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(57) Abstract: The invention provides anti-MIC antibodies and methods of using the same.

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ANTI-MIC ANTIBODIES AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/414,670, which is hereby incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] Not applicable. (N/A)

PARTIES TO A JOINT RESEARCH AGREEMENT

[0003] Not applicable. (N/A)

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0004] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 146392034740SEQLIST.txt, date recorded: October 26, 2017, size: 226 KB).

FIELD OF THE INVENTION

[0005] The present invention relates to anti-MIC antibodies, methods of using the same, and methods for mapping epitopes.

BACKGROUND OF THE INVENTION

[0006] MIC (*e.g.,* MICA, MICB) is a ligand for NKG2D, a receptor that is expressed on most human NK cells, γδ T cells, and CD8+ T cells. While NKG2D ligands are not usually found on healthy tissues, various forms of cellular stress, including DNA damage, upregulate ligand expression, resulting in their frequent detection in multiple solid and hematologic malignancies,including melanoma. Upon binding to MIC, NKG2D activates perforin-dependent cytolysis by NK cells and provides co-stimulation of T cells, resulting in killing of the NKG2D-expressing cells, *e.g.,* tumor cells. However, despite this mechanism, many MIC positive tumors have been identified. Studies have shown that immune escape of tumors is achieved by the shedding of MIC from these tumor cells. Soluble MIC triggers internalization and downregulation of surface NKG2D receptors and impaired function of cytotoxic lymphocytes. Soluble MIC may also stimulate the expansion of regulatory NKG2D+CD4+Foxp3- T cells that may antagonize anti-tumor cytotoxicity through Fas ligand, IL-10, and TGF-β. Sera from cancer patients typically contain elevated levels of the soluble form (sMICA).

[0007] It is clear that there continues to be a need for agents that have clinical attributes that are optimal for development as therapeutic agents. The antibodies described herein meet this need and provide other benefits.

[0008] Mapping the epitope is a critical aspect of characterizing antibodies. Knowledge of the epitope helps to guide design, selection and/or identification of other antibodies with similar properties. Identification of the epitope may also provide insight into the mechanism of binding for an antibody. Existing methods for mapping include x-ray crystallography, array-based oligopeptide screening, the use of synthetic peptides or proteolytic fragments of the antigen in ELISA or competition assays, site directed mutagenesis, phage display libraries of random peptide or antigen fragment sequences, proteolysis of antigens in the presence or absence of antibody with mass spectral analysis of either binding or non-binding fragments, and Shotgun Mutagenesis by Integral Molecular. Many of these methods are expensive and time consuming, requiring many steps before meaningful results are available. Thus there remains a need for improved methods of epitope mapping.

[0009] All references cited herein, including patent applications, patent publications, and UniProtKB/Swiss-Prot Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY OF THE INVENTION

[0010] The invention provides anti-MIC antibodies and methods of using the same. The invention also provides methods for epitope mapping. The epitope mapping methods described herein provide several advantages over methods existing in the art. For example, the methods described herein enable epitope mapping of antibodies using properly folded polypeptide, while other methods map epitopes using short polypeptide fragments that are likely to be misfolded or unfolded. Furthermore, the addition of glycans can be stabilizing to a polypeptide, whereas substitution with alanines frequently disrupts polypeptide structure, causing aggregation and rendering the polypeptide unusable. Moreover, the methods described herein permit faster scanning of a large surface or the entire surface of an antigen and use a fewer number of constructs than in an alanine scan.

[0011] In one aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008. In some embodiments, the antibodies bind to an epitope on human MICA*008 comprising amino acid residues Glu215, His248, and Arg279 of human MICA*008. In other embodiments, the antibodies bind to an epitope on human MICA*008 comprising amino acid residues Gly243 and Arg279 of human MICA*008. In other embodiments, the antibodies bind to an epitope on human MICA*008 comprising amino acid residues His248 and Arg279 of human MICA*008. In other

embodiments, the antibodies bind to an epitope on human MICA*008 comprising amino acid residue His248 of human MICA*008. In other embodiments, the antibodies bind to an epitope on human MICA*008 comprising amino acid residues Arg279 of human MICA*008.

[0012] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 7; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 8; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 9; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 10. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 11, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 12; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 13; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 14.

[0013] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 15; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 15. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 16.

[0014] In another aspect, provided herein are antibodies comprising a VH sequence of SEQ ID NO: 15 and a VL sequence of SEQ ID NO: 16.

[0015] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 19; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 20; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 21; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 23; a

FR-L2 comprising the amino acid sequence of SEQ ID NO: 24; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 25; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 26. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 27, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 28; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 29; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 30.

[0016] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 31; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 32; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 31. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 32.

[0017] In another aspect, provided herein are antibodies comprising a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 32.

[0018] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 33; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 34; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 35; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 36; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 37; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 38. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 39; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 40; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 41; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 42. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 43, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 44; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 45; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 46.

[0019] In another aspect, provided are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of

SEQ ID NO: 47; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 48; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 47. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 48.

[0020] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 47 and a VL sequence of SEQ ID NO: 48.

[0021] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 49; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 50; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 51; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 52; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 53; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 54. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 55; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 56; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 57; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 58. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 59, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 60; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 61; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 62.

[0022] In another aspect, provided are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 63; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 64; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 63. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 64.

[0023] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 63 and a VL sequence of SEQ ID NO: 64.

[0024] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 65; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 66; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 67; a

HVR-L1 comprising the amino acid sequence of SEQ ID NO: 68; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 69; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 70. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 71; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 72; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 73; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 74. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 75, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 76; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 77; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 78.

[0025] In another aspect, the antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 79; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 80; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 79. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 80.

[0026] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 79 and a VL sequence of SEQ ID NO: 80.

[0027] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 81; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 82; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 83; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 84; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 85; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 86. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 87; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 88; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 89; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 90. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 91, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 92; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 93; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 94.

[0028] In another aspect, provided are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 95; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 96; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibody comprises a VH sequence of SEQ ID NO: 95. In other embodiments, the antibody comprises a VL sequence of SEQ ID NO: 96.

[0029] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 95 and a VL sequence of SEQ ID NO: 96.

[0030] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 97; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 98; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 99; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 100; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 101; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 102. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 103; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 104; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 105; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 106. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 107, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 108; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 109; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 110.

[0031] In another aspect, provided here antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 111; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 112; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 111. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 112.

[0032] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 111 and a VL sequence of SEQ ID NO: 112.

[0033] In another aspect, provided here are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 113; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 114; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 115; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 116; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 118. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 119; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 120; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 121; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 122. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 123, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 124; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 125; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 126.

[0034] In another aspect, provided are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 127; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 128; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 127. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 128.

[0035] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 127 and a VL sequence of SEQ ID NO: 128.

[0036] In another aspect, provided here are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 129; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 130; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 131; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 132; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 133; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 134. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 135; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 136; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 137; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 138. In other embodiments, the antibodies further comprise the following heavy chain

variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 139, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 140; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 141; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 142.

[0037] In another aspect, provided are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 143; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 144; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 143. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 144.

[0038] In another aspect, provided are antibodies that comprise a VH sequence of SEQ ID NO: 143 and a VL sequence of SEQ ID NO: 144.

[0039] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 145; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 146; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 147; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 148; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 149; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 150. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 151; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 152; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 153; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 154. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 155, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 156; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 157; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 158.

[0040] In another aspect, provided are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 159; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 160; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some

embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 159. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 160.

[0041] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 159 and a VL sequence of SEQ ID NO: 160.

[0042] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 161; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 162; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 163; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 164; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 165; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 166. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 167; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 168; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 169; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 170. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 171, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 172; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 173; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 174.

[0043] In another aspect, provided are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 175; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 176; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 175. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 176.

[0044] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 175 and a VL sequence of SEQ ID NO: 176.

[0045] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 177; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 178; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 179; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 180; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 181; and a HVR-L3 comprising the amino acid sequence of

SEQ ID NO: 182. In some embodiments, The antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 183; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 184; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 185; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 186. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 187, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 188; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 189; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 190.

[0046] In another aspect, provided are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 191; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 192; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 191. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 192.

[0047] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 191 and a VL sequence of SEQ ID NO: 192.

[0048] In one aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residue Gly243 of human MICA*008. In some embodiments, the antibodies bind to an epitope on human MICA*008 comprising amino acid residues Gly243 and Arg279 of human MICA*008.

[0049] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 209; a HVR-H2 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 210, SEQ ID NO: 215, SEQ ID NO: 216, and SEQ ID NO: 217; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 211;a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 212; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 213; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 214. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 248, SEQ ID NO: 252, SEQ ID NO: 258, SEQ ID NO: 261, and SEQ ID NO: 266; a FR-L2 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 249, SEQ ID NO: 253, SEQ ID NO: 256, SEQ ID NO: 259, SEQ ID NO: 262, and SEQ

ID NO: 264; a FR-L3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 250, SEQ ID NO: 254, SEQ ID NO: 257, SEQ ID NO: 260, SEQ ID NO: 263, SEQ ID NO: 265, and SEQ ID NO: 267; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 251 or SEQ ID NO: 255. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 295, SEQ ID NO: 299, and SEQ ID NO: 303; a FR-H2 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 296, SEQ ID NO: 300, and SEQ ID NO: 304; a FR-H3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 297, SEQ ID NO: 301, SEQ ID NO: 305, SEQ ID NO: 306, SEQ ID NO: 307, SEQ ID NO: 308, SEQ ID NO: 309; SEQ ID NO: 310, SEQ ID NO: 311, SEQ ID NO: 312, SEQ ID NO: 313, and SEQ ID NO: 314; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 298 or SEQ ID NO: 302.

[0050] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residue Gly243 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 369; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 370; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 369. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 370.

[0051] In another aspect, provided herein are antibodies comprising a VH sequence of SEQ ID NO: 369 and a VL sequence of SEQ ID NO: 370.

[0052] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 218; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 219; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 220; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 221; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 222; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 223. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 268 or SEQ ID NO: 272; a FR-L2 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 269, SEQ ID NO: 273, and SEQ ID NO: 275; a FR-L3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 270, SEQ ID NO: 274, and SEQ ID NO: 276; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 255 or SEQ ID NO: 271. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 315, SEQ ID NO: 319, and SEQ ID NO: 323; a FR-H2 comprising the amino acid sequence of SEQ ID

NO: 316 or SEQ ID NO: 320; a FR-H3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 317, SEQ ID NO: 321, SEQ ID NO: 322, and SEQ ID NO: 324; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 302 or SEQ ID NO: 318.

[0053] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residue Gly243 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 415; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 416; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 415. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 416.

[0054] In another aspect, provided herein are antibodies comprising a VH sequence of SEQ ID NO: 415 and a VL sequence of SEQ ID NO: 416.

[0055] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 224; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 225; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 226; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 227; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 228; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 229. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 277 of SEQ ID NO: 280; a FR-L2 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 278, SEQ ID NO: 281, and SEQ ID NO: 283; a FR-L3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 279, SEQ ID NO: 282, and SEQ ID NO: 284; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 251 or SEQ ID NO: 255. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 325, SEQ ID NO: 329, SEQ ID NO: 331, and SEQ ID NO: 333; a FR-H2 comprising the amino acid sequence of SEQ ID NO: 320 or SEQ ID NO: 326; a FR-H3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 327, SEQ ID NO: 330, SEQ ID NO: 332, and SEQ ID NO: 334; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 302 or SEQ ID NO: 328.

[0056] In another aspect, provided are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residue Gly243 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the

amino acid sequence of SEQ ID NO: 429; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 430; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 429. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 430.

[0057] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 429 and a VL sequence of SEQ ID NO: 430.

[0058] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 236; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 237; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 238; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 239; a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 240; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 241. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 288; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 289; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 290; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 271. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 339, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 340; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 341; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 342.

[0059] In another aspect, provided are antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residue Gly243 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 437; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 438; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 437. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 438.

[0060] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 437 and a VL sequence of SEQ ID NO: 438.

[0061] In another aspect, provided herein are antibodies that specifically bind to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs): a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 242; a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 243; a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 244; a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 245; a HVR-L2 comprising the amino

acid sequence of SEQ ID NO: 246; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 247. In some embodiments, the antibodies further comprise the following light chain variable region framework regions (FRs): a FR-L1 comprising the amino acid sequence of SEQ ID NO: 291; a FR-L2 comprising the amino acid sequence of SEQ ID NO: 292; a FR-L3 comprising the amino acid sequence of SEQ ID NO: 293; and a FR-L4 comprising the amino acid sequence of SEQ ID NO: 294. In other embodiments, the antibodies further comprise the following heavy chain variable region FRs: a FR-H1 comprising the amino acid sequence of SEQ ID NO: 343, a FR-H2 comprising the amino acid sequence of SEQ ID NO: 344; a FR-H3 comprising the amino acid sequence of SEQ ID NO: 345; and a FR-H4 comprising the amino acid sequence of SEQ ID NO: 346.

[0062] In another aspect, the antibodies that specifically bind to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residue Gly243 of human MICA*008 and that comprise (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 439; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 440; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibodies comprise a VH sequence of SEQ ID NO: 439. In other embodiments, the antibodies comprise a VL sequence of SEQ ID NO: 440.

[0063] In another aspect, provided are antibodies comprising a VH sequence of SEQ ID NO: 439 and a VL sequence of SEQ ID NO: 440.

[0064] In certain embodiments of antibodies of any one of the preceding aspects, the antibody binds to human MICA with a Kd of about 100 nM or lower.

[0065] In certain embodiments of antibodies of any one of the preceding aspects, the antibody is capable of binding to human MICA*002, human MICA*004 and human MICB*005.

[0066] In certain embodiments of antibodies of any one of the preceding aspects, the antibody is capable of binding to the extracellular domain of human MICA*008, human MICA*002, human MICA*004, human MICA*008 and human MICB*005.

[0067] In certain embodiments of antibodies of any one of the preceding aspects, the antibody is capable of binding to the alpha3 domain of human MICA*008, human MICA*002, human MICA*004, and human MICB*005.

[0068] In certain embodiments of antibodies of any one of the preceding aspects, the antibody is monoclonal.

[0069] In certain embodiments of antibodies of any one of the preceding aspects, the antibody is human, humanized, or chimeric.

[0070] In certain embodiments of antibodies of any one of the preceding aspects, at least a portion of the framework sequence is a human consensus framework sequence.

[0071] In certain embodiments of antibodies of any one of the preceding aspects, the antibody is a full-length antibody.

[0072] In certain embodiments of antibodies of any one of the preceding aspects, the antibody is a bispecific antibody.

[0073] In certain embodiments of antibodies of any one of the preceding aspects, the antibody is an antibody fragment that binds human MICA*008. In some embodiments, the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, single chain variable fragment (scFv), and (Fab')2 fragments.

[0074] In certain embodiments of antibodies of any one of the preceding aspects, the antibody is an IgG class antibody. In some embodiments, the IgG class antibody is an IgG1 subclass antibody. In some embodiments, the IgG class antibody is an IgG2 subclass antibody. In some embodiments, the IgG class antibody is an IgG4 subclass antibody.

[0075] In another aspect, provided herein are isolated nucleic acids encoding the antibodies of the preceding aspects.

[0076] In another aspect, provided herein are vectors comprising the nucleic acids of the preceding aspects.

[0077] In another aspect, provided herein are host cells comprising the vectors of the preceding aspects. In some embodiments, the host cell is prokaryotic, *e.g.,* Escherichia coli. In other embodiments, the host cell is eukaryotic, e.g., a 293 cell, a CHO cell, a yeast cell, or a plant cell.

[0078] In another aspect, provided herein are methods of producing the antibody of any preceding aspects, the method comprising culturing the host cell of any of the preceding aspects in a culture medium. In some embodiments, the methods further comprise recovering the antibody from the host cell or culture medium.

[0079] In another aspect, provided herein are immunoconjugates comprising the antibody of any of the preceding aspects and a cytotoxic agent.

[0080] In another aspect, provided herein are compositions comprising the antibody of any of the preceding aspects. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier, excipient, or diluent. In some embodiments, the composition is a pharmaceutical

composition. In other embodiments, the composition further comprises a PD-1 axis binding antagonist or an additional therapeutic agent.

[0081] In another aspect, provided herein are antibodies of the preceding aspects for use in reducing shedding of MIC, for use in reducing levels of soluble MIC, and for use in reducing both shedding of MIC and levels of soluble MIC.

[0082] In another aspect, provided herein are antibodies of the preceding aspects for use as a medicament.

[0083] In another aspect, provided herein are antibodies of the preceding aspects for use in treating or delaying progression of a cancer in a subject in need thereof. In embodiments of this aspect, the cancer may be epithelial cancer, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, Merkel cell cancer, or other hematologic malignancies.

[0084] In another aspect, provided herein are antibodies of the preceding aspects for use in treating or delaying progression of an immune related disease in a subject in need thereof. In some embodiments of this aspect, the immune related disease is associated with a NKG2D ligand. In further embodiments, the NKG2D ligand is MIC. In other embodiments of this aspect, the immune related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity.

[0085] In another aspect, provided herein are antibodies of the preceding aspects for use in increasing, enhancing, or stimulating an immune response or function in a subject in need thereof.

[0086] In another aspect, provided herein are uses of the antibodies of any of the preceding aspects in the manufacture of a medicament for reducing shedding of MIC. In another aspect, provided herein are uses of the antibodies of the preceding aspects in the manufacture of a medicament for reducing levels of soluble MIC. In another aspect, provided herein are uses of the antibodies of the preceding aspects in the manufacture of a medicament for reducing shedding of MIC and levels of soluble MIC.

[0087] In another aspect, provided herein are uses of the antibodies of the preceding aspects in the manufacture of a medicament for treating or delaying progression of a cancer in a subject in need thereof. In embodiments of this aspect, the cancer may be epithelial cancer, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma,

head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, Merkel cell cancer, or other hematologic malignancies.

[0088] In another aspect, provided herein are uses of the antibodies of the preceding aspects in the manufacture of a medicament for treating or delaying progression of an immune related disease in a subject in need thereof. In some embodiments of this aspect, the immune related disease is associated with a NKG2D ligand. In further embodiments, the NKG2D ligand is MIC. In other embodiments of this aspect, the immune related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity.

[0089] In another aspect, provided herein are uses of the antibodies of the preceding aspects in the manufacture of a medicament for increasing, enhancing, or stimulating an immune response or function in a subject in need thereof.

[0090] In another aspect, provided herein are methods of reducing shedding of MIC in an individual comprising administering to the individual an effective amount of the antibody of any of the preceding aspects to reduce shedding of MIC. In another aspect, provided herein are methods of reducing levels of soluble MIC in an individual comprising administering to the individual an effective amount of the antibody of any of the preceding aspects to reduce levels of soluble MIC. In another aspect, provided herein are methods of reducing shedding of MIC and reducing levels of soluble MIC in an individual comprising administering to the individual an effective amount of the antibody of any of the preceding aspects to reduce shedding of MIC and levels of soluble MIC.

[0091] In another aspect, provided herein are methods for treating or delaying progression of a cancer in a subject, the method comprising administering to the subject an effective amount of the antibody of any of the preceding aspects, thereby treating or delaying the progression of the cancer in the subject. In embodiments of this aspect, the cancer may be epithelial cancer, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, Merkel cell cancer, and other hematologic malignancies.

[0092] In another aspect, provided herein are methods for treating or delaying progression of an immune related disease in a subject, the method comprising administering to the subject an effective amount of the antibody of any of the preceding aspects, thereby treating or delaying the progression of the immune related disease in the subject. In some embodiments of this aspect, the immune related disease is associated with a NKG2D ligand. In further embodiments, the NKG2D ligand is MIC. In

other embodiments of this aspect, the immune related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity.

[0093] In another aspect, provided herein are methods of increasing, enhancing, or stimulating an immune response or function in a subject, the comprising administering to the subject an effective amount of the antibody of any of the preceding aspects, thereby of increasing, enhancing, or stimulating an immune response or function in the subject.

[0094] In certain embodiments of the above methods for reducing shedding of MIC in an individual, treating or delaying progression of a cancer, treating or delaying progression of an immune related disease, or increasing, enhancing or stimulating an immune response of function in a subject, the methods further comprise administering to the subject a PD-1 axis binding antagonist. In some embodiments, the PD-1 axis binding antagonist is administered prior to or subsequent to the administration of the antibody. In other embodiments, the PD-1 axis binding antagonist is administered concurrently with the antibody. The PD-1 axis binding antagonist of these embodiments may be a PD-1 binding antagonist, a PD-L1 binding antagonist, or a PD-L2 binding antagonist.

[0095] In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners. The PD-1 binding antagonist may inhibit the binding of PD-1 to PD L1, inhibit the binding of PD-1 to PD L2, or inhibit the binding of PD-1 to both PD-L1 and PD-L2. In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody, such as MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, BGB-108, and BGB-A317.

[0096] In some embodiments, the PD-1 axis binding antagonist is a PD-L1 binding antagonist. The PD-L1 binding antagonist may inhibit the binding of PD-L1 to PD-1, inhibit the binding of PD-L1 to B7-1, or inhibit the binding of PD-L1 to both PD-1 and B7-1. In some embodiments, the PD-L1 binding antagonist is an anti-PD-L1 antibody, such as MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). In preferred embodiments, the anti-PD-L1 antibody is MPDL3280A.

[0097] In some embodiments, the PD-1 axis binding antagonist is a PD-L2 binding antagonist. IN some embodiments, the PD-L2 binding antagonist is anti-PD-L2 antibody. In other embodiments, the PD-L2 binding antagonist is an immunoadhesin.

[0098] In certain embodiments of the above methods for reducing shedding of MIC in an individual, treating or delaying progression of a cancer, treating or delaying progression of an immune related disease, or increasing, enhancing or stimulating an immune response of function in a subject, the methods further comprise administering to the subject an agent that decreases or inhibits one or more

additional inhibitory co-stimulatory receptors. In some embodiments, the one or more additional inhibitory co-stimulatory receptor is selected from the group consisting of PD-1, CTLA-4, LAG3, TIM3, BTLA, VISTA, B7H4, and MM.

[0099] In certain embodiments of the above methods for reducing shedding of MIC in an individual, treating or delaying progression of a cancer, treating or delaying progression of an immune related disease, or increasing, enhancing or stimulating an immune response of function in a subject, the methods further comprise administering to the subject an additional therapeutic agent. In some embodiments, wherein the additional therapeutic agent is a chemotherapeutic agent.

[0100] In certain embodiments of the above methods for reducing shedding of MIC in an individual, treating or delaying progression of a cancer, treating or delaying progression of an immune related disease, or increasing, enhancing or stimulating an immune response of function in a subject, the antibody of the preceding aspects is administered parenterally, intrapulmonarily, intranasally, intramuscularly, intravenously, intraarterially, intraperitoneally, or subcutaneously.

[0101] In certain embodiments of the above methods for reducing shedding of MIC in an individual, treating or delaying progression of a cancer, treating or delaying progression of an immune related disease, or increasing, enhancing or stimulating an immune response of function in a subject, the subject is a human.

[0102] In another aspect, provided herein are kits comprising the antibody of any of the preceding aspects and a package insert comprising instructions for using the antibody for treating or delaying progression of a cancer in a subject. In another aspect, provided herein are kits comprising the antibody of any of the preceding aspects and a package insert comprising instructions for using the antibody for treating or delaying progression of an immune related disease in a subject. In another aspect, provided herein are kits comprising the antibody of any of the preceding aspect and a package insert comprising instructions for increasing, enhancing, or stimulating an immune response or function in a subject. In certain embodiments of the instructions of the kits of the preceding aspects, the subject is a human.

[0103] In another aspect, provided herein are methods of mapping an epitope of an antibody comprising substituting an unglycosylated amino acid of a polypeptide to generate a glycosylated polypeptide comprising a substituted glycosylated amino acid; determining whether the antibody binds to the glycosylated polypeptide; and identifying at least one of the unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid as part of the epitope if binding of the antibody to the glycosylated polypeptide is reduced compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

[0104] In another aspect, provided herein are methods of mapping an epitope of an antibody comprising substituting an unglycosylated amino acid of a polypeptide to generate a glycosylated polypeptide comprising a substituted glycosylated amino acid; and determining whether the antibody binds to the glycosylated polypeptide, wherein at least one of the unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid is identified as part of the epitope if binding of the antibody to the glycosylated polypeptide is reduced compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

[0105] In another aspect, provided herein are methods of mapping an epitope of an antibody comprising identifying at least one of an unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid as part of the epitope if binding of the antibody to a glycosylated polypeptide comprising a substituted glycosylated amino acid is reduced compared to binding of the antibody to a polypeptide without the substituted glycosylated amino acid, wherein the glycosylated polypeptide is generated by substituting an unglycosylated amino acid of the polypeptide.

[0106] In some embodiments of the preceding methods for mapping an epitope, substituting an unglycosylated amino acid of a polypeptide comprises introducing a glycosylation site in the polypeptide. In some embodiments of any of the preceding methods for mapping an epitope, the epitope is a conformational epitope. In some embodiments of any of the preceding methods for mapping an epitope, the epitope is a linear epitope. In some embodiments of any of the preceding methods for mapping an epitope, the unglycosylated amino acid is on the surface of the polypeptide without the substituted glycosylated amino acid. In some embodiments of any of the preceding methods for mapping an epitope, the substituted glycosylated amino acid comprises an N-linked glycan. In some embodiments of any of the preceding methods for mapping an epitope, the polypeptide comprises a Fc domain. In some embodiments of any of the preceding methods for mapping an epitope, binding of antibody to the glycosylated polypeptide is detected by ELISA. In some embodiments of the method for mapping an epitope, binding of the antibody to the glycosylated polypeptide is reduced by at least 50% compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

[0107] In another aspect, provided herein are methods for binning antibodies comprising mapping the epitope of a first antibody by the preceding methods described herein; mapping the epitope of a second antibody by the methods described herein; and determining that the first antibody and the second antibody are in the same bin if they have the same epitope.

[0108] In another aspect, provided herein are methods for identifying the contact amino acids of an antibody comprising substituting an unglycosylated amino acid of a polypeptide to generate a glycosylated polypeptide comprising a substituted glycosylated amino acid; determining whether the

antibody binds to the glycosylated polypeptide; and identifying at least one of the unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid as one of the contact amino acids of the antibody if binding of the antibody to the glycosylated polypeptide is reduced compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

[0109] In another aspect, provided herein are methods for identifying the contact amino acids of an antibody comprising substituting an unglycosylated amino acid of a polypeptide to generate a glycosylated polypeptide comprising a substituted glycosylated amino acid; and determining whether the antibody binds to the glycosylated polypeptide, wherein at least one of the unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid is identified as one of the contact amino acids of the antibody if binding of the antibody to the glycosylated polypeptide is reduced compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

[0110] In another aspect, provided herein are methods for identifying the contact amino acids of an antibody comprising identifying at least one of an unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid as one of the contact amino acids of the antibody if binding of the antibody to a glycosylated polypeptide comprising a substituted glycosylated amino acid is reduced compared to binding of the antibody to a polypeptide without the substituted glycosylated amino acid, wherein the glycosylated polypeptide is generated by substituting an unglycosylated amino acid of the polypeptide.

[0111] In another aspect, provided herein are antibody of any of the preceding aspects, wherein the epitope is mapped by any of the methods of epitope mapping of the preceding aspects.

[0112] In another aspect, provided herein are antibodies that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, Arg279, Arg213, Ser214, Ala216, Ser217, Asn220, Arg271, Arg240, Gln241, Asp242, Val244, Ser245, Thr281, Ser247, Asp249, Thr250, Trp253, Glu276, Glu277, and Gln278 of human MICA*008. In some embodiments, the antibody binds to an epitope on human MICA*008 comprising a first amino acid residue, a second amino acid residue, and a third amino acid residue; wherein the first amino acid residue is selected from the group consisting of Glu215, Arg213, Ser214, Ala216, Ser217, Asn220, and Arg271 of human MICA*008; the second amino acid residue is selected from the group consisting of His248, Ser247, Asp249, Thr250, and Trp253 of human MICA*008; and the third amino acid residue is selected from the group consisting of Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and Thr281 of human MICA*008. In some embodiments, the antibody binds to an epitope on human

MICA*008 comprising a first amino acid residue and a second amino acid residue; wherein the first amino acid residue is selected from the group consisting of Gly243, Arg240, Gln241, Asp242, Val244, Ser245, Arg279, and Thr281 of human MICA*008; and the second amino acid residue is selected from the group consisting of Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and Thr281 of human MICA*008. In other embodiments, the antibody binds to an epitope on human MICA*008 comprising a first amino acid residue and a second amino acid residue; wherein the first amino acid residue is selected from the group consisting of His248, Ser247, Asp249, Thr250, or Trp253 of human MICA*008; and the second amino acid residue is selected from the group consisting of Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and Thr281 of human MICA*008. In still other embodiments, the antibody binds to an epitope on human MICA*008 comprising amino acid residues selected from the group consisting of His248, Ser247, Asp249, Thr250, and Trp253 of human MICA*008. In still other embodiments, the antibody binds to an epitope on human MICA*008 comprising amino acid residues selected from the group consisting of Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and Thr281 of human MICA*008.

[0113] In another aspect, provided herein are antibodies that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Gly243, Arg240, Gln241, Asp242, Val244, Ser245, Arg 279, and Thr281 of human MICA*008.

[0114] In some embodiments, the antibody binds to an epitope on human MICA*008 comprising a first amino acid residue, and a second amino acid residue, wherein the first residue is selected from the group consisting of: Gly243, Arg240, Gln241, Asp242, Val244, Ser245, Arg 279, and Thr281 of human MICA*008, and the second residue is selected from the group consisting of Arg 279, Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and Thr281 of human MICA*008.

[0115] In another aspect, provided herein are antibodies that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Arg240, Gln241, Val244, Ser245, His248, Glu276, Arg279,Tyr283, Glu285, His290, and Thr292 of human MICA*008.

[0116] In another aspect, provided herein are antibodies that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Arg240, Gln241, Asp242, Gly243, Val244, Ser245, Leu246, Ser247, Asp249, Thr250, Arg279, Tyr283, His290, Ser291, Thr292, and Pro294 of human MICA*008.

[0117] In another aspect, provided herein are antibodies that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Arg240, Asp242, Gly243, Val244, Glu277, Gln278, Arg279, Phe280, Thr281, Tyr283, Glu285, Gly288, Asn289, His290, Ser291, Thr292, Pro294, Val295, Pro296, and Ser297 of human MICA*008.

[0118] In another aspect, provided herein are antibodies that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Asn234, Ile235, Ile236, Leu237, Thr238, Trp239, and Arg240 of human MICA*008.

[0119] In another aspect, provided herein are antibodies that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Val268, Ala269, Thr270, Arg271, Ile272, Cys273, Arg274, Gly275, Glu276, Glu277, Gln278, Arg279, and Phe280 of human MICA*008.

[0120] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE FIGURES

[0121] **FIG. 1A – FIG. 1L**: Amino acid sequences of variable regions of anti-MIC antibodies. Heavy chain HVR - H1, -H2, and -H3, and light chain HVR -L1, -L2, and -L3 sequences are marked. Amino acid positions are numbered according to the Kabat numbering system as described herein. **FIG. 1A:** 3C9.10; **FIG. 1B:** 9C9.5.6; **FIG. 1C:** 1E6.1.3; **FIG. 1D:** 7A3.1.9; **FIG. 1E:** 6E12.5**; FIG. 1F:** 6E1.1.12; **FIG. 1G:** 7D4.6; **FIG. 1H:** 2E5.2.3**; FIG. 1-I:** 20G11; **FIG. 1-J:** 32D2; **FIG. 1K:** 3E11; **FIG. 1L:** 6F8.7**.**

[0122] **FIG. 2** shows MICA/B alpha3 domain alignment, % identity, and % similarity.

[0123] **FIG. 3A – FIG. 3E**: Black spheres indicate the engineered glycosylation site Asparagine that blocked antibody binding whereas grey spheres indicate engineered glycosylation sites that maintained antibody binding. **FIG. 3A**: Bin 1 (3C9.10) epitope maps to "bottom" of MICA. **FIG. 3B**: Bin 2 (6F8, 7D4, 32D2, and 3E11) epitope maps to "bottom and front" of MICA. **FIG. 3C**: Bin 3 (9C9.5.6, 1E6.1.3, 7A3.1.9) epitope maps to "bottom and side" of MICA. **FIG. 3D**: Bin 4 (6E12.5) epitope maps to "side" of MICA. **FIG. 3E**: Bin 5: 20G11 epitope maps to "front" of MICA.

[0124] **FIG. 4A – FIG. 4B** show the melt curves from Differential Scanning Fluorimetry (DSF) for the Glyco variants. **FIG. 4A** shows the fluorescence response (RFU) of all glycosylation variants. **FIG. 4B** shows the first derivative of the melt curves for all the glycosylation variants.

[0125] **FIG. 5** shows the DSF melting temperature (T_m) values for MICA and mIgG2a.

[0126] **FIG. 6** shows a gel confirming that the Glyco mutants are glycosylated.

