an IgH locus allowing expression of heavy chain only antibodies (see for example, WO2022/011457 A1, the entire content of which is incorporated herein by reference). In some embodiments, the transgenic animal may be immunized with a TROP2 antigen.

In an exemplary embodiment, the binding agent or the antigen binding domain(s) of the binding agent may comprise an amino acid sequence of a CDR1, CDR2 and/or CDR3 of a heavy chain variable region and/or an amino acid sequence of a CDR1, CDR2 and/or CDR3 of a light chain variable region.

In another exemplary embodiment, the binding agent or the antigen binding domain(s) of the binding agent may comprise an amino acid sequence of a CDR1, CDR2 and CDR3 of a heavy chain variable region and an amino acid sequence of a CDR1, CDR2 and CDR3 of a light chain variable region.

In an exemplary embodiment, the amino acid sequence of a CDR1, CDR2 and CDR3 of the heavy chain variable region and the amino acid sequence of a CDR1, CDR2 and CDR3 of the light chain variable region are on same polypeptide chain.

Alternatively, the amino acid sequence of CDR1, CDR2 and CDR3 of the heavy chain variable region and the amino acid sequence of CDR1, CDR2 and CDR3 of the light chain variable region are on separate polypeptide chains.

In accordance with the present disclosure, the binding agent or the antigen binding domain(s) of the binding agent may comprise a heavy chain variable region and a light chain variable region of an antibody. In an exemplary embodiment, the heavy chain variable region and the light chain variable region are on same polypeptide chain. In another exemplary embodiment, the heavy chain variable region and the light chain variable region are on separate polypeptide chains.

In accordance with the present disclosure, the CDRs may be identified using the Kabat numbering scheme (e.g., Kabat, J Immunol., 147:1709-19 (1991); Chothia C, Lesk AM, J Mol Biol. Aug 20;196(4):901-17 (1987)).

Alternatively, the CDRs may be identified using the IMGT numbering scheme (e.g., Lefranc, M.-P., The Immunologist, 7, 132-136 (1999)).

In some instances, the CDR1, CDR2, CDR3 and/or FR2 correspond to the Kabat CDR1, CDR2, CDR3 and/or FR2.

In other instances, the CDR1, CDR2, CDR3 and/or FR2 correspond to the IMGT CDR1, CDR2, CDR3 and/or FR2.

In some embodiments, the binding agent is expressed as a polypeptide chain that comprises in a N- to C-terminal fashion, a) an antigen binding domain that includes an antigen binding fragment of a single domain antibody, b) a linker and c) a dimerization domain.

In some embodiments, the binding agent is expressed as a polypeptide chain that comprises in a N- to C-terminal fashion, a) an antigen binding domain of a single domain antibody, b) a linker and c) a dimerization domain.

In some embodiments, the binding agent is expressed as a polypeptide chain that comprises in a N- to C-terminal fashion, a) a dimerization domain, b) a linker and c) an antigen binding domain that includes an antigen binding fragment of a single domain antibody.

In some embodiments, the binding agent is expressed as a polypeptide chain that comprises in a N- to C-terminal fashion, a) a dimerization domain, b) a linker and c) an antigen binding domain of a single domain antibody.

In other embodiments, the binding agent is expressed as a polypeptide chain that comprises in a N- to C-terminal fashion, a) an antigen binding domain of a single domain antibody, b) a linker, c) a dimerization domain, d) a linker and e) an antigen binding domain of a single domain antibody. In some embodiments, the antigen binding domain of a) and the antigen binding domain of e) are different. In some embodiments, the antigen binding domain of a) and the antigen binding domain of e) are the same. In some embodiments, the linker of b) and the linker of d) are the same. In some embodiments, the linker of b) and the linker of d) are different.

In accordance with the present disclosure, the binding agent may comprise additional amino acid sequence or moieties (small molecules, labels and the like) at an N- and/or C-terminus. For example, in accordance with the present disclosure, the binding agent may comprise additional antigen binding domain(s) of single domain antibody(ies) at an N- or C-terminus. The additional antigen binding domain(s) may be separated from the core by one or more linkers.

In some embodiments, the polypeptide chain may comprise additional sequences, including without limitations, additional antigen binding domains or additional linkers.

In some embodiments, the linker is an amino acid sequence of at least 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 amino acid residues in length.

In some embodiment, the linker is non-cleavable.

In some embodiments, the linker comprises one or more units of GGGGS.

In some embodiments, the linker may comprise the formula $(X(PAPAP))_nKA$ wherein n is an integer selected from 1 to 10, wherein X is present or absent and is A. In some embodiments, the linker may comprise the sequence set forth in SEQ ID NO:163.

In some embodiments, the linker is an antibody hinge or a portion thereof. For example, in some embodiments, the linker may comprise human IgG1 hinge sequence or a portion thereof. In some embodiments, the linker may comprise human IgG2 hinge sequence or a portion thereof. In some embodiments, the linker may comprise human IgG3 hinge sequence or a portion thereof. In yet other embodiment, the linker may comprise human IgG4 hinge sequence or a portion thereof.

The binding agent or antigen binding domain may comprise a sequence as set forth herein.

In accordance with the present disclosure, the binding agent, or antigen binding domain(s) of the binding agent may have the amino acid sequence set forth in SEQ ID NO: 143.

SEQ ID NO:143 (KD002 consensus)

X_{1a}VQLX_{1b}ESGGGX_{1c}VQX_{1d}GGSLRLSCAAS**GFPFSSAD**MSWVRQAPGK$X_{1e}X_{1f}$EW VSX_{1g}**INAX**$_{1h}$**GSKT**YYYX_{1i}DX_{1j}VKGRFTISRDNX_{1k}KNTLYLX_{1l}MNX_{1m}L$X_{1n}X_{1o}$EDT AX_{1p}YYX_{1q}C**ARAKLTDTHYVEDY**WGQGTX_{1r}VTVSS.

In an exemplary embodiment, any of X_{1a} to X_{1r} is each independently any (e.g., naturally occurring) amino acid residue.

In another exemplary embodiment, any of X_{1a} to X_{1r} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:23 or SEQ ID NOs:73-93, or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In yet in another exemplary embodiment, any of X_{1a} to X_{1r} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In a further exemplary embodiment, any X_{1a} to X_{1r} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:23 or SEQ ID Nos:73-93.

In another exemplary embodiment, any X_{1a} to X_{1r} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

In yet another exemplary embodiment, any of X_{1a} to X_{1r} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 73, 75, 76, 77, 78, 81, 82, 83, 85, 89 or 91, or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In a further exemplary embodiment, any X_{1a} to X_{1r} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 73, 75, 76, 77, 78, 81, 82, 83, 85, 89 or 91.

In yet a further exemplary embodiment X_{1a} is Q or E; X_{1b} is Q, V or L; X_{1c} is M or L; X_{1d} is V or P; X_{1e} is G or Q; X_{1f} is P, L or R; X_{1g} is Y or V; X_{1h} is D, N, E; X_{1i} is P or A; X_{1j} is S or T; X_{1k} is A or S; X_{1l} is L or Q; X_{1m} is N or S; X_{1n} is K or R; X_{1o} is P or A; X_{1p} is L or V; X_{1q} is R or Y; and/or X_{1r} is Q or M.

In another exemplary embodiment X_{1a} is Q or E; X_{1b} is Q, V or L; X_{1c} is M or L; X_{1d} is V or P; X_{1e} is G or Q; X_{1f} is P, L or R; X_{1g} is Y; X_{1h} is D, X_{1i} is P or A; X_{1j} is S or T; X_{1k} is A or S; X_{1l} is L or Q; X_{1m} is N or S; X_{1n} is K or R; X_{1o} is P or A; X_{1p} is L or V; X_{1q} is R or Y; and/or X_{1r} is Q or M.

In accordance with the present disclosure, the binding agent, or antigen binding domain(s) of the binding agent may have the amino acid sequence set forth in SEQ ID NO:144.

SEQ ID NO:144 (KD005 consensus)

X_{2a}VQLX_{2b}ESGGGL$X_{2c}X_{2d}X_{2e}$GGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKGX_{2f}E WVSX_{2g}**ITSGGTT**YYX_{2h}DX_{2i}VKGRFTISRDNX_{2j}KNTLYLQMNSL$X_{2k}X_{2l}X_{2m}$DTAX_{2n}YX_{2o}C**AKSRLTDSHYVEDA**WGQGTX_{2p}VTVSS

In an exemplary embodiment, any of X_{2a} to X_{2p} is each independently any (e.g., naturally occurring) amino acid residue.

In another exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:26 or SEQ ID NO:94-114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In another exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In yet another exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:26 or SEQ ID NO:94-114.

In a further exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

In yet a further exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 100, 101, 102, 103, 104, 105, 106, 107 or 108 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In an additional exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 100, 101, 102, 103, 104, 105, 106, 107 or 108.

In another exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 100, 102, 103, 104, 105, 106, 108, 112 or 114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In a further exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 100, 102, 103, 104, 105, 106, 108, 112 or 114.

In yet a further exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 105, 112, 113 or 114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In another exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 105, 112, 113 or 114.

In another exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 102 or 105 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In an additional exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 102 or 105.

In another exemplary embodiment, X_{2a} is Q or E; X_{2b} is Q, L or V; X_{2c} is V or I; X_{2d} is E or Q; X_{2e} is A or P; X_{2f} is P, or L; X_{2g} is Y, A or V; X_{2h} is P or A; X_{2i} is S or T; X_{2j} is A or S; X_{2k} is K or R; X_{2l} is P or A; X_{2m} is D or E; X_{2n} is L or V; X_{2o} is R or Y and/or X_{2p} is Q or L.

In yet another exemplary embodiment, X_{2a} is Q or E; X_{2b} is Q or L; X_{2c} is V or I; X_{2d} is E or Q; X_{2e} is A or P; X_{2f} is P, or L; X_{2g} is Y or A; X_{2h} is P or A; X_{2i} is S or T; X_{2j} is A or S; X_{2k} is K or R; X_{2l} is P or A; X_{2m} is D or E; X_{2n} is L or V; X_{2o} is R or Y and/or X_{2p} is Q or L.

In accordance with the present disclosure, the binding agent, or antigen binding domain(s) of the binding agent may have the amino acid sequence set forth in SEQ ID NO:145.

SEQ ID NO: 145 (Consensus from KD001 to KD005 heavy chains)

QVQLQESGGGX$_{3a}$VX$_{3b}$X$_{3c}$GX$_{3d}$SLRLSCAAS**GFX$_{3e}$FSX$_{3f}$X$_{3g}$D**MSWVRQAPGKGPE WVSY**IX$_{3h}$X$_{3i}$X$_{3j}$X$_{3k}$X$_{3l}$X$_{3m}$T**X$_{3n}$YPDSVKGRFTX$_{3o}$SRDNAKNX$_{3p}$LYLX$_{3q}$MNX$_{3r}$LX$_{3s}$ PX$_{3t}$DTALYRC**AX$_{3u}$X$_{3v}$X$_{3w}$LTDX$_{3x}$HYVEDX$_{3y}$**WGQGTQVTVSS

In an exemplary embodiment any of X_{3a} to X_{3y} is each independently any (e.g., naturally occurring) amino acid residue.

In another exemplary embodiment, any of X_{3a} to X_{3y} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In yet another exemplary embodiment, any of X_{3a} to X_{3y} is each independently an amino acid residue most frequently found in the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

In a further exemplary embodiment, X_{3a} is M or L; X_{3b} is Q, H or E; X_{3c} is V, P or A; X_{3d} is G or R; X_{3e} is P or T; X_{3f} is S, N or G; X_{3g} is A, Y or S; X_{3h} is N or T; X_{3i} is A, S or G; X_{3j} is D, G or S; X_{3k} is G or absent; X_{3l} is S or G; X_{3m} is K, N or T; X_{3n} is Y or D; X_{3o} is I or T; X_{3p} is T or M; X_{3q} is L or Q; X_{3r} is N or S; X_{3s} is K or T; X_{3t} is E or D; X_{3u} is R or K; X_{3v} is A or S; X_{3w} is K or R; X_{3x} is T or S; and/or X_{3y} is Y or A.

In yet a further exemplary embodiment, X_{3a} is M or L; X_{3b} is Q, H or E; X_{3c} is V, P or A; X_{3d} is G or R; X_{3e} is P or T; X_{3f} is S, N or G; X_{3g} is A, Y or S; X_{3h} is N or T; X_{3i} is A, S or G; X_{3j} is D, G or S; X_{3k} is G or absent; X_{3l} is S or G; X_{3m} is K, N or T; X_{3n} is Y; X_{3o} is I or T; X_{3p} is T or

M; X_{3q} is L or Q; X_{3r} is N or S; X_{3s} is K or T; X_{3t} is E or D; X_{3u} is R or K; X_{3v} is A or S; X_{3w} is R; X_{3x} is S; and/or X_{3y} is A.

Table 1: Position of amino acid residues with reference to SEQ ID NO:145.

SEQ ID NO: 145 amino acid residues numbering (IMGT CDR position bold underlined). Exemplary interacting residues are indicated in each third row.														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Q	V	Q	L	Q	E	S	G	G	G	X_{3a}	V	X_{3b}	X_{3c}	G
Q	V	Q	L											
16	17	18	19	20	21	22	23	24	25	**26**	**27**	**28**	**29**	**30**
X_{3d}	S	L	R	L	S	C	A	A	S	G	F	X_{3e}	F	S
31	**32**	**33**	34	35	36	37	38	39	40	41	42	43	44	45
X_{3f}	X_{3g}	D	M	S	W	V	R	Q	A	P	G	K	G	P
						V		Q						P
46	47	48	49	50	**51**	**52**	**53**	**54**	**54a**	**55**	**56**	**57**	58	59
E	W	V	S	Y	I	X_{3h}	X_{3i}	X_{3j}	X_{3k}	X_{3l}	X_{3m}	T	X_{3n}	Y
E	W			Y									Y	
60	61	62	63	64	65	66	67	68	69	70	71	72	73	74
P	D	S	V	K	G	R	F	T	X_{3o}	S	R	D	N	A
75	76	77	78	79	80	81	82	83	84	85	86	87	88	89
K	N	X_{3p}	L	Y	L	X_{3q}	M	N	X_{3r}	L	X_{3s}	P	X_{3t}	D
90	91	92	93	94	95	**96**	**97**	**98**	**99**	**100**	**101**	**102**	**103**	**104**
T	A	L	Y	R	C	A	X_{3u}	X_{3v}	X_{3w}	L	T	D	X_{3x}	H
									R	L	T	D	S	H
105	**106**	**107**	**108**	**109**	110	111	112	113	114	115	116	117	118	119
Y	V	E	D	X_{3y}	W	G	Q	G	T	Q	V	T	V	S
Y	V	E	D	A	W	G	Q							

120														
S														

In accordance with the present disclosure, the binding agent, or antigen binding domain(s) of the binding agent may have the amino acid sequence set forth in SEQ ID NO:146.

SEQ ID NO:146 (Consensus for KD001-KD005 and selected variants heavy chains)

X_{4a}VQLX_{4b}ESGGG$X_{4c}X_{4d}X_{4e}X_{4f}$GX_{4g}SLRLSCAASGFX_{4h}FS$X_{4i}X_{4j}$DMX_{4k}WX_{4l}RQAP GK$X_{4m}X_{4n}X_{4o}$WVSX_{4p}I$X_{4q}X_{4r}X_{4s}$G$X_{4t}X_{4u}$TX_{4v}YX_{4w}DX_{4x}VKGRFTX_{4y}SRDNX_{4z}KNX_{5a} LYLX_{5b}MNX_{5c}L$X_{5d}X_{5e}X_{5f}$DTAX_{5g}YX_{5h}CA$X_{5i}X_{5j}X_{5k}$LTDX_{5l}HYVEDX_{5m}WGQGTX_{5n}V TVSS.

In an exemplary embodiment, any of X_{4a} to X_{5n} is each independently any (e.g., naturally occurring) amino acid residue.

In another exemplary embodiment, any of X_{4a} to X_{5n} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs:22-26, SEQ ID Nos:73-114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In yet another exemplary embodiment, any of X_{4a} to X_{5n} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In another exemplary embodiment, any of X_{4a} to X_{5n} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs:22-26, SEQ ID Nos:73-114.

In another exemplary embodiment, any of X_{4a} to X_{5n} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

In a further exemplary embodiment, X_{4a} is Q or E, X_{4b} is Q, L or V, X_{4c} is M or L, X_{4d} is V or I, X_{4e} is Q, H or E, X_{4f} is P, A or V, X_{4g} is G or R, X_{4h} is P or T, X_{4i} is G, S or N, X_{4j} is A or S, X_{4k} is S or H, X_{4l} is V or Y, X_{4m} is G or Q, X_{4n} is P, L or R, X_{4o} is E or V, X_{4p} is Y, A or R, X_{4q} is T or N, X_{4r} is A, S or G, X_{4s} is G, S, D, N or E, X_{4t} is absent or S, X_{4u} is K or T, X_{4v} is Y, D or

S, X_{4w} is P or A, X_{4x} is S or T, X_{4y} is I or T, X_{4z} is S or A X_{5a} is T or M X_{5b} is Q or L, X_{5c} is S or N, X_{5d} is K, R or T, X_{5e} is P or A, X_{5f} is E or D, X_{5g} is V or L, X_{5h} is Y or R, X_{5i} is R or K, X_{5j} is A or S, X_{5k} is K or R, X_{5l} is S or T, X_{5m} is Y or A, and/or X_{5n} is Q, L or M.

In some embodiments, the amino acid substitution is a conservative amino acid substitution.

The present disclosure provides in further aspects and embodiments, a binding agent that comprises one or more antigen binding domains, wherein at least one of the one or more antigen binding domains is capable of binding to human trophoblast cell surface antigen-2 (human TROP2) and comprises an amino acid sequence at least 65% identical to the amino acid sequence set forth in any one of SEQ ID NOs:22-26, or SEQ ID NOs: 71-115.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:22. More particularly, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:22. In an embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence identical to SEQ ID NO:22.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:23. More particularly, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:23. In an embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence identical to SEQ ID NO:23.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:24. More particularly, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 95% identical, at least 99%

identical or identical to SEQ ID NO:24. In an embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence identical to SEQ ID NO:24.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:25. More particularly, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:25. In an embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence identical to SEQ ID NO:25.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:26. More particularly, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:26. In an embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence identical to SEQ ID NO:26.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NOs: 71-115. More particularly, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NOs: 71-115. In an embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence identical to any one of SEQ ID NOs:71-115.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NOs: 73-114. More particularly, the binding agent or antigen binding domain(s) of the binding agent may comprise an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NOs: 73-114. In an embodiment, the binding agent or

antigen binding domain(s) of the binding agent may comprise an amino acid sequence identical to any one of SEQ ID NOs:73-114.

In accordance with the present disclosure, the binding agent or antigen binding domain(s) of the binding agent may have a sequence as set forth herein, wherein the amino acid residue at position 1 is Q, the amino acid residue at position 2 is V, the amino acid residue at position 3 is Q, the amino acid residue at position 4 is L, the amino acid residue at position 37 is V, the amino acid residue at position 39 is Q, the amino acid residue at position 45 is P, the amino acid residue at position 46 is E, the amino acid residue at position 47 is W, the amino acid residue at position 50 is Y, the amino acid residue at position 58 is Y, the amino acid residue at position 99 is R, the amino acid residue at position 100 is L, the amino acid residue at position 101 is T, the amino acid residue at position 102 is D, the amino acid residue at position 103 is S, the amino acid residue at position 104 is H, the amino acid residue at position 105 is Y, the amino acid residue at position 106 is V, the amino acid residue at position 107 is E, the amino acid residue at position 108 is D, the amino acid residue at position 109 is A, the amino acid residue at position 110 is W, the amino acid residue at position 111 is G, and/or the amino acid residue at position 112 is Q and the position is with reference to SEQ ID NO:145 (**Table 1**).

The binding agent, or antigen binding domain(s) of the binding agent may have an amino acid sequence as defined in any one of SEQ ID Nos:22-26, 73-114, or 143 to 146 and may comprise from one to five amino acid deletions or from one to five amino acid additions. In an exemplary embodiment, the deletion or additions are consecutive. In another exemplary embodiment, the deletion or additions are non-consecutives. In yet another exemplary embodiment, the deletion or additions are at the N-terminus. In a further exemplary embodiment, the deletion or additions are at the C-terminus. In yet a further exemplary embodiment, the deletion or additions are outside of an IMGT CDR3 sequence and/or IMGT FR2 sequence. In yet a further exemplary embodiment, the deletion or additions are outside of a Kabat CDR3 sequence and/or Kabat FR2 sequence.

In an exemplary embodiment, the binding agent or antigen binding domain(s) thereof may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:126.

In an exemplary embodiment, the binding agent or antigen binding domain(s) thereof may comprise the amino acid sequence of the FR2 set forth in SEQ ID NO:121.

In another exemplary embodiment, the binding agent or antigen binding domain(s) thereof may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:126 and the amino acid sequence of the FR2 set forth in SEQ ID NO:121.

In accordance with the present disclosure, the amino acid sequence of the CDR3 and the amino acid sequence of the FR2 are on a same polypeptide chain. Alternatively, in accordance with the present disclosure, the amino acid sequence of the CDR3 and the amino acid sequence of the FR2 are on separate polypeptide chains.

In accordance with the present disclosure the amino acid sequence of the CDR3 is as set forth in SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:54 or SEQ ID NO:61.

In accordance with the present disclosure the amino acid sequence of the FR2 is as set forth in SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 or SEQ ID NO:120.

The binding agent or antigen binding domain may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:122.

In accordance with the present disclosure, the amino acid sequence of the CDR1 is as set forth in SEQ ID NO:52, SEQ ID NO:55 or SEQ ID NO:56.

The binding agent or antigen binding domain may comprise the amino acid sequence of the CDR2 set forth in SEQ ID NO:125.

In accordance with the present disclosure, the amino acid sequence of the CDR2 is as set forth in SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:123 or SEQ ID NO:124.

Accordingly, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in any one of SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:54, SEQ ID NO:58 or SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in any one of SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 or SEQ ID NO:120.

In exemplary embodiments, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in any one of SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:54, SEQ ID NO:58 or SEQ ID NO:61, the amino acid sequence of

the FR2 set forth in any one of SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 or SEQ ID NO:120, the amino acid sequence of the CDR1 set forth in any one of SEQ ID NO: 52, SEQ ID NO: 55, SEQ ID NO:59, SEQ ID NO: 52 or SEQ ID NO: 62 and the amino acid sequence of the CDR2 set forth in any one of SEQ ID NO: 47, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO:123 or SEQ ID NO:124.

In an exemplary embodiment, the binding agent or antigen binding domain(s) thereof may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:142.

In an exemplary embodiment, the binding agent or antigen binding domain(s) thereof may comprise the amino acid sequence of the FR2 set forth in SEQ ID NO:175.

In another exemplary embodiment, the binding agent or antigen binding domain(s) thereof may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:142 and the amino acid sequence of the FR2 set forth in SEQ ID NO:175.

In accordance with the present disclosure, the amino acid sequence of the CDR3 and the amino acid sequence of the FR2 are on a same polypeptide chain. Alternatively, in accordance with the present disclosure, the amino acid sequence of the CDR3 and the amino acid sequence of the FR2 are on separate polypeptide chains.

In accordance with the present disclosure the amino acid sequence of the CDR3 is as set forth in SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141.

In accordance with the present disclosure the amino acid sequence of the FR2 is as set forth in SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 or SEQ ID NO:174. In a specific embodiment, the amino acid sequence of the FR2 is as set forth in SEQ ID NO:170 or SEQ ID NO:171.

The binding agent or antigen binding domain may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:128.

In accordance with the present disclosure, the amino acid sequence of the CDR1 is as set forth in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:127.

The binding agent or antigen binding domain may comprise the amino acid sequence of the CDR2 set forth in SEQ ID NO:140.

In accordance with the present disclosure, the amino acid sequence of the CDR2 is as set forth in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138 or SEQ ID NO:139.

Accordingly, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in any one of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141 and the amino acid sequence of the FR2 set forth in any one of SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 or SEQ ID NO:174.

In exemplary embodiments, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in any one of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141, the amino acid sequence of the FR2 set forth in any one of SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 or SEQ ID NO:174, the amino acid sequence of the CDR1 set forth in any one of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:127 and the amino acid sequence of the CDR2 set forth in any one of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138 or SEQ ID NO:139.

In more specific embodiments, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in any one of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141, the amino acid sequence of the FR2 set forth in any one of SEQ ID NO:170 or SEQ ID NO:171,the amino acid sequence of the CDR1 set forth in any one of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:127 and the amino acid sequence of the CDR2 set forth in any one of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138 or SEQ ID NO:139.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:54 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid

sequence of the CDR1 set forth in SEQ ID NO:52 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:53.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:47.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:58 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:56 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:57.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:59 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:60.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48

and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:119 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 124.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 123.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:120 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:5.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:130.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:131.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:133.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:131.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:132.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:133.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:172 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:133.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:134.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:135.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:174 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:127 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:129.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:14.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:137.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:138.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:136.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:137.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:138.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:139.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:173 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:137.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:52, the amino acid sequence of the CDR2 set forth in SEQ ID NO:53, the amino acid sequence of the CDR3 set forth in SEQ ID NO:54 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO:48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:56, the amino acid sequence of the CDR2 set forth in SEQ ID NO:57, the amino acid sequence of the CDR3 set forth in SEQ ID NO:58 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:59, the amino acid sequence of the CDR2 set forth in SEQ ID NO:60, the amino acid sequence of the CDR3 set forth in SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO: 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:119.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:124, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:123, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118.

In another exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:120.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:5, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:130, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:131, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:133, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:131, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:132, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:133, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:133, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:172.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:134, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:135, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:127, the amino acid sequence of the CDR2 set forth in SEQ ID NO:129, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:174.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:14, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:137, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:138, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:136, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:137, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:138, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:139, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the binding agent may comprise the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:137, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:173.

In an exemplary embodiment, the amino acid sequence of the CDR1, CDR2, CDR3 and/or FR2 are on a single polypeptide chain.

In another exemplary embodiment, the amino acid sequence of the CDR3 and the amino acid sequence of the FR2 are on a same polypeptide chain.

In yet another exemplary embodiment, the amino acid sequence of the CDR1, CDR2, CDR3 and FR2 are on a same polypeptide chain.

In yet another exemplary embodiment, the amino acid sequence of the CDR3 and the amino acid sequence of the FR2 are on two polypeptide chains.

The present disclosure also relates to binding agent that is capable of binding to TROP2 comprising one or more antigen binding domains or at least one antigen binding fragment of a single domain antibody, wherein at least one of the one or more antigen binding domains or at least one antigen binding fragment comprises:

a. a heavy chain complementarity determining region 1 (CDRH1) having the amino acid sequence set forth in SEQ ID NO:1, a heavy chain complementarity determining region 2 (CDRH2) having the amino acid sequence set forth in SEQ ID NO:2 and a heavy chain complementarity determining region 3 (CDRH3) having the amino acid sequence set forth in SEQ ID NO:3;

b. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:5 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

c. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:7, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:8 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:9;

d. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:10, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:11 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:12;

e. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:14 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

f. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:16, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:17 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:18;

g. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:19, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:20 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:21;

h. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:46 or in SEQ ID NO:55, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:47 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:48;

i. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:49 or in SEQ ID NO: 62, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:50 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:51;

j. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:52, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:53 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:54;

k. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:56, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:57 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:58;

l. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:59, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:60 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:61;

m. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:63, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:64 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:65;

n. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:66, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:67 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:68;

o. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:127, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:129 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

p. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:130 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

q. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:131 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

r. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:132 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

s. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:133 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

t. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:134 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

u. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:135 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

v. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:136 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

w. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:137 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

x. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:138 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

y. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:139 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

z. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:22;

aa. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:23;

bb. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:24;

cc. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:25;

dd. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:26;

ee. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:27;

ff. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:28;

gg. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs:73 to 114.

In yet other aspects and embodiments, the present disclosure relates to a binding agent that is capable of binding to trophoblast cell surface antigen-2 (TROP2) and that comprises at least one antigen binding fragment of a single domain antibody.

In other exemplary embodiments, the binding agent, or antigen binding domain(s) of the binding agent comprises:

a. a variable region having an amino acid sequence as set forth in SEQ ID NO:22;

b. a variable region having an amino acid sequence as set forth in SEQ ID NO:23;

c. a variable region having an amino acid sequence as set forth in SEQ ID NO:24;

d. a variable region having an amino acid sequence as set forth in SEQ ID NO:25;

e. a variable region having an amino acid sequence as set forth in SEQ ID NO:26;

f. a variable region having an amino acid sequence as set forth in SEQ ID NO:27

g. a variable region having an amino acid sequence as set forth in SEQ ID NO:28 or;

h. a variable region having an amino acid sequence as set forth in any one of SEQ ID Nos:73-114.

In other exemplary embodiments, the binding agent comprises:

a. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:29;

b. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:30;

c. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:31;

d. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:32;

e. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:33;

f. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:34;

g. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:35;

h. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:44 or,

i. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:45.

In further exemplary embodiments, the binding agent comprises:

a. an amino acid sequence as set forth in SEQ ID NO:29;

b. an amino acid sequence as set forth in SEQ ID NO:30;

c. an amino acid sequence as set forth in SEQ ID NO:31;

d. an amino acid sequence as set forth in SEQ ID NO:32;

e. an amino acid sequence as set forth in SEQ ID NO:33;

f. an amino acid sequence as set forth in SEQ ID NO:34;

g. an amino acid sequence as set forth in SEQ ID NO:35;

h. an amino acid sequence as set forth in SEQ ID NO:44 or,

i. an amino acid sequence as set forth in SEQ ID NO:45.

In some embodiments, the antigen binding domain is a heavy chain variable region.

In some embodiments, the heavy chain variable region is a camelid VH or VHH.

In some embodiments, the heavy chain variable region is a human VH.

In some embodiments, the heavy chain variable region is a mouse VH.

In some embodiments, the heavy chain variable region is a rat VH.

In some embodiments, the heavy chain variable region is a humanized VH or VHH. For example, the heavy chain variable region may be a humanized camelid VH or VHH. In other example, the heavy chain variable region may be a humanized mouse VH. In other example, the heavy chain variable region may be a humanized rat VH.

In other embodiments, the antigen binding domain is a heavy chain.

In accordance with the present disclosure, the binding agent may comprise two heavy chain variable regions. Each heavy chain variable regions may be on separate heavy chains. Accordingly, the binding agent may comprise two heavy chains.

The binding agent of the present disclosure may be, for example, a single domain antibody (heavy chain only antibody).

In some exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a heavy chain complementarity determining region 1 (CDRH1) having the amino acid sequence set forth in SEQ ID NO:1, a heavy chain complementarity determining region 2 (CDRH2) having the amino acid sequence set forth in SEQ ID NO:2 and a heavy chain complementarity determining region 3 (CDRH3) having the amino acid sequence set forth in SEQ ID NO:3.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:5 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6.

In yet other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:7, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:8 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:9.

In further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:10, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:11 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:12.

In some exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:14 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:16, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:17 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:18.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:19, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:20 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:21.

In yet other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:46, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:47 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:48.

In yet other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:55, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:47 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:48.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:49, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:50 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:51.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:62, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:50 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:51.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:52, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:53 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:54.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:56, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:57 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:58.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:59, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:60 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:61.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:63, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:64 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:65.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:66, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:67 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:68.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises the CDRH1, CDRH2 and CDRH3 of SEQ ID NO:152.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises the CDRH1, CDRH2 and CDRH3 of SEQ ID NO:153.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises the CDRH1, CDRH2 and CDRH3 of SEQ ID NO:154.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:22.

In some exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:23.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:24.

In further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:25.

In yet further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:26.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:27.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:28.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:71.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:72.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:73.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:74.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:75.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:76.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NOs77.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:78.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:79.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:80.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:81.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:82.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:83.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:84.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:85.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:86.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:87.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:88.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:89.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:90.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:91.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:92.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:93.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:94.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:95.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, v identical to the amino acid sequence set forth in SEQ ID NO:96.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:97.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:98.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:99.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:100.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:101.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:102.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:103.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:104.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:105.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:106.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:107.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:108.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:109.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:110.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:111.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:112.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:113.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% , 99% identical to the amino acid sequence set forth in SEQ ID NO:114.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the variable region of SEQ ID NO: 152.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the variable region of SEQ ID NO: 153.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the variable region of SEQ ID NO: 154.

In further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:29.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:30.

In some exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:31.

In further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:32.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:33.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:34.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% , 99% identical to the amino acid sequence set forth in SEQ ID NO:35.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:44.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:45.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:152.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:153.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:154.

In further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:22.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:23.

In further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:24.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:25.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:26.

In further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:27.

In yet further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:28.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:29.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:73.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:74.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:75.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:76.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:77.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:78.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:79.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:80.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:81.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:82.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:83.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:84.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:85.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:86.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:87.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:88.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:89.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:90.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:91.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:92.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:93.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:94.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:95.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:96.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:97.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:98.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:99.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:100.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:101.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:102.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:103.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:104.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:105.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:106.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:107.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:108.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:109.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:110.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:111.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:112.

In another exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:113.

In additional exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO: 114.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:30.

In yet other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:31.

In further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:32.

In yet further exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:33.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:34.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:35.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:44.

In other exemplary embodiments, the binding agent is a heavy chain only antibody that comprises an amino acid sequence as set forth in SEQ ID NO:45.

In some exemplary embodiments, the heavy chain only antibody comprises a dimerization domain as described herein. For example, the heavy chain only antibody may comprise a constant region of a human IgG or a portion thereof. The heavy chain only antibody may thus be engineered. Accordingly, the heavy chain only antibody may be non-naturally occurring.

In some exemplary embodiments, the constant region or portion thereof is from an IgG1, an IgG2, an IgG3 or an IgG4. For example, the constant region or portion thereof is from a IgG1, an IgG2, an IgG3 or an IgG4 heavy chain.

In some embodiments, the constant region or portion thereof is from a human IgG1. For example, the constant region or portion thereof is from a IgG1 heavy chain.

In some embodiment, the constant region or portion thereof comprises a hinge.

In some embodiment, the constant region or portion thereof comprises a peptidic linker.

In some embodiments, the heavy chain only antibody comprises two heavy chains. The two chains of the heavy chain only antibody may be for example, identical.

In some embodiments, the binding agent or at least one of the one or more antigen binding domains may have an affinity of $\leq 10^{-6}$ M for human TROP2.

In some embodiments, the binding agent or at least one of the one or more antigen binding domains may have an affinity of $\leq 10^{-7}$ M for human TROP2.

In some embodiments, the binding agent or at least one of the one or more antigen binding domains may have an affinity of $\leq 10^{-8}$ M for human TROP2.

In some embodiments, the binding agent or at least one of the one or more antigen binding domains may have an affinity of $\leq 10^{-9}$ M for human TROP2.

In some embodiments, the binding agent or at least one of the one or more antigen binding domains may have an affinity of $\leq 10^{-10}$ M for human TROP2.

In some embodiments, the binding agent or at least one of the one or more antigen binding domains may have an affinity of $\leq 10^{-11}$ M for human TROP2.

In some embodiments, the binding agent or at least one of the one or more antigen binding domains may have an affinity of $\leq 10^{-12}$ M for human TROP2.

In some aspects and embodiments, the heavy chain only antibody has an affinity for human TROP2 in the picomolar range.

In some aspects and embodiments, the KD value of the heavy chain only antibody is lower than 10E-11 for human TROP2.

In an exemplary embodiment, the binding agent or at least one of the one or more antigen binding domains may have an affinity of 1 $x10^{-10}$ M or lower for human TROP2.

In another exemplary embodiment, the binding agent or at least one of the one or more antigen binding domains may have an affinity of at between 1 $x10^{-10}$ M and 1 $x10^{-12}$ for human TROP2.

In another exemplary embodiment, the binding agent or at least one of the one or more antigen binding domains may have an affinity of at between 1 $x10^{-10}$ M and 1 $x10^{-11}$ for human TROP2.

In an exemplary embodiment, the binding agent or at least one of the one or more antigen binding domains may have a dissociation rate of between 1 x 10^{-6} to 1 x 10^{-4} for human TROP2.

In an exemplary embodiment, the binding agent or at least one of the one or more antigen binding domains may have a dissociation rate of between 1 x 10^{-5} to 1 x 10^{-4} for human TROP2.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the present disclosure may have ADCP activity towards cells expressing human TROP2.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the present disclosure may have ADCP activity towards cells expressing human TROP2 and Cluster of Differentiation 47 (CD47) (Uniprot Accession No. Q08722-1). An exemplary and non-limiting embodiment of a CD47 antigen is provided in SEQ ID NO:176.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the present disclosure may have ADCC activity towards cells expressing human TROP2.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the present disclosure may be internalized in cells expressing human TROP2.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the present disclosure may be capable of inhibiting the growth of tumor cells expressing human TROP2.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the present disclosure may be capable of inhibiting the growth of tumor cells expressing human TROP2 and CD47.

Accordingly, the binding agent may comprise one or more antigen binding domains that is capable of binding to CD47.

In an exemplary embodiment, the antigen binding domain that is capable of binding to CD47 may comprise:

a. a CDRH1 having the amino acid sequence set forth in SEQ ID NO156, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:157 and/or a CDRH3 having the amino acid sequence set forth in SEQ ID NO:158, or

b. a CDRH1 having the amino acid sequence set forth in SEQ ID NO159, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:160 and/or a CDRH3 having the amino acid sequence set forth in SEQ ID NO:161.

In an exemplary embodiment, the antigen binding domains that is capable of binding to CD47 may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:155.

In another exemplary embodiment, the antigen binding domains that is capable of binding to CD47 may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:150 or 151.

The present disclosure also relates to a binding agent that is capable of competing with one or more binding agents disclosed herein.

In some embodiments, the binding agent comprises at least one antigen binding domain that is capable of competing with one or more binding agent disclosed herein.

In some embodiments, the binding agent comprises at least one antigen binding fragment of a single domain antibody that is capable of competing with one or more binding agent disclosed herein.

More particularly, the binding agent is capable of competing or comprises one or more antigen binding domains that is capable of competing with a binding agent comprising:

a. a heavy chain complementarity determining region 1 (CDRH1) having the amino acid sequence set forth in SEQ ID NO:1, a heavy chain complementarity determining region 2 (CDRH2) having the amino acid sequence set forth in SEQ ID NO:2 and a heavy chain

complementarity determining region 3 (CDRH3) having the amino acid sequence set forth in SEQ ID NO:3;

b. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:5 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

c. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:7, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:8 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:9;

d. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:10, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:11 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:12;

e. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:14 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

f. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:16, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:17 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:18;

g. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:19, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:20 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:21;

h. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:46 or SEQ ID NO:55, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:47 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:48;

i. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:49 or SEQ ID NO:62, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:50 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:51;

j. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:52, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:53 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:54;

k. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:56, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:57 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:58;

l. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:59, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:60 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:61;

m. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:63, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:64 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:65;

n. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:66, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:67 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:68;

o. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:127, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:129 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

p. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:130 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

q. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:131 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

r. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:132 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

s. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:133 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

t. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:134 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

u. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:135 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

v. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:136 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

w. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:137 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

x. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:138 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

y. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:139 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

z. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:22;

aa. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:23;

bb. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:24;

cc. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:25;

dd. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:26;

ee. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:27;

ff. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:28 and/or;

gg. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs:73 to 114.

In accordance with the present disclosure, the binding agent comprises two antigen binding fragments.

In accordance with the present disclosure, the binding agent comprises two identical antigen binding fragments.

In accordance with the present disclosure, the binding agent comprises two antigen binding domains.

In accordance with the present disclosure, the binding agent comprises two identical antigen binding domains.

In accordance with the present disclosure, the binding agent comprises more than two antigen binding fragments.

In accordance with the present disclosure, the binding agent comprises more than two antigen binding domains.

In accordance with the present disclosure, the binding agent comprises antigen binding fragments of at least two different single domain antibodies.

In some embodiments, the at least two different single domain antibodies have the same specificity.

In other embodiments the at least two different single domain antibodies have different specificities.

In accordance with the present disclosure, the binding agent comprises two antigen binding fragments of same or different single domain antibodies.

In accordance with the present disclosure, the binding agent comprises more than two antigen binding fragments of same and/or different single domain antibodies.

In an exemplary embodiment, the binding agent may comprise a Fc or a portion thereof that is capable of binding to an Fc receptor of monocytes and/or macrophages.

In another exemplary embodiment, the binding agent may comprise a Fc or a portion thereof that is capable of binding to an Fc receptor on CD68+ macrophages.

In accordance with the present disclosure, the binding agent comprises a dimerization domain.

In some embodiments, the dimerization domain allows the formation of homodimers.

In some embodiments, the dimerization domain allows the formation of heterodimers.

In some embodiments, the dimerization domain comprises an amino acid sequence that is contiguous to the amino acid sequence of the antigen binding domain.

In some embodiments, the dimerization domain comprises an immunoglobulin dimerization domain. Other dimerization domains known to a person skilled in the art are contemplated herein including leucine zippers, etc.

In some embodiments, the dimerization domain comprises an IgG, IgM, IgA, IgD or IgE dimerization domain (from human or animal IgGss, IgM, IgAs, IgDs or IgEs).

In some embodiments, the dimerization domain comprises a constant region of an antibody or a portion thereof.

In some embodiments, the constant region or portion thereof is from an IgG1, an IgG2, an IgG3 or an IgG4 heavy chain or combination thereof. For example, the constant region or portion thereof comprises amino acid sequence of an IgG1, an IgG2, an IgG3 or an IgG4 heavy chain or combination thereof.

In some embodiments, the constant region or portion thereof is from a human IgG1 heavy chain.

In some embodiments, the constant region or portion thereof is from a human IgG2 heavy chain.

In some embodiments, the constant region or portion thereof is from a human IgG3 heavy chain.

In some embodiments, the constant region or portion thereof is from a human IgG4 heavy chain.

In some embodiments, the dimerization domain comprises a Fc region of an antibody or a portion thereof.

In some embodiments, the dimerization domain comprises a Fc region or a portion thereof that comprises Fc modification(s) or not.

In other embodiments, the dimerization domain comprises an altered or mutated Fc region or portion thereof. For example, the Fc region or portion thereof may be altered or mutated so as to modify one or more characteristics of the binding agent. In exemplary embodiments, the Fc region or portion thereof is unglycosylated. In other exemplary embodiments, the Fc region or portion thereof comprises one or more of the Fc modification(s) of **Table A**.

In other exemplary embodiments, the Fc region or portion thereof is altered or mutated so as to increase ADCC activity. For example, afucosylation (e.g., N297 in accordance with the EU numbering system) may result in an increased FcRgII binding on NK cells and may potently increase ADCC. Accordingly, in some embodiments, the Fc region or portion thereof may have a reduced number of fucose residues. In other embodiments, the binding agent comprises an afucosylated Fc region or portion thereof. In yet other embodiments, the Fc region or portion thereof of the binding agent lacks fucose residues. In additional embodiments, the Fc region or portion thereof of the binding agent lacks core fucose residues. In further embodiments, the Fc region or portion thereof of the binding agent fully lacks core fucose residues.

In some embodiments, the dimerization domain comprises a CH3 domain.

In some embodiments, the CH3 domain is a natural CH3 domain or a mutated CH3 domain.

In some embodiments, the dimerization domain also comprises a CH2 domain.

In some embodiments, the dimerization domain comprises a CH3 domain of an antibody. The dimerization domain may also comprise a CH2 domain of an antibody.

In some embodiments, the CH2 domain is a natural CH2 domain or a mutated CH2 domain.

In some embodiments, the dimerization domain comprises a natural CH2 domain and a natural CH3 domain.

In some embodiments, the dimerization domain comprises a natural CH2 domain and a mutated CH3 domain. Exemplary embodiments of mutated CH3 domain are provided in PCT/CA2020/051753 filed on December 18, 2020, and published on June 24, 2021, under No. WO2021/119832A1, the entire content of which is incorporated herein by reference.

In some embodiments, the dimerization domain comprises a mutated CH2 domain and a natural CH3 domain.

In some embodiments, the dimerization domain comprises a mutated CH2 domain and a mutated CH3 domain.

In some embodiments, the binding agent is an antibody or an antigen binding fragment thereof or a variant thereof.

In some embodiments, the antibody or an antigen binding fragment thereof is a single domain antibody or an antigen binding fragment thereof or a variant thereof.

In some embodiments, the binding agent is a competing binding agent.

In some embodiments, the binding agent is a competing antibody or antigen binding fragment thereof.

In some embodiments, the binding agent is a competing single domain antibody or antigen binding fragment thereof.

In some embodiments, the binding agent is an antibody-like molecule.

In some embodiments, the binding agent comprises a protein scaffold.

In some embodiments, the binding agent comprises an immune cell modulating agent.

In some embodiments, the antigen binding fragments of single domain antibodies are in tandem.

In some embodiments, the binding agent is naked.

In some embodiments, the binding agent is conjugated to a therapeutic moiety.

In some embodiments, the binding agent is conjugated to a detectable moiety.

In some embodiments, the binding agent is conjugated to a protein allowing an extended half-life.

In some embodiments, the binding agent is attached to a nanoparticle.

In yet other aspects, the binding agent of the present disclosure possesses anti-tumor activity. In some embodiments, the binding agent of the present disclosure may be functional when naked (unconjugated). In other embodiments, the binding agent of the present disclosure possesses antibody-dependent cellular cytotoxicity (ADCC) activity. In other embodiments, the binding agent of the present disclosure possesses antibody-dependent cellular phagocytosis activity. In yet other embodiments, the binding agent of the present disclosure possess activity as an antibody drug conjugate.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may have naked anti-tumor activity to a variety of solid tumors.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may cause tumor regression in *in vivo xenograft* models of multiple tumor types.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may cause tumor growth inhibition in *in vivo xenograft* models of multiple tumor types.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may cause *in vivo* tumor regression of late-stage tumors (large tumors).

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may cause *in vivo* tumor regression of tumors.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may cause tumor regression at very low dose levels.

In some embodiments, the binding agent may have anti-tumor effect at a dose of approximately between 0.01 to 150 mg/kg, 0.01 to 100 mg/kg, 0.01 to 50mg/kg, 0.05 to 150 mg/kg 0.05 to 100 mg/kg, 0.05 to 50 mg/kg, 0.05 to 30 mg/kg, 0.05 to 10 mg/kg, 0.1 to 50mg/kg, 0.1 to 30mg/kg, 0.1 to 10m/kg, or 1.0 to 10 mg/kg.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may cause tumor regression at a dose of 0.1mg/kg once per week.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may cause tumor regression with low frequency dosing.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may cause tumor regression with dosing once every three weeks.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may directly impact viability of tumor cells *in vitro*.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may elicit effector function *in vitro*.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may mediate their *in vivo* anti-tumor efficacy by an effector function-driven mechanism of action.

In some embodiments, the anti-TROP2 sdAbs of the present disclosure may mediate their *in vivo* anti-tumor efficacy by direct anti-tumor activity.

In some embodiments, the antigen binding fragment is partially or fully humanized.

In some embodiments, the antigen binding fragment comprises one or more human frameworks.

In some embodiments, the antigen binding domain is partially or fully humanized.

In some embodiments, the antigen binding domain comprises one or more human frameworks.

In other embodiments, the antigen binding domain comprises one or more humanized frameworks. In further embodiments, the antigen binding domain comprises one or more partially humanized frameworks.

In some embodiments, the level of humanization of a single domain antibody may be calculated based on the sequence identity to the closest human VH germline gene. Accordingly, the percentage of humanization score is based on framework regions 1-3 (FR1, FR2, FR3) and CDR1 and CDR2. In accordance with an embodiment, the sequence of CDR3 and FR4 are not accounted for assessing the level of humanization of a single domain disclosed herein.

In exemplary embodiments, the level of humanization of the antigen binding domain is at least 80%.

In other exemplary embodiments, the level of humanization of the variable region of the single domain antibody of the present disclosure is at least 80%.

Other aspects and embodiments of the present disclosure relate to a composition comprising the binding agent disclosed herein. In some embodiments, the composition of the present disclosure is composed of binding agent that have the same sequence.

Other aspects and embodiments of the present disclosure relate to a pharmaceutical composition comprising the binding agent disclosed herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition of the present disclosure is composed of binding agent that have the same sequence.

Yet other aspects and embodiments of the present disclosure relate to a pharmaceutical composition comprising a conjugated binding agent as disclosed herein and a pharmaceutically acceptable carrier.

Yet other aspects and embodiments of the present disclosure relate to a nucleic acid or set of nucleic acids encoding the binding agent disclosed herein.

Additional aspects and embodiments of the present disclosure relate to a vector comprising a nucleic disclosed herein or a set of vectors each comprising a nucleic acid disclosed herein

Further aspects and embodiments of the present disclosure relate to a cell expressing the binding agent disclosed herein.

In some embodiments, the cells are engineered to express a binding agent such as for example, an antibody or an antigen binding fragment thereof or an antibody-like molecule.

Accordingly, the present disclosure encompasses isolated cells that express an antibody or an antigen binding fragment thereof or an antibody-like molecule.

In other embodiments, the cells are engineered to express a binding agent such as for example, a chimeric antigen receptor (CAR) construct. Accordingly, the present disclosure encompasses T-cells or NK cells that express a chimeric antigen receptor (CAR) construct.

Additional aspects and embodiments of the present disclosure relate to a cell comprising the nucleic acid or the vector disclosed herein.

Further aspects and embodiments of the present disclosure relate to a kit comprising the binding agent disclosed herein.

In some embodiments, the kit may comprise a binding agent that binds to TROP2 and a binding agent that binds to a macrophage marker. For example, the kit may comprise an antibody or an antigen binding fragment thereof that specifically binds to TROP2. In some instances, the kit may comprise a binding agent as described herein. The kit may also comprise an antibody that binds to a macrophage marker, such as for example and without limitations an antibody or antigen binding fragment thereof that binds to CD68 (human CD68).

Yet further aspects and embodiments of the present disclosure relate to a kit comprising the nucleic acid disclosed herein.

Yet further aspects and embodiments of the present disclosure relate to a kit comprising the vector disclosed herein.

Yet further aspects and embodiments of the present disclosure relate to a kit comprising the cells disclosed herein.

In an additional aspect and embodiments, the present disclosure relates to a method of treating a disorder or disease comprising administering the binding agent disclosed herein.

In further aspects and embodiments, the present disclosure relates to a method of treating a disorder or disease comprising administering the composition disclosed herein.

In further aspects and embodiments, the present disclosure relates to a method of treating a disorder or disease comprising administering the pharmaceutical composition disclosed herein.

In some embodiments, the TROP2 binding agent, composition or pharmaceutical composition disclosed herein is administered to a subject having cancer selected from epithetial cancer, lung cancer (such as small cell lung cancer, non small cell lung cancer), myeloma, prostate cancer, breast cancer (such as triple negative breast cancer), rectal cancer, pancreatic cancer, glioblastoma, cervical cancer, colorectal cancer, gastric cancer, ovarian cancer, thyroid cancer, stomach cancer, cancer of the urinary bladder, cancer of the uterus, esophageal cancer, head and neck cancer, blood cancer.

In other embodiments, the TROP2 binding agent, composition or pharmaceutical compositions disclosed herein is administered to a subject having a metastatic cancer.

In accordance with the present disclosure, the binding agent, composition or pharmaceutical composition may be administered to a subject having a tumor expressing TROP2.

Other aspects of the disclosure relate to a method of treating cancer, which comprises administering a drug that targets human TROP2 to a subject having a tumor expressing TROP2 and expressing one or more macrophage markers.

In some instances, the drug may be a binding agent as described herein. In other instances, the binding agent may be Sacituzumab Govitecan, hTINA-1, h7E6, and AR47A6.4.2. In other aspects, the binding agent may be a small molecule.

In some embodiments, the macrophage marker is a pan macrophage marker such as, for example and without limitations, CD68.

Therefore, in accordance with the present disclosure, the binding agent, composition or pharmaceutical composition may be administered to a subject having a tumor expressing TROP2 and a macrophage marker such as CD68.

In some instances, the eligibility of a subject to a treatment targeting TROP2 may be determined. For example, the tumor of the subject may be tested for expression of TROP2 and/or for expression of a macrophage marker prior to the administration of the binding agent, composition or pharmaceutical composition described herein.

In other instances, the efficacy of the treatment may be assessed. For example, the tumor of the subject may be tested for expression of TROP2 and for expression of a macrophage marker

during and/or after treatment with the binding agent, composition or pharmaceutical composition described herein.

In other aspects and embodiments, the present disclosure relates to a method of making the binding agent disclosed herein by transforming cells with one or more vectors comprising the nucleic acid disclosed herein.

In some embodiments, the method may further comprise isolating and/or purifying the binding agent from impurities.

In some embodiments, the method further comprises conjugating the binding agent with a therapeutic moiety, detectable moiety, or a protein allowing an extended half-life or to nanoparticles.

The present disclosure also relates to the use of the binding agent disclosed herein for detecting TROP2 in tumor cells or tumor tissue.

In exemplary embodiments, the binding agent may have the CDR amino acid sequence of SEQ ID NO:152.

In another exemplary embodiments, the binding agent may have the CDR amino acid sequence of SEQ ID NO:153.

In another exemplary embodiments, the binding agent may have the CDR amino acid sequence of SEQ ID NO: 154.

In exemplary embodiments, the binding agent may have an amino acid sequence having at least 80% sequence identity with the variable region of any one of SEQ ID NO:152, SEQ ID NO:153 or SEQ ID NO:154.

In exemplary embodiments, the binding agent may have one or more CDRs of any one of SEQ ID NO:152, SEQ ID NO:153 or SEQ ID NO:154.

In exemplary embodiments, the binding agent may have the CDR1, CDR2 and CDR3 amino acid sequence of any one of SEQ ID NO:152, SEQ ID NO:153 or SEQ ID NO:154.

In exemplary embodiments, the binding agent may have an amino acid sequence identical to the variable region of any one of SEQ ID NO:152, SEQ ID NO:153 or SEQ ID NO:154.

In yet other exemplary embodiments, the binding agent may have an amino acid sequence identical to any one of SEQ ID NO:152, SEQ ID NO:153 or SEQ ID NO:154.

The present disclosure also relates to method for identifying a binding agent capable of binding to human TROP2 and having anti-tumor activity as described herein.

Further scope, applicability and advantages of the present disclosure will become apparent from the non-restrictive detailed description given hereinafter. It should be understood, however, that this detailed description, while indicating exemplary embodiments of the disclosure, is given by way of example only, with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-D: graphs illustrating the tumor volume over time of SCID mice bearing MDA-MB-453 (**Figure 1A**), MDA-MB-231 (**Figure 1B**), BxPC-3 (**Figure 1C**) or T.Tn (**Figure 1D**) treated with the KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs or with PBS.

Figure 1E: histogram showing binding of KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs to MDA-MB-453, MDA-MB-231, BxPC3, and A375 cells compared with AR47A6.4.2 anti-TROP2 monoclonal antibody as analyzed by flow cytometry. Negative control is sdAb that binds HEWL (NC).

Figure 2A: graph of *in vivo* dose/response study of weekly treatment of KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs at doses of 0.5mg/kg, 2mg/kg, 8 mg/kg and 30mg/kg i.p. in MDA-MB-453 implanted NCG mice tumor model. Negative control is PBS.

Figure 2B: graph of *in vivo* weekly treatment of large tumor with KD004 at 8mg/kg, once weekly (QW) i.p. in MDA-MB-453 implanted NCG mice tumor model. Negative control is PBS. Treatment was discontinued at around day 70, and replaced with PBS, and the negative control treated mice were treated with KD004 at 8mg/kg QW.

Figure 2C: graph showing the anti-tumor effect of various treatment regimen with KD004 at a dose of 8mg/kg with the indicated frequencies i.p. in MDA-MB-453 implanted NCG mice tumor model. Negative control is PBS.

Figure 2D: graph showing the anti-tumor effect of various treatment regimen with KD002 or KD005 at a dose of 0.1mg/kg i.p. in MDA-MB-453 implanted NCG mice tumor model. Negative control is PBS.

Figure 2E: Anti-TROP2 *in vivo* pharmacokinetic parameters.

Figure 3: graph showing tumor volume over time of MDA-MB-453 implanted NCG mice treated with KD004 at 8 mg/kg i.p. at the indicated intervals in the presence or absence of human PBMCs. Negative control is PBS.

Figure 4A: histogram showing cell viability of MDA-MB-453 cells following 12 days treatment with 1 μM KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs in comparison with AR47A6.4.2 anti-TROP2 monoclonal antibody. Negative controls include sdAb that binds HEWL (NC) or untreated cells.

Figure 4B image of MDA-MB-453 cells at 4x resolution after 12 days of treatment with KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAb. Negative controls include sdAb that binds HEWL (NC) or untreated cells.

Figure 5A: histogram showing cell viability of MDA-MB-453 cells reflecting the ADCC activity of KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs. Negative controls include untreated cells (No Ab), no effector cells, no target cells or sdAb that binds HEWL (NC). Comparisons includes cells treated with human IgG1 anti-TROP2 antibodies generated with the sequences from clones hTINA-1 and h7E6.

Figure 5B: sdAbs tumor growth inhibition (TGI) was tested *in vivo* in NCG and SCID mice having different effector function background and implanted with either MDA-MB-231 or MDA-MB-453 cells s.c. Administration of KD002 or KD004 was initiated when average tumor volume reached 100mm^3.

Figures 6A-B: histograms illustrating internalization of KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs (**Figure 6A**) or AR47A6.4.2 (**Figure 6B**) in MDA-MB-453 cells after 4 or 24 hours. Negative controls include sdAb that binds HEWL (NC) or medium only.

Figures 6C: graph illustrating internalization of KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs in MDA-MB-453. Internalization was assessed by conjugation of HCAbs with a pH-sensitive probe and monitored by flow cytometry (Em. 560nm).

Figure 6D: Confocal microscopy images of MDA-MB-453 cells showing KD002 and KD005 antibody binding and internalization were taken at 0, 6 and 24 hours. For each time point, one representative cell is shown. The cells were stained with anti-human IgG Fc-FITC and the nuclei were counterstained with DAPI. Images were obtained using confocal fluorescence microscopy. Scale bar, 10μM.

Figures 7A-D: binding of KD001, KD002, KD003, KD004, KD005, KD006 or KD007 anti-TROP2 sdAbs to CHO (**Figure 7A**) or HEK parental cells (**Figure 7C**) or CHO (**Figure 7B**) or HEK cells expressing human TROP2 (**Figure 7D**) compared with negative control (sdAb that binds HEWL (NC)) or comparator monoclonal antibodies AR47A6.4.2, hTINA-1, h7E6 and hRS7 as assessed by flow cytometry.

Figure 7E: Bar graph illustrating binding of KD001, KD002, KD003, KD004, KD005, KD006 or KD007 anti-TROP2 sdAbs to recombinant human EpCAM (rhEpCAM) and to recombinant human IGFBP-1 (rhIGFB-1) proteins.

Figure 8: graph illustrating competition assays performed with either His tag conjugated KD001 (**Figure 8A**), KD002 (**Figure 8B**), KD003 (**Figure 8C**), KD004 (**Figure 8D**) or KD005 (**Figure 8E**) and each of KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs for binding to human recombinant TROP2.

Figure 9A: graph illustrating competition assays between His tag conjugated KD004 and hRS7 or AR47A6.4.2 antibodies for the binding of human recombinant TROP2.

Figure 9B: graph illustrating competition assays between His tagged KD006 and KD007 anti-TROP2 sdAbs for the binding of human recombinant TROP2.

Figure 10A: schematic showing the various constructs used to map the binding domain of KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs.

Figure 10B: picture of immunoblots performed with KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs or with AR47A6.4.2 and hRS7 on human TROP2 extracellular domain (ECD) and human TROP2 cysteine poor domain (CPD). Negative control includes sdAb that binds HEWL (NC).

Figures 11A-E: pictures of immunoblots performed with KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs on different forms of recombinant human TROP2 (non-denaturated, linear, reduced, deglycosylated using PNGase F).

Figures 12A-12D: graph showing binding of KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs on recombinant cynomolgus/Rhesus TROP2 protein (**Figure 12A**), on recombinant human TROP2 protein (**Figure 12B**), on mouse TROP2 protein (**Figure 12C**) or on rat TROP2 protein (**Figure 12D**). Negative control includes sdAb that binds HEWL. Positive control includes anti-TROP2 polyclonal antibody.

Figure 12E: table illustrating affinity of KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs to human TROP2 and to cynomolgus TROP2 by SPR.

Figure 12F: table illustrating affinity of KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs to human TROP2 with ka, kd and KD indicated.

Figure 13A-13B: histogram showing the binding of the indicated TROP2 antibodies at 100 nM to HEK cells transfected with TROP2 constructs with various cysteine-rich domain truncations as indicated.

Figure 13C: graph showing the tumor growth of in the xenograft model of T.Tn in the SCID mice upon treatment with anti-TROP2 antibodies at 8 mg/kg i.p., once per week. Treatment with KD006 led to a tumor growth inhibition of 48.6% in the xenograft model of T.Tn.

Figure 14A: graph of the antibody-mediated cellular phagocytosis activity of IgG1- or IgG4 versions of KD002 and KD004 against MDA-MB-453 tumors.

Figure 14B: graph showing CD47 and TROP2 expression level on MDA-MB-453, MDA-MB-231, and NCI-H292 cells measured by staining with anti-CD47 (Biolegend, cat# 323124) and anti-TROP2 (Biolegend, cat# 363804) antibodies at 1/100 dilutions. Data are shown as fold change of CD47 and TROP2 level relative to the isotype control staining.

Figure 14C-E: graphs showing the dose-response ADCP activity of KD002 and KD004 TROP2 antibodies against the MDA-MB-453 (**Figure 14C**), MDA-MB-231 (**Figure 14D**), and NCI-H292 (**Figure 14E**) cells. The ADCP assays were performed with the Promega ADCP assay kits (Promega, cat# CS314906). Testing antibodies were serial diluted from 1.88 nM with 1/2.2 dilution. TROP2 antibodies KD002 and KD004 showed a high level of ADCP activity against

MDA-MB-453 cells, whereas relatively low ADCP activity on MDA-MB-231 and NCI-H292 cells.

Figure 14F: graph illustrating the ADCP activity of KD002 and KD004 and the corresponding IgG4 version; KD017 and KD018 against MDA-MB-453, MDA-MB-231, and NCI-H292 cells. Assay was performed at the antibody concentration of 1 nM.

Figure 15A: graph illustrating the CD47 expression level on MDA-MB-231 and NCI-H292 cells after CD47 knockdown with siRNA. The CD47 siRNA and control siRNA were used in the knockdown assay (Thermofisher CD47 silencer select pre-designed siRNA (Cat# 4392421) and silencer negative control siRNA (Cat# AM4635).

Figure 15B-C: graphs showing the dose-response ADCP activity of KD002 and KD005 TROP2 antibodies against the MDA-MB-231 (**Figure 15B**), and NCI-H292 cells (**Figure 15C**) upon CD47 knockdown. Testing antibodies were serial diluted from 2.1 nM with 1/2.5 dilution.

Figure 15D: graphs illustrating the ADCP activity of KD002 and KD005 and the corresponding IgG4 version, KD017 and KD019 against the MDA-MB-453, MDA-MB-231, and NCI-H292 cells upon inhibition of CD47 expression with siRNA (siCD47) or not (siNC). Assay was performed at the antibody concentration of 1 nM.

Figure 15E: graphs showing *in vivo* efficacy of TROP2-CD47 bispecific antibodies in the xenograft model of MDA-MB-231 in SCID mice. Mice were treated with KD004 at 8 mg/kg, once *per* week and KD065 and KD066 were administered at the same molarity (11 mg/kg) i.p.

Figure 16A: plots showing the THP-1 differentiated M1 and M2 macrophages upon treatment with PMA and cytokines. THP-1 cells showed upregulation of CD68 after PMA treatment. The M1 cells showed upregulation of CD38 and CD86 after treatment with IFN-γ and LPS. M2 macrophages showed upregulation of CD206 and downregulation of CD38 after treatment with IL-4 and IL-13.

Figure 16B: bar graph showing the percentage of phagocytosed M1 or M2 macrophages upon treatment with KD002, KD004 or KD005 at 10 nM as illustrated by gating on the pHrodo positive and Celltrace Violet positive cells.

Figure 17A: bar graph illustrating the binding of TROP2 antibodies at 25 nM to TROP2 recombinant proteins by ELISA using an anti-IgG4 Fc secondary antibody.

Figure 17B: graph showing the anti-tumor effect of KD002 and KD017 at a dose of 8 mg/kg i.v. in MDA-MB-453 implanted NCG mice tumor model. Negative control is PBS.

Figure 18A: graph showing the dose-response ADCP activity of KD002 and KD005 TROP2 antibodies against the MDA-MB-453 (upper panel), MDA-MB-468 (middle panel), and T.Tn cells (lower panel). The ADCP assays were performed using the Promega ADCP assay kits (Promega, cat# CS314906).

Figure 18B: graph showing the anti-tumor effect of KD002 and KD005 in the MDA-MB-453 (upper panel), MDA-MB-468 (middle panel), and T.T.n (lower panel) xenograft tumor models. Testing antibodies were administered with 8 mg/kg i.p., once per week.

Figure 19A: Photomicrographs of IHC staining of mouse macrophages with F4/80 antibody on the xenograft tumors collected from COLO205, CAPAN1, MDA-MB-231, T.T.n and MDA-MB-453 xenograft models treated with the TROP2 antibodies KD002 and KD005 at the dose level of 8 mg/kg. Tumors were collected at the end of the study.

Figure 19B: Human colon adenocarcinoma tumor tissue microarray with KD067, KD068 or isotype control KD069 antibody. The human multiple tumor tissue microarray was purchased from Novus Biologics (cat# NBP2-42056) and stained with the antibodies at 2 µg/ml.

Figure 19C: Human breast invasive ductal carcinoma tumor tissue microarray with KD067, KD068 or isotype control KD069 antibody. The human multiple tumor tissue microarray was purchased from Novus Biologics (cat# NBP2-42052) and stained with the antibodies at 2 µg/ml.

Figure 19D: Human head and neck tumor tissue microarray with KD067, KD068 or isotype control KD069 antibody (H&N squamous cell carcinoma). The human multiple tumor tissue microarray was purchased from Novus Biologics (cat# NBP2-42056) and stained with the antibodies at 2 µg/ml.

Figure 19E: Human kidney transitional cell carcinoma microarray with KD067, KD068 or isotype control KD069 antibody. The human multiple tumor tissue microarray was purchased from Novus Biologics (cat# NBP2-42052) and stained with the antibodies at 2 µg/ml.

Figure 20A: X-ray crystal structure of the extracellular domain of TROP2 protein with translucent surface rendering. The relative position of the Cystein-Rich Domain (CRD), Thyroglobulin Domain (TY-1), and Cystein-Poor Domain (CPD) are indicated.

Figure 20B: X-ray cocrystal of KD012 with human TROP2 (surface rendering shown). The contact region on TROP2 is shown on the surface of TROP2 in dark grey. The contacts between KD012 and TROP2 occur mainly in the CRD of TROP2 and CDR3 and framework 2 of KD012. CDRs and framework 2 are indicated with arrows.

Figure 20C: X-ray cocrystal structure of KD012 with human TROP2. KD102 is depicted as a ribbon diagram. Selected residues on KD012 are shown, with the interacting residues on TROP2 shown in dark grey.

Figure 20D: shows amino acid residues involved in the interaction between KD012 and human TROP2 (within 4.5 Å; CDRs annotated in accordance with IMGT numbering).

Figure 21A-21B: represents dose-response ELISA binding of KD002 and humanized variants on human TROP2 recombinant protein. TROP2 antibodies were serially diluted from 200 nM to 0.00256 nM with 1/5 dilutions.

Figure 22A-22B: represents dose-response ELISA binding of KD005 and humanized variants on human TROP2 recombinant protein. TROP2 antibodies were serially diluted from 200 nM to 0.00256 nM with 1/5 dilutions.

Figures 23A-23B: bar graphs illustrating the relative light units of KD002 humanized variants compared to KD002 at 0.5nM (**Figure 23A**) or 0.1nM (**Figure 23B)** as measured with the Promega ADCP reporter assays.

Figures 24A-24B: bar graphs showing the relative light units of KD005 and KD005 humanized variants at 0.5nM (**Figure 24A**) and 0.1nM (**Figure 24B**) as measured with the Promega ADCP reporter assays.

Figures 24C-24I: dose-response ADCP assays carried out in MDA-MB-453 cells with KD045 (**Figure 24C**), KD047 (**Figure 24D**), KD048 (**Figure 24E**), KD049 (**Figure 24F**), KD053 (**Figure 24G**), KD059 (**Figure 24H**) and KD063 (**Figure 24I**).

Figures 25-25B: bar graphs showing the relative light units of KD002 humanized variants compared to KD002 at 0.5nM (**Figure 25A**) or 0.1nM (**Figure 25B**) as measured with the Promega ADCC reporter assays.

Figures 26A-26B: bar graphs showing the relative light units of KD005 and KD005 humanized variants at 0.5nM (**Figure 26A**) and 0.1nM (**Figure 26B**) as measured with the Promega ADCC reporter assays.

Figure 27: Alignment between KD001, KD002, KD003, KD004 and KD005 variable regions.

Figure 28: Alignment and consensus sequences of KD002 variants.

Figure 29: Alignment of KD002 variants selected for their ADCC and ADCP activity.

Figure 30: Alignment and consensus sequences of KD005 variants.

Figure 31: Alignment of KD005 variants selected for their binding activity.

Figure 32: Alignment of KD005 variants selected for their ADCP activity.

Figure 33: Alignment of KD005 variants selected for their ADCC activity.

Figure 34: Alignment of KD005 variants selected based on all characteristics.

DETAILED DESCRIPTION

Definitions

The use of the terms "a" and "an" and "the" and similar referents in the context of describing embodiments (especially in the context of the claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

Unless specifically stated or obvious from context, as used herein the term “or” is understood to be inclusive and covers both “or” and “and”.

The term “and/or” where used herein is to be taken as specific disclosure of each of the specified features or components with or without the other.

The terms "comprising", "having", "including", and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to") unless otherwise noted. The term “consisting of” is to be construed as close-ended.

As used herein the term "TROP2" refers to a TROP2 protein or to a polypeptide comprising the TROP2 amino acid sequence. The term "TROP2" encompasses the human TROP2 protein and any protein having at least 80% identity with the human TROP2 protein.

The term "anti- TROP2 antibody" refers to an antibody of any format that binds to the TROP2 protein such as a TROP2 antigen or to a portion thereof and includes without limitations "anti- TROP2 single domain antibody".

The term "binding agent" refers to a molecule that comprises a polypeptidic portion (such as polypeptide chain(s)) and that is capable of specifically binding an antigen or that comprises a domain such as an antigen binding domain that is capable of specifically binding an antigen.

The term "antibody" is used in the broadest sense and encompasses various antibody formats and structures, including any immunoglobulin, monoclonal antibody, polyclonal antibody, bivalent antibody, monovalent antibody, bispecific antibody, multiple specific (multi-specific) antibody, conventional antibody, single domain antibody, single chain antibody, heavy chain only antibody, nanobody, full-length antibody, humanized antibody, chimeric antibody that binds to a specific antigen, and any antigen binding fragment that exhibits the desired antigen binding activity. An antibody can be naturally occurring (native) or the results or recombination technologies.

As used herein the expressions "single domain antibody" and "heavy chain only antibody" are used interchangeably. The term "single domain antibody" includes naturally occurring single domain antibody or heavy chain only antibody as well as single domain antibody in which the naturally occurring constant region is replaced by another dimerization domain such as for example a constant region of a human immunoglobulin (e.g., IgG) as well as variants such as humanized or chimeric single domain antibodies.

