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(71) Applicant: **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, California 94080-4990 (US).

(72) Inventors: **AGARD, Nicholas John**; c/o GENENTECH, INC., 1 DNA Way, South San Francisco, California 94080-4990 (US). **DE SOUSA E MELO, Felipe**; c/o GENENTECH, INC., 1 DNA Way, South San Francisco,

co, California 94080-4990 (US). **LI, Jing**; c/o GENENTECH, INC., 1 DNA Way, South San Francisco, California 94080-4990 (US). **MAREI, Hadir Mohamed Gehad Mohamed Fouad Elsayed**; c/o GENENTECH, INC., 1 DNA Way, South San Francisco, California 94080-4990 (US). **TSAI, Wen-Ting K.**; c/o GENENTECH, INC., 1 DNA Way, South San Francisco, California 94080-4990 (US). **DE SAUVAGE, Frederic Joseph**; c/o GENENTECH, INC., 1 DNA Way, South San Francisco, California 94080-4990 (US). **HE, Jieyan**; c/o GENENTECH, INC., 1 DNA Way, South San Francisco, California 94080-4990 (US). **KEE, Yee Seir**; c/o GENENTECH, INC., 1 DNA Way, South San Francisco, California 94080-4990 (US).

(74) Agent: **DOHERTY, Elizabeth A.** et al.; 125 Cambridge Park Drive, Suite 301, Cambridge, Massachusetts 02140 (US).

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(54) Title: MULTISPECIFIC BINDING PROTEIN DEGRADER PLATFORM AND METHODS OF USE

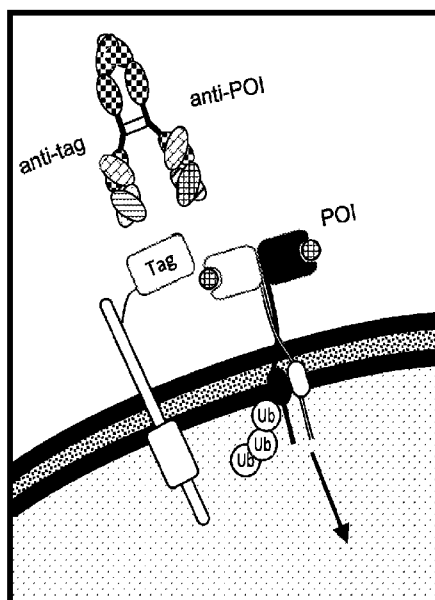


Fig. 2A

(57) Abstract: The present disclosure relates to a multispecific antibody platform for targeted degradation of cell surface proteins. The disclosure relates to multispecific (e.g., bispecific or trispecific) binding molecules such as multispecific antibodies that target at least one transmembrane E3 ubiquitin ligase protein and at least one cell surface protein, for example, a cell surface protein that is intended for degradation, and methods of using the same.



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MULTISPECIFIC BINDING PROTEIN DEGRADER PLATFORM AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/145,336, filed
5 February 3, 2021, and U.S. Provisional Application No. 63/217,470, filed July 1, 2021, and U.S.
Provisional Application No. 63/274,288, filed November 1, 2021, the disclosures of each of
which are hereby incorporated by reference in their entireties.

SEQUENCE LISTING

This application contains a Sequence Listing in computer readable form entitled “01164-
10 0011-00PCT_ST25”, created February 1, 2022, having a size of 501 KB, which is incorporated
by reference herein.

FIELD

The present disclosure relates to a multispecific antibody platform for targeted
degradation of cell surface proteins. The disclosure relates to multispecific (e.g., bispecific or
15 trispecific) binding molecules such as multispecific antibodies that target at least one
transmembrane E3 ubiquitin ligase protein and at least one cell surface protein, for example, a
cell surface protein that is intended for degradation, and methods of using the same.

BACKGROUND

Various platforms have been constructed for the purpose of targeting the degradation of a
20 protein of interest. For example, PROTACs (proteolysis targeting chimeras) are small-molecule
constructs intended to target cytosolic proteins to the 26S proteasome for degradation. They
may include a domain binding to a ubiquitin E3 ligase, a domain binding to a protein targeted for
degradation (i.e., a protein of interest) and a linker molecule to link the two binding domains.
PROTACs, however, target cytosolic protein domains, meaning that this approach cannot be
25 used with certain membrane-bound or cell surface protein targets. (*See, e.g.,* Ahn et al.,
ChemRxiv, doi.org/10.26434/chemrxiv.12736778.v1, posted July 30, 2020.) A LYTAC

(lysosome targeting chimera) construct, for example, is a multivalent construct that binds to a membrane protein of interest and that also includes a ligand such as mannose-6-phosphonate that binds to the cation-independent mannose-6-phosphate receptor (CI-M6PR). Linking the targeted membrane protein to CI-M6PR brings the protein to the lysosome for degradation. (*See Id.*)

5 However, unlike a PROTAC, a LYTAC requires a chemical conjugation, for example, to a mannose-6-phosphonate, which may need to be controlled during manufacturing, such as to avoid product heterogeneity. In addition, since CI-M6PR is expressed in nearly all tissues, this technique may not be useful for selectively degrading a protein in a specific tissue or tissues. A bispecific antibody that binds a cell surface E3 ubiquitin ligase, RNF43, and the cell-surface
10 immune checkpoint protein, PD-L1, was described by Cotton et al. (J. Am. Chem. Soc. 2021, available at <https://dx.doi.org/10.1021/jacs.0c10008>). Lysosomal degradation of PD-L1 in a tumor cell line exposed to the bispecific antibody *in vitro* was demonstrated. WO2021/176034 describes bispecific single chain (VHH) “anti-tag” antibodies that were capable of degrading transiently expressed tagged cell surface proteins in HEK293T cells that also transiently
15 expressed tagged E3 ligases. The general applicability of the approach including efficiency of degrading endogenous cell surface proteins in cells expressing an endogenous E3 ligase and effectiveness *in vivo*, however, remains unknown as well as optimal attributes of a platform that utilizes antibodies against cell surface ligases.

SUMMARY

20 The present disclosure relates to an improved approach to target degradation of membrane-bound and cell surface proteins, which uses multispecific binding proteins, such as multispecific (e.g., bispecific or trispecific) antibodies that bind to at least one transmembrane E3 ubiquitin ligase, such as RNF43 or ZNRF3, or such as RNF128, RNF130, RNF133, RNF148, RNF149, RNF150, RNF167, or ZNRF4, or other cell surface ligases, as well as to a cell surface
25 protein intended for degradation. The multispecific binding proteins use the transmembrane E3 ubiquitin ligase, for example, to target the cell surface protein of interest to lysosomes for degradation. Zinc and RING finger 3 (ZNRF3) and its homolog, RING finger 43 (RNF43), are exemplary transmembrane E3 ubiquitin ligases that normally promote the degradation and turnover of the Frizzled (FZD) and LRP6 receptors on the cell surface (Hao et al., *Nature*
30 485:195-200 (2012); Koo et al., *Nature* 488:665-669 (2012)). RNF43 and ZNRF3 are also members of the Wnt signaling pathway, which may be activated, for example, in certain types of cancers. (See, e.g., Fig. 1C.) Thus, tumor cells from various cancers may express relatively high levels of RNF43 and ZNRF3 in comparison to other cells, which, in some embodiments, may allow for selective degradation of target proteins in tumor cells. A variety of other

transmembrane E3 ubiquitin ligases may also be used as targets for multispecific binding proteins herein. Optimal attributes of the multispecific antibodies are also provided herein, including for example, optimized binding affinities and multispecific formats. In some embodiments, multispecific binding proteins herein are called PROTABs (“proteolysis targeting antibodies”).

In addition to providing a general platform of multispecific binding proteins targeting a transmembrane E3 ubiquitin ligase and a cell surface protein for destruction, the present disclosure also provides a variety of antibody heavy and light chain variable regions targeting RNF43 or ZNRF3 that may be useful in constructing such multispecific antibodies and binding proteins. Binding proteins herein may, in some embodiments, be used therapeutically, for example, to degrade proteins that contribute to disease progression, for example but not limited to, in cells in which the Wnt pathway is activated and that express transmembrane E3 ubiquitin ligases. For example, certain cancers such as colorectal cancer (CRC) are characterized by overexpression of RNF43 and ZNRF3. Thus, multispecific binding proteins herein that target one or both of those ligases may be selectively targeted to cancer cells due to the cells’ overexpression of these proteins. Binding proteins herein may also be used *in vitro*, for example, to degrade a particular membrane protein in cell culture or tissue assays and thereby reduce its level and knock down its associated signaling. For example, certain E3 ubiquitin ligases such as RNF130, RNF149, and RNF167 are expressed in hematopoietic cells, and multispecific binding proteins that target those ligases may be used, in some embodiments, to target hematopoietic cells *in vivo* or *in vitro*. Similarly, E3 ubiquitin ligases such as RNF133 and RNF148 are expressed in testicular cells, and multispecific binding proteins that target those ligases may be used, in some embodiments, to target testicular cells *in vivo* or *in vitro*.

The invention provides, inter alia, multispecific binding proteins that bind to at least a first cell surface target protein and a second cell surface protein, wherein the first cell surface target protein is a transmembrane E3 ubiquitin ligase. In some aspects, a multispecific binding protein reduces the level of the second cell surface protein on the surface of a cell compared to the level observed in the absence of the multispecific binding protein. In certain aspects, a multispecific binding protein reduces the level of the second cell surface protein on the surface of a cell *in vitro* compared to the level observed in the absence of the multispecific binding protein. In some such cases, the multispecific binding protein reduces the level of the second cell surface protein on the surface of a cell *in vitro* as determined by flow cytometry or by luminescence assay. In some cases, a multispecific binding protein reduces the level of the second cell surface protein on the surface of a cell *in vivo* compared to the level observed in the absence of the multispecific binding protein, or both *in vitro* and *in vivo*.

In some aspects, a multispecific binding protein herein is a multispecific antibody. For example, in some aspects, the protein is a bispecific or trispecific antibody, such as a 1+1 FabIgG, a 1+1 FvIgG, a 2+1 FvIgG, 2+1 FabIgG, a one-armed FvIgG, or a one-armed FabIgG. In some such cases, protein comprises IgG Fc regions comprising at least one knob-into-hole
5 modification.

In some aspects, the transmembrane E3 ubiquitin ligase does not have catalytic activity, wherein lack of catalytic activity is determined in a cell surface degradation assay. In other aspects, the transmembrane E3 ubiquitin ligase has catalytic activity, wherein presence of catalytic activity is determined in a cell surface degradation assay.

10 In some aspects, a multispecific binding protein herein binds to a transmembrane E3 ubiquitin ligase selected from: RNF43, ZNRF3, RNF13, RNF128, RNF130, RNF133, RNF148, RNF149, RNF150, RNF167, ZNRF4, RSPRY1, SYVN1, LNX1 isoform 2, and TRIM7 isoform 3. In some aspects, a multispecific binding protein herein binds to a transmembrane E3 ubiquitin ligase selected from: RNF43, RNF128, RNF130, RNF133 RNF149, RNF150, or ZNRF3. In
15 some aspects, the transmembrane E3 ubiquitin ligase is RNF130, RNF133, RNF149, or RNF150. In some aspects, the transmembrane E3 ubiquitin ligase is RNF130, RNF149, or RNF167. In some aspects, the transmembrane E3 ubiquitin ligase is RNF133 or RNF148. In some aspects, the transmembrane E3 ubiquitin ligase is RNF43 or ZNRF3 or both, such that the protein binds to RNF43, ZNRF3, or both RNF43 and ZNRF3, optionally wherein the protein does not block
20 binding between RNF43 and/or ZNRF3 and FZD and/or LRP6.

In some aspects, the multispecific binding protein is a multispecific antibody that binds to RNF43 or ZNRF3; or comprises an antibody heavy chain variable region (VH) comprising (a) CDR-H1 (b) CDR-H2, and (c) CDR-H3, and a light chain variable domain (VL) comprising (d) CDR-L1, (e) CDR-L2, and (f) CDR-L3 that bind to RNF43 or ZNRF3; or comprises a VH and a
25 VL that bind to RNF43 or ZNRF3.

In some aspects, the multispecific binding protein comprises a heavy chain variable domain (VH) comprising a heavy chain complementarity determining region 1 (CDR-H1), CDR-H2, and/or CDR-H3 of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130,
30 RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76,
35 RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131,

ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

In some aspects, the multispecific binding protein comprises a heavy chain variable region (VH) comprising the CDR-H1, CDR-H2, and CDR-H3 any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

In some aspects, the multispecific binding protein comprises a light chain variable domain (VL) comprising a light chain complementarity determining region 1 (CDR-L1), CDR-L2, and/or CDR-L3 of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53,

RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

In some aspects, the multispecific binding protein comprises a light chain variable region (VL) comprising the CDR-L1, CDR-L2, and CDR-L3 any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

In some aspects, the multispecific binding protein comprises a heavy chain variable domain (VH) comprising (a) a heavy chain complementarity determining region 1 (CDR-H1), CDR-H2, and CDR-H3 and (b) a light chain variable domain (VL) comprising (a) a light chain complementarity determining region 1 (CDR-L1), CDR-L2, and CDR-L3 of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145,

RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

In any of the above cases, the heavy chain CDRs and/or the light chain CDRs may be Kabat, Chothia, or McCallum CDRs. The associated VH and VL amino acid and DNA sequences for the listed antibodies above are provided in the sequence table below, with SEQ ID Nos: 33-444.

In some aspects, the multispecific binding protein comprises a heavy chain variable region (VH) comprising an amino acid sequence at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the VH of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16,

RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332, optionally wherein the amino acid sequences of the CDR-H1, CDR-H2, and/or CDR-H3 of the VH are 100% identical to those of the chosen antibody.

In some aspects, the multispecific binding protein comprises a light chain variable region (VL) comprising an amino acid sequence at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the VL of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332, optionally wherein the amino acid sequences of the CDR-H1, CDR-H2, and/or CDR-H3 of the VH are 100% identical to those of the chosen antibody.

In some aspects, the multispecific binding protein comprises a heavy chain variable region (VH) comprising an amino acid sequence of the VH of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265,

ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

In some aspects, the multispecific binding protein comprises a light chain variable region (VL) comprising an amino acid sequence of the VL of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332. As noted above, the VH and VL amino acid and DNA sequences of the above-listed antibodies are in SEQ ID Nos: 33-444 in the sequence table below.

In some aspects, the multispecific binding protein binds to ZNRF3 and/or RNF43, and has a binding affinity for ZNRF3 or RNF43 of less than 50 nM, or less than 10 nM, or less than 1 nM, or less than 0.5 nM, or less than 0.05 nM, or between 50 nM and 10 nM, or between 10 nM and 1 nM, or between 1 nM and 0.5 nM, or between 0.5 nM and 0.05 nM, or between 0.05 nM and 0.01 nM. In some aspects, the protein is a multispecific antibody comprising a heavy chain variable region and/or a light chain variable region of a rat anti-human RNF43 B cell antibody, a rat anti-human ZNRF3 B cell antibody, a rabbit anti-human RNF43 B cell antibody, or a rabbit anti-human ZNRF3 B cell antibody. In some aspects, the protein is a trispecific antibody comprising a 2+1 FvIgG or a 2+1 FabIgG format, wherein the protein binds to both ZNRF3 and

RNF43 and to at least one second cell surface protein, optionally wherein the protein does not block binding of ZNRF3 or RNF43 to FZD and LRP6.

In some aspects, a multispecific binding protein herein may be a multispecific antibody comprising a heavy chain variable region or light chain variable region that is chimeric, or
5 comprising humanized variable regions. In some cases, the protein comprises a wild-type human Fc region. In some cases, the Fc region is an IgG1, IgG2, IgG3, or IgG4 Fc region, optionally either a wild-type human Fc region, or a human Fc region with one or more engineered mutations. In some cases, the protein comprises an Fc region having effector function. In some cases, the protein comprises a human IgG1 Fc region comprising a LALAPG mutation or a
10 substitution at position N297, such as N297G or N297Q, and/or wherein the Fc region lacks effector function.

In some aspects, the second cell surface protein to which the multispecific binding protein binds is a receptor tyrosine kinase, a growth factor receptor, a cytokine, a mucin, a Siglec receptor, or an immune checkpoint modulator, or is HER2, HER3, IGF1R, an EGFR, an FGFR,
15 a VEGFR, a PDGFR, EpCAM, FZD, PD-L1, CTLA4, PD-1, TIM3, LAG3, TIGIT, CEACAM1, CD25, ILT-2, ILT-3, ILT-4, ILT-5, LAIR-1, PECAM-1 (CD31), PILR-alpha, SIRT-1, or SIRP-alpha. In some cases, the second cell surface protein is HER2, EGFR, or IGF1R. In some cases, the multispecific binding protein comprises a heavy chain variable region and a light chain variable region of an anti-HER2 antibody such as 4D5, 7C2, or 2C4 or an anti-IGF1R antibody
20 such as cixutumumab, ganitumab, dalotuzumab, figitumumab, robatumumab, teprotumumab, or istiratumab.

The present disclosure also relates to isolated nucleic acids or sets of nucleic acids (e.g., comprising two or more nucleic acids each encoding a portion of a multimeric protein such as a light chain or a heavy chain or a half antibody), encoding a multispecific binding protein as
25 described herein. The present disclosure also relates to host cells comprising such nucleic acids or sets of nucleic acids. The present disclosure also encompasses methods of producing the multispecific binding proteins herein, comprising culturing a host cell comprising nucleic acids or sets of nucleic acids encoding the protein under conditions suitable for the expression of the protein. Such methods may further comprise recovering the protein from the host cell. The
30 present disclosure further encompasses multispecific binding proteins produced by the methods herein.

A pharmaceutical composition may also be prepared, comprising a multispecific binding protein as described herein and a pharmaceutically acceptable carrier.

The present disclosure also encompasses multispecific binding proteins and related
35 pharmaceutical compositions for use as medicaments, for example, for use in treating cancer, an

autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease in a subject, and/or for use in reducing the level of a cell surface protein in a subject in need thereof, optionally wherein the subject has cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease, and/or for use
5 in increasing an immune response in a subject, such as a subject with cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease. In some aspects, (a) the subject has a mutation in RNF43 and the multispecific binding protein does not bind to or does not activate RNF43, or (b) the subject has a mutation in ZNRF3 and the multispecific binding protein does not bind to or does not activate ZNRF3. In some such cases,
10 where the subject has an RNF43 or ZNRF3 mutation, the subject has a cancer in which the cancer comprises the mutation.

The present disclosure also relates to use of a multispecific binding protein or pharmaceutical composition herein in the manufacture of a medicament for treatment of cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease in a subject; and/or for reducing the level of a cell surface protein in a subject
15 in need thereof, optionally wherein the subject has cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease; and/or for increasing an immune response in a subject, such as a subject with cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease. In some aspects, (a) the subject has a mutation in RNF43 and the multispecific binding protein does not bind to or does not activate RNF43, or (b) the subject has a mutation in ZNRF3 and the multispecific binding protein does not bind to or does not activate ZNRF3. In some such cases,
20 where the subject has an RNF43 or ZNRF3 mutation, the subject has a cancer in which the cancer comprises the mutation.

The present disclosure also relates to methods of treating cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease in a subject in need thereof, comprising administering to the subject an effective amount of a multispecific binding protein or pharmaceutical composition herein. The present disclosure further relates to methods of reducing the level of a cell surface protein in a subject in need
30 thereof, comprising administering to the subject an effective amount of a multispecific binding protein or pharmaceutical composition herein, optionally wherein the subject has cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease. And the disclosure further relates to methods of increasing an immune response in a subject in need thereof, comprising administering to the subject an effective
35 amount of a multispecific binding protein or pharmaceutical composition herein, optionally

wherein the subject has cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease. In some aspects, (a) the subject has a mutation in RNF43 and the multispecific binding protein does not bind to or does not activate RNF43, or (b) the subject has a mutation in ZNRF3 and the multispecific binding protein does not bind to or does not activate ZNRF3. In some such cases, where the subject has an RNF43 or ZNRF3 mutation, the subject has a cancer in which the cancer comprises the mutation. In some cases, the methods further comprise, prior to administering the multispecific binding protein, determining whether the subject has a mutation in RNF43 or ZNRF3, wherein, (a) if the subject has a mutation in RNF43, the multispecific binding protein does not bind to or does not activate RNF43, and (b) if the subject has a mutation in ZNRF3, the multispecific binding protein does not bind to or does not activate ZNRF3.

In some aspects, multispecific binding proteins herein may be used to deplete levels of a cell surface protein on particular cell types by targeting an E3 ubiquitin ligase that is primarily expressed on those cell types. For example, the present disclosure also includes methods of inducing degradation of a cell surface protein on the surface of hematopoietic cells using a multispecific binding protein herein, wherein the transmembrane E3 ubiquitin ligase used in the multispecific protein is RNF130, RNF149, or RNF167. Additionally, the present disclosure also includes methods of inducing degradation of a cell surface protein on the surface of testicular cells using a multispecific binding protein herein, wherein the transmembrane E3 ubiquitin ligase used in the multispecific protein is RNF133 or RNF148. In certain aspects, the multispecific binding protein reduces the level of the cell surface protein on the surface of a particular cell type, such as a hematopoietic or testicular cell, *in vitro* compared to the level observed in the absence of the multispecific binding protein. In some such cases, the multispecific binding protein reduces the level of the cell surface protein on the surface of a particular cell type, such as a hematopoietic or testicular cell, *in vitro* as determined by flow cytometry or by luminescence assay. In some cases, a multispecific binding protein reduces the level of the second cell surface protein on the surface of a particular cell type, such as these, *in vivo* compared to the level observed in the absence of the multispecific binding protein, or alternatively, both *in vitro* and *in vivo*. Accordingly, the present disclosure also includes methods of reducing the level of a cell surface protein on the surface of hematopoietic cells in a cell or tissue sample *in vitro* or *in vivo* in a subject, comprising administering to the cell or tissue sample, or to the subject a multispecific binding protein that targets one or more of RNF130, RNF149, and RNF167. The disclosure also includes methods of reducing the level of a cell surface protein on the surface of testicular cells in a cell or tissue sample *in vitro* or *in vivo* in a

subject, comprising administering to the cell or tissue sample or to the subject a multispecific binding protein that targets one or both of RNF133 and RNF148.

The present disclosure also encompasses kits comprising a multispecific binding protein, nucleic acid, set of nucleic acids, or host cell described herein, and further comprising one or more reagents for expressing or purifying the multispecific binding protein and/or one or more reagents for incubating the protein with a cell or tissue sample *in vitro* to reduce the level of a cell surface protein in the sample. And the disclosure also encompasses methods of reducing the level of a cell surface protein in a cell or tissue sample *in vitro*, comprising incubating the sample with a multispecific binding protein described herein.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1F show that cell surface ligases driven by Oncogenic Wnt can be repurposed for targeted degradation. **Fig. 1A)** Schematic depicting the role of RNF43 in controlling cell surface abundance of frizzled (FZD) receptors through ubiquitination. **Fig. 1B)** Human adenoma primary tissues expression of RNF43 (left panel) and ZNRF3 (right panel) (see Galamb O., *et al.*, Cancer Epidemiol Biomarkers Prev 17(10): 2835-2845, 2008). **Fig. 1C)** pan-TCGA (The Cancer Genome Atlas) data showing expression of RNF43 in various cancer subtypes. Note the high expression in colorectal cancer and other tumor subtypes displaying elevated Wnt activity (red dots indicate tumor tissue; black dots indicate normal tissue). **Fig. 1D)** Doxycycline inducible expression of wild-type (WT) RNF43, but not a mutant, delta RING RNF43 that is deficient in ligase activity, leads to decreased FZDs cell surface expression. **Fig. 1E)** Schematic strategy used to chemically dimerize RNF43 and/or ZNRF3 to a non-natural cell surface with an A/C heterodimerizer, including Receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs). **Fig. 1F)** Immunoprecipitation of tagged RNF43 followed by immunoblotting of tagged HER2 in transiently transfected HEK293T cells after treatment with A/C heterodimerizer.

Figures 2A-2E shows antibody mediated dimerization of gD-tagged cell surface RNF43 or ZNRF3 ligases to HER2. **Fig. 2A)** Schematic depicts dimerization of N-terminus gD-tagged RNF43 or ZNRF3 ligases to protein of interest using an anti-gD/anti-protein of interest (POI) bispecific antibody. **Fig. 2B)** Bispecific antibodies against gD and three distinct HER2 epitopes. **Fig. 2C)** Fluorescence assisted cell sorting (FACS) plot depicting cell surface gD expression of HEK293T cells transiently transfected with either gD tagged RNF43 or ZNRF3. **Fig. 2D)** Western blot of HER2 in HEK293T transiently transfected with gD tagged RNF43, gD tagged ZNRF3 or control ("mock") after treatment with bispecific antibodies targeting gD and HER2 ("gD/HER2"). Actin is used as a loading control. **Fig. 2E)** Western blot of HER2 in MCF7,

KPL4 and SKBr3 cells stably transfected with gD-RNF43 and gD-ZNRF3 after treatment with the depicted bispecific antibodies. Downstream modulation of the HER2 pathway is depicted in SKBr3 using p-Erk. Actin is used as a loading control.

Figures 3A-3E show a mechanism of cell surface clearance and receptor degradation upon RNF43/ZNRF3 dimerization. **Fig. 3A)** Mean fluorescence intensity (MFI) of cell surface HER2 in HT29 cells transfected with gD-ZNRF3 after treatment with various antibodies against gD/HER2 or CD3/HER2. **Fig. 3B)** Western blot of HER2 in HEK293T transiently transfected with gD tagged ZNRF3 after treatment with the depicted bispecific antibodies against gD/HER2 or CD3/HER2 or transfected with a guide RNA driving genetic deletion of HER2. Tubulin is used as a loading control. **Fig. 3C)** Immunoprecipitation of HER2 followed by immunoblotting of ubiquitin in HEK293T transiently transfected with gD tagged RNF43, ZNRF3 or control (“mock”) after treatment with the depicted three different bispecific antibodies against gD and HER2: T (Trastuzumab, 4D5), P (Pertuzumab, 2C4), and 7 (7C2). **Fig. 3D)** Western blot of HER2 in HEK293T transiently transfected with gD tagged RNF43, ZNRF3 that are either proficient (WT RING) or deficient (delta-RING) in ligase activity, or control after treatment with the depicted bispecific antibodies against gD/HER2. Actin is used as a loading control. **Fig. 3E)** Western blot of HER2 in HEK293T transiently transfected with gD tagged RNF43, ZNRF3 or control after treatment with the depicted bispecific antibodies against gD/HER2 and depicted inhibitors (Bortezomib = proteasome inhibitor; E1 activating enzyme inhibitor, BafA = Lysosome inhibitor). Actin is used as a loading control. (T = Trastuzumab (4D5), P = Pertuzumab (2C4), 7 = 7C2)

Figures 4A-4F show phenotypic impact of cell surface ligase mediated receptor degradation. **Fig. 4A)** Immunoprecipitation of tagged RNF43 followed by immunoblotting of tagged IGF1R in transiently transfected HEK293T cells after treatment with AC heterodimerizer. Tubulin is used as a loading control. **Fig. 4B)** Western blot of IGF1R in HT29 cells transfected with a doxycycline (“dox”) inducible gD tagged ZNRF3 after treatment with dox and the depicted bispecific antibodies against gD an various IGF1R epitopes. Tubulin is used as a loading control. **Fig. 4C)** Clonogenic growth assay of HT55 cells transfected with a dox inducible CRISPR-Cas9 and sgRNA targeting IGF1R or non-targeting control (NTC) sgRNA. **Fig. 4D)** In vivo tumor growth of HT55 cells transfected with a dox inducible CRISPR-Cas9 and sgRNA targeting IGF1R or non-targeting control (NTC) sgRNA. **Fig. 4E)** Clonogenic growth assay of HT55 cells transfected with a dox inducible gD-ZNRF3 after treatment with anti-Citxu/gD or anti-Citxu/NIST bispecific antibodies. Licor based quantification of Fig. 4E) is depicted in **Fig. 4F)**.

Figures 5A-5F provide a characterization of RNF43 and ZNRF3 targeted antibodies and related bispecifics. **Fig. 5A)** SPR binding of antibodies discovered from rat or rabbit immunizations to RNF43 or ZNRF3. **Fig. 5B and 5C)** Cross-blocking analysis of a subset of ZNRF3 (Fig. 5B) and RNF43 (Fig. 5C) binding antibodies. **Fig. 5D)** Time course of cixutumumab/hSC37.39 bispecific antibody driving cell surface clearance of IGF-1R at zero to 10 µg/mL concentrations, measured by tracking IGF1R MFI at 1 to 24 hours. **Fig. 5E and Fig. 5F)** Clearance of IGF1R using a panel of ligase/cixutumumab bispecific antibodies binding to human ZNRF3 ("hu ZNRF3"; **Fig. 5E**) or human RNF43 ("hu RNF43"; **Fig. 5F**). Percent clearance is plotted versus monovalent affinity for each of the respective ligases.

Figures 6A-6C show characterization of the degradation potential of novel cell surface ligase antibodies. **Fig. 6A and Fig. 6B)** Mean fluorescence intensity (MFI) of cell surface IGF1R in SW1417 or HT29 cells transfected with gD-ZNRF3 after treatment with various antibodies against IGF1R/ZNRF3 (**Fig. 6A**) or IGF1R/NIST (**Fig. 6B**). **Fig. 6C)** Western blot of IGF1R in parental SW1417 cells after treatment with depicted bispecific antibodies. Actin is used as loading control.

Figures 7A-7F show characterization of the degradation potential of novel formats of cell surface ligase antibodies. **Fig. 7A and Fig. 7B)** show schematics of the tested antibodies. **Fig 7C)-Fig. 7F)** show flow cytometry analysis of IGF1R to assess antibody activity in HT29 cells overexpressing the relevant ligase. **Fig. 7C)** shows results for cixutumumab/anti-RNF43 constructs with one or two cixutumumab (anti-IGFR; "cixu") binding regions, **Fig. 7D)** shows results for cixutumumab/anti-RNF43 constructs with one or two RNF43 binding regions, **Fig. 7E)** shows results for istiratumab/anti-RNF43 constructs with one or two istiratumab (anti-IGFR; "istira") binding regions, and **Fig. 7F)** shows results for istiratumab/anti-RNF43 constructs with one or two RNF43 binding regions.

Figures 8A-8D) show characterization of the degradation potential of additional novel formats of cell surface ligase antibodies. **Fig. 8A)** Schematics of the tested FvIgG or FabIgG format antibodies are shown. **Fig 8B)** Flow cytometry analysis of IGF1R is used to assess activity of the FvIgG formats in HT29 cells overexpressing the relevant ligase. **Fig 8C and Fig. 8D)** Flow cytometry analysis of IGF1R is used to assess activity of the FabIgG formats in HT29 cells overexpressing the relevant ligase (**Fig. 8C** = cixutumumab/anti-RNF43; **Fig. 8D** = istiratumab/anti-RNF43).

Figure 9 shows characterization of the degradation potential of EGFR targeted multispecifics. Flow cytometry analysis of EGFR is used to assess activity of the multispecifics in HT29 cells overexpressing the relevant ligase.

Figures 10A-10F show characterization of degradation potential of novel cell surface ligase antibodies. **Fig. 10A)** Cartoon depicting the structural domains of both RNF43 and ZNRF3, including signal peptide (SP), Transmembrane domain (TM) and ligase domain (RING). **Fig. 10B)** Identification and gD tagging of additional E3 ligases with similar structural characteristics as RNF43 and ZNRF3, including SP and TM. **Fig. 10C)** FACS plot depicts transfection of gD-ZNRF3 in HEK293T cells (FITC positive) and gD cell surface detection using anti-gD antibody (conjugated to APC). **Fig. 10D)** Cell surface MFI of gD signal from depicted gD tagged ligases transiently transfected into HEK293T cells. gD is detected using anti-gD APC conjugated antibody. **Fig. 10E and Fig. 10F)** Western blots of HER2 in HEK293T cells transiently transfected with the depicted dox inducible gD tagged ligases after treatment with anti-HER2/gD bispecific antibody. Western blot for gD and tubulin are used transfection evaluation and loading control, respectively. **Fig. 10E** shows data for ligases RNF13, RNF43, RNF128, RNF130, RNF133, RNF148, and RNF149. **Fig. 10F** shows data for ligases RNF150, RNF167, ZNRF3, LNX1, RSPRY1, SYVN1, and TRIM7.

Figures 11A and 11B show the activation of Wnt/ β -catenin signaling pathway via TCF reporter by ZNRF3 antibodies. **Fig. 11A)** Rat clone ZNRF3 bivalent antibodies triggered minor activation of Wnt/ β -catenin in the presence of 100 ng/mL recombinant Wnt3a in HEK293 cells. Cells without any treatment, with DMSO, and with 100 ng/mL Wnt3a were negative controls; cells treated with 2 μ M GSK3 β or various concentration of RSPO3 (500 ng/ μ L, 10 ng/ μ L, 0.2 ng/ μ L) in combination with 100 ng/mL Wnt3a were positive controls. **Fig. 11B)** Rabbit clone ZNRF3 bivalent antibodies triggered minor activation of Wnt/ β -catenin. Positive and negative controls used are described above. Data shown are from two independent experiments (n=2).

Figures 12A-12F show the kinetics of bispecific antibody-mediated surface IGF1R-HibiT clearance. **Fig. 12A-12C)** Percent cell surface IGF1R-HibiT that remained was evaluated at 0, 4, 8 and 24 hours (h) with bispecific antibodies Cixu/ZNRF3-6 (**Fig. 12A**), Cixu/ZNRF3-55 (**Fig. 12B**) and Cixu/RNF43-67 (**Fig. 12C**) at concentrations of 10 μ g/mL and 1 μ g/mL. A time-dependent clearance was observed. Cixu/RNF43 is a positive control. **Fig. 12D-12F)** Time-dependent clearance was observed using the new format Cixu-RNF43 Fv-IgG (**Fig. 12D**) and Istira-RNF43 Fv-IgG (**Fig. 12E**), and Istira-RNF43 Fv-IgG (**Fig. 12F**) at 10 μ g/mL displayed a “hook effect”.

Figures 13A-13C show cellular toxicity and cell surface clearance evaluated by multiplexing the HiBiT extracellular detection assay with LDH or CellTiter-Glo® assay. **Fig. 13A)** IGF1R-HibiT remained on the cell surface after treatment with cixu/ZNRF3 bispecific antibodies at 1 μ g/mL over 48h. **Fig. 13B)** Lactate dehydrogenase released by HT29 cells treated with cixu/ZNRF3 bispecific antibodies at 1 μ g/mL over 48h was measured using an LDH

cytotoxicity assay. No toxicity was observed with the treatment conditions. **Fig. 13C)** CellTiter-Glo® assay measured luminescence signal (RLU) was performed after detection of IGF1R-HiBiT on the cell surface using the HiBiT extracellular detection system. Similar to the LDH assay, no toxicity was observed with the treatment conditions.

Figures 14A and 14B show lytic detection of the total level of IGF1R-HiBiT. **Fig. 14A)** The HiBiT extracellular system is used to detect the remaining amount of IGF1R-HiBiT on the surface of HT29 cells. The lytic detection shows a total level of IGF1R-HiBiT (extracellular and intracellular). Both systems measure a luminescence signal. Cixu-RNF43 Fv-IgG format caused 20% IGF1R-HiBiT to remain on the cell surface and 80% intact IGF1R-HiBiT.

Cixu/RNF43.hSC37.39 was a positive control. Cixu/NIST, Cixu/Cixu and UT were negative controls. **Fig 14B)** Western-blot shows the total amount of IGF1R_HiBiT or IGF1R in WT HT29 cells upon 24h treatment of new format and bispecific antibodies. Pro IGF1R-HiBiT/IGF1R is the precursor of IGF1R-HiBiT/IGF1R. B-actin is an internal control.

Figures 15A-15G show different, exemplary formats for constructing multispecific antibodies that are compatible with embodiments herein, a “2+1 FabIgG” format (**Fig. 15A**), a “one armed FvIgG” or “OA FvIgG” format (**Fig. 15B**), and a one armed FabIgG” or “OA FabIgG” format (**Fig. 15C**). **Figures 15D-15E** show two different optional versions of a trispecific anti-EGFR-RNF43-ZNRF3 antibody, while **Figures 15F-15G** show two different optional versions of a trispecific anti-IGF1R-RNF43-ZNRF3 antibody.

Figure 16 shows that combinations of bipecifics targeting both RNF43/IGF1R and ZNRF3/IGF1R are more effective at removing IGF1R from the cell surface than either bispecific alone.

Figure 17 shows that RNF43 is homogeneously expressed throughout the tumor mass in a transplanted murine APC mutant colorectal cancer model.

Figures 18A-18B show Western-blot showing the total amount of IGF1R in WT LS180 cells upon 24h treatment of various bispecific antibodies (**Fig. 18A**). Pro IGF1R is the precursor of IGF1R. Tubulin is used as a loading control. Degradation percentage is summarized across various cell lines (**Fig. 18B**).

Figures 19A-19B show Western blot analysis of lysates derived from HT29 cells subjected to the indicated treatments: IGF1 stimulation (+IGF1; 50 ng/ml; 5 minutes), no treatment (-), bivalent antibody treatment (Cixu; 1 µg/ml; 135 minutes), control bispecific antibody (NIST; 1 µg/ml; 135 minutes), RNF43-based bispecific antibody (RNF43-35; 1 µg/ml; 135 minutes) or ZNRF3-based bispecific antibody (ZNRF3-55; 1 µg/ml; 135 minutes) following IgG or IGF1Rβ immunoprecipitation (IP). IGF1R ubiquitylation was evaluated by

immunoblotting IP samples using an antibody against ubiquitin. Immunoprecipitated and total

IGF1R β levels were evaluated using an antibody against IGF1R β and α -TUBULIN was used as a loading control (**Fig. 19 A**). Western blot analysis of lysates derived from parental HEK293T cells or HEK293T cells expressing N-terminally gD and C-terminally FLAG tagged ZNRF3 wild type (WT) or the delta RING mutant (Δ RING) following incubation with HA epitope tag antibody agarose conjugate or agarose-TUBE2. Total and co-precipitated IGF1R levels were evaluated by immunoblotting using an antibody against IGF1R β . ZNRF3 ubiquitylation and expression levels were evaluated using an antibody against the C-terminal FLAG tag and α -TUBULIN was used as a loading control (**Fig. 19B**).

Figure 20 shows Western blot of IGF1R in HEK293T transiently transfected with gD tagged ZNRF3 that is either proficient (WT RING) or deficient (delta-RING) in ligase activity, or control after treatment with the depicted bispecific antibodies against gD/Cixutumumab. Tubulin is used as a loading control.

Figures 21A-21C show distribution of RNF43 expression is plotted against the presence of RNF43 mutations. Particular emphasis is made on the SW48 CRC line that display elevated RNF43 expression and a frameshit variant within the RING domain (**Fig. 21A**). Cell surface expression of RNF43 is showed for a the non expressing line RKO compared to the RING domain mutant SW48 (**Fig. 21 B**). Western blot of IGF1R in SW48 after treatment with the depicted bispecific antibodies against RNF43/IGF1R or ZNRF3/IGF1R (**Fig. 21 C**).

Figures 22A-22C show schematic representation of indels generation within RNF43 (**Fig. 22A**) and ZNRF3 (**Fig. 22 B**) using CRISPR CAS9 system. FACS plot depicts cell surface ligase expression of RNF43 or ZNRF3 in the presence of n-terminal truncation (N-term) or RING indel. Western blot of IGF1R in HT29 cells that are ligase proficient, N-term knock out or RING deficient transiently transfected with gD tagged ZNRF3 that is either proficient after treatment with the depicted bispecific antibodies (**Fig. 22 C**).

Figure 23 shows a Western blot of IGF1R in DLD1 after treatment with the depicted bispecific antibodies against gD/IGF1R and depicted inhibitors (Bortezomib = proteasome inhibitor; E1 activating enzyme inhibitor, BafA = Lysosome inhibitor). Tubulin is used as a loading control, Ubiquitin is used to validate inhibition of the proteasome and E1 activating enzyme. LC3B is used to confirm the inhibition of the lysosome.

Figure 24 shows a Western blot of IGF1R in DLD1 upon 24h treatment of new format and bispecific antibodies show three different, exemplary formats for constructing bispecific antibodies that are compatible with embodiments herein, a “2+1 FabIgG” format (**Fig. 15A**), a “one armed FvIgG” or “OA FvIgG” format.

Figures 25A-25C show Western blot of IGF1R, pIGF1R and downstream component of the IGF1R signaling axis, including pAKT and pS6 in SW48 cells treated with various depicted

antibodies (**Fig. 25A**). Note the near complete inhibition of pAKT signaling upon ligase mediated degradation of IGF1R. Clonogenic outgrowth of SW48 cells 14 days after treatment with depicted antibodies (**Fig. 25B**) and licor based quantification of (**Fig. 25B**) is depicted in (**Fig. 25C**).

Figures 26A-26D show a clonogenic growth assay of HT29 cells transfected with a dox inducible gD-ZNRF3 after treatment with various anti-Citxu/ZNRF3 or control bispecific antibodies. Licor based quantification of **Fig. 26A** (results for gD-ZNRF3 WT-FLAG) and **Fig. 26C** (results for gD-ZNRF3 delta-RING-FLAG mutant) are depicted in **Fig. 26B** and **Fig. 26D**, respectively.

Figure 27 shows a Western blot of PD-L1, in SW48 cells treated with various depicted antibodies. Note the deep degradation seen when ZNRF3/PD-L1 antibodies are used.

Figures 28A-28B show characterization of degradation potential of novel cell surface ligase antibodies. FACS plot depicts cell surface expression of gD-RNF43, RNF13 and RNF128 stable HT29 cells (**Fig. 28 A**). gD cell surface detection using anti-gD antibody (conjugated to APC). **Fig. 28 B**: Cell surface MFI of gD signal from depicted gD tagged ligases stably integrated into HT29 cells. gD is detected using anti-gD APC conjugated antibody.

Figure 29 shows a Western blot of IGF1R in HT29 cells with the depicted dox inducible gD tagged ligases after treatment with anti-IGF1R/gD bispecific antibody. Western blot for gD and tubulin are used transfection evaluation and loading control, respectively.

Figure 30 shows that bispecific antibodies designed to tether endogenous RNF43 or ZNRF3 to IGF1R induce IGF1R target degradation in an SW48 *in vivo* xenograft model.

Figure 31 shows a heatmap depicting expression of indicated cell surface ligases across normal tissues (source GTEx) as described in Example 18. Data is z-scored normalized.

Figure 32 shows Western blot analysis of lysates from doxycycline treated HT29 cells harboring the indicated doxycycline inducible gD-ligase-FLAG expression constructs following 24 hours incubation with gD*IGF1R (Cixu) bispecific PROTAB. Endogenous IGF1R β and exogenous gD-ligase-FLAG protein levels were detected. Data are representative of three independent experiments.

Figure 33 shows further Western blot analysis of lysates from doxycycline treated HT29 cells harboring the indicated doxycycline inducible gD-ligase-FLAG expression constructs following 24 hours incubation with gD*IGF1R (Cixu) bispecific PROTAB. Endogenous IGF1R β and exogenous gD-ligase-FLAG protein levels were detected. Data are representative of three independent experiments.

Figure 34 shows Western blot analysis of lysates from doxycycline treated HT29 cells harboring the indicated doxycycline inducible gD-ligase-FLAG expression constructs following

24 hours incubation with gD*HER2 (4D5) bispecific PROTAB. Endogenous HER2 and exogenous gD-ligase-FLAG protein levels were detected. Data are representative of two independent experiments.

Figure 35 shows Western blot analysis of lysates from doxycycline treated HT29 cells harboring the indicated doxycycline inducible gD-ligase-FLAG expression constructs following 24 hours incubation with gD*PD-L1 (Atezo) bispecific PROTAB. Endogenous PD-L1 and exogenous gD-ligase-FLAG protein levels were detected. Data are representative of two independent experiments.

Figure 36 shows further Western blot analysis of lysates from doxycycline treated HT29 cells harboring the indicated doxycycline inducible gD-ligase-FLAG expression constructs following 24 hours incubation with gD*HER2 (4D5) bispecific PROTAB. Endogenous HER2 and exogenous gD-ligase-FLAG protein levels were detected. Data are representative of two independent experiments.

Figure 37 shows further Western blot analysis of lysates from doxycycline treated HT29 cells harboring the indicated doxycycline inducible gD-ligase-FLAG expression constructs following 24 hours incubation with gD*PD-L1 (Atezo) bispecific PROTAB. Endogenous PD-L1 and exogenous gD-ligase-FLAG protein levels were detected. Data are representative of two independent experiments.

Figures 38A-38L show cell surface clearance of EpCAM (Figs. 38A-38F) and degradation of EpCAM in doxycycline treated HT29 cells harboring the indicated doxycycline inducible gD-ligase expression constructs (Figs. 38G-38J) following 24 hours incubation with 1 μ g/ml and 10 μ g/ml of the gD-EpCAM or control gD-NIST bispecific PROTAB. Fig. 38A-Fig. 38F show %EpCAM clearance assessed by FACS, while Fig. 38G-Fig. 38L show EpCAM degradation assessed by MFI. Fig. 38A and Fig. 38G: HT29 cells; Fig. 38B and Fig. 38H: HT29-gD-RNF43; Fig. 38C and Fig. 38I: HT29-gD-RNF133; Fig. 38D and Fig. 38J HT29-gD-RNF149; Fig. 38E and Fig. 38K: HT29-gD-RNF150; and Fig. 38F and Fig. 38L: HT29-gD-ZNRF3. Fig. 38C and Fig. 38D show that HT29-gD-RNF133 and HT29-gD-RNF149 had the highest clearance of EpCAM (20-30%) compared to the other HT-29-gD-ligases, while Fig. 38I and Fig. 38J show that HT29-gD-RNF133 and HT29-gD-RNF149 had the highest degradation of EpCAM compared to the other HT29-gD-ligases. Similar results showing highest clearance and degradation of HER2, EGFR, and IGF1R by the respective gD-HER2, gD-EGFR, or gD-IGF1R PROTAB in HT29-gD-RNF133 and HT29-gD-RNF149 compared to the other HT-29-gD-ligases were observed (data not shown).

Figures 39A and 39B show HER2 degradation in SW48 following bivalent or PROTAB antibody treatment. Fig. 39A shows level of HER2 degradation by Western blot analysis in

SW48 cells left untreated (-), or subjected to a HER2 bivalent antibody (7C2), a NIST*HER2 (NIST) control bispecific antibody, or HER2 bispecific PROTABs (ZNRF3-6 and RNF43-37.39) for 48 hours. Fig. 39B shows level of HER2 degradation by Western blot analysis in SW48 cells left untreated (-), or subjected to a HER2 bivalent antibody (4D5), NIST*HER2 bispecific antibody (NIST), a ZNRF3*HER2 PROTAB antibody (ZNRF3-6), or a ZNRF3*NIST control bispecific antibody for 48 hours. The level of alpha-tubulin was used as a loading control. Data represent two independent experiments.

DETAILED DESCRIPTION OF THE INVENTION

I. DEFINITIONS

As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

A “transmembrane E3 ubiquitin ligase” refers to a class of protein that comprises at least one cell membrane spanning region and that has E3 ubiquitin ligase activity. A transmembrane E3 ubiquitin ligase has “catalytic activity” herein if it is capable of facilitating the final transfer of ubiquitin from the ubiquitin-conjugating enzymes (E2s) to substrates to alter that substrate function. In some embodiments, catalytic activity may be determined in a cell surface assay (see, e.g., Example 18 below.)

A “protein of interest” or a “protein targeted for degradation” or a “degradation target protein” or the like refer to a protein that is intended to be brought to lysosomes for degradation by the molecules described herein. In some embodiments herein, such a protein intended for degradation is a “cell surface protein.” A “cell surface protein” as used herein broadly refers to a protein that is located at the cell surface, either as a transmembrane protein possessing an extracellular domain, or as a protein that is otherwise localized to the cell surface, such as a protein that is bound to a transmembrane protein.

Proteins such as transmembrane E3 ubiquitin ligases and degradation targets herein may be from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), or domestic mammals (e.g., dogs, cats, horses, livestock such as cattle, pigs, sheep, goats, etc.), avians (e.g., fowl, chickens, turkey), or fish, unless otherwise indicated.

A “multispecific binding protein” as used herein refers to a protein molecule that may be used to bind to a transmembrane E3 ubiquitin ligase and also to a degradation target protein. The protein is “multispecific” because it binds to at least two target proteins (i.e., the transmembrane E3 ubiquitin ligase and the degradation target). In some embodiments, the

binding protein is an antibody, such as a bispecific or multispecific antibody, or is a bispecific or multispecific protein that comprises an antibody or antibody fragment and optionally another specific protein binding domain.

The term “antibody” herein refers to a molecule comprising at least complementarity-determining region (CDR) 1, CDR2, and CDR3 of a heavy chain and at least CDR1, CDR2, and CDR3 of a light chain, wherein the molecule is capable of binding to antigen. The term is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies, diabodies, etc.), full length antibodies, single-chain antibodies, antibody conjugates, and antibody fragments, so long as they exhibit the desired binding activity.

[001] An “isolated” antibody is one that has been separated from a component of its natural environment. In some aspects, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC) methods. For a review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An “antigen” refers to the target of an antibody, i.e., the molecule to which the antibody specifically binds. The term “epitope” denotes the site on an antigen, either proteinaceous or non-proteinaceous, to which an antibody binds. Epitopes on a protein can be formed both from contiguous amino acid stretches (linear epitope) or comprise non-contiguous amino acids (conformational epitope), e.g., coming in spatial proximity due to the folding of the antigen, i.e. by the tertiary folding of a proteinaceous antigen. Linear epitopes are typically still bound by an antibody after exposure of the proteinaceous antigen to denaturing agents, whereas conformational epitopes are typically destroyed upon treatment with denaturing agents.

“Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by common methods known in the art, including those described herein.

In this disclosure, “binds” or “binding” or “specific binding” and similar terms, when referring to a protein and its ligand or an antibody and its antigen target for example, means that the binding affinity is sufficiently strong that the interaction between the members of the binding pair cannot be due to random molecular associations (i.e. “nonspecific binding”). Such binding

typically requires a dissociation constant (K_D) of 1 μ M or less, and may often involve a K_D of 100 nM or less.

An “anti-RNF43 antibody” or a “RNF43-antibody” or an “antibody that specifically binds RNF43” or an “antibody that binds to RNF43” and similar phrases (e.g., an anti-gD antibody or an anti-ZNRF3 antibody, or an anti-HER2 antibody and the like) refer to an antibody that specifically binds to the indicated protein.

The term “heavy chain” refers to a polypeptide comprising at least a heavy chain variable region, with or without a leader sequence. In some embodiments, a heavy chain comprises at least a portion of a heavy chain constant region. The term “full-length heavy chain” refers to a polypeptide comprising a heavy chain variable region and a heavy chain constant region, with or without a leader sequence.

The term “light chain” refers to a polypeptide comprising at least a light chain variable region, with or without a leader sequence. In some embodiments, a light chain comprises at least a portion of a light chain constant region. The term “full-length light chain” refers to a polypeptide comprising a light chain variable region and a light chain constant region, with or without a leader sequence.

The term “hypervariable region” or “HVR” as used herein refers to each of the regions of an antibody variable region which are hypervariable in sequence and which determine antigen binding specificity, for example “complementarity determining regions” (“CDRs”). Generally, antibodies comprise six CDRs: three in the VH (CDR-H1 or heavy chain CDR1, CDR-H2, CDR-H3), and three in the VL (CDR-L1, CDR-L2, CDR-L3). Exemplary CDRs herein include:

(a) “Chothia CDRs”: hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));

(b) “Kabat CDRs”: CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991)); and

(c) “McCallum CDRs”: antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)).

Unless specifically indicated, the CDRs are determined according to Kabat et al., *supra*. One of skill in the art will understand that the CDR designations can also be determined according to McCallum, or any other scientifically accepted nomenclature system.

“Framework” or “FR” refers to the residues of the variable region residues that are not part of the complementary determining regions (CDRs). The FR of a variable region generally consists of four FRs: FR1, FR2, FR3, and FR4. Accordingly, the CDR and FR sequences generally appear in the following sequence in VH (or VL): FR1-CDR-H1(CDR-L1)-FR2- CDR-
 5 H2(CDR-L2)-FR3- CDR-H3(CDR-L3)-FR4. An “acceptor human framework” for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus
 10 framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some aspects, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some aspects, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

The term “variable region” or “variable domain” interchangeably refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three complementary determining regions (CDRs). See, e.g., Kindt et al.
 20 *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007). A variable domain may comprise heavy chain (HC) CDR1-FR2-CDR2-FR3-CDR3 with or without all or a portion of FR1 and/or FR4; and light chain (LC) CDR1-FR2-CDR2-FR3-CDR3 with or without all or a portion of FR1 and/or FR4. That is, a variable domain may lack a portion of FR1 and/or FR4 so long as it retains antigen-binding activity. A single VH or VL domain may be sufficient to
 25 confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The light chain and heavy chain “constant regions” of an antibody refer to additional
 30 sequence portions outside of the FRs and CDRs and variable regions. Certain antibody fragments may lack all or some of the constant regions. From N- to C-terminus, each heavy chain has a variable domain (VH), also called a variable heavy domain or a heavy chain variable region, followed by three constant heavy domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable domain (VL), also called a variable light domain or a
 35 light chain variable region, followed by a constant light (CL) domain.

The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one aspect, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, antibodies produced by host cells may undergo post-translational cleavage of one or more, particularly one or two, amino acids from the C-terminus of the heavy chain. Therefore, an antibody produced by a host cell by expression of a specific nucleic acid molecule encoding a full-length heavy chain may include the full-length heavy chain, or it may include a cleaved variant of the full-length heavy chain. This may be the case where the final two C-terminal amino acids of the heavy chain are glycine (G446) and lysine (K447, numbering according to EU index). Therefore, the C-terminal lysine (Lys447), or the C-terminal glycine (Gly446) and lysine (Lys447), of the Fc region may or may not be present. Thus, a “full-length IgG1” for example, includes an IgG1 with Gly446 and Lys447, or without Lys447, or without both Gly446 and Lys447. Amino acid sequences of heavy chains including an Fc region are denoted herein without C-terminal glycine-lysine dipeptide if not indicated otherwise. In one aspect, a heavy chain including an Fc region as specified herein, comprised in an antibody according to the invention, may comprise Gly446 and Lys447 (numbering according to EU index). In one aspect, a heavy chain including an Fc region as specified herein, comprised in an antibody according to the invention, may comprise Gly446 (numbering according to EU index). Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

“Effector functions” refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor); and B cell activation.

The “class” of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. In certain aspects, the antibody is of the human IgG₁, IgG₂, IgG₃, or IgG₄ isotype. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The light chain of an antibody may be

assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen (i.e. RNF43, ZNRF3, gD, or a degradation target protein) to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv, and scFab); single domain antibodies (dAbs); and multispecific antibodies formed from antibody fragments. For a review of certain antibody fragments, see Holliger and Hudson, *Nature Biotechnology* 23:1126-1136 (2005).

The terms “full length antibody”, “intact antibody”, and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or, in the case of an IgG antibody, having heavy chains that contain an Fc region as defined herein.

The term “multispecific” herein refers to a molecule that can bind to more than one different target or antigen, such as to two or three or more different targets or antigens. The term “bispecific” herein refers to a molecule such as a binding protein or antibody that is able to specifically bind to two different targets or antigens. A “multispecific” or “bispecific” antibody herein may include the appropriate full length heavy and light chains for binding to two different antigens, or it may include appropriate antibody fragments for binding to two different antigens. There are a variety of different platforms for creating multispecific binding proteins that are compatible with this disclosure.

The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing

transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

The term “chimeric” antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

A “humanized” antibody refers to a chimeric antibody comprising amino acid residues from non-human CDRs and amino acid residues from human FRs. In certain aspects, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

An “immunoconjugate” is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent. A “naked antibody” refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical composition.

The term “nucleic acid molecule” or “polynucleotide” includes any compound and/or substance that comprises a polymer of nucleotides. Each nucleotide is composed of a base, specifically a purine- or pyrimidine base (i.e. cytosine (C), guanine (G), adenine (A), thymine (T) or uracil (U)), a sugar (i.e. deoxyribose or ribose), and a phosphate group. Often, the nucleic acid molecule is described by the sequence of bases, whereby said bases represent the primary structure (linear structure) of a nucleic acid molecule. The sequence of bases is typically represented from 5' to 3'. Herein, the term nucleic acid molecule encompasses deoxyribonucleic acid (DNA) including e.g., complementary DNA (cDNA) and genomic DNA, ribonucleic acid (RNA), in particular messenger RNA (mRNA), synthetic forms of DNA or RNA, and mixed polymers comprising two or more of these molecules. The nucleic acid molecule may be linear or circular. In addition, the term nucleic acid molecule includes both, sense and antisense strands, as well as single stranded and double stranded forms. Moreover, the herein described nucleic acid molecule can contain naturally occurring or non-naturally occurring nucleotides. Examples

of non-naturally occurring nucleotides include modified nucleotide bases with derivatized sugars or phosphate backbone linkages or chemically modified residues. Nucleic acid molecules also encompass DNA and RNA molecules which are suitable as a vector for direct expression of an antibody of the invention *in vitro* and/or *in vivo*, e.g., in a host or patient. Such DNA (e.g.,
5 cDNA) or RNA (e.g., mRNA) vectors, can be unmodified or modified. For example, mRNA can be chemically modified to enhance the stability of the RNA vector and/or expression of the encoded molecule so that mRNA can be injected into a subject to generate the antibody *in vivo* (see e.g., Stadler et al, Nature Medicine 2017, published online 12 June 2017, doi:10.1038/nm.4356 or EP 2 101 823 B1).

10 An “isolated” nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

15 “Isolated nucleic acid encoding an antibody” refers to one or more nucleic acid molecules encoding antibody heavy and light chains of antibodies herein (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term “vector”, as used herein, refers to a nucleic acid molecule capable of propagating
20 another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors”.

The terms “host cell”, “host cell line”, and “host cell culture” are used interchangeably and
25 refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells”, which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as
30 screened or selected for in the originally transformed cell are included herein.

“Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity,
35 and not considering any conservative substitutions as part of the sequence identity for the

purposes of the alignment. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, Clustal W, Megalign (DNASTAR) software or the FASTA program package. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Alternatively, the percent identity values can be generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087 and is described in WO 2001/007611.

Unless otherwise indicated, for purposes herein, percent amino acid sequence identity values are generated using the ggsearch program of the FASTA package version 36.3.8c or later with a BLOSUM50 comparison matrix. The FASTA program package was authored by W. R. Pearson and D. J. Lipman (1988), "Improved Tools for Biological Sequence Analysis", PNAS 85:2444-2448; W. R. Pearson (1996) "Effective protein sequence comparison" Meth. Enzymol. 266:227- 258; and Pearson et. al. (1997) Genomics 46:24-36 and is publicly available from www.fasta.bioch.virginia.edu/fasta_www2/fasta_down.shtml or www.ebi.ac.uk/Tools/sss/fasta. Alternatively, a public server accessible at fasta.bioch.virginia.edu/fasta_www2/index.cgi can be used to compare the sequences, using the ggsearch (global protein:protein) program and default options (BLOSUM50; open: -10; ext: -2; Ktup = 2) to ensure a global, rather than local, alignment is performed. Percent amino acid identity is given in the output alignment header.

By "reduce" is meant the ability to cause an overall decrease of 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or greater. In some embodiments, reduce or inhibit can refer to a relative reduction compared to a reference (*e.g.*, reference level of biological activity (*e.g.*, wnt signaling) or binding). In some embodiments, reduce may refer to reduction of the "level" (*i.e.* the amount or concentration) of a cell surface protein on a cell, for example. Degradation of a protein, for example, can result in reduction of the level of that protein observed in a cell or tissue sample, such as by flow cytometry or by qualitative analysis of fluorescence staining.

A multispecific binding protein that "blocks binding of" a transmembrane E3 ubiquitin ligase to a ligand refers to the ability to inhibit the interaction between the ligase and one of its ligands. For example, ligases such as RNF43 and ZNRF3 bind to native ligands such as frizzled (FZD) and LRP6. In some aspects, a multispecific binding protein does not block such binding. Such inhibition may occur through any mechanism, including direct interference with ligand binding, *e.g.*, because of

overlapping binding sites on the ligase for the antibody and one or more ligands, and/or indirect interference with ligand binding, such as allosteric interference with binding, e.g., by causing conformational changes in the ligase that alter ligand affinity.

As used herein, the term “about” refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term “about” generally refers to a range of numerical values (e.g., +/-5-10% of the recited range) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). When terms such as “at least” and “about” precede a list of numerical values or ranges, the terms modify all of the values or ranges provided in the list. In some instances, the term about may include numerical values that are rounded to the nearest significant figure.

Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

In this application, the use of “or” means “and/or” unless stated otherwise. In the context of a multiple dependent claim, the use of “or” refers back to more than one preceding independent or dependent claim in the alternative only. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

Exemplary techniques used in connection with recombinant DNA, oligonucleotide synthesis, tissue culture and transformation (e.g., electroporation, lipofection), enzymatic reactions, and purification techniques are described, e.g., in Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), among other places.

The term “pharmaceutical composition” or “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the pharmaceutical composition would be administered.

A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical composition or formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

An “individual” or “subject” is a human unless otherwise specified. In some cases, where specified, an “individual” or “subject” is a non-human mammal or includes non-human mammals (e.g. “a mammalian subject” or a “non-human mammal subject”). Mammals include,

but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In some cases, non-mammalian animals whose cells express transmembrane E3 ubiquitin ligases can also be specified (e.g., avians or fish).

5 As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of a disease in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or
10 indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some aspects, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

An “effective amount” of an agent, e.g., a pharmaceutical composition, refers to an amount
15 effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer include, but are not limited to, carcinoma, lymphoma (e.g., Hodgkin’s and non-Hodgkin’s
20 lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine
25 carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.

II. MULTISPECIFIC BINDING PROTEINS

In one aspect, the disclosure herein concerns multispecific binding proteins that are
30 capable of specifically binding both at least one first cell surface protein which comprises a transmembrane E3 ubiquitin ligase and also to at least one second cell surface protein. In some embodiments, the second cell surface protein may be intended for degradation (i.e. a degradation target protein). The multispecific binding protein molecules may have a variety of general formats. For example, multispecific binding proteins, in some embodiments, may be bispecific

(i.e. binding to two targets) or in some embodiments, may be able to bind to more than two targets or to more than one site on a target. In some embodiments, multispecific antibodies may be trispecific, i.e., binding to three targets or sites. In other embodiments, they may bind to more than three targets or sites. In some embodiments, multispecific binding proteins are

5 multispecific antibodies, including multispecific antibody fragments as well as multispecific full length antibodies such as full length IgG antibodies. There are various bispecific and trispecific and other multispecific antibody formats compatible with molecules herein, as described below. In other embodiments, multispecific binding proteins herein may comprise other types of specific ligands for the target molecules or a combination of an antibody variable region specific

10 for one target and a different type of protein ligand specific for another target. In some embodiments, the multispecific binding proteins herein are known by the acronym PROTAB.

In some embodiments, the transmembrane E3 ubiquitin ligase is any one of RNF43, ZNRF3, RNF13, RNF128, RNF130, RNF133, RNF148, RNF149, RNF150, RNF167, ZNRF4, RSPRY1, SYVN1, LNX1 isoform 2, or TRIM7 isoform 3. In some embodiments, the

15 transmembrane E3 ubiquitin ligase is a RNF13, RNF43, SYVN1, RNF130, RNF148, RNF149, LNX1_isoform 2, RNF128, RNF133, ZNRF4, RSPRY1, TRIM7_isoform 3, RNF167, RNF150, or ZNRF3 comprising an amino acid sequence selected from any one of SEQ ID Nos: 445-459, respectively. (See the sequence table below.) In some embodiments, the transmembrane E3 ubiquitin ligase is RNF43, RNF128, RNF130, RNF133 RNF149, RNF150, or ZNRF3. In some

20 embodiments, the transmembrane E3 ubiquitin ligase is RNF130, RNF133, RNF149, or RNF150. In some embodiments, the transmembrane E3 ubiquitin ligase is RNF130, RNF149, or RNF167. In some embodiments, the transmembrane E3 ubiquitin ligase is RNF133 or RNF148. In some embodiments, the transmembrane E3 ubiquitin ligase is RNF43 or ZNRF3. In some

25 embodiments, the multispecific binding protein binds to one type of transmembrane E3 ubiquitin ligase, e.g., ZNRF3 or RNF43. In other embodiments, the multispecific binding protein binds to two transmembrane E3 ubiquitin ligases, such as to both RNF43 and ZNRF3. For example, the multispecific binding protein may comprise two different antibody fragments, one recognizing RNF43 and one recognizing ZNRF3, allowing for binding to both ligases.

As shown in Fig. 1C, for example, RNF43 is expressed in a number of cancer cell lines.

30 And both RNF43 and ZNRF3 are members of the Wnt signaling pathway, which is activated in a number of tumor cell lines. As those proteins are expected to be expressed on numerous types of tumors, constructs herein may be particularly useful in reducing the level of one or more target cell surface proteins in tumors in a subject. For example, constructs herein may be useful in reducing the level of target cell surface proteins wherein high levels of such proteins are known

35 to promote cancer growth. In addition, certain target cells, such as cancer cells, may express

high levels of a transmembrane E3 ubiquitin ligase like RNF43 or ZNRF3, which may allow the multispecific binding proteins to preferentially bind to those target cells in comparison to other normal or nondiseased cells that express lower levels of the ligase. As such, constructs herein may be useful in treatment of cancers. And, depending on the cell surface protein chosen for degradation, constructs herein may be useful in increasing an immune response in a subject, such as in a cancer subject.

The disclosure herein also relates to exemplary anti-RNF43 and anti-ZNRF3 antibodies that may be useful in constructing multispecific binding proteins herein, such as multispecific or bispecific antibodies. These are described in more detail below.

In some embodiments, multispecific binding proteins may also block the ability of the normal ligands for the transmembrane E3 ubiquitin ligase to bind to it. Thus, for example, in some embodiments, the proteins may block the ability of FZD or LRP6 to bind to RNF43 or ZNRF3. In other embodiments, the multispecific binding proteins do not block binding of the transmembrane E3 ubiquitin ligase to its endogenous ligands.

In some embodiments, the multispecific binding protein reduces the level of the cell surface protein on the surface of a cell compared to the level observed in the absence of the multispecific binding protein. In some cases, the multispecific binding protein reduces the level of the cell surface protein on the surface of a cell *in vitro*, such as observed by flow cytometry, compared to the level observed in the absence of the multispecific binding protein. In some cases, the multispecific binding protein reduces the level of the cell surface protein on the surface of a cell *in vivo* compared to the level observed in the absence of the multispecific binding protein.

In addition to the transmembrane E3 ubiquitin ligases, multispecific binding proteins of the disclosure may recognize a second cell surface protein, such as a protein that may be targeted for degradation by being brought into close proximity with the transmembrane E3 ubiquitin ligase. There are a wide variety of cell surface proteins that can be targeted for degradation by the instant multispecific binding proteins. Examples include, for instance, receptor tyrosine kinases, growth factor receptors, cytokines including cytokine receptors, mucins, Siglec receptors, and immune checkpoint modulators. Examples of growth factor receptors include, for example, fibroblast growth factor receptors FGFRs, vascular endothelial growth factor receptors VEGFRs, epidermal growth factor receptors EGFRs, and platelet derived growth factor receptors PDGFRs. Various growth factor receptors can be overexpressed in certain cancers, for example. Examples of mucins include, for example, MUC1, MUC2, MUC3A, MUC3B, MUC4, MUC5B, MUC6, MUC7, MUC8, MUC12, MUC13, MUC15, MUC16, MUC17, MUC19, and MUC20 as well as MUC21 and MUC22. Mucins such as MUC1 may be overexpressed in certain cancers.

Examples of Siglec (sialic acid binding immunoglobulin type lectins) receptors include, for example, CD22, CD33, MAG/Siglec-4, Siglec-5, Siglec-7, and Siglec-9. Exemplary cytokine receptors include, for example, members of the TNFR super family such as CD40, CD27, OX40, 4-1BB, and others. Particular example cell surface proteins that could be targeted by multispecific binding proteins herein include, for example, HER2, HER3, IGF1R, an EGFR, an FGFR, a VEGFR, a PDGFR, EpCAM, FZD, PD-L1, CTLA4, PD-1, TIM3, LAG3, TIGIT, CEACAM1, CD25, ILT-2, ILT-3, ILT-4, ILT-5, LAIR-1, PECAM-1 (CD31), PILR-alpha, SIRT1, or SIRP-alpha. Specific example cell surface proteins that could be targeted by multispecific binding proteins herein include, for example, HER2, IGF1R, EGFR, FZLD5, EpCAM, and PD-L1. Depending on the particular target cell surface protein chosen, multispecific binding proteins herein may include antibodies or antibody fragments derived from, for example, an anti-HER2 antibody such as 4D5, 7C2, or 2C4, or an anti-IGF1R antibody such as cixutumumab, ganitumab, dalotuzumab, figitumumab, robatumumab, teprotumumab, or istiratumab.

While reducing the cell surface level of these or other proteins may be useful, for example, in therapeutic treatments, such as for cancer, the present multispecific binding proteins are useful in a wide variety of settings, indeed wherever there is a need to reduce the level of a particular protein at the cell surface by inducing its degradation. For example, selectively reducing the level of a particular cell surface protein in an in vitro cell culture or tissue sample may be useful in a variety of experimental settings where, for example, the impact of that protein on a particular mechanism is to be studied. Accordingly, the cell surface protein targeted for destruction may include a wide variety of cell surface proteins.

A. Exemplary Multispecific Binding Protein Formats

In certain aspects, a multispecific binding protein provided herein is a multispecific antibody, e.g., a bispecific antibody or trispecific antibody. In other aspects, the multispecific binding protein may comprise one or more antigen binding fragments from an antibody coupled with other protein domains, such as ligand binding domains from a non-antibody protein. “Multispecific antibodies” generally are monoclonal antibodies that have binding specificities for at least two different sites, i.e., different epitopes on different antigens or different epitopes on the same antigen. In certain aspects, the multispecific antibody has three or more binding specificities. Multispecific antibodies may be prepared as full length antibodies or antibody fragments. A multispecific molecule that binds to two sites or targets is “bispecific” while one that binds to three sites or targets is “trispecific.” Thus, a bispecific binding protein, such as a bispecific antibody, herein binds to both a transmembrane E3 ubiquitin ligase and to a cell

surface protein. A trispecific binding protein, such as a trispecific antibody, may be constructed to bind, for example, to a cell surface protein and more than one transmembrane E3 ubiquitin ligase, to more than one cell surface protein and to one transmembrane E3 ubiquitin ligase, or to one cell surface protein and one transmembrane E3 ubiquitin ligase but to two sites on either the
5 cell surface protein or the ligase.

For instance, in some aspects, a multispecific binding protein herein may be engineered to bind to RNF43 and to one or more second cell surface proteins. In other aspects, the protein may be engineered to bind to ZNRF3 and to one or more second cell surface proteins. Or the protein may be engineered to bind to both RNF43 and to ZNRF3 and to one or more second cell surface
10 proteins. In yet other aspects, the multispecific binding protein herein may be engineered to bind to RNF13, RNF128, RNF130, RNF133, RNF148, RNF149, RNF150, RNF167, ZNRF4, RSPRY1, SYVN1, LNX1 isoform 2, or TRIM7 isoform 3, and to one or more second cell surface proteins.

Techniques for making multispecific antibodies include, but are not limited to,
15 recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)) and “knob-in-hole” engineering (see, e.g., U.S. Patent No. 5,731,168, and Atwell et al., *J. Mol. Biol.* 270:26 (1997)). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (see, e.g., WO 2009/089004); cross-linking two or
20 more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992) and WO 2011/034605); using the common light chain technology for circumventing the light chain mis-pairing problem (see, e.g., WO 98/50431); using “diabody” technology for making bispecific antibody fragments (see, e.g., Hollinger et al.,
25 *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see, e.g., Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991).

Engineered antibodies with three or more antigen binding sites, including for example, “Octopus antibodies”, or DVD-Ig are also included herein (see, e.g., WO 2001/77342 and WO
30 2008/024715). Other examples of multispecific antibodies with three or more antigen binding sites can be found in WO 2010/115589, WO 2010/112193, WO 2010/136172, WO 2010/145792, and WO 2013/026831. The bispecific antibody or antigen binding fragment thereof also includes a “Dual Acting FAb” or “DAF” comprising an antigen binding site that binds to the cell surface protein and another that binds to the transmembrane E3 ubiquitin ligase
35 (see, e.g., US 2008/0069820 and WO 2015/095539).

Multi-specific antibodies may also be provided in an asymmetric form with a domain crossover in one or more binding arms of the same antigen specificity, i.e. by exchanging the VH/VL domains (see e.g., WO 2009/080252 and WO 2015/150447), the CH1/CL domains (see e.g., WO 2009/080253) or the complete Fab arms (see e.g., WO 2009/080251, WO 2016/016299, also see Schaefer et al, PNAS, 108 (2011) 1187-1191, and Klein et al., MAbs 8 (2016) 1010-20). In one aspect, the multispecific antibody comprises a cross-Fab fragment. The term "cross-Fab fragment" or "xFab fragment" or "crossover Fab fragment" refers to a Fab fragment, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged. A cross-Fab fragment comprises a polypeptide chain composed of the light chain variable region (VL) and the heavy chain constant region 1 (CH1), and a polypeptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL). Asymmetrical Fab arms can also be engineered by introducing charged or non-charged amino acid mutations into domain interfaces to direct correct Fab pairing. See e.g., WO 2016/172485.

Various further molecular formats for multispecific antibodies are known in the art and are included herein (see e.g., Spiess et al., Mol Immunol 67 (2015) 95-106).

A particular type of multispecific antibodies, also included herein, are bispecific antibodies designed to simultaneously bind to a surface antigen on a target cell, e.g., a tumor cell, and to another cell surface protein that can target the surface antigen for lysosomal degradation. Hence, in certain aspects, an antibody provided herein is a multispecific antibody, particularly a bispecific antibody, wherein one of the binding specificities is for the cell surface protein to be degraded and the other binding specificity is for the transmembrane E3 ubiquitin ligase.

Examples of bispecific antibody formats that may be useful for this purpose include, but are not limited to, molecules wherein two scFv molecules are fused by a flexible linker (see, e.g., WO 2004/106381, WO 2005/061547, WO 2007/042261, and WO 2008/119567, Nagorsen and Bäuerle, Exp Cell Res 317, 1255-1260 (2011)); diabodies (Holliger et al., Prot Eng 9, 299-305 (1996)) and derivatives thereof, such as tandem diabodies ("TandAb"; Kipriyanov et al., J Mol Biol 293, 41-56 (1999)); "DART" (dual affinity retargeting) molecules which are based on the diabody format but feature a C-terminal disulfide bridge for additional stabilization (Johnson et al., J Mol Biol 399, 436-449 (2010)), and so-called triomabs, which are whole hybrid mouse/rat IgG molecules (reviewed in Seimetz et al., Cancer Treat Rev 36, 458-467 (2010)). Particular bispecific antibody formats included herein are described in WO 2013/026833, WO 2013/026839, WO 2016/020309; Bacac et al., Oncoimmunology 5(8) (2016) e1203498. Bispecific antibody formats described in the aforementioned documents that bind T cells (e.g., by binding CD3, an invariant component of the T cell receptor complex), can be adapted to bind

the targets as described herein instead of T cells, e.g., by replacing the antibody fragment that binds CD3 with an antibody fragment that binds a cell surface ligase.

In some embodiments, a multispecific antibody herein may have a symmetrical “1+1 FabIgG” or “1+1 FvIgG” bispecific format, comprising two Fv domains recognizing different targets linked to interacting Fc regions, or comprising two Fab regions recognizing different targets linked to interacting Fc regions. Some further examples of bispecific antibody formats herein include those provided in Figs. 15A-15C: 2+1 FabIgG, one-armed FvIgG, and one-armed FabIgG, respectively. These formats are asymmetrical, for example. Illustrations of trispecific antibodies that bind to a cell surface protein target as well as to both RNF43 and ZNFR3 are depicted in Figs. 15D-15G. The 2+1 FabIgG format shown in Figure 15A is described, for example, in WO 2015/095539. Optionally, antibodies such as those above comprise interacting Fc regions comprising at least one “knob-into-hole” modification. For example, a knob-into-hole modification may help to prevent mis-pairing of the Fc regions. Exemplary knob-into-hole modifications include, for instance, substitutions of one or more amino acids on a CH3 or CH2 region on one Fc domain for a larger amino acid than naturally found at that position, along with substitutions of one or more amino acids in close proximity on the partner Fc domain for a smaller amino acid than normally found. (See, e.g., U.S. Patent No. 5,731,168, and Atwell et al., J. Mol. Biol. 270:26 (1997), as well as WO 2009/089004, WO 2012/106587, and U.S. Patent Publication 2009/0182127, for examples). In some embodiments, knob-into-hole modifications may substitutions at amino acid positions 366, 368, and 407 of a human IgG1 Fc, for example. For example, in some embodiments, “knob mutations” may comprise a substitution of T366 in a human IgG1 Fc with a larger amino acid such as W (and optionally one of S354C or Y349C) and an accompanying “hole mutation” on the partner Fc may comprise substitution of T366, L368, and Y407 for smaller amino acids such as T366S, L368A and Y407V (and optionally Y349C or S354C). (See, e.g., Carter, P. et al., Immunotechnol. 2 (1996) 73.) Numbering is according to EU index numbering.

In some aspects, a multispecific binding protein herein may have one of the following formats, or another format tested in the Examples section or depicted in the figures herein:

- a) a 2+1 FabIgG comprising an arm with two Fab regions binding to a transmembrane E3 ubiquitin ligase and an arm with one Fab region binding to a cell surface protein; (see, e.g., Figs. 7B and 15A)
- b) a 2+1 FabIgG comprising an arm with two Fab regions binding to a cell surface protein and an arm with one Fab region binding to a transmembrane E3 ubiquitin ligase; (see, e.g., Figs. 7A and 15A)
- c) a one armed FvIgG comprising an Fv region binding to a cell surface protein and

a Fab region binding to a transmembrane E3 ubiquitin ligase; (see, e.g., Fig. 8A, far left, and 15B);

d) a one armed FvIgG comprising an Fv binding to a transmembrane E3 ubiquitin ligase and a Fab binding to a cell surface protein (see, e.g., Fig. 8A, second from left, and 15B);

5 e) a one armed FabIgG comprising a first Fab region binding to a cell surface protein and a second Fab region binding to a transmembrane E3 ubiquitin ligase; (see, e.g., Fig. 8A, third from left, and 15C);

f) a one armed FabIgG comprising a first Fab region binding to a transmembrane E3 ubiquitin ligase and a second Fab region binding to a cell surface protein (see, e.g., Fig. 8A, far
10 right, and 15C);

g) a 1+1 FabIgG comprising a first Fab region binding to a transmembrane E3 ubiquitin ligase and a second Fab region binding to a cell surface protein; and

h) a 1+1 FvIgG comprising a first Fv region binding to a transmembrane E3 ubiquitin ligase and a second Fv region binding to a cell surface protein. In any of the above, the protein
15 may comprise at least one knob-into-hole modification in the Fc region, for example, which in some embodiments may reduce mis-pairing of the two halves of the IgG molecule.

In certain aspects, a multispecific binding protein such as a multispecific antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody
20 include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl
25 pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary,
30 and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

B. Exemplary Anti-RNF43 and Anti-ZNRF3 Antibodies useful in constructing Multispecific Binding Molecules

In one aspect, the disclosure also provides antibodies that specifically bind to RNF43 and/or ZNRF3 that may be useful in constructing multispecific binding proteins according to the disclosure. These include the murine, rat, or rabbit antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, and ZNRF3-332, described in the Examples below, or humanized versions of those antibodies.

In some embodiments, a multispecific binding protein herein comprises a heavy chain variable domain (VH) comprising (a) a heavy chain complementarity determining region 1 (CDR-H1), CDR-H2, and/or CDR-H3 of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265,

ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332. In some embodiments, the multispecific binding protein comprises a heavy chain variable domain (VH) comprising (a) a heavy chain complementarity determining region 1 (CDR-H1), CDR-H2, and CDR-H3 of any one of the above antibodies.

In some embodiments, the multispecific binding protein comprises a light chain variable domain (VL) comprising (a) a light chain complementarity determining region 1 (CDR-L1), CDR-L2, and/or CDR-L3 of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332. In some embodiments, it comprises a light chain variable domain (VL) comprising (a) a light chain complementarity determining region 1 (CDR-L1), CDR-L2, and CDR-L3 of any one of those antibodies. In some embodiments, the multispecific binding protein comprises all of the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 of one of the above anti-RNF43 or anti-ZNRF3 antibodies.

In some cases, the multispecific binding protein comprises a heavy chain variable region (VH) comprising an amino acid sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the VH of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126,

RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156,
 RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181,
 RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210,
 RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-
 5 35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-
 71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-
 117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172,
 ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231,
 ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265,
 10 ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30,
 ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329,
 ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12,
 RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16,
 RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331,
 15 ZNRF3-333, or ZNRF3-332.

In some cases, the multispecific binding protein comprises a light chain variable region
 (VL) comprising an amino acid sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,
 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the VH of any one of antibodies RNF43-
 104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126,
 20 RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156,
 RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181,
 RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210,
 RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-
 35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-
 25 71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-
 117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172,
 ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231,
 ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265,
 ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30,
 30 ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329,
 ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12,
 RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16,
 RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331,
 ZNRF3-333, or ZNRF3-332.

In some cases, the multispecific binding protein comprises a VH comprising an amino acid sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the VH of any one of above antibodies and also a VL comprising an amino acid sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the VL of any one of above antibodies.

In some cases, the multispecific binding protein comprises a heavy chain variable region (VH) comprising an amino acid sequence of the VH of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332. In some cases, the multispecific binding protein comprises a light chain variable region (VL) comprising an amino acid sequence of the VL of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279,

ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332. In some cases, the protein comprises both the VH and the VL of one of the above antibodies. The sequence table below provides DNA and protein sequences of the VH and VL of the above antibodies, from SEQ ID NO: 33 to SEQ ID NO: 444.

In further embodiments, a multispecific binding protein may be constructed using amino acid sequences provided in one or more of SEQ ID Nos: 1-32 in the sequence table below.

In some embodiments, the multispecific binding protein comprising the above CDRs or VH/VLs has a binding affinity for ZNRF3 or RNF43 of less than 50 nM, or less than 10 nM, or less than 1 nM, or less than 0.5 nM, or less than 0.05 nM, or between 50 nM and 10 nM, or between 10 nM and 1 nM, or between 1 nM and 0.5 nM, or between 0.5 nM and 0.05 nM, or between 0.05 nM and 0.01 nM. In some cases, affinity is measured using a BIACORE® surface plasmon resonance assay, for example, as described in the Examples below.

In some embodiments, the multispecific binding protein binds to both ZNRF3 and RNF43. For example, in some embodiments, the multispecific binding protein is a multispecific antibody that binds to both ZNRF3 and RNF43. In some embodiments, it is a trispecific antibody binding to those ligases and to a cell surface protein targeted for degradation. In some embodiments, it may have a format as shown in Fig. 15A-15G, Fig. 7A-B, or Fig. 8A.

In some embodiments, a multispecific binding protein comprising the above CDRs or VH/VLs is a rat anti-human or rabbit anti-human antibody or murine anti-human antibody. In other embodiments, it is humanized or chimeric. In some embodiments, the multispecific binding protein comprises a wild-type human Fc region, such as from a human IgG1, IgG2, IgG3, or IgG4. In other embodiments, the protein comprises a human IgG1 Fc with one or more amino acid substitutions, such as a LALAPG mutation or a substitution at N297, e.g., N297G or N297Q, for example, to render the Fc effectorless. In some embodiments, the multispecific binding protein does not have effector function. In other embodiments, the multispecific binding protein has effector function. These and other optional constant regions or Fc regions are also discussed further below.

C. Conjugates Comprising Multispecific Binding Proteins

In other embodiments, a multispecific binding protein herein may further be conjugated (chemically bonded) to one or more other molecules, such as a label or a therapeutic agent. A

variety of radioactive isotope labels are available for the production of radioconjugates. Examples include At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Exemplary therapeutic agents include cytotoxic agents, chemotherapeutic agents, drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one aspect, a multispecific binding protein is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more of the therapeutic agents mentioned above. The antibody is typically connected to one or more of the therapeutic agents using linkers. An overview of ADC technology including examples of therapeutic agents and drugs and linkers is set forth in *Pharmacol Review* 68:3-19 (2016). Other potential conjugated therapeutic agents include enzymatically active toxins or fragments thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

Conjugates of a protein and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 94/11026. The linker may be a “cleavable linker” facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The immunoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and
 5 SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

D. Antibody Variants

In certain aspects, amino acid sequence variants of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128,
 10 RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-
 15 74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332 provided herein are contemplated. For example, it may be desirable
 25 to alter the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and
 30 substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a) Substitution, Insertion, and Deletion Variants

In certain aspects, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the CDRs and framework regions of the variable regions. Conservative substitutions are shown in Table A under the heading of “preferred substitutions”. More substantial changes are provided in Table A under the heading of “exemplary substitutions”, and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest, e.g., in the variable and/or constant regions, and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE A

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- 5 (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for a member of another class.

One type of substitutional variant involves substituting one or more hypervariable region
10 residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be
15 conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more CDR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g., binding affinity).

Alterations (e.g., substitutions) may be made in CDRs, e.g., to improve antibody affinity.
20 Such alterations may be made in CDR “hotspots”, i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods*
25 *in Molecular Biology* 178:1-37 (O’Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some aspects of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce
30 diversity involves CDR-directed approaches, in which several CDR residues (e.g., 4-6 residues at a time) are randomized. CDR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain aspects, substitutions, insertions, or deletions may occur within one or more
35 CDRs so long as such alterations do not substantially reduce the ability of the antibody to bind

antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in the CDRs. Such alterations may, for example, be outside of antigen contacting residues in the CDRs. In certain variant VH and VL sequences provided above, each CDR either is unaltered, or contains no
5 more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral
10 or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex may be used to identify contact points between the antibody and antigen. Such contact residues and neighboring
15 residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal
20 insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g., for ADEPT (antibody directed enzyme prodrug therapy)) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

In certain aspects, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the oligosaccharide attached thereto may be
30 altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of

the biantennary oligosaccharide structure. In some aspects, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

In one aspect, antibody variants are provided having a non-fucosylated oligosaccharide, i.e. an oligosaccharide structure that lacks fucose attached (directly or indirectly) to an Fc region. Such non-fucosylated oligosaccharide (also referred to as “afucosylated” oligosaccharide) particularly is an N-linked oligosaccharide which lacks a fucose residue attached to the first GlcNAc in the stem of the biantennary oligosaccharide structure. In one aspect, antibody variants are provided having an increased proportion of non-fucosylated oligosaccharides in the Fc region as compared to a native or parent antibody. For example, the proportion of non-fucosylated oligosaccharides may be at least about 20%, at least about 40%, at least about 60%, at least about 80%, or even about 100% (i.e. no fucosylated oligosaccharides are present). The percentage of non-fucosylated oligosaccharides is the (average) amount of oligosaccharides lacking fucose residues, relative to the sum of all oligosaccharides attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2006/082515, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such antibodies having an increased proportion of non-fucosylated oligosaccharides in the Fc region may have improved Fc γ RIIIa receptor binding and/or improved effector function, in particular improved ADCC function. See, e.g., US 2003/0157108; US 2004/0093621.