[0127] **FIG. 7A** shows the human MICA *008 residues grafted on mouse MILL1. **FIG. 7B** shows the non-MICA*008 residues in the MILL1 chimera.

[0128] **FIG. 8** shows that the Bin 6 and 7 (2E5.2.3 and 6E1.1.12, respectively) epitope maps to the"back and top" of MICA. Black indicates the possible epitope based upon these antibodies binding the MILL1-MICA chimera. Residues not included in the epitope are based on data from engineered glycosylation site mapping, allelic differences between MICA002, 004, 008 and MICB005, since Bin 6 and 7 antibodies bind all four alleles, and residues in the MICA*001 structures (PDB codes 1B3J and 1HYR) predicted to be inward facing or not have any accessible surface area using the solvent accessibility calculation program GETAREA, as described in Fraczkiewicz, R. and Braun, W. (1998) "Exact and Efficient Analytical Calculation of the Accessible Surface Areas and Their Gradients for Macromolecules" *J. Comp. Chem.*, **19**, 319-333.

[0129] **FIG. 9** shows the fold-decrease in affinity from alanine scanning. Fold-decrease greater than 3 is shaded.

[0130] **FIG. 10** shows the 2E5.2.3 epitope with Ala scan data included. Residues in black are the epitope of 2E5.2.3, while residues in gray are not. Residues not included in the epitope are based on data from Alanine scanning, engineered glycosylation site mapping, allelic differences between MICA002, 004, 008 and MICB005, since 2E5.2.3 binds all four alleles, and residues in the MICA*001 structures (PDB codes 1B3J and 1HYR) predicted to be inward facing or not have any accessible surface area using the solvent accessibility calculation program GETAREA, as described in Fraczkiewicz, R. and Braun, W. (1998) "Exact and Efficient Analytical Calculation of the Accessible Surface Areas and Their Gradients for Macromolecules" *J. Comp. Chem.*, **19**, 319-333.Supporting our hypothesis that 2E5.2.3 was predicted to bind the “back” and “top” of MICA, Ala scanning of residues on the “front” showed that 2E5.2.3 did not bind to any of these resdues. This reveals that the epitope for 2E5.2.3 is not on the “front” of MICA, and is on the “back” and “top” of MICA.

[0131] **FIGS. 11A- FIG. 11B** compare the epitope for antibodies mapped by glycosylation engineering and Ala scan. **FIG. 11A**: 6F8; **FIG. 11B**: 7D4.

[0132] FIG. 12 provides a summary of the sequence identifiers for the anti-MIC antibodies described herein.

[0133] FIG. 13A – FIG. 13C show percent reduction of soluble MIC (sMIC) shedding caused by inhibition by titrating combinations of anti-MIC antibodies 1D5, 13A9, and 6E1, added onto cells.

[0134] FIG. 14A – FIG. 14F: Amino acid sequences of variable regions of 1D5, 13A9, 15F11, 6E1 (also called 6E1.1.12), 18G3, and 12H10 anti-MIC antibodies. Heavy chain HVR - H1 (**FIG. 14A**), HVR - H2 (**FIG. 14B**), and HVR - H3 (**FIG. 14C**), and light chain HVR - L1 (**FIG. 14D**), HVR - L2 (**FIG. 14E**), and HVR - L3 (**FIG. 14F**) sequences are marked. Amino acid positions are numbered according to the Kabat numbering system as described herein.

[0135] FIG. 15A - FIG. 15B provide the results of the experiments testing for antibody interference with the shedding inhibition assay. **FIG. 15A** provides the results for the HCC1534 (MICA*004) cells. **FIG. 15B** provides the results for the MEL-JUSO cells (MICA*008).

[0136] FIG. 16A – FIG. 16C depict percent reduction of sMIC signal caused by antibody interference by titrating combinations of anti-MIC antibodies 1D5, 13A9, and 6E1, added onto conditioned media.

[0137] FIG. 17: 52 anti-MICA antibodies were selected from all the antibody campaigns that had the highest binding affinity to MICA*008 alpha-3 domain. Binding affinity values determined by biacore were less than or equal to 10 nM, with a range of 0.5 nM to 10 nM.

[0138] FIG. 18A and FIG. 18B: Waterfall plot showing percent shedding inhibition of MICA*008 (**FIG. 18A**) and binding affinity (KD) for MICA*008 alpha-3 domain vs. percent shedding inhibition of MICA*008 for the 52 anti-MICA antibodies with affinity less than or equal to 10 nM (**FIG. 18B**). The anti-MICA antibodies show an 80-fold range for percent shedding inhibition of MICA*008 from 1 to 81%.

[0139] FIG. 19A shows three unique epitope bins for a set of 52 anti-MICA antibodies determined using Wasatch. Affinity for MICA*008 vs. Wasatch determined epitope bins (**FIG. 19B**) and percent shedding inhibition vs. Wasatch dertermined epitope bins (**FIG. 19C**) are also shown.

[0140] FIG. 20 depicts a Glyco-engineering Epitope Mapping (GEM) method. Single N-linked glycosylation sites are created by changing one or two residues in a protein antigen to achieve the N-X-S/T motif. Multiple antigen variants that each have a unique and newly created N-linked glycosylation site are then assessed for binding activity to an antibody panel using Elisa, Biacore or Wasatch. The epitope is then defined as any site that cannot be bound when glycosylation is present, thus masking the site.

[0141] **FIG. 21A** depicts the location of 7 individual and uniquely engineered N-linked glycosylation sites on the 'front' of MICA*008. Also shown is an analysis of total glycosylation (% fucosylation and % afucosylation) for each glyco-engineered MICA*008 variant **(FIG. 21B). FIG. 21C** depicts unique epitope bins identified by the Glyco-engineering Epitope Mapping (GEM) method based on the Elisa binding characteristics of the 7 glyco-engineered MICA*008 variants to 96 anti-MICA antibodies. **FIG. 21D** is the crystal structure of 1D5 indicating the epitopes of the glyco-engineered MICA variants on the 'front' and 'bottom' of MICA that when blocked by anti-MICA antibodies were most effective at blocking MICA*008 shedding. G243 (location of Glyco14's G243N mutation) and R279 (location of Glyco16's R279N mutation) were the epitopes that allow the best shedding inhibition of MICA*008 by anti-MICA antibodies.

[0142] **FIG. 22A – FIG. 22D** depict renderings of the crystal structure of the 1D5 Fab bound to the MICA*008 α3 domain shown from different angles, with the Fab depicted as a surface and the MICA*008 α3 domain as a ribbon diagram. The "front face" of the MICA*008 α3 domain, defined as the beta sheet of the Ig domain containing the carboxy-terminal strand, and the "back face" of the MICA*008 α3 domain, defined as the beta sheet of the Ig domain containing the amino-terminal strand, are highlighted along with the amino- (N-term) and carboxy-terminals (C-term).

[0143] **FIG. 23A** and **FIG. 23B** are renderings of the crystal structure of the 1D5 Fab CDRs bound to the MICA*008 α3 domain, shown from different angles. The MICA*008 α3 domain is depicted as a surface with the CDRs of the 1D5 Fab as ribbons. Residues defined as part of the 1D5 paratope (within 4.5 Å of the MICA*008 α3 domain) have their side chains shown as sticks.

[0144] **FIG. 24A** and **FIG. 24B** are open book representations of the interface between the 1D5 Fab and the MICA*008 α3 domain, respectively, shown as surfaces. Residues in the 1D5 paratope, defined as being within 4.5 Å of the MICA*008 α3 domain, are highlighted in **FIG. 24A** with their residue numbers. MICA*008 α3 domain residues in the 1D5 epitope, defined as being within 4.5 Å of the 1D5 Fab, are highlighted in **FIG. 24B** with their residue numbers. **FIG. 24C** and **FIG. 24D** are open book representations of the interface between the 1D5 Fab and the MICA*008 α3 domain, respectively, shown as ribbon diagrams. Residues in the 1D5 paratope, defined as being within 4.5 Å of the MICA*008 α3 domain, are highlighted in **FIG. 24C** with their residue numbers and have their side chains shown as sticks. MICA*008 α3 domain residues in the 1D5 epitope, defined as being within 4.5 Å of the 1D5 Fab, are highlighted in **FIG. 24D** with their residue numbers and have their side chains shown as sticks.

[0145] **FIG. 25A** and **FIG. 25B** highlight hydrogen bonds between residues of the LC of 1D5 and the MICA*008 α3 domain. The HC is not depicted for simplicity of viewing the LC interactions. **FIG.**

25C and **FIG. 25D** highlight hydrogen bonds between residues of the HC of 1D5 and the MICA*008 α3 domain. The LC is not depicted for simplicity of viewing the HC interactions.

[0146] **FIG. 26A** and **FIG. 26B** are open book representations of the interface between the 1D5 Fab and the MICA*008 α3 domain, respectively, shown as surfaces with electrostatic surface potentials colored as calculated in Pymol. Residues in the 1D5 paratope, defined as being within 4.5 Å of the MICA*008 α3 domain, are highlighted in **FIG. 26A** with their residue numbers. MICA*008 α3 domain residues in the 1D5 epitope, defined as being within 4.5 Å of the 1D5 Fab, are highlighted in **FIG. 26B** with their residue numbers.

[0147] **FIG. 27A** and **FIG. 27B** show the MICA*008 α3 domain structure depicted as a ribbon diagram or surface representation, respectively, with the 240s loop highlighted. **FIG. 27C** shows the 1D5 Fab structure as a ribbon diagram with the L3 and H3 CDRs highlighted, and small residues of these CDRs such as serine and glycine indicated by residue number. The side chains of these serines are shown as sticks. **FIG. 27D** - **FIG. 27E** are renderings of the crystal structure of the 1D5 Fab CDRs L3 and H3 bound to the MICA*008 α3 domain, shown from different angles to demonstrate the shape complementarity of the convex 240s loop of MICA*008 α3 domain and the concave surface created by the orientation and primary sequence of these CDRs. The MICA*008 α3 domain is depicted as a surface with the 240s loop highlighted and the L3 and H3 CDRs of the 1D5 Fab as ribbon diagrams. Serine residues within L3 and H3 defined as part of the 1D5 paratope (within 4.5 Å of the MICA*008 α3 domain) have their side chains shown as sticks. **FIG. 27F** and **FIG. 27G** are renderings of the crystal structure of the 1D5 Fab LC and HC bound to the MICA*008 α3 domain,

respectively, shown from different angles. Only one chain of the Fab is depicted for simplicity of viewing. The 240s loop of the MICA*008 α3 domain is highlighted. Residues of either the LC or HC CDRs defined as part of the 1D5 paratope (within 4.5 Å of the MICA*008 α3 domain) have their side chains shown as sticks.

[0148] **FIG. 28A – FIG. 28D** are renderings of the crystal structure of the 13A9 Fab bound to MICA*008 α3 domain shown from different angles, with the Fab depicted as a surface and the MICA*008 α3 domain as a ribbon diagram. The "front face" of the MICA*008 α3 domain, defined as the beta sheet of the Ig domain containing the carboxy-terminal strand, and the "back face" of the MICA*008 α3 domain, defined as the beta sheet of the Ig domain containing the amino-terminal strand, are highlighted along with the amino- (N-term) and carboxy-termininals (C-term).

[0149] **FIG. 29A** and **FIG. 29B** are renderings of the crystal structure of the 13A9 Fab CDRs bound to the MICA*008 α3 domain, shown from different angles. The MICA*008 α3 domain is depicted as a surface with the CDRs of the 13A9 Fab as ribbon diagrams. Residues defined as part of the 13A9 paratope (within 4.5 Å of the MICA*008 α3 domain) have their side chains shown as sticks.

[0150] **FIG. 30A – FIG. 30B** are open book representations of the interface between the 13A9 Fab and the MICA*008 α3 domain, respectively, shown as surfaces. Residues in the 13A9 paratope, defined as being within 4.5 Å of the MICA*008 α3 domain, are highlighted in **FIG. 30A** with their residue numbers. MICA*008 α3 domain residues in the 13A9 epitope, defined as being within 4.5 Å of the 13A9 Fab, are highlighted in **FIG. 30B** with their residue numbers. **FIG. 30C – FIG. 30D** are open book representations of the interface between the 13A9 Fab and the MICA*008 α3 domain, respectively, shown as ribbon diagrams. Residues in the 13A9 paratope, defined as being within 4.5 Å of the MICA*008 α3 domain, are highlighted in **FIG. 30C** with their residue numbers and have their side chains shown as sticks. MICA*008 α3 domain residues in the 13A9 epitope, defined as being within 4.5 Å of the 13A9 Fab, are highlighted in **FIG. 30D** with their residue numbers and have their side chains shown as sticks.

[0151] **FIG. 31A** highlights hydrogen bonds between residues of the LC of 13A9 and the MICA*008 α3 domain. The HC is not depicted for simplicity of viewing the LC interactions. **FIG. 31B – FIG. 31D** highlight hydrogen bonds between residues of the HC of 13A9 and the MICA*008 α3 domain. The LC is not depicted for simplicity of viewing the HC interactions.

[0152] **FIG. 32A - FIG. 32B** are open book representations of the interface between the 13A9 Fab and the MICA*008 α3 domain, respectively, shown as surfaces with electrostatic surface potentials colored as calculated in Pymol. Residues in the 13A9 paratope, defined as being within 4.5 Å of the

MICA*008 α3 domain, are highlighted in **FIG. 32A** with their residue numbers. MICA*008 α3 domain residues in the 13A9 epitope, defined as being within 4.5 Å of the 13A9 Fab, are highlighted in **FIG. 32B** with their residue numbers.

[0153] **FIG. 33A** and **FIG. 33B** show the MICA*008 α3 domain structure depicted as a ribbon diagram or surface representation, respectively, with residues 240-245, 277-279, and 288-297 highlighted to demonstrate the ridges and valley they create on the "front face" of the MICA*008 α3 domain. **FIG. 33C** is a rendering of the 13A9 Fab-MICA*008 α3 domain complex structure with the Fab CDRs shown as ribbon diagrams and the MICA*008 α3 domain as a surface. All six CDRs are highlighted with side chains of residues in the paratope (within 4.5 Å of the MICA*008 α3 domain) shown as sticks. Residues 240-245, 277-279, and 288-297 of the MICA*008 α3 domain are also highlighted on the MICA*008 surface to demonstrate that the 13A9 Fab binds across the ridges and valley created by these MICA*008 residues.**FIG. 33D** and **FIG. 33E** are renderings of the crystal structure of the 13A9 CDRs bound to the MICA*008 α3 domain, shown from different angles to demonstrate the shape complementarity of the ridges and valley of residues 240-245, 277-279, and 288-297 of the MICA*008 α3 domain and the side chains of the 13A9 Fab that fill the valley or span

the ridges. Side chains of residues in the 13A9 paratope (within 4.5 Å of the MICA*008 α3 domain) are shown as sticks.

[0154] **FIG. 34A - FIG. 34D** are renderings of the crystal structure of the 6E1 (also called 6E1.1.12) Fab bound to the C273S MICA*008 α3 domain shown from different angles, with the Fab depicted as a surface and the MICA*008 α3 domain as a ribbon diagram. The "front face" of the MICA*008 α3 domain, defined as the beta sheet of the Ig domain containing the carboxy-terminal strand, and the "back face" of the MICA*008 α3 domain, defined as the beta sheet of the Ig domain containing the amino-terminal strand, are highlighted along with the amino- (N-term) and carboxy-termininals (C-term).

[0155] **FIG. 35A** and **FIG. 35B** are renderings of the crystal structure of the 6E1 Fab CDRs bound to the MICA*008 α3 domain, shown from different angles. The MICA*008 α3 domain is depicted as a surface with the CDRs of the 6E1 Fab as ribbon diagrams. Residues defined as part of the 6E1 paratope (within 4.5 Å of the MICA*008 α3 domain) have their side chains shown as sticks. It is noted that CDR L3 does not make any contact with the MICA*008 α3 domain.

[0156] **FIG. 36A** and **FIG. 36B** are open book representations of the interface between the 6E1 Fab and the MICA*008 C273S α3 domain, respectively, shown as surfaces. Residues in the 6E1 paratope, defined as being within 4.5 Å of the MICA*008 C273S α3 domain, are highlighted in **FIG. 36A** with their residue numbers. MICA*008 C273S α3 domain residues in the 6E1 epitope, defined as being within 4.5 Å of the 6E1 Fab, are highlighted in **FIG. 36B** with their residue numbers. **FIG. 36C** and **FIG. 36D** are open book representations of the interface between the 6E1 Fab and the MICA*008 α3 domain, respectively, shown as ribbon diagrams. Residues in the 6E1 paratope, defined as being within 4.5 Å of the MICA*008 α3 domain, are highlighted in **FIG. 36C** with their residue numbers and have their side chains shown as sticks. MICA*008 α3 domain residues in the 6E1 epitope, defined as being within 4.5 Å of the 6E1 Fab, are highlighted in **FIG. 36D** with their residue numbers and have their side chains shown as sticks.

[0157] **FIG. 37A** highlights hydrogen bonds and salt bridges between residues of the LC of 6E1 and the MICA*008 C273S α3 domain. The HC is not depicted for simplicity of viewing the LC interactions. **FIG. 37B** and **FIG. 37C** highlight hydrogen bonds and salt bridges between residues of the HC of 6E1 and the MICA*008 C273S α3 domain. The LC is not depicted for simplicity of viewing the HC interactions.

[0158] **FIG. 38A** and **FIG. 38B** are open book representations of the interface between the 6E1 Fab and the MICA*008 C273S α3 domain, respectively, shown as surfaces with electrostatic surface potentials colored as calculated in Pymol. Residues in the 6E1 paratope, defined as being within 4.5 Å of the MICA*008 C273S α3 domain, are highlighted in 17A with their residue numbers. MICA*008

C273S α3 domain residues in the 6E1 epitope, defined as being within 4.5 Å of the 6E1 Fab, are highlighted in 17B with their residue numbers.

[0159] **FIG. 39A** and **FIG. 39B** show the 6E1 Fab depicted as a ribbon diagram representation in two different orientations, with CDR L1 highlighted to demonstrate the extended length of this CDR. **FIG. 39C** and **FIG. 39D** show the MICA*008 C273S α3 domain from the 6E1 Fab complex structure depicted as a ribbon diagram or surface representation, respectively, with the 250s loop highlighted. **FIG. 39E** and **FIG. 39F** are renderings of the crystal structure of the 6E1 Fab shown as a surface bound to the MICA*008 C273S α3 domain depicted as a ribbon diagram. This interaction is shown from different angles to demonstrate binding of the 250s loop of MICA*008 in the groove generated by the long CDR L1 of 6E1. Side chains of the residues in the 250s loop are shown as sticks. **FIG. 39G** depicts the hydrophobic binding pocket of Leu257 in the 6E1 Fab peptide-binding groove. This pocket is composed of residues from CDRs L1, H1, and H3 of the 6E1 Fab and the side chains of these residues are shown as sticks. **FIG. 39H** depicts the hydrophillic binding pocket of Asp255 in the 6E1 Fab peptide-binding groove. This pocket is composed of residues from CDRs L1, L2, and H3 of the 6E1 Fab and the side chains of these residues are shown as sticks.

[0160] **FIG. 40A** is a rendering of the crystal structures of the 1D5 Fab-MICA*008 α3 domain complex, the 13A9 Fab-MICA*008 α3 domain complex, and 6E1 Fab-MICA*008 C273S α3 domain complex, superimposed on one another with respect to MICA, showing that 1D5 and 13A9 have overlapping epitopes on the "front face" of the MICA*008 α3 domain whereas 6E1 binds to the "back face" of the α3 domain. **FIG. 40B** is a surface representation of the MICA*008 α3 domain with the 1D5 Fab epitope (MICA*008 α3 domain residues within 4.5 Å of the 1D5 Fab), the 13A9 Fab epitope (MICA*008 α3 domain residues within 4.5 Å of the 13A9 Fab), and both the 1D5 and 13A9 epitopes combined, mapped onto the surface of the MICA*008 α3 domain highlighted in color. **FIG. 40C** is a ribbon diagram representation of the MICA*008 α3 domain with the 1D5 Fab epitope (MICA*008 α3 domain residues within 4.5 Å of the 1D5 Fab), the 13A9 Fab epitope (MICA*008 α3 domain residues within 4.5 Å of the 13A9 Fab), and both the 1D5 and 13A9 epitopes combined, mapped onto the ribbon diagram of the MICA*008 α3 domain. Side chains of residues in the defined epitopes are shown as sticks and colored.

[0161] **FIG. 41A** depicts the sequence of the MICA*001 α3 domain (residues 204-297) and stalk region (residues 298-313, underlined). Reported MICA*001 cleavage sites from C1R cells (Waldhauer, I. *et al.*, (2008) Cancer Research 68:6368) and TRAMP C2 cells (Wang, X. *et al.* (2009) BBRC 387:476) are indicated within the protein sequence by down- and up-pointing arrows, respectively. The homologous residues of these reported cleavage sites are mapped onto the crystal structure of the MICA*008 α3 domain (taken from the 13A9 Fab-MICA*008 α3 domain complex structure) rendered as either a ribbon diagram or surface representation. Cleavage sites are highlighted

in color and the side chains are shown as sticks in the ribbon diagram. **FIG. 41B** shows a side-by-side comparison of the 1D5, 13A9, and 6E1 Fab epitopes mapped onto the surface of the MICA*008 α3 domain structure on the left with the reported cleavage sites mapped onto the surface of the MICA*008 α3 domain structure on the right. The boundaries of the 1D5, 13A9 and 6E1 Fab epitopes are also outlined with dashed lines on the MICA*008 α3 domain structures depicting the cleavage sites. The Fab epitopes were defined as MICA*008 α3 domain residues within 4.5 Å of the respective Fabs. The cleavages sites are those reported by Waldhauer, I. *et al.*, (2008) Cancer Research 68:6368 and Wang, X. *et al.* (2009) BBRC 387:476.

[0162] FIG. 42 depicts samples after reduction, deglycosylation, and alkylation from either MICA*008 α3 domain antigen alone unexposed to the laser (MICA, unexposed), MICA*008 α3 domain antigen alone exposed to the laser (MICA, exposed), MICA*008:1D5 Fab complex unexposed (Complex, unexposed) and MICA*008:1D5 Fab complex exposed (Complex, exposed) were run on a 4-20% Tris-Glycine gel. The boxed regions indicate the bands containing MICA*008 α3 domain that were excised for tryptic and chymotryptic digest and subsequent mass spectrometric analysis.

[0163] Sequence coverage obtained from tryptic (**FIG. 43A**) and chymotryptic (**FIG. 43B**) digest of the MICA*008 α3 domain from the MICA*008:1D5 Fab complex, exposed sample is shown. Residue numbering starts at the beginning of the α3 domain construct where the first two residues G1 and S2 are cloning artifacts and the start of the α3 domain is at T3 corresponding to residue Thr204 of the full-length MICA*008 protein. The end of the α3 domain is S96 corresponding to residue Ser297 of the full-length MICA*008 protein. This is followed by residues G97-S99 (cloning artifacts) and H100-H107 (His8 purification tag). Peptide alignment to MicA sequence was performed using Byonic software (Protein Metrics, Palo Alto, CA). Peak intensity is denoted by the color scale shown in the figure.

[0164] FIG. 44 shows percent change in oxidation when the MICA*008 α3 domain is bound in a complex with the 1D5 Fab relative to MICA*008 α3 domain alone is plotted against the MICA*008 α3 domain construct residue numbers. Residue numbering starts at the beginning of the α3 domain construct where the first two residues G1 and S2 are cloning artifacts and the start of the α3 domain is at T3 corresponding to residue Thr204 of the full-length MICA*008 protein. The end of the α3 domain is S96 corresponding to residue Ser297 of the full-length MICA*008 protein. The greatest change in oxidation is seen for residue W38 (Trp239 of the full-length MICA*008 protein) and Ile71 and Phe79 (Ile272 and Phe280 of the full-length MICA*008 protein).

[0165] FIG. 45 depicts MICA*008 α3 domain peptides that showed significant change in oxidation protection in the presence of the bound 1D5 Fab compared to the MICA*008 α3 domain alone sample are mapped onto the crystal structure of the MICA*008 α3 domain determined above (from the

complex of MICA*008 α3 domain bound to 1D5). These include N33-R39 (Asn234-Arg240 of the full-length MICA*008 protein) and V67-F79 (Val268-Phe280).

[0166] **FIG. 46A** shows a side-by-side comparison of the 1D5, 13A9, and 6E1 Fab epitopes mapped onto the surface of the MICA*008 α3 domain structure on the left with the glycoengineered variants mapped onto the surface of the MICA*008 α3 domain structure on the right. The boundaries of the 1D5, 13A9 and 6E1 Fab epitopes are also outlined with dashed lines on the MICA*008 α3 domain structures depicting the cleavage sites. The Fab epitopes were defined as MICA*008 α3 domain residues within 4.5 Å of the respective Fabs. The table shows the relative binding of the Fabs to the various glycoengineered variants with + indicating binding is unaffected, - indicating binding is completely lost, and +/- indicating binding is partially lost relative to the wild-type MICA*008 α3 domain. **FIG. 46B** is a rendering of the 1D5 Fab-MICA*008 α3 domain and 13A9 Fab-MICA*008 α3 domain complex structures shown from the same orientation with the Fabs depicted as surfaces and the MICA*008 α3 domains shown as ribbon diagrams. The table shows the relative binding of the Fabs to the various glycoengineered variants with + indicating binding is unaffected, - indicating binding is completely lost, and +/- indicating binding is partially lost relative to the wild-type MICA*008 α3 domain. The two mutations, Glyco14 (G243N) and Glyco16 (R279N), which affecting binding of the 1D5 and 13A9 Fabs to different degrees, are shown as spheres and highlighted with arrows. **FIG. 46C** depicts the 1D5 epitope determined by glycosylation engineering, FPOP, alanine scanning, and X-ray crystallography mapped onto the crystal structure of the MICA*008 α3 domain shown as a ribbon diagram. Residues identified as part of the epitope by each method are highlighted in color and the residue numbers are indicated. Epitope residues determined by glycosylation engineering of MICA*008 were defined as showing a loss in 1D5 binding upon mutation to Asn and introduction of an *N*-linked glycan, resulting in an ELISA OD_{450} signal below 0.5. Epitope residues determined by FPOP were defined as showing decreased oxidation within a tryptic or chymotryptic peptide of MICA*008 upon 1D5 Fab binding, relative to the same MICA*008 peptides in the absence of the Fab. Epitope residues determined by alanine scanning of MICA*008 were defined as showing a 3-fold decrease in 1D5 binding affinity (K_D measured by SPR) when mutated to alanine, relative to wild-type MICA*008. Epitope residues determined by X-ray crystallography were defined as being within 4.5 Å of the 1D5 Fab.

[0167] **FIG. 47A** depicts the epitopes of Dana Farber's CM33322 Ab4, CM33322 Ab28, and CM33322 Ab29, Innate Pharma's 16A8, and the University of Washington's H9 anti-MICA/B antibodies mapped on to the sequence (**FIG. 47A**) and structure (**FIG. 47B**) of the MICA*008 α3 domain. The epitopes of these previously described antibodies map predominantly to the "back face" of the MICA*008 α3 domain. In contrast, the epitopes of 1D5 and 13A9 map to the "front face" of the MICA*008 α3 domain.

[0168] **FIG. 48** depicts NKG2D-Fc vs. anti-MICA/B concentration, showing that antibody 1D5 does not interfere with MIC-NKG2D interactions.

[0169] **FIG. 49A** depicts the crystal structure of the 1D5 Fab bound to the MICA*008 α3 domain determined at 1.3 Å resolution. 1D5 binds to the "front face" of the α3 domain containing the C-terminal beta strand. **FIG. 49B** depicts the epitope of 1D5, defined as MICA residues within 4.5 Å of the Fab, on the surface of the "front face" of the MICA*008 α3 domain. Reported cleavage sites are shown with the boundary of the 1D5 epitope also indicated.

[0170] **FIG. 50A** illustrates the timeline of tumor inoculation in BALB/c SCID mice and dosing regimen of anti-MIC 1D5 antibody. **FIG. 50B** depicts tumor volumes by caliper measurement in various treatment groups. **FIG. 50C** and **FIG. 50D** depict the number of NK cells and the proportion of Granzyme B-positive NK cells, respectively, in harvested tumors following treatment with anti-MIC 1D5 antibody.

[0171] **FIG. 51A** shows that anti-MIC (1D5 antibody) treatment stabilized MICA surface expression in B16 cells engineered to express MICA (B16-MICA002) in a dox dependent manner. **FIG. 52B** depicts the anti-tumor response of a combination treatment of anti-MIC 1D5 and anti-PD-L1 in B16-MICA002.

DETAILED DESCRIPTION OF THE EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

[0172] Before describing the invention in detail, it is to be understood that this invention is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0173] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0174] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0175] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0176] The term **"MIC"** as used herein, refers to any native MHC Class I related chain A or B from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and

rats), unless otherwise indicated. The term encompasses "full-length," unprocessed MIC as well as any form of MIC that results from processing in the cell. The term also encompasses naturally occurring variants of MIC, for example, splice variants or allelic variants, including MICA alleles 002, 004, 008 and MICB allele 005. Other alleles of MICA encompassed by the term include MICA*001, MICA*005, MICA*006, MICA*007, MICA*009, MICA*010, MICA*011, MICA*012, MICA*013, MICA*014, MICA*015, MICA*016, MICA*017, MICA*018, MICA*019, MICA*020, MICA*022, MICA*023, MICA*024, MICA*025, MICA*026, MICA*027, MICA*028, MICA*029, MICA*030, MICA*031, MICA*032, MICA*033, MICA*034, MICA*035, MICA*036, MICA*037, MICA*038, MICA*039, MICA*040, MICA*041, MICA*042, MICA*043, MICA*044, MICA*045, MICA*046, MICA*047, MICA*048, MICA*049, MICA*050, MICA*051, MICA*052, MICA*053, MICA*054, MICA*055 and MICA*056. Other alleles of MICB encompassed by the term include MICB*001, MICB*002, MICB*003, MICB*004, MICB*006, MICB*007, MICB*008, MICB*009N, MICB*010, MICB*011, MICB*012, MICB*013, MICB*014, MICB*015, MICB*016, MICB*018, MICB*019, MICB*020, MICB*021N and MICB*022. A listing of alleles is available at hla.alleles/org/alleles/classo.html. The amino acid sequence of an exemplary human **MICA allele 008** (including the signal sequence) is shown in **SEQ ID NO: 193**. All amino acid numbering in the application for MICA *008 is with respect to a human MICA*008 sequence including the signal sequence. Further description of MIC is provided in Stephens et al., Trends Immunol. 2001 Jul;22(7):378-85. **FIG. 2** shows MICA/B alpha3 domain alignment, percent identity, and percent similarity.

[0177] The terms "**anti-MIC antibody", "an antibody that binds to MIC" and "an antibody that specifically binds to MIC"** refer to an antibody that is capable of binding MIC with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting MIC. In one embodiment, the extent of binding of an anti-MIC antibody to an unrelated, non-MIC protein is less than about 10% of the binding of the antibody to MIC as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to MIC has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g., 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-MIC antibody binds to an epitope of MIC that is conserved among MIC from different species.

[0178] 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 framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, 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 embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0179] **"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 (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0180] An **"affinity matured"** antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

[0181] The term **"antibody"** herein 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), and antibody fragments so long as they exhibit the desired antigen-binding activity.

[0182] An **"antibody fragment"** refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen 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 multispecific antibodies formed from antibody fragments.

[0183] An **"antibody that binds to the same epitope"** as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

[0184] 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.

[0185] 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., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[0186] The term **"cytotoxic agent"** as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids

(vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

[0187] **"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.

[0188] An **"effective amount"** of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0189] 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 embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. 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.

[0190] **"Framework"** or **"FR"** refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0191] 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 having heavy chains that contain an Fc region as defined herein.

[0192] The terms **"host cell," "host cell line," and "host cell culture"** are used interchangeably and 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 screened or selected for in the originally transformed cell are included herein.

[0193] 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.

[0194] A **"human consensus framework"** is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra.* In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

[0195] A **"humanized"** antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., 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.

[0196] The term **"hypervariable region" or "HVR"** as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and/or form structurally defined loops ("hypervariable loops") and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

[0197] (a) 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));

[0198] (b) 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));

[0199] (c) 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)); and

[0200] (d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

[0201] In one embodiment, HVR residues comprise those identified in **FIG. 1A-FIG. 1L** or elsewhere in the specification.

[0202] Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

[0203] An **"immunoconjugate"** is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0204] An **"individual" or "subject"** is a mammal. 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 certain embodiments, the individual or subject is a human.

[0205] An **"isolated"** antibody is one which has been separated from a component of its natural environment. In some embodiments, 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). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

[0206] 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.

[0207] **"Isolated nucleic acid encoding an anti-MIC antibody"** refers to one or more nucleic acid molecules encoding antibody heavy and light chains (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.

[0208] 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 to be used 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.

[0209] 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 formulation.

[0210] **"Native antibodies"** refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. 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.