A "naturally occurring single domain antibody" includes antibodies produced by camelids (camelid antibodies) or by shark. "Naturally occurring single domain antibody(ies)" also encompass antibody(ies) that are produced by transgenic animals modified to express heavy chain only antibodies. Exemplary embodiments of transgenic animals are provided in international application No. PCT/CA2021/050951 filed on July 21, 2021, and published on January 20, 2022, under No. WO2022/011457, the entire content of which is incorporated herein by reference.

It is to be understood herein that any of the terms "KD001", "KD002", "KD003", "KD004", "KD005", "KD006", KD007", "KD008", "KD009", "KD010", "KD011", "KD012", "KD013", "KD014", "KD015", "KD016", "KD017", "KD018", "KD019", "KD020", "KD021", "KD022", "KD023", "KD024", "KD025", "KD026", "KD027", "KD028", "KD029", "KD030", "KD031", "KD032", "KD033", "KD034", "KD035", "KD036", "KD037", "KD038", "KD039", "KD040", "KD041", "KD042", "KD043", "KD044", "KD045", "KD046", "KD047", "KD048", "KD049", "KD050", "KD051", "KD052", "KD053", "KD054", "KD055", "KD056", "KD057", "KD058", "KD059", "KD060", "KD061", "KD062", "KD063", "KD064", "KD067", "KD068" or "KD069" refer to a single domain antibody that comprises two heavy chains, each having the corresponding heavy chain amino acid sequence set forth in Table 15, 16, 18 or 19 or, each having the corresponding variable region amino acid sequence set forth in Table 15, 16, 18 or 19.

As such, in some embodiments any one of KD001, KD002, KD003, KD004, KD005, KD006, KD007, KD008, KD009, KD010, KD011, KD012, KD013, KD014, KD015, KD016, KD017, KD018, KD019, KD020, KD021, KD022, KD023, KD024, KD025, KD026, KD027, KD028, KD029, KD030, KD031, KD032, KD033, KD034, KD035, KD036, KD037, KD038, KD039, KD040, KD041, KD042, KD043, KD044, KD045, KD046, KD047, KD048, KD049, KD050, KD051, KD052, KD053, KD054, KD055, KD056, KD057, KD058, KD059, KD060, KD061, KD062, KD063, KD064, KD067, KD068 or KD069 may have the corresponding variable region amino acid sequence set forth in Table 15, 16, 18 or 19 and a constant region that differs from that of the heavy chain amino acid sequence set forth in Table 15, 16, 18 or 19.

In other embodiments, any one of KD001, KD002, KD003, KD004, KD005, KD006, KD007, KD008, KD009, KD010, KD011, KD012, KD013, KD014, KD015, KD016, KD017, KD018, KD019, KD020, KD021, KD022, KD023, KD024, KD025, KD026, KD027, KD028, KD029, KD030, KD031, KD032, KD033, KD034, KD035, KD036, KD037, KD038, KD039, KD040, KD041, KD042, KD043, KD044, KD045, KD046, KD047, KD048, KD049, KD050, KD051, KD052, KD053, KD054, KD055, KD056, KD057, KD058, KD059, KD060, KD061, KD062, KD063, KD064, KD067, KD068 or KD069 may have the corresponding variable region amino acid sequence set forth in Table 15, 16, 18 or 19 and a constant region allowing formation of homodimers as described herein.

In yet other embodiments, any one of KD001, KD002, KD003, KD004, KD005, KD006, KD007, KD008, KD009, KD010, KD011, KD012, KD013, KD014, KD015, KD016, KD017, KD018, KD019, KD020, KD021, KD022, KD023, KD024, KD025, KD026, KD027, KD028, KD029, KD030, KD031, KD032, KD033, KD034, KD035, KD036, KD037, KD038, KD039, KD040, KD041, KD042, KD043, KD044, KD045, KD046, KD047, KD048, KD049, KD050, KD051, KD052, KD053, KD054, KD055, KD056, KD057, KD058, KD059, KD060, KD061, KD062, KD063, KD064, KD067, KD068 or KD069 may have the corresponding variable region amino acid sequence set forth in Table 15, 16, 18 or 19 and a constant region that binds to a macrophage Fc receptor.

The terms "KD065" or "KD066" refer to a binding agent that comprises two chains, each having the corresponding amino acid sequence of the polypeptide chain set forth SEQ ID NO:150 or SEQ ID NO:151 respectively or each having the corresponding variable region amino acid sequence of the polypeptide chain.

In some embodiments, any one of KD065 or KD066 may have the corresponding variable region amino acid sequence of KD065 or KD66 and a different hinge and/or dimerization domain (e.g., CH2 and/or CH3) than KD065 or KD66.

The term "subject in need" as used herein refer to a subject having, suspected of having a disorder or disease associated with TROP2, TROP2 expression (e.g., expression in tissue, cells or serum) or TROP2 overexpression. The term "subject in need" also refers to a subject having, suspected of having a disorder or disease that may benefit from treatment with a binding agent targeting TROP2. A "subject in need" include a subject having cancer or suspected of having cancer.

The term "treatment" for purposes of this disclosure refers to both therapeutic treatment and prophylactic or tumor prevention measures. Subject in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. Subject in need of treatment thus include subjects that already have cancer as well as those prone to have cancer or those in whom cancer is to be managed.

The term "natural" as used in the context of a sequence refers to a sequence that occurs in nature.

The term "about" or "approximately" with respect to a given value means that variation in the value is contemplated. In some embodiments, the term "about" or "approximately" shall generally mean a range within +/- 10 percent, within +/- 5 percent, within +/- 4 percent, within +/- 3 percent, within +/- 2 percent or within +/- 1 percent of a given value or range.

It is to be understood herein, that expressions referring to ranges of values in the format such as "from A to B", include each individual value and any sub-range comprised and including such ranges. For example, the expression "from 1 to 10" includes sub-ranges such as and without limitations, "from 2 to 10", "from 2 to 9", "from 3 to 6", "from 5 to 7" and any individual values comprised between and including 1 and 10, *i.e.,* 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

It is to be understood herein that the term "at least" with respect to a given value intends to include the value and superior values. For example, the term "at least 80%" includes "at least 80%", "at least 81%", "at least 82%", "at least 83%", "at least 84%", "at least 85%", "at least 86%", "at least 87%", "at least 88%","at least 89%", "at least 90%", "at least 91%", "at least 92%", "at least 93%", "at least 94%", "at least 95%", "at least 96%", "at least 97%", "at least 98%", "at least 99%", "at least 99.1%", "at least 99.2%", at least 99.3%", at least 99.4%", at least 99.5%", at least 99.6%", at least 99.7%", at least 99.8%", at least 99.9%", and 100%.

As used herein, the term "humanized" means that the binding agent such as an antibody or antigen binding fragment comprises CDRs or CDR amino acid residues derived from non-human animal antibodies, FR regions derived from human antibodies, and when applicable, the constant regions derived from human antibodies.

As used herein the expression "fully humanized framework region" means that the amino acid sequence of all framework regions of a given binding agent such as an antibody or antigen binding fragment is identical to that of a human antibody variable region e.g., a human germline antibody variable region sequence).

As used herein the expression "partially humanized" means that the amino acid sequence of one or more or all framework regions of the binding agent such as an antibody or antigen binding fragment is not totally identical to that of a human antibody variable region such as a germline human antibody variable region (e.g., a human germline antibody variable region sequence).

In some instances, a humanized, fully humanized or partially humanized binding agent such as an antibody or antigen binding fragment may comprises CDRs or CDR amino acid residues of a human antibody variable region (e.g., a human germline antibody variable region sequence).

A "partially humanized framework region" thus encompasses, for example and without limitations, a human framework region that contains back-mutations reintroducing an original amino acid at its original location. A "partially humanized framework region" also encompasses, for example and without limitations, a human framework region in which one or more amino acid residue has been replaced, added or deleted.

As used herein, the term "affinity" refers to the strength of non-covalent interaction between a binding agent or antigen binding domain(s) thereof and an antigen. "Affinity" is represented, for example, by K_D value, i.e., the ratio of dissociation rate to association rate (k_{off}/k_{on}) when the binding between the antigen and the binding agent (e.g., antibody such as single domain antibody) reaches equilibrium. The affinity may be determined by using any conventional method known in the art, including but are not limited to, surface plasmon resonance method, microscale thermophoresis method, HPLC-MS method and flow cytometry (such as FACS) method. An antibody generally has a K_D value of $\leq 10^{-6}$ M (e.g., $\leq 5x10^{-7}$ M, $\leq 2x10^{-7}$ M, $\leq 10^{-7}$ M, $\leq 5x10^{-8}$ M, $\leq 2x10^{-8}$ M, $\leq 10^{-8}$ M, $\leq 5x10^{-9}$ M, $\leq 4x10^{-9}$M, $\leq 3x10^{-9}$M, $\leq 2x10^{-9}$ M, or $\leq 10^{-9}$ M and any value $\leq 10^{-6}$). Preferablly, an antibody has a K_D value in the nanomolar range or lower (e.g., $\leq 9x10^{-9}$ M, $\leq 8x10^{-9}$ M, $\leq 7x10^{-9}$ M, $\leq 6x10^{-9}$ M, $\leq 5x10^{-9}$ M, $\leq 4x10^{-9}$ M, $\leq 3x10^{-9}$ M, $\leq 2x10^{-9}$ M, $\leq 1x10^{-9}$ M or lower e.g., $\leq 1x10^{-10}$ M, $\leq 1x10^{-11}$ M, $\leq 1x10^{-12}$ M). Even more preferablly, an antibody has a K_D value in the picomolar range or lower (e.g., $\leq 9x10^{-12}$ M, $\leq 8x10^{-12}$ M, $\leq 7x10^{-12}$ M, $\leq 6x10^{-12}$ M, $\leq 5x10^{-12}$ M, $\leq 4x10^{-12}$ M, $\leq 3x10^{-12}$ M, $\leq 2x10^{-12}$ M, $\leq 1x10^{-12}$ M or lower).

The term "specific binding" or "specifically binds" as used herein refers to a non-random binding reaction between two molecules, such as for example between an antibody and an antigen. Specific binding can be characterized in binding affinity, A K_D value of $\leq 10^{-6}$ M (e.g., $\leq 5x10^{-7}$ M, $\leq 2x10^{-7}$ M, $\leq 10^{-7}$ M, $\leq 5x10^{-8}$ M, $\leq 2x10^{-8}$ M, $\leq 10^{-8}$ M, $\leq 5x10^{-9}$ M, $\leq 4x10^{-9}$M, $\leq 3x10^{-9}$M, $\leq 2x10^{-9}$ M, or $\leq 10^{-9}$ M and any value $\leq 10^{-6}$) can indicate specific binding between a binding agent (e.g., antibody such as single domain antibody) and TROP2 (e.g., human TROP2).

As used herein, the term "competing for binding", "compete for binding", "compete(s) with" or "competing with" refers to the ability of a first antibody or antigen-binding fragment thereof (e.g., single domain antibody or antigen binding fragment thereof) to inhibit the binding interaction between TROP2 and a second anti-TROP2 antibody (e.g., a single domain antibody or antigen binding fragment thereof) to any detectable degree.

As used herein the term "epitope" as used herein refers to the specific group of atoms or amino acid residues on an antigen to which an antibody (e.g., single domain antibody) binds. Two antibodies may bind the same or a closely related epitope within an antigen if they exhibit competitive binding for the antigen. An epitope can be linear or conformational (i.e., including amino acid residues spaced apart). For example, if an antibody or antigen binding fragment blocks binding of a reference antibody (e.g., single domain antibody) to the antigen by at least 85%, or at least 90%, or at least 95%, then the antibody or antigen-binding fragment may be considered to bind the same/closely related epitope as the reference antibody. For example, a monoclonal, chimeric, human or humanized antibody or an antigen binding fragment thereof may compete with a single domain antibody of the present disclosure for binding to human TROP2.

As used herein, the term "sequence identity" of the present invention indicates the degree of identity between two nucleic acid or two amino acid sequences when best compared and compared when a mutation such as substitution, insertion or deletion is appropriate. The sequence identity can be at least 85 %, 90 % or 95 %, preferably at least 95 %. Non-limiting examples include 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 95 %, 96 %, 97 %, 98 %, 99 %, and 100 %.

The term "antigen binding fragment(s)" as used in the expression "antibody or antigen binding fragment thereof" or "single domain antibody or antigen binding fragment thereof" refers to a fragment of the antibody or single antibody that encompasses the antigen binding domain and that may incorporate or not, other portion(s) of the antibody or single domain antibody such as for example amino acid residues of the hinge region, amino acid residues of a constant region, portion of a Fc region. Regardless of structure, an antigen binding fragment binds to the same antigen that is recognized by the complete antibody (e.g., single domain antibody).

An antigen binding fragment of a single domain antibody includes for example the CDRs and sequences that encompass the CDRs such as the entire variable region or portion thereof or the entire heavy chain or portion thereof and may include or not, other portion(s) of the antibody.

The term "antigen binding domain" relates to the portion of an antibody that is involved in antigen binding and comprises for example, one or more CDRs, one or more framework regions (FR) or the entire variable region. The term "antigen binding domain" in the context of a single domain antibody thereof relates to the portion of single domain antibody that is involved in antigen binding and comprises for example, one or more of CDR1 (CDRH1), CDR2 (CDRH2) or CDR3 (CDRH3), one or more framework regions FR1, FR2, FR3, FR4 or the entire variable region (VH or VHH). The term "antigen binding domain" " in the context of a native antibody thereof relates to the portion of a native antibody that is involved in antigen binding and comprises for example, one or more of CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 or CDRL3, one or more light chain or heavy chain framework regions FR1, FR2, FR3, FR4 or one or both entire variable regions (heavy chain variable region (VH) and/or light chain variable region (VL)).

TROP2 antigens

Antibodies or antigen binding fragments of the present disclosure may be obtained, for example, by immunizing an animal with a TROP2 antigen.

Alternatively, the antibodies or antigen binding fragments of the present disclosure may be obtained from a library of antibodies.

Binding agents that bind to TROP2 may be identified by *in silico*, *in vitro* and/or *in vivo* methods. Several *in vitro* methods rely on the binding of candidate antibodies or antigen binding fragments thereof to an antigen. As such anti-TROP2 antibodies or antigen binding fragments thereof may be identified by methods involving binding to the TROP2 antigen disclosed herein and/or using assays disclosed herein.

In an embodiment, the TROP2 antigen is a human TROP2, a TROP2 homologue, a TROP2 variant having a sequence at least 80% identical to human TROP2 thereof or a fragment thereof.

In an exemplary embodiment, the TROP2 homologue is cynomolgus TROP2.

In another exemplary embodiment, the TROP2 homologue is Rhesus TROP2.

In some embodiments, the TROP2 antigen comprises the extracellular domain of TROP2 (e.g., of human TROP2, TROP2 homologue or variant thereof).

In some embodiments, the TROP2 antigen comprises amino acid residues 27-274 of human TROP2, or of a TROP2 homologue or a fragment thereof. Accordingly, the TROP2 antigen may comprise a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-274 or a fragment thereof.

In other embodiments, the TROP2 antigen comprises amino acid residues 27-146 of human TROP2, or of a TROP2 homologue or a fragment thereof. Accordingly, the TROP2 antigen may comprise a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-146 or a fragment thereof.

In yet other embodiments, the TROP2 antigen comprises amino acid residues 27-73 of human TROP2, or of a TROP2 homologue or a fragment thereof. Accordingly, the TROP2 antigen may comprise a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-73 or a fragment thereof.

In accordance with the present disclosure, the TROP2 antigen may comprise at least 10 amino acid residues of human TROP2.

More particularly, the TROP2 antigen is a fragment of at least 10 amino acid residues of a human TROP2 amino acid sequence consisting of amino acid residues 27-146.

In accordance with the present disclosure the TROP2 antigen comprises between 10 to 110 amino acid residues, including for example between 10 to 100 amino acid residues, between 10 to 90 amino acid residues, between 10 to 80 amino acid residues, between 10 to 70 amino acid residues, between 10 to 60 amino acid residues, between 10 to 50 amino acid residues, between 10 to 40 amino acid residues, between 10 to 30 amino acid residues, 10 to 29 amino acid residues, between 10 to 28 amino acid residues, between 10 to 27 amino acid residues, between 10 to 26 amino acid residues, between 10 to 25 amino acid residues, between 10 to 24 amino acid residues, between 10 to 23 amino acid residues, between 10 to 22 amino acid residues, between 10 to 21 amino acid residues, between 10 to 20 amino acid residues, between 10 to 19 amino acid residues, between 10 to 18 amino acid residues, between 10 to 17 amino acid residues, between 10 to 16 amino acid residues, between 10 to 15 amino acid residues, between 10 to 14 amino acid residues,

between 10 to 13 amino acid residues, between 10 to 12 amino acid residues between 10 to 11 amino acid residues of a human TROP2 amino acid sequence consisting of amino acid residues 27-146.

For instances, the TROP2 antigen comprises at least 10 amino acid residues of a human TROP2 amino acid sequence consisting of amino acid residues 27-73.

In accordance with the present disclosure the TROP2 antigen comprises between 10 to 45 amino acid residues, including for example between 10 to 40 amino acid residues, between 10 to 35 amino acid residues, between 10 to 30 amino acid residues, 10 to 29 amino acid residues, between 10 to 28 amino acid residues, between 10 to 27 amino acid residues, between 10 to 26 amino acid residues, between 10 to 25 amino acid residues, between 10 to 24 amino acid residues, between 10 to 23 amino acid residues, between 10 to 22 amino acid residues, between 10 to 21 amino acid residues, between 10 to 20 amino acid residues, between 10 to 19 amino acid residues, between 10 to 18 amino acid residues, between 10 to 17 amino acid residues, between 10 to 16 amino acid residues, between 10 to 15 amino acid residues, between 10 to 14 amino acid residues, between 10 to 13 amino acid residues, between 10 to 12 amino acid residues between 10 to 11 amino acid residues of a human TROP2 amino acid sequence consisting of amino acid residues 27-73.

In some instances, the TROP2 antigen comprises a human TROP2 amino acid sequence consisting of amino acid residues 27-56 of human TROP2 or a fragment thereof.

In other instances, the TROP2 antigen comprises a human TROP2 amino acid sequence consisting of amino acid residues 27-66 of human TROP2 or a fragment thereof.

In yet other instances, the TROP2 antigen comprises a human TROP2 amino acid sequence consisting of amino acid residues 47-56 of human TROP2 or a fragment thereof.

In other instances, the TROP2 antigen comprises a human TROP2 amino acid sequence consisting of amino acid residues 60-66 of human TROP2 or a fragment thereof.

In yet other instances, the TROP2 antigen comprises a human TROP2 amino acid sequence consisting of amino acid residues 47-66 of human TROP2 or a fragment thereof.

For example, the TROP2 antigen comprises a human TROP2 amino acid sequence consisting of amino acid residues 27-56, 27-66, 28-66, 29-66, 30-66, 31-66, 32-66, 33-66, 34-66, 35-66, 36-66, 37-66, 38-66, 39-66, 40-66, 41-66, 42-66, 43-66, 44-66, 45-66, 46-66 or 47-66.

In some instances, the TROP2 antigen may exclude the cysteine poor (CPD) domain of TROP2.

An exemplary and non-limiting embodiment of a TROP2 antigen is provided in SEQ ID NO:164.

Binding agents

Exemplary embodiments of binding agents of the present disclosure includes a polypeptide chain disclosed herein.

Exemplary embodiments of binding agents of the present disclosure includes antibodies or antigen binding fragments thereof.

Exemplary embodiments of binding agents therefore include without limitations, native antibodies or antigen binding fragments thereof or single domain antibodies or antigen binding fragments thereof or variants thereof such as for example, humanized, chimeric or human antibodies or antigen binding fragments thereof or humanized or chimeric single domain antibodies or antigen binding fragments thereof.

Single domain antibodies or antigen binding fragments thereof disclosed herein are particularly contemplated as well as binding agents that comprise one or more antigen binding domain(s) of one or more single domain antibodies disclosed herein.

Single domain antibodies that compete with one or more single domain antibodies (having an amino acid sequence as disclosed herein) for the binding of a TROP2 antigen are also encompassed by the present disclosure.

Other exemplary embodiments of binding agents of the present disclosure include binding agents comprising antigen binding domain(s) such as antibody-like molecules (including single chain antibody), protein scaffold molecules and immune cell modulating agents.

Accordingly, exemplary embodiments of binding agents of the present disclosure include antibody-like molecules that comprise one or more antigen binding domain(s) of one or more single antibodies disclosed herein.

Additional exemplary embodiments of binding agents of the present disclosure include protein scaffold molecules that comprise one or more antigen binding domain(s) of one or more single antibodies disclosed herein.

Yet additional exemplary embodiments of binding agents of the present disclosure comprises immune cell modulating agents that comprise one or more antigen binding domain(s) of one or more single antibodies disclosed herein.

Binding agents including for example and without limitations, antibodies or antigen binding fragments thereof as well as single domain antibodies or antigen binding fragments thereof that competes with of a single domain antibody having an amino acid sequence disclosed herein are also encompassed by the present disclosure.

The present disclosure thus relates to a binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains comprises an antigen binding fragment thereof that is capable of binding to trophoblast cell surface antigen-2 (TROP2).

In some embodiments, the binding agent of the present disclosure is capable of binding to the extracellular domain (ECD) of TROP2.

In some embodiments, the binding agent of the present disclosure is capable of binding to the cysteine-rich domain and/or to the thyroglobulin type-1 domain of TROP2.

In some embodiments, the binding agent of the present disclosure is capable of binding to an epitope comprising amino acids of the extracellular domain of TROP2.

In some embodiments, the binding agent of the present disclosure is capable of binding to an epitope comprising amino acids of the cysteine-rich domain and/or of the thyroglobulin type-1 domain of TROP2.

In some embodiments, the binding agent of the present disclosure is capable of binding to an epitope including amino acid residues comprised within amino acid sequence 27-274 of TROP2 or a fragment thereof.

In some embodiments, the binding agent of the present disclosure is not capable of binding to the cysteine-poor domain (CPD) of TROP2.

In some embodiments, the binding agent of the present disclosure is not capable of binding to the amino acid 146 to 274 of TROP2.

Antibodies and antigen binding fragments

In some embodiments, the binding agent is an anti-TROP2 antibody or an antigen binding fragment thereof. It is to be understood herein that the following represent exemplary and non-limiting embodiments of antibodies or antigen binding fragments thereof.

Antibodies or antigen binding fragments thereof (or their sequence) may be obtained from immunization of an animal (including a transgenic animal) with a TROP2 antigen as disclosed herein. For example, single domain antibodies or antigen binding fragments thereof (or their sequence) may be obtained by immunizing an animal such as a dromedary, a camel, a llama, an alpaca, a rabbit with a TROP2 antigen (e.g., ECD or fragments thereof). Alternatively, the sequence of a single domain antibody or antigen binding domain thereof may be obtained by immunizing a transgenic animal that is able to express single domain antibodies (see international application No. PCT/CA2021/050951 filed on July 12, 2021, and published on January 20, 2022, under No. WO2022/011457 A1 the entire content of which is incorporated herein by reference).

In some instances, an antibody or antigen binding fragment thereof (or its sequence) is obtained from serum or tissue of immunized animals such as bone marrow, spleen and/or lymph nodes.

An hybridoma that results from fusion of cells of an immunized animal with a malignant and immortal plasma cell such as myeloma cells lacking hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) an enzyme allowing cells to grow in HAT medium (hypoxanthine-aminopterin-thymidine) may be generated. Spleen cells are particularly useful to generate hybridomas. The antibody or antigen binding fragment thereof may be isolated from an hybridoma.

The nucleic acid sequence of an antibody or antigen binding fragment thereof may be obtained by PCR amplification and sequencing from cells or tissue able to express the antibody or antigen binding fragment thereof. The amino acid sequence information is obtained from the nucleic acid sequence. Due to inherent degeneracy of the genetic code, a nucleic acid molecule encoding the same amino acid sequence but having a different nucleic acid sequence compared to the original nucleic acid sequence may be generated.

Once a nucleic acid sequence encoding the amino acid sequence of an antibody is obtained, recombinant expression of an antibody is rendered possible by transfection of one or more expression vector(s) into immortalized mammalian cell lines.

Alternatively, the sequence of an antibody of antigen binding fragment thereof may also be obtained by screening an established antibody library for sequences that have desired features such as for example, homology or identity with a reference antibody.

Antibodies of the present disclosure includes native or intact antibodies that specifically bind to TROP2. A native or intact antibody includes an antibody that is recombinantly expressed, isolated from immunized animals or from an hybridoma.

Exemplary embodiments of binding agents of the present disclosure include antibodies or antigen binding fragments thereof that specifically bind to at least one epitope comprising amino acids of the extracellular domain (ECD) of TROP2. Other exemplary embodiments of binding agents of the present disclosure include antibodies or antigen binding fragments thereof that specifically bind to at least one epitope comprising amino acids of the cysteine rich domain of TROP2. In some embodiments, the antibodies or antigen binding fragments thereof bind to a TROP2 antigen disclosed herein.

In an embodiment, the antibodies of the present disclosure specifically bind to human TROP2. In another embodiment, the antibodies of the present disclosure specifically bind to cynomolgus TROP2. In another embodiment, the antibodies of the present disclosure specifically bind to Rhesus TROP2.

More particularly, the antibodies of the present disclosure specifically bind to the ECD or the CRD of human TROP2.

A native or intact antibody comprises two heavy (H) chains and two light (L) chains. Mammalian heavy chains are classified as alpha, delta, epsilon, gamma, and mu, each heavy chain consists of a variable region (VH) and a first, second, third, and optionally fourth constant region (CH1, CH2, CH3, CH4 respectively); mammalian light chains are classified as λ or κ, while each light chain consists of a variable region (VL) and a constant region. The antibody has a "Y" shape, with the stem of the Y consisting of the second and third constant regions of two heavy chains bound together via disulfide bonding. Each arm of the Y includes the variable region and first constant region of a single heavy chain bound to the variable and constant regions of a single light chain. The variable regions of the light and heavy chains are responsible for antigen binding. The variable regions in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain CDRs including CDRL1, CDRL2, and CDRL3, heavy chain CDRs including CDRH1, CDRH2, CDRH3). CDR boundaries for the antibodies and antigen-binding fragments disclosed herein may be defined or identified by the conventions of Kabat, IMGT, Chothia, or Al-Lazikani. The three CDRs are interposed between flanking stretches known as framework regions (FRs) (light chain FRs including LFR1, LFR2, LFR3, and LFR4, heavy chain FRs including HFR1, HFR2, HFR3, and HFR4), which are more highly conserved than the CDRs and form a scaffold to support the highly variable loops. The constant regions of the heavy and light chains are not involved in antigen-binding, but exhibit various effector functions. Antibodies are assigned to classes based on the amino acid sequences of the constant regions of their heavy chains. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of alpha, delta, epsilon, gamma, and mu heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as IgG1 (gamma1 heavy chain), IgG2 (gamma2 heavy chain), IgG3 (gamma3 heavy chain), IgG4 (gamma4 heavy chain), IgA1 (alpha1 heavy chain), or IgA2 (alpha2 heavy chain).

Antibodies of the present disclosure includes chimeric antibodies that specifically bind to TROP2. A chimeric antibody is a recombinant protein that contains the variable region(s) including the complementarity determining regions (CDRs) of an antibody derived from one or more species, such as a rodent antibody, while the constant domains of the antibody molecule are derived from those of another species, such as a human antibody.

Antibodies of the present disclosure includes humanized antibodies that specifically bind to TROP2. A humanized antibody is a recombinant protein in which the CDRs or CDR residues from an antibody from one species; e.g., a rodent antibody, are transferred from the heavy and light variable chains of the rodent antibody into human heavy and light variable regions (e.g., framework region sequences). The constant domains of the antibody molecule are derived from those of a human antibody. In certain embodiments, a limited number of framework region amino acid residues from the parent (rodent) antibody may be substituted into the human antibody framework region sequences. In other examples, as simply transferring mouse CDRs into human FRs often results in a reduction or even loss of antibody affinity, additional modification might be required in order to restore the original affinity of the murine antibody. This can be accomplished by the replacement of one or more some human residues in the FR regions with their murine counterparts to obtain an antibody that possesses good binding affinity to its epitope. In some embodiment the humanized antibody is a humanized single domain antibody. In some embodiments, a humanized antibody that competes with a single domain antibody disclosed herein is encompassed by the present disclosure.

Antibodies of the present disclosure includes human antibodies that specifically binds to TROP2. A fully human antibody can be obtained from a transgenic non-human animal. Methods for producing fully human antibodies using either combinatorial approaches or transgenic animals transformed with human immunoglobulin loci are known in the art. In one alternative, the phage display technique may be used to generate human antibodies. The fully human antibodies are expected to exhibit even fewer side effects than chimeric or humanized antibodies and to function in vivo as essentially endogenous human antibodies. In some embodiments, a human antibody that competes with a single domain antibody disclosed herein is encompassed by the present disclosure.

Antibodies of the present disclosure includes single domain antibodies (sdAbs) that specifically binds to TROP2. As used herein the expressions "single domain antibody" and "heavy chain only antibody" are used interchangeably. The term "single domain antibody" is used in its broad sense and includes naturally occurring single domain antibodies or variants thereof such as humanized single domain antibodies or chimeric single domain antibodies. A single domain antibody is said to be chimeric when it possesses a constant region of another species. In an

embodiment, the single domain antibody is a chimeric single domain antibody. In some embodiments, the chimeric single domain antibody comprises a humanized variable region and a human constant region. In another embodiment, the chimeric single domain antibody comprises the variable region of a native single domain antibody.

A single domain antibody (sdAb) is usually composed of two heavy chains. A single domain antibody is about 12-15 kDa in size (about 110 amino acids in length). A sdAb can selectively bind to target antigens, like full-size antibodies, and have similar affinities for antigens. However, because of their much smaller size, they may be capable of better penetration into solid tumors. The smaller size also contributes to the stability of the sdAb, which is more resistant to pH and temperature extremes than full size antibodies (Van Der Linden et al., 1999, Biochim Biophys Act 1431:37-46). Single-domain antibodies were originally developed following the discovery that camelids (camels, alpacas, llamas) possess fully functional antibodies without light chains (e.g., Hamsen et al., 2007, Appl Microbiol Biotechnol 77:13-22). Naturally occurring single domain antibodies consist of a single variable region (VH or VHH) and two constant domains (CH2 and CH3). The variable region of naturally occurring single domain antibodies may be cloned and fused to a constant region of a naturally occurring antibody heavy chain. In some embodiments, the variable region of a naturally occurring single domain antibody is replaced with that of a human IgG heavy chain constant region which comprise CH1, CH2 and CH3 domains and optionally CH4 domain and may optionally comprise a hinge.

The antigen binding property of a single domain antibody is conferred by the heavy chain variable region. As such, a single heavy chain or even a single heavy variable region (VH or VHH) may be sufficient for specific binding to an antigen. However, a single domain antibody is usually composed of two heavy chains. The two heavy chains may be assembled via a dimerization domain such as for example a constant region of a class G human immunoglobulin. The two heavy chains may also be expressed as a single polypeptide chain.

Accordingly, in some embodiments, binding agents include antibodies and antigen-binding fragments thereof such as, for example and without limitations, single domain antibodies from camelids or sharks, human antibodies including IgGs (including human IgG1, human IgG2, human IgG3, human IgG4), human IgMs, human IgAs (including human IgA1 and human IgA2), human IgEs, human IgDs, animal antibodies including for example, IgGs (IgG1, IgG2a, IgG2b, IgG2c,

IgG3, IgG4), IgMs, IgAs, IgEs and IgDs and variants (e.g., chimeric, humanized or human) having a similar format.

In other embodiments, binding agents of the present disclosure encompass antigen-binding fragments such as, for example and without limitations, Fab, Fab', $F(ab')_2$, complementarity determining regions, variable regions including VHs, VHHs, VLs, and the like.

An antigen binding fragment can be prepared by known techniques such as by proteolytic hydrolysis of a full-length antibody or by expression in E. coli or another host of the DNA coding for the fragment. An antigen binding fragment can be obtained by pepsin or papain digestion of full-length antibodies by conventional methods. For example, antigen binding fragment can be produced by enzymatic cleavage of antibodies with pepsin to provide an approximate 100 kD fragment denoted $F(ab')_2$. A $F(ab')_2$ fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce an approximate 50 Kd Fab' monovalent fragment. Alternatively, an enzymatic cleavage using papain produces two monovalent Fab fragments and an Fc fragment directly.

In some embodiments, antibodies that binds to TROP2 can be bispecific or multispecific. A "multispecific antibody" is an antibody that can bind simultaneously to at least two targets that are of different structure, e.g., two or more different antigens, two or more different epitopes on the same antigen, or a hapten and/or an antigen or epitope. A "multivalent antibody" is an antibody that can bind simultaneously to at least two targets that are of the same or different structure. Valency indicates how many antigen binding domains the antibody has; i.e., monovalent, bivalent, trivalent or multivalent. Multivalency provides an antibody with an advantage of multiple interactions in binding to an antigen, thus increasing the avidity of binding to the antigen. Specificity indicates how many antigens or epitopes an antibody is able to bind; i.e., monospecific, bispecific, trispecific, multispecific. Using these definitions, a native antibody (e.g., an IgG) is bivalent because it has two antigen binding domains (each binding domain being composed of amino acid residues from the light chain and heavy chain variable regions) but is monospecific because each antigen binding domain binds to the same epitope. Multispecific, multivalent antibodies include antibodies that have more than one antigen binding domain of different specificity. A "bispecific antibody" is an antibody that can bind simultaneously to two targets

which are of different structure. Bispecific antibodies (bsAb) and bispecific antibody fragments (bsFab) may have at least antigen binding domain that specifically binds to TROP2.

In addition to recombinant technologies, numerous methods to produce bispecific or multispecific antibodies are known, as disclosed, for example, in U.S. Pat. No. 7,405,320, the Examples section of which is incorporated herein by reference. Bispecific antibodies can be produced by the quadroma method, which involves the fusion of two different hybridomas, each producing a monoclonal antibody recognizing a different antigenic site. Another method for producing bispecific antibodies uses heterobifunctional cross-linkers to chemically tether two different monoclonal antibodies.

Single domain antibodies

An exemplary embodiment of binding agent of the present disclosure is a single domain antibody. It is to be understood herein that the following represent exemplary and non-limiting embodiments of single domain antibodies or antigen binding fragments thereof.

The single domain antibody possesses unique binding features. For example, the complementarity determining region 3 (CDR3) of the sdAb disclosed herein forms a loop fitting into a groove within the cysteine rich domain (CRD) of the extracellular domain (ECD) of TROP2. Amino acid residues of the CDR3 and of the framework region 2 (FR2) interact with amino acid residues of TROP2 cysteine rich domain and together form a clip around the CRD. These unique properties provide a high affinity of sdAbs for TROP2 and a slow dissociation rate. Surprisingly, amino acid residues of the complementarity determining region 1 (CDR1) and of the complementarity determining region 2 (CDR2) do not appear to interact with amino acid residues of the CRD.

The single domain antibodies of the present disclosure also possess anti-cancer activity such as antibody-dependent cellular cytotoxicity (ADCC) and/or antibody-dependent cellular phagocytosis (ADCP) activity. The single domain antibodies of the present disclosure may also be conjugated for therapeutic, or detection uses.

Exemplary embodiments of binding agents of the present disclosure include single domain antibodies or antigen binding fragments thereof that specifically bind to at least one epitope comprising amino acids of the extracellular domain (ECD) of TROP2. Other exemplary

embodiments of binding agents of the present disclosure include single domain antibodies or antigen binding fragments thereof that specifically bind to at least one epitope comprising amino acids of the cysteine rich domain of TROP2. In some embodiments, the single domain antibodies or antigen binding fragments thereof bind to a TROP2 antigen disclosed herein.

In an embodiment, the single domain antibodies of the present disclosure specifically bind to human TROP2. In another embodiment, the single domain antibodies of the present disclosure specifically bind to cynomolgus TROP2. In another embodiment, the single domain antibodies of the present disclosure specifically bind to Rhesus TROP2.

More particularly, the single domain antibodies of the present disclosure specifically bind to the ECD or the CRD of human TROP2.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:54 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:52 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:53.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:47.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:58 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:56 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:57.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of

the CDR1 set forth in SEQ ID NO:59 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:60.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:119 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 124.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 123.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:120 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:5.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:130.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:131.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:133.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:131.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:132.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:133.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:172 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:133.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:134.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:4 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:135.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:174 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:127 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:129.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:14.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:137.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:138.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:136.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:137.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:138.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:139.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:173 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:137.

In some embodiments single domain antibody or antigen binding fragment thereof of the present disclosure comprises the three CDRs of the single domain antibody disclosed herein.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises a CDR1 having the amino acid sequence set forth in SEQ ID NO:1, a heavy chain complementarity determining region 2 (CDRH2) having the amino acid sequence set forth in SEQ ID NO:2 and a heavy chain complementarity determining region 3 (CDRH3) having the amino acid sequence set forth in SEQ ID NO:3.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:5 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:7, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:8 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:9.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:10, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:11 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:12.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:14 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:16, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:17 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:18.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:19, a CDRH2

having the amino acid sequence set forth in SEQ ID NO:20 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:21.

In other exemplary embodiments, the single domain antibody comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:46, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:47 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:48.

In other exemplary embodiments, the single domain antibody comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:55, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:47 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:48.

In other exemplary embodiments, the single domain antibody comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:49, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:50 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:51.

In other exemplary embodiments, the single domain antibody comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:62, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:50 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:51.

In other exemplary embodiments, the single domain antibody comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:52, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:53 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:54.

In other exemplary embodiments, the single domain antibody comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:56, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:57 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:58.

In other exemplary embodiments, the single domain antibody comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:59, a CDRH2 having the amino acid sequence

set forth in SEQ ID NO:60 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:61.

In other exemplary embodiments, the single domain antibody comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:63, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:64 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:65.

In other exemplary embodiments, the single domain antibody comprises a CDRH1 having the amino acid sequence set forth in SEQ ID NO:66, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:67 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:68.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:123 and the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:124 and the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:130 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:6.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:131 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:6.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid

sequence of the CDR2 set forth in SEQ ID NO:132 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:6.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:133 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:6.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:134 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:6.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:135 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:6.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:127, the amino acid sequence of the CDR2 set forth in SEQ ID NO:129 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:6.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:14 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:15.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:136 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:15.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino

acid sequence of the CDR2 set forth in SEQ ID NO:137 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:15.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:138 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:15.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:139 and the amino acid sequence of the CDR3 set forth in SEQ ID NO:15.

In some embodiments single domain antibody or antigen binding fragment thereof of the present disclosure comprises the CDR1, CDR2, CDR3 and the FR2 amino acid sequence of the single domain antibody disclosed herein.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:52, the amino acid sequence of the CDR2 set forth in SEQ ID NO:53, the amino acid sequence of the CDR3 set forth in SEQ ID NO:54 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO:48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:56, the amino acid sequence of the CDR2 set forth in SEQ ID NO:57, the amino acid sequence of the CDR3 set forth in SEQ ID NO:58 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:59, the amino acid sequence of the CDR2 set forth in SEQ ID NO:60, the amino acid sequence of the CDR3 set forth in SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO: 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:119.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:124, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:123, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:120.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:5, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:130, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:131, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:133, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:131, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:132, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:133, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:133, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:172.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:134, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:4, the amino acid sequence of the CDR2 set forth in SEQ ID NO:135, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:127, the amino acid sequence of the CDR2 set forth in SEQ ID NO:129, the amino acid sequence of the CDR3 set forth in SEQ ID NO:6 and the amino acid sequence of the FR2 set forth in SEQ ID NO:174.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:14, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:137, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:138, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:170.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:136, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:137, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:138, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:139, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:13, the amino acid sequence of the CDR2 set forth in SEQ ID NO:137, the amino acid sequence of the CDR3 set forth in SEQ ID NO:15 and the amino acid sequence of the FR2 set forth in SEQ ID NO:173.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the variable region of the single domain antibody disclosed herein.

In further exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:22.

In other exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:23.

In further exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:24.

In additional exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:25.

In other exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:26.

In further exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:27.

In yet further exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence as set forth in SEQ ID NO:28.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:29.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:71.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:72.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:73.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:74.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:75.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:76.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:77.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:78.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:79.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:80.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:81.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:82.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:83.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:84.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:85.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:86.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:87.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:88.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:89.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:90.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:91.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:92.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:93.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:94.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:95.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:96.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:97.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:98.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:99.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:100.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:101.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:012.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:103.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:104.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:105.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:106.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:107.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:108.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:109.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:110.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:111.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:112.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:113.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:114.

In additional exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:115.

In another exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises the heavy chain of the single domain antibody disclosed herein.

In exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:30.

In yet other exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:31.

In further exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:32.

In yet further exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:33.

In other exemplary embodiments, the single domain antibody that an amino acid sequence as set forth in SEQ ID NO:34.

In other exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:35.

In other exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:44.

In other exemplary embodiments, the single domain antibody comprises an amino acid sequence as set forth in SEQ ID NO:45.

Binding agents comprising antigen binding domain(s) of sdAbs

Binding agents of the present disclosure comprise one or more antigen binding domain(s) of an antibody or antigen binding fragment thereof that binds to TROP2. For example, the binding agents of the present disclosure comprise one or more antigen binding domain(s) of a single domain antibody disclosed herein. It is to be understood herein that the following represent exemplary and non-limiting embodiments of binding agents comprising antigen binding domain(s) of single domain antibodies.

In some embodiments, the binding agent of the present disclosure, comprises one or more antigen binding domains that specifically bind to trophoblast cell surface antigen-2 (TROP2). In some embodiments, the binding agent of the present disclosure, comprises one or more antigen binding domains that binds to a different target than TROP2.

Exemplary embodiments of binding agents of the present disclosure include those that comprise one or more antigen binding domains that specifically bind to at least one epitope comprising amino acids of the extracellular domain (ECD) of TROP2. Other exemplary embodiments of binding agents of the present disclosure include those that comprise one or more antigen binding domains that specifically bind to at least one epitope comprising amino acids of the cysteine rich domain of TROP2. In some embodiments, the binding agent comprise one or more antigen binding domains that specifically bind to a TROP2 antigen disclosed herein.

In an embodiment, the binding agent of the present disclosure comprises one or more antigen binding domains that specifically bind to human TROP2. In another embodiment, the binding agent of the present disclosure comprises one or more antigen binding domains that specifically bind to cynomolgus TROP2. In another embodiment, the binding agent of the present disclosure comprises one or more antigen binding domains that specifically bind to Rhesus TROP2.

More particularly, the binding agent of the present disclosure comprises one or more antigen binding domains that specifically bind to the ECD or the CRD of human TROP2.

In other embodiments, the binding agents of the present disclosure comprise one or more antigen binding domain(s) of a native antibody (CDRs, and/or VL and VH) that binds to TROP2.

The CDRs or variable regions (VL and VH) of antibodies and antigen binding fragments thereof may be expressed as a single polypeptide chain to generate single chain Fv (scFV) molecules and variants. Numerous formats are contemplated as indicated below.

As well, the CDRs or variable region of multiple single domain antibodies may be expressed as a single polypeptide chain thereby providing multivalence and/or multispecificity to the binding agent. Moreover, multiple polypeptide chains may be assembled to increase the diversity or avidity of interactions with TROP2. Numerous formats are contemplated as indicated below.

Accordingly, antigen binding fragments or antigen binding domain(s) of the single domain antibodies disclosed herein may be used to generate binding agents that are monospecific, multispecific, monovalent or multivalent.

For example, the variable region or CDRs of a single domain antibody can be fused with a Fc, CH3 or CH2-CH3 domain of an antibody to allow homodimerization thereby generating a multivalent binding agent.

Alternatively, multiple copies of the variable region or CDRs of a single domain antibody can be fused in tandem with required spacers or linkers to generate a multivalent and monospecific binding agent.

For example, the variable regions or CDRs of two single domain antibodies can be fused with a Fc, CH3 or CH2-CH3 domain to allow heterodimerization thereby generating a multivalent and multispecific binding agent.

Alternatively, the variable regions or CDRs of two or more single domain antibodies may be fused in tandem with required spacers or linkers to generate a multivalent and multispecific binding agent.

Binding agents of the present disclosure may have a format of an antibody or antigen binding fragment thereof, an antibody-like molecule (native antibody fused with a single domain antibody or with an antigen binding domain of a sdAb, Fc-, CH3- fusions and the like), a fusion with protein scaffolds, immune cell modulating agents and the like.

Binding agents also include immune cell modulating agents such as for example, dual-affinity retargeting molecules (DARTs), chimeric antigen receptors (CAR) constructs, bispecific T cell engagers construct (BiTEs), bispecific killer cell engagers (BiKEs), trispecific killer cell engagers (TriKEs) containing scFvs or VHHs.

Binding agents also encompass fusion with protein scaffolds, including ankyrin repeat proteins, Z-domain of Staphylococcus protein-A, Type-III fibronetin, knottin and the like. Exemplary embodiments of binding agents include monospecific-, bispecifics (symmetric or asymmetric) trispecific-, or multispecific antibodies as well as monovalent, bivalent, trivalent or multivalent antibodies, single chain FVs (scFVs) and derivatives such diabody, triabody, tetrabody, tandem di-scFvs, tandem tri-scFvs, scFV-Fc, minibody (scFV-CH3), tandem diabody, di-diabody, bibody and the like, VH or VHHs and derivatives such as, tandem bispecific or multispecific VHH, bivalent VHH-Fc fusions, VHH-hinge-CH2-CH3 fusions, bivalent CH3 fusions, VHH pentabody, decabody and the like.

A single chain Fv molecule (scFv) comprises a VL domain and a VH domain. The VL and VH domains associate to form a target binding site. These two domains are further covalently linked by a peptide linker (L). A scFv molecule is denoted as either VL-L-VH if the VL domain is the N-terminal part of the scFv molecule, or as VH-L-VL if the VH domain is the N-terminal part of the scFv molecule. Methods for making scFv molecules and designing suitable peptide linkers are described in U.S. Pat. No. 4,704,692, U.S. Pat. No. 4,946,778, R. Raag and M. Whitlow, "Single Chain Fvs." FASEB Vol 9:73-80 (1995) and R. E. Bird and B. W. Walker, Single Chain Antibody Variable Regions, TIBTECH, Vol 9: 132-137 (1991).

In some embodiments, the binding agents of the present disclosure may have a format as disclosed in PCT/CA2020/051753 filed on December 18, 2020 and published on June 24, 2021 under No. WO2021/119832A1, the entire content of which is incorporated herein by reference) such as for example, as formula Ia, formula Ib, formula Ic, formula II, formula III, formula IV, formula V, formula VI, formula VII or formula VIII and the like or formula I, formula II, formula III, formula IIIa and formula IIIb, formula IV, formula V, formula VI, formula VII or formula VIII disclosed herein.

The binding agents of the present disclosure may be formed by the assembly of two polypeptide chains having the same configuration (with same or different amino acid sequence) or

having different configurations where the same or different configurations include the configuration set forth in formula I, formula II, formula III, formula IIIa and formula IIIb, formula IV, formula V, formula VI, formula VII or formula VIII disclosed herein.

In some embodiments, the binding agents of the present disclosure is monospecific. In some embodiments, the binding agents of the present disclosure is multispecific.

The binding agents of the present disclosure (including antibodies or antigen binding fragments thereof) may be monospecific, multispecific, monovalent or multivalent.

A binding agent that comprises two or more antigen binding domains of a single domain antibody is considered multivalent. A multivalent single domain antibody can be monospecific (if all antigen binding domains bind to exactly the same epitope) or multispecific (if the antigen binding domains bind to different epitopes on same target or to different epitopes or targets). A binding agent that comprises only one antigen binding domain is considered monovalent.

Production of binding agents such as antibodies or antigen binding fragments, may involve cloning procedures and recombinant expression. The antigen binding domain sequence (e.g., sequence of the variable light chain and variable heavy chain of an intact antibody or the sequence of the variable heavy chain of a single domain antibody) may be obtained by a variety of molecular cloning procedures, such as RT-PCR, 5'-RACE, and cDNA library screening. The sequence of interest may be cloned into an expression vector(s), the expression vector(s) transfected into cells and the binding agent isolated and/or purified. In the case of an intact antibody, the light chain and the heavy chain can be cloned into a single expression vector or into two separate expression vectors. Co-transfection of the separate expression vectors is necessary for the intact antibody to assemble properly. In the case of a single domain antibody, a single expression vector (expressing the heavy chain) is sufficient.

Monospecific binding agents encompass binding agents that are specific for a single epitope of a given antigen.

An exemplary embodiment of a monospecific binding agent includes a binding agent that comprise one antigen binding fragment of a single domain antibody. Another exemplary embodiment of a monospecific binding agent includes a binding agent that comprise more than one antigen binding fragments of a single domain antibody, but the antigen binding fragments have

identical CDRs and framework regions. Yet another exemplary embodiment of a binding agent includes a binding agent that comprise more than one antigen binding fragments of single domain antibodies, but the variable regions have identical CDRs and different framework regions. A further exemplary embodiment of a monospecific binding agent includes a binding agent that comprise antigen binding fragments of single domain antibodies that differ in the amino acid sequence of one or more of their CDRs (e.g., conservative substitution in one or more CDRs) without affecting their ability to bind to the same antigen or epitope.

Multispecific binding agents encompass binding agents that are specific for more than one epitope (of the same antigen or different antigens) or to more than one antigen. For example, a multispecific polypeptide chain or binding agent may thus have more than one variable region of a single domain antibody, at least two of which bind to different antigens or epitopes.

The binding agents of the present disclosure may thus be bispecific, trispecific, tetraspecific, pentaspecific, hexaspecific etc. In some embodiments, each variable region may be specific for a given antigen. In some embodiments, two or more antigen binding fragments of a given binding agent may be specific for the same or different antigens. In some embodiments, three or more antigen binding fragments of a given binding agent may be specific for the same or different antigens. In some embodiments, four or more antigen binding fragments of a given binding agent may be specific for the same or different antigens. In some embodiments, five or more antigen binding fragments of a given binding agent may be specific for the same or different antigens. In some embodiments, six or more antigen binding fragments of a given binding agent may be specific for the same or different antigens. The specificity may depend on the number of antigen binding fragments present in a given binding agent.

Other exemplary non-limiting embodiments of multispecific binding agents include those having two antigen binding fragments of different specificities. Yet other exemplary non-limiting embodiments of multispecific binding agents include those having more than two antigen binding fragments that bind to two different antigens, proteins or to two different epitopes on the same antigens or proteins.

In some embodiments, the binding agent of the present disclosure is monovalent.

In some embodiments, the binding agent of the present disclosure is multivalent.

Exemplary non-limiting embodiments of multivalent binding agents include binding agents composed of multivalent polypeptide chains. Other non-limiting exemplary embodiments of multivalent binding agents include binding agents composed of more than one monovalent polypeptide chain.

In accordance with the present disclosure, a bispecific binding agent may be bivalent or multivalent depending on the number of variable regions that it contains. Exemplary non-limiting embodiments of bispecific binding agents include those comprising two identical bispecific polypeptide chains that form a dimer.

Exemplary and non-limiting embodiments of bispecific binding agents are provided herein.

In some embodiments, the bispecific binding agent comprises an antigen binding domain that binds to TROP2 and comprises formula II (see PCT/CA2020/051753 filed on December 18, 2020, and published on June 24, 2021, under No. WO2021/119832A1 the entire content of which is herein incorporated by reference).

In other embodiments, the binding agent comprises a polypeptide chain that comprises in a N- to C-terminal fashion, a) an antigen binding domain of a single domain antibody, b) a linker, c) a dimerization domain, d) a linker and e) an antigen binding domain of a single domain antibody, at least one of the antigen binding domain of a) or e) is an antigen binding domain that binds to TROP2.

In some embodiments, the antigen binding domain of a) and the antigen binding domain of e) are different.

In some embodiments, the antigen binding domain of a) and the antigen binding domain of e) are the same.

In some embodiments, the linker of b) and the linker of d) are the same.

In some embodiments, the linker of b) and the linker of d) are different.

In an exemplary embodiment, the antigen binding domain of a) comprises an antigen binding domain of a single domain antibody that specifically binds to human TROP2.

In another exemplary embodiment, the antigen binding domain of a) comprises an antigen binding domain of a single domain antibody that specifically binds to CD47 such as for example human CD47.

In an exemplary embodiment, the antigen binding domain of e) comprises an antigen binding domain of a single domain antibody that specifically binds to human TROP2.

In an exemplary embodiment, the antigen binding domain of e) comprises an antigen binding domain of a single domain antibody that specifically binds to CD47, such as for example human CD47.

In some embodiments, the bispecific binding agent comprises two polypeptide chains. The two polypeptide chains are non-covalently assembled by amino acid residues of the CH2 and/or CH3 domain contained therein so as to obtain a bispecific and tetravalent binding agent.

In an exemplary embodiment, the binding agent or antigen binding domain(s) of the present disclosure may be capable of inhibiting the growth of tumor cells expressing human TROP2 and CD47.

In exemplary embodiments, the binding agent comprises one or more antigen binding domains that is capable of binding to CD47.

In another exemplary embodiments, the binding agent comprises one or more antigen binding domains that is capable of binding to TROP2 and one or more antigen binding domains that is capable of binding to CD47.

In an exemplary embodiment, the antigen binding domain that is capable of binding to CD47 comprises:

a. a CDRH1 having the amino acid sequence set forth in SEQ ID NO156, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:157 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:158, or

b. a CDRH1 having the amino acid sequence set forth in SEQ ID NO159, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:160 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:161.

In an exemplary embodiment, the antigen binding domain that is capable of binding to CD47 comprises an amino acid sequence as set forth in SEQ ID NO:155.

In an exemplary embodiment, the binding agent is capable of binding to CD47 and TROP2 and comprises an amino acid sequence as set forth in SEQ ID NO:155 and the amino acid sequence of at least one antigen binding domain of at least one single domain antibody disclosed herein.

In other exemplary embodiments, the binding agent is capable of binding to CD47 and TROP2 and comprises an amino acid sequence as set forth in SEQ ID NO:155 and the amino acid sequence of an antigen binding domain of at least one single domain antibody set forth in any one of SEQ ID NOs:22-26 or SEQ ID Nos:73-114.

In another exemplary embodiment, the binding agent is capable of binding to CD47 and TROP2 and comprises an amino acid sequence as set forth in SEQ ID NO:150 or in SEQ ID NO:151.

Variants

Variants of the sequences disclosed herein are also encompassed by the present disclosure.

Binding agent of the present disclosure therefore encompass antibody variants (e.g., single domain antibody variants), variants of antibody-like molecule, variants of protein scaffold, variants of immune cell modulating agent. In addition, binding agents of the present disclosure may comprise an antigen binding fragment or an antigen binding domain of an antibody variant such as an antigen binding fragment or an antigen binding domain of a single domain antibody variant.

The present disclosure more particularly encompasses variants of the single domain antibody disclosed herein as well as binding agents that comprise one or more antigen binding domain of a single domain antibody variant. Variants of the binding agents disclosed herein may have the same format as the binding agent disclosed herein. As such, variants of the binding agents encompassed by the present disclosure may be multispecific and/or multivalent.

Variants encompassed by the present disclosure include those which may comprise an insertion of one or more amino acid residues at one or more position, a deletion of one or more amino acid residues at one or more position or a substitution of one or more amino acid residues at one or more position (conservative or non-conservative substitutions).

For example, naturally occurring residues are divided into groups based on common side chain properties. Conservative substitutions may be made by exchanging an amino acid from one of the groups listed below (group 1 to 6) for another amino acid of the same group. Non-conservative substitutions will entail exchanging a member of one of these groups for another.

(group 1) hydrophobic: norleucine, methionine (Met), Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile)

(group 2) neutral hydrophilic: Cysteine (Cys), Serine (Ser), Threonine (Thr), Asparagine (Asn), Glutamine (Gln),

(group 3) acidic: Aspartic acid (Asp), Glutamic acid (Glu)

(group 4) basic: Histidine (His), Lysine (Lys), Arginine (Arg)

(group 5) residues that influence chain orientation: Glycine (Gly), Proline (Pro); and

(group 6) aromatic: Tryptophan (Trp), Tyrosine (Tyr), Phenylalanine (Phe)

Other exemplary embodiments of conservative substitutions are shown in Table 2 under the heading of "preferred substitutions". If such substitutions result in an undesired property, then more substantial changes, denominated "exemplary substitutions" in Table 2, or as further described below in reference to amino acid classes, may be introduced and the products screened.

One of skill in the art will recognize that certain amino acids are less positively charged, are neutral, are negatively charged or have a reduced charge in comparison to other amino acids. Amino acids can be categorized based on net charge as indicated by an amino acid's isoelectric point. The isoelectric point is the pH at which the average net charge of the amino acid molecule is zero. When pH>pI, an amino acid has a net negative charge, and when the pH<pI, an amino acid has a net positive charge. In some embodiments, the measured pI value for an antibody is between about 3 and 9 (e.g. 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, and 9) and any values in between. In some embodiments, the measured pI value for an antibody is between about 4 and 7 (e.g. 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0), and any values in between. Exemplary isoelectric points of amino acids are shown in Table 2 below. Generally amino acids with positive electrically charged side chains include, for example, Arginine (R), Histidine (H), and Lysine (K). Amino acids with negative electrically charged side chains include, for example, Aspartic Acid

(D) and Glutamic Acid (E). Amino acids with polar properties include, for example, Serine (S), Threonine (T), Asparagine (N), Glutamine (Q), and Cysteine (C), Tyrosine (Y) and Tryptophan (W). Non-polar amino acids include, for example, Alanine (A), Valine (V), Isoleucine (I), Leucine (L), Methionine (M), Phenylalanine (F), Glycine (G) and Proline (P).

In some embodiments, the isoelectric point of an antibody is modified via amino acid substitution. *See*, *e.g.* US20110076275. In some embodiments, modifying the isoelectric point of a polypeptide comprising an antibody results in a change in the antibody's half-life.

Table 2. Exemplary amino acid substitutions

Original residue	Exemplary substitution	Conservative substitution	pI (isoelectric point)
Ala (A)	Val, Leu, Ile	Val	6.0
Arg (R)	Lys, Gln, Asn	Lys	10.76
Asn (N)	Gln, His, Lys, Arg, Asp	Gln	5.41
Asp (D)	Glu, Asn	Glu	2.77
Cys (C)	Ser, Ala	Ser	5.07
Gln (Q)	Asn; Glu	Asn	5.65
Glu (E)	Asp, Gln	Asp	3.22
Gly (G)	Ala	Ala	5.97
His (H)	Asn, Gln, Lys, Arg,	Arg	7.59
Ile (I)	Leu, Val, Met, Ala, Phe, norleucine	Leu	6.02
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile	5.98
Lys (K)	Arg, Gln, Asn	Arg	9.74
Met (M)	Leu, Phe, Ile	Leu	5.74
Phe (F)	Leu, Val, Ile, Ala, Tyr	Tyr	5.48
Pro (P)	Ala	Ala	6.30
Ser (S)	Thr	Thr	5.58
Thr (T)	Ser	Ser	5.60
Trp (W)	Tyr, Phe	Tyr	5.89
Tyr (Y)	Trp, Phe, Thr, Ser	Phe	5.66

Original residue	Exemplary substitution	Conservative substitution	pI (isoelectric point)
Val (V)	Ile, Leu, Met, Phe, Ala, norleucine	Leu	5.96

Generally, the degree of similarity and identity between variable chains is determined herein using the Blast2 sequence program (Tatiana A. Tatusova, Thomas L. Madden (1999), "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250) using default settings, i.e., blastp program, BLOSUM62 matrix (open gap 11 and extension gap penalty 1; gapx dropoff 50, expect 10.0, word size 3) and activated filters.

Percent identity will therefore be indicative of amino acids which are identical in comparison with the original peptide and which may occupy the same or similar position.

Percent similarity will be indicative of amino acids which are identical and those which are replaced with conservative amino acid substitution in comparison with the original peptide at the same or similar position.

Variants of the present disclosure may therefore comprise a sequence that is at least 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical with that of an original or reference sequence or a portion of an original sequence.

In an exemplary embodiment, mutations in one or more CDRs, variable region or constant region may be performed by amino acid substitution. Exemplary embodiments of substitutions include conservative amino acid substitutions or non-conservative amino acid substitutions. Exemplary substitutions are provided in **Table 2**. Other exemplary substitutions are provided in **Table 13**. Yet other exemplary substitutions are provided in **Table 14**.

In some embodiments, polypeptide chains having an amino acid sequence which is at least 75%, 80% 85%, 90%, 95%, 99% identical or less than 100% identical to a given amino acid sequence, may have amino acid substitutions, additions or deletions that are generally located outside of the complementarity determining regions.

In some embodiments, a variant may have at least 80% sequence identity with a sequence disclosed herein. In other embodiments, a variant may have at least 85% sequence identity with a

sequence disclosed herein. In yet embodiments, a variant may have at least 90% sequence identity with a sequence disclosed herein. In further embodiments, a variant may have at least 95% sequence identity with a sequence disclosed herein. In other embodiments, a variant may have at least 99% sequence identity with a sequence disclosed herein.

In some embodiments, variation may occur in one or more CDR. The mutations may occur for example, in CDR amino acid residues that are not involved in the interaction with TROP2. In an exemplary embodiment, amino acid residues of CDR1 and/or CDR2 may be mutated. In other exemplary embodiments, amino acid residues of CDR3 may be mutated. CDR mutations that improve affinity with TROP2 are particularly contemplated.

In other embodiments, variation may occur in the framework regions. The mutations may occur for example, in the framework amino acid residues that are not involved in the interaction with TROP2. In an exemplary embodiment, amino acid residues of FR1 and FR3 and/or FR4 may be mutated. In other exemplary embodiments, amino acid residues of FR2 may be mutated. FR2 mutations that improve affinity with TROP2 are particularly contemplated.

In other embodiments, the variations may occur in the constant region. In an exemplary embodiment, the constant region may be mutated so as to modulate ADCC activity. In another exemplary embodiment, the constant region may be mutated so as to modulate ADCP activity. In another exemplary embodiment, the constant region may be mutated so as to improve ADCP activity. In yet other exemplary embodiment, the constant region may be mutated so as to improve internalization. In yet other exemplary embodiment, the constant region may be mutated so as to improve stability.

Exemplary and non-limiting embodiments of mutations in the constant region (e.g., Fc region) that improves one or more effector function are encompassed by the present disclosure, exemplary embodiments of which, are provided in the **Table A** (List of Mutations Antibodies (Basel). 2020 Nov 17;9(4):64, the entire content of which is incorporated herein by reference).

Table A

	Effector Function vs. WT			
Fc Modification(s)	ID	ADCC	ADCP	Reference

S298A/E333A/K334A	AAA	+	n.d.	R. J. Biol. Chem. 2001, 276, 6591–6604.
S239D/I332E	DE	++	+	Proc. Natl. Acad. Sci. USA 2006, 103, 4005–401
S239D/A330L/I332E	DLE	+++	+	Proc. Natl. Acad. Sci. USA 2006, 103, 4005–401
G236A	G236A	-	+	Mol. Cancer Ther. 2008, 7, 2517–2527.
G236A/S239D/I332E	ADE	+	+	Mol. Cancer Ther. 2008, 7, 2517–2527.
F243L/R292P/Y300L/V305I/P396L	LPLIL	++	n.d.	Cancer Res. 2007, 67, 8882–8890.
L235V/F243L/R292P/Y300L/P396L	VLPLL	+	n.d.	Breast Cancer Res. 2011, 13, R123.
P247I/A339Q		++		Immunol. Cell Biol. 2020, 98, 287–304.
Afucosylation (Potelligent)*	Potelligent	++	n.d.	Biotechnol. Bioeng. 2004, 87, 614–622.
*POTELLIGENT Technology involves the reduction of the amount of fucose in the carbohydrate structure of an antibody using a proprietary fucosyl transferase-knockout CHO cell line. (-) : no change; (+) : 2 fold increase; (++) : 2–9.99 fold increase; (+++):10–99.99; (nd): no data.				

Exemplary and non-limiting embodiments of single domain antibody variants are provided herein. Binding agents comprising one or more antigen binding domain(s) of one or more single domain antibody variants are also encompassed by the present disclosure.

In an aspect of the present disclosure, single domain antibody variants include those having the amino acid sequence set forth in SEQ ID NO:143-146.

In accordance with the present disclosure, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence set forth in SEQ ID NO: 143.

SEQ ID NO:143 (KD002 consensus)

X_{1a}VQLX_{1b}ESGGGX_{1c}VQX_{1d}GGSLRLSCAAS**GFPFSSAD**MSWVRQAPGK$X_{1e}X_{1f}$EW VSX_{1g}**INAX**$_{1h}$**GSKT**YYAX_{1i}DX_{1j}VKGRFTISRDNX_{1k}KNTLYLX_{1l}MNX_{1m}L$X_{1n}X_{1o}$EDT AX_{1p}YX_{1q}C**ARAKLTDTHYVEDY**WGQGTX_{1r}VTVSS.

In an exemplary embodiment, any of X_{1a} to X_{1r} is each independently any (e.g., naturally occurring) amino acid residue.

In another exemplary embodiment, any of X_{1a} to X_{1r} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:23 or SEQ ID NOs:73-93, or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In yet in another exemplary embodiment, any of X_{1a} to X_{1r} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In a further exemplary embodiment, any X_{1a} to X_{1r} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:23 or SEQ ID Nos:73-93.

In another exemplary embodiment, any X_{1a} to X_{1r} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

In yet another exemplary embodiment, any of X_{1a} to X_{1r} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 73, 75, 76, 77, 78, 81, 82, 83, 85, 89 or 91, or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In a further exemplary embodiment, any X_{1a} to X_{1r} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 73, 75, 76, 77, 78, 81, 82, 83, 85, 89 or 91.

In yet a further exemplary embodiment X_{1a} is Q or E; X_{1b} is Q, V or L; X_{1c} is M or L; X_{1d} is V or P; X_{1e} is G or Q; X_{1f} is P, L or R; X_{1g} is Y or V; X_{1h} is D, N, E; X_{1i} is P or A; X_{1j} is S or T; X_{1k} is A or S; X_{1l} is L or Q; X_{1m} is N or S; X_{1n} is K or R; X_{1o} is P or A; X_{1p} is L or V; X_{1q} is R or Y; and/or X_{1r} is Q or M.

In another exemplary embodiment X_{1a} is Q or E; X_{1b} is Q, V or L; X_{1c} is M or L; X_{1d} is V or P; X_{1e} is G or Q; X_{1f} is P, L or R; X_{1g} is Y; X_{1h} is D, X_{1i} is P or A; X_{1j} is S or T; X_{1k} is A or S; X_{1l} is L or Q; X_{1m} is N or S; X_{1n} is K or R; X_{1o} is P or A; X_{1p} is L or V; X_{1q} is R or Y; and/or X_{1r} is Q or M.

In accordance with the present disclosure, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence set forth in SEQ ID NO:144.

SEQ ID NO:144 (KD005 consensus)

X_{2a}VQLX_{2b}ESGGGL$X_{2c}X_{2d}X_{2e}$GGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKGX_{2f}E WVSX_{2g}**ITSGGTT**YYX_{2h}DX_{2i}VKGRFTISRDNX_{2j}KNTLYLQMNSL$X_{2k}X_{2l}X_{2m}$DTAX_{2n}YX_{2o}C**AKSRLTDSHYVEDA**WGQGTX_{2p}VTVSS

In an exemplary embodiment, any of X_{2a} to X_{2p} is each independently any (e.g., naturally occurring) amino acid residue.

In another exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:26 or SEQ ID NO:94-114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In another exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In yet another exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:26 or SEQ ID NO:94-114.

In a further exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

In yet a further exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 100, 101, 102, 103, 104, 105, 106, 107 or 108 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In an additional exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 100, 101, 102, 103, 104, 105, 106, 107 or 108.

In another exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 100, 102, 103, 104, 105, 106, 108, 112 or 114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In a further exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 100, 102, 103, 104, 105, 106, 108, 112 or 114.

In yet a further exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 105, 112, 113 or 114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In another exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 105, 112, 113 or 114.

In another exemplary embodiment, any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 102 or 105 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In an additional exemplary embodiment, any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 102 or 105.

In another exemplary embodiment, X_{2a} is Q or E; X_{2b} is Q, L or V; X_{2c} is V or I; X_{2d} is E or Q; X_{2e} is A or P; X_{2f} is P, or L; X_{2g} is Y, A or V; X_{2h} is P or A; X_{2i} is S or T; X_{2j} is A or S; X_{2k} is K or R; X_{2l} is P or A; X_{2m} is D or E; X_{2n} is L or V; X_{2o} is R or Y and/or X_{2p} is Q or L.

In yet another exemplary embodiment, X_{2a} is Q or E; X_{2b} is Q or L; X_{2c} is V or I; X_{2d} is E or Q; X_{2e} is A or P; X_{2f} is P, or L; X_{2g} is Y or A; X_{2h} is P or A; X_{2i} is S or T; X_{2j} is A or S; X_{2k} is K or R; X_{2l} is P or A; X_{2m} is D or E; X_{2n} is L or V; X_{2o} is R or Y and/or X_{2p} is Q or L.

In accordance with the present disclosure, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence set forth in SEQ ID NO:145.

SEQ ID NO: 145 (Consensus from KD001 to KD005 heavy chain)
QVQLQESGGGX$_{3a}$VX$_{3b}$X$_{3c}$GX$_{3d}$SLRLSCAAS**GFX$_{3e}$FSX$_{3f}$X$_{3g}$D**MSWVRQAPGKGPE
WVSY**IX$_{3h}$X$_{3i}$X$_{3j}$X$_{3k}$X$_{3l}$X$_{3m}$T**X$_{3n}$YPDSVKGRFTX$_{3o}$SRDNAKNX$_{3p}$LYLX$_{3q}$MNX$_{3r}$LX$_{3s}$
PX$_{3t}$DTALYRC**AX$_{3u}$X$_{3v}$X$_{3w}$LTDX$_{3x}$HYVEDX$_{3y}$**WGQGTQVTVSS

In an exemplary embodiment any of X$_{3a}$ to X$_{3y}$ is each independently any (e.g., naturally occurring) amino acid residue.

In another exemplary embodiment, any of X$_{3a}$ to X$_{3y}$ is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In yet another exemplary embodiment, any of X$_{3a}$ to X$_{3y}$ is each independently an amino acid residue most frequently found in the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

In a further exemplary embodiment, X$_{3a}$ is M or L; X$_{3b}$ is Q, H or E; X$_{3c}$ is V, P or A; X$_{3d}$ is G or R; X$_{3e}$ is P or T; X$_{3f}$ is S, N or G; X$_{3g}$ is A, Y or S; X$_{3h}$ is N or T; X$_{3i}$ is A, S or G; X$_{3j}$ is D, G or S; X$_{3k}$ is G or absent; X$_{3l}$ is S or G; X$_{3m}$ is K, N or T; X$_{3n}$ is Y or D; X$_{3o}$ is I or T; X$_{3p}$ is T or M; X$_{3q}$ is L or Q; X$_{3r}$ is N or S; X$_{3s}$ is K or T; X$_{3t}$ is E or D; X$_{3u}$ is R or K; X$_{3v}$ is A or S; X$_{3w}$ is K or R; X$_{3x}$ is T or S; and/or X$_{3y}$ is Y or A.