Examples of cell lines capable of producing antibodies with reduced fucosylation include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US 2003/0157108; and WO 2004/056312, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87:614-622 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO 2003/085107), or cells with reduced or abolished activity of a GDP-fucose synthesis or transporter protein (see, e.g., US2004259150, US2005031613, US2004132140, US2004110282).

In a further aspect, antibody variants are provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function as described above. Examples of such antibody variants are described, e.g., in Umana

et al., Nat Biotechnol 17, 176-180 (1999); Ferrara et al., Biotechn Bioeng 93, 851-861 (2006); WO 99/54342; WO 2004/065540, WO 2003/011878.

Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

c) Fc region variants

In certain aspects, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG₁, IgG₂, IgG₃ or IgG₄ Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions.

In some aspects, an antibody herein has effector function. In other aspects, an antibody herein lacks effector function. In certain aspects, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC)) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g., Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)).

Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad.*

Sci. USA 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. *et al.*, *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. *et al.*, *Int'l. Immunol.* 18(12):1759-1769 (2006); WO 2013/120929 A1).

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields *et al.*, *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

In certain aspects, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

In certain aspects, an antibody variant comprises an Fc region with one or more amino acid substitutions which diminish FcγR binding, e.g., substitutions at positions 234 and 235 of the Fc region (EU numbering of residues). In one aspect, the substitutions are L234A and L235A (LALA). In certain aspects, the antibody variant further comprises D265A and/or P329G in an Fc region derived from a human IgG₁ Fc region. In one aspect, the substitutions are L234A, L235A and P329G (LALAPG) in an Fc region derived from a human IgG₁ Fc region. (See, e.g., WO 2012/130831). In another aspect, the substitutions are L234A, L235A and D265A (LALA-DA) in an Fc region derived from a human IgG₁ Fc region.

In some aspects, the antibodies may have a modification at position N297 to reduce or eliminate ADCC activity, such as N297G or N297Q. In some such cases, the antibody lacks effector function.

In some aspects, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie *et al.* *J. Immunol.* 164: 4178-4184 (2000).

Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934 (Hinton et al.). Those antibodies comprise an Fc region with one or more
 5 substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 252, 254, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (See, e.g., US Patent No. 7,371,826; Dall'Acqua, W.F., et al. *J. Biol. Chem.* 281 (2006) 23514-23524).

10 Fc region residues critical to the mouse Fc-mouse FcRn interaction have been identified by site-directed mutagenesis (see e.g. Dall'Acqua, W.F., et al. *J. Immunol.* 169 (2002) 5171-5180). Residues I253, H310, H433, N434, and H435 (EU index numbering) are involved in the interaction (Medesan, C., et al., *Eur. J. Immunol.* 26 (1996) 2533; Firan, M., et al., *Int. Immunol.* 13 (2001) 993; Kim, J.K., et al., *Eur. J. Immunol.* 24 (1994) 542). Residues I253, H310, and
 15 H435 were found to be critical for the interaction of human Fc with murine FcRn (Kim, J.K., et al., *Eur. J. Immunol.* 29 (1999) 2819). Studies of the human Fc-human FcRn complex have shown that residues I253, S254, H435, and Y436 are crucial for the interaction (Firan, M., et al., *Int. Immunol.* 13 (2001) 993; Shields, R.L., et al., *J. Biol. Chem.* 276 (2001) 6591-6604). In Yeung, Y.A., et al. (*J. Immunol.* 182 (2009) 7667-7671) various mutants of residues 248 to 259
 20 and 301 to 317 and 376 to 382 and 424 to 437 have been reported and examined.

In certain aspects, an antibody variant comprises an Fc region with one or more amino acid substitutions, which reduce FcRn binding, e.g., substitutions at positions 253, and/or 310, and/or 435 of the Fc-region (EU numbering of residues). In certain aspects, the antibody variant
 25 comprises an Fc region with the amino acid substitutions at positions 253, 310 and 435. In one aspect, the substitutions are I253A, H310A and H435A in an Fc region derived from a human IgG1 Fc-region. See, e.g., Grevys, A., et al., *J. Immunol.* 194 (2015) 5497-5508.

In certain aspects, an antibody variant comprises an Fc region with one or more amino acid substitutions, which reduce FcRn binding, e.g., substitutions at positions 310, and/or 433, and/or 436 of the Fc region (EU numbering of residues). In certain aspects, the antibody variant
 30 comprises an Fc region with the amino acid substitutions at positions 310, 433 and 436. In one aspect, the substitutions are H310A, H433A and Y436A in an Fc region derived from a human IgG1 Fc-region. (See, e.g., WO 2014/177460 A1).

In certain aspects, an antibody variant comprises an Fc region with one or more amino acid substitutions which increase FcRn binding, e.g., substitutions at positions 252, and/or 254, and/or
 35 256 of the Fc region (EU numbering of residues). In certain aspects, the antibody variant

comprises an Fc region with amino acid substitutions at positions 252, 254, and 256. In one aspect, the substitutions are M252Y, S254T and T256E in an Fc region derived from a human IgG₁ Fc-region. See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

The C-terminus of the heavy chain of the antibody as reported herein can be a complete C-terminus ending with the amino acid residues PGK. The C-terminus of the heavy chain can be a shortened C-terminus in which one or two of the C terminal amino acid residues have been removed. In one preferred aspect, the C-terminus of the heavy chain is a shortened C-terminus ending PG. In one aspect of all aspects as reported herein, an antibody comprising a heavy chain including a C-terminal CH3 domain as specified herein, comprises the C-terminal glycine-lysine dipeptide (G446 and K447, EU index numbering of amino acid positions). In one aspect of all aspects as reported herein, an antibody comprising a heavy chain including a C-terminal CH3 domain, as specified herein, comprises a C-terminal glycine residue (G446, EU index numbering of amino acid positions).

d) Cysteine engineered antibody variants

In certain aspects, it may be desirable to create cysteine engineered antibodies, e.g., THIOMABTM antibodies, in which one or more residues of an antibody are substituted with cysteine residues. In particular aspects, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541, 8,30,930, 7,855,275, 9,000,130, or WO 2016040856.

e) Antibody Derivatives

In certain aspects, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers,

polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

E. Recombinant Methods and Compositions

Proteins herein may be produced using recombinant methods and compositions, e.g., as described in US 4,816,567. For these methods one or more isolated nucleic acid(s) encoding an antibody are provided.

In case of a native antibody or native antibody fragment two nucleic acids are required, one for the light chain or a fragment thereof and one for the heavy chain or a fragment thereof. Such nucleic acid(s) encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chain(s) of the antibody). These nucleic acids can be on the same expression vector or on different expression vectors.

In case of a bispecific antibody with heterodimeric heavy chains four nucleic acids are required, one for the first light chain, one for the first heavy chain comprising the first heteromonomeric Fc-region polypeptide, one for the second light chain, and one for the second heavy chain comprising the second heteromonomeric Fc-region polypeptide. The four nucleic acids can be comprised in one or more nucleic acid molecules or expression vectors. Such nucleic acid(s) encode an amino acid sequence comprising the first VL and/or an amino acid sequence comprising the first VH including the first heteromonomeric Fc-region and/or an amino acid sequence comprising the second VL and/or an amino acid sequence comprising the second VH including the second heteromonomeric Fc-region of the antibody (e.g., the first and/or second light and/or the first and/or second heavy chains of the antibody). These nucleic acids can be on the same expression vector or on different expression vectors, normally these nucleic acids are located on two or three expression vectors, i.e. one vector can comprise more than one of these nucleic acids. Examples of these bispecific antibodies are CrossMabs (see, e.g., Schaefer, W. et al, PNAS, 108 (2011) 11187-1191). For example, one of the heteromonomeric heavy chain comprises the so-called “knob mutations” (T366W and optionally one of S354C or Y349C) and the other comprises the so-called “hole mutations” (T366S, L368A and Y407V and

optionally Y349C or S354C) (see, e.g., Carter, P. et al., Immunotechnol. 2 (1996) 73) according to EU index numbering.

In one aspect, isolated nucleic acids encoding an antibody or other component of a multispecific binding protein as used in the methods as reported herein are provided.

5 In one aspect, a method of making a multispecific binding protein is provided, wherein the method comprises culturing a host cell comprising nucleic acid(s) encoding the antibody or components of the protein, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody or other protein components from the host cell (or host cell culture medium).

10 For recombinant production of an antibody, nucleic acids encoding the antibody, e.g., as described above, are isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acids may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody) or produced by
15 recombinant methods or obtained by chemical synthesis.

Suitable host cells for cloning or expression of protein-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., US 5,648,237, US 5,789,199, and
20 US 5,840,523. (See also Charlton, K.A., In: Methods in Molecular Biology, Vol. 248, Lo, B.K.C. (ed.), Humana Press, Totowa, NJ (2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are
25 suitable cloning or expression hosts for protein-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized”, resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gemgross, T.U., Nat. Biotech. 22 (2004) 1409-1414; and Li, H. et al., Nat. Biotech. 24 (2006) 210-215.

Suitable host cells for the expression of (glycosylated) antibody are also derived from
30 multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures can also be utilized as hosts. See, e.g., US 5,959,177, US 6,040,498, US 6,420,548, US 7,125,978, and US 6,417,429 (describing PLANTIBODIESTM technology for
35 producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham, F.L. et al., J. Gen Virol. 36 (1977) 59-74); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, J.P., Biol. Reprod. 23 (1980) 243-252); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells (as described, e.g., in Mather, J.P. et al., Annals N.Y. Acad. Sci. 383 (1982) 44-68); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR- CHO cells (Urlaub, G. et al., Proc. Natl. Acad. Sci. USA 77 (1980) 4216-4220); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki, P. and Wu, A.M., Methods in Molecular Biology, Vol. 248, Lo, B.K.C. (ed.), Humana Press, Totowa, NJ (2004), pp. 255-268.

In one aspect, the host cell is eukaryotic, e.g., a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell).

F. Pharmaceutical Compositions

In a further aspect, provided are pharmaceutical compositions comprising any of the multispecific binding proteins provided herein, e.g., for use in any of the below therapeutic methods. In one aspect, a pharmaceutical composition comprises any of the proteins provided herein and a pharmaceutically acceptable carrier. In another aspect, a pharmaceutical composition comprises any of the proteins provided herein and at least one additional therapeutic agent, e.g., as described below.

Pharmaceutical compositions of proteins as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized compositions or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as histidine, phosphate, citrate, acetate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight

(less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX[®], Halozyme, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody compositions are described in US Patent No. 6,267,958. Aqueous antibody compositions include those described in US Patent No. 6,171,586 and WO 2006/044908, the latter compositions including a histidine-acetate buffer.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Pharmaceutical compositions for sustained-release may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules.

The pharmaceutical compositions to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

G. Therapeutic Methods and Routes of Administration

Any of the multispecific binding proteins provided herein may be used in therapeutic methods. For example, they may be useful in any therapeutic method in which it is beneficial to reduce the level of a particular cell surface protein in a subject by targeting it for lysosomal degradation, and in which it is beneficial to do so in cells that readily express a transmembrane E3 ubiquitin ligase.

In one aspect, an multispecific binding protein for use as a medicament is provided. In further aspects, an multispecific binding protein for use in treating a disease such as cancer is provided. Examples of cancer include, but are not limited to, carcinoma, lymphoma (*e.g.*, Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular
5 examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer,
10 liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease.

In certain aspects, an multispecific binding protein for use in a method of treatment is provided. In certain aspects, the invention provides an multispecific binding protein for use in a
15 method of treating an individual having a disease such as cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease, comprising administering to the individual an effective amount of the multispecific binding protein. In one such aspect, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent (*e.g.*, one, two, three, four, five, or six additional
20 therapeutic agents), *e.g.*, as described below.

In further aspects, the invention provides an multispecific binding protein for use in reducing the level of a cell surface protein in a subject that is targeted by the multispecific binding protein. In some cases, the subject may have a disease such as cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease.

25 In a further aspect, the invention provides for the use of an multispecific binding protein in the manufacture or preparation of a medicament. In one aspect, the medicament is for treatment of a disease such as cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease. In a further aspect, the medicament is for use in a method of treating a disease such as cancer, an autoimmune condition, an inflammatory
30 condition, a neurodegenerative condition, or an infectious disease, comprising administering to an individual having cancer an effective amount of the medicament. In one such aspect, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described below. In a further aspect, the medicament is for in reducing the level of a cell surface protein in a subject that is targeted by the multispecific

binding protein. In some cases, the subject may have a disease such as cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease.

In a further aspect, the invention provides a method for treating a disease such as cancer. In one aspect, the method comprises administering to an individual having such cancer, an
5 autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease, an effective amount of an multispecific binding protein. In one such aspect, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below.

In a further aspect, the invention provides a method for in reducing the level of a cell
10 surface protein in a subject that is targeted by the multispecific binding protein. In some cases, the subject may have a disease such as cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease. For example, in some aspects, multispecific binding proteins herein may be used to reduce levels of a cell surface protein on particular cell types by targeting an E3 ubiquitin ligase that is primarily expressed on
15 those cell types. For example, the transmembrane E3 ubiquitin ligases RNF130, RNF149, and RNF167 are expressed on hematopoietic cells and multispecific binding proteins targeting those ligases could be used in some embodiments to reduce levels of cell surface proteins on those cells. Additionally, the transmembrane E3 ubiquitin ligases RNF133 and RNF148 are expressed on testicular cells and multispecific binding proteins targeting those ligases could be used in
20 some embodiments to reduce levels of cell surface proteins on those cells.

In a further aspect, the invention provides pharmaceutical compositions comprising any of the multispecific binding proteins provided herein, e.g., for use in any of the above therapeutic methods. In one aspect, a pharmaceutical composition comprises any of the multispecific
25 binding proteins provided herein and a pharmaceutically acceptable carrier. In another aspect, a pharmaceutical composition comprises any of the multispecific binding proteins provided herein and at least one additional therapeutic agent, e.g., as described below.

In other aspects of the uses and methods of treatment herein, a subject may have a mutation in the RNF43 or ZNRF3 protein. For example, in certain cancers, such as colorectal
30 cancer and endometrial cancer, among others, the RNF43 protein has been found to be mutated. *See, e.g., Y.J. van Herwaarden et al., Histopathology 78: 749-758 (2021).* In such cases, a multispecific binding protein comprising an anti-RNF43 component may be less effective in reducing the level of a targeted cell surface protein. However, a multispecific binding protein comprising an anti-ZNRF3 component may readily lead to reduction in the level of the targeted
35 cell surface protein. (See, e.g., Example 29 below and Figs. 21-22.) Thus, in some

embodiments, where a subject has been determined previously to have an RNF43 or ZNRF3 mutation, for example, where a subject has a cancer comprising cancer cells having a mutated RNF43 or ZNRF3, the subject may be administered a multispecific binding protein that does not bind to or does not activate the RNF43 or ZNRF3 protein. Thus, for example, if a subject has an RNF43 mutation, a multispecific binding protein provided to that subject may comprise an anti-ZNRF3 component, and vice versa. Accordingly, in some aspects, treatment methods and uses may also comprise, prior to administering the multispecific binding protein, determining whether the subject has a mutation in RNF43 or ZNRF3, wherein, (a) if the subject has a mutation in RNF43, the multispecific binding protein does not bind to or does not activate RNF43, and (b) if the subject has a mutation in ZNRF3, the multispecific binding protein does not bind to or does not activate ZNRF3. Such mutations may be identified, for example, at the protein level by techniques such as immunohistochemistry (IHC), or they may be identified at the nucleic acid level by techniques such as reverse-transcription polymerase chain reaction (RT-PCR), whole genome sequencing, exome sequencing, or in situ hybridization or the like. Because such mutations may be present in certain cancers, in some such aspects, the subject may be a cancer subject.

Proteins herein can be administered alone or used in a combination therapy. For instance, the combination therapy includes administering a multispecific binding protein and administering at least one additional therapeutic agent (e.g. one, two, three, four, five, or six additional therapeutic agents). Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate pharmaceutical compositions), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents.

A multispecific binding protein of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g., by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

H. Kits and Articles of Manufacture and Other Methods of Use

In another aspect of the disclosure, multispecific binding molecules herein may be used in vitro, i.e. in the laboratory to modify the level of a cell surface protein of a cell culture sample or tissue sample. For instance, there are numerous instances in which it may be beneficial to assay

the behaviour of a cell or tissue sample in which the level of a particular cell surface signaling molecule is artificially reduced. Employing such a multispecific binding molecule may provide a relatively simply way to modulate the level of such a protein.

Thus, the present disclosure also encompasses methods of reducing the level of a cell surface protein in a cell or tissue sample in vitro, comprising incubating the sample with the multispecific binding protein. The present disclosure also encompasses kits for this purpose. Kits may comprise the multispecific binding protein, optionally also with instructions for use, appropriate buffers, and/or labeling molecules. Kits may also comprise nucleic acids, vectors, or host cells that comprise polynucleotides encoding the multispecific binding protein, so that the protein may, for example, be produced for use in such methods.

III. EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1: Development and characterization of rat anti-human cell surface ligase rat B cell antibodies or rabbit B cell antibodies

Rats and rabbits were chosen for the generation of cell surface ligase antibodies because of certain advantages provided over alternative methods, e.g., screening of phage libraries. For example, antibodies obtained from animals may have greater specificity and better pharmacokinetic properties than phage-derived antibodies leading to better therapeutic candidates than phage-derived antibodies. Sprague Dawley rats (Charles River, Hollister, CA) were immunized with a priming dose of 100 µg human (RNF43 or ZNRF3) protein solubilized in detergent mixed with MPL+TDM adjuvant (Sigma-Aldrich, St. Louis, MO), CFA (Sigma-Aldrich, St. Louis, MO) or mixed with a combination of TLR agonists: 50ug MPL (Sigma-Aldrich), 20ug R848 (Invivogen, San Diego, CA), 10ug PolyI:C (Invivogen), and 10ug CpG (Invivogen) divided among multiple sites. New Zealand white rabbits were also immunized with a mixture of the same proteins solubilized in detergent mixed with CFA (Sigma-Aldrich, St. Louis, MO). For additional protein boosts, the rats and rabbits received half amount of the priming dose protein diluted in PBS. Rats and rabbits were dosed every two weeks. Polyclonal antisera from these rats and rabbits were purified and tested by ELISA for binding to human RNF43 and human ZNRF3. For the rats, multiple lymph nodes were harvested three days after the last immunization that showed detectable FACS reactivity against human RNF43 or human ZNRF3. IgM-negative B-cells from these rats were purified from whole lymphocytes using

magnetic separation (Miltenyi Biotec, San Diego, CA) and stained with anti-rat IgM antibody (Jackson ImmunoResearch, West Grove, PA), anti-rat CD45RA (Biolegend, San Diego, CA), anti-rat CD8a (Biolegend, San Diego, CA), and labeled human RNF43-Alex 633 or human ZNRF3-Alex633 by Lightning-Link Alex 633 Antibody Labeling kit (Novus, Centennial, CO).

5 Rat B-cells showing minimal rat IgM expression while binding to the human RNF43 or human ZNRF3 protein were sorted and deposited into 96-well plates containing a culture medium containing feeder cells and supplemented with cytokines using a FACS Aria III sorter (BD, Franklin Lakes, NJ). For the rabbits, the blood was collected three days after the last immunization that showed detectable FACS reactivity against human RNF43 and human

10 ZNRF3. IgG-positive B cells from these rabbits were purified from the whole blood using magnetic separation (Miltenyi Biotec, San Diego, CA) and stained with anti-rabbit IgG antibody (Southern Biotech, Birmingham, AL) and labeled human RNF43-Alex 633 or human ZNRF3-Alex633 by Lightning-Link Alex 633 Antibody Labeling kit (Novus, Centennial, CO). Rabbit B-cells showing maximum rabbit IgG expression while binding to the human RNF43 or human

15 ZNRF3 protein were sorted and deposited into 96-well plates containing a culture medium containing feeder cells and supplemented with cytokines using a FACS Aria III sorter (BD, Franklin Lakes, NJ). Supernatants were screened by ELISA against human RNF43 or human ZNRF3 seven days after sorting. Supernatants demonstrating human RNF43 or human ZNRF3 binding were tested by FACS for binding to human RNF43 or human ZNRF3 expressed on the surface of gD-hRNF43 or binding to human ZNRF3 expressed on the surface of gD-hZNR3.

20 RNA was extracted from B-cells that showed RNF43 or ZNRF3 FACS binding for molecular cloning and recombinant expression. Recombinant antibodies were tested by FACS for binding to human RNF43 expressed on the surface of gD-hRNF43 or binding to human ZNRF3 expressed on the surface of gD-hZNR3.

25 **Example 2: Recombinant antibody generation.**

DNA encoding antibody heavy and light chain variable domains was generated by gene synthesis. The synthesized genes fragments were inserted into mammalian expression vectors containing the corresponding heavy or light constant domains. Species and isotypes included human IgG1 and murine IgG2a. Some variable domain sequences were edited to remove
30 apparent unpaired cysteine residues and NX[S/T] N-glycosylation motifs. Recombinant antibodies were produced by transient transfection of Expi293 cells with mammalian expression vectors encoding the antibody heavy chain and light chain. Heavy chain and light chain were encoded on separate vectors, and were transfected using a 1:1 ratio of heavy chain expression vector to light chain expression vector. Antibodies were purified from the cell culture

supernatant by affinity chromatography. In some cases, antibodies underwent an additional purification step based on SEC.

Bispecific antibodies were generated using knob-into-hole technology (Ridgway *et al.*, *Prot Eng.* 1996) using human IgG1 or murine IgG2a backbones typically including mutations to reduce effector function (e.g. L234A, L235A, P329G, and/or N297G). Abs containing either knobs or holes were expressed and purified prior to assembly into bispecific format essentially as previously described (Williams *et al.*, *Biotechnol Prog.*, 2015). In some cases, mutations were introduced to enable bispecific expression in a single cell including appropriate light-chain pairing (Dillon *et al.*, *mAbs*, 2017).

Example 3: Affinity and epitope determination for recombinant antibodies.

Antibodies were screened for binding to recombinant human RNF43 and ZNRF3 ECDs using a Biacore® 8k instrument (GE Life Sciences). Briefly, antibodies diluted to 1 µg/ml in 1XHBSP buffer (Cytiva, BR100368) were captured on a Sensor Chip Protein A (GE Life Sciences) using a flow rate of 10 µl/minute and a contact time of 60 seconds. Binding of recombinant human RNF43 and ZNRF3 extracellular domain to the captured antibodies was analyzed at 25°C using a single cycle kinetics method with a flow rate of 30 µl/minute, a contact time of 180 seconds and a dissociation time of 600 seconds. The concentration of recombinant human RNF43 and ZNRF3 extracellular domain in the single cycle kinetics were 0, 0.8, 4, 20, and 100 nM. Between cycles, the chip was regenerated using 10 mM Glycine HCl pH 1.5 injected for 30 seconds at 30 µl/minute. Data were evaluated using Biacore 8K Evaluation software (GE Life Sciences). Kinetic constants were obtained using a 1:1 binding model with the parameter RI set to zero. Multiple antibodies targeting RNF43 and ZNRF3 were shown to have sub-nanomolar affinities as shown in Tables 1-4. Cell surface ligase antibodies with sub-nanomolar affinities may provide advantages in certain instances, for example, in the degradation efficiency which is correlated with the durability of the ternary complex as shown in Fig. 5D.

Table 1 Binding of rat clones to huRNF43

Clone	Affinity (nM)
RNF43-71	0.03
RNF43-25	
RNF43-38	4.93
RNF43-56	1.84
RNF43-38	8.66
RNF43-33	62.97

RNF43-35	0.07
RNF43-90	0.29
RNF43-69	16.8
RNF43-75	4.65
RNF43-61	0.43
RNF43-74	0.35
RNF43-31	0.13
RNF43-41	0.37
RNF43-67	0.59
RNF43-80	0.48
RNF43-86	17.27
RNF43-53	3.3
RNF43-186	
RNF43-108	
RNF43-181	8.59E-02
RNF43-196	0.114
RNF43-216	0.291
RNF43-130	0.134
RNF43-136	1.91
RNF43-177	6.47
RNF43-116	1.42
RNF43-104	9.12
RNF43-145	0.504
RNF43-156	0.209
RNF43-224	0.584
RNF43-168	1.36
RNF43-176	5.71
RNF43-200	0.539
RNF43-206	0.58
RNF43-220	1.57
RNF43-128	N.B.
RNF43-126	1.75
RNF43-217	1.49
RNF43-170	76
RNF43-179	31.7
RNF43-221	51.6
RNF43-180	2.81
RNF43-129	1.17
RNF43-107	0.341
RNF43-117	19.8

RNF43-152	17.8
RNF43-201	9.9
RNF43-106	15.6
RNF43-123	13.4
RNF43-210	13.4

Table 2 Binding of rat clones to huZNF3	
Clone	Affinity (nM)
ZNRF3-171	1.01
ZNRF3-101	0.07
ZNRF3-117	0.2
ZNRF3-170	
ZNRF3-163	0.61
ZNRF3-195	0.27
ZNRF3-6	0.12
ZNRF3-128	3.16
ZNRF3-35	
ZNRF3-90	0.27
ZNRF3-131	2.39
ZNRF3-17	0.04
ZNRF3-55	0.02
ZNRF3-172	0.21
ZNRF3-179	0.11
ZNRF3-182	0.06
ZNRF3-30	0.12
ZNRF3-305	7.18
ZNRF3-312	0.611
ZNRF3-279	1.66
ZNRF3-275	0.0565
ZNRF3-314	0.104
ZNRF3-301	0.0974
ZNRF3-269	0.312
ZNRF3-219	1.9
ZNRF3-265	0.0286
ZNRF3-253	0.483
ZNRF3-254	0.104
ZNRF3-300	0.239
ZNRF3-222	N.B.
ZNRF3-287	0.191
ZNRF3-322	N.B.
ZNRF3-247	0.161
ZNRF3-296	0.0371

ZNRF3-223	0.0443
ZNRF3-231	0.0783
ZNRF3-244	6.85
ZNRF3-270	0.0424
ZNRF3-329	0.283
ZNRF3-255	0.26
ZNRF3-237	5.3

Table 3: Binding of rabbit clones to huRNF43	
Clone	Affinity (nM)
RNF43-13	99.6
RNF43-19	34.2
RNF43-9	1.8
RNF43-2	N.B.
RNF43-21	10.3
RNF43-22	0.5
RNF43-23	85
RNF43-6	10.3
RNF43-14	0.8
RNF43-17	6.7
RNF43-15	8
RNF43-5	1.1
RNF43-11	27.7
RNF43-16	37.8
RNF43-20	N.B.

Table 4: Binding of rabbit clones to huZNRF3	
Clone	Affinity (nM)
ZNRF3-331	0.2
ZNRF3-332	1.6
ZNRF3-333	8.7

Example 4: HTP epitope binning.

- 5 Antibodies generated by animal immunization were reformatted into hIgG1 backbones and binning was performed using the CFM2/MX96 SPR system (Wasatch Microfluidics, now Carterra), equipped with DA v6.19.3, IBIS SUIT, SprintX & Carterra Epitope Tool software. 10

µg/ml antibodies were immobilized on an SPR sensor prism CMD 200 M (Xantec Bioanalytics) by amine coupling using a 10 mM sodium acetate pH 4.5 immobilization buffer. Immobilization was performed with the CFM2 instrument and the sensor prism was then transferred to the IBIS MX96 instrument for SPR-based competition analysis. Immobilized antibodies were exposed first to 100 nM recombinant human RNF43 or ZNRF3 extracellular domain and then to 10 µg/ml antibody in solution, using an HBS-EP running buffer (10 mM HEPES, 150 mM NaCl, 0.05% Tween 20, pH 7.4, 1 mM EDTA). 10 mM glycine/HCl pH 1.7 were used as the regeneration buffer. Epitope bin results are shown in **Figure 5b** and indicate several different RNF43 and ZNRF3 sandwiching profiles indicating binding to multiple different epitopes on the antigens.

Example 5: Assessing target cell-surface clearance by flow cytometry

Antibodies were assessed for their ability to remove target antigens from the cell surface of multiple target cells. Cell lines included SW1417, HT29, and engineered HT-29 cell lines including ones engineered to express N-terminally tagged ligases. N-terminal tags included an epitope tag (gD) or a HiBit tag (Promega) for quantitation of tagged proteins through the Nano-glo HiBit Extracellular Detection System (Promega). Cell lines were pools or clonal and optionally included ligase expression on a doxycycline inducible promoter. Cells were plated into 96-well plates at 50,000-120,000 cells/wells and grown in ATCC recommended media (+/- doxycycline) in the presence of various concentrations of antibody. After 24 hours (or the indicated period of time), media was removed, cells were washed with PBS (150 µL), and detached from the well surface by addition of accutase (Millipore) (100 µL) at 37°C for 10 min. Accutase activity was quenched by addition of 100 µL of RPMI + 10% heat inactivated Fetal bovine serum with penicillin, streptomycin, and glutamine, cells were centrifuged for 4 min @ 1200 rpm, and the supernatant was discarded. Cells were resuspended in 150 µL FACS buffer (PBS with 0.5% BSA and 0.05% sodium azide) for 10 min at 4 °C, centrifuged and supernatant discarded. Cells were incubated with 40 µL of staining antibodies (2 µg/ml final concentration) for 45 min at 4 °C, washed twice in 150 µL of FACS buffer and resuspended in 40 µL of FACS buffer for analysis. Staining antibodies include xIGF-1R mIgG1 APC (1H7, ebioscience catalog # 17-8849-42) and xRNF43 37.17 APC (in-house generated reagent). Background / negative control staining antibodies include NISTMab hIgG1APC and xRagweed mIgG1 APC (both in-house generated reagents).

Flow cytometry analysis was performed on a BD FACSCelesta™ Flow Cytometer using the High Throughput Sampler (HTS) in high throughput mode. Each sample was first resuspended twice with 10 µL of resuspension volume. Then 10 µL of sample was aspirated from each well and flow through the cytometer at the flow rate of 180 µL/min. Between each samples

the system was washed with 400 ul (wash volume) of buffer. The SSC, FSC, and APC signals were measured. Using Flowjo FACS analysis software, cells were gated for single cell based on the SSC and FSC profile. Mean Fluorescent Intensity (MFI) of APC signal as a measure of IGF-1R level on the cell surface.

5 **Example 6: Genetic induced dimerization of ligases to cell surface receptors**

The effect of chemically induced dimerization of RNF43 or ZNRF3 with HER2 was assessed using the iDimerize system (Takara Bio). Briefly, HEK293T cells were reverse transfected with pBind plasmids to induce the constitutive co-expression of RNF43-DmrA-HA and HER2-DmrC-FLAG or ZNRF3-DmrA-HA and HER2-DmrC-FLAG. After 24 hours, cells
10 were either left untreated or treated with 500 nM A/C heterodimerizer (TakaraBio; REF 635057) to induce ligase-target dimerization and cells were incubated for an additional 24 hours. Cells were then lysed in GST lysis buffer [25mM Tris•HCl, pH 7.2, 150mM NaCl, 5mM MgCl₂, 1% NP-40 and 5% glycerol, 1 % Halt protease & phosphatase inhibitor cocktail]. Cleared lysates were subjected to an HA immunoprecipitation (IP) by incubating lysates with 25 µl Pierce HA
15 epitope tag antibody agarose conjugate (2-2.2.14) (Thermo Scientific; REF 26182) for 3 hours at 4 °C. For input samples, 20 µl were used from the prepared IP samples prior to incubation. Beads were washed following incubation and prepared for Western blot analysis.

Example 7: Western blot and Immunoprecipitation

HT29, HEK293T and SW1417 cells were plated in 6 well dish (Corning, cat #3516) at a
20 density of 500,000 cells per well and let adhere overnight. Cells were treated with bispecific antibodies diluted at 1ug/mL in RPMI-1640 media supplemented with FBS, L-glutamine, and Pen/ Strep for 40 hours. Where applicable, cells were pre-treated with doxycycline (500ng/ml), Bortezomib (200nM, Selleck, cat# S1013), E1 inhibitor (MLN7243, cat# S8341, 100nM), or Bafilomycin A (Tocris - Cat. No. 1334, 50nM). Following 24-48 hour incubation, cells were
25 lysed in RIPA buffer supplemented with Phosphatase/ Protease inhibitor (Thermo Fisher, Cat #78442) for 20 minutes on ice. Lysates were clarified and normalized, and 20ug of each sample was run on 4-12% Bis-Tris gel (Invitrogen, Cat #WG1403) using BOLT MOPS SDS running buffer at 90V for approximately 2 hours. The gel was transferred to Nitrocellulose membrane using iBlot2 system using iBlot2 Nitrocellulose Regular stacks (Invitrogen, Cat #IB23001) and
30 transferred using protocol P0 (1 minute at 20V, 4 minutes at 23V, and 2 minutes at 25V). Blots were blocked with 5% milk in TBST and blocked for 1 hour at room temperature. Blots were transferred to 5% milk in TBST containing antibodies for IGF1R-beta (Cell signaling, #3027), Vinculin (Cell signaling, cat #13901), and incubated at 4 degrees overnight. Blots were washed 4 times for 5 minutes each with TBST before being incubated in 5% milk containing IRDye

secondary antibody (Li-Cor, Cat #926-32211) for 1 hour at room temperature. Blots were washed 4 times for 5 minutes each and developed using the Li-Cor system.

Example 8: Characterization of cell surface ligases driven by Wnt signaling activity

RNF43 and ZNRF3 are Wnt canonical targets that control the turnover of the Wnt ligands receptors FZD and LRP at the cell surface (Fig. 1A). This is achieved through ubiquitination and consequently cell surface clearance of FZDs/LRP by RNF43/ZNRF3 (Fig. 1A). To evaluate the impact of oncogenic Wnt signaling on RNF43 and ZNRF3 expression, we made use of publicly available gene expression profiles from various colorectal cancer patients data sets, where Wnt signaling is constitutively hyperactive. Both cell surface ligases displayed elevated expression in colorectal adenoma samples compared to healthy control normal mucosa (Galamb O., *et al.*, Dis Markers, 2008. GSE4183; Fig 1B). In addition, colorectal cancer gene expression analysis derived from the cancer genome atlas (TCGA) demonstrated elevated expression of RNF43 in CRC compared to other tumor types and normal colon (Fig 1C). Importantly, in situ hybridization of *Rnf43* in murine colon cancer models demonstrated broad and homogeneous expression of the ligase within the entire tumor mass (Fig 17). The canonical role of RNF43 and ZNRF3 was validated using transfection of an inducible plasmid that conditionally expresses either ligases into HPAF-II cells upon addition of doxycycline (dox). Dox mediated over expression of WT RNF43 leads to cell surface clearance of FZD receptors in a ligase dependent manner (Fig. 1D). To probe the degradative potential of RNF43 and ZNRF3 beyond their natural substrates, namely FZDs and LRP5/6, we took advantage of the idimerize system. HEK293T were transfected with pBind plasmids to induce the constitutive co-expression of RNF43-DmrA-HA and HER2-DmrC-FLAG. 24hr after addition of the AC heterodimerizer, an increase binding between RNF43 and HER2 was detected through immunoprecipitation of RNF43 followed by immunoblotting of HER2 (Fig. 1E left panel). This increase in ligase-target binding was function as it resulted in HER2 target degradation (Fig. 1E right panel).

Example 9: Expression and dimerization of tagged RNF43 and ZNRF3 ligases to HER2

We further evaluated the ability of both cell surface ligases to degrade receptors when engaged with bispecific antibodies. N-terminally gD tagged ligases were generated, and dimerized to HER2 by using bispecific targeting three different epitopes of HER2 (4D5, 7C2, and 2C4) (Fig 2A-B) and gD. Cell surface expression of both ligases was confirmed by flow cytometry (Fig. 2C) and gD based dimerization through either HER2 epitopes led to HER2 degradation, which was dependent on the ligase expression (Fig. 2D). We further validated this degradation potential in additional HER2 expressing breast cancer cell lines, including the HER2

amplified KPL4 cell line. Importantly, HER2 degradation also impacted downstream signaling as evidenced by a decrease in pERK activity (Fig. 2E).

Example 10: Antibody dependency of cell surface clearance

Bispecific antibodies were generated as described above targeting three different epitopes of HER2 (4D5, 7C2, and 2C4) (Fendly *et al. Canc Res* 1990) and either the gD epitope tag or an irrelevant antigen (human CD3). Impact of these bispecific antibodies was assessed on HT-29 cells expressing doxycycline inducible gD-tagged ZNRF3 or RNF43, as described above.

Different antibodies showed different levels of clearance of HER2 from the cell surface (Fig. 3A). Monovalent binding of 2C4/CD3 was sufficient to drive partial clearance of HER2, though substantially less than is seen with the 2C4/gD bispecific Ab. To understand the impact of cell surface clearance on HER2 degradation we investigated HER2 levels by Western blot. Similar to what was observed by flow cytometry, ligase dimerization to HER2 was required for HER2 degradation. Importantly, monovalent binding to HER2, using anti-2C4/CD3 did not result in substantial HER2 degradation, suggesting that cell surface receptor clearance does not

necessarily lead to receptor degradation and ligase dimerization is required (Fig. 3B). To substantiate that claim, we probed the ubiquitination status of HER2 through

Immunoprecipitation. Ubiquitin smears were detected on HER2 after treatment with the various HER2/gD bispecific antibodies only in the presence of gD tagged ligases (Fig. 3C). Furthermore, ligase activity was required for HER2 degradation as deletion of the RING domain from either ZNRF3, and to a lesser extent RNF43, prevented HER2 degradation after ligase dimerization (Fig. 3D). Conversely, inhibition of the E1 Ubiquitin activating enzyme also rescued degradation, which we found was dependent on lysosomal rather than proteasomal activity (Fig. 3E).

Example 11: Cell surface ligase mediated degradation is applicable to multiple receptors

Chemical induced dimerization was used to probe the degradative potential of RNF43 to additional cell surface receptors, in particular IGF1R, which, like HER2, is a receptor tyrosine kinase. Similarly to what was observed with HER2, addition of the AC hetero-dimerizer to HEK293T cells transfected with a pBind plasmid co-expressing RNF43-DmrA-HA and IGF-1R-DmrC-FLAG, led to a dose dependent increase in binding between the two receptors (Fig. 4A left panel). Consequently, this increase in binding between ligase-target resulted in a dose dependent degradation of IGF-1R (Fig. 4A right panel). Bispecific antibodies were generated using variable regions targeting IGF-1R and gD and assessed for their ability to degrade IGF1R in HT-29 cells expressing a doxycycline inducible gD tagged ZNRF3. Western blot analysis of HT-29 cells treated with 1ug/ml of the depicted antibodies was performed 24hr after treatment.

For most bispecific antibodies, gD-ZNRF3 dimerization resulted in IGF1R degradation that was equivalent to the decrease in protein expression observed in a conditional KO made using CRISPr Cas9 and sgRNA specific for IGF1R (Fig. 4B). The phenotypic consequence of ligase mediated degradation of cell surface receptors was further evaluated using HT55 colon cancer
5 cells. These cells rely on IGF1R for survival both *in vitro* (Fig. 4C) and *in vivo* (Fig. 4D). In line with the viability defect observed after genetic mediated KO of IGF-1R, HT55 cells treated for seven days with bispecific antibodies targeting IGF-1R and gD displayed a decrease in clonogenic growth, that was dependent on the expression of the ligase (Fig. 4E, 4F).

Example 12: Kinetics of clearance from the cell surface.

10 Bispecific antibodies were generated using variable regions targeting IGF-1R (cixutumumab) and RNF43 (hSC37.39, US20170073430A1) and assessed for their ability to degrade IGF-1R in HT-29 cells with doxycycline induced overexpression of RNF43 by flow cytometry (Fig. 5D). One hour following antibody administration, cells treated with higher levels of the bispecific (0.1 ug/ml or greater) showed modestly higher levels of cell-surface
15 IGF1R. By three hours, this trend had reversed with decreased cell surface IGF1R, and by eight hours the trend had saturated. Continuous treatment out to 96 hours showed no further clearance of target.

Example 13: Assessment of RNF43 and ZNRF3 bispecific antibodies

A subset of RNF43 and ZNRF3 antibodies discovered as described above were
20 reformatted into bispecific antibodies targeting the respective ligases and IGF1R (cixutumumab) (i.e., "PROTABs"). Activity of these ligases was assessed by their ability to drive cell surface clearance of IGF1R from HT29 cells with doxycycline induced expression of the corresponding ligase by flow cytometry (Fig. 6A). Antibodies targeting the ligases with high monovalent affinities towards the ligases (greater than approximately 1nM) showed saturating levels of
25 IGF1R clearance when paired with cixutumumab, while lower affinity antibodies showed reduced activity (Fig. 6B). Further evaluation of the ZNRF3-IGF-1R PROTAB was done on SW1417 cells which express high endogenous level of ZNRF3 and RNF43. Flow cytometry revealed a dose dependent cell surface clearance of IGF-1R in these cells after treatment with a ZNRF3-IGF-1R antibody (Fig. 6A). Western blot confirmed cell degradation of IGF-1R in
30 SW1417 after with an RNF43-IGF-1R antibody (Fig. 6C).

Without being bound by theory, the correspondence of ligase affinity to efficiency of degradation is hypothesized to be due to the durability of the ternary complex between IGF1R, the antibody and the ligase. Biochemical estimates suggested that at least four ubiquitins must be transferred to the target to enable efficient degradation, and thus a long-lived complex is

necessary to enable multiple catalytic cycles. The durability of the overall complex was dictated by binding affinities of both sides of the bispecific antibody. For ligase antibodies much tighter than 1 nM, the affinity of cixutumumab (~5 nM) was the primary determinant in the half-life of the complex, thus the efficiency of degradation saturates. For ligase Abs weaker than 1 nM, we observed reduced clearance corresponding to the affinity of the ligase arm. Interestingly, no strong dependence of epitope was seen for the ligase antibodies, so for RNF43 and ZNRF3, the specific geometry of interaction did not strongly impact the efficiency of degradation. This is potentially different than what we saw for HER2 antibodies paired with an anti-gD Tag antibody (Figs. 2A-2B) where changing the epitope of the HER2 targeted antibody appeared to influence degradation efficiency. Again without being bound by theory, we hypothesize this is due to the relatively micro-differentiations in epitope on the single extracellular protease-associated domain for the ligases, versus gross changes in epitopes targeting different domains for HER2. For bispecific ligase antibodies with other (non-cixutumumab) arms, the affinity at which this saturation takes place may thus vary corresponding to the affinity of that arm.

Example 14: Impact of bivalent binding on target degradation.

To investigate the impact of receptor clustering on target clearance, antibodies targeting two copies of the anti-RNF43 antibody or the anti-IGF1R antibody were generated in 2+1 Fab-IgG format (Figures 7A-7B). Activity of these antibodies was assessed by their ability to drive cell surface clearance of IGF1R from HT29 cells with doxycycline induced expression of the corresponding ligase by flow cytometry (Figs. 7C-7F). Antibodies binding two copies of IGF1R and one copy of RNF43 saturated clearance at levels similar to the corresponding traditional knob-in-hole (1+1) bispecific antibody (Fig. 7C and Fig. 7E). Antibodies binding two copies of the ligase drove enhanced clearance compared to the corresponding traditional knob-in-hole (1+1) bispecific antibody (Fig. 7D and Fig. 7F). These antibodies also reduced the level of cell-surface RNF43, presumably due to auto-ubiquitination in *trans* (data not shown).

Example 15: Impact of binding distance on target degradation.

We hypothesized that the distance between the ligase and target might influence the efficiency of target clearance. To investigate this phenomenon, bispecific antibodies in the one-armed FabIgG (OA-FabIgG) and one-armed FvIgG (OA-FvIgG) format were generated (Fig. 8A). Activity of these antibodies was assessed by their ability to drive cell surface clearance of IGF1R from HT29 cells with doxycycline induced expression of the corresponding ligase by flow cytometry (Figs. 8B-8C). Both OA-FvIgG and OA-FvIgG formats resulted in enhanced degradation compared to the corresponding 1+1 bispecific. For the Fab-IgG format similar results were seen with both cixutumumab and isitiramab as the anti-IGF1R antibody. In addition, the results shown in Fig. 8 suggest that the efficiency of degradation can be fine-tuned

or optimized by varying the location of each antigen binding domain, e.g, by placing the ligase antigen binding domain either in the external position (the Fv in OA-FvIgG or the Fab in OA-FabIgG) or the internal position.

Example 16: Combination of valency and mimized distance

5 2+1 Fab-IgG and FvIgG antibodies comprised of 2x RNF37.39 and 1x cixutumumab are generated placing the cixutumumab Fab at the internal position. Assessment of these antibodies in the cell surface clearance assay described in Example 5 above finds that they clear IGF1R more efficiently than the corresponding traditional knob-in-hole (1+1) bispecific antibody. This data was further validated using DLD1 and looking at total IGF1R level using western blot
10 analysis (Fig. 24).

Example 17: Cell surface ligase-mediated clearance of EGFR

 Multispecific antibodies were generated targeting EGFR (using cetuximab or D1.5 variable domains), and RNF43 or an irrelevant antigen. Activity of these antibodies was assessed by their ability to drive cell surface clearance of EGFR from HT29 cells with
15 doxycycline induced expression of the corresponding ligase by flow cytometry (Fig. 9). Both 1+1 bispecific antibodies targeting both the ligase and EGFR resulted in efficient clearance. Consistent with the results described in Example 14, the 2+1 FabIgG targeting 2x RNF43 and 1x EGFR resulted in more complete clearance.

Example 18: Identification and characterization of novel cell surface ligases

20 To identify putative cell surface ligases the domain structure of RNF43 and ZNRF3 was evaluated (Fig. 10A). This revealed the presence of an N-terminal Signal Peptide (SP) that has been associated with membrane integration. As such, a list of known E3 ubiquitin ligases was evaluated using both UNIPROT and the Signal-P software to identify ligases with SP. Among the identified proteins, we evaluated the following 15 ligases which include both single and
25 multiple transmembrane domain ligases: RNF13, RNF43, RNF128, RNF130 (having two transmembrane domains, Fig. 10B), RNF133, RNF148, RNF149, RNF150, RNF167, ZNRF3, ZNRF4, LYNX1, RSPRY1, SYVN1 (having five transmembrane domains, Fig. 10B), and TRIM7.

 Doxycycline-inducible pBind plasmids with IRES-eGFP encoding all 15 ligases with an
30 N-terminal gD tag were generated (Fig. 10B). Cell surface presentation of each ligase was evaluated using FACS analysis. Briefly, HEK293T cells were transiently transfected with the respective ligase plasmids. After 24 hours cells were treated with 1 ug/ml doxycycline for an additional 24 hours to induce ligase expression. Live cells were then prepared for FACS analysis by staining using an anti-gD primary antibody followed by an APC secondary antibody. To

evaluate doxycycline induction the IRES-eGFP signal was examined (Fig. 10C). The APC median florescence intensity (MFI) was also quantified from three independent experiments for each sample as outlined (Fig. 10D). As assay controls, parental and mock transfected cells were also evaluated as well as HEK293T cells constitutively expressing gD-tagged Fzd8, a known cell surface protein.

In parallel to the FACS analysis, HEK293T transfected cells were also used to evaluate the degradative potential of each putative cell surface ligase. Following 24 hours doxycycline induction, cells were treated with 10 ig/ml gD+HER2 (7C2) bispecific antibody for 24 hours. Cells were then lysed and prepared for Western blot analysis to examine the total levels of HER2 upon bispecific antibody treatment. (Fig. 10E and 10F.) Assessment of these cell surface ligases in the standard cell degradation assay finds that numerous cell surface ligases can be dimerized to HER2 and lead to HER2 efficient degradation.

Additional experiments were carried out as follows. Fig. 10D shows that RNF128, RNF130, RNF133, RNF149 and RNF150 exhibited detectable cell surface expression while RNF13, RNF148, and RNF167 expression was substantially lower. To assess whether cell-surface expression broadly enabled degradation of non-natural targets, we treated cells with gD*IGF1R PROTABs, and monitored IGF1R levels. Western blot analysis confirmed that colocalizing validated cell surface E3 ubiquitin ligases to IGF1R drives degradation, as shown in Fig. 32. Conversely, ligases with undetectable cell surface expression by flow cytometry were unable to induce target degradation as shown in Fig. 33. We also generated bispecific antibodies that target gD and either HER2 or programmed death-ligand 1 (PD-L1), two therapeutically relevant cancer targets. Similar to IGF1R, degradation of HER2 and PD-L1 occurred upon engagement of various ligases as shown in Figs. 34 and 35, but no degradation was detected using ligases with undetectable cell surface expression as shown in Figs. 36 and 37.

Figure 31 shows that several of the newly identified cell surface E3 ligases showed discrete tissue expression patterns, providing possibilities for tissue specific degradation based on choice of particular E3 ligase. For example, RNF130, RNF149 and RNF167 displayed apparent elevated expression in the hematopoietic compartment, and these ligases could be used to target cells of hematopoietic origin, while RNF133 and RNF148 expression was mainly restricted to the testis and these ligases could be used to target testicular cells.

The catalytic activity of an E3 ubiquitin ligase is considered essential to facilitate the final transfer of ubiquitin from the ubiquitin-conjugating enzymes (E2s) to substrates to alter that substrate function. To test the importance of catalytic activity, catalytically active and inactive versions of each individual ligase can be used in degradation assays to assess whether or not catalytic activity is required for each ligase to mediate degradation of cell surface receptors.

Preliminary data suggests that certain ligases may require catalytic activity while other certain ligases may not. A separate cell line was used to validate cell surface expression of newly identified ligases. HT29 cells were transfected with each individual ligase, selected with puromycin for stable integration. Stable lines were treated with 1 ug/ml doxycycline for an 24 hours to induce ligase expression. Live cells were then prepared for FACS analysis by staining using an anti-gD primary antibody followed by an APC secondary antibody. The percentage of APC positive cells was also quantified from three independent experiments for each sample as outlined (Fig. 27A, B).

Example 19: Cell surface clearance of diverse substrates

Bispecific antibodies are generated targeting the gD epitope and exemplary receptor tyrosine kinases, HER2, IGF1R, and EGFR, as well as various structurally distinct cell-surface proteins, including multipass transmembrane domain proteins, e.g., FZLD5, proteins having large extracellular and intracellular domains, e.g., EpCAM, and proteins having an immunoglobulin domain with a short intracellular domain, e.g., PD-L1. Antibodies at 10 ug/ml are added to cells overexpressing ligases tagged with an N-terminal gD tag. Western blot analysis reveals addition of the bispecific antibody drives degradation of the target antigen. An example is depicted in Fig. 29 using HT29 cells that stably express various cell surface ligases. Dimerization of several ligases to IGF1R using gD/IGF1R antibody result in profound receptor degradation.

Example 20: Assessing Wnt signaling pathway activation by TOPBrite TCF reporter assay

The Wnt-dependent reporter Nano-Glo Dual® luciferase system (Promega), containing a control promotor that expresses *Renilla* luciferase and a TCF promotor that expresses firefly luciferase, was used to evaluate the activation of Wnt/ β -Catenine by rat or rabbit mutlispecific antibodies targeting ZNRF3. HEK293 cells transiently transfected with plasmids containing a control promoter and TCF promoter were plated in Corning® 96-well Flat Clear Bottom White Polystyrene TC-treated Microplates at 100,000 cells per well and incubated in RPMI media with 10% FBS, 1% penicillin-streptomycin, 1x L-glutamine and 40 μ g/mL hygromycin B (Thermo) overnight. On the following day, rat or rabbit clone ZNRF3 antibodies at 0.1 mg/mL and recombinant human Wnt3a at 100 ng/mL (Abcam) were added to the cells. GSK3 β inhibitor at 2 uM or RSPO3 at 500, 10 and 0.2 ng/ μ L in the presence of 100 ng/mL Wnt3a was used as a positive control. DMSO or 100 ng/mL Wnt3a treated cells were negative controls. Upon 24 hours (h) incubation, cell media was replaced with fresh media and an equal volume of Dual-Glo® reagent was added to each well followed by gentle mixing. After 10 minutes incubation,

the firefly luminescence signal was measured using GloMax® Discover Microplate Reader at 0.3 second integration time (Promega). Then an equal volume of Dual-Glo Stop&Glo® reagent was added to each well and the *Renilla* luminescence signal was measured after 10 minutes incubation using the same setting. The relative ratio was calculated from the ratio of firefly to *Renilla* luminescence and then further normalized by taking a ratio of an untreated sample. Results from ZNRF3 antibodies are shown in Figs. 11A-11B.

Example 21: Assessing IGF1R cell-surface clearance by NanoLuc® luciferase complementation assay

A HiBiT tag was introduced to the N-terminus of IGF1R by a CRISPR-Cas9 system on HT29 cells. Single clones with the highest NanoLuc® luciferase luminescence readout were selected and verified by Sanger sequencing. Cells were plated onto Corning® 96-well Flat Clear Bottom White Polystyrene TC-treated Microplates at 100,000 cells per well and incubated in ATCC recommended media in the presence of various concentrations of antibodies. After 24 hours (or the indicated period of time), the cells were washed with 100 µL PBS once and then replenished with 100 µL fresh media. The detection reagent was prepared by diluting the LgBiT protein at a ratio of 1:100 and the substrate at a ratio of 1:50 into a desired volume of detection buffer supplied in the detection kit. A 100 µL of the detection reagent then was added to the cells in 100 µL fresh media. For Nano-Glo® HiBiT Extracellular Detection System which detects the surface level of IGF1R, detection was performed upon 10 min incubation at room temperature with gentle mixing using a plate shaker. Percent IGF1R clearance was calculated using the equation % Clearance = (Untreated samples RLU- treated samples RLU)/Untreated samples RLU *100. RNF43 and ZNRF3 multispecific antibodies were assessed in this cell line as well (Tables 5-7). A subset of samples were run in two separate runs, in which case both results are included.

Table 5: Surface IGF1R clearance by bispecific RNF43-IGF1R antibodies

Short Name	%IGF1R clearance at 1 ug/ml
cixu/RNF43-71	54%
cixuRNF43-25	61%
cixu/RNF43-33	25%
cixu/RNF43-35	73%
cixu/RNF43-90	72%
cixu/RNF43-69	42%

Short Name	%IGF1R clearance at 1 ug/ml
cixu/RNF43-75	52%
cixu/RNF43-31	60%
cixu/RNF43-41	50%
cixu/RNF43-80	60%
cixu/RNF43-130	71%
cixu/RNF43-177	42%
cixu/ RNF43-104	44%
cixu/RNF43-156	62%
cixu/RNF43-168	69%
cixu/RNF43-176	16%
cixu/RNF43-170	33%
cixu/RNF43-221	7%
cixu/RNF43-180	51%
cixu/RNF43-107	48%
cixu/RNF43-201	24%
cixu/RNF43-106	21%
cixu/RNF43-210	22%
cixu/xRNF43 37.39	40%
cixu/RNF43-90	77%
cixu/RNF43-75	28%
cixu/RNF43-61	29%
cixu/RNF43-31	51%
cixu/RNF43-41	30%
cixu/RNF43-80	47%
cixu/RNF43-86	36%
cixu/RNF43-53	66%
cixu/ RNF43-186	50%
cixu/RNF43-181	76%
cixu/RNF43-196	67%
cixu/RNF43-216	65%
cixu/RNF43-130	71%
cixu/RNF43-116	50%
cixu/RNF43-104	38%

Short Name	%IGF1R clearance at 1 ug/ml
cixu/RNF43-145	74%
cixu/RNF43-156	77%
cixu/RNF43-224	70%
cixu/RNF43-176	63%
cixu/RNF43-200	68%
cixu/RNF43-206	19%
cixu/RNF43-220	63%
cixu/RNF43-128	4%
cixu/RNF43-126	62%
cixu/RNF43-217	61%
cixu/RNF43-170	33%
cixu/RNF43-179	27%
cixu/RNF43-221	23%
cixu/RNF43-180	66%
cixu/RNF43-129	61%
cixu/RNF43-107	69%
cixu/RNF43-117	38%
cixu/RNF43-152	20%
cixu/RNF43-201	26%
cixu/RNF43-106	18%
cixu/RNF43-123	24%
cixu/RNF43-210	9%
Cixutumumab/RNF43.h SC37.39	68%
RNF43.37.39 / NIST	-9%
Cixu / NIST	12%

Table 6: Surface IGF1R clearance by bispecific ZNRF3-IGF1R antibodies

Short Name	%IGF1R clearance at 1 ug/ml
cixu/ZNRF3-172	60%
cixu/ZNRF3-182	60%
cixu/ZNRF3-279	34%

cixu/ZNRF3-314	51%
cixu/ZNRF3-269	49%
cixu/ZNRF3-219	9%
cixu/ZNRF3-300	50%
cixu/ZNRF3-222	21%
cixu/ZNRF3-322	59%
cixu/ZNRF3-270	66%
cixu/ZNRF3-237	3%
cixu/ZNRF3-171	-3%
cixu/ZNRF3-55	55%
cixu/ZNRF3-6	52%
Cixu/gD	-42%
Cixu /NIST	2%

Table 7: Surface IGF1R clearance by new format bispecific antibodies

Short Name	%IGF1R clearance at 1 ug/mle
2+1 Cixu-Cixu/RNF43.37.39	49%
2+1 Cixu-Cixu/NIST	37%
2+1 Istira-Istira-RNF43.37.39	49%
2+1 Istira-Istira/NIST	7%
2+1 RNF43-RNF43.39.39/Cixu	57%
2+1 RNF43-RNF43.39.39/Istira	-5%
2+1 RNF43-RNF43.39.39/NIST	47%
RNF43-RNF43.hsc37.39 hIgG1	3%
Cixu-RNF43 Fv-IgG	75%
RNF43-Cixu Fv-IgG	30%
Istira-RNF43 Fv-IgG	71%
RNF43-Istira Fv-IgG	40%
xIGF1R.Cixu.hIgG1	18%
xIGF1R.Istira.hIgG1	41%

Tables 5-7 show the percent surface IGF1R-HibiT clearance. Table 5 shows that rat Cixu/RNF43 bispecific antibodies triggered cell surface IGF1R-HibiT clearance in HT29 at a concentration of

1 µg/mL. Table 6 shows that rat Cixu/ZNRF3 bispecific antibodies triggered cell surface IGF1R-HibiT clearance in HT29 at a concentration of 1 µg/mL. Table 7 shows that new format antibodies including Fv-IgGs and 2+1 trispecific format triggered cell surface IGF1R-HibiT clearance in HT29 at a concentration of 1 µg/mL.

5 **Example 22: Monitoring IGF1R surface clearance kinetics using NanoLuc® luciferase complementation assay**

HibiT tagged IGF1R HT29 cells were seeded overnight at 100,000 cells per well in a Corning® 96-well Flat Clear Bottom White Polystyrene TC-treated Microplates and incubated in ATCC recommended media. 1 µg/mL or 10 µg/mL bispecific and new format antibodies were added to the cells on the second day and incubated at various time points: 0, 4, 8, 24 hours. The luminescence signal of IGF1R-HibiT was detected using the HibiT extracellular system following the description written in the previous example. Percent IGF1R was calculated using the equation $\% \text{IGF1R} = (1 - (\text{Untreated samples RLU} - \text{treated samples RLU}) / \text{Untreated samples RLU}) * 100$. Assessment of a variety of multispecific antibodies showed different rates of degradation (Figs. 12A-12F).

Example 23: Multiplex NanoLuc luciferase complementation assay with cell viability assay

HiBiT tagged IGF1R HT29 cells were seeded overnight at 100,000 cells per well in a Corning® 96-well Flat Clear Bottom White Polystyrene TC-treated Microplates and incubated in ATCC recommended media. 1 µg/mL bispecific antibodies were added to the cells on the second day and incubated for 24 h. Cell media containing lactate dehydrogenase were collected for LDH cytotoxicity assay (Promega) before the addition of the HiBiT extracellular detection reagent. About 2-5 µL of the media was diluted 300x in LDH storage buffer and then mixed with an equal volume of LDH detection reagent. LDH lytic reagent was added to the control samples for maximum LDH detection. The reaction was incubated at room temperature for 30-60 minutes and the luminescence signal was measured using GloMax® Discover Microplate Reader at 1 second integration time. The % cytotoxicity was calculated using the equation: $\% \text{Cytotoxicity} = 100 \times ((\text{Experiment LDH release} - \text{Medium background}) / (\text{Maximum LDH release control} - \text{Medium background}))$. Post HiBiT extracellular detection, cells were washed twice with PBS and replenished with fresh media. CellTiter-glo® reagents (Promega) was then added to the cells in an equal volume and incubated for 10-30 min before the luminescence detection using GloMax® Discover Microplate Reader at 1 second integration time (Promega). While various multi-specifics showed variable influences on target degradation, no impact was seen on cell

death as determined by LDH assay or the number of cells as determined by Cell Titer-go (Figs. 13A-13C).

Example 24: Assessing IGF1R degradation using NanoLuc® luciferase complementation assay

5 HibiT tagged IGF1R HT29 cells were seeded overnight at 100,000 cells per well and then treated with various bispecific and new format antibodies at 1 µg/mL. Upon 24 h incubation, the cells were incubated with the HibiT lytic detection reagent for the total amount of IGF1R-HibiT. The lytic detection reagent was prepared by diluting the LgBiT protein at a ratio of 1:100 and the substrate at a ratio of 1:50 into a desired volume of detection buffer supplied in the detection kit.

10 The luminescent signal was measured using GloMax® Discover Microplate Reader at 1 second integration time after 20 minutes incubation with the lytic detection reagent. Percent IGF1R was calculated using the equation mentioned in the previous example (Fig. 14A). Western blot was also used to determine the total amount of IGF1R after 24 h antibody treatment. HT29 IGF1R-HiBiT tagged and wild type cells (WT) were treated with various antibodies as indicated (1.

15 Cixu-RNF43 Fv-Ig, 2. Cixutumumab/RNF43.hSC37.39, 3. Cixu/NIST, 4. Cixu/Cixu) at 1 µg/mL for 24 h. Untreated cells (5. UT) were used as a control. 20 µg of total proteins were loaded in each lane and the target protein is probed with anti IGF1 receptor antibody (Abcam EPR19322) which recognizes both pro IGF1R (~200 kDa) and IGF1R (~95 kDa). β-actin is shown as a protein loading control (Fig. 14B). Western blot analysis shows that the HiBit line

20 has predominantly Pro-IGF1R which is intracellular and not subject to degradation, resulting in limited degradation in the lytic HibiT assay. The extracellular mature IGF1R is more abundant in the WT HT-29 cells and is efficiently degraded by the ligase bispecifics as shown by Western blot.

Example 25: Trispecific antibodies for enhanced degradation of IGF1R and EGFR

25 Preliminary data showed that the combination of two traditional knob-in-hole bispecifics (RNF43/Cixu and ZNRF3/Cixu bispecifics) results in increased clearance. Because it would be advantageous, e.g., for ease of manufacture and administration to subjects, to create a single molecule having all the desired antigen binding domains, the 2+1 FabIgG format can be used. Figures 15D-15G shows exemplary trispecific antibody degraders using the 2+1 FabIgG format.

30 Trispecific antibodies are generated targeting RNF43 (RNF43.37.39), ZNRF3 (ZNRF3-6), and IGF1R (Cixutumumab) or EGFR (Cetuximab). Antibody format is a Fab-IgG/IgG with the receptor tyrosine kinase antibody placed at the internal position and ligase antibodies placed at the alternatively at the two external positions. This arrangement may optimize the proximity to

each anti-ligase antigen binding domain and further improve the efficiency of degradation. The Fab-IgG half-antibody incorporates LC-pairing mutations (Dillon *et al.* mAbs, 9:2, 213-230, 2017) to allow efficient pairing of the LC arm. The FabIgG and IgG half antibodies are assembled using methods well-known to those in the art. Assessment of these antibodies in the cell surface clearance assay described in Example 5 finds that they clear IGF1R more efficiently than either of the corresponding 1+1 bispecifics in cells with substantial expression of each.

Example 26: Monitoring IGF1R surface clearance after combinations of bispecifics.

The activity of multiple bispecific antibodies showed saturated at clearance of <100% for HER2, EGFR, and IGF1R. We hypothesized that simultaneously addressing one target with two different ligases might allow us to enhance clearance, due to different rate-limiting steps. To investigate this hypothesis HibiT tagged IGF1R HT29 cells were seeded overnight at 100,000 cells per well in a Corning® 96-well Flat Clear Bottom White Polystyrene TC-treated Microplates and incubated in ATCC recommended media. 1 µg/mL of a single bispecific antibody (targeting IGF1R and ZNRF3 or RNF43) or combination of two bispecific antibodies (the first targeting IGF1R and RNF43, and the second targeting IGF1R and ZNRF3) were added to the cells on the second day and incubated for 24 hours. The luminescence signal of IGF1R-HibiT was detected using the HibiT extracellular system following the description written in the previous example. Percent IGF1R was calculated using the equation $\%IGF1R = (1 - (\text{Untreated samples RLU} - \text{treated samples RLU}) / \text{Untreated samples RLU}) * 100$. Two different combinations of bispecifics, resulted in clearance of IGF1R that was more efficient than the saturating levels of either individual bispecific (Figure 16).

Example 27: Degradation of IGF1R across multiple CRC lines

A subset of RNF43 and ZNRF3 antibodies discovered as described above were reformatted into bispecific antibodies targeting the respective ligases and IGF1R (cixutumumab). Activity of these ligases was assessed by their ability to drive whole cell degradation of IGF1R in DLD1 cell and in a large panel of colon cancer cell lines (Fig. 18A, B). Of note, KM-12, RKO, GP2D and JHH7 cells do not display appreciable ligase expression and consequently serve as negative control (Fig. 18B).

Example 28: Assessment of target ubiquitylation upon bivalent/bispecific antibody treatment

To evaluate the impact of bispecific antibody treatment on target (IGF1R) ubiquitylation, parental HEK293T cells or HEK293T cells harboring a doxycycline inducible construct of

ZNRF3 wild type (WT) or the delta RING mutant (Δ RING) were subjected to a TUBE2 pulldown and samples were assessed by Western blot analysis. In brief, 10 million cells were plated in 10 cm dishes in the presence of doxycycline (1 μ g/ml or 31.25 ng/ml) to induce the expression of N-terminally gD and C-terminally FLAG tagged ZNRF3 WT or ZNRF3 Δ RING, respectively. Following 24 hours of doxycycline induction cells were subjected to a media exchange (+ doxycycline) and treated with gDxCixutumumab (IGF1R) bispecific antibody (0.5 μ g/ml). After two hours of bispecific antibody treatment at 37 °C cells were lysed in 500 μ l GST lysis buffer [25mM Tris•HCl, pH 7.2, 150mM NaCl, 5mM MgCl₂, 1% NP-40 and 5% glycerol, 1 % Halt protease & phosphatase inhibitor cocktail, 50 μ M PR-619, 5 mM 1,10 phenanthroline, 10 μ M MG132]. Cleared lysates were divided equally and incubated with either 20 μ l/sample prewashed Pierce HA epitope tag antibody agarose conjugate (2-2.2.14) (Thermo Scientific; REF 26182) for non-specific pulldown evaluation or 20 μ l/sample prewashed agarose-TUBE2 beads (Life Sensors; REF UM402) to enrich polyubiquitinated proteins. Lysate-bead mixtures were incubated for 2 hours and 30 minutes at 4 °C. For input samples, 30 μ l were used from the prepared pulldown samples prior to incubation. Beads were washed following incubation and prepared for Western blot analysis (Fig. 19B).

Furthermore, IGF1R ubiquitylation following IGF1 stimulation (R&D systems; REF 291-G1), bivalent antibody treatment (CixutumumabXCixutumumab), control bispecific antibody treatment (CixutumumabXNIST) or ligase-based bispecific antibody treatment (CixutumumabXRNF43-35; CixutumumabXZNRF3-55) was evaluated in HT29 cells. Briefly, 20 million cells were plated in 15 cm dishes. Cells were subjected to serum starvation 48 hours post plating. Following 22 hours serum starvation, cells were subjected to a media exchange (serum starved media) concomitant with bivalent or bispecific antibody treatment (1 μ g/ml). Cells were incubated for 2 hours and 15 minutes at 37 °C. For IGF1 stimulation, cells were treated with 50 ng/ml IGF1 5 minutes prior to cell lysis. Following incubation, cells were scraped in 800 μ l PBS and cell pellets were resuspended in 1.2 ml GST lysis buffer [25mM Tris•HCl, pH 7.2, 150mM NaCl, 5mM MgCl₂, 1% NP-40 and 5% glycerol, 1 % Halt protease & phosphatase inhibitor cocktail, 10 mM NEM, 1 mM PMSF]. Cleared lysates were divided equally and incubated with 25 μ l/sample prewashed Dynabeads protein G (ThermoFisher Scientific; REF 10004D) conjugated with either rabbit (DA1E) mAb IgG XP isotype control (cell signaling; REF 3900) for non-specific bait protein precipitation or IGF-1 receptor β (D23H3) XP rabbit mAb (cell signaling; REF 9750) for IGF1R β immunoprecipitation at 1.87 μ g/sample. Lysate-bead mixtures were incubated for 24 hours at 4 °C. For input samples, 60 μ l were used from the

prepared pulldown samples prior to incubation. Beads were washed following incubation and prepared for Western blot analysis (Fig. 19A).

Example 29: Validation of ligase activity requirements across cell lines and targets

To substantiate the claim described above for HER2 in Example 10, we probed whether the ligase activity ligase was also required for degradation of additional targets. As demonstrated in Fig. 20, deletion of the RING domain from ZNRF3 prevented IGF1R degradation after ligase dimerization after treatment with various ZNRF3/IGF1R bispecific antibodies. Furthermore, we took advantage of naturally occurring RNF43 mutations in a small subset of colon cancers. We selected SW48 as it has a frameshift deletion within the RING domain of RNF43 (Fig. 21A) yet it maintains cell surface ligase expression (Fig 21B). Dimerization of RNF43/IGF1R bispecific antibodies in that cell line had no impact on IGF1R level, demonstrating that RING and the c-terminus of RNF43 is required for ligase activity. Of note, ZNRF3 was still competent for IGF1R degradation when a ZNRF3/IGF1R antibody was used. To further substantiate this claim, we generated endogenous ligase knock out for both RNF43 or ZNRF3 in HT29 cells (Fig. 22 A). Expectedly, RNF43/IGF1R treatment of RNF43 KO had no impact on IGF1R level while ZNRF3/IGF1R treatment could still degrade the receptor. Importantly, RING knock out did not impact either ligase cell surface expression (Fig. 22A FACS panel), but degradation was also rescued, demonstrating that RING/C-terminus presence is required for full degradative potential of either ligase (Fig. 22 B).

Interestingly, inhibition of the E1 Ubiquitin activating enzyme also rescued degradation, which we found was dependent on both lysosomal and proteasomal activity (Fig. 23) for IGF1R. This is different than what was observed for HER2 where only lysosomal activity was required for degradation of that receptor. Hence, this data suggests that the route of degradation is dictated by the target rather than the ligase itself.

Example 30: Biological consequence of degradation on cell signaling and growth

The biological impact of degradation over receptor binding was assessed by looking at modulation of IGF1R signaling. SW48 treated with ZNRF3/IGF1R bispecific antibodies display deep IGF1R degradation. Importantly, this degradation also impacted further downstream signaling as revealed by a complete loss of AKT activity (phosphorylation of AKT) and a decrease phosphorylation of S6, two key downstream effectors of IGF1R (Fig. 25 A). Importantly, the decrease in AKT and S6 activity was superior in ligase treated cells compared to the IGF1R bivalent, demonstrating that degradation provide deeper pathway inhibition than classical blocking antibodies. This decrease in IGF1R signaling also translated functionally as

evidenced by a decrease in cell growth in SW48 cells treated with ZNRF3/IGF1R (Fig. 25 B, C). In line with the above, the ligase domain was required to impact cell growth since ZNRF3 RING deficient HT29 failed to impact cell growth while WT ZNRF3 dimerized to IGF1R resulted in slower growth in these cells (Fig. 26A-D).

5 **Example 31: Endogenous degradation of additional substrate**

Bispecific antibodies are generated targeting the PD-L1 receptor and exemplary ligase antibodies described above. As depicted in Fig. 27, treatment of SW48 cells with ZNRF3/PDL1 revealed profound and near complete degradation of PD-L1 demonstrating the breadth of degradation across multiple substrates.

10 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Example 32. *In vivo* Assessment

15 An attractive advantage of the PROTAB technology is that it may overcome the challenges shared by small molecule intracellular degraders, such as limited bioavailability and cell permeability, which can limit their *in vivo* activity.

Approximately 10^6 SW48 cells were resuspended in PBS basal media, admixed with 50% Matrigel (Corning) to a final volume of 200 μ l, and injected subcutaneously in the left flank of
20 NSG mice (NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ (NSG) (colony 005557). Mice were purchased from the Jackson Laboratory). Females of 6 to 12 weeks old were used for experiments. Tumor dimensions were measured using calipers and tumor volume was calculated as $0.523 \times \text{length} \times \text{width} \times \text{width}$. Animals were humanely euthanized according to the following criteria: clinical signs of persistent distress or pain, significant body-weight loss ($>20\%$), tumor
25 size exceeding $2,500 \text{ mm}^3$, or when tumors ulcerated. Maximum tumor size permitted by the Institutional Animal Care and Use Committee (IACUC) is $3,000 \text{ mm}^3$ and in none of the experiments was this limit exceeded. When tumors reached $\sim 400 \text{ mm}^3$, animals were randomized to receive a single intraperitoneal injection of the PROTABs. All mice were euthanized 72 hr after injection and tumors were collected for further processing.

30 The lumen implantation procedure has previously been described (de Sousa E Melo F *et al.* Modeling Colorectal Cancer Progression Through Orthotopic Implantation of Organoids. Methods Mol Biol 2171, 331-346 (2020)). Briefly, mice were anesthetized by isoflurane inhalation and injected intraperitoneally (i.p.) with buprenorphine at 0.05 to 0.1 mg/kg. A blunt-

ended hemostat (Micro-Mosquito, No. 13010-12, Fine Science Tools) was inserted ~1 cm into the anus. The hemostat was angled toward the mucosa and opened slightly such that a single mucosal fold could be clasped by closing the hemostat to the first notch. The hemostat was retracted from the anus, exposing the clasped exteriorized mucosa. A 10 µL of solution
5 containing 50,000 cells admixed with 50% matrigel (Corning) in PBS was directly injected into the colonic mucosae. After reversing the prolapse, the hemostat was then released.

In vivo activity was assessed in SW48 tumor bearing mice (Fig.30). The upper portion of Figure 30 shows a schematic representation of the SW48 xenograft model used to analyze the effect of a Cixutuzumab (anti-IGF1R)-based bivalent antibody, NIST* IGF1R (Cixu) bispecific
10 antibody or the indicated ZNRF3*IGF1R (Cixu) bispecific PROTAB *in vivo*. Following three weeks of SW48 implantation, animals were subjected to antibody treatment. Tumors were collected, homogenized and analyzed biochemically 72 hours post antibody treatment.

The lower portion of Figure 30 shows Western blot analysis of SW48 *in vivo* tumor lysates derived from mice left untreated (-) or subjected to a Cixu bivalent antibody,
15 NIST*IGF1R (Cixu) or a ZNRF3-55*IGF1R (Cixu) bispecific PROTAB for 72 hours. Data are representative of four animals per treatment group. Endogenous IGF1Rβ levels were detected. GAPDH was used as a loading control.

The results demonstrate that a single dose of a ZNRF3-based IGF1R PROTABS drove substantial degradation of IGF1R. Intriguingly, IGF1R degradation was partially suppressed at
20 the highest dose (Fig.30), suggestive of a hook effect that has been reported with some PROTACs (Kannt A *et al.* Expanding the arsenal of E3 ubiquitin ligases for proximity-induced protein degradation. Cell Chem Biol 28, 1014-1031. (2021); Khan, S *et al.* PROteolysis Targeting Chimeras (PROTACs) as emerging anticancer therapeutics. Oncogene 39, 4909–4924 (2020)).

IV. SEQUENCES

The table below provides exemplary sequences referred to in this disclosure.