[0211] The term **"package insert"** is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0212] **"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, and not considering any conservative substitutions as part of the sequence identity. 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, ALIGN or Megalign (DNASTAR) software. 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. For purposes herein, however, % amino acid sequence identity values are 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. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0213] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

[0214] 100 times the fraction X/Y

[0215] where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0216] The term **"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 formulation would be administered.

[0217] A **"pharmaceutically acceptable carrier"** refers to an ingredient in a pharmaceutical 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, diluent, stabilizer, or preservative.

[0218] 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 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 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 embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

[0219] The term **"variable region" or "variable domain"** 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 hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to 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).

[0220] The term **"vector,"** as used herein, refers to a nucleic acid molecule capable of propagating 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."

[0221] **"Chemotherapeutic agent"** includes chemical compounds useful in the treatment of cancer. Examples of chemotherapeutic agents include erlotinib (TARCEVA®, Genentech/OSI Pharm.), bortezomib (VELCADE®, Millennium Pharm.), disulfiram, epigallocatechin gallate , salinosporamide A, carfilzomib, 17-AAG (geldanamycin), radicicol, lactate dehydrogenase A (LDH-A), fulvestrant (FASLODEX®, AstraZeneca), sunitib (SUTENT®, Pfizer/Sugen), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), finasunate (VATALANIB®, Novartis), oxaliplatin (ELOXATIN®, Sanofi), 5-FU (5-fluorouracil), leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), Lonafamib (SCH 66336), sorafenib (NEXAVAR®, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), AG1478, alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including topotecan and irinotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); adrenocorticosteroids (including prednisone and prednisolone); cyproterone acetate; 5α-reductases including finasteride and dutasteride); vorinostat, romidepsin, panobinostat, valproic acid, mocetinostat dolastatin; aldesleukin, talc duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlomaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin γ1I and calicheamicin ω1I (Angew Chem. Intl. Ed. Engl. 1994 33:183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® (doxorubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin,

esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamnol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL (paclitaxel; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, Ill.), and TAXOTERE® (docetaxel, doxetaxel; Sanofi-Aventis); chloranmbucil; GEMZAR® (gemcitabine); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0222] Chemotherapeutic agent also includes (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, iodoxyfene , 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifine citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestanie, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide and goserelin; buserelin, tripterelin, medroxyprogesterone acetate, diethylstilbestrol, premarin,

fluoxymesterone, all transretionic acid, fenretinide, as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors; (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; (vii) ribozymes such as VEGF expression inhibitors (e.g., ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; PROLEUKIN®, rIL-2; a topoisomerase 1 inhibitor such as LURTOTECAN®; ABARELIX® rmRH; and (ix) pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0223] Chemotherapeutic agent also includes antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), pertuzumab (OMNITARG®, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixia), and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth). Additional humanized monoclonal antibodies with therapeutic potential as agents in combination with the compounds of the invention include: apolizumab, aselizumab, atlizumab, bapineuzumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, ustekinumab, visilizumab, and the anti–interleukin-12 (ABT-874/J695, Wyeth Research and Abbott Laboratories) which is a recombinant exclusively human-sequence, full-length IgG1 λ antibody genetically modified to recognize interleukin-12 p40 protein.

[0224] Chemotherapeutic agent also includes "EGFR inhibitors," which refers to compounds that bind to or otherwise interact directly with EGFR and prevent or reduce its signaling activity, and is alternatively referred to as an "EGFR antagonist." Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US Patent No. 4,943, 533, Mendelsohn et al.) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); IMC-11F8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF or Panitumumab (see WO98/50433, Abgenix/Amgen); EMD 55900

(Stragliotto et al. Eur. J. Cancer 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF-alpha for EGFR binding (EMD/Merck); human EGFR antibody, HuMax-EGFR (GenMab); fully human antibodies known as E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6. 3 and E7.6. 3 and described in US 6,235,883; MDX-447 (Medarex Inc); and mAb 806 or humanized mAb 806 (Johns et al., J. Biol. Chem. 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, e.g., EP659,439A2, Merck Patent GmbH). EGFR antagonists include small molecules such as compounds described in US Patent Nos: 5,616,582, 5,457,105, 5,475,001, 5,654,307, 5,679,683, 6,084,095, 6,265,410, 6,455,534, 6,521,620, 6,596,726, 6,713,484, 5,770,599, 6,140,332, 5,866,572, 6,399,602, 6,344,459, 6,602,863, 6,391,874, 6,344,455, 5,760,041, 6,002,008, and 5,747,498, as well as the following PCT publications: WO98/14451, WO98/50038, WO99/09016, and WO99/24037. Particular small molecule EGFR antagonists include OSI-774 (CP-358774, erlotinib, TARCEVA® Genentech/OSI Pharmaceuticals); PD 183805 (CI 1033, 2-propenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-quinazolinyl]-, dihydrochloride, Pfizer Inc.); ZD1839, gefitinib (IRESSA®) 4-(3'-Chloro-4'-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline, AstraZeneca); ZM 105180 ((6-amino-4-(3-methylphenyl-amino)-quinazoline, Zeneca); BIBX-1382 (N8-(3-chloro-4-fluoro-phenyl)-N2-(1-methyl-piperidin-4-yl)-pyrimido[5,4-d]pyrimidine-2,8-diamine, Boehringer Ingelheim); PKI-166 ((R)-4-[4-[(1-phenylethyl)amino]-1H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol); (R)-6-(4-hydroxyphenyl)-4-[(1-phenylethyl)amino]-7H-pyrrolo[2,3-d]pyrimidine); CL-387785 (N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide); EKB-569 (N-[4-[(3-chloro-4-fluorophenyl)amino]-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide) (Wyeth); AG1478 (Pfizer); AG1571 (SU 5271; Pfizer); dual EGFR/HER2 tyrosine kinase inhibitors such as lapatinib (TYKERB®, GSK572016 or N-[3-chloro-4-[(3 fluorophenyl)methoxy]phenyl]-6[5[[[2methylsulfonyl)ethyl]amino]methyl]-2-furanyl]-4-quinazolinamine).

[0225] Chemotherapeutic agents also include "tyrosine kinase inhibitors" including the EGFR-targeted drugs noted in the preceding paragraph; small molecule HER2 tyrosine kinase inhibitor such as TAK165 available from Takeda; CP-724,714, an oral selective inhibitor of the ErbB2 receptor tyrosine kinase (Pfizer and OSI); dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 and EGFR-overexpressing cells; lapatinib (GSK572016; available from Glaxo-SmithKline), an oral HER2 and EGFR tyrosine kinase inhibitor; PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibit Raf-1 signaling; non-HER targeted TK inhibitors such as imatinib mesylate (GLEEVEC®, available from Glaxo SmithKline); multi-targeted tyrosine kinase inhibitors such as sunitinib (SUTENT®, available from Pfizer); VEGF receptor tyrosine kinase inhibitors such as vatalanib (PTK787/ZK222584, available from Novartis/Schering AG); MAPK extracellular regulated kinase I inhibitor CI-1040

(available from Pharmacia); quinazolines, such as PD 153035,4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrimidopyrimidines; pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lamber); antisense molecules (e.g. those that bind to HER-encoding nucleic acid); quinoxalines (US Patent No. 5,804,396); tryphostins (US Patent No. 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-HER inhibitors such as CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); imatinib mesylate (GLEEVEC®); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxinib (Pfizer); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone), rapamycin (sirolimus, RAPAMUNE®); or as described in any of the following patent publications: US Patent No. 5,804,396; WO 1999/09016 (American Cyanamid); WO 1998/43960 (American Cyanamid); WO 1997/38983 (Warner Lambert); WO 1999/06378 (Warner Lambert); WO 1999/06396 (Warner Lambert); WO 1996/30347 (Pfizer, Inc); WO 1996/33978 (Zeneca); WO 1996/3397 (Zeneca) and WO 1996/33980 (Zeneca).

[0226] Chemotherapeutic agents also include dexamethasone, interferons, colchicine, metoprine, cyclosporine, amphotericin, metronidazole, alemtuzumab, alitretinoin, allopurinol, amifostine, arsenic trioxide, asparaginase, BCG live, bevacuzimab, bexarotene, cladribine, clofarabine, darbepoetin alfa, denileukin, dexrazoxane, epoetin alfa, elotinib, filgrastim, histrelin acetate, ibritumomab, interferon alfa-2a, interferon alfa-2b, lenalidomide, levamisole, mesna, methoxsalen, nandrolone, nelarabine, nofetumomab, oprelvekin, palifermin, pamidronate, pegademase, pegaspargase, pegfilgrastim, pemetrexed disodium, plicamycin, porfimer sodium, quinacrine, rasburicase, sargramostim, temozolomide, VM-26, 6-TG, toremifene, tretinoin, ATRA, valrubicin, zoledronate, and zoledronic acid, and pharmaceutically acceptable salts thereof.

[0227] Chemotherapeutic agents also include hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, triamcinolone acetonide, triamcinolone alcohol, mometasone, amcinonide, budesonide, desonide, fluocinonide, fluocinolone acetonide, betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, fluocortolone, hydrocortisone-17-butyrate, hydrocortisone-17-valerate, aclometasone dipropionate, betamethasone valerate, betamethasone dipropionate, prednicarbate, clobetasone-17-butyrate, clobetasol-17-propionate, fluocortolone caproate, fluocortolone pivalate and fluprednidene acetate; immune selective anti-inflammatory peptides (ImSAIDs) such as phenylalanine-glutamine-glycine (FEG) and its D-isomeric form (feG) (IMULAN BioTherapeutics, LLC); anti-rheumatic drugs such as azathioprine, ciclosporin (cyclosporine A), D-penicillamine, gold salts, hydroxychloroquine, leflunomideminocycline, sulfasalazine, tumor necrosis factor alpha (TNFα) blockers such as etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), golimumab (Simponi), Interleukin 1 (IL-1) blockers such as anakinra (Kineret), T cell costimulation blockers such as abatacept (Orencia),

Interleukin 6 (IL-6) blockers such as tocilizumab (ACTEMERA®); Interleukin 13 (IL-13) blockers such as lebrikizumab; Interferon alpha (IFN) blockers such as Rontalizumab; Beta 7 integrin blockers such as rhuMAb Beta7; IgE pathway blockers such as Anti-M1 prime; Secreted homotrimeric LTa3 and membrane bound heterotrimer LTa1/β2 blockers such as Anti-lymphotoxin alpha (LTa); radioactive isotopes (e.g., At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu); miscellaneous investigational agents such as thioplatin, PS-341, phenylbutyrate, ET-18- OCH3, or farnesyl transferase inhibitors (L-739749, L-744832); polyphenols such as quercetin, resveratrol, piceatannol, epigallocatechine gallate, theaflavins, flavanols, procyanidins, betulinic acid and derivatives thereof; autophagy inhibitors such as chloroquine; delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; acetylcamptothecin, scopolectin, and 9-aminocamptothecin); podophyllotoxin; tegafur (UFTORAL®); bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine; perifosine, COX-2 inhibitor (e.g. celecoxib or etoricoxib), proteosome inhibitor (e.g. PS341); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASARTM); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATINTM) combined with 5-FU and leucovorin.

[0228] Chemotherapeutic agents also include non-steroidal anti-inflammatory drugswith analgesic, antipyretic and anti-inflammatory effects. NSAIDs include non-selective inhibitors of the enzyme cyclooxygenase. Specific examples of NSAIDs include aspirin, propionic acid derivatives such as ibuprofen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin and naproxen, acetic acid derivatives such as indomethacin, sulindac, etodolac, diclofenac, enolic acid derivatives such as piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam and isoxicam, fenamic acid derivatives such as mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, and COX-2 inhibitors such as celecoxib, etoricoxib, lumiracoxib, parecoxib, rofecoxib, rofecoxib, and valdecoxib. NSAIDs can be indicated for the symptomatic relief of conditions such as rheumatoid arthritis, osteoarthritis, inflammatory arthropathies, ankylosing spondylitis, psoriatic arthritis, Reiter's syndrome, acute gout, dysmenorrhoea, metastatic bone pain, headache and migraine, postoperative pain, mild-to-moderate pain due to inflammation and tissue injury, pyrexia, ileus, and renal colic.

[0229] In some embodiments, an antigen binding polypeptide (e.g., antibody) or complex described herein may be administered in conjunction with a PARP inhibitor (e.g., Olaparanib, Rucaparib,

Niraparib, Cediranib, BMN673, Veliparib), Trabectedin, nab-paclitaxel (albumen-bound paclitaxel, ABRAXANE), Trebananib, Pazopanib, Cediranib, Palbociclib, everolimus, fluoropyrimidine (e.g., FOLFOX, FOLFIRI), IFL, regorafenib, Reolysin, Alimta, Zykadia, Sutent, Torisel (temsirolimus), Inlyta (axitinib, Pfizer), Afinitor (everolimus, Novartis), Nexavar (sorafenib, Onyx / Bayer), Votrient, Pazopanib, axitinib, IMA-901, AGS-003, cabozantinib, Vinflunine, Hsp90 inhibitor (e.g., apatorsin), Ad-GM-CSF (CT-0070), Temazolomide, IL-2, IFNa, vinblastine, Thalomid, dacarbazine, cyclophosphamide, lenalidomide, azacytidine, lenalidomide, bortezomid (VELCADE), amrubicine, carfilzomib, pralatrexate, and/or enzastaurin.

[0230] The term **"PD-1 axis binding antagonist"** refers to a molecule that inhibits the interaction of a PD-1 axis binding partner with either one or more of its binding partner, so as to remove T-cell dysfunction resulting from signaling on the PD-1 signaling axis – with a result being to restore or enhance T-cell function (*e.g.*, proliferation, cytokine production, target cell killing). As used herein, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

[0231] The term **"PD-1 binding antagonist"** refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to one or more of its binding partners. In a specific aspect, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1 and/or PD-L2. For example, PD-1 binding antagonists include anti-PD-1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-1 with PD-L1 and/or PD-L2. In one embodiment, a PD-1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-1 so as render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In a specific aspect, a PD-1 binding antagonist is MDX-1106 (nivolumab) described herein. In another specific aspect, a PD-1 binding antagonist is MK-3475 (pembrolizumab) described herein. In another specific aspect, a PD-1 binding antagonist is CT-011 (pidilizumab) described herein. In another specific aspect, a PD-1 binding antagonist is AMP-224 described herein.

[0232] The term **"PD-L1 binding antagonist"** refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1, B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, the PD-L1 binding antagonists include anti-PD-L1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit,

abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1, B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L1 so as to render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, a PD-L1 binding antagonist is an anti-PD-L1 antibody. In a specific aspect, an anti-PD-L1 antibody is YW243.55.S70 described herein. In another specific aspect, an anti-PD-L1 antibody is MDX-1105 described herein. In still another specific aspect, an anti-PD-L1 antibody is MPDL3280A described herein. In still another specific aspect, an anti-PD-L1 antibody is MEDI4736 described herein.

[0233] The term **"PD-L2 binding antagonist"** refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In some embodiments, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to one or more of its binding partners. In a specific aspect, the PD-L2 binding antagonist inhibits binding of PD-L2 to PD-1. In some embodiments, the PD-L2 antagonists include anti-PD-L2 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In one embodiment, a PD-L2 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L2 so as render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, a PD-L2 binding antagonist is an immunoadhesin.

[0234] The term **"NKG2D ligand"** refers to any molecule which is capable of binding to the NKG2D receptor and activating the receptor such that it triggers killing of the NKG2D-expressing cell. Exemplary NKG2D ligands include MICA, MICB, and related splice variants and alleles.

[0235] The term **"reducing shedding of MIC"** or grammatical variants thereof refer to a reduction in the release of membrane-bound MIC from MIC-expressing cells. In certain embodiments, the reduction in shedding of MIC reduces levels of soluble MIC such that there is a reduction in the effects of soluble MIC, including those that impair the immune system and permit cancer and other immune related diseases to develop and progress. In certain embodiments, the shedding of MIC is reduced by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%. 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% compared to shedding of MIC in the absence of antibodies described herein.

[0236] The term **"reducing levels of soluble MIC"** or grammatical variants thereof refers to reducing levels of non-membrane bound MIC. In certain embodiments, the reduction of soluble MIC is such that there is a reduction in the effects of soluble MIC, including those that impair the immune system and permit cancer and other immune related diseases to develop and progress. For example, the

reduction of soluble MIC may reduce downregulation of NKG2D induced by soluble MIC. The reduction in levels of soluble MIC may arise from multiple mechanisms, including a reduction in shedding of MIC and/or binding of soluble MIC by an antibody described herein. In certain embodiments, the level of soluble MIC is reduced by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%. 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% compared to levels of soluble MIC in the absence of antibodies described herein.

[0237] **"Tumor immunity"** refers to the process in which tumors evade immune recognition and clearance. Thus, as a therapeutic concept, tumor immunity is "treated" when such evasion is attenuated, and the tumors are recognized and attacked by the immune system. Examples of tumor recognition include tumor binding, tumor shrinkage and tumor clearance.

[0238] **"Antibody-dependent cell-mediated cytotoxicity" or "ADCC"** refers to a form of cytotoxicity in which secreted immunoglobulin bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g. NK cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. 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 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 or U.S. Patent No. 6,737,056 (Presta), may be performed. Useful effector cells for such assays include PBMC and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-656 (1998).

[0239] The term **"biomarker"** as used herein refers to an indicator, *e.g.*, predictive, diagnostic, and/or prognostic, which can be detected in a sample. The biomarker may serve as an indicator of a particular subtype of a disease or disorder (*e.g.*, cancer) characterized by certain, molecular, pathological, histological, and/or clinical features. In some embodiments, a biomarker is a gene. Biomarkers include, but are not limited to, polynucleotides (*e.g.*, DNA, and/or RNA), polynucleotide copy number alterations (*e.g.*, DNA copy numbers), polypeptides, polypeptide and polynucleotide modifications (*e.g.* posttranslational modifications), carbohydrates, and/or glycolipid-based molecular markers. The terms **"cancer"** and **"cancerous"** refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include, but not limited to, squamous cell cancer (*e.g.*, epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of

the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain, as well as head and neck cancer, and associated metastases.

[0240] **"Complement dependent cytotoxicity" or "CDC"** refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass), which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed. Polypeptide variants with altered Fc region amino acid sequences (polypeptides with a variant Fc region) and increased or decreased C1q binding capability are described, e.g., in US Patent No. 6,194,551 B1 and WO 1999/51642. See also, e.g., Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

[0241] The term "**diagnosis**" is used herein to refer to the identification or classification of a molecular or pathological state, disease or condition (e.g., cancer). For example, "diagnosis" may refer to identification of a particular type of cancer. "Diagnosis" may also refer to the classification of a particular subtype of cancer, e.g., by histopathological criteria, or by molecular features (e.g., a subtype characterized by expression of one or a combination of biomarkers (e.g., particular genes or proteins encoded by said genes)).

[0242] As used herein, **"delaying progression of a disease"** means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0243] **"Expression"** generally refers to the process by which information (*e.g.*, gene-encoded

[0244] and/or epigenetic) is converted into the structures present and operating in the cell. Therefore, as used herein, "expression" may refer to transcription into a polynucleotide, translation into a polypeptide, or even polynucleotide and/or polypeptide modifications (*e.g.*, posttranslational

modification of a polypeptide). Fragments of the transcribed polynucleotide, the translated polypeptide, or polynucleotide and/or polypeptide modifications (*e.g.*, posttranslational modification of a polypeptide) shall also be regarded as expressed whether they originate from a transcript generated by alternative splicing or a degraded transcript, or from a post-translational processing of the polypeptide, *e.g.*, by proteolysis. "Expressed genes" include those that are transcribed into a polynucleotide as mRNA and then translated into a polypeptide, and also those that are transcribed into RNA but not translated into a polypeptide (for example, transfer and ribosomal RNAs).

[0245] A **"growth inhibitory agent"** when used herein refers to a compound or composition which inhibits growth of a cell either in vitro or in vivo. In one embodiment, growth inhibitory agent is growth inhibitory antibody that prevents or reduces proliferation of a cell expressing an antigen to which the antibody binds. In another embodiment, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., The Molecular Basis of Cancer, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W.B. Saunders, Philadelphia, 1995), e.g., p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0246] As used herein, **"in conjunction with"** refers to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" refers to administration of one treatment modality before, during, or after administration of the other treatment modality to the individual.

[0247] The word **"label"** when used herein refers to a detectable compound or composition. The label is typically conjugated or fused directly or indirectly to a reagent, such as a polynucleotide probe or an antibody, and facilitates detection of the reagent to which it is conjugated or fused. The label may itself be detectable (*e.g.*, radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which results in a detectable product.

[0248] The term **"polynucleotide,"** when used in singular or plural, generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified

RNA or DNA. Thus, for instance, polynucleotides as defined herein include, without limitation, single- and double-stranded DNA, DNA including single- and double-stranded regions, single- and double-stranded RNA, and RNA including single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or include single- and double-stranded regions. In addition, the term "polynucleotide" as used herein refers to triple- stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. The term "polynucleotide" specifically includes cDNAs. The term includes DNAs (including cDNAs) and RNAs that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritiated bases, are included within the term "polynucleotides" as defined herein. In general, the term "polynucleotide" embraces all chemically, enzymatically and/or metabolically modified forms of unmodified polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells.

[0249] By **"radiation therapy"** is meant the use of directed gamma rays or beta rays to induce sufficient damage to a cell so as to limit its ability to function normally or to destroy the cell altogether. It will be appreciated that there will be many ways known in the art to determine the dosage and duration of treatment. Typical treatments are given as a one-time administration and typical dosages range from 10 to 200 units (Grays) per day.

[0250] The term **"sample,"** as used herein, refers to a composition that is obtained or derived from a subject and/or individual of interest that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. For example, the phrase "disease sample" and variations thereof refers to any sample obtained from a subject of interest that would be expected or is known to contain the cellular and/or molecular entity that is to be characterized. Samples include, but are not limited to, primary or cultured cells or cell lines, cell supernatants, cell lysates, platelets, serum, plasma, vitreous fluid, lymph fluid, synovial fluid, follicular fluid, seminal fluid, amniotic fluid, milk, whole blood, blood-derived cells, urine, cerebro-spinal fluid, saliva, sputum, tears, perspiration, mucus, tumor lysates, and tissue culture medium, tissue extracts such as homogenized tissue, tumor tissue, cellular extracts, and combinations thereof.

II. COMPOSITIONS AND METHODS OF USE

[0251] In one aspect, the invention is based, in part, on identification of anti-MIC antibodies that reduce shedding of MIC and or reduce levels of soluble MIC. In certain embodiments, antibodies that bind to MIC are provided. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of cancer and immune related disease.

A. Exemplary Anti-MIC Antibodies

[0252] In one aspect, the invention provides isolated antibodies that bind to MIC. In certain embodiments, an anti-MIC antibody reduces shedding of MIC and/or levels of soluble MIC.

In one aspect, the invention provides an anti-MIC antibody that binds to an epitope on human MICA*008 including, but not limited to Glu215, Gly243, His248, Arg279, Arg213, Ser214, Ala216, Ser217, Asn220, Arg271, Arg240, Gln241, Asp242, Val244, Ser245, Thr281, Ser247, Asp249, Thr250, Trp253, Glu276, Glu277, and/or Gln278 of human MICA*008.

[0253] In some embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to a first amino acid residue, a second amino acid residue, and a third amino acid residue; wherein the first amino acid residue is Glu215, Arg213, Ser214, Ala216, Ser217, Asn220, or Arg271; the second amino acid residue is His248, Ser247, Asp249, Thr250, or Trp253; and the third amino acid residue is Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, or Thr281.

[0254] In other embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to a first amino acid residue and a second amino acid residue; wherein the first amino acid residue is Gly243, Arg240, Gln241, Asp242, Val244, Ser245, Arg279, or Thr281; and the second amino acid residue is Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, or Thr281.

[0255] In still other embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to a first amino acid residue and a second amino acid residue; wherein the first amino acid residue is His248, Ser247, Asp249, Thr250, or Trp253; and the second amino acid residue is Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, or Thr281.

[0256] In other embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to amino acid residue His248, Ser247, Asp249, Thr250, and/or Trp253.

[0257] In other embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to amino acid residues Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and/or Thr281.

[0258] In other embodiments, the antibody binds to an epitope on the "back and top" of MIC. In one embodiment, the antibody may bind to residues Val205, Pro206, Met208, Thr212, Gly219, Thr222, Thr224, Arg226, Ser228, Tyr231, Pro232, Gln241, Asp242, Thr250, any amino acid between Asp255 to Gly262, Tyr264, Gln265, Trp267, Arg271, Gly275, Glu277, Gly288, Asn289, and/or His290 of human MICA*008. In another embodiment, the antibody may bind to Val205, Pro206,

Met208, Thr212, Gly219, Thr222, Thr224, Arg226, Ser228, Tyr231, Pro232, Asp242, any amino acid between Asp255 to Gly262, Tyr264, Gln265, Trp267, and/or Arg271 of human MICA*008.

[0259] In another aspect, the antibody binds to an epitope on human MICA*008 including, but not limited to amino acid residues Gly243, Arg240, Gln241, Asp242, Val244, Ser245, Arg 279, and/or Thr281.

[0260] In some embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to a first amino acid residue, and a second amino acid residue; wherein the first amino acid residue is Gly243, Arg240, Gln241, Asp242, Val244, Ser245, Arg 279, or Thr281; and the second amino acid residue is Arg 279, Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, or Thr281.

[0261] In some embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to amino aid residue Gly243.

[0262] In some embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to amino aid residues Gly243, and Arg279.

[0263] In other embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to amino acid residues Arg240, Gln241,Val244, Ser245, His248, Glu276, Arg279,Tyr283, Glu285, His290, and/or Thr292.

[0264] In other embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to amino acid residues Arg240, Gln241, Asp242, Gly243, Val244, Ser245, Leu246, Ser247, Asp249, Thr250, Arg279, Tyr283, His290, Ser291, Thr292, and/or Pro294.

[0265] In other embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to amino acid residues Arg240, Asp242, Gly243, Val244, Glu277, Gln278, Arg279, Phe280, Thr281, Tyr283, Glu285, Gly288, Asn289, His290, Ser291, Thr292, Pro294, Val295, Pro296, and/or Ser297.

[0266] In other embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to amino acid residues Asn234, Ile235, Ile236, Leu237, Thr238, Trp239, and/or Arg240. In other embodiments, the antibody binds to an epitope on human MICA*008 including, but not limited to amino acid residues Val268, Ala269, Thr270, Arg271, Ile272, Cys273, Arg274, Gly275, Glu276, Glu277, Gln278, Arg279, and/or Phe280.

[0267] In another aspect, the invention provides an anti-MIC antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 1, 17, 33 ,49, 65, 81, 97, 113, 129, 145, 161, or 177**; (b) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, or 178**; (c) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, or 179**; (d) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, or 180**; (e) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 5, 21, 37, 53,**

69, 85, 101, 117, 133, 149, 165, or 181; and (f) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, or 182**.

[0268] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 1, 17, 33 ,49, 65, 81, 97, 113, 129, 145, 161, or 177**; (b) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, or 178**; and (c) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, or 179**. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, or 179**. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, or 179** and HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, or 182**. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, or 179**; HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, or 182**; and HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, or 178**. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 1, 17, 33 ,49, 65, 81, 97, 113, 129, 145, 161, or 177**; (b) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, or 178**; and (c) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, or 179**.

[0269] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, or 180**; (b) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 5, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, or 181**; and (c) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, or 182**. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, or 180**; (b) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 5, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, or 181**; and (c) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, or 182.**

[0270] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 1, 17, 33 ,49, 65, 81, 97, 113, 129, 145, 161, or 177**; (ii) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, or 178**; and (iii) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, or 179**; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 4, 20,**

36, 52, 68, 84, 100, 116, 132, 148, 164, or 180; (ii) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 5, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, or 181**; and (iii) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, or 182.**

[0271] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 1, 17, 33 ,49, 65, 81, 97, 113, 129, 145, 161, or 177**; (b) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, or 178**; (c) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, or 179**; (d) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, or 180**; (e) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 5, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, or 181**; and (f) HVR-L3 comprising an amino acid sequence selected from **SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, or 182**.

[0272] In another aspect, an anti-MIC antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of **SEQ ID NO: 15, 31, 47, 63, 79, 95, 111, 127, 143, 159, 175, or 191**. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-MIC antibody comprising that sequence retains the ability to bind to MIC. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in **SEQ ID NO: 15, 31, 47, 63, 79, 95, 111, 127, 143, 159, 175, or 191**. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-MIC antibody comprises the VH sequence in **SEQ ID NO: 15, 31, 47, 63, 79, 95, 111, 127, 143, 159, 175, or 191**, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 1, 17, 33 ,49, 65, 81, 97, 113, 129, 145, 161, or 177**, (b) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, or 178**, and (c) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, or 179**.

[0273] In another aspect, an anti-MIC antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of **SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, or 192**. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-MIC antibody comprising that sequence retains the ability to bind to MIC. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in **SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, or 192**. In certain embodiments, the substitutions, insertions, or deletions occur in

regions outside the HVRs (i.e., in the FRs). Optionally, the anti-MIC antibody comprises the VL sequence in **SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, or 192**, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, or 180**; (b) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 5, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, or 181**; and (c) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, or 182**.

[0274] In another aspect, an anti-MIC antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in **SEQ ID NO: 15, 31, 47, 63, 79, 95, 111, 127, 143, 159, 175, or 191** and **SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, or 192**, respectively, including post-translational modifications of those sequences.

[0275] In any of the above embodiments, an anti-MIC antibody is humanized. In one embodiment, an anti-MIC antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In another embodiment, an anti-MIC antibody comprises HVRs as in any of the above embodiments, and further comprises a VH and/or VL comprising a FR sequence as disclosed in **FIGS. 1A – 1L.** In some embodiments, the anti-MIC antibodies described above further comprise at least one, two, three or four light chain variable region framework regions (FRs) selected from (a) a FR-L1 comprising the amino acid sequence of **SEQ ID NO: 7, 23, 39, 55, 71, 87, 103, 119, 135, 151, 167 or 183**; (b) a FR-L2 comprising the amino acid sequence of **SEQ ID NO: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, or 184**; (c) a FR-L3 comprising the amino acid sequence of **SEQ ID NO: 9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, or 185**; and (d) a FR-L4 comprising the amino acid sequence of **SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186**. In other embodiments, the anti-MIC antibodies decribed above further comprise the following heavy chain variable region FRs: a) a FR-H1 comprising the amino acid sequence of **SEQ ID NO: 11, 27, 43, 59, 75, 91, 107, 123, 139, 155, 171, or 187**; b) a FR-H2 comprising the amino acid sequence of **SEQ ID NO: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, or 188**; c) a FR-H3 comprising the amino acid sequence of **SEQ ID NO: 13, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173 or 189**; and d) a FR-H4 comprising the amino acid sequence of **SEQ ID NO: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, or 190**.

[0276] Exemplary antibodies (3C9.10, 7D4.6, 6F8.7, 32D2, 3E11, 9C9.5.6, 1E6.1.3, 7A3.1.9, 6E12.5, 20G11, 6E1.1.12, and 2E5.2.3) are described in **FIG. 1A – FIG. 1L**, **FIG. 12**, the Examples and the Sequence section of the Detailed Description.

[0277] Exemplary antibody variants of 1D5, 13A9, 15F11, 6E1, 18G3, 12H10 are described in **FIG. 14A - FIG. 14F**, the Examples and the Sequence section of the Detailed Description.

[0278] In one aspect, the invention provides an anti-MIC antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 209, 218, 224, 236, or 242**; (b) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 210, 215, 216, 217, 219, 225, 237, or 243**; (c) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 211, 220, 226, 238, or 244**; (d) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 212, 221, 227, 239, or 245**; (e) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 213, 222, 228, 240, or 246**; and (f) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 214, 223, 229, 241, or 247**.

[0279] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 209, 218, 224, 236, or 242**; (b) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 210, 215, 216, 217, 219, 225, 237, or 243**; and (c) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 211, 220, 226, 238, or 244**. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 211, 220, 226, 238, or 244**. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 211, 220, 226, 238, or 244** and HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 214, 223, 229, 241, or 247**. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 211, 220, 226, 238, or 244**; HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 214, 223, 229, 241, or 247**; and HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 210, 215, 216, 217, 219, 225, 237, or 243**. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 209, 218, 224, 236, or 242**; (b) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 210, 215, 216, 217, 219, 225, 237, or 243**; and (c) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 211, 220, 226, 238, or 244**.