In yet a further exemplary embodiment, X$_{3a}$ is M or L; X$_{3b}$ is Q, H or E; X$_{3c}$ is V, P or A; X$_{3d}$ is G or R; X$_{3c}$ is P or T; X$_{3f}$ is S, N or G; X$_{3g}$ is A, Y or S; X$_{3h}$ is N or T; X$_{3i}$ is A, S or G; X$_{3j}$ is D, G or S; X$_{3k}$ is G or absent; X$_{3l}$ is S or G; X$_{3m}$ is K, N or T; X$_{3n}$ is Y; X$_{3o}$ is I or T; X$_{3p}$ is T or M; X$_{3q}$ is L or Q; X$_{3r}$ is N or S; X$_{3s}$ is K or T; X$_{3t}$ is E or D; X$_{3u}$ is R or K; X$_{3v}$ is A or S; X$_{3w}$ is R; X$_{3x}$ is S; and/or X$_{3y}$ is A.

In accordance with the present disclosure, the single domain antibody or antigen binding fragment thereof comprises the amino acid sequence set forth in SEQ ID NO:146.

SEQ ID NO:146 (Consensus for KD001-KD005 and selected variants heavy chains)
X$_{4a}$VQLX$_{4b}$ESGGGX$_{4c}$X$_{4d}$X$_{4e}$X$_{4f}$GX$_{4g}$SLRLSCAASGFX$_{4h}$FSX$_{4i}$X$_{4j}$DMX$_{4k}$WX$_{4l}$RQAP
GKX$_{4m}$X$_{4n}$X$_{4o}$WVSX$_{4p}$IX$_{4q}$X$_{4r}$X$_{4s}$GX$_{4t}$X$_{4u}$TX$_{4v}$YX$_{4w}$DX$_{4x}$VKGRFTX$_{4y}$SRDNX$_{4z}$KNX$_{5a}$

$LYLX_{5b}MNX_{5c}LX_{5d}X_{5e}X_{5f}DTAX_{5g}YX_{5h}CAX_{5i}X_{5j}X_{5k}LTDX_{5l}HYVEDX_{5m}WGQGTX_{5n}V$ TVSS.

In an exemplary embodiment, any of X_{4a} to X_{5n} is each independently any (e.g., naturally occurring) amino acid residue.

In another exemplary embodiment, any of X_{4a} to X_{5n} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs:22-26, SEQ ID Nos:73-114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In yet another exemplary embodiment, any of X_{4a} to X_{5n} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof.

In another exemplary embodiment, any of X_{4a} to X_{5n} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs:22-26, SEQ ID Nos:73-114.

In another exemplary embodiment, any of X_{4a} to X_{5n} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

In a further exemplary embodiment, X_{4a} is Q or E, X_{4b} is Q, L or V, X_{4c} is M or L, X_{4d} is V or I, X_{4e} is Q, H or E, X_{4f} is P, A or V, X_{4g} is G or R, X_{4h} is P or T, X_{4i} is G, S or N, X_{4j} is A or S, X_{4k} is S or H, X_{4l} is V or Y, X_{4m} is G or Q, X_{4n} is P, L or R, X_{4o} is E or V, X_{4p} is Y, A or R, X_{4q} is T or N, X_{4r} is A, S or G, X_{4s} is G, S, D, N or E, X_{4t} is absent or S, X_{4u} is K or T, X_{4v} is Y, D or S, X_{4w} is P or A, X_{4x} is S or T, X_{4y} is I or T, X_{4z} is S or A X_{5a} is T or M X_{5b} is Q or L, X_{5c} is S or N, X_{5d} is K, R or T, X_{5e} is P or A, X_{5f} is E or D, X_{5g} is V or L, X_{5h} is Y or R, X_{5i} is R or K, X_{5j} is A or S, X_{5k} is K or R, X_{5l} is S or T, X_{5m} is Y or A, and/or X_{5n} is Q, L or M.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR3 is as set forth in SEQ ID NO:126.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146,

wherein the amino acid sequence of the CDR3 is as set forth in SEQ ID NO:126 and the amino acid sequence of the FR2 is as set forth in SEQ ID NO:121. In accordance with the present disclosure, the amino acid sequence of the CDR3 and the amino acid sequence of the FR2 are on a same polypeptide chain. Alternatively, in accordance with the present disclosure, the amino acid sequence of the CDR3 and the amino acid sequence of the FR2 are on separate polypeptide chains.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the CDR3 amino acid sequence is as set forth in SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:54 or SEQ ID NO:61.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the FR2 amino acid sequence is as set forth in SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 or SEQ ID NO:120.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR1 is as set forth in SEQ ID NO:122.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the CDR1 amino acid sequence is as set forth in SEQ ID NO:52, SEQ ID NO:55 or SEQ ID NO:56.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR2 is as set forth in SEQ ID NO:125.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the CDR2 amino acid sequence is as set forth in SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:123 or SEQ ID NO:124.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146,

wherein the amino acid sequence of the CDR3 is as set forth in any one of SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:54, SEQ ID NO:58 or SEQ ID NO:61 and the amino acid sequence of the FR2 is as set forth in any one of SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 or SEQ ID NO:120.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR3 is as set forth in any one of SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:54, SEQ ID NO:58 or SEQ ID NO:61, the amino acid sequence of the FR2 is as set forth in any one of SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 or SEQ ID NO:120, the amino acid sequence of the CDR1 is as set forth in any one of SEQ ID NO: 52, SEQ ID NO: 55, SEQ ID NO:59, SEQ ID NO: 52 or SEQ ID NO: 62 and the amino acid sequence of the CDR2 is as set forth in any one of SEQ ID NO: 47, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO:123 or SEQ ID NO:124.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR3 is as set forth in SEQ ID NO:142.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the FR2 is as set forth in SEQ ID NO:175.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR3 is as set forth in SEQ ID NO:142 and the amino acid sequence of the FR2 is as set forth in SEQ ID NO:175.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the CDR3 amino acid sequence is as set forth in SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146,

wherein the FR2 amino acid sequence is as set forth SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 or SEQ ID NO:174. In a specific embodiment, the amino acid sequence of the FR2 is as set forth in SEQ ID NO:170 or SEQ ID NO:171.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR1 is as set forth in SEQ ID NO:128.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the CDR1 amino acid sequence is as set forth in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:127.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR2 is as set forth in SEQ ID NO:140.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the CDR2 amino acid sequence is as set forth in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138 or SEQ ID NO:139.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR3 is as set forth in any one of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141 and the amino acid sequence of the FR2 is as set forth in any one of SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 or SEQ ID NO:174.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR3 is as set forth in any one of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141, the amino acid sequence of the

FR2 is as set forth in any one of SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 or SEQ ID NO:174, the amino acid sequence of the CDR1 is as set forth in any one of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:127 and the amino acid sequence of the CDR2 is as set forth in any one of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138 or SEQ ID NO:139.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof may have the amino acid sequence as defined in any one of SEQ ID NOs: 143 to 146, wherein the amino acid sequence of the CDR3 is as set forth in any one SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141, the amino acid sequence of the FR2 is as set forth in any one of SEQ ID NO:170 or SEQ ID NO:171, the amino acid sequence of the CDR1 is as set forth in any one of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:127 and the amino acid sequence of the CDR2 is as set forth in any one of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138 or SEQ ID NO:139.

In an aspect of the present disclosure, single domain antibody variants include those comprising an amino acid sequence at least 65%, 70%, 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence of the antigen binding domains, variable region or single domain antibodies exemplified herein.

In some embodiments, single domain antibody variants include variants of KD001 (having a variable region as set forth in SEQ ID NO:22). In exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:22.

Other exemplary embodiments of single domain antibody variants include variants of KD002 (having a variable region as set forth in SEQ ID NO:23). In some exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:23. In

other exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence as set forth in any one of SEQ ID NO:71-93.

In some embodiments, single domain antibody variants include variants of KD003 (having a variable region as set forth in SEQ ID NO:24). In additional exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:24.

In some embodiments, single domain antibody variants include variants of KD004 (having a variable region as set forth in SEQ ID NO:25). In further exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:25.

Other exemplary embodiments of single domain antibody variants include variants of KD005 (having a variable region as set forth in SEQ ID NO:26). In some exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:26.

In some exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence as set forth in any one of SEQ ID NO:73-93.

In other exemplary embodiments, the single domain antibody comprises a variable region having an amino acid sequence as set forth in any one of SEQ ID NO:94-114.

Other exemplary embodiments of single domain antibody variants include those comprising an amino acid sequence at least 65% identical to the amino acid sequence set forth in any one of SEQ ID NOs:22-26, or SEQ ID NOs: 73-114.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:22. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:22.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least

80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:23. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:23.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:24. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:24.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:25. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:25.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:26. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:26.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:71. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:71.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least

80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:72. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:72.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:73. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:73.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:74. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:74.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:75. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:75.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:76. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:76.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least

80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:77. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:77.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:78. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:78.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:79. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:79.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:80. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:80.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:81. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:81.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least

80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:82. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:82.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:83. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:83.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:84. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:84.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:85. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:85.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:86. More particularly, the single domain antibody or antigen binding fragment thereof comprises may comprise an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:86.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises may comprise an amino acid sequence at least 70% identical, at least 75%

identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:87. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:87.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:88. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:88.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:89. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:89.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:90. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:90.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:91. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:91.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least

80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:92. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:92.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:93. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:93.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:94. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:94.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:95. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:95.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:96. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:96.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least

80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:97. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:97.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:98. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:98.

In an exemplary embodiment the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:99. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:99.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:100. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:100.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:101. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:101.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least

80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:102. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:102.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:103. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:103.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:104. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:104.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:105. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:105.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:106. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:106.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least

80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:107. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:107.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:108. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:108.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:109. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:109.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:110. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:110.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:111. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:111.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least

80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:112. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:112.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:113. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:113.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:114. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:114.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to SEQ ID NO:115. More particularly, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 95% identical, at least 99% identical or identical to SEQ ID NO:115.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:29.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:30.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:31.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:32.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:33.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:34.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:35.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:44.

In an exemplary embodiment, the single domain antibody or antigen binding fragment thereof comprises an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:45.

In accordance with the present disclosure, the single domain antibody or antigen binding fragment thereof comprises a sequence as set forth herein, wherein the amino acid residue at position 37 is V, the amino acid residue at position 39 is Q, the amino acid residue at position 45 is P, the amino acid residue at position 47 is W, the amino acid residue at position 50 is Y, the amino acid residue at position 58 is Y, the amino acid residue at position 99 is R, the amino acid residue at position 100 is L, the amino acid residue at position 101 is T, the amino acid residue at position 102 is D, the amino acid residue at position 103 is S, the amino acid residue at position 104 is H, the amino acid residue at position 105 is Y, the amino acid residue at position 106 is V,

the amino acid residue at position 107 is E, the amino acid residue at position 108 is D, the amino acid residue at position 109 and/or the position is with reference to SEQ ID NO:145 (**Table 1**).

In accordance with the present disclosure, the single domain antibody or antigen binding fragment thereof comprises a sequence as set forth herein, wherein the amino acid residue at position 1 is Q, the amino acid residue at position 2 is V, the amino acid residue at position 37 is V, the amino acid residue at position 39 is Q, the amino acid residue at position 45 is P, the amino acid residue at position 47 is W, the amino acid residue at position 50 is Y, the amino acid residue at position 58 is Y, the amino acid residue at position 99 is R, the amino acid residue at position 100 is L, the amino acid residue at position 101 is T, the amino acid residue at position 102 is D, the amino acid residue at position 103 is S, the amino acid residue at position 104 is H, the amino acid residue at position 105 is Y, the amino acid residue at position 106 is V, the amino acid residue at position 107 is E, the amino acid residue at position 108 is D, the amino acid residue at position 109 is A, the amino acid residue at position 110 is W and/or the amino acid residue at position 112 is Q and the position is with reference to SEQ ID NO:145 (**Table 1**).

In accordance with the present disclosure, the single domain antibody or antigen binding fragment thereof comprises a sequence as set forth herein, wherein the amino acid residue at position 1 is Q, the amino acid residue at position 2 is V, the amino acid residue at position 3 is Q, the amino acid residue at position 4 is L, the amino acid residue at position 37 is V, the amino acid residue at position 39 is Q, the amino acid residue at position 45 is P, the amino acid residue at position 46 is E, the amino acid residue at position 47 is W, the amino acid residue at position 50 is Y, the amino acid residue at position 58 is Y, the amino acid residue at position 99 is R, the amino acid residue at position 100 is L, the amino acid residue at position 101 is T, the amino acid residue at position 102 is D, the amino acid residue at position 103 is S, the amino acid residue at position 104 is H, the amino acid residue at position 105 is Y, the amino acid residue at position 106 is V, the amino acid residue at position 107 is E, the amino acid residue at position 108 is D, the amino acid residue at position 109 is A, the amino acid residue at position 110 is W, the amino acid residue at position 111 is G, and/or the amino acid residue at position 112 is Q and the position is with reference to SEQ ID NO:145 (**Table 1**).

Yet other exemplary embodiments of single domain antibody variants include single domain antibody or antigen binding fragment thereof having an amino acid sequence as defined

in any one of SEQ ID Nos:22-26, 73-114, or 143 to 146 and may comprise from one to five amino acid deletions or from one to five amino acid additions. In an exemplary embodiment, the deletion or additions are consecutive. In another exemplary embodiment, the deletion or additions are non-consecutives. In yet another exemplary embodiment, the deletion or additions are at the N-terminus. In a further exemplary embodiment, the deletion or additions are at the C-terminus. In yet a further exemplary embodiment, the deletion or additions are outside of an IMGT CDR3 sequence and/or IMGT FR2 sequence. In yet a further exemplary embodiment, the deletion or additions are outside of a Kabat CDR3 sequence and/or Kabat FR2 sequence.

Exemplary embodiments of binding agent include for example and without limitation binding agent variants such as bispecific binding agent variants.

For example, a bispecific binding agent variant may comprise one or more antigen binding domains of one or more single domain antibody variants.

In an exemplary embodiment, the binding agent is a bispecific binding agent variant that comprises an antigen binding domain that specifically binds to CD47 and that comprises an amino acid sequence at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to the amino acid sequence set forth in SEQ ID NO:155.

In an exemplary embodiment, the bispecific binding agent variant is capable of binding to CD47 and TROP2 and comprises an amino acid sequence at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% to the amino acid sequence set forth in SEQ ID NO:155 and the amino acid sequence of an antigen binding domain of a single domain antibody or of a single domain antibody variant as disclosed herein.

In other exemplary embodiments, the bispecific binding agent variant is capable of binding to CD47 and TROP2 and comprises an amino acid sequence at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% to the amino acid sequence set forth in SEQ ID NO:155 and the amino acid sequence of an antigen binding domain of a single domain antibody disclosed as set forth in any one of SEQ ID NOs:22-26 or SEQ ID Nos:73-114.

In another exemplary embodiment, the bispecific binding agent variant is capable of binding to CD47 and TROP2 and comprises an amino acid sequence at least 80% identical, at least

85% identical, at least 90% identical, at least 95% identical, at least 99% to the amino acid sequence set forth in SEQ ID NO:150 or in SEQ ID NO:151.

The bispecific binding agent variant may comprise:

a CDRH1 having the amino acid sequence set forth in SEQ ID NO156, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:157 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:158, or

b. a CDRH1 having the amino acid sequence set forth in SEQ ID NO159, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:160 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:161.

Competing binding agents

Binding agents of the present disclosure also encompass those that compete with a binding agent, or a single domain antibody disclosed herewith. Single domain antibodies that compete with one or more single domain antibody or antigen binding fragment thereof having an amino acid sequence as disclosed herein are provided.

In an exemplary embodiment the competing binding agent is capable of competing with a binding agent as disclosed herein.

In an exemplary embodiment the competing binding agent comprises one or more antigen binding domains that are capable of competing with the binding agent disclosed herein.

In an exemplary embodiment, a competing binding agent or competing single domain antibody may be selected based on any type of binding assays (FACS, ELISA, SPR etc.) using a TROP2 antigen as described herein.

In another exemplary embodiment, a competing binding agent or competing single domain antibody may be selected based on screening assays described herein.

In some exemplary embodiments, the competing binding agent or competing single domain antibody comprises an amino acid sequence at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical to the amino acid sequence set forth in any one of SEQ ID NOs:22-26, or SEQ ID NOs: 73-114.

In other exemplary embodiments, the competing binding agent or competing single domain antibody comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:126, the amino acid sequence of the FR2 set forth in SEQ ID NO:121.

In yet other exemplary embodiments, the competing binding agent or competing single domain antibody comprises:

a. the amino acid sequence of the CDR3 set forth in SEQ ID NO:54 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:52 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:53;

b. the amino acid sequence of the CDR3 set forth in SEQ ID NO:48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:47;

c. the amino acid sequence of the CDR3 set forth in SEQ ID NO:58 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:56 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:57;

d. the amino acid sequence of the CDR3 set forth in SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:59 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:60;

e. the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50,

f. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

g. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

h. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

i. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:119 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

j. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 124;

k. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 123;

l. the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50;

m. the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50;

n. amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50;

o. amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:120 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50;

p. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:127, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:129 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

q. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:130 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

r. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:131 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

s. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:132 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

t. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:133 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

u. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:134 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

v. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:135 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

w. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:136 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

x. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:137 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

y. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:138 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15 or,

z. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:139 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15.

In other exemplary embodiments, the competing binding agent or competing single domain antibody comprises:

a. the amino acid sequence of the CDR1 set forth in SEQ ID NO:52, the amino acid sequence of the CDR2 set forth in SEQ ID NO:53, the amino acid sequence of the CDR3 set forth in SEQ ID NO:54 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

b. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO:48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

c. the amino acid sequence of the CDR1 set forth in SEQ ID NO:56, the amino acid sequence of the CDR2 set forth in SEQ ID NO:57, the amino acid sequence of the CDR3 set forth in SEQ ID NO:58 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

d. the amino acid sequence of the CDR1 set forth in SEQ ID NO:59, the amino acid sequence of the CDR2 set forth in SEQ ID NO:60, the amino acid sequence of the

CDR3 set forth in SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

e. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

f. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

g. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

h. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118;

i. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:119;

j. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 124, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

k. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 123, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

l. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

m. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

n. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 or,

o. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:120.

In yet other exemplary embodiments, the competing binding agent or competing single domain antibody comprises the amino acid sequence as defined in any one of SEQ ID NO:143-146 and the amino acid residue at position 1 is Q, the amino acid residue at position 2 is V, the amino acid residue at position 3 is Q, the amino acid residue at position 4 is L, the amino acid residue at position 37 is V, the amino acid residue at position 39 is Q, the amino acid residue at position 45 is P, the amino acid residue at position 46 is E, the amino acid residue at position 47 is W, the amino acid residue at position 50 is Y, the amino acid residue at position 58 is Y, the amino acid residue at position 99 is R, the amino acid residue at position 100 is L, the amino acid residue at position 101 is T, the amino acid residue at position 102 is D, the amino acid residue at position 103 is S, the amino acid residue at position 104 is H, the amino acid residue at position 105 is Y, the amino acid residue at position 106 is V, the amino acid residue at position 107 is E, the amino acid residue at position 108 is D, the amino acid residue at position 109 is A, the amino acid residue at position 110 is W, the amino acid residue at position 111 is G, and/or the amino acid residue at position 112 is Q and the position is with reference to SEQ ID NO:145 (**Table 1**).

In yet other exemplary embodiments, the competing binding agent or competing single domain antibody has an affinity of $\leq 10^{-6}$ M for human TROP2.

In other exemplary embodiments, the competing binding agent or competing single domain antibody has anti-tumor activity.

In other exemplary embodiments, the competing binding agent or competing single domain antibody competes with a single domain antibody comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:26.

In other exemplary embodiments, the competing binding agent or competing single domain antibody competes with a single domain antibody comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:33.

In other exemplary embodiments, the present disclosure provides an antibody or an antigen binding fragment thereof that specifically binds to an epitope comprising amino acid residues of amino acids 27-146 of TROP2; and competes for binding to TROP2 with a single domain antibody that specifically binds to an epitope formed by amino acid residues of amino acids 27-146 of TROP2.

Nucleic acids, vectors, kits, cells and method of making binding agents

The present disclosure also relates to a nucleic acid molecule or vector encoding a binding agent or a binding agent variant as disclosed herein.

In some embodiments, the nucleic acid molecule or vector encodes an antibody or an antigen binding fragment thereof or a variant thereof.

In some embodiments, the nucleic acid molecule or vector encodes a single domain antibody or an antigen binding fragment thereof or a variant thereof.

In some embodiments, the nucleic acid molecule or vector encodes a competing binding agent.

In some embodiments, the nucleic acid molecule or vector encodes a competing antibody or antigen binding fragment thereof.

In some embodiments, the nucleic acid molecule or vector encodes a competing single domain antibody or antigen binding fragment thereof.

In some embodiments, the nucleic acid molecule or vector encodes an antibody-like molecule.

In some embodiments, the nucleic acid molecule or vector encodes a protein scaffold.

In some embodiments, the nucleic acid molecule or vector encodes an immune cell modulating agent.

Nucleic acid molecules of the present disclosure may be single-stranded or double-stranded. The nucleic acid molecules disclosed herein may comprises deoxyribonucleotides, ribonucleotides, modified deoxyribonucleotides or modified ribonucleotides. The nucleic acid molecules of the present disclosure may comprise for example DNA.

In some embodiments, the nucleic acid sequence is as set forth in **Table 22**.

Due to the inherent degeneracy of the genetic code, DNA sequences that encode the same, substantially the same or a functionally equivalent amino acid sequence may be produced and used. As such, in some embodiments, the nucleic acid sequence may be a variant of the nucleic acid sequence set forth in **Table 22**. In some embodiments, the nucleic acid variant may be at least 80%, at least 85%, at least 905, at least 95%, at least 99% identical to the nucleic acid sequence set forth in **Table 22** and encodes an amino acid sequence of a binding agent, antigen binding domain or a portion disclosed herein.

The nucleotide sequences of the present disclosure may be engineered using methods generally known in the art in order to alter the nucleotide sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth. Codon-optimized nucleic acids encoding the polypeptide chains described herein are encompassed by the present disclosure.

The polypeptide chains and binding agents disclosed herein may be made by a variety of methods familiar to those skilled in the art, including by recombinant DNA methods or by *in vitro* transcription/translation.

Generally, the polypeptide chains described herein are expressed from nucleic acid sequences inserted into an expression vector, *i.e.*, a vector that contains the elements for transcriptional and translational control of the inserted coding sequence in a particular host. These

elements may include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' un-translated regions.

A variety of expression vector/host cell systems known to those of skill in the art may be used to express the polypeptide chains or binding agent described herein. In the event, that the binding agent is composed of distinct polypeptide chains, each of such polypeptide chain may be provided by separate expression vectors or by a unique expression vector. In accordance with the present disclosure, the two chains of a binding agent may be encoded by a single vector or by separate vectors (vector set).

Polypeptides are often expressed in mammalian cells. For long-term production of recombinant proteins, a stable expression system may be used in which the DNA segment is incorporated into the host cell genome or maintained in an episomal form by the use of selectable markers. A host cell type may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed polypeptide in the desired fashion. Different host cells that have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and W138) are available commercially and from the American Type Culture Collection (ATCC) and may be chosen to ensure the correct modification and processing of the expressed polypeptide.

Other types of expression system can be used. These include, for example, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with baculovirus vectors; plant cell systems transformed with viral or bacterial expression vectors; or animal cell systems.

The present disclosure therefore relates to isolated cells transformed or transfected with a vector, nucleic acid, sets of vectors or sets of nucleic acids encoding the binding agents (antibodies or antigen binding fragments thereof such as single domain antibodies) described herein. The present disclosure therefore also relates to isolated cells capable or expressing the binding agents (antibodies or antigen binding fragments such as single domain antibodies) thereof disclosed herein.

The present disclosure also relates to a method of making binding agents (e.g., antibodies or antigen binding fragments thereof such as single domain antibodies). The method may comprise

providing a cell (e.g., a mammalian cell) with a vector or sets of vectors encoding the binding agent (e.g., antibodies or antigen binding fragments thereof such as single domain antibodies) disclosed herein and allowing expression.

The method may also comprises purifying the binding agents (e.g., antibodies or antigen binding fragments such as single domain antibodies) thereof from cells or cell debris.

In some embodiments, the method of manufacture allows to reach a purity level of at least 80%, of at least 85%, at least 90%, at least 99% (of dimers).

In some embodiments, the purity level is between 80.0% to 99.9%.

In some embodiments, the purity level is between 95.0% to 99.9%.

In some embodiments, the purity level is at least 90.0 +/- 5.0%.

In some embodiments, the purity level is at least 95.0 +/- 5.0%.

In some embodiments, the purity level is 99.0 +/- 1.0%.

In some embodiments, the titer of the antibodies or antigen binding fragments thereof (e.g., single domain antibody) produced by cells may be 0.1 g/L or more. In some instances, the titer of the antibodies or antigen binding fragments thereof produced by cells may be 0.5 g/L or more. In some instances, the titer of the antibodies or antigen binding fragments thereof produced by cells may be 1 g/L or more. In some instances, the titer of the antibodies or antigen binding fragments thereof produced by cells may be 2 g/L or more. In some instances, the titer of the antibodies or antigen binding fragments thereof produced by cells may be 3 g/L or more. In some instances, the titer of the antibodies or antigen binding fragments thereof produced by cells may be 4 g/L or more.

The method may also comprise a step of isolating and/or purifying the antibodies or antigen binding fragments thereof from impurities.

Conjugates

In some embodiments, the binding agent of the present disclosure may be conjugated with a therapeutic moiety or with a detectable moiety.

Although, the binding agents of the present disclosure may have activity against tumor cells when unconjugated, the present disclosure also encompasses a conjugated form of such binding agents.

Binding agents described herein may, in some instances, have no or low activity against tumor cells when unconjugated. Binding agents may thus be conjugated with a therapeutic moiety so as to deliver the therapeutic moiety to TROP2-expressing cells.

The antibodies or antigen binding fragments thereof of the present disclosure (e.g., single domain antibodies or antigen binding fragments thereof) may be conjugated, for example, with a therapeutic moiety (for therapeutic purposes) or with a detectable moiety (i.e., for detection or diagnostic purposes) or to a protein allowing an extended half-life or is attached to nanoparticle. In some instances, therapeutic or detectable moieties may be linked to at least one amino acid residues of the antibodies or antigen binding fragments thereof.

In an exemplary embodiment, the antibodies or antigen binding fragments thereof of the present disclosure is conjugated with a therapeutic moiety such as for example and without limitation, a chemotherapeutic, a cytokine, a cytotoxic agent, an anti-cancer drug (e.g., small molecule), and the like.

Therapeutic moiety may include, for example and without limitation, Yttrium-90, Scandium-47, Rhenium-186, Iodine-131, Iodine-125, and many others recognized by those skilled in the art (e.g., lutetium (e.g., Lu^{177}), bismuth (e.g., Bi^{213}), copper (e.g., Cu^{67})), 5-fluorouracil, adriamycin, irinotecan, taxanes, pseudomonas endotoxin, ricin, auristatins (e.g., monomethyl auristatin E, monomethyl auristatin F), maytansinoids (e.g., mertansine, batansine), tubulysin B analogues (e.g., Tub196), camptothecin, exatecan derivatives (e.g., Dxd), belotecanderivatives, irinotecan or irinotecan metabolites (e.g., SN-38) and other toxins.

In another exemplary embodiment, the antibodies or antigen binding fragments thereof of the present disclosure is conjugated with a detectable moiety including for example and without limitation, a moiety detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical and/or other physical means. A detectable moiety may be coupled either directly and/or indirectly (for example via a linkage, such as, without limitation, a DOTA or NHS linkage) to the polypeptide chain or binding agent using methods well known in the art. A wide variety of detectable moieties may be used, with the choice depending on the sensitivity required, ease of conjugation, stability requirements and available instrumentation. A suitable detectable moiety includes, but is not limited to, a fluorescent label, a radioactive label (for example, without limitation, ^{125}I, In^{111}, Tc^{99}, I^{131} and including positron emitting isotopes for PET scanner etc), a

nuclear magnetic resonance active label, a luminescent label, a chemiluminescent label, a chromophore label, an enzyme label (for example and without limitation horseradish peroxidase, alkaline phosphatase, etc.), quantum dots and/or a nanoparticle. Detectable moiety may cause and/or produce a detectable signal thereby allowing for a signal from the detectable moiety to be detected.

Pharmaceutical compositions

Pharmaceutical compositions comprising binding agents of the present disclosure are also encompassed by the present disclosure.

In some embodiments, the pharmaceutical composition comprises a binding agent variant.

In some embodiments, the pharmaceutical composition comprises an antibody or an antigen binding fragment thereof or a variant thereof.

In some embodiments, the pharmaceutical composition comprises a single domain antibody or an antigen binding fragment thereof or a variant thereof.

In some embodiments, the pharmaceutical composition comprises a competing binding agent.

In some embodiments, the pharmaceutical composition comprises a competing antibody or antigen binding fragment thereof.

In some embodiments, the pharmaceutical composition comprises a competing single domain antibody or antigen binding fragment thereof.

In some embodiments, the pharmaceutical composition comprises an antibody-like molecule.

In some embodiments, the pharmaceutical composition comprises a protein scaffold.

In some embodiments, the pharmaceutical composition comprises an immune cell modulating agent or cells expressing the immune cell modulating agent.

Pharmaceutical compositions may therefore comprise the antibodies or antigen binding fragments thereof of the present disclosure (e.g., single domain antibodies or antigen. Binding fragments thereof).

The pharmaceutical composition of the present disclosure may also comprise a pharmaceutically acceptable carrier.

In some embodiments, the pharmaceutical composition comprises conjugated binding agents.

In some embodiments, the pharmaceutical composition comprises conjugated antibodies or antigen binding fragments thereof as disclosed herein (e.g., single domain antibodies or antigen binding fragment thereof). In some embodiments, the pharmaceutical composition comprises antibodies or antigen binding fragments thereof (e.g., single domain antibodies or antigen binding fragment thereof) conjugated with a therapeutic moiety. In some embodiments, the pharmaceutical composition comprises antibodies or antigen binding fragments thereof (e.g., single domain antibodies or antigen binding fragment thereof) conjugated with a detectable label.

In addition to the active ingredients, a pharmaceutical composition may contain pharmaceutically acceptable carriers comprising water, PBS, salt solutions, gelatins, oils, alcohols, and other excipients and auxiliaries that facilitate processing of the active compounds into preparations that may be used pharmaceutically. In other instances, such preparations may be sterilized.

As used herein, "pharmaceutical composition" means therapeutically effective amounts of the agent together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvant and/or carriers. A "therapeutically effective amount" as used herein refers to that amount which provides a therapeutic effect for a given condition and administration regimen. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (e.g., Tris-HCl., acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween™ 20, Tween™ 80, Pluronic F68, bile acid salts). Solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Such compositions will

influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils). Also encompassed by the disclosure are particulate compositions coated with polymers (e.g., poloxamers or poloxamines). Other embodiments of the compositions of the disclosure incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal, oral, vaginal, rectal routes. In one embodiment the pharmaceutical composition is administered parenterally, paracancerally, transmucosally, transdermally, intramuscularly, intravenously, intradermally, subcutaneously, intraperitonealy, intraventricularly, intracranially and intratumorally.

Further, as used herein "pharmaceutically acceptable carrier" or "pharmaceutical carrier" are known in the art and include, but are not limited to, 0.01-0.1 M or 0.05 M phosphate buffer or 0.8 % saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's orfixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, collating agents, inert gases and the like.

For any compound, the therapeutically effective dose may be estimated initially either in cell culture assays or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the concentration range and route of administration. Such information may then be used to determine useful doses and routes for administration in humans. These techniques are well known to one skilled in the art and a therapeutically effective dose refers to that amount of active ingredient that ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating and contrasting the ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the

population) statistics. Any of the pharmaceutical compositions described above may be applied to any subject in need of therapy, including, but not limited to, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and especially humans.

The pharmaceutical compositions described herein may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In an exemplary embodiment, the pharmaceutical composition comprises a single domain antibody or an antigen binding fragment thereof selected from KD001, KD002, KD003, KD004, KD005, KD006, KD007, KD008, KD009, KD010, KD011, KD012, KD013, KD014, KD015, KD016, KD017, KD018, KD019, KD020, KD021, KD022, KD023, KD024, KD025, KD026, KD027, KD028, KD029, KD030, KD031, KD032, KD033, KD034, KD035, KD036, KD037, KD038, KD039, KD040, KD041, KD042, KD043, KD044, KD045, KD046, KD047, KD048, KD049, KD050, KD051, KD052, KD053, KD054, KD055, KD056, KD057, KD058, KD059, KD060, KD061, KD062, KD063 or KD064.

In some embodiment, the pharmaceutical composition comprises a single domain antibody or antigen binding fragment thereof selected from the group of **Figure 27**.

In some embodiment, the pharmaceutical composition comprises a single domain antibody or antigen binding fragment thereof selected from the group of **Figure 28**.

In some embodiment, the pharmaceutical composition comprises a single domain antibody or antigen binding fragment thereof selected from the group of **Figure 29**.

In some embodiment, the pharmaceutical composition comprises a single domain antibody or antigen binding fragment thereof selected from the group of **Figure 30**.

In some embodiment, the pharmaceutical composition comprises a single domain antibody or antigen binding fragment thereof selected from the group of **Figure 31**.

In some embodiment, the pharmaceutical composition comprises a single domain antibody or antigen binding fragment thereof selected from the group of **Figure 32**.

In some embodiment, the pharmaceutical composition comprises a single domain antibody or antigen binding fragment thereof selected from the group of **Figure 33**.

In some embodiment, the pharmaceutical composition comprises a single domain antibody or antigen binding fragment thereof selected from the group of **Figure 34**.

In an exemplary embodiment, the pharmaceutical composition comprises KD065 or an antigen binding fragment thereof.

In an exemplary embodiment, the pharmaceutical composition comprises KD066 or an antigen binding fragment thereof.

In another exemplary embodiment, the pharmaceutical composition comprises a single domain antibody or an antigen binding fragment thereof selected from KD067, KD068 or KD069 or an antigen binding fragment thereof.

In some embodiment, the pharmaceutical composition comprises KD001 or a binding agent that comprises the antigen binding domain of KD001. In other embodiment, the pharmaceutical composition comprises a variant of KD001 or a binding agent that comprises the antigen binding domain of a KD001 variant. In some embodiment, the pharmaceutical composition comprises a humanized version of KD001 or a binding agent that comprises the antigen binding domain of humanized version of KD001. In some embodiment, the pharmaceutical composition comprises a single domain antibody capable of competing with KD001 or a binding agent that comprises the antigen binding domain of a single domain antibody capable of competing with KD001.

In some embodiment, the pharmaceutical composition comprises KD002 or a binding agent that comprises the antigen binding domain of KD002. In other embodiment, the pharmaceutical composition comprises a variant of KD002 or a binding agent that comprises the antigen binding domain of a KD002 variant. In some embodiment, the pharmaceutical composition comprises a humanized version of KD002 or a binding agent that comprises the antigen binding domain of humanized version of KD002. In some embodiment, the pharmaceutical composition comprises a single domain antibody capable of competing with KD002 or a binding agent that comprises the antigen binding domain of a single domain antibody capable of competing with KD002.

In some embodiment, the pharmaceutical composition comprises KD003 or a binding agent that comprises the antigen binding domain of KD003. In other embodiment, the pharmaceutical

composition comprises a variant of KD003 or a binding agent that comprises the antigen binding domain of a KD003 variant. In some embodiment, the pharmaceutical composition comprises a humanized version of KD003 or a binding agent that comprises the antigen binding domain of humanized version of KD003. In some embodiment, the pharmaceutical composition comprises a single domain antibody capable of competing with KD003 or a binding agent that comprises the antigen binding domain of a single domain antibody capable of competing with KD003.

In some embodiment, the pharmaceutical composition comprises KD004 or a binding agent that comprises the antigen binding domain of KD004. In other embodiment, the pharmaceutical composition comprises a variant of KD004 or a binding agent that comprises the antigen binding domain of a KD004 variant. In some embodiment, the pharmaceutical composition comprises a humanized version of KD004 or a binding agent that comprises the antigen binding domain of humanized version of KD004. In some embodiment, the pharmaceutical composition comprises a single domain antibody capable of competing with KD004 or a binding agent that comprises the antigen binding domain of a single domain antibody capable of competing with KD004.

In some embodiment, the pharmaceutical composition comprises KD005 or a binding agent that comprises the antigen binding domain of KD005. In other embodiment, the pharmaceutical composition comprises a variant of KD005 or a binding agent that comprises the antigen binding domain of a KD005 variant. In some embodiment, the pharmaceutical composition comprises a humanized version of KD005 or a binding agent that comprises the antigen binding domain of humanized version of KD005. In some embodiment, the pharmaceutical composition comprises a single domain antibody capable of competing with KD005 or a binding agent that comprises the antigen binding domain of a single domain antibody capable of competing with KD005.

In some embodiment, the pharmaceutical composition comprises KD006 or a binding agent that comprises the antigen binding domain of KD006. In other embodiment, the pharmaceutical composition comprises a variant of KD006 or a binding agent that comprises the antigen binding domain of a KD006 variant. In some embodiment, the pharmaceutical composition comprises a humanized version of KD006 or a binding agent that comprises the antigen binding domain of humanized version of KD006. In some embodiment, the pharmaceutical composition comprises a single domain antibody capable of competing with KD006 or a binding agent that comprises the antigen binding domain of a single domain antibody capable of competing with KD006.

In some embodiment, the pharmaceutical composition comprises KD007 or a binding agent that comprises the antigen binding domain of KD007. In other embodiment, the pharmaceutical composition comprises a variant of KD007 or a binding agent that comprises the antigen binding domain of a KD007 variant. In some embodiment, the pharmaceutical composition comprises a humanized version of KD007 or a binding agent that comprises the antigen binding domain of humanized version of KD007. In some embodiment, the pharmaceutical composition comprises a single domain antibody capable of competing with KD007 or a binding agent that comprises the antigen binding domain of a single domain antibody capable of competing with KD007.

In some embodiment, the pharmaceutical composition comprises KD048 or a binding agent that comprises the antigen binding domain of KD048. In other embodiment, the pharmaceutical composition comprises a variant of KD048 or a binding agent that comprises the antigen binding domain of a KD048 variant. In some embodiment, the pharmaceutical composition comprises a humanized version of KD048 or a binding agent that comprises the antigen binding domain of humanized version of KD048. In some embodiment, the pharmaceutical composition comprises a single domain antibody capable of competing with KD048 or a binding agent that comprises the antigen binding domain of a single domain antibody capable of competing with KD048.

In some embodiment, the pharmaceutical composition comprises KD053 or a binding agent that comprises the antigen binding domain of KD053. In other embodiment, the pharmaceutical composition comprises a variant of KD053 or a binding agent that comprises the antigen binding domain of a KD053 variant. In some embodiment, the pharmaceutical composition comprises a humanized version of KD053 or a binding agent that comprises the antigen binding domain of humanized version of KD053. In some embodiment, the pharmaceutical composition comprises a single domain antibody capable of competing with KD053 or a binding agent that comprises the antigen binding domain of a single domain antibody capable of competing with KD053.

Any one of KD001-KD007, including variants (e.g., including humanized version), competing single domain antibodies thereof or binding agent comprising same may comprise a single polypeptide chain, two polypeptide chains or more than two polypeptide chains.

In some instances, the pharmaceutical composition may comprise a conjugated form of a single domain antibody or an antigen binding fragment thereof selected from KD001, KD002, KD003, KD004, KD005, KD006, KD007, KD008, KD009, KD010, KD011, KD012, KD013,

KD014, KD015, KD016, KD017, KD018, KD019, KD020, KD021, KD022, KD023, KD024, KD025, KD026, KD027, KD028, KD029, KD030, KD031, KD032, KD033, KD034, KD035, KD036, KD037, KD038, KD039, KD040, KD041, KD042, KD043, KD044, KD045, KD046, KD047, KD048, KD049, KD050, KD051, KD052, KD053, KD054, KD055, KD056, KD057, KD058, KD059, KD060, KD061, KD062, KD063 or KD064 or their corresponding variants, competing single domain antibodies thereof or binding agent comprising same.

Method of use

The present disclosure also relates to the use of binding agents disclosed herein (or pharmaceutical composition comprising same) for treatment of disorders or diseases.

In some embodiments, the method comprises administering a binding agent or a variant thereof or a pharmaceutical composition comprising same to a subject in need.

In some embodiments, the binding agent is an antibody or an antigen binding fragment thereof or a variant thereof and the method comprises administering the antibody or an antigen binding fragment thereof or variant thereof or a pharmaceutical composition comprising same to a subject in need.

In some embodiments, the binding agent is a single domain antibody or an antigen binding fragment thereof or a variant thereof and the method comprises administering the single domain antibody or antigen binding fragment thereof or a variant thereof or a pharmaceutical composition comprising same to a subject in need.

In some embodiments, the binding agent is a competing binding agent and the method comprises administering the competing binding agent or a pharmaceutical composition comprising same to a subject in need.

In some embodiments, the binding agent is a competing antibody or antigen binding fragment thereof and the method comprises administering the competing antibody or antigen binding fragment thereof or a pharmaceutical composition comprising same to a subject in need.

In some embodiments, the binding agent is a competing single domain antibody or antigen binding fragment thereof and the method comprises administering the competing single domain antibody or antigen binding fragment thereof or a pharmaceutical composition comprising same to a subject in need.

In some embodiments, the binding agent is an antibody-like molecule and the method comprises administering the antibody-like molecule or a pharmaceutical composition comprising same to a subject in need.

In In some embodiments, the binding agent comprises a protein scaffold and the method comprises administering the protein scaffold or a pharmaceutical composition comprising same to a subject in need.

In some embodiments, the binding agent is an immune cell modulating agent and the method comprises administering an immune cell modulating agent or a pharmaceutical composition comprising same to a subject in need or cells modified to express the immune cell modulating agent thereof.

The binding agents of the present disclosure may be used for treatment of disorders or diseases. In some embodiments, the binding agent of the present disclosure is a single domain antibody be used for treating disorders or diseases.

In some embodiments, the binding agent may be used to target therapeutics and/or diagnostics to a target cell, circulating protein or tissue. Accordingly, in some embodiments, the binding agent of the present disclosure is a single domain antibody used to target therapeutics and/or diagnostics to a target cell, circulating protein or tissue.

In some embodiments, the binding agent may be conjugated with a therapeutic moiety and used for therapeutic methods. Accordingly, in some embodiments, the binding agent of the present disclosure is a single domain antibody used for therapeutic methods.

In some embodiments, the binding agent may be conjugated with a detectable moiety and used for detection or diagnostic methods. Accordingly, in some embodiments, the binding agent of the present disclosure is a single domain antibody used for detection or diagnostic methods.

In some embodiments, the binding agent (e.g., antibody or antigen binding fragment thereof such as, for example, single domain antibody or antigen binding fragment thereof) of the present disclosure may be used for targeting tumors *in vivo*.

In some embodiments, the binding agent (e.g., antibody or antigen binding fragment thereof such as, for example, single domain antibody or antigen binding fragment thereof) may be used for promoting tumor regression and/or reducing tumor volume *in vivo*.

The binding agent of the present disclosure (e.g., antibody or antigen binding fragment thereof such as, for example, single domain antibody or antigen binding fragment thereof) may thus be used for cancer treatment.

The method of the present disclosure may comprise a step of administering the binding agent (e.g., antibody or antigen binding fragment thereof such as, for example, single domain antibody or antigen binding fragment thereof) or a pharmaceutical composition comprising the binding agent to an individual in need.

In some embodiments, the binding agent (e.g., antibody or antigen binding fragment thereof such as, for example, single domain antibody or antigen binding fragment thereof) is administered in combination with a chemotherapeutic.

In accordance with the present disclosure, the individual in need may be a human. Further in accordance with the present disclosure, the individual in need may be an animal.

In some embodiment, treatment of disorders or diseases that are caused or associated with expression of TROP2 are particularly contemplated. In some embodiment, treatment of disorders or diseases that are caused or associated with overexpression of TROP2 are also particularly contemplated.

In some embodiments, the disorder or disease may be cancer.

In some embodiments, the binding agent of the present disclosure (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) is administered intravenously, intra-muscularly, or by fusion.

In some embodiment, the antibodies or antigen binding fragments thereof (e.g., single domain antibody or antigen binding fragment thereof) is administered intravenously.

In some embodiment, the antibodies or antigen binding fragments thereof (e.g., single domain antibody or antigen binding fragment thereof) is administered intra-muscularly.

In some embodiment, the antibodies or antigen binding fragments thereof (e.g., single domain antibody or antigen binding fragment thereof) is administered by infusion.

In some embodiments, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) is administered at a dose of approximately between 0.01 to 150 mg/kg, 0.01 to 100 mg/kg, 0.01 to 50mg/kg, 0.05 to 150 mg/kg, 0.05 to 100 mg/kg, 0.05 to 50 mg/kg, 0.05 to 30 mg/kg, 0.05 to 10 mg/kg, 0.1 to 50mg/kg, 0.1 to 30mg/kg, 0.1 to 10m/kg, or 1.0 to 10 mg/kg.

In some embodiments, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) is administered at a dose of approximately between 0.05 to 100 mg/kg.

In some embodiments, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) is administered at a dose of approximately between 0.05 to 50 mg/kg.

In other embodiments, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) is administered at a dose of approximately between 0.05 to 30 mg/kg.

In yet other embodiments, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) is administered at a dose of approximately between 0.05 to 10 mg/kg.

In yet other embodiments, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) is administered at a dose of approximately between 0.1 to 10 mg/kg.

Accordingly, in exemplary embodiments, the dose of the binding agent may be approximately 0.1 mg/kg. In other exemplary embodiments, the dose of the binding agent may be approximately 0.2 mg/kg. In yet other exemplary embodiments, the dose of the binding agent may

be approximately 0.3 mg/kg. In other exemplary embodiments, the dose of the binding agent may be approximately 0.4 mg/kg. In other exemplary embodiments, the dose of the binding agent may be approximately 0.5 mg/kg. In yet other exemplary embodiments, the dose of the binding agent may be approximately 1 mg/kg. In further exemplary embodiments, the dose of the binding agent may be approximately 2 mg/kg. In yet further exemplary embodiments, the dose of the binding agent may be approximately 3 mg/kg. In additional exemplary embodiments, the dose of the binding agent may be approximately 4 mg/kg. In other exemplary embodiments, the dose of the binding agent may be approximately 5 mg/kg. In yet other exemplary embodiments, the dose of the binding agent may be approximately 6 mg/kg. In further exemplary embodiments, the dose of the binding agent may be approximately 7 mg/kg. In yet further exemplary embodiments, the dose of the binding agent may be approximately 8 mg/kg. In additional exemplary embodiments, the dose of the binding agent may be approximately 9 mg/kg. In other exemplary embodiments, the dose of the binding agent may be approximately 10 mg/kg. In yet other exemplary embodiments, the dose of the binding agent may be more than 10 mg/kg.

Accordingly, in exemplary embodiments, the dose of the single domain antibody may be approximately 0.1 mg/kg. In other exemplary embodiments, the dose of the single domain antibody may be approximately 0.2 mg/kg. In yet other exemplary embodiments, the dose of the single domain antibody may be approximately 0.3 mg/kg. In other exemplary embodiments, the dose of single domain antibody may be approximately 0.4 mg/kg. In other exemplary embodiments, the dose of the single domain antibody may be approximately 0.5 mg/kg. In yet other exemplary embodiments, the dose of the single domain antibody may be approximately 1 mg/kg. In further exemplary embodiments, the dose of the single domain antibody may be approximately 2 mg/kg. In yet further exemplary embodiments, the dose of the single domain antibody may be approximately 3 mg/kg. In additional exemplary embodiments, the dose of the single domain antibody may be approximately 4 mg/kg. In other exemplary embodiments, the dose of single domain antibody may be approximately 5 mg/kg. In yet other exemplary embodiments, the dose of the single domain antibody may be approximately 6 mg/kg. In further exemplary embodiments, the dose of the single domain antibody may be approximately 7 mg/kg. In yet further exemplary embodiments, the dose of the single domain antibody may be approximately 8 mg/kg. In additional exemplary embodiments, the dose of the single domain antibody may be approximately 9 mg/kg. In other exemplary embodiments, the dose of the single domain antibody may be approximately

10 mg/kg. In yet other exemplary embodiments, the dose of the single domain antibody may be more than 10 mg/kg.

In accordance with the present disclosure, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) may be administered once weekly, more often than once a week (e.g., twice a week), once every two weeks, once every three weeks, once every month, or once every two months.

In accordance with the present disclosure, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) may be administered once weekly.

In accordance with the present disclosure, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) may be administered more often than once a week (e.g., twice a week).

Also in accordance with the present disclosure, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) may be administered once every two weeks.

In accordance with the present disclosure, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) may be administered once every three weeks.

In accordance with the present disclosure, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) may be administered once every month.

The binding agent of the present disclosure particularly encompasses single domain antibodies which are administered to a subject having a disorder or disease associated with TROP2 expression or with TROP2 overexpression.

In some embodiments, the anti-TROP2 single domain antibodies disclosed herein is administered to a subject having cancer selected from epithetial cancer, lung cancer (such as small cell lung cancer, non small cell lung cancer), myeloma, prostate cancer, breast cancer (such as triple negative breast cancer), rectal cancer, pancreatic cancer, glioblastoma, cervical cancer, colorectal cancer, gastric cancer, ovarian cancer, thyroid cancer, stomach cancer, cancer of the urinary bladder, cancer of the uterus, esophageal cancer, head and neck cancer, blood cancer.

In other embodiments, the anti-TROP2 single domain antibodies disclosed herein is administered to a subject having a metastatic cancer.

In an exemplary embodiment, the method comprises administering a single domain antibody or an antigen binding fragment thereof or a pharmaceutical composition comprising the single domain antibody or an antigen binding fragment thereof selected from KD001, KD002, KD003, KD004, KD005, KD006, KD007, KD008, KD009, KD010, KD011, KD012, KD013, KD014, KD015, KD016, KD017, KD018, KD019, KD020, KD021, KD022, KD023, KD024, KD025, KD026, KD027, KD028, KD029, KD030, KD031, KD032, KD033, KD034, KD035, KD036, KD037, KD038, KD039, KD040, KD041, KD042, KD043, KD044, KD045, KD046, KD047, KD048, KD049, KD050, KD051, KD052, KD053, KD054, KD055, KD056, KD057, KD058, KD059, KD060, KD061, KD062, KD063 or KD064 or an antigen binding fragment thereof.

In some embodiment, the method comprises administering a single domain antibody or an antigen binding fragment thereof or a pharmaceutical composition comprising the single domain antibody or an antigen binding fragment thereof selected from the group of **Figure 27**.

In some embodiment, the method comprises administering a single domain antibody or an antigen binding fragment thereof or a pharmaceutical composition comprising the single domain antibody or an antigen binding fragment thereof selected from the group of **Figure 28**.

In some embodiment, the method comprises administering a single domain antibody or an antigen binding fragment thereof or a pharmaceutical composition comprising the single domain antibody or an antigen binding fragment thereof selected from the group of **Figure 29**.

In some embodiment, the method comprises administering a single domain antibody or an antigen binding fragment thereof or a pharmaceutical composition comprising the single domain antibody or an antigen binding fragment thereof selected from the group of **Figure 30**.

In some embodiment, the method comprises administering a single domain antibody or an antigen binding fragment thereof or a pharmaceutical composition comprising the single domain antibody or an antigen binding fragment thereof selected from the group of **Figure 31**.

In some embodiment, the method comprises administering a single domain antibody or an antigen binding fragment thereof or a pharmaceutical composition comprising the single domain antibody or an antigen binding fragment thereof selected from the group of **Figure 32**.

In some embodiment, the method comprises administering a single domain antibody or an antigen binding fragment thereof or a pharmaceutical composition comprising the single domain antibody or an antigen binding fragment thereof selected from the group of **Figure 33**.

In some embodiment, the method comprises administering a single domain antibody or an antigen binding fragment thereof or a pharmaceutical composition comprising the single domain antibody or an antigen binding fragment thereof selected from the group of **Figure 34**.

In an exemplary embodiment, the method comprises administering KD065 or an antigen binding fragment thereof or a pharmaceutical composition comprising KD065 or an antigen binding fragment thereof.

In an exemplary embodiment, the method comprises administering KD066 or an antigen binding fragment thereof or a pharmaceutical composition comprising KD066 or an antigen binding fragment thereof.

In another exemplary embodiment, the pharmaceutical composition comprises a single domain antibody or an antigen binding fragment thereof selected from KD067, KD068 or KD069 or an antigen binding fragment thereof.

In an exemplary embodiment, the method comprises administering a single domain antibody or an antigen binding fragment thereof or a pharmaceutical composition comprising the single domain antibody or an antigen binding fragment thereof selected from KD067, KD068 or KD069 or an antigen binding fragment thereof.

In some embodiment, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) used in the method of treatment is KD001, comprises KD001 or comprises the antigen binding domain of KD001. In some embodiment, the binding agent used in the method of treatment is a KD001 variant, comprises a KD001 variant or comprises the antigen binding domain of a KD001 variant. In some embodiment, the binding agent used in the method of treatment is a humanized version of KD001, comprises a humanized version of KD001 or comprises the antigen binding domain of a humanized version of KD001. In some embodiment, the binding agent used in the method of treatment is single domain antibody capable of competing with KD001, comprises a single domain antibody capable of competing with KD001 or comprises the antigen binding domain of a single domain antibody capable of competing with KD001.

In some embodiment, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) used in the method of treatment is KD002, comprises KD002 or comprises the antigen binding domain of KD002. In other embodiment, the binding agent used in the method of treatment is a variant of KD002, comprise a variant of KD002 or comprises the antigen binding domain of a KD002 variant. In some embodiment, the binding agent used in the method of treatment is a humanized version of KD002, comprises a humanized version of KD002 or comprises the antigen binding domain of humanized version of KD002. In some embodiment, the binding agent used in the method of treatment is a single domain antibody capable of competing with KD002, comprises a single domain antibody capable of competing with KD002 or comprises the antigen binding domain of a single domain antibody capable of competing with KD002.

In some embodiment, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) used in the method of treatment is KD003, comprises KD003 or comprises the antigen binding domain of KD003. In other embodiment, the binding agent used in the method of treatment is a variant of KD003, comprise a variant of KD003 or comprises the antigen binding domain of a KD003 variant. In some embodiment, the binding agent used in the method of treatment is a humanized version of KD003, comprises a humanized version of KD003 or comprises the antigen binding domain of humanized version of KD003. In some embodiment,

the binding agent used in the method of treatment is a single domain antibody capable of competing with KD003, comprises a single domain antibody capable of competing with KD003 or comprises the antigen binding domain of a single domain antibody capable of competing with KD003.

In some embodiment, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) used in the method of treatment is KD004, comprises KD004 or comprises the antigen binding domain of KD004. In other embodiment, the binding agent used in the method of treatment is a variant of KD004, comprise a variant of KD004 or comprises the antigen binding domain of a KD004 variant. In some embodiment, the binding agent used in the method of treatment is a humanized version of KD004, comprises a humanized version of KD004 or comprises the antigen binding domain of humanized version of KD004. In some embodiment, the binding agent used in the method of treatment is a single domain antibody capable of competing with KD004, comprises a single domain antibody capable of competing with KD004 or comprises the antigen binding domain of a single domain antibody capable of competing with KD004.

In some embodiment, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) used in the method of treatment is KD005, comprises KD005 or comprises the antigen binding domain of KD005. In other embodiment, the binding agent used in the method of treatment is a variant of KD005, comprise a variant of KD005 or comprises the antigen binding domain of a KD005 variant. In some embodiment, the binding agent used in the method of treatment is a humanized version of KD005, comprises a humanized version of KD005 or comprises the antigen binding domain of humanized version of KD005. In some embodiment, the binding agent used in the method of treatment is a single domain antibody capable of competing with KD005, comprises a single domain antibody capable of competing with KD005 or comprises the antigen binding domain of a single domain antibody capable of competing with KD005.

In some embodiment, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) used in the method of treatment is KD006, comprises KD006 or comprises the antigen binding domain of KD006. In other embodiment, the binding agent used in the method of treatment is a variant of KD006, comprise a variant of KD006 or comprises the

antigen binding domain of a KD006 variant. In some embodiment, the binding agent used in the method of treatment is a humanized version of KD006, comprises a humanized version of KD006 or comprises the antigen binding domain of humanized version of KD006. In some embodiment, the binding agent used in the method of treatment is a single domain antibody capable of competing with KD006, comprises a single domain antibody capable of competing with KD006 or comprises the antigen binding domain of a single domain antibody capable of competing with KD006.

In some embodiment, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) used in the method of treatment is KD007, comprises KD007 or comprises the antigen binding domain of KD007. In other embodiment, the binding agent used in the method of treatment is a variant of KD007, comprise a variant of KD007 or comprises the antigen binding domain of a KD007 variant. In some embodiment, the binding agent used in the method of treatment is a humanized version of KD007, comprises a humanized version of KD007 or comprises the antigen binding domain of humanized version of KD007. In some embodiment, the binding agent used in the method of treatment is a single domain antibody capable of competing with KD007, comprises a single domain antibody capable of competing with KD007 or comprises the antigen binding domain of a single domain antibody capable of competing with KD007.

In some embodiment, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or antigen binding fragment thereof) used in the method of treatment is KD048, comprises KD048 or comprises the antigen binding domain of KD048. In other embodiment, the binding agent used in the method of treatment is a variant of KD048, comprise a variant of KD048 or comprises the antigen binding domain of a KD048 variant. In some embodiment, the binding agent used in the method of treatment is a humanized version of KD048, comprises a humanized version of KD048 or comprises the antigen binding domain of humanized version of KD048. In some embodiment, the binding agent used in the method of treatment is a single domain antibody capable of competing with KD007, comprises a single domain antibody capable of competing with KD048 or comprises the antigen binding domain of a single domain antibody capable of competing with KD048.

In some embodiment, the binding agent (including for example and without limitations, an antibody or antigen binding fragment thereof such as, for example, a single domain antibody or

antigen binding fragment thereof) used in the method of treatment is KD053, comprises KD053 or comprises the antigen binding domain of KD053. In other embodiment, the binding agent used in the method of treatment is a variant of KD053, comprise a variant of KD053 or comprises the antigen binding domain of a KD053 variant. In some embodiment, the binding agent used in the method of treatment is a humanized version of KD053, comprises a humanized version of KD053 or comprises the antigen binding domain of humanized version of KD053. In some embodiment, the binding agent used in the method of treatment is a single domain antibody capable of competing with KD007, comprises a single domain antibody capable of competing with KD053 or comprises the antigen binding domain of a single domain antibody capable of competing with KD053.

In some embodiments, the binding agent is a single domain antibody and is used at a dose of approximately between 0.1 to 10 mg/kg once a week.

In some embodiments, KD001 or KD001 variant (such as humanized KD001 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once a week.

In some embodiments, KD001 or KD001 variant (such as humanized KD001 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every two weeks.

In some embodiments, KD001 or KD001 variant (such as humanized KD001 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every three weeks.

In some embodiments, KD002 or KD002 variant (such as humanized KD002 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once a week.

In some embodiments, KD002 or KD002 variant (such as humanized KD002 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every two weeks.

In some embodiments, KD002 or KD002 variant (such as humanized KD002 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every three weeks.

In some embodiments, KD003 or KD003 variant (such as humanized KD003 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once a week.

In some embodiments, KD003 or KD003 variant (such as humanized KD003 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every two weeks.

In some embodiments, KD003 or KD003 variant (such as humanized KD003 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every three weeks.

In some embodiments, KD004 or KD004 variant (such as humanized KD004 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once a week.

In some embodiments, KD004 or KD004 variant (such as humanized KD004 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every two weeks.

In some embodiments, KD004 or KD004 variant (such as humanized KD004 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every three weeks.

In some embodiments, KD005 or KD005 variant (such as humanized KD005 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once a week.

In some embodiments, KD005 or KD005 variant (such as humanized KD005 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every two weeks.

In some embodiments, KD005 or KD005 variant (such as humanized KD005 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every three weeks.

In some embodiments, KD006 or KD006 variant (such as humanized KD006 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once a week.

In some embodiments, KD006 or KD006 variant (such as humanized KD006 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every two weeks.

In some embodiments, KD006 or KD006 variant (such as humanized KD006 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every three weeks.

In some embodiments, KD007 or KD007 variant (such as humanized KD007 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once a week.

In some embodiments, KD007 or KD007 variant (such as humanized KD007 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every two weeks.

In some embodiments, KD007 or KD007 variant (such as humanized KD007 variant) is used at a dose of approximately between 0.1 to 10 mg/kg once every three weeks.

In some instances, the method of the present disclosure comprises administering a pharmaceutical composition comprising a conjugated form of a single domain antibody or an antigen binding fragment thereof selected from KD001, KD002, KD003, KD004, KD005, KD006, KD007, KD008, KD009, KD010, KD011, KD012, KD013, KD014, KD015, KD016, KD017, KD018, KD019, KD020, KD021, KD022, KD023, KD024, KD025, KD026, KD027, KD028, KD029, KD030, KD031, KD032, KD033, KD034, KD035, KD036, KD037, KD038, KD039, KD040, KD041, KD042, KD043, KD044, KD045, KD046, KD047, KD048, KD049, KD050, KD051, KD052, KD053, KD054, KD055, KD056, KD057, KD058, KD059, KD060, KD061, KD062, KD063 or KD064 or their corresponding variants, competing single domain antibodies thereof or binding agent comprising same.

The binding agent (e.g., antibodies or antigen binding fragments thereof) of the present disclosure may be used for detection purposes.

Detection of a particular target may be performed *in vitro* by contacting a sample, containing or suspected of containing the target with the binding agent (e.g., antibodies or antigen binding fragments thereof) described herein and quantifying a signal associated with positive or negative binding using a detection apparatus.

The sample may originate from a mammal (e.g., a human). The sample may be a tissue sample obtained from the mammal or a cell culture supernatant.

In some embodiments, the sample may be a serum sample, a plasma sample, a blood sample, semen or ascitic fluid obtained from the mammal.

Detection of a particular target may be performed *in vivo* by administering the antibodies or antigen binding fragments thereof to an individual and quantifying a signal associated with positive or negative binding using a detection apparatus.

Upon detecting the presence of the target in the sample or in the individual, a drug (e.g., antibody, small molecule, antibodies or antigen binding fragments thereof disclosed herein) may be administered to the individual.

Companion Diagnostic

The present application also provides a test and medical device that may be used as a companion diagnostic.

The test may be used to evaluate the expression of TROP2 in tumor or tumor cells and/or the presence of macrophages in a tumor.

In some embodiments, the test may be carried out prior to treating a subject with a drug targeting TROP2. Accordingly, the test may be carried out to determine whether a subject would be eligible for treatment with the drug.

In other embodiments, the test may be carried out during or subsequent to treatment with a drug targeting TROP2. Accordingly, the test may be carried out to determine whether the subject respond to treatment or not and/or to monitor ongoing treatment.

In some embodiments, the test may be used as a companion diagnostic to treatment with any type of drug targeting TROP2 such as for example and without limitation, small molecules and biologics including the binding agents disclosed herein or any therapeutic antibodies against TROP2.

The test may be particularly used as a companion diagnostic to treatment with a binding agent described herein.

The test may help identify subjects who are most likely to benefit from treatment with the binding agent disclosed herewith.

In some embodiments, the test may be used to determine the level of TROP2 expressing cells in a tumor. In other embodiments, the test may be used to determine the level of TROP2 expression in tumor cells. In another embodiment, the test may be used to determine the presence of macrophages in a tumor.

In some embodiments, the test is based on immunodetection of markers. Accordingly, the test uses a binding agent targeting TROP2 and optionally a binding agent targeting one or more macrophage markers.

In an exemplary embodiment, the test is an immunohistochemical assay. In another exemplary embodiment, the test is an immunocytochemical assay. In yet other embodiments, the test is a flow cytometry assay. In yet other embodiments, the test is a Western blot. In yet other embodiments, the assay is an ELISA.

In other embodiments, the test is based on detection of nucleic acids. Accordingly, the test may comprise nucleic acid molecules that are capable of specific hybridization with nucleic acids encoding TROP2 or their complement thereof and optionally nucleic acid molecules that are capable of binding to one or more macrophages markers.

In some embodiments, the test is based on *in situ* hybridization.

In another embodiment, the test is based on polymerase chain reaction.

The test may be carried out, for example, on a tumor biopsy obtained from a patient and the level of selected biomarker expression may assessed. In an embodiment, expression of TROP2 is determined and may be scored as low, moderate or high by IHC staining with the TROP2 binding agent. In another embodiment, the human macrophage infiltration, is determined and may be scored as low, moderate or high by IHC staining. The characterization of TROP2 expression as low, moderate and high may be determined, for example, by a trained pathologist.

Based on the **Figure 16B** indicating that both M1 and M2 macrophages lead to phagocytosis, a pan macrophage marker may be used to evaluate the macrophage infiltration level and the potential efficacy prediction. In an exemplary embodiment, the presence of macrophages in tumors may be assessed with the pan marker CD68.

The binding agent or pharmaceutical composition of the present disclosure may be administered to patients carrying tumors with high TROP2/high CD68 expression, low CD68/high TROP2 expression, low CD68/low TROP2 expression.

The present disclosure therefore relates to a kit that comprise a binding agent that specifically binds to TROP2 and a binding agent that binds to a macrophage marker.

The kit may more particularly comprise a binding agent that specifically binds to TROP2 and a binding agent that binds to a pan macrophage marker. Exemplary embodiments of pan macrophages markers include for example and without limitations, CD68, CD80, and CD11c.

Screening assay

Binding agents having same or similar property(ies) as any one of KD001-KD005 and/or KD001-KD005 such as KD002 variants or KD005 variants can be identify by performing one or more of the assays described herein.

For example, candidate binding agents may be submitted to binding assays (e.g., ELISA, FACS etc.) for identifying those that bind to the same or overlapping region of TROP2 as any one of KD001, KD002, KD003, KD004, KD005, KD002 variants or KD005 variants.

The binding assay may measure positive binding to a specific region of TROP2 or to specific amino acid residues of TROP2 or loss of binding when a specific region of TROP2 is deleted or when specific amino acid residues of TROP2 are mutated or deleted.

The binding assays may be carried out on recombinant polypeptides or on cells expressing the polypeptides.

In some embodiments, a binding agent may be identified by contacting a candidate binding agent with a polypeptide comprising a sequence at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical or identical to the human TROP2 amino acid sequence, or a fragment thereof or with a cell expressing such polypeptide or fragment thereof.

Due to their high level of identity, a binding agent that binds to human TROP2 may be identified by contacting a candidate binding agent with a polypeptide comprising a cynomolgus TROP2 amino acid sequence or a fragment thereof or with a cell expressing such polypeptide or fragment thereof.

In an exemplary embodiment, the candidate binding agent is contacted with a polypeptide comprising a sequence consisting of amino acid residues 27-73 of human TROP2 or of cynomolgus TROP2 or a fragment thereof or with a cell expressing such polypeptide or fragment thereof.

In an exemplary embodiment, the candidate binding agent is contacted with a polypeptide comprising a sequence consisting of amino acid residues 27-56 of human TROP2 or of cynomolgus TROP2 or a fragment thereof or with a cell expressing such polypeptide or fragment thereof.

In an exemplary embodiment, the candidate binding agent is contacted with a polypeptide comprising a sequence consisting of amino acid residues 27-66 of human TROP2 or of cynomolgus TROP2 or a fragment thereof or with a cell expressing such polypeptide or fragment thereof.

The present disclosure therefore relates to a method of identifying a binding agent that comprises a) contacting a candidate binding agent with a polypeptide comprising i) a TROP2 amino acid sequence consisting of amino acid residues 27-146 of human TROP2 or of cynomolgus

TROP2, ii) a polypeptide comprising a sequence at least 80% identical to the TROP2 amino acid sequence of i) or iii) a fragment of i) or ii) thereof and b) selecting a binding agent that binds to the polypeptide. The method of the present disclosure may also be carried out by contacting a candidate binding agent with cell expressing such polypeptide or fragment thereof.

The binding agent may also be selected for not being capable of binding to a polypeptide that comprises a human TROP2 amino acid sequence consisting of amino acid residues 147-274 or a fragment thereof or for having a reduced binding to said polypeptide compared to the full human TROP2 polypeptide.

For example, the binding agent may be selected for not being capable of binding to one or more polypeptide comprising a human TROP2 amino acid sequence carrying from 5 to 70 amino acid deletions in the amino acid sequence defined by residues 1-73 of human TROP2 or for having a reduced binding to said polypeptide compared to a human TROP2 polypeptide having no such deletion.

For example, the binding agent may be selected for not being capable of binding to one or more polypeptide selected from polypeptides comprising a human TROP2 amino acid sequence carrying deletion of amino acid residues 27-36, 27-44, 27-56, 27-66 or 27-73 or for having a reduced binding to said polypeptide compared to a human TROP2 polypeptide having no such deletion.

More particularly, the binding agent may be selected for not being capable of binding to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 149.

Binding agents may also be selected based on their ability to compete with one or more of binding agents or antigen binding domains disclosed herein. More particularly, binding agents may also be identified based on their ability to compete with any one of KD001, KD002, KD003, KD004, KD005, KD002 variants or KD005 variants.

In some embodiments, the binding agent or antigen binding domain does not compete with Sacituzumab, hTINA-1, h7E6, and/or AR47A6.4.2.

For example, the method of the present disclosure may comprise a) performing a competition assay between a candidate binding agent and the binding agent of any one of SEQ ID NO:22-26 or SEQ ID NO:94-114 for binding to i) human TROP2, ii) cynomolgus TROP2, iii) a

polypeptide comprising a sequence at least 80% identical to human TROP2, iv) a polypeptide comprising a sequence at least 80% identical to cynomolgus TROP2 or v) a fragment of any one of i) to iv) thereof and b) selecting a competing binding agent.

In an exemplary embodiment, the competition assay is performed with a polypeptide comprising a sequence consisting of amino acid residues 27-146 of human TROP2 or of cynomolgus TROP2 or a fragment thereof.

In an exemplary embodiment, the competition assay is performed with a polypeptide comprising a sequence consisting of amino acid residues 27-73 of human TROP2 or of cynomolgus TROP2 or a fragment thereof.

In an exemplary embodiment, the competition assay is performed with a polypeptide comprising a sequence consisting of amino acid residues 27-56 of human TROP2 or of cynomolgus TROP2 or a fragment thereof.

In an exemplary embodiment, the competition assay is performed with a polypeptide comprising a sequence consisting of amino acid residues 27-66 of human TROP2 or of cynomolgus TROP2 or a fragment thereof.

In a particular embodiment, binding agents that compete with any one of KD001, KD002, KD003, KD004, KD005, KD002 variants or KD005 variants are selected. In other particular embodiments, binding agents that compete with KD002 and/or KD005 are particularly selected.

In an exemplary embodiment, the TROP2 amino acid sequence used in the binding experiments and/or in the competition assay is a human TROP2 amino acid sequence.

In an exemplary embodiment, the TROP2 amino acid sequence used in the binding experiments and/or in the competition assay is a cynomolgus TROP2 amino acid sequence.

In an exemplary embodiment, the TROP2 fragment used in the binding experiments and/or in the competition assay is a fragment of at least 10 amino acid residues.

In an exemplary embodiment, the TROP2 fragment used in the binding experiments and/or in the competition assay is a fragment of between 10 to 30 amino acid residues, including for example a fragment of between 10 to 25 amino acid residues, a fragment of between 10 to 20 amino acid residues, a fragment of between 10 to 15 amino acid residues etc.

In an embodiment, the human TROP2 amino acid sequence used in the binding experiments and/or in the competition assay may include for example, amino acid residues 47-56 of human TROP2.