SEQ ID NO	DESCRIPTION	SEQUENCE
1	gD.gD.mIgG2a.LALAPG.AV-Hole (heavy chain (HC) DNA including signal peptide (SP))	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCAGAGGTTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCT CACTCCGTTTGTCTGTGCCGCTTCTGGCTACTCCATCACCTCCGACTTTGCC TGGAACTGGGTCCGTCAAGCCCCGGGTAAAGGGCCTGGAATGGGTGGATACAT TAGTTACTCTGGAACCACTAGCTATAACCTAGCCTGAAGTCCCGTATCACTA TAAGTCGCGACAATTCCAAAACACATTTACCTGCAGATGAACAGCCTGCGT GCTGAGGACACTGCCGTCTATTATTGTGCTCGAGAAAACACTATGGCCGTTTC TCACGTTGGGTACTTTCGACGTCTGGGGTCAAGGAACCCCTGGTCACCGTCTCGA GTGCCAAAACAACAGCCCCATCGGTCTATCCACTGGCTCCTGTGTGTGGAGAT ACAACCTGGCTCCTCGGTGACTCTAGGATGCCTGGTCAAGGGTTATTTCCCTGA GCCAGTGACCTTGACCTGGAACCTCTGGATCCCTGTCCAGTGGTGTGCACACCT TCCCAGCTGTCCTGCAGTCTGACCTCTACACCCTCAGCAGCTCAGTGACTGTA ACGTCGAGCACCTGGCCCAGCCAGTCCATCACCTGCAATGTGGCCACCCGGC AAGCAGCACCAAGGTGGACAAGAAAATTGAGCCCAGAGGACCCACAATCAAGC CCTGTCTCCATGCAAATGCCAGCACCTAACGCCGCGGGTGGACCATCCGTC TTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATCTCCCTGAGCCCCAT AGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCA GCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGA GAGGATTACAACAGTACTCTACGCGTGGTCAAGTCCCTCCCATCCAGCACCA GGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCG GAGCGCCCATCGAGAGAACCATCTCAAACCCAAAGGGTCAAGAGCTCCA CAGGTATATGTCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTAC TCTGACCTGCGCTGTACAGACTTCATGCCTGAAGACATTTACGTGGAGTGG CCAACAACGGGAAAAACAGAGCTAAACTACAAGAACACTGAACAGTCTGGAC TCTGATGGTTCTTACTTCATGGTTAGCAAGCTGAGAGTGGAAAAGAAGAACTG GGTGGAAAGAAATAGCTACTCTCTGTTCAAGTGGTCCACGAGGGTCTGCACAATC ACCACACGACTAAGAGCTTCTCCCGGACTCCGGGTAAATGA
2	gD.gD.mIgG2a.LALAPG.AV-Hole (light chain (LC) DNA including SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGGAAGTACA TTCAGATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCTCTGTGGGCG ATAGGGTCACCATCACCTGCCGTGCCAGTGGTCTGTGACAGCTATGGTAAC AGCTTCATCCACTGGTATCAGCAGAAACCAGGAAAAGCTCCGAACTACTGAT TTACCGGGCCTCGGACCTGGAGTCTGGAGTCCCTTCTCGCTTCTCTGGATCCG GTTCTGGGACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCAGAAGACTTC GCAACTTATTACTGTGAGCAAAATTACGCGGATCCGTTTACATTTGGACAGGG TACCAAGGTGGAGATCAAGCGCGCTGATGCTGCACCAACTGTATCCATCTTCC CACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCTGTGCTTCTTG AACAACTTCTACCCCAAGACATCAATGTCAAGTGGAAAGATGATGGCAGTGA ACGACAAAATGGCGTCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCA CCTACAGCATGAGCAGCACCTCACGTTGACCAAGGACGAGTATGAACGACAT AACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAA GAGCTTCAACAGGAATGAGTGTGA
3	gD.gD.mIgG2a.LALAPG.AV-Hole heavy chain (mature sequence)	EVQLVESGGG LVQPGGSLRL SCAASGYSIT SDFAWNVRQ APGKGLEWVG YISYSGTTSY NPSLKSRTI SRDNSKNTFY LQMNSLRAED TAVYYCAREN YYGRSHVGYF DVWQGTLVT VSSAKTTAPS VYPLAPVCGD TTGSSVTLGC LVKGYFPEPV TLTWNSGSL SGVHTFPAVL QSDLYTLSS VTVTSSTWPS QSITCNVAHP ASSTKVDKKI EPRGPTIKPC PPCKCPAPNA AGGPSVFIFP PKIKDVLMI LSPIVTCVVV DVSEDDPDVQ ISWVFNNEV HTAQQTQTHRE DYNSTLRVVS ALPIQHQDWM SGKEFKCKVN NKDLGAPIER TISKPKGSVR APQVYVLP EEEMTKKQVT LTCAVTDFMP EDIYVEWTNN GKTELNYKNT EPVLDSDGSY FMVSKLRVEK KNWVERNSYS CSVVHEGLHN HHTTKSFSRT PGK
4	gD.gD.mIgG2a.LALAPG.AV-Hole light	DIQMTQSPSS LSASVGDRTV ITCRASASVD SYGNSFIHWY QQKPGKAPKL LIYRASDLES GVPSTRFSGSG SGTDFTLTIS SLQPEDFATY YCQQNYADPF TFGQGTKVEI KRADAAPTVS

SEQ ID NO	DESCRIPTION	SEQUENCE
	chain (mature sequence)	IFPPSSEQLT SGGASVVCFL NNFYPKDINV KWKIDGSEKQ NGVLNSWTDQ DSKDSTYSMS STLTLTKDEY ERHNSYTCEA THKTSTSPIV KSFNRNEC
5	xRNF43.hSC37.39.mIgG2a.LAL APG.AV-Hole.AX (HC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCACAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAGAAGCCCGGCGCCT CCGTGAAGGTGTCTTGCAGGCTCCGGCTACACCTTACCACCTACACCATC CACTGGGTGAGGCAGGCCCCCGGCCAGGGCTGGAGTGGATGGGCTACATCAA CCCCAGGTCCGGCTACACCGAGTACAACCAGAAGTTCCAGGACAGGGTGACCA TGACCAGGGACACCTCCACCTCCACCGTGTACATGGAGCTGTCTCCCTGAGG TCCGAGGACACCGCCGTGTACTACTGCGCCAGGTCTACGAGTTCTGGGGCCA GGGCACCAACCGTGACCGTCTCGAGTGCCAAAACAACAGCCCCATCGGTCTATC CACTGGCTCCTGTGTGTGGAGATACAACCTGGCTCCTCGGTGACTCTAGGATGC CTGGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACCTGGATC CCTGTCCAGTGGTGTGCACACCTTCCAGCTGTCTTGCAGTCTGACCTCTACA CCCTCAGCAGCTCAGTGACTGTAACGTCGAGCACCTGGCCCAGCCAGTCCATC ACCTGCAATGTGGCCCCACCGGCAAGCAGCACCAAGGTGGACAAGAAAATTGA GCCCAGAGGACCCACAATCAAGCCCTGTCTCCATGCAATGCCAGCACCTA ACGCCGCGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTA CTCATGATCTCCCTGAGCCCATAGTACATGTGTGGTGGATGTGAGCGA GGATGACCCAGATGTCCAGATCAGCTGGTTGTGAACAACGTGGAAGTACACA CAGCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTCTACGCGTGGTC AGTGCCCTCCCATCCAGCACAGGACTGGATGAGTGGCAAGGAGTTCAAATG CAAGGTCAACAACAAGACCTCGGAGCGCCATCGAGAGAACCATCTCAAAC CCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCCTCCACCAGAGAA GAGATGACTAAGAAACAGGTCACTCTGACCTGCGCTGTACAGACTTCATGCC TGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAACTACA AGAACACTGAACCACTCTGGACTCTGATGGTTCTTACTTCTATGGTTAGCAAG CTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGTACTCTCTGTTCAGT GGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTC CGGGTAAATGA
6	xRNF43.hSC37.39.mIgG2a.LAL APG.AV-Hole.AX (LC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCAGAGATCGTGATGACCCAGTCCCCCGCCACCCTGTCCGTGTCCCCGGCG AGAGGGCCACCCTGTCTTGCAGGCTCCAGAACGTGGGCATCAACGTGGCC TGGTATCAGCAGAAGCCCCGGCCAGGCCCCAGGGCCCTGATCTACTCCGCCTC CTACAGGTACTCCGGCATCCCCCGCCAGGTTCTCCGGCTCCGGCTCCGGCACCG AGTTCACCTGACCATCTCTCTCCCTGCAGTCCGAGGACTTCGCCGTGTACTAC TGCCACCACTACAAGACCTACCCCTACACCTTCCGGCGCGGTACCAAGCTGGA GATCAAGCGCGCTGATGCTGCACCAACTGTATCCATCTTCCACCATTCCAGTG AGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACCTCTAC CCCAAGACATCAATGTCAAGTGGAGATTGATGGCAGTGAACGACAAAATGG CGTCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGA GCAGCACCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACC TGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAG GAATGAGTGTG
7	xRNF43.hSC37.39.mIgG2a.LAL APG.AV-Hole.AX mature HC	EVQLVQSGAE VKKPGASVKV SCKASGYFT TYTIHWVRQA PGQGLEWMGY INPRSGYTEY NQKFQDRVIM TRDTSTSTVY MELSSLRSED TAVYYCARSY EFWGQGTIVT VSSAKTTAPS VYPLAPVCGD TTGSSVTLGC LVKGYFPEPV TLTWNSGSL SGVHTFPAVL QSDLYTLSSS VVTSSSTWPS QSITCNVAHP ASSTKVDKKI EPRGPTIKPC PCKCPAPNA AGGPSVFIFP PKIKDVLMS LSPIVTCVVV DVSEDDPDVQ ISWVFNVEV HTAQQTQTHRE DYNSTLRVVS ALPIQHQQDWM SGKEFKCKVN NKDLGAPIER TISKPKGSVR APQVYVLPPEEEMTKKQVT LTCAVTDFMP EDIYVEWTNN GKTELNYKNT EPVLDSDGSY FMVSKLRVEK KNWVERNSYS CSVVHEGLHN HHTTKSFSRT PGK
8	xRNF43.hSC37.39.mIgG2a.LAL APG.AV-Hole.AX mature LC	EIVMTQSPAT LSVSPGERAT LSKASQNVG INVAWYQQKRP GQAPRALIYS ASYRYSGIPA RFGSGSGSGTE FTLTISSLQS EDFAVYYCHQ YKTPYTFGG GTKLEIKRAD AAPTVISIFPP SSEQLTSGGA SVVCFLNNFY PKDINVKWKI DGSEKQNGVL

SEQ ID NO	DESCRIPTION	SEQUENCE
		NSWTDQDSKD STYSMSSTLT LTKDEYERHN SYTCEATHKT STSPIVKSFN RNEC
9	NIST.mIgG2a.L ALAPG.AV-Hole (HC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCACAGGTGACCTGAGGGAGTCCGGCCCCGCCCTGGTGAAGCCACCCAGA CCCTGACCTGACCTGCACCTTCTCCGGCTTCTCCCTGTCCACCGCCGGCATG TCCGTGGGCTGGATCAGGCAGCCCCCGCAAGGCCCTGGAGTGGCTGGCCGA CATCTGGTGGGACGACAAGAAGCACTACAACCCCTCCCTGAAGGACAGGCTGA CCATCTCCAAGGACACCTCCAAGAACCAGGTGGTGTGAAGGTGACCAACATG GACCCCGCCGACACCGCCACCTACTACTGCGCCAGGGACATGATCTTCAACTT CTACTTCGACGTGTGGGGCCAGGGCACCACCGTGACCGTCTCGAGTGCCAAAA CAACAGCCCCATCGGTCTATCCACTGGCTCCTGTGTGTGGAGATACAACCTGGC TCCTCGGTGACTCTAGGATGCCTGGTCAAGGGTTATTTCCCTGAGCCAGTGAC CTTGACCTGGAACCTCTGGATCCCTGTCCAGTGGTGTGCACACCTTCCCAGCTG TCCTGCAGTCTGACCTCT ACACCCCTCAGCAGCTCAGTGACTGTAACGTGAGCACCTGGCCCAGCCAGTCC ATCACCTGCAATGTGGCCACCCGGCAAGCAGCACCAAGGTGGACAAGAAAAAT TGAGCCCAGAGGACCCACAATCAAGCCCTGTCTCCATGCAAATGCCAGCAC CTAACGCCCGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGAT GTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAG CGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAGTAC ACACAGCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTCTACGCGTG GTCAGTGGCCCTCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAA ATGCAAGGTCAACAACAAAGACCTCGGAGCGCCCATCGAGAGAACCATCTCAA AACCCAAAGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCTCCACAGAA GAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCGCTGTACAGACTTCAT GCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACT ACAAGAACACTGAACAGTCTTGACTCTGATGGTTCTTACTTCATGGTTAGC AAGCTGAGAGTGGAAAAGAAGAAGTGGGTGGAAAGAAATAGCTACTCTCTGTTT AGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGA CTCCGGGTAAATGA
10	NIST.mIgG2a.L ALAPG.AV-Hole (LC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCAGACATCCAGATGACCCAGTCCCCCTCCACCCTGTCCGCTCCGTGGGCG ACAGGGTGACCATCACCTGCTCCGCTCCTCCAGGGTGGGCTACATGCACTGG TATCAGCAGAAGCCCCGCAAGGCCCCCCAAGCTGCTGATCTACGACACCTCCAA GCTGGCCTCCGGCGTGCCCTCCAGGTTCTCCGGCTCCGGCTCCGGCACCAGT TCACCCTGACCATCTCCTCCCTGCAGCCCGACGACTTCGCCACCTACTACTGC TTCCAGGGCTCCGGCTACCCCTTACCTTCGGCGGCGGTACCAAGTGGAGAT CAAGCGCGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGC AGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACCTTACCCCC AAAGACATCAATGTCAAGTGAAGATTGATGGCAGTGAACGACAAAATGGCGT CCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCA GCACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGT GAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAA TGAGTGTGGA
11	NIST.mIgG2a.L ALAPG.AV-Hole (HC mature)	QVTLRESGPA LVKPTQTLTL TCTFSGFSLs TAGMSVGWIR QPPGKALEWL ADIWWDKKH YNPSLKDRLT ISKDTSKNQV VLKVTNMDPA DTATYYCARD MIFNFYFDVW GQGTTVTVSS AKTTAPSVYP LAPVCGDITG SSVTLGCLVK GYFPEPVTLT WNSGSLSSGV HTFPAVLQSD LYTLSSSVTV TSSTWPSQSI TCNVAHPASS TKVDKKIEPR GPTIKPCPPC KCPAPNAAGG PSVFI FPPKI KDVLMISLSP IVTCVVVDVS EDDPDVQISW FVNNVEVHTA QTQTHREDYN STLRVVSALP IQHQDWMGSK EFKCKVNNKD LGAPIERTIS KPKGSVRAPQ VYVLPPEEEE MTKKQVTLTC AVTDFMPEDI YVEWTNNGKT ELNYKNTEPV LSDSGSYFMV SKLRVEKKNW VERNYSYCSV VHEGLHNHHT TKFSRTPG
12	NIST.mIgG2a.L ALAPG.AV-Hole (LC mature)	DIQMTQSPST LSASVGDRTV ITCSASSRVG YMHYQQKPG KAPKLLIYDT SKLASGVPSR FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG TKVEIKRADA APTVSIFPPS SEQLTSGGAS VVCFLNNFYP KDINVKWKID GSERQNGVLN

SEQ ID NO	DESCRIPTION	SEQUENCE
		SWTDQDSKDS TYSMSSTLTL TKDEYERHNS YTCEATHKTS TSPIVKSFNR NEC
13	HER2.4D5VH-mIgG2aCH1-4D5VH.mIgG2a.LALAPG.Knob (HC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCAGAAGTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCT CACTCCGTTTGTCTGTGCAGCTTCTGGCTTCAACATTAAAGACACCTATATA CACTGGGTGCGTCAGGCCCCGGGTAAGGGCCTGGAATGGGTTGCAAGGATTTA TCCTACGAATGGTTATACTAGATATGCCGATAGCGTCAAGGGCCGTTTCACTA TAAGCGCAGACACATCCAAAAACACAGCCTACCTGCAGATGAACAGCCTGCGT GCTGAGGACACTGCCGTCTATTATTGTTCTAGATGGGGAGGGGACGGCTTCTA TGCTATGGACTACTGGGGTCAAGGAACCTGGTCACCGTCTCGAGTGCCAAGA CCACCGCCCCCAGCGTGTACCCCTGGCCCCCGTGTGCGGGACACCAACCGGC AGCAGCGTGACCCTGGGCTGCCTGGTGAAGGGCTACTTCCCCGAGCCCCGTGAC CCTGACCTGGAACAGCGGCAGCCTGAGCAGCGGCGTGCACACCTTCCCCGCCG TGCTGCAGAGCGACCTGTACACCTGAGCAGCAGCGTGACCCTGACCAGCAGC ACCTGGCCCCAGCCAGAGCATCACCTGCAACGTGGCCCCACCCGCCAGCAGCAC CAAGGTGGACAAGAAGATCGAGCCCAGAGCCCCACCATCAAGCCCCAGGTGC AGCTGGTGGAGAGCGGCGGGCGGCTGGTGCAGCCCCGGCGGCAGCCTGAGACTG AGCTGCGCCGCCAGCGGCTTCAACATCAAGGACACCTACATCCACTGGGTGAG ACAGGCCCCCGCAAGGGCCTGGAGTGGGTGGCCAGAATCTACCCACCAACG GCTACACCAGATACGCCGACAGCGTGAAGGGCAGATTACCATCAGCGCCGAC ACCAGCAAGAACACCGCCTACCTGCAGATGAACAGCCTGAGAGCCGAGGACAC CGCCGTGTACTACTGCAGCAGATGGGGCGGCGACGGCTTCTACGCCATGGACT ACTGGGGCCAGGGCACCTGGTGACCGTCTCGAGTGCCAAAACAACAGCCCCA TCGGTCTATCCACTGGCTCCTGTGTGTGGAGATACAACCTGGCTCCTCGGTGAC TCTAGGATGCCTGGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGA ACTCTGGATCCCTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCTGCAGTCT GACCTCTACACCTCAGCAGCTCAGTGACTGTAACGTGAGCAGCTGGCCAG CCAGTCCATCACCTGCAATGTGGCCACCCGGCAAGCAGCACCAGGTGGACA AGAAAATTGAGCCCAGAGGACCCACAATCAAGCCCTGTCTCCATGCAATGC CCAGCACCTAACGCCGCGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGAT CAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGG ATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTG GAAGTACACACAGCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTCT ACGCGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATGAGTGGCAAGG AGTTCAAATGCAAGGTCAACAACAAGACCTCGGAGCGCCCATCGAGAGAACC ATCTCAAAACCCAAAGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCCTCC ACCAGAAGAAGAGATGACTAAGAAACAGGTCACTCTGTGGTGATGGTGCAG ACTTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAG CTAAACTACAAGAACACTGAACCAGTCTTGACTCTGATGGTTCTTACTTCAT GTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGCTACT CCTGTTCAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTC TCCCGGACTCCGGGTAAATGA
14	HER2.4D5VH-mIgG2aCH1-4D5VH.mIgG2a.LALAPG.Knob (LC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCAGATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCG ATAGGGTCACCATACCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCC TGGTATCAACAGAAACAGGAAAAGCTCCGAAACTACTGATTCTCTCGGCATC CTTCCTCTACTCTGGAGTCCCTTCTCGCTTCTCTGGATCCAGATCTGGGACGG ATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTAC TGTCAGCAACATTATACTACTCCTCCACGTTCCGACAGGGTACCAAGCTGGA GATCAAGCGCGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTG AGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACCTCTAC CCCAAAGACATCAATGTCAAGTGAAGATTGATGGCAGTGAACGACAAAATGG CGTCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGA GCAGCACCTCAGCTTGACCAAGGACGAGTATGAACGACATAACAGCTATACC TGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAG GAATGAGTGTGA
15	HER2.4D5VH-mIgG2aCH1-4D5VH.mIgG2a.LALAPG.Knob (LC DNA with SP)	EVQLVESGGG LVQPGGSLRL SCAASGFNIK DTYIHWVRQA PGKLEWVAR IYPTNGYTRY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCSRWG GDGFYAMDYW GQGLTVTVSS AKTTAPSVYP LAPVCGDITG SSVTLGCLVK GYFPEPVTLT

SEQ ID NO	DESCRIPTION	SEQUENCE
	LALAPG.Knob (HC mature)	WNSGSLSSGV HTFPAVLQSD LYTLSSSVTV TSSTWPSQSI TCNVAHPASS TKVDKKIEPR GPTIKPEVQL VESGGGLVQP GGSLRLSCAA SGFNIKDTYI HWVRQAPGKG LEWVARIYPT NGYTRYADSV KGRFTISADT SKNTAYLQMN SLRAEDTAVY YCSRWGGDGF YAMDYWGQGT LVTVSSAKTT APSVYPLAPV CGDTTGSSVT LGCLVKGYFP EPVTLTWNSG SLSSGVHTFP AVLQSDLYTL SSSVTVTSSST WPSQSITCNV AHPASSTKVD KKIEPRGPTI KPCPPCKCPA PNAAGGPSVF IFPPKIKDVL MISLSPIVTC VVVDVSEDDP DVQISWFVNN VEVHTAQQT HREDYNSTLR VVSALPIQHQ DWMSGKEFKC KVNNDLGLAP IERTISKPKG SVRAPQVYVL PPPEEEMTKK QVTLLWCMVD FMPEDIYVEW TNNGKTELNY KNTEPVLDSG GSYFMYSKLR VEKKNWVERN SYSCSVVHEG LHNHHTTKSF SRTPGK
16	HER2.4D5VH-mIgG2aCH1-4D5VH.mIgG2a.LALAPG.Knob (LC mature)	DIQMTQSPSS LSASVGDRTV ITCRASQDVN TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFGSGRSGTD FTLTISSLQP EDFATYYCQQ HYTTPPTFGQ GTKLEIKRAD AAPTVISIFPP SSEQLTSGGA SVVCFLNNFY PKDINVKWKI DGSEKQNGVL NSWTDQDSK STYSMSSTLT LTKDEYERHN SYTCEATHKT STSPIVKSFN RNEC
17	HER2.2C4VH-mIgG2aCH1-2C4VH.mIgG2a.LALAPG.Knob (HC DNA with SP)	ATGGGATGGTCATGTATCATCTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCAGAGGTGCAGCTGGTGGAGAGCGGCGGCGGCTGGTGCAGCCAGGCGGCA GCCTGCGCCTGAGCTGCGCCGCCAGCGGCTTCACCTTCACCGACTACACCATG GACTGGGTGCGCCAGGCCCCAGGCAAGGGCTGGAGTGGGTGGCCGACGTGAA CCCAAACAGCGCGGCAGCATCTACAACAGCGCTTCAAGGGCCGCTTCACCC TGAGCGTGGACCGCAGCAAGAACACCCCTGTACCTGCAGATGAACAGCCTGCGC GCCGAGGACACCGCCGTGTACTACTGCGCCCGCAACCTGGGCCCCAAGCTTCTA CTTCGACTACTGGGGCCAGGGCACCCCTGGTGACCGTCTCGAGTGCCCAAGACCA CCGCCCCAGCGTGTACCCCTGGCCCGCGTGTGCGCGCAGACACACCGGCAGC AGCGTGACCCCTGGGCTGCCTGGTGAAGGGCTACTTCCCCGAGCCCGTGACCC GACCTGGAACAGCGGCAGCCTGAGCAGCGCGGTGCACACCTTCCCCGCCGTGC TGCAGAGCGACCTGTACACCCCTGAGCAGCAGCGTGACCGTGACCAGCAGCACC TGGCCCGAGCAGAGCATCACCTGCAACGTGGCCCAACCCCGCCAGCAGCACC GGTGGACAAGAAGATCGAGCCCGAGAGGCCCAACCATCAAGCCCGAGGTGCAGC TGGTGGAGAGCGGCGGCGGCGCTGGTGCAGCCCGGCGGCAGCCTGAGACTGAGC TGCGCCCGCAGCGGCTTCACCTTACCGACTACACCATGGACTGGGTGAGACA GGCCCCGGCAAGGGCCTGGAGTGGGTGGCCGACGTGAACCCCAACAGCGGCG GCAGCATCTACAACAGAGATTCAAGGGCAGATTACCCCTGAGCGTGGACAG AGCAAGAACACCCCTGTACCTGCAGATGAACAGCCTGAGAGCGAGGACACCGC CGTGTACTACTGCGCCAGAAACCTGGGCCCCAGCTTCTACTTCGACTACTGGG GCCAGGGCACCCCTGGTGACCGTCTCGAGTGCCAAAACAACAGCCCCATCGGTC TATCCACTGGCTCCTGTGTGTGGAGATACAACCTGGCTCCTCGGTGACTCTAGG ATGCCCTGGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACCTCTG GATCCCTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCTCTGCAGTCTGACCTC TACACCCTCAGCAGCTCAGTGACTGTAACGTGAGCAGCCTGGCCCGAGCAGTC CATCACCTGCAATGTGGCCCAACCGGCAAGCAGCACAAGGTGGACAAGAAAA TTGAGCCCGAGGACCCACAATCAAGCCCTGTCTCCATGCAATGCCAGCA CCTAACGCGCGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGA TGTAATCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGA GCGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTA CACACAGCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTCTACGCGT GGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCA AATGCAAGGTCAACAACAAAGACCTCGGAGCGCCCATCGAGAGAACCATCTCA AAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGA AGAAGAGATGACTAAGAAACAGGTCACTCTGTGGTGCATGGTACAGACTTCA TGCTTGAAGACATTTACGTGGAGTGGACCAACAACGGAAGGAGAGCTAAAC TACAAGAACACTGAACAGTCTGGACTCTGATGGTTCTTACTTCATGTACAG CAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAAGAAATAGCTACTCCTGTT CAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGG ACTCCGGGTAATGA

SEQ ID NO	DESCRIPTION	SEQUENCE
18	HER2.2C4VH-mIgG2aCH1-2C4VH.mIgG2a.LALAPG.Knob (LC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACATTTCAGACATCCAGATGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTGGGCGACCGCGTGACCATCACCTGCAAGGCCAGCCAGGACGTGAGCATCGGCGTGGCC TGGTATCAGCAGAAGCCAGGCAAGGCCCCAAAGCTGCTGATCTACAGCGCCAGCTACCGCTACACCGGCGTGCCAAGCCGCTTCAGCGGCAGCGGCAGCGGCACCG ACTTCACCGTGACCATCAGCAGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCCAGCAGTACTACATCTACCCATACACCTTCGGCCAGGGTACCAAGCTGGA GATCAAGCGCGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTG AGCAGTTAACATCTGGAGGTGCCCTCAGTCGTGTGCTTCTTGAACAACTTCTAC CCCAAAGACATCAATGTCAAGTGGGAAGATTGATGGCAGTGAACGACAAAATGG CGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGA GCAGCACCGCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACC TGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAG GAATGAGTGTGA
19	HER2.2C4VH-mIgG2aCH1-2C4VH.mIgG2a.LALAPG.Knob (HC mature)	EVQLVESGGG LVQPGGSLRL SCAASGFTFT DYTMDWVRQA PGKGLEWVAD VNPNSGGSIY NQRFKGRFTL SVDRSKNTLY LQMNSLRAED TAVYYCARNL GPSFYFDYWG QGTLVTVSSA KTTAPSVYPL APVCGDTTGS SVTLGCLVKG YFPEPVTLTW NSGSLSSGVH TFPAVLQSDL YTLSSSVTVT SSTWPSQSIT CNVAHPASST KVDKKIEPRG PTIKPEVQLV ESGGGLVQPG GSLRLSCAAS GFTFTDYTMD WVRQAPGKGL EWVADVNPNS GGSIYNQRFK GRFTLSVDRS KNTLYLQMNS LRAEDTAVYY CARNLGPSFY FDYWGQGLV TVSSAKTTAP SVYPLAPVCG DTTGSSVTLG CLVKGYFPEP VTLTWNSGSL SSGVHTFPAV LQSDLYTLSS SVTVTSSTWP QSITCNVAH PASSTKVDKK IEPRGPTIKP CPPCKCPAPN AAGGPSVFIF PPKIKDVLMI SLSPIVTCVV VDVSEDDPDV QISWFVNVE VHQAQTQTHR EDYNSTLRVV SALPIQHQDW MSGKEFKCKV NNKDLGAPIE RTISKPKGSV RAPQVYVLPP PEEEMTKKQV TLWCMVDFM PEDIYVEWTN NGKTELNYKN TEPVLDSGDS YFMYSKLRVE KKNWVERNSY SCSVVHEGLH NHHTTKSFSR TPGK
20	HER2.2C4VH-mIgG2aCH1-2C4VH.mIgG2a.LALAPG.Knob (LC mature)	DIQMTQSPSS LSASVGRVT ITCKASQDVS IGVAWYQQKP GKAPKLLIYS ASYRYTGVPs RFGSGSGTD FTLTISSLQP EDFATYYCQQ YYIYPYTFGQ GTKLEIKRAD AAPTVISIFPP SSEQLTSGGA SVVCFLNNFY PKDINVWKI DGSERQNGVL NSWTDQSKD STYSMSSTLT LTKDEYERHN SYTCEATHKT STSPIVKSFN RNEC
21	HER2.7C2VH-mIgG2aCH1-7C2VH.mIgG2a.LALAPG.Knob (HC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACATTTCACAGGTCCAAGTGCAGCAGCCTGGGGCTGAACTGGTGAGGCCTGGAGCTT CAGTGAAGCTGTCTGCAAGGCTTCTGGCTACTCCTTCACCGGCTACTGGATG AACTGGTTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGCATGATTCA TCCTTCCGATAGTGAAATTAGGGCAAATCAGAAATTACAGGACAAGGCCACAT TGACTGTAGACAAGTCCTCCACCACAGCCTACATGCAACTCAGCAGCCGACAT CTGAGGACTCTGCGGTCTATTACTGTGCACGAGGGACTTACGACGGAGGCTT TGAATACTGGGGCCAAGGCACCACTCTCACAGTCTCGAGTGCCAAGACCACCG CCCCCAGCGTGTACCCCTGGCCCCGTGTGCGGCGACACCACCGGCAGCAGC GTGACCCCTGGGCTGCCTGGTGAAGGGCTACTTCCCCGAGCCCCGTGACCCTGAC CTGGAACAGCGGCAGCCTGAGCAGCGGCTGCACACCTTCCCCGCCGTGCTGC AGAGCGACCTGTACACCCTGAGCAGCAGCGTGACCGTGACCAGCAGCACCTGG CCCAGCCAGAGCATCACCTGCAACGTGGCCACCCCGCCAGCAGCACCAAGGT GGACAAGAAGATCGAGCCAGAGGCCCCACCATCAAGCCCCAGGTGCAGCTGC AGCAGCCCCGCGCCGAGCTGGTGAGACCCGCGCCAGCGTGAAGCTGAGCTGC AAGGCCAGCGGTACAGCTTCACCGGCTACTGGATGAACTGGCTGAAGCAGAG ACCCGGCCAGGGCCTGGAGTGGATCGGCATGATCCACCCAGCGACAGCGAGA TCAGAGCCAACCAGAAGTTACAGACAAGGCCACCCTGACCGTGGACAAGAGC AGCACCACCGCTACATGCAGCTGAGCAGCCCCACCAGCAGGACAGCGCGT GTACTACTGCGCCAGAGGCACCTACGACGCGGCTTTCAGTACTGGGGCCAGG GCACCACCTGACCGTCTCGAGTGCCAAAACAACAGCCCCATCGGTCTATCCA CTGGCTCCTGTGTGTGGAGATACAACCTGGCTCCTCGGTGACTCTAGGATGCCT GGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAAGTCTGGATCCC

SEQ ID NO	DESCRIPTION	SEQUENCE
		TGTCCAGTGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACC CTCAGCAGCTCAGTGACTGTAACGTCGAGCACCTGGCCCAGCCAGTCCATCAC CTGCAATGTGGCCACCCGGCAAGCAGCACCAAGGTGGACAAGAAAATTGAGC CCAGAGGACCCACAATCAAGCCCTGTCTCCATGCAAATGCCAGCACCTAAC GCCGCGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACT CATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGG ATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACA GCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTCTACGCGTGGTCAG TGCCCTCCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCA AGGTCAACAACAAAGACCTCGGAGCGCCCATCGAGAGAACATCTCAAAACCC AAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGA GATGACTAAGAAACAGGTCACCTCTGTGGTGCATGGTCACAGACTTCATGCCTG AAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTACAAG AACACTGAACCAGTCTGGACTCTGATGGTTCTTACTTCATGTACAGCAAGCT GAGAGTGGAAAAGAAGAACTGGGTGGAAAAGAAATAGTACTCCTGTTCAAGTGG TCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCG GGTAAATGA
22	HER2.7C2VH- mIgG2aCH1- 7C2VH.mIgG2a. LALAPG.Knob (LC DNA with SP)	ATGGGATGGTCATGTATCATCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCAGACATCGTGCTGACCCAGAGCCAGCCAGCCTGGTGGTGAGCCTGGGCC AGCGCGCCACCATCAGCTGCCGCGCCAGCCAGAGCGTGAGCGGCAGCCGCTTC ACCTACATGCACTGGTATCAGCAGAAGCCAGGCCAGCCACCAAAGCTGCTGAT CAAGTACGCCAGCATCCTGGAGAGCGCGGTGCCAGCCCGCTTCAGCGCGGCG GCAGCGGCACCGACTTCACCCTGAACATCCACCCAGTGGAGGAGGACGACACC GCCACCTACTACTGCCAGCAGCTGGGAGATCCCACCATGGACCTTCGGCGG CGGTACCAAGCTGGAGATCAAGCGCGCTGATGCTGCACCAACTGTATCCATCT TCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTC TTGAACAACCTTCTACCCCAAAGACATCAATGTCAAGTGAAGATTGATGGCAG TGAACGACAAAATGGCGTCTTGAACAGTTGGACTGATCAGCAGCAGCAAGACA GCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGA CATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGT CAAGAGCTTCAACAGGAATGAGTGTGA
23	HER2.7C2VH- mIgG2aCH1- 7C2VH.mIgG2a. LALAPG.Knob (HC mature)	QVQLQQPGAE LVRPGASVKL SCKASGYSFT GYWMNWLKQR PGQGLEWIGM IHPSDSEIRA NQKFRDKATL TVDKSSTTAY MQLSSPTSED SAVYYCARGT YDGGFEYWGQ GTTLTVSSAK TTAPSVYPLA PVCDDTTGSS VTLGCLVKGY FPEPVTLTNW SGSLSSGVHT FPAVLQSDLY TLSSSVTVTS STWPSQSITE NVAHPASSTK VDKKIEPRGP TIKPQVQLQQ PGAEIVRPGA SVKLSCKASG YSFTGYWMNW LKQRPQGQLE WIGMIHPSDS EIRANQKFRD KATLTVDKSS TTAYMQLSSP TSEDSAVYYC ARGTYDGGFE YWGQGTTLTV SSAKTTAPSV YPLAPVCGDT TGSSVTLGCL VKGYFPEPVT LTWNSSGSLSS GVHTFPAVLQ SDLYTLSSSV TVTSSTWPSQ SITCNVAHPA SSTKVDKKIE PRGPTIKPCP PCKCPAPNAA GGPSVFIFPP KIKDVLMLISL SPIVTCVVVD VSEDDPDVQI SWFVNNNEVH TAQTQTHRED YNSTLRVSA LPIQHQQDWS KGEFKCKVNN KDLGAPIERT ISKPKGSVRA PQVYVLPPE EEMTKKQVTL WCMVTDPMPE DIYVEWTNNG KTELNYKNTE PVLDSGGSYF MYSKLRVEKK NWVERNSYSC SVVHEGLHNH HTTKSFSTRP GK
24	HER2.7C2VH- mIgG2aCH1- 7C2VH.mIgG2a. LALAPG.Knob (LC mature)	DIVLTQSPAS LVSLSGQRAT ISCRASQSVS GSRFTYMHWY QQKPGQPPKL LIKYASILES GVPARFSGGG SGTDFTLNIH PVEEDDTATY YCQHSWEIPP WTFGGGTKLE IKRADAAPTV SIFPPSSEQL TSGGASVCF LNNFYPKDIN VKWKIDGSER QNGVLNSWTD QDSKDSTYSM SSTLTLLTKDE YERHNSYTCE ATHKTSTSPI VKSFNRNEC
25	IGF-1R.xIGF- 1Rcixutumumab VH.mIgG2a.LAL APG.Knob (HC DNA with SP)	ATGGGATGGTCATGTATCATCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCAGAGGTGCAGCTGGTGACAGCGCGCGGAGGTGAAGAAGCCCGGACAG GCGTGAAGGTGAGCTGCAAGGCCAGCGCGGCACCTTCAGCAGCTACGCCATC AGCTGGGTGAGACAGGCCCCCGGCCAGGGCCTGGAGTGGATGGCGGCATCAT CCCCATCTTCGGCACCGCCAACCTACGCCCAGAAGTTCCAGGGCAGAGTGACCA TCACCGCCGACAAGAGCACCAGCACCGCCTACATGGAGCTGAGCAGCCTGAGA

SEQ ID NO	DESCRIPTION	SEQUENCE
		AGCGAGGACACCGCCGTGTACTACTGCGCCAGAGCCCCCTGAGATTCCTGGA GTGGAGCACCAGGACCCTACTACTACTACTACATGGACGTGTGGGGCAAGG GCACCACCGTGACCGTCTCGAGTGCCAAAACAACAGCCCCATCGGTCTATCCA CTGGCTCCTGTGTGTGGAGATACTGGCTCCTCGGTGACTCTAGGATGCCT GGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAAGTCTGGATCCC TGTCCAGTGGTGTGCACACCTTCCCAGCTGCCTGCAGTCTGACCTCTACACC CTCAGCAGCTCAGTGACTGTAACGTCGAGCACCTGGCCCAGCCAGTCCATCAC CTGCAATGTGGCCACCCGGCAAGCAGCACCAAGGTGGACAAGAAAATTGAGC CCAGAGGACCCACAATCAAGCCCTGTCTCCATGCAAATGCCAGCACCTAAC GCCGCGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACT CATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGG ATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACA GCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTCTACGCGTGGTCAG TGCCCTCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCA AGGTCAACAACAAAGACCTCGGAGCGCCATCGAGAGAACCATCTCAAACCC AAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCCTCCACCAGAAGAAGA GATGACTAAGAAACAGGTCACCTCTGTGGTGCATGGTCACAGACTTCATGCCTG AAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTACAAG AACACTGAACCAGTCTTGAGTCTGATGGTTCTTACTTCATGTACAGCAAGCT GAGAGTGGAAAAGAAGAACTGGGTGGAAAAGAAATAGCTACTCCTGTTCAAGTGG TCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCG GGTAAATGA
26	IGF-1R.xIGF-1Rcixutumumab VH.mIgG2a.LAL APG.Knob (LC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCAAGCAGCGAGCTGACCCAGGACCCCGCCGTGAGCGTGGCCCTGGGCCAGA CCGTGAGAATCACCTGCCAGGGCGACAGCCTGAGAAGCTACTACGCCACCTGG TACCAGCAGAAGCCCGGCCAGGCCCCCATCCTGGTGATCTACGGCGAGAACAA GAGACCCAGCGGCATCCCCGACAGATTCAGCGGCAGCAGCAGCGGCAACACCG CCAGCCTGACCATCACCGGCGCCAGGCCAGGACGAGGACGAGCCGACTACTACTGC AAGAGCAGAGACGGCAGCGGCCAGCACCTGGTGTTCGGCGGCGGCCACCAAGCT GACCGTGCTTGCCAGCCCAAGTCCACTCCCACTCTCACCCTGTTTCCACCCT CGAGTGAGGAGCTCAAGGAAAACAAAGCCCACTGGTGTGTCTGATTTCCAAC TTTTCCCGAGTGGTGTGACAGTGGCCTGGAAGGCAAATGGTACACCTATCAC CCAGGGTGTGGACACTTCAAATCCCACCAAGAGGGCAACAAGTTTCATGGCGA GCAGCTTCTACATTTGACATCGGACCAGTGGAGATCTCACAACAGTTTACC TGTCAAGTTACACATGAAGGGGACACTGTGGAGAAGAGTCTGTACAGTGTCTGA CTGTCTCTGA
27	IGF-1R.xIGF-1Rcixutumumab VH.mIgG2a.LAL APG.Knob (HC mature)	EVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG IIPIFGTANY AQKFQGRVTI TADKSTSTAY MELSSLRSED TAVYYCARAP LRFLEWSTQD HYYYYYMDVW GKGTTTVTVSS AKTTAPSVYP LAPVCGDTTG SSVTLGCLVK GYFPEPVTLT WNSGSLSSGV HTFPAVLQSD LYTLSSSVTV TSSTWPSQSI TCNVAHPASS TKVDKKIEPR GPTIKPCPPC KCPAPNAAGG PSVFIFPPKI KDVLMI SLSP IVTCVVVDVS EDDPDVQISW FVNNVEVHTA QTQTHREDYN STLRVVSALP IQHQDWMSGK EFKCKVNNKD LGAPIERTIS KPKGSVRAPQ VYVLPPEEE MTKKQVTLWC MVTDFMPEDI YVEWTNNGKT ELNYKNTEPV LDSDSYFMY SKLRVEKKNW VERNYSYSCSV VHEGLHNHHT TKSFSRTPGK
28	IGF-1R.xIGF-1Rcixutumumab VH.mIgG2a.LAL APG.Knob (LC mature)	SSELTQDPAV SVALGQTVRI TCQGDSLRSY YATWYQQKPG QAPILVIYGE NKRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCKSR DSGSQHLVFG GGTKLTVLGQ PKSTPTLTVF PPSSEELKEN KATLVCLISN FSPSGVTVAW KANGTPITQG VDTSNPTKEG NKFMASSFLH LTSQWRSHN SFTCQVTHEG DTVEKSLSRA DCL
29	N1ST.mIgG2a.LALAPG.Knob (HC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACA TTCACAGGTGACCTGAGGGAGTCCGGCCCCGCCCTGGTGAAGCCACCCAGA CCCTGACCCTGACCTGCACCTTCTCCGGCTTCTCCCTGTCCACCGCCGGCATG TCCGTGGGCTGGATCAGGCAGCCCCCGCAAGGCCCTGGAGTGGCTGGCCGA CATCTGGTGGGACGACAAGAAGCACTACAACCCCTCCCTGAAGGACAGGCTGA CCATCTCCAAGGACACCTCCAAGAACCAGGTGGTGTGAAGGTGACCAACATG

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		GACCCCGCCGACACCGCCACCTACTACTGCGCCAGGGACATGATCTTCAACTTCTACTTCGACGTGTGGGGCCAGGGCACCACCGTGACCGTCTCGAGTGCCAAAA CAACAGCCCCATCGGTCTATCCACTGGCTCCTGTGTGTGGAGATACAACTGGCTCCTCGGTGACTCTAGGATGCCTGGTCAAGGGTTATTTCCCTGAGCCAGTGACTTGACCTGGAACCTCTGGATCCCTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCTGTCAGTCTGACCTCTACACCCTCAGCAGCTCAGTGACTGTAACGTCGAGCACCTGGCCGACCGAGTCCATCACCTGCAATGTGGCCACCCGGCAAGCAGCAC CAAGGTGGACAAGAAAATTGAGCCCAGAGGACCCACAATCAAGCCCTGTCCCTCATGCAAATGCCAGCACCTAACGCCGCGGGTGGACCATCCGTCTTCATCTTCCTCCAAAGATCAAGGATGTACTCATGATCTCCCTGAGCCCCAAGTGCATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAGGATTAC AACAGTACTCTACGCGTGGTCAAGTCCCTCCCATCCAGCACCCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCGGAGCGCCCATCGAGAGAACCATCTCAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCACTCTGTGGTGCATGGTCCAGACTTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTACAAGAACACTGAACCAGTCCCTGGACTCTGATGGTTCTTACTTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGGTAAATGA
30	NIST.mIgG2a.L ALAPG.Knob (LC DNA with SP)	ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACATTTCAGACATCCAGATGACCCAGTCCCCCTCCACCCTGTCCGCTCCGTGGGCGACAGGGTGACCATCACCTGCTCCGCTCCTCCAGGGTGGGCTACATGCACTGGTATCAGCAGAAGCCCGCAAGGCCCCCAAGCTGCTGATCTACGACACCTCCAA GCTGGCTCCGGCGTGCCCTCCAGGTTCTCCGGCTCCGGCTCCGGCACCAGGTTTACCCCTGACCATCTCCTCCCTGCAGCCCGACGACTTCGCCACCTACTACTGCTTCCAGGGTCCGGCTACCCCTTACCTTCGGCGGCGGTACCAAGGTGGAGATCAAGCGCGCTGATGCTGCACCAACTGTATCCATCTTCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACCTTACCCCCAAAGACATCAATGTCAAGTGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAA TGAGTGTGA
31	NIST.mIgG2a.L ALAPG.Knob (HC mature)	QVTLRESGPA LVKPTQTLTL TCTFSGFSLs TAGMSVGWIR QPPGKALEWL ADIWDDKKH YNPSLKDRLT ISKDTSKNQV VLKVTNMDPA DTATYYCARD MIFNFYFDVW GQGTTVTVSS AKTTAPSVYP LAPVCGDTTG SSVTLGCLVK GYFPEPVTLT WNSGSLSSGV HTFPAVLQSD LYTLSSSVTV TSSTWPSQSI TCNVAHPASS TKVDKKIEPR GPTIKPCPPC KCPAPNAAGG PSVFI FPPKI KDVLMISLSP IVTCVVVDVS EDDPDVQISW FVNNVEVHTA QTQTHREDYN STLRVVSALP IQHQDWMGSK EFKCKVNNKD LGAPIERTIS KPKGSVRAPQ VYVLPPEEE MTKKQVTLWC MVTDFMPEDI YVEWTNNGKT ELNYKNTEPV LDSDGSYFMY SKLRVEKKNW VERNYSYCSV VHEGLHNHHT TKSFSRTPGK
32	NIST.mIgG2a.L ALAPG.Knob (LC mature)	DIQMTQSPST LSASVGDRVIT ITCSASSRVG YMHYQOKPG KAPKLLIYDT SKLASGVPSR FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGG TKVEIKRADA APTVSIFPPS SEQLTSGGAS VVCFLNNFYP KDINVWKID GSERQNGVLN SWTDQDSKDS TYSMSSTLTL TKDEYERHNS YTCEATHKTS TSPIVKSFNK NEC
33	RNF43-104 (variable heavy chain (VH) DNA)	CAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGCAGCCCTCACAGACCTTGTCTCTACCTGCAGTGTCTCTGGGTTTTTCATTAAACCAGCTTCCATGTGCACTGGGTTTCAGACGCTCCAGGAAAAGGTCTGGAGTGGATTAATGTGGAGT AATGGAGACACATCATATAATTCAGATCTCAAATCCCGACTGAGCATCAGCAGGGACACCTCCAAGAGTCAAGTCTTCTTAAAAATGCACAGTCTGCAGACTGAAGACACAGCCACTTACTACTGTGCCAGAACAGATGTATACCACGGATTGCGTGCTATGGATGCCTGGGGTCAGGGAACCTCAGTCACTGTCTCTCTCA

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34	RNF43-104 (variable light chain (VL) DNA)	GACATCCAGATGACACAGTCTCCGTCATTTCTGTCTGCATCTCTTGGAACAGCATCACCATCACTTGCCATGCCAGTCAGTTCATCAAGGGTTGGTTAGCCTGGTACCAACAAAAGTCAGGGAATGCTCCTGAACTGTTAATTTATAAGTCATCTAGCTGCATTGAGGGGTTCCATCAAGATTGAGTGGCAGCGGATCTGGAACAGATTATATTCTACTATCAGCAACCTACAGCCTGAAGATATTGCCACTTATTACTGTCAGCATTATCAAGGCTTTCCGCTCACGTTGCGTTCTGGGACCAAGCTGGAGATCAAA
35	RNF43-104 (VH protein)	QVQLKESGPGVLVQPSQTLSTLCSVSGFSLTSFHVHWRQPPGKGLEWMGLMWSNGDTSYNSDLKSRSLISRDTSKSKQVFLKMHSLQTEDTATYYCARTDVYHGFAMDAGWGQTSVTVSS
36	RNF43-104 (VL protein)	DIQMTQSPSFLSASLGNISITITCHASQFIKGLAWYQQKSGNAPELLIYKSSSLHSGVPSRFSGSGSGTDYILTISNLPEDIATYYCQHYQGFPPLTFGSGTKLEIK
37	RNF43-106 (VH DNA)	CAGATCCAGTTGGGACAGTCTGGACCTGAGCTGACGAAGCCTGGAGAGTCAGTGAAGATCTCCTGCAAGGCTTCTGGGTATACCTTCTCAGACTATCCAATACACTGGGTGAAACAGGCTCCAGGAAAGGGCTTGAAGTGGATGGGCTGGATCAACACCTATACTGGGAAGCCAACATTTGCTGATGACTTCAAAGGACGGTTTGTCTTCTCTTTGGAAGCCTCTGCCAGCACTGCAAATTTGCAGATCAGCAACCTCAAAAATGAGGACACGGCTACATATTTCTGTGCTCTGGGCCCATTTCTACAGTCTTCTATGCTATGGATGCCTGGGGTCAAGGAACCTCAGTCACTGTCTCCTCA
38	RNF43-106 (VL DNA)	GACATCCAGATGACCCAGTCTCCTTCATTCCTGTCTGCATCTGTGGGAGACAGAGTCACTATCAACTGCAAAGCAAGTCAGAATATTAACAAGTATTTAACTGGTATCAGCAAAAGCTTGGAGAAGCTCCCAAACGCCTGATATATAATACAAACAATTTGCAAACAGGCATCCCATCAAGGTTGAGTGGCAGTGGATCTGGTACAGATTACACACTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCCACATATTTCTGCTTGCAGCATAATAGTTTTCCGTACACGTTTGGAGCTGGGACCAAGCTGGAAGCTGAAA
39	RNF43-106 (VH prot)	QIQLGQSGPELTKPGESVKISCKASGYTFSDYPIHWVKQAPGKGLKWMGWINTYTGKPTFADDFKGRFVFSLEASASTANLQISNLKNEATATYFCAALGPISITVFYAMDAGWGQTSVTVSS
40	RNF43-106 (VL prot)	DIQMTQSPSFLSASVGDRTINCKASQNKYLNWYQQKLGAEAPKRLIYNTNNLQTGIPSRFSGSGSGTDYTLTISLQPEDFATYFCLQHNSFPYTFGAGTKLELK
41	RNF43-107 (VH DNA)	GAGGTGCAGCCGGTGGAGTCTGGGGGCGGCTTGGTGCAGCCTGGAAGTCCCTAAAACCTCTCCTGCCCAGCCTCAGGATTCATTTTCAGTAACATATGACATGGCCTGGGTCCGCCAGGCTCCAACGAAGGGTCTGGAGTGGGTGCGTTCCATTAGTCCCTAGTGGTGGTAGAACTTACTATCGCGACTCCGTGAAGGGCCGATTCACTATTTCCAGAGATAATTCAAAAAGTACCTTACTTGCAAATGGACAGTCTGAGATCTGAGGACACCGCCACTTTTACTGTGCAACACATCGGTACTACAGTTACTTTGATTTCTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
42	RNF43-107 (VL DNA)	GACATCCAGATGACACAGTCTCCAGCTTCCCTGTCTGCATCTCTGGGAGAAACATCTCCATCGATTGTGCAACAAGTGAAGGACATTTACAATAATTTAGCGTGGTATCAGCAGAAGTCAGGGAGATCTCCTCAGCTCCTGATCTCTGCTACAAATAGGTTGCAAGATGGGGTCCCATCACGGTTCAGTGGCAGTGGTTCTGGCACACAGTATTCTCTCAAGATCAGCGGCATGCAACCTGAAGATGAAGGGGTTTATTTCTGCTACAGGGTCCAAGTATCCGTATACGTTTGGAACTGGGACCAAGCTGGAAGCTGAAA
43	RNF43-107 (VH prot)	EVQPVESGGGLVQPGRSRLKLSCPASGFTFSNYDMAWVRQAPTKGLEWVASISPSGGRTYYRDSVKGRFTISRDNKSTLYLQMDSLRSEDATATFYCATHRYYSYFD FWGQGVMTVSS
44	RNF43-107 (VL prot)	DIQMTQSPASLSASLGETISIDCRTSEDIYNNLAWYQQKSGRSPQLLISATNRLQDGVPSRFSGSGSGTDYSLKISGMQPEDEGVYFCLQGSKYPTFTGTGKLELK
45	RNF43-108 (VH DNA)	CAGATACAACTGCAGCAATCTGGGGCTGAACTGGTGAAGCCTGGGTCCCTCAGTGACAGTTTCTGCAAGGCTTCTGGCTACACCTTCAACAGTAACCTTTATGCACTGGATAAGACAGCAGCCTGGCAATGGCCTTGAGTGGATTGGGTGGATTTATCCTGGCGATGGTGATGCAGAGACCAATCGAAAGTTCAATGGGAAGGCAACACTCAC TGCAGACAAATCCTCCACCACAGCCTACTTGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTTCTGTGCAAGAGGAGTGATGACGTCGTTCTGGTAC TTTGACTTCTGGGGCCAGGAACCATAGTCACCGTGTCTCA

SEQ ID NO	DESCRIPTION	SEQUENCE
46	RNF43-108 (VL DNA)	GATGTCCAGATGACCCAGTCTCCATATAATCTTGCTGCCTCTCCTGGAGAAAGTGTTTCCATCAATTGCAAGGCAAGTAAGAGCATTAGTAAGTATTTAGCCTGGTATCAACAGAAACCTGGGAAAACAAATAAGATTCTAATCTACGATGGGTCAACTTTGCAATCTGGAATTCATCGAGGTTCACTGGCAGTGGATCTGGTACAGATTTCACTCTACCATCAGAGGCTGGAGCCTGAAGATTTTGGACTCTATTACTGTC AACAGCATAATGAACAGCCACCTACGTTTCGGCTCGGGACGAGGTTGGAAATAAAA
47	RNF43-108 (VH prot)	QIQ LQQSGAELVKPGSSVTVSCKASGYTFTSNFMHWIRQQPGNGLEWIGWIYPGDGDAETNRKFNKGATLTADKSSTTAYLQLSSLTSEDSAVYFCARGVMTSFWYFDFWGP GTIVTVSS
48	RNF43-108 (VL prot)	DVQMTQSPYNLAASPGESVSINCKASKSISKYLAWYQQKPGKTNKILIIDGSTLQSGIPSRFSGSGSGTDFTLTIRGLEPEDFGLYYCQQHNEQPPFTFGSGTRLEIK
49	RNF43-116 (VH DNA)	CAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGCAAAGCCTGGCTCTTCAGTGAAGATTTCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTATATAAGCTGGATAAAGCAGACGACTGGACAGGACCTTGAGTATATTGGATATGTTAATACGAGAAGTGGAGGTACTAACTACAATGAGAAGTTC AAGGTCAAGGCCACATTGAC TG TAGACAATTCTCCAGCACAGCCTTCATGCAACTCAGCAGCCTGACACCTGACGACTCTGCGGTCTATTACTGTGCAAGAGGCAGAACGGACTGGGGCCAAGGC ACTCTGGTCACTGTCTCTTCA
50	RNF43-116 (VL DNA)	GACATCCAGATGACCCAGTCTCCTTCACTCCTGTCTGCATCTGTGGGAGACAGAGTCACTGTAAACTGCAAAGCAAGTCAGAATATTTATAAGAACTTAGAGTGGTAGCAGCAAAGCATGGAGAAGCTCCAAAACCTCCGATATATTATACAAACAATTGCAAAACGGGCATCTCATCAAGGTTCACTGGCAGTGGATCTGGTACAGATTA CACACTCACCATCAGCAGCCTGCAGCCTGAAGATGTTGCCACATATTACTGCTTTCAGTATAACAGCGGGTACACGTTTGGAGCTGGGACCAAGCTGGAAGTGAAG
51	RNF43-116 (VH prot)	QVQLQQSGAELAKPGSSVKISCKASGYTFTSYIISWIKQT TGGDLEYIGYVNT RSGGTNYNEKFKVKATLTVDNSSSTAFMQ LSSLTPDDSAVYYCARGRTDWGQGTLTVTVSS
52	RNF43-116 (VL prot)	DIQMTQSPSLLSASVGDRTVNCKASQNIYKNLEWYQQKHGEAPKLLIYYTNNLQTGISSRFSGSGSGTDYTLTISLQPEDVATYYCFQYNSGYTFGAGTKLELK
53	RNF43-117 (VH DNA)	CAGGTTTCTCTGAAAGAGTCTGGCCCTGGGATGTTGCAGCCCTCCAGACCCCTCAGTCTGACTTGCACTTTCTCTGAGTTTCTACGAGCACTTATGGTTTGGGTGTGGGCTGGATTGCTCAGCCTTCAGGGAAGGGTCTGGAGTGGCTGGCGAACATTGGTGGGGATGATGATAAGTACTACAATCCATCTCTGAAAAACCGCTCACAATCTCCAAGGACACCTCCAACAACCAAGCATTCTCAAGATCACCAATGTGGACA CTGCTGATACTGCCACGTACTACTGTGCTCGGATAGACGGCAGGTGGCACTACTTTGATTACTGGGGCCAAGGAGACAGGGTCATAGTCTCCTCA
54	RNF43-117 (VL DNA)	GATATTGTCTGACCCAGACTCCATCCATATTGTCTGCTACCATTGGACAATCGGTCTCCATCTCTTGCAAGTCAACTCAGAGTCTCTTAGATAGTATGGAAACA CTTATTTATATTGGTTCTCTACAGAGGCCAGGCCATCTCCACAGCGTCTAATTATTTGGTATCCAACCTGGGATCTGGGGTCCCCAACAGGTTCACTGGCAGTGGGTCAGGAACAGATTTCACTCAAAATCAGTGGAGTGGAGGCTGAGGATTTGGGATTTTATTACTGCATGCAAGGTGCCATACTCCGCTCACGTTTCGGTTCTGGGACCAAGCTGGAATCAAA
55	RNF43-117 (VH prot)	QVSLKESGPGMLQPSQTLSTCTFSEFSLSTYGLGVGWIRQPSGKGLEWLANIWWDDDKYYNP SLKNRLTISKDTSNNQAF LKITNVD TADTATYYCARIDGRWHYFDYWGQGDRIVSS
56	RNF43-117 (VL prot)	DIVLTQTPSILSATIGQSVSISCRSTQSLSDSGNTYLYWFLQRPQGSPQRILIYLSNLRGSGVPNRFSGSGSGTDFTLTKISGVEAEDLGFYYCMQGAHTPLTFGSGTKLEIK
57	RNF43-123 (VH DNA)	CAGATCCAGTTGGTACAGTCTGGACCTGAGCTGAAGAAGCCTGGAGAGTCAGTGAAGATCTCCTGCAAGGCTTCTGGGTATACCTTCACAGACTATCCAATGCACTGGGTGAAACAGGCTCCAGGAAAGGGCTTGAAGTGGATGGGCTGGATCAACACCTATACTGGGAAGCCAACATATGCTGATGACTTCAAAGGACGGTTTGTCTTCTCTTTGGAAAGCCTCTGCCAACACTGCAATTTACAGATCAGCAACCTCAAAAATGACGACACGGCTACATATTTCTGTGCTCTGGGCCCCATTTCTACAGCTCTTCTATGCTGTGGATGCCTGGGCTCAAGGAACCTCAGTCACTGTCTCCTCA
58	RNF43-123 (VL DNA)	GACATCCAGATGACCCAGTCTCCTTCATTCCSTGTCTGCATCTGTGGGAGACAGAGTCACTATCAACTGCAAAGCAAGTCAGAATATTAACAAGTACTTAAACTGGT

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		ATCAGCAAAAGGTTGGAGAAGCTCCCAAGCGCCTGATGTATAATACAAACAAT TTGCAAACAGGCATCCCATCAAGGTTTCAGTGGCAGTGGATCTGGTACAGATTA CACACTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCCACATATTTCTGCT TGCAGCATAATAGTTTTCCGTACACGTTTGGACCTGGGACCGAGCTGGAACCTG AAA
59	RNF43-123 (VH prot)	QIQLVQSGPELKKPGESVKISCKASGYTFTDYPMHVVKQAPGKGLKWMGWINT YTGKPTYADDFKGRFVFSLEASANTANLQISNLKNDTDTATYFCALGPISITVEFY AVDAWGQGTSTVTSS
60	RNF43-123 (VL prot)	DIQMTQSPSFLSASVGDRTINCKASQNINKYLNWYQQKVGEPKRLMYNTNN LQTGIPSRFSGSGSGTDYTLTISSLQPEDFATYFCLQHNSFPYTFGPGTELEL K
61	RNF43-126 (VH DNA)	GAGGTGCAGCTTCAGGAGTCAGGACCTGGCCTTGTGAAACCCTCACAGTCACT CTCCCTCACCTGTTCTGTCACTGGTTTCTCCATCACCAGTAGTCATAGATGGA ACTGGATCCGGAAGTTCCAGGAAATAAACTGGAGTGGATGGGATATATAAAC AGTGCAGGCAGCACTAACTATAACCCGTCTCTCAAAGTCTGAATCTCCATTAC TAGAGACACATCCAAGAATCAGTTCCTTCTGCAGGTGAACTCTGTAACACTG AGGACACAGCCACTTATTACTGTACGAGATTAGGACTACAGTATCGGGACTTT GATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
62	RNF43-126 (VL DNA)	GACATCCAGATGACACAGTCTCCAGCTTCCCTGTCTGCATCTCTGGGAGAAAC TATCTCCATCGAATGTGCGAGCAAGTACGACATTTACAGTAATTTAGCGTGCT ATCAGCAGAAGTCAGGAAATCTCCTCAGCTCCTGATCTATGCTGCAAAATAGG TTGAAAGATGGGGTCCCATCACGGTTCAGTGGCAGTGGATCTGGCACACAGTA TTCTCTCAAGATCAGCGGCATGCAAACTGAAGATGAAGGGGATTATTTCTGTC TACAGGCTTCCAAGTTTCCGCTCACGTTCCGTTCTGGGACCAAGCTGGAGATC AAA
63	RNF43-126 (VH prot)	EVQLQESGPGLVKPSQSLSLTCSVTGFSITSSHRWNWIRKFPNGKLEWMGYIN SAGSTNYPNPSLKSRI SITRDTSKNQFFLQVNSVTTEDTATYYCTRLGLQYRDF DYWGQGVMTVSS
64	RNF43-126 (VL prot)	DIQMTQSPASLSASLGETISIECRASDDIYSNLAWYQQKSGKSPQLLIYAANR LKDGVPSRFSGSGSGTDYSLKISGMQTEDEGDYFCLQASKFPLTFGSGTKLEI K
65	RNF43-128 (VH DNA)	GAGGTGCAGCTTCAGGAGTCAGGACCTGGCCTTGTGAAACCCTCTCAGTCACT CTCCCTCACCTGTTCTGTCACTGGTTTCTCCATCACCAGTCAATTATAAATGGA ACTGGATCCGGAAGTTCCCAAGAAATAAACTGGAGTGGATGGGATATATAGAC AATTCAGGCAGCACTAACTACATCCCGTCTCTCAAAGTCTGAATCTCCATTAC TAGAGACACATCCAAGAATCAGTTCCTTCTGCAGGTGAACTCTGTAACACTG AGGACACAGCCACTTATTACTGTGCGAGGGACTGCCTGGACTGGGGCCAAGGA GTCATGGTCACAGTCTCCTCA
66	RNF43-128 (VL DNA)	GATGTTGTGTGACCCAGACTCCACCACTTTATCGGCTACCATTGGACAATC AGTCTCCATCTCTTGCAAGTCAAGTCAGAGTCTCTTACATAGTAATGGAAACA CCTATTTACATTGGTTGATACAGAGGCCAGGCCAATCTCCACAGCTTCTAATT TACTTGGTATCCAGACTGGAATCTGGGGTCCCCAACAGGTTCACTGGCAGTGG GTCAGGAAGTGAATTTCTCAGTCAAATCAGTGGAGTAGAGGCTGAGGATTTGG GAATTTATTAAGTGTGTGCAAGATACCCATACTCCGTGGACGTTCCGTGGAGGC ACCAAGCTGGAATTGAAA
67	RNF43-128 (VH prot)	EVQLQESGPGLVKPSQSLSLTCSVTGFSITSHYKWNWIRKFPNKGLEWMGYID NSGSTNYPNPSLKSRI SITRDTSKNQFFLQVNSVTTEDTATYYCARDCLDWGQG VMVTVSS
68	RNF43-128 (VL prot)	DVVLQTQTPPTLSATIGQSVSISCRSSQSLHNSNGNTYHLHWLIQRPQSPQLLI YLVSRLESQVPSRFSGSGSGTDFTSVKISGVEAEDLGIYYCVQDTHTPWTFGGG TKLELK
69	RNF43-129 (VH DNA)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGAAGGTCCAT GAAACTCTCCTGTGCAGCCTCAGGATTCACTTTCAGTAAGTATGACATGGCCT GGGTCCGCCAGGCTCCAAAGAAGGGTCTGGAGTGGGTGCAAGTATTACTTAT GATGTTAGTAGCACTTACTATCGAGACTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATGCAAAAAGCACCTATATTTGCAAAATGGACAGTCTGAGGTCTG AGGACACGGCCACTTATTACTGTACAACATTTGGATCGGGCGGGTTATTACTAT GATGGTTATGGCTTCTATTGGTTTGCCAACTGGGGCCAAGGCACCTCGGTAC TGTCTCTTCA

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70	RNF43-129 (VL DNA)	GACATTCAGATGACCCAGTCTCCATCCTCCATGTCTGTGTCTCTGGGAGACACAGTCACTATTACTTGCCGGGCAAGTCAGGACGTTAAAATTTATGTAACTGGTTCCAGCAGAAACCAGGGAAATCTCCAGACAAATGATTTACCGTGCAACGAAC TTGGCAGATGGGGTCTCATCAAGGTTGAGCGGCAGTAGGTCTGGATCAGATTA TTTATTACCATCAGCAGCCTGGAGTCTGAAGATGTGGCAGACTATCACTGTC TACAGTATGATGAGTATCCTCCAACGTTGCGTCTCTGGGACCAAGGTGGAGATC AAA
71	RNF43-129 (VH prot)	EVQLVESGGGLVQPGRSMKLSCAASGFTFSNYDMAWVRQAPKKGLEWVASITY DVSSTYYRDSVKGRFTISRDNASTLYLQMDSLRSEDATATYYCTTLDRAGYYY DGYGFYWFANWQGGLVTVSS
72	RNF43-129 (VL prot)	DIQMTQSPSSMSVSLGDTVTITCRASQDVKIYVNWVQKPKGKSPRQMIYRATN LADGVSSRFSGSRSGSDYLFITISSLESEDVADYHCLQYDEYPPPTFGSGTKVEI K
73	RNF43-130 (VH DNA)	CAGGTTTCAGCTGGTCCAATCTGGGGCTGAGGTGGTGAAGCCTGGGTCTCAGT GAAAATTTCTGCAAGGCTTCTGGCTACACCTTCACCGGTAACCTTTATCACT GGATAAACAGCAGCCTGGCAATGGCCTTGAGTGGATTGGGTGGATTTATCCT GGAGATGGTGATACAACATACAATCAGAAGTTGAGTGGGAAGGCAACACTCAC TGCAGACAAGTCTCCAGCACAGCCTATATGCAGCTCAGCAACCTGACATCTG AGGACTCTGCAGTCTATTTCTGTGCAAAGTGGAGGGACCTGAGCAGTGATTGG TTTGCTTACTGGGGCCAAGGCACCTCTGGTCACTGTCTCTCTCC
74	RNF43-130 (VL DNA)	GACATTGTGATGACCCAGTCTCCAGTCTCCCTGACTGTGTGAGAAGGAGAGAT GGTCAACATAAACTGCAAGTCCAGTCAGAGTCTTTTATCCAGTGGAAACCAAA AGAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCAAGACTACTG ATCTACTTTACATCCACTAGACAATCAGGGGTCCCTGATCGCTTCATAGGCAG TGGATCTGGGACAGACTTCACTCTGACCATCAGCGATGTGCAGGCTGAAGACC TGGCAGATTATTACTGCCTGCAGCATTACAGCTATCCGAACACGTTTGGAGCT GGGACCAAGTTGGAAGTGAAG
75	RNF43-130 (VH prot)	QVQLVQSGAEVVKPGSSSVKISCKASGYTFGTNFIHWIKQQPGNGLEWIGWIYP GDGDTTYNQKFSKGATLTADKSSSTAYMQLSLNLTSEDSAVYFCAKWRDLSSDW FAYWQGGLVTVSS
76	RNF43-130 (VL prot)	DIVMTQSPVSLTVSEGEVMTINCKSSQSLLSSGNQKNYLAWYQQKPGQSPRLI IYFTSTRQSGVPDRFIGSGSGTDFTLTISDVQAEDLADYYCLQHYSPNTFGA GTKLELK
77	RNF43-136 (VH DNA)	CAGGTTTCAGCTGCAGCAATCTGGGGCTGAATTAGTGAAGCCTGGGTCTCAGT GAAAATTTCTGCAAGGCTTCTGGCTACACCTTCACCAAGTAAGTTTATGCACT GGATAAAGCAGCAGCCTGGAAGTGGCCTTGAGTGGATTGGGTGGATTTATCCT GAACATGGTAATACTAAGTACAATCAAAGTTGATGGGAAGGCAACACTCAC TGCAGACAAATCCTCCAGCACAGCCTATATGCAACTCAGCAGCCTGACATCTG GGGACTCTGCAGTCTATTTCTGTGCAAGACAAAGCAGCAGGGGCCCTATGCT ATGGATGCCTGGGGTCAAGGAACCTCAGTCACTGTCTCTCTCA
78	RNF43-136 (VL DNA)	GACATCCAAATGACACAGTCTCCAGTTTCCCTGTCTGCATCTCTTGGACAAAC TGTCTCCATCGAATGTCTAACAAGTGAGGGCATTCCCATTGATTTAGCGTGGT ATCAGCAGAAGTCAGGGAAATCTCCTCAACTCCTTATCTATGCTGCAAGTAGG TCACAAGACGGGGTCCCATCACGGTTCAGTGGCAGTGGATCTGGCACACGGTA TTCTCTCAAGATCAGCGGCATGCAACCTGAAGATGAAGCAGATTATTTCTGTC AACAGACTTACAAGTATCCATTACGTTGCGCTCAGGGACGAGGTTGGAAATA AAA
79	RNF43-136 (VH prot)	QVQLQQSGAELVKPGSSSVKISCKASGYTFSTKFMHWIKQQPGSGLEWIGWIYP EHGNTKYNQKFDGKATLTADKSSSTAYMQLSSLTSGDSAVYFCARQSSQGAYA MDAWGQGTSTVTVSS
80	RNF43-136 (VL prot)	DIQMTQSPVSLASLQGVTSIECLTSEGIPIDLAWYQQKSGKSPQLLIYAASR SQDGVPSRFSGSGSGLTRYSLKISGMQPEDEADYFCQQTYYKYPFTFGSGTRLEI K
81	RNF43-145 (VH DNA)	GAGGTGCAGCTGAAGGAATCAGGACCTGGTCTGGTGCAGCCCTCACAGACCT GTCCCTCACCTGCACTGTCTCTGGATTCTCATTAACGGACTACAGTGTATACT GGGTTCGCCAGCCTCCAGGAAAAGTCTGGAGTGGATGGGAGTAATGTGGAGT GGTGGAACCACAGCATATAATTGAGCTCTCAAATCCCAGCTGAGCATCAGCAG GGACACCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGCTGCAAACTGAAG ACACAGCCATTTACTACTGTACCAGAGGGGGCAAAATATGATGGTACTTATTAC

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		TACGGCTGGTACTTTGACTTCTGGGGCCAGGAACCATGGTCACCGTGTCTCTC A
82	RNF43-145 (VL DNA)	GACGTTGTGTGACTCAGTCTCCAGCCACCCTGTCTGTGACTCCAGGAGAAAAG AATCAGTCTCTCCTGCCGGGCCAGCGAGAGTGTTGACACTTATCTATACTGGT ATCAGCAAAAGCCAAATGAGTCTCCAAGGCTTCTCATTAAGTATGCTTCCCAG TCCATCTCTGGAATACCCTCCAGGTTCACTGGCAGTGGATCTGGGACAGATTT CACTCTCAGTATCAATGGAGTAGAGCTTGAAGATTTATCAATTTATTACTGTC AACAAGGTAACAGCTTGCCCCCTCACGTTCCGGCTCAGGGACGAAGTTGGAAATA AAA
83	RNF43-145 (VH prot)	EVQLKESGPGLVQPSQTLSTLTCTVSGFSLTDYSVYWVRQPPGKLEWMGMVWS GGTTAYNSALKSRLSISRDTSKSQVFLKMNSLQTEDTAIYYCTRGGKYDGTYY YGWYFDFWGPMTMTVSS
84	RNF43-145 (VL prot)	DVVLTSQSPATLSVTPGERISLSCRASESDTYLYWYQQKPNESPRLLIKYASQ SISGIPSRFSGSGSGTDFTLSINGVELEDLSIYYCQQGNSLPLTFGSGTKLEI K
85	RNF43-152 (VH DNA)	CAGGTTACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGCCCTCCCAGACCCT CAGTCTGACTTGACACCTTCTCTGGGTCTTCACTGAGCACTTATGGTATGGGTG TGGGCTGGATTCTGCAGCCTTCAGGGAAGGGTCTGGAGTGGCTGGCAAACATT TGGTGGGATGATGATAAGTACTACAATCCATCTCTGAAAAACCGGCTCACAAT CTCCAAGGACACCTCCAACAACCAATCATTCCTCAAGATCGCCAATGTGGACA CTGCAGATACTGCCACATACTACTGTGCTCGGATAGACGGTACTTATAACTAC TTTGATTATTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
86	RNF43-152 (VL DNA)	GATGTTGTGTTGACCCAGACTCCATCCAGACTGTCTGCTACCATTGGACAATC GGTCTCCATCTCTTGCAAGTCAAGTCAAGTCTCTTAGATAGTTATGGAAACA CCTATTTATCTTGGTTCCTACAGAGGCCAGGCCAATCTCCACAGCGTCTAATT TTTTTGGTGTCCAACCTGGGATCTGGGGTCCCCAACAGGGTCAGTGGCAGTGG GTCAGGAAGTGAATTCACACTCAAATCAGTGGAGTGGAGGCTGAGGATTTGG GAGTTTATTACTGCATGCAAACCTACCCATGATCCTCTCACGTTCCGGTCTGGG ACCAAGCTGGAGATCAAA
87	RNF43-152 (VH prot)	QVTLKESGPGILQPSQTLSTLTCTFSGSSLSTYGMGVGWIRQPSGKLEWLANI WWDDDKYYNPSLKNRLTISKDTSNNQSFLLKIANVDTADTATYYCARIDGTINY FDYWGQGVMTVSS
88	RNF43-152 (VL prot)	DVVLTSQSPATLSVTPGERISLSCRASESDTYLYWYQQKPNESPRLLIKYASQ FLVSNLGSVGNRVSGSGSGTDFTLKISGVEAEDLGVIYCMQTHDPLTFGSG TKLEIK
89	RNF43-156 (VH DNA)	GAGGTGCAGCTGAAGGAATCAGGACCTGGTCTGGTGCAGCCCTCACAGACCCT GTCCCTCACCTGCACTGTCTCTGGATTCTCATTAACGGACTACAGTGTATACT GGGTTCCGCGAGCCTCCAGGAAAAGGTCTGGAGTGGATGGGAGTAATGTGGAGA GGTGAACCAAGCATATAATTGAGTCTCAAATCCCGACTGAGCATCAGCAG GGACACCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGAAG ACACAGCCATTTACTACTGTACCAGAGGGGGCAAATATGATGGTACTTATTAC TACGGCTGGTACTTTGACTTCTGGGGCCAGGAACCATGGTCACCGTGTCTCTC A
90	RNF43-156 (VL DNA)	GACGTTGTGTGACTCAGTCTCCAGCCACCCTGTCTGTGACTCCAGGAGAAAAG AATCAGTCTCTCCTGCCGGGCCAGCGAGAGTGTTGACACTTATCTATACTGGT ATCAGCAAAAACCAATGAGTCTCCAAGGCTTCTCATTAAGTATGCTTCCCAG TCCATCTCTGGAATACCCTCCAGGTTCACTGGCAGTGGATCTGGGACAGATTT CACTCTCAGTATCAATGGAGTAGAGCTTGAAGATTTATCAATTTATTACTGTC AACAAGGTAACAGCTTGCCCCCTCACGTTCCGGCTCAGGGACGAAGTTGGAAATA AAA
91	RNF43-156 (VH prot)	EVQLKESGPGLVQPSQTLSTLTCTVSGFSLTDYSVYWVRQPPGKLEWMGMVWR GGTTAYNSALKSRLSISRDTSKSQVFLKMNSLQTEDTAIYYCTRGGKYDGTYY YGWYFDFWGPMTMTVSS
92	RNF43-156 (VL prot)	DVVLTSQSPATLSVTPGERISLSCRASESDTYLYWYQQKPNESPRLLIKYASQ SISGIPSRFSGSGSGTDFTLSINGVELEDLSIYYCQQGNSLPLTFGSGTKLEI K
93	RNF43-168 (VH DNA)	CAGGTGCAGCTGAAGGAGTCAAGACCTGGTCTGGTGCAGCCCTCACAGACTTT GTCTCTCACCTGCAGTGTCTCTGGGTTCTCACTAAACAGGTATCATGTAAGCT GGGTTCCGCGAGCCTCCAGGAAAAGGTCTGGAGTGGATGGGAGTAGTAGTGGACT GGTGAAGCACAGCATATAATTCACTTCTCAAATCCCGACTGAGCATCAGCAG

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		GGACATCTCCAAGAGCCAAGTTTTCTTAAAAATGATCAGTCTGCAAACCTGAAG ACACAGCCACTTACTACTGTGTGTCAGTCAATACTACGGGTATACCTCCTACTTT GATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
94	RNF43-168 (VL DNA)	GACATCCAGATGACACAGTCTCCTGCTTCCCTGTCTGCGTCTCCGGAAGAAAT TGTCACGGTCACATGCCAGGCAAGCCAGGACATTGATAATTGGTTAACATGGT ATCAGCAGAAACCAGGAAAATCTCCTCAACTCCTGATCTATAGTGCACCAGC TTGGCAGACGGGATCCCATCAAGGTTGAGCGGCAGTAGATCTGGTACACAGTA TTCTCTTAAAAATCGACAGACTACAGGTTGAAGATACTGGAATCTATTACTGTC TACAGCGTTATGGTAATCCGTGGACGTTCCGTGGAGGCACCAAGCTGGAATTG AAA
95	RNF43-168 (VH prot)	QVQLKESGPGVLVQPSQTLSTCTVSGFSLNRYHVSWSVRQPPGKGLEWMGVVWT GGSTAYNSLLKSRLSISRDISKSQVFLKMISLQTEDTATYYCVSQYYGYTSYF DYWGQGVMTVSS
96	RNF43-168 (VL prot)	DIQMTQSPASLSASPEEIVTVTCQASQDIDNWLTYQQKPGKSPQLLIYSATS LADGIPSRFSGSRSGTQYSLKIDRLQVEDTGIYYCLQRYGNPWTFGGGTKLEL K
97	RNF43-170 (VH DNA)	GAGGTACAACCTGGTGGAGTCTGGGGGAGGCTTAGTGCCGCCTGGAAGGTCCCT GAAACTCTCCTGTGTAGCCTCAGGATTCACCTTCAATGACTATTACATGGCCT GGGTCCGCCAGGCTCCAACGAAGGCTCTGGAGTGGGTGCGCATCCATTAGTTAT GAGGGTCGTGCACTTACTATGGAGACTCCGTGAAGGGCCGATTCATATCTC CAGAGATAATGCAAAAAGCACCTTATATCTGCAAATGAATAGTCTGAGGCTG ATGACACGGCCACTTATTATTGTGCAACACATAGCAACTATATCTTTGATTAC TGGGGCCAGGAGTCATGGTCACAGTCTCCTCA
98	RNF43-170 (VL DNA)	GATATCCGGATGACACAGTCTCCAGCTTCCCTGTCTGCATCTCTGGGAGAAAC TGTC AACATCGAATGTCTAGCAAGTGAGGACATTTACAGTGATTTTGCATGGT TTCAGCAGAAGCCAGGGCAATCTCCTCTGCTCCTGATCTATAATGGAATAGT TTGCAAAATGGGGTCCCTTCACGGTTTAGTGGCAGTGGATCTGGCACACAGTA TTCTCTAAAAATCAACAGCCTCCAATCTGAAGATGTGCGGACTTATTTCTGTC AACAAATATAACAATTATCCTCCGACGTTCCGTGGAGGCACCAAGCTGGAATTG AAA
99	RNF43-170 (VH prot)	EVQLVESGGGLVPPGRSLKLSVCVASGFTFNDYYMAWVRQAPTKGLEWVASISY EGRRTYYGDSVKGRFTISRDNASTLYLQMNSLRSDDTATYYCATHSNYIFDY WGQGVMTVSS
100	RNF43-170 (VL prot)	DIRMTQSPASLSASLGTVNIECLASEDIYSDFAWFQQKPGQSPLLLIYNGNS LQNGVPSRFSGSGSGTQYSLKINSLSQSEDAVATYFCQQYNNYPPTFGGGTKLEL K
101	RNF43-176 (VH DNA)	CAGGTGCAGCTGAAGGAGTCAGGACCTGGTCTGGTGCAGCCCTCACAGACTTT GTCTCTCACCTGCACTGTCTCTGGGTTCTCACTAACAGCTATCATGTAAGCT GGGTTCCGCCAGCCTCCAGAAAAGTCTGGAGTGGATGGGAGTAATATGGACT GGTGGAAGCACAGCATATAATTCACCTCTCAAATCCCGACTGAGCATCAGCAG GGACATCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACCTGAAG ACACAGCCACTTACTACTGTGCCAGTCAATACTACGGGTATACCTCCTATTTT GATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
102	RNF43-176 (VL DNA)	GACATCCAGATGACACAGTCTCCTGTTTCCCTGTCTGCGTCTCCGGAAGAAAT TGTCACGATCACATGCCAGGCAAGCCAGGACATTGATAATTGGTTAACATGGT ATCAGCAGAAACCAGGGAAATCTCCTCAACTCCTGATCTATAGTGCACCAGC TTGGCAGACGGGATCCCATCAAGGTTGAGCGGCAGTAGATCTGGTACACAGTA TTCTCTTAAGATCAGCAGACTACAGATTGAAGATACTGGGATCTATTACTGTC TACAGCGTTATGGTAATCCGTGGACGTTCCGTGGAGGCACCAAGCTGGAATTG AAA
103	RNF43-176 (VH prot)	QVQLKESGPGVLVQPSQTLSTCTVSGFSLTSYHVSWSVRQPPGKGLEWMGVIWT GGSTAYNSLLKSRLSISRDISKSQVFLKMNSLQTEDTATYYCASQYYGYTSYF DYWGQGVMTVSS
104	RNF43-176 (VL prot)	DIQMTQSPVSLASPEEIVTITCQASQDIDNWLTYQQKPGKSPQLLIYSATS LADGIPSRFSGSRSGTQYSLKISRLQIEDTGIYYCLQRYGNPWTFGGGTKLEL K
105	RNF43-177 (VH DNA)	CAGGTCAAGCTGCTGCAGTCTGGGGCTGCACTGGTGAAGCCTGGGGACTCTGC GAAGATGTCTTGCAAAGCTTCTGGTTATACATTCAGTACTCCATTATACACT GGGTGAAACAGAGTCATGGAAGAGCCTTGAGTGGATTGGTTATATTAACTCT TACAGTGGTGGTATTACAGTACAATGAAAAGTTCAAGAACAAGGCCACATTGAC

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		TGTAGACAAATCCAGCAGCACAGCCTATGTGGAGTTTAGCAGATTGACATCTG AAGATTCTGCAATCTATTACTGTGCAAGAGAAATTCGGGGTATGGATGCCTGG GGTCAAGGAACCTTCAGTCACTGTCTCCTCA
106	RNF43-177 (VL DNA)	GACATCGTGTGACTCAGTCTCCAGCCACCCTGTCTGTGACTCCAGGAGAGAG TGTGAGTCTCTCCTGCAGGGCCAGTCAGGGTATTAGCACTAGTTTACTGCTGGT ATCAGCAAAAATCAACTGAGTCTCCAAGGCTTCTCATCAAGTATGCTTCCCAG TCCGTCTCTGGAATCCCCTCCAGGTTCACTGGCAGTGGATCAGGGACAGATTT CACTCTCAGTATCAACAGAGTAGAATCTGAAGATTTTTCACTTTATTACTGTC AACAGAGTTACAACCTTGCCCCGCACGTTTGGACCTGGGACCAAGCTGGAACCTG AAA
107	RNF43-177 (VH prot)	QVKLLQSGAALVKPGDSAKMSCKASGYTFTDSIIHWVKQSHGKSLEWIGYINP YSGGIQYNEKFKNKATLTVDKSSSTAYVEFSRLTSEDSAIYYCAREIRGMDAW GQGTSTVTVSS
108	RNF43-177 (VL prot)	DIVLTQSPATLSVTPGESVSLSCRASQGISTSLHWYQQKSTESPRLLIKYASQ SVSGIPSRFSGSGSGTDFTLSINRVESEDFSVYYCQQSYNLPRTFGPGTKLEL K
109	RNF43-179 (VH DNA)	GAGGTACAACCTGGTGGAGTCTGGGGGAGGCTTAGTGACAGCCTGGAAGGTCCCT GAAACTCTCCTGTGTAGCCTCAGGATTCACCTTCAATGACTATTACATGGCCT GGGTCCGCCAGGCTCCAAAGAAGGGTCTGGAGTGGGTGCGCATCCATTAATTAT GAGGGTAGTCGCACTTACTATGGAGACTCCGTGAAGGGCCGATTCATATCTC CAGAGATAATGCAAAAAGCACCTTATATCTGCAAATGAATAGTCTGAGGTCTG AGGACACGGCCACTTATTATTGTGCAACACATAGCAACTATATCTTTGATAAT TGGGGCCAGGGAGTCATGGTCACAGTCTCCTCA
110	RNF43-179 (VL DNA)	GATATCCGGATGACACAGTCTCCAGCTTCCCTGTCTGCATCTCTGGGAGAAAC TGTC AACATCGAATGTCTAGCAAGTGAGGACATTTACAGTGATTTTGCATGGT TTCAGCAGAAGCCAGGGCAATCTCCTCTGCTCCTGATCTATAATGGAATAAT TTGCAAAATGGGGTCCCTTCACGGTTTAGTGGCAGTGGATCTGGCACACAGTA TTCTCTAAAAATCAACAGCCTGCAATCTGAAGATGTGCGCACTTATTTCTGTC AACAAATATAACAATTATCCTCCGACGTTCCGTGGAGGCACCAAACTGGAATTG AAA
111	RNF43-179 (VH prot)	EVQLVESGGGLVQPGRSLKLSCVASGFTFNDYYMAWVRQAPKKGLEWVASINY EGSRYYGDSVKGRFTISRDNASTLYLQMNSLRSEDTATYYCATHSNYIFDN WGQGVMTVTVSS
112	RNF43-179 (VL prot)	DIRMTQSPASLSASLGETVNI ECLASEDIYSDFAWFQQKPGQSPLLLIYNGNN LQNGVPSRFSGSGSGTQYSLKINSLSQSEDEVATYFCQQYNNYPPTFGGGTKLEL K
113	RNF43-180 (VH DNA)	GAGGCGCAATTGGTGGAGTCTGGGGGAGGCTTAGTGCGGCCTGGAAGGTCCCT GAAACTCACCTGTGCAGCCTCAGGATTCACCTTTCAGTCACTATGACATGGCCT GGGTCCGCCAGGCTCCAGCAGGGGTCTGGAGTGGGTGCGCATCCATTGGTCTCT GGTGGTGGTCTCACTCACTATCGAGACTCCGTAAAGGGCCGATTCAGTCTCTC CAGAGATCATGCAGAAAGCAGCCTATACCTGCAAATGGACAGTCTGAGGTCTG AGGACACGGCCACTTATTACTGTGCAAGACATTACGGGTGGTCTACTTTGAT TACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
114	RNF43-180 (VL DNA)	GACATCCAGATGACACAGTCTCCGTCATTTCTGTCTGCATCTCTTGGAGACAG CATCACCATCACTTGCCATGCCAGTCAGAACATCAAGGGTTGGTTAGCCTGGT ATCAACAAAAATCGGGGAATGCTCCTGAACTGTTGATTTATACGGCTTCTGGC CTGCGATCAGGGGTCCATCAAGATTCAGTGGCAGCGGATCTGGAACAGATTA TATTCTCACTATCAGCAACCTACAGCCTGAAGATTTTGCCACTTATTATTGTC AGCATTTATCAAAGCTTTCCGAACACGTTTGGAGCCGGGACCAAGCTGGAACCTG AGA
115	RNF43-180 (VH prot)	EAQLVESGGGLVLRPGRSLKLTC AASGFTF SHYDMAWVRQAPARGLEWVASIGP GGGLTHYRDSVKGRFTVSRDHAESSLYLQMDSLRSEDTATYYCARHYGWSYFD YWGQGVMTVTVSS
116	RNF43-180 (VL prot)	DIQMTQSPSPFLSASLGDSITITCHASQNIKGWLAWYQQKSGNAPELLIYTASG LRSGVPSRFSGSGSGTDYILTISNLPEDFATYYCQHYQSFPNTFGAGTKLEL R
117	RNF43-181 (VH DNA)	CAGATACAGCTGCAGCAATCTGGGGCTGAACTGGTGAAGCCTGGGTCTCAGT GAAAATTTCTGCAAGTCTTCTGCCCTACAGGTTACACAGTAACCTTTATCACT GGATAAGACAGCAGCCTGGCAATGGCCTTGAGTGGATTGGGTGGATTATCCT GGCGATGGTGATGCAGAGAACAATCAAAGTTCAATGGGAAGGTAATACTCAC

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		TGCAGACAAATCGTCCAGCACAGCCTATATGCAGCTCAGCAGCCTGACATCTG AGGACTCTGCGGTCTATTTCTGTGCAAGAGGAGTGATGACGTCGTTGTGGTAT TTTGACTTTTGGGGCCAGGAACCATGGTCACCGTGTCTCA
118	RNF43-181 (VL DNA)	GATGTCCAGATGACCCAGTCTCCTTATAATCTTGCTGCCTCTCCTGGAGAAAG TGTTTCCATCAATTGCAAGGCAAGTAAGAGCATTAGTAAGTATTTAGCCTGGT ATCAACAGAAACCTGGGAAAACAAATAAGCTTCTTATCTACGATGGGTCCACT TTGCAATCTGGAATTCATCGAGGTTCACTGGCAGTGGATCTGGTACAGATTT CACTCTCACCATCAGAAGCCTGGAGCCTGAAGATTTTGGGCTCTATTACTGTC AACAGCATCATGAACAGCCACCTACGTTCCGGCTCGGGGACGAAGTTGGAAATA AAA
119	RNF43-181 (VH prot)	QIQQLQQSGAELVKPGSSVKISCKSSAYRFTSNFIHWIRQQPGNGLEWIGWIYP GDGDAENNQKFNKGKILTADKSSSTAYMQLSSLTSEDSAVYFCARGVMTSLWY FDFWGPMTMTVSS
120	RNF43-181 (VL prot)	DVQMTQSPYNLAASPGESVSINCKASKSISKYLAWYQQKPGKTNKLLIYDGST LQSGIPSRFSGSGSGTDFTLTIRSLPEPFDGLYYCQQHHEQPPFTFGSGTKLEI K
121	RNF43-186 (VH DNA)	CAGGTACAGCTACAGCAATCAGGGACTGAACTGGTGAAGCCTGCGTCTCAGT GAAAATTTCTGCAAGGCTTCTGGCTACACCTTCACCACTAACTATATGCACT GGATAAGGCAGCAGCCTGGAAATGGCCTTGAGTGGATTGGGTGGATTTATCCT GGAAATGATAATACTAAGTACAATCAAAAGTTTCGATGGGAAGGCAACACTCAC TGCAGACAAATCCTCCAGCACAGCCTATATGCAGCTCAGCAGCCTGACATCTG AGGACTCTGCAGTCTATTTCTGTGCAAGAGGGGGATTTCGACTACGGAGGGTAT TGGTTTGCTTACTGGGGCCAGGGCACTCCAGTCACTGTCTCTTCA
122	RNF43-186 (VL DNA)	GATATCCGGATGACACAGTCTCCAGCTTCCCTGTCTGCATCTCTGGGAGAAAC TGTC AACATCGAATGTCTATCAAGTGAAGACATTTACAGTGAATTAGCATGGT ATCAGCAGAAGCCAGGGAAATCTCCTCAGCTCCTGATGTATAATGGAATAGC TTGCAAAATGGGGTCCCTTCACGGTTTAGTGGCAGTGGATCTGGCACACAATA TTCTCTAAAAATAAACAGCCTGCAATCTGAAGATGTGCGCACTTATTTCTGTC AACAAATATAGAAATTATCCATTACGTTCCGGCTCAGGGACGAAGTTGGAAATA AAA
123	RNF43-186 (VH prot)	QVQLQQSGTELVKPASSVKISCKASGYTFITNFMHWIRQQPGNGLEWIGWIYP GNDNTKYNQKFDGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGGFYDGGY WFAWVGQTPVTVSS
124	RNF43-186 (VL prot)	DIRMTQSPASLSASLGETVNI ECLSSEDIYSELAWYQQKPGKSPQLLMYNGNS LQNGVPSRFSGSGSGTQYSLKINSLSQSEDAVATYFCQQYRNPFTFGSGTKLEI K
125	RNF43-187 (VH DNA)	CAGGTCCAGCTGCAGCAATCTGGGGCTGAACTGGTGAAGCCTGGGTCTCAGT GAAGATTTCTGCAAGGCTTCTGGCTACACCTTCACCACTAACTTTATGCACT GGATAAACAGCAGCCTGGAAATGGCTTTGAGTGGATTGGGTGGATTTATCCT GGAGATGGTGATACAGACTACAATCAAAAGTTCAATGGGAGGGCAACACTCAC TGCAGACAAATCCTCCAGCACAGCCTATATGCAGCTCAGCAGCCTGACATCTG AGGACTCTGCAGTCTATTTCTGTGCAAGATGGGCGGGCCATCGTGAAGATTGG TTTCTTACTGGGGCCAAGGCACCTCTGGTCACTGTCTCTTCA
126	RNF43-187 (VL DNA)	GACATTGTGATGACCCAATCTCCATCCTCCCTGGCTGTGTGTCAGCAGGAGAGAC GGTCACTATAAACTGCAAGTCCAGTCAGAGTCTTTTATCCAGTGGAAACCAAA AGAACTACTTGGCCTGGTACCAGCAGAAACAGGGCAGTCTCTAACTGCTG ATCTACTTGGCATCCACTAGGGAATCTGGAGTCCCTGATCGCTTCATAGGCAG TGGATCTGGGACAGACTTCACCTGACCATCAGCAGTGTGCAGGCTGAAGATC TGGCAGACTATTACTGTGAGCAGCATACACCTATCCGAACACGTTTGGACCT GGGACCAAGTTGGAAGTGA
127	RNF43-187 (VH prot)	QVQLQQSGAELVKPGSSVKISCKASGYTFITNFMHWIKQQPGNGFEWIGWIYP GDGDTDYNQKFNKRATLTADKSSSTAYMQLSSLTSEDSAVYFCARWAGHREDW FPYWGQGLTVTVSS
128	RNF43-187 (VL prot)	DIVMTQSPSSLAIVSAGETVTINCKSSQSLLSSGNQKNYLAWYQQKPGQSPKLL IYLASTRESGVPDRFIFGSGSGTDFTLTISVQAEDLADYYCQQHYTYPNFTFGP GTKLELK
129	RNF43-196 (VH DNA)	CAGGTACAGCTGCAGCAATCTGGGGCTGAACTGGTGAAGCCTGGGTCTCAGT GAAAATTTCTGCAAGGCTTCTGGCTTACCTTCACCACTAACTTTATGCACT GGATAAACAGCAGCCTGGAAATGGCCTTGAGTGGATTGGGTGGATTTATCCT GGAGATGGTGATACAGACTACAATCAAAAGTTCAATGGGAAGGCAACTCTCAC