[0280] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 212, 221, 227, 239, or 245**; (b) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 213, 222, 228, 240, or 246**; and (c) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 214, 223, 229, 241, or 247**. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 212, 221, 227, 239, or 245**; (b) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 213, 222, 228, 240, or 246**; and (c) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: SEQ ID NO: 214, 223, 229, 241, or 247.**

[0281] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 209, 218, 224, 236, or 242**; (ii) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 210, 215, 216, 217, 219, 225, 237, or 243**; and (iii) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 211, 220, 226, 238, or 244**; and (b) a VL domain comprising at

least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 212, 221, 227, 239, or 245**; (ii) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 213, 222, 228, 240, or 246**; and (iii) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: SEQ ID NO: 214, 223, 229, 241, or 247.**

[0282] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 209, 218, 224, 236, or 242**; (b) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 210, 215, 216, 217, 219, 225, 237, or 243**; (c) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 211, 220, 226, 238, or 244**; (d) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 212, 221, 227, 239, or 245**; (e) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 213, 222, 228, 240, or 246**; and (f) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 214, 223, 229, 241, or 247**.

[0283] In another aspect, an anti-MIC antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of **SEQ ID NO: 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, or 439**. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-MIC antibody comprising that sequence retains the ability to bind to MIC. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in **SEQ ID NO: 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, or 439**. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-MIC antibody comprises the VH sequence in **SEQ ID NO: 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, or 439**, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of **SEQ ID NO: 209, 218, 224, 236, or 242**; (b) HVR-H2 comprising the amino acid sequence of **SEQ ID NO: 210, 215, 216, 217, 219, 225, 237, or 243**; and (c) HVR-H3 comprising the amino acid sequence of **SEQ ID NO: 211, 220, 226, 238, or 244**.

[0284] In another aspect, an anti-MIC antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of **SEQ ID NO: 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, or**

440. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-MIC antibody comprising that sequence retains the ability to bind to MIC. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in **SEQ ID NO: 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, or 440**. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-MIC antibody comprises the VL sequence in **SEQ ID NO: 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, or 440**, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of **SEQ ID NO: 212, 221, 227, 239, or 245**; (b) HVR-L2 comprising the amino acid sequence of **SEQ ID NO: 213, 222, 228, 240, or 246**; and (c) HVR-L3 comprising the amino acid sequence of **SEQ ID NO: 214, 223, 229, 241, or 247**.

[0285] In another aspect, an anti-MIC antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in **SEQ ID NO: 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, or 439** and **SEQ ID NO: 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, or 440**, respectively, including post-translational modifications of those sequences.

[0286] In any of the above embodiments, an anti-MIC antibody is humanized. In one embodiment, an anti-MIC antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In another embodiment, an anti-MIC antibody comprises HVRs as in any of the above embodiments, and further comprises a VH and/or VL comprising a FR sequence as disclosed in **FIG. 14A – FIG. 14F.** In some embodiments, the anti-MIC antibodies described above further comprise at least one, two, three or four light chain variable region framework regions (FRs) selected from (a) a FR-L1 comprising the amino acid sequence of **SEQ ID NO: 248, 252, 258, 261, 266, 268, 272, 277, 280, 288, or 291**; (b) a FR-L2 comprising the amino acid sequence of **SEQ ID NO: 249, 253, 256, 259, 262, 264, 269, 273, 275, 278, 281, 283, 289, or 292**; (c) a FR-L3 comprising the amino acid sequence of **SEQ ID NO: 250, 254, 257, 260, 263, 265, 267, 270, 274, 276, 279, 282, 284, 290, or 293**; and (d) a FR-L4 comprising the amino acid sequence of **SEQ ID NO: 251, 255, 271, or 294**. In

other embodiments, the anti-MIC antibodies decribed above further comprise the following heavy chain variable region FRs: a) a FR-H1 comprising the amino acid sequence of **SEQ ID NO: 295, 299, 303, 315, 319, 323, 325, 329, 331, 333, 339, or 343**; b) a FR-H2 comprising the amino acid sequence of **SEQ ID NO: 296, 300, 304, 316, 320, 326, 340, or 344**; c) a FR-H3 comprising the amino acid sequence of **SEQ ID NO: 297, 301, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 317, 321, 322, 324, 327, 330, 332, 334, 341, or 345**; and d) a FR-H4 comprising the amino acid sequence of **SEQ ID NO: 298, 302, 318, 328, 342, or 346.**

[0287] In a further aspect, the anti-MIC antibody is capable of binding to human MICA*002, human MICA*004 and human MICB*005. In some embodments, the anti-MIC antibody is capable of binding to the extracellular domain of human MICA*008, human MICA*002, human MICA*004 and human MICB*005. In some embodiments, the anti-MIC antibody is capable of binding to the alpha3 domain of human MICA*008, human MICA*002, human MICA*004 and human MICB*005.

[0288] In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-MIC antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-MIC antibody provided herein.

[0289] In a further aspect of the invention, an anti-MIC antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-MIC antibody is an antibody fragment, e.g., a Fab, Fab', Fab'-SH, Fv, single chain variable fragment (scFv), and (Fab') fragment. In another embodiment, the antibody is a full length antibody, e.g., an intact IgG class antibody or IgG1 isotype or other antibody class or isotype as defined herein.

[0290] In a further aspect, an anti-MIC antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

1. Antibody Affinity

[0291] In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

[0292] In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA). In one embodiment, an RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc

#269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 µl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0293] According to another embodiment, Kd is measured using a BIACORE® surface plasmon resonance assay. For example, an assay using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) is performed at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). In one embodiment, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 µl/min. Association rates (kon) and dissociation rates (koff) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio koff/kon. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 106 M-1 s-1 by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25oC of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

[0294] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')$_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of*

Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

[0295] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0296] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see,* e.g., U.S. Patent No. 6,248,516 B1).

[0297] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

[0298] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0299] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0300] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34

(2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0301] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

[0302] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[0303] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HuMab® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VelociMouse® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0304] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562

(2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0305] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

[0306] Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0307] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0308] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[0309] In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for MIC and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of MIC. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express MIC. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0310] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or 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)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *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).

[0311] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

[0312] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to MIC as well as another, different antigen (see, US 2008/0069820, for example).

7. Antibody Variants

[0313] In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve 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 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

[0314] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in **Table 1** under the heading of "preferred substitutions." More substantial changes are provided in **Table 1** 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 and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 1: Amino Acid Substitutions.

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

[0315] 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) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0316] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0317] One type of substitutional variant involves substituting one or more hypervariable region 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 conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0318] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "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 in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments 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 diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR 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.

[0319] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs 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 HVRs. Such alterations may, for example, be outside of antigen contacting residues in the HVRs. In certain embodiments of the variant VH and

VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0320] 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 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 to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0321] 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 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) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

[0322] In certain embodiments, 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.

[0323] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be 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 embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0324] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high

mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, 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 fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, 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 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[0325] Antibodies variants are further 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. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). 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 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

[0326] In certain embodiments, 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.

[0327] In certain embodiments, 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 and 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 Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. 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)).

[0328] 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).

[0329] 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).)

[0330] In certain embodiments, 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).

[0331] In some embodiments, 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).

[0332] 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 US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more 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, 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 (US Patent No. 7,371,826).

[0333] 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.

d) Cysteine engineered antibody variants

[0334] In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, 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. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

e) Antibody Derivatives

[0335] In certain embodiments, 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, propropylene glycol homopolymers, prolypropylene 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.

[0336] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

[0337] Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-MIC antibody described herein is provided. Such nucleic acid may 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 chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-MIC antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under in a culture medium conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0338] For recombinant production of an anti-MIC antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid 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).

[0339] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, such as *Escherichia coli*, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo,

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.

[0340] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-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 Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

[0341] Suitable host cells for the expression of glycosylated antibody are also derived from 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.

[0342] Plant cell cultures can also be utilized as hosts. *See,* e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[0343] 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 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); 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 et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); 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 and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

C. Assays

[0344] Anti-MIC antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Binding assays and other assays

[0345] In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

[0346] In another aspect, competition assays may be used to identify an antibody that competes with any of the anti-MIC antibodies described herein for binding to MIC. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by any of the anti-MIC antibodies described herein. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

[0347] In an exemplary competition assay, immobilized MIC is incubated in a solution comprising a first labeled antibody that binds to MIC (e.g., any of the anti-MIC antibodies described herein) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to MIC. The second antibody may be present in a hybridoma supernatant. As a control, immobilized MIC is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to MIC, excess unbound antibody is removed, and the amount of label associated with immobilized MIC is measured. If the amount of label associated with immobilized MIC is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to MIC. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

2. Activity assays

[0348] In one aspect, assays are provided for identifying anti-MIC antibodies thereof having biological activity. Biological activity may include, *e.g.*, reducing the shedding of MIC and/or reducing the level of soluble MIC. Antibodies having such biological activity in vivo and/or in vitro are also provided.

[0349] In certain embodiments, an antibody of the invention is tested for such biological activity. In some embodiments, the reduction in shedding of MIC is tested by assaying and comparing levels of surface MIC on MIC-expressing cells in the presence or absence of antibody. Levels of cell surface MIC can detected using either qualitative (e.g., immunohistochemistry) or quantitative (e.g., flow cytometry) methods known to those of skill for detecting surface polypeptides. An exemplary assay is described in Example 14 of WO2015/085210. In other embodiments, a reduction in the level of soluble MIC is tested by assaying and comparing levels of MIC found in the culture media of MIC-expressing cells in the presence or absence of antibody using, for example, an ELISA. An exemplary assay is described in the examples herein as well as Example 8 of WO2013/117647.

[0350] In certain embodiments, the shedding of MIC is reduced by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%. 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% compared to shedding of MIC in the absence of antibodies described herein.

[0351] In certain embodiments, the reduction in levels of soluble MIC is reduced by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% compared to the reduction in levels of soluble MIC in the absence of antibodies described herein.

[0352] Other biological activities of interest for the antibodies described herein include any that would enhance the antibody's efficacy for treating or delaying progression of cancer or an immune related disease and/or increasing, enhancing, or stimulating an immune response or function in a subject. Such biological activities may include enhancement of NK and T cell cytolytic function,, inhibition of NKG2D downregulation, ability to reduce serum levels of MIC *in vivo*, and inhibition of tumor growth. Assays for these activities are well known in the art. Exemplary assays are described in WO2015/085210. In certain embodiments, the antibodies described herein enhance the biological activity by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%. 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% compared to the level of biological activity in the absence of antibodies described herein.

D. Immunoconjugates

[0353] The invention also provides immunoconjugates comprising an anti-MIC antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or 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.

[0354] In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0355] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment 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.

[0356] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} 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.

[0357] Conjugates of an antibody 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 WO94/11026. 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.

[0358] The immunuoconjugates 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 SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

E. Methods and Compositions for Diagnostics and Detection

[0359] In certain embodiments, any of the anti-MIC antibodies provided herein is useful for detecting the presence of MIC in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as a sample of a tumor from a biopsy, surgical specimen or a fine needle aspirate. In some embodiments, the biological sample is ascites, urine, blood, plasma or serum.

[0360] In one embodiment, an anti-MIC antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of MIC in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-MIC antibody as described herein under conditions permissive for binding of the anti-MIC antibody to MIC, and detecting whether a complex is formed between the anti-MIC antibody and MIC. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-MIC antibody is used to select subjects eligible for therapy with an anti-MIC antibody, e.g. where MIC is a biomarker for selection of patients. Exemplary disorders that may be diagnosed using an antibody of the invention include cancer, such as an epithelial cancer (*e.g.,* melanoma).

[0361] In certain embodiments, labeled anti-MIC antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

F. Pharmaceutical Formulations and Compositions

[0362] Pharmaceutical formulations and compositions of an anti-MIC antibody 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 formulations 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 phosphate, citrate, 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 dextrins; 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 insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, 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.

[0363] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0364] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide an additional medicament (examples of which are provided herein). Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0365] 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-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0366] Sustained-release preparations 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.

[0367] The formulations 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 Compositions

[0368] Any of the anti-MIC antibodies provided herein may be used in therapeutic methods.

[0369] In one aspect, an anti-MIC antibody for use as a medicament is provided. In further aspects, an anti-MIC antibody for use in treating or delaying progression of cancer or an immune related disease (*e.g.*, immune related disease associated with a NKG2D ligand, unresolved acute infection, chronic infection, tumor immunity) or increasing, enhancing or stimulating an immune response or function is provided. In certain embodiments, an anti-MIC antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-MIC antibody for use in a method of treating an individual having cancer or an immune related disease (*e.g.*, immune related disease

associated with a NKG2D ligand, unresolved acute infection, chronic infection, tumor immunity or in need of increasing, enhancing or stimulating an immune response or function comprising administering to the individual an effective amount of the anti-MIC antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further embodiments, the invention provides an anti-MIC antibody for use in reducing shedding of MIC and/or reducing the level of soluble MIC. In certain embodiments, the invention provides an anti-MIC antibody for use in a method of reducing shedding of MIC and/or reducing the level of soluble MIC in an individual comprising administering to the individual an effective of the anti-MIC antibody to reducing shedding of MIC and/or reducing the level of soluble MIC. An "individual" according to any of the above embodiments is preferably a human.

[0370] In a further aspect, the invention provides for the use of an anti-MIC antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treating or delaying progression of cancer or an immune related disease (*e.g.*, immune related disease associated with a NKG2D ligand, unresolved acute infection, chronic infection, tumor immunity) or increasing, enhancing or stimulating an immune response or function. In a further embodiment, the medicament is for use in a method of treating or delaying progression of cancer or an immune related disease (*e.g.*, Immune related disease associated with NKG2D ligand, unresolved acute infection, chronic infection, tumor immunity) or increasing, enhancing or stimulating an immune response or function comprising administering to an individual having cancer or an immune related disease (*e.g.*, immune related disease associated with NKG2D ligand, unresolved acute infection, chronic infection, tumor immunity) or in need of increasing, enhancing or stimulating an immune response or function an effective amount of the medicament. In one such embodiment, 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 embodiment, the medicament is for reducing shedding of MIC and/or reducing the level of soluble MIC. In a further embodiment, the medicament is for use in a method of reducing shedding of MIC and/or reducing the level of soluble MIC.in an individual comprising administering to the individual an amount effective of the medicament to reducing shedding of MIC and/or reducing the level of soluble MIC. An "individual" according to any of the above embodiments may be a human.

[0371] In a further aspect, the invention provides a method for treating in treating or delaying progression of cancer or an immune related disease (*e.g.*, immune related disease associated with NKG2D ligand, unresolved acute infection, chronic infection, tumor immunity) or increasing, enhancing or stimulating an immune response or function. In one embodiment, the method comprises administering to an individual having such cancer or an immune related disease (*e.g.*, Immune related disease associated with NKG2D ligand, unresolved acute infection, chronic infection, tumor immunity) or in need of increasing, enhancing or stimulating an immune response or function an

effective amount of an anti-MIC antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human.

[0372] In a further aspect, the invention provides a method for reducing shedding of MIC and/or reducing the level of soluble MIC in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-MIC antibody reducing shedding of MIC and/or reducing the level of soluble MIC. In one embodiment, an "individual" is a human.

[0373] In embodiments of the therapeutic methods described above, the cancer is any epithelial cancer, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, Merkel cell cancer, or other hematologic malignancies.

[0374] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-MIC antibodies provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-MIC antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-MIC antibodies provided herein and at least one additional therapeutic agent, e.g., as described in the below Combination Therapies section.

[0375] An antibody 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. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0376] Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1

to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0377] For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1mg/kg-10mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (*e.g.* such that the patient receives from about two to about twenty, or *e.g.* about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0378] It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-MIC antibody.

Combination Therapies

[0379] Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, concurrently and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of the anti-MIC antibody and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other. Antibodies of the invention can also be used in combination with radiation therapy.

[0380] In some embodiments, an antibody provided herein may be administered in conjunction with a chemotherapy or chemotherapeutic agent. In some embodiments, an antibody provided herein may be administered in conjunction with a radiation therapy or radiotherapeutic agent. In some embodiments,

an antibody provided herein may be administered in conjunction with a targeted therapy or targeted therapeutic agent. In some embodiments, an antibody provided herein may be administered in conjunction with an immunotherapy or immunotherapeutic agent, for example a monoclonal antibody.

[0381] In some embodiments, an antibody provided herein may be administered in conjunction with a PD-1 axis binding antagonist. A PD-1 axis binding antagonist includes but is not limited to a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. Alternative names for "PD-1" include CD279 and SLEB2. Alternative names for "PD-L1" include B7-H1, B7-4, CD274, and B7-H. Alternative names for "PD-L2" include B7-DC, Btdc, and CD273. In some embodiments, PD-1, PD-Ll , and PD-L2 are human PD-1 , PD-Ll and PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect the PD-1 ligand binding partners are PD-Ll and/or PD-L2. In another embodiment, a PD-Ll binding antagonist is a molecule that inhibits the binding of PD-Ll to its binding partners. In a specific aspect, PD-Ll binding partners are PD-1 and/or B7- 1. In another embodiment, the PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to its binding partners, such as an anti-PD-L2 antibody or immunoadhesin. In a specific aspect, a PD-L2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of MDX-1106 (nivolumab, OPDIVO), Merck 3475 (MK-3475, pembrolizumab, KEYTRUDA), CT- 011 (Pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, BGB-108, and BGB-A317. In some embodiments, the PD-1 binding antagonist is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP-224. In some embodiments, the PD-Ll binding antagonist is anti-PD-Ll antibody. In some embodiments, the anti-PD-Ll binding antagonist is selected from the group consisting of YW243.55.S70, MPDL3280A (atezolizumab), MEDI4736 (durvalumab), MDX-1105, and MSB0010718C (avelumab). MDX-1105, also known as BMS-936559, is an anti-PD-Ll antibody described in WO2007/005874. Antibody YW243.55.S70 (heavy and light chain variable region sequences shown in SEQ ID Nos. 20 and 21, respectively) is an anti-PD-Ll described in WO 2010/077634 Al . MDX-1106, also known as MDX-1106-04, ONO-4538, BMS-936558 or nivolumab, is an anti-PD-1 antibody described in WO2006/121168. Merck 3475, also known as MK-3475, SCH-900475 or pembrolizumab, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT, hBAT-1 or pidilizumab, is an anti- PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PD-L2- Fc fusion soluble receptor described in WO2010/027827 and WO201 1/066342. In some embodiments, the anti-PD-1 antibody is MDX- 1106. Alternative names for "MDX- 1106" include MDX-1 106-04, ONO-4538,

BMS-936558 or nivolumab. In some embodiments, the anti-PD-1 antibody is nivolumab (CAS Registry Number: 946414-94-4).

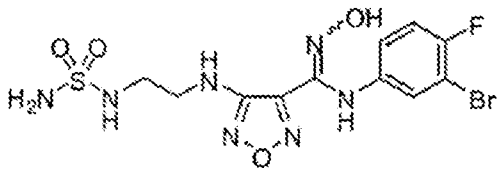
[0382] In some embodiments, an antibody provided herein may be administered in conjunction with an agonist directed against an activating co-stimulatory molecule. In some embodiments, an activating co-stimulatory molecule may include CD40, CD226, CD28, GITR, CD137, CD27, HVEM, or CD127. In some embodiments, the agonist directed against an activating co-stimulatory molecule is an agonist antibody that binds to CD40, CD226, CD28, OX40, GITR, CD137, CD27, HVEM, or CD127. In some embodiments, an antibody provided herein may be administered in conjunction with an antagonist directed against an inhibitory co-stimulatory molecule. In some embodiments, an inhibitory co-stimulatory molecule may include CTLA-4 (also known as CD152), PD-1, TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO, TIGIT, MICA/B, or arginase. In some embodiments, the antagonist directed against an inhibitory co-stimulatory molecule is an antagonist antibody that binds to CTLA-4, PD-1, TIM-3, BTLA, VISTA, LAG-3 (e.g., LAG-3-IgG fusion protein (IMP321)), B7-H3, B7-H4, IDO, TIGIT, MICA/B, or arginase.

[0383] In some embodiments, an antibody provided herein may be administered in conjunction with an antagonist directed against CTLA-4 (also known as CD152), *e.g.,* a blocking antibody. In some embodiments, an antibody provided herein may be administered in conjunction with ipilimumab (also known as MDX-010, MDX-101, or Yervoy®). In some embodiments, an antibody provided herein may be administered in conjunction with tremelimumab (also known as ticilimumab or CP-675,206). In some embodiments, an antibody provided herein may be administered in conjunction with an antagonist directed against B7-H3 (also known as CD276), *e.g.,* a blocking antibody. In some embodiments, an antibody provided herein may be administered in conjunction with MGA271. In some embodiments, an antibody provided herein may be administered in conjunction with an antagonist directed against a TGF beta, *e.g.,* metelimumab (also known as CAT-192), fresolimumab (also known as GC1008), or LY2157299.

[0384] In some embodiments, an antibody provided herein may be administered in conjunction with a treatment comprising adoptive transfer of a T cell (*e.g.,* a cytotoxic T cell or CTL) expressing a chimeric antigen receptor (CAR). In some embodiments, an antibody provided herein may be administered in conjunction with UCART19. In some embodiments, an antibody provided herein may be administered in conjunction with WT128z. In some embodiments, an antibody provided herein may be administered in conjunction with KTE-C19 (Kite). In some embodiments, an antibody provided herein may be administered in conjunction with CTL019 (Novartis). In some embodiments, an antibody provided herein may be administered in conjunction with a treatment comprising adoptive transfer of a T cell comprising a dominant-negative TGF beta receptor, *e.g,* a dominant-negative TGF beta type II receptor. In some embodiments, an antibody provided herein may be administered in conjunction with a treatment comprising a HERCREEM protocol (see, e.g., ClinicalTrials.gov Identifier NCT00889954).

[0385] In some embodiments, an antibody provided herein may be administered in conjunction with an antagonist directed against CD19. In some embodiments, an antibody provided herein may be administered in conjunction with MOR00208. In some embodiments, an antibody provided herein may be administered in conjunction with an antagonist directed against CD38. In some embodiments, an antibody provided herein may be administered in conjunction with daratumumab.

[0386] In some embodiments, an antibody provided herein may be administered in conjunction with an agonist directed against CD137 (also known as TNFRSF9, 4-1BB, or ILA), *e.g.,* an activating antibody. In some embodiments, an antibody provided herein may be administered in conjunction with urelumab (also known as BMS-663513). In some embodiments, an antibody provided herein may be administered in conjunction with an agonist directed against CD40, *e.g.,* an activating antibody. In some embodiments, an antibody provided herein may be administered in conjunction with CP-870893 or RO7009789. In some embodiments, an antibody provided herein may be administered in conjunction with an agonist directed against OX40 (also known as CD134), *e.g.,* an activating antibody. In some embodiments, an antibody provided herein may be administered in conjunction with an agonist directed against CD27, *e.g.,* an activating antibody. In some embodiments, an antibody provided herein may be administered in conjunction with CDX-1127 (also known as varlilumab). In some embodiments, an antibody provided herein may be administered in conjunction with an antagonist directed against indoleamine-2,3-dioxygenase (IDO). In some embodiments, with the IDO antagonist is 1-methyl-D-tryptophan (also known as 1-D-MT). In some embodiments, the IDO antagonist is an IDO antagonist shown in WO2010/005958 (the contents of which are expressly incorporated by record herein). In some embodiments the IDO antagonist is 4-({2-[(Aminosulfonyl)amino]ethyl}amino)-*N*-(3-bromo-4-fluorophenyl)-*N'*-hydroxy-1,2,5-oxadiazole-3-carboximidamide (*e.g.,* as described in Example 23 of WO2010/005958). In some embodiments the IDO antagonist is



In some embodiments, the IDO antagonist is INCB24360. In some embodiments, the IDO antagonist is Indoximod (the D isomer of 1-methyl-tryptophan). In some embodiments, an antibody provided herein may be administered in conjunction with an antibody-drug conjugate. In some embodiments, the antibody-drug conjugate comprises mertansine or monomethyl auristatin E (MMAE). In some embodiments, an antibody provided herein may be administered in conjunction with an anti-NaPi2b antibody-MMAE conjugate (also known as DNIB0600A, RG7599 or lifastuzumab vedotin). In some embodiments, an antibody provided herein may be administered in conjunction with trastuzumab emtansine (also known as T-DM1, ado-trastuzumab emtansine, or KADCYLA®, Genentech). In some embodiments, an antibody provided herein may be administered in conjunction with an anti-

MUC16 antibody-MMAE conjugate, DMUC5754A. In some embodiments, an antibody provided herein may be administered in conjunction with an anti-MUC16 antibody-MMAE conjugate, DMUC4064A. In some embodiments, an antibody provided herein may be administered in conjunction with an antibody-drug conjugate targeting the endothelin B receptor (EDNBR), *e.g.,* an antibody directed against EDNBR conjugated with MMAE. In some embodiments, an antibody provided herein may be administered in conjunction with an antibody-drug conjugate targeting the lymphocyte antigen 6 complex, locus E (Ly6E), *e.g.,* an antibody directed against Ly6E conjugated with MMAE, (also known as DLYE5953A). In some embodiments, an antibody provided herein may be administered in conjunction with polatuzumab vedotin. In some embodiments, an antibody provided herein may be administered in conjunction with an antibody-drug conjugate targeting CD30. In some embodiments, an antibody provided herein may be administered in conjunction with ADCETRIS (also known as brentuximab vedotin). In some embodiments, an antibody provided herein may be administered in conjunction with polatuzumab vedotin.

[0387] In some embodiments, an antibody provided herein may be administered in conjunction with an angiogenesis inhibitor. In some embodiments, an antibody provided herein may be administered in conjunction with an antibody directed against a VEGF, *e.g.,* VEGF-A. In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab (also known as AVASTIN®, Genentech). In some embodiments, an antibody provided herein may be administered in conjunction with an antibody directed against angiopoietin 2 (also known as Ang2). In some embodiments, an antibody provided herein may be administered in conjunction with MEDI3617. In some embodiments, an antibody provided herein may be administered in conjunction with an antibody directed against VEGFR2. In some embodiments, an antibody provided herein may be administered in conjunction with ramucirumab. In some embodiments, an antibody provided herein may be administered in conjunction with a VEGF Receptor fusion protein. In some embodiments, an antibody provided herein may be administered in conjunction with aflibercept. In some embodiments, an antibody provided herein may be administered in conjunction with ziv-aflibercept (also known as VEGF Trap or Zaltrap®). In some embodiments, an antibody provided herein may be administered in conjunction with a bispecific antibody directed against VEGF and Ang2. In some embodiments, an antibody provided herein may be administered in conjunction with RG7221 (also known as vanucizumab). In some embodiments, an antibody provided herein may be administered in conjunction with an angiogenesis inhibitor and in conjunction with a PD-1 axis binding antagonist (e.g., a PD-1 binding antagonist such as an anti-PD-1 antibody, a PD-L1 binding antagonist such as an anti-PD-L1 antibody, and a PD-L2 binding antagonist such as an anti-PD-L2 antibody). In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and a PD-1 axis binding antagonist (e.g., a PD-1 binding antagonist such as an anti-PD-1 antibody, a PD-L1 binding antagonist such as an anti-PD-L1 antibody, and a PD-L2 binding antagonist such as an anti-PD-L2 antibody). In some embodiments, an antibody provided herein may be administered in

conjunction with bevacizumab and MDX-1106 (nivolumab, OPDIVO). In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and Merck 3475 (MK-3475, pembrolizumab, KEYTRUDA). In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and CT- 011 (Pidilizumab). In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and MEDI-0680 (AMP-514). In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and PDR001. In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and REGN2810. In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and BGB-108. In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and BGB-A317. In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and YW243.55.S70. In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and MPDL3280A. In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and MEDI4736. In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and MDX-1105. In some embodiments, an antibody provided herein may be administered in conjunction with bevacizumab and MSB0010718C (avelumab).

[0388] In some embodiments, an antibody provided herein may be administered in conjunction with an antineoplastic agent. In some embodiments, an antibody provided herein may be administered in conjunction with an agent targeting CSF-1R (also known as M-CSFR or CD115). In some embodiments, an antibody provided herein may be administered in conjunction with anti-CSF-1R antibody (also known as IMC-CS4 or LY3022855) In some embodiments, an antibody provided herein may be administered in conjunction with anti-CSF-1R antibody, RG7155 (also known as RO5509554 or emactuzumab). In some embodiments, an antibody provided herein may be administered in conjunction with an interferon, for example interferon alpha or interferon gamma. In some embodiments, an antibody provided herein may be administered in conjunction with Roferon-A (also known as recombinant Interferon alpha-2a). In some embodiments, an antibody provided herein may be administered in conjunction with GM-CSF (also known as recombinant human granulocyte macrophage colony stimulating factor, rhu GM-CSF, sargramostim, or Leukine®). In some embodiments, an antibody provided herein may be administered in conjunction with IL-2 (also known as aldesleukin or Proleukin®). In some embodiments, an antibody provided herein may be administered in conjunction with IL-12. In some embodiments, an antibody provided herein may be administered in conjunction with IL27. In some embodiments, an antibody provided herein may be administered in conjunction with IL-15. In some embodiments, an antibody provided herein may be administered in conjunction with ALT-803. In some embodiments, an antibody provided herein may be administered in conjunction with an antibody targeting CD20. In some embodiments, the antibody targeting CD20 is obinutuzumab (also known as GA101 or Gazyva®) or rituximab. In some

embodiments, an antibody provided herein may be administered in conjunction with an antibody targeting GITR. In some embodiments, the antibody targeting GITR is TRX518. In some embodiments, the antibody targeting GITR is MK04166 (Merck).

[0389] In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of Bruton's tyrosine kinase (BTK). In some embodiments, an antibody provided herein may be administered in conjunction with ibrutinib. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of Isocitrate dehydrogenase 1 (IDH1) and/or Isocitrate dehydrogenase 2 (IDH2). In some embodiments, an antibody provided herein may be administered in conjunction with AG-120 (Agios).

[0390] In some embodiments, an antibody provided herein may be administered in conjunction with obinutuzumab and a PD-1 axis binding antagonist (e.g., a PD-1 binding antagonist such as an anti-PD-1 antibody, a PD-L1 binding antagonist such as an anti-PD-L1 antibody, and a PD-L2 binding antagonist such as an anti-PD-L2 antibody).

[0391] In some embodiments, an antibody provided herein may be administered in conjunction with a cancer vaccine. In some embodiments, the cancer vaccine is a peptide cancer vaccine, which in some embodiments is a personalized peptide vaccine. In some embodiments the peptide cancer vaccine is a multivalent long peptide, a multi-peptide, a peptide cocktail, a hybrid peptide, or a peptide-pulsed dendritic cell vaccine (see, e.g., Yamada et al., Cancer Sci, 104:14-21, 2013). In some embodiments, an antibody provided herein may be administered in conjunction with an adjuvant. In some embodiments, an antibody provided herein may be administered in conjunction with a treatment comprising a TLR agonist, *e.g.,* Poly-ICLC (also known as Hiltonol®), LPS, MPL, or CpG ODN. In some embodiments, an antibody provided herein may be administered in conjunction with tumor necrosis factor (TNF) alpha. In some embodiments, an antibody provided herein may be administered in conjunction with IL-1. In some embodiments, an antibody provided herein may be administered in conjunction with HMGB1. In some embodiments, an antibody provided herein may be administered in conjunction with an IL-10 antagonist. In some embodiments, an antibody provided herein may be administered in conjunction with an IL-4 antagonist. In some embodiments, an antibody provided herein may be administered in conjunction with an IL-13 antagonist. In some embodiments, an antibody provided herein may be administered in conjunction with an IL-17 antagonist. In some embodiments, an antibody provided herein may be administered in conjunction with an HVEM antagonist. In some embodiments, an antibody provided herein may be administered in conjunction with an ICOS agonist, *e.g.,* by administration of ICOS-L, or an agonistic antibody directed against ICOS. In some embodiments, an antibody provided herein may be administered in conjunction with a treatment targeting CX3CL1. In some embodiments, an antibody provided herein may be administered in conjunction with a treatment targeting CXCL9. In some embodiments, an antibody provided herein may be administered in conjunction with a treatment targeting CXCL10. In some embodiments, an antibody provided herein may be administered in conjunction with a treatment

targeting CCL5. In some embodiments, an antibody provided herein may be administered in conjunction with an LFA-1 or ICAM1 agonist. In some embodiments, an antibody provided herein may be administered in conjunction with a Selectin agonist.

[0392] In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of B-Raf. In some embodiments, an antibody provided herein may be administered in conjunction with vemurafenib (also known as Zelboraf®). In some embodiments, an antibody provided herein may be administered in conjunction with dabrafenib (also known as Tafinlar®). In some embodiments, an antibody provided herein may be administered in conjunction with encorafenib (LGX818).

[0393] In some embodiments, an antibody provided herein may be administered in conjunction with an EGFR inhibitor. In some embodiments, an antibody provided herein may be administered in conjunction with erlotinib (also known as Tarceva®). In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of EGFR-T790M. In some embodiments, an antibody provided herein may be administered in conjunction with gefitinib. In some embodiments, an antibody provided herein may be administered in conjunction with afatinib. In some embodiments, an antibody provided herein may be administered in conjunction with cetuximab (also known as Erbitux®). In some embodiments, an antibody provided herein may be administered in conjunction with panitumumab (also known as Vectibix®). In some embodiments, an antibody provided herein may be administered in conjunction with rociletinib. In some embodiments, an antibody provided herein may be administered in conjunction with AZD9291. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of a MEK, such as MEK1 (also known as MAP2K1) and/or MEK2 (also known as MAP2K2). In some embodiments, an antibody provided herein may be administered in conjunction with cobimetinib (also known as GDC-0973 or XL-518). In some embodiments, an antibody provided herein may be administered in conjunction with trametinib (also known as Mekinist®). In some embodiments, an antibody provided herein may be administered in conjunction with binimetinib.