In another embodiment, the human TROP2 amino acid sequence used in the binding experiments and/or in the competition assay may include for example, amino acid residues 60-66 of human TROP2.

In yet another embodiment, the human TROP2 amino acid sequence used in the binding experiments and/or in the competition assay may include for example, amino acid residues 47-66 of human TROP2.

The candidate binding agent may comprise the antigen binding domain of an antibody or antigen binding fragment thereof, of an antibody-like molecule, of a fusion with protein scaffolds or of an immune cell modulating agent or the like.

Alternatively, the candidate binding agent may be selected from an antibody or antigen binding fragment thereof, an antibody-like molecule, a fusion with protein scaffolds or an immune cell modulating agent.

In an exemplary embodiment, the candidate binding agent may comprise an antibody having a light chain variable region and a heavy chain variable region or an antigen binding domain of said antibody.

In another exemplary embodiment, the candidate binding agent may comprise a heavy chain only antibody or an antigen binding domain of said heavy chain only antibody.

In some embodiments, the binding agent is selected for its affinity for human TROP2.

In some embodiments, the binding agent is selected for its affinity for cynomolgus TROP2.

In some embodiments, the binding agent is selected for having an affinity for human TROP2 that is similar (with +/- 25% variation) to that of KD001, KD002, KD003, KD004, KD005 or to a variable region thereof.

In other embodiments, the binding agent is selected for having an affinity for human TROP2 that is identical or superior to that of KD001, KD002, KD003, KD004, KD005 or to a variable region thereof.

In some embodiments, the binding agent is selected for having a dissociation rate that is similar (with +/- 25% variation) to that of KD001, KD002, KD003, KD004, KD005 or to a variable region thereof for human TROP2.

In some embodiments, the binding agent is selected for having a dissociation rate that is identical or lower to that of KD001, KD002, KD003, KD004, KD005 or to a variable region thereof for human TROP2.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has an affinity of $\leq 10^{-6}$ M.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has an affinity of $\leq 10^{-7}$ M.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has an affinity of $\leq 10^{-8}$ M.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has an affinity of $\leq 10^{-9}$ M.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has an affinity of $\leq 10^{-10}$ M.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has an affinity of $\leq 10^{-11}$ M.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has an affinity of at least 1×10^{-10} M for human TROP2.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has an affinity of between 1×10^{-10} M and 1×10^{-12} for human TROP2.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has an affinity of between 1×10^{-10} M and 1×10^{-11} for human TROP2.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has a dissociation rate of between 1 x 10^{-6} to 1 x 10^{-4}.

In some embodiments, the candidate binding agent or at least one of the one or more antigen binding domains of the candidate binding agent has a dissociation rate of between 1 x 10^{-5} to 1 x 10^{-4}.

In accordance with the present disclosure, the affinity may be measured on recombinant human TROP2 protein or on cells expressing human TROP2 protein. The affinity may be measured on full length human TROP2 protein.

The binding agents can also be submitted to *in vitro* or *in vivo* assays to assess function. Exemplary assays include internalization, ADCC, ADCP, cell viability, *in vitro* tumor growth inhibition experiments and/or *in vivo* tumor growth inhibition experiments as described herein.

In some embodiments, the binding agent is selected for *in vitro* potency against tumor cells.

In some embodiments, the binding agent is selected for *in vivo* potency against tumor cells.

Binding agents having similar (+/- 25%) or better activity as any one of KD001, KD002, KD003, KD004, KD005, KD002 variants or KD005 variants are particularly selected. However, Binding agents having reduced activity compared with any one of KD001, KD002, KD003, KD004, KD005, KD002 variants or KD005 variants (e.g., 10% activity, 20% activity, 30% activity, 40% activity, 50% activity, between 50% and 75% activity, between 50% and 85% activity, between 50% and 95% activity of a reference antibody) may still be functional and may be selected.

In some embodiments, the binding agent may be selected for having at least one pharmacokinetic parameter similar to that of any one of KD001, KD002, KD003, KD004 or KD005.

In some embodiments, the binding agent may be selected for having at least one pharmacokinetic parameter equivalent to that of any one of as any one of KD001, KD002, KD003, KD004, KD005, KD002 variants or KD005 variants.

In some embodiments, the binding agent may be selected for having a sequence as set forth in any one of SEQ ID NOs:143-147.

EXAMPLES

Example 1- Animal immunization and immune library construction

Single domain antibodies were generated by immunizing transgenic mouse carrying CH1 deletions at an immunoglobulin heavy chain (IgH) locus (see international application No. PCT/CA2021/050951 filed on July 12, 2021, and published on January 20, 2022, under No. WO2022/011457 A1 the entire content of which is incorporated herein by reference) with the extracellular domain of human TROP2 protein (aa 1-274) comprising His tag (e.g., SEQ ID NO:164) (Sino Biological, Cat. No. 10428-H08H: GenBank Acc. No. NP_002344.2)).

Sequences of single-domain antibodies were uncovered from clone picking after phage display panning of reconstituted immune libraries against the antigens.

DNA fragments encoding VHHs or VHs were generally subcloned into constructs comprising a constant region of a human antibody (e.g., human IgG1 Fc) for expression and *in vitro* and *in vivo* testing.

Example 2- Production and purification of sdAbs.

sdAbs were expressed in cells and isolated as protein dimers. The sdAbs identified in the experimental section and/or Figures as KD001 to KD068 are composed of two heavy chains. Unless, specifically mentioned otherwise each of KD001 to KD068 have the corresponding heavy chain amino acid sequence set forth in in Table 15, 16, 18 or 19.

Protein dimers (e.g., homodimers) were expressed in 2.5 mL or 400 mL culture volume from the DNA construct using the ExpiCHO™ Expression System (Thermo Fisher, Cat. no. A29133) or the Expi293™ Expression System (Thermo Fisher, Cat. no. A14635).

Freshly thawed ExpiCHO-s cells were allowed to recover in culture for two or more passages before transfection. Cells were then passaged every 3-4 days until they reach $4x10^6$-$6x10^6$ cells/mL at which time they were diluted to $2x10^5$-$3x10^5$ cells/mL in ExpiCHO™ Expression Medium pre-warmed to 37°C. The day prior to transfection, cells were diluted to 3 $x10^6$- $4x10^6$ cells/mL and on the day of transfection, cells were further diluted to $6.x10^6$ cells/mL. 1 µg of DNA/ mL of culture volume was diluted with cold OptiPRO™ medium (100 µL for 2.5 mL of culture volume; 16 mL for 400 mL of culture volume). ExpiFectamine™ CHO Reagent (8 µL for 2.5 mL of culture volume; 1280 µL for 400 mL of culture volume) was added to medium containing DNA

and incubated with ExpiFectamine™/DNA complexes at room temperature for 1-5 min. Then the DNA complex was transferred to culture (at $6x10^6$ cells/mL) while swirling. The cells were incubated at 37°C under 8% CO_2 and 80% humidity with shaking (INFORS HT shaker, 125 rpm). 18-22h after onset of transfection, ExpiCHO™ feed (0.6 mL for 2.5 mL of culture volume; 96 mL for 400 mL of culture volume) and ExpiCHO™ enhancer (15 µL for 2.5 mL of culture volume; 2.4 mL for 400 mL of culture volume) were added to the cells. The cells were returned to INFORS HT incubator set at 37°C under 8% CO_2 and 80% humidity with shaking at 125 rpm (25 mm orbit). 8 days post-transfection, supernatants were clarified by centrifugation at 4000 x g for 30 min. Supernatants were filter-sterilized using a Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units 1000 mL filter unit (Thermo Scientific, Cat. no. 567-0020) and were stored at 4°C or frozen for later analysis.

Freshly thawed Expi293F cells were allowed to recover in culture for two or more passages before transfection. Cells were then passaged every 3-4 days until they reach $3x10^6$-$5x10^6$ cells/mL at which time they were diluted to $3x10^5$-$5x10^5$ cells/mL in Expi293™ Expression Medium pre-warmed to 37°C. The day prior to transfection, cells were diluted to $2.5x10^6$-$3x10^6$ and on the day of transfection, cells were further diluted to $3x10^6$ viable cells/ mL. 1 µg of DNA / mL of culture volume was diluted with Opti-MEM™ I Reduced Serum medium to get a final volume of 150 µL for 2.5 mL of culture volume and 24 mL for 400 mL of culture volume. ExpiFectamine™ 293 Reagent (8 µL for 2.5 mL of culture volume; 1.3 mL for 400 mL of culture volume) was added to medium Opti-MEM™ I Reduced Serum medium (140 µL for 2.5 mL of culture volume; 22.5 mL for 400 mL of culture volume) to incubate at room temperature for 5 minutes. Diluted ExpiFectamine™ was added to diluted DNA and incubate for 15 minutes at room temperature. ExpiFectamine™/ DNA solution was transferred to culture drop by drop (at $3x10^6$ cells/mL) while swirling. The cells were incubated at 37°C under 8% CO_2 and 80% humidity with overnight shaking (INFORS HT shaker, 125 rpm). 18-22h after onset of transfection, ExpiFectamine™ 293 Transfection Enhancer 1 (15 µL for 2.5 mL of culture volume; 2.4 mL for 400 mL of culture volume) and ExpiFectamine™ 293 Transfection Enhancer 2 (50 µL for 2.5 mL of culture volume; 24 mL for 400 mL of culture volume) were added to the cells. The cells were returned to INFORS HT incubator set at 37°C under 8% CO_2 and 80% humidity with shaking at 125 rpm (25mm orbit). 5 days post-transfection, supernatants were clarified by centrifugation at 4000 x g for 30 min. Supernatants were filter-sterilized using a Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units

1000 mL filter unit (Thermo Scientific, Cat. no. 567-0020) and were stored at 4°C or frozen for later analysis.

Proteins are purified using 3-mL MabSelect SuRe resin (GE Healthcare, Cat. No. 17-5438-02) with gravity columns or 40-mL MabSelect SuRe resin with AKTA PURE (GE Healthcare, Piscataway, NJ) depending on the supernatant volume. Resin was incubated with 0.5 NaOH for one hour and equilibrated with Tris-base buffer pH 7.4 (50 mM Tris-HCl, 150 mM NaCl, pH 7.4) prior injection. Supernatant was applied on gravity columns or the at 5 mL/min on 40-mL column. Resin column was washed with 3 CV (column volume) with Tris-base buffer pH 7.4 at flow rate of 10 mL/min. Protein was eluted with 3 CV of 0.1M citrate acid pH 3 at 10 mL/min. Fractions identified with protein from the visual output of the chromatogram (absorbance at 280 nm) were pooled together. Pooled fractions were neutralized with 1 M Tris-HCl pH 9 to achieve the pH ~ 5-6 before transferring into PBS (Phosphate-buffered saline) pH 6 buffer prepared from PBS 10X pH 7.2 (15 mM Potassium Phosphate monobasic 1552 mM Sodium Chloride 27 mM Sodium Phosphate dibasic, ThermoFisher, Cat. no. 70013073) or 20 mM Histidine 7% Sucrose 0.02% Polysorbate80 pH 5.5.

Buffer exchange was carried out by sample concentrators for proteins purified from gravity columns or either by dialysis or by desalting column for proteins purified from AKTA PURE. Proteins purified from gravity columns were concentrated with sample concentrator VivaSpin 2, 50 kDa MWCO (GE Healthcare, Cat. no. 28932257) by centrifugation at 3,500-4,000 x g at 4°C then, diluted with PBS pH 6 to achieve 4-fold and repeated until sample reached 200-fold. Dialysis was carried out in 4L of PBS pH 6 overnight at 4°C using 7 kDa molecular weight cut-off dialysis tubing (ThermoFisher, Cat. no. 68799). On the other hand, desalting column was incubated with 0.5 NaOH overnight and equilibrated with PBS pH 6. Volume of 15 mL of neutralized protein sample was loaded into the HiPrep 26/10 desalting column (GE Healthcare, Cat. no. 17-5087-02) at 0.5 mL/min then, protein was eluted with 2 CV of PBS pH 6. Loading and elution steps were repeated until no neutralized protein sample from elution of affinity column remained. Fractions identified with protein from the visual output of the chromatogram (absorbance at 280 nm) were pooled together.

Sample was filter-sterilized using a Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units 150 mL filter unit (Thermo Scientific, Cat. no. 565-0010). Final protein sample was

quantified by optical density at 280 nanometer and tested for endotoxin level with Endosafe® LAL Reagent cartridges (Charles River Cat. no. PTS2005). Final protein sample was analyzed on SDS-PAGE gels under reducing or non-reducing conditions (see section SDS PAGE and Western Blotting).

Example 3- *In vivo* anti-tumor effect

The anti-tumor effect of the sdAbs of the present disclosure was tested in established tumor models in mice.

NCG mice (5-6 weeks old, female) were purchased from Charles River Laboratories (St. Constant, QC). CB-17 Fox Chase SCID mice (6-8 weeks old, female) were purchased either from Charles River Laboratories (St. Constant, QC) or from UHN (Toronto, ON). Mice were housed in a pathogen-free environment at the animal facility in UHN. The animal work was conducted according to the guidelines of the Canadian Council on Animal Care (CCAC) and the Animal Use Protocol approved by the Animal Care Committee at UHN.

Briefly, SCID mice or NCG mice were implanted subcutaneously with 5 or 10 million tumor cells. Animals were randomized into experimental groups when tumor volumes reached 100 mm^3 and were then treated (arrows) with the human IgG1 fusion construct of the KD001-KD005 or with PBS (negative control). Tumor volume was measured twice per week.

Antibody efficacy was tested in SCID or in NCG mice bearing MDA-MB-453, MDA-MB-231, BxPC-3, T.Tn tumors or MDA-MB-468 tumors. In **Figure 1A**, the NCG mice were treated with testing articles at 8 mg/kg, once per week for 8 doses. In **Figures 1B** to **1D**, the SCID mice were treated with testing articles at 8 mg/kg, once per week for 8 doses. In **Figure 2A**, the NCG mice were treated with testing articles at 0.5, 2, 8, 30 mg/kg once per week for 8 doses. The PBS control arm were treated with testing articles at 8 mg/kg in **Figure 2B**. In **Figure 2C**, the NCG mice were treated with KD004 at 8 mg/kg with different dosing frequencies, including twice per week, every two weeks, and every three weeks. In **Figure 2D**, the SCID mice were treated with testing articles at 0.1 mg/kg once per week for 6 doses. In **Figure 3**, the NCG mice were engrafted with or without 10 million PBMC and then treated with KD004 at 8 mg/kg, twice per week for 8 doses. In **Figure 13C**, SCID mice were treated with testing articles at 8 mg/kg once per week for 6 doses. In **Figure 15E**, SCID mice were treated with testing articles at 8 mg/kg once per week for 6 doses.

No signs of toxicity were observed throughout the studies. All sdAb-treated mice had significantly smaller tumors compared to the PBS-treated animals (**Figures 1A-1D**).

The binding of anti-TROP2 sdAbs to several cancer cell lines including MDA-MB-453, MDA-MB-231, BxPC3, and A375 cells were analyzed by flow cytometry (**Figure 1E**). 1×10^5 cells were incubated with an Fc receptor blocking antibody (1/100 dilution) for 10 minutes at room temperature. Anti-TROP2 antibodies were diluted in FACS staining buffer to 10 nM and added and incubated with cells for 30 minutes on ice. After washing three times with FACS staining buffer, FITC-conjugated secondary antibodies recognizing the human Fc region (Biolegend) were mixed with cells. After staining for 30 minutes, cells were washed three times, followed by resuspension into 100 µL of 7-Amino-Actinomycin D (7-AAD) solution (1/100 dilution). Cells were analyzed by BD FACSCanto™ II Flow cytometer (BD Bioscience). Data were analyzed with FlowJo via gating on single cells and live cells.

As illustrated in **Figure 1E**, all the tested anti-TROP2 antibodies exhibited similar binding to tested cancer cell lines. All the tested anti-TROP2 antibodies showed the highest binding to BxPC3 cells, intermediate binding to MDA-MB-453 cells, and weak binding to MDA-MB-231 cells. None of the TROP2 antibodies bound to low TROP2 expression cell lines A375, illustrating the binding specificity of tested antibodies to TROP2. The binding of TROP2 sdAbs showed comparable binding to MDA-MB-231, MDA-MB453, and BxPC3 as AR47A6.4.2 TROP2 antibody (see Truong *et al.*, 2008).

Example 4- Dose-response, schedule, regimen and pharmacokinetics

In **Figure 2 A** and **2B**, all the NCG mice were humanized with human PBMCs (10 million cells, i.v.) the day before treatment initiated, represented by the first time point in the graphs. In **Figures 2 C** and **2D**, the NCG mice were not humanized with human PBMCs. Human PBMC cells (10 million cells/mouse were injected i.v.) were received from HemaCare Corporation (Northridge, CA). Tumor cells were implanted in NCG mice in a similar fashion to SCID mice. In all models, tumor volumes were measured using vernier calipers and the mice were weighed one or two times weekly. Tumor volume was calculated using the formula: ½ (Length × Width^2).

As illustrated in **Figure 2A**, the *in vivo* dose/response study indicates that weekly treatment of sdAbs KD004 (0.5mg/kg, 2mg/kg, 8 mg/kg and 30mg/kg) prevents the growth of MDA-MB-

453 implanted in NCG mice (5 million cells s.c.). No signs of toxicity were observed when animals were treated with higher doses.

Late intervention with anti-TROP2 therapy caused tumor regression in tumors of very large size (average 500mm^3) when dosed with KD004 at 8mg/kg, once weekly (QW) in the MDA-MB-453 tumor model in NCG mice. **(Figure 2B)**.

Potent tumor regression was also achieved even when anti-TROP2 sdAb KD004 was administered once every three weeks in the MDA-MB-453 tumor model, in NCG mice, at a dose of 8mg/kg **(Figure 2C)**.

Treatment with KD002 or KD005 at a dose of 0.1mg/kg (i.p.) in MDA-MB-453 implanted NCG mice tumor model also led to tumor regression **(Figure 2D)**. The data indicated a strong anti-tumor activity of KD002 and KD005 in the pre-clinical MDA-MB-453 model. Such a strong activity may be explained by the higher binding affinity and potent ADCP activity mediated by macrophages.

Different groups of NCG mice were administered a single dose of KD004 at 2, 8 or 30mg/kg dose level. Blood samples were collected at different time points and serum was prepared. Level of KD004 in serum was measured by ELISA and the pharmacokinetic parameters were calculated using the WinNonLin software. The anti-TROP2 *in vivo* pharmacokinetic parameters show that the half-life of KD004 is approximately 60 hours when administered at a dose of 8-30 mg/kg i.v. in NCG mice **(Figure 2E)**.

As illustrated in **Figure 3**, the sdAb of the present disclosure possess anti-tumor activity as a naked antibody but may also induce ADCC.

The mechanism of action of the sdAbs of the present disclosure was further investigated.

Example 5- Cell viability

Cell viability of MDA-MB-453 cells following 12 days treatment with 1 μM TROP2 sdAbs was analyzed using the Promega CellTitre-Glo luminescent assay **(Figures 4A-4B)**. **Figure 4A**. MDA-MB-453 cells were seeded in 96-well plates at 5 x 10^3 cells per well and incubated overnight at 37°C, 5% CO_2. Cells were treated with 1 μM TROP2 sdAbs for 12 days. Following treatment cell viability was assessed using CellTitre-Glo luminescent assay (Promega) according to manufacturer's instructions. Negative control wells were treated with isotype control sdAb (sdAb

with VHH specific for HEWL). AR47A6.4.2 TROP2 antibody was used for comparison purposes. Control wells containing cells which did not receive any treatment were used for signal normalization and calculation of percentage cell viability following treatment. Background luminescence was determined using wells containing medium without cells. Values represent the mean ± S.D. of three replicates for each treatment. **Figure 4B** shows cells imaged at 4x resolution after 12 days of treatment with TROP2 sdAb. Images were captured using EVOS XL Core Imaging System.

As shown in **Figures 4A-4B**, the TROP2 sdAbs of the present disclosure (KD001, KD002, KD003, KD004 and KD005) directly inhibited MDA-MB-453 cell viability more than 50% compared to AR47A6.4.2 TROP2 antibody (which in turn did not directly affect cell viability) and the negative control (sdAb with VHH specific for HEWL).

Example 6- ADCC activity

The ADCC activity of sdAbs was further characterized using the Promega ADCC reporter assay kit (Promega, G7015). In brief, the target cells MDA-MB-453 cells were collected and using TrypLE and resuspended at the concentration of 3 million/mL. 12,500 cells were seeded into each well and incubated overnight. The next day, testing antibodies were diluted into the assay medium, with a final testing concentration of 1 μg/mL. The ADCC effector cells were thawed gently in a 37°C water bath and resuspended into the assay medium. The ratio between effector cells and target cells used was 1:10. Thus 1,250 effector cells were transferred to each well. Plates were incubated at 37°C incubator for 24 hours. 24 hours later, plates were equilibrated to room temperature. The Bio-Glo luciferase assay substrate was added to each well. Incubate the plate at room temperature for 5-30 minutes. Luminescence signal was measured by SpectraMax i3x plate reader. Data were analyzed by signal fold change upon untreated control wells.

As shown in **Figure 5A**, the tested anti-TROP2 sdAbs showed high ADCC capability against MDA-MB-453 cells. This ADCC reporter assay also indicates that KD001, KD002, KD003, KD004 or KD005 anti-TROP2 sdAbs are capable of inducing ADCC comparable to the human IgG1 anti-TROP2 antibodies generated with the sequences from clones hTINA-1 and h7E6.

NCG and SCID mice were implanted with either MDA-MB-231 or MDA-MB-453 cells s.c.. Anti-TROP2 sdAb (KD002 or KD004) administration was initiated when the average tumor

volume reached 100mm^3. As illustrated in **Figure 5B**, sdAbs *in vivo* activity is similar in mouse strains with different effector function components.

Example 7- Internalization

The internalization assay was divided into two steps. First, the anti-TROP2 sdAbs were conjugated with Promega pHAb Amine reactive dye (Promega, G9841) using on-bead antibody conjugation. Second, the internalization assay was carried out and analyzed by flow cytometry.

For pHAb amine-reactive dye labeling, anti-TROP2 sdAbs were conjugated to the protein A beads. anti-TROP2 sdAbs were mixed with protein A beads and incubated for 60 minutes at room temperature. The antibody/bead mix was then washed with the washing buffer and resuspended in the amine/thiol conjugation buffer. The pHAb amine-reactive dye was added into the tubes containing antibody/bead mix with a 20 molar excess of dye and incubated at room temperature for 60 minutes. Tubes were placed in the magnetic stand and supernatant was discarded. Beads were washed another two times with washing buffer before elution into the elution buffer. Eluted antibody was resuspended into the neutralization buffer from the kit.

The internalization assay was performed with MDA-MB-453 cells in a flow-based format. The MDA-MB-453 cells were collected and diluted to 300,000 cells/mL. 100 µL of cell solution (30,000) was transferred to the 96-well plates. Cells were incubated in the incubator overnight. The next day, pH conjugated antibodies were diluted to 10 µg/mL concentration. 50 µL of the pH conjugated antibodies were distributed into the corresponding wells and incubated to the indicated timepoints (4 hours and 24 hours). Plates were washed 1x with flow buffer (DPBS containing 2% serum) and cells were resuspended in 100 µL of flow buffer containing 7-AAD at 1:100 dilution. Plates were analyzed by BD FACSCanto II. Antibody internalized cells were gated based on 7-AAD negative and PE positive cells.

As illustrated in **Figure 6A** and **Figure 6B**, the positive control anti-TROP2 antibody AR47A6.4.2 showed internalization at 4 hours after incubation. The internalization of anti-TROP2 sdAbs was detected at 4 hours for KD002, KD003, and KD004. Increased internalization was observed at 24 hours for KD002, KD003, and KD004. KD002 showed the highest internalization among the anti-TROP2 sdAbs. Interestingly, KD005 was not internalized although it bound a similar epitope to the other anti-TROP2 sdAbs.

As illustrated in **Figure 6C,** which represents the results from a dose-response internalization experiment in MDA-MB-231 cells, it was observed that each of the anti-TROP2 sdAbs KD001-KD005 (0.125nM-128nM range) display a unique dose-response internalization profile. Some sdAbs internalized only to a very small extent (e.g., KD005), whereas others showed internalization levels comparable to that of monoclonal antibodies known to internalize efficiently (e.g., KD002). Internalization was assessed by conjugation of sdAbs with a pH-sensitive probe and monitored by flow cytometry (Em. 560nm) after 24 hours. This internalization process was also visualized by microscopy as illustrated by **Figure 6D**. MDA-MB-453 cells were treated with αTROP2 sdAbs and internalization was monitored at 0 hours (after 15 minutes at 4°C), 6 hours or 24 hours by confocal microscopy. Cells were stained with anti-human IgG Fc antibody and counterstained for nuclei (DAPI). Isotype negative control HCAb (NC) showed no binding to cells. Whereas anti-TROP2 sdAb staining is restricted to membrane at 0h, and at 6 and 24 hours punctate green staining is observed (arrowheads) revealing antibody internalization.

These results indicate that the sdAbs of the present disclosure may be used as a naked antibody or as an antibody drug conjugate.

Example 8- Specificity, epitope characterization and domain binding

The binding of anti-TROP2 sdAbs to cells was assessed by flow cytometry. To determine the binding specificity of anti-TROP2 sdAbs, the CHO and HEK parental cells were transiently transfected with human TROP2 by lipofectamine. 24 hours after transfection, cells were collected and stained with anti-TROP2 antibodies. In brief, 1×10^5 cells were incubated with an Fc receptor blocking antibody (1/100 dilution) for 10 minutes at room temperature. Anti-TROP2 antibodies were diluted in FACS staining buffer to 100 nM and added and incubated with cells for 30 minutes on ice. After washing three times with FACS staining buffer, APC-conjugated secondary antibodies recognizing the human Fc region (Biolegend) were mixed with cells. After staining for 30 minutes, cells were washed three times, followed by resuspension into 100 µL of 7-Amino-Actinomycin D (7-AAD) solution (1/100 dilution). Cells were analyzed by BD FACSCanto™ II Flow cytometer (BD Bioscience). Data were analyzed with FlowJo via gating on single cells and live cells.

As illustrated in **Figures 7A- 7D**, all anti-TROP2 antibodies did not bind to the HEK and CHO parental cells, which is similar to the negative control. After TROP2 transient transfection,

all anti-TROP2 sdAbs showed specific binding to the HEK-TROP2 and CHO-TROP2 cells as also observed for comparator antibodies, AR47A6.4.2, hTINA-1, h7E6, hRS7 (see Cardillo *et al.* 2011).

Also as illustrated in **Figure 7E**, binding of anti-TROP2 sdAbs is highly specific, as they did not bind to the highly related EpCAM and IGFBP-1 proteins, by ELISA.

The epitope characterization was assessed by competitive ELISA (**Figure 8** and **Figure 9**). Microplates were coated with human recombinant TROP2 at 4 °C overnight. After washing, plates were blocked with blocking buffer (PBS + 2% BSA), for 1 hour at room temperature. Meanwhile, the dilution for anti-TROP2 VHH sdAbs was prepared with blocking buffer making two different dilutions depending on the tag. For sdAbs with His tag, the antibody was diluted to 28571.4 pM. For sdAbs without tag, antibodies were serially diluted from 2857142.9 pM to 0.29 pM (8 dilutions, 10X). After that, equal volume of the his-tagged sdAbs and serial diluted non-tagged sdAbs were mixed, to make the final concentration of his-tagged sdAbs 14285.7 pM and non-tagged sdAbs from 1428571.4 pM to 0.14 pM. After washing two times, the mixed antibody contents were added into the wells and the plates were incubated for 2 hours at room temperature. The HRP-conjugated anti-6x His was diluted to 1:5000 and added to the wells after washing three times. Any unbound conjugate was removed by washing after 1 hour incubation at room temperature, and the colorimetric reaction was performed by adding the same volume of Supersignal ELISA Pico Luminol/Enhancer and SuperSignal ELISA Pico Stable Peroxide Solution and detected on SpectraMax i3x Microplate Reader. Positive and negative controls were included in each plate.

As illustrated in **Figures 8A- 8E**, anti-TROP2 sdAbs KD001, KD002, KD003, KD004, and KD005 compete with each other for binding to human TROP2 recombinant protein, suggesting they bind to very similar epitopes binding of TROP2 or bind to overlapping epitopes.

Anti-TROP2 sdAb KD004 did not compete with hRS7 and AR47A6.4.2 for binding to human TROP2 recombinant protein, as illustrated in **Figure 9A**, implying that KD004 binds different epitopes from hRS7 and AR47A6.4.2.

Anti-TROP2 sdAbs KD006 and KD007 did not compete for binding to human TROP2 recombinant protein (**Figure 9B**), implying that KD006 binds a different epitope from KD007. KD006 and KD007 do not compete with KD001-KD005 in these experiments (data not shown).

In order to decipher the domain specificity of TROP2 sdAbs, immunoblots were performed with various TROP2 constructs including the extracellular domain of TROP2 and the cysteine poor domain of TROP2 (**Figure 10A**).

Recombinant protein of human TROP2 extracellular domain (ECD) (Sino Biological, Cat. No. 10428-H08H) was incubated with PNGase F enzyme (NEB, Cat. No. P0704) for the deglycosylation reaction under both denatured and non-denatured conditions as *per* the manufacturer's protocol. Samples were prepared for SDS-PAGE analysis under reduced conditions premixed with NuPAGE™ sample reducing agent (ThermoFisher Cat. No. NP0004) or non-reducing conditions without reducing agent.

Samples of recombinant proteins of human TROP2 extracellular domain (ECD) (Sino Biological, Cat. no. 10428-H08H) and human TROP2 cysteine poor domain (CPD) were prepared for SDS-PAGE analysis under reducing or non-reducing conditions with NuPAGE™ LDS Sample Buffer (ThermoFisher Cat. no NP0007) with NuPAGE™ Sample Reducing Agent (ThermoFisher Cat. no. NP0004) or without reducing agent. Samples under reducing condition were denatured by heating at 70°C for 10 minutes. Samples (16 µL) were loaded onto 4-12% Bis-Tris mini-gels (1.5 mm, 15 wells) or 250 ng of samples were loaded onto 14% Tris-glycine mini-gels alongside a BSA standard. Electrophoresis was conducted using a X-Cell SureLock™ mini-gel device at 125 volts for approximately 1 hour. Gels were stained using GelCode™ staining reagent (Thermo Fisher, Cat. no. 24594).

Electrophoresis was conducted using a X-Cell SureLock™ mini-gel device at 125 volts for 1 hour. Proteins were transferred to nitrocellulose membranes using the iBlot™ system (Thermo Fisher, Cat. no. IB301031) according to the manufacturer's instructions. Membranes were blocked by incubation in PBS/0.1% Tween™ 20/5%Milk for 1 hour at room temperature with shaking, followed by incubation with 1µg/mL of Anti-TROP2 antibodies or the isotype control (IgG1 isotype) in 20 mL of PBS/0.1% Tween™ 20/ 5% milk. Membranes were washed three times for 10 minutes with PBS/0.1% Tween™ 20. Membranes were incubated with HRP-conjugated goat anti-human IgG antibody (Sigma-Aldrich, AP113P) diluted at 1:10000 in blocking buffer for 1 hour at room temperature with shaking. Membranes were washed three times for 10 minutes with PBS/0.1% Tween™ 20. The signal was visualized using Super Signal™ West Pico PLUS (Thermo

Fisher, Cat. no. 34080) according to the manufacturer's instructions. Images were captured using the Azure Biosystem imaging system.

As illustrated in **Figure 10B**, all sdAbs tested bound to the extracellular domain of human TROP2 but did not bind to the cysteine poor domain of human TROP2 contrary to other MAbs such as AR47A6.4.2 or hRS7.

The epitope recognized by the sdAbs of the present disclosure involves the CRD/TY-1 region of TROP2.

Western blots using recombinant human TROP2 in different forms (non-denatured, linear, reduced, deglycosylated using PNGase F) confirm that the sdAbs of the present disclosure effectively bind to TROP2 (**Figures 11A-11E**).

The specificity of sdAb for different TROP2 species was also investigated.

TROP2 full-length extracellular domain recombinant proteins including cynomolgus/Rhesus (**Figure 12A**), human (**Figure 12B**), mouse (**Figure 12C**), and rat (**Figure 12D**) were coated on 96-well ELISA plates at the concentration of 1 μg/mL. Plates were covered with adhesive plate cover and stored at 4°C overnight. The next day, plates were washed 3x with washing buffer (PBS with 0.1% Tween™ 20). Plates were blocked with 2% fat-free BSA in washing buffer for 1 hour at 37°C. During the incubation time, the anti-TROP2 sdAbs were diluted in the blocking buffer to the indicated concentrations. In brief, the anti-TROP2 sdAbs starting concentration was 1000 nM and serial diluted 1 in 5 to 0.02048 pM. After blocking, the blocking buffer was removed from the wells and 100 μL of anti-TROP2 sdAbs dilutions were transferred to the corresponding wells. Plates were covered with adhesive plates cover and incubated at 37°C for 1 hour. During the primary antibody incubation, the secondary antibody (goat anti-human IgG, HRP conjugated) was diluted 1 in 5000 with the blocking buffer. Plates were washed 3x with blocking buffer, followed by secondary antibody incubation for another 1 hour at 37°C. After the secondary antibody incubation, plates were washed 3x with washing buffer and added with 100 μL of ELISA chemiluminescent substrate (Thermofisher). The luminescence signal was detected by SpectraMax i3x at 425 nM.

As shown in **Figures 12A-12D**, the human anti-TROP2 sdAbs showed cross-reactivity to the cynomolgus/ Rhesus TROP2, and a lower binding activity to mouse and rat TROP2 by ELISA.

All anti-TROP2 sdAbs have very high affinity, as shown by surface plasmon resonance (SPR) assay, both for human and cynomolgus TROP2 (low pM) (**Figure 12E**). All anti-TROP2 sdAbs showed a very slow dissociation rate as indicated by the kd rate (**Figure 12F**).

In order to determine the region of TROP2 targeted by the anti-TROP2 antibodies, the binding of anti-TROP2 antibodies to cells expressing TROP2 truncation was assessed by flow cytometry.

To determine the effect of TROP2 truncations on the binding ability of anti-TROP2 sdAbs, the HEK parental cells were transiently transfected with human TROP2 mutants by lipofectamine. 24 hours after transfection, cells were collected and stained with anti-TROP2 antibodies (**Figure 13A** and **Figure 13B**). In brief, 1×10^5 cells were incubated with an Fc receptor blocking antibody (1/100 dilution) for 10 minutes at room temperature. Anti-TROP2 antibodies were diluted in FACS staining buffer to 100 nM and added and incubated with cells for 30 minutes on ice. After washing three times with FACS staining buffer, APC-conjugated secondary antibodies recognizing the human Fc region (Biolegend, Cat# 410712) were mixed with cells. After staining for 30 minutes, cells were washed three times, followed by resuspension into 100 µL of 7-Amino-Actinomycin D (7-AAD) solution (BD Biosciences, Cat# 559925).

As illustrated in **Figure 13A** and **Figure 13B**, clone AR47A6.4.2, hRS7, hTINA-1, h7E6 and KD006 binding to TROP2 was not affected by TROP2 truncation in the cysteine-rich domain. KD001, KD002, KD003, KD004, and KD005 lost their binding to TROP2 on HEK cells when TROP2 27-56 regions were truncated, implying that KD001-KD005 binding to TROP2 involves the TROP2 27-56 region.

Different from KD001 to KD005, KD006 and KD007 bound to another region to TROP2 proteins. Similar to other benchmark TROP2 antibodies, KD006 was able to bind to all TROP2 CRD truncations, implying that binding of these antibodies to TROP2 involves the TY-1 and/or CPD region. KD007 did not bind to any of the TROP2 CRD truncations, indicating that KD007 binding to TROP2 may involve TROP2 N-terminal 1-27 amino acids.

Treatment with KD006 led to a tumor growth inhibition of 48.6% in the xenograft model of T.Tn whereas KD007 did not show efficacy in this model (**Figure 13C**).

Example 9- ADCP

The ADCP assays were performed with the Promega ADCP kits (cat# CS314906). In brief, 200,000 target cells, including MDA-MB-453, MDA-MB-231 (, NCI-H292, MDA-MB-468, T.Tn tumor cells were seeded into the 96-well plates overnight. The next day, testing antibodies were serial diluted (1 in 2 dilutions starting from 2 nM) into the RPMI 1640 culture medium. 25 µL of the serial diluted testing antibody was added to the wells containing appropriate adherent target cells.

THP-1 cells (ADCP Reporter Bioassay Effector Cells) were taken from -140°C storage and transferred to the bench on dry ice. The effector cells were thawed in a 37°C water bath until cells are just thawed (about 2 - 3 minutes). While thawing, the vial was gently agitated and visually inspected. The cell suspension was mixed in the vial by pipetting up and down and 0.5 ml cells was transferred to the tube containing 4.5 ml assay buffer. The tube was mixed by gently inverting. The ADCP Reporter Bioassay Effector Cells (THP-1) suspension were transferred to a sterile reagent reservoir and 25 µl of the cell suspension was dispensed to all the wells containing target cells and antibodies. The lid was placed on the plates and the plate was incubated for 4 hours at 37°C in a CO2 incubator. 4 hours later, the Bio-Glo-NL™ Luciferase assay reagent was freshly prepared and reconstituted and equilibrated at room temperature. Using a manual multichannel pipette, 75µl of io-Glo-NL™ Luciferase assay reagent was added to the assay plates while avoiding creating any bubbles. The plate was incubated at ambient temperature for 5–30 minutes and then luminescence was measured using a plate reader with glow-type luminescence read capabilities.

Experiments with KD002 and KD004 as well as corresponding versions in which the IgG1 Fc portion of KD002 and KD004 (KD017 and KD018) was replaced for an IgG4 Fc, were tested for antibody-dependent cellular phagocytosis (ADCP). As illustrated in **Figure 14A**, the anti-TROP2 sdAbs IgG1 but not the IgG4 version elicited ADCP *in vitro* in a dose-response test (0.01nM to 1nM range). Antibodies' stock (20 nM) was serially diluted and added to the wells containing target and effector reporter THP-1 cells. MDA-MB-453 cells (target) were seeded at 20,000 cell/well overnight. The ADCP effector cells (THP-1 cells) were dispensed at an E:T ratio of 3:2.

Since CD47 is a checkpoint for macrophage ADCP activity, we assessed the level of CD47 expression in various cancer cells lines expressing TROP2 to evaluate the impact on the efficacy of anti-TROP2 sdAbs.

As illustrated in **Figure 14B**, MDA-MB-453 cells showed the lowest CD47 expression, whereas the level of cell surface CD47 expression in NCI-H292 and MDA-MB-231 is similar. The level of TROP2 expression is higher in NCI-H292, followed by MDA-MB-453 and MDA-MB-231.

The ADCP assays were performed with the Promega ADCP kits (cat# CS314906). 200,000 target cells, including MDA-MB-453 (**Figure 14C** and **Figure 18A-upper panel**), MDA-MB-231 (**Figure 14D**), NCI-H292 (**Figure 14E**), MDA-MB-468 (**Figure 18A-middle panel**) or T.Tn tumor cells (**Figure 18A-lower panel**) were seeded into the 96-well plates overnight. The next day, testing antibodies (KD002 or KD004) were serial diluted (1 in 2.2 dilutions starting from 2 nM) into RPMI 1640 culture medium. 25 µL of the serial diluted testing antibody was added to the wells containing appropriate adherent target cells.

As illustrated in **Figures 14C-14E**, TROP2 antibodies KD002 and KD004 showed a high level of ADCP activity against MDA-MB-453 cells and lower ADCP activity on MDA-MB-231 and NCI-H292 cells.

As shown in the **Figure 14C**, the EC50 of the two antibodies KD002 and KD004 are close to each other at around 0.1 nM, but MDA-MB-453 there was a higher RLUmax than the MDA-MB-231 and NCI-H292 cells, which may be explained by the lower expression of CD47 on MDA-MB-453 cells.

As shown in **Figure 14F**, IgG4 versions of the KD002 and KD004 antibodies (KD017 and KD018) have lost ADCP activity against MDA-MB-453, MDA-MB-231 and NCI-H292 cells in a similar experiment. These *in vitro* data suggest the ADCP mechanism of the TROP2 antibodies may be dependent on the Fc receptor.

Expression of CD47 was knocked down using small interfering RNA (siRNA) and similar ADCP assay were performed. MDA-MB-231 cells and NIC-H292 cells were transfected with CD47 siRNA (Thermofisher, Cat# 4392421) or control siRNA (Thermofisher, Cat# AM4635) by the lipofectamine transfection kits. 48 hours after transfection, cells were collected and CD47 expression level was determined by flow cytometry. Once CD47 knockdown was confirmed, cells were used as the target cells in ADCP assays.

Expression level of CD47 and TROP2 on NCI-H292, MDA-MB-453 and MDA-MB-231 cells after CD47 knockdown was determined (**Figure 15A**). 1×10^5 cells were incubated with an Fc receptor blocking antibody (1/100 dilution) for 10 minutes at room temperature. Diluted APC-conjugated anti-CD47, PE-conjugated anti-TROP2 antibodies (Biolegend) (1/100 dilution) were mixed with cells. After staining for 30 minutes, cells were washed three times, followed by resuspension into 100 µL of 7-Amino-Actinomycin D (7-AAD) solution (1/100 dilution).

Cells were analyzed by BD FACSCanto™ II Flow cytometer (BD Bioscience). Data were analyzed with FlowJo via gating on single cells and live cells.

As illustrated in **Figure 15A**, transfection with CD47 siRNA led to a significant reduction of CD47 expression in MDA-MB-231 and NCI-H292 cells.

As illustrated in **Figures 15B-15D**, CD47 knockdown by siRNA increased ADCP activity (phagocytosis) of KD002 and KD005 in both MDA-MB-231 and NCI-H292 cells. In MDA-MB-231 KD005 appears to have higher ADCP activity than KD002 and also show ADCP activity even without inhibition of CD47.

Moreover, as illustrated in **Figure 15D**, in comparison to KD002 and KD005, the IgG4 versions of KD002 and KD005 antibodies (KD017 and KD019) have lost ADCP activity against MDA-MB-231 and NCI-H292 tumor cells even when CD47 expression is inhibited.

These results indicated that knockdown of CD47 significantly enhanced the maximal signal of the ADCP assay. Since CD47 is a checkpoint for macrophage ADCP activity, the higher expression of CD47 on MDA-MB-231 and NCI-H292 may explain the relatively low ADCP activity. This is consistent with data showing that higher CD47 expression led to the lower ADCP activity.

The *in vivo* ADCP mechanism and the potential enhancing ADCP activity was also verified with an anti-CD47 antibody. Briefly two bispecific antibodies comprising either KD011 at the N-terminus and KD070 at the C-terminus of Fc or KD011 at the C- terminus and KD070 at the N-terminus of Fc were generated. Both KD065 and KD066 showed increased anti-tumor activity compared to KD004 that does not include the anti-CD47 module (**Figure 15E**).

These data suggest that potency of anti-TROP2 antibodies may be increased by inhibiting CD47. Bispecific or multispecific antibodies comprising an anti-TROP2 antigen binding domain

and a CD47 antigen binding domain may therefore be used for treating cancer associated with TROP2 expression.

THP-1 differentiation

THP-1 cells were differentiated into macrophages in the six-well plates. Two million cells were plated in the six-well plates and stimulated with PMA (Sigma, P8139-1MG) at the concentration of 150 nM for 24 hours. Cells were washed with PBS and treated with cytokines for 72 hours in the cell culture medium. For M1 cell differentiation, cells were treated with 20 ng/ml IFN-gamma (Cedarlane, 285-IF-100) and 10 pg/ml LPS (Cedarlane, 437628-1MG). For M2 cell differentiation, cells were treated with 20 ng/ml IL-4 (Cedarlane, 204-IL-010) and 20 ng/ml IL-13 (Cedarlane, 213-ILB-010).

Before running macrophage phagocytosis assay, cells were collected with TrypLE and washed twice with PBS. The target cells MDA-MB-453 were stained with pHrodo SE (Invitrogen, P36600) at 120 ng/ml for 30 minutes at room temperature. The M1 or M2 macrophages were stained with Celltrace Violet (Invitrogen, C34557 A) at 5 μM for 30 minutes at room temperature. Cells. Cells were washed with PBS and resuspended in the RPMI complete medium. M1 or M2 macrophages were mixed with MDA-MB-453 cells at the E:T ratio of 2:1 and treated with the indicated antibodies for 4 hours. After the incubation, cells were washed with PBS and resuspended in the FACS buffer with 1/100 dilution of 7-AAD. Cells were analyzed by the FACS CantoII cytometer and double positive cells were gated as the phagocytosed macrophages.

Figure 16A shows that THP-1 cells differentiate into M1 and M2 macrophages upon treatment with PMA and cytokines. THP-1 cells showed upregulation of CD68 after PMA treatment. The M1 cells showed upregulation of CD38 and CD86 after treatment with IFN-γ and LPS. M2 macrophages showed upregulation of CD206 and downregulation of CD38 after treatment with IL-3 and IL-13.

As illustrated in **Figure 16B**, both M1 and M2 macrophages lead to phagocytosis of target cells MDA-MB-453 by KD002 and KD005.

Example 10- *In vivo* mechanism of action

The mechanism of action was further investigated *in vivo*.

As illustrated in **Figure 17A** KD017 which is the IgG4 version of KD002 was detected by the secondary antibody while as expected, the IgG1 version KD002 was not. KD017 is thus able to bind to recombinant human TROP2. However, as illustrated in **Figure 17B**, KD017 has lost the anti-tumor effect in the MDA-MB-453 xenograft tumor model. These data suggest that KD002 anti-tumor function is a Fc receptor-dependent activity. Since the NCG mice only has myeloid cells but lack all B cell, T cell and NK cells, this Fc-dependent cellular activity is likely mediated by macrophage ADCP.

As illustrated in **Figure 18A**, TROP2 antibodies KD002 and KD005 showed a high level of ADCP activity against MDA-MB-453 cells (upper panel) and MDA-MB-468 cells (middle panel) and a medium level of ADCP activity against T.T.n cells (lower panel). Corresponding to the higher *in vitro* ADCP activity, KD002 and KD005 treatment led to complete tumor regression in the MDA-MB-453 (upper panel), and MDA-MB-468 (middle panel) xenograft model (**Figure 18B**). KD002 and KD005 led to a partial response in T.Tn xenograft tumor model with a tumor growth inhibition of 60% (**Figure 18B-lower panel**).

For mouse macrophage IHC staining, tumor samples including COLO205, CAPAN1, MDA-MB-231, T.Tn and MDA-MB-453 were collected from the xenograft models and formalin-fixed paraffin-embedded. Samples were stained with the mouse macrophage marker F4/80 followed by the secondary antibody-HRP (**Figure 19A**). There was a correlation between the degree of macrophage infiltration and the potency of TROP2 antibodies. In the MDA-MB-453 xenograft model in which TROP2 antibodies treatment led to a complete regression, macrophage showed massive infiltration into the tumors confirmed by IHC. In the MDA-MB-231 and T.Tn xenograft model in which TROP2 antibodies showed a 50%-60% tumor growth inhibition, macrophage exhibited a medium level of macrophage infiltration. COLO205 and CAPAN1 xenograft model showed little macrophage infiltration and no tumor growth inhibition (TGI).

CD47 showed a higher expression in most of the tumors confirmed by IHC. However, overall, we observed a correlation between TROP2 antibodies efficacy and macrophage infiltration (**Figure 19A**).

These data indicate that the TROP2 antibody potency involves macrophage infiltration into the tumors. TROP2 expression and macrophage infiltration could represent clinical biomarkers to achieve optimal treatment result.

Example 11- TROP2 expression in selected tumors

TROP2 IHC staining

To understand the TROP2 expression prevalence across different cancer types, we stained TROP2 expression with TROP2 antibodies at 2 mg/ml on the FFPE cancer tissue microarrays (Novus Biologics, cat# NBP2-42052 & NBP2-42056) and frozen colon cancer microarray (Biochain, T6235090) (**Figures 19B-19E**). In the Novus Biologics FFPE cancer tissue microarrays, TROP2 antibody showed high positive staining on ovary cancer (71%), bladder cancer (100%), breast cancer (100%), uterus cancer (83.3%), head and neck cancer (78%), and stomach cancer (77.8%) (**Table 3**). We confirmed the head and neck cancer by staining with Biomax head and neck cancer microarrays containing over 150 patient samples (85% positive). The trop2 staining on FFPE colon cancer microarray was low, which might be due to the antigen retrieval method. To verify the colon cancer prevalence, we stained with frozen colon cancer arrays and showed 94% positive by TROP2 antibody staining. These data suggested the positive TROP2 epitope, a potential TROP2 biomarker by IHC staining and clinical indication of cancer types for the clinical trials and treatment.

Table 3- IHC Summary of KD005/ All Tumors TMAs

Arrays	Organ	No. of positive Tumors/Total (%)	IHC Score		
			+	++	+++
TMA #1* NBPS-45052 & 45056	Ovary	5/7 (71.4)	2	1	2
	Bladder	8/8 (100)	0	3	5
	Breast	8/8 (100)	4	0	4
	Kidney	1/13 (7.7)	0	0	1
	Uterus	5/6 (83.3)	1	3	1
	Lung	7/9 (100)	2	3	4
	Colon**	3/8 (37.5)	1	0	2
	Esophagus	7/9 (77.8)	0	1	6

Table 3- IHC Summary of KD005/ All Tumors TMAs

Arrays	Organ	No. of positive Tumors/Total (%)	IHC Score		
			+	++	+++
	Liver	2/9 (22.2)	1	0	1
	Rectum	2/9 (22.2)	1	1	0
	Stomach	7/9 (77.8)	2	4	1
	Total Positive	57/89 (64)	14	16	27
TMA #2* (H&N) Biomax HN602 & HN601d	Head and Neck	101/119 (85)	40	53	8
TMA Frozen Array Biochain T6235090	Colon Cancer	33/35 (94)	7	15	11

Example 12- Crystallization and structure determination

Crystallization

Crystals were obtained by the vapor diffusion method at 18°C, by mixing equal volumes of protein plus well solution.

The TROP2 ECD:VHH 3572 crystals grew in 55.5 mM MES, 45.5 mM Imidazole, 10 mM D-Galactose, 10 mM D-Glucose, 10 mM D-Mannose, 10 mM D-Xylose, 10 mM L-Fucose, 10 mM N-acetyl-D-glucosamine, 7% (w/v) PEG 20,000 and 14% (w/v) PEG 500 MME. For cryoprotection, crystals were generally transferred to a solution of mother liquor with the precipitant increased to 10% (w/v) PEG 20,000 and 20% (w/v) PEG 500 MME.

Data sets were collected at the European Synchrotron Radiation Facility (beamline MASSIF-1). Data was processed using XDS (Kabsch, W. (2010a/b) Acta Cryst D66, 125-132) and AIMLESS (Evans, P.R. and Murshudov, G.N. (2013) Acta Cryst D69, 1204-1214) from the CCP4 Suite (Winn, M., et al. (2011) Acta Cryst D67, 235-242).

The crystal structure was solved by molecular replacement using Phaser (McCoy et al., (2005) Acta Cryst D 61, 458-64), using atomic models generated using Alphafold2-multimer (Evans et al., (2022) bioRxiv doi:10.1101/2021.10.04.463034). Atomic models were built using Coot (Emsley, P. & Cowtan, K. Coot, (2004) Acta Cryst D60, 2126-32) and refined with Refmac (Murshudov, et al., (1997) Acta Cryst D53, 240-255) Co-crystals of TROP2 ECD and VHH M100Y were refined to 1.75 Å resolution after merging three datasets (**Table 4**).

Table 4

	TROP2 + 3572
Data Collection	
Beamline	ESRF MASSIF-1
Wavelength	0.965459
Space Group	P 3_221
Cell Dimensions a, b, c (Å), α, β, γ (o)	81.5, 81.5, 113.6, 90, 90, 120
Resolution (Å)	70.56 – 1.75
Rmerge	0.150 (3.923)
CC 1/2	0.994 (0.293)
I/σI	7.2 (0.8)
Completeness (%)	100.0 (99.9)
Redundancy	9.4 (6.6)
Refinement	
No. of Reflections	412573
No. of Unique	43744

Rfactor / Rfree (%)	19.9 (25.8)
Wilson *B*-factors (Å)	39.11
B-factors (Å)	
Protein	52.23
R.M.S. Deviations	
Bond lengths (Å)	0.0079
Bond angles (°)	1.458

As can be seen from **Figures 20A-20C**, the CDR3 and FR2 region of the variable region of KD005 (i.e., KD012) appears to surround a knob formed by the cysteine-rich domain of TROP2. The CDR3 of KD012 forms a clip fitting into a groove within the CRD of the TROP2 protein which may explain the high-affinity binding and slow dissociation rate. Furthermore, the framework region 2 of the KD012 sdAb also contributes to the binding to TROP2 which appears to be an unusual feature.

Since KD001, KD002, KD003, KD004 and KD005 compete with each other, it is expected that they all form a similar structure and have similar binding affinity. It is also unique that the framework 2 of KD012 contribute to the binding to TROP2 protein.

These structural data supported the high affinity of these anti-TROP2 sdAbs and potent *in vivo* efficacy.

The co-crystal structured revealed amino acid residues of KD012 and TROP2 that are involved in this interaction (**Figure 20D**).

Table 5A shows the amino acid residues of the variable region of KD005 (i.e., corresponding to KD012: SEQ ID NO: 6) that interact with human TROP2.

Table 5A

Type	Position KD012	Location KD012	Residues KD012	Position TROP2	Location TROP2	Residues TROP2	Energy	Distance
D	1	FR1	Gln1	80	TY-1	Asp109	-0.22	3.81
D	1	FR1	Gln1	81	TY-1	Pro110	-0.1	3.9
D	1	FR1	Gln1	82	TY-1	Glu111	-0.27	3.86

Type	Position KD012	Location KD012	Residues KD012	Position TROP2	Location TROP2	Residues TROP2	Energy	Distance
D	2	FR1	Val2	81	TY-1	Pro110	-0.31	4.01
DH	2	FR1	Val2	82	TY-1	Glu111	-6.95	3.82
D	37	FR2	Val37	27	CRD	Arg56	-0.98	4.13
D	39	FR2	Gln39	31	CRD	Ser60	0.01	4.26
D	45	FR2	Pro45	27	CRD	Arg56	1.14	4.09
D	45	FR2	Pro45	31	CRD	Ser60	-0.01	4.2
D	45	FR2	Pro45	32	CRD	Gly61	0.94	3.95
DH	47	FR2	Trp47	18	CRD	Asp47	-5.09	3.68
D	47	FR2	Trp47	19	CRD	Gly48	0.66	3.92
DA	47	FR2	Trp47	20	CRD	Pro49	-4.89	3.97
D	47	FR2	Trp47	25	CRD	Gln54	1.92	3.87
D	47	FR2	Trp47	27	CRD	Arg56	0.7	4.09
D	50	FR2	Tyr50	20	CRD	Pro49	2.66	3.93
D	58	FR3	Tyr58	20	CRD	Pro49	0.71	4.23
D	58	FR3	Tyr58	21	CRD	Gly50	1.96	3.91
DIH	99	CDR3	Arg99	36	CRD	Asp65	-19.2	3.47
D	100	CDR3	Leu100	20	CRD	Pro49	-0.54	4.35
D	100	CDR3	Leu100	23	CRD	Arg52	-1.27	4.03
DH	101	CDR3	Thr101	23	CRD	Arg52	-9.92	3.69
D	101	CDR3	Thr101	36	CRD	Asp65	-0.68	4.06
D	102	CDR3	Asp102	23	CRD	Arg52	-0.04	3.92
D	103	CDR3	Ser103	2	CRD	Gln31	-0.02	4.13
D	103	CDR3	Ser103	23	CRD	Arg52	-0.43	4.03
D	103	CDR3	Ser103	24	CRD	Cys53	-0.12	3.85
DH	104	CDR3	His104	6	CRD	Thr35	-4.23	3.51
D	104	CDR3	His104	23	CRD	Arg52	0.28	4.02
DH	104	CDR3	His104	24	CRD	Cys53	-1.17	3.91
DH	104	CDR3	His104	26	CRD	Cys55	-0.97	3.93
DA	104	CDR3	His104	37	CRD	Cys66	0.07	3.88
D	105	CDR3	Tyr105	19	CRD	Gly48	0.32	4.49
D	105	CDR3	Tyr105	20	CRD	Pro49	2.68	4.06
D	105	CDR3	Tyr105	23	CRD	Arg52	1.8	4.11
DH	105	CDR3	Tyr105	24	CRD	Cys53	-5.53	3.87
D	105	CDR3	Tyr105	25	CRD	Gln54	0.77	4.13
D	105	CDR3	Tyr105	27	CRD	Arg56	1.5	3.69
D	105	CDR3	Tyr105	34	CRD	Ala63	0.75	4.1
D	105	CDR3	Tyr105	35	CRD	Val64	0.03	4.13
D	106	CDR3	Val106	34	CRD	Ala63	-0.3	3.97
D	106	CDR3	Val106	35	CRD	Val64	-0.27	4.09

Type	Position KD012	Location KD012	Residues KD012	Position TROP2	Location TROP2	Residues TROP2	Energy	Distance
D	106	CDR3	Val106	36	CRD	Asp65	-0.64	4.16
DIH	107	CDR3	Glu107	27	CRD	Arg56	-37.12	3.27
D	107	CDR3	Glu107	32	CRD	Gly61	0.02	4.16
D	107	CDR3	Glu107	33	CRD	Met62	-1.04	3.89
DH	107	CDR3	Glu107	34	CRD	Ala63	-9.04	3.9
D	108	CDR3	Asp108	33	CRD	Met62	-0.27	4.43
D	108	CDR3	Asp108	81	TY-1	Pro110	0.01	4.29
D	108	CDR3	Asp108	82	TY-1	Glu111	-0.47	3.81
D	109	CDR3	Ala109	33	CRD	Met62	-0.79	4.25
D	109	CDR3	Ala109	81	TY-1	Pro110	-0.68	3.78
D	109	CDR3	Ala109	82	TY-1	Glu111	0.01	4.2
D	110	FR4	Trp110	27	CRD	Arg56	-0.26	4.37
D	110	FR4	Trp110	31	CRD	Ser60	-0.65	3.79
D	110	FR4	Trp110	32	CRD	Gly61	-0.48	4.11
D	110	FR4	Trp110	33	CRD	Met62	-5.73	4.1
DH	110	FR4	Trp110	81	TY-1	Pro110	-3.73	3.94
D	112	FR4	Gln112	86	TY-1	Lys115	-0.09	4.22

Table 5B

D	distance	VdW distance interaction energies.
A	arene	Arene interactions in the list, these include π:π, π-H, and π:cation contacts
I	ionic	Ionic bond contacts in the list
H	hbond	Hydrogen bond contacts

KD102 annotated according to IMGT numbering

This data is one representative example of protein-protein interactions determined from the co-crystal structure done in triplicate. Slight variation in the distance and energy values are likely due to the energy minimization step performed in QuickPrep in MOE.

Example 13 – Antibody humanization and characterization

ELISA binding assay

Human TROP2 recombinant proteins were coated on 96-well ELISA plates at the concentration of 1 µg/mL. Plates were covered with adhesive plate cover and stored at 4°C overnight. The next day, plates were washed 3x with washing buffer (PBS with 0.1% Tween™ 20). Plates were blocked with 2% fat-free BSA in washing buffer for 1 hour at 37°C. During the incubation time, the TROP2 reference antibodies, as well as the humanized variants, were diluted

in the blocking buffer to the indicated concentrations. In brief, the anti-TROP2 sdAbs starting concentration was 200 nM and serial diluted 1 in 5 to 0.00256 nM. After blocking, the blocking buffer was removed from the wells and 100 µL of anti-TROP2 sdAbs dilutions were transferred to the corresponding wells. Plates were covered with adhesive plates cover and incubated at 37°C for 1 hour. During the primary antibody incubation, the secondary antibody (goat anti-human IgG, HRP conjugated) was diluted 1 in 5000 with the blocking buffer. Plates were washed 3x with blocking buffer, followed by secondary antibody incubation for another 1 hour at 37°C. After the secondary antibody incubation, plates were washed 3x with washing buffer and added with 100 µL of ELISA chemiluminescent substrate (Thermofisher). The luminescence signal was detected by SpectraMax i3x at 425 nM.

As illustrated in **Figure 21**, all humanized variants of KD002 showed similar binding to KD002 except for KD021 and KD020, which showed reduced binding to recombinant human TROP2 protein in this assay. EC50 values are provided in **Table 6**.

Table 6

KD002 variants	EC50 (nM)
KD002	0.07
KD020	N/A
KD021	4.30
KD022	0.07
KD023	0.08
KD024	0.09
KD025	0.11
KD026	0.10
KD027	0.09
KD028	0.14
KD029	0.16
KD030	0.12
KD031	0.11

KD002 variants	EC50 (nM)
KD032	0.15
KD033	0.13
KD034	0.13
KD035	0.12
KD036	0.13
KD037	0.18
KD038	0.26
KD039	0.15
KD040	0.05
KD041	0.15
KD042	0.07

As illustrated in **Figures 22A** and **22B**, all humanized variants showed similar binding to KD005 except for KD064, which showed reduced binding EC50 and maximum binding signal in this assay. Since the amino acid changes in KD064 are at the framework 2, this implies the importance of framework binding to TROP2 in addition to the CDR3. EC50 values and % EC50 value compared to KD005 are presented in **Table 7**.

Table 7

Code name	EC50 (nM)	EC50 % to KD005	RMax
KD002	0.055	0.6875	199906.5
KD005	0.08	1	202140.5
KD043	0.09	1.125	195093
KD044	0.08	1	200020
KD045	0.09	1.125	206168.5
KD046	0.08	1	206369.5
KD047	0.14	1.75	207446.5
KD048	0.11	1.375	173921.5

KD049	0.14	1.75	212138
KD050	0.1	1.25	211592
KD051	0.12	1.5	211126
KD052	0.07	0.875	189730
KD053	0.1	1.25	198757
KD054	0.07	0.875	195032.5
KD055	0.08	1	194513
KD056	0.09	1.125	190663.5
KD057	0.09	1.125	183824.5
KD058	0.1	1.25	183885.5
KD059	0.1	1.25	188315.5
KD060	0.11	1.375	190929
KD061	0.12	1.5	191304
KD062	0.14	1.75	189381
KD063	0.12	1.5	184553
KD064	0.14	1.75	167056.5

ADCP assays were carried out as described above. Results of assays for ADCP activity for KD002 and humanized variants are presented in **Figures 23A** and **23B**. Antibody concentration of 0.1 nM and 0.5 nM were selected as 0.1 nM is close to the EC50 and 0.5 nM is close to the plateau observed. As illustrated in **Figures 23A** and **23B**, the humanized variants KD020 and KD021 had a significantly reduced ADCP activity, consistent with the binding data. Some of the amino acid substitution in KD020 are located in the framework 2 and may help explaining the decreased binding as framework 2 contributes to TROP2 binding as revealed by the structure data.

Results of assays for ADCP activity for KD005 and humanized variants are presented in **Figures 24A** and **24B**. The antibody concentration of 0.1 nM and 0.5 nM were selected as 0.1 nM is close to the EC50 and 0.5 nM is close to the plateau. At concentration of 0.1 nM, the majority of KD005 humanized variants exhibited better ADCP activity than the reference antibody KD005 except for the KD064. At concentration of 0.5 nM, the activity appeared essentially equivalent

except for the KD064. These results are consistent with the binding data as KD064 significantly reduced the binding to TROP2 after framework2 mutation.

However, dose-response ADCP analysis **Figures 24C-24I** carried out on selected KD005 humanized variants (KD045, KD047, KD048, KD049, KD053, Kd059 and KD063) revealed that some had slightly ADCP better activity, while others had similar ADCP activity or lower ADCP activity.

The ADCC activity of the humanized variants was evaluated as described above. Results of ADCC activity for KD002 and KD002 humanized variants are presented in **Figures 25A** and **25B**. Antibody concentration of 0.1 nM and 0.5 nM were selected as 0.1 nM is close to the EC50 and 0.5 nM is close to the plateau. At 0.5 nM concentration, the majority of humanized variants had similar ADCC activity to KD002 except for KD020 and KD021. The explanation is that the ADCC activity showed saturation at 0.5 nM close to the plateau. ADCC activity at 0.1 nM showed greater variability than at 0.5 nM. The humanized variants KD022, KD027, KD025, KD026, and KD024 exhibited better ADCC activity than KD002. Whereas KD028, KD020 and KD021 have a significantly reduced ADCC activity, which was consistent with the binding data.

Results of ADCC activity for KD005 and KD005 humanized variants are presented in **Figures 26A** and **26B**. Antibody concentration of 0.1 nM and 0.5 nM were selected as 0.1 nM is close to the EC50 and 0.5 nM is close to the plateau. At the concentration of 0.1 nM, the majority of KD005 humanized variants exhibited similar or better ADCC activity than the reference antibody KD005 except for the KD064. This is consistent with the binding data as KD064 significantly reduced the binding to TROP2 after framework2 mutation.

Based on the above results KD002 and KD005 humanized variants were ranked according to binding data, ADCP activity and/or ADCC activity.

For example, based on ELISA binding data, it appears that KD056, KD057, KD050, KD055, KD043, KD045, KD052, KD054, KD044, KD046 have a EC50 that is similar to or better than KD005, whereas KD054, KD043, KD053, KD044, KD045, KD046, KD047, KD051, KD050 and KD049 have a Rmax that is similar to or better than KD005.

Therefore, based on binding data, it appears that KD043, KD044, KD045, KD046, KD047, KD049, KD050, KD051, KD052, KD053, KD054, KD055, KD056, and KD057 represent the best binders.

The binding EC50 and the maximum binding signal (Rmax) of selected KD005 humanized variants based on the ELISA binding curve is presented in **Table 8**. The binding EC50 and Rmax were normalized to the reference antibody KD005.

Table 8

Variant Designation	EC50 (% KD005)	Variant Designation	Rmax (% KD005)
KD056	1.21	KD054	0.964
KD057	1.15	KD043	0.965
KD050	1.12	KD053	0.983
KD055	1.12	KD054	0.990
KD043	1.01	KD005	1
KD045	1.01	KD045	1.0199
KD005	1	KD046	1.0209
KD052	0.93	KD047	1.0262
KD054	0.93	KD051	1.0445
KD044	0.9	KD050	1.0468
KD046	0.9	KD049	1.0495

Based on ADCP assays performed herein, it appears that KD043, KD044, KD045, KD046, KD048, KD049, KD051, KD052, KD053, KD054, KD055, KD057, KD061, and KD063 have the best ADCP activity.

The ADCP activity of KD005 humanized variants at 0.1 nM and 0.5 nM are presented in **Table 9** and **Table 10**. The data was normalized to the reference antibody KD005.

Table 9: ADCP activity measured at 0.1 nM and 0.5 nM relative to KD005 ranked based on highest value at 0.1nM.

	0.5 nM (% KD005)	0.1 nM (% KD005)
KD063	96.1	189.9
KD043	108.1	189.0
KD046	72.1	186.4
KD049	92.8	169.1
KD044	96.4	165.0
KD061	83.8	164.7
KD045	87.3	163.5
KD052	98.2	162.6
KD053	97.6	161.7
KD054	94.9	160.5
KD005	100.0	100.0

Table 10: ADCP activity measured at 0.1 nM and 0.5 nM relative to KD005 ranked based on highest value at 0.5nM.

	0.5 nM (% KD005)	0.1 nM (% KD005)
KD051	113.1	126.9
KD043	108.1	189.0
KD057	103.9	96.6
KD005	100.0	100.0
KD052	98.2	162.6
KD053	97.6	161.7
KD044	96.4	165.0
KD063	96.1	189.9
KD054	94.9	160.5
KD055	93.0	157.9
KD049	92.8	169.1

5

Based on ADCC assay performed at 0.1 nM and 0.5 nM (EC50 and % Emax relative to KD005), it appears that KD043, KD044, KD045, KD046, KD047, KD048, KD049, KD050, KD051, KD053, KD054, KD061, KD062, and KD063 have the best ADCC activity.

Tables 11 and **Table 12** summarize the ADCC activity of KD005 humanized variants at 0.1 nM and 0.5 nM. The data was normalized to the reference antibody KD005.

Table 11: ADCC activity measured at 0.1 nM and 0.5 nM relative to KD005 ranked based on highest value at 0.1nM.

	0.5 nM (% KD005)	0.1 nM (% KD005)
KD050	96.3	214.3
KD046	127.8	199.9
KD048	124.2	154.5
KD061	95.5	151.4
KD062	97.5	150.1
KD045	98.3	145.7
KD063	98.7	140.1
KD043	98.8	139.8
KD044	105.4	127.3
KD054	90.3	126.8
KD005	100.0	100.0

Table 12: ADCC activity measured at 0.1 nM and 0.5 nM relative to KD005 ranked based on highest value at 0.5 nM.

	0.5 nM (%KD005)	0.1 nM (%KD005)
KD046	127.8	199.9
KD048	124.2	154.5
KD051	120.0	124.5
KD044	105.4	127.3

KD047	102.5	115.5
KD049	100.8	115.7
KD005	100.0	100.0
KD043	98.8	139.8
KD063	98.7	140.1
KD053	98.3	110.8
KD045	98.3	145.7

Example 14 – Sequence analysis

Co-crystallization of the variable region of KD005 with human TROP2 reveals that amino acid residues of CDRH3 and FR2 are in close contact with the CRD region of TROP2 (see **Table 5 and Figures 20B-20D**). Amino acid residues of TY-1 (74-146) appear to be involved in the interaction but to a lesser extent, whereas it appears that there is no significant interaction with amino acid residues of the CPD (147-274).

The competition assays and FACS analysis indicate that KD001-KD005 all bind to the same or overlapping regions of TROP2. Therefore, KD001, KD002, KD003 and KD004 may likely bind some or all interacting amino acid residues of human TROP2 identified for KD005.

Since KD002 variants have similar properties as KD002, it is expected that KD002 variants also bind to the CRD region of TROP2. As well, since KD005 variants have similar properties as KD002, it is expected that KD005 variants also bind to the CRD region of TROP2.

An alignment between the variable regions of KD001-KD005 (**Figure 27**: SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26) was generated. The alignment was used to generate an exemplary consensus sequence (SEQ ID NO:145) carrying 25 amino acid substitutions out of 120 or 121 positions.

KD001-KD005 are therefore approximately 80% identical to each other over the entire length of the polypeptide.

As illustrated in **Figure 27**, the variable region of KD001-KD005 have at least 80% sequence identity to each other over the entire length of the polypeptide.

In the alignment of **Figure 27** as well as in other alignments presented herein a colon (:) indicates conservation between groups of strongly similar properties as below - roughly equivalent to scoring > 0.5 in the Gonnet PAM 250 matrix (**Table 13**). A period (.) indicates conservation between groups of weakly similar properties as below - roughly equivalent to scoring =< 0.5 and > 0 in the Gonnet PAM 250 matrix (**Table 14**). For example, it may be possible to substitute an amino acid residue identified as being part of a group in **Table 13** for another of the same group. Similarly, it may be possible to substitute an amino acid residue identified as being part of a group in **Table 14** for another of the same group.

An empty space indicates that the amino acid may be a non-conservative amino acid substitution.