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		TGCAGACAAATCCTCCAGCACAGCCTATATGGAGCTCAGCAGCCTGACATCTG AGGACTCTGCAGTCTATTTCTGTGCAAGAGCGTACTACGGTAGCTACTGGTAC TTTGACTTCTGGGGCCAGGAACCAAGGTCACCGTGTCTCA
130	RNF43-196 (VL DNA)	GATGTCCAGATGACCCAGTCTCCACATAATCTTGCTGCCTCTCCTGGAGAAAG TGTTTCCATCAATTGCAAGGCAAGTAAGAGCATTAGCAAGTATTTAGCCTGGT ATCAGCAGAAACCTGGGAAAGTAAAAAGCTTCTTATCTACGGTGGGTCAACT TTGCAATCTGGAATTCCATCGAGGTTCAAGTGGCAGTGGATCTGGTACAGATTT CACTCTCACCATCAGCAGCCTGGAGCCTGAAGATTTTGGACTCTATTACTGTC AACAGCATAATGAATACCCACCGACGTTCCGTGGAGGCACCAGGCTGGAATTG CAA
131	RNF43-196 (VH prot)	QVQLQQSGAELVKPGSSVKISCKASGFTFTSNFMHWIKQQPGNGLEWIGWIYP GDGDTDYNQKFNKATLTADKSSSTAYMELSSLTSEDSAVYFCARAYYSYWY FDWGPQTKVTVSS
132	RNF43-196 (VL prot)	DVQMTQSPHNLAASPGESVSINCKASKSISKYLAWYQQKPGKVKLLIYGGST LQSGIPSRFSGSGSGTDFTLTITSSLEPEDFGLYYCQQHNEYPPFTGGGTRLEL Q
133	RNF43-200 (VH DNA)	CAGGTGCAGCTGAAGGAGTCAGGACCTGGTCTGGTGCAGCCCTCACAGACTTT GTCTCTCACCTGCACTGTCTCTGGGTTCTCACTAACAGCTATCATATAAGCT GGGTTCCGCGAGCCTCCAGGAAAAGGTCCTGGAGTGGATGGGAGTAATATGGACT GGTGGAAGCACAGCATATAATTCACCTCTCAAATCCCGACTGAGCATCAGCAG GGACATCTCCAAGAGCCAAGTCTTCTTAAAAATGAACAGTCTGCAAGCTGAAG ACACAGCCACTTACTACTGTGCCGGATCATACTACGGGTATACCTCCTACTTT GATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCTCTCA
134	RNF43-200 (VL DNA)	GACATCCAGATGACACAGTCTCCTGCTTCCCTGTCTGCGTCTCCGGAAGAAAT TGTCACGATCACATGCCAGGCAAGCCAGGACATTGATAATTGGTTAGCATGGT ATCAGCAGAAACCAGGGAAATCTCCTCAACTCCTGATCTATAGTGAACCCAGG TTGGCAGACGGGATCCCATCAAGGTTCAAGCGGAGTAGATCTGGTATACAGTA TTCTCTTAAGATCAACAGACTGCAGGTTGAAGATACTGGAATCTATTACTGTC TACAGCGTTTTGGTAATCCTTGGACGTTCCGTGGAGGCACCAAGCTGGAATTG AAA
135	RNF43-200 (VH prot)	QVQLKESGPGVLVPSQTLSTCTVSGFSLTSYHISWVRQPPGKGLEWMGVIWT GGSTAYNSLLKSRLSISRDISKSQVFLKMNSLQAEDTATYYCAGSYGYTSYF DYWGQGVMTVSS
136	RNF43-200 (VL prot)	DIQMTQSPASLSASPEEIVTITCQASQDIDNWLAWYQQKPGKSPQLLIYSATR LADGIPSRFSGSRSGIQYSLKINRLQVEDTGIYYCLQRFGNPWTFGGGTKLEL K
137	RNF43-201 (VH DNA)	CAGGTTACTCTGAAAGAGTATGGCCCTGGGATATTGCAGCCCTCCCAGACCCT CAGTCTGACTTGCATTTCTCTGGGTTTTCACTGAGGACTTATGGTATGGGTG TGGGCTGGATTGCTCAGCCTTCAAGGAAGGCTCTGGAGTGGCTGACAAATATT TGGTGGGATGATGATAAGTACTACAATCCATCTCTGAAAAACCGGCTCACAAT CTCCAAGGACACCTCCAACAACCAAGCATTCCTCAAGATCACCATGTGGACA CTGAGGATAGTGCCACATACTATTGTGCTCGGATAGACGGTACTTTTAACTAC TTTGATTATTGGGGCCAAGGAGTCATGGTCACAGTCTCTCTCA
138	RNF43-201 (VL DNA)	GATGTTGTGCTGACCCAGACTCCTTCCAGATTGTCTGCTACCATTGGACAATC GGTCTCCATCTCTTGCAGGTCAAGTCAGAGTCTCTTAGATAGTGATGGAAACA CCTATTTATATTGGTTCCTACAGAGGCCAGGCCAATCTCCACAGCGTCAATT TATTTGGTATCCAACCTGGGATCTGGGGTCCCCAACAGGGTCAGTGGCAGTGG GTCAGGAACAGATTTACACTCAAAATCAGTGGAGTGGAGGCTGAGGATTTGG GAATTTATTACTGCTTACAAGCTACCCATGCTCCTCTCACGTTCCGGTCTGGG ACCAAGCTGGAGATCAAA
139	RNF43-201 (VH prot)	QVTLKEYGPGILQPSQTLSTCTFSGFSLRTYGMGVGWIRQPSGKLEWLTNI WWDDDKYYNPSLKNRLTISKDTSNNQAFLEKITNVDTEDSATYYCARIDGTFNY FDYWGQGVMTVSS
140	RNF43-201 (VL prot)	DVVLVTQTPSRLSATIGQSVSISCRSSQSLLDSGNTYLYWFLQRPQSPQRLI YLVSNLGSVPNRVSGSGSGTDFTLKISGVEAEDLGIYYCLQATHAPLTFGSG TKLEIK
141	RNF43-206 (VH DNA)	CAGGTGCAGCTGAAGGAGTCAGGACCTGGTCTGGTGCAGCCCTCACAGACTTT GTCTCTCACCTGCACTGTCTCTGGGTTCTCACTAACAGTTTTTCATATAAGCT GGGTTCCGCGAGCCTCCAGGAAAAGGTCCTGGAGTGGATGGGAATAATATGACT GGTGAAGCACAGCATATAATTCACCTTCTCAAATCCCGACTGAGCATCAGCAG

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		GGACATCTCCAAGAGCCAAGTCTTCTTAAAAATGAACAGTCTGCAAACCTGAAG ACACAGCCACTTACTACTGTGCCGGATCATATCACGGGTATACCTCCTACTTT GATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
142	RNF43-206 (VL DNA)	GACATCCAGATGACTCAGTCTCCTGCTTCCCTGTCTGCGTCTCCGGAAGAAAT TGTCACGATCACATGCCAGGCAAGCCAGGACATTGATAATTGGTTAGCATGGT ATCAGCAGCAACCAGGGAAATCTCCTCAACTCCTGATCTATAGTGCAACTAGC TTGGCAGACGGGATCCCATCAAGGTTGAGCGGCAGTAGATCTGGTACACAGTA TTCTCTTAAGATCAACAGACTACAGGTTGAAGATACTGGAATCTATTACTGTC TACAGCGTTATAGTAATCCTTGGACGTTCCGTGGAGGCACCAAGCTGGAATTG AAA
143	RNF43-206 (VH prot)	QVQLKESGPGVLVQPSQTLSTCTVSGFSLTSFHI SWVRQPPGKGLEWMGI IWT GGSTAYNSLLKSRLSISRDISKSQVFLKMNSLQTEDTATYYCAGSYHYGTSYF DYWGQGVMVTVSS
144	RNF43-206 (VL prot)	DIQMTQSPASLSASPEEIVTITCQASQDIDNWLAWYQQQPGKSPQLLIYSATS LADGIPSRFSGSRSGTQYSLKINRLQVEDTGIYYCLQRYSNPWTFGGGTKLEL K
145	RNF43-210 (VH DNA)	CAGATCCAGTTGGTACAGTCTGGACCTGAGCTGAAGAAGCCTGAAGAGTCAGT GAAGATCTCCTGCAAGGCTTCTGGGTATATCTTCACAGACTATTTAATACACT GGGTGAAGCAGGCTCCAGGAGAGGGCTTGAAGTGGATGGGCTGGATCAACACC TATACCTGGGAAGCCAACATATGCTGATGACTTCAAAGGACGGTTTGTCTTTTC TTTGGAAAGCCTCTGCCAGCACTGCAATTTTGCAGATCAGCAACCTCAAAATG AGGACACGGCTACATATTTCTGTGCTCTGGGCCCCATTACTACAGTCGTCTAT GTCATGGATGCCTGGGGTCAAGGAACCTCAGTCACTGTCTCCTCA
146	RNF43-210 (VL DNA)	GACATCCAGATGACCCAGTCTCCTTCATTCCTGTCTGCATCTGTGGGAGACAG AGTCACATCAACTGCAAAGCAAGTCAGAATATTAACAAGTACTTAACTGGT ATCAGCAAAAGCTTGGAGAAGCTCCCAAACGCCTGATATATAATACAAACAAT TTGCAAACAGACATCCCATCAAGGTTGAGTGGCAGTGGATCTGGTGCAGATTA CACACTCACCATCAACAGCCTGCAGCCTGAAGATTTTGCCACATATTTCTGCT TGCAGCATAATAGTTTTCCGTACACGTTTGGAGCTGGGACCAAACTGGAACCTG AAA
147	RNF43-210 (VH prot)	QIQLVQSGPELKKPEESVKISCKASGYIFTDYLIHWVKQAPGEGLEKWMGWINT YTGKPTYADDFKGRFVFSLEASASTAILQISNLKNEATATYFCALGPITTVVY VMDAWQGQTSVTVSS
148	RNF43-210 (VL prot)	DIQMTQSPSFLSASVGDRTINCKASQNINKYLNWYQQKLGEAPKRLIYNTNN LQTDIPSRFSGSGSGADYTLTINSLQPEDFATYFCLQHNSFPYTFGAGTKLEL K
149	RNF43-213 (VH DNA)	CAGGTGCAGCTGAAGGAGTCAGGACCTGGTCTGGTGCAGCCCTCACAGACTTT GTCTCTCACCTGCACTGTCTCTGGGTTCTCACTAACAGGTATCATGTAAGCT GGGTTCCGCGAGCCTCCAGAAAAGTCTGGAGTGGATGGGAGTAATATGGACT GGTGGAAAGCACAGCATATAATTCATTCTCAAATCCCGACTGAGCATCAGCAG GGACATCTCCAAGAGCCAAGTTTCTTAAAAATGAACAGTCTGCAAACCTGAAG ACACAGCCACTTACTACTGTGCCAGTCAATACTACGGGTATACCTCCTACTTT GATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCGCA
150	RNF43-213 (VL DNA)	GACATCCAGATGACACAGTCTCCTGCTTCCCTGTCTGCGTCTCCGGAAGAAAT TGTCACGATCACATGCCAGGCAAGCCAGGACATTGCTAATTGGTTAGCATGGT ATCAGCAGAAACCAGGGAAATCTCCTCAACTCCTGATCTATAGTGCAACCAAC TTGGCAGACGGGATCCCATCAAGGTTGAGCGGCAGTAGATCTGGTACACAGTA TTCTCTTAAGATCAGCAGACTACAGGTTGAAGATACTGGAGTCTATTACTGTC TACAGCGTTATGGTAATCCGTGGACGTTCCGTGGAGGCACCAACCTGGAATTG AAA
151	RNF43-213 (VH prot)	QVQLKESGPGVLVQPSQTLSTCTVSGFSLTRYHVSWSVRQPPGKGLEWMGVIWT GGSTAYNSLLKSRLSISRDISKSQVFLKMNSLQTEDTATYYCASQYYGTSYF DYWGQGVMVTVSA
152	RNF43-213 (VL prot)	DIQMTQSPASLSASPEEIVTITCQASQDIANWLAWYQQKPGKSPQLLIYSATN LADGIPSRFSGSRSGTQYSLKISRLQVEDTGVIYCLQRYGNPWTFGGGTNLEL K
153	RNF43-217 (VH DNA)	GAGGTGCAGCTTCAGGAGTCAGGACCTGGCCTTGTGAAACCCTCACAGTCACT CTCCCTCACCTGTTCTGTCACTGGTTTCTCCATCACCAGTAGTCATAGATGGA ACTGGATCCGGAAGTTCCAGGAAATAAACTGGAGTGGATGGGATATATAAAC AGTGCAGGCAGCACTAACTATAACCCGCTCTCTCAAAGTCGAGTCTCCATTAC

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		TAGAGACGCATCCAAGAATCAGTTCTTCCTGCAGGTGAACTCTGTAACACTG AGGACACAGCCACTTATTACTGTACGAGATTAGGACTACAGTATCGGGACTTT GATTACTGGGGCCAGGGAGTCATGGTCACAGTCTCCTCA
154	RNF43-217 (VL DNA)	GACATCCAGATGACACAGTCTCCAGCTTCCCTGTCTGCATCTCTGGGAGAAAC TATCTCCATCGAATGTGAGCAAGTGACGACATTTACAGTAATTTAGCGTGGT ATCAGCAGAAGTCAGGGAAATCTCCTCAGCTCCTGATCTATGCTGCAATAGG TTGAAAGATGGGGTCCCATCACGGTTCAGTGGCAGTGGGTCTGGCACACAGTA TTCTCTCAAGATCAGCGGCATGGAACTGAAGATGAAGGGGATTATTTCTGTC TACAGGCTTCCAAGTTTCCGCTCACGTTCCGTTCTGGGACCAAGCTGGAGATC AAA
155	RNF43-217 (VH prot)	EVQLQESGPGLVKPSQSLSLTCSVTGFSITSSHRWNWIRKFPGNKLEWMGYIN SAGSTNYPNLSKRSVITRDASKNQFFLQVNSVTTEDTATYYCTRLGLQYRDF DYWGQGVMTVSS
156	RNF43-217 (VL prot)	DIQMTQSPASLSASLGETISIECRASDDIYSNLAWYQQKSGKSPQLLIYAANR LKDGVPSRFSGSGSGTQYSLKISGMETEDEGDYFCLQASKFPLTFGSGTKLEI K
157	RNF43-221 (VH DNA)	GAGGTGCAGCTGGTGGAGTCTGGCGGAGGCTTAGTGCAGCCTGGAAGGTCCCT AAACTCTCCTGTACAGCCTCAGGATTCAGTCTCAGTGACTATGCCATGGCCT GGGTCCGCCAGGCTCCAGAGAAAGTCTGGAATGGGTGCAACCATAAGTTAT GATGGTAGTAGAACTTACTATGGAGGCTCCGTGAAGGCCGATTCACTATCTC CAGAGATAATGCAGAAAATATCCTATACCTGCAAATGGACAGTCTGAGGCTG CGGACACGGCCACTTATTATTGTGCAAACCTCAGCAACTCCCCATTACTGGGGC CAAGGAGTCATGGTCACAGTCTCCTCA
158	RNF43-221 (VL DNA)	GATGTTGTGATGACCCAGACTCCAGCGTCTTTGTGCGTTGCCATTGGACAACC AGCCTCCATCTCTTGCAAGTCAAGCCAGAGCCTCTTAGATACTAGTCGAAAGA CATTTTTGAATTGGATATTACAGAGGCCCGGCCAGTCTCAAAGCGCCTAATC TATCAGGTTTCCAACTGTACTCAGAGGTTCTGATAGGTTCAAGTGGCAGTGG ATCAGAGACAGAGTTTACTCTTAAATCAGCAGAGTGGAGGCTGAGGATTTGG GAGTGTATTACTGCTGGCAAGGTACACATTTCCGGACACGTTTGGAGCTGGG ACCAAGCTGGAAGTGAAG
159	RNF43-221 (VH prot)	EVQLVESGGGLVQPGRSLKLSCTASGFTLSDYAMAWVRQAPKGLWVATISY DGSRTYYGGSVKGRFTISRDNENILYLQMDSLRSADTATYYCANSATPHYWG QGVMVTVSS
160	RNF43-221 (VL prot)	DVVMVTQTPASLSLAIGQPASISCKSSQSLLDTSRKTFNLNWLQRPQSPKRLI YQVSKLYSEVPDRFSGSGSETEFTLKISRVEAEDLGVYYCWQGHFPTDFGAG TKLELK
161	RNF43-224 (VH DNA)	GAGGTGCAGCTGAAGGAATCAGGACCTGGTCTGGTGCAGCCCTCACAGACCCT GTCCCTCACCTGCACTGTCTCTGGATTCTCATTGTGCGGACTACAGTGACTACT GGGTTCGCCAGCCTCCAGGAAAAGTCTGGAGTGGATGGGAGTTATGTGGAGT GGTGGAAACACAGCATATAATTACAGCTCTCAAATCCCGACTGAGCATCAGCAG GGACACCTCCAAGAACCAAGTTCTCTTAGAAATGAACAGTCTGCAAACGAAG ACACAGCCATTTACTACTGTACCAGAGGGGGCAAATATGATGGTACTTATTAC TACGGCTGGTACTTTGACTTCTGGGGCCAGGAACCATGGTCACCGTGTCTCTC A
162	RNF43-224 (VL DNA)	GACGTTGTGCTGACTCAGTCTCCAGCCACCCTGTCTGTGACTCCAGGAGAAAG AATCAGTCTCTCCTGTGCGGGCCAGCGAGAGTGTGACACTTATCTATCCTGGT ATCAGCAAAAACCAATGAGTCTCCAAGGCTTCTCATTAAAGTATGCTTCCAG TCCATCTCTGGAATTCCTCCAGGTTCAAGTGGCAGTGGATCTGGGACAGATTT CACTCTCAGTATCAATGGAGTAGAGCTTGAAGATTTATCAATTTATTACTGTC AACAGGGTAACAGCTTGCCCCCTCACGTTCCGCTCAGGGACGAAGTTGGAAATA AAA
163	RNF43-224 (VH prot)	EVQLKESGPGLVQPSQTLSTCTVSGFSLSDYSVHWVRQPPGKLEWMGMVWS GGTTAYNSALKSRLSISRDTSKNQVLEMNLSLQTEDTAIYYCTRGKYDGTYY YGWYFDFWGPMTMTVSS
164	RNF43-224 (VL prot)	DVVLTSQSPATLSVTPGERISLSCRASEVDYLSWYQQKPNESPRLLIKYASQ SISGIPSRFSGSGSGTDFTLSINGVELEDLSIYYCQQGNSLPLTFGSGTKLEI K
165	RNF43-25 (VH DNA)	CAGGTCAAGCTGCTGCAGTCTGGGGCTGCACTGGTGAAGCCTGGGACTCTGT GAAGATGTCTTGCAAAGCTTCTGGTTATACATTCACTGACTACATTATACATT GGGTGAAGCAGAGTCATGGAAGGAGCCTTGAGTGGATTGCTTATATTAATCCT

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		TACAGTGGTAGTACTAATACTACAATGAAAAGTTCAAGAGCAAGGCCACATTGAC TGTAGACAGATCCAACAACACAGCCTATATGGAGTTTAGCAGATTGACATCTG AGGATTCTGCAATCTACTACTGTGCAAGAAAGAACTACGGAGGTTATGGAGTT ATGGATGCCTGGGGTCAAGGAGCTTCAGTCACTGTCTCTCTCA
166	RNF43-25 (VL DNA)	GACATCCAGATGACCCAGTCTCCTTCACTCCTGTCTGCATCTGTGGGAGACAG AGTCACTCTCAGCTGCAAAGCAAGTCAGAATATTTATAAGAACTTAGCCTGGC ATCAGCAAAAGCTTGGAGAAGCTCCCAAACCTCTGATTTATTATGCAAACACC TTGCAAACGGGCATCCCATCAAGGTTCAAGTGGCAGTGGATTTGGTACAGATTT CACACTCACAATCAGCAGCCTGCAGCCTGAAGATGTTGCCACATATTTCTGCC AGCAGTCTTCTAGCGGGTGGACGTTTCGGTGGAGGCCAAGCTGGAATTGAAA
167	RNF43-25 (VH prot)	QVKLLQSGAALVKPGDSVKMSCKASGYTFDYIIHWVKQSHGRSLEWIAIYNP YSGSTNYNEKFKSKATLTVDRSNNTAYMEFSRLTSEDSAIYYCARKNYGGYGV MDAWGQGASVTVSS
168	RNF43-25 (VL prot)	DIQMTQSPSLLSASVGDRTLSCKASQNIYKNLAWHQQLGEAPKLLIYYANT LQTGIPSRFSGSGFGTDFTLTITSSLPEDVATYFCQQSSSGWTFGGGTKELELK
169	RNF43-31 (VH DNA)	GCGGTACAGCTGGTGGAGTCTGGGGGAGGCTCAGTGCAGCCTGGAAGTCCAT GCGGCTCTCCTGCGCAGCCTCAGGATTCAGTTTTCAGTAACATGACATGACCT GGGTCCGCCAGGCTCCAACGAAGGGTCTGGAGTGGGTGCGATCCATTACTTAT GATGGTGGTAGTACTTACTCTCGAGACTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATGCAAAAAGCACCCCTATACCTGCAAATGGACAGTCTGAGGTCTG AGGACACGGCCACTTATTACTGTACAACAGATCGGGGTAGATACCTACCTTAC TACTTTGATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCGCA
170	RNF43-31 (VL DNA)	GATGTTGTGCTGACACAACTCCAGTTTCCCTGTCTGTACACTTGGAGATCA AGCTTCTATATCTTGCAGGTCTAGTCAGAGCCTGGAATATAGTGATGGATATA GTTATTTGGAATGGTACCTACAGAAGCCAGGCCAGTCTCCACAGCTCCTCATC TATGAAGTTTCCAGCCGATTTTCTGGGGTCCCGACAGGTTTATTGGCAGTGG GTCAGGGACAGATTTACCCCTCAAGATCAGCAGAGTAGAGCCTGAGGACTTGG GAGTTTATTACTGCTTCCAAGCTATACATGATCCCACGTTTGGAGCTGGGACC AAGCTGGAAGTGA
171	RNF43-31 (VH prot)	AVQLVESGGGVQPGSRMRLSCAASGFTFSNYDMTWVRQAPTGLEWVASITY DGGSTYSRDSVKGRFTISRDNKSTLYLQMDSLRSEDATYYCTTDRGRYLPY YFDYWGGQVMVTVSA
172	RNF43-31 (VL prot)	DVVLVTQTPVSLSVTLGDAQASISCRSSQSLEYS DGYSYLEWYLQKPGQSPQLLI YEVSSRFSGVPDRFISGSGTDFTLKISRVEPEDLGVIYCFQAIHDPFTFGAGT KLELEK
173	RNF43-33 (VH DNA)	CAGGAGCAGCTGAAGGAGTCAGGACCTGGTCTGGTGCAGCCCTCACAGACCT GTCCCTCACCTGCACTGTCTCTGGGTTCTCATTAACCAGCTATAATGTACACT GGGTTTCGACAGCCTCCAAGAAAGGGTCTGGAGTGGATGGGAAGAATGAGATAT AATGGAGACACATCATATAATTCAGCTCTCAAATCCAGACTGAGCATTAGCAG GGACACCTCCAAGAAGCAAGTTTTCTTAAAAATGAACAGTCTGCAAACCTGATG ACACAGGCACTTACTACTGTGCCAGAGGGCCCTCTGGGATTACTGGGGCCAA GGAGTCATGGTCACAGTCTCTCTCA
174	RNF43-33 (VL DNA)	GATGTTGTGCTGACCCAGACTCCACCCACTTTATCGGCTACCATTTGGACAATC AGTCTCCGTCTCTTGCAGGTCAAGTCAGAGTCTCTTACATAGTAATGGAAACA CCTATTTAAATTGGTTGCTACAGAGGCCAGGCCAACCTCCACAACCTTCTAATT TATTTGGTATCCAGACTGGAATCTGGGGTCCCCAACAGGTTCAAGTGGCAGTGG GTCAGGAAGTGAATTCACACTCAAATCAGTGGAGTAGAGGCTGAGGATTTGG GAGTTTATTACTGCGTGCAAAGTACCCATGCTCCGTACACGTTTGGAGCTGGG ACCAAGCTGGAAGTGA
175	RNF43-33 (VH prot)	QEQLKESGPGVLVQPSQTLSTCTVSGFSLTSYNVHWVRQPPRKGLEWMGRMR NGDTSYNSALKSRLSISRDTSKNQVFLKMNSLQTDGTYYCARGPLWDYWGQ GVMVTVSS
176	RNF43-33 (VL prot)	DVVLVTQTPPTLSATIGQSVSVSCRSSQSLLHSNGNTYLNWLLQRPQPPQLLI YLVSRLESVGNRFSGSGTDFTLKISGVEAEDLGVIYCVQSTHAPYTFGAG TKLELEK
177	RNF43-35 (VH DNA)	CAGGTACAGTTGAAGGAGTCAGGACCTGGTCTGGTGCAGCCCTCGCAGACCT GTCCCTCACCTGCACTGTCTCTGGGTTCTCACTAACCACTACAGTGTCCACT GGGTTTCGCCAACATTGAGAAAGAATCTGGAATGGATGGGAAGAATGTGACT GCTGGAGACACATCATATAATTCAGCGTTCACATCCCGACTGAACATCTTCAG GGACACCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACCTGAGG

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		ACACAGGCACTTACTACTGTGCCAGATCCTCCTATACTTCGGGTACCCTTTT GATTCCTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
178	RNF43-35 (VL DNA)	GACACTGTACTGACCCAGTCTCCTGCTTTGGCTGTGTCTCCAGGAGAGAGGGT TACCATCTCCTGTAGGGCCAGTGAAAGTGTCACTAACTTATGCACTGGTACC AACAGAGACCAGGACAACAACCCCACTCCTCATCTATCTAACATCACACCTA GCATCTGGGGTCCCTGCCAGGTTCACTGGCAGTGGGTCTGGGACAGACTTCAC CCTCACCATTGATCCTGTGGAGGCTGATGACACTGCAACCTATTACTGTCAGC AGAGTCGGAATGATCCACGTTTGGAGCTGGGACCAAGCTGGAAGTAAA
179	RNF43-35 (VH prot)	QVQLKESGPGLVQPSQTLSTCTVSGFSLTTYSVHWVRQHSKGNLEWMGRMWT AGDTSYNSAFTSRNLNIFRDTSKSKQVFLKMNSLQTEDTGTYTCARSSYTSGYPF DSWGQGVMTVSS
180	RNF43-35 (VL prot)	DTVLTQSPALAVSPGERVTISCRASESVSKLMHWYQQRPGQQPQLLIYLTSHL ASGVPARFSGSGSGTDFTLTIDPVEADDTATYYCQQSRNDPTFGAGTKLELK
181	RNF43-38 (VH DNA)	GAAGTACAGCTTGTGGAGTCTGGAGGAGGCTTGGTGCAACCTGGGACTTCTCT GAAAATCTCTTGTGTAGCCTCGGGATTCACTTTCAGTGACTACTGGATGAGCT GGGTTCGCCAGACTCCTGGAAAGACCATGGAGTGGATTGGAGATATTAAATCT GATGGCAGTTACACAACTATGCACCATCCCTAAAGAATCGATTACAAATCTC CAGAGACAATGCCAAGAGCACCTGTACCTGCAGATGAACAATGTGAGATCTG AAGACACAGCCACTTATTACTGTACTAGAGATGCATATAGGTACAACCTACGTC CACTATGGTTTGGATGCCTGGGGCCAAGGAGCTTCAGTCACTGTCTCCTCA
182	RNF43-38 (VL DNA)	CAGTTTGTGCTTACTCAGCCAACTCTGTGTCTGCGAATCTCGGAAGCACAGT CAAACGTCTTGCAATCGCAGCACTGGTAACATTGGAAGCAATTATGTGAGCT GGTACCAGCAGCATGAGGGAAGATCTCCCACTATGCTTTACAGGGATGGT AAGAGACCAGATGGAATTCCTGACAGGTTCTCTGGCTCCATTGACGGATCTTC CAACTCAGCCCTCCTGACAATCAACAATGTGCAGACTGAAGATGAAGTGAAGT ACTTCTGTCACTCTTACAGTAGTGGTATTAAGTATATTTTTCGGCGGTGGAACC AAGCTCACTGTCTCA
183	RNF43-38 (VH prot)	EVQLVESGGGLVQPGTSLKISCVASGFTFSQDYWMSWVRQTPGKTMIEWIGDIKS DGSYTNYPASLKNRFTISRDNASTLYLQMNVRSEDTATYYCTRDAYRNYV HYGLDAWGQGASVTVSS
184	RNF43-38 (VL prot)	QFVLTQPNVSVANLGSITVKLSNRSTGNIGSNYVSWYQQHEGRSPPTMLYRDG KRPDGIIPDRFSGSIDGSSNSALLTINNVQTEDEADYFCQSYSSGIKYIFGGGT KLTVL
185	RNF43-41 (VH DNA)	GAGGTGCACCTGGTGGAGTCTGGGGGAGGCTTAGTGACGCTGGAAGGTCCGT GAACTCTCCTGTACAGCCTCAGGATTCAGTTTCAGTTACTATGACATGGCCT GGGTCCGCCAGGCTCCAACGAAGGGTCTGGAGTGGGTTCGCATCCCTTACTTAT GATGGTAGTAGCACTTACTATCGAGACTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATGCAAAAAGCACCTTATACCTGCAATGGACAGTCTGAGGTCTG AGGACACGGCCACTTATTACTGTACAACAGATCGGGGTAGATACCTACCTAC CACTTTGATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
186	RNF43-41 (VL DNA)	GATGTTGTGTTGACACAACTCCAGTTTCCCTGTCTGTACACTTGGAGATCA AGCTTCTATATCTTGACAGGTCTAGTCAGAGCCTGGAATATAGTGATGGATACA CTTATTTGGAATGGTACCTACAGAAGCCAGGCCAGTCTCCACAGCTCCTCATC TATGAAGTTCCACCCGATTTTCTGGGGTCCCAGACAGGTTCAATTGGCAGTGG GTCAGGGACAGATTTACCCCTTAAGATCAGCAGAGTAGAGCCTGAGGACTTGG GAGTTTATTACTGCTTCCAAGCTACACATGATCCCACGTTTGGAGCTGGGACC AAGCTCGAACTGAAA
187	RNF43-41 (VH prot)	EVHLVESGGGLVQPGRSVKLSCTASGFTFSYDMAWVRQAPTKGLEWVASLTY DGSSTYYRDSVKGRFTISRDNASTLYLQMDSLRSEDTATYYCTTDRGRYLPY HFDYWGQGVMTVSS
188	RNF43-41 (VL prot)	DVVLVTQTPVSLSVTLGDAQSISCRSSQSLEYSDGYTYLEWYLQKPGQSPQLLI YEVSTRFSGVPDRFIGSGSGTDFTLKISRVEPEDLGVYYCFQATHDPTFGAGT KLELK
189	RNF43-53 (VH DNA)	GAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTAGTGACGCTGGAAGGTCCCT GAACTCTCCTGTGGAGCCTCAGGATTCAGTTTCAGTACCTATTACATGGTCT GGGTCCGCCAGGCTCCAACGAAGGGTCTGGAGTGGGTTCGCATCCATTAGTCTC AATGGTGGTAACACTTACTATCCAGACTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATGCAAAAAGCACCTTATACCTGCAATGGACAGTCTGAGGTCTG AGGACACGGCCACTTATTACTGTGCAACAGATGGGTGCGTATTACTATGATGGT TATCGGTGGCTTGCTTACTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA

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190	RNF43-53 (VL DNA)	CAGTTTGTGCTTACTCAGCCAAACTCTGTGTCTACGAATCTCGGAAGCACAGT CAAACGTCTTGCAAGCGCAGCACTGGTAACATTGGAAGCAATTATGTGAGCT GGTACCAGCAGCATGAGGGAAGATCTCCCACCACTCTGATTTATAGGGATGAT AAGAGACCAGATGGAGTTCCTGACAGGTTCTCTGGCTCCATTGACAGATCTTC CAACTCAGCCCTCCTGACAATCAATAATGTGCAGACTGAAGATGAAGCTGGCT ACTTCTGTCACTCTTTCAGTAGTGGTCTGTTTATTTTCGGCGGTGGAACCAAG CTCACTGTCTCA
191	RNF43-53 (VH prot)	EVQLVESGGGLVQPGRSLKLSCGASGFSFSTYYMVVWRQAPTKGLEWVASISP NGGNTYYPDSVKGRFTISRDNASTQYLQMDSLRSEDATATYYCATDGSYYDYG YRWLAYWGQGLVTVSS
192	RNF43-53 (VL prot)	QFVLTQPNVSTNLGSTVKLSCKRSTGNIGSNYVSWYQQHEGRSPPTLIYRDD KRPDGVDPDRFSGSIDRSSNSALLTINNVTQTEDEAGYFCQSFSSGLFIFGGGTK LTVL
193	RNF43-56 (VH DNA)	GAAGTACAGCTTGTGGAGTCTGGAGGAGGCTTGGTGCAACCTGGGACTTCTCT GAAAATCTCTTGTGTAGCCTCGGGATTCACTTTCAGTGACTACTGGATGAGCT GGGTTCGCCAGACTCCTGGAAGGACCATGGAGTGGATTGGAGATATTAAATAT GATGGCAGTTACACAACTATGCACCATCCCTAAAGAATCGATTACAAATCTC CAGAGACAATGCCAAGAGTATCCTGTACCTGCAGATGAACAATGTGAGATCTG AGGACACAGCCACTTATTACTGTACTAGAGATGCATATGGGTACAACATACGTC CACTATGGTTTGGATGCCTGGGGCCAAGGAGCTTCAGTCACTGTCTCTCTCA
194	RNF43-56 (VL DNA)	CAGTTTGTGCTTACTCAGCCAAACTCTGTGTCTTCAATCTCGGAAGCACAGT CAAACGTCTTGCAATCGCAGCACTGGTAACATTGGAAGCAATTATGTGAGTT GGTACCAGCAACATGAGGGAAGATCTCCCACCACTATGATTTATAGGGATGAT AAGAGACCAGATGGAATTCCTGACAGGTTCTCTGGCTCCATTGACGGATCTTC CAACTCGGCCCTCCTGACAATCAACAATGTGCAGACTGAAGATGAAGCTGACT ACTTCTGTCACTCTTACAGTAGTGATATTAAGTATATTTTTCGGCGGTGGAACC AAGCTCACTGTCTCA
195	RNF43-56 (VH prot)	EVQLVESGGGLVQPGTSLKISCVASGFTFSDYWMSWVRQTPGRMTEWIGDIKY DGSYTNYPAPSLKNRFTISRDNASILYLQMNVRSEDATATYYCTRDAGYNYV HYGLDAWGQGASVTVSS
196	RNF43-56 (VL prot)	QFVLTQPNVSSNLGSTVKLSNCRSTGNIGSNYVSWYQQHEGRSPPTMIYRDD KRPDGIPDRFSGSIDGSSNSALLTINNVTQTEDEADYFCQSYSSDIKYIFGGGT KLTVL
197	RNF43-61 (VH DNA)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGACGCTGGAAGGTCCCT GACACTCTCTGTGCAGCCTCAGGATTCATTTTCAGTGACTATTACATGGCCT GGGTCCGCCAGGCTCCAAAGAAGGGTCTGGAGTGGGTGCGATCCATTACTTAT GAGGGCAGTAGTACCTACTACGGAGACTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATACAAAAGCACCCATATCTGCTAATGAACAGTCTGAGGTCTG ACGACTCGGCCACTTACTATTGCGCAAGACATGGGGCACACTATACATATAAT TACGCCTACTACTTTGATTACTGGGGCCAGGAGTCATGGTCACAGTCTCCTC A
198	RNF43-61 (VL DNA)	GACATCATGTGACTCAGTCTCCAACCTACCCTGTCTGTGATTCCAGGAGAGAA CATCAGTCTCTCCTGCAGGGCCAGTCAGAGTGTTGGTACTAACTTACATTGGT ATCAGCAAAAACCAAGTGAGTCTCCAAGGGTCTCATCAACTATGCTTCCAG TCCATCTCTGGAATCCCTCCAGGTTCACTGGCAGTGGATCAGGGACAGATTT CACTCTCAATATTAACAGAGTAGAGCCTGAAGATTCTTCAGTTTATTACTGTC AACAGAGTAAGAGCTGGCCATTACGTTCCGGCTCAGGAACGAAGTTGGAAATA AAA
199	RNF43-61 (VH prot)	EVQLVESGGDLVQPGRSLTLSCAASGFI FSDYYMAVWRQAPKKGLEWVASITY EGSSTYYGDSVKGRFTISRDNKSTLYLLMNSLRSDSATYYCARHGAHYTYN YAYYFDYWQGVMTVSS
200	RNF43-61 (VL prot)	DIMLTQSPTTSLVPIGENISLSCRASQSVGTNLHWYQQKPSESPRVLINIASQ SISGIPSRFSGSGSGTDFTLNINRVEPEDSSVYYCQQSKSWPFTFGSGTKLEI K
201	RNF43-67 (VH DNA)	GCGGTGCAGCTGGTGGAGTCTGGGGGAGGCTCAGTGCAGCCTGGAAGGTCCAT GAGGCTCTCCTGCGCAGCCTCAGGATTCATTTTCAGTAACATATGACATGACCT GGGTCCGCCAGGCTCCAACGAAGGGTCTGGAGTGGGTGCGATCCATTACTTCT GATGGTGGTAGTACTTACTCTCGAGACTCCGTGAAGGGCCGATTCATATCTC CAGAGATAATGCAAAAAGCACCCATATACCTGCAAAATGGACAGTCTGAGGTCTG

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		AGGACACGGCCACTTATTACTGTACAACAGATCGGGGTAGATACCTACCCTACTACTTTGATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCGCA
202	RNF43-67 (VL DNA)	GATGTTGTGCTGACACAACTCCAGTTTCCCTGTCTGTACAGTTGGAGATCAAGCTTCTATATCTTGACAGGTCTAGTCAGAGCCTGGAATATAGTGATGGATATAGTTATTTGGAATGGTACCTACAGAAGCCAGGCCAGTCTCCACAGCTCCTCATCTATGAAGTTCCAGCCGATTTTCTGGGGTCCCAGACAGGTTTCATTGGCAGTGGTTCAGGGACAGATTTACCCCTCAAGATCAGCAGAGTAGAGCCTGAGGACTTGGAGTTTATTACTGCTTCCAAGCTATACATGATCCCACGTTTGGAGCTGGGACC AAGCTGGAAGTGA
203	RNF43-67 (VH prot)	AVQLVESGGGSVQPGSRMRLS CAASGFTFSNYDMTWVRQAPTKGLEWVASITS DGGSTYSRDSVKGRFTISRDNASTLYLQMDSLRSEDATATYYCTTDRGRYLPY YFDYWGGQVMVTVSA
204	RNF43-67 (VL prot)	DVVLVTQTPVSLSVTVGDQASISCRSSQSLEYS DGYSYLEWYLQKPGQSPQLLI YEVSRRFSGVPDRFIGSGSGTDFTLKISRVEPEDLGVIYCFQAIHDPFTGAGT KLELK
205	RNF43-69 (VH DNA)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAAGTCCCTGAAACTCTCCTGTGCAGCCTCAGGATTCACCTTTCAGTGACTATTACATGGCCTGGGTCCGCCAGGCTCCAAAGAAGGGTCTGGAGTGGGTGCGATCCATTACTTATGAGGGTAGTAGCACTTACTATGGAGACTCCGTGAAGGGCCGATTCACTATCTCAGAGATAATGCAAAAAGCACCCATACCTGCAAATGAACAGTCTGAGGTCTGAGGACACGGCCACTTTTATTGTGCAAGACATCTGGACTATGGGTATGACTACGCCTGGTACTTTGACTTCTGGGGCCCAGGAACCATGGTCACCGTGTCTCTCA
206	RNF43-69 (VL DNA)	GACATCGTGTGACTCAGTCTCCAACCACCCTGTCTGTGACTCCAGGAGAGACAGTCAGTCTCTCCTGCAGGACTAGCCATAGTATTGGCACAATCTACACTGGTATCAGCAAAAACAAATGAGTCTCCAAGGCTTCTCATCAAGTATGCTTCCAGTCCATCTCTGGGATCCCCTCCAGGTTCAAGTGCAGTGGATCAGGGACAGATTTTACTCTCAACATCAACAATGTGGAGTTTGATGATGTCTCAAGTTATTTTGTCAACAGACTCAAAGCTGGCCCCCTCACGTTCCGTTCTGGGACCAAGCTGGAGATCAA
207	RNF43-69 (VH prot)	EVQLVESGGGLVQPGSRSLKLS CAASGFTFSDYYMAWVRQAPKKGLEWVASITY EGSSTYYGDSVKGRFTISRDNASTLYLQMNSLRSEDATATFYCARHLDYGYDYAWYFDWGPVTMTVSS
208	RNF43-69 (VL prot)	DIVLTQSPPTLSVTPGETVSLSCRTSHSIGTNLHWYQQKTNESPRLLIKYASQSIGIPSRFSASGSGTDFTLNINNVEFDDVSSYFCQQTQSWPLTFGSGTKLEIK
209	RNF43-71 (VH DNA)	CAGGTCAAGCTGCTGCAGTCTGGGGCTGCACTGGTGAAGCCTGGGGACTCTGTGAAGATGTCTTGCAAAGCTTCTGGTTATACATTCAGTGACCACATTATACACTGGGTGAAGCAGAGTCATGGAAAGAGCCTTGAGTGGATTGGTTATATTAATCCTTACAGTGGTAGTACTAACAATGAAAAGTTCAAGAGCAAGGCCACATTGACTGTAGACAGATCCAACAACACAGCCTATATGGAGTTTAGCAGATTGACATCTGAGGATTCTGCAATCTATTACTGTGCAAGAAAGAACTACGGAGGTTATGGAGTTATGGATGCCTGGGGTCAAGGAGCTTCAGTCACTGTCTCTCTCA
210	RNF43-71 (VL DNA)	GACATCCAGATGACCCAGTCTCCTTCACTCCTGTCTGCATCTGTGGGAGACAGAGTCAGTCTCAGCTGCAAAGCAAGTCAGAATATTTATAAGAACTTAGCCTGGCATCAGCAAAAGCTTGGAGAAGCTCCCAAACCTCCTGATTTATTATGCAACAGC TTGCAAACGGGCATCCCATCAAGGTTCAAGTGCAGTGGATTTGGTACAGATTA CACACTCACCATCAGCAGCCTGCAGCCTGAAGATGTTGCCACATATTTCTGCC AGCAGTCTTCTAGCGGGTGGACGTTCCGGTGGAGGCACCAAGCTGGAATTGAAA
211	RNF43-71 (VH prot)	QVKLLQSGAALVKPGDSVKMSCKASGYTFDTHIIHWVQKSHGKSLIEWIYINP YSGSTNYNEKFKSKATLTVDRSNNTAYMEFSRLTSEDSAIYYCARKNYGGYGVMDAWGQGASVTVSS
212	RNF43-71 (VL prot)	DIQMTQSPSLLSASVGDRTLSCKASQNIYKNLAWHQQLGEAPKLLIYYANS LQTGIIPSRFSGSGFGTDYTLTISLSLPEDVATYFCQQSSSGWTFGGGTKLELK
213	RNF43-74 (VH DNA)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGCAGCCTGGAAGTCCCTGACACTCTCCTGTGCAGCCTCAGGATTCACCTTTCAGTGACTATTACATGGCCTGGGTCCGCCAGGCTCCAAAGAAGGGTCTGGAGTGGGTGCGATCCATTACTTATGAGGGCAGTAGTACCTACTACGGAGACTCCGTGAAGGGCCGATTCACTATCTCAGAGATAATACAAAAGCACCCATATATCTGCTAATGAACAGTCTGAGGTCTGACGACACGGCCACTTACTATTGCGCAAGACATGGGGCACACTATACATATAAT

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		TACGCCTACTACTTTGATTACTGGGGCCAGGGAGTCATGGTCACAGTCTCCTC A
214	RNF43-74 (VL DNA)	GACATCATGCTGACTCAGTCTCCAACCTACCCGTGTCTGTGATTCCAGGAGAGAA CATCAGTCTCTCCTGCAGGGCCAGTCAGAGTGTGGTACTAACTTACATTGGT ATCAGCAAAAACCAAGTGAGTCTCCAAGGGTCTCATCAACTATGCTTCCCAG TCCATCTCTGGAATCCCCCTCCAGGTTCACTGGCAGTGGATCAGGGACAGATTT CACTCTCAATATTAACAGAGTAGAGCCTGAAGATTCTTCAGTTTATTACTGTC AACAGAGTAAGAGCTGGCCATTACGTTTCGGCTCAGGCACGAAGTTGGAATA AAA
215	RNF43-74 (VH prot)	EVQLVESGGDLVQPGRSLTLSCAASGFTFSDYYMAWVRQAPKKGLEWVASITY EGSSTYYGDSVKGRFTISRDNKSTLYLLMNSLRSDDTATYYCARHGAHYTYN YAYYFDYWGGQVMVTVSS
216	RNF43-74 (VL prot)	DIMLTQSPPTLSVIPGENISLSCRASQSVGTNLHWYQQKPSSESPRLINIASQ SISGIPSRFSGSGSGTDFTLNINRVEPEDSSVYYCQQSKSWPFTFGSGTKLEI K
217	RNF43-75 (VH DNA)	GAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAAGGTCCCT GAACTCTCCTGTGCAGCCTCAGGATTCACCTTCAGTGACCATTACATGGCCT GGGTCCGCCAGGCTCCAAAGAAGGGTCTGGAGTGGGTGCGATCCATTACTTAT GAGGGTAGTAGCACTTACTATGGAGACTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATGCAAAAAGCACCCCTATACCTGCAAATGAACAGTCTGAGGTCTG AGGACACGGCCACTTTTATTGTGCAAGACATCTGGACTATGGGTATGACTAC GCCTGGTCCCTTGACTTCTGGGGCCCAGGAACCATGGTCACCGTGTCTCTCA GACATCGTGTGACTCAGTCTCCAATCATCCTGTCTGTGACTCCAGGAGAGAC AGTCAGTCTCTCCTGCAGGACTAGCCATAGTATTGGCACAATCTACACTGGT ATCAGCAAAAACAAATGAGTCTCCAAGGCTTCTCATCAACTATGCTTCCCAG TCCATCTCTGGGATCCCCCTCCAGGTTCACTGCCAGTGGATCAGGGACAGATTT TACTCTCAACATCAACAATGTGGAGTTTGAAGATGTGCGAAGTTATTATTGTC AACAGACTCAAAGGTGGCCCCCTCACGTTTCGGTCTGGGACCAAGCTGGAGATC AAA
218	RNF43-75 (VL DNA)	GACATCGTGTGACTCAGTCTCCAATCATCCTGTCTGTGACTCCAGGAGAGAC AGTCAGTCTCTCCTGCAGGACTAGCCATAGTATTGGCACAATCTACACTGGT ATCAGCAAAAACAAATGAGTCTCCAAGGCTTCTCATCAACTATGCTTCCCAG TCCATCTCTGGGATCCCCCTCCAGGTTCACTGCCAGTGGATCAGGGACAGATTT TACTCTCAACATCAACAATGTGGAGTTTGAAGATGTGCGAAGTTATTATTGTC AACAGACTCAAAGGTGGCCCCCTCACGTTTCGGTCTGGGACCAAGCTGGAGATC AAA
219	RNF43-75 (VH prot)	EVQLVESGGGLVQPGRSLKLSCAASGFTFSDHYMAWVRQAPKKGLEWVASITY EGSSTYYGDSVKGRFTISRDNKSTLYLQMNLSLRSEDATFYCARHLDYGYDY AWSFDWGPVTMTVSS
220	RNF43-75 (VL prot)	DIVLTQSPPIILSVTPGETVLSLSCRTSHSIGTNLHWYQQKTNESPRLLINIASQ SISGIPSRFSASGSGTDFTLNINNVEFEDVASYYCQQTQRWPLTFGSGTKLEI K
221	RNF43-76 (VH DNA)	GAAGTACAGCTTGTGGAGTCTGGAGGAGGCTTGGTGCAACCTGGGACTTCTCT GAAAATCTCTTGTGTAGCCTCGGGATTCACCTTCAGTGACTACTGGATGAGCT GGGTTCGCCAGACTCCTGGAAAGACCATGGAGTGGATTGGAGATATTAAATCT GATGGCAGTTACACAACTATGCACCATCCCTAAAGAATCGATTCACAATCTC CAGAGACAATGCCAAGAGCACCCCTGTACCTGCAGATGAACAATGTGAGATCTG AAGACACAGCCACTTATTACTGTACTAGAGATGCATATAGGTACAACCTACGTC CACTATGGTTTGGATGCCTGGGGCCAAGGAGCTTCAGTCACTGTCTCTCTCA
222	RNF43-76 (VL DNA)	CAGTTTGTGCTTACTCAGCCAACTCTGTGTCTTCGAATCTCGGAAGCACAGT CAAACGTGTCTTGAATCGCAGCACTGGTAACATTGGAAGCAATTATGTGAGCT GGTACCAGCAGCATGAGGGAAGATCTCCCACTACTATGATTTACAGGGATGGT AAGAGACCAGATGGAATTCCTGACAGGTTCTCTGGCTCCATTGACGGATCTTC CAACTCAGCCCTCCTGACAATCAACAATGTGCAGACTGAAGATGAAGCTGACT ACTTCTGTCAGTCTTACAGTAGTGGTATTAAGTATATTTTTCGGCGGTGGAACC AAGCTCACTGTCTCTA
223	RNF43-76 (VH prot)	EVQLVESGGGLVQPGTSLKISCVASGFTFSDYWMSWVRQTPGKTMWIGDIKS DGSYTNYPKSLKNRFTISRDNKSTLYLQMNVRSEDATYYCTRDAYRYNYV HYGLDAWGQASVTVSS
224	RNF43-76 (VL prot)	QFVLTQPNVSSNLGSTVKLSNCRSTGNIGSNYVSWYQQHEGRSPPTMIYRDG KRPDGIIPDRFSGSIDGSSNSALLTINNVTQTEADYFCQSYSSGIKYIFGGGT KLTVL
225	RNF43-80 (VH DNA)	GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTAGTCCAGCCTGGAAGGTCCCT GAAAGTCTCCTGTTTAGCCTCTGGATTCAACTTCGGTGACTTTGGAATGAATT GGATTTCGCCAGGCTCCAGGGAAGGGCTGGAGTGGGTTTCATATATTAGTAGT ACCAGTGGTACAATCTACTATGCGGACACTGTGAAGGGTCGCTTACCATTTC CAGAGATAATGCCAAGAACACCCTTTATCTCCAACCTGAGCAGTCTGAGGTCTG

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		AGGACACCGCCTTGTATTACTGTGCAAGACCGGCGGGAAGGTACTTTGATTAC TGGGGCCAAGGAGTCTTGGTCACAGTCTCCTCA
226	RNF43-80 (VL DNA)	GACATTGTGATGACCCAATCTCCATCCTCTCTGGCTGTGTGTCAGCAGGAGAGAC GGTCACTATAGACTGCAAGTCCAATCAGAGTCTTTTATACAGTGGAAACCAAA AGAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAACTGCTG ATCTACTGGACTTCTACTAGGCAATCTGGTGTCCCTGATCGCTTCATAGGCAG TGGATCTGGGACAGAGTTCACTCTGACCATCAGCAGTGTGCAGGCAGAAGATC TGGCAATATATTACTGTGACAGTATTATCACACTCCATTACGTTTCGGCTCA GGGACGAAATTGGAAATAAAA
227	RNF43-80 (VH prot)	EVQLVESGGGLVQPGRSLKVSCLASGFNFGDFGMNWIRQAPKGLEWVSYISS TSGTIYYADTVKGRFTISRDNAKNTLYLQLSSLRSEDALYYCARPAGRYFDY WGQGVLVTVSS
228	RNF43-80 (VL prot)	DIVMTQSPSSLAVSAGETVTIDCKSNQSLLYSGNQKNYLAWYQQKPGQSPKLL IYWTSTRQSGVPDRFIGSGSGTEFTLTISVQAEDLAIYYCQYYHTPFTFGS GTKLEIK
229	RNF43-86 (VH DNA)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAAGGTCCCT GAAACTCTCCTGTGCAGCCTCAGGATTCACCTTCACTAACTATTACATGGCCT GGGTCCGCCAGGCTCCAACGAAGGGTCTGGAGTGGGTGCGCTCCATCAGTCTC AGTGGTGATACCACTTACTATCCAGACTCCGTGAAGGGCCGATTCACTGTCTC CAGAGATAATGCAAAAAGTACCCAATACCTGCAAATGGACAATCTGAGGTCTG AGGACACGGCCACTTATTACTGTGCAAGAGACGCATATACATACTATGGATAT AAGGGCTGGTACTTTGACTTCTGGGGCCAGGAACCATGGTCACCGTGTCTCTC A
230	RNF43-86 (VL DNA)	CAGTTTGTGCTTACTCAGCCAACTCTGTGTCTACGAATCTCGGAAGTGCAGT CAAACGTGTCTTGCAAGCGCAGCACTGATGACATTGGAAGCAATTATGTGAAC GGTACCAGCAGCATGAGGGAAGATCTCCACCACTATGATTTATAGGGATGAT AAGAGACCAGATGGAGTTCCTGACAGGTTCTCTGGCTCCATTGACAGATCTTC CAACTCAGCCCTCCTGACAATCAATAATGTGCAGACTGAAGATGAGGCTGACT ACTTCTGTGCTTACAGTAGTGGTATTTATATTTTCGGCGGTGGAACCAAG CTCACTGTCCTG
231	RNF43-86 (VH prot)	EVQLVESGGGLVQPGRSLKLSAASGFTFTNYMAWVRQAPTKGLEWVASISP SGDTTYYPDSVKGRFTVSRDNAKSTQYLQMDNLRSEDATYYCARDAYTTYGY KGWYFDFWGPMTMTVSS
232	RNF43-86 (VL prot)	QFVLTQPNVSTNLGSAVKLSCKRSTDDIGSNYVNWYQQHEGRSPPTMIYRDD KRPDGVPRDFSGSIDRSSNSALLTINNVQTEADYFCQSYSSGIYIFGGGTK LTVL
233	RNF43-90 (VH DNA)	CAGGTACAGTTGAAGGAGTCAGGACCTGGTCTGGTGCAGCCCTCACAGACCTT GTCCCTCACCTGCACTGTCTCTGGATTCTCACTTACCAGTACAGTGTACACT GGGTTTCGCCAACATTGGAAGAACCGTCTGGAATGGATGGGAAGAATGTGAGT GCTGGAGACACATCATTGTAATTCAGCGTTCACATCCCGACTGAACATCACCAG GGACACCTCCAAGAGCCAAGTTTCTTAAAAATGAACAGTCTGCAAACGAAG ACACAGGCACTTACTACTGTGTGATCCTTCTATAATGCGGGGTATCCTTTT GATTACTGGGGCCAGGGAGTCATGGTCACAGTCTCCTCA
234	RNF43-90 (VL DNA)	GACACTGTACTGACCCAGTCTCCTGCTTTGGCTGTGTCTCCAGGAGAGAGGGT TACTATCTCCTGTAGGGCCAGTGATAGTGTCAATAACTTATGAACGGTATC AACAGAAACCAGGACAGCGACCCAACTCCTCATCTATCTGGCATCACACCTA GCATCTGGGGTCCCTGCCAGGTTCACTGGCAGTGGGTCTGGGACAGACTTCAC CCTCACCATTGATCCTGTGGAGACTGATGACACTGCAACCTATTACTGTGACG AGAGTAGGAATGATCCCACGTTTGGACCTGGGACCAAGCTGGAACCTGAAA
235	RNF43-90 (VH prot)	QVQLKESGPGLVQPSQTLSTCTVSGFSLTSYSVHWVRQHSNRLWGMGRMWS AGDTSFNSAFTSRLNITRDTSKSVFLKMNSLQTEDTGTYTCVRSFYNAGYPF DYWGQGVMTVSS
236	RNF43-90 (VL prot)	DTVLTQSPALAVSPGERVTISCRASDSVIKLMNWYQQKPGQRPKLLIYLASHL ASGVPARFSGSGSGTDFTLTIDPVETDDTATYYCQQRNDPTFGPGTKLELK
237	ZNRF3-101 (VH DNA)	CAGGTGCAACTGAAGGAGTCAGGACCTGGTCTGGTGCAGCCACACAGACCTT GTCCCTCACCTGCACTGTCTCTGGGTTTTCACTGACCAGCTCTACTGTAAGCT GGGTTTCGCCAACCTCCAGGAAAGGGTCTGGAGTGGATTGCAGCAATATCAAGT GCTGAAAACACATATTATAATTCAGGTCTCAAATCCCGACTGAGCATCAGCAG GGACACCTCCAAAAGCCAAGTTTCTTAAAAATGAACAGTCTGCAAACGAAG

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		ACACAGCCATGTACTTCTGTGCCGGCGGTAGAACTACTGGAGACCCTCTTTGAT TCCTGGGGCCAAAGGAGTCATGGTCACAGTCTCCTCA
238	ZNRF3-101 (VL DNA)	GACATTGTGCTGACCCAGTCTCCTGCTTTGGCTGTGTCTCTAGGGCAGAGGGC CACCATCTCTTGCAAGACCAACCAGAATGTCGATTTTTATGGCAAACTTATA TGCACCTGGTACCAACAGAAACGGGGACAACAACCCAAACTCCTCATCTATTTA GCATCCAACTTAGCATCTGGGATCCCTGCCAGGTTCACTGGTAGAGGGTCTGG GACAGACTTCACCCCTCACCATTGATCCTGTGGCGACTGATGATACTGCAACCT ATTACTGTCAGCAGACTAGGAGTCTTCTCCACGTTTGGAGCTGGGACCAAG CTGGAAGTAAAA
239	ZNRF3-101 (VH prot)	QVQLKESGPGLVQPTQTLSLTCTVSGFSLTSSTVSWVRQPPGKLEWIAAIISS AENTYYNSGLKSRLSISRDTSKSQVFLKMNSLQTEDTAMVFCAGGRILETLFD SWGQGVMTVSS
240	ZNRF3-101 (VL prot)	DIVLTQSPALAVSLGQRATISCKTNQNVDFYGYKTYMHWYQQKRGQQPKLLIYL ASNLAGIPARFSGRSGTDFTLTIDPVATDDTATYYCQQTRSLPPTFGAGTK LELK
241	ZNRF3-117 (VH DNA)	GAGATACAGCTGCAGGAGTCAGGACCTGGCCTTGTGAAACCTTCACAGTCACT CTCCCTCACCTGTTCTGTCACTGGTTACACCATTACAGTGATTATGATTGGA GCTGGATCCGGAAGTTCACAGGAAATAAAATGGAGTGGATGGGATACATAAAC TACAGTGGTAGCACTAACTACAACCCATCGCTCAAAAGTCGAATCTCCTTTAC CAGAGACACATCCAAGAATCAGTTCTTCTGCACTTGAATCTATAACTACTG ACGATACAGCCACATATTACTGTGCAAGATATGGTCCATACTACGGGTTTTC GACTGGTACTTTGACTTCTGGGGCCCGGGAACCATGGTCACCGTGTCTCTCA
242	ZNRF3-117 (VL DNA)	GATGTCCAGATGACCCAGTCTCCGTCTTATCTTGCTGCGTCTCCTGGAGAAAAG TGTTTCCATCAGTTGCAAGGCAAGTCAGAGCATTAGTAATTATTTAGCCTGGT ATCAACAGAAACCTGGAAAAACATATAGGCTTCTTATCTACTCTGGGTCAACT TTGCAATCTGGAATCCATCAAGGTTCACTGGCAGTGGATCTGGTACAGATTT CACTCTCACCATCAGAAGCCTGGAGCCTGAAGATTTTGGACTCTATTACTGTC AACAGTATTATGAAAAACCGCTCACGTTCCGTTCTGGGACCAAGCTGGAGATC AAA
243	ZNRF3-117 (VH prot)	EIQQLQESGPGVLKPSQSLSLTCSVTGYTITSDYDWSWIRKFPNGKMEWMGYIN YSGSTNYPNLSKSRISFTRDTSKNQFFLQLNSITDDTATYYCARYGPYYGFS DWYFDWGPMTMTVSS
244	ZNRF3-117 (VL prot)	DVQMTQSPSYLAASPGESVSI SCKASQSI SNYLAWYQQKPGKTYRLLIYSGST LQSGTPSRFSGSGSGTDFTLTIRSLPEDFGLYYCQQYYEKPLTFGSGTKLEI K
245	ZNRF3-128 (VH DNA)	GAGGTACAGCTGGTGGAGTCTGGAGGAGGCTTAGTGCAGCCTGGAAAGTCCCT GAAACTCTCCTGTTTCAGCCTCTGGATTACATTCAGTAGCTATGGCATGCACT GGATTCGCCAAGCTCCAGGAAAGGGCTAGATTGGGTTGCATACATTAGTACT AACAGCGCTACAGTCTATGCAGACGCTGTGAAGGGCCGGTTCCACTCTCCAG AGACAATGCAAAGAACACCTGTACCTACAACCTCAACAGTCTGAAGTCTGAGG ACACTGCCATCTATTACTGTGCAAGAAGCGGCTATACCACCTATATGCCCTTT ACTTACTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA
246	ZNRF3-128 (VL DNA)	GATGTTTTGATGACACAACTCCAGTCTCCCTGCCTGTCAGCCTTGGAGGTCA AGTCTCTATCTCTTGCCGGTCAAGTCAGAGCCTTGTACACAGTGATGGAAACA CCTACTTGCAATTGGTATCTGCAGAAGCCAGGCCAGTCTCCACAGCTCCTCATC TATCGGGTTTCCAACAGATTTTCTGGGGTGTGACAGAGTTCAGTGGCAGTGG GTCAGGGACAGATTTACCCTCAAGATCAGCAGAGTAGAGCCTGACGACTTGG GAATTTATTACTGCTTACAACTACACATTTTCTCACGTGGACGTTCCGGTGA GGCACCAAGCTGGAATTGAAA
247	ZNRF3-128 (VH prot)	EVQLVESGGGLVQPGKSLKLSASGFTFSSYGMHWIRQAPGKGLDWVAYIST NSATVYADAVKGRFTISRDNKNTLYLQLNSLKSIEDTAIYYCARSGYTTYMPF TYWGQGLTVTVSS
248	ZNRF3-128 (VL prot)	DVLMTQTTPVSLPVSLGGQVSI SCRSSQSLVHSDGNTYLHWYLPKPGQSPQLLI YRVSNRFSVSDRFSGSGSGTDFTLKISRVEPDDLGIYYCLQTHFLTWTFGG GTKLELK
249	ZNRF3-131 (VH DNA)	GAGGTGCAGGTGGTGGAGTCTGGGGGAGGCCTAGTGCAGCCTGGAAGATCCCT GAAAGTCTCCTGTGCAGCCTCAGGATTCACTTTCAGTAGATATGGCATGGCTT GGGTCCGCCAGGCTCCGACGAAGGCTCTGGAGTGGGTGCGATCCATTAGTACT GGTGGTGAACCACTTACTATCGAGACTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATGTAAAAACACCCTATACCTGCAAATGGACAGTCTGAGGTCTG

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		AGGACACGGCCACTTATTATTGTGCAAGACATGGGCGGGTACTAAATTACTTTGATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCTTTA
250	ZNRF3-131 (VL DNA)	GACATCCAGATGACACAGTCTCCAGCTTCCCTGTCTGCATCTCTGGGAGAAAC TGTCTCCATCGAATGTCTAACAAGTGAGGACATATACACTTATTTAGCATGGT ATCAGCAGAAGCCAGGGAAATCTCCTCACCTCCTGATCTTTGCTGCAAGTAGG TTGCAAGATGGGGTCCCATCACGGTTCAGTGGCAGTGGATCTGGCACACAGTA TTCTCTCAAGATCAGCGGCATGCAACCTGAAGATGAAGGGGATTATTTCTGTC TACAGGGTCCAAGTTTCCGTACACGTTTGGAGCTGGGACCAAGCTGGACCTG AAA
251	ZNRF3-131 (VH prot)	EVQVVESGGGLVQPGRSLKVS CAASGFTFSRYGMAWVRQAPTKGLEWVASIST GGENTYYRDSVKGRFTISRDNVKNLTLYLQMDSLRSEDATATYYCARHGRVLNYF DYWGQGVMTVSL
252	ZNRF3-131 (VL prot)	DIQMTQSPASLSASLGETVSI ECLTSEDIYTYLAWYQQKPKSPHLLI FAASR LQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDYFCLQGSKFYPYTFGAGTKLDL K
253	ZNRF3-163 (VH DNA)	GAGGTGCAGTTGGAGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAAGGTCCAT GAAATTCCTCTGTGCAGCCTCAGGATTCACCTTCAGTAACATATTACATGGCCT GGGTCCGCCAGGCTCCATCGAAGGGTCTGGAGTGGGTGCGATCCATTAGTACT GGTGGAGGTGATGTTTACTATCGAGACTCCGTGAAGGGCCGATTACGATCTC CAGAGATAATGCAAAAAGCACCCCTATACCTGCAAATGGACAGTCTGAGGTCTG AGGACACGGCCACTTATTACTGTGCAAGACATTCGGGGGGTAGAATTCGGGCC CGGGGTCACTACTTTGATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTC A
254	ZNRF3-163 (VL DNA)	GACATCCAGATGACACAGTCTCCAGCTTCCCTGTCTGCATCTCTTGGAGAAAC TGTCTCCATCGAATGTCTAGCAAGTGAGGACATTTCCAATGATTTAGCGTGGT ATCAGCAGAAGTCAGGGAAATCTCCTCAGGTCCTGATCTATGGTGTAAAGCAGG TTGCAAGACGGGGTCCCATCACGGTTCAGTGGCAGTGGATCTGGCACACGGTA TTCTCTCAAGATCAGCGGCATGCAACCTGAAGATGAAGCAGATTATTTCTGTC AACAGAGTTACAAGTATCCGTACACGTTTGGAGCTGGGACCAAGCTGGAAGTG AAA
255	ZNRF3-163 (VH prot)	EVQLEESGGGLVQPGRSMKFSCAASGFTFSNYMAWVRQAPSKGLEWVASIST GGGDVYYRDSVKGRFTISRDNKSTLYLQMDSLRSEDATATYYCARHSGGRIRAR GHYFDYWGQGVMTVSS
256	ZNRF3-163 (VL prot)	DIQMTQSPASLSASLGETVSI ECLASEDISNDLAWYQQKSGKSPQVLIYGVSR LQDGVPSRFSGSGSGTRYSLKISGMQPEDEADYFCQQSYKYPYTFGAGTKLEL K
257	ZNRF3-17 (VH DNA)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCT GAACTCTCCTGTGCAGCCTCAGGATTCACCTTCAGTAATTATGACATGGCCT GGGTCCGCCAGGCTCCAACGAAGGGTCTGGAGTGGGTGCGATCCATTAGTACT GGTGGTGGTAAACTTACTATCGAGACTCCGTGAAGGGCCGACTCACTATCTC CAGAGATAATGCAAAAACACCCAATACCTGCAAATGGACAGTCTGAGGTCTG AGGACACGGCCACTTATTACTGTGCAAGACTCGGTCCGGCATACTCCGGGGAG TGGTTTGCTTACTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA
258	ZNRF3-17 (VL DNA)	GACACTGTGCTGACCCAGTCTCCTGCTTTGGCTGTGTCTCCAGGAGAGAGGGT TACCATCTCCTGTGGGGCCAGTGAAGGTGTCAATACACGTATGCACTGGTACC AACAGAGATCAGGACAACAACCCAACTCCTCATCTACGGTTCCTCCAACCTA GATTCTGGAGTCCCTGACAGGTTCACTGGCAGTGGGTCTGGGACAGACTTTAC CCTCACCATCGATCCTGTGGAGGCTAAAGATGCTGCAACCTATTTCTGTGAGC AGAGTTGGAATGTTCCGAACACGTTTGGAGCTGGGACCAAACTGGAAGTGA AAA
259	ZNRF3-17 (VH prot)	EVQLVESGGGLVQPGGSLKLS CAASGFTFSNYDMAWVRQAPTKGLEWVASIST GGGKTYRDSVKGRLTISRDNKNTQYLQMDSLRSEDATATYYCARLGPAYSGE WFAYWQGTLVTVSS
260	ZNRF3-17 (VL prot)	DTVLTQSPALAVSPGERVTIS CGASEGVNTRMHWYQQRSGQPKLLIYGSSNL DSGVPDRFSGSGSGTDFTLTIDPVEAKDAATYFCQQSWNPNTFGAGTKLEL K
261	ZNRF3-170 (VH DNA)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAAGGACCT GAACTCTCCTGTGCAGCCTCAGGATTCACCTTCAGTGACTATTACATGGCCT GGGTCCGCCAGGCTCCAAAGAAGGGTCTGGAGTGGGTGCGATCCATTAGTTCT GGGGGTTTTAGTATTTATTATGGAGACTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATGCAAAAACACCCCTATACCTGCAAATGAACAGTCTGAGTCTG

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		AGGACACGGCCACTTATTATTGTGCAAGACATACGATTAGTACGGCCTTTTTT GATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
262	ZNRF3-170 (VL DNA)	GACACTGTGCTGACCCAGTCTCCTGCTTTGGCTGTGTCTCCAGGAGAGAGGGT TACCATCTCCTGTAAGGCCAATGAAAATGTCAGTACACGTATGCACTGGTACC AACAGAGACCAGGACAGCGACCCAACTCCTCATCTATAAAGCATCAAACCTA GGATCTGGGGTCCCTGCCAGGTTTCAGTGGCAGTGGGTCTGGGACAGACTTCAC CCTCACCATTGATCCTGTGGAGGCTGATGACACTGCAACCTATTTCTGTACAGC AGAGTTGGAATGGTCCCTCCGTGGACGTTCCGTGGAGGCACCAAGCTGGAGTTG AAA
263	ZNRF3-170 (VH prot)	EVQLVESGGGLVQPGRITLKLSCAASGFTFSDDYMAWVRQAPKKGLEWVASISS GGFSIYYGDSVKGRFTISRDNAKTTLYLQMNSLRSEDTATYYCARHTISTAEFF DYWGQGVMVTVSS
264	ZNRF3-170 (VL prot)	DTVLTQSPALAVSPGERVTISCKANENVSTRMHYQQRPGQRPKLLIYKASNL GSGVPARFSGSGSGTDFTLTIDPVEADDTATYFCQQSWNGPPWTFGGGTKLEL K
265	ZNRF3-171 (VH DNA)	CAGGTCCAGTTGCACCAATCTGGAAGTGAAGTGGCCAGACCTGGGACTTCAGT GAAGCTGTCTGCAAGGCTTCTGGCTATACCTTCAGCAGGTTTTATATTAAGT GGGTGAAGAAGAGGCCTGGACAGGGCCTTGAGTGGATCGGATATATTGATCCT GGAAATGGTGGTACTTTCCACAGTGAGAAGTTCAGGACAAAGCCTCACTTAC TGCAGACACATCTTCAGCAGCCTACATGGTACTGGGACGCTGGCACCTG AAGACTCTGCATTCTATTTCTGTGTAAGGGGTACTATAGCAGCTATGATTGG TTTGCTTACTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA
266	ZNRF3-171 (VL DNA)	GACATCCAGTTGACCCAGTCTCCATCCTCCATGTCTGCATCTCTGGGAGACAG AGTCAGTCTTACTTGCCAGTCAAGCCAGGGCATTGGAAATTATTTAAGCTGGT ACCAGCATAAACCAGGGGAACCTCCTAAGCCTATACTTTATTATGCAACCAAC TTGGCAGATGGGGTCCCATCAAGATTCAGTGGCAGTAGGTCTGGGTGAGATTA TTCTCTCACCATCAGCAGCCTGGAGTCTGAAGATACAGCAATCTATTACTGTC TACAGTATGATGAGTACCTTTACACGTTTGGACCTGGGACCAAGCTGGAAGTGA AGA
267	ZNRF3-171 (VH prot)	QVQLHQSGTELRPGTSVKLSCKASGYTFSRFYINWVKRPGQGLEWIGYIDP GNGGTFHSEKFKDKASLTADTSSSTAYMVLGSLAPEDSAFYFCVRGYYSSYDW FAYWGQGLVTVSS
268	ZNRF3-171 (VL prot)	DIQLTQSPSSMSASLGDRVSLTCQSSQGIGNYLSWYQHKPGEPPKPILYATN LADGVPSRFSRSGSGSDYSLTISSEDTATYYCLQYDEYPYTFPGTKLEL R
269	ZNRF3-172 (VH DNA)	GAGGTGCAGCTGGCGGAGTCTGGGGGAGGCTTAGAGCAGCCTGGAAGGTCCCT GAAACTCTCCTGTGCAGCCTCAGGATTCATTTTCAGTAACTATGACATGGCCT GGGTCCGCCAGGCTCCAACGAAGGCTCTGGAGTGGGTGCGATCCATTATTGCT AGTGGTGGTACTACTTATTATCGAGACTCCGTGAAGGGCCGATTCAGTGTCTC CAGAGATAATGCAAAAAGCACCCCTATACCTACAAATGGACAGTCTGAGGTCTG AGGACACGGCCACTTATTACTGTGCAAGACATGGGGTCCGTAGCTACGATTGG TTTGCTGACTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA
270	ZNRF3-172 (VL DNA)	GACATTGTCTTGACCCAGTCTCCTGCTTTGGCTGTGTCTCTAGGGCAGAGGGC CACAATCTCCTGTAGGGCCAGCCAAAGTGTCACTATATCTGGATTTAATCTTA TGCAGTGGTACCAACAGAAACCAGGACAAACCAAGCTCCTCATCTATCGT GCATCCAACCTAGCATTTGGGATCCCTGCCAGGTTTCAGTGGCAGTGGGTCTGG GACAGACTTCACCCTCACCATCAATCCTGTGCAGGCTGATGATATTACAACCT ATTACTGTCAGCAAAGTAGGAAGTCTCGGACGTTCCGTGGAGGCACCAACTG GAATTGAAA
271	ZNRF3-172 (VH prot)	EVQLAESGGGLEQPGRSRLKLSCAASGFTFSNYDMAWVRQAPTKGLEWVASIIA SGGTYYRDSVKGRFTVSRDNASTLYLQMDSLRSEDTATYYCARHGVGSYDW FADWGQGLVTVSS
272	ZNRF3-172 (VL prot)	DIVLTQSPALAVSLGQRATISCRASQSVTISGFNLMHWYQKPGQPKLLIYR ASNLAFIGIPAREFSGSGSGTDFTLTINPVQADDITYYCQSRKSRFTFGGGLK ELK
273	ZNRF3-179 (VH DNA)	GAGGTGCAGCTGGCGGAGTCTGGGGGAGGCTTAGAGCAGCCTGGAAGGTCCCT GAAACTCTCCTGTGCAGCCTCAGGATTCATTTTCAGTAACTATGACATGGCCT GGGTCCGCCAGGCTCCAACGAAGGCTCTGGAGTGGGTGCGATCCATTATTACT AGTGGTGATACTACTTATTATCGAGACTCCGTGAAGGGCCGATTCAGTGTCTC CAGAGATAATGCAAAAAGCACCCCTATACCTGCAAAATGGACAGTCTGAGGTCTG

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		AGGACACGGCCACTTATCACTGTGCAAGACATGGGGTCGGTAGCTACGATTGG TTTGCTGACTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA
274	ZNRF3-179 (VL DNA)	GACATTGTCTTGACCCAGTCTCCTGCTTTGGCTGTGTCTCTAGGGCAGAGGGC CACAATCTCCTGTAGGGCCAGCCAAAGTGTCACTATATCTGGATATAATCTTA TGCACGTGGTACCAACAGAAACCAGGACAACAACCCAAACTCCTCATCTATCGT GCATCCAACCTAGCATTGTTGGGATCCCTGCCAGGTTCACTGGCAGTGGGTCTGG GACAGACTTCACCCCTCACCATCAATCCTGTGCAGGCTGATGATATTACAACT ATTACTGTCAGCAGAGTAGGAAGTCTCGGACGTTCCGTGGAGGCACCAAAGT GAATTGAAA
275	ZNRF3-179 (VH prot)	EVQLAESGGGLEQPGRSCLKLSAASGFTFSNYDMAWVRQAPTKGLEWVASIIT SGDTTYRDSVKGRFTVSRDPAKSTLYLQMDSLRSEDATATYHCAHGVGSYDW FADWGQGLTVTVSS
276	ZNRF3-179 (VL prot)	DIVLTQSPALAVSLGQRATISCRASQSVTISGYNLMHWYQQKPGQPKLLIYR ASNLAFGIPARFSGSGSGTDFTLTINPVQADDITTYCQQSRKSRFTGGGTKL ELK
277	ZNRF3-182 (VH DNA)	GAGGTGCAGCTGGCGGAGTCTGGGGGAGGCTTAGAGCAGCCTGGAAGTCCCT GAACTCTCCTGTGCAGCCTCAGGATTCACCTTCAGTAACCTATGACATGGCCT GGGTCCGCCAGGCTCCAACGAAGGGTCTGGAGTGGGTGCGATCCATTATTA AGTGGTGATACTTCTTATTATCGAGACTCCGTGAAGGGCCGATTCACTGTCTC CAGAGATAATGCAAAAAGCACCCCTATACCTGCAAATGGACAGCTGAGGTCTG AGGACACGGCCACTTATTACTGTGCAAGACATGGGGTCGGTAGCTACGATTGG TTTGCTGACTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA
278	ZNRF3-182 (VL DNA)	GACATTGTCTTGACCCAGTCTCCTGCTTTGGCTGTGTCTCTAGGGCAGAGGGC CACAATCTCCTGTAGGGCCAGCCAAAGTGTCACTATATCTGGATTTAATCTTA TGCACGTGGTACCAACAGAAACCAGGACAACAACCCAAACTCCTCATCTATCGT GCATCCAACCTAGCATTGTTGGGATCCCTGCCAGGTTCACTGGCAGTGGGTCTGG GACAGACTTCACCCCTCACCATCAATCCTGTGCAGGCTGATGATTTTACAACT ATTACTGTCAGCAGAGTAGGAAGTCTCGGACGTTCCGTGGAGGCACCAAAGT GAATTGAAA
279	ZNRF3-182 (VH prot)	EVQLAESGGGLEQPGRSCLKLSAASGFTFSNYDMAWVRQAPTKGLEWVASI SGDTSYYRDSVKGRFTVSRDPAKSTLYLQMDSLRSEDATATYCARHGVGSYDW FADWGQGLTVTVSS
280	ZNRF3-182 (VL prot)	DIVLTQSPALAVSLGQRATISCRASQSVTISGFNLMHWYQQKPGQPKLLIYR ASNLAFGIPARFSGSGSGTDFTLTINPVQADFTTYCQQSRKSRFTGGGTKL ELK
281	ZNRF3-195 (VH DNA)	GAGGTACAGCTGGTGGAGTCTGGAGGAGGCTTAGTGACGCTGGAAGTCCCT GAACCTCTCCTGTTCAGCCTCTGGATTCCCATTCAGTACCTATGGCATGCACT GGATTCGCCAAGCTCCAGGAAAGGGCTAGATTGGGTGTCATACATTTCTTAT AACAGCGGTACAGTCTATGACAGCGCTGTGAAGGGCCGGTTCACTATCTCAG AGACAATGCAAAGAACCCCTGTACCTGCAACTCAACAGTCTGAAGTCTGAAG ACACTGCCATCTATTACTGTGCAAGAGGTTACTATGATGGTTATTATCGTTAC TGGGGCCAAGGAGTCATGGTCACAGTCTCCTCG
282	ZNRF3-195 (VL DNA)	GACATTGTGATGACCCAGTCTCCATTCTCCCTGGCTGTGTGACAGAGGAGAT GGTCACTATAAACTGCAAGTCCAGTCAGAGTCTTTTATCCAGTGGAAACAAA AGAACTACTTGGCCTGGTACCAGCAGAGACCAGGGCAGTCTCCTAACTACTG ATCTACCATGCATCCACTAGGCAATCAGGGGTCCTGATCGCTTCATTGGCAG TGGATCTGGGACAGACTTCACCTCTGACCATCAGCGATGTGCAGGCTGAAGACC TGGCAGATTATTACTGCCTACAACATTACAGCTCTCCACGTTCCGCTCAGGG ACGAAGTTGGAGATAAAA
283	ZNRF3-195 (VH prot)	EVQLVESGGGLVQPGNSLNLSASGFPFSTYGMHWIRQAPGKGLDWVAYISY NSGTVYADAVKGRFTISRDNKNTLYLQNLKSLKSEDTAIYYCARGYYDGYRY WGQGVMTVTVSS
284	ZNRF3-195 (VL prot)	DIVMTQSPFSLAVSEGEVMTINCKSSQSLSSGNQKNYLAWYQQRPGQSPKLL IYHASTRQSGVPDRFISGSGTDFTLTISDVQAEDLADYYCLQHYSSPTFGSG TKLEIK
285	ZNRF3-219 (VH DNA)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGACGCTGGAAGGTCCCT GAAAGTCTCCTGTGCAGCCTCAGGATTCACCTTCAGTACCTTTGCAATGGCCT GGGTGCGCCAGGCTCCAAGAAGGGTCTGGAGTGGGTGCAACCATTAATCTCT GGTGGTAGTAACACTTACTATCCAGACTCCGTGAAAGGCCGATTCACTATCTC CAGAGATAATGCAAAAAGCACCCCTATACCTGCAAATGGACAGTCTGAGGTCTG

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		AGGACACGGCCAATTATTACTGTGCAAAGTGCGGCTACCCGGGTATAACGTCTTTGATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
286	ZNRF3-219 (VL DNA)	GACATCCAGATGACCCAGTCTCCTTCATTCTGTCTGCATCTGTGGGAGACAGAGTCTCTATCAACTGCAAAGCAAGTCAGAATATTGGCAAGTACTTAACTGGTATCAGCAAGAGCTTGGAGAAGCTCCCAAACGCCTGATATATAATAACAAACAATTGCAAACAGGCATCCCATCAAGGTTCAAGTGGCAGTGGATCTGGTACAGATTACACTCACCATCAGCGGCCTGCAGCCTGAAGATTTTGCCACATATTTCTGCTTGCAGCATAATGGTTTTCCGCTCACGTTCCGTTCTGGGACCAAGCTGGAGATCAAA
287	ZNRF3-219 (VH prot)	EVQLVESGGGLVQPGRSLKVS CAASGFTFSTFAMAWVRQAPKKGLEWVATITP GGSNTYYPDSVKGRFTISRDNASTLYLQMDSLRSEDNTANYCYCAKCGYPGITS FDYWGQGVMVTVSS
288	ZNRF3-219 (VL prot)	DIQMTQSPSPFLSASVGRVSI NCKASQNI GKYLNWYQQELGEAPKRLIYNTNN LQTGIPSRFSGSGSGTDYTLTISGLQPEDFATYFCLQHNGFPLTFGSGTKLEIK
289	ZNRF3-222 (VH DNA)	GAAGTGCAGCTGGTGGAGTCTGGTGGAGGCCTAGTGCAGCCGGGAAGGTCCCTGAAACTCTCCTGTGCAGTCTCAGGATTCACCTTCAGTAACATATGGCATGGCCTGGGTCCGCCAGACTCCAACGAAGGGGCTGGAGTGGGTGCGAACCATTACTTATGATGGTAGTACCACTTACTATCGAGACTCCGTGAAGGGCCGATTCACTATCTCAGAGATAATGCAAAGAGCGCCCTATCCCTGCAAATGGACAGTCTGAGGTCTGAGGACACGGCCACTTATCACTGTGCAAGGCAGGGTTTGGGGTACCCGTTTGCTCTACTGGGGTCAAGGCACCTCTGGTCACTGTCTCTTCC
290	ZNRF3-222 (VL DNA)	GATGTTGTGATGACACAACTCCAGTCTCCCTGCCTGTCAGCCTTGGAGGTCAAGCCTCTATCTCTTGCCGGACAAGTCAGAGCCTGGTACATAGTAATGGAAACA CCTACTTGCAATTGGTACCTGCAGAAGCCAGGCCAGTCTCCACAGCTCCTCATCTATCGGGTTTCCAACAGATTCTCTGGGGTGCCAGACAGGTTCAAGTGGCAGTGGGTCAGGGACAGATTTACCCTCAAGATCAGTAGAATAGAGCCTGAGGACTTGGGAGATTATTACTGCTTACAAAGTACACATTTCCGTACACGTTTGGAGCTGGGACCAAGCTGGAAGTAAAA
291	ZNRF3-222 (VH prot)	EVQLVESGGGLVQPGRSLKLSCAVSGFTFSNYGMAWVRQTPKGLEWVATITY DGSTTYRDSVKGRFTISRDNKALS LQMDSLRSEDNTATYHCAHQGLYPFV YWGQGT LVT VSS
292	ZNRF3-222 (VL prot)	DVVMQTQTPVSLPVSLGGQASISCRTSQSLVHSNGNTYLHWY LQKPGQSPQLLI YRVSNRFSGVPRFSGSGSGTDFTLKISRIEPEDLGDY YCLQSTHPYTFGAGTKLELK
293	ZNRF3-223 (VH DNA)	GAGGTACAGCTGGTGGAGTCTGGAGGAGGCTTAGTGCAGCCTGGAGAGTCCCTGAAACTCTCCTGTTCAGCCTCTGGATTACATTCAGTAACATATGGCATGCACTGGATACGCCAAGTTCAGGAAAGGGGCTAGATTGGGTTGCATACATTACTAATAGCGGCGGTTCACTCTATGCAGCCGCTGTGAAGGGCCGTTCACTATCCAGAGACAATGCAAAGAACACCTGTACCTGGAATCAACAGTCTGAAGTCTGAGGACACTGCCATCTATCACTGTGCAAGAGGTTATAGTTACCCGGGACCTTACTGGGCGGAGGCACTCTGGTCAAGGTTCTCTTCA
294	ZNRF3-223 (VL DNA)	GATATTCAAGTGACTCAATCTCCATCCTCCCTCTTGGCATCTCTAGGAGAGAGAGTCACTATCACATGCCAGACAGGTCAAGCATTAAACAATTACCTAAACTGGTATCAGCAGAGGCCAGGACAAGCTCCTCTACTCTTGATCTATTTTGCAACCAAGTTGTCAGACTGGCGTGCCATCAAGGTTCAAGTGGCCAATATTCTGGGAGAAGTTTCACTCTAACCATCACTAGCCTGGAGCCAGAAGATATTGCAAATTATTTTGTCTGCAACATTACAGTCTCCGTACACGTTTGGAGCTGGGACCAAGCTGGAAGTGAAGA
295	ZNRF3-223 (VH prot)	EVQLVESGGGLVQPGESLKLSCSASGFTFSNYGMHWIRQVPKGKLDWVAYITN SGGSLYAAAVKGRFTISRDNKNTLYLELTSLKSEDTAIYHCARGYSYPGPYW GRGTLVT VSS
296	ZNRF3-223 (VL prot)	DIQVTQSPSSLLASLGERVTITCQTGQSINNYLNWYQQRPQAPLLLIYFATS LQTVPSRFSQYSGRSFTLTITSLPEPIANYFCLQHYSPPYTFGAGTKLELR
297	ZNRF3-231 (VH DNA)	GAGGTACAGCTGGTGGAGTCTGGAGGAGGCTTAGTGCAGCCTGGAGAGTCCCTGAAACTCTCCTGTTCAGCCTCTGGATTACATTCAGTAACATATGGAATGCACTGGATACGCCAAGTTCAGGAAAGGACTAGATTGGGTTGCATACATTAGTAATAGCGGCGGTTCACTCTATGCAGACGCTGTGAAGGGCCGTTCACTATCCAGAGACAATGCAAAGAACACCTATACCTGGAATCAACAGTCTGAAGTCTGAGG