[0394] In some embodiments, an antibody provided herein may be administered in conjunction an inhibitor of B-Raf (e.g., vemurafenib or dabrafenib) and an inhibitor of MEK (e.g., MEK1 and/or MEK2 (e.g., cobimetinib or trametinib). In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of ERK (e.g., ERK1/2). In some embodiments, an antibody provided herein may be administered in conjunction with GDC-0994). In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of B-Raf, an inhibitor of MEK, and an inhibitor of ERK1/2. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of EGFR, an inhibitor of MEK, and an inhibitor of ERK1/2. In some embodiments, an antibody provided herein may be administered in conjunction with one or more MAP kinase pathway inhibitor. In some embodiments, an antibody

provided herein may be administered in conjunction with CK127. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of K-Ras.

[0395] In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of c-Met. In some embodiments, an antibody provided herein may be administered in conjunction with onartuzumab (also known as MetMAb). In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of anaplatic lymphoma kinase (ALK). In some embodiments, an antibody provided herein may be administered in conjunction with AF802 (also known as CH5424802 or alectinib). In some embodiments, an antibody provided herein may be administered in conjunction with crizotinib. In some embodiments, an antibody provided herein may be administered in conjunction with ceritinib. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of a phosphatidylinositol 3-kinase (PI3K). In some embodiments, an antibody provided herein may be administered in conjuction with buparlisib (BKM-120). In some embodiments, an antibody provided herein may be administered in conjunction with pictilisib (also known as GDC-0941). In some embodiments, an antibody provided herein may be administered in conjunction with buparlisib (also known as BKM-120). In some embodiments, an antibody provided herein may be administered in conjunction with perifosine (also known as KRX-0401). In some embodiments, an antibody provided herein may be administered in conjunction with a delta-selective inhibitor of a phosphatidylinositol 3-kinase (PI3K). In some embodiments, an antibody provided herein may be administered in conjunction with idelalisib (also known as GS-1101 or CAL-101). In some embodiments, an antibody provided herein may be administered in conjunction with taselisib (also known as GDC-0032). In some embodiments, an antibody provided herein may be administered in conjunction with BYL-719. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of an Akt. In some embodiments, an antibody provided herein may be administered in conjunction with MK2206. In some embodiments, an antibody provided herein may be administered in conjunction with GSK690693. In some embodiments, an antibody provided herein may be administered in conjunction with ipatasertib (also known as GDC-0068). In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of mTOR. In some embodiments, an antibody provided herein may be administered in conjunction with sirolimus (also known as rapamycin). In some embodiments, an antibody provided herein may be administered in conjunction with temsirolimus (also known as CCI-779 or Torisel®). In some embodiments, an antibody provided herein may be administered in conjunction with everolimus (also known as RAD001). In some embodiments, an antibody provided herein may be administered in conjunction with ridaforolimus (also known as AP-23573, MK-8669, or deforolimus). In some embodiments, an antibody provided herein may be administered in conjunction with OSI-027. In some embodiments, an antibody provided herein may be administered in conjunction with AZD8055. In some embodiments, an antibody provided herein may be administered in conjunction with INK128. In some embodiments, an antibody provided herein may be administered

in conjunction with a dual PI3K/mTOR inhibitor. In some embodiments, an antibody provided herein may be administered in conjunction with XL765. In some embodiments, an antibody provided herein may be administered in conjunction with GDC-0980. In some embodiments, an antibody provided herein may be administered in conjunction with BEZ235 (also known as NVP-BEZ235). In some embodiments, an antibody provided herein may be administered in conjunction with BGT226. In some embodiments, an antibody provided herein may be administered in conjunction with GSK2126458. In some embodiments, an antibody provided herein may be administered in conjunction with PF-04691502. In some embodiments, an antibody provided herein may be administered in conjunction with PF-05212384 (also known as PKI-587).

[0396] In some embodiments, an antibody provided herein may be administered in conjunction with an agent that selectively degrades the estrogen receptor. In some embodiments, an antibody provided herein may be administered in conjunction with GDC-0927. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of HER3. In some embodiments, an antibody provided herein may be administered in conjunction with duligotuzumab. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of LSD1. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of MDM2. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of BCL2. In some embodiments, an antibody provided herein may be administered in conjunction with venetoclax. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of CHK1. In some embodiments, an antibody provided herein may be administered in conjunction with GDC-0575. In some embodiments, an antibody provided herein may be administered in conjunction with an inhibitor of activated hedgehog signaling pathway. In some embodiments, an antibody provided herein may be administered in conjunction with ERIVEDGE.

[0397] In some embodiments, an antibody provided herein may be administered in conjunction with radiation therapy. In some embodiments, an antibody provided herein may be administered in conjunction with gemcitabine. In some embodiments, an antibody provided herein may be administered in conjunction with nab-paclitaxel (ABRAXANE). In some embodiments, an antibody provided herein may be administered in conjunction with trastuzumab. In some embodiments, an antibody provided herein may be administered in conjunction with TVEC. In some embodiments, an antibody provided herein may be administered in conjunction with IL27. In some embodiments, an antibody provided herein may be administered in conjunction with cyclophosphamide. In some embodiments, an antibody provided herein may be administered in conjunction with an agent that recruits T cells to the tumor. In some embodiments, an antibody provided herein may be administered in conjunction with lirilumab (IPH2102/BMS-986015). In some embodiments, an antibody provided herein may be administered in conjunction with Idelalisib. In some embodiments, an antibody provided herein may be administered in conjunction with an antibody that targets CD3 and CD20. In

some embodiments, an antibody provided herein may be administered in conjunction with REGN1979. In some embodiments, an antibody provided herein may be administered in conjunction with an antibody that targets CD3 and CD19. In some embodiments, an antibody provided herein may be administered in conjunction with blinatumomab.

[0398] In some embodiments, an antibody provided herein may be administered in conjunction with an oncolytic virus. In some embodiments, an antibody provided herein may be administered in conjunction with carboplatin and nab-paclitaxel. In some embodiments, an antibody provided herein may be administered in conjunction with carboplatin and paclitaxel. In some embodiments, an antibody provided herein may be administered in conjunction with cisplatin and pemetrexed. In some embodiments, an antibody provided herein may be administered in conjunction with cisplatin and gemcitabine. In some embodiments, an antibody provided herein may be administered in conjunction with FOLFOX. In some embodiments, an antibody provided herein may be administered in conjunction with FOLFIRI.

[0399] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody provided herein can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies provided herein can also be used in combination with radiation therapy.

H. Articles of Manufacture

[0400] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the conditions of choice described herein. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection

(BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0401] It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-MIC antibody.

III. METHODS FOR EPITOPE MAPPING

[0402] In one aspect, the invention is based on Applicants' discovery that introduction of glycosylation on an antigen can block antibody binding and provide information on the antigen epitope. Without wishing to be bound by theory, Applicants believe that glycosylation of specific amino acids of a polypeptide can disrupt antibody binding and accordingly, that reduced binding of an antibody to a glycosylated polypeptide compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid indicates that at least one of the previously unglycosylated amino acid or surface-exposed amino acids in its structural proximity, *e.g.*, amino acids within 1, 2, 3, 4, or 5 Angstroms and accessible to antibodies, are a part of an epitope or a contact amino acid of the antibody. In some aspects, the methods described herein relate to mapping an epitope of an antibody. In other aspects, the methods described herein relate to identifying the contact amino acids of an antibody.

[0403] In some embodiments, the methods comprise (a) substituting an unglycosylated amino acid of a polypeptide to generate a glycosylated polypeptide comprising a substituted glycosylated amino acid; (b) determining whether the antibody binds to the glycosylated polypeptide; and (c) identifying at least one of the unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid as part of the epitope or one of the contact amino acids of the antibody if binding of the antibody to the glycosylated polypeptide is reduced compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

[0404] In some embodiments, the methods comprise (a) substituting an unglycosylated amino acid of a polypeptide to generate a glycosylated polypeptide comprising a substituted glycosylated amino acid; and (b) determining whether the antibody binds to the glycosylated polypeptide, wherein at least one of the unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid is identified as part of the epitope or one of the contact amino acids of the antibody if binding of the antibody to the glycosylated polypeptide is reduced compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

[0405] In some embodiments, the methods comprise identifying at least one of an unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid as part of the epitope or one of the contact amino acids of the antibody if binding of the antibody to a glycosylated polypeptide comprising a substituted glycosylated amino acid is reduced compared to

binding of the antibody to a polypeptide without the substituted glycosylated amino acid, wherein the glycosylated polypeptide is generated by substituting an unglycosylated amino acid of the polypeptide.

[0406] The methods described herein can be used to map the epitope or identify the contact amino acids for any polypeptide that can be expressed in a recombinant expression host with glycosylation machinery. The polypeptide may be a full-length protein, a fragment of a protein, and/or a protein fused to another domain to facilitate expression and folding, *e.g.*, a Fc region. The epitope or contact amino acids to be mapped or identified may be linear or conformational.

[0407] In some embodiments, substitution of the unglycosylated amino acid of the polypeptide is accomplished by introducing or creating a glycosylation site in the polypeptide. The glycosylation site may be for any form of glycosylation, including, but not limited to, N-linked glycosylation, O-linked glycosylation, C-mannosylation, and glypiation. In eukaryotes, glycosylation sites for N-linked glycosylation are defined by a consensus sequence (N-X-S/T (standard sequons); N-X-C, N-G, N-X-V (non-standard sequons) (Moremen et al. *Nature Reviews Molecular Cell Biology* **13,** 448–462 (2012)); X=any residue except P). For other types of glycosylation, bioinformatics software may be used to predict glycosylation sites *e.g.*, Caragea et al. *BMC Bioinformatics* **8,** 438 (2007). Methods for engineering glycosylation sites via site-directed mutagenesis are well known in the art. Introduction of N-linked glycosylation sites and confirmation of glycosylation by increased MW as measured by SDS-PAGE are described in Eggink et al. *J. Virol.* **88,** 699–704 (2014). Methods are also described in Chandramouli et al. "Structure of HCMV glycoprotein B in the postfusion conformation bound to a broadly neutralizing human antibody" *Nature Communications,* in press. In some embodiments, to minimize the number of mutations needed for glycosylation, the glycosylation site is introduced at the site of a partial glycosylation motif. For N-linked glycosylation, a partial glycosylation motif in the primary sequence of the polypeptide is present when an asparagine residue is not followed by a proline, or when serine and threonine residues are not preceded by proline.

[0408] The substituted glycosylated amino acid in the methods described herein may comprise any type of glycan that can be linked to an amino acid and disrupts antibody binding. For example, a N-linked glycan, an O-linked glycan, a C-linked mannose, or a glycolipid.

[0409] In some embodiments, the unglycosylated amino acid is on the surface of the polypeptide without the substituted glycosylated amino acid. Any means of predicting or visualizing the structure of a polypeptide, such as a crystal structure or homology model, can be used to identify surface exposed positions to ensure properly folded material. Homology models can be created by modeling software such as SwissModel, MOE (CCG), Bioluminate (Schrödinger).

[0410] Binding of the antibody to the glycosylated polypeptide may be detected by any means known in the art to detect binding between antibodies and polypeptides, including ELISA and other methods described in the Assay section of this disclosure. Binding of the antibody to the glycosylated polypeptide may be reduced by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%. 50%, 55%, 60%, 65%,

70%, 75%, 80%, 85%, 90%, 95%, or 100% compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

[0411] In some embodiments, the methods of epitope mapping and identifying contact amino acids of the antibody also include steps to ensure that the substitution of the unglycosylated amino acid with a substituted glycosylated amino acid does not alter the overall integrity or structure of the polypeptide. Exemplary steps include testing for binding of the glycosylated polypeptide to control antibodies that bind outside of the engineered glycosylation area. Other exemplary steps include performing biophysical techniques such as differential scanning fluorimetry (DSF) (Niesen et al. *Nat Protoc* **2,** 2212–2221 (2007)), differential scanning calorimetry (DSC) Makhatadze, G. I. (1998) *Characterization of Recombinant Proteins, Chapter 7: Measuring Protein Thermostability by Differential Scanning Calorimetry,* 7.9.1–7.9.14. In *Current Protocols in Protein Science b*y John Wiley & Sons, Inc.. doi:10.1002/0471140864.ps0709s12/pdf**,** circular dichroism (CD) (Greenfield et al. *Nat Protoc* **1,** 2876–2890 (2006)) to confirm the polypeptide still properly folds after substitution of the unglycosylated amino acid with a substituted glycosylated amino acid. In other embodiments, the methods of epitope mapping and identifying contact amino acids of the antibody also include steps to ensure that the substitution of the unglycosylated amino acid with a glycosylated amino acid is successful, for example, by mass-spectrometric assays, gel-shift assays or a Western. In some embodiments, subsequent to mapping of the epitope or identifying contact amino acids, alanine scanning is used to fine map the epitope.

[0412] Methods for binning antibodies are also provided. The methods comprise mapping an epitope or identifying contact amino acids of a first antibody by any of the methods described above, mapping an epitope or identifying contact amino acids of a second antibody by any of the methods described above, and determining that the first antibody and the second antibody are in the same bin if they have the same epitope or contact amino acids.

[0413] Antibodies described herein, where the epitope is mapped by the above methods or the contact amino acids are identified by the above methods, are also provided.

[0414] The specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLES

[0415] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various

modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: Generation of Anti-MICA/B Antibodies

[0416] This Example describes the strategies that were used to generate anti-MICA/B antibodies.

Legacy Immunization Protocol

[0417] A panel of antibodies that selectively bind human MICA were generated using MICA fused to Fc (in house) as an immunogen. Ten BALB/c mice were each injected at the footpad with MICA-Fc protein resuspended with 2-component Ribi in PBS. All subsequent boosts contained MICA-Fc protein resuspended with Ribi in PBS. Nine rounds of boosts followed every 3 to 4 days at footpad.

GH Immunization Protocol

[0418] A panel of antibodies that selectively bind human MICA were generated using MICA alpha 3 domains (alleles 002, 004, 008 and MICB 005) fused to murine IgG2a (in house) as an immunogen. Five BALB/c mice were each initially injected at the base of tail with 50 ug MICA protein (mixture of alleles) resuspended with Complete Freund's Adjuvant (CFA) in PBS. All subsequent boosts contained mixtures of MICA alleles resuspended with Incomplete Freund's Adjuvant (IFA) in PBS. Five rounds of boosts followed every two weeks at different sites (hock 16 ug/mouse, IP 8 ug/mouse, subcutaneous 8 ug/mouse, and/or base of tail 8 ug/mouse).

GG Immunization Protocol

[0419] A panel of antibodies that selectively bind human MICA were generated using MICA alpha 3 domains (allelles 002, 004, 008 and MICB 005) fused to murine IgG2a (in house) as an immunogen. Five BALB/c mice were each initially injected at the hock and IP with 6 and 3 ug of MICA002 alpha 3 domain fused to muIgG2a protein (mixture of alleles) resuspended with cocktail adjuvant (cocktail consisting of poly I:C+monophosphoryl lipid A (MBL)+R848+CpG) in PBS per mouse respectively. All subsequent boosts contained sequential immunization of different MICA alpha 3 domains fused to muIgG2a (alleles 004, 008 and MICB005) resuspended with cocktail adjuvant in PBS. Fifteen rounds of boosts followed every 3-4 days at different sites (hock 6 ug/mouse, IP 3 ug/mouse, subcutaneous 3 ug/mouse, and/or base of tail 3 ug/mouse).

HZ Immunization Protocol

[0420] A panel of antibodies that bind human MICA were generated using MICA/B ECD domains (allelles 002, 004, 008 and MICB 005) with a Flag tag (in house). Five BALB/c mice were each initially injected at the base of tail with 50 ug MICA/B protein (mixture of alleles) resuspended with cocktail adjuvant (poly I:C+monophosphoryl lipid A (MBL)+R848+CpG) in PBS. All subsequent boosts contained mixtures of MICA alleles resuspended with cocktail adjuvant in PBS. Mice were

injected once a week at different sites (hock 16 ug/mouse, IP 8 ug/mouse, subcutaneous 8 ug/mouse, and/or base of tail 8 ug/mouse).

Hybridoma Fusing And Development

[0421] For all of the above described immunizations, three days after the final boost, animals which showed positive serum titers by ELISA were sacrificed, and a single cell suspension of splenocytes was fused with the mouse myeloma cell line P3X63Ag.U.1 (American Type Culture Collection, Manassas, VA) using electrofusion (Cyto Pulse Sciences, Inc., Glen Burnie, MD). Fused hybridoma cells were selected from unfused splenic, popliteal node or myeloma cells using hypoxanthin-aminopterin-thymidine (HAT) selection in Medium D from the ClonaCell® hybridoma selection kit (StemCell Technologies, Inc., Vancouver, BC, Canada). Hybridoma cells were cultured in Medium E from the ClonaCell® hybridoma selection kit, and cell culture supernatants were used for further characterization and screening.

Example 2: Binding Assays

[0422] This Example describes identification of hybridoma clones that bind to human MICA/B alleles, as measured by ELISA. Antibodies that bind ELISA were further tested using Biacore.

ELISA

[0423] Enzyme-linked immunosorbent assays (ELISAs) were used to determine if hybridoma clones described as above were producing monoclonal antibodies that bound to human MICA/B alleles. Nunc Maxisorp plates were coated with human MICA alleles 002, 004, 008 or MICB 005 alpha 3 domain (in-house) at 2 ug/ml in 50 mM sodium carbonate buffer, pH 9.6 overnight at 4°C. Supernatants from mouse derived hybridomas were added to the plates after blocking for a hour with a 1X PBS buffer containing 0.5% BSA and 0.05% Tween 20. After incubation, plates were washed multiple times with wash buffer (1X PBS containing 0.05% Tween 20) and secondary antibody (goat anti-mouse IgG Fc conjugated to HRP, Sigma) was added. Plates were then washed multiple times with wash buffer again before adding either BioFX TMB Microwell 1 Component Peroxidase. After several minutes of incubation, the reaction was stopped with BioFX Stop Reagent for TMB Microwell. Plates were read on a Spectra MAX 340 plate reader (Molecular Devices; Sunnyvale, CA) at 630 nm.

[0424] The sequence of the MICA*008 alpha3 construct is provided below:

>MICA008.muIgG2a (a3 domain in bold). Note: This was only used for the glyco variants.

G**STVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGN GTYQTWVATRICRGEEQRFTCYMEHSGNHSTHPVPS**GNSRAQVTDKKIEPRGPTIKPCPPC KCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQT HREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPP

EEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKK NWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK (SEQ ID: 194)

His-tagged MICA proteins used for ELISA and Biacore.

[0425] The sequence of the MICA*008 alpha3 construct is provided below:

>MICA008.His (a3 domain in bold)

GS**TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGN GTYQTWVATRICRGEEQRFTCYMEHSGNHSTHPVPS**GNSHHHHHHHH (SEQ ID: 195)

The sequence of the MICA*002 alpha3 construct is provided below:

>MICA002.His (a3 domain in bold)

GS**TVPPMVNVTRSEASEGNITVTCRASGFYPWNITLSWRQDGVSLSHDTQQWGDVLPDG N GTYQTWVATRICQGEEQRFTCYMEHSGNHSTHPVPS**GNSHHHHHHHH (SEQ ID: 196)
The sequence of the MICA*004 alpha3 construct is provided below:

>MICA004.His (a3 domain in bold)

GS**VPPMVNVTRSEASEGNITVTCRASSFYPRNITLTWRQDGVSLSHDTQQWGDVLPDGN G TYQTWVATRICQGEEQRFTCYMEHSGNHSTHPVPS**GNSHHHHHHHH (SEQ ID: 197)

The sequence of the MICB*005 alpha3 construct is provided below:

>MICB005.His (a3 domain in bold)

GS**TVPPMVNVTCSEVSEGNITVTCRASSFYPRNITLTWRQDGVSLSHNTQQWGDVLPDG N GTYQTWVATRIRQGEEQRFTCYMEHSGNHGTHPVPS**GNSHHHHHHHH (SEQ ID: 198)

Table 2. ELISA data for anti-MICA antibodies and binding to the alpha 3 domain of MICA alleles.

	MICA*008 a3	MICA*002 α3	MICA*004 α3	MICB*005 α3
3C9.10	-	+	+	+
7D4.6	+	+	+	+
6F8.7	+	+	+	+
32D2	+	+	+	+
3E11	+	+	+	+
9C9.5.6	-	+	+	+
1E6.1.3	+	+	+	+
7A3.1.9	+	+	+	+
6E12.5	+	+	+	+
20G11	+	+	+	+
6E1.1.12	+	+	+	+
2E5.2.3	+	+	+	+

1D5	+	+	+	+
15F11	+	+	+	+
13A9	+	+	+	+
12H10	+	+	+	+
18G3	+	+	+	+

Note: ELISA values >0.5 are designated with a plus sign. ELISA values <0.5 are designated with a minus sign.

Biacore

[0426] The binding kinetics of the anti-MICA/B antibodies were measured using surface plasmon resonance (SPR) on a Biacore T200 instrument (GE Healthcare). Anti-murine Fc or anti-human Fc (GE Healthcare) was immobilized on a CM5 sensor chip via amine-based coupling using manufacturer provided protocol. Anti-MICA/B antibody was captured by the anti- Fc and the MICA/B alleles were passed over. Antibody binding was measured to human MICA 002, 004, 008 and MICB 005 alpha 3 domains (His tagged, in-house). Sensograms for binding of MICA/B were recorded using an injection time of 2-3 minutes with a flow rate of 30 ml/min, at a temperature of 25°C, and with a running buffer of 10mM HEPES, pH 7.4, 150 mM NaCl, and 0.005% Tween 20. After injection, disassociation of the alpha 3 domain from the antibody was monitored for 10 minutes in running buffer. The surface was regenerated between binding cycles with a 60 ul injection of 10 mM Glycine HCl pH 1.7. After subtraction of a blank which contained running buffer only, sensograms observed for MICA binding to anti-MICA/B antibody were analyzed using a 1:1 Langmuir binding model with software supplied by the manufacturer to calculate the kinetics and binding constants. The data was analyzed using a 1:1 binding model. Sensograms of MICA alleles binding to captured anti-MICA antibody were used to calculate the dissociation constant (Kd). Kinetic constants from these data are provided in **Table 3**. Final bin assignments for each clone are also shown (see Example 6 for further details regarding final bin assignments). **FIG. 3A-FIG. 3E** depict structural models showing where the epitopes for each bin are located on MICA.

Table 3: Kinetic Constants for Anti-MICA Antibodies Binding to its Ligands

Clone	huMICA*002 alpha 3-his	huMICA*004 alpha 3-his	huMICA*008 alpha 3-his	huMICB*005 alpha 3-his	Final Bin
	Kd (nM)	Kd (nM)	Kd (nM)	Kd (nM)	
3C9.10	81	60	no binding	45	1
9C9.5.6	16	18	no binding	11	3
1E6.1.3	24	37	IC	18	3
7A3.1.9	22	8.7	IC	16	3
6E12.5	4.5	3	15.1	IC	4
6E1.1.12	2.8	8.7	6.8	10	6
7D4.6	3.5	8.9	10	20	2
2E5.2.3	0.66	1.96	1.8	2.7	7
6F8.7	2.4	6.2	8.4	14.5	2
20G11	1.76	1.06	0.77	1.05	5
3E11	7.7	5	2.5	6.8	2
32D2	3.18	2.85	1.94	2.82	2
15F11	3.05	1.22	1.02	3.28	2
13A9	0.50	1.55	0.94	0.97	8
12H10	25.8	7.02	1.48	10.7	9
18G3	10.4	4.03	2.03	6.18	9
1D5	0.67	0.49	0.42	1.09	2

Note: IC: inconclusive since weak binding was observed under experimental conditions despite good capture of monoclonal antibody

Example 3: Epitope Mapping by Octet Competition

Methods

[0427] Antibody epitope bins were determined using the Octet (Fortebio). MICA alpha 3 domain (allele 004, His tag) protein was biotinylated using EZ-LINK NHS-PEG4 Biotin (Pierce). Streptavidin biosensors tips (Fortebio) were used to capture biotinylated MICA protein (180 seconds in 10 ug/ml solution). Baseline was stabilized for 60 seconds before primary antibody (10 ug/ml) was allowed to associate for 300 seconds with captured protein. Panel of secondary antibodies at 5 ug/ml were then allowed to associate with the antigen and primary antibody complex for additional 300 seconds.

Results

[0428] Signals were recorded for each binding event and compared to determine antibody bins. No additional binding upon addition of second antibody indicated overlapping epitope bin. Additional binding upon addition of second antibody indicated separate epitope bin. The results show 3 bins.

Table 4. Epitope mapping by antibody competition with Octet shows 3 bins.

	Octet Bin
3C9.10	1
7D4.6	1
6F8.7	1
32D2	N.D.
3E11	N.D.
9C9.5.6	1
1E6.1.3	1
7A3.1.9	1
6E12.5	1
20G11	N.D.
6E1.1.12	3
15F11	N.D.
13A9	N.D.
12H10	N.D.
18G3	N.D.
1D5	N.D.
2E5.2.3	2

Note: N.D. = not determined.

Example 4: Epitope Mapping by Glycosylation Site Engineering

[0429] This Example illustrates the use of single engineered N-linked glycosylation sites on an antigen to block antibody binding and give information on the antigen epitope. Specifically, this Example shows mapping of an epitope by substituting an unglycosylated amino acid of MICA*008 with a glycosylated asparagine or a threonine residue that introduces an N-linked glycosylation site and testing for antibody binding to a MICA*008 Fc fusion having the glycosylated asparagine. MICA*008 was engineered by introducing single or multiple N-linked glycosylation sites to mask

different antigenic regions. These constructs were used to probe the MICA*008 antigen binding sites of various anti-MICA antibodies.

[0430] A comparative structural study of asparagines in human, mouse, fly, plant and yeast showed that a high percentage of asparagines in N-X-S/T, where X≠P, motifs implicated in N-glycosylation are localized within a turn/loop and are solvent-exposed at the protein surface. The glycosylation site engineered variants were designed with this in mind and engineered into surface exposed residues in loops or near turns (Lam et al., "Structure-based Comparative Analysis and Prediction of N-linked Glycosylation Sites in Evolutionarily Distant Eukaryotes" Genomics Proteomics Bioinformatics (2013) 11(2):96-104).

Methods

Glycosylation Engineering

[0431] A single residue in MICA*008 was changed to Asn or Thr to introduce a new glycosylation site, which has a sequence motif of N-X-S/T, where X≠P. Seven constructs with individual mutations and a hyperglycosylated construct that contained five engineered N-linked glycosylation sites were made. Only residues that were surface exposed in the crystal structures of MICA*001 (Protein Data Bank structures 1B3J and 1HYR) were mutated. These MICA*008 engineered glycosylation site variants were expressed as murine IgG2a Fc fusions, expressed in 293S mammalian cells, and purified by MabSelect Sure.

[0432] The following variants were made (numbering is with respect to human MICA*008 sequence with signal sequence):

Glyco4: E215N.G243N.H248N.R279N

Glyco11: R202N

Glyco12: E215N

Glyco13: I236T

Glyco14: G243N

Glyco15: H248N

Glyco16: R279N

Glyco17: C-terminal insert of N298.G299.S300

[0433] Sequences of glycosylation-engineered sites on MICA*008.α3.murine IgG2a Fc fusion proteins are provided below. Only the MICA*008.α3 domain is shown with the mutations in bold and underlined, without the sequence for the murine IgG2a Fc fusion.

>MICA.008.a3.T204-S297.mIgG2a.Wild-type

TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGNGTYQ TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS (SEQ ID: 199)

>MICA.008.a3.T204-S297.E215N.G243N.H248N.R279N.C-terminal insert of N298.G299.S300.mIgG2a.Glyco4 (Hypergylcosylated)

TVPPMVNVTRS**N**ASEGNITVTCRASSFYPRNIILTWRQD**N**VSLS**N**DTQQWGDVLPDGNGTYQ TWVATRICRGEEQ**N**FTCYMEHSGNHSTHPVPS**NGS** (SEQ ID: 200)

>MICA.008.a3.R202-S297.R202N mIgG2a.Glyco11

NRTVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGNGT YQTWVATRICRGEEQRFTCYMEHSGNHSTHPVPS (SEQ ID: 201)

>MICA.008.a3.T204-S297.E215N.mIgG2a.Glyco12

TVPPMVNVTRS**N**ASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGNGTYQ TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS (SEQ ID: 202)

>MICA.008.a3.T204-S297.I236T.mIgG2a.Glyco13

TVPPMVNVTRSEASEGNITVTCRASSFYPRNI**T**LTWRQDGVSLSHDTQQWGDVLPDGNGTYQ TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS (SEQ ID: 203)

>MICA.008.a3.T204-S297.G243N.mIgG2a.Glyco14

TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQD**N**VSLSHDTQQWGDVLPDGNGTYQ TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS (SEQ ID: 204)

>MICA.008.a3.T204-S297.H248N.mIgG2a.Glyco15

TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLS**N**DTQQWGDVLPDGNGTYQ TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS (SEQ ID: 205)

>MICA.008.a3.T204-S297.R279N.mIgG2a.Glyco16

TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGNGTYQ TWVATRICRGEEQ**N**FTCYMEHSGNHSTHPVPS (SEQ ID: 206)

>MICA.008.a3.T204-S297.C-terminal insert of N298.G299.S300.mIgG2a.Glyco17

TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGNGTYQ TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS**NGS** (SEQ ID: 207)

[0434] Hybridoma derived clones binding to all four MICA/B alleles alpha 3 domains were tested by ELISA for binding to the MICA*008 alpha 3 glycosylation variants fused to murine IgG2a Fc fusion. Nunc maxisorp plates were coated with 2 ug/ml of glycosylation variants as previously described. Cloned and recombinantly expressed hybridoma monoclonal antibodies were added to the plates after blocking with a 1X PBS buffer containing 0.5% BSA and 0.05% Tween 20. After incubation, plates were washed multiple times with wash buffer (1X PBS containing 0.05% Tween 20) and secondary antibody (goat anti-human IgG Fc conjugated to HRP) was added. Plates were then washed multiple times with wash buffer again before adding either BioFX TMB Microwell 1 Component Peroxidase or tetramethylbenzidine (TMB) substrate (KPL; Gaithersburg, MD). After several minutes of incubation, the reaction was stopped with BioFX stop solution or 1 N solution of HCl, respectively. Plates were read on a Spectra MAX 340 plate reader at either 630 or 450 nm respectively (Molecular Devices; Sunnyvale, CA).

Differential Scanning Fluorimetry (DSF)

[0435] Differential Scanning Fluorimetry (DSF) is a method used to measure the thermal stability of a protein. In this method, a SYPRO Orange fluorescent dye is combined with a protein and heated up slowly. When the protein unfolds, the exposed hydrophobic surface binds to the dye giving a fluorescent signal. The fluorescence signal is measured at every temperature during the heating process and the highest signals are correlated to a protein melt.

Protein stability measurements by differential scanning fluorimetry

[0436] Protein stability was determined using a Biorad CFX96 Real-Time System (Biorad, USA) with a final dilution of 1:500 of the Sypro Orange dye stock (Molecular Probes, USA). Fluorescence of a 25 µL sample (0.5 mg/ml) in PBS was recorded from 20-100 °C (0.2 °C increments, 10 seconds hold per step). The muIgG2a melt corresponded to the second transition or peak seen with the MICA.mIgG2a Glyco variants (**FIG. 4A, FIG. 5**). The MICA melt was assigned to the first transition or peak (**FIG. 4A, FIG. 5**).

Gel Shift Assay

[0437] 6 ug of each MICA.mIgG2a Glyco variant was run on a 4-12% Bis-Tris Gel (Life Technologies) using MES-SDS running buffer (50 mM MES, 50 mM TRIS Base pH 7.3, 0.1 % SDS, 1 mM EDTA). Proteins bands were stained with GelCode Blue Safe Protein Stain (Thermo Fisher Scientific). The Glyco-engineered variants show shifts that are upward or distinct compared to wild-type (WT) MICA*008.mIgG2a indicating that they have differing glycosylation states.