Table 13 (:)
STA
NEQK
NHQK
NDEQ
QHRK
MILV
MILF
HY
FYW

Table 14 (.)
CSA
ATV
SAG
STNK
STPA
SGND
SNDEQK
NDEQHK
NEQHRK
FVLIM
HFY

Upon analysis of the sequence, it appears that KD001-KD005 all have an identical IMGT FR2 amino acid sequence, but different IMGT CDRH1, IMGT CDRH2 and IMGT CDRH3 or different combinations of IMGT CDRH1, IMGT CDRH2 and IMGT CDRH3.

For example, KD001, KD004 and KD005 have identical IMGT CDRH1 (SEQ ID Nos: 52, 59 and 62 are identical) whereas KD002 and KD003 have unique IMGT CDRH1 sequences.

KD001 and KD005 have identical IMGT CDRH2 (SEQ ID Nos: 50 and 53 are identical) whereas KD002, KD003 and KD004 have unique IMGT CDRH2 sequences.

KD002 and KD003 have identical IMGT CDRH3 (SEQ ID NO:48 and 58 are identical) and KD001 and KD004 have identical IMGT CDRH3, whereas KD005 has a unique IMGT CDRH3.

There is a high level of variability between KD001-KD005 IMGT CDRH1 indicating that this region may not be involved in binding as also illustrated by the co-crystallization experiments.

There is also substantial variability in IMGT CDRH2 indicating that this region may not be involved in binding as also illustrated by the co-crystallization experiments.

There is, however a high level of similarity between the IMGT CDRH3 of KD001-KD005. An alignment between the different IMGT CDRH3 shows that 9/14 amino acid residues are identical and 4/14 amino acid residues are conservative amino acid substitutions. Nevertheless, these alignments show that some level of variation may be tolerated in CDRH3 as well.

The amino acid sequence of KD002 and KD005 variants was also analyzed.

The IMGT CDRH1 (SEQ ID NO:55) and IMGT CDRH3 (SEQ ID NO:48) of KD002 variants is identical to that of KD002.

The CDRH2 of most variants is identical to that of KD002 (SEQ ID NO:47) except for 2 variants having a sequence illustrated in SEQ ID:123 and SEQ ID NO:124 that each includes one amino acid substitution.

Several KD002 variants have an IMGT FR2 identical to that of KD002 (SEQ ID NO:116) while others have one or two amino acid substitutions. Variations in KD002 IMGT FR2 are illustrated in SEQ ID NO: 117, SEQ ID NO: 118 and SEQ ID NO:119.

The IMGT CDRH1, CDRH2 and CDRH3 of KD005 variants are identical to that of KD005.

Several KD005 variants have an IMGT FR2 identical to that of KD005 while others have one or two have one or two amino acid substitutions. Variations in KD005 IMGT FR2 are illustrated in SEQ ID NO: 117, SEQ ID NO: 118 and SEQ ID NO:120.

An alignment between the IMGT FR2 sequences of all tested variants shows that 14/17 amino acid residues are identical. The binding and/or function of these variants was not affected by these substitutions suggesting that FR2 can sustain some level of variation.

However, the amino acid substitutions introduced in the IMGT FR2 of KD064 lead to a reduced binding and activity. The co-crystallization experiment confirms that amino acid residues 37, 45, 47, and 50 of IMGT FR2 are involved in binding with human TROP2. Based on the results obtained for KD064, amino acid substitution V/Y at position 37 and amino acid substitution P/R at position 45 may affect binding.

Overall, the results presented herein show that CDRH3 and FR2 are important for binding to TROP2 but that some level of variation may be tolerated and that CDRH1 and CDRH2 do not appear to be involved in binding to human TROP2.

An alignment between the entire variable region of KD002 (SEQ ID NO:23) and the entire variable region of exemplary embodiments of KD002 variants (**Figure 28)** was made. The alignment was used to generate an exemplary consensus sequence (SEQ ID NO:143) carrying 18 amino acid substitutions out of the 121 positions.

KD002 and KD002 variants are therefore approximately 85% identical to each other over the entire length of the polypeptide.

KD002 variants selected for their ADCC and ADCP activity are also illustrated in **Figure 29**.

An alignment between the variable region of KD005 (SEQ ID NO:26) and the variable region of exemplary embodiments of KD005 variants (**Figure 30**) was also made. The alignment was used to generate an exemplary consensus sequence (SEQ ID NO:144) carrying 16 amino acid substitutions out of the 120 positions.

KD005 and KD005 variants are therefore approximately 85% identical to each other over the entire length of the polypeptide.

KD005 variants selected for their binding activity are illustrated in **Figure 31**.

KD005 variants selected for their ADCP activity are illustrated in **Figures 24C-24I** and in **Figure 32**.

KD005 variants selected for their ADCC activity are illustrated in **Figure 33**.

KD005 variants selected for their binding activity, ADCP activity and ADCC activity are illustrated in **Figure 34**.

Since they have similar activity and sequences, an alignment between the variable regions of KD001-KD005 (SEQ ID NOs:22-26 and variants (SEQ ID NOs: 94-115) was generated. The alignment was used to generate an exemplary consensus sequence (SEQ ID NO:146) carrying 40 amino acid substitutions out of 121 positions.

All variants are therefore approximately 65% identical to each other over the entire length of the polypeptide. In addition to the embodiments described and provided in this disclosure, the following non-limiting embodiments are particularly contemplated.

Example 15- Dose range finding intravenous toxicity study in cynomolgus monkey

The potential toxicity of the TROP2 testing articles was evaluated in the naïve cynomolgus monkeys. All procedures and protocols were carried out under the institutional guidelines of Sinclair Research Center in Missouri, USA. The design is in an accelerated, increasing dosing study. The naïve cynomolgus monkey was first administered intravenously with KD002 or KD005 at the dosing level of 25 mg/kg. If the animal tolerated the first 25 mg/kg dose well, the next dose, 100 mg/kg, was given one week later. If the animal also tolerated 100 mg/kg dose, another two repeating dose of 100 mg/kg were given to the animal once *per* week.

During the study, animals were examined by the veterinary staff as warranted by clinical signs or other changes. In-cage observations were performed twice daily in the AM and PM (with at least 4 hours between observations) during the in-life study period to assess general health, mortality, or moribundity. Each animal underwent a physical examination, including assessment of behavior, general body condition, condition of major body systems, and skin/ coat. Detailed clinical observations were performed at least five times following each dose at 1, 2, 6, 12, and 24 hours post dose and daily on non-dosing days starting after each animal's first dosing event. Serum samples for hematology and clinical chemistry were collected at 2 and 7 days after each dose.

For both KD002 and KD005 testing articles, there were no instances of moribundity or unscheduled deaths during the study. All animals underwent a physical examination and were deemed satisfactory for study inclusion. There were no test article-related effects on body weight

relative to dose group. Throughout the dosing phase, all animals had body weights remained with 10% of their weights in the acclimation phase. There were no instances of inappetence either during the study. There were no test article-related findings for any hematology parameters for both KD002 and KD005 studies. All the serum chemistry parameters were in the normal range during the dosing phase to the acclimation phase values for each animal.

Based on the above observations, 100 mg/kg of KD002 and KD005 once weekly for up to at least three doses were well tolerated in cynomolgus non-human primates.

In addition to the embodiments described and provided in this disclosure, the following non-limiting embodiments are contemplated.

1. A binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2).

2. A single domain antibody that specifically binds to at least one epitope comprising amino acid residues of the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2).

3. A binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2) and comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:22.

4. A binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2) and comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:23.

5. A binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2) and comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:24.

6. A binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to the extracellular domain

(ECD) of trophoblast cell surface antigen-2 (TROP2) and comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:25.

7. A binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2) and comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:26.

8. A binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2) and comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:27.

9. A binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2) and comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:28.

10. The binding agent of any of the preceding embodiments, wherein the one or more antigen binding domains are one or more antigen binding domains of a single domain antibody.

11. The binding agent of any of the preceding embodiments, wherein the binding agent is a single domain antibody.

12. The binding agent of any of the preceding embodiments, wherein the one or more antigen binding domains comprise a CDR3 amino acid sequence as set forth in SEQ ID NO:126 or in SEQ ID NO:142.

13. The binding agent of any of the preceding embodiments, wherein the one or more antigen binding domains comprise a FR2 amino acid sequence as set forth in SEQ ID NO:121 or in SEQ ID NO: 175.

14. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:145 and wherein any of X_{3a} to X_{3y} is each independently any amino acid residue.

15. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:145 and wherein:
    a. any of X_{3a} to X_{3y} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;
    b. wherein any of X_{3a} to X_{3y} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

16. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:145 and wherein X_{3a} is M or L; X_{3b} is Q, H or E; X_{3c} is V, P or A; X_{3d} is G or R; X_{3e} is P or T; X_{3f} is S, N or G; X_{3g} is A, Y or S; X_{3h} is N or T; X_{3i} is A, S or G; X_{3j} is D, G or S; X_{3k} is G or absent; X_{3l} is S or G; X_{3m} is K, N or T; X_{3n} is Y or D; X_{3o} is I or T; X_{3p} is T or M; X_{3q} is L or Q; X_{3r} is N or S; X_{3s} is K or T; X_{3t} is E or D; X_{3u} is R or K; X_{3v} is A or S; X_{3w} is K or R; X_{3x} is T or S; and/or X_{3y} is Y or A.

17. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:145 and wherein X_{3a} is M or L; X_{3b} is Q, H or E; X_{3c} is V, P or A; X_{3d} is G or R; X_{3e} is P or T; X_{3f} is S, N or G; X_{3g} is A, Y or S; X_{3h} is N or T; X_{3i} is A, S or G; X_{3j} is D, G or S; X_{3k} is G or absent; X_{3l} is S or G; X_{3m} is K, N or T; X_{3n} is Y; X_{3o} is I or T; X_{3p} is T or M; X_{3q} is L or Q; X_{3r} is N or S; X_{3s} is K or T; X_{3t} is E or D; X_{3u} is R or K; X_{3v} is A or S; X_{3w} is R; X_{3x} is S; and/or X_{3y} is A.

18. The binding agent of any of the preceding embodiments, wherein at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:144 and wherein any of X_{2a} to X_{2p} is each independently any amino acid residue.

19. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:144 and wherein,

a. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:26 or SEQ ID NO:94-114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof

b. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof;

c. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:26 or SEQ ID NO:94-114 or;

d. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

20. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:144 and wherein:

a. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 100, 101, 102, 103, 104, 105, 106, 107 or 108 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;

b. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 100, 101, 102, 103, 104, 105, 106, 107 or 108.

21. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:144 and wherein:

a. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 100, 102, 103,

104, 105, 106, 108, 112 or 114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;

b. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 100, 102, 103, 104, 105, 106, 108, 112 or 114.

22. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:144 and wherein:

a. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 105, 112, 113 or 114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;

b. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 105, 112, 113 or 114.

23. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:144 and wherein:

a. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 102 or 105 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;

b. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 102 or 105.

24. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:144 and wherein X_{2a} is Q or E; X_{2b} is Q, L or V; X_{2c} is V or I; X_{2d} is E or Q; X_{2e} is A or P; X_{2f} is P, or L; X_{2g} is Y, A or V; X_{2h} is P or A; X_{2i} is S or T;

X_{2j} is A or S; X_{2k} is K or R; X_{2l} is P or A; X_{2m} is D or E; X_{2n} is L or V; X_{2o} is R or Y and/or X_{2p} is Q or L.

25. The binding agent of any of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:144 and wherein X_{2a} is Q or E; X_{2b} is Q or L; X_{2c} is V or I; X_{2d} is E or Q; X_{2e} is A or P; X_{2f} is P, or L; X_{2g} is Y or A; X_{2h} is P or A; X_{2i} is S or T; X_{2j} is A or S; X_{2k} is K or R; X_{2l} is P or A; X_{2m} is D or E; X_{2n} is L or V; X_{2o} is R or Y and/or X_{2p} is Q or L.

26. The binding agent of any one of the preceding embodiments, wherein the amino acid residue at position 1 is Q, the amino acid residue at position 2 is V, the amino acid residue at position 3 is Q, the amino acid residue at position 4 is L, the amino acid residue at position 37 is V, the amino acid residue at position 39 is Q, the amino acid residue at position 45 is P, the amino acid residue at position 46 is E, the amino acid residue at position 47 is W, the amino acid residue at position 50 is Y, the amino acid residue at position 58 is Y, the amino acid residue at position 99 is R, the amino acid residue at position 100 is L, the amino acid residue at position 101 is T, the amino acid residue at position 102 is D, the amino acid residue at position 103 is S, the amino acid residue at position 104 is H, the amino acid residue at position 105 is Y, the amino acid residue at position 106 is V, the amino acid residue at position 107 is E, the amino acid residue at position 108 is D, the amino acid residue at position 109 is A, the amino acid residue at position 110 is W, the amino acid residue at position 111 is G, and/or the amino acid residue at position 112 is Q and the position is with reference to SEQ ID NO:145 (**Table 1**)

27. The binding agent of any one of the preceding embodiments, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the CDR3 set forth in any one of SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:54, SEQ ID NO:58 or SEQ ID NO:61, the amino acid sequence of the FR2 set forth in any one of SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 or SEQ ID NO:120 and optionally the amino acid sequence of the CDR1 set forth in any one of SEQ ID NO: 52, SEQ ID NO: 55, SEQ ID NO:59, SEQ ID NO: 52 or SEQ ID NO: 62 and/or the amino

acid sequence of the CDR2 set forth in any one of SEQ ID NO: 47, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO:123 or SEQ ID NO:124 or;

28. The binding agent of any one of the preceding embodiments, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the CDR3 set forth in any one of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141, the amino acid sequence of the FR2 set forth in any one of SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 or SEQ ID NO:174 and optionally the amino acid sequence of the CDR1 set forth in any one of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:127 and/or the amino acid sequence of the CDR2 set forth in any one of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138 or SEQ ID NO:139.

29. The binding agent of any one of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains has an affinity of $\leq 10^{-6}$ M for TROP2.

30. The binding agent of any one of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains has an affinity of $\leq 10^{-10}$ M for human TROP2.

31. The binding agent of any one of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains has an affinity of at between 1×10^{-10} M and 1×10^{-12} for human TROP2.

32. The binding agent of any one of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains has a dissociation rate of between 1×10^{-6} to 1×10^{-4}.

33. The binding agent of any of the preceding embodiments, wherein the binding agent comprises partially humanized framework regions.

34. The binding agent of any of the preceding embodiments, wherein the binding agent comprises fully humanized framework regions.

35. The binding agent of any one of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains has ADCP activity towards cells expressing human TROP2.

36. The binding agent of any one of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains has ADCP activity towards cells expressing human TROP2 and CD47.

37. The binding agent of any one of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains has ADCC activity towards cells expressing human TROP2.

38. The binding agent of any one of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains is internalized in cells expressing human TROP2.

39. The binding agent of any one of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains is capable of inhibiting the growth of tumor cells expressing human TROP2.

40. The binding agent of any one of the preceding embodiments, wherein the binding agent or at least one of the one or more antigen binding domains is capable of inhibiting the growth of tumor cells expressing human TROP2 and CD47.

41. The binding agent of any one of the preceding embodiments, wherein the binding agent comprises one or more antigen binding domains that specifically binds to CD47.

42. The binding agent of any one of the preceding embodiments, wherein the binding agent comprises a Fc portion that is capable of binding to an Fc receptor of monocytes and/or macrophages.

43. The binding agent of any one of the preceding embodiments, wherein the binding agent comprises a dimerization domain.

44. The binding agent of any of the preceding embodiments, wherein the binding agent is naked.

45. The binding agent of any of the preceding embodiments, wherein the binding agent is conjugated to a therapeutic moiety.

46. The binding agent of any of the preceding embodiments, wherein the binding agent is conjugated to a detectable moiety.

47. The binding agent of any one of the preceding embodiments, wherein the binding agent or one or more of the antigen binding domains specifically binds to human TROP2.

48. The binding agent of any one of the preceding embodiments, wherein the binding agent comprises two antigen binding domains or more.

49. The binding agent of any one of the preceding embodiments, wherein the binding agent is monospecific.

50. The binding agent of any one of the preceding embodiments, wherein the binding agent is bispecific.

51. The binding agent of any one of the preceding embodiments, wherein the binding agent is multispecific.

52. The binding agent of any one of the preceding embodiments, wherein the binding agent is capable of competing with a single domain antibody comprising the amino acid sequence set forth in SEQ ID NO:26.

53. The binding agent of any one of the preceding embodiments, wherein the binding agent specifically binds to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-146 or a fragment thereof.

54. The binding agent of any one of the preceding embodiments, wherein the binding agent specifically binds to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-73 or a fragment thereof.

55. The binding agent of any one of the preceding embodiments, wherein the binding agent does not significantly bind to or is not capable of binding to a) a polypeptide that comprises a human TROP2 amino acid sequence consisting of amino acid residues 147-274 or a fragment thereof, b) a human TROP2 amino acid sequence comprising deletion of amino acid residues 27-36, 27-44, 27-56, 27-66 or 27-73 or has a reduced binding to said

polypeptide compared to the full human TROP2 polypeptide and/or a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 149.

56. A single domain antibody that competes with the binding agent of any one of the preceding embodiments.

57. A composition comprising the binding agent or single domain antibody of any of the preceding embodiments.

58. A pharmaceutical composition comprising the binding agent or single domain antibody of any of the preceding embodiments and a pharmaceutically acceptable carrier.

59. A nucleic acid or vector encoding the binding agent or single domain antibody of any of the preceding embodiments.

60. A cell expressing the binding agent or single domain antibody of any of the preceding embodiments.

61. A cell comprising the nucleic acid or the vector of any of the preceding embodiments.

62. A kit comprising the binding agent or single domain antibody of any of the preceding embodiments.

63. A kit comprising the nucleic acid or the vector of any of the preceding embodiments.

64. A method of treating a disorder or disease comprising administering the binding agent or single domain antibody of any one of the preceding embodiments or a pharmaceutical composition comprising the binding agent or single domain antibody of any one of the preceding embodiments.

65. The method of the preceding embodiments, wherein the disorder or disease is cancer, solid tumor, epithetial cancer, lung cancer, metastatic lung cancer, small cell lung cancer, non-small cell lung cancer, myeloma, prostate cancer, breast cancer, triple negative breast cancer, rectal cancer, pancreatic cancer, glioblastoma, cervical cancer, colorectal cancer, gastric cancer, ovarian cancer, thyroid cancer, stomach cancer, urinary bladder cancer, cancer of the uterus, esophegeal cancer, blood cancer, or head and neck cancer.

66. The method of any of the preceding embodiments, wherein the binding agent or single domain antibody is administered at a dose of approximately between 0.05 to 150 mg/kg.

67. The method of any of the preceding embodiments, wherein the binding agent or single domain antibody is administered once weekly.

68. The method of any of the preceding embodiments, wherein the binding agent or single domain antibody is administered once every two weeks, once every three weeks, once every month or less frequently.

69. The method of any of the preceding embodiments, wherein the binding agent or single domain antibody is administered by intra-veinous injection, by intra-muscular injection or by infusion.

70. The method of any of the preceding embodiments, wherein the binding agent or single domain antibody is administered to a subject having a tumor or tumor cells expressing TROP2 and optionally expressing a macrophages marker.

The embodiments and examples described herein are illustrative and are not meant to limit the scope of the claims. Variations of the foregoing embodiments, including alternatives, modifications and equivalents, are intended by the inventors to be encompassed by the claims.

Citations listed in the present application are incorporated herein by reference.

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SEQUENCE TABLES

Table 15: anti-TROP2 sequences
(KD001-KD005 and exemplary CDR sequences are provided)

Anti-TROP2	CDR1	CDR2	CDR3
KD008 and KD001 Kabat CDRs	GSDMS (SEQ ID NO:1)	YITSGGTTYYPDSVKG (SEQ ID NO:2)	ARLTDSHYVEDA (SEQ ID NO:3)
KD008 and KD001 IMGT CDRs	GFTFSGSD (SEQ ID NO: 52)	ITSGGTT (SEQ ID NO: 53)	AKARLTDSHYVEDA (SEQ ID NO: 54)
KD008 (VHH) (KD001 variable region) (SEQ ID NO:22)	QVQLQESGGGMVHPGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSGGTTYYPDSVKGRFTISRDNAKNTLYLQMNSLTPDDTALYRCAKARLTDSHYVEDAWGQGTQVTVSS		
KD001 heavy chain (SEQ ID NO:29)	QVQLQESGGGMVHPGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSGGTTYYPDSVKGRFTISRDNAKNTLYLQMNSLTPDDTALYRCAKARLTDSHYVEDAWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK		
KD009 and KD002 Kabat CDRs	SADMS (SEQ ID NO:4)	YINADGSKTYYPDSVKG (SEQ ID NO:5)	AKLTDTHYVEDY (SEQ ID NO:6)
KD009 and KD002 IMGT CDRs	GFPFSSADMS (SEQ ID NO:46) or GFPFSSAD (SEQ ID NO:55)	INADGSKT (SEQ ID NO:47)	ARAKLTDTHYVEDY (SEQ ID NO:48)

Anti-TROP2	CDR1	CDR2	CDR3
KD009 (VHH) (KD002 variable region) (SEQ ID NO:23)	QVQLQESGGGMVQVGGSLRLSCAASGFPFSSADMSWVRQAPGK GPEWVSYINADGSKTYYPDSVKGRFTISRDNAKNTLYLLMNNLKP EDTALYRCARAKLTDTHYVEDYWGQGTQVTVSS		
KD002 heavy chain (SEQ ID NO:30)	QVQLQESGGGMVQVGGSLRLSCAASGFPFSSADMSWVRQAPGK GPEWVSYINADGSKTYYPDSVKGRFTISRDNAKNTLYLLMNNLKP EDTALYRCARAKLTDTHYVEDYWGQGTQVTVSSEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK		
KD010 and KD003 Kabat CDRs	NYDMS (SEQ ID NO:7)	YINAGGSNTDYPDS VKG (SEQ ID NO:8)	AKLTDTHYVEDY (SEQ ID NO:9)
KD010 and KD003 IMGT CDRs	GFTFSNYD (SEQ ID NO: 56)	INAGGSNT (SEQ ID NO: 57)	ARAKLTDTHYVED Y (SEQ ID NO: 58)
KD010 (VHH) (KD003 variable region) (SEQ ID NO:24)	QVQLQESGGGMVQPGGSLRLSCAASGFTFSNYDMSWVRQAPGK GPEWVSYINAGGSNTDYPDSVKGRFTISRDNAKNMLYLLMNNLK PEDTALYRCARAKLTDTHYVEDYWGQGTQVTVSS		

Anti-TROP2	CDR1	CDR2	CDR3
KD003 heavy chain (SEQ ID NO:31)	QVQLQESGGGMVQPGGSLRLSCAASGFTFSNYDMSWVRQAPGK GPEWVSYINAGGSNTDYPDSVKGRFTISRDNAKNMLYLLMNNLK PEDTALYRCARAKLTDTHYVEDYWGQGTQVTVSSEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK		
KD011 and KD004 Kabat CDRs	GSDMS (SEQ ID NO:10)	YITGSGTTYYPDSV KG (SEQ ID NO:11)	ARLTDSHYVEDA (SEQ ID NO:12)
KD011 and KD004 IMGT CDRs	GFTFSGSD (SEQ ID NO: 59)	ITGSGTT (SEQ ID NO:60)	AKARLTDSHYVED A (SEQ ID NO:61)
KD011 (VHH) (KD004 variable region) (SEQ ID NO:25)	QVQLQESGGGLVQPGRSLRLSCAASGFTFSGSDMSWVRQAPGKG PEWVSYITGSGTTYYPDSVKGRFTTSRDNAKNMLYLQMNSLKPD DTALYRCAKARLTDSHYVEDAWGQGTQVTVSS		
KD004 heavy chain (SEQ ID NO:32)	QVQLQESGGGLVQPGRSLRLSCAASGFTFSGSDMSWVRQAPGKG PEWVSYITGSGTTYYPDSVKGRFTTSRDNAKNMLYLQMNSLKPD DTALYRCAKARLTDSHYVEDAWGQGTQVTVSSEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK		

Anti-TROP2	CDR1	CDR2	CDR3
KD012 and KD005 Kabat CDRs	GSDMS (SEQ ID NO:13)	YITSGGTTYYPDSVKG (SEQ ID NO:14)	SRLTDSHYVEDA (SEQ ID NO:15)
KD012 and KD005 IMGT CDRs	GFTFSGSDMS (SEQ ID NO:49) or GFTFSGSD (SEQ ID NO:62)	ITSGGTT (SEQ ID NO:50)	AKSRLTDSHYVEDA (SEQ ID NO:51)
KD012 (VHH) (KD005 variable region) (SEQ ID NO:26)	QVQLQESGGGLVEAGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSGGTTYYPDSVKGRFTISRDNAKNTLYLQMNSLKPDDTALYRCAKSRLTDSHYVEDAWGQGTQVTVSS		
KD005 heavy chain (SEQ ID NO:33)	QVQLQESGGGLVEAGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSGGTTYYPDSVKGRFTISRDNAKNTLYLQMNSLKPDDTALYRCAKSRLTDSHYVEDAWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	
KD015 heavy chain (SEQ ID NO:44) IgG4 version of KD002	QVQLQESGGGMVQVGGSLRLSCAASGFPFSSADMSWVRQAPGKGPEWVSYINADGSKTYYPDSVKGRFTISRDNAKNTLYLLMNNLKPEDTALYRCARAKLTDTHYVEDYWGQGTQVTVSSESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK		

Anti-TROP2	CDR1	CDR2	CDR3
KD016 heavy chain (SEQ ID NO:45) IgG4 version of KD004	QVQLQESGGGLVQPGRSLRLSCAASGFTFSGSDMSWVRQAPGKG PEWVSYITGSGTTYYPDSVKGRFTTSRDNAKNMLYLQMNSLKPD DTALYRCAKARLTDSHYVEDAWGQGTQVTVSSESKYGPPCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK		
KD017 heavy chain (SEQ ID NO: 155)	QVQLQESGGGMVQVGGSLRLSCAASGFPFSSADMSWVRQAPGK GPEWVSYINADGSKTYYPDSVKGRFTISRDNAKNTLYLLMNNLKP EDTALYRCARAKLTDTHYVEDYWGQGTQVTVSSESKYGPPCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK		
KD018 heavy chain (SEQ ID NO: 156)	QVQLQESGGGLVQPGRSLRLSCAASGFTFSGSDMSWVRQAPGKG PEWVSYITGSGTTYYPDSVKGRFTTSRDNAKNMLYLQMNSLKPD DTALYRCAKARLTDSHYVEDAWGQGTQVTVSSESKYGPPCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK		

Anti-TROP2	CDR1	CDR2	CDR3
KD019 heavy chain (SEQ ID NO:162	QVQLQESGGGLVEAGGSLRLSCAASGFTFSGSDMSWVRQAPGKG PEWVSYITSGGTTYYPDSVKGRFTISRDNAKNTLYLQMNSLKPDD TALYRCAKSRLTDSHYVEDAWGQGTQVTVSSESKYGPPCPPCPAP EAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK		

Table 16: anti-TROP2 sequences

(KD006, KD007: Exemplary CDR sequences are provided)

KD013 and KD006 Kabat CDRs	TKAMG (SEQ ID NO:16)	AISESGSTYYADSVKD (SEQ ID NO:17)	KWYSGSFDDTRSYDY (SEQ ID NO:18)
KD013 and KD006 IMGT CDRs	VRTFSTKA (SEQ ID NO:63)	ISESGST (SEQ ID NO:64)	GVKWYSGSFDDTRSYDY (SEQ ID NO:65)
KD013 (VHH) (KD006 variable region) (SEQ ID NO:27)	QVQLQESGGGLVQAGNSLRLSCVASVRTFSTKAMGWFRQAPGKEREFTAAISESGSTYYADSVKDRFTISRDNVKNTVYLQMNSLKPEDTAVYYCGVKWYSGSFDDTRSYDYWGQGTQVTVSS		
KD006 heavy chain (SEQ ID NO:34)	QVQLQESGGGLVQAGNSLRLSCVASVRTFSTKAMGWFRQAPGKEREFTAAISESGSTYYADSVKDRFTISRDNVKNTVYLQMNSLKPEDTAVYYCGVKWYSGSFDDTRSYDYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK		
KD014 and KD007 Kabat CDRs	FYAMA (SEQ ID NO:19)	TITAYGNTNYADSVKG (SEQ ID NO:20)	NLPSASHNY (SEQ ID NO:21)
KD014 and KD007 IMGT CDRs	GFAFSFYA (SEQ ID NO:66)	ITAYGNT (SEQ ID NO:67)	NANLPSASHNY (SEQ ID NO:68)
KD014 (VHH) (KD007 variable region) (SEQ ID NO:28)	QVQLQESGGGLVQPGGSLRLSCAASGFAFSFYAMAWYRQAPGKQRDMVATITAYGNTNYADSVKGRFTTSRDNAKNTVYLQMNSLKPEDTAVYYCNANLPSASHNYWGQGTQVTVSS		

KD007 heavy chain (SEQ ID NO:35)	QVQLQESGGGLVQPGGSLRLSCAASGFAFSFYAMAWYRQAPGKQ RDMVATITAYGNTNYADSVKGRFTTSRDNAKNTVYLQMNSLKPE DTAVYVCNANLPSASHNYWGQGTQVTVSSEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Table 17: anti-CD47 sequences

KD070 **Kabat CDRs**	VNDIR (SEQ ID NO:156)	RITGGGRTDYADSVKG (SEQ ID NO:157)	WGRGY (SEQ ID NO:158)
KD070 **IMGT CDRs**	RFDFSVND (SEQ ID NO:159)	ITGGGRT (SEQ ID NO:160)	WGRGY (SEQ ID NO:161)
KD070 (VHH) Variable region (SEQ ID NO:155)	QVQLQESGGGLVQPGGSLRLSCAASRFDFSVNDIRWYRQAPGNE RELVARITGGGRTDYADSVKGRFTISRDNAKNTVYLQMNNLKPE DTAVYYCWGRGYWGQGTQVTVSS		

Table 18: KD002 and variants amino acid sequence

Code No.	**SEQUENCE** **IMGT CDRs: bold and underlined** **FR2: twice underlined**
KD002 heavy chain (SEQ ID NO:30)	QVQLQESGGGMVQVGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGK GPEWVSY**INADGSKT**YYPDSVKGRFTISRDNAKNTLYLLMNNLK PEDTALYRC**ARAKLTDTHYVEDY**WGQGTQVTVSSEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
KD020 variable region (SEQ ID NO:71)	EVQLVESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MHWVRQAPGKG LVWVSR**INADGSKT**SYADTVKGRFTISRDNAKNTLYLQMNSLRA EDTAVYYC**ARAKLTDTHYVEDY**WGQGTLVTVSS
KD021 variable region (SEQ ID NO :72)	EVQLVESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSY**INADGSKT**SYADTVKGRFTISRDNAKNTLYLQMNSLRA EDTAVYYC**ARAKLTDTHYVEDY**WGQGTLVTVSS
KD022 variable region (SEQ ID NO :73)	EVQLVESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG PEWVSY**INADGSKT**YYADTVKGRFTISRDNAKNTLYLQMNSLRA EDTAVYYC**ARAKLTDTHYVEDY**WGQGTQVTVSS
KD023 variable region (SEQ ID NO :74)	QVQLVESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG PEWVSY**INADGSKT**YYPDTVKGRFTISRDNAKNTLYLQMNSLRA EDTAVYYC**ARAKLTDTHYVEDY**WGQGTQVTVSS
KD024 variable region (SEQ ID NO :75)	QVQLVESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG PEWVSY**INADGSKT**YYPDTVKGRFTISRDNAKNTLYLQMNSLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTQVTVSS

Code No.	SEQUENCE IMGT CDRs: bold and underlined FR2: twice underlined
KD025 variable region (SEQ ID NO :76)	QVQLVESGGGLVQVGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGK GPEWVSY**INADGSKT**YYPDTVKGRFTISRDNAKNTLYLQMNSLK PEDTAVYRC**ARAKLTDTHYVEDY**WGQGTQVTVSS
KD026 variable region (SEQ ID NO :77)	QVQLQESGGGMVQVGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGK GPEWVSY**INADGSKT**YYPDTVKGRFTISRDNAKNTLYLQMNSLK PEDTAVYRC**ARAKLTDTHYVEDY**WGQGTQVTVSS
KD027 variable region (SEQ ID NO :78)	QVQLQESGGGMVQVGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGK GPEWVSY**INADGSKT**YYPDSVKGRFTISRDNAKNTLYLQMNSLK PEDTAVYRC**ARAKLTDTHYVEDY**WGQGTQVTVSS
KD028 variable region (SEQ ID NO :79)	EVQLLESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSV**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD029 variable region (SEQ ID NO :80)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG PEWVSY**INADGSKT**YYPDTVKGRFTISRDNSKNTLYLQMNNLRA EDTALYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD030 variable region (SEQ ID NO :81)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSY**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD031 variable region (SEQ ID NO :82)	EVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSY**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS

Code No.	SEQUENCE IMGT CDRs: bold and underlined FR2: twice underlined
KD032 variable region (SEQ ID NO :83)	QVQLLESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSY**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD033 variable region (SEQ ID NO :84)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSV**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD034 variable region (SEQ ID NO :85)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSY**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD035 variable region (SEQ ID NO :86)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG PEWVSY**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD036 variable region (SEQ ID NO :87)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSY**INADGSKT**YYPDTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD037 variable region (SEQ ID NO :88)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSY**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNNLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD038 variable region (SEQ ID NO :89)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSY**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTALYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS

Code No.	SEQUENCE IMGT CDRs: bold and underlined FR2: twice underlined
KD039 variable region (SEQ ID NO :90)	QVQLQESGGGMVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGK GLEWVSY**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYRC**ARAKLTDTHYVEDY**WGQGTQVTVSS
KD040 variable region (SEQ ID NO :91)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKQ REWVSY**INADGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD041 variable region (SEQ ID NO :92)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSY**INAEGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS
KD042 variable region (SEQ ID NO :93)	QVQLQESGGGLVQPGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKG LEWVSY**INANGSKT**YYADTVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYRC**ARAKLTDTHYVEDY**WGQGTMVTVSS

Table 19: KD005 and variants amino acid sequence

Code	**SEQUENCE** **IMGT CDRs: bold and underlined** **FR2: twice underlined**
KD005 heavy chain (SEQ ID NO:33)	QVQLQESGGGLVEAGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGK GPEWVSY**ITSGGTT**YYPDSVKGRFTISRDNAKNTLYLQMNSLKP DDTALYRC**AKSRLTDSHYVEDA**WGQGTQVTVSSEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
KD043 variable region (SEQ ID NO:94)	EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSA**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD044 variable region (SEQ ID NO:95)	EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD045 variable region (SEQ ID NO:96)	EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD046 variable region (SEQ ID NO:97)	QVQLLESGGGLVQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG PEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD047 variable region (SEQ ID NO:98)	QVQLLESGGGLVQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG PEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS

Code	SEQUENCE IMGT CDRs: bold and underlined FR2: twice underlined
KD048 variable region(SEQ ID NO:99)	QVQLQESGGGLVQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG PEWVSY**ITSGGTT**YYPDTVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYC**AKSRLTDSHYVEDA**WGQGTQVTVSS
KD049 variable region (SEQ ID NO:100)	QVQLQESGGGLVQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG PEWVSY**ITSGGTT**YYPDTVKGRFTISRDNSKNTLYLQMNSLRAED TAVYRC**AKSRLTDSHYVEDA**WGQGTQVTVSS
KD050 variable region (SEQ ID NO:101)	QVQLQESGGGLVQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG PEWVSY**ITSGGTT**YYPDTVKGRFTISRDNSKNTLYLQMNSLKPDD TAVYRC**AKSRLTDSHYVEDA**WGQGTQVTVSS
KD051 variable region (SEQ ID NO:102)	QVQLQESGGGLVQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG PEWVSY**ITSGGTT**YYPDTVKGRFTISRDNAKNTLYLQMNSLKPD DTALYRC**AKSRLTDSHYVEDA**WGQGTQVTVSS
KD052 variable region (SEQ ID NO:103)	EVQLVESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSV**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD053 variable region (SEQ ID NO:104)	QVQLQESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG PEWVSY**ITSGGTT**YYPDTVKGRFTISRDNSKNTLYLQMNSLRAD DTALYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD054 variable region (SEQ ID NO:105)	QVQLQESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD055 variable region (SEQ ID NO:106)	EVQLQESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS

Code	SEQUENCE IMGT CDRs: bold and underlined FR2: twice underlined
KD056 variable region (SEQ ID NO:107)	QVQLVESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD057 variable region (SEQ ID NO:108)	QVQLQESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSV**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD058 variable region (SEQ ID NO:109)	QVQLQESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD059 variable region (SEQ ID NO:110)	QVQLQESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG PEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD060 variable region (SEQ ID NO:111)	QVQLQESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSY**ITSGGTT**YYPDTVKGRFTISRDNSKNTLYLQMNSLRAED TAVYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD061 variable region (SEQ ID NO:112)	QVQLQESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAD DTAVYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS
KD062 variable region (SEQ ID NO:113)	QVQLQESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTALYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS

Code	SEQUENCE IMGT CDRs: bold and underlined FR2: twice underlined
KD063 variable region (SEQ ID NO:114)	QVQLQESGGGLIEPGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKG LEWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYRC**AKSRLTDSHYVEDA**WGQGTQVTVSS
KD064 variable region (SEQ ID NO:115)	QVQLQESGGGLIQPGGSLRLSCAAS**GFTFSGSD**MSWYRQAPGKG REWVSY**ITSGGTT**YYADTVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYRC**AKSRLTDSHYVEDA**WGQGTLVTVSS

Table 20A-D: Alignment of IMGT CDRs or FR2 sequences of KD001-KD005 and variants

Table 20A

IMGT CDRH1	KD001-KD005 original sequences GFTFSGSD (SEQ ID NOs: 52, 59 and 62) GFPFSSAD (SEQ ID NO:55) GFTFSNYD (SEQ ID NO: 56)
IMGT CDRH1 Alignment, Consensus, Examples	Alignment 52 GFTFSGSD 8 56 GFTFSNYD 8 55 GFPFSSAD 8 ** **. * Consensus GFX$_{7a}$FSX$_{7b}$X$_{7c}$D (SEQ ID NO:122) Examples a) Wherein any one of X$_{7a}$ to X7$_c$ is any amino acid. b) Wherein X$_{7a}$ is any amino acid; Wherein X$_{7b}$ is G, N, S or D and/or Wherein X$_{7c}$ is any amino acid. c) Wherein X$_{7a}$ is T or P; Wherein X$_{7b}$ is G, N or S and/or Wherein X$_{7c}$ is S, Y or A.

Table 20B

IMGT CDRH2	KD001-KD005 original sequences INADGSKT (SEQ ID NO:47) ITSGGTT (SEQ ID NOs:50 and 53) INAGGSNT (SEQ ID NO: 57) ITGSGTT (SEQ ID NO:60) KD002 Variants INANGSKT (SEQ ID NO: 123) INAEGSKT (SEQ ID NO: 124)
IMGT CDRH2 Alignment, Consensus, Examples	Alignment 47 INADGSKT 8 124 INANGSKT 8 123 INAEGSKT 8 57 INAGGSNT 8 50 ITSGGTT- 7 60 ITGSGTT- 7 *.. *:. Consensus $IX_{8a}X_{8b}X_{8c}GX_{8d}X_{8e}X_{8f}$ (SEQ ID NO:125) Examples a) Wherein any one of X_{8a} to X_{8f} is any amino acid. b) Wherein X_{8a} is S, T, N or K; Wherein X_{8b} is A, S or G; Wherein X_{8c} is any amino acid; Wherein X_{8d} is S, T or A; Wherein X_{8e} is S, T, N or K and/or Wherein X_{8f} is any amino acid or absent. c) Wherein X_{8a} is N or T; Wherein X_{8b} is A, S or G; Wherein X_{8c} is D, N, E, G or S; Wherein X_{8d} is S or T; Wherein X_{8e} is K, N or T and/or Wherein X_{8f} is T or absent.

Table 20C

IMGT CDRH3	KD001-KD005 original sequences ARAKLTDTHYVEDY (SEQ ID NOs:48 and 58) AKSRLTDSHYVEDA (SEQ ID NO:51) AKARLTDSHYVEDA (SEQ ID NO: 54) AKARLTDSHYVEDA (SEQ ID NO:61)
IMGT CDRH3 Alignment, Consensus, Examples	Alignment 48 ARAKLTDTHYVEDY 14 51 AKSRLTDSHYVEDA 14 54 AKARLTDSHYVEDA 14 61 AKARLTDSHYVEDA 14 *:::***:***** Consensus $AX_{9a}X_{9b}X_{9c}LTDX_{9d}HYVEDX_{9e}$ (SEQ ID NO:126) Examples a) Wherein any one of X_{9a} to X_{9e} is any amino acid. b) Wherein X_{9a} is Q, H, R or K; Wherein X_{9b} is S, T or A; Wherein X_{9c} is Q, H, R or K; Wherein X_{9d} is S, T or A and/or Wherein X_{9e} is any amino acid or absent. c) Wherein X_{9a} is R or K; Wherein X_{9b} is A or S; Wherein X_{9c} is K or R; Wherein X_{9d} is T or S and/or Wherein X_{9e} is Y, A or absent.

Table 20D

IMGT FR2	KD001-KD005 original sequences (e.g., aa 34-50 of SEQ ID NO:33) MSWVRQAPGKGPEWVSY (SEQ ID:116) KD002 variants MSWVRQAPGKGLEWVSY (SEQ ID: 117) MSWVRQAPGKGLEWVSV (SEQ ID: 118) MSWVRQAPGKQREWVSY (SEQ ID: 119) KD005 variants MSWVRQAPGKGLEWVSA (SEQ ID: 120) MSWVRQAPGKGLEWVSV (SEQ ID: 118) MSWVRQAPGKGLEWVSY (SEQ ID: 117)
IMGT FR2 Alignment, Consensus, Examples	Alignment 119 MSWVRQAPGKQREWVSY 17 120 MSWVRQAPGKGLEWVSA 17 118 MSWVRQAPGKGLEWVSV 17 116 MSWVRQAPGKGPEWVSY 17 117 MSWVRQAPGKGLEWVSY 17 ********** **** Consensus MSWVRQAPGK$X_{6a}X_{6b}$EWVSX_{6c} (consensus SEQ ID NO:121) Examples a) Wherein any one of X_{6a} to X_{6c} is any amino acid. b) Wherein X_{6a} is Q or G; Wherein X_{6b} is R, L or P and/or Wherein X_{6c} is Y, A or V.

Table 21A-C: Alignment of Kabat CDRs or FR2 sequences of KD001-KD005 and variants

Table 21A

Kabat CDRH1	KD001-KD005 original sequences GSDMS (SEQ ID NOs:1, 10 and 13) SADMS (SEQ ID NO:4) NYDMS (SEQ ID NO:7) KD002 variants SADMH (SEQ ID NO:127)
Kabat CDRH1 Alignment, Consensus, Examples	Alignment 7 NYDMS 5 1 GSDMS 5 4 SADMS 5 127 SADMH 5 . ** Consensus $X_{10a}X_{10b}DMX_{10c}$ (SEQ ID NO:128) Examples a) Wherein any one of X_{10a} to X_{10c} is any amino acid. b) Wherein X_{10a} is S, G, N or D; Wherein X_{10b} is any amino acid and/or Wherein X_{10c} is any amino acid. c) Wherein X_{10a} is N, G or S; Wherein X_{10b} is Y, S or A and/or Wherein X_{10c} is S or H.

Table 21B

Kabat CDRH2	KD001-KD005 original sequences YITSGGTTYYPDSVKG (SEQ ID NOs:2 and 14) YINADGSKTYYPDSVKG (SEQ ID NO:5) YINAGGSNTDYPDSVKG (SEQ ID NO:8) YITGSGTTYYPDSVKG (SEQ ID NO:11) KD002 variants RINADGSKTSYADTVKG (SEQ ID NO:129) YINADGSKTSYADTVKG (SEQ ID NO:130) YINADGSKTYYPDTVKG (SEQ ID NO:131) VINADGSKTYYADTVKG (SEQ ID NO:132) YINADGSKTYYADTVKG (SEQ ID NO:133) YINAEGSKTYYADTVKG (SEQ ID NO:134) YINANGSKTYYADTVKG (SEQ ID NO:135) KD005 variants AITSGGTTYYADTVKG (SEQ ID NO:136) YITSGGTTYYADTVKG (SEQ ID NO:137) YITSGGTTYYPDTVKG (SEQ ID NO:138) VITSGGTTYYADTVKG (SEQ ID NO:139)
Kabat CDRH2 Alignment, Consensus, Examples	Alignment 11 YI-TGSGTTYYPDSVKG 16 2 YI-TSGGTTYYPDSVKG 16 138 YI-TSGGTTYYPDTVKG 16 136 AI-TSGGTTYYADTVKG 16 135 YI-TSGGTTYYADTVKG 16 139 VI-TSGGTTYYADTVKG 16 8 YINAGGSNTDYPDSVKG 17 129 RINADGSKTSYADTVKG 17 130 YINADGSKTSYADTVKG 17 5 YINADGSKTYYPDSVKG 17 131 YINADGSKTYYPDTVKG 17 135 YINANGSKTYYADTVKG 17 134 YINAEGSKTYYADTVKG 17 132 VINADGSKTYYADTVKG 17 133 YINADGSKTYYADTVKG 17 * : ...* * *:***

	Consensus $X_{11a}IX_{11b}X_{11c}X_{11d}X_{11e}X_{11f}X_{11g}TX_{11h}YX_{11i}DX_{11j}VKG$ (SEQ ID NO: 140) Examples a) Wherein any one of X_{11a} to X_{11j} is any amino acid. b) Wherein X_{11a} is any amino acid; Wherein X_{11b} is any amino acid or absent; Wherein X_{11c} is S, T or A; Wherein X_{11d} is any amino acid; Wherein X_{11e} is S, A or G; Wherein X_{11f} S, A or G; Wherein X_{11g} is S, T, N or K; Wherein X_{11h} is any amino acid; Wherein X_{11i} is any amino acid; Wherein X_{11j} is S, T, or A. c) Wherein X_{11a} is Y, A, V or R; Wherein X_{11b} is absent or N; Wherein X_{11c} is T or A; Wherein X_{11d} is G, S, D, N or E; Wherein X_{11e} is S or G; Wherein X_{11f} is G or S; Wherein X_{11g} is T, N or K; Wherein X_{11h} is Y, D or S; Wherein X_{11i} is P or A and/or Wherein X_{11j} is S or T.

Table 21C

Kabat CDRH3	KD001-KD005 original sequences ARLTDSHYVED (SEQ ID NO:3) AKLTDTHYVEDY (SEQ ID NOs:6 and 9) ARLTDSHYVEDA (SEQ ID NO:12) SRLTDSHYVED (SEQ ID NO:15) KD005 variants SRLTDSHYVEDA (SEQ ID NO: 141)
Kabat CDRH3 Alignment, Consensus, Examples	Alignment 6 AKLTDTHYVEDY 12 3 ARLTDSHYVED- 11 12 ARLTDSHYVEDA 12 15 SRLTDSHYVED- 11 140 SRLTDSHYVEDA 12 ::***:***** Consensus $X_{12a}X_{12b}$LTDX_{12c}HYVEDX_{12d} (SEQ ID NO:142) Examples a) Wherein any one of X_{12a} to X_{12d} is any amino acid. b) Wherein X_{12a} is S, T or A; Wherein X_{12b} is Q, H, R or K; Wherein X_{12c} is S, T or A and/or Wherein X_{12d} is any amino acid or absent. c) Wherein X_{12a} is A or S; Wherein X_{12b} is K or R; Wherein X_{12c} is T or S and/or Wherein X_{12d} is Y, A or absent.

Table 21D

Kabat FR2	WVRQAPGKGPEWVS (SEQ ID NO:170) WVRQAPGKGLEWVS (SEQ ID NO:171) WVRQAPGKQREWVS (SEQ ID NO:172) WYRQAPGKGREWVS (SEQ ID NO:173) WVRQAPGKGLVWVS (SEQ ID NO:174)
Kabat FR2 Alignment, Consensus, Examples	Alignment 173 WYRQAPGKGREWVS 172 WVRQAPGKQREWVS 174 WVRQAPGKGLVWVS 170 WVRQAPGKGPEWVS 171 WVRQAPGKGLEWVS * ****** *** Consensus WX13aRQAPGKX13bX13cX13dWVS (SEQ ID NO:175) Examples a) Wherein any one of X13a to X13d is any amino acid. b) Wherein X13a is V or Y; wherein X13b is G or Q; wherein X13b is P, L or R and; wherein X13d is E or V c) Wherein X13a is V; wherein X13b is G or Q and; wherein X13b is P, L or R wherein X13d is E

SEQ ID NO:36 Human IgG1 hinge AA sequences:
EPKSCDKTHTCPPCP

SEQ ID NO:37 Human IgG1 CH2 AA sequences:
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

SEQ ID NO:38 Human IgG1 CH3 AA sequences:
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:39 human TROP2
MARGPGLAPPPLRLPLLLLVLAAVTGHTAAQDNCTCPTNKMTVCSPDGPGGRCQCRAL
GSGMAVDCSTLTSKCLLLKARMSAPKNARTLVRPSEHALVDNDGLYDPDCDPEGRFKA
RQCNQTSVCWCVNSVGVRRTDKGDLSLRCDELVRTHHILIDLRHRPTAGAFNHSDLDA
ELRRLFRERYRLHPKFVAAVHYEQPTIQIELRQNTSQKAAGDVDIGDAAYYFERDIKGES
LFQGRGGLDLRVRGEPLQVERTLIYYLDEIPPKFSMKRLTAGLIAVIVVVVVALVAGMA
VLVITNRRKSGKYKKVEIKELGELRKEPSL

SEQ ID NO:40 cynomolgus TROP2
MARGPGLAPPPLRLPLLLLLLAAVTGHTAAQDNCTCPTNKMTVCSPDGPGGRCQCRAL
GSGVAVDCSTLTSKCLLLKARMSAPKNARTLVRPNEHALVDNDGLYDPDCDPEGRFKA
RQCNQTSVCWCVNSVGVRRTDKGDLSLRCDELVRTHHILIDLRHRPTAGAFNHSDLDA
ELRRLFRERYRLHPKFVAAVHYEQPTIQIELRQNTSQKAAGDVDIGDAAYYFERDVKGE
SLFQGRGGLDLRVRGEPLQVERTLIYYLDEIPPKFSMKRLTAGLIAVIVVVVVALVAGVA
VLVISNRRKSGKYKKVEIKELGELRKEPSL

SEQ ID NO:41 mouse TROP2
MARGLDLAPLLLLLLAMATRFCTAQSNCTCPTNKMTVCDTNGPGGVCQCRAMGSQVL
VDCSTLTSKCLLLKARMSARKSGRSLVMPSEHAILDNDGLYDPECDDKGRFKARQCNQ
TSVCWCVNSVGVRRTDKGDQSLRCDEVVRTHHILIELRHRPTDRAFNHSDLDSELRRLF
QERYKLHPSFLSAVHYEEPTIQIELRQNASQKGLRDVDIADAAYYFERDIKGESLFMGRR
GLDVQVRGEPLHVERTLIYYLDEKPPQFSMKRLTAGVIAVIAVVSVAVVAGVVVLVVT
KRRKSGKYKKVELKELGEMRSEPSL

SEQ ID NO:42 Human IgG4 hinge AA sequences:

ESKYGPPCPPCP

SEQ ID NO:34 Human IgG4 CH2 AA sequences:
APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK

SEQ ID NO:43 Human IgG4 CH3 AA sequences:
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO:143 (KD002 consensus)
X_{1a}VQLX$_{1b}$ESGGGX$_{1c}$VQX$_{1d}$GGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKX$_{1e}$X$_{1f}$EWVSX$_{1g}$
INAX$_{1h}$**GSKT**YYX$_{1i}$DX$_{1j}$VKGRFTISRDNX$_{1k}$KNTLYLX$_{1l}$MNX$_{1m}$LX$_{1n}$X$_{1o}$EDTAX$_{1p}$YX$_{1q}$C**A**
RAKLTDTHYVEDYWGQGTX$_{1r}$VTVSS

SEQ ID NO:144 (KD005 consensus)
X_{2a}VQLX$_{2b}$ESGGGLX$_{2c}$X$_{2d}$X$_{2e}$GGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKGX$_{2f}$EWVSX$_{2g}$
ITSGGTTYYX$_{2h}$DX$_{2i}$VKGRFTISRDNX$_{2j}$KNTLYLQMNSLX$_{2k}$X$_{2l}$X$_{2m}$DTAX$_{2n}$YX$_{2o}$C**AKSRL**
TDSHYVEDAWGQGTX$_{2p}$VTVSS

SEQ ID NO: 145 (Consensus from KD001 to KD005)
QVQLQESGGGX$_{3a}$VX$_{3b}$X$_{3c}$GX$_{3d}$SLRLSCAAS**GFX$_{3e}$FSX$_{3f}$X$_{3g}$D**MSWVRQAPGKGPEWVSY
IX$_{3h}$X$_{3i}$X$_{3j}$X$_{3k}$X$_{3l}$X$_{3m}$TX$_{3n}$YPDSVKGRFTX$_{3o}$SRDNAKNX$_{3p}$LYLX$_{3q}$MNX$_{3r}$LX$_{3s}$PX$_{3t}$DTALYR
C**AX$_{3u}$X$_{3v}$X$_{3w}$LTDX$_{3x}$HYVEDX$_{3y}$**WGQGTQVTVSS

SEQ ID NO:146 (Consensus for KD001-KD005 and selected variants)
X_{4a}VQLX$_{4b}$ESGGGX$_{4c}$X$_{4d}$X$_{4e}$X$_{4f}$GX$_{4g}$SLRLSCAASGFX$_{4h}$FSX$_{4i}$X$_{4j}$DMX$_{4k}$WX4$_{l}$RQAPGKX$_{4m}$
X$_{4n}$X$_{4o}$WVSX$_{4p}$IX$_{4q}$X$_{4r}$X$_{4s}$GX$_{4t}$X$_{4u}$TX$_{4v}$YX$_{4w}$DX$_{4x}$VKGRFTX$_{4y}$SRDNX$_{4z}$KNX$_{5a}$LYLX$_{5b}$MNX$_{5c}$
LX$_{5d}$X$_{5e}$X$_{5f}$DTAX$_{5g}$YX$_{5h}$CAX$_{5i}$X$_{5j}$X$_{5k}$LTDX$_{5l}$HYVEDX$_{5m}$WGQGTX$_{5n}$VTVSS.

SEQ ID NO:147 (TROP2 delta 27-36)
MARGPGLAPPPLRLPLLLLVLAAVTGPTNKMTVCSPDGPGGRCQCRALGSGMAVDCST
LTSKCLLLKARMSAPKNARTLVRPSEHALVDNDGLYDPDCDPEGRFKARQCNQTSVCW
CVNSVGVRRTDKGDLSLRCDDLVRTHHILIDLRHRPTAGAFNHSDLDAELRRLFRERYR
LHPKFVAAVHYEQPTIQIELRQNTSQKAAGEVDIGDAAYYFERDIKGESLFQGRGGLDL

RVRGEPLQVERTLIYYLDEIPPKFSMKRLTAGLIAVIVVVVVALVAGMAVLVITNRRKSG
KYKKVE IKELGELRKE PSL

SEQ ID NO:148 (TROP2 delta 27-44)
MARGPGLAPPPLRLPLLLLVLAAVTGSPDGPGGRCQCRALGSGMAVDCSTLTSKCLLLK
ARMSAPKNARTLVRPSEHALVDNDGLYDPDCDPEGRFKARQCNQTSVCWCVNSVGVR
RTDKGDLSLRCDDLVRTHHILIDLRHRPTAGAFNHSDLDAELRRLFRERYRLHPKFVAA
VHYEQPTIQIELRQNTSQKAAGEVDIGDAAYYFERDIKGESLFQGRGGLDLRVRGEPLQ
VERTLIYYLDEIPPKFSMKRLTAGLIAVIVVVVVALVAGMAVLVITNRKSGKYKKVEIKE
LGELRKEPSL

SEQ ID NO:149 (TROP2 delta 27-56)
MARGPGLAPPPLRLPLLLLVLAAVTGALGSGMAVDCSTLTSKCLLLKARMSAPKNART
LVRPSEHALVDNDGLYDPDCDPEGRFKARQCNQTSVCWCVNSVGVRRTDKGDLSLRC
DDLVRTHHILIDLRHRPTAGAFNHSDLDAELRRLFRERYRLHPKFVAAVHYEQPTIQIEL
RQNTSQKAAGEVDIGDAAYYFERDIKGESLFQGRGGLDLRVRGEPLQVERTLIYYLDEIP
PKFSMKRLTAGLIAVIVVVVVALVAGMAVLVITNRRKSGKYKKVEIKELGELRKEPSL

SEQ ID NO:150 (KD065: TROP2-CD47 bispecific, TROP2 VHH at the N-terminal- variable regions underlined)
QVQLQESGGGLVQPGRSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITGSGTTY
YPDSVKGRFTTSRDNAKNMLYLQMNSLKPDDTALYRCAKARLTDSHYVEDAWGQGT
QVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGKAPAPAPAPAPAPAPAPAPAPKAQVQLQESGGGLVQPGGSLRLSCAASRFDFSVNDI
RWYRQAPGNERELVARITGGGRTDYADSVKGRFTISRDNAKNTVYLQMNNLKPEDTA
VYYCWGRGYWGQGTQVTVSS

SEQ ID NO:151 (KD066: TROP2-CD47 bispecific, TROP2 VHH at the C-terminal- - variable regions underlined)
QVQLQESGGGLVQPGGSLRLSCAASRFDFSVNDIRWYRQAPGNERELVARITGGGRTD
YADSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCWGRGYWGQGTQVTVSSEPKS

CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKAPAPAPA
PAPAPAPAPAPAPKAQVQLQESGGGLVQPGRSLRLSCAASGFTFSGSDMSWVRQAPGK
GPEWVSYITGSGTTYYPDSVKGRFTTSRDNAKNMLYLQMNSLKPDDTALYRCAKARLT
DSHYVEDAWGQGTQVTVSS

SEQ ID NO:152: KD067 (variable region 1-140- mouse hinge- mouse constant region -IMGT CDRH1, CDRH2 and CDRH3 in bold and underlined)

QVQLQESGGGMVQVGGSLRLSCAAS**GFPFSSAD**MSWVRQAPGKGPEWVSY**INADGSK**
TYYPDSVKGRFTISRDNAKNTLYLLMNNLKPEDTALYRC**ARAKLTDTHYVEDY**WGQG
TQVTVSSVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQ
FSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIE
KTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYK
NTQPIMNTNGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK

SEQ ID NO:153: KD068 (variable region 1-139- mouse hinge- mouse constant region -IMGT CDRH1, CDRH2 and CDRH3 in bold and underlined)

QVQLQESGGGLVEAGGSLRLSCAAS**GFTFSGSD**MSWVRQAPGKGPEWVSY**ITSGGTT**
YYPDSVKGRFTISRDNAKNTLYLQMNSLKPDDTALYRC**AKSRLTDSHYVEDA**WGQGT
QVTVSSVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFS
WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKT
ISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNT
QPIMNTNGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK

SEQ ID NO:154: KD069 (variable region 1-146- mouse hinge- mouse constant region -IMGT CDRH1, CDRH2 and CDRH3 in bold and underlined)

DVQLVESGGGSVQAGGSLRLSCAAS**GSTDSIEY**MTWFRQAPGKAREGVAA**LYTHTGN**
TYYTDSVKGRFTISQDKAKNMAYLRMDSVKSEDTAIYTC**GATRKYVPVRFALDQSSY**
DYWGQGTQVTVSSVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISK
DDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSA
AFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQ

PAENYKNTQPIMNTNGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHS PGK

SEQ ID NO:163 (linker)

APAPAPAPAPAPAPAPAPAPKA

SEQ ID NO:164 (amino acid sequence 1 to 274 of human TROP2)
MARGPGLAPPPLRLPLLLLVLAAVTGHTAAQDNCTCPTNKMTVCSPDGPGGRCQCRAL GSGMAVDCSTLTSKCLLLKARMSAPKNARTLVRPSEHALVDNDGLYDPDCDPEGRFKA RQCNQTSVCWCVNSVGVRRTDKGDLSLRCDELVRTHHILIDLRHRPTAGAFNHSDLDA ELRRLFRERYRLHPKFVAAVHYEQPTIQIELRQNTSQKAAGDVDIGDAAYYFERDIKGES LFQGRGGLDLRVRGEPLQVERTLIYYLDEIPPKFSMKRLT

Table 22: Exemplary nucleotide sequence encoding the heavy chains of KD001-KD005

Anti-TROP2	Nucleotide Sequence
KD001 Heavy chain SEQ ID NO:165	CAGGTGCAATTGCAAGAGTCTGGCGGCGGAATGGTGCACCCTG GTGGATCTCTGAGACTGTCTTGTGCCGCCTCCGGCTTCACCTTC TCTGGCTCTGATATGTCCTGGGTCCGACAGGCTCCTGGCAAGG GACCTGAATGGGTGTCCTACATCACCTCCGGCGGCACCACCTA CTATCCCGACTCTGTGAAGGGCAGATTCACCATCTCTCGGGAC AACGCCAAGAACACCCTGTACCTGCAGATGAACTCTCTGACCC CTGACGACACAGCCCTGTACAGATGTGCCAAGGCCAGACTGAC CGACAGCCACTACGTGGAAGATGCTTGGGGCCAGGGAACACA GGTCACCGTGTCTAGTGAACCCAAGTCCTGCGACAAGACCCAC ACCTGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACCTTC CGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATCT CTCGGACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCTCA CGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGTG GAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTAC AACTCCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACC AGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCA ACAAGGCCCTGCCTGCTCCTATCGAAAAGACCATCTCCAAGGC

Anti-TROP2	Nucleotide Sequence
	CAAGGGCCAGCCTAGGGAACCCCAGGTTTACACCCTGCCTCCA AGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGCC TGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGGGA GAGCAATGGCCAGCCTGAGAACAACTACAAGACAACCCCTCCT GTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCCAAGCTGAC AGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCTCCTGC TCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGT CCCTGTCTCTGTCCCCTGGCAAA
KD002 Heavy chain SEQ ID NO:166	CAGGTGCAATTGCAAGAGTCTGGCGGCGGAATGGTGCAAGTCG GCGGATCTCTGAGACTGTCTTGTGCCGCCTCTGGCTTCCCTTTC TCCTCCGCCGATATGTCCTGGGTCCGACAGGCTCCTGGAAAGG GACCTGAGTGGGTGTCCTACATCAACGCCGACGGCTCCAAGAC CTACTATCCCGACTCTGTGAAGGGCAGATTCACCATCTCTCGGG ACAACGCCAAGAACACCCTGTACCTGCTGATGAACAACCTGAA GCCTGAGGACACAGCCCTGTACAGATGCGCCAGAGCCAAGCTG ACCGACACACACTACGTGGAAGATTACTGGGGCCAGGGCACAC AGGTCACCGTGTCTAGTGAACCCAAGTCCTGCGACAAGACCCA CACCTGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACCTT CCGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATC TCTCGGACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCTC ACGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGT GGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTA CAACTCCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCAC CAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCC AACAAGGCCCTGCCTGCTCCTATCGAAAAGACCATCTCCAAGG CCAAGGGCCAGCCTAGGGAACCCCAGGTTTACACCCTGCCTCC AAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTG

Anti-TROP2	Nucleotide Sequence
	CCTGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGG GAGAGCAATGGCCAGCCTGAGAACAACTACAAGACAACCCCT CCTGTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCCAAGCT GACAGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCTCC TGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGA AGTCCCTGTCTCTGTCCCCTGGCAAA
KD003 Heavy chain SEQ ID NO:167	CAGGTGCAATTGCAAGAGTCTGGCGGCGGAATGGTGCAGCCTG GTGGATCTCTGAGACTGTCTTGTGCCGCCTCCGGCTTCACCTTC TCCAACTACGACATGTCCTGGGTCCGACAGGCTCCTGGAAAGG GACCTGAGTGGGTGTCCTACATCAACGCTGGCGGCTCCAACAC CGACTATCCCGACTCTGTGAAGGGCAGATTCACCATCTCTCGG GACAACGCCAAGAACATGCTGTACCTGCTGATGAACAACCTGA AGCCTGAGGACACAGCCCTGTACAGATGCGCCAGAGCCAAGCT GACCGACACACACTACGTGGAAGATTACTGGGGCCAGGGCAC ACAGGTCACCGTGTCTAGTGAACCCAAGTCCTGCGACAAGACC CACACCTGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACC TTCCGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGA TCTCTCGGACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCT CACGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCG TGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGT ACAACTCCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCA CCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCC AACAAGGCCCTGCCTGCTCCTATCGAAAAGACCATCTCCAAGG CCAAGGGCCAGCCTAGGGAACCCCAGGTTTACACCCTGCCTCC AAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTG CCTGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGG GAGAGCAATGGCCAGCCTGAGAACAACTACAAGACAACCCCT CCTGTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCCAAGCT GACAGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCTCC

Anti-TROP2	Nucleotide Sequence
	TGCTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGA AGTCCCTGTCTCTGTCCCCTGGCAAA
KD004 Heavy chain SEQ ID NO:168	CAGGTGCAATTGCAAGAGTCTGGCGGCGGACTGGTGCAGCCTG GAAGATCTCTGAGACTGTCTTGTGCCGCCTCCGGCTTCACCTTC TCTGGCTCTGATATGTCCTGGGTCCGACAGGCTCCTGGCAAGG GACCTGAATGGGTGTCCTACATCACCGGCTCCGGCACCACCTA CTATCCCGACTCTGTGAAGGGCAGATTCACCACCTCCAGAGAC AACGCCAAGAACATGCTGTACCTGCAGATGAACTCCCTGAAGC CTGACGACACAGCCCTGTACAGATGCGCCAAGGCCAGACTGAC CGACAGCCACTACGTGGAAGATGCTTGGGGCCAGGGAACACA GGTCACCGTGTCTAGTGAACCCAAGTCCTGCGACAAGACCCAC ACCTGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACCTTC CGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATCT CTCGGACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCTCA CGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGTG GAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTAC AACTCCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACC AGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCA ACAAGGCCCTGCCTGCTCCTATCGAAAAGACCATCTCCAAGGC CAAGGGCCAGCCTAGGGAACCCCAGGTTTACACCCTGCCTCCA AGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGCC TGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGGGA GAGCAATGGCCAGCCTGAGAACAACTACAAGACAACCCCTCCT GTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCCAAGCTGAC AGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCTCCTGC TCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGT CCCTGTCTCTGTCCCCTGGCAAA
KD005 Heavy chain SEQ ID NO:169	CAGGTGCAATTGCAAGAGTCTGGCGGCGGACTGGTGGAAGCTG GTGGATCTCTGAGACTGTCTTGTGCCGCCTCCGGCTTCACCTTC TCTGGCTCTGATATGTCCTGGGTCCGACAGGCTCCTGGCAAGG GACCTGAATGGGTGTCCTACATCACCTCCGGCGGCACCACCTA CTATCCCGACTCTGTGAAGGGCAGATTCACCATCTCTCGGGAC AACGCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGAAGC CTGACGACACAGCCCTGTACAGATGCGCCAAGTCTCGGCTGAC CGACAGCCACTACGTGGAAGATGCTTGGGGCCAGGGAACACA GGTCACCGTGTCTAGTGAACCCAAGTCCTGCGACAAGACCCAC ACCTGTCCTCCATGTCCTGCTCCAGAACTGCTCGGCGGACCTTC CGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATCT CTCGGACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCTCA CGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGTG GAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTAC AACTCCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACC

Anti-TROP2	Nucleotide Sequence
	AGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCA ACAAGGCCCTGCCTGCTCCTATCGAAAAGACCATCTCCAAGGC CAAGGGCCAGCCTAGGGAACCCCAGGTTTACACCCTGCCTCCA AGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGCC TGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGGGA GAGCAATGGCCAGCCTGAGAACAACTACAAGACAACCCCTCCT GTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCCAAGCTGAC AGTGGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCTCCTGC TCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGAAGT CCCTGTCTCTGTCCCCTGGCAAA

SEQ lD NO:176 (CD47 Antigen)

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKW

KFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVT

ELTREGETIIELKYRVVSWFSP

CLAIMS:

1. A binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2) and comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:26.
2. The binding agent of claim 1, wherein the binding agent is capable of competing with a single domain antibody comprising the amino acid sequence set forth in SEQ ID NO:26.
3. The binding agent of claim 1 or 2, wherein the one or more antigen binding domains are one or more antigen binding domains of a single domain antibody.
4. The binding agent of any one of the preceding claims, wherein the one or more antigen binding domains comprise a CDR3 amino acid sequence as set forth in SEQ ID NO:126 or in SEQ ID NO:142.
5. The binding agent of any one of the preceding claims, wherein the one or more antigen binding domains comprise a FR2 amino acid sequence as set forth in SEQ ID NO:121 or in SEQ ID NO: 175.
6. The binding agent of any one of the preceding claims, wherein the binding agent comprises two antigen binding domains or more.
7. The binding agent of any one of claims 1 to 6, wherein the binding agent is monospecific.
8. The binding agent of any one of claims 1 to 6, wherein the binding agent is bispecific.
9. The binding agent of any one of claims 1 to 6, wherein the binding agent is multispecific.
10. The binding agent of any one of the preceding claims, wherein the binding agent is a single domain antibody.
11. The binding agent of any one of the preceding claims, wherein the binding agent has activity against tumor cells.
12. A single domain antibody that competes with the binding agent of any one of claims 1-11.

13. A binding agent comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2).

14. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains specifically binds to the cysteine-rich domain and/or to the thyroglobulin type-1 domain of TROP2.

15. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains specifically binds to a polypeptide comprising a TROP2 amino acid sequence consisting of amino acid residues 27-146 or a fragment thereof.

16. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains specifically binds to a polypeptide comprising a TROP2 amino acid sequence consisting of amino acid residues 27-73 or a fragment thereof.

17. The binding agent of claim 15 or 16, wherein the fragment thereof comprises amino acid residues 27-56, 27-66, 47-56, 60-66 or 47-66 of TROP2.

18. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains does not significantly bind to or is not capable of binding to a polypeptide that comprises a TROP2 amino acid sequence consisting of amino acid residues 147-274 or a fragment thereof.

19. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains is not capable of binding to one or more polypeptide selected from polypeptides comprising a TROP2 amino acid sequence comprising deletion of amino acid residues 27-36, 27-44, 27-56, 27-66 or 27-73 or has a reduced binding to said polypeptide compared to the full TROP2 polypeptide.

20. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains is not capable of binding to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 149.

21. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains comprises CDR1, CDR2, CDR3, and/or FR2 or combination thereof of a single domain antibody.

22. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains comprises CDR3 of a single domain antibody.

23. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains comprises FR2 and CDR3 of a single domain antibody.

24. The binding agent of claim 23, wherein FR2 and CDR3 are on same polypeptide chain.

25. The binding agent of claim 23, wherein FR2 and CDR3 are on separate polypeptide chains.

26. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains comprises a variable region of a single domain antibody.

27. The binding agent of any one of claims 1 to 26, wherein the binding agent or at least one of the one or more antigen binding domains comprises a camelid VH or VHH.

28. The binding agent of any one of claims 1 to 26, wherein the binding agent or at least one of the one or more antigen binding domains comprises a mouse VH.

29. The binding agent of any one of claims 1 to 26, wherein the binding agent or at least one of the one or more antigen binding domains comprises a human VH.

30. The binding agent of any one of claims 1 to 26, wherein the binding agent or at least one of the one or more antigen binding domains comprises a humanized VH or VHH.