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		ACACTGCCATCTATCACTGTGCAAGAGGTTATAGTTACCCGGGACCTTACTGGG GCCAAGGCACTCTGGTCACGGTCTCTTCA
298	ZNRF3-231 (VL DNA)	GATATTCAAGTGACTCAATCTCCATCCTCCCTCTTGGCATCTCTAGGAGAGAG AGTCACTATCACATGCCAGACAGGTGAGAGTATTAACAATTACCTGAACTGGT ATCAGCAGAGGCCAGGACAAGCTCCTCTACTCTTGATCTATTTTGCACCAGT TTGCAGACTGGCGTGCCATCAAGGTTGAGTGGCCAATATTCTGGGAGAGGTTT CACTCTAACCATCACTAGCCTGGAGCCAGAAGATATTGCAAATTATTTTGTCT TGCAACATTACAGTCTCCTCCGTACACGTTTGGAGCTGGGACCAAGCTGGAAGTGA AGA
299	ZNRF3-231 (VH prot)	EVQLVESGGGLVQPGESLKLSCSASGFTFSNYGMHWIRQVPGKGLDWVAYISN SGGSLYADAVKGRFTISRDNKNTLYLELNSLKSEDTAIYHCARGYSYPGPYW GQGT LVTVSS
300	ZNRF3-231 (VL prot)	DIQVTSQSSLLASLGERVTITCQTGQSINNYLNWYQQRPQGAPLLLIYFATS LQTVPSRFSQYSGRSFTLTITSLEPEDIANYFCLQHYSPPYTFGAGTKLEL R
301	ZNRF3-237 (VH DNA)	CAGATCCAGTTGGTGCAGTCTGGACCTGAGCTGAAGAAGCCTGGAGAGTCAGT GAAGATCTCTGCAAGGCTTCTGGGTATACCTTCACAGACTATGCAATGCACT GGGTGAAACAGGTTCCAGGAAAGGGCTTGAAGTGGATGGGCTGGATCAACACC TATACTGGGAAGCCAACATATGCTGATGACTTCAAAGGACGGTTTGTCTTCTC TTTGGAAGCCTCTGCCAGCACTGTAAATTTGCAGATCAGCAACCTCAAAAATG AGGACACGGCTAATGTTTTCTGTGCAAGAGGGGGTTATTATCACGTGGTTGG TTTGCTTACTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA
302	ZNRF3-237 (VL DNA)	GATATTGTGATGACACAACTCCAGTCTCCCTGTCTGTGAGCCTTGGAGGTCA AGTCTCTATCTCTTGCCGGTCTAGTCAGAGCTTTGTTTCATAGTGATGGAAATA GCTATTTGAGTTGGTACCTGCAGAAGCCCGCCAATCTCCACAGCTCCTCATT TATGAGGTTTCCAACCGATTATCTGGGGTCCCAGATAGGTTTCAGTGGTAGTGG TTCAGGGACAGATTTACCCCTCAAGATCAGCAGAGTAGAGCATGATGATTTGG GAGTTTATTACTGCGGCCAAGCTTCAAAAATCCGCTCACGTTCCGTTCTGGG ACCAAGCTGGAGATCAAA
303	ZNRF3-237 (VH prot)	QIQLVQSGPELKKPGESVKISCKASGYTFDYAMHWVKQVPGKGLKWMGWINT YTGKPTYADDFKGRFVFSLEASASTVNLQISNLKNEDTANVFCARGGLLSRGW FAYWGQGT LVTVSS
304	ZNRF3-237 (VL prot)	DIVMTQTPVSLSVSLGGQVSISCRSSQS FVHSDGNSYLSWYLQKPGQSPQLLI YEVSNRLSGVPDRFSGSGSDFTLKISRVEHDDLGVYYCGQASKIPLTFGSG TKLEIK
305	ZNRF3-244 (VH DNA)	GAGGTACAGCTGGTGGAGTCTGGAGGAGGCTTAGTGACGCTGGAAAGTCCCT GAACTCTCCTGTTCAGCCTCTGGATTACATTCAGTAGCTATGGCATGCACT GGATACGCCAACTCCAGGAAAGGGACTAGATTGGGTTGCATACATTAGTACT AACAGCGGTACAGTCTATGCAGACGCTGTGAAGGGCCGGTTCCACATCTCCAG AGACAATGCAAAGAACACCTGTACCTGCAACTCAACAGTCTGCAGTCTGAAG ACACTGCCATCTATTACTGTGCAAGAGGTTATAACTACCCGGGAGCTTACTGG GGCCAAGGCACTCTGGTCACTGTCTCTTCA
306	ZNRF3-244 (VL DNA)	GATATTCAAGTGACTCAATCTCCATCCTCCCTCTTGGCATCTCTAGGAGAGAG AGTCACTATCACATGCCAGACAAGTCAGAGCATTAGCAATAACCTAAACTGGT ATCAGCAGAAACCAGGACAAGCTCCTATGCTCTTGATCTATTATGCAACCAGT TTGCAGACTGGCATGCCATCAAGGTTGAGTGGCCAATATTCTGGGAGAGGTTT CACTCTAACCATCACTAGCCTGGAGCCAGAAGATATTGCAAATTATTTTGTCT TGCAACATTACAATCCTCCGTACACGTTTGGAGCTGGGACCAAGCTGGAAGTGA AAA
307	ZNRF3-244 (VH prot)	EVQLVESGGGLVQPGKSLKLSCSASGFTFSNYGMHWIRQTPGKGLDWVAYIST NSGTVYADAVKGRFTISRDNKNTLYLQLNSLQSEDTAIYYCARGYNYPGAYW GQGT LVTVSS
308	ZNRF3-244 (VL prot)	DIQVTSQSSLLASLGERVTITCQTSQSISNNLNWYQQKPGQAPMLLIYYATS LQTMPSRFSQYSGRSFTLTITSLEPEDIANYFCLQHYNPPYTFGAGTKLEL K
309	ZNRF3-247 (VH DNA)	GAGGTGCAGCTGGTGGGGTCTGGTGAAGGCTTAGTACAGCCTGGAAGTTCCAT GAACTCTCATGTGTAGCCTCTGGATTCACTTTCAGTAACCTCTGGCATGAACT GGATTGACAGGCTCCAAAGAAGGGCTGGAATGGATAGCACTGATTTATTAT ACTAACACATACTATGCAGACTCTGTGAAGGGTCGATTACCATCTCCAGAGA CAATTCTAAGAACACCCTGTACCTGGAAATGAACAGTCTGAGATCTGAGGACA

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		CAGCCATGTATTACTGTGCAAAAAGGGGTCTAACTGGGAGCGTTGATTACTGGG GCCAAGGAGTCATGGTCACAGTCTCCTCA
310	ZNRF3-247 (VL DNA)	GACATCCAGATGACCCAGTCTCCTTCATTCTGTCTGCATCTGTGGGAGACAG AGTCACTATCAACTGCAAAGCAAGTCAGAATATTAACAGGTACTTAACTGGT ATCAGCAGAAGCTTGGAGAAGCTCCCAAACGCCTAATATATGATACAAGCAAT TTGCAAACGGGCATTCCATCAAGGTTCAAGTGGCAGTGGGTCTGGTACAGATTT CACACTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCCACATATTTCTGCT TGCAGCATGATAGTTTTCCCAACACGTTTGGAACTGGGACCAAGCTGGAGCTG AAA
311	ZNRF3-247 (VH prot)	EVQLVGSGEGLVQPGSSMKLSCVASGFTFSNSGMNWIRQAPKKGLEWIALIYY TNTYYADSVKGRFTISRDN SKNTLYLEMNSLRSED TAMYYCAKRLTGSDVYD WGQVMVTVSS
312	ZNRF3-247 (VL prot)	DIQMTQSPSPFLSASVGDRTINCKASQINIRYLNWYQQKLGEPKRLIYDTSN LQTGIPSRFSGSGSGTDFTLTISSLQPEDFATYFCLQHDSFPNTFGTGKLEL K
313	ZNRF3-253 (VH DNA)	GAGGTGCAGCTTG TAGAGTCTGGGGGCGGTTAGTGCAGCCTGAAAGGTCCAT GAAACTCTCCTGTGCAGCCTCAGGATTCATTTTCAGTA ACTATGGCATGGCCT GGGTCCGCCAGGCTCCAACGAAGGGGCTGGAGTGGGTTGCAACCATTAATTTCT GATGGTAGTGGCACTTACTATCGAGGCTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATGCAAAAAGCACCCCTATATCTACAAATGAACAGTCTGAGGTCTG ACGACACGGCCACTTATTACTGTACAAGAGGTCGGATGCTACGGATCATTAGC TTCTTTGATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCTTCA
314	ZNRF3-253 (VL DNA)	GACATCCAGATGACACAGTCTCCAGCTTCCCTGTCAGCATCTCTTGGAGAAAC TGTCTCCATCGAATGTCTAGCAAGTGAGGACATTGCCAATGATTTAGCGTGGT ATCAACAGAAGTCAGGGAAATCTCCTCAGGTCTGATCTATGCTGCAAGTAGG GTGCAAGACGGGGTCCCATCACGGTTCAGTGGCAGTGGATCTGGCACACGGTA TTCTCTCAAGATCACCGACATGCAACCTGAAGATGTGGCAGATTATTTCTGTC AACAGAGTTACGGCTTTCCGCTCACGTTCCGTTCTGGGACCAAGCTAGAGATC AAA
315	ZNRF3-253 (VH prot)	EVQLVESGGGLVQPERSMKLSCAASGFTFSNYGMAWVRQAPTKGLEWVATINS DGSGETYYRGSVKGRFTISRDN AKSTLYLQMNSLRSDDTATYYCTRGRMLRIIS FFDYWGQVMVTVSS
316	ZNRF3-253 (VL prot)	DIQMTQSPASLSASLGTVSIECLASEDIANDLAWYQQKSGKSPQVLIYAASR VQDGVPSRFSGSGSGTRYSLKITDMQPEDVADYFCQQSYGFPLTFSGTKLEI K
317	ZNRF3-254 (VH DNA)	GAGGTGCAACTGGTAGAGTCTGGGGGCGGTCTAGTGCAGCCTGAAAGGTCCAT GAAACTCTCCTGTGCAGCCTCAGGATTCATTTCAATAACTATGGCATGGCCT GGGTCCGCCAGACTCCAACGAAGGGACTGGAATGGGTTGCAACCATTAATTTCT GATGGTAGTGGCACTACTATCGAGGCTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATGCAAAAAGGCACCCCTATATCTACAAATGCACAATCTGAGGTCTG ACGACACGGCCACTTATTACTGTGCACGAGGTGGGATACTTCGGATCATTAACC TACTTTGATTCTCTGGGGCCAAGGAGTCGTGGTCACAGTCTCCTCA
318	ZNRF3-254 (VL DNA)	GACATCCAGATGACACAGTCTCCAGCTTCCCTGTCAGCATCTCTTGGAGAAAG TGTCTCCATCGAATGTCTAGCAAGTGAGGACATTGCCAATGATTTAGCGTGGT ATCAACAGAAGTCAGGGAGATCTCCTCAGGTCTGATCCATGCTGCAAGTAGG GTGCAAGACGGGGTCCCATCACGGTTCAGTGGCAGTGGATCTGGCACACGGTA CTCTCTCAAGATCACCGACATGCAACCTGAAGATGTAGCAGATTATTTCTGTC AACAGAGTTACGGCTTTCCGCTCACGTTCCGTTCTGGGACCAAGCTAGAGATC AAA
319	ZNRF3-254 (VH prot)	EVQLVESGGGLVQPERSMKLSCAASGFTFNNGMAWVRQTPTKGLEWVATINS DGSGETHYRGSVKGRFTISRDN AKGTLYLQMHNLRSDDTATYYCARGRIILRIIT YFDSWGQGVVTVSS
320	ZNRF3-254 (VL prot)	DIQMTQSPASLSASLGESVSIIECLASEDIANDLAWYQQKSGRSPQVLIHAASR VQDGVPSRFSGSGSGTRYSLKITDMQPEDVADYFCQQSYGFPLTFSGTKLEI K
321	ZNRF3-255 (VH DNA)	GAGGTGCAGCTGGTGGAGTCGGGGGGAGGCTTAGTGCAGCCTGGAAGGTCCCT GAAACTCTCCTGCGCAGCCTCAGGATTCATTTTCAGTAGCTTTACAATGGCCT GGGTCCGCCAGGCTCCAAGAAGGGTCTGGAGTGGGTGCGCATTCATTAGTTCT GGTGGTGGTGGCACTTACTATCCAGATTCCGTGAAGGGCAGATTTACTATCTC CAGAGATAATGCAAAAAGCACCCCTATACCTGCAAAATGGACAGTCTGAGATCTG

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		AGGACACGGCCAGTTACTATTGTGCAACCGGGGGTTTGCTTACTGGGGCCAG GGCACTCTGGTCATTGTCTCTTCA
322	ZNRF3-255 (VL DNA)	GACATCCAGATGACCCAGTCTCCATCTTCCCTGCCTGCATCTCTGGGAGACAG AGTCACTATTACTTGCCGGGCAAGTCAAGACATTGGAAGTTATTTAAGATGGT TCCAGCAGAAACCGGGGAAGTCTCCTAGGCTTATGATTTATGGTGAACACAGC TTGGCAAATGGGGTCCCATCAAGGTTCAAGTGGCAGTAGGTCTGGGTGAGATTA TTCTCTGACCATCAACAACCTGGAGTCTGAAGATATGGCTATTTATTACTGTC TGCAGCATAATGAGTTTCCGTACACGTTTGGAACTGGGACCAAGCTGGAAGTGA AAA
323	ZNRF3-255 (VH prot)	EVQLVESGGGLVQPGRSLKLSAASGFTFSSFTMAWVRQAPKKGLEWVASISS GGGGTYYPDSVKGRFTISRDNASTLYLQMDSLRSEDATSYCATGGFAYWGQ GTLVIVSS
324	ZNRF3-255 (VL prot)	DIQMTQSPSSLPASLGDRVTITCRASQDIGSYLRWFQQKPKGSPRLMIYGATS LANGVPSRFSRSGSDYSLTINNLESEDMAIYYCLQHNEFPYTFGTGKLEL K
325	ZNRF3-265 (VH DNA)	GAGGTGCAGTTGGTAGAGTCTGGGGGCGGTTTAGTGCAGCCTGGAAGGTCCAT GAAACTCTCCTGTGTAGCCTCAGGATTCAGTTTCAGTAAATATGGCATGGCCT GGGTCCGCCAGGCTCCGACGAAGGCTTTGGAGTGGGTTGCAATCATTAGTCAG GATGGTAGTAGAATTTACTATGGAGGCTCCGTGAAGGGCCGATTCACTCTCTC CAGAGATAATGAAAAAAGCACCCACACCTGCACATGAACAGTCTGAGGTCTG AGGACACGGCCACTTATTATTGTGCAAGAGGAAGTATACTACGGATGATAACT TTCTTTGATTATTGGGGCCAAGGAGTCACGGTCACAGTCTCCTCA
326	ZNRF3-265 (VL DNA)	GACATCCAGATGACTCAGTCTCCAGCTTCCCTGTCTGCATCTCTTGGAGAAAC TGTCTCCATCGAATGTCTAGCAAGTGAGGGCATTTCGAATGATTTAGCGTGGT ATCAGCAGAAAGTCAGGGAAATCTCCTCGGCTCCTGATCTATGCTGCAAGTAGG TTGCAAGACGGGGTCCCATCACGGTTCAGTGGCAGTGGATCTGACACACGGTT TTCTCTCAAGATCAGCGGCATGCACCCCTGAAGATGAAGCAGATTATTTCTGTC AACAGAGTTACGGTTATCCGCTCACGTTCCGTTCTGGGACCAAGCTGGAGATC AAA
327	ZNRF3-265 (VH prot)	EVQLVESGGGLVQPGRSMKLSCVASGFSSFSKYGMAWVRQAPT KALEWVAII SQ DGSRIYYGGSVKGRFTLSRDNEKSTLHLHMSLRSEDATYYCARGSILRMIT FFDYWGQGVTVTVSS
328	ZNRF3-265 (VL prot)	DIQMTQSPASLSASLGSETVSI ECLASEGISNDLAWYQQKSGKSPRLLIYAASR LQDGVPSRFSRSGSDTRFSLKISGMHPED EADYFCQQSYGYPLTFGSGTKLEI K
329	ZNRF3-269 (VH DNA)	GAGATACAACCTTCAGGAGTCAGGACCTGGCCTTGTGAAACCTTCACAGTCACT CTCCCTCACTTGTCTGTCACTGATGACACCATTACAGTGGTTATGATTGGA GCTGGATCCGGAAGTTCCCGGGAATAAGATGGAGTGGATGGGGACATAAGC AGCAGCGGGCACACGAATAACACCTTCGCTCCAGATTGCGATCTCCATAAC TAGAGACACATCCAAGAATCAGTTCTTTCTGCAGTTGAACTCTGTAACAACTG AGGATTCAGCCACCTATTATTGTGCTAGAGGCGGTTTTTTATTTCTTTTACT TTCTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA
330	ZNRF3-269 (VL DNA)	GACATCCAGATGACCCAGTCTCCATCCTCCCTGCCTGCATCTCTGGGAGAGAG AGTCACCATCAGTTGCAGAGCAAGTCAGGTTCTTAACAATAATTTAAACTGGT ATCAGCAGAAACCAGATGGAACGATTAAACCCCTACTCTACTACACATCCAAT TTACAATCTGGTGTCCCATCAAGGTTCAAGTGGCAGTGGGTCTGGGACAGATTA TTCTCTCACCATCACCAGCCTTGACCCCTGAAGATTTTGCAATATATTACTGCC AACAGGATGCTAGTTTTCTCCTCCGACGTTCCGTTGGCGGCACCAAGCTGGAATTG AAA
331	ZNRF3-269 (VH prot)	EIQLQESGPGVLKPSQSLSLTCSVTDDTITSGYDWSWIRKFPGNKMEWMGDIS SSGHTNYPNLSQIRISITRDTSKNQFFLQLNSVTTEDSATYYCARGGFLFPFT FWGQGT LVTVSS
332	ZNRF3-269 (VL prot)	DIQMTQSPSSLPASLGERTVITSCRASQVLNNLNWYQQKPDGTIKPLLYTNS LQSGVPSRFSRSGSGSDYSLTITSLDPEDFAIYYCQQDASFPPTFGGKLEL K
333	ZNRF3-270 (VH DNA)	GAGGTACAGCTGGTGGAGTCTGGAGGAGGCTTAGTGCAGCCTGGAGAGTCCCT GAAACTCTCCTGTTCAAGCTCTGGATTACATTCAGTAATATGGCATGCACT GGATACGCCAAGTTCCAGGAAAGGGCTAGATTGGGTTGCATACATTACTAAT AGCGGCGGTTCACTCTATGCAGCGCTGTGAAGGGCGGTTACCATCTCCAG AGACAATGCAAAGAACACCCTGTACCTGGAACCTCACCAGTCTGAAGCCTGAGG

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		ACACTGCCATCTATCACTGTGCAAGAGGTTACAGTTATCCGGGACCTTACTGGGCGGAGGCACTCTGGTCACGGTCTCTTCA
334	ZNRF3-270 (VL DNA)	GATATTCAAGTGACTCAATCTCCATCCTCCCTCTTGGCATCTCTAGGAGAGAGAGTCACTATCACATGCCAGACAGGTGAGAGCATTAAACAATTACCTAACTGGTATCAGCAGAGGCCAGGACAAGCTCCTCTACTCTTGATCTATTTTGCACCAGTTTGCAGACTGGCGTGCCATCAAGGTTCAAGTGGCCAATATTCTGGGAGAGGTTTCACTCTAACCATCACTAGCCTGGAGCCAGAAGATATTGCAAATTATTTTGTCTGCAGCATTACAGTCTCCGTACACGTTTGGAGCTGGGACCAAGCTGGAAGTGAAGA
335	ZNRF3-270 (VH prot)	EVQLVESGGGLVQPGESESLKLSASGFTFSNYGMHWIRQVPGKGLDWVAYITN SGGSLYAAAVKGRFTISRDNKNTLYLELTSLPEDTAIYHCARGYSYPGPYWGRGTLVTVSS
336	ZNRF3-270 (VL prot)	DIQVTSQSPSSLLASLGERVTITCQTGQSINNYLNWYQQRPGQAPLLLLIYFATS LQTVPSRFSGQYSGRSFTLTITSLEPEDIANYFCLQHYSPPYTFGAGTKLELR
337	ZNRF3-275 (VH DNA)	CAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTTGTCAGCCCTCACAGACCTGTCCCTCACCTGCACTGTCTCTGGGTTCTCATTTAACTACTATAATGTTCACTGGCTTCGACAGCCTCCAGGAAAGGGTCTGGAGTGGTGGGAGTAATCTGGAGTGGTGGAAACAGAGATTACAATTGAGTCTCAAACCCCGACTGCGCATCAGCAGGACACCTCCAAGACCCAAGTTTTCTTAACATTGAACAGTCTGCAGACTGAGGACACAGGCATTTACTATTGTACAGAAATGTGGGGCTATGGGTACAACCCCTTTGATCACTGGGGCCAAGGAGTCATGGTCACTGCTCTCTCA
338	ZNRF3-275 (VL DNA)	GACATCCAGATGACTCAGTCTCCGTCACTTCTGTCTGCATCTCTTGGAGACAA CATCACCATCACTTGCCATGCCAGTCAGGACATCAAGGGTTGGTTAGCCTGGTACCAACAAAAGTCAGGGAATGCTCCTGAACTGTTGATTTATAAGGCATCTAGCCTGCAATCAGGGGTTCATCAAGATTCAGTGGCGGCGGATCTGGAACAGAATA TGTTCTCACTATCAGCAACCTACAGCCTGACGATATTGCCACTTATTACTGTCAGCTTTATCACAGCTTTCATTACGTTTGGCTCAGGGACGAAGTTGGAAACA AAA
339	ZNRF3-275 (VH prot)	QVQLKESGPGLVQPSQTLSTCTVSGFSFNYYNVHWLRQPPGKGLEWLGLVWS GGNRDYNSALKPRLRISRDTSKTQVFLTLNSLQTEDTGIYYCTRMWGYGNPF DHWGQGVMTVSS
340	ZNRF3-275 (VL prot)	DIQMTQSPSFLSASLGDNITITCHASQDIKGLAWYQQKSGNAPELLIYKASS LQSGVPSRFSGGGSGTEYVLTISNLPDDIATYYCQLYHSFPTFGSGTKLET K
341	ZNRF3-279 (VH DNA)	CAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTGAAGCCTGGGACTTCTGTGAGGCTGTCTCTGCAAGGCTTCTGGCTACACCTTTACTCACAACCATATGAACTGGATAAAGCAGACGACTGGACAGGGCCTTGAGTGGATTGGAATTATTAATCCGGGAAGTGGAGTACTAATTACAATGTGAGGTTCAAGGGCAAGGCCACATTGAC TGTAGACACATCCTCCAGCACAGCCTTCATGCAACTCAGCAGCCTGACACCTGAGGACTCTGCGGTCTATTACTGTGCAAGAAACCTTATTACTATAGCAGGCCCCGTTTGCTTACTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA
342	ZNRF3-279 (VL DNA)	GACACTGTACTGACCCAGTCTCCTGCTTTGACTGTGTCTCCAGGAGAGAGGGT TTCCATCTCCTGTAGGGCCAGTGAAGGGTCAAGTTCATATATACACTGGTACC AACAGAAACCAGGACAGCAGCCAACTCCTCATCTATAAAGCATCAAACCTA GCATCTGGGGTCCCTGCCAGGTTCAAGTGGCAGTGGGTCTGGGACAGACTTCAC CCTCACCATTGATCCTGTGGAGGCTGATGACACTGCAACCTATTTCTGTGTCAGC AGAGTTGGATTGATCACACGTTTGGAACTGGGACCAAGCTGGAACCTGAAA
343	ZNRF3-279 (VH prot)	QVQLQQSGAELVKPGTSLVLSCKASGYTFTHNHMNWIKQTTGQGLEWIGIINP GSGGTNYNVRFKQKATLTVDTSSTAFMQLSSLTPEDSAVYYCARNLITIAGP FAYWGQGLTVTVSS
344	ZNRF3-279 (VL prot)	DTVLTQSPALTVSPGERVVISCRASEGVSSYIHWYQQKPGQPKLLIYKASNL ASGVPARFSGSGSDFTLTIDPVEADDTATYFCQQSWIDHTFGTGKLELK
345	ZNRF3-287 (VH DNA)	GAGGTGCAGTTGGTGGGGTCTGGTGGAGGCTTGGTGCAGCCTGGAAGGTCTCTGAAAATCTCCTGTGCAGCCTCAGGATTCACCTTTCAGTTACTATGGCATGGCCTGGGTCCGCCAGGCTCCAACGAGGGGGCTGGAGTGGGTGCAACCATTACTTATAATGGTTCTAAGTCTTACTATCGAGACTCCGTGAAGGGCCGATTCACTATCTCAGAGAGTATGCAAAAAGCACCTTATACCTCCAATGGACGATCTGACGTCTG AAGACACGGCCACTTATTTCTGTGCAAGACGGACTACGGGTATGCTCTTTGAT TACTGGGGCCAAGGAGTCTTGGTCACTGCTCTCTCA

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346	ZNRF3-287 (VL DNA)	CAGGCTGTCCTTACTCAGCCAAACTCTGTGTCTATGTCTCTAGGAAGCACAGT CAAGCTGTCTTGCACACTCAACTCTGGTAACATAGAAAACAACCTTTGTGCACT GGTACCAACAATATGAGGGAAGATCTCCCACCACTATGATTTATAATGATGAT AAGAGACCGGATGGTGTCCCTGACAGGTTCTCTGGCTCCATTGACAGCTCTTC CAACTCAGCCTTCCTGACAATCAATAATGTGAAATTGGAGATGAAGCTATCT ACTTCTGTCAATCTTTTGTAGTAGTATTCATATTTTCGGCGGTGGAACCAAG CTCACTGTCCTA
347	ZNRF3-287 (VH prot)	EVQLVSGSGGLVQPGRSLKISCAASGFTFSYYGMAWVRQAPTRGLEWVATITY NGSKSYRDSVKGRFTISREYAKSTLYLQMDLTS EDTATYFCARRTGM LFD YWGQGV LTVSS
348	ZNRF3-287 (VL prot)	QAVLTQPN SVMSLGSTVKLSCTLNSGNIENNFVHWYQQYEGRSPTTMIYND KRPDGV PDRFSGSIDSSNSAFLTINNVEIGDEAIYFCHSFVSSIHFGGGTK LTVL
349	ZNRF3-296 (VH DNA)	GAGGTACAGCTGGTGGAGTCTGGAGGAGGCTTAGTGCAGCCTGGAAAGTCCCT GAGACTCTCCTGTTCAGCCTCTGGATTACATTACAGTAAGTATGGCCTGCACT GGATACGCCAAGCTCCAGGAAAGGACTAGATTGGCTTGGATATAGTAGTAGT AACAGCGGTACAGTCTATGGAGACGCTGTGAAGGGCCGGGTCAACATCTCCAG AGACAATGCAAGAACACCCTGTACCTGCAACTCAACAGTCTGAAGTCTGAAG ACACTGCCATCTATTATTGTGCAAGAGGCTATAGCAGCTCCCCGTTTGCTTAC TGGGGCCAAGGCACCTCTGGTCACTGTCTCTTCA
350	ZNRF3-296 (VL DNA)	GACATTGTGCTGACCCAGTCTCCTGCTTTGGCTGTGTCTCTAGGCCAGAGAGT CACCATCTCATGCAAAGCCAGCCAGAATGTCGATGGTTTTGGCATTGGTTATA TGCCTGGTACCAACAGAAACCAGGACAGAAACCCAACTCCTCATCTTTGCA GGATCCAACCTAGCATCTGGGATCCCTGCCAGGTTCACTGGCAGTGGGTCTGG GACAGACTTCAACCTCACCATCGATCCTGTGGAGACTGATGATATTGCAACCT ATTACTGTGAGCAGAAATAAGATTTTCCCTCACGTTCCGTTCTGGGTCCAGG CTGGAGATCACA
351	ZNRF3-296 (VH prot)	EVQLVESGGGLVQPGLSLRLSCSASGFTFSNYGLHWIRQAPGKGLDWLGYSS NSGTVYGD AVKGRVTISRDNAKNTLYLQLNSLKSEDTAIYYCARGYSSSPFAY WGQGT LTVSS
352	ZNRF3-296 (VL prot)	DIVLTQSPALAVSLGQRVTISCKASQNV DGF GIGYMHWYQQKPGQPKLLI FA GSNLA SGIPARFSGSGSDFTLTIDPVETDDIATYYCQQNKDFPLTFGSGSR LEIT
353	ZNRF3-30 (VH DNA)	GAGGTGCAGCTGGCGGAGTCTGGGGGAGGCTTAGAGCAGCCTGGAAGTCCCT GAACTCTCCTGTGCAGCCTCAGGATTCATTTACAGTAAGTATGACATGGCCT GGGTCCGCCAGGCTCCAACGAGGGGTCTGGAGTGGGTGCGATCCATTCCTACT AGTGGTGGTACTACTTATTATCGAGACTCCGTGAAGGGCCGATTCACTGTCTC CAGAGATAATGCAAAAAGCACCTTACCTGCAATGGACAGTCTGAGGTCTG AGGACACGGCCACTTATTACTGTGCAAGACATGGGGTCGGTAGCTACGATTGG TTTGCTGACTGGGGCCAAGGCACCTCTGGTCACTGTCTCTTCA
354	ZNRF3-30 (VL DNA)	GACATTGTCTTGACCCAGTCTCCTGCTTTGGCTGTGTCTCTAGGGCAGAGGGC CACAATCTCCTGTAGGGCCAGCCAAAGTGTCACTATATCTGGATATAATCTTA TGCCTGGTACCAACAGAAACCAGGACAACAACCCAACTCCTCATCTATCGT GCATCCAACCTAGCATTTGGGATCCCTGCCAGGTTCACTGGCAGTGGGTCTGG GACAGACTTCAACCTCACCATCAATCCTGTGCAGGCTGATGATATTACGACCT ATTACTGTGAGCAGAGTAGGAAGTCTCGGACGTTCCGTGGAGGCACGAGACTG GAATTGAAA
355	ZNRF3-30 (VH prot)	EVQLAESGGGLEQPGRLKLSCAASGFTFSNYDMAWVRQAPTRGLEWVASILT SGGTTYRDSVKGRFTVSRDNASTLYLQMDSLRSEDTATYYCARHGVGSYDW FADWQGT LTVSS
356	ZNRF3-30 (VL prot)	DIVLTQSPALAVSLGQRATISCRASQSVTISGYNLMHWYQQKPGQPKLLIYR ASNLA FGI PARFSGSGSDFTLTINPVQADDITYYCQQSRKSRFTGGGTRL ELK
357	ZNRF3-300 (VH DNA)	GAGGTGCAGCTGGTAGAGTCTGGGGGCGGTTTAGTACAGCCTGAAAGTCCAT GAACTCTCCTGTGCAGCCTCAGGATTCATTTACAGTAAGTATGGCATGGCCT GGGTCCGCCAGGCTCCAACGAAGGGGCTGGAGTGGGTGCAACCATTAATCTC GATGGTAGTGGCACTTACTATCGAGGCTCCGTGAAGGGCCGATTCACTATCTC CAGAGATAATGCAAGAAACACCCTATACCTAGAAATGAACAGTCTGCGGTCTG ACGACACGGCCACTTATTACTGTACAAGAGGTCGGATACTACGATTATTACC TACTTTGATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA

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358	ZNRF3-300 (VL DNA)	GACATCCAGATGACACAGTCTCCAGCTTCCCTGTGAGCATCTGTTGGAGAAACCGTCTCCATCGAATGTCTAGCAAGTGAGGACATTGCCAATGATTTAGCGTGGTATCAGCAGAAGTCAGGGAAATCTCCTCAGGTCTGATCTATGCTGCAAGTAGGTGCAAGACGGGGTCCCATCACGGTTCAGTGGCAGTGGATCTGGCACACGATA TTCTCTCAGGATCAGCGACATGCAACCTGAAGATGTAGCAGATTATTTCTGTC AACAGAGTTACAGCTTTCCGCTCACGTTCCGATCTGGGACCAAGCTGGAGATC AAA
359	ZNRF3-300 (VH prot)	EVQLVESGGGLVQPERSMKLSCAASGFTFSNYGMAWVRQAPTKGLEWVATINS DSGSTYYRGSVKGRFTISRDNARNTLYLEMNSLRSDDTATYYCTRGRILRIIT YFDYWGGQVMVTVSS
360	ZNRF3-300 (VL prot)	DIQMTQSPASLSASVGETVSI ECLASEDIANDLAWYQQKSGKSPQVLIYAASRVQDGVPSRFSGSGSGTRYSLRISDMQPEDVADYFCQQSYSFPLTFGSGTKLEIK
361	ZNRF3-301 (VH DNA)	CAGGTACAGCTGAAGGAGTCAGGACCTGGGCTGGTGCAGCCCTCACAGACCTGTCCCTCACCTGCAGTGTCTCTGGGTTCTACTAACCAGCTACAGTGTACTGGGTTGCGCCAGCACTCAGGAAAGAGTCTGGAATGGATGGGAAGAATATGGAGTGATGGAGACACATCATATAATTCAGCGTTCACATCCCAGCTGAGCATCAGCAGGGACACCTCCAAGAGCCAAGTTTCTTAAAAATGAACAGTCTCCAACTGAGGACACAGGCACTTACTACTGTGACAGATCGGGTGGGAGCCCTTCTTTGATTAT TGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
362	ZNRF3-301 (VL DNA)	CAGTTTGTGCTTACTCAGTCAAACCTCTGTGTCTACGAATCTCGGAAGCACAGTCAAACCTGTCTTGCGAGCGCAGCATTGGTAACATTGGAAGCAATTATGTGAATTGGTACCAGCAGTATGAGGGAAGATCTCCACCAGTATGATTTATAAGTTTGATCAGAGACCTGATGGAGTTCCTGACAGGTTCTCTGGCTCCATTGACAGATCTTCCAATTCAGTCTCCTGACAATCAATAATGTGCAGACTGAAGATGAAGCTGACTACTTCTGTGAGTCTTACAGTAGTGATATGTATATTTTCGGCGGTGGAACCAAGCTCACTGTCTCA
363	ZNRF3-301 (VH prot)	QVQLKESGPGLVQPSQTLSTCTVSGFSLTSYSVHWVRQHSKSLSEWMGRIWSDGDTSYNSAFTSRSLISRDTSKSQVFLKMNSLQTEDTGTYYCDRSGGSPFFDYWGQGVMMVTVSS
364	ZNRF3-301 (VL prot)	QFVLTQSNVSTNLGSTVKLSCERSIGNIGSNYNWYQQYEGRSPPTMIYKFDQRPDGVPRFSGSIDRSSNSVLLTINNVQTEDEADYFCQSYSSDMYIFGGGTKLTVL
365	ZNRF3-305 (VH DNA)	GAGGTCTGTGTCAGCAGTCAGGCGCTGAGCTTGTGAGACCTGGGTCTCAGTCAAGATTTCTTGCAAGACTTCTGGCTACACCTTTACAGATTACTATTTGCACTGGGTGAAGCAGAGGCCTGAACAGGGCCTGGTATGGATAGGACGGATTAATCCTATAAATGGAAAACTGTTTGTATAGAGAAGTTCAAACCAAGGCCACACTGAC TGCAGATAAATCGTCCAACACAGCCTACATGGAACCTCAGCAGCCTGACATCTGAAGACACCGCAACCTATTTTGTACTAGAGAGGGCTTGCTTACTGGGGCCAA GGCCTCTGGTCACTGTCTCTTCA
366	ZNRF3-305 (VL DNA)	GACATCCAGATGACCCAGTCTCCATCGTCCCTGCCTGCATCTCTGGGAGAGAGAGTCAACATCAGTTGTAGAGCAAGTCAGGGTATTAGCAATAATTTAACTGGTATCAGCAGAAACAGATGGAACGATTAAACCCCTGATCTACTACACATCCAATTTACAATCTGGTGTCCCATCAAGGTTCAAGTGGCAGTGGGTCTGGGACAGATTA TTCTCTCACCATCGGCAGCCTTGAGCCTGAAGATTTTACAATGTATTACTGCC AACAGGATGCTAGTTTTCTCCACGTTTGAGAGCTGGGACCAAGCTGGAAGCTG AAA
367	ZNRF3-305 (VH prot)	EVLLQQSGAELVRPGSSVKISCKTSGYTFDYYLHWVKQRPEQGLVWIGRINP INGKTVCIEKFKTKATLTADKSSNTAYMELSSLTSEDATYFCTRGGGLAYWQG GTLVTVSS
368	ZNRF3-305 (VL prot)	DIQMTQSPSSLPASLGERVTISCRASQGISNNLNWYQQKPDGTIKPLIYYTSNLQSGVPSRFSGSGSGTDYSLTIGSLEPEDFTMYCQQDASFPPTFGAGTKLELK
369	ZNRF3-312 (VH DNA)	CAGGTACAACTGCAGCAGTCTGGAGCTGAGTTGGTGAAGCCTGGGTCTCAGTGAAGATGTCTGCAAGGCTTCTGGCTACAGTTTCAACACCTACTATATCTATTGGATAAATCAGAGGCCTGGACAGGGCCTTGAGTGGATTGGGCGTATTGGTCTT GGAAGTGGAGATACTGATTACAATGAGAAATCAAGGGCAAGGCCACATTAC TGTAGACAAATTTTCCAACACAGCCTACCTGCAACTCAGCAGCTGACACCTG AGGACACTGCGGTCTATTACTGTGCAAGATGGGACTACGGGTATAGGGGGTTT GCTTACTGGGGCCAGGGCACTCTGGTCACTGTCTCTTCA

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370	ZNRF3-312 (VL DNA)	GATGTTGTGATGACACAAACTCCAGTCTCCCTGTCTGTCAGCCTTGGAGGTCA AGTCTCTATCTCTTGCCGGTCTAGTCAGAGCTTTGTACACAGTGATGGAAATA CCTATTTGAATTGGTACCTGCAGACGCCCCGGCCAATCTCCACAACCTCTCATC TATAAGGTTTCCAACCGATTATCTGGGGTCCCAGATAGGTTCAAGTGGTAGTGG TTCAGGGACATATTTACCCTCAAGATCAGCAGAGTCGAGCATGATGATTGG GAGTTTATACTGCGGCCAGGCTTCAAAAATTCGCTCACGTTCCGTTCCGGG ACCAAGCTGGAGATCAAA
371	ZNRF3-312 (VH prot)	QVQLQQSGAELVKPGSSVKMSCKASGYSFTYYIYWINQRPQGLEWIGRIGL GSGD TDYNEKFKGKATFTVDKFSNTAYLQLSSLTPEDTAVYYCARWDYGYRGF AYWGQGT LVT VSS
372	ZNRF3-312 (VL prot)	DVVM TQTPVSLSVSLGGQVSI SCRSSQS FVHSDGNTYLNWYLQTPGQSPQLLI YKVS NR LSGVPDRFSGSGSYFTLKISRVEHDDLGVYYCGQASKIPLTFGSG TKLEIK
373	ZNRF3-314 (VH DNA)	CAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTTGTGCAGCCCTCACAGACCT GTCCCTCACCTGCAATGTCTCTGGTTTCTCATTTAACTACTATAATGTACACT GGGTTCGACAGCCTCCAGGAAAGGGTCTGGAGTGGTGGGAGTAATCTGGAGT GGTGGAAACACAGATTACAATTCAGCTCTCAAACCCCGACTGCGCATCAGCAG GGACACCTCCAAGAGCCAAGTTTCTTAACATTGAACAGTCTGCAGACTGAGG ACACAGGCATTACTATTGTAACAGAATGTGGGGCTATGGGTATAACCCCTTT GATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
374	ZNRF3-314 (VL DNA)	GACATCCAGATGACTCAGTCTCCGTCATTTCTGTCTGCATCTCTTGGAAACAA CATCACCATCACTTGCCATGCCAGTCAGGACATCAAGGGTGGTTAGCCTGGT ACCAACAAAAGTCAGGGAATGCTCCTGAACTGTTGATTTATAAGACATCTAGC CTGCAATCAGGGGTTCCATCAAGATTCAAGTGGCAGCGGATCTGGAACAGAATA TGTTCTCACTATCAGCAACCTACAGCCTGACGATATTGCCACTTATTACTGTC AGCTTTATCACAGCTTTCCATTACGTTCCGGCTCAGGGACGAAGTTGGAAATA AAA
375	ZNRF3-314 (VH prot)	QVQLKESGPGLVQPSQTLSTLCNVSGFSFNYYNVHWRQPPGKLEWLGVIWS GGNTDYN SALKPRLRISRDTSKSQVFLTLNSLQTEDTGIYYCNRMWGYGNPF DYWGQGVMTVSS
376	ZNRF3-314 (VL prot)	DIQMTQSPSFSLASLGNNITITCHASQDIKGLAWYQQKSGNAPELLIYKTSS LQSGVPSRFSGSGSGTEYVLTISNLPDDIATYYCQLYHSFPFTFGSGTKLEI K
377	ZNRF3-322 (VH DNA)	GAGGTGCACCTGGTGGAGTCTGGGGGAGGCTAGTGCAGCCTGGAAGGTCTCT GACACTATCCTGTGTTGCCTCTGGATTACATTCATAAAGTGGTGGATGACCT GGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGCATCCATTTCTCAC ACTGGTGGTAGCACCTCCTATCCAGACTCTGTGAAGGGCCGTTTCACTATCTC CAGAGATAATGCAAAAAGTACCTTACCTGCAATGACCAGTCTGAGGTCTG ACGATACGGCCACTTATTACTGTACAAGATGGGGACCCATACTACGGGGCTTT GATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
378	ZNRF3-322 (VL DNA)	GATGTTGTGCTGACCCAGACTCCACCCACTTTATCGGCTACCATTGGACAATC CGTCTCCATCTCTTGACAGGTCAAGTCAGAGTCTCTTACATATTAATGGAAACA CCTATGTAAATTGGTTGGTACAGAGGCCAGGCCAACCTCCACAACCTCTATTT TATTTGGTATCCAGACTGGAATCTGGGGTCCCCAACAGGTTCAAGTGGCAGTGG GTCAGGAAGTGAATTCACACTCAAATCAGTGGAGTAGAGGCTGAGGATTTGG GAGTTTATTACTGCGTGCAAAGTACCCATGCTCCTCCGACGTTCCGGTGGAGGC ACCAAGCTGGAATTGAAA
379	ZNRF3-322 (VH prot)	EVHLVESGGGLVQPGRSLT LSCVASGFTFNNWMTWIRQAPGKLEWVASISH TGGSTSY PDSVKGRFTISRDNASTLYLQMTSLRSDDTATYYCTRWGPILRGF DYWGQGVMTVSS
380	ZNRF3-322 (VL prot)	DVVL TQTPPTLSATIGQSVSISCRSSQSL LHINGNTYVNWLVQRPQPPQLLF YLVSRLES GVPNRFSGSGSGTDFTLKISGVEAEDLGVIYCVQSTHAPPTFGGG TKLELK
381	ZNRF3-329 (VH DNA)	GAGGTACAGCTGGTGGAGTCTGGAGGAGGCTTAGTGCAGCCTGGAAAGTCCCT GAAACTCTCTGTTCAGCCTCTGGATTACATTCAGTAGCCATGGCATGCACT GGATACGCCAAGCTCCAGGAAAGGGGCTAGATTGGGTTGCATACATTAGTAAT AACAGCGGTACAATCTATTACCGCTGTGAGGGGCGGTTCCACATCTCCAG AGACAATTCAAAGAACACCTGTTCCTACAACCTCAACAGTCTGAAGCTGAAG ACACTGCCATCTATTACTGTGCAAGAGGTTATAACTACCCGGGAGCTTACTGG GGCCAAGGCACTCTGGTCACTGTCTCTTCA

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382	ZNRF3-329 (VL DNA)	GATATTCAAGTGACTCAATCTCCATCCTCCCTCTTGGCATCTCTAGGAGAGAGAGTCACTATCACATGCCAGACAAGTCAGAGCATTAGAAATTACCTAAACTGGTATCAGCAGAAACCAGGACAAGTTCCCATACTCTTGATCTATTATGCAACCAGTTTGCAGACTGACATGCCATCAAGGTTCAAGTGGCCAATATTCTGGGAGAAGTTTCACTCTCACCATCACTAGCCTGGAGCCAGAAGACATTGCAAATTATTTTGTCTGCAGCATTACAGTGTTCCGTACACGTTTGGAGCTGGGACCAAGCTGGAAGTAAA
383	ZNRF3-329 (VH prot)	EVQLVESGGGLVQPGKSLKLSKSCASGFTFSSHGMHWIRQAPGKGLDWVAYISNNSGTIYSAAVRGRFTISRDNKNTLFLQLNSLKSEDTAIYYCARGYNYPGAYWGQGTLLTVSS
384	ZNRF3-329 (VL prot)	DIQVTQSPSSLASLGERVTITCQTSQSIRNYLNWYQQKPGQVPILLIYYATS LQTDMPSRFSGQYSGRSFTLTITSLEPEDIANYFCLQHYSPYPTFGAGTKLELK
385	ZNRF3-35 (VH DNA)	GAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAAGGTCCATGAAACTCTCCTGTGCAGCCTCAGGATTCACCTTCAGTAAGTATTACATGGCCTGGGTCCGCCAGGCTCCAAGGAAGGGTCTGGAGTGGGTTCGCATCCATTAGTACTGGTGGTGGTAACACTTACTATCGAGAATACGTGAGGGGCCGATTCACTATCTCAGAGATAATGCAAAAAGCACCTATATCTGCAATGGACAGTCTGAGGTCTGAGGAAACGGCCACTTATTACTGTGCAAGACATTCCGGGGGTATAATCCGGGCCCGGGGTCACTACTTTGATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA
386	ZNRF3-35 (VL DNA)	GACATCCAGATGACACAGTCTCCAGCTTCCCTGTCTGCATCTCTTGGAGAAAATGTCTCCATCGAATGTCTAGCAAGTGAGGGCATTTCGAATGATTTAGCGTGGTATCAGCAGAAAGTCAGGGAAATCTCCTCAGGTCTGATCTCTGCTGCAAGTAGGTTGCAAGACGGGGTCCCATCACGGTTCAGTGGCAGTGGATCTGGCACACGGTATTCTCTCAAGATCAGCGGCATGCAACCTGAAGACGAAGCAGATTATTTCTGTCAACAGAGTTACACGTATCCGTACACGTTTGGAGCTGGGACCAAGCTGGAAGTAAA
387	ZNRF3-35 (VH prot)	EVQLVESGGGLVQPGSRMKLSCAASGFTFSNYMMAWVRQAPRKGLEWVASISTGGGNTYYREYVRGRFTISRDNKSTLYLQMDSLRSEETATYYCARHSGGIIRARGHYFDYWGQGMVTVSS
388	ZNRF3-35 (VL prot)	DIQMTQSPASLSASLGEIVSIECLASEGISNDLAWYQQKSGKSPQVLISAASRLQDGVPSRFSGSGSSTRYSLKISGMQPEDEADYFCQQSYTYPYPTFGAGTKLELK
389	ZNRF3-55 (VH DNA)	GAGGTGCACCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCAGGATTCACCTTCAGTAAGTATGACATGGCCTGGGTCCGCCAGGCTCCAACGAGGGGTCTGGAGTGGGTTCGCTTCATTAGTCTCTGGTGGTGGAAAACTTACTATCGAGACTCCGTGAAGGGCCGACTCACTATCTCAGAAACAATGCAGAAAAATACCCAATACCTGCAAATTGACAGTCTGAGGTCTGAAGACACGGCCACTTATTACTGTTCAAGACTCGGTCCGGCATACTCCGGAGAA TGGTTTGCTTACTGGGGCCAAGGCACTCTGGTCACTGTCTCTTCA
390	ZNRF3-55 (VL DNA)	GACACTGTGCTGACCCAGTCTCCTGCTTTGACTGTGTCTCCAGGAGACAAAAT TACCATCTCCTGTAGGGCCAGTGAAGGTGTCAATACACGTATACACTGGTACC AACAGAAATCAGGACAGCAACCCAACTCCTCATCTACGGTGCATCCAACCTTA GATTCTGGAGTCCCTGACAGGTTCAAGTGGCAGTGGGTTTGGGACAGACTTTAC CCTCACCATCGATCCTGTTGAGGCTAGTGATACTGCCACCTATTTCTGTGTCAGC AGAGTTGGAATGTTCCGCACACGTTTGGAGGTGGGACCAAACTGGAAGTAAAA
391	ZNRF3-55 (VH prot)	EVHLVESGGGLVQPGGSLKLSCAASGFTFSNYDMAWVRQAPTRGLEWVASISP GGGKTYRDSVKGRLTISRNNQENTQYLQIDSLRSEDATATYYCSRLLGPAYSGE WFAIYWGQGTLLTVSS
392	ZNRF3-55 (VL prot)	DTVLTQSPALTVSPGDKITISCRASEGVNTRIHWYQQKSGQQPKLLIYGASNL DSGVPDRFSGSGFGTDFTLTIDPVEASDTATYFCQQSWNPHTFGGGTKLELK
393	ZNRF3-6 (VH DNA)	GAGGTACAGTGGTGGAGTCTGGAGGAGGCTTAGTGCCGCCTGGAAAGTCCCTGAAACTCTCCTGTTCAGCCTCTGGATTCCCATTCAGTAAGTATGGCATGCACT GGATTCGCCAAGCTCCAGGAAAGGGGCTTGATTGGGTTGGATACATTAGTAGT AATAGTGGTACAATCTATGCAGACGCTGTGAAGGGCCGGTTCACCATCTCCAG AGACAATGCAAGAACACCCTGTACCTGCTAATCAACAGTCTGAAGTCTGAAG AACTGCCATGTATTACTGTGCAAGAGGTTATTTTGATGGTTATTATCGTTTC TGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA

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394	ZNRF3-6 (VL DNA)	GACATTGTGATGACCCAGTCTCCATTCTCCCTGGCTGTGTGTCAGAAGGAGACAT GGTCACTATAATGTGCAGGTCCAGTCAGAGTCTTCTATCCAGTGGAAACCAAA AGAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAACTACTG ATCTACCATGCATCCACTAGACAATCAGGGGTCCCTGATCGCTTCATAGGCAG TGGATCTGGGACAGACTTCACTCTGACCATCAGCGATGTGCAGGCTGAAGACC TGGCAGATTATTACTGCCTGCAGCATTACAGTTCTCCACGTTCCGGCTCAGGG ACGAAGTTGGAAATAAAA
395	ZNRF3-6 (VH prot)	EVQLVESGGGLVPPGKSLKLSASGFPFSNYGMHWIRQAPGKGLDWVGYISS NSGTIYADAVKGRFTISRDNAKNTLYLLINSLKSEDTAMYYCARGYFDGYRFR WGQGVMTVSS
396	ZNRF3-6 (VL prot)	DIVMTQSPFSLAVSEGDMVTIMCRSSQSLSSGNQKNYLAWYQQKPGQSPKLL IYHASTRQSGVPDRFIGSGSGTDFTLTISDVQAEDLADYYCLQHYSSPTFGSG TKLEIK
397	ZNRF3-90 (VH DNA)	GAGGTGCAGTTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAAGGTCCAT GAAGCTCTCCTGTGCAGCCTCAGGATTCATTTCAGTAATACTATTACATGGCCT GGGTCCGCCAGGCTCCAAGGAAGGGTCTGGAGTGGGTGCGATCCATTACTACT GGTGGAACTAACTTACTATCGAGACTCCGTGAGGGGCCGATTCACTATCTC CAGAGATAATGCAAAAAGCACCTTATACCTGCAATGGACAGTCTGAGGTCTG AGGAAACGGCCACTTATTACTGTGCAAGACATTCCGGGGGGTTTAATTCGGGCC CGGGGCCACTACTTTCATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTC A
398	ZNRF3-90 (VL DNA)	GACATCCAGATGACACAGTCTCCAGCTTCCCTGTCTGCATCTCTTGGAGAAAT TGTCTCCATCGAATGTCTAGCAAGTGAGGGCATTTCGAATGATTTAGCGTGGT ATCAGCAGAAAGTCAGGGAAATCTCCTCAGGTCTTGGTCTATGCTGCAAGTAGG TTGCAAGACGGGGTCCCATCACGGTTCAGTGGCAGTGGATCTGGCACACGGTA TTCTCTCAAGATCAGCGGCATGCAACCTGAAGATGAAGCAGATTATTTCTGTC AACAGAGTTACACGTATCCGTACACGTTTGGAGCTGGGACCAAGCTGGAAGTGA AAA
399	ZNRF3-90 (VH prot)	EVQLVESGGGLVQPGSRMKLSAASGFTFSNYMAWVRQAPRKGLEWVASITTT GGTNTYYRDSVRGRFTISRDNASTLYLQMDSLRSEETATYYCARHSGGLIRA RGHYFHYWGQGVMTVSS
400	ZNRF3-90 (VL prot)	DIQMTQSPASLSASLGEIVSIECLASEGISNDLAWYQQKSGKSPQVLVYASR LQDGVPSRFRSGSGSGTRYSLKISGMQPEDEADYFCQQSYTYPYTFGAGTKLEL K
401	RNF43-1 (VH prot)	QSVEESGGRLVTPGTPLILTCTVSGIDLRYNMNWRQAPGKGLEWYIGYIYYG TGTTYASWVKGRFTISKSTTTVDLKITSPTTEDTATYFCASGDFWGPGLVTV VSS
402	RNF43-1 (VL prot)	DIVMTQTPASVEAAVGGTVTINCQASQSISSYLAWYQQKPGQPPKLLIYASN LESGVPSRFRKSGSGSGTQFTLTISDLECAATAATYYCQAYYSSITGWAFGGTEV VVK
403	RNF43-24 (VH prot)	QEQLVESGGGLVQPGESLTLSCKASGFSFSTGYDMCWVRQAPGKGLEWIGCIG TSSGDTGYASWAKGRFTISKASSTTVTLQMTSLTAADTATYFCARGFSLWGPG TLVTVSS
404	RNF43-24 (VL prot)	AQVLTQTASPVSAAVGGTVTINCQSSQSVYSNNRLGWYQQKPGQPPRLLIYQT SKLASGVPSRFRKSGSGSGTQFTLTISDLECDAAATYYCLGIYDCSSADCTAFGG GTEVVVK
405	RNF43-8 (VH prot)	QSVEESGGRLVTPGTPLTLTCTVSGFSLSSYAMAWVRQAPGEGLEWIGTINRA GNTYYASWAKGRFTISKSTTTVDLKVTSPTTEDTATYFCARSLYSTNNLWGPG TLVTVSS
406	RNF43-8 (VL prot)	AIVMTQTPSSKSVPGGTVTISQASESLYDNDLSWFQQKPGQPPKLLIYQA SSLASGVPSRFRKSGSGSGTQFTLTISDVVCGDAATYYCAGYAGSSTDGVAFGGG TEVVVK
407	RNF43-12 (VH prot)	QEQLVESGGGLVKPGASLTLTCKASGFSLSNGDVMCWVRQAPGKGLEWIGCIG ISNNDRAAYASWAKGRFTISKSTTTVTLQTTSLTAADSATYFCARYLASEYY NLWGPGTLVTGSS
408	RNF43-12 (VL prot)	AYDMTQTPASVEAAVGGTITINCQASESISSWLAWYQQKPGQPPKLLIYKAST LASGVSSRFEKSGSGTEFTLTISDLECAATAATYYCQOGASWSNVENTFGGGTE VVVK

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409	RNF43-20 (VH prot)	REQLVESGGGLVQPEGSLLTLTKASGDFSSNVMCWVRQAPGKGLEWIGCIYT GSGSTAYASWAKGRFTITRSTSLNTVTLQMTSLTAADTATYFCARYVSGGEYY NLWGPGLTVTVSS
410	RNF43-20 (VL prot)	ALVMTQTPASVEAAVGGTVTIKCQASQSISSYLNWYQQKPGQPPKLLIYKAST LASGVSSRFKSGSGGTQFTLTISDLECADAAATYYCQQGSSWNSIENIFGGGTE VVVK
411	RNF43-11 (VH prot)	QEQLVESGGGLVQPEGSLLTLTKASGDFSSNVMCWVRQAPGKGLEWIGCIYT GSSGSTYYASWAKGRFTITRSTSLNTVTLQMTSLTAADTATYFCARYVSGGEY YNLWGPGLTVTVSS
412	RNF43-11 (VL prot)	ALVMTQTPASVEAAVGGTVTINCQASESISNWLAWYQQKPGQPPKLLIYKAST LASGVSSRFKSGSGGTQFTLTISDLECADAAATYYCQQGSSWINIEDVFGGGTE VVVK
413	RNF43-23 (VH prot)	QEQVVESGGGLVQPEGSLLTLCKSGGDFSGNVLCWVRQAPGKGLEWIGCIAT GIGSTAYASWAKGRFTITRGTSLNTVDLKMSTSLTAADTATYFCARYVSGSEYY NFWGPGLTVTVSS
414	RNF43-23 (VL prot)	AYDMTQTPASVEAVGGTVTIKCQASQSISSFLAWYQQKPGQPPMLLIYKAST LASGVPSRFKSGSGGTQFTLTISGVECADAAATYYCQQGYSWNGVDNTFGGGTE VVVK
415	RNF43-6 (VH prot)	QSLEESGGRLVTPGTPLTLTCTVSGFSLSAFGMNWVRQAPGKGLEWIGYISAG GSAVYPNWAEGRFTISKASTTTLKMTSLTETDATTYFCVRGGYVGSTNSLWG PGTLTVTVSS
416	RNF43-6 (VL prot)	AAVLTQTPSPVSAAVGGTVSISCSQSSKSVHNNNLAWYQQKPGQPPKLLIYGA STLASGVPSRFKSGSGGTQFTLTISDVQCDDAAATYYCSGGYSSIIDNGFGGGT DVVVE
417	RNF43-5 (VH prot)	QSVEESGGRLVTPGTPLTLTCTVSGFSLNTYAMGWVRQAPGKGLEWIGTIGSS GSTYYATWAKGRFTVSKTSTTVDLKITSPTTEDTATYFCVRGADGLNYYPIWG PGTLTVTVSL
418	RNF43-5 (VL prot)	AQVLTQTPSSVSAAVGGTVTINCQASQSVYNKNLAWYQQKPGQPPKLLIYDA SDLASGVSSRFKSGSGGTQFTLTISGVQCDDAAATYYCQGEFNCDKGCNAFGG GTEVVVK
419	RNF43-15 (VH prot)	QEQLVESGGDLVKPGASLLTLCTASRFSFSDDDVICWVRQAPGKGLEWIGCIA TINGNTAYATWAKGRFTSSKTSSTTVTLQMTSLTAADTATYFCARWDTGSEHV TLWGPGLTVTVSS
420	RNF43-15 (VL prot)	AYDMTQTPASVEAVGGTVTIKCQASQSISSYLAZYQQKPGQPPKPLIYEASK LASGVPSRFSGSGSGGTQFTLTISGVQCADAATYYCQQGASWSNVNDVFGGGTE VVVK
421	RNF43-2 (VH prot)	QSLEESGGDLVKPGASLLTLCTASGFSFSSSYMCWVRQAPGKGLELIGCGFT GRHYTYASWAKGRFTISKSSSTTVTLQMTSLTAADTATYFCARKSGSDYDYF DLWGPGLTVTVSS
422	RNF43-2 (VL prot)	AYDMTQTPASVEAVGGTVTIKCQASQSISSYLSWYQQKPGQRPKLLIYRAST LASGVSSRFKSGSGGTFTLTISDLECADAAATYYCQQGYVSSNINNFGGGTE VVVK
423	RNF43-16 (VH prot)	QSVEESGGRLVTPGTPLTLTCTVSGFFLSSYYMSWVRQAPGKGLEWIGYIYAG SGSTYYASWAKGRFTISKSTTVDLKITSPTTEDTATYFCVRDINGGDYGMDF WGPGLTVTVSS
424	RNF43-16 (VL prot)	DIVMTQTPASVSAAVGGTVTIKCQASESIDSLAWYQQKPGQRPKLLIYAASN LASGVSSRFKSGSGGTEYSLTISGVQCDDAAATYYCQCTYGSVTSNTYGIAGFGG GTEVVVK
425	RNF43-14 (VH prot)	QSVEESGGRLVTPGTPLTLTCTVSGIDLSSYAMGWVRQAPGKGLEWIGFIYAS GSTYYASWAKGRFTISKSTTVDLKITSPTTEDTATYFCARDSSETINYFNIW GPGLTVTVSL
426	RNF43-14 (VL prot)	NIIVMTQTPSPVSGAVGGTVTIKCQASQSISSYLNWYQQKPGQPPKLLIYRAST LASGVSSRFKSGSGGTFTLTISDLECADAAATYYCQQGYIISYLDNAFGGGTE VVVK
427	RNF43-17 (VH prot)	QSVEESGGRLVTPGTPLTLTCTGSGFSLSNYAMGWVRQAPGKGLEWIGFIYAS GSTWYASWAKGRFTISKSTTVDLKITSPTTEDTATYFCCRDATTTYDRDLW GQGTLLTVSS
428	RNF43-17 (VL prot)	AFELTQTPSSVEAAVGGTVTIKCQASQSISSYLNWYQQKPGQPPKLLIYRAST LPSGVPSRFKSGSGGTFTLTISGVECADAAATYYCQQGYSVTNLDEAFGGGTE VVVK

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429	RNF43-22 (VH prot)	QEQLLEESGGDLVKPGASLTLTCTASGFSVNSYYMCWVRQAPGKGLEWIIACIN TGSSGSTYYASWAKGRFTISKTSSTTVTLQMTTLTAADTATYFCARGYAGYAG YGQFNLWGPGLVTVSS
430	RNF43-22 (VL prot)	AYDMTQTPASVEVAVGGTVTIKCQASQSISSYLAWYQQKPGQRPKVLIIYDVSK LASGVPSRFKSGSGSGTQFTLTISGVECADEAATYYCQQGYMSNVNDNTFGGGTE VVVK
431	RNF43-9 (VH prot)	QSLEESGGDLVQPEGSLTLTCTASGFSFSSSCYMCWVRQAPGKGLEWIIACINT GSSGSTYYASWAKGRFTISKTSSTTVTLQMTILTAADTATYFCARGYAGYTG GQFNLWGPGLVTVSS
432	RNF43-9 (VL prot)	QIVMTQTPASVEAAVGGTVTIKCQASQSISSYLAWYQQKPGQRPKLLIYDASK LASGVPSRFKSGSGSGTQFTLTISGVECADEAATYYCQQGYTMNNVDNAFGGGTE VVVK
433	RNF43-21 (VH prot)	QSLEESGGDLVKPGASLTLTCTASGFSFSSSYMCWVRQAPGKGLEWIIACIYA GSSGSTYYASWAKGRFTVSKTSSTTVTLQMTSLTAADTATYFCARYTYGSGSD SYFNLWGPGLVTVSS
434	RNF43-21 (VL prot)	AYDMTQTPASVEAAVGGTVTIQCQASQSIGSYLAWYQQKPGQPPKLLIYRAST LSSGVPSRFKSGSGSGTQFTLTISDLECADEAATYYCQQGYSITNLDNGFGGGTE VVVK
435	RNF43-13 (VH prot)	QSLEESGGGLVKPGGLTLTLTCKASGIDFSGYYMCWVRQAPGKGLEWIIACIYT TSGSAWYASWAKGRFTISITSLNLTVTLQMTSLTATDTATYFCVRYANYGGYN YPEFGVLWGPGLVTVSS
436	RNF43-13 (VL prot)	AYDMTQTPASVEVAVGGTVTIKCQASEDIYNLLAWYQQKPGQPPKLLIYRASK LASGVPSRFSGSGSGTEFTLTISSVQCDDAATYYCQQGYSISNVENPFGGGTE VVVK
437	RNF43-19 (VH prot)	QQQLEESGGGLVKPGGLTLTLTCKASGIDFSTYYMCWVRQAPGKGLEWIIACIY TGDGNTYYASWAKGRFTISKTSSTTVTLQMTSLTAADTATYFCARDLAGSSYY SGYYFKLWGPGLVTVSS
438	RNF43-19 (VL prot)	DVVMTQTPASVSEPVGGTVTIKCQASQSIGSNLAWYQQKPGQPPKLLIYSVSN LASGVSSRFKGSRSGETEYTLTISDLECADEAATYYCQSGYYGSTYAFGGGTEVV VK
439	ZNRF3-331 (VH prot)	QSVEESGGRLVTPGTPLTLTCTVSGFSLSSYAMGWVRQAPGKGLEWIGIIYAG DTTYYASWAKGRFTISETSTTVDLKITSPTTEDTATYFCARKSDDSDSYLLW GPGLVTVSS
440	ZNRF3-331 (VL prot)	AYDMTQTPASVEVAVGGTVTIKCQASQSISSYLNWYQQKRGQPPKLLIYDAST LASGVPSRFSGSGSGTEFTLTISGVQCDDAATYYCQQGWSSTNIDNIFGGGTE VVVK
441	ZNRF3-333 (VH prot)	QSLEESGGGLVQPEGSLTLTCTASGIDFSARSYMCWVRQAPGKGLEWVACIYT GSSGSTYYANWAKGRFTISKTSSTTVTLRMTSLTAADTASYFCARGAYVGYGF AAFTLWGPGLVTVSS
442	ZNRF3-333 (VL prot)	DIVMTQTPASVEAAVGGTVTINCQASQSISSYLAWYQQKPGQPPKLLIYYASN LESGVPSRFKSGSGSGTQFTLTISDLECADEAATYYCQAYYSSITGWAFFGGTEV VVK
443	ZNRF3-332 (VH prot)	QSLEESGGGLVQPEGSLTLTCTASGIDFSTRNYMCWVRQAPGKGLEWVGCIYT SSGGTYASWVNGRFTISKTSSTTVTLRMTSLTAADTATYFCARGGYVGYGYA TYFNLWGPGLVAVSS
444	ZNRF3-332 (VL prot)	DVVMTQTPASVSEPVGGTVTIKCQASQSISSYLSWYQQKPGQPPKLLIYYVSN LESGVPSRFKSGSGSGTEFTLTISDLECADEAATYYCQNYSSSSSGWAFGGGTEV VVK
445	Human RNF13 (O43567; UNQ16056)	MLLSIGMLMLSATQVYITLTVQLFAFLNLLPVEAGKYALADASLKMADPNRFR GKDLPLVGSIDILAYNFENASQTFDDLPAFGRYRLPAEGLKGFLLINSKPENACE PIVPPPVKDNSSGTFIVLIRRLDCNFDIKVLNAQRAGYKAAIVHNVDSDDLIS MGSNDIEVLKKIDIPSVFIGESSANS LKDEFTYEKGHLLVPEFSLPLEYYL IPFLIIVGICLILIVIFMITKVFQDRHRARNRLRKDQLKKLPVHKFKKGDEY DVCAICLDEYEDGDKLRILPCSHAYHCKCVDPLTKTKKTCPVCKQKVVP SQG DSDSDTDSSQEENEVTEHTPLLRPLASVSAQSFGALSESRSHQNMTESSDYEE DDNEDTDSSDAENEINEHDVVVQLQPNGERDYNIAINTV
446	Human RNF43 (Q68DV7; UNQ9671)	MSGGHQLQLAALWPWLLMATLQAGKYALADASLKMADPNRFRGKDLPLVLSGF GRTGLVLAAAVESERSAEQKAIIRVIPLKMDPTGKLNLTLEGVFAGVAEITPA EGKLMQSHPLYLCLNASDDDNLEPGFISIVKLESPPRAPRCLSLASKARMAGE RGASAVLFDITEDRAAAEQQLQPLGLTWPVVLIIWGNDAEKLMEFVYKNQKAHV

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		RIELKEPPAWPDYDVWILMTVVGTIFVILASVLRIRCRPRHSRPDPLQQRTA WAI SQLATRRYQASCRQARGEWPDSGSSCSSAPVCAICLEEFSEGQELRVI SC LHEFHRNCVDPWLHQHRTCLCMFNITEGDSFSQSLGSPRSYQEPGRRLHLIR QHPGHAHYHLPAAYLLGSPRSASAVARPPRPGFLLPSQEPGMGPRHHRFPRAAHP RAPGEQQRLAGAQHPYAQGWGLSHLQSTSQHAAACPVPLRRARPDPSSSGSGES YCTERSGYLADGPASDSSSGPCHGSSSDSVVNCTDISLQGVHGSSTFCSLS SDFDPLVYCSPKGDPQRVDMQPSVTSRPRSLLDSVVPTGETQVSSHVHYHRHRH HHYKKRFQWHGRKPGPETGVPQSRPPIPRTPQPEPPSPDQOVTRNSAAPS RLSNPQCPRALPEPAPGPVDASSICPSTSSLFNLQKSSLSARHPQRKRGGPS EPTPGSRPQDATVHPACQIFPHYTPSVAYPWSPEAHP LICGPPGLDKRLLPET PGPCYSNSQPVWLCLTPRQPLEPHPPGEGPSEWSSDTAEGRPCPYHQCQVLSA QPGSEEELEELCEQAV
447	Human SYVN1 (Q86TM6; UNQ22255)	MFRTAVMMAASLALTGAVVAHAGKYALADASLKMADPNRFRGKDLPLVLSYYL KHQFYPTVVYLTKSSPSMAVLYIQAFVLVFLLGKVMGKVFFGQLRAAEMEHL ERSWYAVTETCLAFVFRDDFSRPFVALFTLLFLKCFHWAEDRVDFMERS NISWLFHCRIVSLMFLGLDFLVSHAYHSILTRGASVQLVFGFEYAILMTM VLTIFIKYVLHSDVLDQSENPDNKAVMYLYTELFTGFIKVLLYMAFMTIMIKV HTFPLFAIRPMYLA MRQFKKAVTDAIMSRAIRNMNTLYPDATPEELQAMD CII CREEMVTGAKRLPCNHI FHTSCLRSWFQRQQTCTCRMDVLRASLPAQSP PPPEPADQGPAPHPPLLPQPPNFPQGLLPFPFPPGMFPLWPPMGFPFPVPP PPSSGEAVAPPSTSAALS RPSGAATTTAAGTSATAASATASGPGSGSAPEAG PAPGFPFPFPWMGMPLPPPFAPFPMPVPPAGFAGLTPEELRALEGHERQHLEA RLQSLRNIHTLLDAAMLQINQYLTVLASLGPPRPATSVNSTEETATTVVAAS STSI PSSEATTPTPGASPPAPEMERPPAPESVGTEEMPEDGE PDAAELRRRL QKLESPVAH
448	Human RNF130 (Q86XS8; UNQ4694)	MSCAGRAGPARLAALALLTCSLWPARAGKYALADASLKMADPNRFRGKDLPLV GSDNASQEYYTALINVTVQEPGRGAPLTFRIDRGRYGLDSPKAEVRGQVLAPL PLHGVADHLGCDPQTRFFVPPNI KQWIALLRGNCTFKEKISR AAFHNAVAVV IYNNKSKEEPTMTHTPGTGDI IAVMITELRGKDI LSYLEKNI SVQMTI AVGT MPPKNFSRGS L VFVSISFIVLMI ISSAWLI FYFIQKIRYTNARDRNQRLGDA AKKAI SKLTTRTVKKGDKETDPDFDHCAVCI ESYKQNDVVRI LPCKHVFHKSC VDPWLSEHCTCPMCKLNILKALGIVPNLPCTDNVAFDMERLRTQAVNRRSAL GDLAGDNSLGLPLRTSGISPLPQDGELTPRTGEINIAVTKEWFIIASFLLS ALTLCYMIIRATASLNANEVEWF
449	Human RNF148 (Q8N7C7; UNQ34666)	MSFLRITPSTHSSVSSGLLRLSIFLLSFPDSNGGKYALADASLKMADPNRFR GKDLPLVLSKAIWTAHLNITFQVGN EITSELGESGVFGNHSPLERVSGVVALP EGWNQNACHPLTNF SRPKQADSWLALIERGGCTFTHKINVAEKGANGV I IYN YQGTGSKVFPMSHQGTENIVAVMISNLKGMELH SIQKGVYVTVII EVGRMHM QWVSHYIMYLF TFLAATIAFYLD CVWRLT PRVPNSFTRRRS QIKTDVKKAI DLQRLV LKEGDEELDLNEDNCVVC FDTYKPDVVRI LTC KHFFHKACIDP WLL AHRTPMCKCDILKT
450	Human RNF149 (Q8NC42; UNQ4467)	MAWRRREASVGARGVLALALLALALCVP GARGGKYALADASLKMADPNRFRGK DLPVLGSRAL EWFSAV VNI EYVDPQTNLTVWSVSESGRFGDSSPKEGAHGLVG VPWAPGGDLEGCAPDTRFFVPEPGGRGAAPWVALVARGGCTFKDKVLVAARN ASAVVLYNEERYGNITLPM SHAGTGNIVVIMISYPKGREILELVQKGPVTMT IGVGTRHVQEFISGQSVVFVAIAFITMMIISLAWLI FYIYIQRFLYTGSIQS SHRKETKKVIGQLLLHTVKHGEKGI DVDAENCAVCI ENFKVKDI IRI LPCKHI FHRICIDPWL LDHRTCPMCKLDVIKALGYWGE PGDVQEMPAPESPPGRDPAAN LSLALPDDDGSDSSPPSASP AESEPQC DPSFKGDAGENTALLEAGRSDSRHG GPIS
451	Human LNX1- isoform2 (Q8TBB; UNQ 574)	MKALLLVLPWLS PAGKYALADASLKMADPNRFRGKDLPLVLSNYIDNVGNLH FLYSELCKGASHYGLTKDRKRSQDGC PDGCASLTATAPSPEVSAAATISLMT DEPGLDNPAYVSSAEDGQPAISPVD SGRSNRTRARPFERSTIRSRSFKKINRA LSVLRRTKSGSAVANHADQGRENSENTTAP EVFPRLYHLIPDGEITSIKINRV DPSELSIRLVGGSETPLVHII IQHIYRDGVIARDGRLLPGDII LKVNMDIS NVPHNYAVRLLRQPCQVLWLTVMREQKFRSRNNGQAPDAPRPRD DS FHVILNK SSPEEQ LGIKLVRKVDEPGVFI FNVLDGGVAYRHGQLEENDRVLAINGHDLRY GSPESAHLIQASERRVHLVVS RQVRQ RSPDI FQ EAGWNSNGSWSPGPGERSN TPKPLHPTITCHEKVNIQKDPGESLGMTVAGGASHREWDLP IYI SVEPGGV ISRDGRIKTGDILLNVDGVELTEVSRSEAVALLKRTSSSIVLKALEVKEYEPQ

SEQ ID NO	DESCRIPTION	SEQUENCE
		EDCSSPAALDSNHNMAPPSDWSPSWVMWLELPRCLYNCKDIVLRRNTAGSLGF CIVGGYEEYNGNKPFFIKSIVEGTPAYNDGRIRCGDILLAVNGRSTSGMIHAC LARLLKELKGRITLTIVSWPGTFL
452	Human RNF128 (Q8TEB7; UNQ8231)	MGPPPGAGVSCRGGCGFSRLAWCFLLALSPQAPGSRGGKYALADASLKMADP NRFRGKDL PVLGSAEAVWTAYLNVSWRVPHTGVNRTVWELSEEGVYGQDSPLE PVAGVLVPPDGPALNACNPHTNFTVPTVWGSTVQVSWLALIQRGGGCTFADK IHLAYERGASGAVIFNFPGRTRNEVIMSHPGAVDIVAIMIGNLKGTILQSIQ RGIQVTMVEVGGKKGHPWVNHYISIFFVSVSFFIITAATVGYFIFYSARRLRNA RAQSRKQRLKADAKKAIGRLQLRTLKQGDKEIGPDGDSCAVCIELYKPNDLV RILTCNHI FHKTCVDPWLLLEHRTCPMCKCDILKALGIEVDVEDGVSLSQVPVS NEI SNSASSHEEDNRSETASSGYASVQGTDEPPLEEHVQSTNESLQLVNHEAN SVAVDVI PHVDNPTFEEDETPNQETAVREIKS
453	Human (RNF133 (Q8WVZ7; UNQ34667)	MHLLKVGTVWRNNTASSWLMKFSVLWLVSQNCRCRAGKYALADASLKMADPNRFR GKDL PVLGSSVVMAYMNI SFHVGNHVLSSELGETGVFGRSSTLKRVRAGVIVPP EGKIQNACNPNTIFSRSKYSETWLALIERGGCTFTQKIKVATEKGASGVIIYN VPGTGNQVPMFHFQAFEDVVVMIGNLKGTETFLIKKGVLTITAVVEVGRKHI IWMNHVLSFVIVTTATLAYFIFYHIHRLCLARIQNRWQRLTDTLQNTFGQL QLRVVKEGDEEINPNGDSCVICFERYPNDIVRILTCKHFFHKNCIDPWILPH GTCPICKDILKVLGIQVVVENGTEPLQVLSNELPETLSPSEETNNEVSPA GTSKVIHVEENPTSQNNDIQPHSVVEDVHPS
454	Human ZNRF4 (Q8WWF5; UNQ12510)	MPLCRPEHLMPRASRPVVAASLPLSHAGKYALADASLKMADPNRFRGKDL PVL GSVIPTQLPSRPGHRPPGRPRRCPKASCLPPVGPSSSTQAKRVTMGWPRPGR ALVAVKALLVLSLLQVPAQAVVRVLEDNSSSVDFADLPALFGVPLAPEGIRG YLMEVKPANACHPIEAPRLGNRSLGAIVLIRYDCTFDLKVLAQVAGFEAAI VHNHSDDLVSMTHVYEDLRGQIAIPSVFVSEAASQDLRVILGCNKSAHALLL PDDPPCHDLGCHPVLTVSWVLGCTLALVVSFAFFVLNHLWLWAQACCShRRPVK TSTCQKAQVRTFTWHNDLCAICLDEYEEGDQLKILPCSHTYHCKCIDPWFSQA PRRSCPVCQKQSVAAEDSFDDSTTYSFRDEDPSPGHRPPIWAIQVQLRSRRLE LLGRASPCHCSTTSLEAEYTTVSSAPPEAPGQ
455	Human RSPRY1 (Q96DX4; UNQ328)	MIVFGWAVFLASRSLGGKYALADASLKMADPNRFRGKDL PVLGSGQLLLTLEE HIAHFLGTGGAATTMGNSCICRDDSGTDDSDVTQQQQAENSAPVPTADTRSQPR DPVRPPRRGRGPHEPRKKQNV DGLVLDTLAVIRTLVDNDQEPYSMITLHEM AETDEGWLDVQSLIRVIPLDPLGPAVITLLLDECPLPTKDALQKLTEILNL NGEVACQDSSHPAKHRNTSAVLGCLAELAGPASIGLLSPGILEYLLQCLKLQ SHPTVMLFALIALEKFAQTS ENKLTISESSISDRLVTLESWANDPDYLRQVG FCAQWSLDNLFLEKEGRQLTYEKNLSSIRAMLNSNDVSEYLIKISPHGLEARCD ASSFESVRCTFCVDAGVWYEVTVTSVGMQIGWATRDSKFLNHEGYGIGDDE YSCAYDGCRLI WYNARSKPHIHPCWKEGDTVGFLLDLNEKQMIFFLNGNQLP PEKQVFSSTVSGFFAAASFMSYQQCEFNFGAKPFKYPPSMKFSTFNDYAFLLTA EEKIILPRHRLALLKQVSIRENCCSLCCDEVADTQLKPCGHS DCLCMDALQL ETCPLCRKEIVSRIRQISHIS
456	Human TRIM7 isoform 3 (Q9C029-4; UNQ 20983)	MTQATGQMLCLGKYALADASLKMADPNRFRGKDL PVLGSHVQVPLQLLLLGQK QMAAEQEKVGAEFQALRAFLVEQEGRLGRLEELSREVAQKQENLAQLGVEI TQLSKLSSQIQETAQKPD LDFLQEFKSTLSRCSNVP GPKPTTVSSEMKNKVVN VSLKTFVLKGM LKKFKEDLRGELEKEEKVELTLD PDTANPRLILSLDLKGVR GERAQDLPNHPCRFDNTNRVLASCGFSSGRHHWEVEVGSKDGWAFGVARES VRKGLTPFTPEEGVWALQLNGGQYWAVTSPERSPLSCGHL SRVRVALDLEVGA VSFYAVEDMRHLYTFRVNFQERVFP LFSVCSTGTYLRIWP
457	Human RNF167 (Q9H6Y7; UNQ16055)	MHPAAFPLPVVVA AVLWGAAPTRGGKYALADASLKMADPNRFRGKDL PVLGSL IRATSDHNASMD FADLPALFGATLSQEG LQGF LVEAHPDNACSPIAPPPAPV NGSVFIALRRFDCNFDLKVLAQKAGYGA AVVHNVNSNELLNMVWNSEEIQQ QIWI PSVFIGERSSEYLRALFVYEKGARVLLVPDNTFPLGYLI PFTGIVGLL VLAMGAVMIARCIQHRKRLQRNRLTKEQLKQIP THDYQKGDQYDVCAICLDEY EDGDKLRVLP CAHAYHSRCVDPWLTQTRKTCPICKQPVHRGPGDEDQEEETQG QEEGDEGEPRDHPASERTPLLGSSPTLPTSFGSLAPAPLVFPGPSTDPPLSP SSPVILV
458	Human RNF150 (Q9ULK6; UNQ11523)	MAMSLIQACCSLALSTWLLSFCFVHLLCLDFTVAGKYALADASLKMADPNRFR GKDL PVLGSEKEEYTA FVNITYAEPAPDPGAGAAGGGGAELHTEKTECGRYG EHS PKQDARGEVVMASAHDR LACDPNTKFAAPTRGKNWIALIPKGNCTYRDK IRNAFLQNASAVVIFNVGSNTNETITMPHAGVEDIVAIMIPEPKGKEIVSLLE

SEQ ID NO	DESCRIPTION	SEQUENCE
		RNITVTMYITIGTRNLQKYVSRTSVVFVSISFIVLMIISLAWLVFYIQRFRY ANARDRNQRRLGDAAKKAI SKLQIRTIKKGDKETESDFDNCVCI EGYKPNDV VRILPCRHLFHKSCVDPWLLDHRTCPMCKMNI LKALGI PPNADCMDDLPTDFE GSLGGPPTNQITGASDTTVNESSVTLDPAVRTVGALQVVQDTPDPI PQEGDVIF TTNSEQEP AVSSDS DISLIMAMEVGLSDVELSTDQDCEEVKS
459	Human ZNRF3 (Q9ULT6; UNQ28198)	MRPRSGGRPGATGRRRRRLRRRPRGLRCSRLPPPPPLP LLLGLLLAAAGPGAA RAGKYALADASLKMADPNRFRGKDL PVLGSKETA FVEVVL FESSPSGDYTTYT TGLTGRFSRAGATLSAEGEIVQMHP LGLCNNDEEDLYEYGWVG VVKLEQPEL DPKPCLTVLGAKRAVQRGATAVIFDVSENPEAIDQLNQ SEDPLKRPVVYVK GADAIKLMNIVNKQKVARARIQHRPPRQPTEYFDMGIFLAFFVVVSLVCLILL VKIKLKQRRSQNSMNRLAVQALEKMETRKFNSKSKGRREGSCGALDTLSSSST SDCAICLEKYIDGEELRVI PCTHRFHRKCVDPWLLQHHTCPHCRHNIEQKGN PSAVCVETSNLSRGRQQRVTLPVHYPRVHRTNAI PAYPTRTSMDSHGNPVTL LTMDRHGEQSLYSPQTPAYIRSYPPLHLDHSLAAHRCGLEHRAYS PAHPFRRP KLSGRSFSKAACFSQYETMYQHYYFQGLSYPEQEGQSPPSLAPRGPARAFPPS GSGSLLFPTVVHVAPP SHLES GSTSSFS CYHGHRSVCSGYLADCPGSDSSSSS SSGQCHC SSSDSVVDCTEVS NQGVY GSCSTFRSSLSSDYDPFIYRSRSPCRAS EAGGSGSSGRGPALCFEGSPPEELPAVHSHGAGRGEPPWGPASPSGDQVSTC SLEMNYSSNSSLEHRGPNSSTSEVGLEASPGAAPDLRRTWKGGHELPSCACCC EPQPS PAGPSAGAAGSSTLFLGPHLYEGSGPAGGEPQSGSSQGLYGLHPDHL P RTDGVKYEGLPCCFYEEKQVARGGGGGSGCYTEDYSVSVQYTLTEEP PPGCYP GARDLSQRIPIIPEDVDCDLGLP SDCQGTHSLGSGWGGTRGPDTPRPHRGLGAT REEERALCCQARALLRPGCPPEEAGAVRANFP SALQDTQESSTTATEAAGPRS HSADSSSPGA

WHAT IS CLAIMED IS:

1. A multispecific binding protein that binds to at least a first cell surface target protein and a second cell surface protein, wherein the first cell surface target protein is a transmembrane E3 ubiquitin ligase.

2. The multispecific binding protein of claim 1, wherein the multispecific binding protein reduces the level of the second cell surface protein on the surface of a cell compared to the level observed in the absence of the multispecific binding protein.

3. The multispecific binding protein of claim 2, wherein the multispecific binding protein reduces the level of the second cell surface protein on the surface of a cell *in vitro* compared to the level observed in the absence of the multispecific binding protein.

4. The multispecific binding protein of claim 3, wherein the multispecific binding protein reduces the level of the second cell surface protein on the surface of a cell as determined by flow cytometry or by luminescence assay.

5. The multispecific binding protein of claim 2, 3, or 4, wherein the multispecific binding protein reduces the level of the second cell surface protein on the surface of a cell *in vivo* compared to the level observed in the absence of the multispecific binding protein.

6. The multispecific binding protein of any one of claims 1-5, wherein the multispecific binding protein is a multispecific antibody.

7. The multispecific antibody of claim 6, which is a bispecific or trispecific antibody, such as a 1+1 FabIgG, a 1+1 FvIgG, a 2+1 FvIgG, 2+1 FabIgG, a one-armed FvIgG, or a one-armed FabIgG.

8. The multispecific binding protein of claim 6 or 7, wherein the protein comprises IgG Fc regions comprising at least one knob-into-hole modification.

9. The multispecific binding protein of any one of claims 1-8, wherein the transmembrane E3 ubiquitin ligase does not have catalytic activity, wherein lack of catalytic activity is determined in a cell surface degradation assay.

10. The multispecific binding protein of any one of claims 1-8, wherein the transmembrane E3 ubiquitin ligase has catalytic activity, wherein presence of catalytic activity is determined in a cell surface degradation assay.

11. The multispecific binding protein of any one of claims 1-10, wherein the transmembrane E3 ubiquitin ligase is RNF43, ZNRF3, RNF13, RNF128, RNF130, RNF133, RNF148, RNF149, RNF150, RNF167, ZNRF4, RSPRY1, SYVN1, LNX1 isoform 2, or TRIM7 isoform 3.

12. The multispecific binding protein of any one of claims 1-10, wherein the transmembrane E3 ubiquitin ligase is RNF43, RNF128, RNF130, RNF133 RNF149, RNF150, or ZNRF3.

13. The multispecific binding protein of any one of claims 1-10, wherein the transmembrane E3 ubiquitin ligase is RNF130, RNF133, RNF149, or RNF150.

14. The multispecific binding protein of any one of claims 1-10, wherein the transmembrane E3 ubiquitin ligase is RNF130, RNF149, or RNF167.

15. The multispecific binding protein of any one of claims 1-10, wherein the transmembrane E3 ubiquitin ligase is RNF133 or RNF148.