Mass Spectrometry

[0438] 5 µg of each protein sample were diluted with 50 mM ammonium bicarbonate pH 8, reduced with 10 mM dithiothreitol at 37°C for 1 hour, and alkylated with 10 mM iodoacetamide at room temperature for 20 minutes. Each sample was separately digested overnight with trypsin (Promega) and chymotrypsin (Thermo Fisher Scientific) at 1:50 enzyme:substrate ratio at 37°C. Peptide digests were quenched with 2% trifluoroacetic acid and subjected to C18 stage-tip clean up. Samples were injected via an auto-sampler onto a 75 µm × 100 mm column (BEH, 1.7 µm, Waters Corp) at a flow rate of 1 µL/min using a NanoAcquity UPLC (Waters). A gradient from 98% solvent A (water + 0.1% formic acid) to 80% solvent B (acetonitrile + 0.1% formic acid) was applied over 40 min. Samples were analyzed on-line via nanospray ionization into a hybrid LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific). Data was collected in data dependent mode with the parent ion being analyzed in the FTMS and the top 8 most abundant ions being selected for fragmentation and analysis in the LTQ. Tandem mass spectrometric data was analyzed using PepFinder software (Thermo Fisher Scientific). Fixed carbamidomethylation on cysteine and variable oxidation on methionine were included in the database search against the protein sequence with a precursor mass tolerance of 20 ppm

and a fragment ion tolerance of 0.8 Da. A CHO N-linked glycan library within the software was also included in the database search for glycosylation site mapping and label-free quantitation.

Results

[0439] If reduced binding to a glycosylation variant was observed, then the potential epitope was presumed to include the glycosylated residue and residues that are structurally within 5 Angstroms of the glycosylated residue.

[0440] It is difficult to predict the size that one N-linked glycosylation can cover due to the varying widths and lengths seen for glycosylation, and the large amount of flexibility, which introduces more size uncertainty. For example, the size of the carbohydrate at each of six N-glycosylation sites in the plasma phospholipid transfer protein ranged from 3.14 to 4.2 kDa as measured by mass spectrometry (Albers et al., "Impact of site-specific N-glycosylation on cellular secretion, activity and specific activity of the plasma phospholipid transfer protein ", Biochimica et Biophysica Acta (2001) 1814(7): 908-11.). However, the data with two antibodies comparing two glycosylation engineered variants and the Ala scanning data suggest that interactions structurally within 5 Angstroms of the Asn in the engineered glycosylation site could be valid epitopes. This is a structural prediction based on a small dataset and using the MICA*001 structure (PDB code 1HYR).

[0441] Listed below are the amino acid residues that are predicted to be within 5Å of each glyco-engineered variant based on the 1HYR structure using Pymol. Residues that were predicted to not have any accessible surface area based upon both PDB structures 1HYR and 1B3J were not included. Mutation of amino acid positions of interest to Asn in the structural model did not alter the results of this analysis.

- Glyco4 variant (E215N.G243N.H248N.R279N): Arg213, Ser214, Ala216, Ser217, Asn220, Arg240, Gln241, Asp242, Gly243, Val244, Ser245, Ser247, Asp249, Thr250, Trp253, Arg271, Glu276, Glu277, Gln278, Arg279, or Thr281.
- Glyco12 variant (Glu215): Arg213, Ser214, Ala216, Ser217, Asn220, or Arg271.
- Glyco14 variant (Gly243): Arg240, Gln241, Asp242, Val244, Ser245, Arg279, or Thr281.
- Glyco15 variant (His248): Ser247, Asp249, Thr250, or Trp253.
- Glyco16 variant (Arg279): Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, or Thr281.

[0442] Using this information, potential epitopes were identified. For example, if reduced binding to the Glyco12 variant was observed, then the epitope is presumed to include at least one of Glu215, Arg213, Ser214, Ala216, Ser217, Asn220, or Arg271.

[0443] Proper folding of glycosylation mutants was confirmed by Differential Scanning Fluorometry (DSF). **FIG. 4A and FIG. 4B** show melt curves and their derivatives for all glycosylation variants. Glyco12 and Glyco16 have a large amount of unfolded material at the start of DSF. The Glyco4 (hyperglycosylated) MICA peak shifts to the right suggesting that it is more stable and that the peak of the mIgG2a melt is masking the MICA melt. Additionally, it has the lowest amount of unfolded

material at the start of DSF compared to the other Glyco variants. **FIG. 5** shows the DSF melting temperature (T_m) values for MICA and mIgG2a. Glyco12 and Glyco16 likely have a combination of folded and unfolded species because we can see positive binding by ELISA, suggesting that some of the material is folded. Glyco4 (hyperglycosylated) is likely more stable than WT. Glycosylation of the MICA*008 Glyco variants is confirmed by gel shift in **FIG. 6**. All of the Glyco variants have shifts that are higher than or distinct from wild-type (WT) MICA*008.mIgG2a. Tryptic peptide mass spectrometry was performed to analyze glycosylation on the glycosylation variants of MICA*008 alpha3 domain. We did not have coverage of the tryptic peptides for the Glyco variants 11, 14, 15 or 16 (**FIG. 3D**). However, we did detect the tryptic peptides for Glyco12, 13 and 17, and they all showed increased N-linked glycosylation compared to their WT MICA*008 tryptic peptide counterpart (**FIG. 3D**). With the addition of the glycosylation site for Glyco12, we saw >99% total glycosylation on the tryptic peptide compared to the WT peptide that had 38% glycosylation and one native glycosylation site. Glyco13 introduced an N-linker glycosylation site in a peptide that had no other sites and went from 0 to 99% total glycosylation. With the addition of the glycosylation site for Glyco17, we saw 86% total glycosylation on the tryptic peptide compared to the WT peptide that had 68% glycosylation and one native glycosylation site.

[0444] Based on these experiments, antibodies were separated into 8 bins and the residues comprising the epitopes were determined. See **Table 5**, located after Example 5 and **FIG. 3A-FIG. 3E**, which are structural models showing where the epitopes for each bin are located on MICA. Bin 1 (3C9.10) epitope maps to "bottom" of MICA. Bin 2 (6F8, 7D4, 32D2, 3E11, 1D5, 15F11) epitope maps to "bottom and front" of MICA. Bin 3 (9C9.5.6, 1E6.1.3, 7A3.1.9) epitope maps to "bottom and side" of MICA. Bin 4 (6E12.5) epitope maps to "side" of MICA. Bin 5 (20G11) epitope maps to "front" of MICA. Bin 8 (12H10 and 18G3) epitope maps to "bottom and front" of MICA.

Example 5: Further Study of Epitopes for 6E1.1.12 and 2E5.2.3 using the MILL1-MICA*008 Chimeras

Methods

[0445] MICA*008 alpha 3 domain was blasted against the non-redundant protein database using NCBI blast and the top hit was the murine MILL1 protein. The residues from MICA*008 surrounding the epitope NGTYQT on the "back" as well as residues in the interface where beta2 microglobulin binding site is found on MILL1 were grafted onto the murine MILL1 sequence. This was done as an alternative strategy to give information about the binding on the "back" of MICA.

[0446] The sequence of the MILL1-MICA*008 chimera.murine IgG2a Fc fusion protein is provided below:

>MILL1-MICA chimera.murine IgG2a Fc fusion
GSTVPPMVTVTSRNYPVGRVTLTCRASSPYPRNITLVWLQDGKPVQQKTFRSGDVLPDGN
GTYQTWVSIRVLPGQEPQFSCNLRHGNHSIMQTAVGNSRAQVTDKKIEPRGPTIKPCPPC
KCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTA

QTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQ
VYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMY
SKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK (SEQ ID: 208)

[0447] **FIG. 7A** shows the MICA *008 residues grafted on MILL. **FIG. 7B** shows the non-MICA *008 residues of the MILL chimera.

Results

[0448] Two antibodies, 6E1.1.12 and 2E5.2.3, bind to the MILL1-MICA*008 chimera protein and all the glycosylation site engineered variants of MICA*008, indicating that they bind an epitope on the "back" of the antigen (Bin 6). See **Table 5** below. **FIG. 8** indicates in black the possible epitope based upon these antibodies binding the MILL1-MICA chimera. Darker grey regions indicate sites that are *not* the epitope. Such sites were identified based on the glycosylation site mapping. They were also identified based on the allelic differences between MICA002, 004, 008 and MICB005, since 6E1.1.12 and 2E5.2.3 bind to all four alleles. The model shows that the epitope for these antibodies is on the "back and top" of the antigen. The residues in black are Val205, Pro206, Met208, Thr212, Gly219, Thr222, Thr224, Arg226, Ser228, Tyr231, Pro232, Gln241, Asp242, Thr250, amino acids between Asp255 and Gly262, Tyr264, Gln265, Trp267, Arg271, Gly275, Glu277, Gly288, Asn289, and His290 of human MICA*008. The 13A9 antibody is unique in that it binds to the MILL1-MICA*008 chimera protein and all the glycosylation site engineered variants of MICA*008, except the hyperglycosylated variant Glyco4 (Bin 7). The Elisa value was 0.9 for Glyco14 suggesting that there is largely decreased binding at this region, even though the >0.65 Elisa cut-off catergorized this as positve binding. Additionally, the Elisa value of Glyco16 was 2.2, which is also suggests some decreased binding compared to the other Glyco variants that all had Elisa values of 3.8 or 3.9. Therefore, the 13A9 antibody seems to share some binding on both the "front" and "back" of MICA*008.

Table 5. ELISA binding to glycosylation variants 4 and 11-17 and MILL1-MICA chimera.

	4	11	12	13	14	15	16	17	Epitope	MILL1-MICA chimera	Glyco-engi-neering Bin
3C9.10	-	+	-	+	+	-	-	+	**E215**: R213, S214, A216, S217, N220, R271; **H248**: S247, D249, T250, W253; **R279**: R240, Q241,D242, G243, E276, E277, N278, T281	-	1
7D4.6	-	+	+	+	-	+	-	+	**G243**: R240, Q241, D242, V244, S245, R279, T281;	-	2

	4	11	12	13	14	15	16	17	Epitope	MILL1-MICA chimera	Glyco-engi-neering Bin
									R279: R240, Q241, D242, G243, E276, E277, N278, T281		
6F8.7	-	+	+	+	-	+	-	+	**G243**: R240, Q241, D242, V244, S245, R279, T281; **R279**: R240, Q241,D242, G243, E276, E277, N278, T281	-	2
32D2	-	+	+	+	-	+	-	+	**G243**: R240, Q241, D242, V244, S245, R279, T281; **R279**: R240, Q241,D242, G243, E276, E277, N278, T281	-	2
3E11	-	+	+	+	-	+	-	+	**G243**: R240, Q241, D242, V244, S245, R279, T281; **R279**: R240, Q241,D242, G243, E276, E277, N278, T281	-	2
15F11	-	+	+	+	-	+	-	+	**G243**: R240, Q241, D242, V244, S245, R279, T281; **R279**: R240, Q241,D242, G243, E276, E277, N278, T281	-	2
1D5	-	+	+	+	-	+	-	+	**G243**: R240, Q241, D242, V244, S245, R279, T281; **R279**: R240, Q241,D242, G243, E276, E277, N278, T281	-	2
9C9.5.6	-	+	+	+	+	-	-	+	**H248**: S247, D249, T250, W253; **R279**: R240, Q241,D242, G243, E276, E277, N278, T281	-	3
1E6.1.3	-	+	+	+	+	-	-	+	**H248**: S247, D249, T250, W253; **R279**: R240, Q241,D242, G243, E276, E277, N278, T281	-	3
7A3.1.9	-	+	+	+	+	-	-	+	**H248**: S247, D249, T250, W253;	-	3

	4	11	12	13	14	15	16	17	Epitope	MILL1-MICA chimera	Glyco-engi-neering Bin
									R279: R240, Q241,D242, G243, E276, E277, N278, T281		
6E12.5	-	+	+	+	+	-	+	+	**H248**: S247, D249, T250, W253	-	4
20G11	-	+	+	+	+	+	-	+	**R279**: R240, Q241,D242, G243, E276, E277, N278, T281	-	5
6E1.1.12	+	+	+	+	+	+	+	+	'back & top': V205, P206, M208, T212, G219, T222, T224, R226, S228, Y231, P232, Q241, D242, T250, D255- G262, Y264, Q265, W267, R271, G275, E277, G288, N289, H290	+	6
2E5.2.3	+	+	+	+	+	+	+	+	'back & top': V205, P206, M208, T212, G219, T222, T224, R226, S228, Y231, P232, Q241, D242, T250, D255- G262, Y264, Q265, W267, R271, G275, E277, G288, N289, H290	+	6
13A9	-	+	+	+	+	+	+	+	'back & top': V205, P206, M208, T212, G219, T222, T224, R226, S228, Y231, P232, Q241, D242, T250, D255- G262, Y264, Q265, W267, R271, G275, E277, G288, N289, H290	+	7
12H10	-	+	+	+	-	+	+	+	**G243**: R240, Q241, D242, V244, S245, R279, T281	-	8
18G3	-	+	+	+	-	+	+	+	**G243**: R240, Q241, D242, V244, S245, R279, T281	-	8

[0449] In **Table 5**, ELISA values >0.65 are shown as positive binding. Final bin assignments are also shown. Residues in bold indicate glycosylation sites; non-bolded residues listed after the glycosylation site are within 5 Angstroms. Epitopes are presumed to comprise at least one of the glycosylated residues or one of the residues within 5 Angstroms of the glycosylated residue.

Example 6: Final Epitope Bin Assignments

[0450] Fina l bin assignment based on Octet competition and glycosylation site engineering shows 7 bins.

Table 6. Final bin assignment.

	Octet Bin	Glycosylation Site Engineering Bin	Final Bin
3C9.10	1	1	1
7D4.6	1	2	2
6F8.7	1	2	2
32D2	N.D.	2	2
3E11	N.D.	2	2
1D5	N.D.	2	2
15F11	N.D.	2	2
9C9.5.6	1	3	3
1E6.1.3	1	3	3
7A3.1.9	1	3	3
6E12.5	1	4	4
20G11	N.D.	5	5
6E1.1.12	3	6	6
2E5.2.3	2	6	7
13A9	N.D.	7	8
12H10	N.D.	8	9
15F11	N.D.	8	9

Note: N.D. = not determined. ELISA values >0.5 are shown.

Example 7: Alanine Scanning

[0451] This example maps the epitopes of 6F8.7 (Final Bin 2), 7D4.6 (Final Bin 2), 2E5.2.3 (Final Bin 7) and 1D5 (Final Bin 2) using alanine scanning of the MICA *008 allele.

Methods

[0452] The binding kinetics of the anti-MICA/B antibodies binding to the MICA alanine variants was measured using surface plasmon resonance (SPR) on a T200 instrument (GE Healthcare). Anti-murine Fc (GE Healthcare) was immobilized on a CM5 sensor chip via amine-based coupling using manufacturer provided protocol. MICA*008.mIgG2a alanine variants were captured by the anti-murine Fc and the anti-MICA/B antibodies as Fabs were passed over. Fab binding was measured to

human MICA*008 alpha 3 domains (mIgG2a tagged, in-house). Sensograms for binding of anti-MICA/B Fabs were recorded using an injection time of 2 minutes with a flow rate of 30 μL/min, at a temperature of 25°C, and with a running buffer of 10mM HEPES, pH 7.4, 150 mM NaCl, 3mM EDTA and 0.005% Tween 20. After injection, disassociation of the Fab from the alpha 3 domain was monitored for 5 minutes in running buffer. The surface was regenerated between binding cycles with a 70 μL injection of 10 mM Glycine HCl pH 1.7. After subtraction of a blank which contained running buffer only, sensograms observed for anti-MICA/B Fab binding to MICA were analyzed using a 1:1 Langmuir binding model with software supplied by the manufacturer to calculate the kinetics and binding constants. Sensograms of anti-MICA/B antibody binding to captured MICA*008
alanine variants were used to calculate the dissociation constant (Kd). The Kd values were then normalized to wild-type MICA*008 and the data are reported as fold-decrease in affinity and provided in **FIG. 9**.

Results

FIG. 9 shows the fold-decrease in affinity from alanine scanning. Fold-decrease greater than 3-fold is shaded. The Ala scan of 2E5.2.3 (Final Bin 7) with the residues in the front confirms the MILL1-MIC chimera data suggesting that the antibody binds to the "back" and not the "front" of MIC. **FIG. 10** shows the 2E5.2.3 epitope with Ala scan data included. The residues in black are Val205, Pro206, Met208, Thr212, Gly219, Thr222, Thr224, Arg226, Ser228, Tyr231, Pro232, Asp242, the amino acids between Asp255 and Gly262, Tyr264, Gln265, Trp267, and Arg271 of human MICA*008. **FIG. 11A** compares the epitope for 6F8 mapped by glycosylation engineering and Ala scan. **FIG. 11B** compares the epitope for 7D4 mapped by glycosylation engineering and Ala scan.

Discussion

[0453] The epitopes that were mapped by glycosylation engineering agreed very well with the results from the Ala scanning Biacore experiments for antibodies 6F8, 7D4 and 1D5. For both 6F8, 7D4 and 1D5, we identified Glyco engineered residues G243N (from Glyco14) and R279N (from Glyco16) as MICA epitopes. From Ala scanning, we identified 2 residues within 5Å of G243 and 2 residues within 5Å of R279 as epitopes for both 6F8, 7D4. For 1D5, we identified 4 residues within 5Å of G243 and 1 residue within 5Å of R279 as epitopes. We identified R279 as an epitope for 6F8, 7D4 and 1D5. However, G243A had a low Rmax in the Biacore experiment for the 2E5.2.3 control and the other antibodies suggesting that the protein was misfolded, so the Ala scan was not informative. Though we were able to identify R240 and V244, residues within 5Å of G243, as epitopes for 6F8, 7D4 and 1D5. In addition to the alanine variant G243A, D242A, L246A, T281A, and H293A could not be assessed due to poorly folded MICA*008 protein.

[0454] Glyco engineering using an ELISA is a quick way to identify regions on the antigen surface that are important for binding. Although there may be limitations to the regions you can cover with

glycosylation, it is a rapid and straightforward method to getting antigen epitope information. Ala scanning an antigen can give very detailed information about the epitope, but it requires many constructs, some of which may fold poorly, and takes more time using Biacore. This is especially cumbersome when mapping an epitope of a large or multi-domain protein. In this case, glycosylation engineering of an antigen would be particularly useful to identify the binding domain or region of the antigen without having to express all Ala variants or separate antigen domains. Once a binding domain or region is identified that is important for binding to the antibody, Ala scan could then be performed on a smaller subset of surface exposed residues.

Example 8: MICA Shedding Inhibition and Interference Assay

[0455] The anti-MIC antibodies were tested for MIC shedding inhibition from MICA*004 and MICA*008 shedding cell lines.

Methods

[0456] To analyze the MICA*004 and MICB*005 alleles, the lung cancer cell line HCC1534 (UTSW) was used. For the MICA*008, the melanoma cell line MEL-JUSO (DSMZ) was used. To examine bona fide cell shedding inhibition, samples reflecting both the effect of antibodies on cell secretion of MIC and samples reflecting antibody interference with the MIC ELISA assay were analyzed. Briefly, cells were cultured in RPMI1640 medium supplemented with 10% FBS and 2mM Glutamax (Gibco) for at least 24 hours (post thaw) in T150 flasks (Corning). Cells were harvested when cell density reached 70-80% confluency and respuspended in culture media at the concentration of 250,000 cells/ml. Forty microliters of cell suspension (10,000 cells total) were added to each well of a 384-well polystyrene tissue culture plate (Greiner). Additional plates were seeded with the same number of cells to serve as supernatant (sup) for the interference assay.

Antibody preparation for combination inhibition

[0457] Antibodies (mIgG2a, CHO-derived) were normalized to 40ug/mL (4X concentration) in culture media for top concentration, and then serially diluted with a 4-fold, 7-point dilution scheme. Diluted 4X antibodies were combined in a checkerboard manner (horizontal/vertical) in a 1:1 ratio (final 20ug/mL [2X] each Ab concentration).

Antibody preparation for concentration-effect analysis

[0458] Antibodies (hIgG1) were normalized to 20ug/mL (2X concentration) in culture media for top concentration, and then serially diluted with a 4-fold, 8 point dilution scheme.

Establishment of shedding inhibition assay

[0459] 40uL of 2X antibody samples were added to the cell culture plate containing 10,000 cells per well, yielding 80uL of volume with a final top concentration of 10ug/mL (1X). Any remaining wells had 40uL of media added to serve as a control (80uL final volume). Samples incubated at 37C/5%CO_2. After 24 hours incubation, the cell culture supernatant were harvested into a 384-well deep-well polypropylene block (Greiner) and frozen at -80C for future analysis (or analyzed the same day). For interference supernatant, cultured media was harvested into a reservoir to pool and was also frozen.

MIC ELISA assay

[0460] ELISA plates were coated with either anti-MICA AMO1 (MBL) for MICA*004 and MICA*008 or anti-MICB 236511 (R&D) for MICB*005. Following overnight incubation, samples were washed 3 times and blocked using casein blocking buffer (Sigma). For inhibition sample analysis, sup samples were thawed the day of the assay (or used immediately after harvesting) and were then were then diluted in sample buffer (PBS 7.4 with 0.5% BSA, 10 ppm Proclin, 0.05% Tween20, 0.25% CHAPS, 5mM EDTA, 0.35M NaCl, 10ppm Proclin) and added to the ELISA plate. For interference sample analysis, pooled conditioned media was spiked with testing antibody and incubated at room temperature for at least 1 hour with gentle agitation. The antibody-spiked media was then added to the ELISA plate. Serially diluted recombinant MIC proteins were also added to the plate (independent of sup) to establish a standard curve for sample quantification.

[0461] Plates were then sealed and incubated at room temperature for 2 hours with gentle agitation. The primary antibodies used were as follows: Bio-8C5.6 (in-house Genentech) for MICA: **FIG. 13A- FIG. 13C**; Bio-6D4 (MBL): **FIG. 15A- FIG. 15B**, and **Table 7**; Bio-8C5.6 (in-house Genentech) for MICA: **FIG. 16A - FIG. 16C**, and **Table 8 – Table 10**; and Bio-7E3 (in-house Genentech) for MICB assay (**FIG. 13A - FIG. 13C; FIG. 15A - FIG. 15C; FIG. 16A – FIG. 16C**; and **Table 8 – Table 10**. These antibodies were diluted in Assay Diluent (PBS pH7.4, 0.5% BSA, 0.05% Tween20, 10ppm Proclin) and incubated with 50ug/mL of non-specific mouse IgG (Equitech-Bio) for 1 hour at room temperature. After sample incubation, plates were washed 6 times, and 25uL of primary antibody was added. Plates were then incubated for 1 hour at room temperature with gentle agitation. After incubation, Streptavidin Poly-HRP80 (Fitzgerald) was diluted in universal casein diluent (Fitzgerald) to 90ng/mL. Plates were washed 6 times and 25uL of diluted Streptavidin Poly-HRP80 were added to each well and incubated for 1 hour at room temperature with gentle agitation. After incubation, plates were washed 6 times and 25uL of TMB (Moss) was added to each well, and incubated with gentle agitation for 15 minutes. 25uL of 1M Phosphoric Acid was then added to each well and ELISA plates were read at 450/620nm wavelength.

Data analysis method

[0462] ODs were processed using 5-parameter curve fitting (1/Y weighting) of the standard material to quantitate the amount of soluble MICA/B (sMIC) in each sample using the corresponding allele standard. Duplicate samples were averaged together, and all non-treated samples were averaged for that particular plate. Percent inhibition of each treated sample was defined as 1-(sMIC in treated sample)/(sMIC in untreated sample average).

[0463] Interference samples were processed in the same way. If the antibody interfered with the MICA/MICB quantification, the MICA/MICB concentration in the antibody spiked condition media would deviate significantly from the untreated condition media (control). If the MICA/MICB amount

was higher than the control, it was defined as positive interference. If the MICA/MICB amount was lower than the control, it was defined as negative interference.

Results

[0464] Results are provided in **Table 7**. For the MICA 008 allele, antibodies in Final Bins 9 and 2 followed by Bin 5 and 7, have the highest % maximum inhibition values and the lowest EC50 values are seen in Bins 2 and 9. Antibodies in Final Bins 1 and 3, however, show poor inhibition of shedding for MICA 008 allele. This inability to block shedding could be due to the low affinity of the antibodies. For the MICA*004 allele, antibodies in Final Bins 2, 9, 5, 7, followed by Bins 8 and 6 show the highest % maximum inhibition values and the lowest EC50 values are seen for Bins 2 and 9. For MICB 005 allele, a smaller set of data is available however, Bin 8 followed by Bin 7 has the highest % maximum inhibition values and the lowest EC50 values are seen in Bins 9, 2, 5 and 8.

[0465] MICA*004 shedding inhibition in HCC1534 cells is shown in **FIG. 13A**. Maximum inhibition of shedding by 1D5 or 13A9 alone was approximately 60%, while maximum inhibition of shedding by 6E1 was approximately 50%. Combining 1D5 with 13A9 did not show additive or synergistic effect. The combination of 1D5 and 6E1, however, further decreased the amount of sMIC in the samples by approximately 10% compared to backgound, indicating a minor additive/synergistic effect. Likewise, the combination of 13A9 and 6E1 also enhanced shedding inhibition.

[0466] MICA*008 shedding inhibition in MEL-JUSO cells is shown in **FIG. 13B**. Maximum inhibition of shedding by 1D5 alone was approximately 70-75%, while maximum inhibition of shedding by 13A9 alone was approximately 40%, and maximum inhibition of 6E1 was approximately 35-50%. There was no additive or synergistic effect in shedding inhibition when 1D5 and 13A9 were combined. The activity of 1D5 was generally maintained in the presence of 13A9. The combination of 1D5 and 6E1, however, further decreased the amount of sMIC in the samples by approximately 5-10% showing a minor additive/synergistic effect. Similarly, the combination of 13A9 and 6E1 showed a 5-10% increase in shedding inhibition.

[0467] MICB*005 shedding inhibition in HCC1534 cells is shown in **FIG. 13C**. Maximum inhibition of shedding by 1D5 alone was approximately 50%, while maximum inhibition by 13A9 reached 75%, and maximum inhibition of shedding by 6E1 was approximately 45-50%. The strong shedding inhibition activity of 13A9 was diminished by the addition of 1D5 as combining 1D5 and 13A9 at 10 ug/ml only showed 53% of shedding inhibition. In addition, there was no additive or synergistic effect in shedding inhibition. The combination of 1D5 and 6E1, however, significantly enhanced shedding inhibition by approximately 20-30% demonstrating an additive/synergistic effect. Combination of 6E1 to 13A9 did not show additive or synergistic effect.

Table 7: EC50 and Percent Maximum Inhibition Values.

Name	MICA*008	MICA*008	MICA*004	MICA*004	MICB*005	MICB*005	Final Bin
	MEL-JUSO	MEL-JUSO	HCC1534	HCC1534	HCC1534	HCC1534	
	EC50 (ug/mL)	% max inhibition	EC50 (ug/mL)	% max inhibition	EC50 (ug/mL)	% max inhibition	
3C9.10	Inactive	<10	50	24	N/A	N/A	1
7D4.6	Inactive	26	0.185	63	N/A	N/A	2
6F8.7	0.163	47.5	0.251	70	N/A	N/A	2
3E11	0.265	70	0.309	55	N/A	N/A	2
9C9.5.6	Inactive	<10	3.176	44	N/A	N/A	3
1E6.1.3	Inactive	20	0.681	46	N/A	N/A	3
7A3.1.9	Inactive	<10	0.411	50	N/A	N/A	3
6E12.5	0.074	28	0.329	37	N/A	N/A	4
6E1.1.12	0.355	42.2	0.265	65	N/A	N/A	6
2E5.2.3	0.195	61	0.107	72	N/A	N/A	7
2E5.2.3	0.1122	60	0.2304	80%	0.1067	60	7
6E1.1.12	0.0941	47	0.1565	73%	0.0791	55	6
15F11	0.0165	74	0.0485	86%	0.0736	59	2
32D2	0.0303	71	0.0592	76%	0.0781	54	2
13A9	0.4888	47	0.1656	66%	0.0530	79	8
18G3	0.0191	75	0.0120	74%	0.0124	48	9
12H10	0.0103	81	0.0689	76%	0.1394	54	9
20G11	0.0285	70	0.1234	69%	0.0169	51	5
1D5	0.0087	77	0.0435	81%	0.0128	54	2

Note: Data highligted in gray was performed on a separate day, using anti-MICA antibodies purified from CHO instead of HEK293 cells.

[0468] Assay interference was tested by diluting the antibody at different concentrations, then combining them and incubating for >1hr with HCC1534 or MEL-JUSO cell culture supernatant, followed by quantification of shed MICA/MICB. If the antibody interfered with the MICA/MICB quantification, the MICA/MICB concentration would deviate significantly from the no antibody control. If the MICA/MICB amount was higher then the control, this would be positive interference (leading to a positive % recovery/negative % inhibition, possibly masking activity). If the MICA/MICB amount was lower than the control, this would be negative interference (leading to a positive % inhibition/false positive). Both positive and negative interference were observed to varying degrees (**Table 8** and **FIG. 15A –FIG. 15B**). There was some positive interference at high concentrations for certain antibodies, potentially due to the presence of co-purified MICA from the HEK293 host cells (**Table 8, FIG. 15A-FIG. 15B, and FIG. 16A-FIG. 16C**). Thus, shedding inhibition activity from listed antibodies did not appear to be due to assay interference artifacts.

[0469] When anti-MICA antibodies purified from CHO were used, no co-purified MICA was present. As mIgG2a CHO-derived material was used, the only significant interference observed was negative,

possibly leading to more potent activity than what was truly occurring in the biological mechanism. MICA*004 assay interference in HCC1534 cells is shown in **FIG. 16A**. For all antibodies, interference was lower than inhibition, therefore antibody actively inhibited shedding and results were not solely artifacts from assay interference. For the combination of 1D5 and 13A9, some interference (~25-35% above background) was observed across most samples, with a slight reduction at lower antibody concentrations. This interference was observed with single antibodies. For the combination of 1D5 and 6E1, there was some interference (~20-35% above background) at high amounts of 6E1 (highest with moderate amounts of 1D5), and this interference was diluted out with 6E1. This interference was not observed at significant levels with single antibodies. For the combination of 13A9 and 6E1, some antibody interference (~10-15% above background) was observed at high concentrations of 13A9 and 6E1, and seemed to dilute out, with 6E1 interference appearing to have a more significant impact than 13A9 in combination with 6E1.

[0470] MICA*008 assay interference in MEL-JUSO cells is shown in **FIG. 16B**. No significant antibody interference was observed on either allele by any antibodies, therefore, any effects were predominantly biological.

[0471] MICB*005 assay interference in HCC1534 cells is shown in **FIG. 16C**. There was some minor interference (20-30% inhibition) from 1D5 and 13A9. Interference was less at higher concentrations of combined antibodies. Therefore, most of 13A9's activity is biological, while much of 1D5's activity is interference. There was minimal interference from 1D5 or 6E1 alone, but when combined there was about 30-40% inhibition, therefore, some of the activity observed is partially due to interference. With the combination of 6E1 and 13A9, combination interference did not follow clear titration trends. Since most interference observed was around 25-30%, much of the activity was determined to be true inhibition.

Table 8: MICA/B Interference.

Name	HCC1534 (MICA*004) Interference)	MEL-JUSO (MICA*008) Interference)	HCC1534 (MICB*005 Interference)
3C9.10	None	None	N/A
7D4.6	Positive Interference	None	N/A
6F8.7	Positive Interference	Positive Interference	N/A
3E11	None	N/A	N/A
9C9.5.6	None	None	N/A
1E6.1.3	None	None	N/A
7A3.1.9	Positive Interference	Positive Interference	N/A
6E12.5	Positive Interference	Positive Interference	N/A
6E1.1.12	Positive Interference	Positive Interference	N/A
2E5.2.3	None	None	N/A
2E5.2.3	33%	No	32%
6E1.1.12	28%	No	25%
15F11	23%	No	22%
32D2	N/A	No	N/A
13A9	16%	No	20%
18G3	28%	No	26%
12H10	23%	No	15%
20G11	21%	No	18%
1D5	18%	No	15%

Note: Data highlighted in gray was performed on a separate day, using anti-MICA antibodies purified from CHO instead of HEK293 cells.

[0472] Summary comparison of anti-MIC antibodies on sMIC shedding inhibition activity using low-interference assay formats (AMO1/8C5 for MICA and 236511/7E3 for MICB) are provided in **Table 9**.

Table 9: Shedding Inhibition of Anti-MIC Antibodies across MIC Alleles

Ab	PANC-1 (MICA*002)		HCC1534 (MICA*004)		MEL-JUSO (MICA*008)		HCC1534 (MICB*005)	
	Max % Inhibition	EC50 (ug/mL)	Max % Inhibition	EC50 (ug/mL)	Max % Inhibition	EC50 (ug/mL)	Max % Inhibition	EC50 (ug/mL)
1D5	48%	0.0342	65%	0.0136	74%	0.0207	59%	0.0386
13A9	66%	0.4180	56%	0.5990	50%	0.7945	76%	0.0349
6E1	23%	Not possible	52%	0.3298	45%	0.1050	58%	0.0724
15F11	54%	0.0752	64%	0.0375	73%	0.0255	60%	0.1016

[0473] Summary comparison of anti-MIC antibodies' interference on conditioned media using low-interference assay formats (AMO1/8C5 for MICA and 236511/7E3 for MICB) are provided in **Table 10**.