31. The binding agent of any one of claims 21 to 30, wherein the CDR1, CDR2, CDR3 and/or FR2 correspond to the IMGT CDR1, CDR2, CDR3 and/or FR2.

32. The binding agent of any one of claims 21 to 30, wherein the CDR1, CDR2, CDR3 and/or FR2 correspond to the Kabat CDR1, CDR2, CDR3 and/or FR2.

33. The binding agent of any one of claims 1 to 32, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:143 wherein any of X_{1a} to X_{1r} is each independently any amino acid residue.

34. The binding agent of claim 33, wherein:

a. any of X_{1a} to X_{1r} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:23 or SEQ ID NOs:73-93, or conservative amino acid substitutions or non-conservative amino acid substitutions thereof;

b. any of X_{1a} to X_{1r} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof;

c. wherein any X_{1a} to X_{1r} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:23 or SEQ ID Nos:73-93 or;

d. wherein any X_{1a} to X_{1r} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

35. The binding agent of claim 33, wherein;

a. any of X_{1a} to X_{1r} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 73, 75, 76, 77, 78, 81, 82, 83, 85, 89 or 91, or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;

b. wherein any X_{1a} to X_{1r} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 73, 75, 76, 77, 78, 81, 82, 83, 85, 89 or 91.

36. The binding agent of claim 33, wherein X_{1a} is Q or E; X_{1b} is Q, V or L; X_{1c} is M or L; X_{1d} is V or P; X_{1e} is G or Q; X_{1f} is P, L or R; X_{1g} is Y or V; X_{1h} is D, N, E; X_{1i} is P or A; X_{1j} is S or T; X_{1k} is A or S; X_{1l} is L or Q; X_{1m} is N or S; X_{1n} is K or R; X_{1o} is P or A; X_{1p} is L or V; X_{1q} is R or Y; and/or X_{1r} is Q or M.

37. The binding agent of claim 33, wherein X_{1a} is Q or E; X_{1b} is Q, V or L; X_{1c} is M or L; X_{1d} is V or P; X_{1e} is G or Q; X_{1f} is P, L or R; X_{1g} is Y; X_{1h} is D, X_{1i} is P or A; X_{1j} is S or T; X_{1k} is A or S; X_{1l} is L or Q; X_{1m} is N or S; X_{1n} is K or R; X_{1o} is P or A; X_{1p} is L or V; X_{1q} is R or Y; and/or X_{1r} is Q or M.

38. The binding agent of any one of claims 1 to 32, wherein wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:144 wherein any of X_{2a} to X_{2p} is each independently any amino acid residue.

39. The binding agent of claim 38, wherein:

a. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:26 or SEQ ID NO:94-114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof

b. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof;

c. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:26 or SEQ ID NO:94-114 or;

d. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

40. The binding agent of claim 38, wherein:

a. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 100, 101, 102, 103, 104, 105, 106, 107 or 108 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;

b. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 100, 101, 102, 103, 104, 105, 106, 107 or 108.

41. The binding agent of claim 38, wherein:

a. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 100, 102, 103, 104, 105, 106, 108, 112 or 114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;

b. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 100, 102, 103, 104, 105, 106, 108, 112 or 114.

42. The binding agent of claim 38, wherein:

a. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 105, 112, 113 or 114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;

b. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 105, 112, 113 or 114.

43. The binding agent of claim 38, wherein:

a. any of X_{2a} to X_{2p} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 102 or 105 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;

b. wherein any X_{2a} to X_{2p} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs: 94, 95, 96, 97, 102 or 105.

44. The binding agent of claim 38, wherein X_{2a} is Q or E; X_{2b} is Q, L or V; X_{2c} is V or I; X_{2d} is E or Q; X_{2e} is A or P; X_{2f} is P, or L; X_{2g} is Y, A or V; X_{2h} is P or A; X_{2i} is S or T; X_{2j} is

A or S; X_{2k} is K or R; X_{2l} is P or A; X_{2m} is D or E; X_{2n} is L or V; X_{2o} is R or Y and/or X_{2p} is Q or L.

45. The binding agent of claim 38, wherein X_{2a} is Q or E; X_{2b} is Q or L; X_{2c} is V or I; X_{2d} is E or Q; X_{2e} is A or P; X_{2f} is P, or L; X_{2g} is Y or A; X_{2h} is P or A; X_{2i} is S or T; X_{2j} is A or S; X_{2k} is K or R; X_{2l} is P or A; X_{2m} is D or E; X_{2n} is L or V; X_{2o} is R or Y and/or X_{2p} is Q or L.

46. The binding agent of any one of claims 1 to 32, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:145 wherein any of X_{3a} to X_{3y} is each independently any amino acid residue.

47. The binding agent of claim 46, wherein:
    a. any of X_{3a} to X_{3y} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof or;
    b. wherein any of X_{3a} to X_{3y} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

48. The binding agent of claim 46, wherein X_{3a} is M or L; X_{3b} is Q, H or E; X_{3c} is V, P or A; X_{3d} is G or R; X_{3e} is P or T; X_{3f} is S, N or G; X_{3g} is A, Y or S; X_{3h} is N or T; X_{3i} is A, S or G; X_{3j} is D, G or S; X_{3k} is G or absent; X_{3l} is S or G; X_{3m} is K, N or T; X_{3n} is Y or D; X_{3o} is I or T; X_{3p} is T or M; X_{3q} is L or Q; X_{3r} is N or S; X_{3s} is K or T; X_{3t} is E or D; X_{3u} is R or K; X_{3v} is A or S; X_{3w} is K or R; X_{3x} is T or S; and/or X_{3y} is Y or A.

49. The binding agent of claim 46, wherein X_{3a} is M or L; X_{3b} is Q, H or E; X_{3c} is V, P or A; X_{3d} is G or R; X_{3e} is P or T; X_{3f} is S, N or G; X_{3g} is A, Y or S; X_{3h} is N or T; X_{3i} is A, S or G; X_{3j} is D, G or S; X_{3k} is G or absent; X_{3l} is S or G; X_{3m} is K, N or T; X_{3n} is Y; X_{3o} is I or T; X_{3p} is T or M; X_{3q} is L or Q; X_{3r} is N or S; X_{3s} is K or T; X_{3t} is E or D; X_{3u} is R or K; X_{3v} is A or S; X_{3w} is R; X_{3x} is S; and/or X_{3y} is A.

50. The binding agent of any one of claims 1 to 32, wherein the binding agent or at least one of the one or more antigen binding domains comprises the amino acid sequence set forth in SEQ ID NO:146 wherein any of X_{4a} to X_{5n} is each independently any amino acid residue.

51. The binding agent of claim 50, wherein:

a. any of X_{4a} to X_{5n} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NOs:22-26, SEQ ID Nos:73-114 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof

b. any of X_{4a} to X_{5n} is each independently an amino acid residue found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 or SEQ ID NO:26 or conservative amino acid substitutions or non-conservative amino acid substitutions thereof;

c. wherein any of X_{4a} to X_{5n} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NOs:22-26, SEQ ID Nos:73-114 or;

d. wherein any of X_{4a} to X_{5n} is each independently an amino acid residue most frequently found at a corresponding position in any one of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 to SEQ ID NO:26.

52. The binding agent of claim 50, wherein X_{4a} is Q or E, X_{4b} is Q, L or V, X_{4c} is M or L, X_{4d} is V or I, X_{4e} is Q, H or E, X_{4f} is P, A or V, X_{4g} is G or R, X_{4h} is P or T, X_{4i} is G, S or N, X_{4j} is A or S, X_{4k} is S or H, X_{4l} is V or Y, X_{4m} is G or Q, X_{4n} is P, L or R, X_{4o} is E or V, X_{4p} is Y, A or R, X_{4q} is T or N, X_{4r} is A, S or G, X_{4s} is G, S, D, N or E, X_{4t} is absent or S, X_{4u} is K or T, X_{4v} is Y, D or S, X_{4w} is P or A, X_{4x} is S or T, X_{4y} is I or T, X_{4z} is S or A X_{5a} is T or M X_{5b} is Q or L, X_{5c} is S or N, X_{5d} is K, R or T, X_{5e} is P or A, X_{5f} is E or D, X_{5g} is V or L, X_{5h} is Y or R, X_{5i} is R or K, X_{5j} is A or S, X_{5k} is K or R, X_{5l} is S or T, X_{5m} is Y or A, and/or X_{5n} is Q, L or M.

53. A binding agent that comprises one or more antigen binding domains, wherein at least one of the one or more antigen binding domains specifically binds to trophoblast cell surface antigen-2 (TROP2) and comprises an amino acid sequence at least 65%, at least 70%, at

least 75%, at least 80% identical to the amino acid sequence set forth in any one of SEQ ID NOs:22-26, or SEQ ID NOs: 71-115.

54. The binding agent of any one of the preceding claims, wherein the at least one of the one or more antigen binding domains comprises an amino acid sequence at least 85% identical to any one of SEQ ID NOs:22-26, or SEQ ID NOs: 73-114.

55. The binding agent of any one of the preceding claims, wherein the at least one of the one or more antigen binding domains comprises an amino acid sequence at least 90% identical to any one of SEQ ID NOs:22-26, or SEQ ID NOs: 73-114.

56. The binding agent of any one of the preceding claims, wherein the at least one of the one or more antigen binding domains comprises an amino acid sequence at least 95% identical to any one of SEQ ID NOs:22-26, or SEQ ID NOs: 73-114.

57. The binding agent of any one of the preceding claims, wherein the at least one of the one or more antigen binding domains comprises an amino acid sequence at least 99% identical to any one of SEQ ID NOs:22-26, or SEQ ID NOs: 73-114.

58. The binding agent of any one of the preceding claims, wherein the at least one of the one or more antigen binding domains comprises an amino acid sequence identical to any one of SEQ ID NOs:22-26, or SEQ ID NOs: 73-114.

59. The binding agent of any one of the preceding claims, wherein the amino acid sequence comprises from one to five amino acid deletions or from one to five amino acid additions.

60. The binding agent of claim 59, wherein the deletion or additions are consecutive.

61. The binding agent of claim 59, wherein the deletion or additions are non-consecutives.

62. The binding agent of any one of claims 59 to 61, wherein the deletion or additions are at the N-terminus.

63. The binding agent of any one of claims 59 to 61, wherein the deletion or additions are at the C-terminus.

64. The binding agent of any one of claims 59 to 61, wherein the deletion or additions are outside of an IMGT CDR3 sequence and/or IMGT FR2 sequence.

65. The binding agent of any one of the preceding claims, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:126.

66. The binding agent of claim 65, wherein the amino acid sequence of the CDR3 is as set forth in SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:54 or SEQ ID NO:61.

67. The binding agent of any one of the preceding claims, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the FR2 set forth in SEQ ID NO:121.

68. The binding agent of claim 67, wherein the amino acid sequence of the FR2 is as set forth in SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 or SEQ ID NO:120.

69. The binding agent of any one of the preceding claims, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:122.

70. The binding agent of claim 69, wherein the amino acid sequence of the CDR1 is as set forth in SEQ ID NO:52, SEQ ID NO:55 or SEQ ID NO:56.

71. The binding agent of any one of the preceding claims, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the CDR2 set forth in SEQ ID NO:125.

72. The binding agent of claim 71, wherein the amino acid sequence of the CDR2 is as set forth in SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:123 or SEQ ID NO:124.

73. The binding agent of any one of the preceding claims, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the CDR3 set forth in any one of SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:54, SEQ ID NO:58 or SEQ ID NO:61, the amino acid sequence of the FR2 set forth in any one of SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 or SEQ ID NO:120, the amino acid sequence of the CDR1 set forth in any one of SEQ ID NO: 52, SEQ ID NO: 55, SEQ ID NO:59, SEQ ID NO: 52 or SEQ ID NO: 62 and the amino acid sequence of the CDR2 set

forth in any one of SEQ ID NO: 47, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO: 57, SEQ ID NO: 60, SEQ ID NO:123 or SEQ ID NO:124.

74. The binding agent of any one of claims 1-73, wherein the binding agent or at least one of the one or more antigen binding domains comprises:

a. the amino acid sequence of the CDR3 set forth in SEQ ID NO:54 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:52 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:53;

b. the amino acid sequence of the CDR3 set forth in SEQ ID NO:48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:47;

c. the amino acid sequence of the CDR3 set forth in SEQ ID NO:58 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:56 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:57;

d. the amino acid sequence of the CDR3 set forth in SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:59 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:60;

e. the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50,

f. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

g. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

h. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

i. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:119 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

j. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 124;

k. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 123;

l. the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50;

m. the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50;

n. amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50 or,

o. amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:120 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

75. The binding agent of any one of claims 1 to 73, wherein the binding agent or at least one of the one or more antigen binding domains comprises:

a. the amino acid sequence of the CDR1 set forth in SEQ ID NO:52, the amino acid sequence of the CDR2 set forth in SEQ ID NO:53, the amino acid sequence of the CDR3 set forth in SEQ ID NO:54 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

b. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO:48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

c. the amino acid sequence of the CDR1 set forth in SEQ ID NO:56, the amino acid sequence of the CDR2 set forth in SEQ ID NO:57, the amino acid sequence of the CDR3 set forth in SEQ ID NO:58 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

d. the amino acid sequence of the CDR1 set forth in SEQ ID NO:59, the amino acid sequence of the CDR2 set forth in SEQ ID NO:60, the amino acid sequence of the CDR3 set forth in SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

e. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the

CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

f. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

g. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

h. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118;

i. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:119;

j. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 124, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

k. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 123, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

l. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the

CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

m. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

n. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 or,

o. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:120.

76. The binding agent of any one claims 1-64, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:142.

77. The binding agent of claim 76, wherein the amino acid sequence of the CDR3 is as set forth in SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141.

78. The binding agent of any one claims 1-64, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the FR2 set forth in SEQ ID NO:175.

79. The binding agent of claim 78, wherein the amino acid sequence of the FR2 is as set forth in SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 or SEQ ID NO:174.

80. The binding agent of any one claims 1-64, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the CDR1 set forth in SEQ ID NO:128.

81. The binding agent of claim 80, wherein the amino acid sequence of the CDR1 is as set forth in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:127.

82. The binding agent of any one claims 1-64, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the CDR2 set forth in SEQ ID NO:140.

83. The binding agent of claim 82, wherein the amino acid sequence of the CDR2 is as set forth in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138 or SEQ ID NO:139.

84. The binding agent of any one claims 1-64, wherein the at least one of the one or more antigen binding domains comprises the amino acid sequence of the CDR3 set forth in any one of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 or SEQ ID NO:141, the amino acid sequence of the FR2 set forth in any one of SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 or SEQ ID NO:174, the amino acid sequence of the CDR1 set forth in any one of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7 or SEQ ID NO:127 and the amino acid sequence of the CDR2 set forth in any one of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138 or SEQ ID NO:139.

85. The binding agent of any one of the preceding claims, wherein the amino acid residue at position 37 is V, the amino acid residue at position 39 is Q, the amino acid residue at position 45 is P, the amino acid residue at position 47 is W, the amino acid residue at position 50 is Y, the amino acid residue at position 58 is Y, the amino acid residue at position 99 is R, the amino acid residue at position 100 is L, the amino acid residue at position 101 is T, the amino acid residue at position 102 is D, the amino acid residue at position 103 is S, the amino acid residue at position 104 is H, the amino acid residue at position 105 is Y, the amino acid residue at position 106 is V, the amino acid residue at position 107 is E, the amino acid residue at position 108 is D, the amino acid residue at position 109 and/or the position is with reference to SEQ ID NO:145 (**Table 1**).

86. The binding agent of any one of the preceding claims, wherein the amino acid residue at position 1 is Q, the amino acid residue at position 2 is V, the amino acid residue at position 37 is V, the amino acid residue at position 39 is Q, the amino acid residue at position 45 is P, the amino acid residue at position 47 is W, the amino acid residue at position 50 is Y, the amino acid residue at position 58 is Y, the amino acid residue at position 99 is R, the amino acid residue at position 100 is L, the amino acid residue at position 101 is T, the amino acid residue at position 102 is D, the amino acid residue at position 103 is S, the amino acid residue at position 104 is H, the amino acid residue at position 105 is Y, the amino acid residue at position 106 is V, the amino acid residue at position 107 is E, the amino acid residue at position 108 is D, the amino acid residue at position 109 is A, the amino acid residue at position 110 is W and/or the amino acid residue at position 112 is Q and the position is with reference to SEQ ID NO:145 (**Table 1**).

87. The binding agent of any one of the preceding claims, wherein the amino acid residue at position 1 is Q, the amino acid residue at position 2 is V, the amino acid residue at position 3 is Q, the amino acid residue at position 4 is L, the amino acid residue at position 37 is V, the amino acid residue at position 39 is Q, the amino acid residue at position 45 is P, the amino acid residue at position 46 is E, the amino acid residue at position 47 is W, the amino acid residue at position 50 is Y, the amino acid residue at position 58 is Y, the amino acid residue at position 99 is R, the amino acid residue at position 100 is L, the amino acid residue at position 101 is T, the amino acid residue at position 102 is D, the amino acid residue at position 103 is S, the amino acid residue at position 104 is H, the amino acid residue at position 105 is Y, the amino acid residue at position 106 is V, the amino acid residue at position 107 is E, the amino acid residue at position 108 is D, the amino acid residue at position 109 is A, the amino acid residue at position 110 is W, the amino acid residue at position 111 is G, and/or the amino acid residue at position 112 is Q and the position is with reference to SEQ ID NO:145 (**Table 1**)

88. The binding agent of any one of claims 21 to 87, wherein the amino acid sequence of the CDR1, CDR2, CDR3 and/or FR2 are on a single polypeptide chain.

89. The binding agent of any one of claims 21 to 88, wherein the amino acid sequence of the CDR3 and the amino acid sequence of the FR2 are on a same polypeptide chain.

90. The binding agent of any one of claims 21 to 89, wherein the amino acid sequence of the CDR1, CDR2, CDR3 and FR2 are on a same polypeptide chain.

91. The binding agent of any one of claims 21 to 89, wherein the amino acid sequence of the CDR3 and the amino acid sequence of the FR2 are on two polypeptide chains.

92. A binding agent that specifically binds to TROP2 comprising one or more antigen binding domains, wherein at least one of the one or more antigen binding domains comprises:

a. a heavy chain complementarity determining region 1 (CDRH1) having the amino acid sequence set forth in SEQ ID NO:1, a heavy chain complementarity determining region 2 (CDRH2) having the amino acid sequence set forth in SEQ ID NO:2 and a heavy chain complementarity determining region 3 (CDRH3) having the amino acid sequence set forth in SEQ ID NO:3;

b. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:5 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

c. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:7, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:8 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:9;

d. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:10, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:11 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:12;

e. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:14 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

f. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:16, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:17 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:18;

g. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:19, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:20 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:21;

h. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:46 or in SEQ ID NO:55, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:47 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:48;

i. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:49 or SEQ ID NO:62, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:50 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:51;

j. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:52, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:53 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:54;

k. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:56, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:57 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:58;

l. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:59, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:60 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:61;

m. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:63, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:64 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:65;

n. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:66, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:67 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:68;

o. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:127, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:129 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

p. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:130 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

q. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:131 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

r. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:132 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

s. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:133 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

t. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:134 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

u. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:4, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:135 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:6;

v. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:136 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

w. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:137 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

x. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:138 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

y. a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:139 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;

z. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:22;

aa. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:23;

bb. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:24;

cc. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:25;

dd. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:26;

ee. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:27;;

ff. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in SEQ ID NO:28 or;

gg. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% identical to the amino acid sequence set forth in any one of SEQ ID NOs:73 to 114.

93. The binding agent of any one of the preceding claims, wherein the binding agent comprises two antigen binding domains.

94. The binding agent of any one of the preceding claims, wherein the binding agent comprises two identical antigen binding domains.

95. The binding agent of claim 93 or 94, wherein each of the two antigen binding domains are on separate polypeptide chains.

96. The binding agent of any one of the preceding claims, wherein the binding agent comprises more than two antigen binding domains.

97. The binding agent of any one of the preceding claims, wherein the binding agent comprises antigen binding domains of at least two different single domain antibodies.

98. The binding agent of claim 97, wherein the at least two different single domain antibodies have the same specificity.

99. The binding agent of claim 97, wherein the at least two different single domain antibodies have different specificities.

100. The binding agent of any one of the preceding claims, wherein the binding agent comprises two antigen binding domains of same or different single domain antibodies.

101. The binding agent of any one of the preceding claims, wherein the binding agent comprises more than two antigen binding domains of same and/or different single domain antibodies.

102. The binding agent of any one of the preceding claims, wherein the binding agent comprises a Fc or Fc portion that is capable of binding to an Fc receptor of monocytes and/or macrophages.

103. The binding agent of claim 102, wherein the binding agent comprises a Fc or Fc portion that is capable of binding to an Fc receptor of M1 and/or M2 macrophages.

104. The binding agent of any one of the preceding claims, wherein the binding agent comprises a dimerization domain.

105. The binding agent of claim 104, wherein the dimerization domain comprises a constant region of an antibody or a portion thereof.

106. The binding agent of claim 105, wherein the constant region or portion thereof is an IgG1, an IgG2, an IgG3 or an IgG4 constant region or a portion thereof.

107. The binding agent of claim 105, wherein the constant region or portion thereof is a human IgG1, an IgG2, an IgG3 or an IgG4 constant region or a portion thereof.

108. The binding agent of claim 105, wherein the constant region or portion thereof is a human IgG1 constant region or a portion thereof.

109. The binding agent of any of the preceding claims, wherein the binding agent is or comprises an antibody or an antigen binding fragment thereof.

110. The binding agent of any of the preceding claims, wherein the binding agent is or comprises a single domain antibody or an antigen binding fragment thereof.

111. The binding agent of any of the preceding claims, wherein the binding agent is an antibody or an antigen binding fragment thereof that comprises a single domain antibody or an antigen binding fragment thereof.

112. The binding agent of any of the preceding claims, wherein the binding agent is or comprises an antibody-like molecule.

113. The binding agent of any of the preceding claims, wherein the binding agent is or comprises a protein scaffold.

114. The binding agent of any of the preceding claims, wherein the binding agent is or comprises an immune cell modulating agent.

115. The binding agent of any of the preceding claims, wherein the antigen binding domains are in tandem.

116. The binding agent of any one of the preceding claims, wherein the binding agent comprises:

a. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% to the amino acid sequence set forth in SEQ ID NO:22;

b. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% to the amino acid sequence set forth in SEQ ID NO:23;

c. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% to the amino acid sequence set forth in SEQ ID NO:24;

d. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% to the amino acid sequence set forth in SEQ ID NO:25;

e. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% to the amino acid sequence set forth in SEQ ID NO:26;

f. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% to the amino acid sequence set forth in SEQ ID NO:27;

g. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% to the amino acid sequence set forth in SEQ ID NO:28 or;

h. a variable region having an amino acid sequence at least 75%, 80% 85%, 90%, 95%, 99% to the amino acid sequence set forth in any one of SEQ ID Nos:73-114.

117. The binding agent of any one of the preceding claims, wherein the binding agent comprises:

a. a variable region having an amino acid sequence as set forth in SEQ ID NO:22;

b. a variable region having an amino acid sequence as set forth in SEQ ID NO:23;

c. a variable region having an amino acid sequence as set forth in SEQ ID NO:24;

d. a variable region having an amino acid sequence as set forth in SEQ ID NO:25;

e. a variable region having an amino acid sequence as set forth in SEQ ID NO:26;

f. a variable region having an amino acid sequence as set forth in SEQ ID NO:27;

g. a variable region having an amino acid sequence as set forth in SEQ ID NO:28 or;

h. a variable region having an amino acid sequence as set forth in any one of SEQ ID Nos:73-114.

118. The binding agent of any one of the preceding claims, wherein the binding agent comprises:

a. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:29;

b. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:30;

c. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:31;

d. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:32;

e. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:33;

f. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:34;

g. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:35;

h. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:44, or,

i. an amino acid sequence at least 75%, 80% 85%, 90%, 95% identical to the amino acid sequence set forth in SEQ ID NO:45.

119. The binding agent of any one of the preceding claims, wherein the binding agent comprises:

a. an amino acid sequence as set forth in SEQ ID NO:29;

b. an amino acid sequence as set forth in SEQ ID NO:30;

c. an amino acid sequence as set forth in SEQ ID NO:31;

d. an amino acid sequence as set forth in SEQ ID NO:32;

e. an amino acid sequence as set forth in SEQ ID NO:33;

f. an amino acid sequence as set forth in SEQ ID NO:34;

g. an amino acid sequence as set forth in SEQ ID NO:35;

h. an amino acid sequence as set forth in SEQ ID NO:44 or,

i. an amino acid sequence as set forth in SEQ ID NO:45.

120. The binding agent of any of the preceding claims, wherein the binding agent is naked.

121. The binding agent of any of the preceding claims, wherein the binding agent is conjugated to a therapeutic moiety.

122. The binding agent of any of the preceding claims, wherein the binding agent is conjugated to a detectable moiety.

123. The binding agent of any of the preceding claims, wherein the binding agent is conjugated to a protein allowing an extended half-life.

124. The binding agent of any of the preceding claims, wherein binding agent is attached to a nanoparticle.

125. The binding agent of any one of the preceding claims, wherein TROP2 is human TROP2.

126. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains specifically binds to human TROP2.

127. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains is capable of binding to a denatured form of human TROP2.

128. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains is capable of binding to a reduced form of human TROP2.

129. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains is capable of binding to a deglycosylated form of human TROP2.

130. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains has an affinity of $\leq 10^{-6}$ M for human TROP2.

131. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains has an affinity of $\leq 10^{-10}$ M for human TROP2.

132. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains has an affinity of at between 1 x10^{-10} M and 1 x10^{-12} for human TROP2.

133. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains has a dissociation rate of between 1 x 10^{-6} to 1 x 10^{-4}.

134. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains is also capable of binding cynomolgus TROP2.

135. The binding agent of any of the preceding claims, wherein binding agent is also capable of binding cynomolgus TROP2.

136. The binding agent of any of the preceding claims, wherein the binding agent preferentially binds human TROP2 over mouse TROP2 or has a better affinity for human TROP2 over mouse TROP2.

137. The binding agent of any of the preceding claims, wherein the binding agent preferentially binds human TROP2 over rat TROP2 or has a better affinity for human TROP2 over rat TROP2.

138. The binding agent of any one of the preceding claims, wherein the antigen binding domain is or comprises a heavy chain variable region.

139. The binding agent of any one of the preceding claims, wherein the binding agent comprises two heavy chain variable regions.

140. The binding agent of claim 139, wherein each heavy chain variable regions are on separate heavy chains.

141. The binding agent of any one of the preceding claims, wherein the antigen binding domain is or comprises a heavy chain.

142. The binding agent of any one of the preceding claims, wherein the binding agent comprises two heavy chains.

143. The binding agent of any one of the preceding claims, wherein the binding agent is a single domain antibody comprising two heavy chains and wherein each heavy chain comprises an identical antigen binding domain.

144. The binding agent of claims 110 or 143, wherein the single domain antibody comprises at least one heavy chain comprising:

a. a variable region having an amino acid sequence as set forth in SEQ ID NO:22;

b. a variable region having an amino acid sequence as set forth in SEQ ID NO:23;

c. a variable region having an amino acid sequence as set forth in SEQ ID NO:24;

d. a variable region having an amino acid sequence as set forth in SEQ ID NO:25;

e. a variable region having an amino acid sequence as set forth in SEQ ID NO:26;

f. a variable region having an amino acid sequence as set forth in SEQ ID NO:27;

g. a variable region having an amino acid sequence as set forth in SEQ ID NO:28

h. a variable region having an amino acid sequence as set forth in any one of SEQ ID Nos:73 to 114;

i. an amino acid sequence as set forth in SEQ ID NO:29;

j. an amino acid sequence as set forth in SEQ ID NO:30;

k. an amino acid sequence as set forth in SEQ ID NO:31;

l. an amino acid sequence as set forth in SEQ ID NO:32;

m. an amino acid sequence as set forth in SEQ ID NO:33;

n. an amino acid sequence as set forth in SEQ ID NO:34;

o. an amino acid sequence as set forth in SEQ ID NO:35;

p. an amino acid sequence as set forth in SEQ ID NO:44 or,

q. an amino acid sequence as set forth in SEQ ID NO:45.

145. The binding agent of any one of claims 139 to 144, wherein each heavy chains comprise a constant region of a human IgG or a portion thereof.

146. The binding agent of claim 145, wherein the constant region or portion thereof is from a human IgG1.

147. The binding agent of claim 145, wherein the constant region or portion thereof is from a human IgG4.

148. The binding agent of any of the preceding claims, wherein the binding agent comprises partially humanized framework regions.

149. The binding agent of any of the preceding claims, wherein the binding agent comprises fully humanized framework regions.

150. The binding agent of any of the preceding claims, wherein all framework regions are humanized.

151. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains has ADCP activity towards cells expressing human TROP2.

152. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains has ADCP activity towards cells expressing human TROP2 and CD47.

153. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains has ADCC activity towards cells expressing human TROP2.

154. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains is internalized in cells expressing human TROP2.

155. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains is capable of inhibiting the growth of tumor cells expressing human TROP2.

156. The binding agent of any one of the preceding claims, wherein the binding agent or at least one of the one or more antigen binding domains is capable of inhibiting the growth of tumor cells expressing human TROP2 and CD47.

157. The binding agent of any one of the preceding claims, wherein the binding agent comprises one or more antigen binding domains that specifically binds to CD47.

158. The binding agent of claim 157, wherein the antigen binding domains that specifically binds to CD47 comprises:

a. a CDRH1 having the amino acid sequence set forth in SEQ ID NO156, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:157 and/or a CDRH3 having the amino acid sequence set forth in SEQ ID NO:158, or

b. a CDRH1 having the amino acid sequence set forth in SEQ ID NO159, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:160 and/or a CDRH3 having the amino acid sequence set forth in SEQ ID NO:161.

159. The binding agent of claim 158, wherein the antigen binding domains that specifically binds to CD47 comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:155.

160. The binding agent of claim 158 or 159, wherein the binding agent comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:150 or 151.

161. A single domain antibody that specifically binds to at least one epitope comprising amino acid residues of the extracellular domain (ECD) of trophoblast cell surface antigen-2 (TROP2).

162. The single domain antibody of any one of claims 110, 143, 144 or 161, wherein the single domain antibody specifically binds to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-146 or a fragment thereof.

163. The single domain antibody of claim 162, wherein the single domain antibody specifically binds to a polypeptide comprising a human TROP2 amino acid sequence consisting of amino acid residues 27-73 or a fragment thereof.

164. The single domain antibody of claim 162, wherein the fragment thereof comprises amino acid residues 27-56, 27-66, 47-56, 60-66 or 47-66 of human TROP2.

165. The single domain antibody of any one of claims 161 to 164, wherein the single domain antibody does not significantly bind to or is not capable of binding to a) a polypeptide that comprises a human TROP2 amino acid sequence consisting of amino acid residues 147-274 or a fragment thereof, b) a human TROP2 amino acid sequence comprising deletion of amino acid residues 27-36, 27-44, 27-56, 27-66 or 27-73 or has a reduced binding to said polypeptide compared to the full human TROP2 polypeptide and/or a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 149.

166. The single domain antibody of any one of claims 161 to 165, wherein the single domain antibody comprises a heavy chain complementarity determining region 3 (CDRH3) having the amino acid sequence set forth in SEQ ID NO:51 and a framework region 2 (FR2) having the amino acid sequence set forth in SEQ ID NO:116.

167. A single domain antibody that competes with the binding agent of any one of claims 1 to 142 or the single domain antibody of any one of claims 161 to 166.

168. A binding agent that competes with the binding agent or single domain antibody of any of the preceding claims or that comprises one or more antigen binding domains that competes with the binding agent or single domain antibody of any of the preceding claims.

169. The binding agent or single domain antibody of claim 167 or 168, wherein the competing binding agent or competing single domain antibody comprises the amino acid sequence of the CDR3 set forth in SEQ ID NO:126, the amino acid sequence of the FR2 set forth in SEQ ID NO:121.

170. The binding agent or single domain antibody of any one of claims 167 to 169, wherein the competing binding agent or competing single domain antibody comprises an amino acid sequence as set forth in any one of claims 33-58.

171. The binding agent or single domain antibody of any one of claims 167 to 170, wherein the competing binding agent or competing single domain antibody comprises:

a. the amino acid sequence of the CDR3 set forth in SEQ ID NO:54 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:52 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:53;

b. the amino acid sequence of the CDR3 set forth in SEQ ID NO:48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:47;

c. the amino acid sequence of the CDR3 set forth in SEQ ID NO:58 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:56 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:57;

d. the amino acid sequence of the CDR3 set forth in SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:59 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:60;

e. the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50,

f. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

g. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

h. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

i. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:119 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 47;

j. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 124;

k. the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:55 and/or the amino acid sequence of the CDR2 set forth in 123;

l. the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50;

m. the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50;

n. amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50 or,

o. amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:120 and optionally the amino acid sequence of the CDR1 set forth in SEQ ID NO:62 and/or the amino acid sequence of the CDR2 set forth in SEQ ID NO:50.

172. The binding agent or single domain antibody of any one of claims 167 to 170, wherein the competing binding agent or competing single domain antibody comprises:

a. the amino acid sequence of the CDR1 set forth in SEQ ID NO:52, the amino acid sequence of the CDR2 set forth in SEQ ID NO:53, the amino acid sequence of the CDR3 set forth in SEQ ID NO:54 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

b. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in SEQ ID NO:47, the amino acid sequence of the CDR3 set forth in SEQ ID NO:48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

c. the amino acid sequence of the CDR1 set forth in SEQ ID NO:56, the amino acid sequence of the CDR2 set forth in SEQ ID NO:57, the amino acid sequence of the CDR3 set forth in SEQ ID NO:58 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

d. the amino acid sequence of the CDR1 set forth in SEQ ID NO:59, the amino acid sequence of the CDR2 set forth in SEQ ID NO:60, the amino acid sequence of the CDR3 set forth in SEQ ID NO:61 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

e. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

f. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

g. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set

forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

h. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118;

i. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 47, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:119;

j. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 124, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

k. the amino acid sequence of the CDR1 set forth in SEQ ID NO:55, the amino acid sequence of the CDR2 set forth in 123, the amino acid sequence of the CDR3 set forth in SEQ ID NO: 48 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

l. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:116;

m. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, the amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:117;

n. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, amino acid sequence of the

CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:118 or,

o. the amino acid sequence of the CDR1 set forth in SEQ ID NO:62, the amino acid sequence of the CDR2 set forth in SEQ ID NO:50, amino acid sequence of the CDR3 set forth in SEQ ID NO:51 and the amino acid sequence of the FR2 set forth in SEQ ID NO:120.

173. The binding agent or single domain antibody of any one of claims 167 to 172, wherein the competing binding agent or competing single domain antibody has an affinity of $\leq 10^{-6}$ M for human TROP2.

174. The binding agent or single domain antibody of any one of claims 167 to 173, wherein the competing binding agent or competing single domain antibody has anti-tumor activity.

175. The binding agent or single domain antibody of any of the preceding claims, wherein the binding agent or single domain antibody competes with a single domain antibody comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:26.

176. The binding agent or single domain antibody of any of the preceding claims, wherein the binding agent or single domain antibody competes with a single domain antibody comprising a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:33.

177. The binding agent or single domain antibody of any of the preceding claims, wherein the binding agent or single domain antibody comprises an Fc region or portion thereof that comprises Fc modification(s) or not.

178. The binding agent or single domain antibody of any of the preceding claims, wherein the binding agent or single domain antibody comprises a Fc region or portion thereof that lacks core fucose residues or that is afucosylated.

179. A composition comprising the binding agent or single domain antibody of any of the preceding claims.

180. A pharmaceutical composition comprising the binding agent or single domain antibody of any of the preceding claims and a pharmaceutically acceptable carrier.

181. A nucleic acid or set of nucleic acids encoding the binding agent or single domain antibody of any of the preceding claims.

182. A vector comprising the nucleic acid of claim 181.

183. A cell expressing the binding agent or single domain antibody of any of the preceding claims.

184. A cell comprising the nucleic acid of claim 181 or the vector of claim 182.

185. A kit comprising the binding agent or single domain antibody of any of the preceding claims.

186. A kit comprising the nucleic acid of claim 181, or the vector of claim 182 or the cell of claim 183 or 184.

187. A method of treating a disorder or disease comprising administering the binding agent or single domain antibody of any one of the preceding claims or a pharmaceutical composition comprising the binding agent or single domain antibody of any one of the preceding claims.

188. The method of claim 187, wherein the disorder or disease is associated with TROP2 expression or overexpression.

189. The method of claim 188, wherein the disorder or disease is cancer, solid tumor, epithetial cancer, lung cancer, metastatic lung cancer, small cell lung cancer, non-small cell lung cancer, myeloma, prostate cancer, breast cancer, triple negative breast cancer, rectal cancer, pancreatic cancer, glioblastoma, cervical cancer, colorectal cancer, gastric cancer, ovarian cancer, thyroid cancer, stomach cancer, urinary bladder cancer, cancer of the uterus, esophegeal cancer, blood cancer, or head and neck cancer.

190. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered at a dose of approximately between 0.05 to 150 mg/kg.

191. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered at a dose of approximately between 0.05 to 100 mg/kg.

192. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered at a dose of approximately between 0.05 to 50 mg/kg.

193. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered at a dose of approximately between 0.05 to 30 mg/kg.

194. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered at a dose of approximately between 0.05 to 10 mg/kg.

195. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered once weekly.

196. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered once every two weeks.

197. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered once every three weeks.

198. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered once every month.

199. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered by intra-veinous injection.

200. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered by intra-muscular injection.

201. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered by infusion.

202. The method of any of the preceding claims, wherein the binding agent or single domain antibody is a naked single domain antibody.

203. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered to a subject having a tumor or tumor cells expressing TROP2 and optionally expressing macrophages marker.

204. The method of any of the preceding claims, wherein the binding agent or single domain antibody is administered to a subject having a tumor or tumor cells expressing TROP2 and a macrophage marker.

205. The method of claim 203 or 204, wherein the macrophage marker is CD68, CD80, or CD11c.

206. A method of making the binding agent or single domain antibody of any one of the preceding claims wherein the method comprises transforming cells with one or more vectors comprising the nucleic acid or set of nucleic acid of claim 181.

207. The method of claim 206, wherein the binding agent or single domain antibody is purified and/or isolated.

208. The method of claim 207, wherein the method further comprises conjugating the binding agent or single domain antibody with a therapeutic moiety, detectable moiety, a protein allowing an extended half-life or is attached to nanoparticle.

209. Use of the binding agent or single domain antibody of any of the preceding claims for detecting TROP2 in tumor cells or tumor tissue.

210. The use as defined in claim 209, wherein the binding agent or single domain antibody comprises an amino acid sequence having at least 80% identity with the variable region of any one of SEQ ID NO:152, SEQ ID NO:153 or SEQ ID NO:154.

211. The use as defined in claim 209, wherein the binding agent or single domain antibody comprises an amino acid sequence identical to the variable region of any one of SEQ ID NO:152, SEQ ID NO:153 or SEQ ID NO:154.

212. The use as defined in claim 209, wherein the binding agent or single domain antibody comprises an amino acid sequence identical to any one of SEQ ID NO:152, SEQ ID NO:153 or SEQ ID NO:154.

213. A method of treating a subject having cancer, the method comprising administering a drug targeting human TROP2 to a subject having a tumor expressing TROP2 and expressing one or more macrophage markers.

214. The method of claim 213, wherein the drug is a binding agent or single domain antibody or an antigen binding fragment thereof as defined in any one of claims 1-178.

215. The method of claim 213, wherein the drug is an antibody or an antigen binding fragment thereof that specifically binds to TROP2.

216. The method of claim 214 or 215, wherein the binding agent, single domain antibody or antigen binding fragment thereof or the antibody or an antigen binding fragment thereof is conjugated.

217. The method of any one of claims 213 to 216, wherein the macrophage marker is CD68, CD80, or CD11c.

218. A kit comprising a binding agent that specifically binds to TROP2 and a binding agent that binds to a macrophage marker.

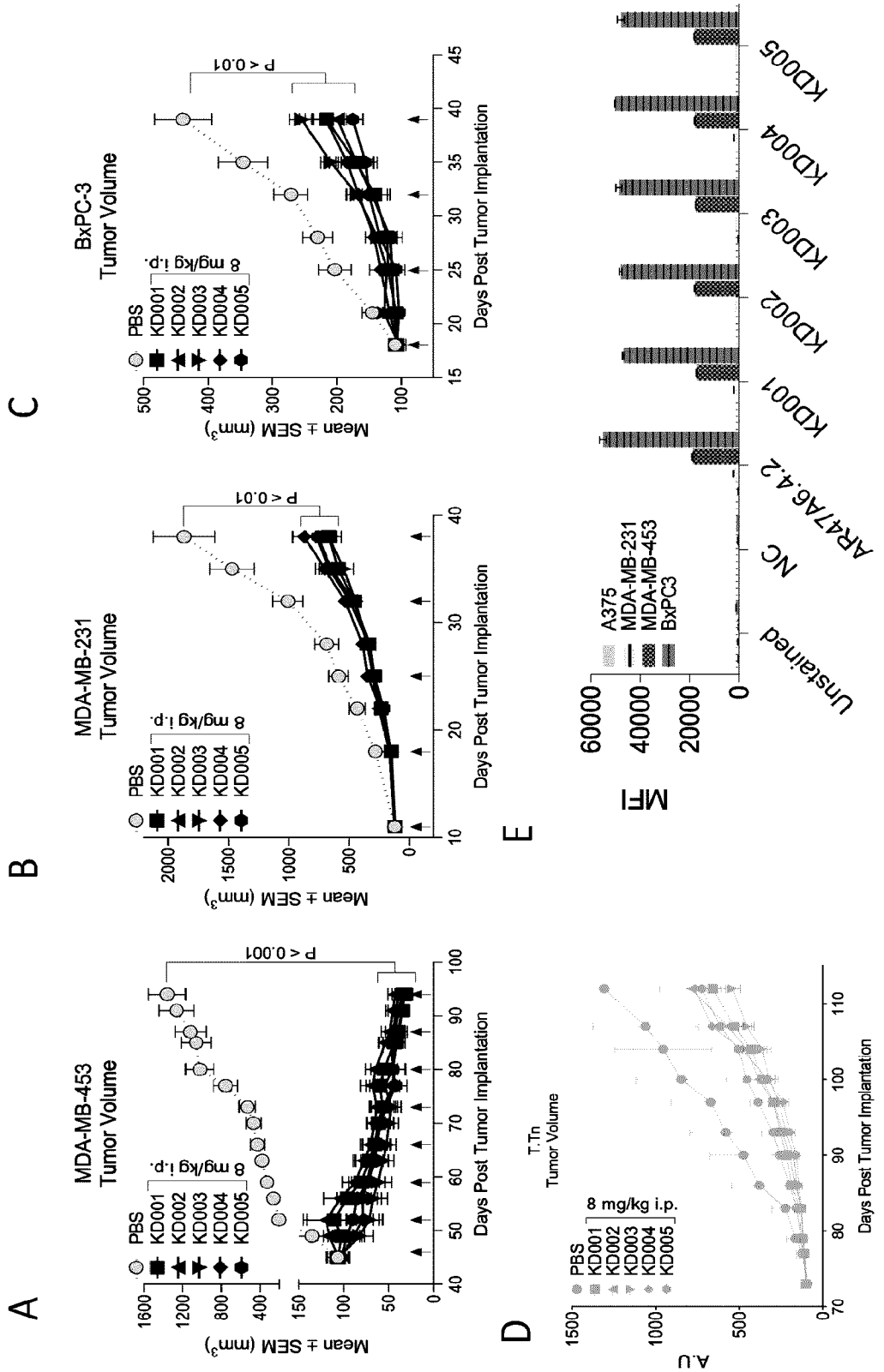
219. The kit of claim 218, wherein the binding agent that specifically binds to TROP2 is an antibody or an antigen binding fragment thereof.

220. The kit of claim 218 or 219, wherein the macrophage marker is a pan macrophage marker.

221. The kit of any one of claims 218 to 220, wherein the binding agent that binds to a macrophage marker is an antibody or an antigen binding fragment thereof that binds CD68, CD80, or CD11c.

222. The kit of any one of claims 220 to 221, wherein the kit is for use as companion diagnostic for the treatment of cancer.

Figure 1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KD002
KD003
KD004
KD005
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P < 0.001
Mean ± SEM (mm³)
Days Post Tumor Implantation
B
MDA-MB-231
Tumor Volume
P < 0.01
C
BxPC-3
Tumor Volume
D
T.Tn
Tumor Volume
A.U
E
MFI
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KD003
KD004
KD005

Figure 2

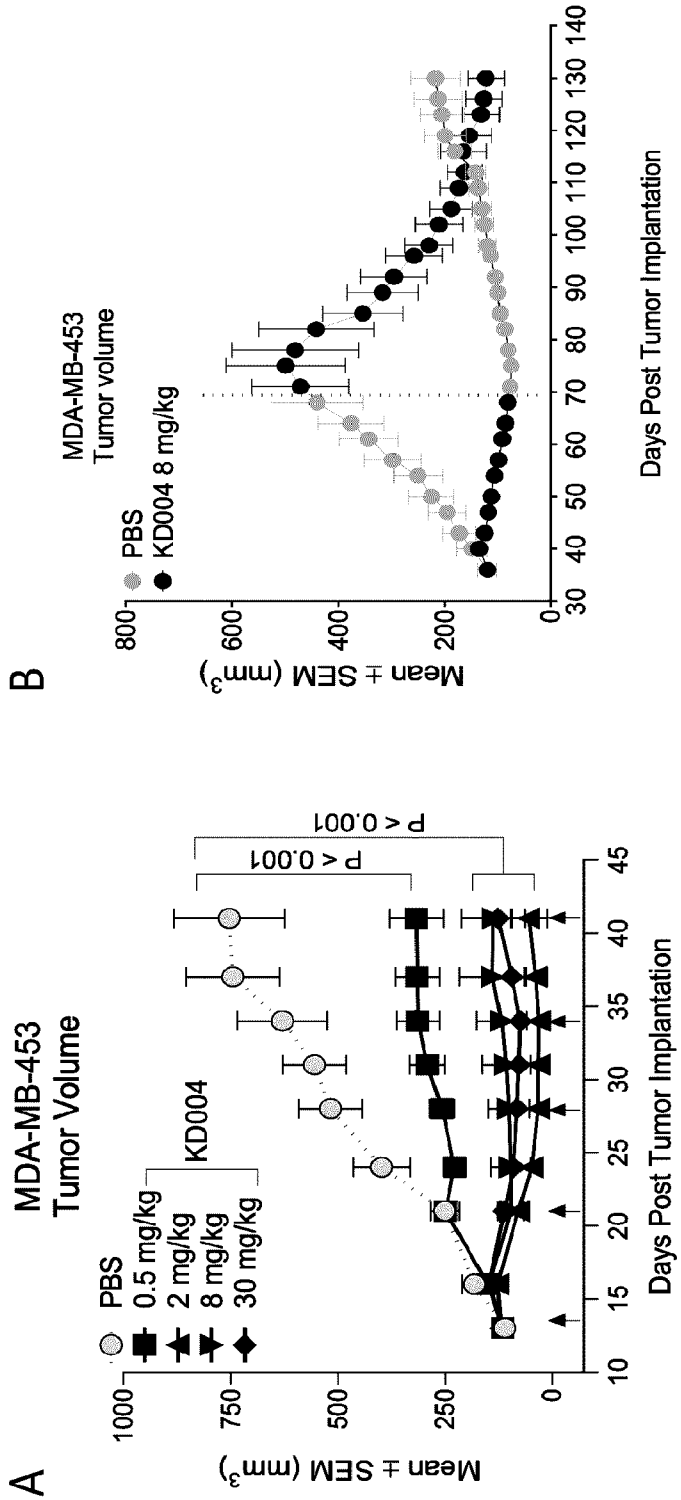
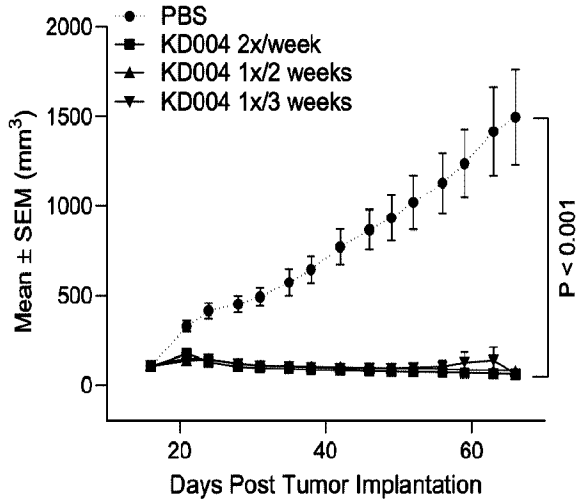
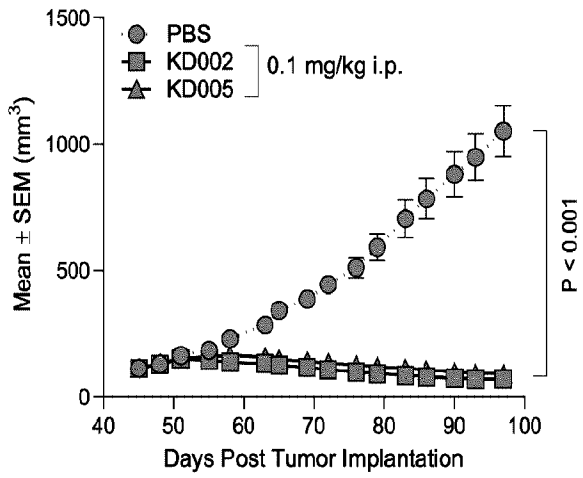

A
MDA-MB-453
Tumor Volume
PBS
0.5 mg/kg
2 mg/kg
8 mg/kg
30 mg/kg
KD004
P < 0.001
P < 0.001
Mean ± SEM (mm³)
Days Post Tumor Implantation
B
MDA-MB-453
Tumor volume
PBS
KD004 8 mg/kg
Mean ± SEM (mm³)
Days Post Tumor Implantation

Figure 2

C



D



E

Mouse model	Dosage (mg/kg)	Route	Cmax (ug/ml)	tmax (hours)	AUC (ug/ml*hr)	$t_{1/2}$ (hours)
NCG	30	I.V	1096.583	0.08	13108	61.2
	8	I.V	320.221	0.08	5174.79	62.1
	2	I.V	64.89	0.08	581.58	22.9

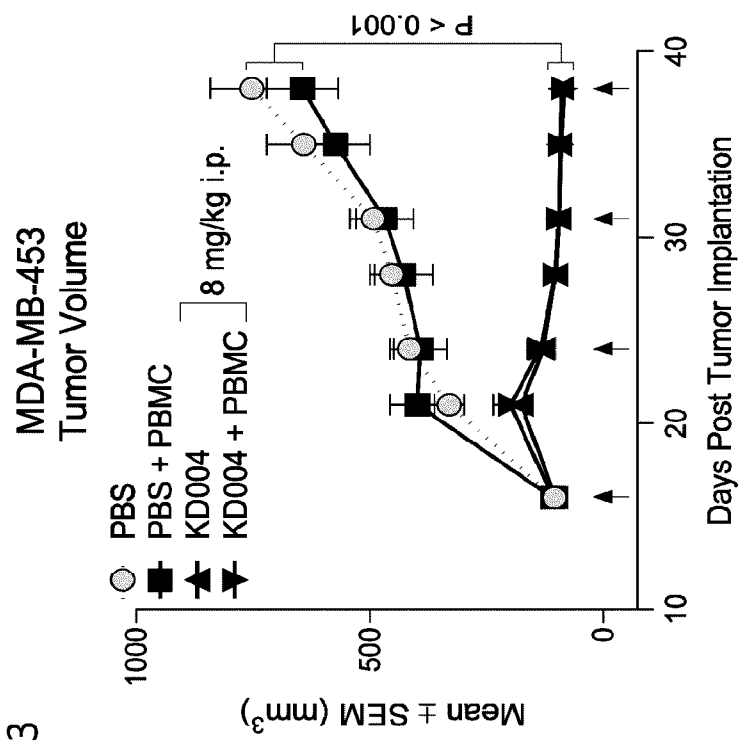

MDA-MB-453
Tumor Volume
PBS
PBS + PBMC
KD004
KD004 + PBMC
8 mg/kg i.p.
P < 0.001
Mean ± SEM (mm³)
1000
500
0
10
20
30
40
Days Post Tumor Implantation

Figure 3

Figure 4

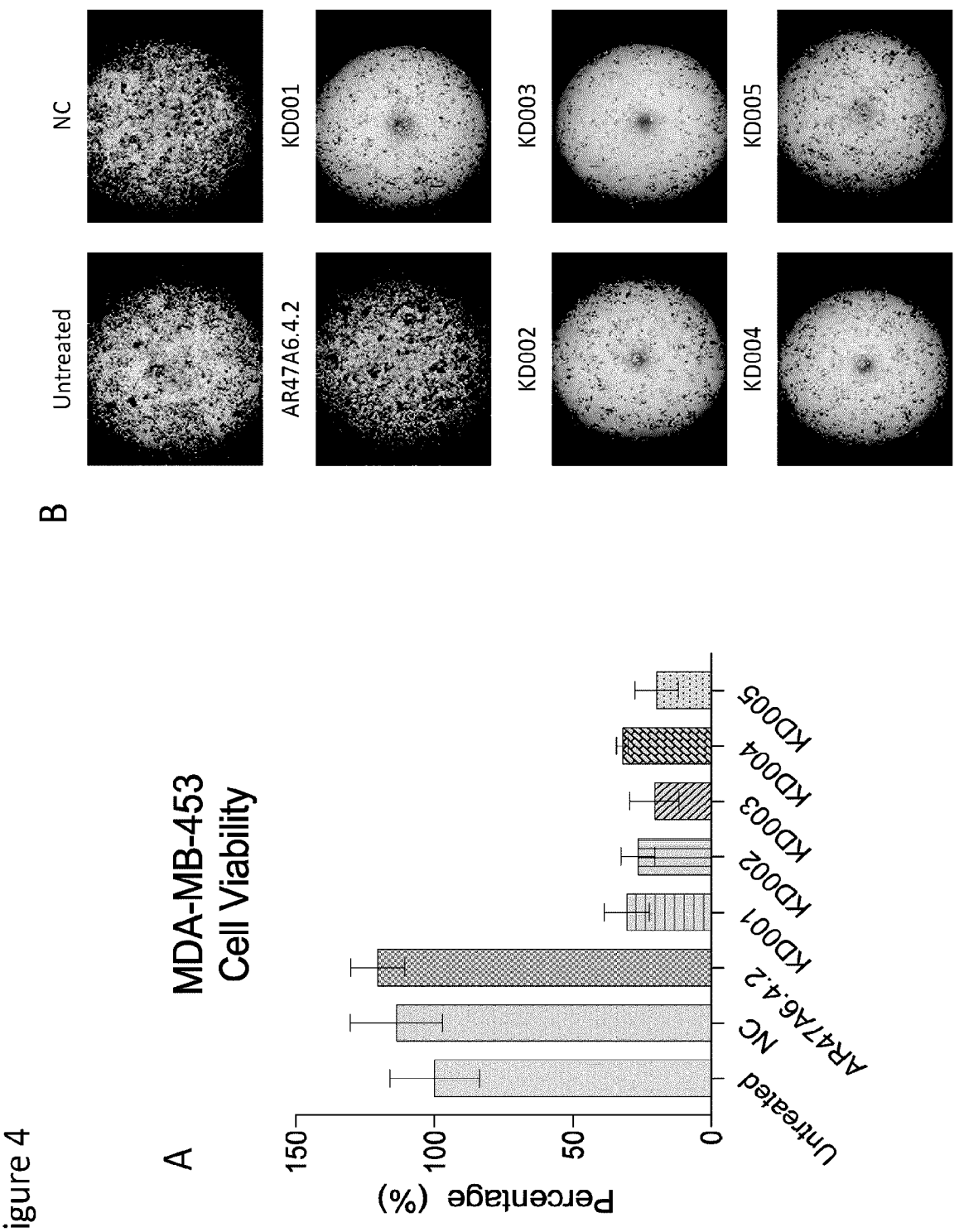
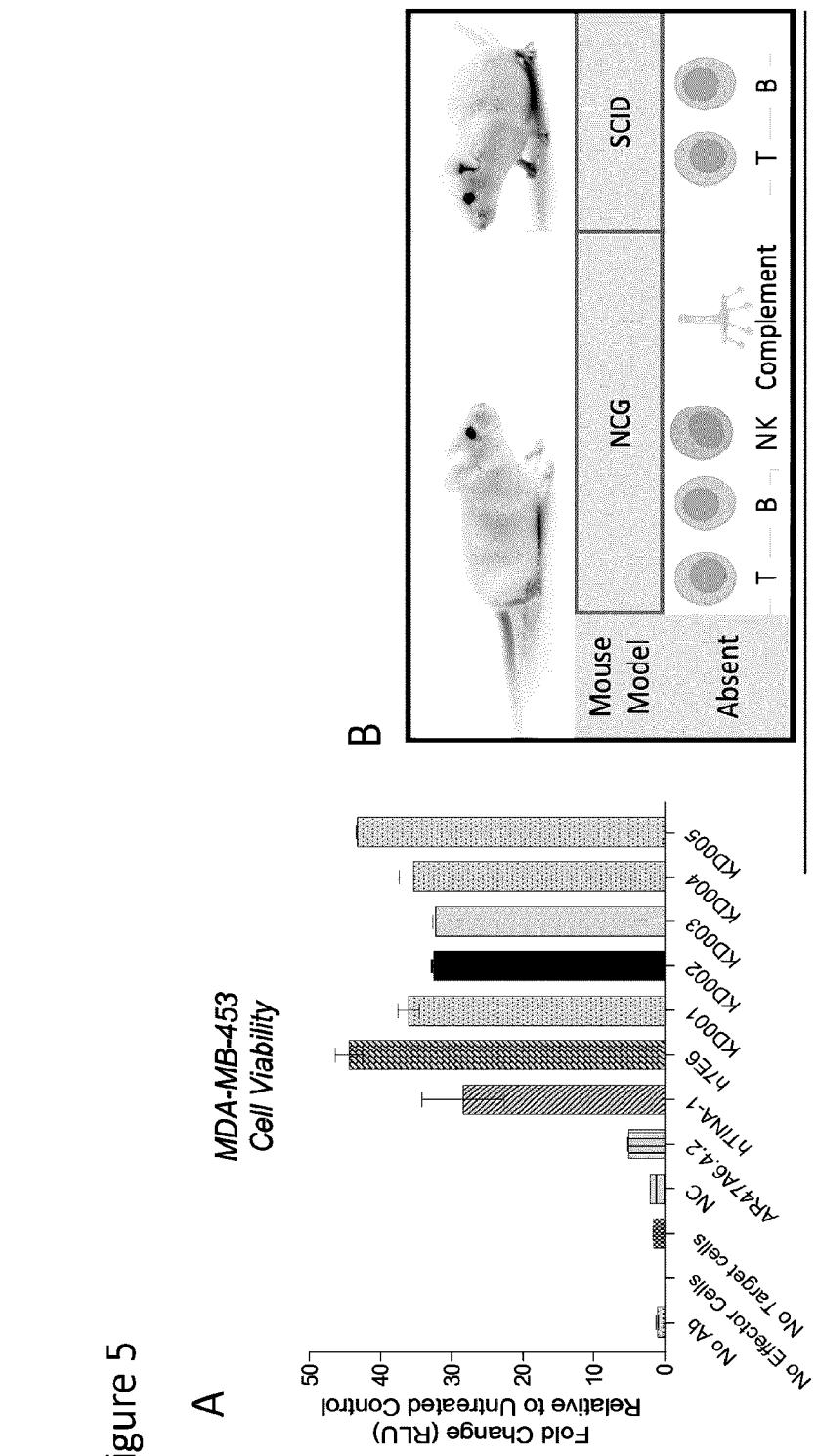

A
MDA-MB-453
Cell Viability
Percentage (%)
150
100
50
0
Untreated
NC
AR47A6.4.2
KD001
KD002
KD003
KD004
KD005
B
Untreated
NC
AR47A6.4.2
KD001
KD002
KD003
KD004
KD005

Figure 5



Cancer cell line	Model	Group	Dose (mg/kg)	freq. (week)	TGI (%)	P-val (TT)
MDA-MB-453	SCID	KD004	8	1	108.86	0.001
	NCG	KD004	8	1	105.12	0.004
MDA-MB-231	SCID	KD002	8	1	62.65	0.004
	NCG	KD002	8	1	40.38	0.002

Figure 6

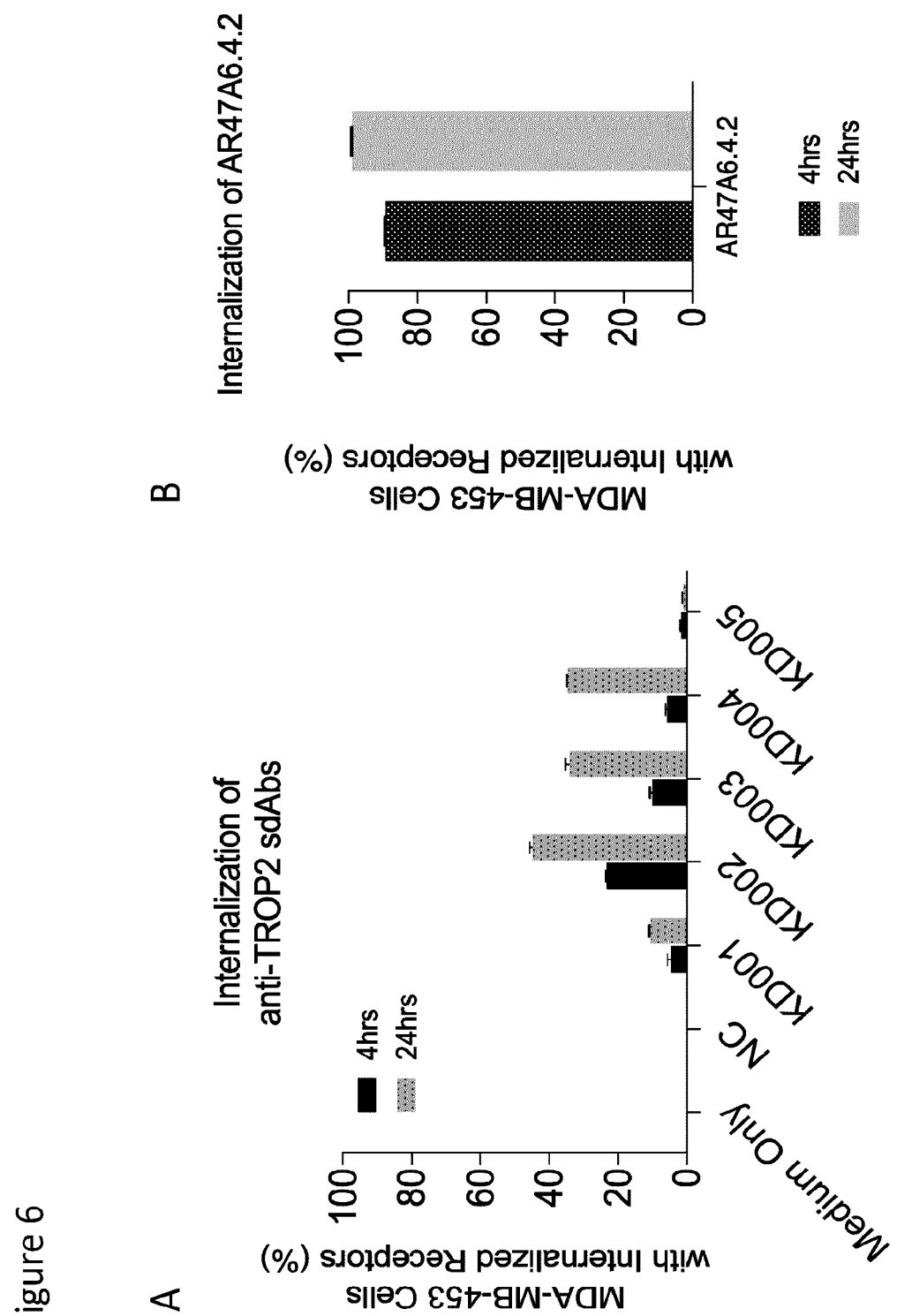
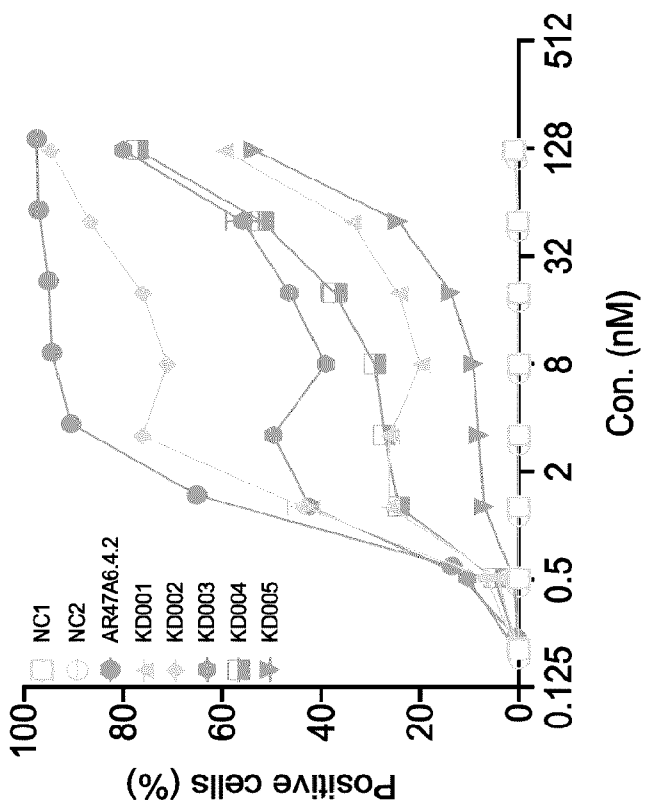

A
Internalization of anti-TROP2 sdAbs
4hrs
24hrs
MDA-MB-453 Cells with Internalized Receptors (%)
100
80
60
40
20
0
Medium Only
NC
KD001
KD002
KD003
KD004
KD005
B
Internalization of AR47A6.4.2
MDA-MB-453 Cells with Internalized Receptors (%)
AR47A6.4.2
4hrs
24hrs

Figure 6

C


Positive cells (%)
100
80
60
40
20
0
0.125
0.5
2
8
32
128
512
Con. (nM)
NC1
NC2
AR47A6.4.2
KD001
KD002
KD003
KD004
KD005

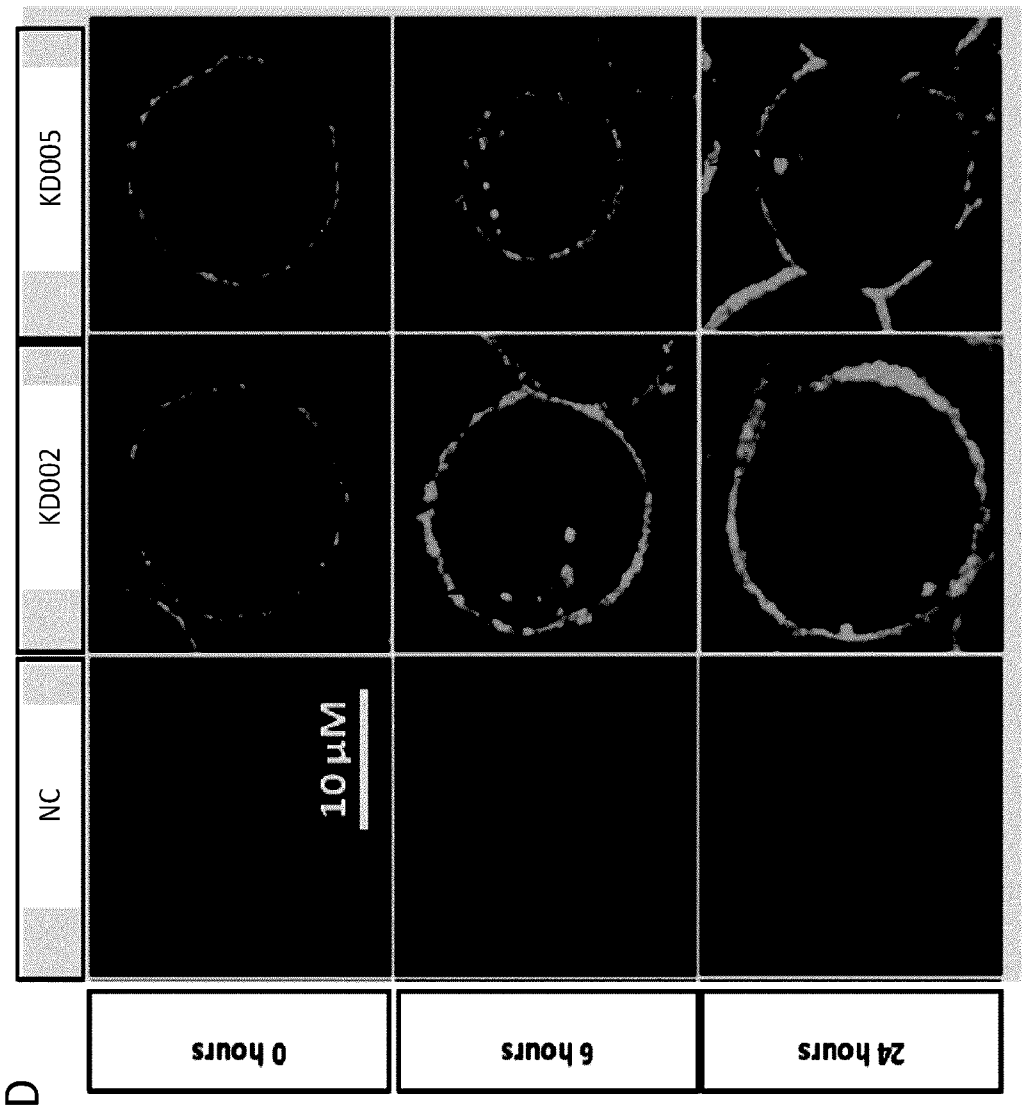
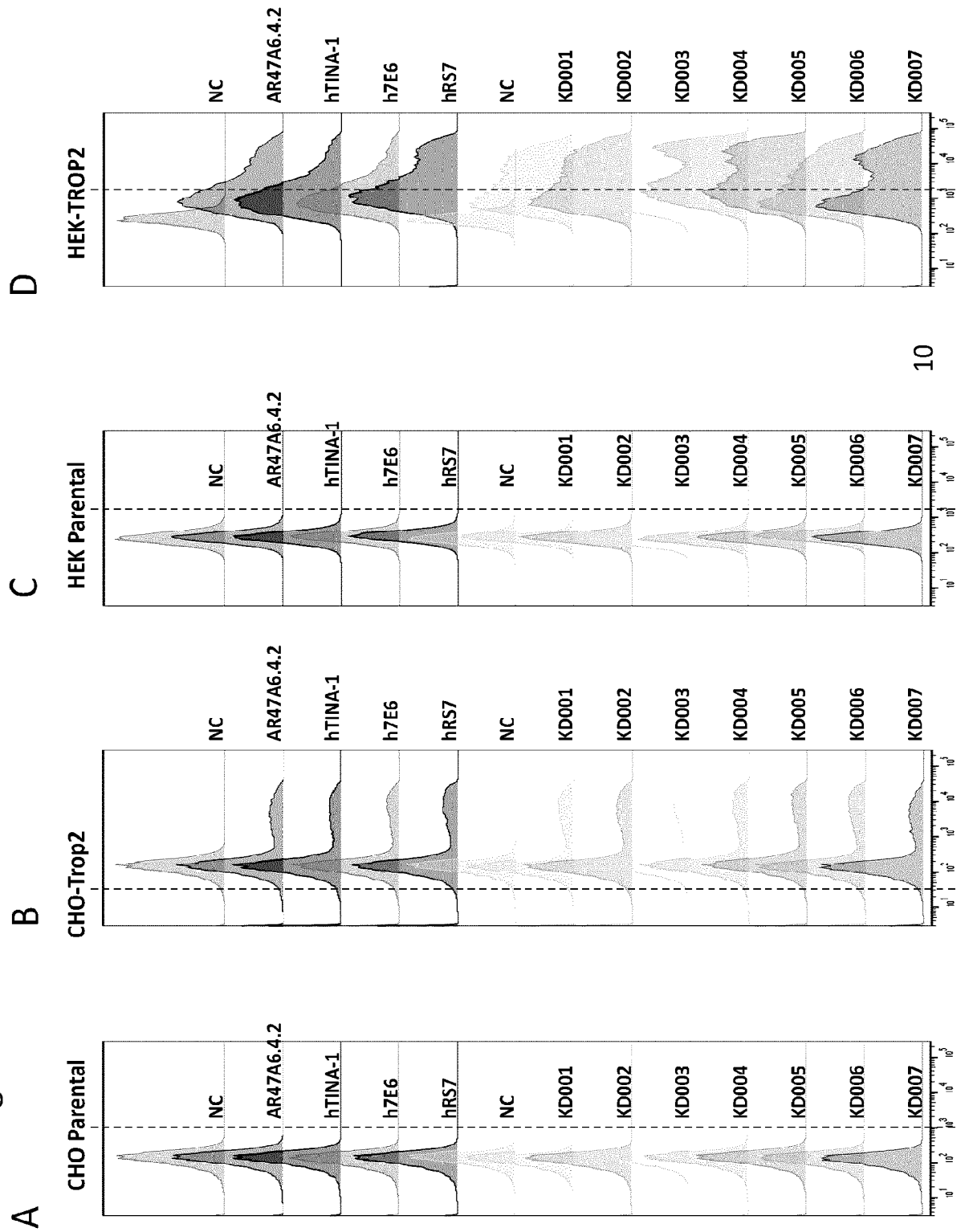