16. The multispecific binding protein of any one of claims 1-10, wherein the protein binds to RNF43, ZNRF3, or both RNF43 and ZNRF3, optionally wherein the protein does not block binding between RNF43 and/or ZNRF3 and FZD and/or LRP6.

17. The multispecific binding protein of any one of claims 1-10 or 16, wherein the multispecific binding protein is a multispecific antibody that binds to RNF43 or ZNRF3; or comprises an antibody heavy chain variable region (VH) comprising (a) CDR-H1 (b) CDR-H2, and (c) CDR-H3, and a light chain variable domain (VL) comprising (d) CDR-L1, (e) CDR-L2, and (f) CDR-L3 that bind to RNF43 or ZNRF3; or comprises a VH and a VL that bind to RNF43 or ZNRF3.

18. The multispecific binding protein of claim 16 or 17, wherein the protein comprises a heavy chain variable domain (VH) comprising a heavy chain complementarity determining region 1 (CDR-H1), CDR-H2, and/or CDR-H3 of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16,

RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

19. The multispecific binding protein of any one of claims 16-18, wherein the multispecific binding protein comprises a heavy chain variable region (VH) comprising the CDR-H1, CDR-H2, and CDR-H3 any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

20. The multispecific binding protein of claim 16 or 17, wherein the protein comprises a light chain variable domain (VL) comprising a light chain complementarity determining region 1 (CDR-L1), CDR-L2, and/or CDR-L3 of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301,

ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

21. The multispecific binding protein of any one of claims 16-17 or 20, wherein the multispecific binding protein comprises a light chain variable region (VL) comprising the CDR-L1, CDR-L2, and CDR-L3 any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

22. The multispecific binding protein of any one of claims 16-21, wherein the protein comprises a heavy chain variable domain (VH) comprising (a) a heavy chain complementarity determining region 1 (CDR-H1), CDR-H2, and CDR-H3 and (b) a light chain variable domain (VL) comprising (a) a light chain complementarity determining region 1 (CDR-L1), CDR-L2, and CDR-L3 of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76,

RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

23. The multispecific binding protein of any one of claims 18-22, wherein the heavy chain CDRs and/or the light chain CDRs are Kabat, Chothia, or McCallum CDRs.

24. The multispecific binding protein of any one of claims 16-23, wherein the multispecific binding protein comprises a heavy chain variable region (VH) comprising an amino acid sequence at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the VH of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

25. The multispecific binding protein of any one of claims 16-24, wherein the multispecific binding protein comprises a light chain variable region (VL) comprising an amino acid sequence at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99%

identical to the VL of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

26. The multispecific binding protein of any one of claims 16-25, wherein the multispecific binding protein comprises a heavy chain variable region (VH) comprising an amino acid sequence of the VH of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17,

RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

27. The multispecific binding protein of any one of claims 16-26, wherein the multispecific binding protein comprises a light chain variable region (VL) comprising an amino acid sequence of the VL of any one of antibodies RNF43-104, RNF43-106, RNF43-107, RNF43-108, RNF43-116, RNF43-117, RNF43-123, RNF43-126, RNF43-128, RNF43-129, RNF43-130, RNF43-136, RNF43-145, RNF43-152, RNF43-156, RNF43-168, RNF43-170, RNF43-176, RNF43-177, RNF43-179, RNF43-180, RNF43-181, RNF43-186, RNF43-187, RNF43-196, RNF43-200, RNF43-201, RNF43-206, RNF43-210, RNF43-213, RNF43-217, RNF43-221, RNF43-224, RNF43-25, RNF43-31, RNF43-33, RNF43-35, RNF43-38, RNF43-41, RNF43-53, RNF43-56, RNF43-61, RNF43-67, RNF43-69, RNF43-71, RNF43-74, RNF43-75, RNF43-76, RNF43-80, RNF43-86, RNF43-90, ZNRF3-101, ZNRF3-117, ZNRF3-128, ZNRF3-131, ZNRF3-163, ZNRF3-17, ZNRF3-170, ZNRF3-171, ZNRF3-172, ZNRF3-179, ZNRF3-182, ZNRF3-195, ZNRF3-219, ZNRF3-222, ZNRF3-223, ZNRF3-231, ZNRF3-237, ZNRF3-244, ZNRF3-247, ZNRF3-253, ZNRF3-254, ZNRF3-255, ZNRF3-265, ZNRF3-269, ZNRF3-270, ZNRF3-275, ZNRF3-279, ZNRF3-287, ZNRF3-296, ZNRF3-30, ZNRF3-300, ZNRF3-301, ZNRF3-305, ZNRF3-312, ZNRF3-314, ZNRF3-322, ZNRF3-329, ZNRF3-35, ZNRF3-55, ZNRF3-6, ZNRF3-90, RNF43-1, RNF43-24, RNF43-8, RNF43-12, RNF43-20, RNF43-11, RNF43-23, RNF43-6, RNF43-5, RNF43-15, RNF43-2, RNF43-16, RNF43-14, RNF43-17, RNF43-22, RNF43-9, RNF43-21, RNF43-13, RNF43-19, ZNRF3-331, ZNRF3-333, or ZNRF3-332.

28. The multispecific binding protein of any one of claims 16-27, wherein the protein has a binding affinity for ZNRF3 or RNF43 of less than 50 nM, or less than 10 nM, or less than 1 nM, or less than 0.5 nM, or less than 0.05 nM, or between 50 nM and 10 nM, or between 10 nM and 1 nM, or between 1 nM and 0.5 nM, or between 0.5 nM and 0.05 nM, or between 0.05 nM and 0.01 nM.

29. The multispecific binding protein of any one of claims 16-28, wherein the protein is a multispecific antibody comprising a heavy chain variable region and/or a light chain variable region of a rat anti-human RNF43 B cell antibody, a rat anti-human ZNRF3 B cell antibody, a rabbit anti-human RNF43 B cell antibody, or a rabbit anti-human ZNRF3 B cell antibody.

30. The multispecific binding protein of any one of claims 16-29, wherein the protein is a trispecific antibody comprising a 2+1 FvIgG or a 2+1 FabIgG format, wherein the protein binds to both ZNRF3 and RNF43 and to at least one second cell surface protein, optionally wherein the protein does not block binding of ZNRF3 or RNF43 to FZD and LRP6.

31. The multispecific binding protein of any one of claims 1-30, wherein the protein is a multispecific antibody comprising a heavy chain variable region or light chain variable region that is chimeric, or wherein the variable regions are humanized.

32. The multispecific binding protein of any one of claims 1-7 or 9-31, wherein the protein comprises a wild-type human Fc region.

33. The multispecific binding protein of claim 32, wherein the Fc region is an IgG1, IgG2, IgG3, or IgG4 Fc region.

34. The multispecific binding protein of any one of claims 1-33, wherein the protein comprises an Fc region having effector function.

35. The multispecific binding protein of any one of claims 1-32, wherein the protein comprises a human IgG1 Fc region comprising a LALAPG mutation or a substitution at position N297, such as N297G or N297Q, and/or wherein the Fc region lacks effector function.

36. The multispecific binding protein of any one of claims 1-35, wherein the second cell surface protein is a receptor tyrosine kinase, a growth factor receptor, a cytokine, a mucin, a Siglec receptor, or an immune checkpoint modulator, or is HER2, HER3, IGF1R, an EGFR, an FGFR, a VEGFR, a PDGFR, EpCAM, FZD, PD-L1, CTLA4, PD-1, TIM3, LAG3, TIGIT, CEACAM1, CD25, ILT-2, ILT-3, ILT-4, ILT-5, LAIR-1, PECAM-1 (CD31), PILR-alpha, SIRL-1, or SIRP-alpha.

37. The multispecific binding protein of claim 36, wherein the second cell surface protein is HER2, EGFR, or IGF1R.

38. The multispecific binding protein of any one of claims 1-37, wherein the protein comprises a heavy chain variable region and a light chain variable region of an anti-HER2 antibody such as 4D5, 7C2, or 2C4 or an anti-IGF1R antibody such as cixutumumab, ganitumab, dalotuzumab, figitumumab, robatumumab, teprotumumab, or istiratumab.

39. An isolated nucleic acid or set of nucleic acids encoding the multispecific binding protein of any of claims 1 to 38.

40. A host cell comprising the nucleic acid or set of nucleic acids of claim 39.

41. A method of producing the multispecific binding protein of any one of claims 1-38, comprising culturing the host cell of claim 40 under conditions suitable for the expression of the protein.

42. The method of claim 41, further comprising recovering the protein from the host cell.

43. A multispecific binding protein produced by the method of claim 42.

44. A pharmaceutical composition comprising the multispecific binding protein of any of claims 1 to 38 and a pharmaceutically acceptable carrier.

45. The multispecific binding protein of any one of claims 1 to 38 or the pharmaceutical composition of claim 44 for use as a medicament.

5 46. The multispecific binding protein of any one of claims 1 to 38 or the pharmaceutical composition of claim 44 for use in treating cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease in a subject.

10 47. The multispecific binding protein of any one of claims 1 to 38 or the pharmaceutical composition of claim 44 for use in reducing the level of a cell surface protein in a subject in need thereof, optionally wherein the subject has cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease.

15 48. The multispecific binding protein of any one of claims 1 to 38 or the pharmaceutical composition of claim 44 for use in increasing an immune response in a subject, such as a subject with cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease.

49. The multispecific binding protein according to any one of claims 46-48, wherein (a) the subject has a mutation in RNF43 and the multispecific binding protein does not bind to or does not activate RNF43, or (b) the subject has a mutation in ZNRF3 and the multispecific binding protein does not bind to or does not activate ZNRF3.

20 50. Use of the multispecific binding protein of any one of claims 1 to 38 or the pharmaceutical composition of claim 44 in the manufacture of a medicament for treatment of cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease in a subject.

25 51. Use of the multispecific binding protein of any one of claims 1 to 38 or the pharmaceutical composition of claim 44 in the manufacture of a medicament for reducing the level of a cell surface protein in a subject in need thereof, optionally wherein the subject has cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease.

30 52. Use of the multispecific binding protein of any one of claims 1 to 38 or the pharmaceutical composition of claim 44 in the manufacture of a medicament for increasing an immune response in a subject, such as a subject with cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease.

53. The use according to any one of claims 50-52, wherein (a) the subject has a mutation in RNF43 and the multispecific binding protein does not bind to or does not activate RNF43, or (b) the subject has a mutation in ZNRF3 and the multispecific binding protein does not bind to or does not activate ZNRF3.

5 54. A method of treating cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease in a subject in need thereof, comprising administering to the subject an effective amount of the multispecific binding protein of any one of claims 1 to 38 or the pharmaceutical composition of claim 44.

10 55. A method of reducing the level of a cell surface protein in a subject in need thereof, comprising administering to the subject an effective amount of the multispecific binding protein of any one of claims 1 to 38 or the pharmaceutical composition of claim 44, optionally wherein the subject has cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease.

15 56. A method of increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of the multispecific binding protein of any one of claims 1 to 38 or the pharmaceutical composition of claim 44, optionally wherein the subject has cancer, an autoimmune condition, an inflammatory condition, a neurodegenerative condition, or an infectious disease.

20 57. The method according to any one of claims 54-56, wherein (a) the subject has a mutation in RNF43 and the multispecific binding protein does not bind to or does not activate RNF43, or (b) the subject has a mutation in ZNRF3 and the multispecific binding protein does not bind to or does not activate ZNRF3.

25 58. The method according to any one of claims 54-56, wherein the method further comprises, prior to administering the multispecific binding protein, determining whether the subject has a mutation in RNF43 or ZNRF3, wherein, (a) if the subject has a mutation in RNF43, the multispecific binding protein does not bind to or does not activate RNF43, and (b) if the subject has a mutation in ZNRF3, the multispecific binding protein does not bind to or does not activate ZNRF3.

30 59. The multispecific binding protein, use or method of any one of claims 49, 53, 57, or 58, wherein the subject has cancer, and wherein the cancer comprises a mutation in RNF43 or ZNRF3.

60. A method of reducing the level of a cell surface protein on the surface of hematopoietic cells in a cell or tissue sample *in vitro* or *in vivo* in a subject, comprising

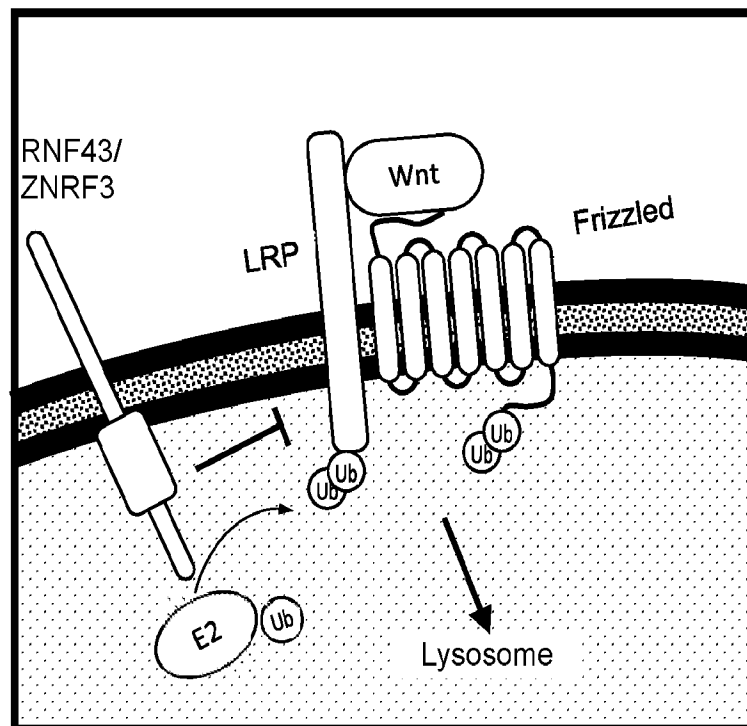
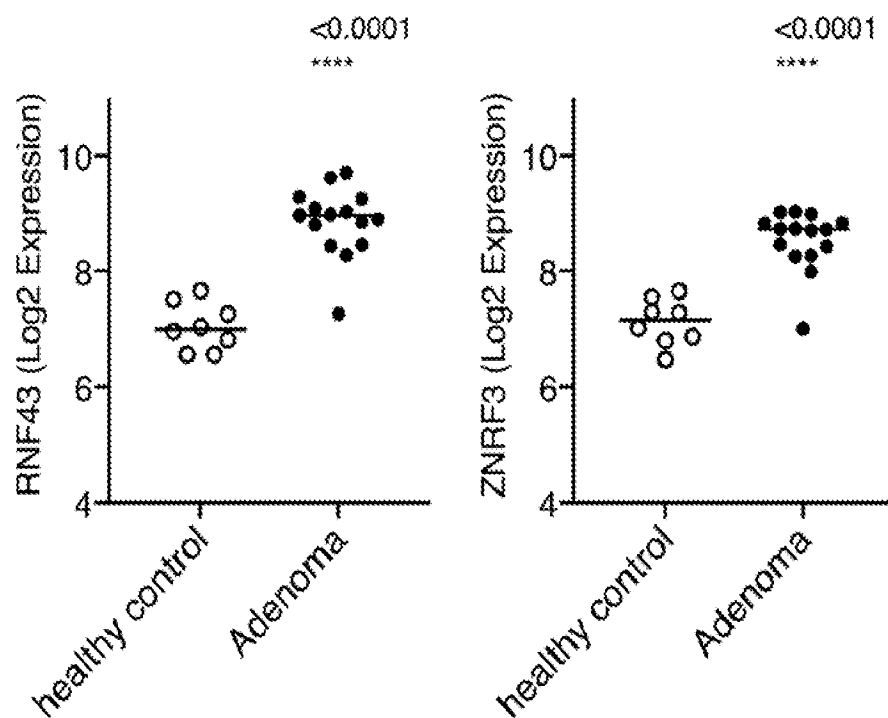
administering to the cell or tissue sample or to the subject, respectively, a multispecific binding protein according to claim 14.

61. A method of reducing the level of a cell surface protein on the surface of testicular cells in a cell or tissue sample *in vitro* or *in vivo* in a subject, comprising administering to the cell
5 or tissue sample or to the subject, respectively, a multispecific binding protein according to claim 15.

62. A kit comprising the multispecific binding protein of any one of claims 1-38 or the nucleic acid or set of nucleic acids of claim 39 or the host cell of claim 40, and further
10 comprising one or more reagents for expressing or purifying the protein and/or one or more reagents for incubating the protein with a cell or tissue sample *in vitro* to reduce the level of a cell surface protein in the sample.

63. A method of reducing the level of a cell surface protein in a cell or tissue sample *in vitro*, comprising incubating the sample with the multispecific binding protein of any one of claims 1-38 or the kit of claim 62.

15

*Fig. 1A**Fig. 1B*

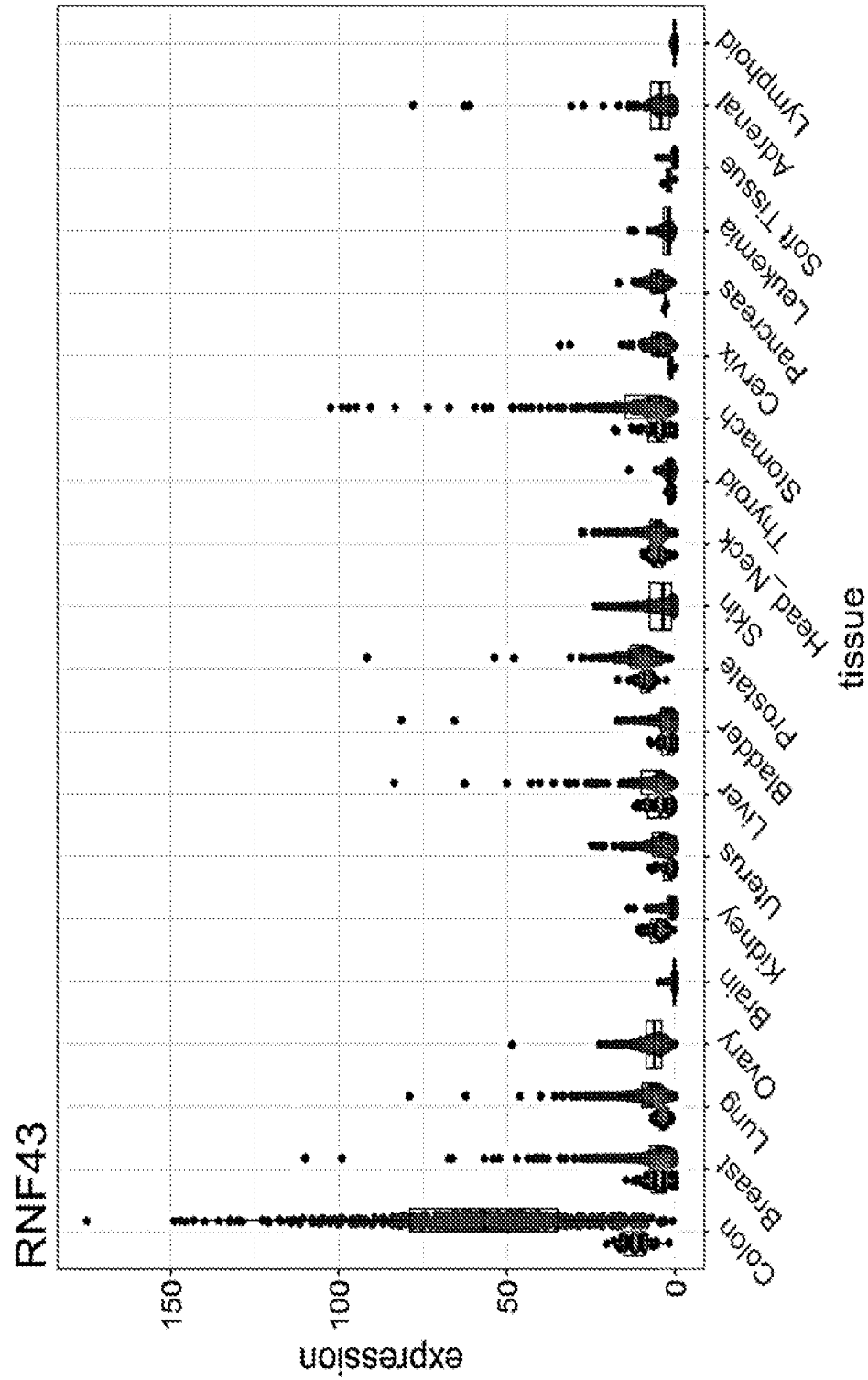


Fig. 1C

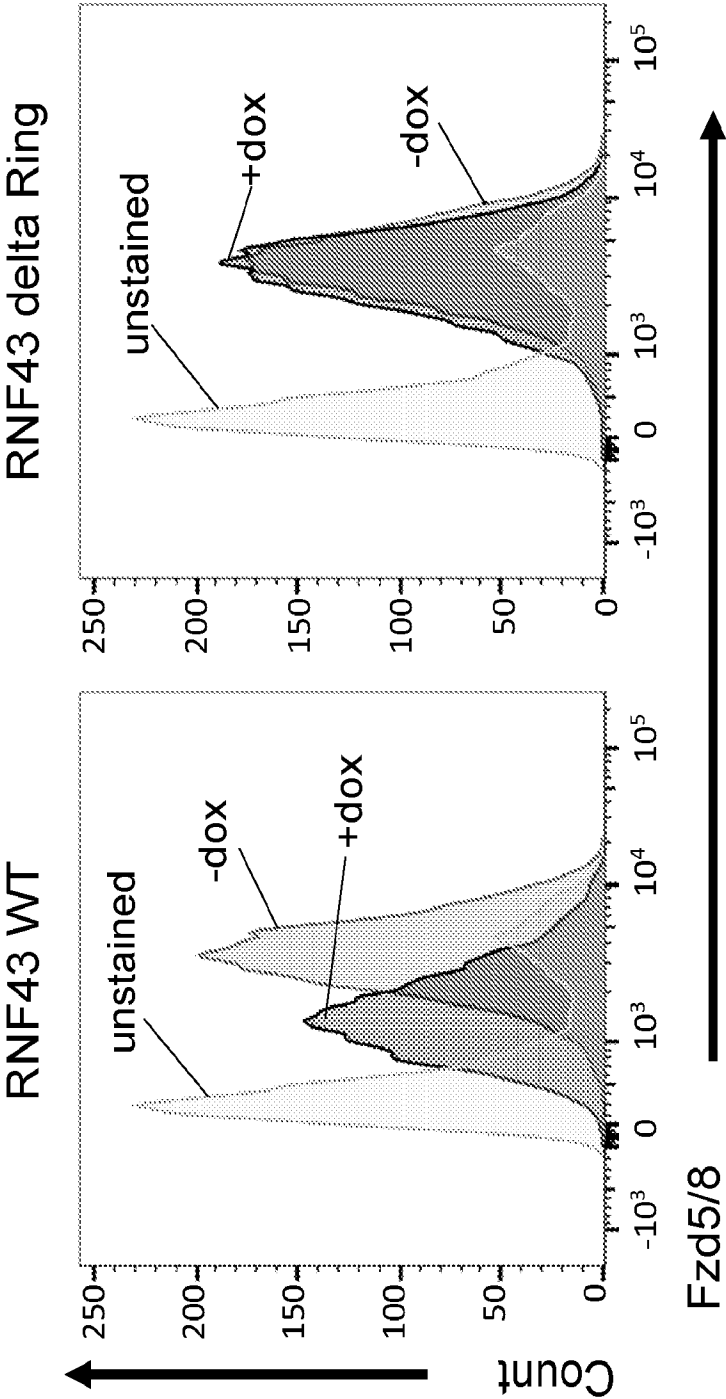


Fig. 1D

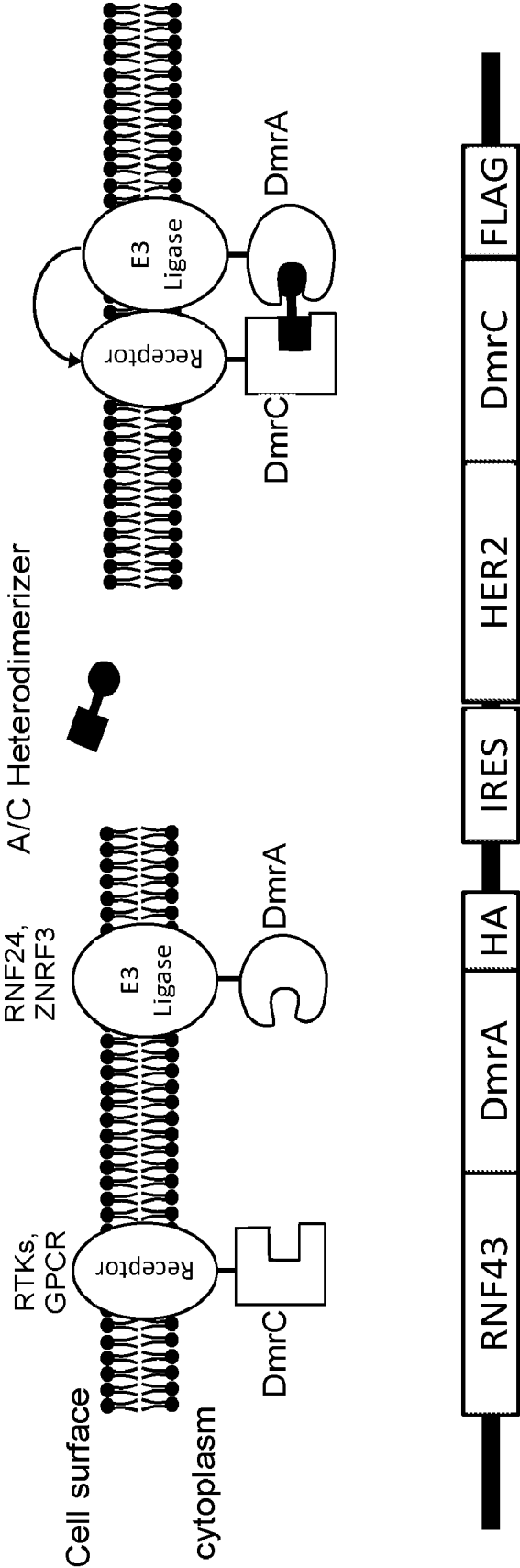
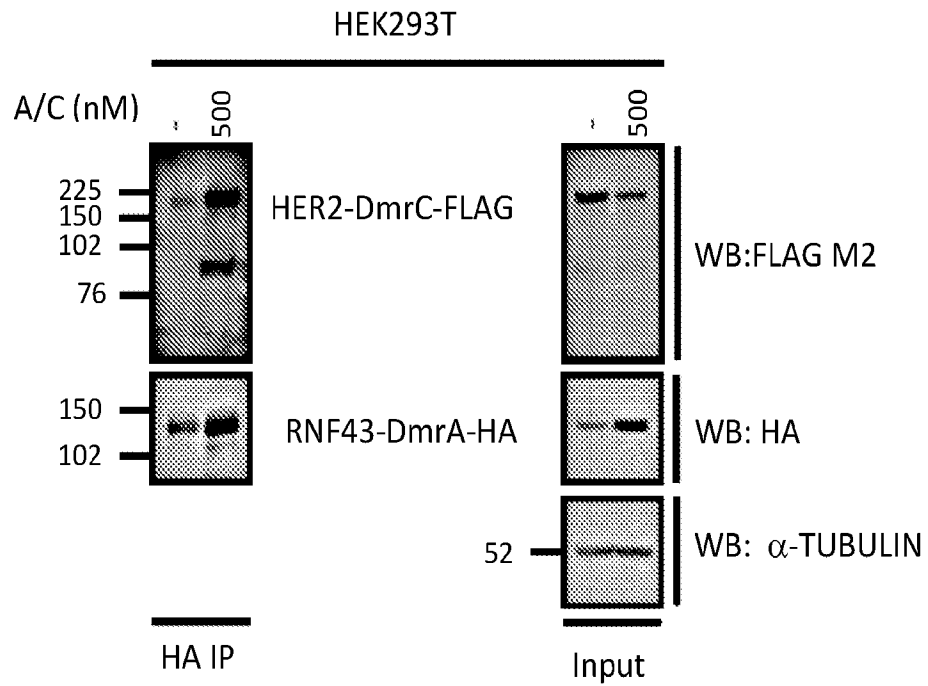
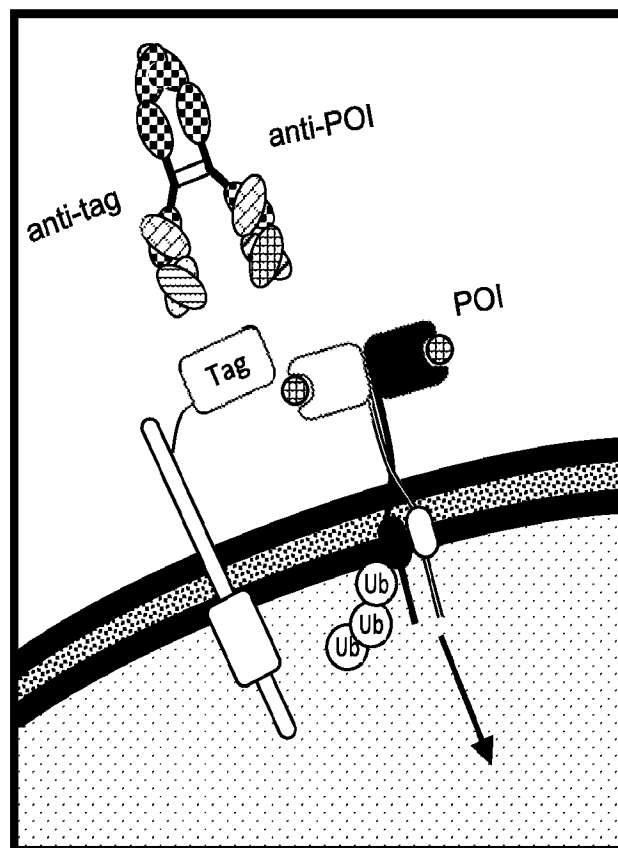


Fig. 1E

*Fig. 1F**Fig. 2A*

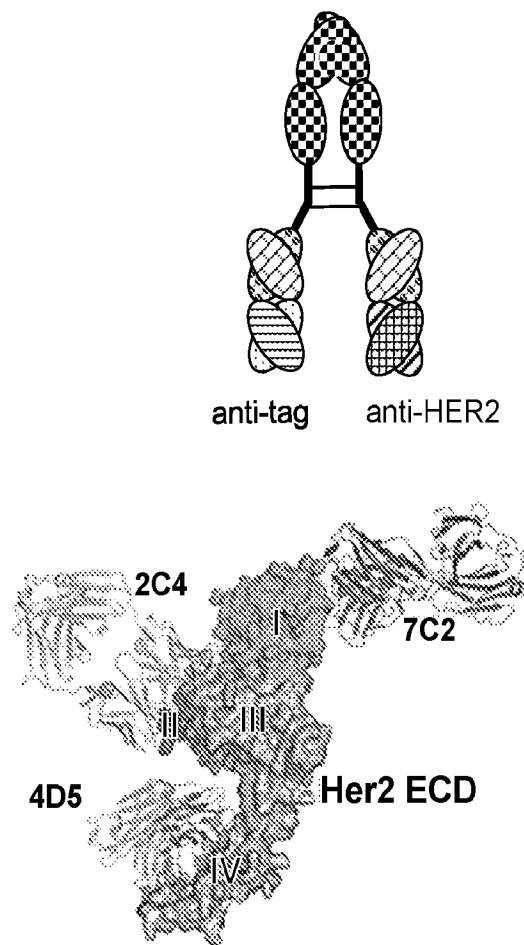


Fig. 2B

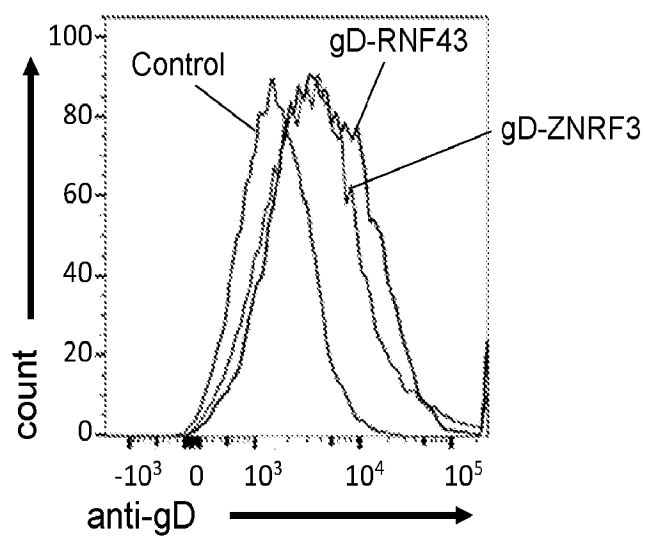
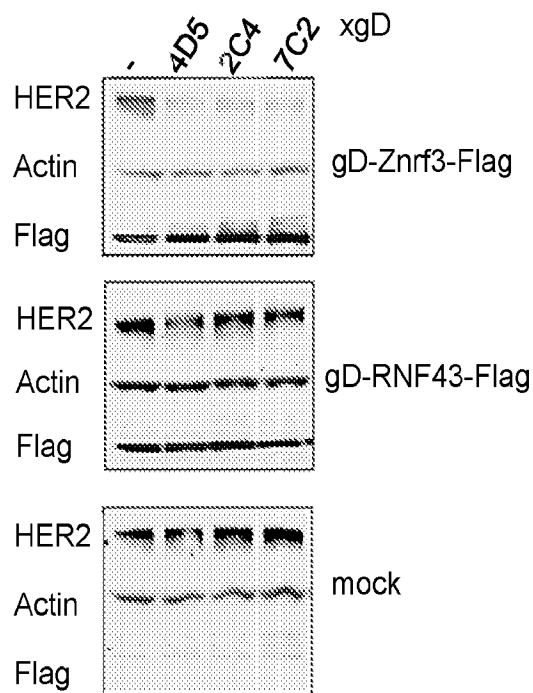
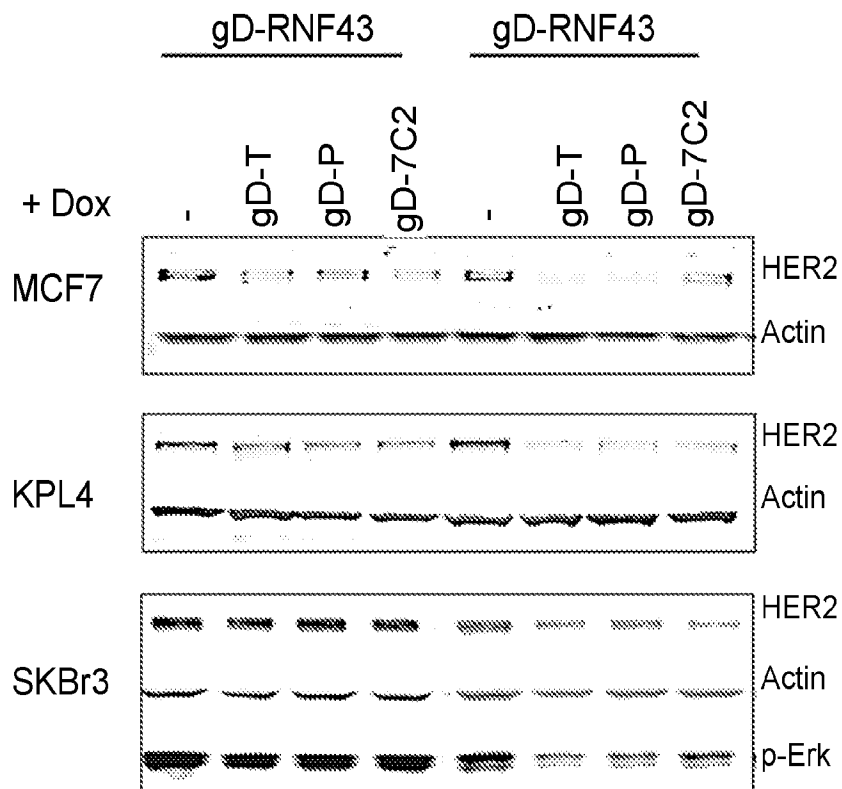
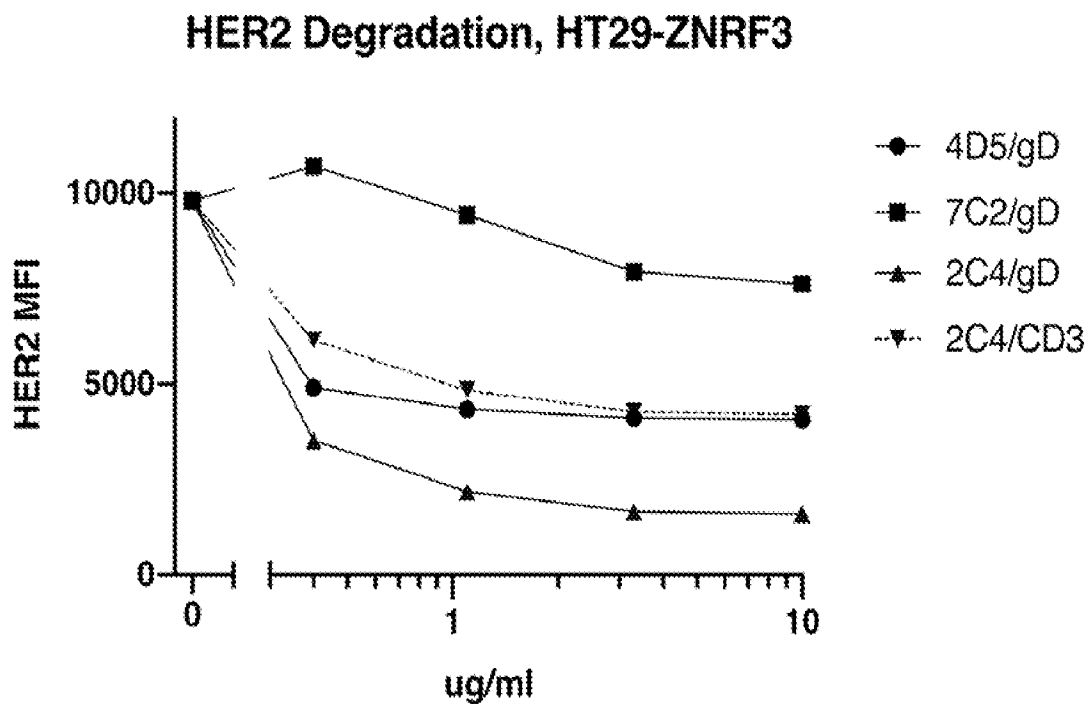
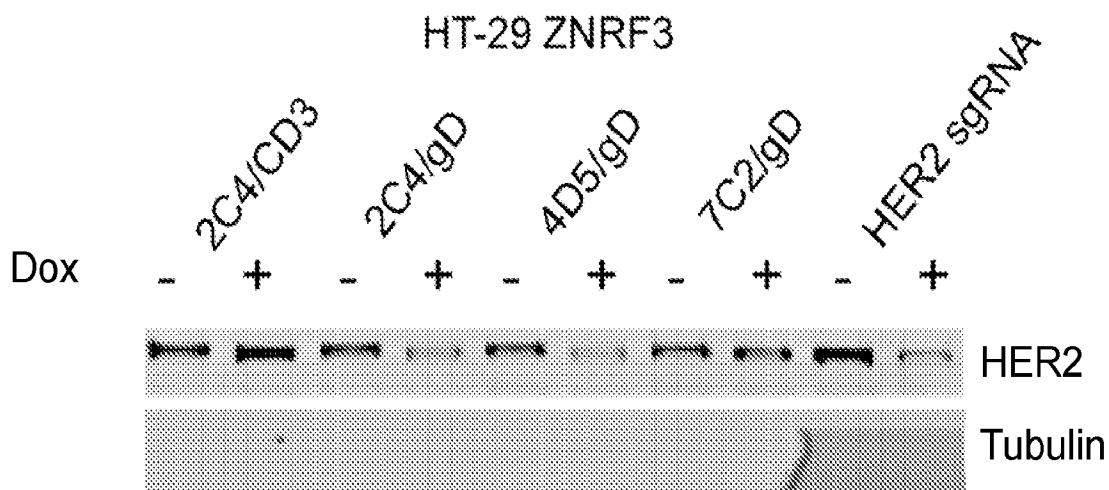
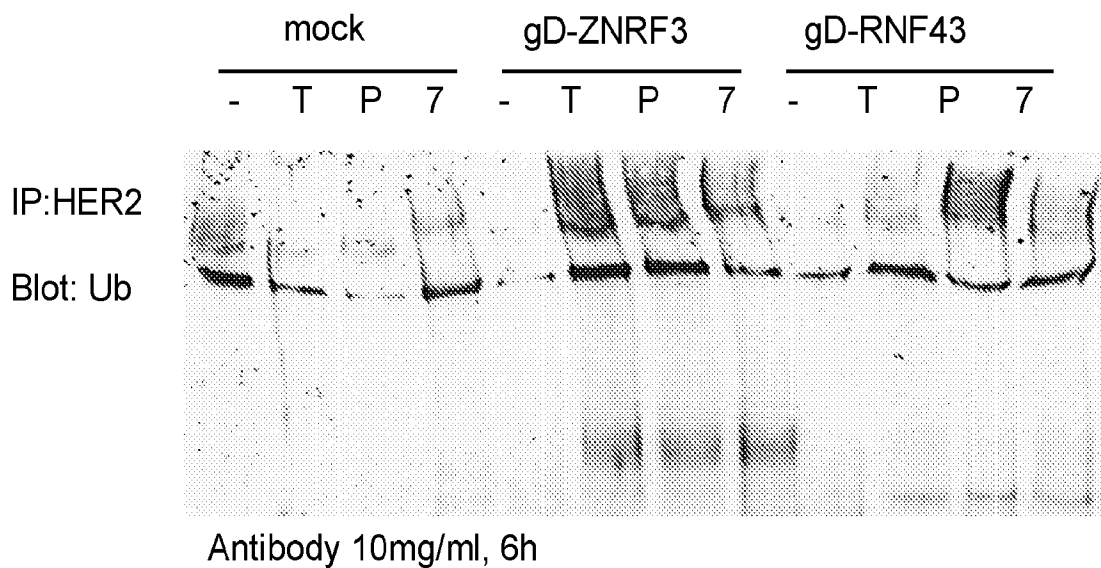
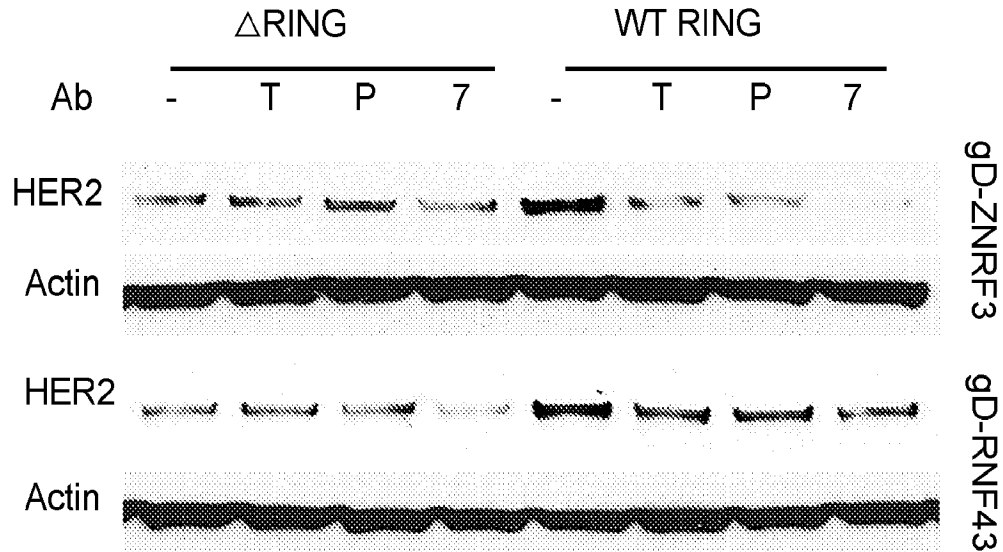
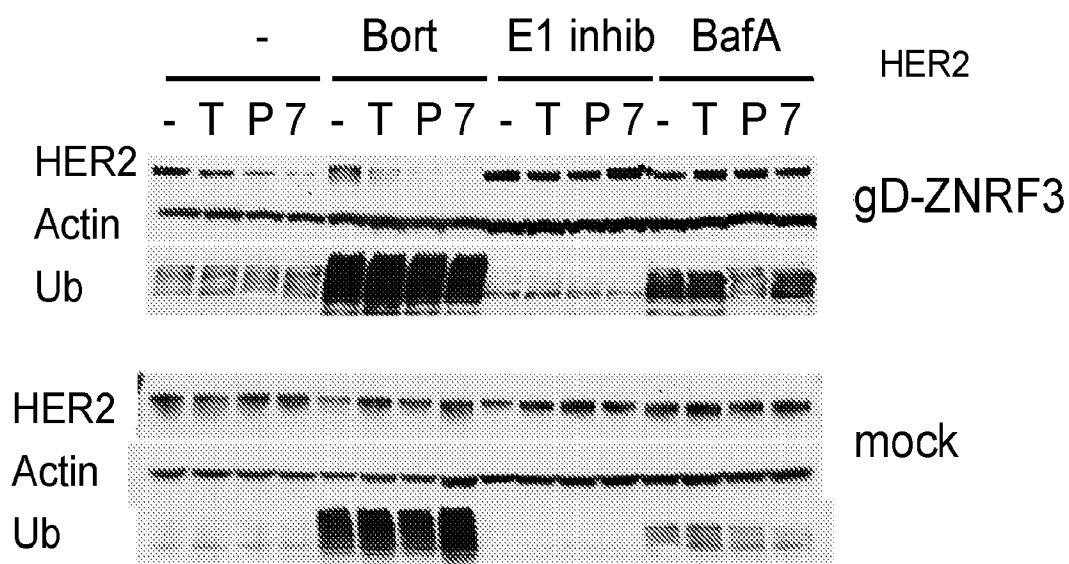
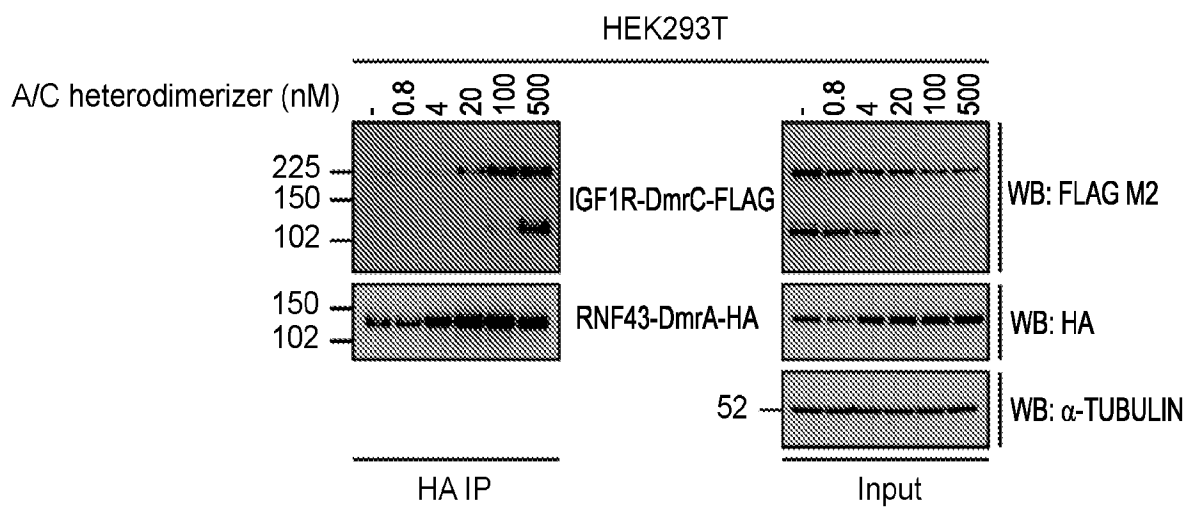


Fig. 2C

**Fig. 2D****Fig. 2E**

*Fig. 3A**Fig. 3B*

**Fig. 3C****Fig. 3D**

*Fig. 3E**Fig. 4A*

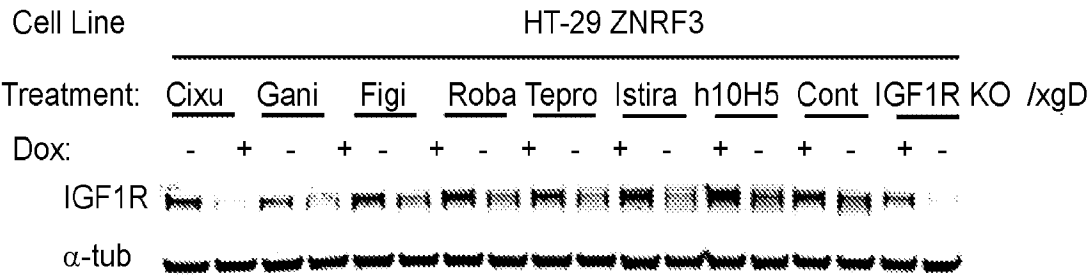


Fig. 4B

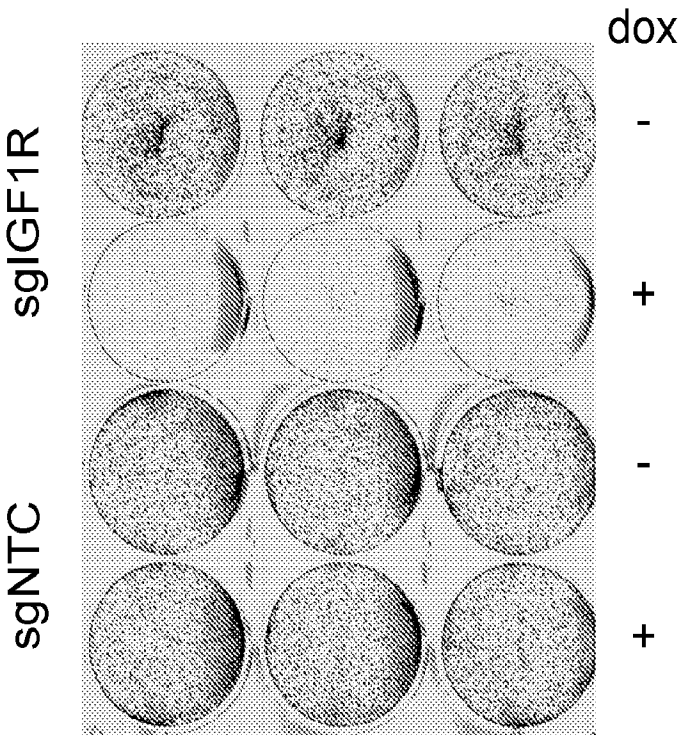
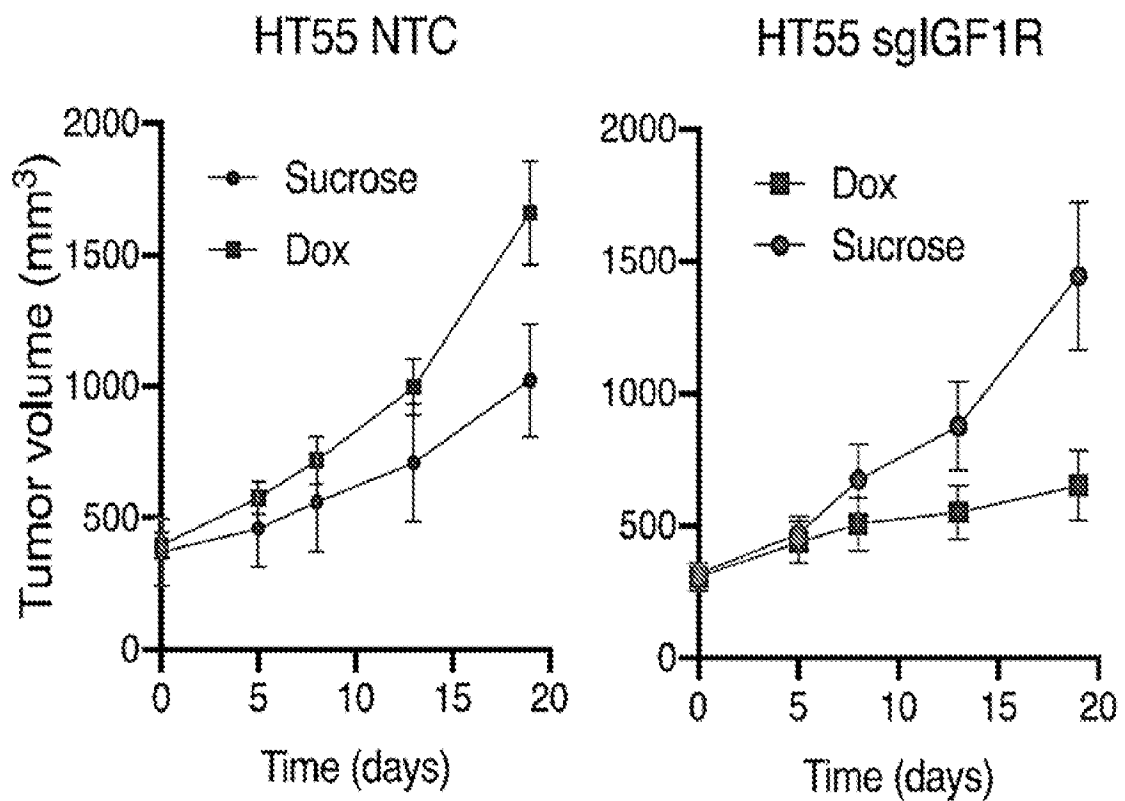
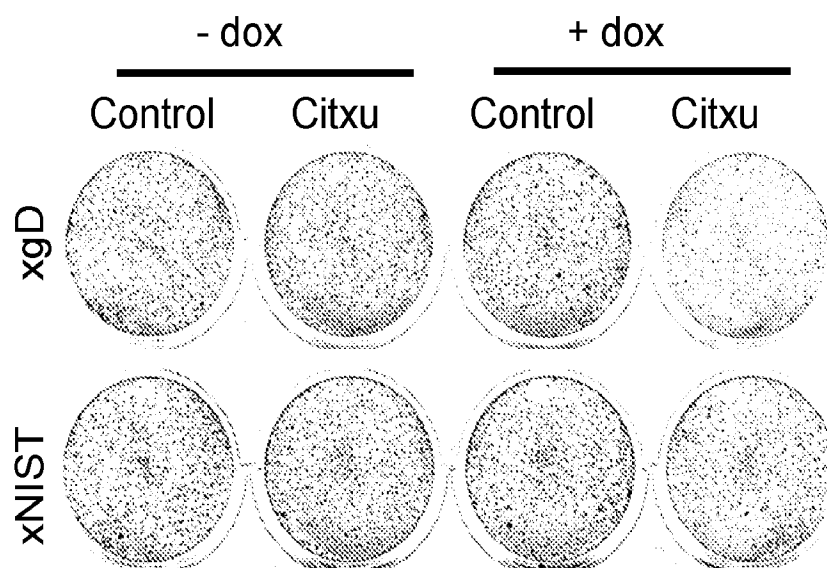
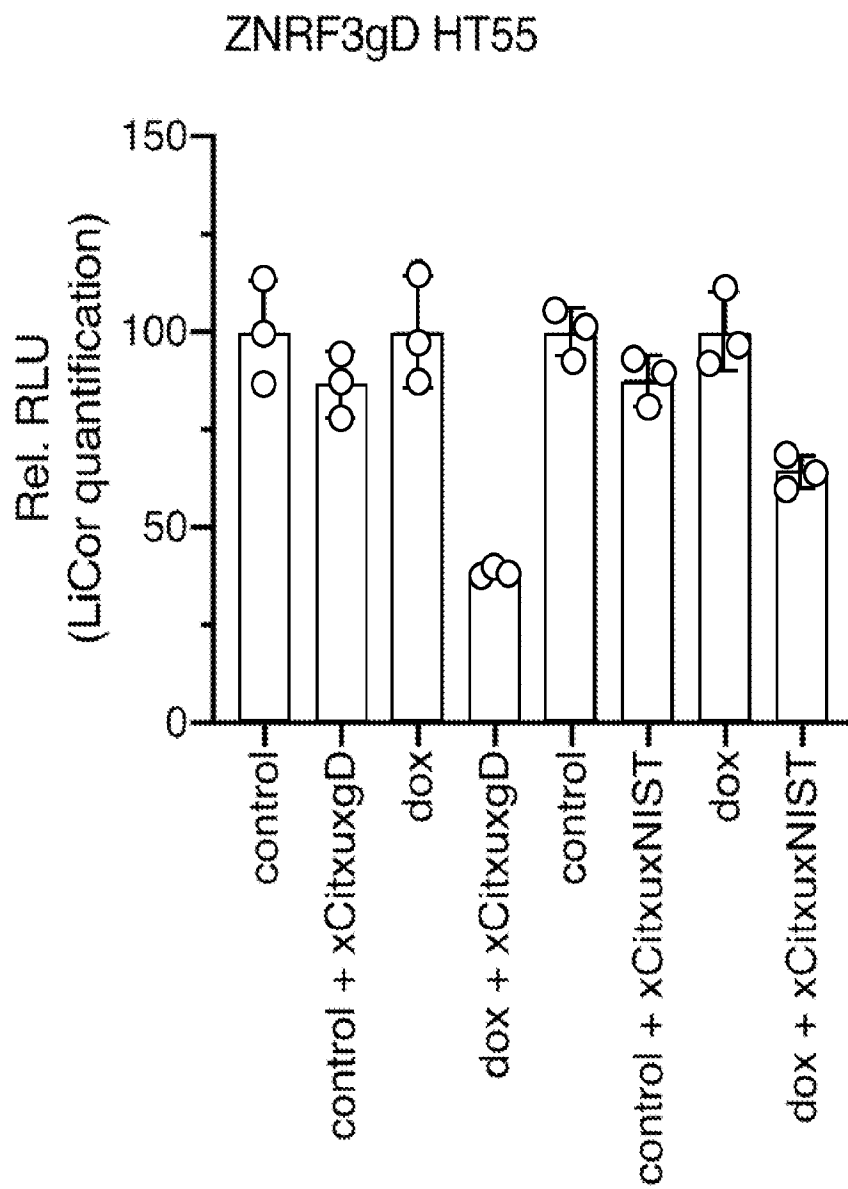
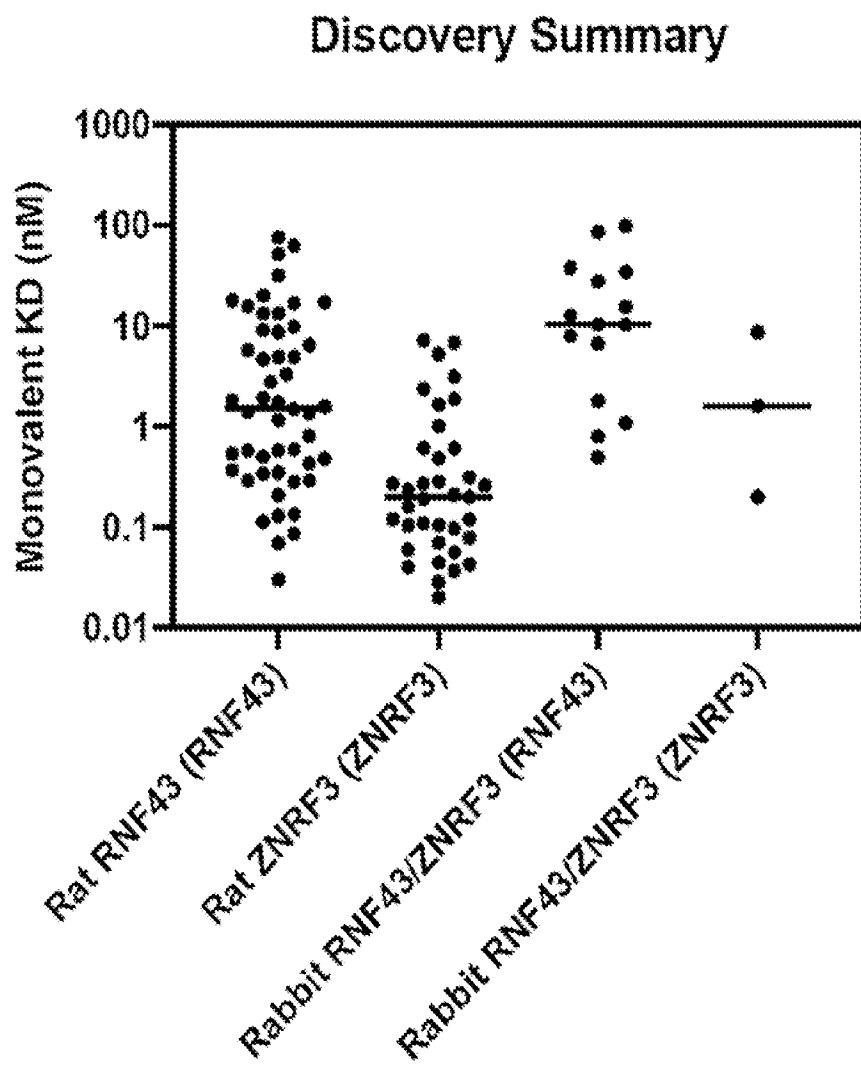


Fig. 4C

**Fig. 4D****Fig. 4E**

*Fig. 4F*

*Fig. 5A*

ZNRF3

Bins	Bins	ZNRF3- 171.hlgG1	ZNRF3- 101.hlgG1	ZNRF3- 117.hlgG1	ZNRF3- 163.hlgG1	ZNRF3- 195.hlgG1	ZNRF3- 6.hlgG1	ZNRF3- 128.hlgG1	ZNRF3- 35.hlgG1	ZNRF3- 90.hlgG1	ZNRF3- 131.hlgG1	ZNRF3- 17.hlgG1	ZNRF3- 55.hlgG1	ZNRF3- 172.hlgG1	ZNRF3- 179.hlgG1	ZNRF3- 182.hlgG1	ZNRF3- 30.hlgG1	ZNRF3- 331	ZNRF3- 332	ZNRF3- 333
	ZNRF3- 171.hlgG1		0.59	0.39	0.41	0.31	0.49	0.73	0.41	0.53	0.37	0.24	0.25	0.55	0.47	0.62	0.51	0.11	0.02	0.07
	ZNRF3- 101.hlgG1	0.32		0.22	0.04	0.11	0.07	0.09	0.04	-0.01	0.1	0.01	0.01	0.06	0.09	0.18	0.11	0.6	0.12	0.03
	ZNRF3- 117.hlgG1	0.37	0.24		-0.01	0.07	0.18	0.41	-0.06	-0.09	0.19	0.07	0.14	0.18	-0.05	0.2	-0.04	-0.1	-0.07	-0.07
	ZNRF3- 163.hlgG1	0.8	-0.03	0.19		-0.03	-0.03	-0.04	-0.03	-0.04	-0.04	-0.04	-0.05	-0.04	-0.04	-0.04	0.53	-0.03	-0.03	-0.03
	ZNRF3- 195.hlgG1	0.59	0.09	0.2	-0.05		-0.06	-0.07	-0.17	-0.11	-0.14	-0.22	-0.08	0.17	0.11	0.16	-0.18	0.63	-0.09	0.12
	ZNRF3- 6.hlgG1	0.67	-0.01	0.48	-0.01	-0.01		-0.02	-0.02	-0.02	-0.02	-0.04	-0.03	-0.02	0	-0.01	-0.01	0.5	0	0
	ZNRF3- 128.hlgG1	0.52	-0.09	0.25	-0.09	-0.08	-0.11		-0.1	-0.12	-0.11	-0.14	-0.14	-0.12	-0.12	-0.14	-0.14	0.12	-0.1	-0.09
	ZNRF3- 35.hlgG1	0.77	-0.03	-0.13	-0.03	-0.03	-0.04	-0.05		-0.05	-0.04	-0.05	-0.05	-0.05	-0.04	-0.05	-0.05	0.47	-0.04	-0.04

Fig. 5B

ZNRFF3

Bins	Bins	ZNRFF3-171.hlgG1	ZNRFF3-101.hlgG1	ZNRFF3-117.hlgG1	ZNRFF3-163.hlgG1	ZNRFF3-195.hlgG1	ZNRFF3-6.hlgG1	ZNRFF3-128.hlgG1	ZNRFF3-35.hlgG1	ZNRFF3-90.hlgG1	ZNRFF3-131.hlgG1	ZNRFF3-17.hlgG1	ZNRFF3-55.hlgG1	ZNRFF3-172.hlgG1	ZNRFF3-179.hlgG1	ZNRFF3-182.hlgG1	ZNRFF3-30.hlgG1	ZNRFF3-331	ZNRFF3-332	ZNRFF3-333
	ZNRFF3-90.hlgG1	0.19	0.03	-0.04	-0.01	0.04	0.01	0.04	0.03		0.13	0.02	0.01	0.19	0.27	0.46	0.29	0.56	0.04	0.15
	ZNRFF3-131.hlgG1	0.73	-0.05	0.36	-0.04	-0.04	-0.05	-0.06	-0.05	-0.06		-0.07	-0.07	-0.06	-0.06	-0.07	-0.06	0.47	-0.05	-0.04
	ZNRFF3-17.hlgG1	0.55	-0.02	0.44	-0.02	-0.01	-0.02	-0.02	-0.02	-0.02	-0.02		-0.03	-0.02	-0.02	-0.03	-0.03	0.38	0.77	1
	ZNRFF3-55.hlgG1	0.5	-0.01	0.33	-0.01	-0.01	-0.0	-0.02	-0.02	-0.02	-0.02	-0.02		-0.02	-0.02	-0.02	-0.02	0.27	0.46	0.59
	ZNRFF3-172.hlgG1	0.51	-0.02	0.05	-0.16	0.15	0.09	0.11	-0.02	-0.01	0.29	0.12	0.31		0.55	0.61	0.15	0.5	0.34	0.85
	ZNRFF3-179.hlgG1	486.7	2.08	-0.68	9.92	7.3	16.41	1.62	1.35	1.09	3.05	-0.49	0.03	-31.42		15.17	-6.76	-3.09	9.59	-23.84
	ZNRFF3-182.hlgG1	0.49	-0.13	-0.17	-0.48	0.58	0.11	0.06	-0.35	-0.08	0.82	-0.64	1.65	4.21	1.45		16.93	0.55	0.13	1.67
	ZNRFF3-30.hlgG1	0.47	-0.09	-0.51	-0.35	0.14	-0.14	0.14	-0.49	-0.1	1.56	-0.91	-7.49	5.57	5.93	11.28		-0.72	2.63	2.07
	ZNRFF3-331	0.8	0.94	-0.04	0.78	0.63	0.96	1.4	0.91	1.05	0.97	0.68	0.72	1.1	1.02	1.15	1.09		1.38	1.54
	ZNRFF3-332	0.32	-0.04	-0.05	-0.04	-0.04	-0.04	-0.04	-0.05	-0.04	-0.04	-0.04	0.7	0.76	-0.04	-0.04	-0.05	0.95		-0.03
	ZNRFF3-333	0.53	-0.03	-0.04	-0.04	-0.03	-0.03	-0.03	-0.04	-0.03	-0.03	1.15	1.18	-0.03	-0.04	-0.03	-0.03	1.26	-0.04	

Fig. 5B (cont.)

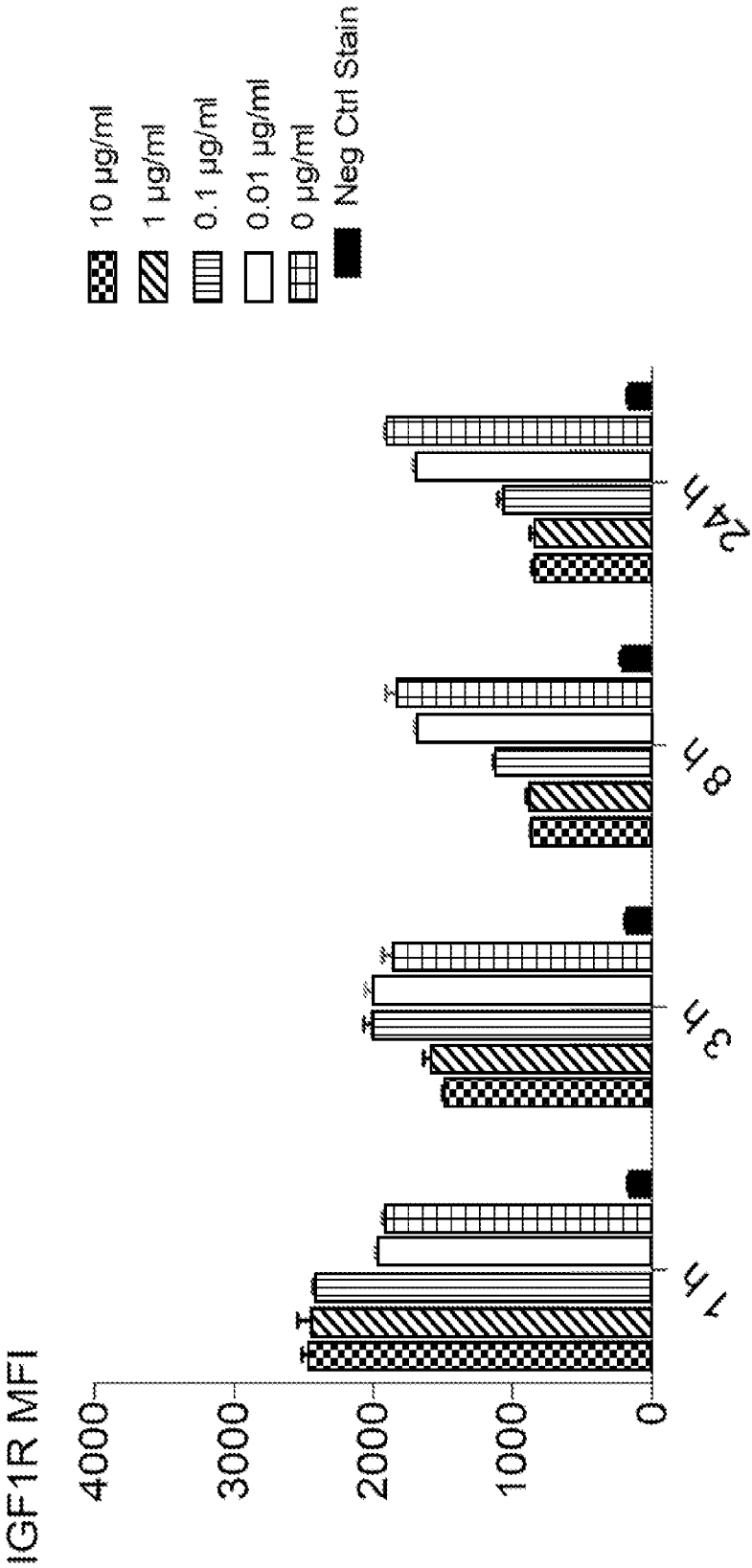
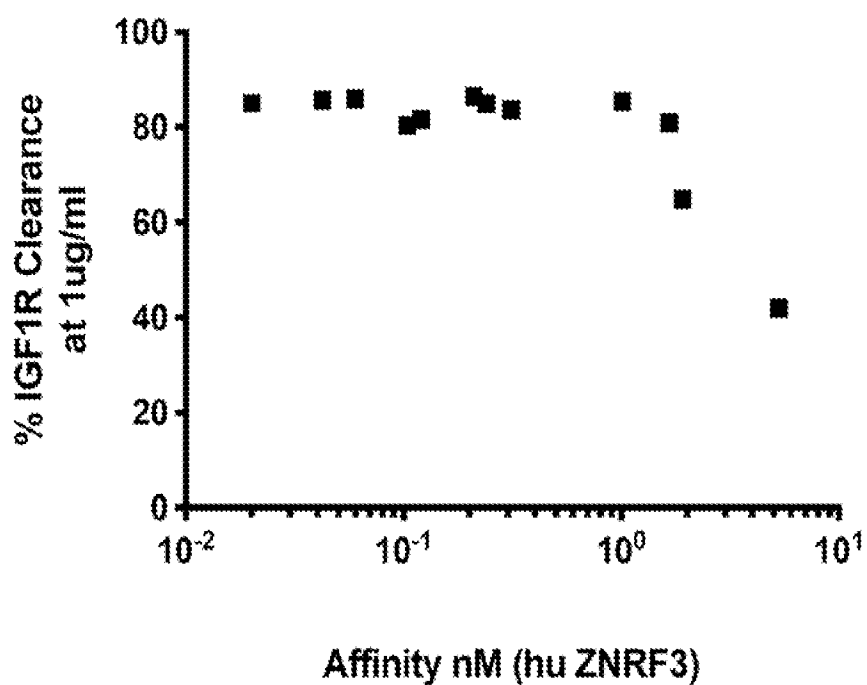
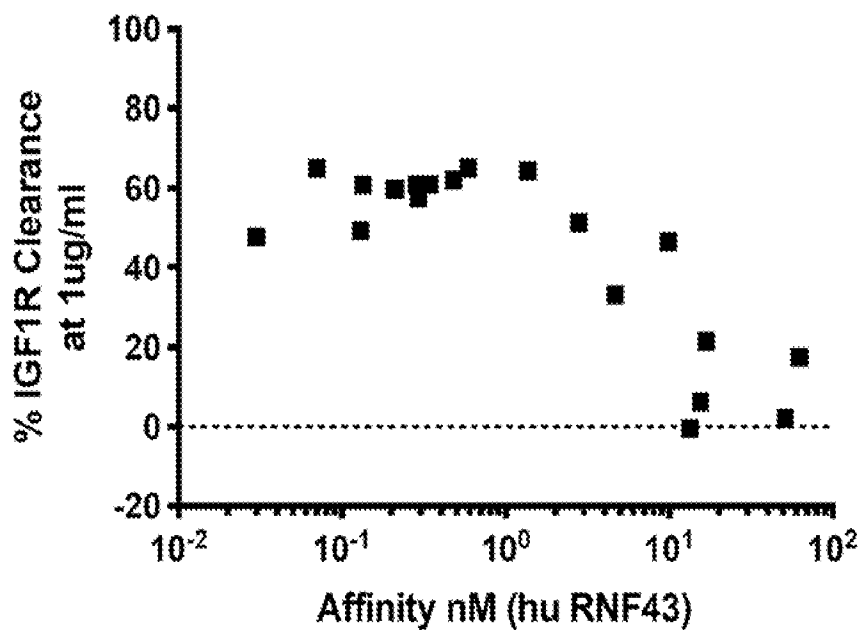