Table 10: Interference of Anti-MIC Antibodies across MIC Alleles

Ab	PANC-1 (MICA*002)	HCC1534 (MICA*004)	MEL-JUSO (MICA*008)	HCC1534 (MICB*005)
	% reduction due to interference at 10ug/mL	% reduction due to interference at 10ug/mL	% reduction due to interference at 10ug/mL	% reduction due to interference at 10ug/mL
1D5	24%	26%	14%	31%
13A9	24%	21%	6%	33%
6E1	26%	23%	13%	17%
15F11	ND	24%	15%	21%

[0474] As shown in **Table 9** and and **Table 10,** anti-MIC antibodies 1D5, 13A9, and 15F11 all demonstrate shedding inhibition activity above interference levels on PANC-1, HCC1534, and MEL-JUSO on all alleles. Anti-MIC antibody 6E1 demonstrates shedding inhibition activity above interference on HCC1534 and MEL-JUSO, but no non-interference activity on PANC-1. 1D5 & 15F11 demonstrated superior shedding inhibition activity on MICA*004 and MICB*008 alleles, while 13A9 demonstrated superior shedding inhibition on alleles MICA*002 and MICB*005.

Example 9: Affinity and Shedding Inhibition Properties of anti-MICA/B antibodies

[0475] This Example analyzes the relationship between MIC epitopes and affinity and shedding inhibition properties of anti-MICA/B clones. Anti-MICA/B clones binding to the alpha 3 domain from the HZ campaign and selected clones from earlier campaigns were tested for binding, antibody bins and inhibition of shedding. Bins were assigned based on this set of clones.

Wasatch Binning Method

[0476] A 96 X 96 array-based SPR imaging system (Carterra USA) was used to epitope bin a panel of monoclonal antibodies. Purified monoclonal hybridoma antibodies were diluted at 10 ug/ml in 10 mM sodium acetate buffer pH 4.5. Using amine coupling, antibodies were directly immobilized onto a SPR sensorprism CMD 200M chip (XanTec Bioanalytics, Germany) using a Continuous Flow Microspotter (Carterra, USA) to create an array of antibodies. For binning analysis, the IBIS MX96 SPRi (Carterra USA) was used to evaluate binding to the immobilized antibodies. The experiment was performed at 25°C in a running buffer of 10mM HEPES, pH 7.4, 150 mM NaCl, 3mM EDTA, and 0.005% Tween 20 (HBS-TE). Antigen was first injected for 4 minutes at 100 nM and was followed by a second 4 minute injection of purified antibody at 10 ug/ml in a running buffer of HBS-TE. The surface was regenerated between cycles with 10 mM Glycine pH 2.0. The binding data was processed using the Wasatch binning software tool.

Results

[0477] A total of 96 anti-MICA/B clones were found to bind ECD and alpha-3 domain of MICA alleles *002, *004 and 008, as well as MICB005 by an ELISA screen (data not shown). ELISA values of >0.5 were deemed as 'binding'. These clones were then tested for binding to alpha-3 domain versions of MICA and MICB by Biacore, and 52 clones selected that showed binding to MICA*008 alpha-3 domain with affinity values </= 10 nM (**FIG. 17**). Next, these 52 anti-MICA/B antibodies were tested for % shedding inhibition of MICA*008 (**FIG. 18A**). Although these antibodies bind well to MICA*008 (</= 10 nM), they inhibit shedding of MICA*008 from 1%-81%. Further dissecting this large range, 6 antibodies displayed 1-20% shedding inhibition of MICA*008, 7 antibodies from 21-40%, 26 antibodies from 41-60%, and 13 antibodies from 61-81%. Twenty-six anti-MICA antibodies inhibited MICA*008 shedding >/= 50%, while 3 antibodies inhibited shedding >/= 75%.

[0478] To understand the determinants responsible for better shedding inhibition properties of the anti-MICA antibodies, the correlation between shedding inhibition and affinity was analyzed. Affinity (KD) was plotted for MICA*008 and % shedding inhibition of MICA*008 for the 52 anti-MICA antibodies (**FIG. 18B**). This data showed no correlation between MICA*008 affinity and % shedding inhibition. Next, the correlation between affinity and a particular epitope on MICA*008 was examined. A binning experiment was performed using Wasatch and three unique epitope bins for the set of 52 anti-MICA antibodies were determined (**FIG. 19A**). Bin 1 and Bin 3 were unique, while Bin 2 had some overlap with Bin 1 and Bin 3. Bin 1 contained antibodies that bound similarly to 1D5, while Bin 3 contained antibodies that had binding similar to 6E1.1.12. MICA*008 Wasatch epitope bins were plotted and compared to binding affinity for the MICA antibodies (**FIG. 19B**). Bin 1 had 33 anti-MICA antibodies with an affinity range of 0.5 – 8.4 nM, Bin 2 had 4 anti-MICA antibodies with an affinity range of 2.1 nM to 10 nM, while Bin 3 had 15 anti-MICA antibodies with an affinity range of 0.47 – 6.8 nM. Although Wasatch determined three distinct MICA*008 epitope bins, the affinity ranges within these bins (particularly Bins 1 and 3) were similar, indicating that there a lack of correlation between affinity and epitope bin.

[0479] Finally, the correlation of shedding inhibition to a certain MICA*008 epitope for the 52 anti-MICA antibodies was analyzed. MICA*008 epitope bins by Wasatch and the percent (%) shedding inhibition were plotted (**FIG. 19C**). Bin 1 had 33 anti-MICA antibodies with a median % shedding inhibition of 51% and a range of 16-81%. Bin 2 had 4 anti-MICA antibodies with a % shedding inhibition median of 39% and a range of 26-42%. Bin 3 had 15 anti-MICA antibodies with a % shedding inhibition median of 47% and a range of 1-64%. The median % shedding inhibition values of Bin 1 and Bin 3 were similar and both had about a 65% range difference. However, a correlation between MICA*008 epitope to ability to inhibit shedding was found to exist. Bin 1 contained the 10 antibodies with the highest shedding inhibition values, while Bin 3 had the three antibodies with the lowest shedding inhibition ability (**FIG. 19C**). Due to the large range of percent shedding inhibition values in Bin 1, a higher resolution epitope mapping method was needed to identify the particular

epitope that was correlated to the best shedding inhibition. This allowed screening of antibodies to select for the desired MICA epitope.

[0480] To address the need for a higher resolution and higher throughput epitope mapping method, a Glyco-engineering Epitope Mapping or GEM method was developed (**FIG. 20**). This method engineered protein antigen sequences to create a single N-linked glycosylation site (N-X-S/T, where X ≠ P). This was achieved by changing an N-X-X site to N-X-S/T or by changing a X-X-S/T site to N-X-S/T. Additionally, a few amino acids could be added to the N- or C-terminally to create a new N-linked glycosylation site. Once a panel of the glyco-engineered antigen variants was created and purified, they were screened for binding to antibodies using either ELISA or Biacore/Wasatch. Positive and negative binding was then established and the epitope was defined as the site or sites where no binding was observed due to the epitope being masked by glycosylation.

[0481] For MICA*008, single N-linked glycosylation sites on the 'front' of MICA*008 alpha3 domain were introduced on 7 separate MICA*008 varaints (**FIG. 21A**). Such variants were designed based on the crystal structure of MICA*001 (Protein Data Bank structures 1B3J and 1HYR) and the 7 residues that were mutated were all surface exposed in the crystal structures. Mass spectrometry on the MICA*008 glyco-engineered variants was performed to analyze glycosylation (**FIG. 21B**). We did not have coverage of the tryptic peptides for the Glyco variants 11, 14, 15 or 16. However, we did detect the tryptic peptides for Glyco12, 13 and 17, and they all showed increased N-linked glycosylation compared to their WT MICA*008 tryptic peptide counterpart (**FIG. 21B**). With the addition of the glycosylation site for Glyco12, we saw >99% total glycosylation on the tryptic peptide compared to the WT peptide that had 38% glycosylation and one native glycosylation site. Glyco13 introduced an N-linked glycosylation site in a peptide that had no other sites and went from 0 to 99% total glycosylation. With the addition of the glycosylation site for Glyco17, we saw 86% total glycosylation on the tryptic peptide compared to the WT peptide that had 68% glycosylation and one native glycosylation site.

[0482] The binding of these 7 MICA glycosylation variants was tested with all 96 anti-MICA/B clones that bound all 4 MICA/B alleles by ELISA. 11 unique epitope bins were identified based on the binding characteristics to the 7 glyco-engineered variants (**FIG. 21C**). The highest number of antibodies were observed in Bin 6 – not binding any of the glyco-engineered MICA variants, Bin 2 – binding to glyco-engineered variants 14 and 16, and in Bin 5 – binding to glyco-engineered variant 16. Further examination of the anti-MICA antibodies that inhibit shedding > 70% showed that the top antibodies that block shedding fell into only 3 bins (**FIG. 21C**). These bins included epitopes with glyco-engineered variants Glyco14, Glyco16 or both Glyco14 and Glyco16. Therefore, antibodies that block epitopes on the 'front' and 'bottom' of MICA were most effective at blocking MICA*008 shedding (**FIG. 21D**).

Example 10: Crystallography for anti-MIC antibodies

[0483] This Example characterizes the crystal structures of anti-MIC 1D5, 13A9, and 6E1.1.12 antibodies.

Expression and Purification of Fabs

[0484] All Fabs were expressed in *E. coli* as chimeras containing murine variable domains and human kappa CL and human IgG1 CH1 constant domains. Cells were pelleted, resuspended in 25 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, and lysed by passing twice through a microfluidizer. DNA was precipitated by the addition of 0.4% polyethyleneimine (PEI) and incubation for 1 hour to up to overnight at 4 °C with stirring. Lysates were cleared by centrifugation at 15,000 x g for 1 hour followed by filtration through a 0.22 μm filter.

[0485] The 1D5 Fab was purified by affinity chromatography using GammaBind Plus Sepharose (GE Healthcare). After affinity capture, the column was washed with equilibration buffer (25 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA), equilibration buffer plus 0.1 % Triton X-100 + 0.1 % Triton X-114, equilibration buffer again, followed by low salt buffer (25 mM succinate, pH 6.0). The Fab was eluted with 150 mM acetic acid, pH 2.7 and immediately neutralized with 1/10 volume of 1 M Tris, pH 9.0. The 1D5 Fab was further purified by cation-exchange chromatography using an SP Sepharose High Performance column (GE Healthcare) pre-equilibrated in buffer A (20 mM sodium citrate, pH 5.0) and eluted with a 0-40 % gradient of buffer B (20 mM sodium citrate, pH 5.0, 1 M NaCl) over 10 column volumes (CVs). After cation-exchange chromatography the 1D5 Fab was dialyzed into 25 mM Tris, pH 7.5, 150 mM NaCl prior to complex formation.

[0486] The 13A9 Fab was purified by affinity chromatography using GammaBind Plus Sepharose (GE Healthcare) as described above followed by size exclusion chromatography using a HiLoad Superdex 200 pg column (GE Healthcare). After size-exclusion chromatography the 13A9 Fab was dialyzed into 25 mM Tris, pH 7.5, 150 mM NaCl, prior to complex formation.

[0487] The 6E1.1.12 Fab was also purified by affinity chromatography using GammaBind Plus Sepharose (GE Healthcare) followed by cation-exchange chromatography as described above, except a 0-20% gradient of buffer B (20 mM sodium citrate, pH 5.0, 1 M NaCl) over 20 CVs was used for elution. Two peaks were observed upon elution from the SP Sepharose High Performance column. Mass spectrometry demonstrated that the major peak contained Fab with a light chain (LC) and heavy chain (HC) matching their respective theoretical masses. The minor peak contained the expected mass of the LC, however the HC was 17 Da smaller than the theoretical mass, suggesting formation of a pyroglutamate on the amino terminus of a fraction of the protein. The peak containing the pyroglutamate was a minor fraction of the total protein therefore the peak containing the glutamine at the amino terminus was used for crystallization. After cation-exchange chromatography the 6E1.1.12 Fab was dialyzed into 25 mM Tris, pH 7.5, 150 mM NaCl prior to complex formation.

Expression and Purification of the Human MICA*008 α3 Domain

[0488] Both the wild-type human MICA*008 α3 domain and a C273S mutant were generated. The C273S mutant was designed to remove the unpaired cysteine from the α3 domain and block covalent dimerization through disulfide bond formation that was observed with a small fraction of the wild-type protein.

[0489] The human MICA*008 α3 domain containing an amino-terminal, thrombin-cleavable His_6 tag was co-expressed with *Streptomyces plicatu* EndoH in *Trichoplusia ni* (*T. ni*) cells using a bacculovirus expression system. DNA encoding residues T204-S297 of human MICA*008 and residues F2-P313 of *Streptomyces plicatu* EndoH were cloned separately into a slightly modified version of pAcgp67 (BD Biosciences). Transfer vectors were co-transfected with BestBac linearized viral DNA (Expression Systems, LLC) into *Spodoptera frugiperda* (*Sf*9) cells using Cellfectin (Invitrogen) to produce recombinant baculovirus. Viruses were amplified twice to prepare the stocks used for protein expression. *T. ni* Pro cells were inoculated into 2 L of ESF921 serum-free, protein-free media (Expression Systems) at 2 x 10^6 cells/mL and infected with 20 mL of virus at a ratio of 1:1 MICA:EndoH. Kifunensine was dissolved in 10 mL of ESF921 media, sterile filtered, and added at infection to a final concentration of 1 mg/L to prevent complex *N*-linked glycan formation. Cells were grown in 5 L Thomson Optimum Growth™ flasks at 27 °C in a shaker with a 2 inch throw at 130 RPM. Forty-eight hours post infection cultures were adjusted with Tris and salts to a final concentration of 50 mM Tris, pH 8.0, 5 mM $CaCl_2$, 1 mM $NiCl_2$ and allowed to mix for 30 minutes to precipitate excess free amino acids in the media. Cells and precipitated amino acids were removed from the culture supernatant by centrifugation at 300 RCF for 15 minutes. The supernatant containing the secreted MICA*008 protein was filtered through a 0.2 μm filter and was adjusted to pH 7.0 prior to purification.

[0490] All MICA*008 purification steps were done at 4 °C. The pH-adjusted supernatant was loaded over a Ni Sepharose Excel (GE Healthcare) column equilibrated in buffer A (50 mM sodium phosphate, pH 8.0, 300 mM NaCl). The column was washed with 2.5% buffer B (50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 400 mM imidazole), Triton X-114 buffer (50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 0.1% Triton X-114), followed by 2.5% buffer B. Protein was eluted with a step gradient of 100% buffer B and then dialyzed into 50 mM sodium phosphate, pH 8.0, 150 mM NaCl.

[0491] The His_6 tag was removed by cleavage with 15 units of thrombin (GE Healthcare) per mg of MICA*008 protein for 16 hours at room temperature with rotation and the cleavage reaction was monitored by mass spectrometry. An additional 5 units of thrombin per mg of MICA*008 was added and the reaction was allowed to proceed for an additional 24 hours at room temperature with rotation. The protein was filtered through a 0.22 μm filter to remove precipitation prior to further purification.

[0492] The untagged MICA*008 α3 domain was separated from any remaining tagged protein by reverse affinity purification using a Ni Sepharose Excel (GE Healthcare) column with collection of the

flow through. The column was washed with 50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 20 mM imidazole and the washes were also collected. Both the flow through and washes were further purified over a HiLoad Superdex 75 pg column (GE Healthcare) to remove thrombin and high molecular weigh aggregates.

[0493] A final polishing step over a Mono S GL column (GE Healthcare) with a 0-100% buffer B (20 mM sodium acetate, pH 5.0, 1 M NaCl) gradient over 30 CVs was performed to remove minor amounts of remaining His_6-tagged MICA*008. Fractions containing untagged MICA*008 α3 domain were pooled and dialyzed into 25 mM Tris, pH 7.5, 150 mM NaCl prior to complex formation.

Purification of Fab-MICA*008 α3 Domain Complexes

[0494] Fab-MICA*008 complexes were formed from a two-fold molar excess of the MICA*008 α3 domain incubated at 4 °C overnight with the various Fabs. Complexes containing the 1D5 or 13A9 Fab were formed with the wild type MICA*008 α3 domain, whereas the 6E1.1.12 complex was formed with the C273S mutant MICA*008 α3 domain. Complexes were purified from the excess MICA*008 α3 domain by size-exclusion chromatography using a HiLoad Superdex 75 pg column (GE Healthcare) in 25 mM Tris, pH 7.5, 150 mM NaCl. Fractions containing the complex were pooled and concentrated to 20 mg/mL for crystallization screening trials.

Crystallization and X-ray Data Collection of Fab-MICA*008 α3 Domain Complexes

[0495] Crystals of the 1D5 Fab-MICA*008 complex grew over 30 days at 4 °C in vapor diffusion hanging drops from a 1:1 mixture of protein (20 mg/mL in 25 mM Tris, pH 7.5, 150 mM NaCl) and well solution (0.1 M sodium acetate, pH 4.6, 25% PEG4000). Crystals were cryo-protected using well solution, flash frozen, and stored in liquid nitrogen. Diffraction data were collected under cryo-cooled conditions (100 K) at the Advanced Photon Source (APS) beamline SER-CAT 22-ID (Argonne National Laboratory, IL) at a wavelength of 1.00 Å (**Table 11**).

[0496] Crystals of the 13A9 Fab-MICA*008 complex grew overnight at 19 °C in vapor diffusion hanging drops from a 2:1 mixture of protein (20 mg/mL in 25 mM Tris, pH 7.5, 150 mM NaCl) and well solution (0.01 M $ZnSO_4$, 0.1 M MES, pH 6.5, 25% PEG 550 MME). These crystals were crushed with a Seed Bead kit (Hampton Research), diluted 1:500 in well solution and used for streak seeding into a 1:1 mixture of protein (20 mg/mL in 25 mM Tris, pH 7.5, 150 mM NaCl) and well solution containing a lower concentration of PEG 550 MME (0.01 M $ZnSO_4$, 0.1 M MES, pH 6.5, 16-18% PEG 550 MME). Seeded crystals grew over three days at 19 °C in vapor diffusion hanging drops. Crystals were cryo-protected in 25% glycerol, 0.01 M $ZnSO_4$, 0.1 M MES, pH 6.5, 15% PEG 550 MME, flash frozen, and stored in liquid nitrogen. Diffraction data were collected under cryo-cooled conditions (100 K) at the Advanced Light Source (ALS) beamline 5.0.2 (Lawrence Berkeley National Laboratory, CA) at a wavelength of 1.00 Å (**Table 11**).

[0497] Crystals of the 6E1.1.12 Fab-MICA*008 C273S complex grew over five days at 19 °C in vapor diffusion hanging drops from a 1:1 mixture of protein (20 mg/mL in 25 mM Tris, pH 7.5, 150 mM NaCl) and well solution (0.1 M tri-sodium citrate, 15% isopropanol, 15% PEG 4000). Crystals were cryo-protected using well solution with the addition of 25% glycerol, flash frozen, and stored in liquid nitrogen. Diffraction data were collected under cryo-cooled conditions (100 K) at the Stanford Synchrotron Radiation Lightsource (SSRL) beamline 12-2 (SLAC National Accelerator Laboratory, CA) at a wavelength of 0.97946 Å (**Table 11**).

Table 11. X-Ray Data Collection and Refinement Statistics

Data Collection	**1D5 Fab – MICA*008 α3**	**13A9 Fab – MICA*008 α3**	**6E1.1.12 Fab – MICA*008 C273S α3**
Beamline	APS SER-CAT 22-ID	ALS 5.0.2	SSRL 12-2
Wavelength (Å)	1.00	1.00	0.97946
Detector	Rayonix 300HS	Pilatus 6M	Pilatus 6M
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions a, b, c (Å) α, β, γ (°)	 58.69, 50.18, 88.63 90.00, 90.85, 90.00	 51.55, 61.83, 172.37 90.00, 90.00, 90.00	 66.50, 89.79, 89.72 90.00, 90.00, 90.00
Resolution (Å)	35.00-1.30 (1.32-1.30)	35.00-1.90 (1.93-1.90)	35.00-2.21 (2.26-2.21)
R_{merge}	0.059 (0.56)	0.085 (0.49)	0.090 (0.33)
$\langle I/\sigma I \rangle$	23.9 (1.4)	18.2 (2.2)	10.5 (1.8)
CC1/2 (highest bin)	(0.793)	(0.724)	(0.591)
Completeness (%)	99.6 (96.4)	95.0 (74.6)	76.0 (59.4)
Redundancy	3.5 (2.9)	5.2 (4.0)	3.7 (1.7)
Observed reflections	443,217	219,704	97,888
Unique reflections	126,363	42,120	26,155
Refinement			
Resolution (Å)	35.00-1.30	35.00-1.90	35.00-2.05
Number of reflections	120,157	40,023	24,785
Reflections in R_{free} set	6,152	2,050	1,353
Fab-MICA complexes in the ASU	1	1	1
R_{work}/R_{free} (%)	18.84/20.54	20.84/24.68	22.34/27.46
Mean *B*-factor (Å^2) Protein Water Glycerol Sulfate Zinc Total solvent All atoms	 13.83 32.41 N/A N/A N/A 32.41 16.24	 19.76 38.02 52.85 64.49 33.63 40.70 21.28	 25.39 33.36 N/A N/A N/A 33.36 25.51
Wilson *B*-factor (Å^2)	14.66	23.66	25.87
No. atoms Protein Water Glycerol Sulfate	 4,186 625 0 0	 4,008 260 48 5	 4,043 61 0 0

Data Collection	1D5 Fab – MICA*008 α3	13A9 Fab – MICA*008 α3	6E1.1.12 Fab – MICA*008 C273S α3
Zinc	0	1	0
Total solvent	625	314	61
All atoms	4,811	4,322	4,104
R.m.s. deviations			
Bond lengths (Å)	0.007	0.006	0.006
Bond angles (°)	1.30	1.12	1.13
Ramachandran plot (%)			
Favored	98.9	97.7	97.5
Allowed	1.1	2.3	2.5
Outliers	0	0	0
Rotamer outliers (%)	1.25	0.91	0.89

Note: *Values in parentheses are for highest-resolution shell.

[0498] The resolution for the 6E1.1.12 Fab-MICA*008 C273S α3 domain complex was called at 2.21 Å where completeness > 50%, but data to 2.05 Å, where CC1/2 > 0.5, were used in refinement. Mean *B*-factors were calculated using Baverage in CCP4 and Wilson *B*-factors calculated using Xtriage in PHENIX. Ramachandran statistics and rotamer outliers were calculated using PDB Tools in PHENIX.

X-ray Structure Determination of Fab-MICA*008 α3 Domain Complexes

[0499] Data were integrated and scaled using HKL2000. The 1D5 Fab-MICA*008 crystal belonged to the $P2_1$ space group with one Fab-MICA complex in the asymmetric unit (ASU). The 13A9 Fab-MICA*008 crystal belonged to the $P2_12_12_1$ space group with one Fab-MICA complex in the ASU. The 6E1.1.12 Fab-MICA*008 C273S crystal was also found to be space group $P2_12_12_1$ with one Fab-MICA complex in the ASU (**Table 11**).

[0500] All structures were determined by molecular replacement using PHASER-MR in PHENIX. For each complex five sequential molecular replacement searches were performed with each of the Fab constant domains (CL and CH1), Fab variable domains (VL and VH), and the MICA α3 domain as individual search models as described below.

[0501] All of the Fabs used in this study contain human kappa CL and human IgG1 CH1 constant domains. The individual CL (residues 108-212 of chain A) and CH1 (residues 121-220 of chain B) domains from the Fab fragment of Herceptin (Protein Data Bank (PDB) accession 1NZ8) were used as search models.

[0502] The 1D5 Fab VL domain is derived from the murine IGKV4-91*01 germline gene and residues 1-108 of chain L from PBD accession 4M1G were used as a search model. The 1D5 Fab VH domain is derived from the murine IGHV1S135*01 germline gene and residues 1-113 of chain H from PDB accession 1F3D were used as a search model.

[0503] The 13A9 Fab VL domain is derived from the murine IGKV12-41*01 germline gene and residues 1-107 of chain A from PDB accession 1A2Y were used as a search model. The 13A9 Fab VH domain is derived from the murine IGHV1-54*02 germline gene and residues 2-113 of chain B from PDB accession 4J8R were used as a search model.

[0504] The 6E1.1.12 VL domain is derived from the murine IGKV1-117*01 germline gene and residues 1-107 of chain J from PDB accession 1F3D were used as a search model. The 6E1.1.12 Fab VH domain is derived from the murine IGHV1-84*01 germline gene and residues 1-113 of chain B from PDB accession 1CT8 were used as a search model.

[0505] For the 1D5 Fab-MICA*008 complex structure residues 181-274 of chain C from MICA*001 PDB accession 1HYR was used as a search model. For subsequent structures of the 13A9 Fab-MICA*008 and 6E1.1.12 Fab-MICA*008 complexes, residues 204-297 of MICA*008 from the 1D5 Fab-MICA*008 complex was used as a search model for the α3 domain.

[0506] Upon obtaining molecular replacement solutions, models were built using COOT and refined in REFMAC and PHENIX. Mean B-factors were calculated using Baverage in CCP4 and Wilson B-factors calculated using Xtriage in PHENIX. Ramachandran statistics and rotamer outliers were calculated using PDB Tools in PHENIX. Contacts were analyzed with Contact/NCONT and PISA in CCP4. All figures were generated with PYMOL.

[0507] The resolution of the 1D5 Fab-MICA*008 complex structure was 1.30 Å and the model was refined to an R_{free} value of 20.5%. The final model contains residues 1-214 of the 1D5 LC, residues 2-216 of the 1D5 HC, and residues 204-297 of the MICA*008 α3 domain, with residues 215-220 of MICA*008 disordered in the structure. Ramachandran statistics indicate 98.9% of residues lie in favored regions, 1.1% in allowed regions, and there are no outliers (**Table 11**).

[0508] The resolution of the 13A9 Fab-MICA*008 complex structure was 1.90 Å and the model was refined to an R_{free} value of 24.7%. The final model contains residues 1-214 of the 13A9 LC, residues 1-217 of the 13A9 HC, and residues 204-297 of the MICA*008 α3 domain. Ramachandran statistics indicate 97.7% of residues lie in favored regions, 2.3% in allowed regions, and there are no outliers (**Table 9**).

[0509] The resolution of the 6E1.1.12 Fab-MICA*008 C273S complex structure was called at 2.21 Å where completeness was still above 50% (59.4%), however data to 2.05 Å resolution, where CC1/2 was still greater than 0.5, were used in the refinement. The model was refined to an R_{free} value of 27.5%. The final model contains residues 1-212 of the 6E1.1.12 LC, residues 2-214 of the 6E1.1.12 HC, and residues 204-297 of the MICA*008 α3 C273S domain. Ramachandran statistics indicate 97.5% of residues lie in favored regions, 2.5% in allowed regions, and there are no outliers (**Table 11**).

Structural Analysis of the 1D5 Fab-MICA*008 α3 Domain Complex

[0510] The structure of the 1D5 Fab in complex with the MICA*008 α3 domain reveals that 1D5 binds to the "front face" of the α3 domain, defined as the beta sheet of the immunoglobulin (Ig) domain containing the carboxy-terminal beta strand (**FIG. 22A-FIG. 22D**). Approximately 1526 $Å^2$ of surface area is buried at the interface of the 1D5 Fab and the MICA*008 α3 domain, 934 $Å^2$ of which comes from contacts with the HC and 526 $Å^2$ from the LC. Residues from all six CDRs make interactions with MICA*008, with the largest number of contacts coming from CDR H1 followed by CDRs L3 and H3 (**FIG. 23A-FIG. 23B**). The epitope of 1D5 is defined as residues of the MICA*008

α3 domain within 4.5 Å of the 1D5 Fab in the structure of the complex (**Table 12** and **FIG. 24A-FIG. 24D**). Similarly the paratope of 1D5 is defined as residues of the 1D5 Fab within 4.5 Å of the MICA*008 α3 domain (**Table 12** and **FIG. 24A-FIG. 24D**).

Table 12. Epitope residues of MICA*008 and their corresponding paratope residues of 1D5

MICA*008α3 Epitope Residues	1D5 LC Paratope Residues	1D5 HC Paratope Residues
Arg240		Ser31, Gln32
Gln241	Tyr32, Gly91, Ser92	
Asp242	Gly91, Leu94, Leu96	Asn33, Tyr35, Tyr50, Ser95, Asn99
Gly243		Gln32, Asn33, Ser95, Gly96
Val244		Tyr35, Ser95, Gly96, Ser97, Ser98, Asn99
Ser245	Arg50	Gly96
Leu246	Tyr32	
Ser247	His31, Arg50	
Asp249	His31	
Thr250	His31	
Arg279	Gly91, Ser92, Ser93, Leu94	Tyr50
Tyr283		Ser31
His290		Ala28, Thr30, Ser31, Tyr53
Ser291		Tyr53
Thr292		Thr30, Glu52, Tyr53, Asn54
Pro294		Asn54

Note: The 1D5 epitope was defined as MICA*008 α3 domain residues within 4.5 Å of the 1D5 Fab. The 1D5 paratope was defined as 1D5 residues within 4.5 Å of the MICA*008 α3 domain. Distances were calculated with Contact/NCONT in CCP4.

[0511] 1D5 interacts with MICA*008 α3 domain using a combination of both polar and non-polar interactions. The polar interactions observed include both hydrogen bonds (**FIG. 25A-FIG. 25D**) and electrostatic interactions (**FIG. 26A-FIG. 26B**). The side chain of Ser247 of MICA*008 makes an intramolecular hydrogen bond with the side chain of Asp249 (**FIG. 25A**). This positions Asp249 to make an electrostatic interaction with His31 of CDR L1 (**FIG. 26A-FIG. 26B**). The 1D5 LC also contributes two hydrogen bonds with the main chain carbonyls of Gly91 and Ser92 of CDR L3 both forming hydrogen bonds with two amines from the guanidinium group of the side chain of Arg279 in MICA*008 (**FIG. 25B**). Several hydrogen bonds and polar interactions also come from the HC of 1D5. Two hydrogen bonds come from residues in CDR H1 with the main chain carbonyl of Ser31 of H1 forming a hydrogen bond with the side chain of Arg240 in MICA*008 (**FIG. 25C**) and the side chain hydroxyl of Tyr35 of H1 forming a hydrogen bond with the main chain carbonyl of Asp242 of MICA*008 (**FIG. 25D**). Tyr50 of CDR H2 also makes a hydrogen bond with the side chain carboxyl of Asp242 of MICA*008 (**FIG. 25D**). In addition the side chain carbonyl of Asn54 of CDR H2 forms a polar interaction with the main chain carbonyl of T292 of MICA*008 (**FIG. 25C**) and the main chain carbonyl of Gly96 of CDR H3 makes a hydrogen bond with the main chain amide of Ser245 of MICA*008 (**FIG. 25D**). The non-polar interactions observed include van der Waals interactions and

shape complementarity. The 240s loop of MICA*008 protrudes out from the "front face" of the Ig domain creating a convex surface on the α3 domain (**FIG. 27A-FIG. 27B**). The 1D5 Fab uses shape complementarity at the interface of the LC and HC to accommodate this surface. Both CDRs L3 and H3 are involved in binding the 240s loop and both contain a string of residues with small side chains such as serine or no side chain such as glycine. These CDRs splay away from the LC/HC interface with the serine side chains pointed away from the MICA*008 interface creating a depression in which the convex surface of the MICA*008 binds (**FIG. 27C-FIG. 27E**). The nature of the CDR L3 and H3 amino acid composition as well as their conformations results in a concave surface that complements the convex nature of the 240s loop. Whereas the side chains and overall loop conformations of CDRs L3 and H3 bend away from the MICA*008 interface, CDRs L1, L2, H1, and H2 and their side chains tend to point towards the interface filling in concave regions of the MICA*008 α3 domain surface (**FIG. 27F-FIG. 27G**).