Figure 6

Figure 7


A
CHO Parental
B
CHO-Trop2
C
HEK Parental
D
HEK-TROP2
NC
AR47A6.4.2
hTINA-1
h7E6
hRS7
KD001
KD002
KD003
KD004
KD005
KD006
KD007
10

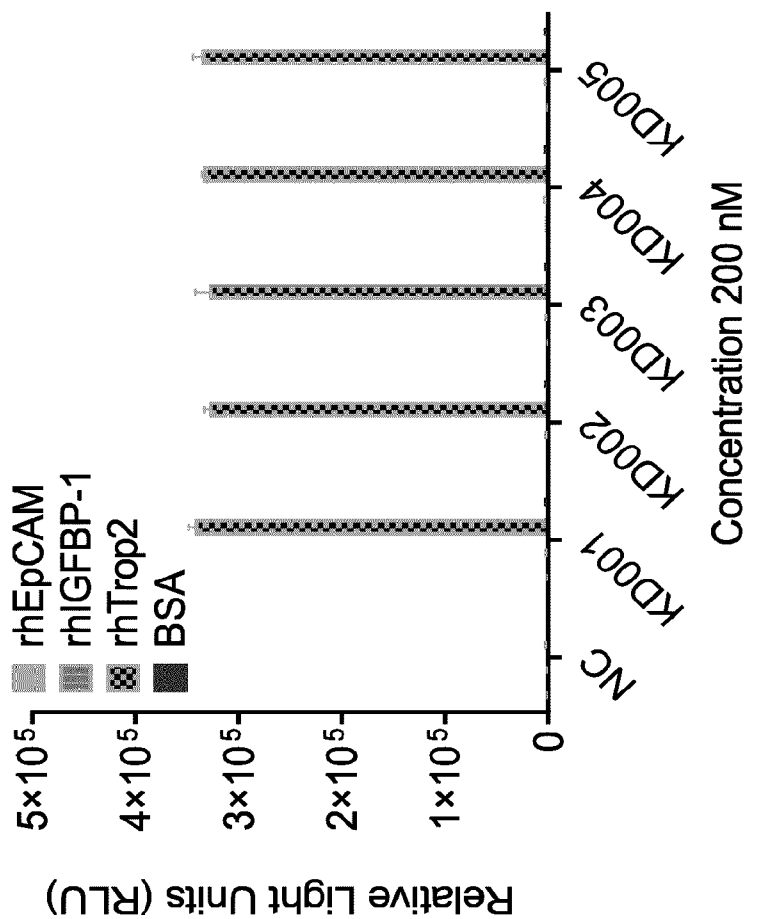

rhEpCAM
rhIGFBP-1
rhTrop2
BSA
Relative Light Units (RLU)
5×10^5
4×10^5
3×10^5
2×10^5
1×10^5
0
NC
KD001
KD002
KD003
KD004
KD005
Concentration 200 nM

Figure 7E

Figure 8

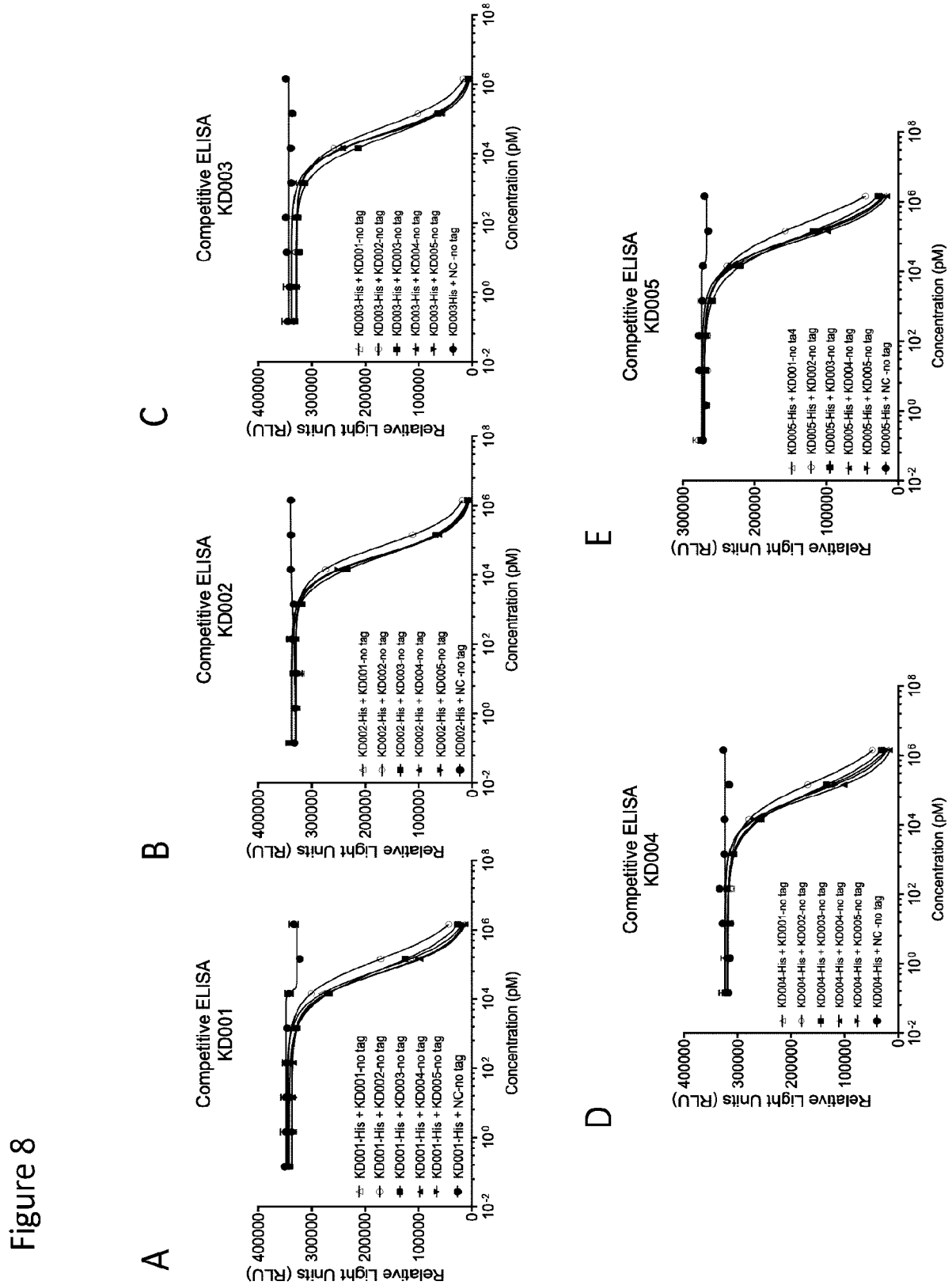

A
Competitive ELISA
KD001
Relative Light Units (RLU)
Concentration (pM)
KD001-His + KD001-no tag
KD001-His + KD002-no tag
KD001-His + KD003-no tag
KD001-His + KD004-no tag
KD001-His + KD005-no tag
KD001-His + NC-no tag
B
Competitive ELISA
KD002
Relative Light Units (RLU)
Concentration (pM)
KD002-His + KD001-no tag
KD002-His + KD002-no tag
KD002-His + KD003-no tag
KD002-His + KD004-no tag
KD002-His + KD005-no tag
KD002-His + NC -no tag
C
Competitive ELISA
KD003
Relative Light Units (RLU)
Concentration (pM)
KD003-His + KD001-no tag
KD003-His + KD002-no tag
KD003-His + KD003-no tag
KD003-His + KD004-no tag
KD003-His + KD005-no tag
KD003His + NC -no tag
D
Competitive ELISA
KD004
Relative Light Units (RLU)
Concentration (pM)
KD004-His + KD001-nc tag
KD004-His + KD002-nc tag
KD004-His + KD003-nc tag
KD004-His + KD004-nc tag
KD004-His + KD005-nc tag
KD004-His + NC -no tag
E
Competitive ELISA
KD005
Relative Light Units (RLU)
Concentration (pM)
KD005-His + KD001-no tag4
KD005-His + KD002-no tag
KD005-His + KD003-no tag
KD005-His + KD004-no tag
KD005-His + KD005-no tag
KD005-His + NC -no tag

Figure 9

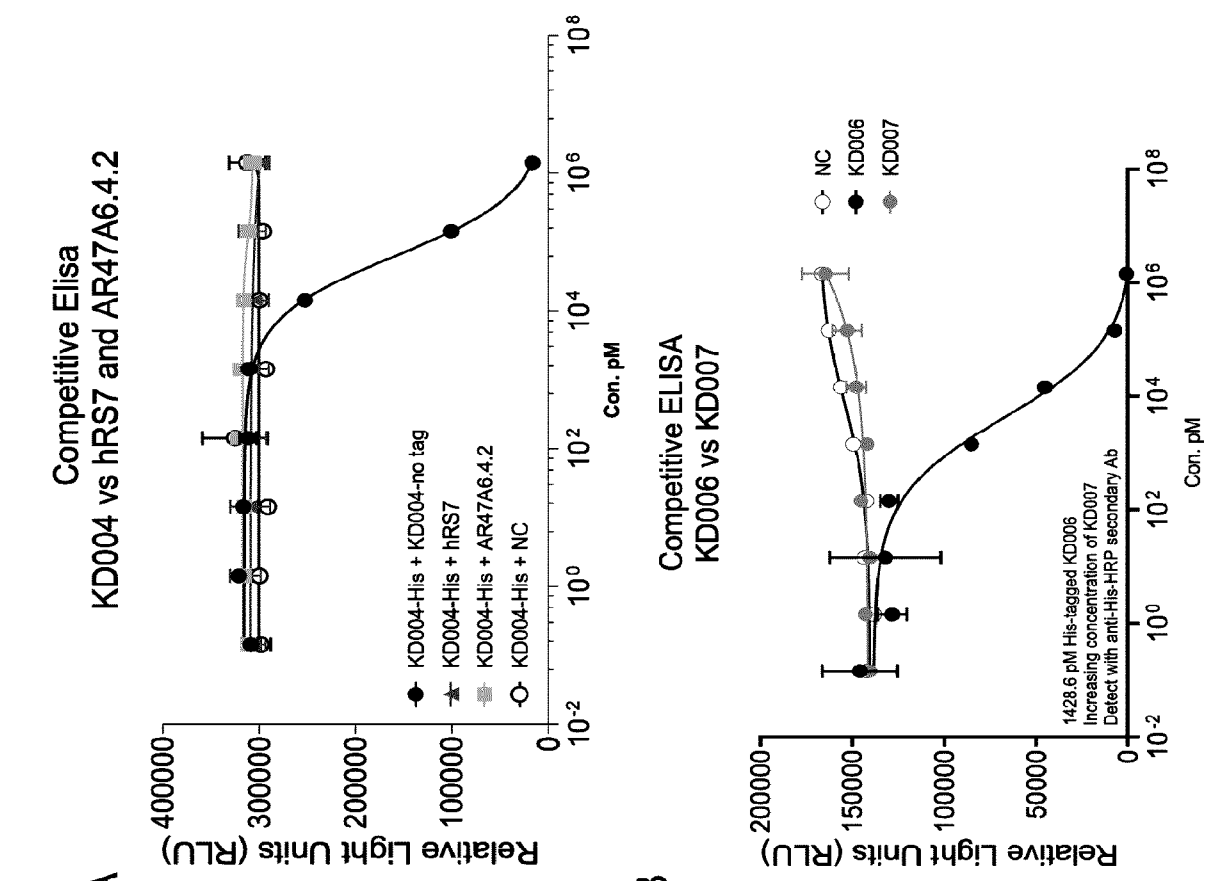

A
Competitive Elisa
KD004 vs hRS7 and AR47A6.4.2
Relative Light Units (RLU)
400000
300000
200000
100000
0
KD004-His + KD004-no tag
KD004-His + hRS7
KD004-His + AR47A6.4.2
KD004-His + NC
10-2
100
102
104
106
108
Con. pM
B
Competitive ELISA
KD006 vs KD007
Relative Light Units (RLU)
200000
150000
100000
50000
0
NC
KD006
KD007
1428.6 pM His-tagged KD006
Increasing concentration of KD007
Detect with anti-His-HRP secondary Ab
10-2
100
102
104
106
108
Con. pM

Figure 10

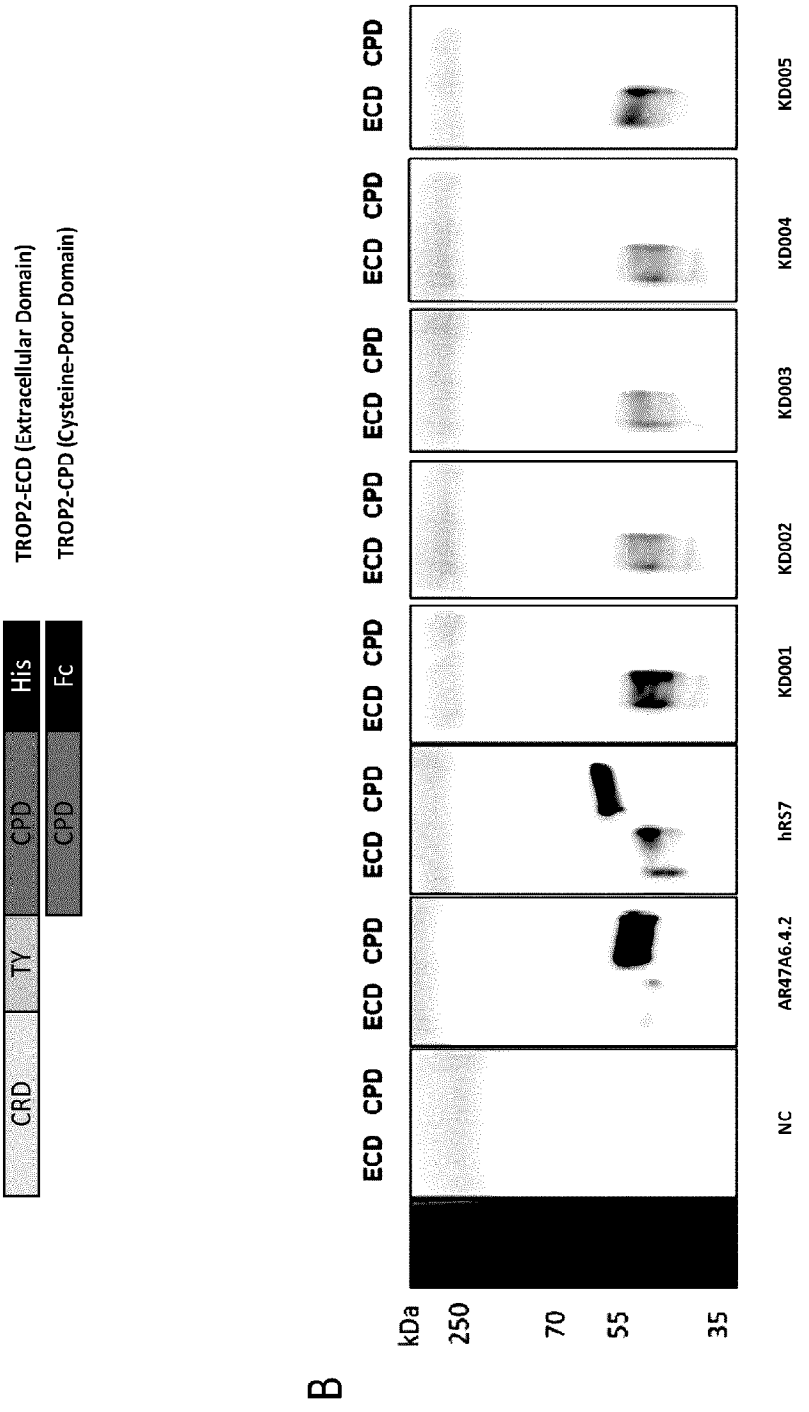

A
CRD
TY
CPD
His
CPD
Fc
TROP2-ECD (Extracellular Domain)
TROP2-CPD (Cysteine-Poor Domain)
B
kDa
250
70
55
35
ECD CPD
NC
ECD CPD
AR47A6.4.2
ECD CPD
hRS7
ECD CPD
KD001
ECD CPD
KD002
ECD CPD
KD003
ECD CPD
KD004
ECD CPD
KD005

Figure 11

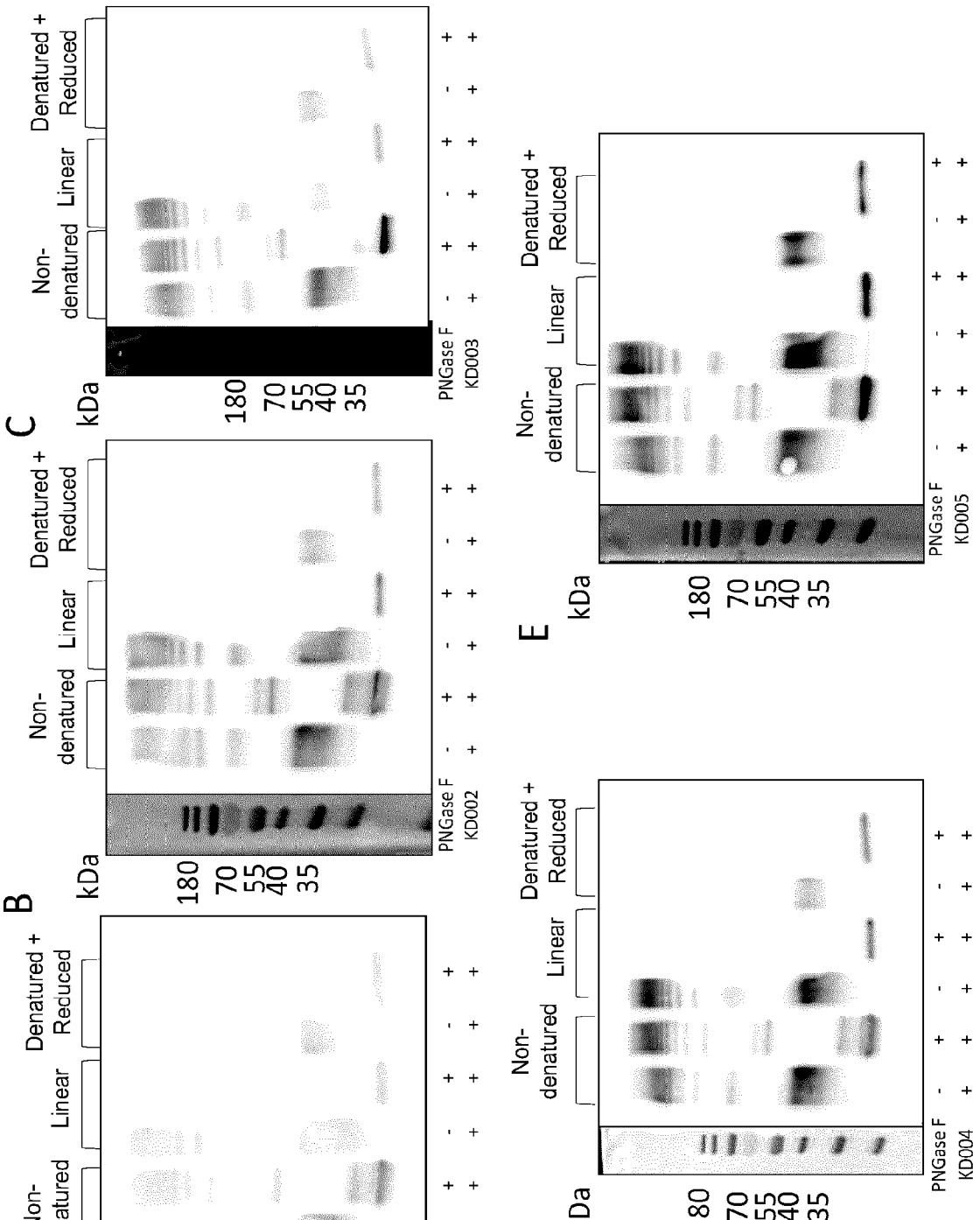

A
B
C
D
E
kDa
180
70
55
40
35
Non-denatured
Linear
Denatured + Reduced
PNGase F
KD001
KD002
KD003
KD004
KD005

Figure 12

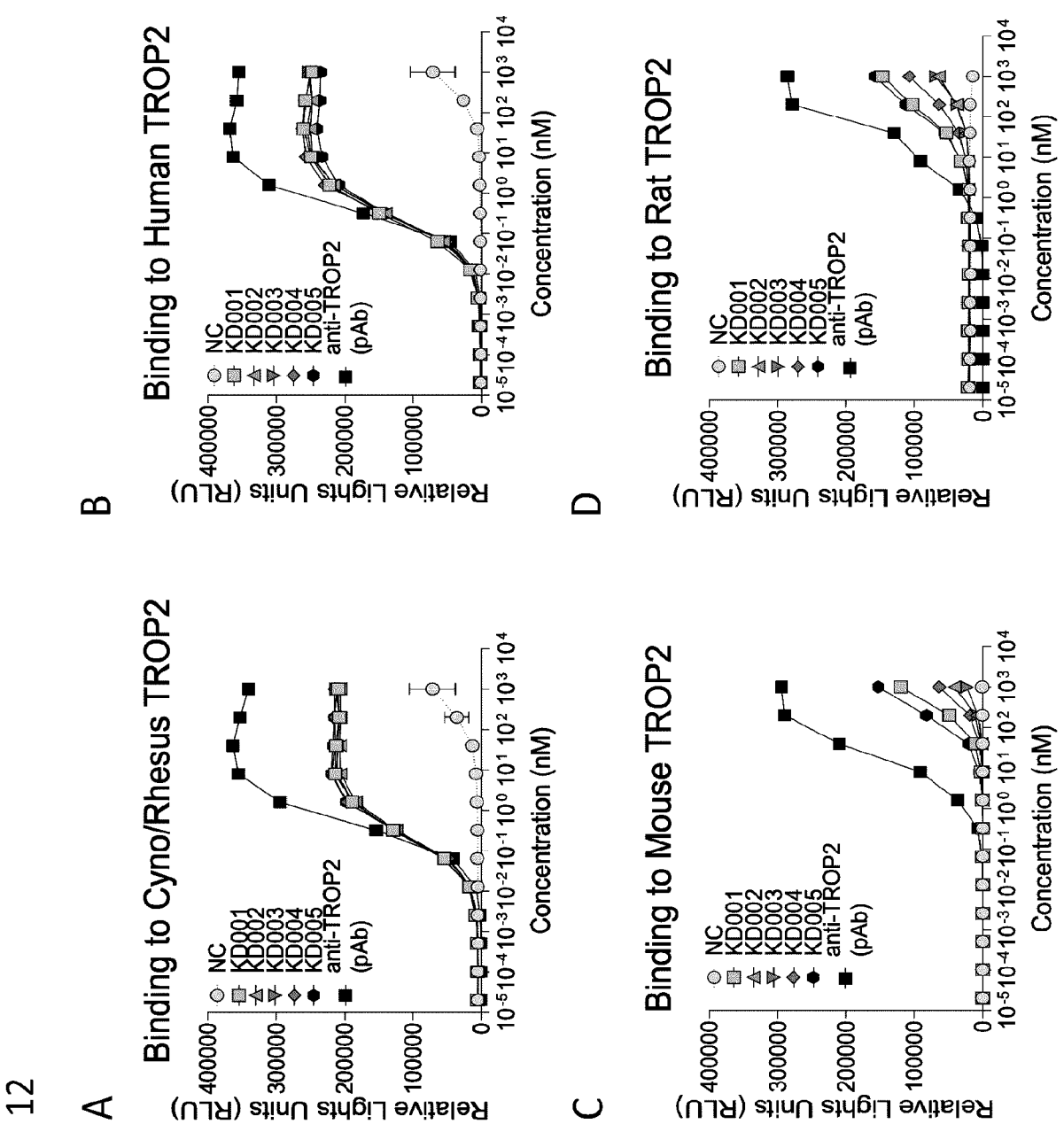

A
Binding to Cyno/Rhesus TROP2
B
Binding to Human TROP2
C
Binding to Mouse TROP2
D
Binding to Rat TROP2
NC
KD001
KD002
KD003
KD004
KD005
anti-TROP2 (pAb)
Relative Lights Units (RLU)
Concentration (nM)

Figure 12E

Antibodies	Human TROP2 KD (M)	Cynomolgus TROP2 KD (M)
KD001	8.26E-11	2.57E-11
KD002	4.32E-11	8.11E-11
KD003	8.93E-11	1.69E-10
KD004	2.94E-11	3.58E-11
KD005	2.68E-11	5.97E-11

Figure 12F

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
KD001	9,13E+05	7,54E-05	8,26E-11
KD002	1,32E+06	5,69E-05	4,32E-11
KD003	1,06E+06	4,88E-05	4,59E-11
KD004	1,51E+06	4,42E-05	2,94E-11
KD005	1,59E+06	4,27E-05	2,68E-11

Figure 13A

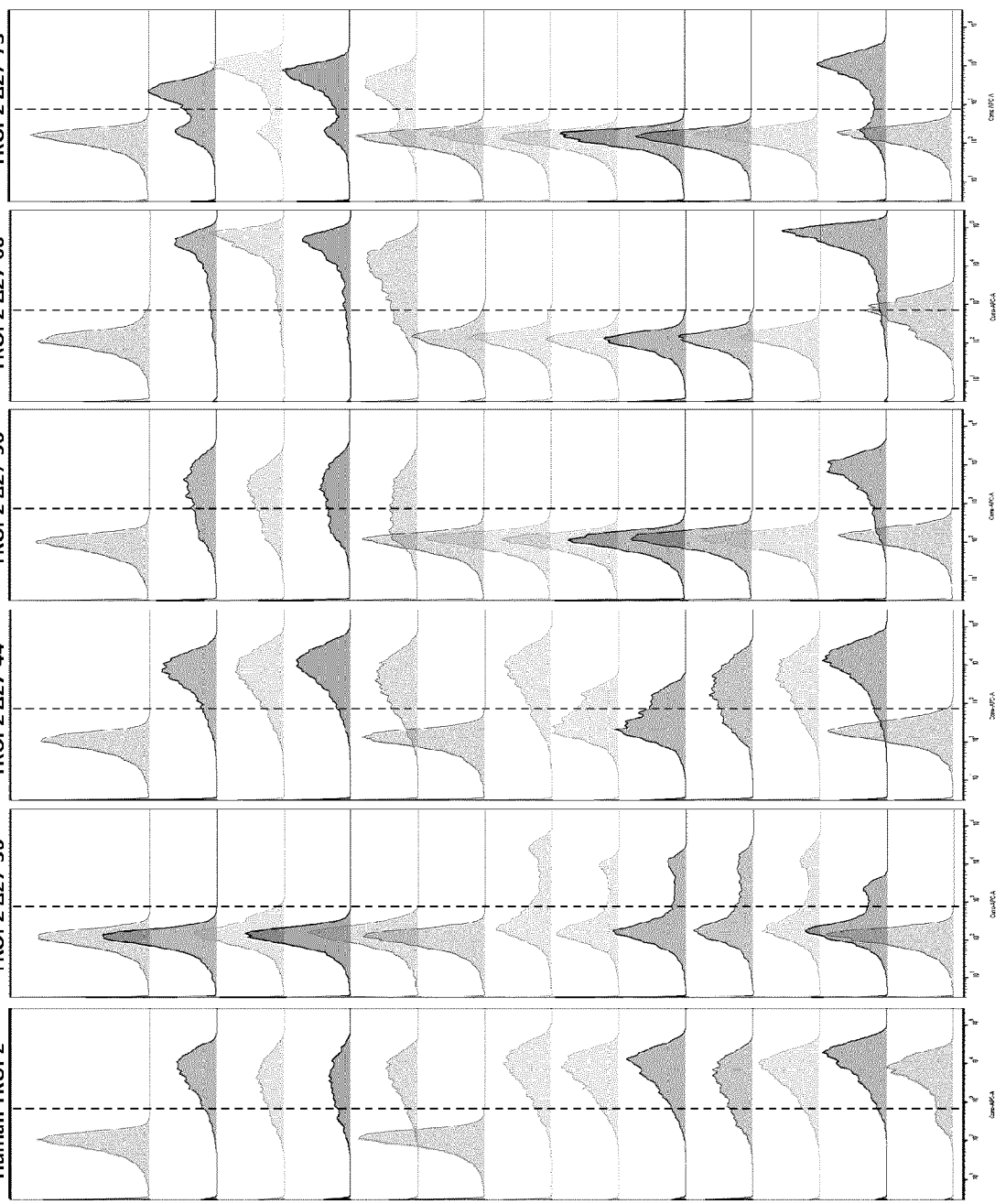
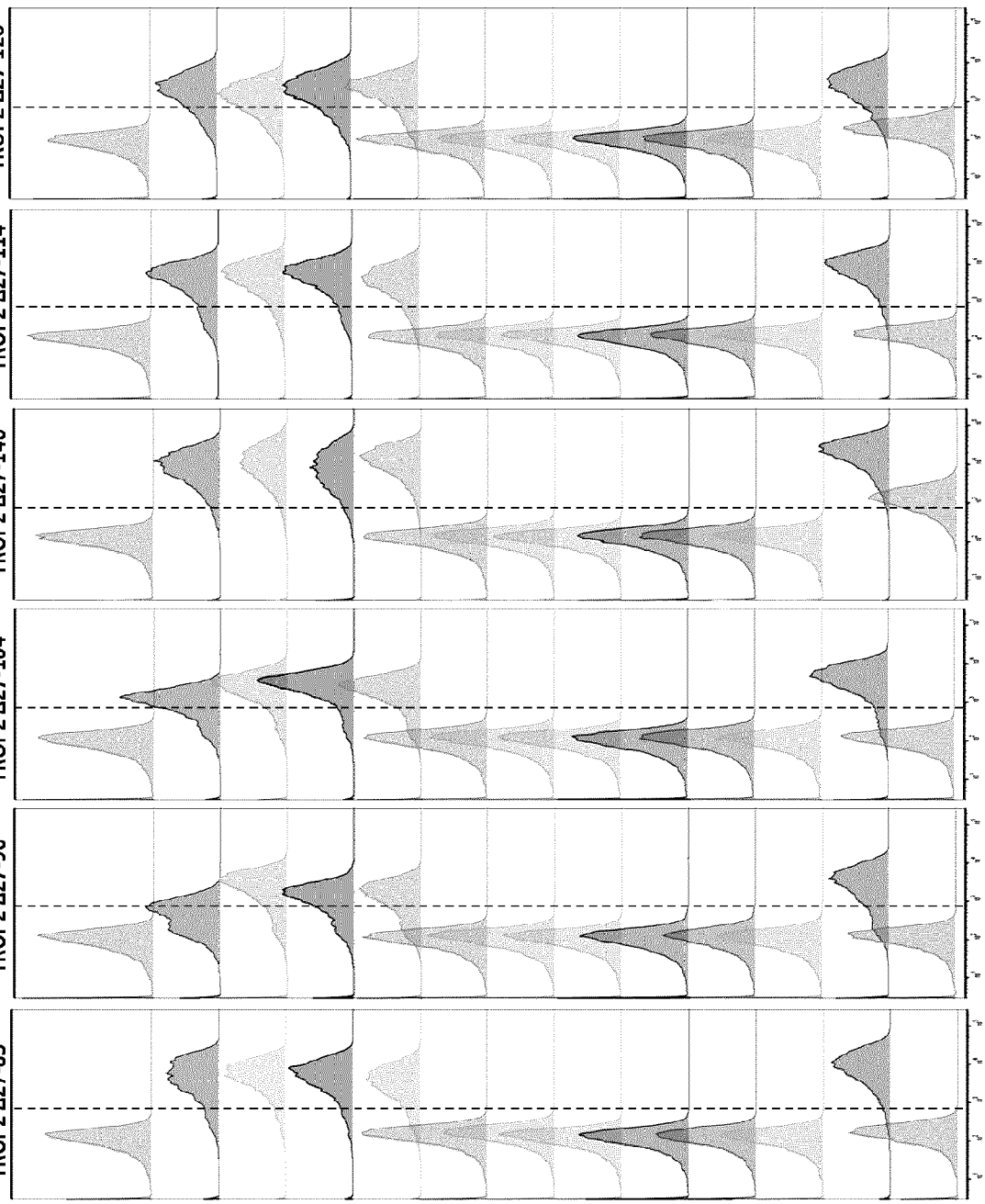

HEK-Human TROP2
HEK-TROP2 Δ27-36
HEK-TROP2 Δ27-44
HEK-TROP2 Δ27-56
HEK-TROP2 Δ27-66
HEK-TROP2 Δ27-73
IgG isotype
AR47A6.4.2
hRS7
hTINA-1
h7E6
NC
KD001
KD002
KD003
KD004
KD005
KD006
KD007

Figure 13B


HEK-TROP2 Δ27-85
HEK-TROP2 Δ27-96
HEK-TROP2 Δ27-104
HEK-TROP2 Δ27-146
HEK-TROP2 Δ27-114
HEK-TROP2 Δ27-126
IgG isotype
AR47A6.4.2
hRS7
hTINA-1
h7E6
NC
KD001
KD002
KD003
KD004
KD005
KD006
KD007

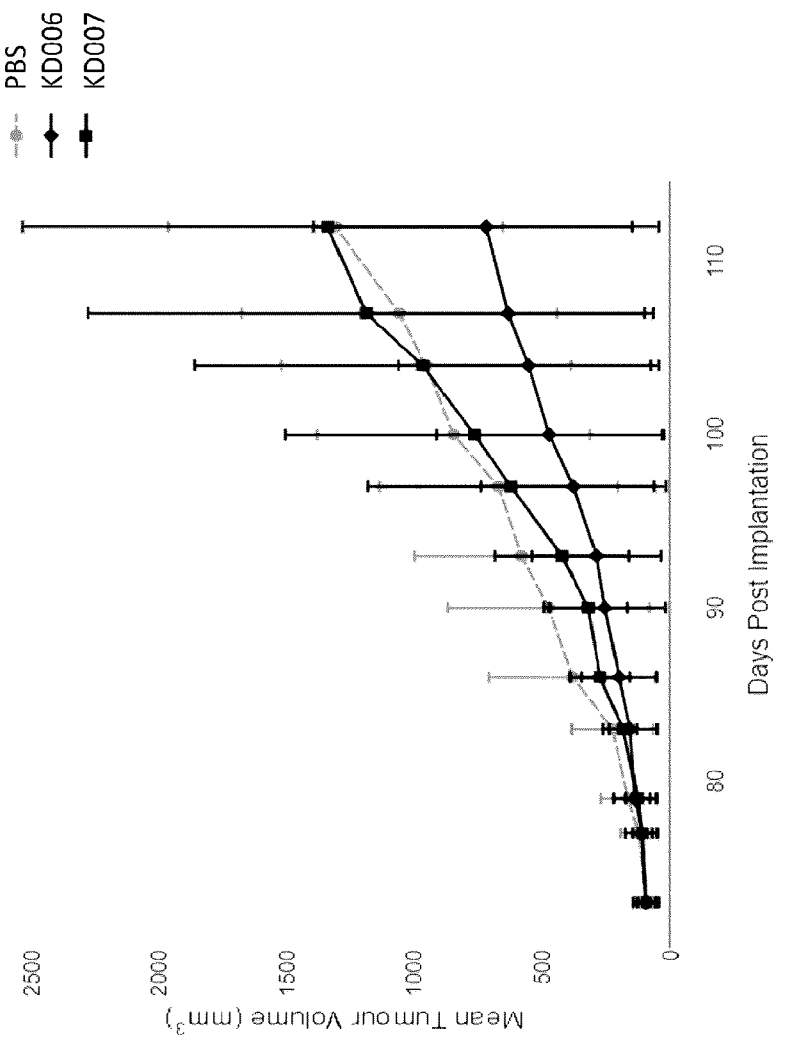

PBS
KD006
KD007
Mean Tumour Volume (mm3)
2500
2000
1500
1000
500
0
80
90
100
110
Days Post Implantation

Figure 13C

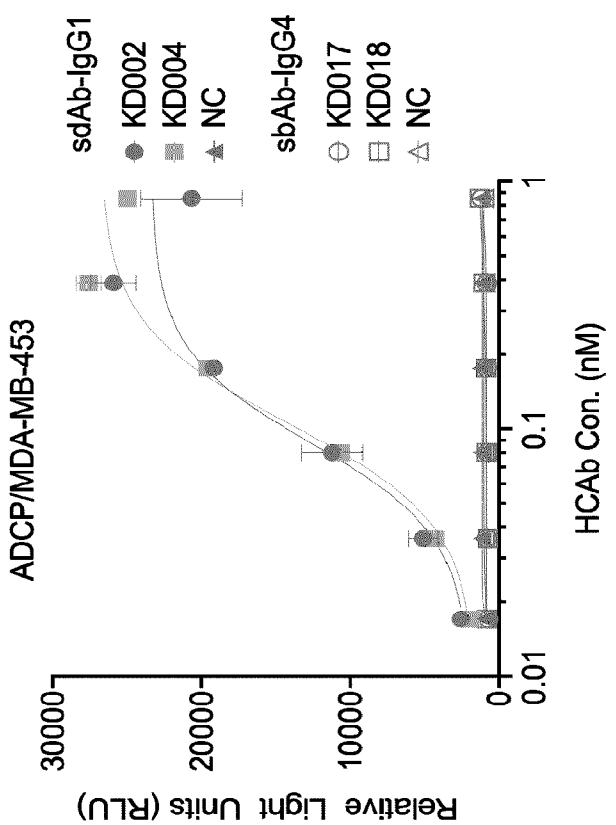
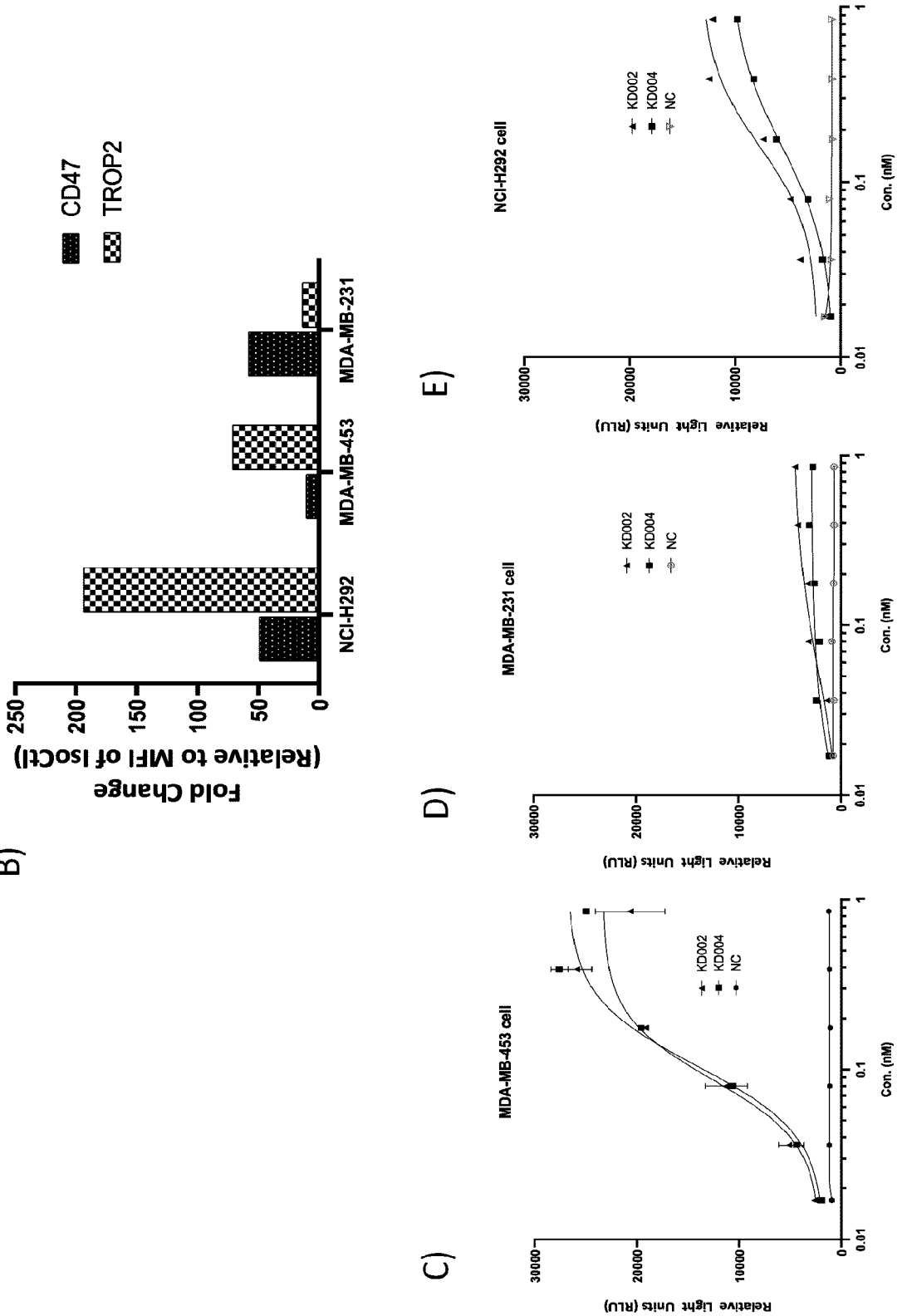

ADCP/MDA-MB-453
sdAb-IgG1
KD002
KD004
NC
sbAb-IgG4
KD017
KD018
NC
Relative Light Units (RLU)
30000
20000
10000
0
0.01
0.1
1
HCAb Con. (nM)

Figure 14A

Figure 14


B)
CD47
TROP2
Fold Change (Relative to MFI of IsoCtl)
250
200
150
100
50
0
NCI-H292
MDA-MB-453
MDA-MB-231
C)
MDA-MB-453 cell
KD002
KD004
NC
Relative Light Units (RLU)
30000
20000
10000
0
0.01
0.1
1
Con. (nM)
D)
MDA-MB-231 cell
KD002
KD004
NC
Relative Light Units (RLU)
30000
20000
10000
0
0.01
0.1
1
Con. (nM)
E)
NCI-H292 cell
KD002
KD004
NC
Relative Light Units (RLU)
30000
20000
10000
0
0.01
0.1
1
Con. (nM)

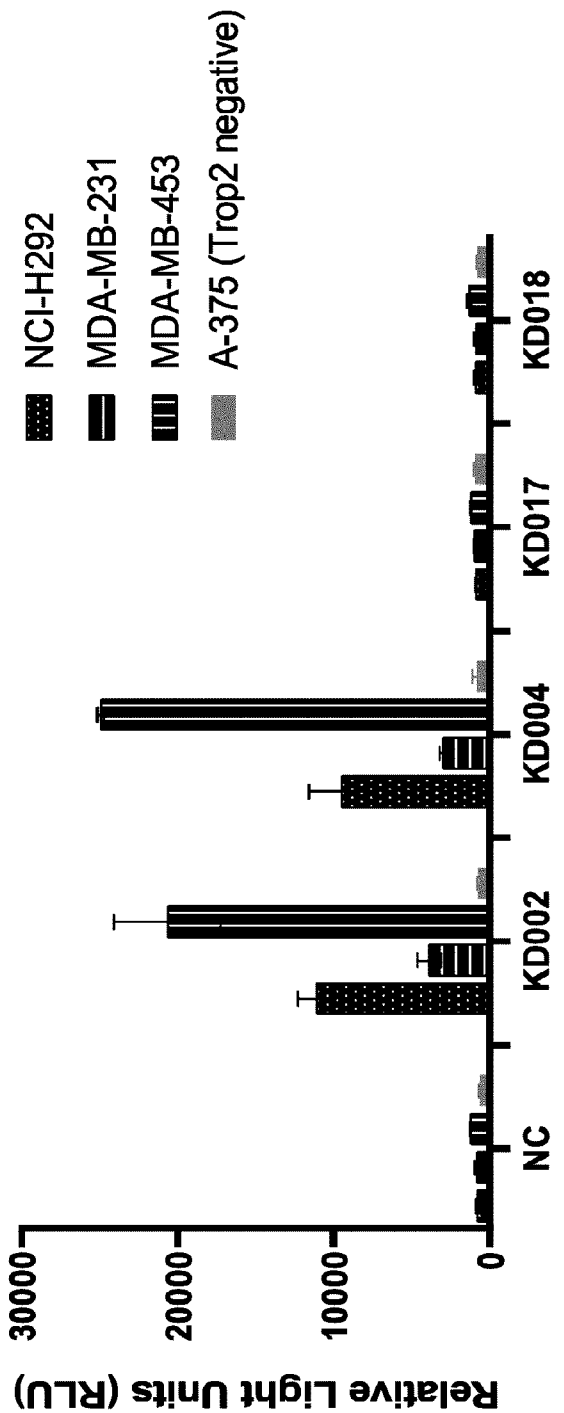

NCI-H292
MDA-MB-231
MDA-MB-453
A-375 (Trop2 negative)
Relative Light Units (RLU)
30000
20000
10000
0
NC
KD002
KD004
KD017
KD018

Figure 14F

Figure 15A

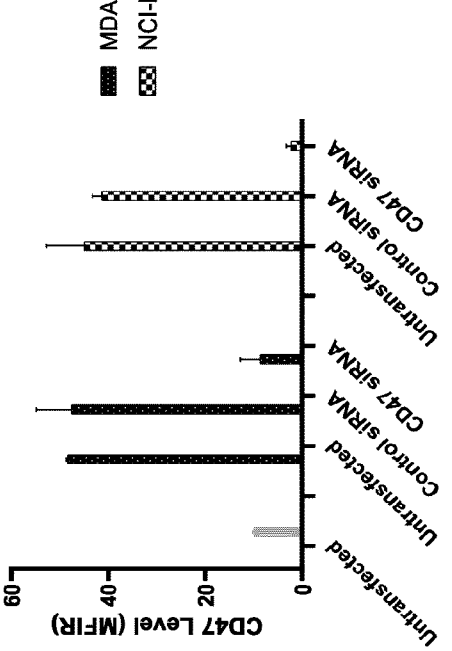


Figure 15B

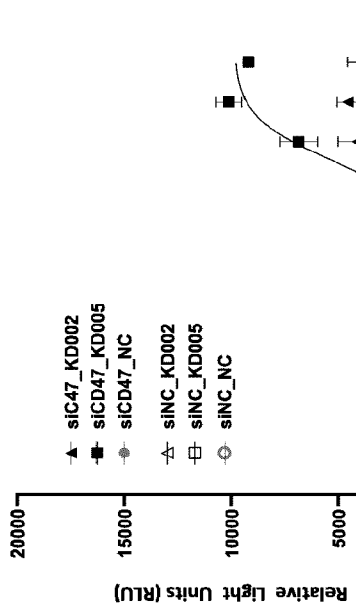


Figure 15C

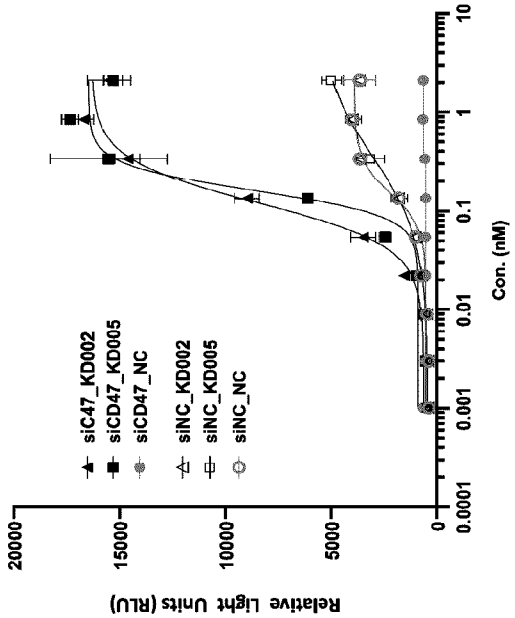
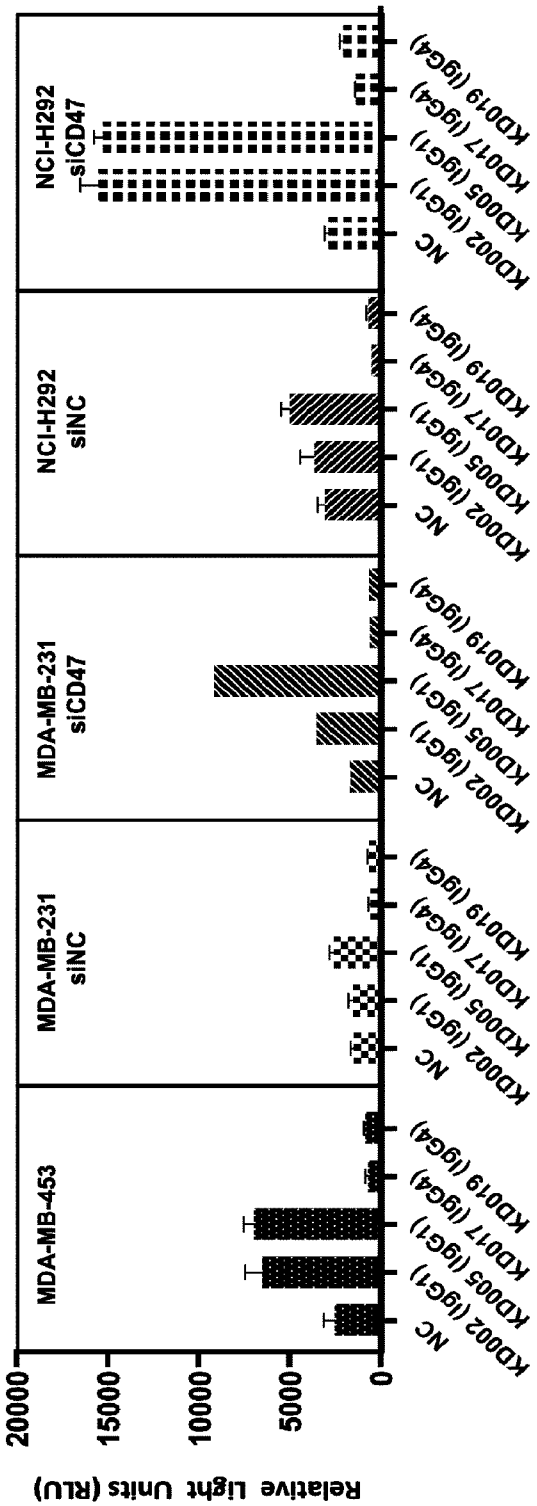


Figure 15D


Relative Light Units (RLU)
20000
15000
10000
5000
0
MDA-MB-453
MDA-MB-231 siNC
MDA-MB-231 siCD47
NCI-H292 siNC
NCI-H292 siCD47
NC
KD002 (IgG1)
KD005 (IgG1)
KD017 (IgG4)
KD019 (IgG4)

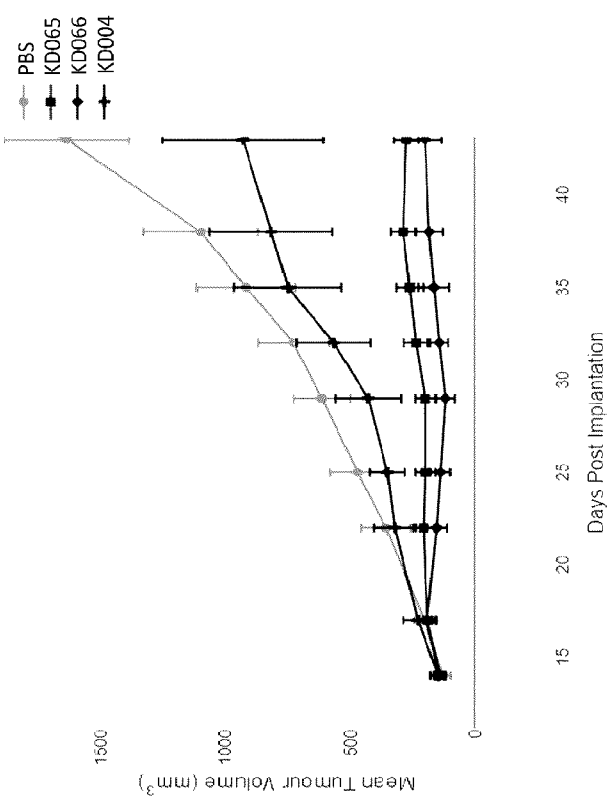
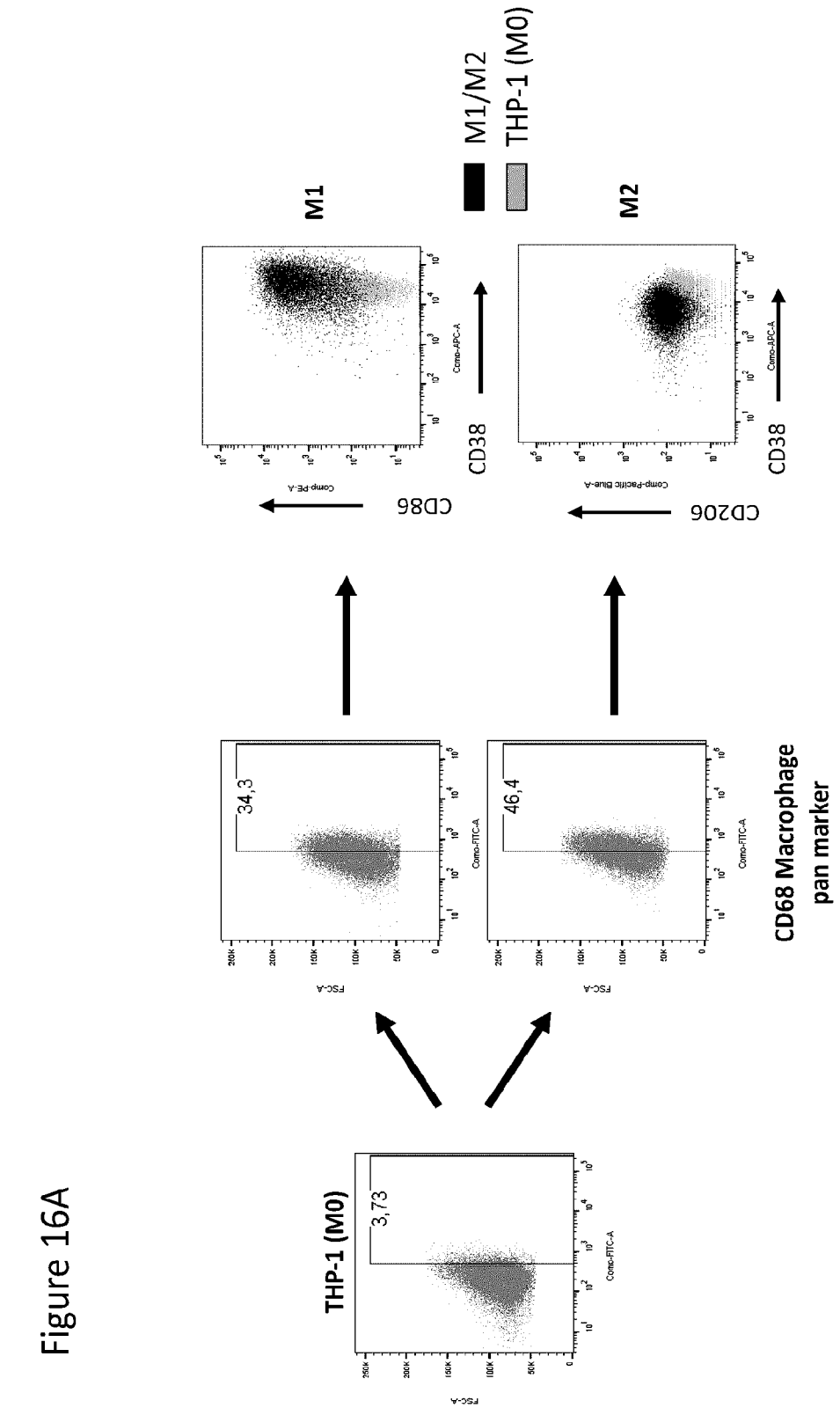

PBS
KD065
KD066
KD004
Mean Tumour Volume (mm³)
1600
1000
500
0
15
20
25
30
35
40
Days Post Implantation

Figure 15E

Figure 16A


THP-1 (M0)
3,73
CD68 Macrophage
pan marker
34,3
46,4
M1/M2
THP-1 (M0)
M1
M2
CD86
CD206
CD38
CD38

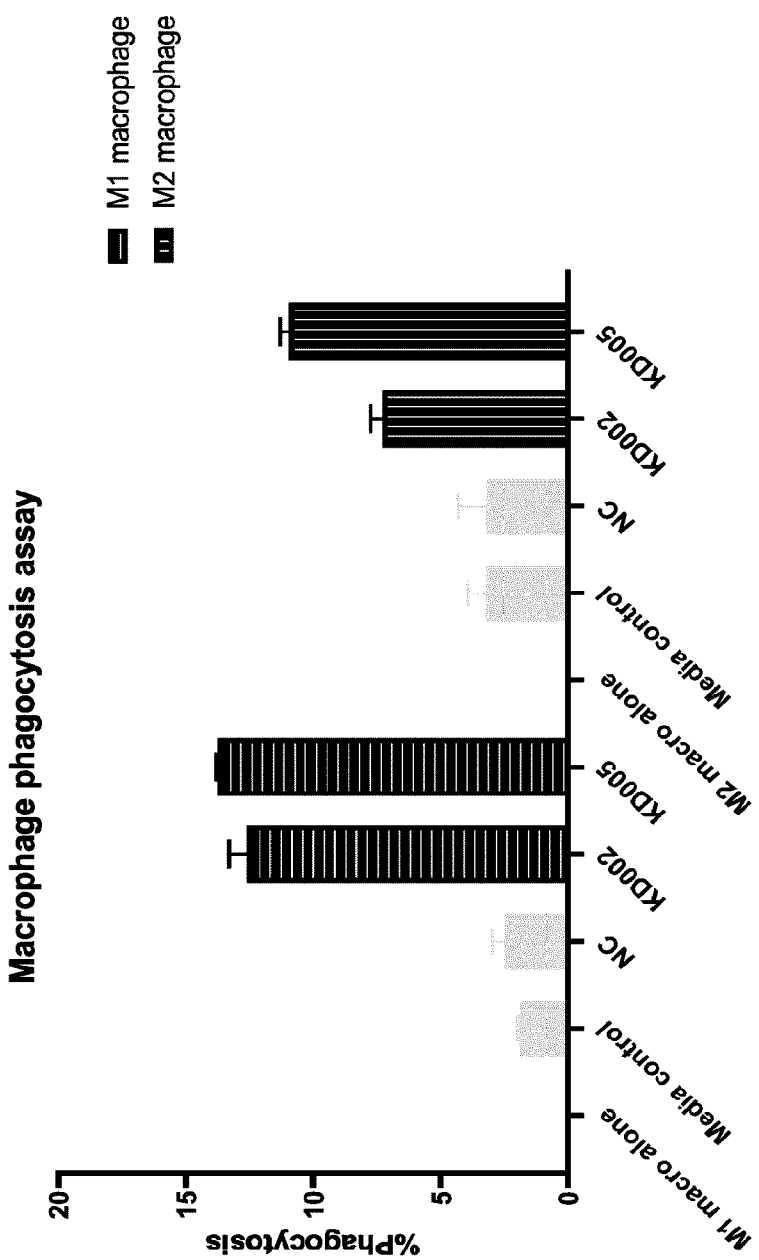

Macrophage phagocytosis assay
M1 macrophage
M2 macrophage
%Phagocytosis
0
5
10
15
20
M1 macro alone
Media control
NC
KD002
KD005
M2 macro alone
Media control
NC
KD002
KD005

Figure 16B

Figure 17A

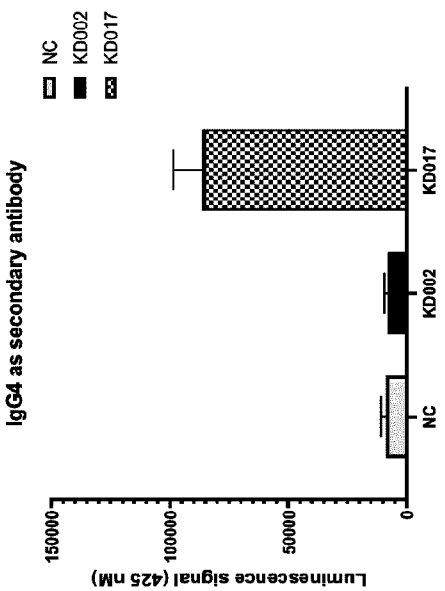
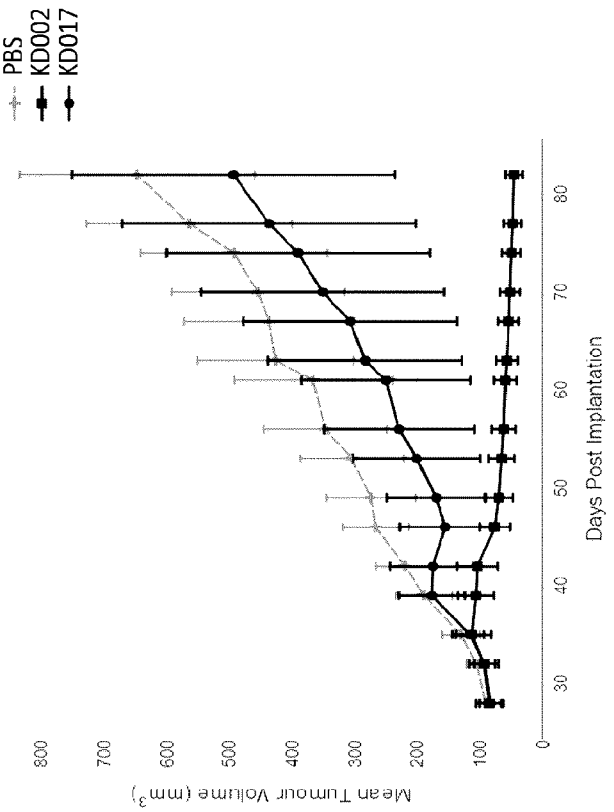


Figure 17B



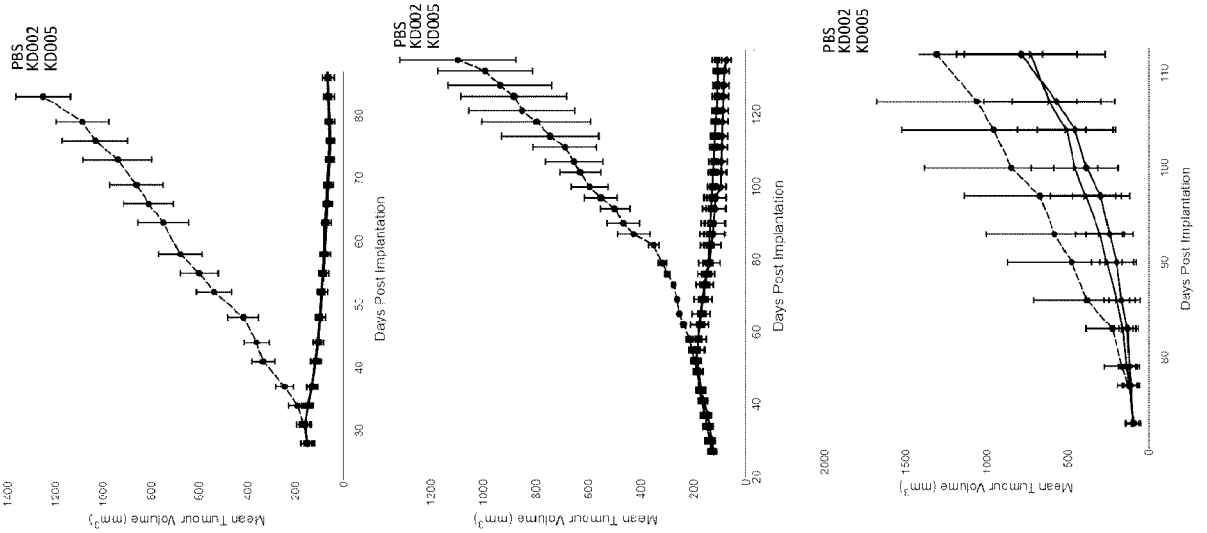


Figure 18B

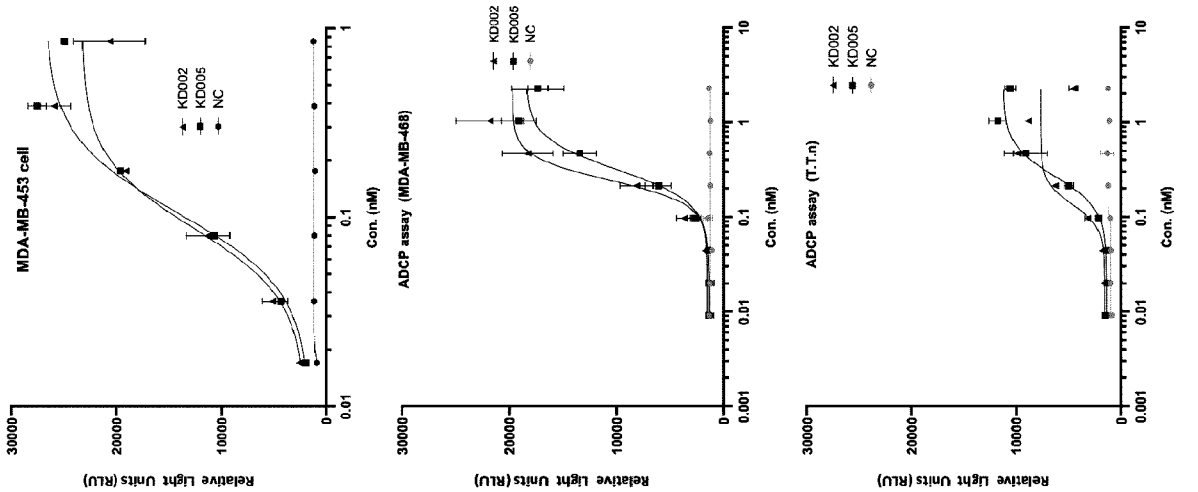


Figure 18A

Figure 19A

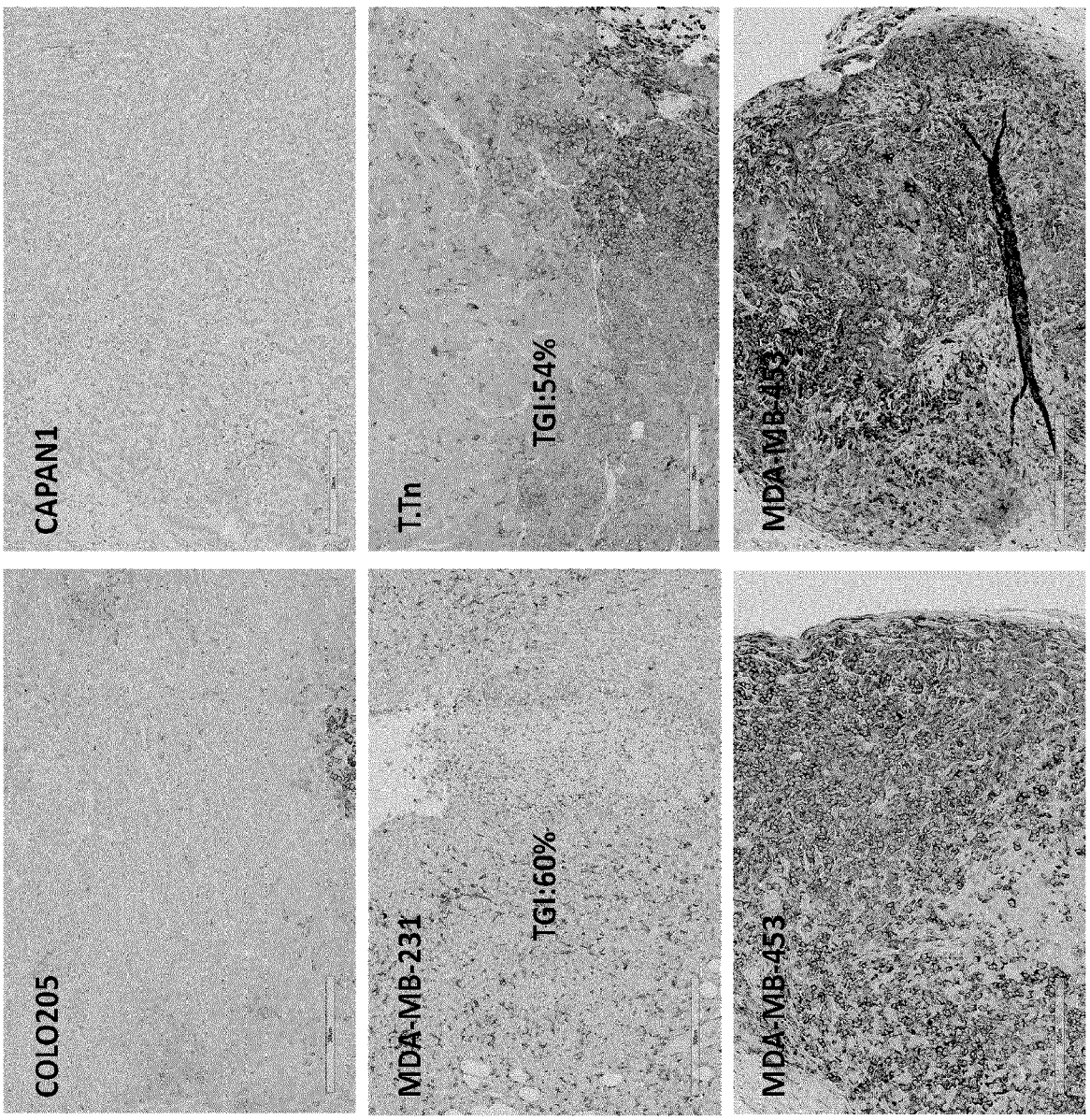

No TGI
TGI
Regression
COLO205
CAPAN1
MDA-MB-231
TGI:60%
T.Tn
TGI:54%
MDA-MB-453
MDA-MB-453