Fig. 5D

*Fig. 5E**Fig. 5F*

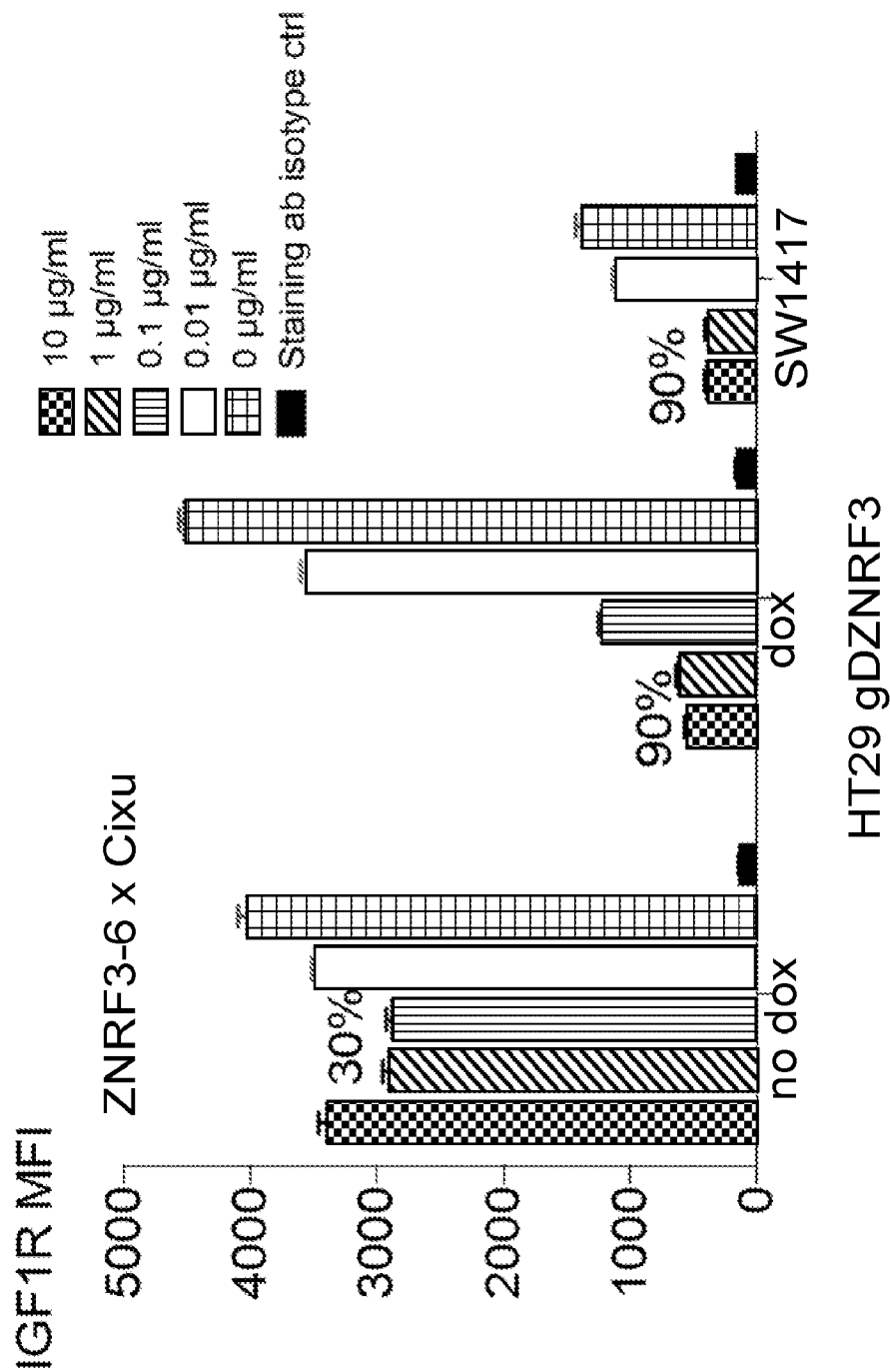


Fig. 6A

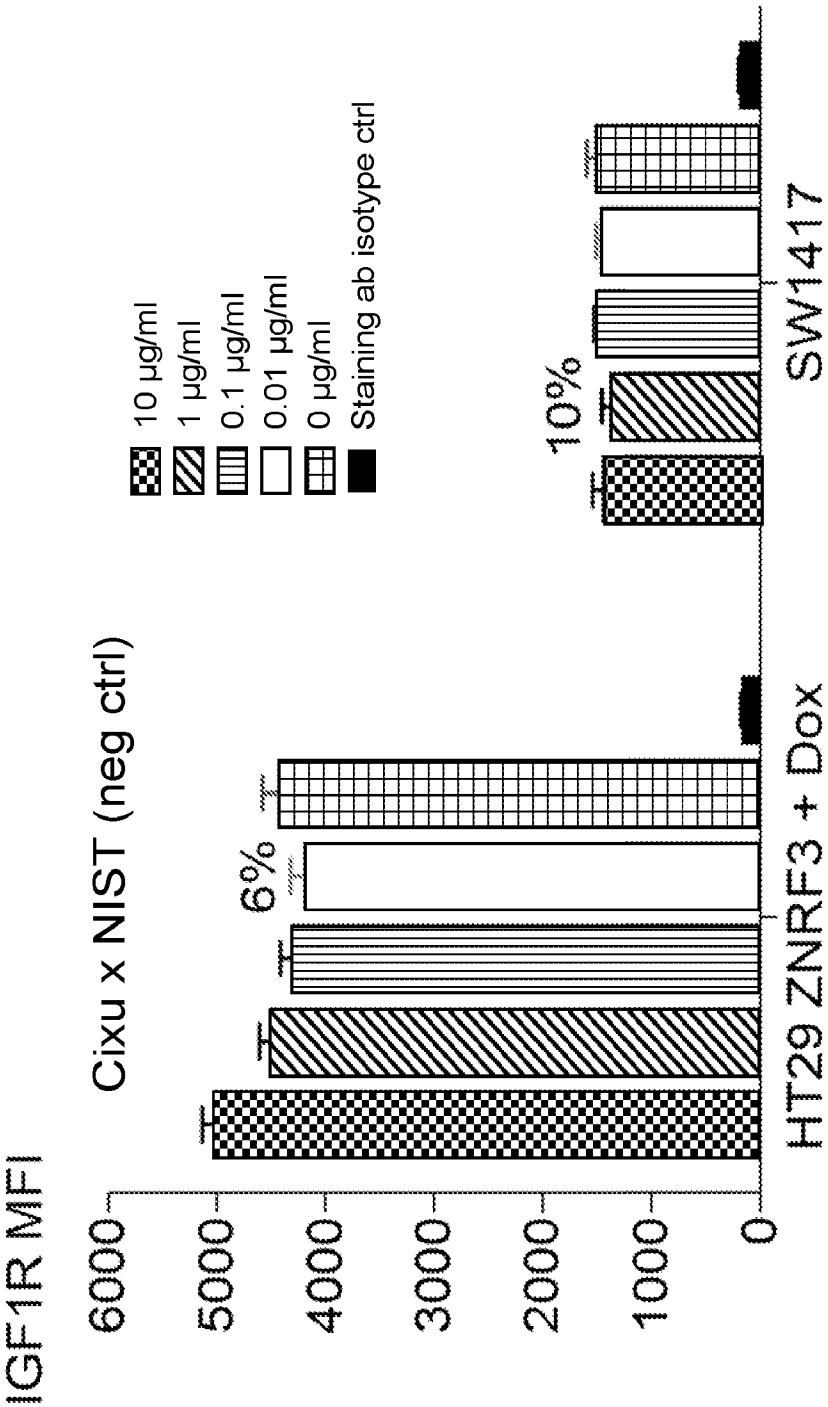


Fig. 6B

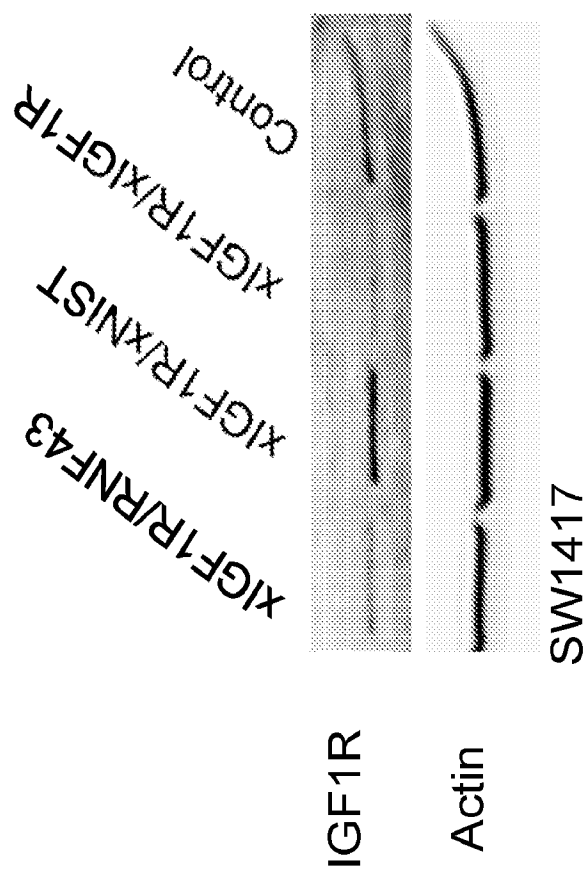


Fig. 6C

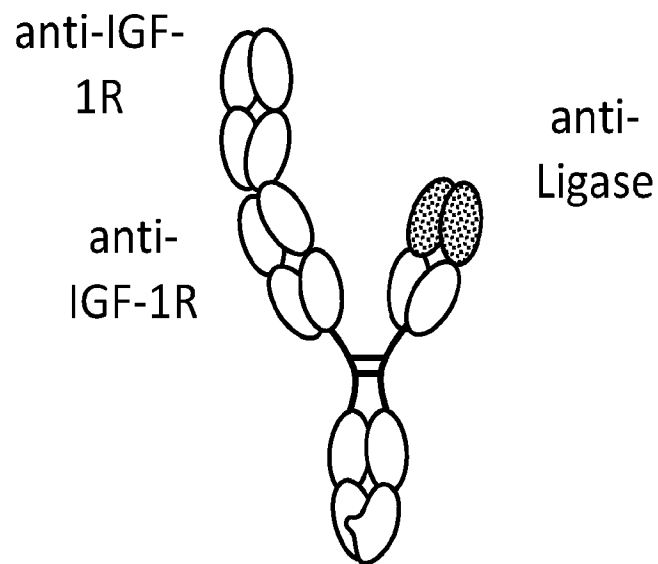


Fig. 7A

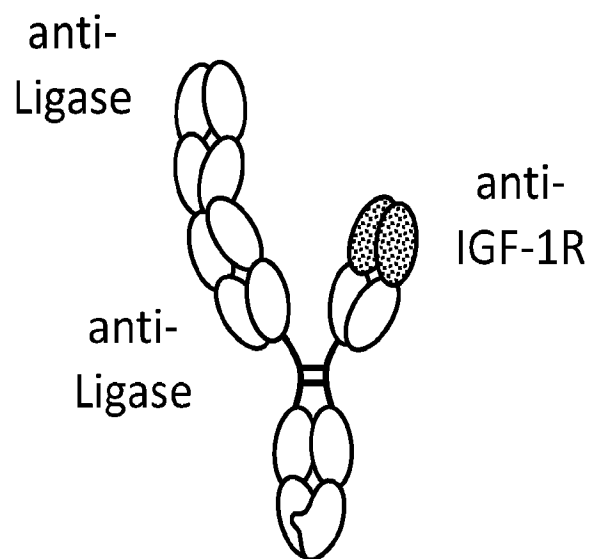


Fig. 7B

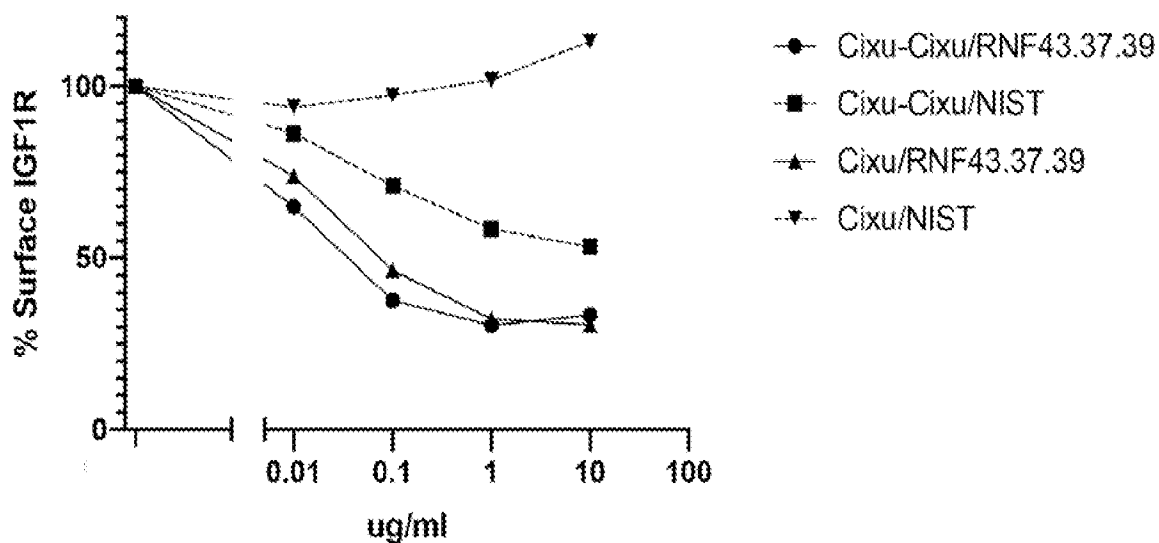


Fig. 7C

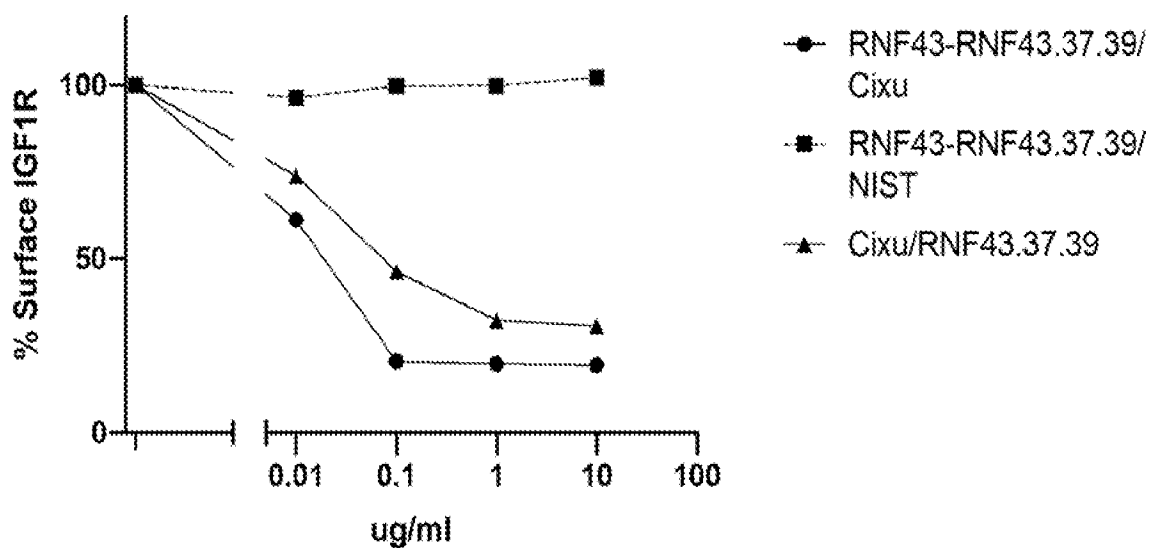


Fig. 7D

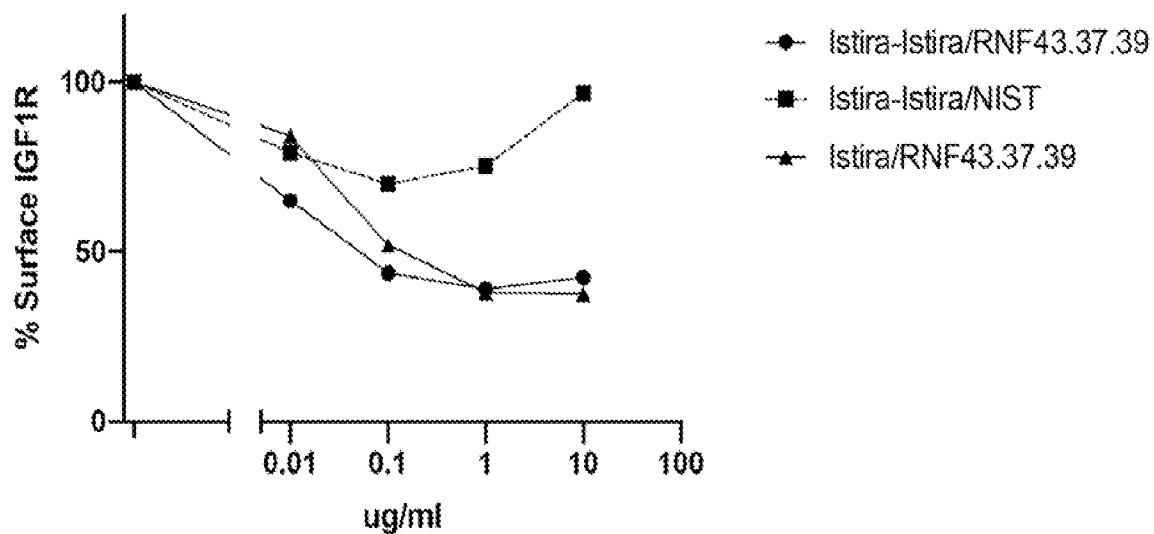


Fig. 7E

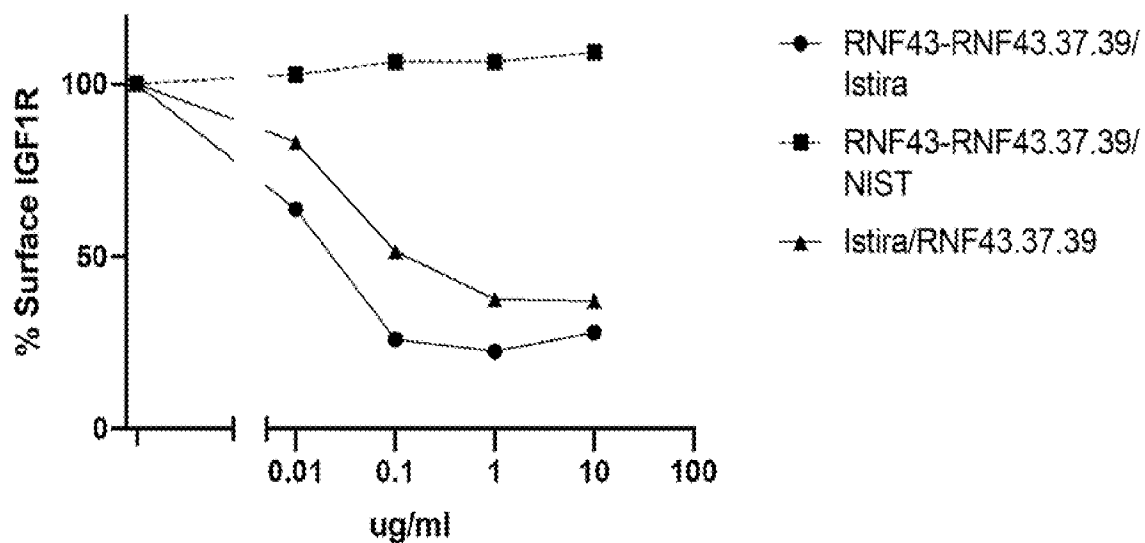


Fig. 7F

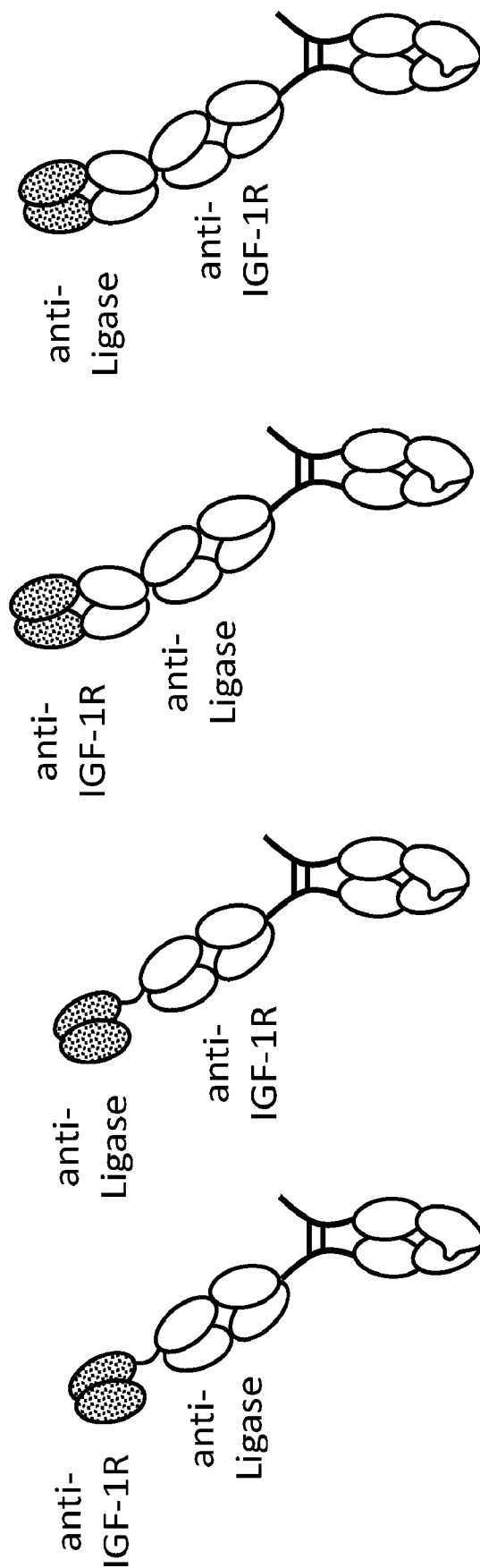
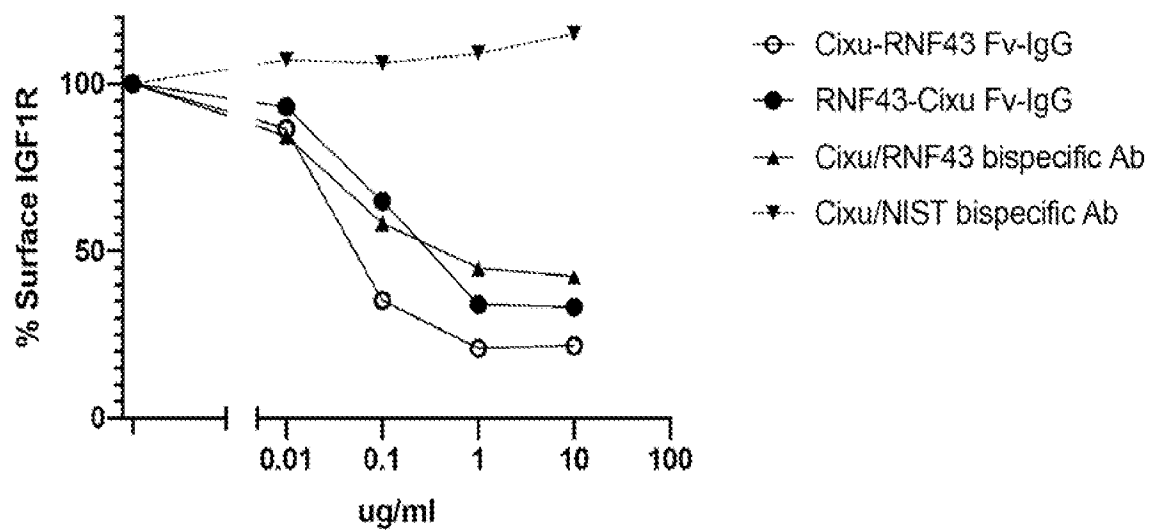
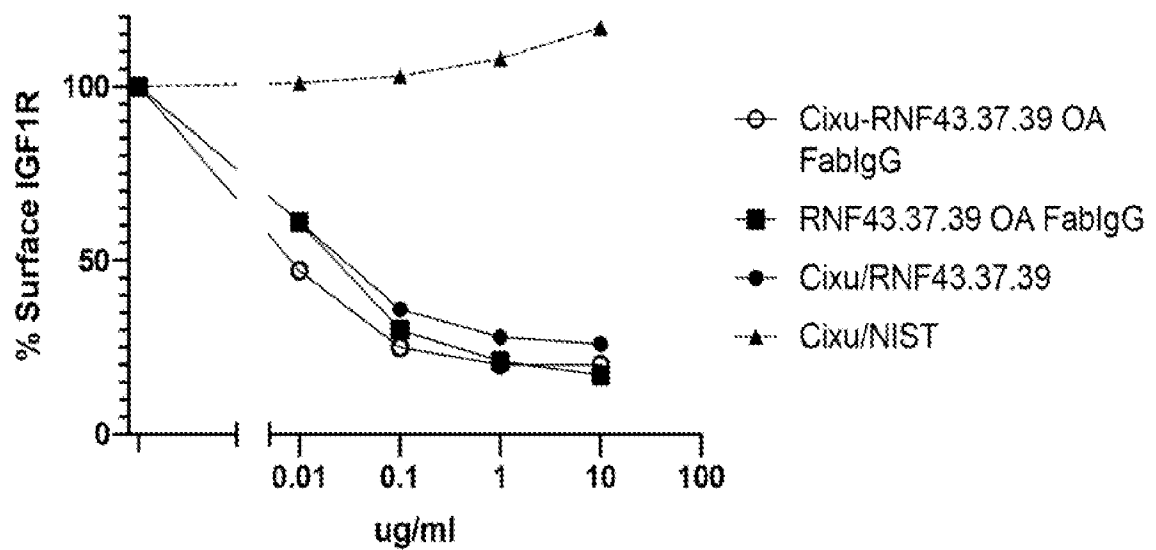


Fig. 8A

*Fig. 8B**Fig. 8C*

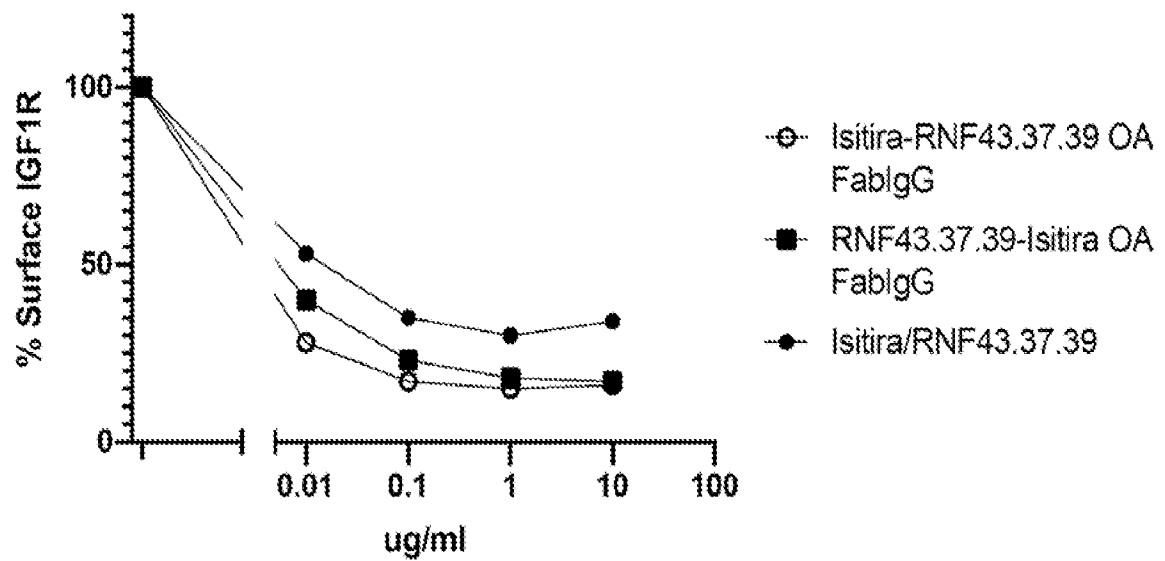


Fig. 8D

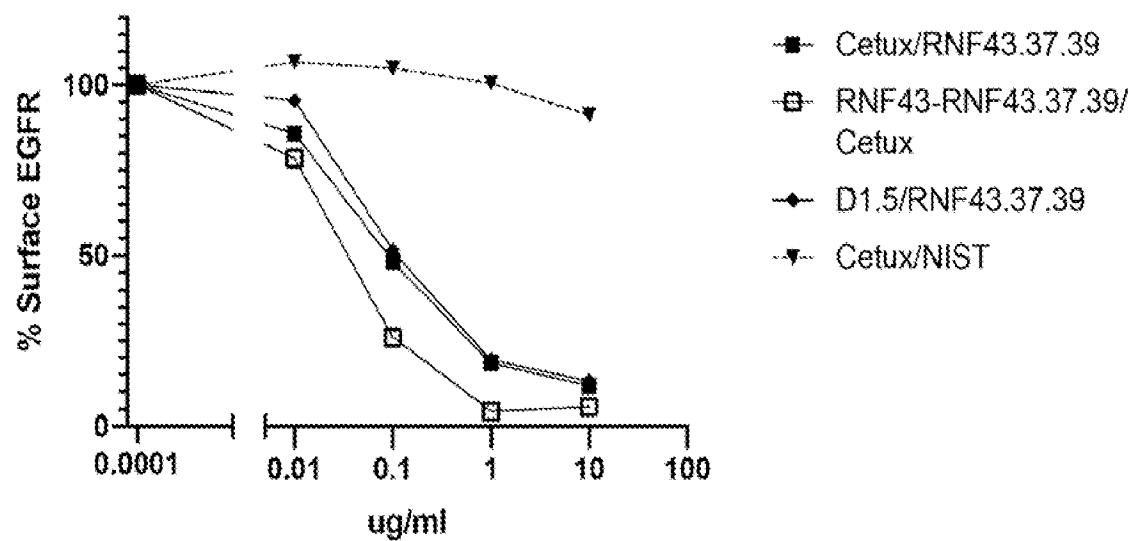


Fig. 9

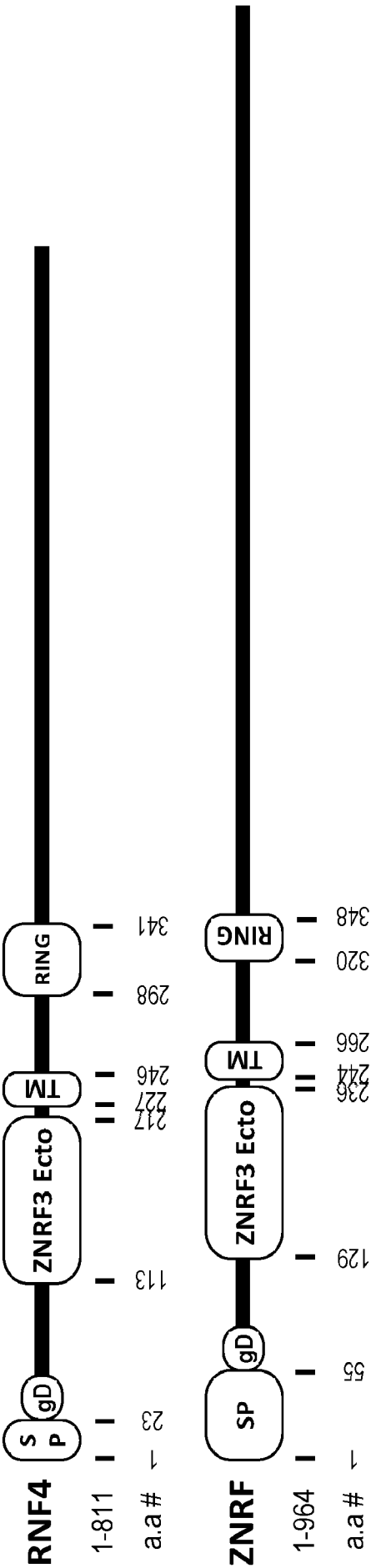
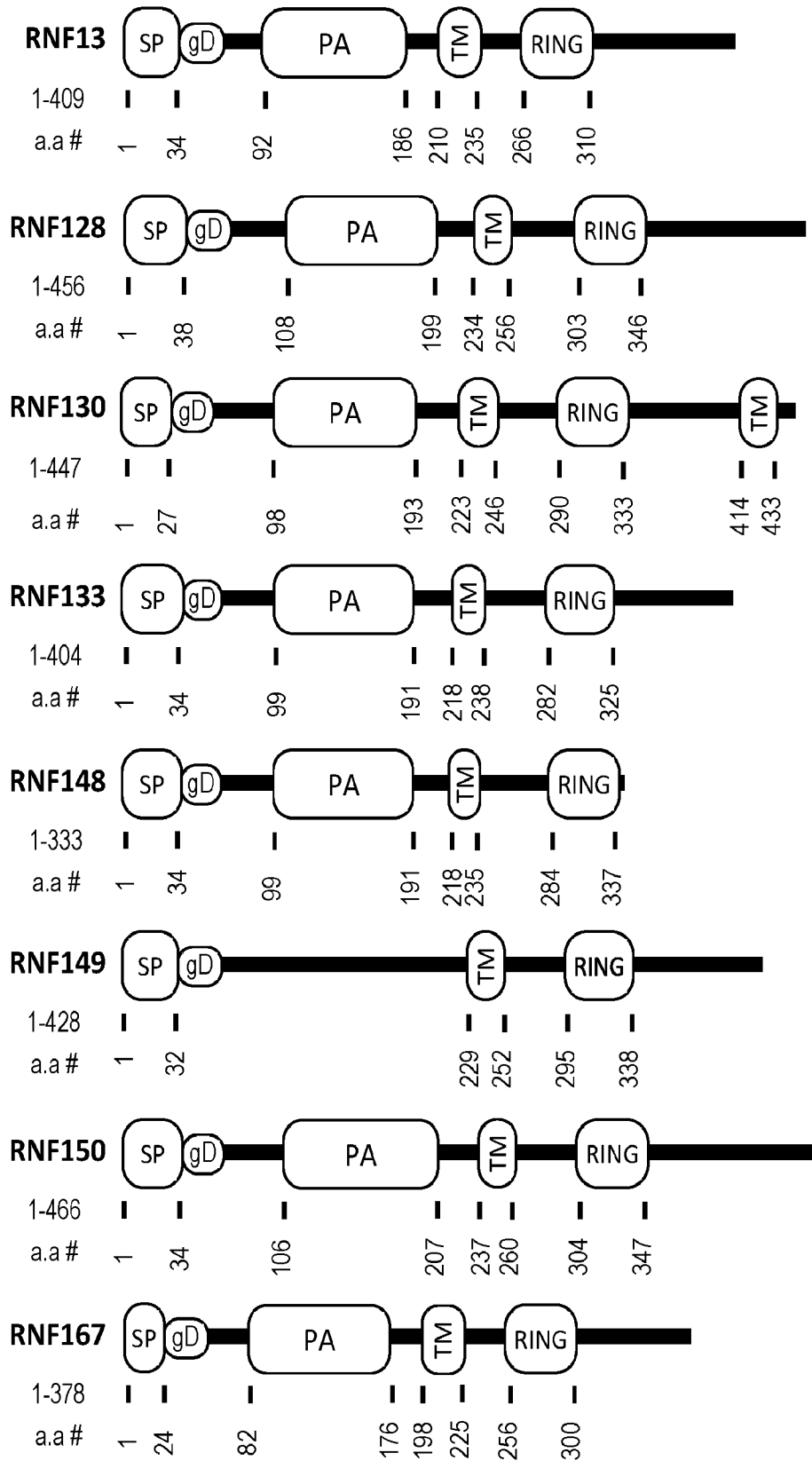


Fig. 10A

**Fig. 10B**

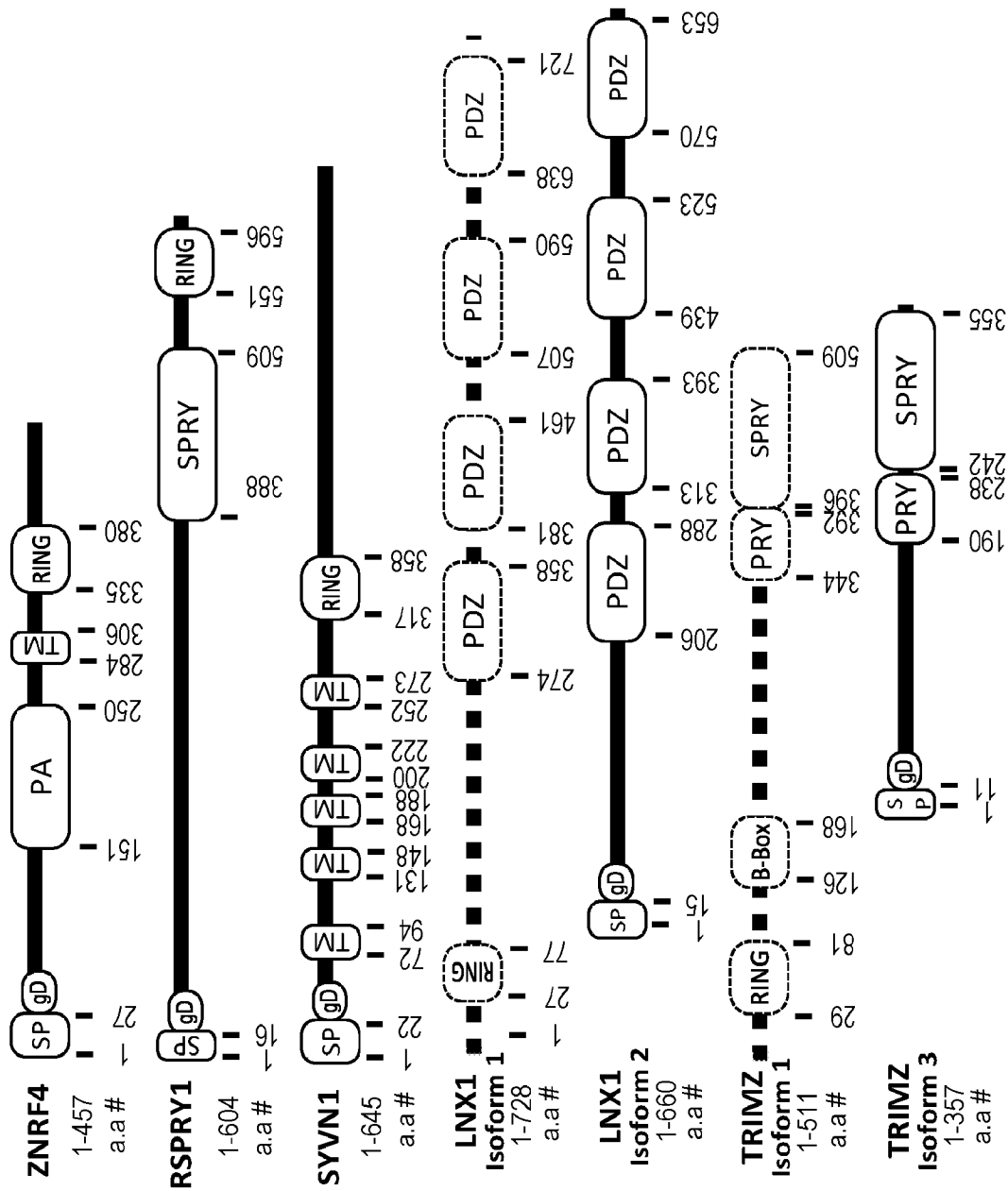


Fig. 10B (cont.)

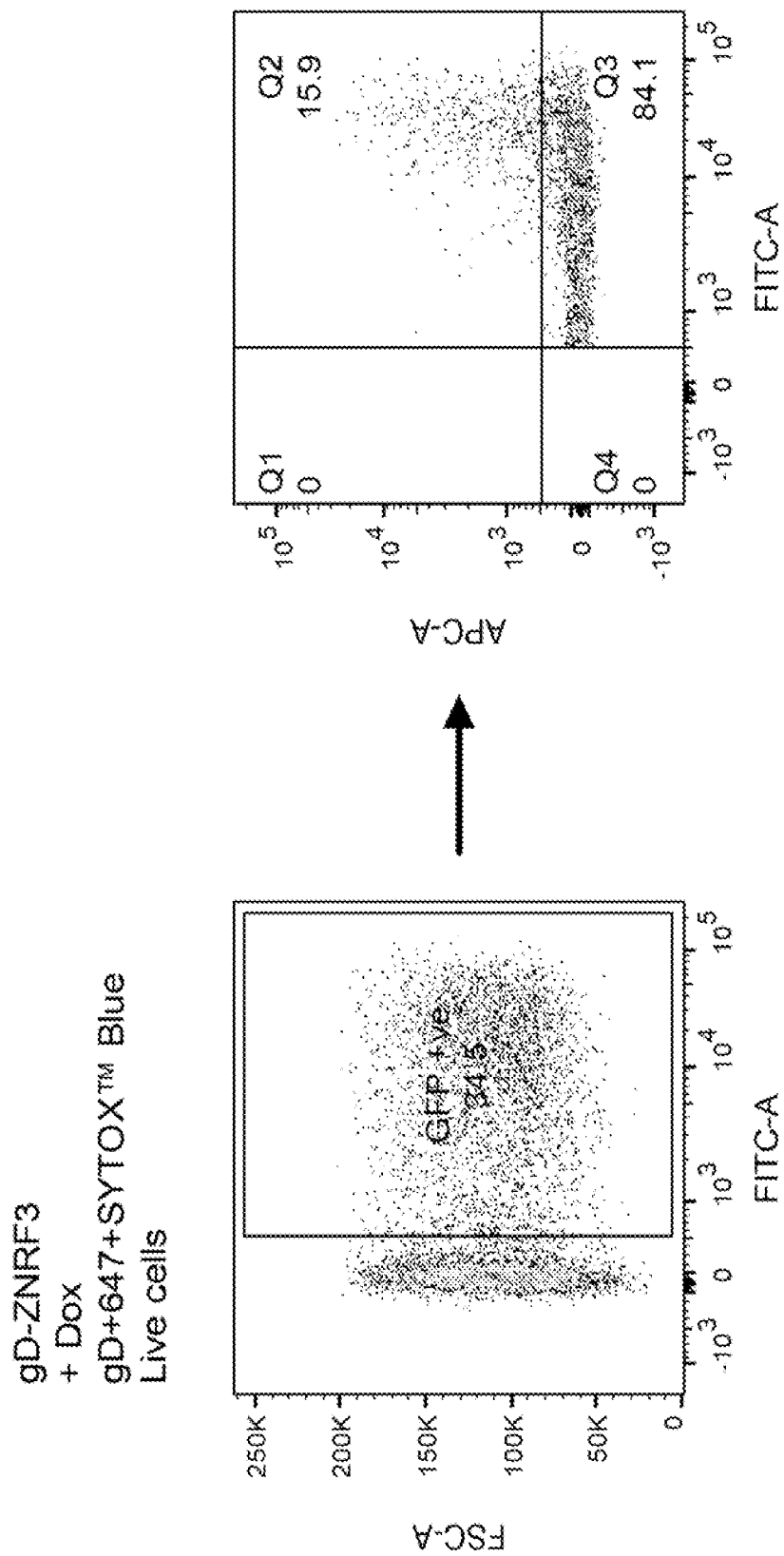


Fig. 10C

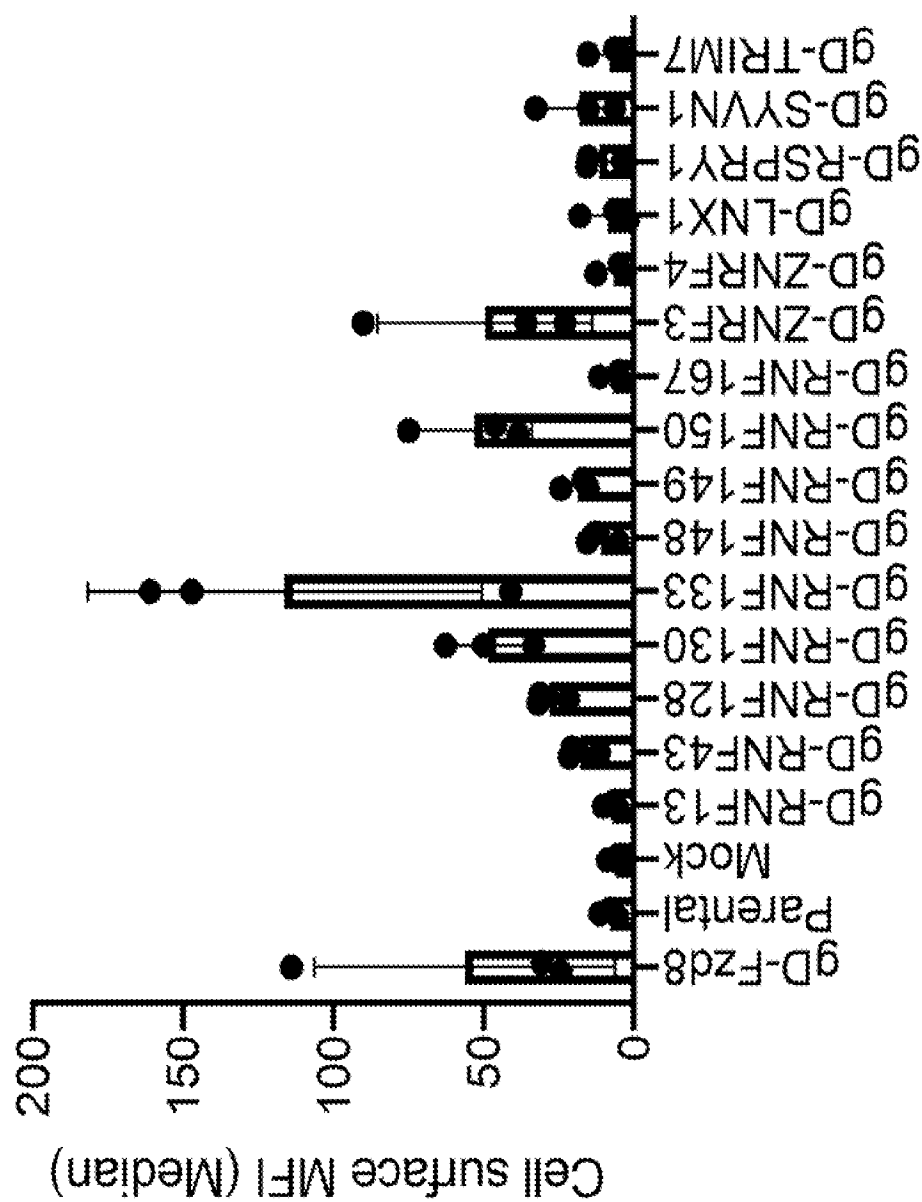


Fig. 10D

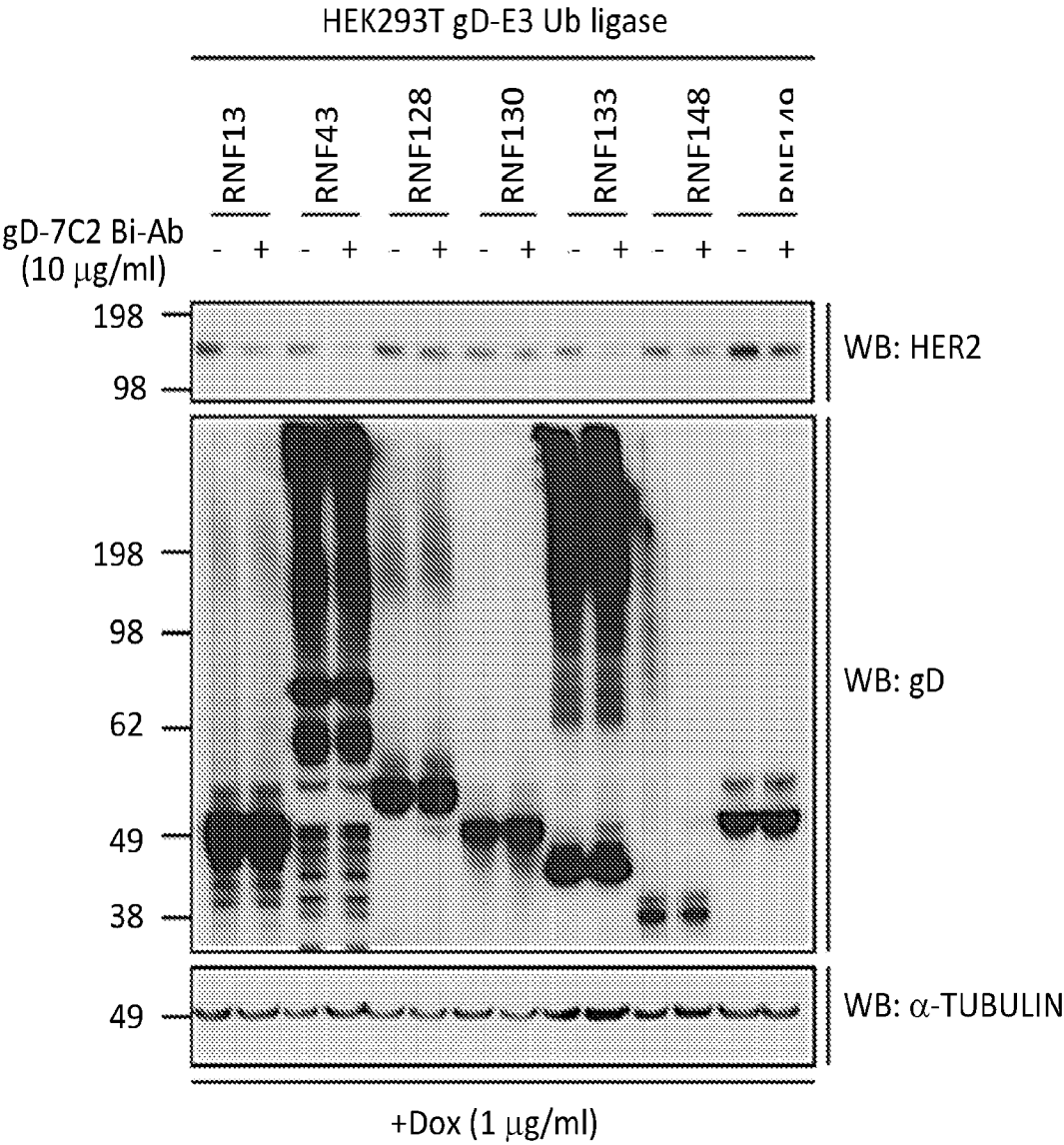


Fig. 10E

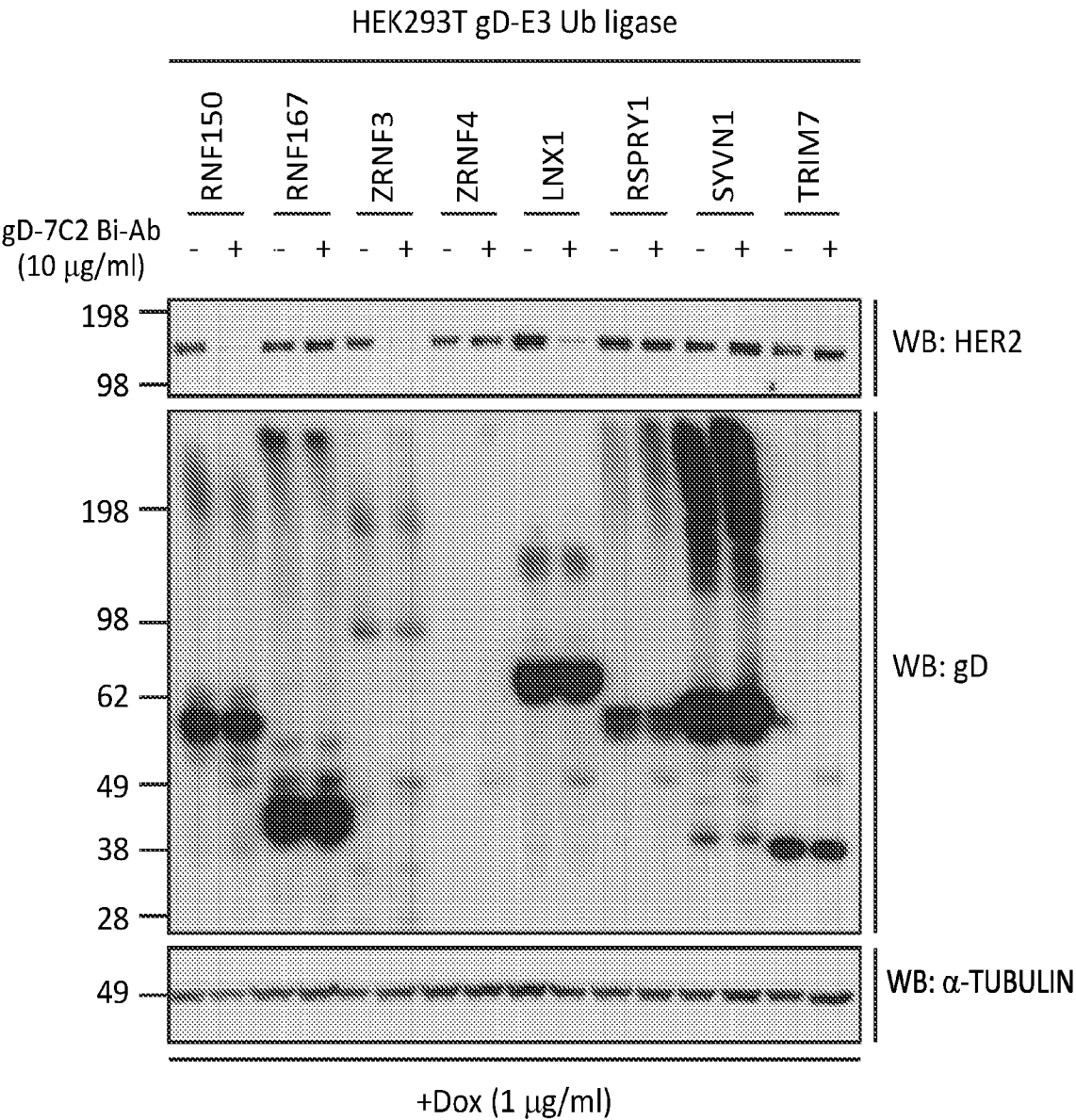


Fig. 10F

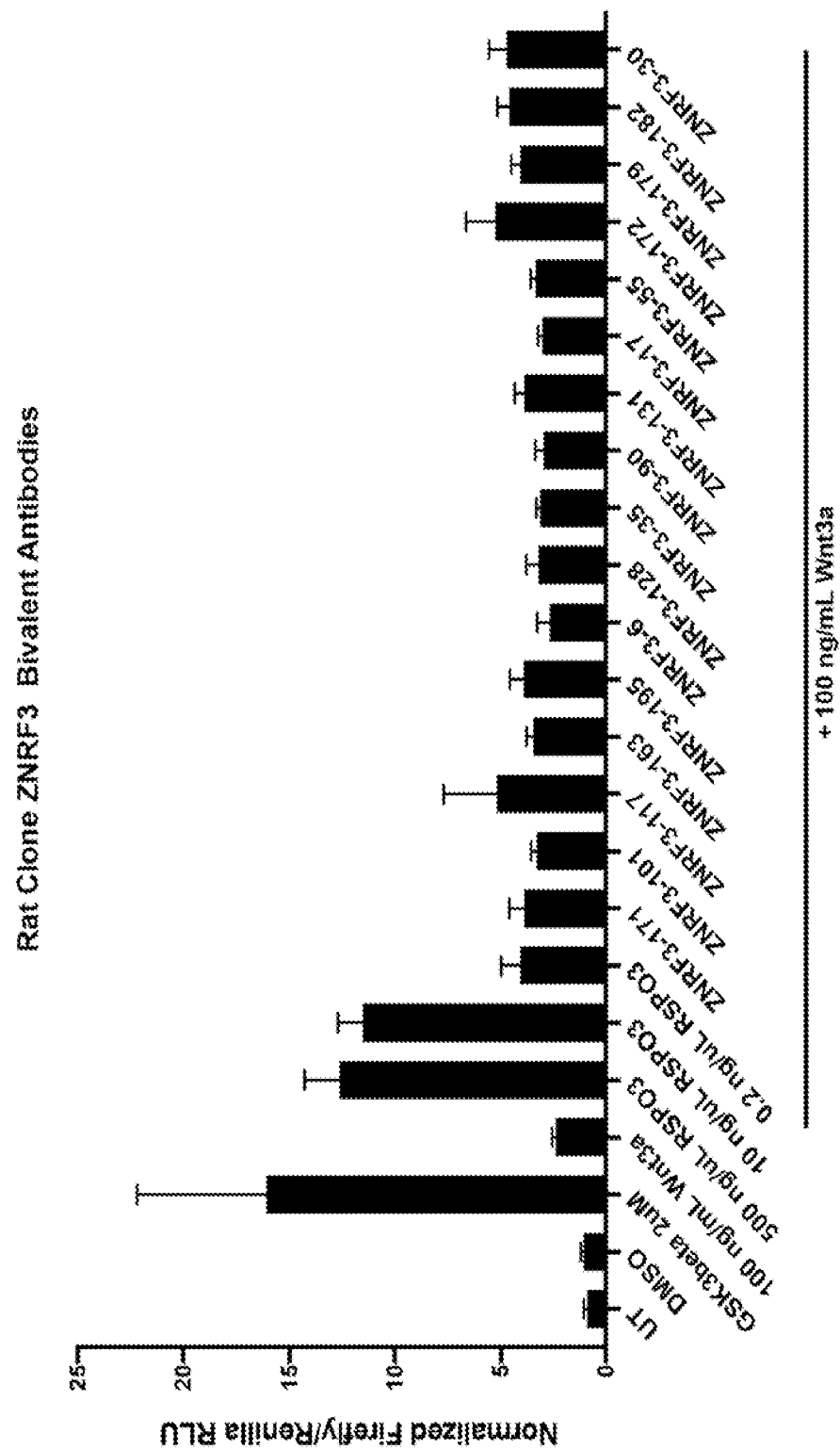
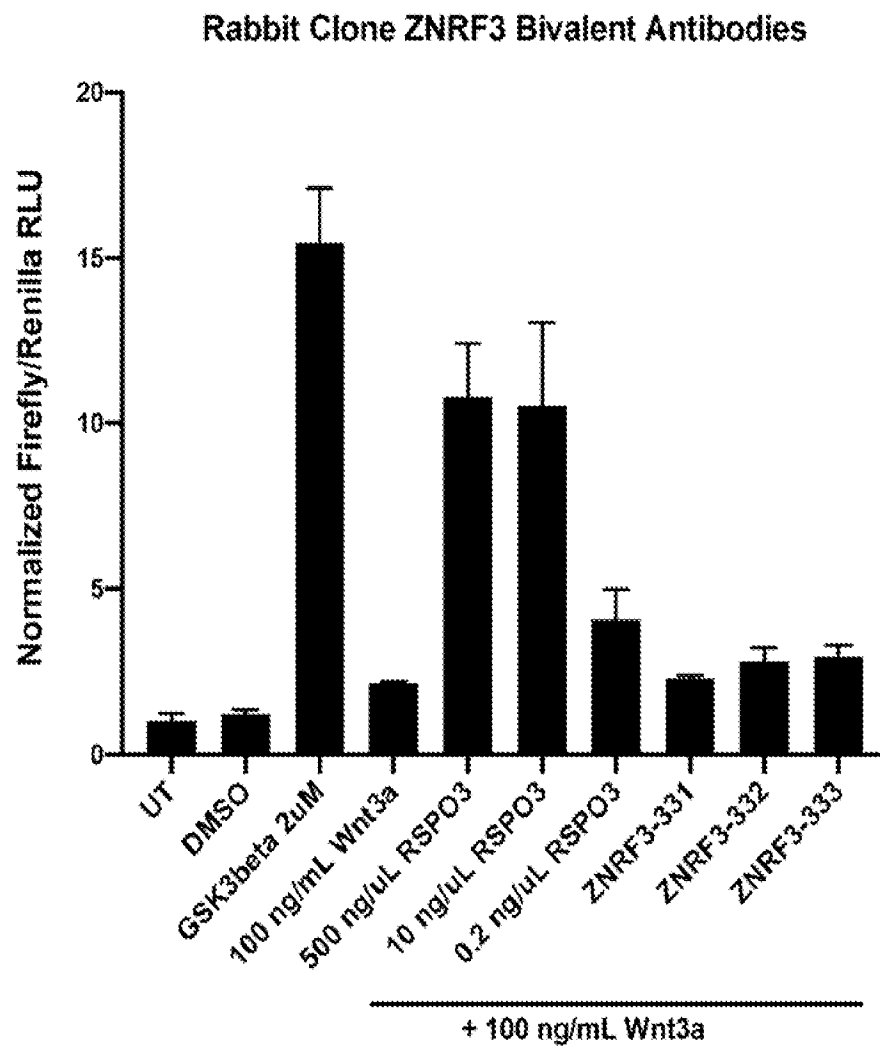
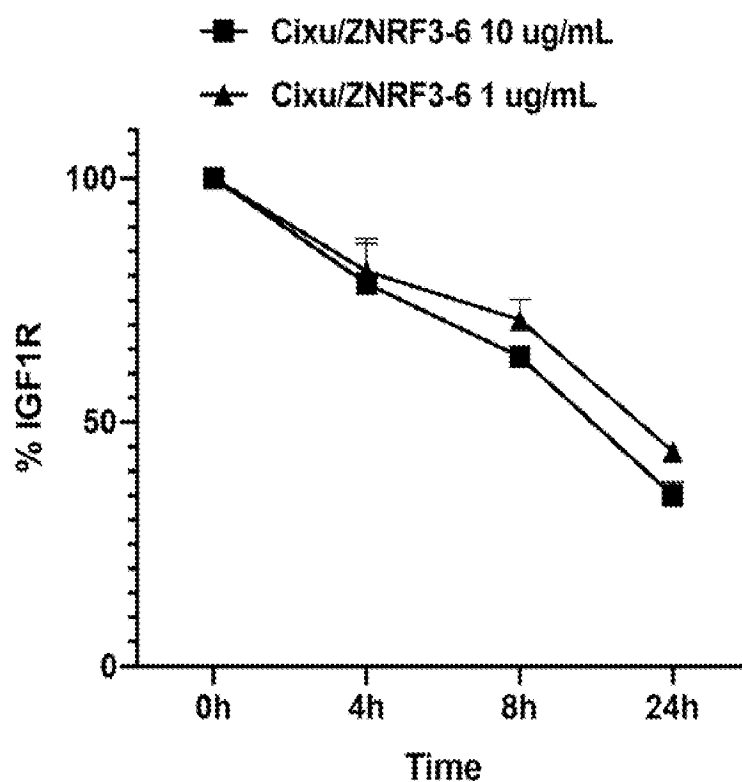
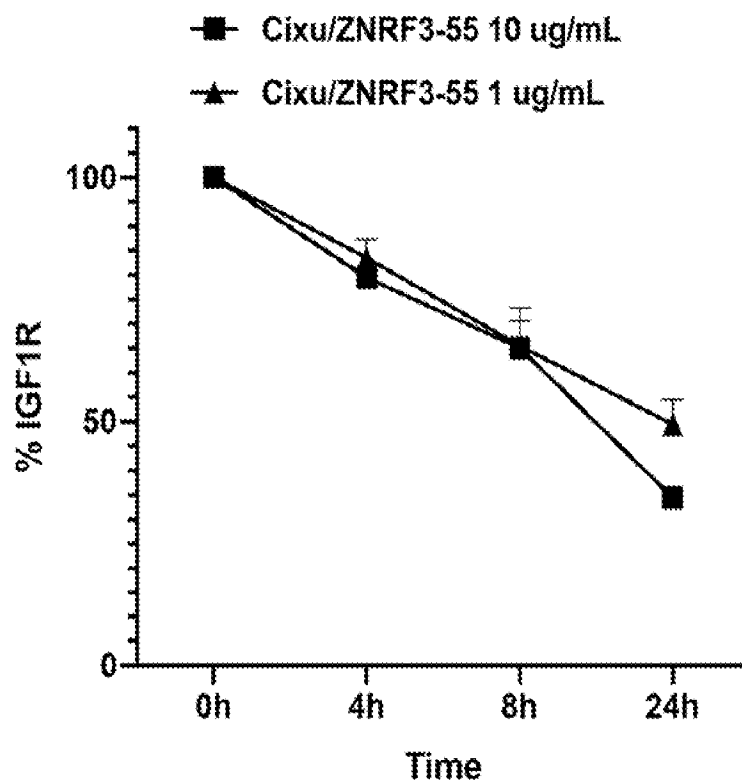
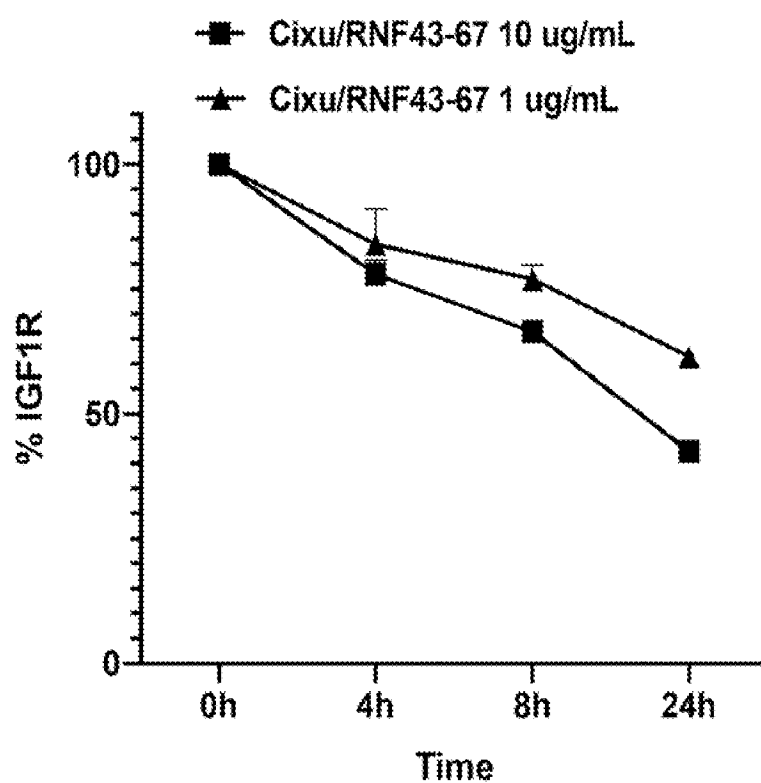
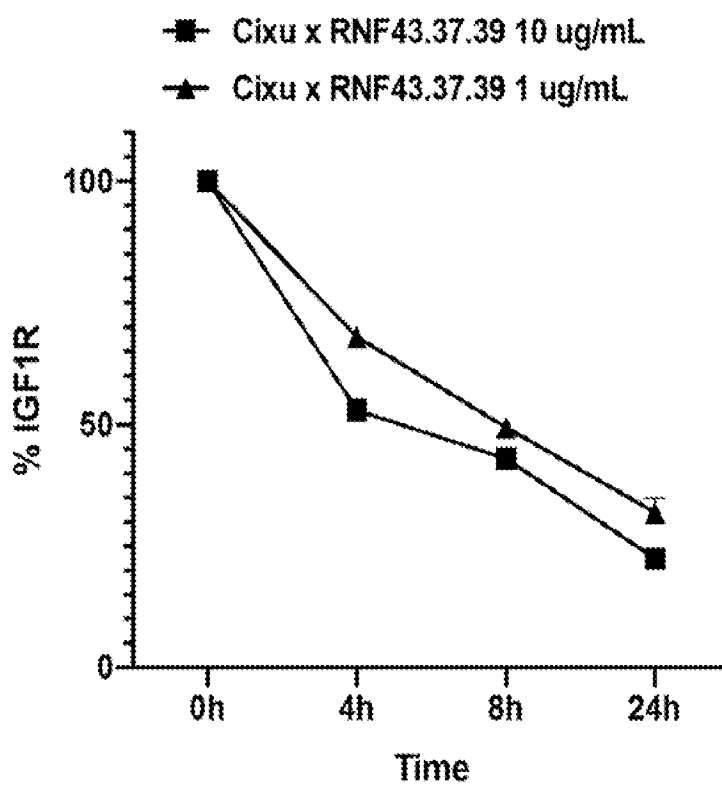
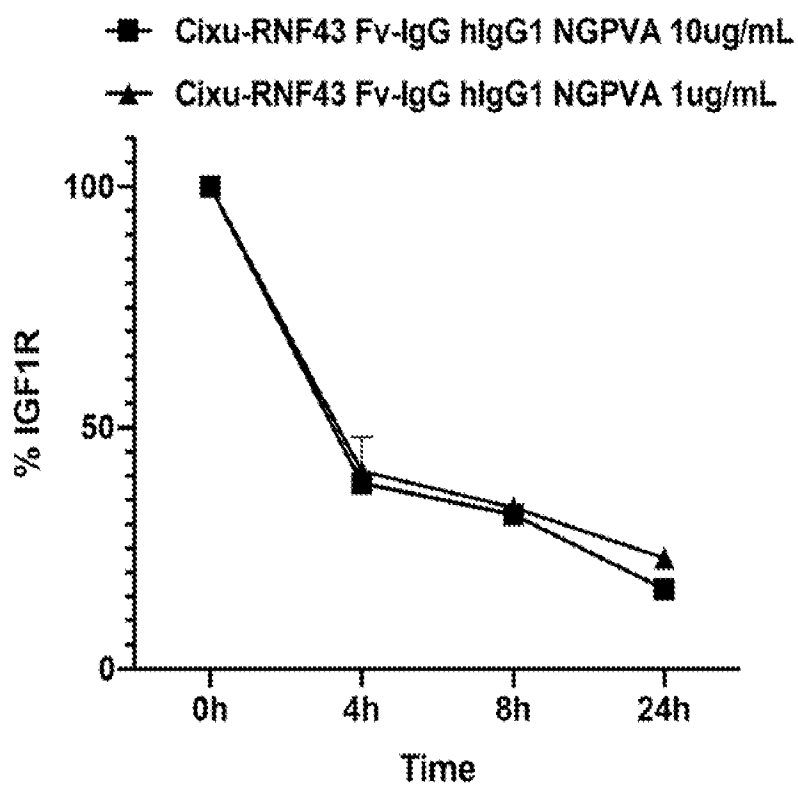
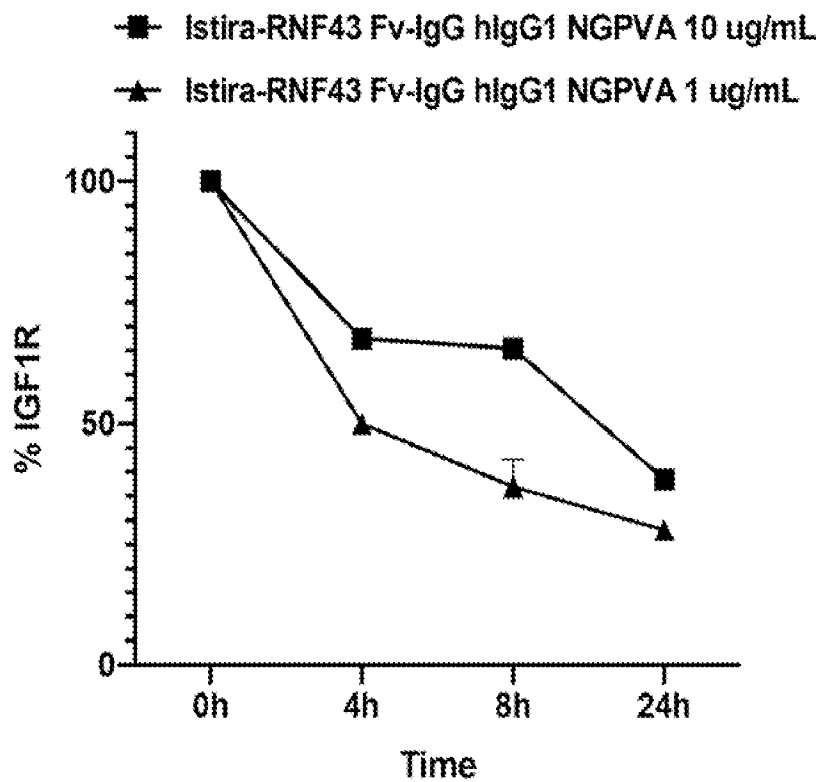


Fig. 11A

**Fig. 11B**

*Fig. 12A**Fig. 12B*

*Fig. 12C**Fig. 12D*

*Fig. 12E**Fig. 12F*

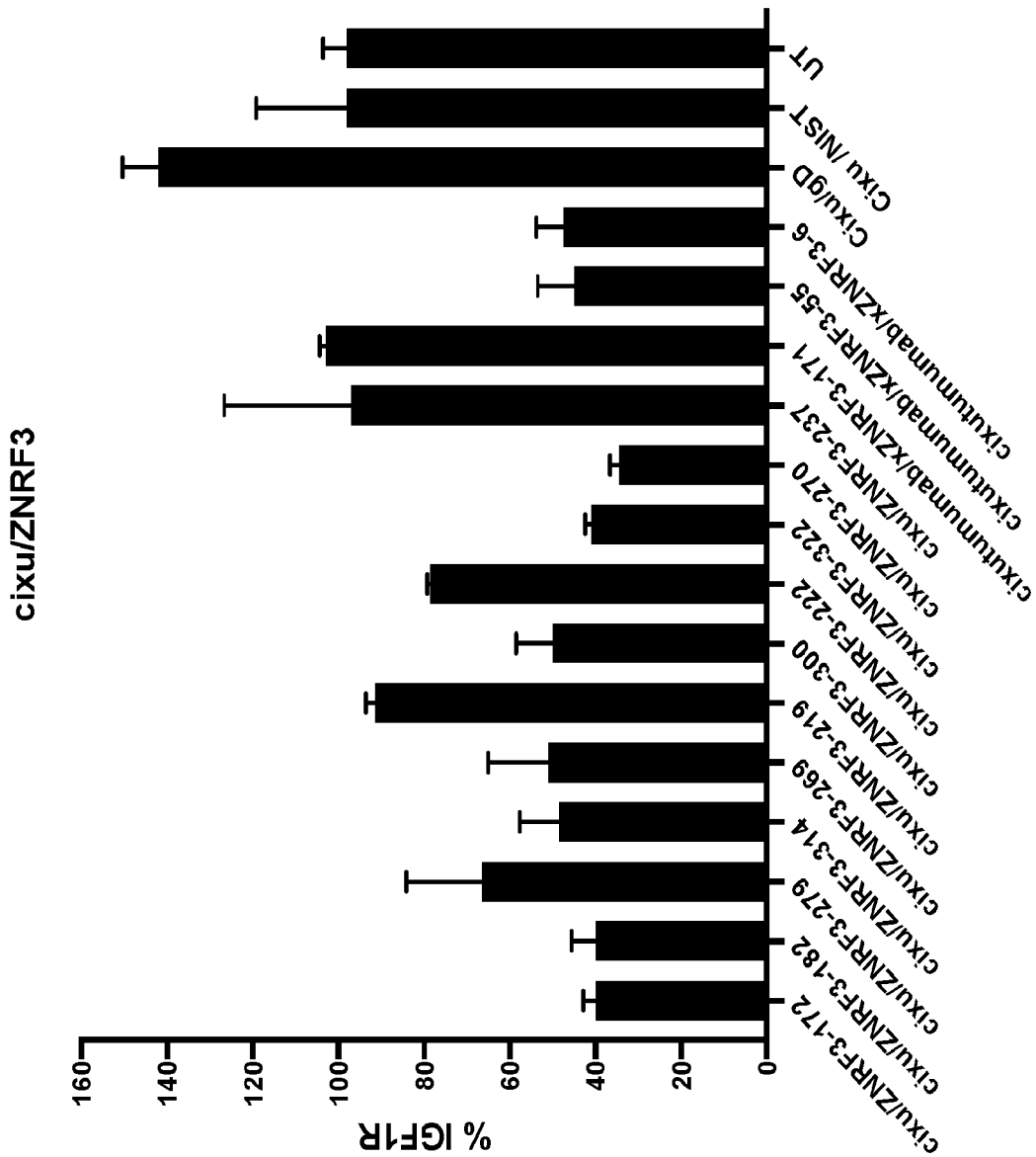


Fig. 13A

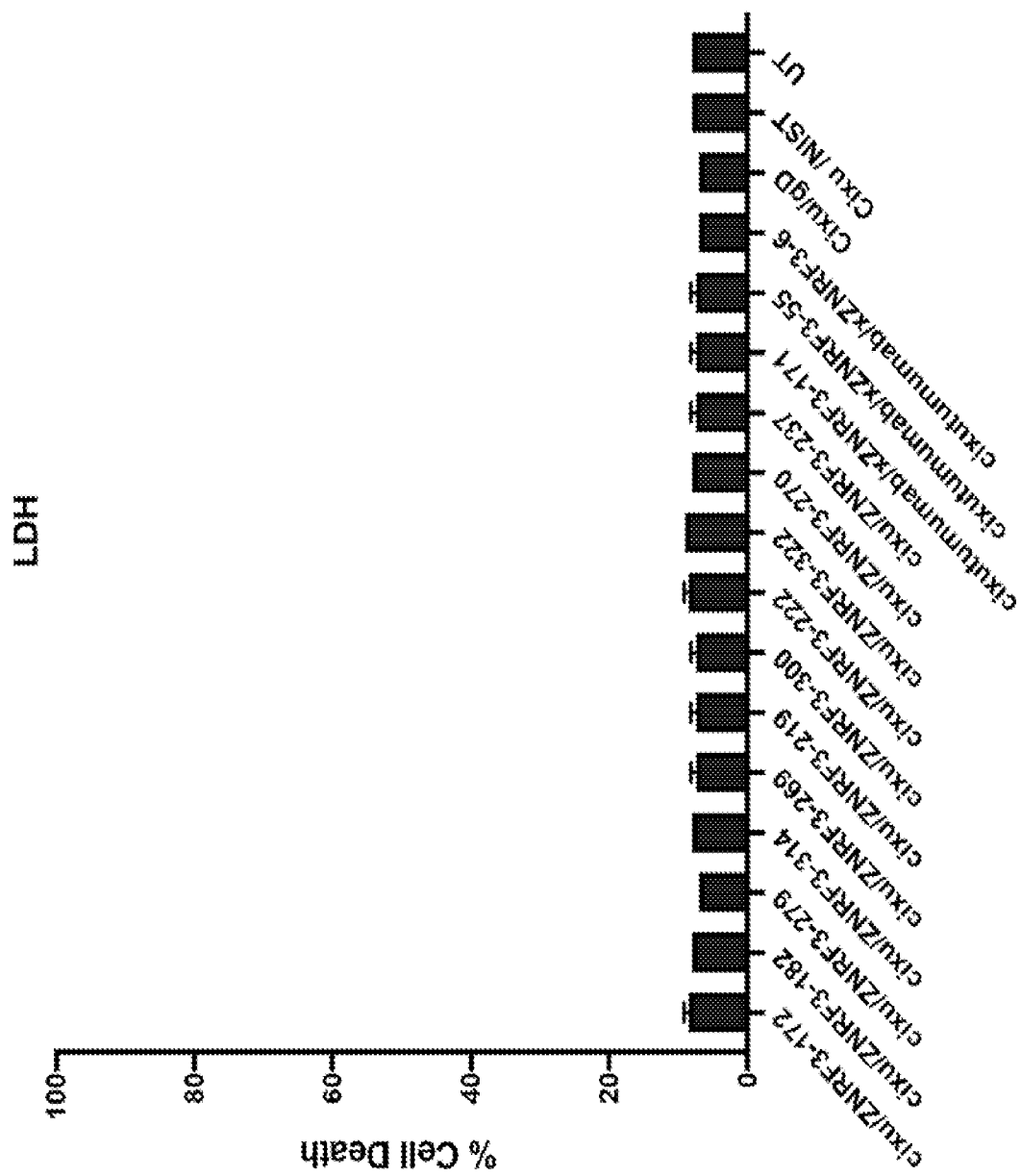
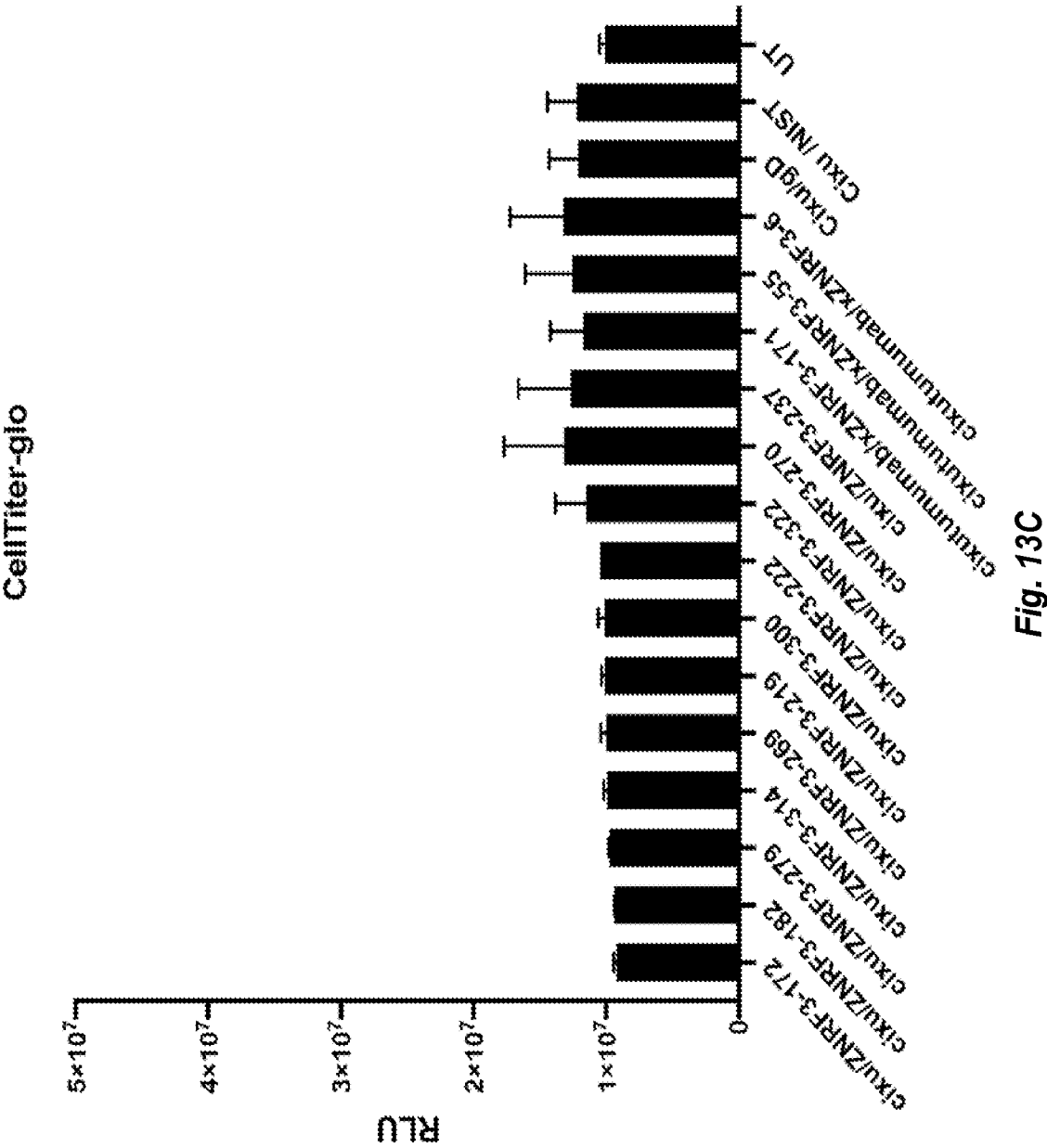


Fig. 13B



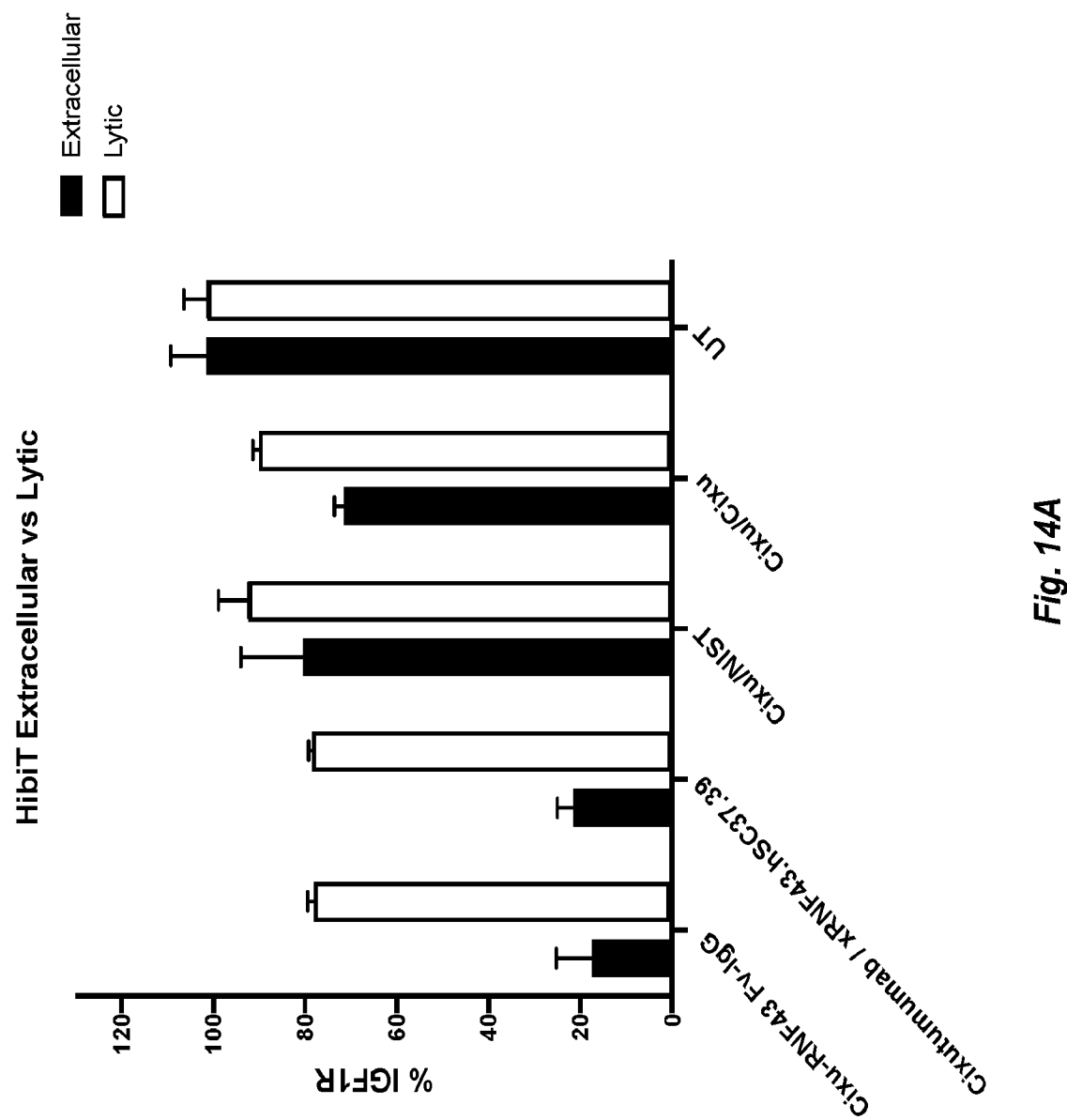


Fig. 14A

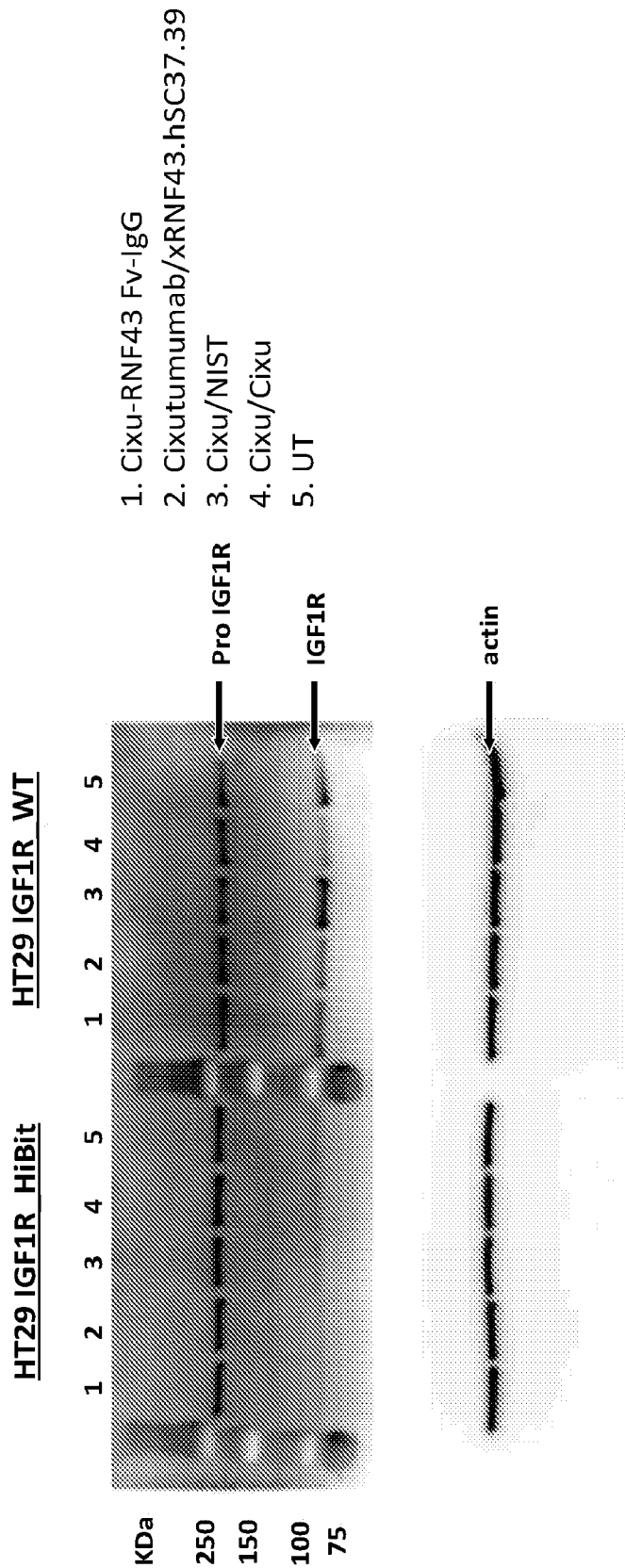
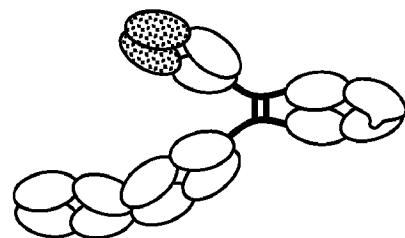
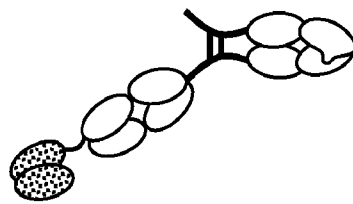


Fig. 14B



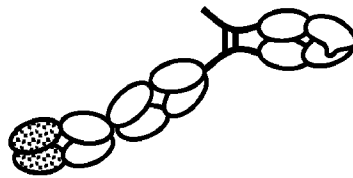
2+1 FabIgG

Fig. 15A



One armed FvIgG
(OA-FvIgG)

Fig. 15B



One armed FabIgG
(OA-FabIgG)

Fig. 15C

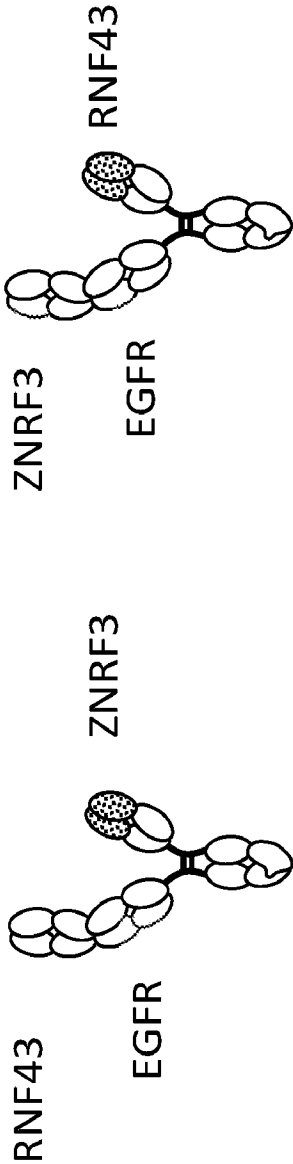


Fig. 15D

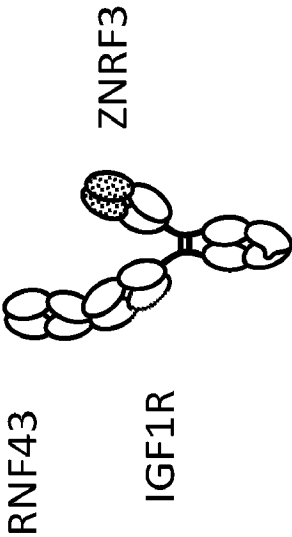


Fig. 15F

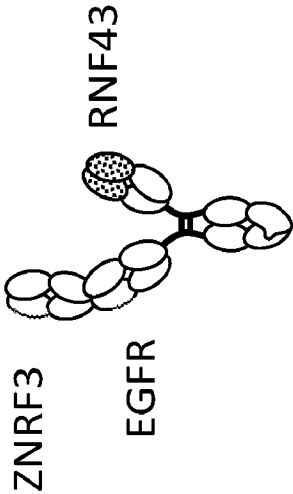


Fig. 15E

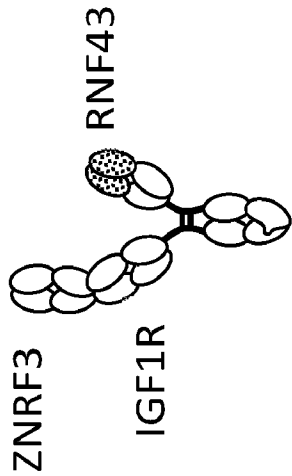
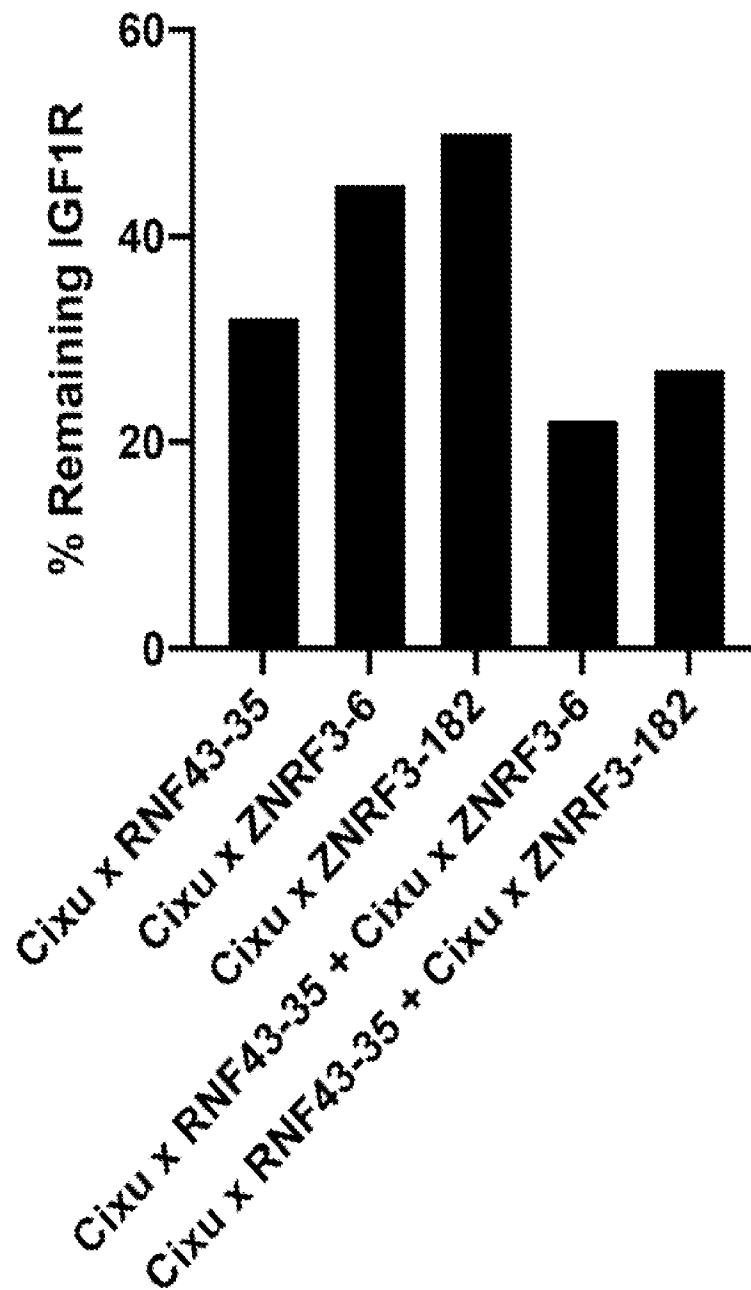


Fig. 15G

*Fig. 16*

murine APC mutant CRC

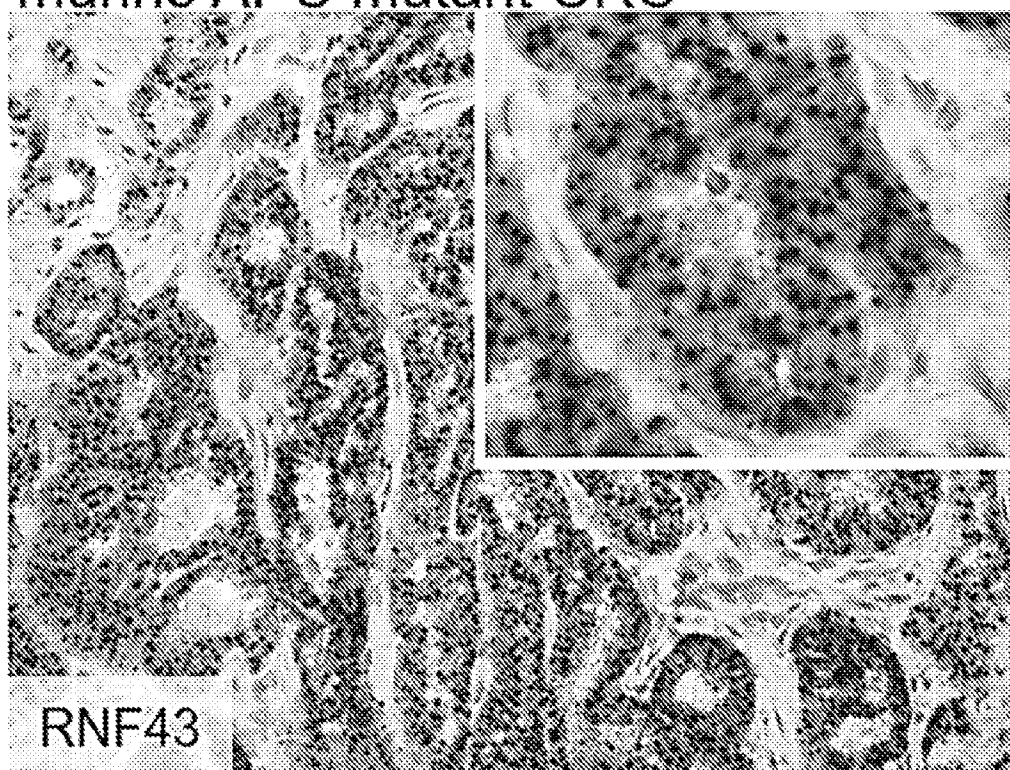


Fig. 17

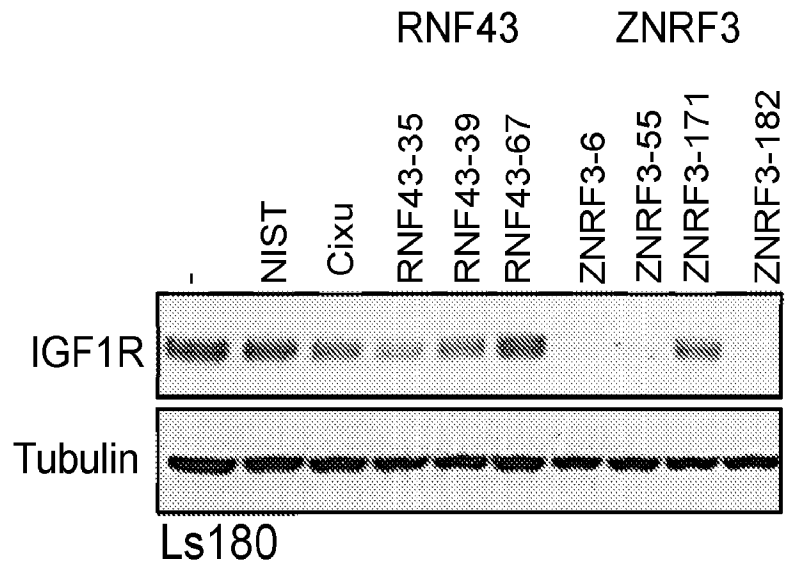


Fig. 18A

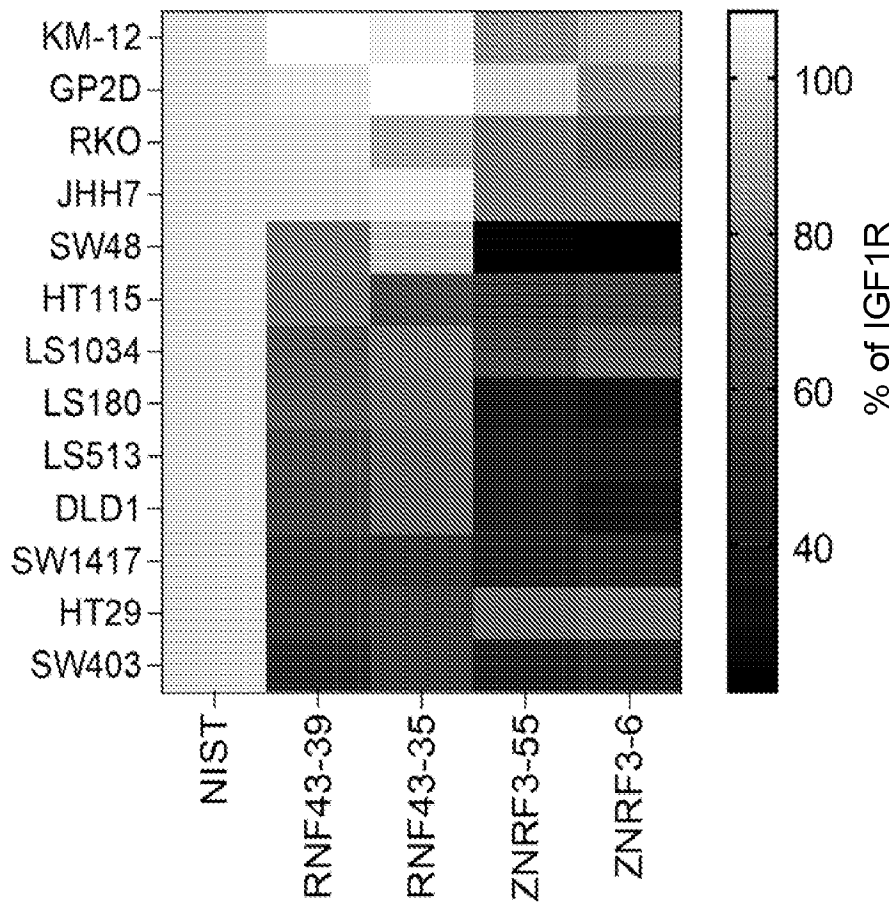


Fig. 18B

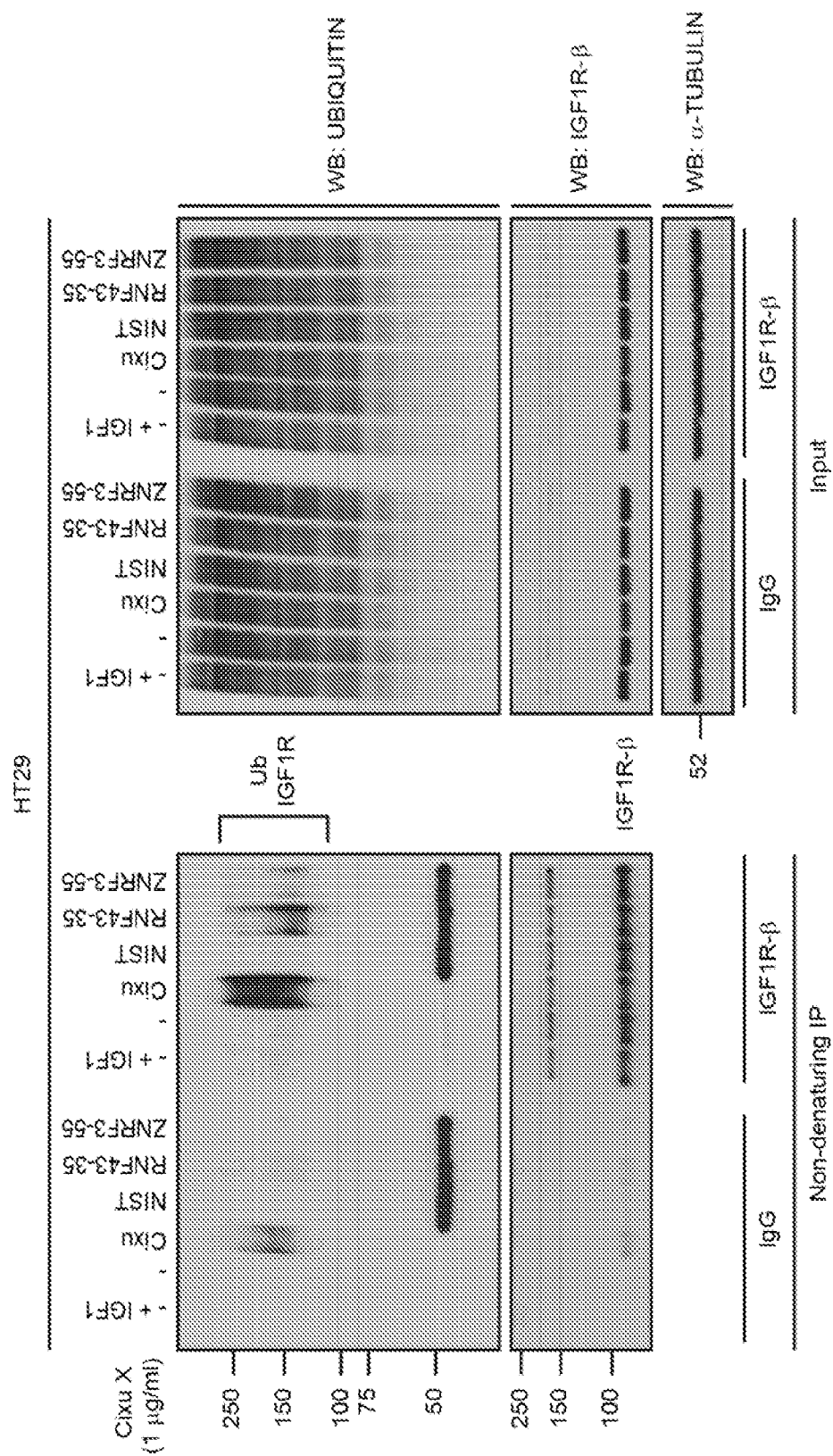


Fig. 19A

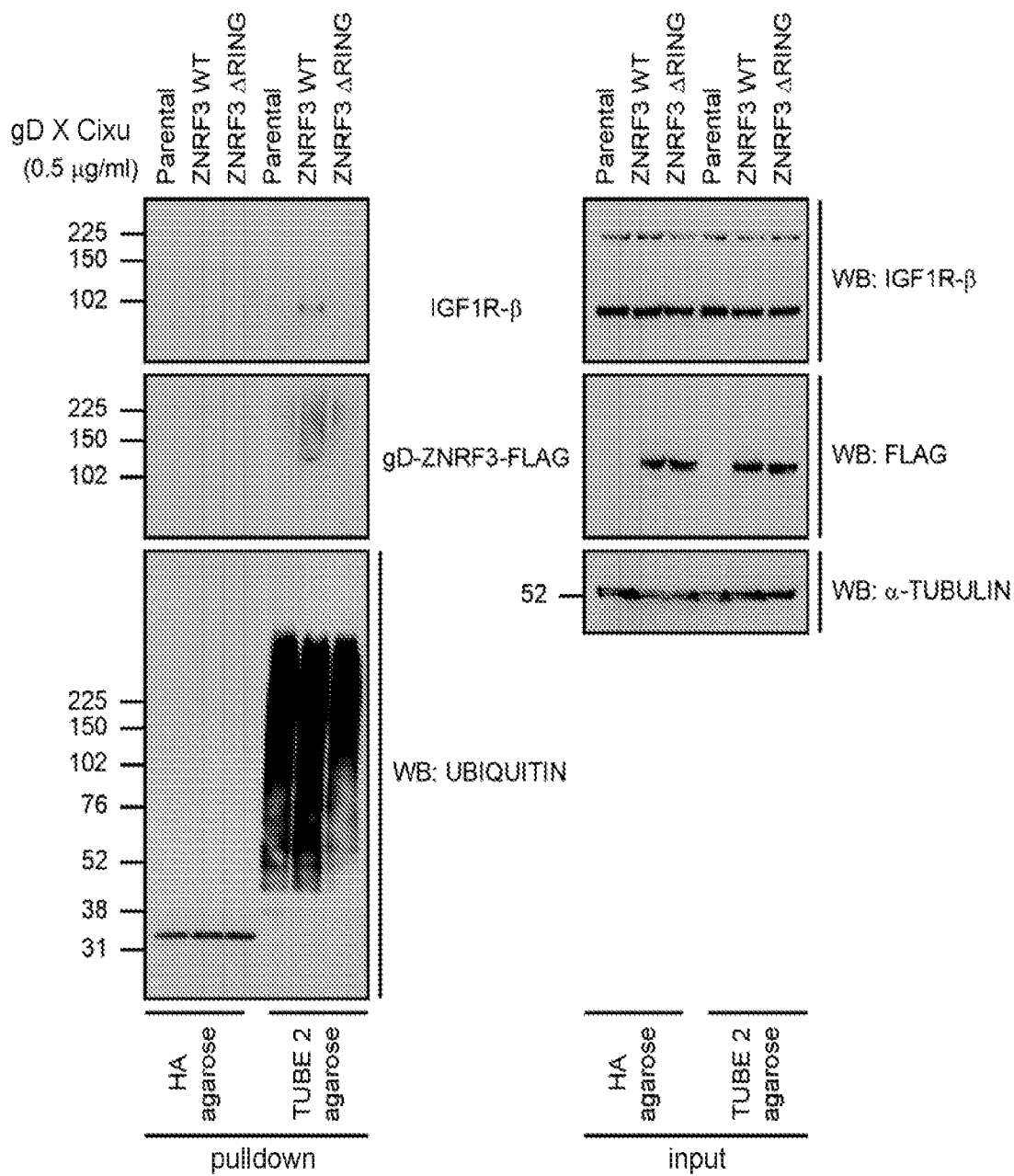


Fig. 19B

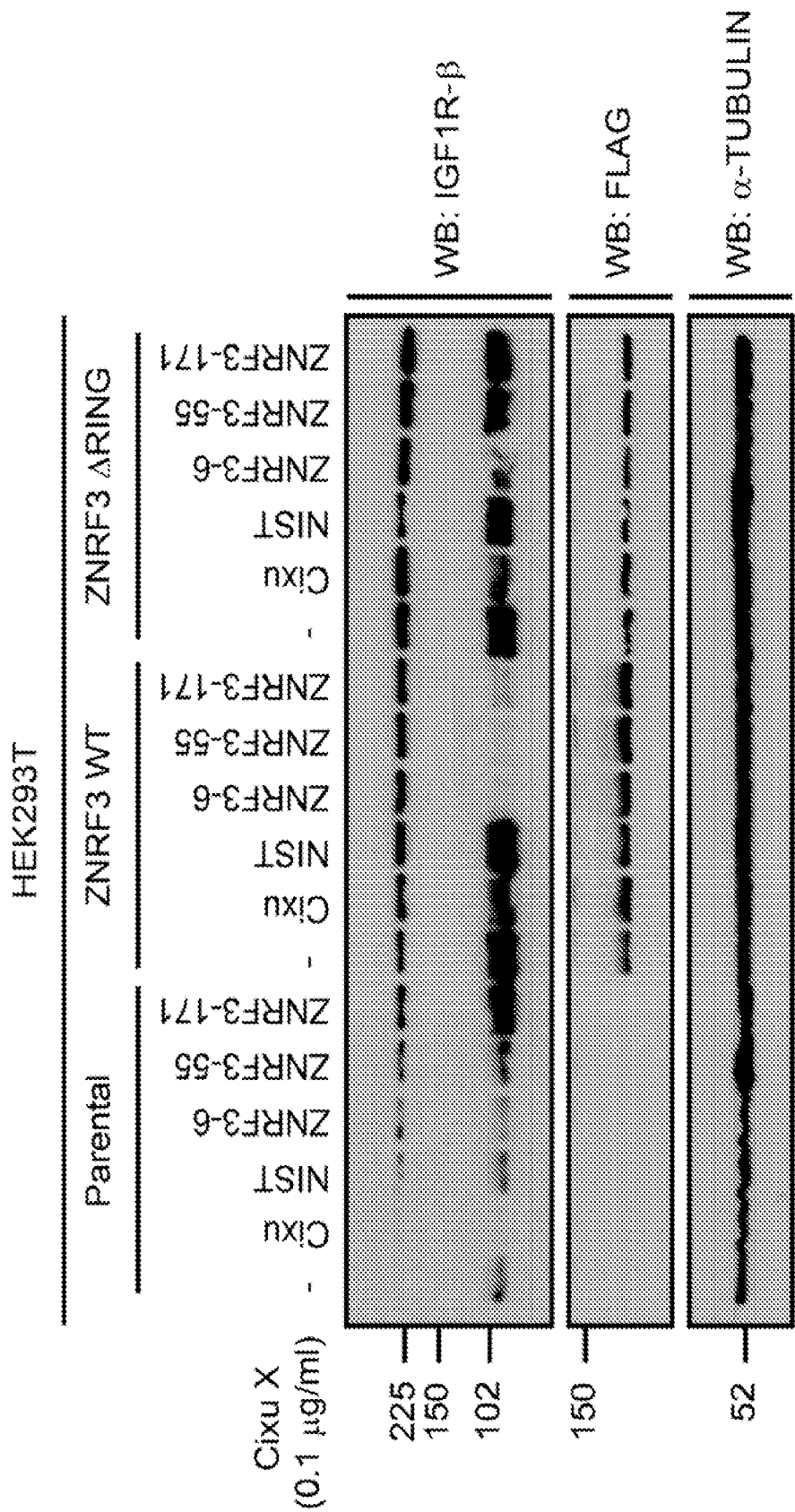


Fig. 20

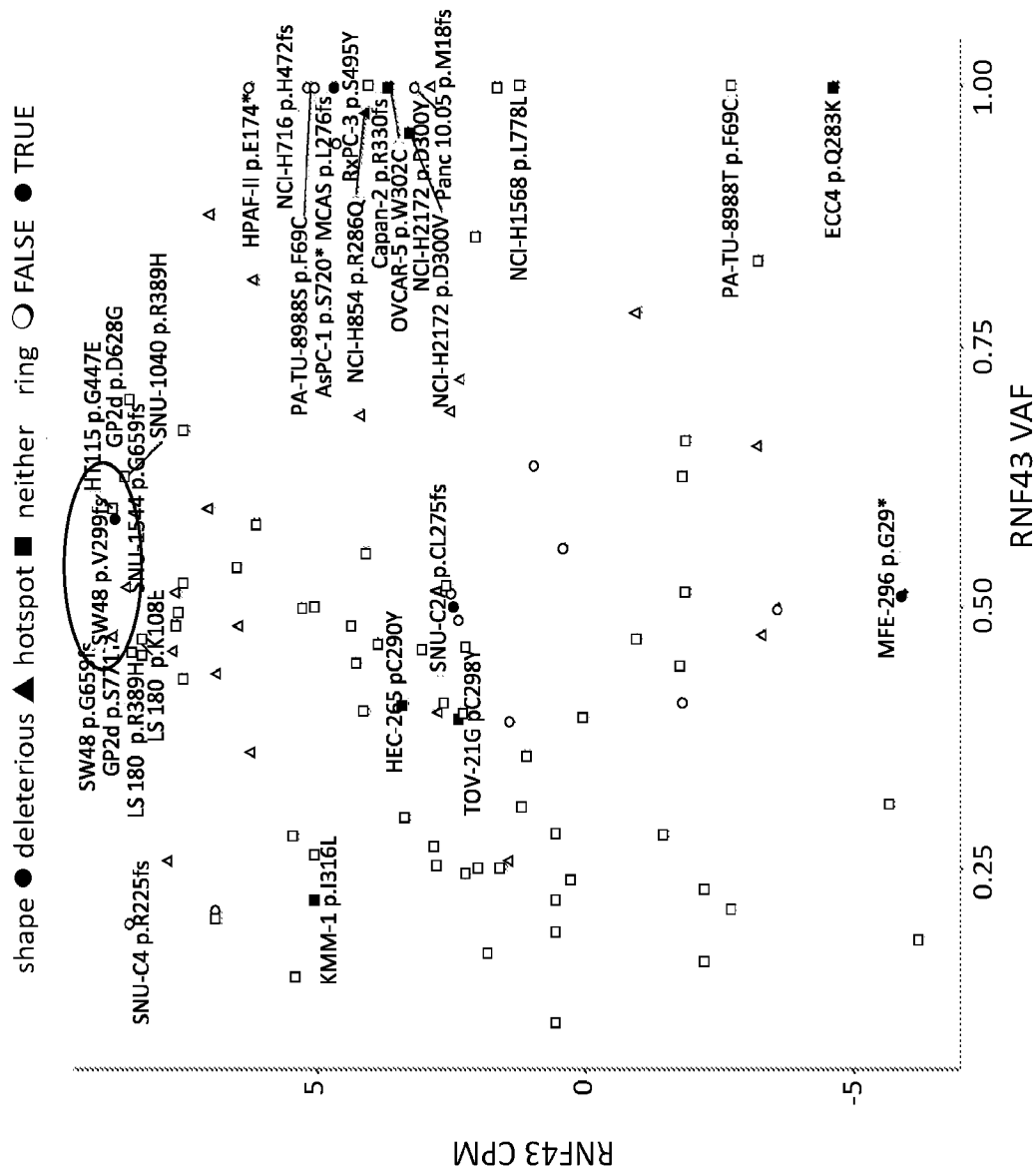


Fig. 21A

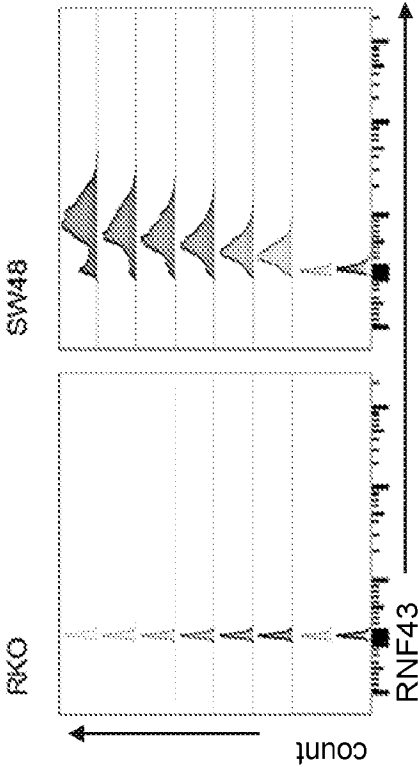


Fig. 21B

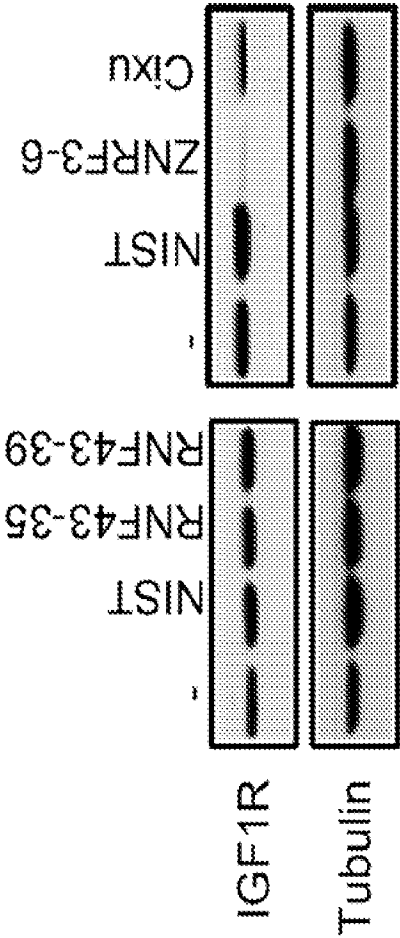


Fig. 21C

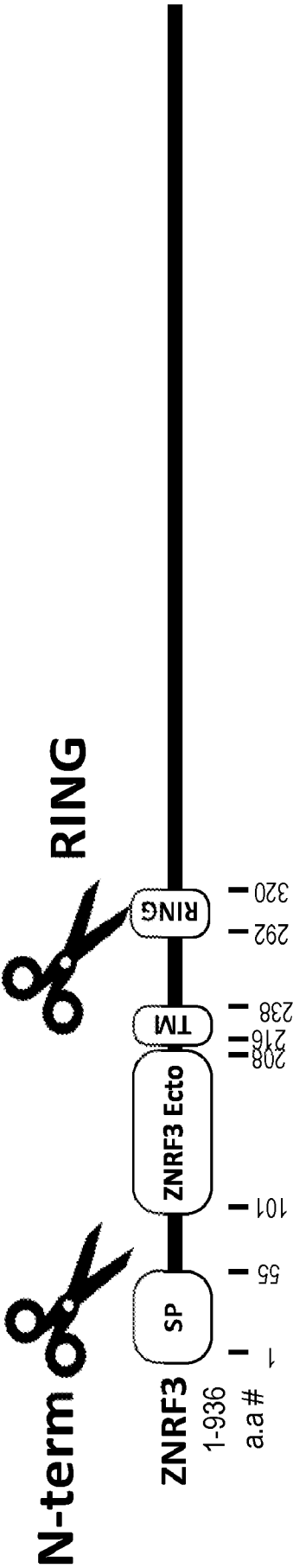


Fig. 22A

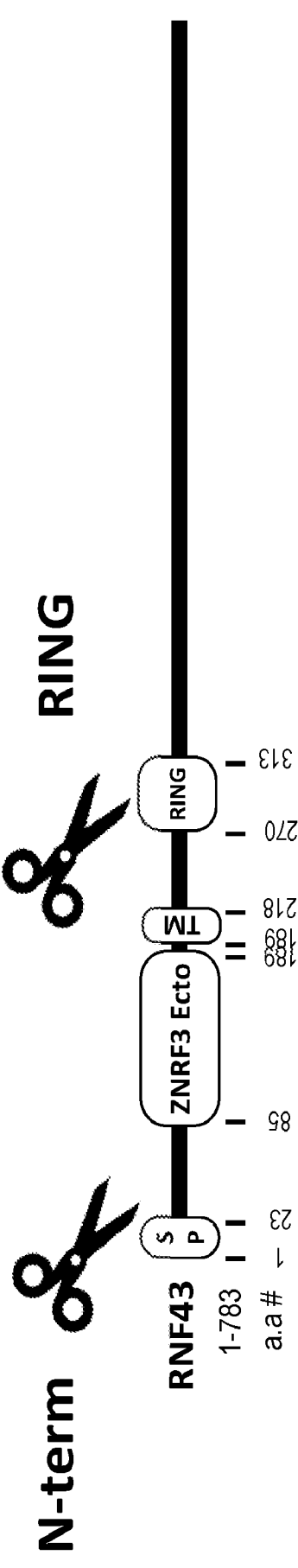


Fig. 22B

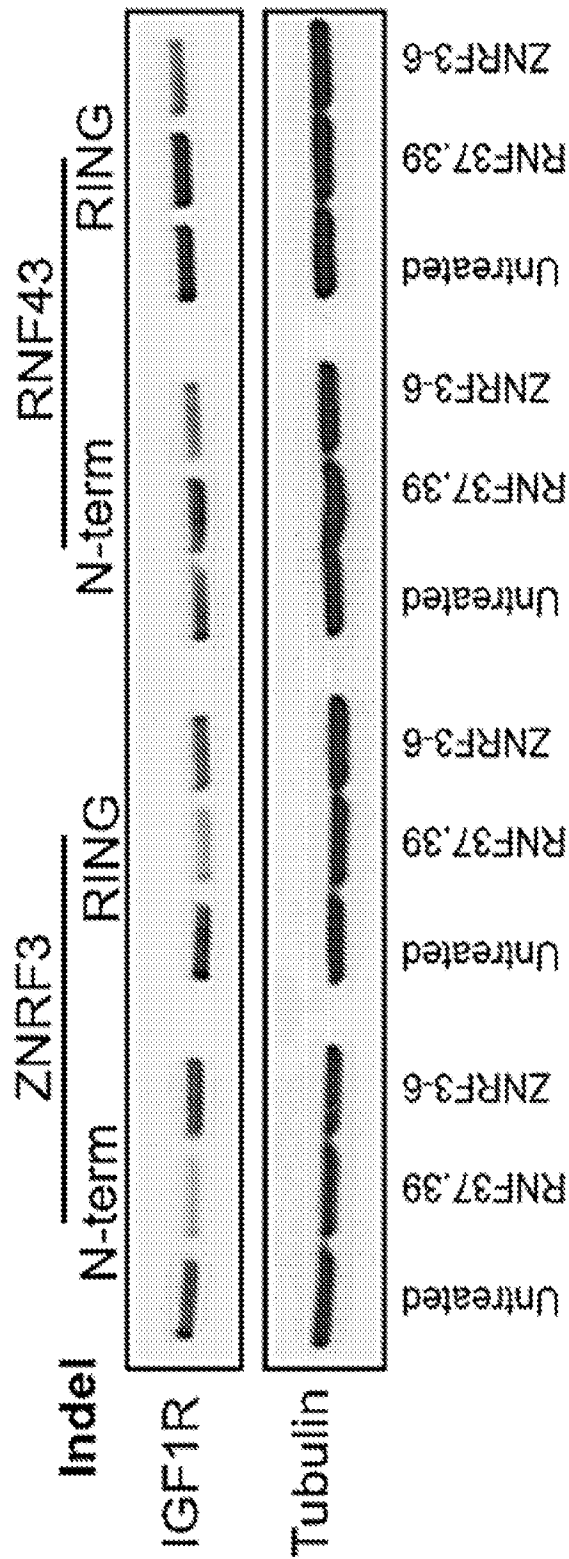


Fig. 22C

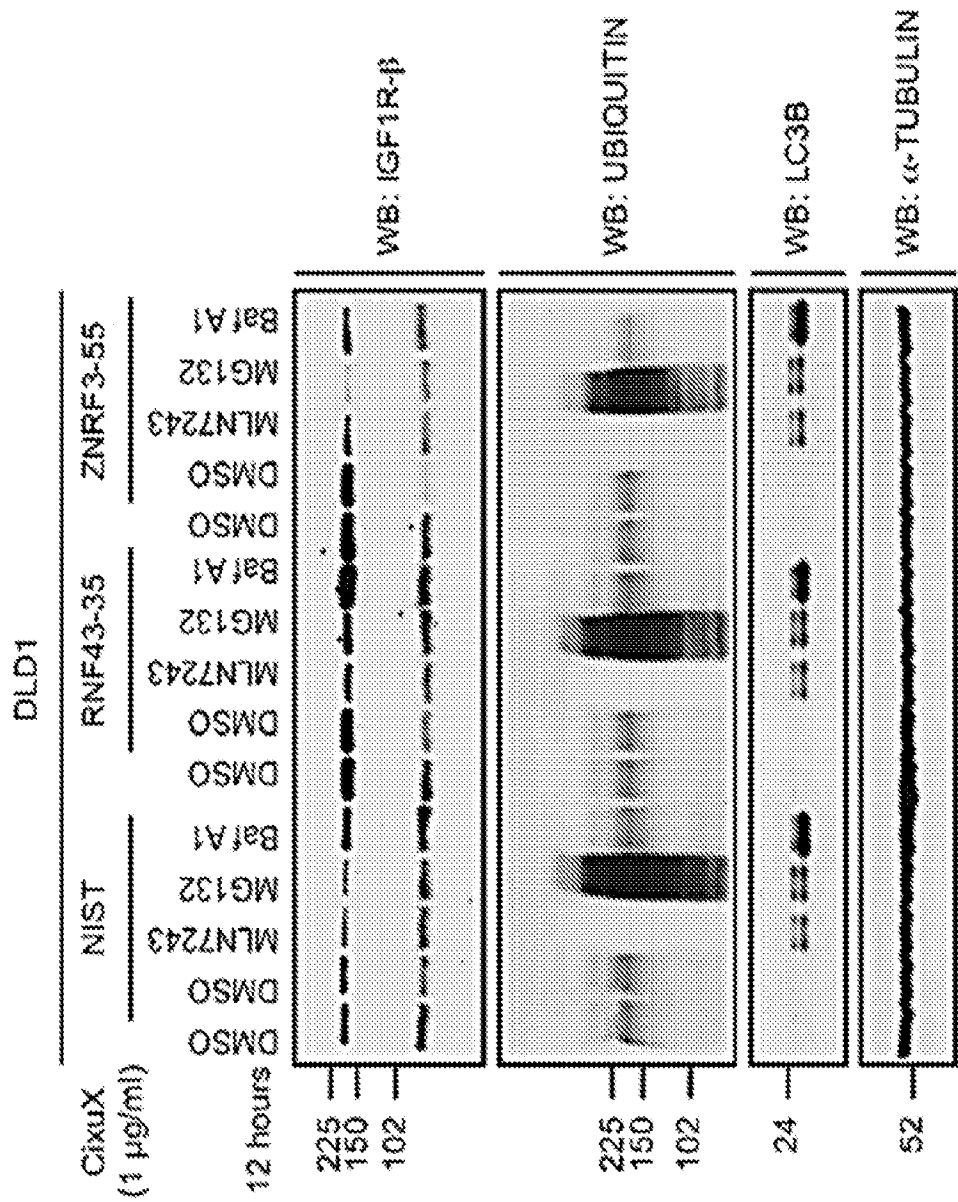


Fig. 23

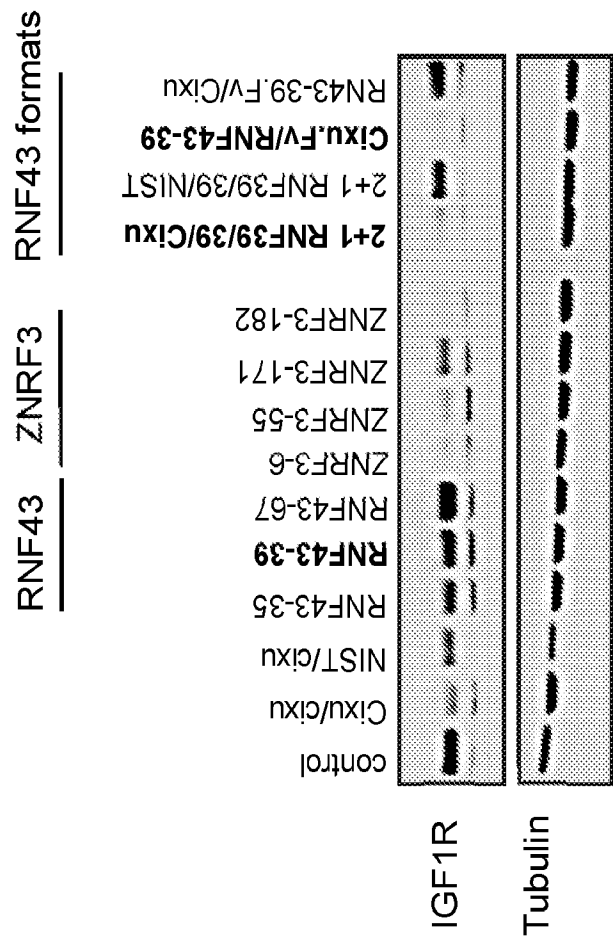


Fig. 24

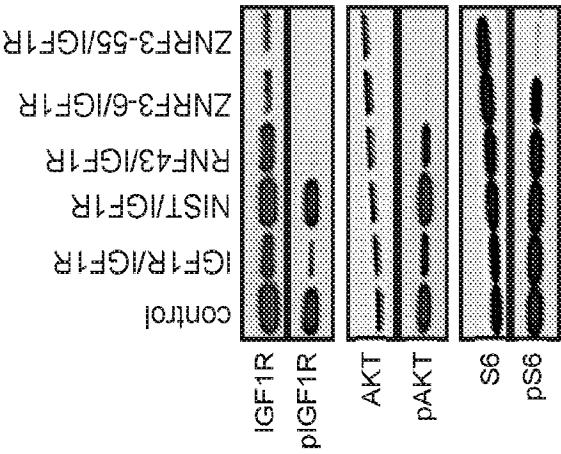


Fig. 25A

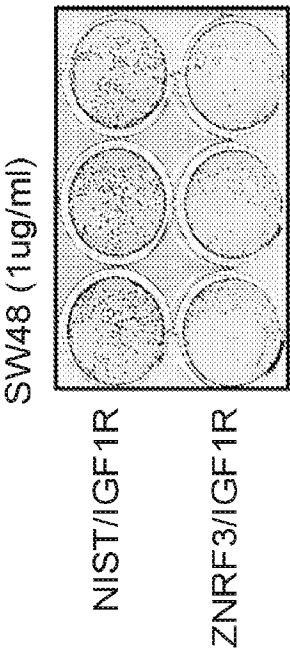


Fig. 25B

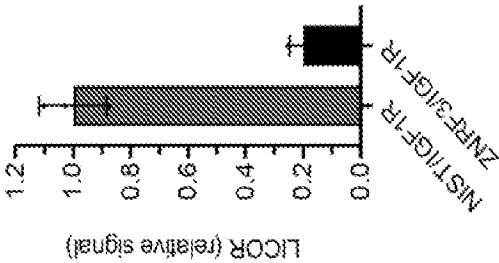


Fig. 25C

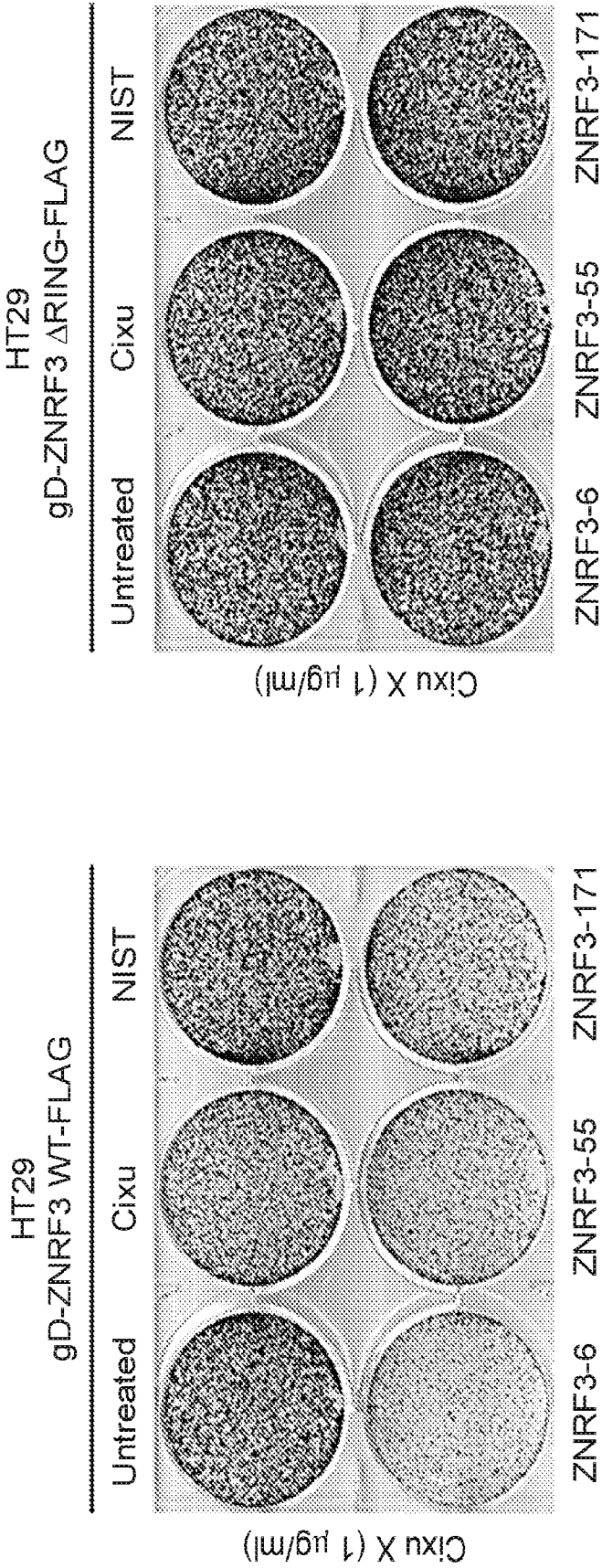


Fig. 26C

Fig. 26A

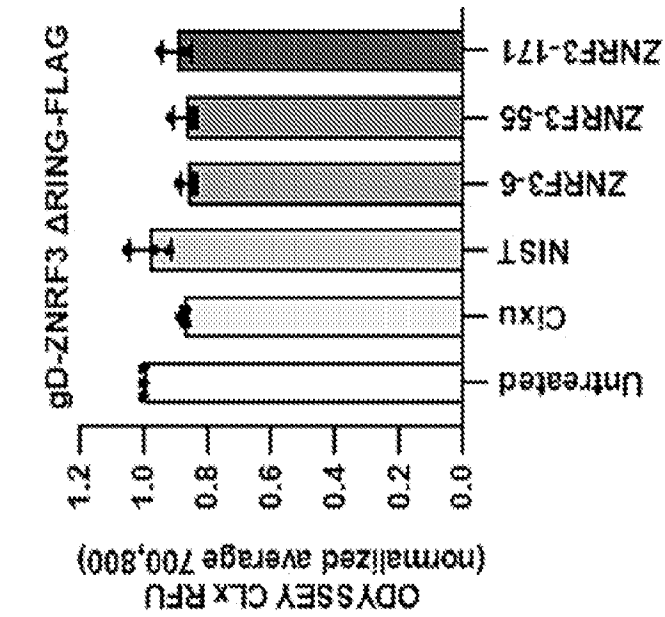


Fig. 26D

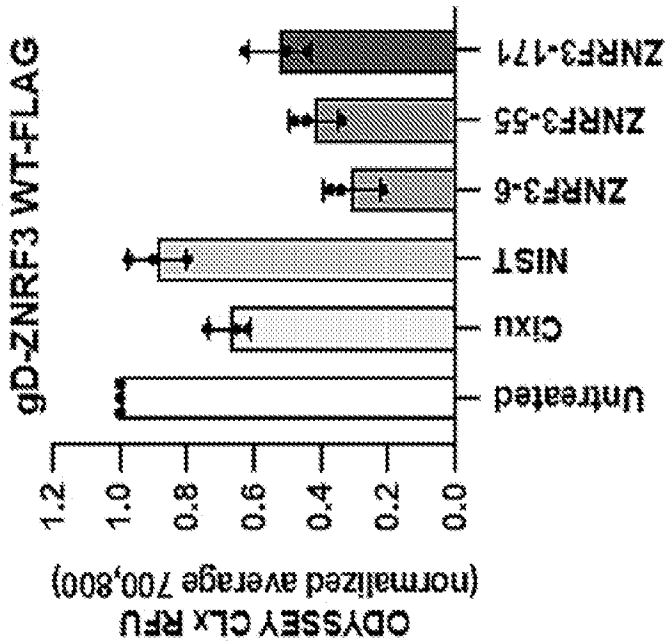


Fig. 26B

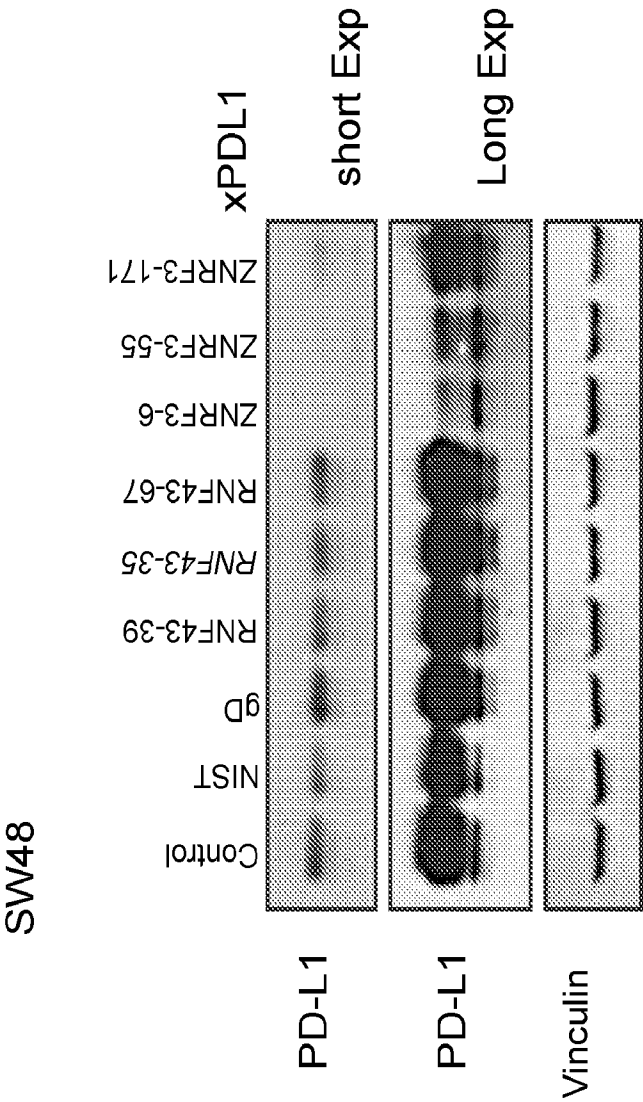


Fig. 27

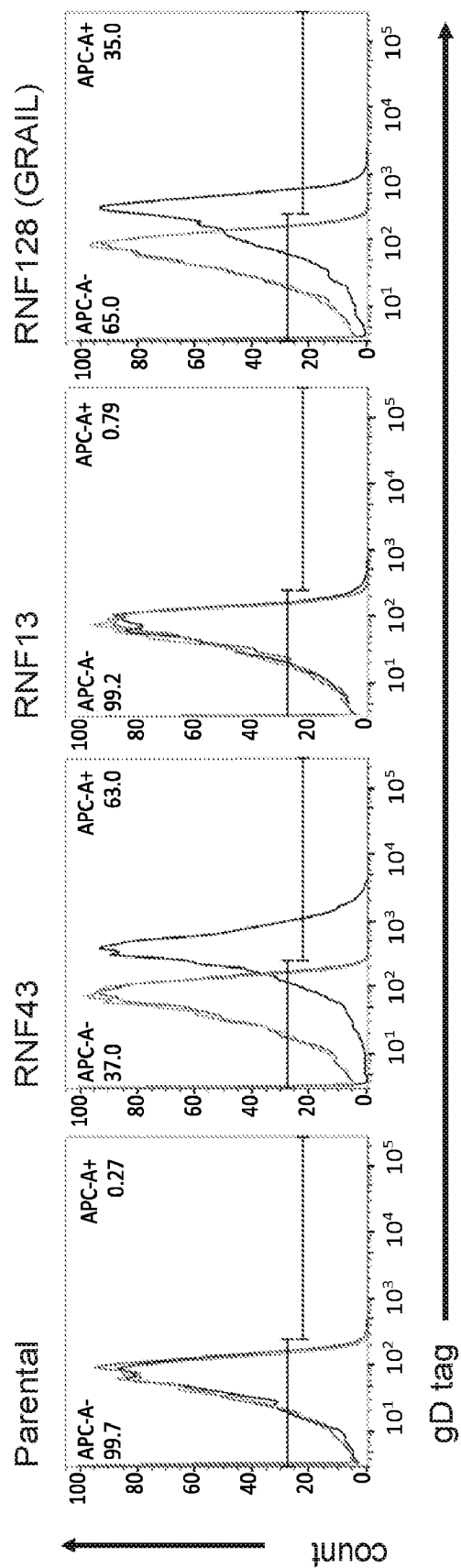


Fig. 28A

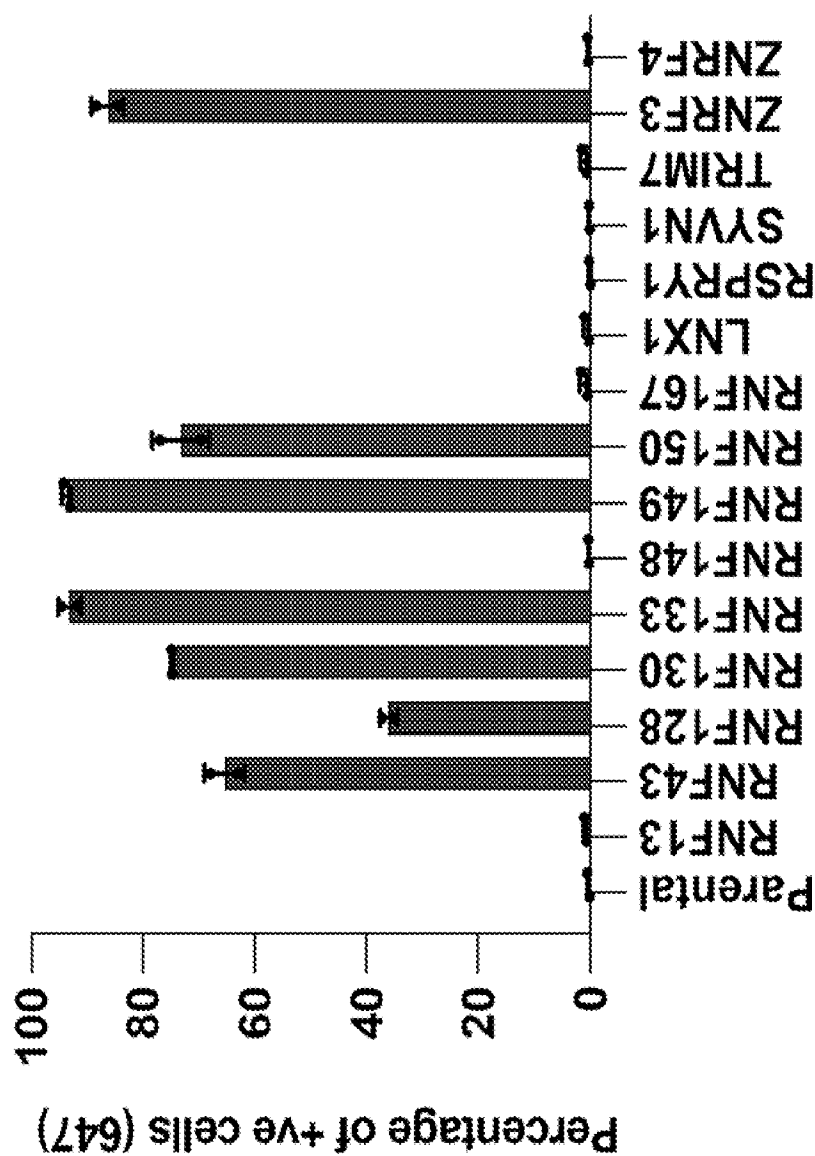


Fig. 28B

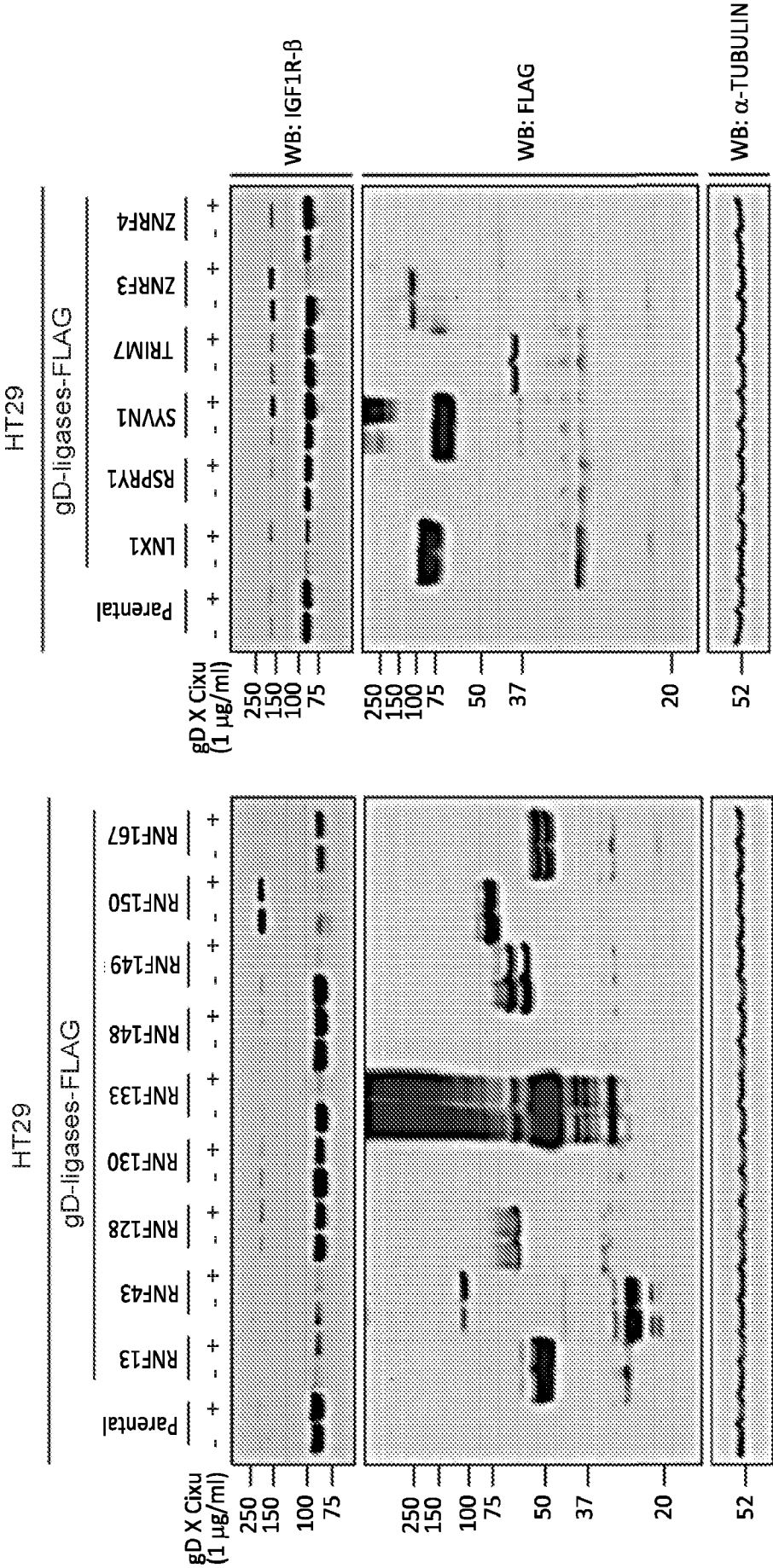


Fig. 29

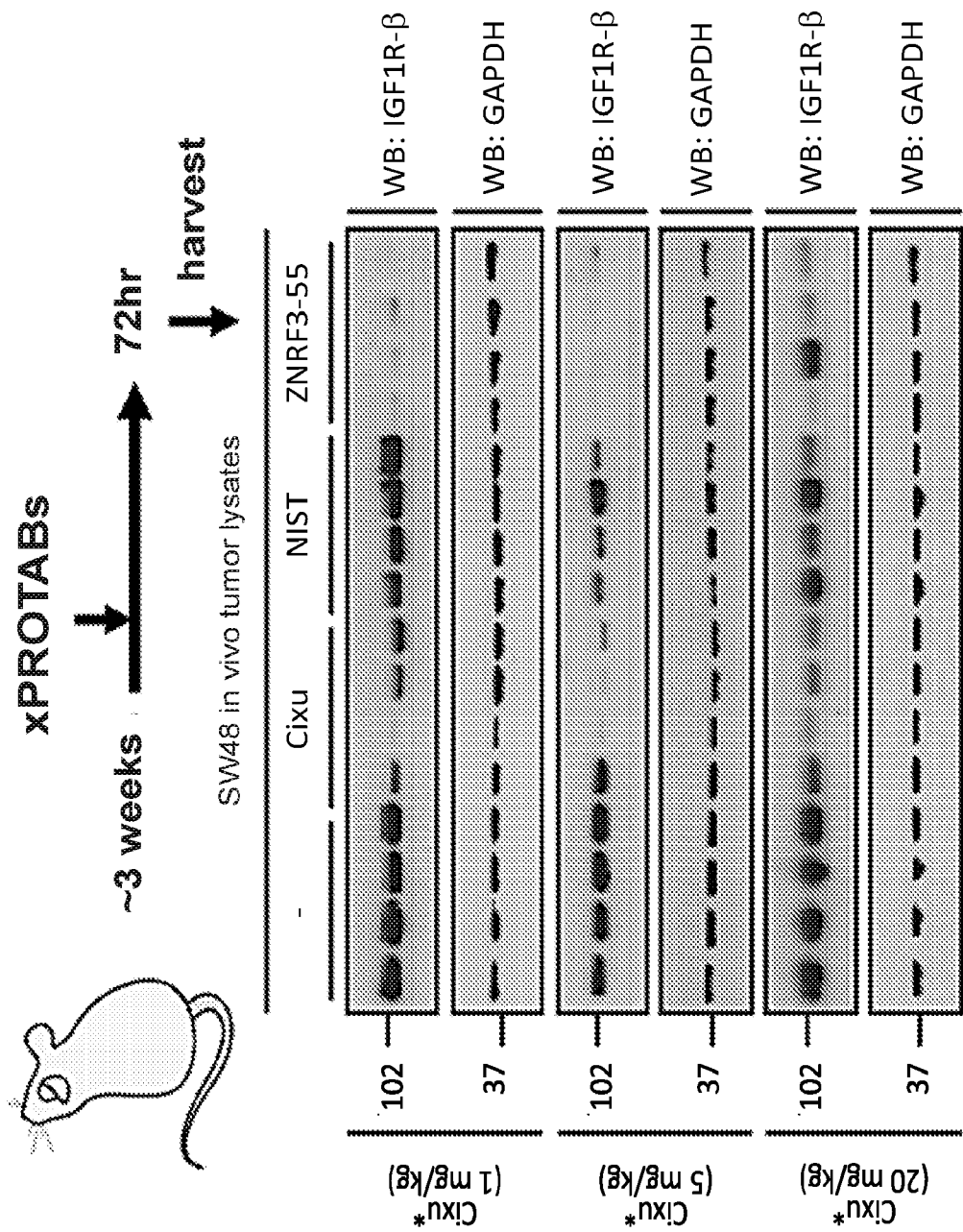
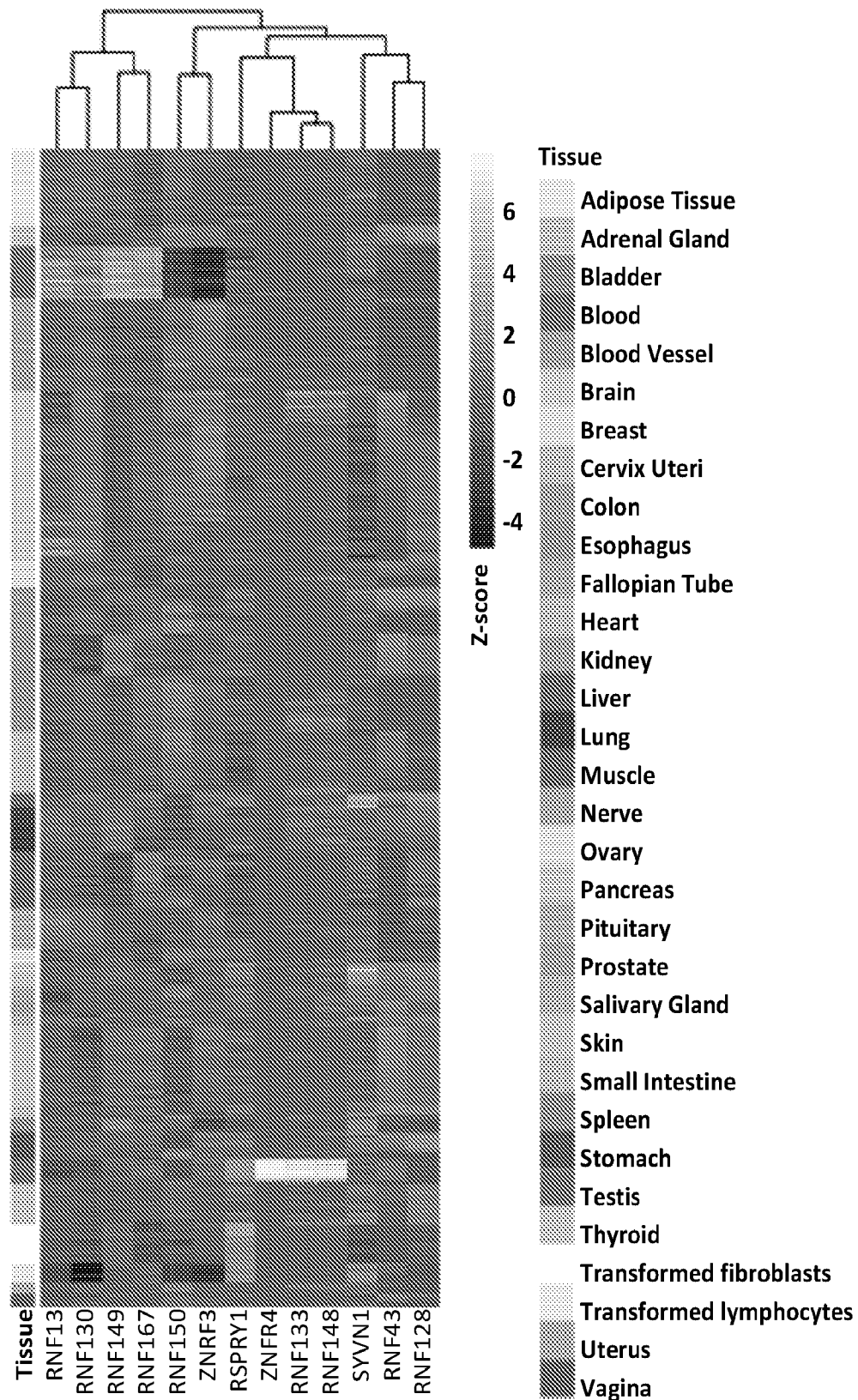


Fig. 30

*Fig. 31*

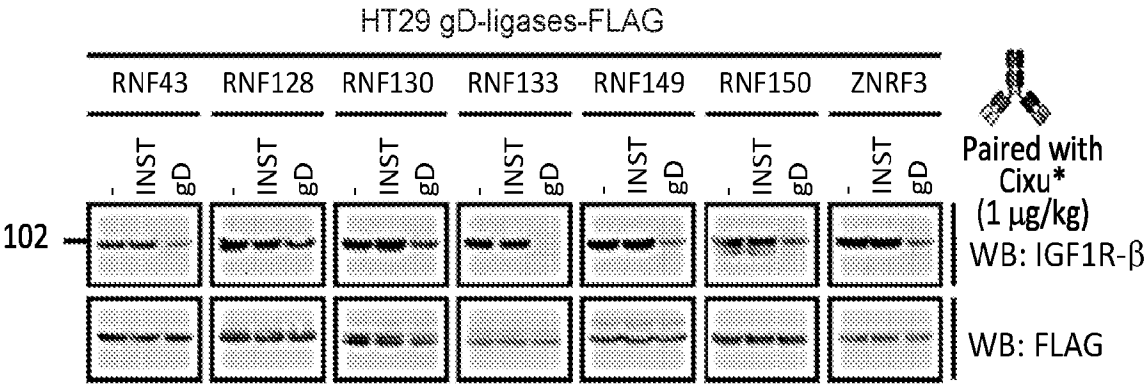


Fig. 32

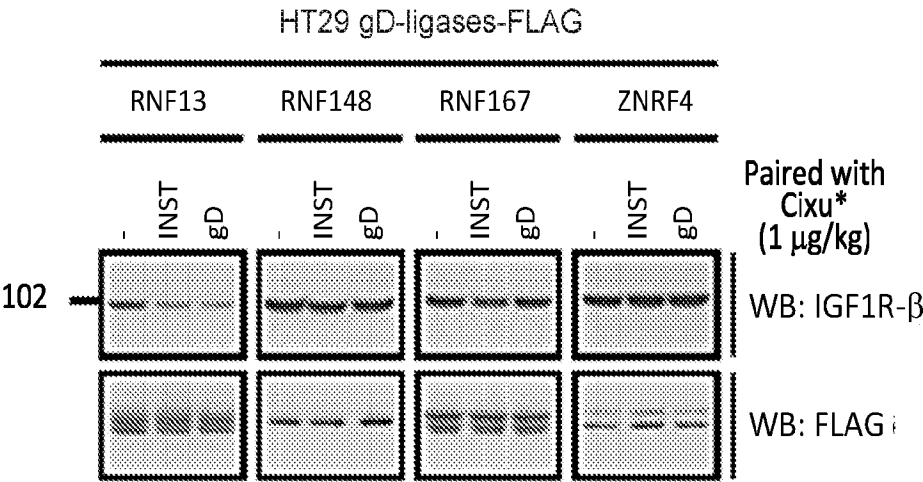


Fig. 33

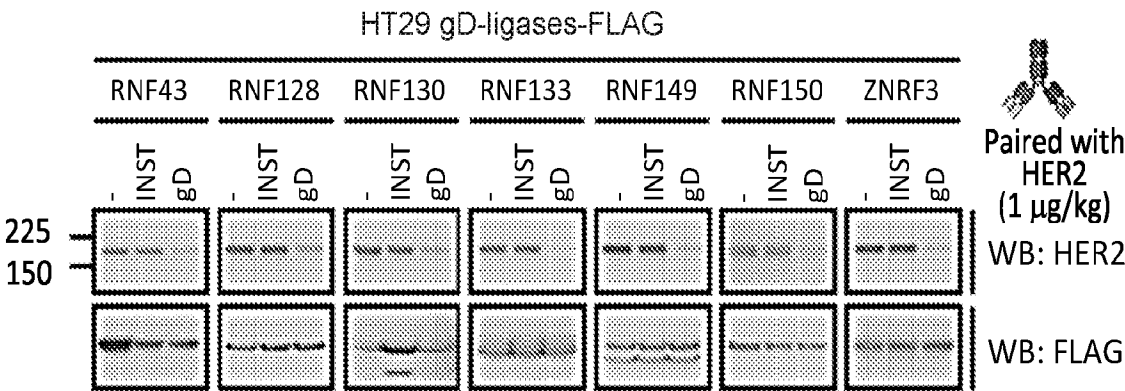


Fig. 34

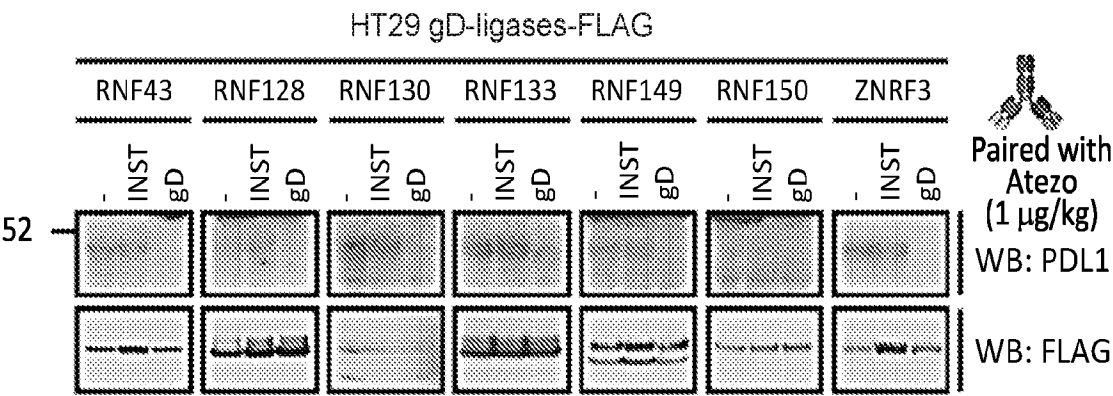


Fig. 35

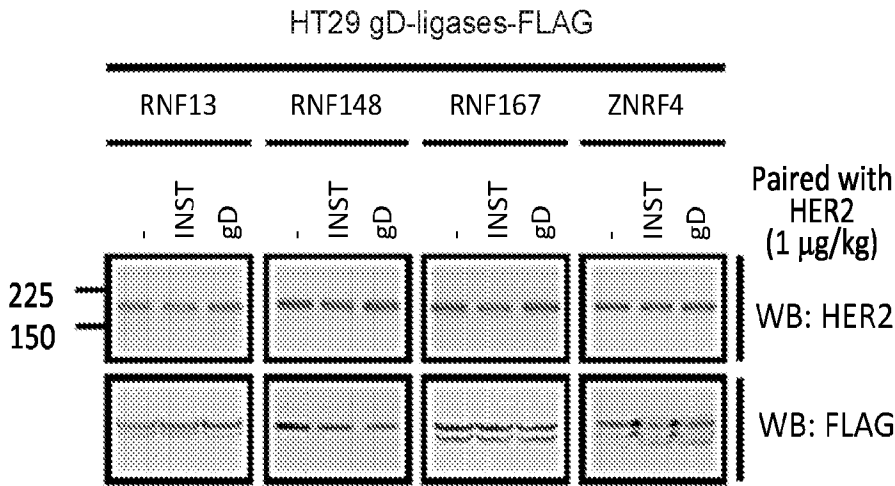


Fig. 36

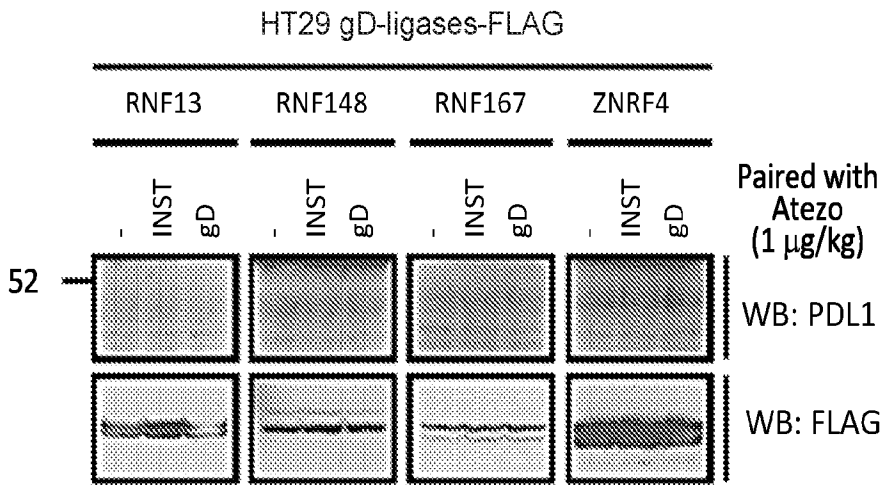


Fig. 37

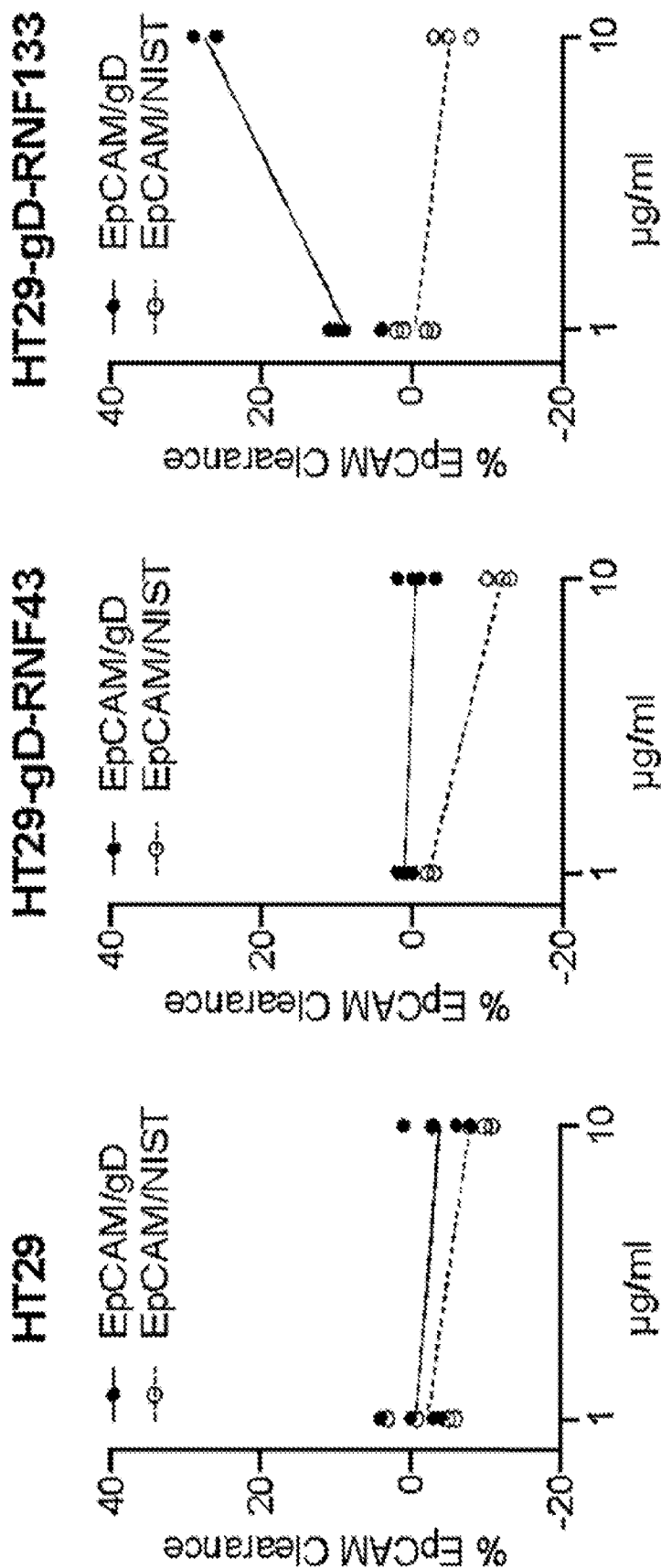


Fig. 38C

Fig. 38B

Fig. 38A

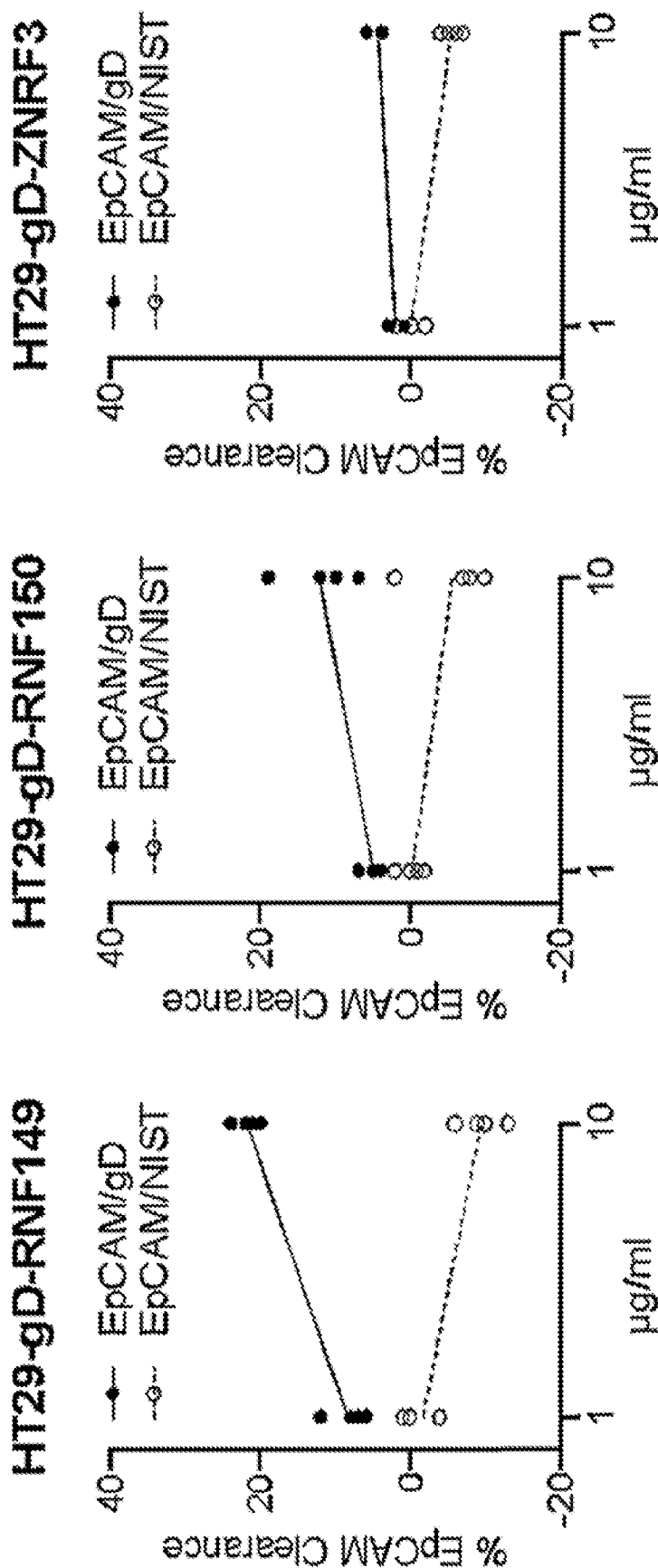


Fig. 38F

Fig. 38E

Fig. 38D

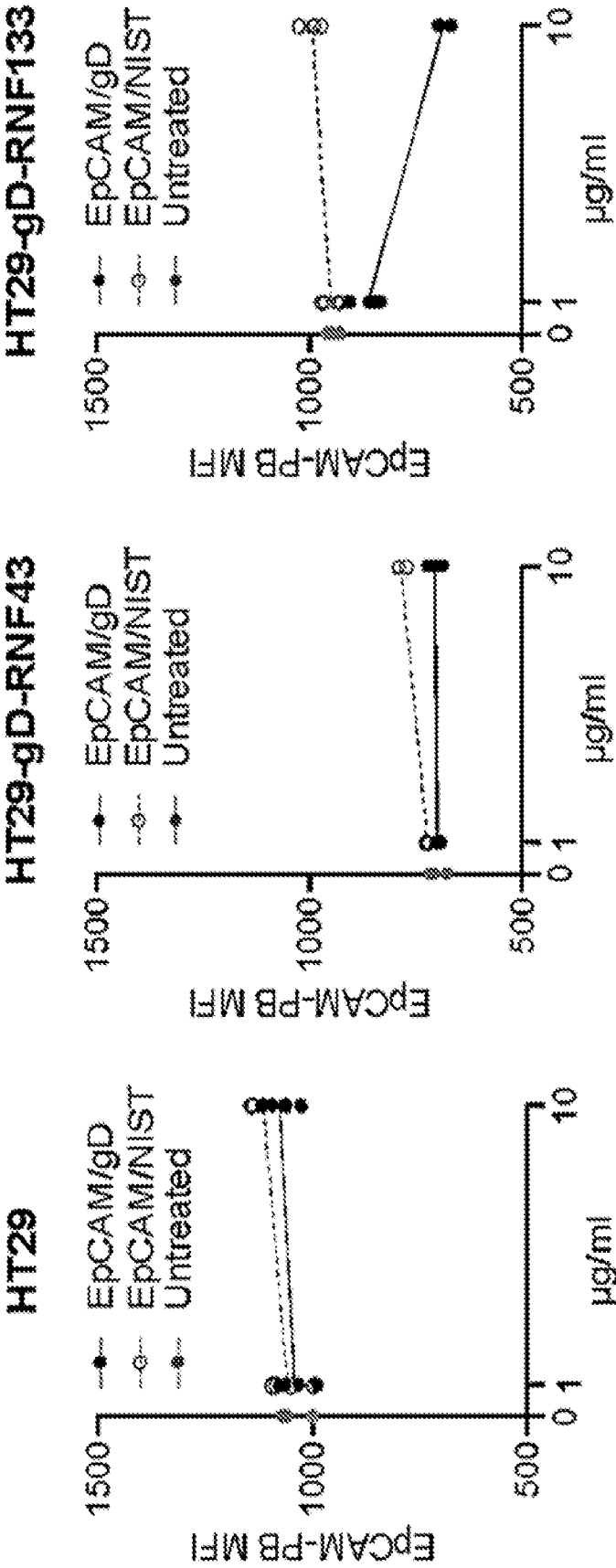


Fig. 38G

Fig. 38H

Fig. 38I

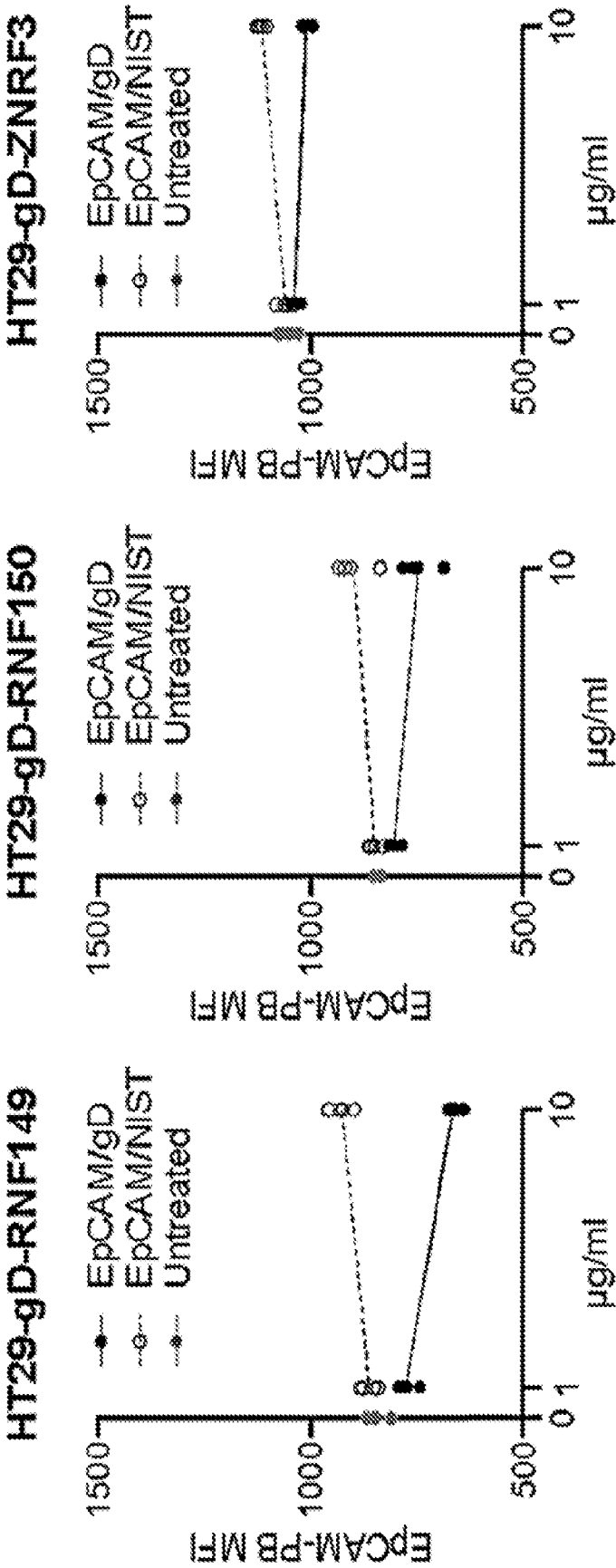


Fig. 38J

Fig. 38K

Fig. 38L

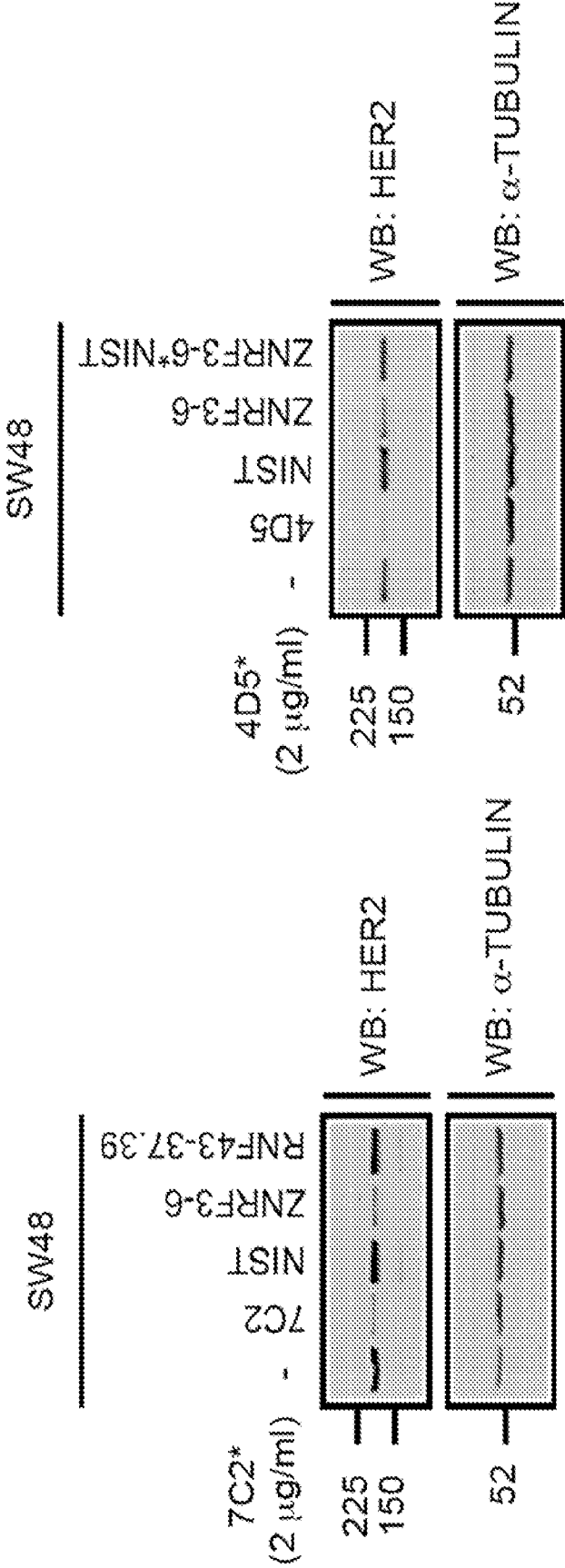


Fig. 39A

Fig. 39B

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/014938

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/40 C07K16/28

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COTTON ADAM D. ET AL: "Development of Antibody-Based PROTACs for the Degradation of the Cell-Surface Immune Checkpoint Protein PD-L1", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, [Online] vol. 143, no. 2, 20 January 2021 (2021-01-20), pages 593-598, XP055906993, ISSN: 0002-7863, DOI: 10.1021/jacs.0c10008 Retrieved from the Internet: URL:https://pubs.acs.org/doi/pdf/10.1021/jacs.0c10008> abstract; figures 1-4; compounds R3, AC-1 page 595, left-hand column - page 596 ----- -/--	1, 6-8, 11, 12, 16, 17, 28-63



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 April 2022

Date of mailing of the international search report

04/07/2022

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2

NL - 2280 HV Rijswijk

Tel. (+31-70) 340-2040,

Fax: (+31-70) 340-3016

Authorized officer

Schulz, Regine

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/014938

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/139950 A1 (H LEE MOFFITT CANCER CT & RES [US]) 2 July 2020 (2020-07-02)	1, 6, 7, 11, 12, 16, 17, 29, 31-52, 54-63
A	abstract; claims 1, 5, 7 - 10, 14 -18; figures 1-5 page 14, paragraph [0074] - page 16, line [0085]; sequences SEQ ID NOs: 1-11 page 25, paragraph [0124] -----	8, 28, 30
X	WO 2013/054307 A2 (NOVARTIS AG [CH]) 18 April 2013 (2013-04-18) abstract page 3, paragraph [0011] - paragraph [0012] -----	1, 6, 7, 11, 12, 16, 17, 39-44, 63
A	WO 2015/164392 A2 (STEMCENTRX INC [US]) 29 October 2015 (2015-10-29) abstract; sequence SEQ ID NO: 68 -----	26

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/014938

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/014938

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
1, 6-8, 11, 12, 16, 17, 26-63 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 2-5, 9, 10, 18-25(completely); 1, 6-8, 11-17, 26-63(partially)

Claims 2 - 5, and 9 - 10 attempt to define the subject-matter in terms of the result to be achieved, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result. They so lack clarity, support and disclosure in the sense of Art. 5 PCT and Art. 6 PCT that they cannot be searched as such.

Claims 18 - 27 refer exclusively to in-house names/internal designations, which have no meaning in the state of the art. Section IV of the description (cf. application: p. 87 - 131) provides a list of constructs and their primary sequences (as well as reference to the SEQ ID NOs) but does not indicate the respective HCDRs and LCDRs - to which claims 18 - 23 refer. It is however, incumbent to Applicants to point them out, and not to expect the ISA to determine them.

Any antibody must generally be defined by its combination of six HCDRs and LCDRs or cognate VH/VL pairs. None of the claims 18 - 27 properly defines the antibodies claimed. Consequently, said claims cannot be searched.

Claim 26 and 27 can be searched if combined, that is, if the antibody comprises both the VH and VL first mentioned in said claim - due to the lack of unity discussed below.

Claim 1 is searched. Claims 6 - 8, 11 - 17, 26/27, and 28 - 63 are searched as dependent on / relating to claim 1 and excluding any non-searched subject-matter.

Optional features and preferred features referred to as 'optionally' being present or referred to as 'such as' are not limiting and therefore not searched as such.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) PCT declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 6-8, 11, 12, 16, 17, 26-63(all partially)

a multispecific binding protein directed to the first combination of VH and VL mentioned in claim 26/27, i.e. comprising RNF43-104 VH and RNF43-104 VL, of SEQ ID NOs: 35, 36, as well as derived products and/or uses

2-124. claims: 1, 6-8, 11-17, 26-63(all partially)

as for invention 1, but concerning the other 123 coonstructs RNF43-106 through ZNRF-332 as listed in claims 26/27; as well as derived products and/or uses thereof.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/014938

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