Figure 19B

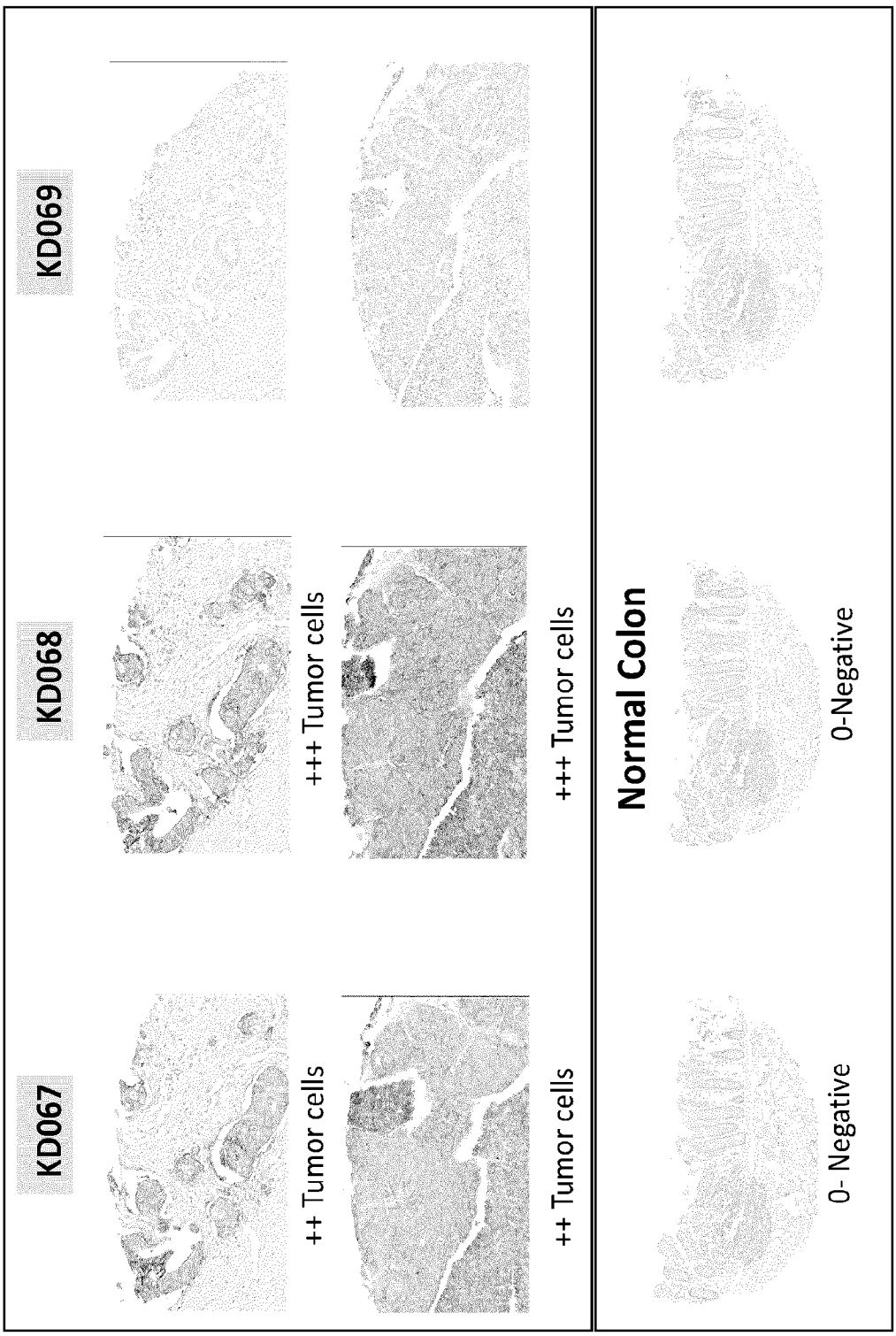

KD067
KD068
KD069
++ Tumor cells
+++ Tumor cells
++ Tumor cells
+++ Tumor cells
Normal Colon
0- Negative
0-Negative

Figure 19C

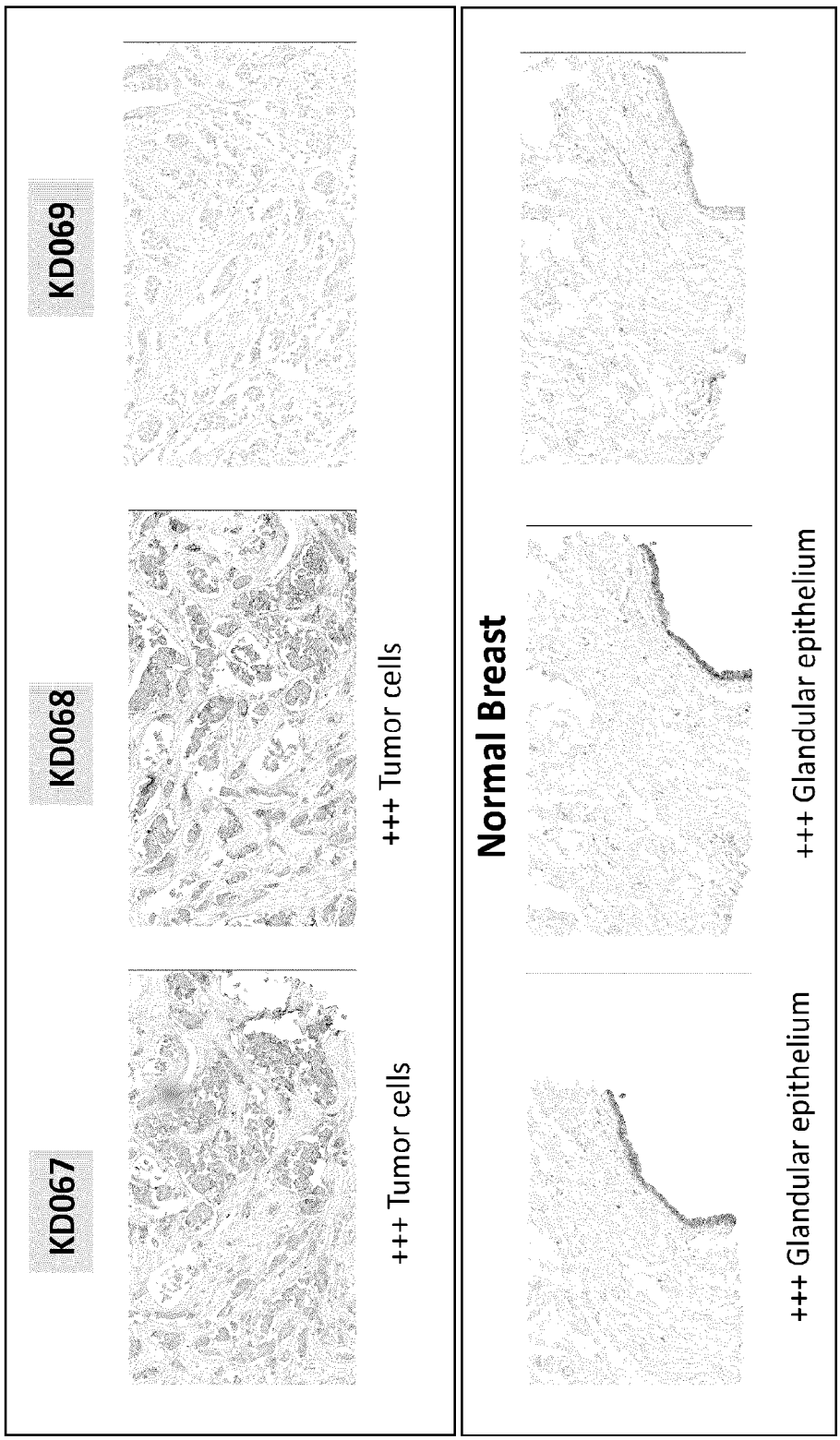

KD067
KD068
KD069
+++ Tumor cells
+++ Tumor cells
Normal Breast
+++ Glandular epithelium
+++ Glandular epithelium

Figure 19D

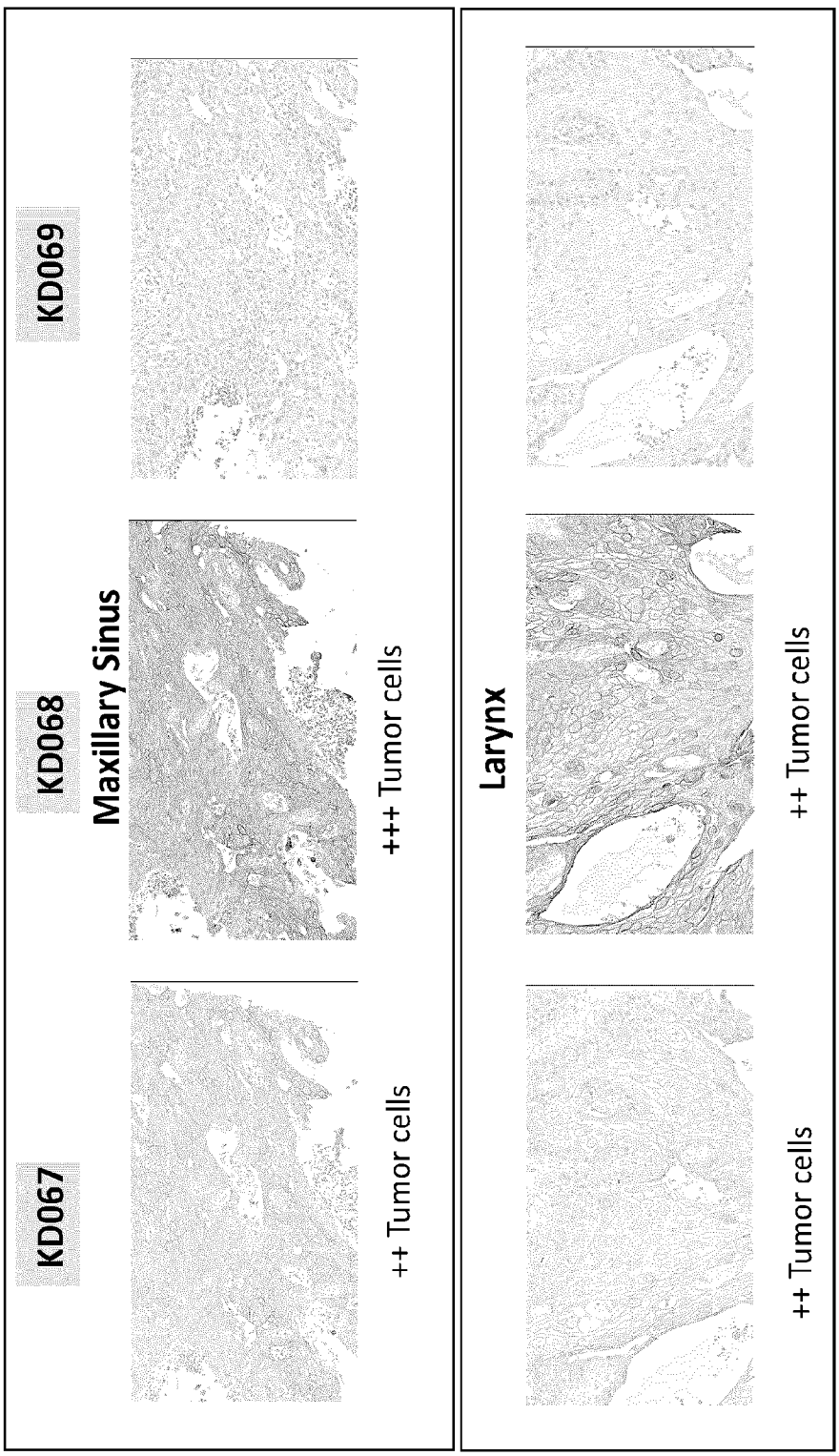
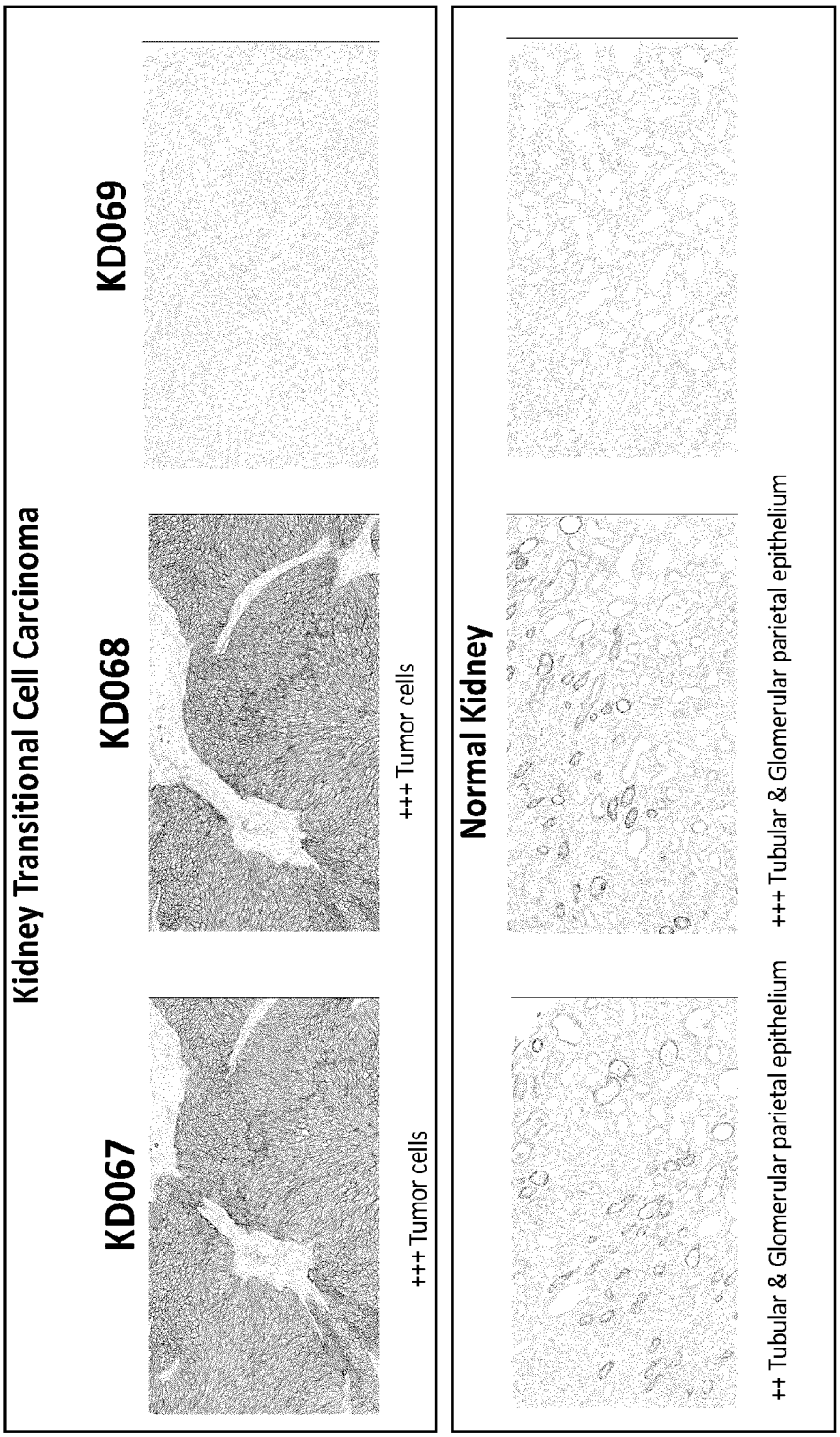

KD067
KD068
KD069
Maxillary Sinus
++ Tumor cells
+++ Tumor cells
Larynx
++ Tumor cells
++ Tumor cells

Figure 19E


Kidney Transitional Cell Carcinoma
KD067
KD068
KD069
+++ Tumor cells
+++ Tumor cells
Normal Kidney
++ Tubular & Glomerular parietal epithelium
+++ Tubular & Glomerular parietal epithelium

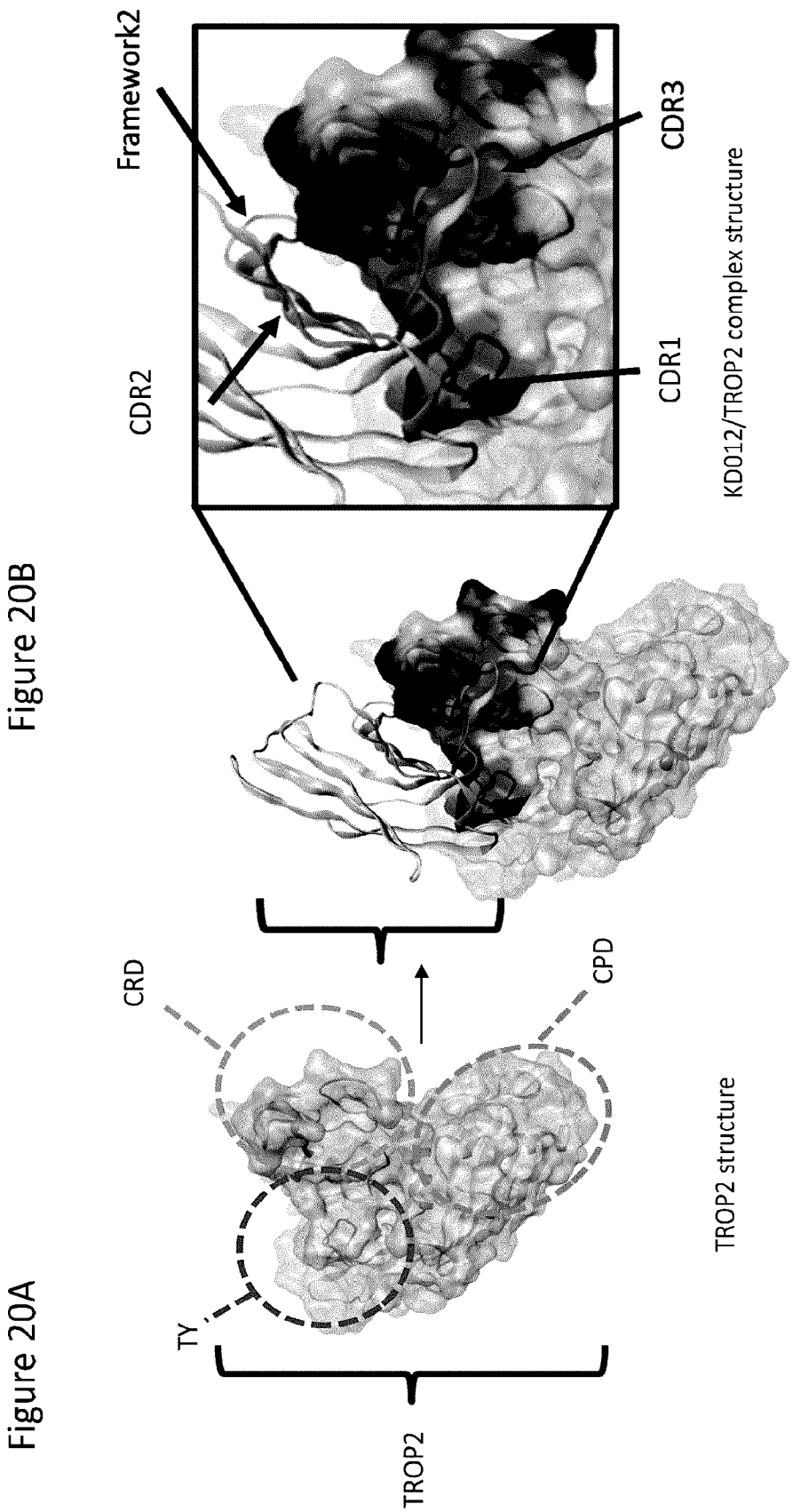


Figure 20B

Figure 20A

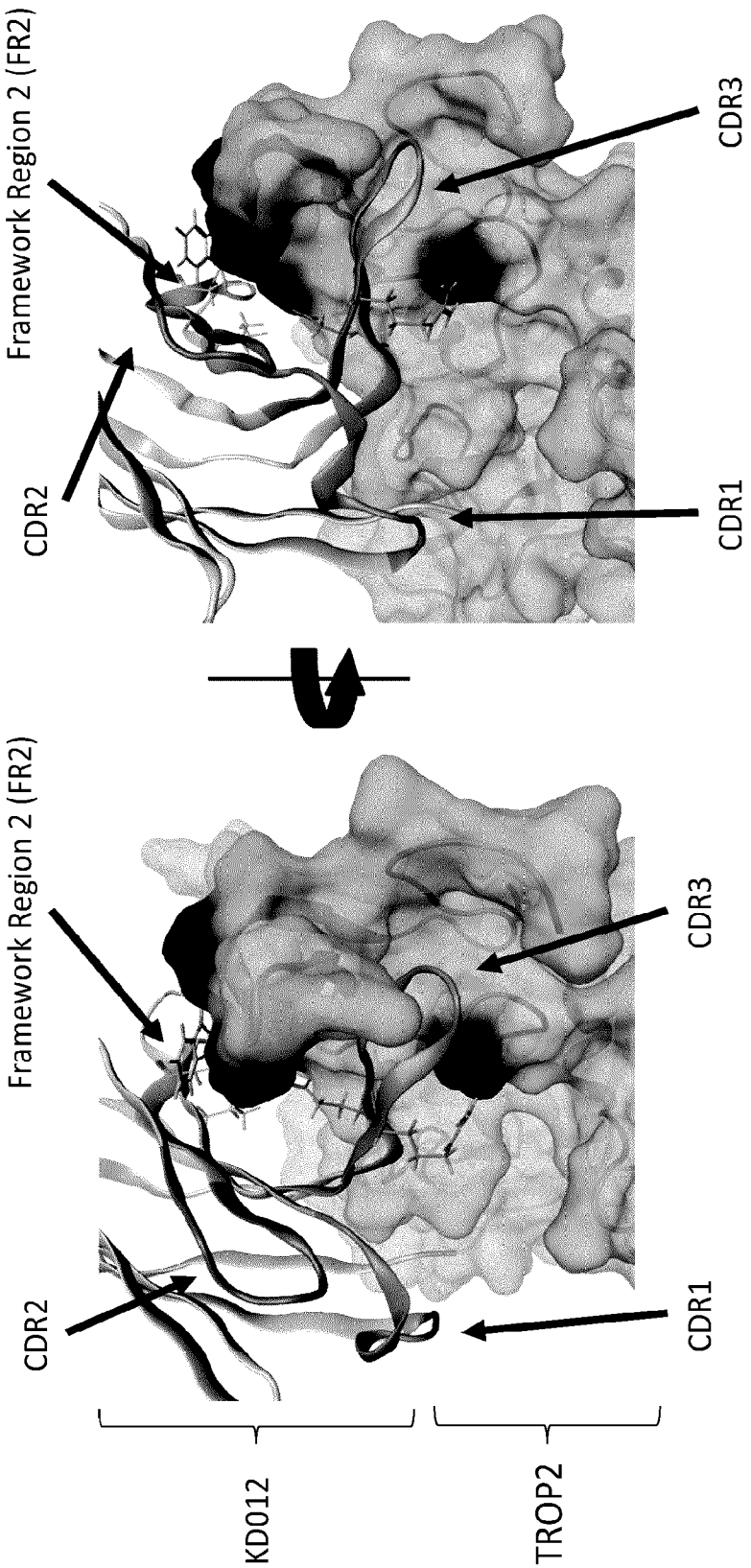

Framework Region 2 (FR2)
CDR2
CDR3
CDR1
Framework Region 2 (FR2)
CDR2
CDR3
CDR1
KD012
TROP2

Figure 20C

Figure 20D
A)

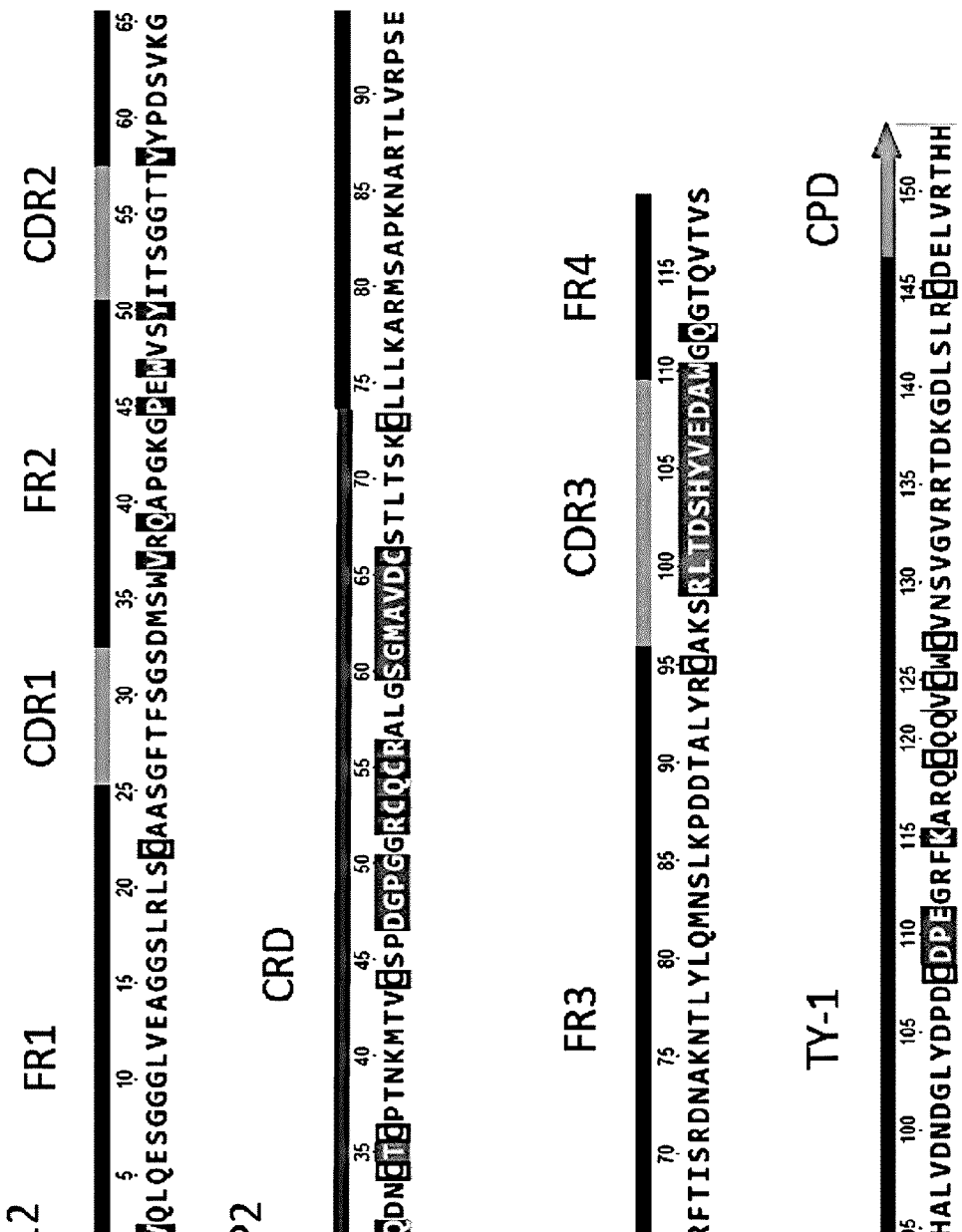


Figure 21

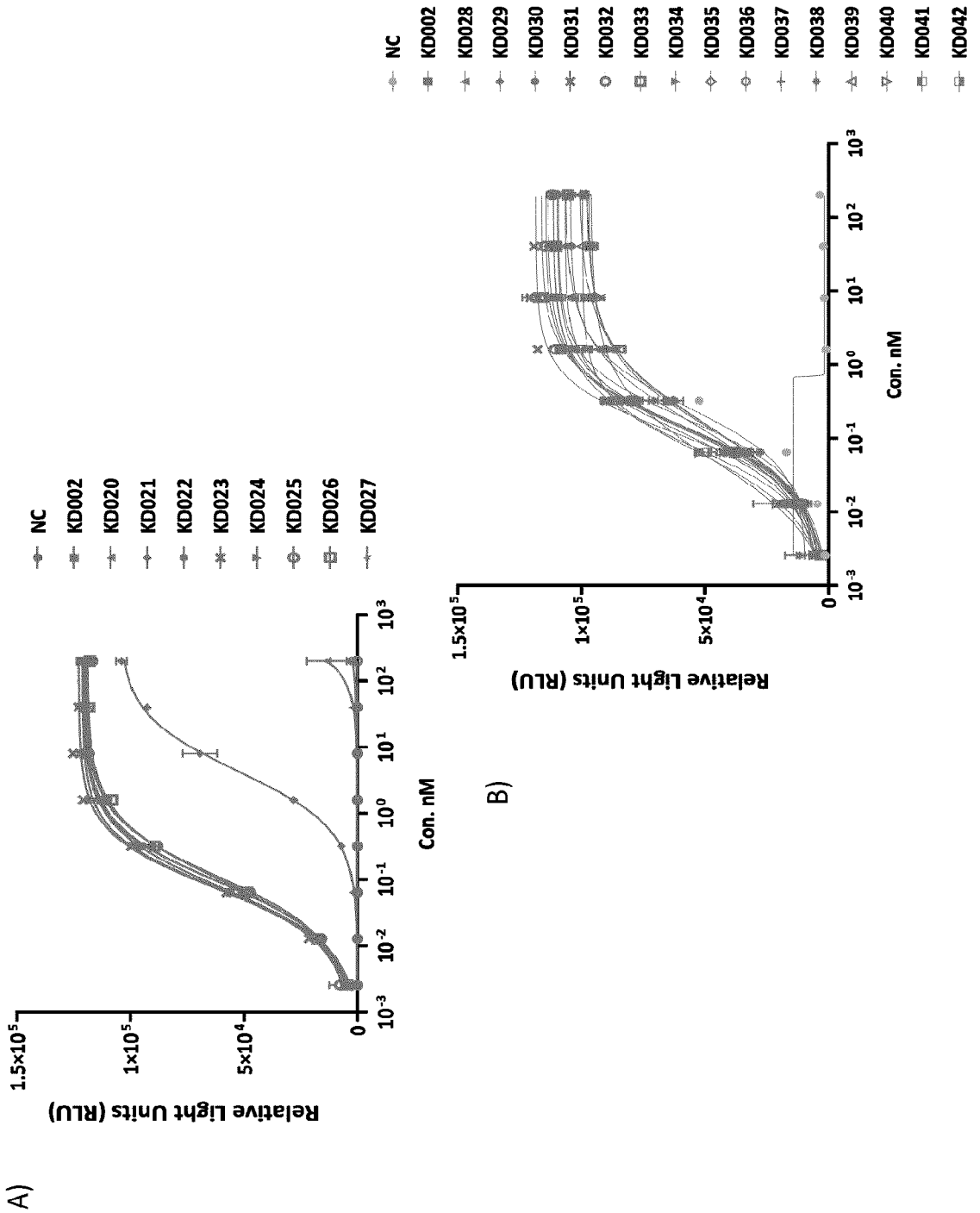


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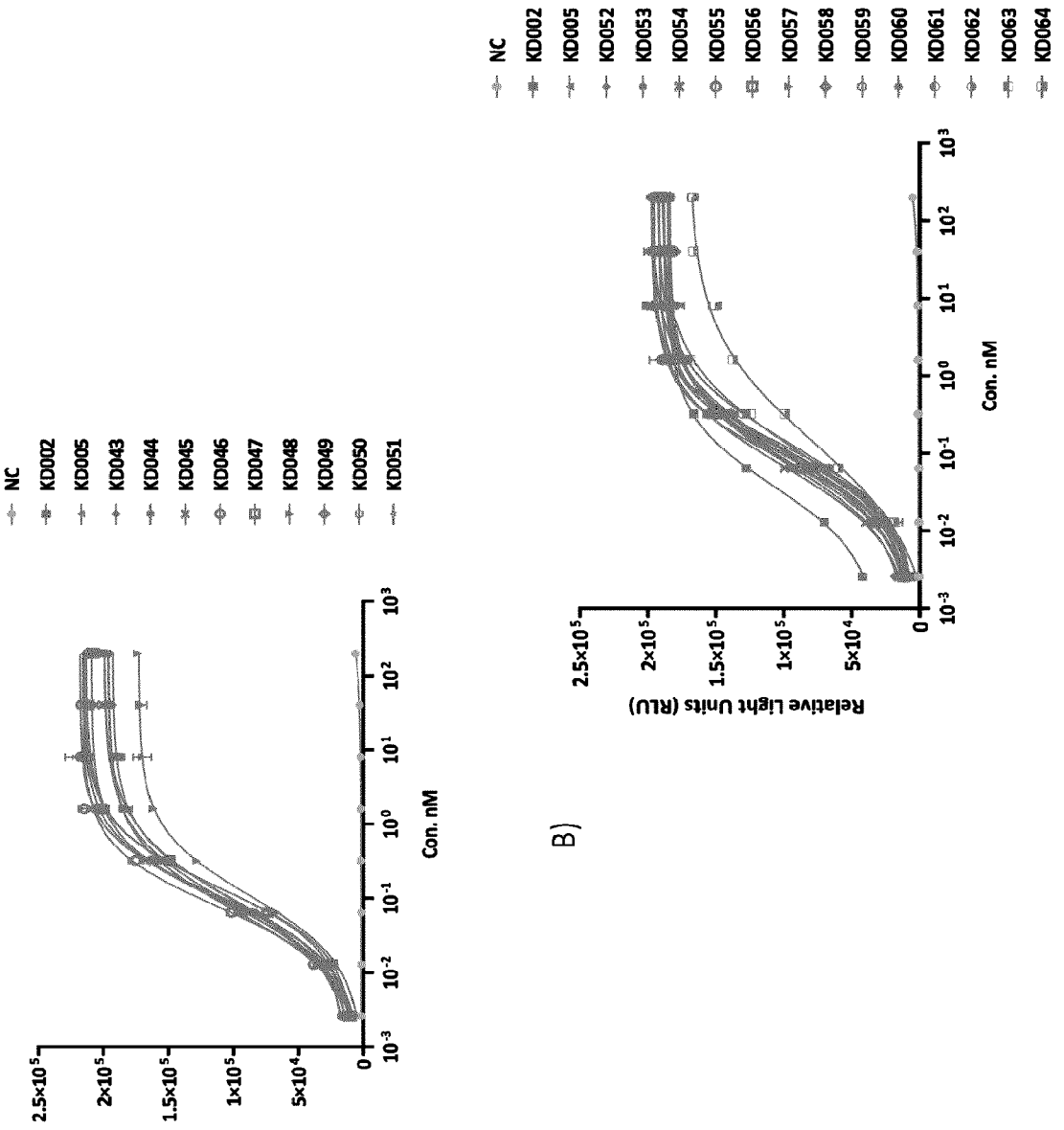
Figure 22



CONFIDENTIAL

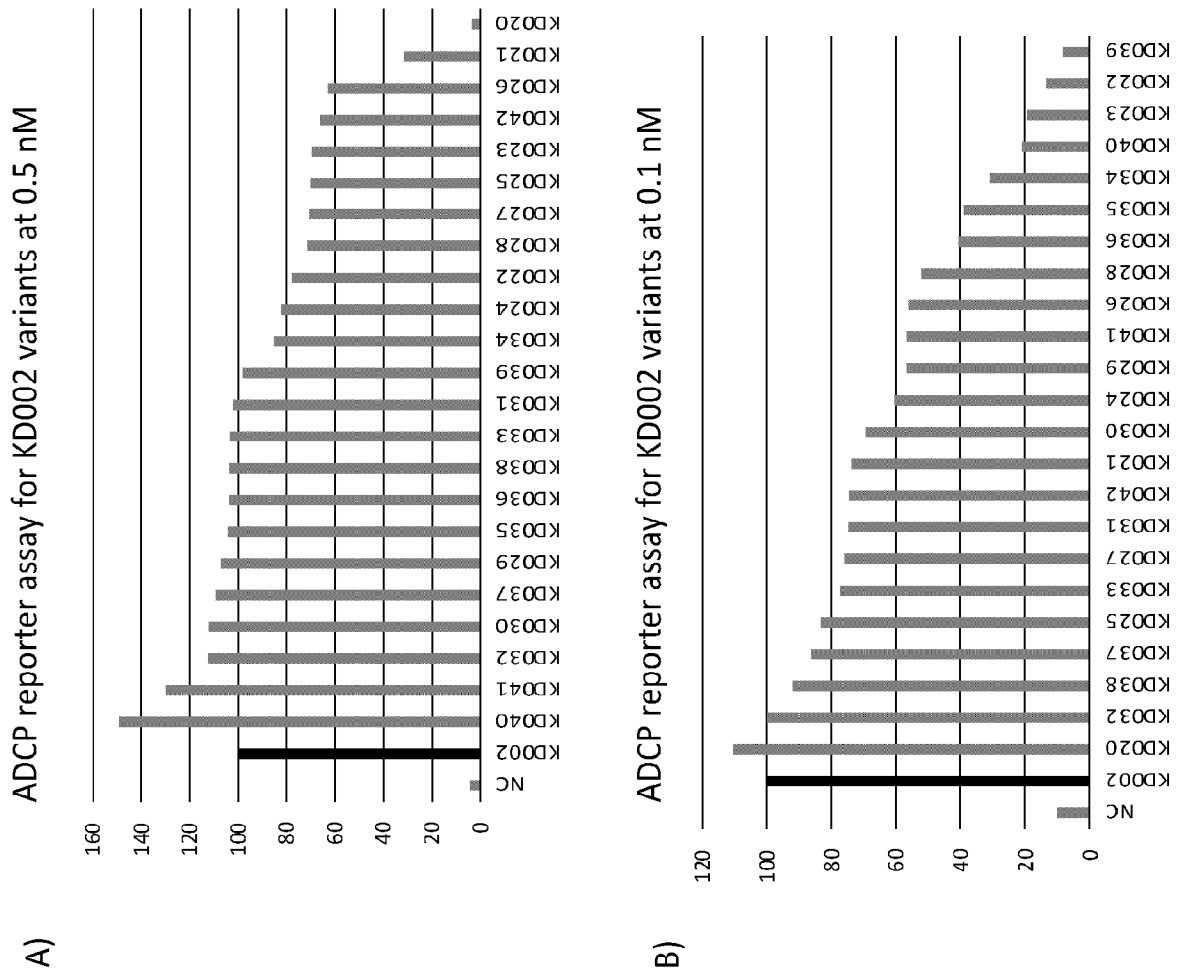

A)
ADCP reporter assay for KD002 variants at 0.5 nM
160
140
120
100
80
60
40
20
0
NC
KD002
KD040
KD041
KD032
KD030
KD037
KD029
KD035
KD036
KD038
KD033
KD031
KD039
KD034
KD024
KD022
KD028
KD027
KD025
KD023
KD042
KD026
KD021
KD020
B)
ADCP reporter assay for KD002 variants at 0.1 nM
120
100
80
60
40
20
0
NC
KD002
KD020
KD032
KD038
KD037
KD025
KD033
KD027
KD031
KD042
KD021
KD030
KD024
KD029
KD041
KD026
KD028
KD036
KD035
KD034
KD040
KD023
KD022
KD039

Figure 23

Figure 24

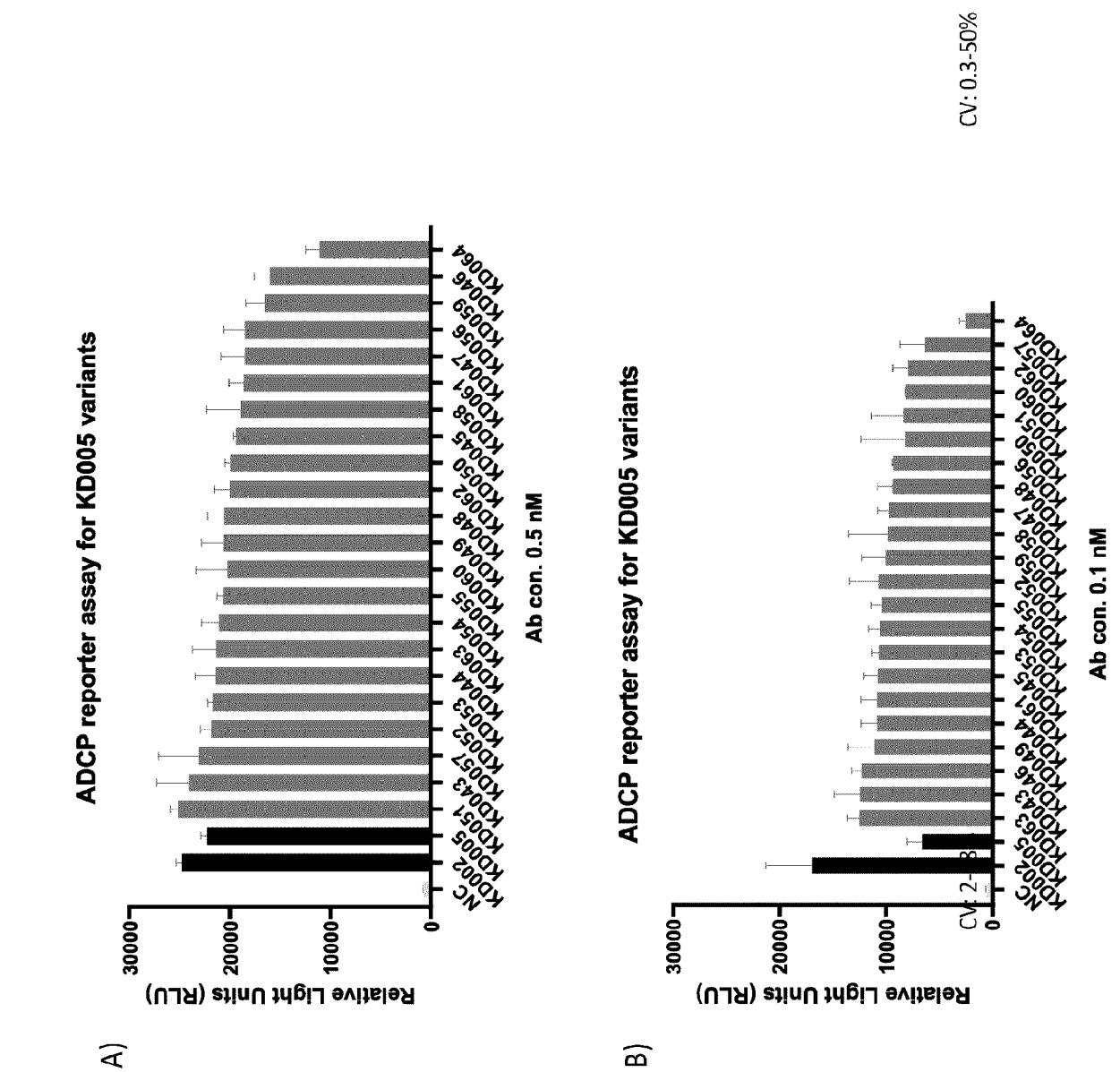

A)
ADCP reporter assay for KD005 variants
Relative Light Units (RLU)
30000
20000
10000
0
NC
KD002
KD005
KD051
KD043
KD057
KD052
KD053
KD044
KD063
KD054
KD055
KD060
KD049
KD048
KD062
KD050
KD045
KD058
KD061
KD047
KD056
KD059
KD046
KD064
Ab con. 0.5 nM
B)
ADCP reporter assay for KD005 variants
Relative Light Units (RLU)
30000
20000
10000
0
CV: 2-8%
CV: 0.3-50%
NC
KD002
KD005
KD063
KD043
KD046
KD049
KD044
KD061
KD045
KD053
KD054
KD055
KD052
KD059
KD058
KD047
KD048
KD056
KD050
KD060
KD051
KD062
KD057
KD064
Ab con. 0.1 nM

Figure 24

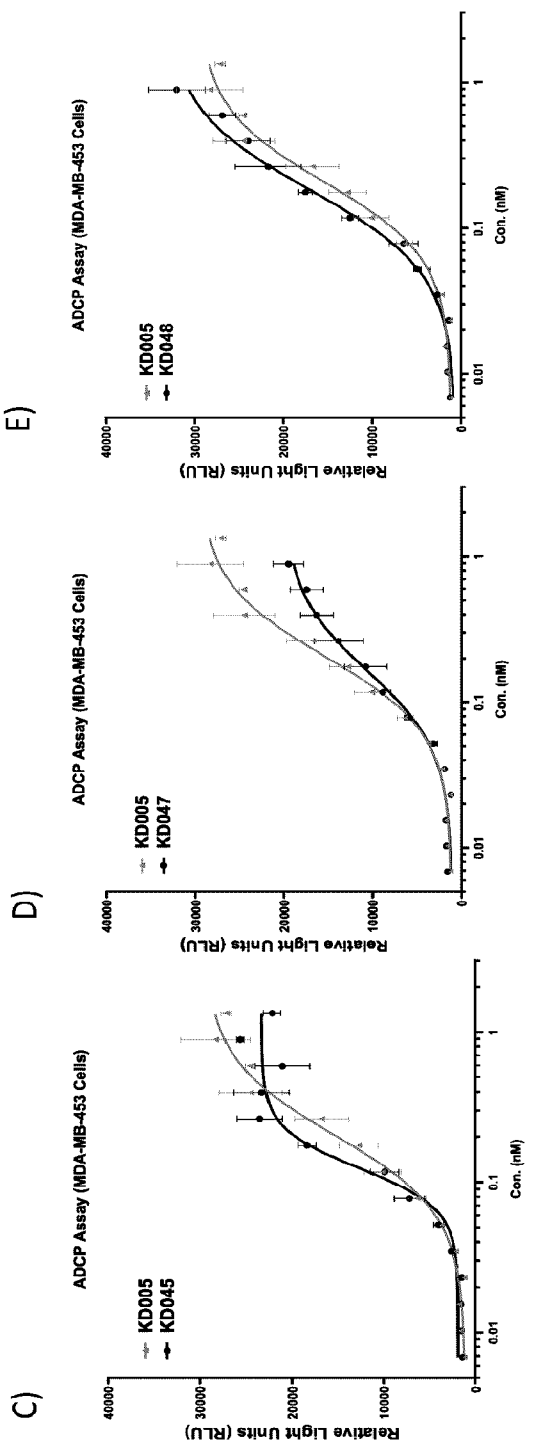

C)
ADCP Assay (MDA-MB-453 Cells)
KD005
KD045
Relative Light Units (RLU)
Con. (nM)
D)
ADCP Assay (MDA-MB-453 Cells)
KD005
KD047
Relative Light Units (RLU)
Con. (nM)
E)
ADCP Assay (MDA-MB-453 Cells)
KD005
KD048
Relative Light Units (RLU)
Con. (nM)

Figure 24

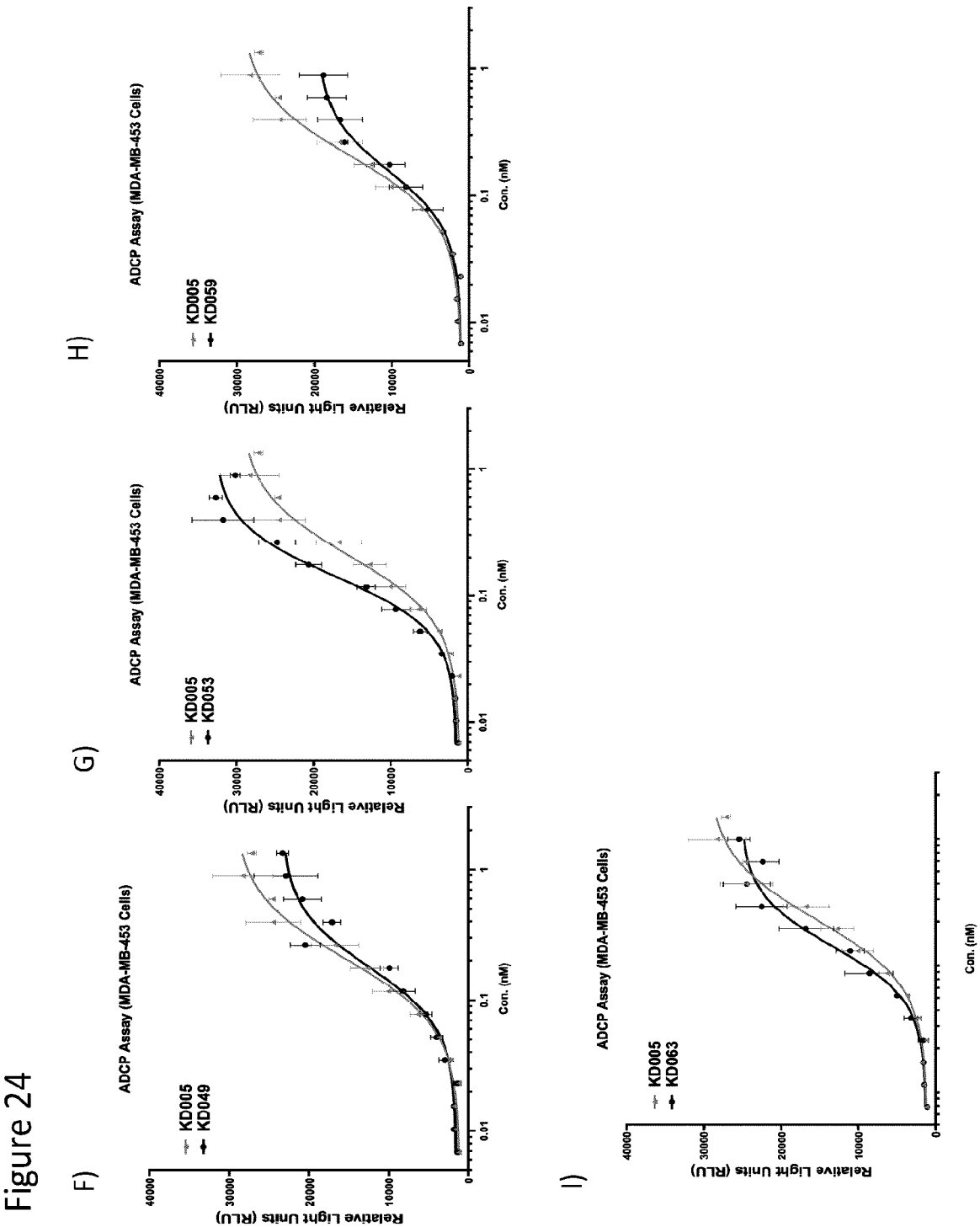

F)
G)
H)
I)
ADCP Assay (MDA-MB-453 Cells)
KD005
KD049
KD053
KD059
KD063
Relative Light Units (RLU)
Con. (nM)
0
10000
20000
30000
40000
0.01
0.1
1

Figure 25

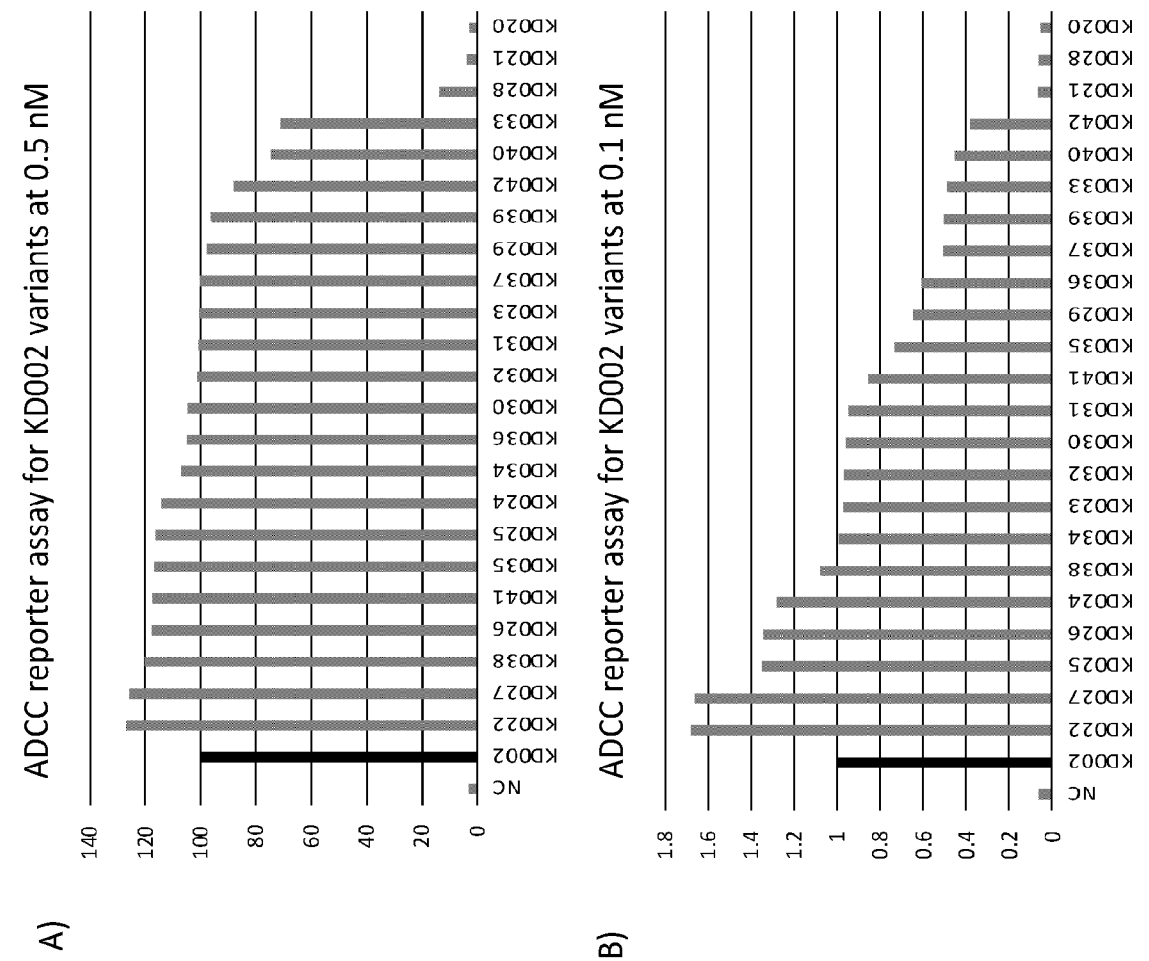

A)
ADCC reporter assay for KD002 variants at 0.5 nM
140
120
100
80
60
40
20
0
NC
KD002
KD022
KD027
KD038
KD026
KD041
KD035
KD025
KD024
KD034
KD036
KD030
KD032
KD031
KD023
KD037
KD029
KD039
KD042
KD040
KD033
KD028
KD021
KD020
B)
ADCC reporter assay for KD002 variants at 0.1 nM
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
NC
KD002
KD022
KD027
KD025
KD026
KD024
KD038
KD034
KD023
KD032
KD030
KD031
KD041
KD035
KD029
KD036
KD037
KD039
KD033
KD040
KD042
KD021
KD028
KD020

Figure 26

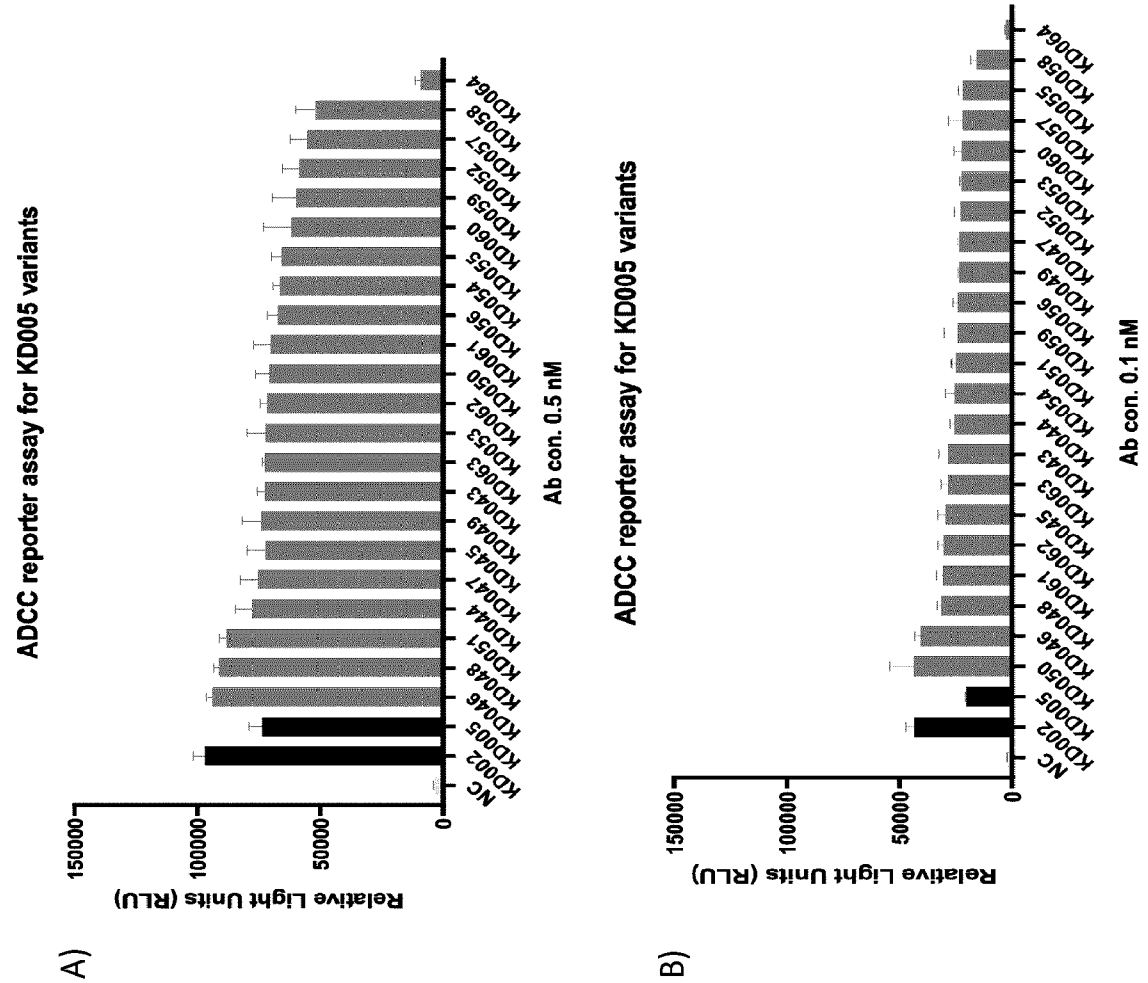


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Figure 27

```
SEQID23      QVQLQESGGGMVQVGGSLRLSCAASGFPFSSADMSWVRQAPGKGPEWVSYINADGSKTYY   60
SEQID24      QVQLQESGGGMVQPGGSLRLSCAASGFTFSNYDMSWVRQAPGKGPEWVSYINAGGSNTDY   60
SEQID22      QVQLQESGGGMVHPGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSG-GTTYY   59
SEQID26      QVQLQESGGGLVEAGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSG-GTTYY   59
SEQID25      QVQLQESGGGLVQPGRSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITGS-GTTYY   59
             **********:*. * *********** **. ******************... ..* *

SEQID23      PDSVKGRFTISRDNAKNTLYLLMNNLKPEDTALYRCARAKLTDTHYVEDYWGQGTQVTVS   120
SEQID24      PDSVKGRFTISRDNAKNMLYLLMNNLKPEDTALYRCARAKLTDTHYVEDYWGQGTQVTVS   120
SEQID22      PDSVKGRFTISRDNAKNTLYLQMNSLTPDDTALYRCAKARLTDSHYVEDAWGQGTQVTVS   119
SEQID26      PDSVKGRFTISRDNAKNTLYLQMNSLKPDDTALYRCAKSRLTDSHYVEDAWGQGTQVTVS   119
SEQID25      PDSVKGRFTTSRDNAKNMLYLQMNSLKPDDTALYRCAKARLTDSHYVEDAWGQGTQVTVS   119
             ********* ******* *** **.*.*:********:::***:***** **********

SEQID23      S     121
SEQID24      S     121
SEQID22      S     120
SEQID26      S     120
SEQID25      S     120
```

Figure 28

CODE	SEQ ID	Sequence of variable region
KD002	23	QVQLQESGGGMVQVGGSLRLSCAASGFPFSSADMSWVRQAPGKGPEWVSYINADGSKTYYPDSVKGRFTISRDNAKNTLYLLMNNLKPEDTALYRCARAKLTDTHYVEDYWGQGTQVTVSS
KD022	73	E***V*****L**P**********A*T**********Q**S*RA****V*Y**********
KD023	74	****V*****L**P**********T**********Q**S*RA****V*Y**********
KD024	75	****V*****L**P**********T**********Q**S*RA****V**********
KD025	76	****V*****L**********T**********Q**S*******V**********
KD026	77	**********T**********Q**S*******V**********
KD027	78	**********Q**S*******V**********
KD028	79	E***L*****L**P**********L****V*********A*T**********S******Q**S*RA****V*Y**********M*****
KD029	80	**********L**P**********T**********S******Q****RA**********M*****
KD030	81	**********L**P**********L**********A*T**********S******Q**S*RA****V**********M*****
KD031	82	E*********L**P**********L**********A*T**********S******Q**S*RA****V**********M*****
KD032	83	****L*****L**P**********L**********A*T**********S******Q**S*RA****V**********M*****
KD033	84	**********L**P**********L****V*********A*T**********S******Q**S*RA****V**********M*****
KD034	85	**********L**P**********L**********A*T**********S******Q**S*RA****V*Y**********M*****
KD035	86	**********L**P**********A*T**********S******Q**S*RA****V**********M*****
KD036	87	**********L**P**********L**********T**********S******Q**S*RA****V**********M*****
KD037	88	**********L**P**********L**********A*T**********S******Q****RA****V**********M*****
KD038	89	**********L**P**********L**********A*T**********S******Q**S*RA**********M*****
KD039	90	**********P**********L**********A*T**********S******Q**S*RA****V**********
KD040	91	**********L**P**********QR**********A*T**********S******Q**S*RA****V**********M*****
KD041	92	**********L**P**********L********E******A*T**********S******Q**S*RA****V**********M*****
KD042	93	**********L**P**********L********N******A*T**********S******Q**S*RA****V**********M*****
	143	XVQLXESGGGXVQXGGSLRLSCAASGFPFSSADMSWVRQAPGKXXEWVSXINAXGSKTYYXDXVKGRFTISRDNXKNTLYLXMNXLXXEDTAXYXCARAKLTDTHYVEDYWGQGTXVTVSS

Figure 29

CODE	SEQ ID	Sequence of variable region
KD002	23	QVQLQESGGGMVQVGGSLRLSCAASGFPFSSADMSWVRQAPGKGPEWVSYINADGSKTYYPDSVKGRFTISRDNAKNTLYLLMNNLKPEDTALYRCARAKLTDTHYVEDYWGQGTQVTVSS
KD022	73	E***V*****L**P**********A*T**********Q**S*RA****V*Y**********
KD024	75	****V*****L**P**********T**********Q**S*RA****V**********
KD025	76	****V*****L**********T**********Q**S*******V**********
KD026	77	**********T**********Q**S*******V**********
KD027	78	**********Q**S*******V**********
KD030	81	**********L**P**********L**********A*T**********S******Q**S*RA****V**********M*****
KD031	82	E*********L**P**********L**********A*T**********S******Q**S*RA****V**********M*****
KD032	83	****L*****L**P**********L**********A*T**********S******Q**S*RA****V**********M*****
KD034	85	**********L**P**********L**********A*T**********S******Q**S*RA****V*Y**********M*****
KD038	89	**********L**P**********L**********A*T**********S******Q**S*RA**********M*****
KD040	91	**********L**P**********QR**********A*T**********S******Q**S*RA****V**********M*****

Figure 30

CODE	SEQ ID	Sequence of variable region
KD005	26	QVQLQESGGGLVEAGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSGGTTYYPDSVKGRFTISRDNAKNTLYLQMNSLKPDDTALYRCAKSRLTDSHYVEDAWGQGTQVTVSS
KD043	94	E***L*******QP*****************************L****A*********A*T**********S**********RAE***V*Y*********************L*****
KD044	95	E***L*******QP*****************************L**************A*T**********S**********RAE***V*Y*********************L*****
KD045	96	E***L*******QP*****************************L**************A*T**********S**********RAE***V**********************L*****
KD046	97	****L*******QP***A*T**********S**********RAE***V*Y*********************L*****
KD047	98	****L*******QP***A*T**********S**********RAE***V**********************L*****
KD048	99	************QP***T**********S**********RAE***V*Y**************************
KD049	100	************QP***T**********S**********RAE***V***************************
KD050	101	************QP***T**********S**********V***************************
KD051	102	************QP***T**
KD052	103	E***V******IQP*****************************L****V*********A*T**********S**********RAE***V*Y*********************L*****
KD053	104	***********IQP***T**********S**********RA**************************L*****
KD054	105	***********IQP*****************************L**************A*T**********S**********RAE***V**********************L*****
KD055	106	E**********IQP*****************************L**************A*T**********S**********RAE***V**********************L*****
KD056	107	****V******IQP*****************************L**************A*T**********S**********RAE***V**********************L*****
KD057	108	***********IQP*****************************L****V*********A*T**********S**********RAE***V**********************L*****
KD058	109	***********IQP*****************************L**************A*T**********S**********RAE***V*Y********************L*****
KD059	110	***********IQP***A*T**********S**********RAE***V**********************L*****
KD060	111	***********IQP*****************************L**************T**********S**********RAE***V**********************L*****
KD061	112	***********IQP*****************************L**************A*T**********S**********RA****V**********************L*****
KD062	113	***********IQP*****************************L**************A*T**********S**********RAE************************L*****
KD063	114	***********I*P*****************************L**************A*T**********S**********RAE***V****************************
	144	XVQLXESGGGLXXGGSLRLSCAASGFTFSGSDMSWVRQAPGKGXEWVSXITSGGTTVYXDXVKGRFTISRDNXKNTLYLQMNSLXXXDTAXYXCAKSRLTDSHYVEDAWGQGTXVTVSS

Figure 31

CODE	SEQ ID	Sequence of variable region
KD005	26	QVQLQESGGGLVEAGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSGGTTYYPDSVKGRFTISRDNAKNTLYLQMNSLKPDDTALYRCAKSRLTDSHYVEDAWGQGTQVTVSS
KD043	94	E***L*******QP*****************************L****A*********A*T**********S**********RAE***V*Y*******************L*****
KD044	95	E***L*******QP*****************************L*************A*T**********S**********RAE***V*Y*******************L*****
KD045	96	E***L*******QP*****************************L*************A*T**********S**********RAE***V**********************L*****
KD046	97	****L*******QP**A*T**********S**********RAE***V*Y*******************L*****
KD047	98	****L*******QP**A*T**********S**********RAE***V**********************L*****
KD049	100	************QP**T**********S**********RAE***V***************************
KD050	101	************QP**T**********S**********V***************************
KD051	102	************QP**T**
KD052	103	E***V******IQP*****************************L****V*********A*T**********S**********RAE***V*Y*******************L*****
KD053	104	***********IQP**T**********S**********RA************************L*****
KD054	105	***********IQP*****************************L*************A*T**********S**********RAE***V**********************L*****
KD055	106	E**********IQP*****************************L*************A*T**********S**********RAE***V**********************L*****
KD056	107	****V******IQP*****************************L*************A*T**********S**********RAE***V**********************L*****
KD057	108	***********IQP*****************************L****V*********A*T**********S**********RAE***V**********************L*****

Figure 32

CODE	SEQ ID	Sequence of variable region
KD005	26	QVQLQESGGGLVEAGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSGGTTYYPDSVKGRFTISRDNAKNTLYLQMNSLKPDDTALYRCAKSRLTDSHYVEDAWGQGTQVTVSS
KD043	94	E***L*******QP*****************************L****A*********A*T**********S**********RAE***V*Y*******************L*****
KD044	95	E***L*******QP*****************************L*************A*T**********S**********RAE***V*Y*******************L*****
KD045	96	E***L*******QP*****************************L*************A*T**********S**********RAE***V**********************L*****
KD046	97	****L*******QP**************************************A*T**********S**********RAE***V*Y*******************L*****
KD049	100	************QP**************************************T**********S**********RAE***V***************************
KD051	102	************QP**************************************T**
KD052	103	E***V******IQP*****************************L****V*********A*T**********S**********RAE***V*Y*******************L*****
KD053	104	***********IQP**************************************T**********S**********RA*************************L*****
KD054	105	***********IQP*****************************L*************A*T**********S**********RAE***V**********************L*****
KD055	106	E**********IQP*****************************L*************A*T**********S**********RAE***V**********************L*****
KD057	108	***********IQP*****************************L****V*********A*T**********S**********RAE***V**********************L*****
KD061	112	***********IQP*****************************L*************A*T**********S**********RA****V**********************L*****
KD063	114	***********I*P*****************************L*************A*T**********S**********RAE***V***************************

Figure 33

CODE	SEQ ID	Sequence of variable region
KD005	26	QVQLQESGGGLVEAGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSGGTTYYPDSVKGRFTISRDNAKNTLYLQMNSLKPDDTALYRCAKSRLTDSHYVEDAWGQGTQVTVSS
KD043	94	E***L*******QP*****************************L****A*********A*T**********S**********RAE***V*Y*******************L*****
KD044	95	E***L*******QP*****************************L*************A*T**********S**********RAE***V*Y*******************L*****
KD045	96	E***L*******QP*****************************L*************A*T**********S**********RAE***V**********************L*****
KD046	97	****L*******QP***A*T**********S**********RAE***V*Y*******************L*****
KD047	98	****L*******QP***A*T**********S**********RAE***V**********************L*****
KD048	99	************QP***T**********S**********RAE***V*Y**************************
KD049	100	************QP***T**********S**********RAE***V**************************
KD050	101	************QP***T**********S**********V**************************
KD051	102	************QP***T**
KD053	104	***********IQP***T**********S**********RA*************************L*****
KD054	105	***********IQP*****************************L*************A*T**********S**********RAE***V**********************L*****
KD061	112	***********IQP*****************************L*************A*T**********S**********RA****V**********************L*****
KD062	113	***********IQP*****************************L*************A*T**********S**********RAE*************************L*****
KD063	114	***********I*P*****************************L*************A*T**********S**********RAE***V**************************

Figure 34

CODE	SEQ ID	Sequence of variable region
KD005	26	QVQLQESGGGLVEAGGSLRLSCAASGFTFSGSDMSWVRQAPGKGPEWVSYITSGGTTYYPDSVKGRFTISRDNAKNTLYLQMNSLKPDDTALYRCAKSRLTDSHYVEDAWGQGTQVTVSS
KD043	94	E***L*******QP*****************************L****A*********A*T**********S**********RAE***V*Y*******************L*****
KD044	95	E***L*******QP*****************************L*************A*T**********S**********RAE***V*Y*******************L*****
KD045	96	E***L*******QP*****************************L*************A*T**********S**********RAE***V**********************L*****
KD046	97	****L*******QP***A*T**********S**********RAE***V*Y*******************L*****
KD051	102	************QP***T**
KD054	105	***********IQP*****************************L*************A*T**********S**********RAE***V**********************L*****

FIG.2