Structural Analysis of the 13A9 Fab-MICA*008 α3 Domain Complex

[0512] The structure of the 13A9 Fab in complex with the MICA*008 α3 domain reveals that 13A9 binds to the "front face" of the α3 domain, defined as the beta sheet of the Ig domain containing the carboxy-terminal beta strand (**FIG. 28A-FIG. 28D**). Approximately 1750 $Å^2$ of surface area is buried at the interface of the 13A9 Fab and the MICA*008 α3 domain, 1086 $Å^2$ of which comes from contacts with the HC and 664 $Å^2$ from the LC. Residues from all six CDRs make interactions with MICA*008, with the largest number of contacts coming from CDR H2 followed by CDRs L3 and H3 (**FIG. 29A-FIG. 29B**). The epitope of 13A9 is defined as residues of the MICA*008 α3 domain within 4.5 Å of the 13A9 Fab in the structure of the complex (**Table 13** and **FIG. 30A-FIG. 30D**). Similarly the paratope of 13A9 is defined as residues of the 13A9 Fab within 4.5 Å of the MICA*008 α3 domain (**Table 13** and **FIG. 30A-FIG. 30D**). 13A9 interacts with MICA*008 α3 domain using a combination of both polar and non-polar interactions. The polar interactions observed include both hydrogen bonds (**FIG. 31A-FIG. 31D**) and electrostatic interactions (**FIG. 32A-FIG. 32B**). Residues from both the LC and HC form hydrogen bonds with MICA*008. Three hydrogen bonds come from CDR L1 of 13A9. The side chain of His30 makes a hydrogen bond with the main chain amide of Ser297, the main chain carbonyl group of Ser31 makes a hydrogen bond with the side chain of Gln278, and the side chain of Tyr32 forms a hydrogen bond with the main chain carbonyl of Val295 (**FIG. 31A**). An additional hydrogen bond from the LC comes from Tyr50 of CDR L2 where the side chain makes a hydrogen bond with the main chain carbonyl of Gln278 (**FIG. 31A**). A total of nine hydrogen bonds come from the HC of 13A9. The main chain carbonyl of Asn31 of CDR H1 forms a hydrogen bond with the side chain of Arg240 (**FIG. 31B**). CDR H2 forms five hydrogen bonds with the MICA*008 α3 domain (**FIG. 31C**). The side chain of Asn52 makes a hydrogen bond with the side chain of Tyr283. The side chain of Ser54 makes a hydrogen bond with the side chain of Glu285. The main chain carbonyl of Ala56 makes a hydrogen bond with the side chain of His290. Finally, Thr57 makes two hydrogen

bonds with its main chain amide forming a bond with the main chain carbonyl of G288 and the main chain carbonyl of Thr57 making a hydrogen bond with the main chain amide of His290. CDR H3 of 13A9 makes three hydrogen bonds with MICA*008, but uses only a single residue to make these bonds (**FIG. 31D**). The side chain of Asn98 makes hydrogen bonds with the main chain carbonyls of Gln278 and of Phe280, as well as with the side chain of Thr281. The non-polar interactions of 13A9 and MICA*008 include van der Waals interactions and shape complementarity. The start of the 240s loop (residues 240-245), the end of the 270s loop (residues 277-279), and the carboxy-terminal beta strand (residues 288-297) of the MICA*008 α3 domain protrude out from the "front face" of the Ig domain creating two ridges with a valley between them (**FIG. 33A-FIG. 33B**). 13A9 binds using all six of its CDRs to span the ridges and valley (**FIG. 33C**). Tyr32 of CDR L1, Tyr96 of CDR L3, Leu33 of CDR H1, Ala56 of CDR H2, and Phe95 and Asn98 of CDR H3 bind with their side chains pointing into the valley creating a complementary convex surface for recognition of the "front face" of the MICA*008 α3 domain (**FIG. 33D-FIG. 33E**). Side chains of residues such as His30 and Ser31 of CDR L1, Tyr50 of CDR L2, Trp92 and Thr94 of CDR L3, Asn31 and Tyr32 of CDR H1, Asn52, Ser54, and Asn58 of CDR H2, and Leu96 of CDR H3 fold over the ridges making significant van der Waals interactions with MICA*008 (**FIG. 33D-FIG. 33E**).

Table 13. Epitope residues of MICA*008 and the corresponding paratope residues of 13A9

MICA*008 α3 Epitope Residues	**13A9 LC Paratope Residues**	**13A9 HC Paratope Residues**
Arg240		Asn31, Tyr32, Leu33, Asn52
Asp242		Tyr32, Leu96, Gly97
Gly243		Asn31, Tyr32, Phe95, Leu96, Gly97
Val244		Asn31, Tyr32
Glu277	Tyr32	
Gln278	His30, Ser31, Tyr32, Tyr50	Gly97, Asn98
Arg279	Tyr50	Gly97, Asn98
Phe280		Asn98
Thr281		Phe95, Asn98
Tyr283		Leu33, Asn52, Ser54, Ala56,
Glu285		Asn52, Ser54, Ala56
Gly288		Ala56, Thr57
Asn289		Ala56, Thr57
His290		Leu33, Ala50, Ile51, Asn52, Ala56, Thr57, Asn58
Ser291		Asn58
Thr292	Thr94, Tyr96	Leu33, Asn58
Pro294	Tyr32, Phe91, Trp92	Asn98
Val295	Tyr32, Trp92	
Pro296	His30, Trp92	
Ser297	His30	

Note: The 13A9 epitope was defined as MICA*008 α3 domain residues within 4.5 Å of the 13A9 Fab. The 13A9 paratope was defined as 13A9 residues within 4.5 Å of the MICA*008 α3 domain. Distances were calculated with Contact/NCONT in CCP4.

Structural Analysis of the 6E1.1.12 Fab-MICA*008 C273S α3 Domain Complex

[0513] The structure of the 6E1.1.12 Fab in complex with the MICA*008 C273S α3 domain reveals that 6E1.1.12 binds to the "back face" of the α3 domain, defined as the beta sheet of the Ig domain containing the amino-terminal beta strand (**FIG. 34A-FIG. 34D**). Approximately 1416 $Å^2$ of surface area is buried at the interface of the 6E1.1.12 Fab and the MICA*008 α3 domain, 1046 $Å^2$ of which comes from contacts with the HC and 370 $Å^2$ from the LC. Residues from five of the six CDRs make interactions with MICA*008, with the largest number of contacts coming from CDR H3 followed by CDRs L1 and H2, and no contacts from CDR L3 (**FIG. 35A-FIG. 35B**). The epitope of 6E1.1.12 is defined as residues of the MICA*008 C273S α3 domain within 4.5 Å of the 6E1.1.12 Fab in the structure of the complex (**Table 14** and **FIG. 36A-FIG. 36D**). Similarly the paratope of 6E1.1.12 is defined as residues of the 6E1.1.12 Fab within 4.5 Å of the MICA*008 C273S α3 domain (**Table 14** and **FIG. 36A-FIG. 36D**).

Table 14. Epitope residues of MICA*008 C273S and the corresponding paratope residues of 6E1.1.12

MICA*008 α3 Epitope Residues	**6E1.1.12 LC Paratope Residues**	**6E1.1.12 HC Paratope Residues**
Thr224		Tyr98
Arg226		Asp31, Tyr98
Arg233	Ser27E, Asn28	
Trp253		Ser100A
Gly254		Tyr97, Ser100A
Asp255	Asn28, Asn30, Tyr32, Lys50	Tyr97, Ser100A, Gly100B
Val256	Asn28	
Leu257	His27D, Asn28, Tyr32	Tyr33, His95, Tyr97, Trp100D
Pro258		Tyr33, Trp50
Asp259		Trp50, Tyr52, Thr54, Gly56
Gly260		Trp50, Gly56, Ser57
Asn261		Thr54, Gly56
Gln265		Tyr33, Tyr52, Tyr97
Thr266		Tyr97
Trp267		Tyr97, Tyr98, Ser100A

Note: The 6E1.1.12 epitope was defined as MICA*008 C273S α3 domain residues within 4.5 Å of the 6E1.1.12 Fab. The 6E1.1.12 paratope was defined as 6E1.1.12 residues within 4.5 Å of the MICA*008 C273S α3 domain. Distances were calculated with Contact/NCONT in CCP4.

[0514] 6E1.1.12 interacts with MICA*008 α3 domain using a combination of both polar and non-polar interactions. The polar interactions observed include hydrogen bonds, salt bridges, pi-cation interactions, and electrostatic interactions (**FIG. 37A-FIG. 37C, FIG. 38A-FIG. 38B**). Residues from both the LC and HC form hydrogen bonds with MICA*008. Two residues in CDR L1 make hydrogen bonds with MICA*008. The side chain of Asn28 forms a hydrogen bond with the main chain carbonyl of Val256 of MICA*008 and the side chain of Tyr32 makes a hydrogen bond with the side chain of Asp255 of MICA*008 (**FIG. 37A**). The positively charged Lys50 of CDR L2 forms a salt bridge with

the negatively charged Asp255 of MICA*008 (**FIG. 37A**). The negatively charged Asp31 of CDR H1 forms a salt bridge with the positively charged Arg226 of MICA*008 (**FIG. 37B**). The side chain of Tyr33 of CDR H1 forms a hydrogen bond with the side chain of Gln265 and with the main chain carbonyl of Pro258 of MICA*008 (**FIG. 37B**). The side chain of Thr54 makes a hydrogen bond with the main chain carbonyl of Asp259 (**FIG. 37B**). Tyr97 of CDR H3 makes three hydrogen bonds. The main chain carbonyl of Tyr97 forms a hydrogen bond with Trp267 of MICA*008 and the side chain of Tyr97 forms two hydrogen bonds with the main chain carbonyls of Asp255 and Gln265 of MICA*008 (**FIG. 37C**). The side chain of Ser100A of CDR H3 also forms a hydrogen bond with the main chain amide of Asp255. Finally Tyr98 forms a pi-cation interaction with Arg226 of MICA*008 (**FIG. 37C**). The non-polar interactions observed include van der Waals interactions and shape complementarity. CDR L1 of 6E1.1.12 has a five amino acid insertion after residue 27 (residues 27A-27E) creating a much longer L1 loop than typically found in most antibodies. This results in CDR L1 protruding out farther than all other CDRs of 6E1.1.12, which creates a groove between CDR L1 and CDR H3 (**FIG. 39A-FIG. 39B**). A longer CDR L1 is often seen in antibodies that bind peptide antigens, with the peptide binding in the cleft created by CDRs L1 and H3. The 250s loop of MICA*008 (residues 254-260) creates a ridge across the "back face" of the α3 domain (**FIG. 39C-FIG. 39D**). The 250s loop binds in the groove between CDR L1 and CDR H3 of 6E1.1.12 mimicking a peptide antigen (**FIG. 39E-FIG. 39F**). In particular the side chains of Asp255 and Leu257 point into the cleft and make interactions with residues from both CDRs L1 and H3. Leu257 binds in a hydrophobic pocket formed by residues His27D, Asn28, Tyr32 from the LC and Tyr33, His95, Tyr97, Trp100D from the HC (**FIG. 39G**). This pocket is heavily lined with aromatic residues. Asp255 binds into a pocket formed by residues Asn28, Asn30, Tyr32, Lys50 of the LC and Tyr97, Ser100A, Gly100B of the HC (**FIG. 39H**) and forms a hydrogen bond with Tyr32 and a salt bridge with Lys50 of the LC (**FIG. 37A**).

Comparison of the 1D5 Fab-MICA*008 α3 Domain, 13A9 Fab-MICA*008 α3 Domain, and 6E1.1.12 Fab-MICA*008 C273S α3 Domain Complex Structures

[0515] Both 1D5 and 13A9 bind to the "front face" of MICA*008, as defined by the defined as the beta sheet of the Ig domain containing the carboxy-terminal beta strand, whereas 6E1.1.12 binds to the "back face" of MICA*008, defined as the beta sheet of the Ig domain containing the amino-terminal beta strand (**FIG. 40A**). Comparison of the epitopes (defined as residues within 4.5 Å of their respective Fabs) of 1D5 and 13A9 revealed that they have partially overlapping epitopes (**FIG. 40B-FIG. 40C**). Analysis of the reported MICA cleavage sites in the literature (*Cancer Res (2008); 68: 6368 and BBRC (2009); 387: 476*) revealed that all cleavage sites in the α3 domain mapped to the "front face" of the domain (**FIG. 41A**). Comparisons of the epitopes defined by crystallography with the reported cleavage sites demonstrated that the 1D5 and 13A9 epitopes overlapped with these cleavage sites found exclusively on the "front face" of the α3 domain (**FIG. 41B**). Binding of 1D5 or 13A9 to the MICA α3 domain may create steric hindrance and block access of proteases required for

cleavage of MICA. In contrast, 6E1.1.12 binds on the "back face" of MICA and away from any reported cleavage sites (**FIG. 41B**). Defining the epitopes helps explain why 1D5 and 13A9 are potent blockers of MICA*008 cleavage and shedding, whereas 6E1.1.12 is a relatively weak blocker of cleavage and shedding.

MicA Oxidative Footprinting by Fast Photochemical Oxidation of Proteins (FPOP)

[0516] The epitope of the 1D5 Fab on MICA*008 was mapped by oxidative footprinting using the Fast Photochemical Oxidation of Proteins (FPOP) method. The 1D5 Fab was expressed and purified as described above for crystallography. The MICA*008 α3 domain (residues Thr204-Ser297) was expressed with a C-terminal His8 tag in CHO DP12 cells. The CHO DP12 cell line is a derivative of the CHO-K1 cell line (ATCC number CCL-61) selected for large-scale production. The MICA*008 α3 domain expression plasmid was transiently transfected using PEI as previously described (Wong, A.W. *et al.* (2010) Biotechnol Bioeng 106:751-763). Supernatants were harvested 14 days after transfection and the secreted MICA*008 α3 domain was purified over a Ni Sepharose Excel (GE Healthcare) column followed by a HiLoad Superdex 75 pg column (GE Healthcare) as described above for crystallography.

[0517] A 1.5:1 molar ratio of 1D5 Fab to MICA*008 α3 domain antigen was prepared in PBS with a total protein concentration of 2.35 mg/mL. The solution was incubated at 37 °C for 30 minutes. Size exclusion chromatography was performed to ensure complete complex formation. For the unbound MICA*008 α3 domain antigen a 2.35 mg/mL solution was prepared in 1 mL PBS. Scavenger Arginine was added at 30 mM final concentration to the protein solutions. The unbound MICA*008 α3 domain or the MICA*008 α3 domain:1D5 Fab complex were flow-mixed with 30 mM peroxide and labeling was performed using the fast photochemical oxidation of proteins (FPOP) methodology previously described (Zhang, Y., et al. (2015) J Am Soc Mass Spectrom 26:526-529; Zhang, Y., *et al.* (2017) J Am Soc Mass Spectrom 28:850-858; Li, J. *et al.* (2017) Anal Chem 89:2250-2258). Briefly, 15 μL/min of MICA solutions were mixed with 30 mM H_2O_2 at a rate of 7.5 μL/min through a micro-tee mixer (Cobert Associates Lab, St. Louis, MO) for rapid mixing prior to light exposure. The oxidation reaction was initiated by pulsing a focused 248 nm KrF excimer laser (GAM Laser Inc., Orlando, FL) at 70 mJ/pulse at a rate of 7 Hz through a 3.0 mm exposure window of 150 μm i.d. fused-silica tubing (Polymicro Technologies, Phoenix, AZ). Oxidized samples were collected in a tube containing a quench solution with Methionine (Sigma Aldrich) and catalase (Sigma Aldrich) in a
final concentration of 40 mM and 1 μM, respectively. Samples were frozen and stored at -80°C before proteolysis and mass spectrometry. To account for background oxidation, an unexposed sample was obtained and compared to three oxidized replicates for each protein state. The sequence and duration of sample collection for FPOP experiments is shown in **Table 15**.

Table 15. Time Table for Labeling Triplicate Samples

Start Time (min)	End time (min)	Duration (min)	Action	Laser Status	Collection
0	2	2	Start Pump (Laser Off)	Off	Waste
2	8	6	Control (No Laser)	Off	Collection Vial
8	10	2	Turn on Laser	On	Waste
10	16	6	Sample 1 Collection	On	Collection Vial
16	22	6	Sample 2 Collection	On	Collection Vial
22	28	6	Sample 3 Collection	On	Collection Vial
28	End		End of Run	Off	Waste
			Change Capillary Position	Off	Waste

[0518] Upon thawing, samples were reduced, deglycosylated with PNGaseF, and alkylated with iodoacetamide then separated on SDS-PAGE (**FIG. 42**). The band at the 15 kDa region corresponding to the molecular weight of the MICA*008 α3 domain was excised from the MICA*008 α3 domain alone and the MICA*008:1D5 Fab complex samples, both unexposed and exposed. Samples were digested with trypsin and chymotrypsin at a 1:500 enzyme:substrate ratio. Digests were C18 stage tip cleaned, dried, and reconstituted in 100 uL of 0.1% Formic acid containing 2% acetonitrile. 1 uL was injected *via* auto-sampler onto a 75 μm×100 mm column (BEH, 1.7 micron, Waters Corp) at a flow rate of 1 μL/min using a NanoAcquity UPLC (Waters Corp). A gradient from 98% solvent A (water +0.1% formic acid) to 80% solvent B (acetonitrile +0.08% formic acid) was applied over 60 min. Samples were analyzed on-line *via* nanospray ionization into a hybrid Elite Orbitrap mass spectrometer (Thermo Fisher Scientific). Resultant spectra were interrogated against a theoretical tryptic and chymotryptic digestion of the protein sequence of the MICA*008 α3 domain construct used. Unmodified and oxidized species for each tryptic peptide were identified from the fragmentation spectra. Oxidation of Cysteine, Tryptophan, Tyrosine, Methionine, Phenylalanine, Histidine, Arginine, Leucine and Isoleucine were quantified by measuring areas under the curve of the matching modified ions versus total occurrence of a given peptide. Oxidation events for both the complex and the unbound MICA*008 α3 domain were then mapped onto the sequence of the MICA*008 α3 domain. Regions of protection were observed as having a significant change between the two values. Triplicate samples were averaged for the final percent oxidation and error bars represent the standard deviation of the three measurements.

[0519] A significant change in oxidation protection was observed for Trp239 within the MICA*008 α3 domain tryptic peptide containing residues Asn234-Arg240 and for residues Ile272 and Phe280 within the MICA*008 α3 domain chymotryptic peptide Val268-Phe280 (**FIG. 43A-B, FIG.44, FIG. 45**).

Comparison of Epitope Mapping Results from Glycosylation Engineering, Alanine Scanning, FPOP, and X-ray Crystallography

[0520] Comparison of the 1D5 epitope determined by glycosylation engineering, alanine scanning, FPOP and X-ray crystallography reveal consistent results between these methods.

[0521] The 1D5 antibody shows a complete loss in binding to the MICA*008 α3 domain containing either the glycosylation variant Glyco14 (G243N) or Glyco16 (R279N), but is unaffected by Glyco11 (R202N), Glyco12 (E215N), Glyco13 (I236T), Glyco15 (H248N), and Glyco17 (C-terminal addition of residues N298, G299, S300). Comparison of the epitope of 1D5 determined by X-ray crystallography with the location of the glycosylation site variants demonstrates that Glyco14 (G243N) and Glyco16 (R279N) are part of the 1D5 epitope, whereas Glyco11 (R202N), Glyco12 (E215N), Glyco13 (I236T), Glyco15 (H248N), and Glyco17 (carboxy-terminal addition of residues N298, G299, S300) fall outside of the 1D5 epitope (**FIG. 46A**).

[0522] The 13A9 antibody shows a partial loss in binding to the MICA*008 α3 domain containing either the glycosylation variant Glyco14 (G243N) or Glyco16 (R279N), but is unaffected by Glyco11 (R202N), Glyco12 (E215N), Glyco13 (I236T), Glyco15 (H248N), and Glyco17 (C-terminal addition of residues N298, G299, S300). Comparison of the epitope of 13A9 determined by X-ray crystallography with the location of the glycosylation site variants demonstrates that Glyco14 (G243N) and Glyco16 (R279N) are part of the 13A9 epitope, whereas Glyco11 (R202N), Glyco12 (E215N), Glyco13 (I236T), Glyco15 (H248N), and Glyco17 (carboxy-terminal addition of residues N298, G299, S300) fall outside of the 13A9 epitope (**FIG. 46A**). Residues N298, G299, and S300 added to the carboxy-terminus are not present in the crystal structure therefore Glyco17 is indicated in **FIG. 46A** by highlighting the carboxy-terminal residue S297, immediately preceding the added glycoslation site. Glycosylation at N298 does not affect binding of 13A9.

[0523] The 6E1.1.12 antibody demonstrated no loss of binding to any of the glycosylation variants tested (Glyco11 (R202N), Glyco12 (E215N), Glyco13 (I236T), Glyco14 (G243N), Glyco15 (H248N), Glyco16 (R279N), or Glyco17 (carboxy-terminal addition of residues N298, G299, S300)). Comparison of the epitope of 6E1.1.12 determined by X-ray crystallography with the location of the glycosylation site variants demonstrates that all glycosylation variants (Glyco11 (R202N), Glyco12 (E215N), Glyco13 (I236T), Glyco14 (G243N), Glyco15 (H248N), Glyco16 (R279N), and Glyco17 (carboxy-terminal addition of residues N298, G299, S300) fall outside of the 6E1.1.12 epitope (**FIG. 46A**).

[0524] All of the epitopes determined by glycosylation engineering are consistent with the epitopes determined by X-ray crystallography. Moreover, glycosylation engineering can discriminate between subtle difference in epitopes such as those of 1D5 and 13A9. As indicated above, X-ray crystal structure analysis demonstrates that 1D5 and 13A9 contain partially overlapping epitopes (**FIG. 40A-FIG. 40C**). Binding of both antibodies was affected by the Glyco14 (G243N) and Glyco16 (R279N) variants, however to different extents. Whereas 1D5 binding was completely abolished by either the

Glyco14 (G243N) or Glyco16 (R279N) variants, 13A9 demonstrated only a partial loss in binding to either of these two variants. Comparison of the X-ray crystal structures reveals that whereas the Glyco14 (G243N) and Glyco16 (R279N) variants are at the center of the binding interface with the 1D5 Fab, they are on the periphery of the binding interface with the 13A9 Fab (**FIG. 46B**). Glycosylation at positions 243 and 279 of MICA*008 would introduce a bulky sugar at the heart of the 1D5 epitope/paratope interaction that would disrupt binding, whereas the same sugars could potentially be accommodated by bending to the side of the 13A9 epitope/paratope away from the Fab, causing only a partial loss in binding.

[0525] Comparison of the epitope of 1D5 determined by FPOP to the epitope determined by the crystal structure reveals overlapping results (**FIG. 46C**). The FPOP epitope was defined by oxidation protection conferred to residue Trp239 in the tryptic peptide Asn234-Arg240 and Phe280 in the chymotryptic peptide Val268-Phe280 by binding of the 1D5 Fab to the MICA*008 α3 domain. These two FPOP peptides include residues Arg279 and Arg240 that are defined as part of the 1D5 epitope from the co-crystal structure (**Table 10** and **FIG. 24A-FIG. 24D**).

[0526] Comparison of the epitope of 1D5 determined by alanine scanning to the epitope determined by the crystal structure also reveals highly overlapping results (**FIG. 46C**). Residues Arg240, Gln241, Val244, Ser245, Asp249, Thr250, Arg279, Tyr283, His290, and Thr292 identified as part of the 1D5 epitope by alanine scanning were also identified as part of the epitope in the crystal structure. Residues Asp242, Gly243, and Leu246 are part of the 1D5 epitope determined by the crystal structure, however they could not be tested in the alanine scan. These residues when mutated to alanine resulted in misfolded protein, as inferred by a lack of binding to these mutants by an antibody known to bind to the opposite face of the MICA*008 α3 domain (on the "back face"). Only three residues, Ser247, Ser291 and Pro294 identified as part of the epitope by the crystal structure were not part of the epitope determined by alanine scanning. All of these residues are on the periphery of the epitope in the crystal structure (**FIG. 24A-FIG. 24D**). Four additional residues, His248, Glu276, Glu285, and Ser287 were identified as part of the 1D5 epitope by alanine scanning that were not found in the epitope determine by the crystal structure. These residues are all found along the periphery of the epitope determined in the co-crystal structure (**FIG. 46C**).

[0527] While crystallography is the gold standard for epitope determination as it provides very high-resolution sequence information about the binding interface between two proteins, it is very labor intensive and low throughput. Alanine scanning, while also providing binding resolution at the level of individual residues, is only slightly less labor intensive than crystallography as many individual alanine point mutants must be expressed, purified and characterized. FPOP is still higher throughput yet, however the sequence resolution is compromised by the fact that binding residues are identified within linear peptides and the peptide fragments are determined by the presence or absence of protease cleavage sites in the primary amino acid sequence. Additionally, not all amino acids are equally susceptible to oxidation and side chains are more susceptible than the main chain peptide backbone.

Therefore, not all contact residues in an epitope will be identified by FPOP as would be in a crystal structure.

[0528] Cross-blocking studies of pair-wise combinations of antibodies is the highest throughput method of epitope mapping but suffers from low-resolution sequence information. Glycosylation engineering combines the higher throughput of methods such as cross-blocking or FPOP with the high-resolution sequence information of alanine scanning and crystallography. Glycosylation engineering provides a rapid, high throughput method to map epitopes of large panels of antibodies with resolution at the individual residue level.

Comparison of the 1D5 and 13A9 Epitopes with Previously Described Epitopes

[0529] All of the previously described anti-MICA/B epitopes that block shedding map to the "back face" of MICA/B defined as the beta sheet of the Ig domain containing the amino-terminal beta strand. These include Dana Farber's CM33322 Ab4, CM33322 Ab28, and CM33322 Ab29, Innate Pharma's 16A8, and the University of Washington's H9 anti-MICA/B antibodies (**FIG. 47A-FIG. 47B**). This is in contrast to the 1D5 and 13A9 epitopes that map to the "front face" of MICA/B defined as the beta sheet of the Ig domain containing the carboxy-terminal beta strand (**FIG. 47A-FIG. 47B**).

Example 11: Biacore Anti-MICA/B Humanized Variants Kinetics

[0530] The binding kinetics of humanized variants of 1D5, 13A9, and 15F11 anti-MICA/B antibodies were measured using surface plasmon resonance (SPR) on a Biacore 8K instrument (GE Healthcare). A Protein A sensorchip (GE Healthcare) was used following manufacturer provided protocols to capture humanized anti-MICA/B antibodies. The MICA/B alleles were passed over. Antibody binding was measured to human MICA 002, 004, 008 and MICB 005 alpha 3 domains (His tagged, in-house). Sensograms for binding of MICA/B were recorded using an injection time of 2 minutes with a flow rate of 30 ml/min, at a temperature of 25°C, and with a running buffer of 10mM HEPES, pH 7.4, 150 mM NaCl, 3mM EDTA, and 0.005% Tween 20. After injection, disassociation of the alpha 3 domain from the antibody was monitored for 10 minutes in running buffer. The surface was regenerated between binding cycles with a 60 ul injection of 10 mM Glycine HCl pH 1.5. After subtraction of a blank which contained running buffer only, sensograms observed for MICA binding to anti-MICA/B antibody were analyzed using a 1:1 Langmuir binding model with software supplied by the manufacturer to calculate the kinetics and binding constants. The data was analyzed using a 1:1 binding model. Sensograms of MICA alleles binding to captured anti-MICA antibody were used to calculate the dissociation constant (Kd). Kinetic constants from these data are provided in **Table 16**.

Table 16: Kinetic Constants for Anti-MICA Antibodies Binding to its Ligands

Clone	huMICA*002 alpha 3-his	huMICA*004 alpha 3-his	huMICA*008 alpha 3-his	huMICB*005 alpha 3-his

	Kd (nM)	Kd (nM)	Kd (nM)	Kd (nM)
1D5	0.83	0.63	0.61	0.95
1D5v1	1.4	1.17	0.96	1.96
1D5v2	1.69	1.34	0.89	2.69
1D5v3	1.21	0.96	0.85	1.78
1D5v4	6.63	6.20	7.45	16.19
1D5v5	2.59	2.13	1.64	3.99
1D5v6	3.69	2.91	2.61	6.15
1D5v7	2.21	1.67	1.70	3.31
1D5v8	11.33	9.41	9.08	18.82
1D5v9	1.31	0.95	0.87	1.51
1D5v10	1.44	1.08	1.05	1.98
1D5v11	1.09	0.83	0.78	1.38
1D5v12	4.64	3.91	3.66	9.16
1D5v13	1.20	0.91	0.85	1.48
1D5v14	1.62	1.23	1.16	2.14
1D5v15	1.06	0.81	0.76	1.26
1D5v16	4.09	3.42	3.21	8.37
1D5v17	0.91	0.68	0.64	1.01
1D5v18	1.04	0.79	0.75	1.41
1D5v19	1.16	0.88	0.83	1.51
1D5v20	1.14	0.88	0.83	1.57
1D5v21	1.35	1.08	0.99	1.98
1D5v22	6.46	5.45	4.98	13.25
1D5v23	1.11	0.90	0.58	1.65
1D5v24	1.21	0.90	1.41	1.62
1D5v25	1.22	0.96	0.93	1.84
1D5v26	3.90	3.17	3.12	7.15
1D5v27	1.85	1.52	1.44	3.31

Clone	huMICA*002 alpha 3-his	huMICA*004 alpha 3-his	huMICA*008 alpha 3-his	huMICB*005 alpha 3-his
	Kd (nM)	Kd (nM)	Kd (nM)	Kd (nM)
1D5v28	1.19	0.87	0.84	1.49
1D5v29	2.01	1.58	1.65	3.86
13A9	4.82	6.77	2.35	2.35
13A9v1	5.87	8.37	5.60	5.12
13A9v2	4.44	5.32	2.56	3.15
13A9v3	4.97	4.79	3.85	3.15
13A9v4	3.93	3.69	2.72	2.40
13A9v5	4.27	5.52	3.68	3.17
13A9v6	3.94	3.77	2.80	2.45
15F11	2.21	1.31	0.79	2.28
15F11v1	12.80	5.96	6.52	16.10
15F11v2	5.05	2.74	3.17	6.71
15F11v3	12.74	5.70	5.56	14.10
15F11v4	4.75	2.58	2.58	5.72
15F11v5	6.05	3.11	3.20	7.80
15F11v6	5.47	2.91	2.94	6.62
6E1.1.12	1.49	3.95	3.72	2.96

Example 12: Anti-alpha 3 MICA/B antibody modulation of MIC-NKG2D pathway in *in vivo* tumor models

[0531] This Example describes the effect of anti-MIC (1D5 antibody) modulation in *in vitro* and *in vivo* assays.

Introduction

[0532] MICA (MHC class I Chain Related Gene A) expression is induced in response to cellular stress and in tumor cells, MICA is induced by DNA damage. MICA binds to the activating receptor NKG2D on CD8+ T and NK cells. NKG2D engagement then induces cytolytic activity in NK cells and costimulates CD8+ T cells. Tumor cells cleave cell surface MICA and the reduced surface MIC expression impairs NKG2D-mediated tumor cell killing. Shed MICA may interfere with NKG2D-mediated binding to tumor cells. A proposed hypothesis for the mechanism of action of anti-MIC antibodies is that anti-MIC antibodies prevent shedding, increasing surface expression and recognition by cytotoxic cells

Results

[0533] As described in Example 8, cell shedding assays were performed using various cell lines: MEL-JUSO, HCC1534, and PANC-1. Cells were incubated at 37°C in medium containing anti-MIC 1D5 antibodies for 24 hours. Subsequently, cell supernatants were harvested and diluted (if needed), analyzed in the relevant ELISA format (MICA or MICB), and quantitated using the corresponding MIC ECD material. Percent shedding inhibition was calculated using the formula: 1-([sMIC] ab-treated cell sub)/([sMIC] untreated cells) where sMIC represented soluble MICA or soluble MICB. EC50 was also determined from these data. **Table 17** displays antibody affinity, IC50 and percent maximum inhibition for each cell shedding assay.

Table 17: Cleavage inhibition activity of antibody 1D5

1D5	MICA*008	MICB*005	MICA*002	MICA*004
	MEL-JUSO cell shedding assay	HCC1534 cell shedding assay	PANC-1 cell shedding assay	HCC1534 cell shedding assay
Affinity (nM)	0.54	2.28	0.82	0.56
IC50 (mg/mL)	0.008234	0.0293	0.02906	0.0247
Max inhibition	81%	52%	53%	70%

[0534] Next, MIC-NKG2D interactions were evaluated using NKG2D-Fc, anti-Fc antibodies, and anti-MIC antibodies. As shown in **FIG. 48,** blocking antibody 8C5 disrupted NKG2D binding in a dose-dependent manner whereas the 1D5 antibody did not interfere with MIC-NKG2D interactions.

[0535] The crystal structure of the 1D5 Fab bound to the MICA*008 α3 domain indicated that 1D5 binds to the "front face" of the α3 domain containing the C-terminal beta strand (**FIG. 49A**). Addionally, the epitope of 1D5, defined as MICA residues within 4.5 Å of the Fab, as well as reported cleavage sites were identified on the surface of the "front face" of the MICA*008 α3 domain (**FIG. 49B**). Taken together, these findings suggest an overlap in the 1D5 Fab-MICA*008 α3 Domain Complex of the 1D5 Epitope and MICA Cleavage Sites

[0536] The anti-tumor effect of 1D5 treatment in xenograft tumor models propagated from HCC1534 cells in BALB/c SCID mice was then analyzed. As shown in **FIG. 50A**, 10 million HCC1534 were used for tumor inoculation in each mouse (n = 10 mice/treatment group). Anti-MIC 1D5 IgG2a, Anti-MIC 1D5 IgG1, or anti-gp120 (control) were then administered TIW for four weeks to each group. Subsquently, PK samples were collected every 7 days for 21 days. Tumor volume by caliper measurement indicated prevention efficacy with anti-MIC 1D5 IgG2a treatment, but not 1D5 IgG1, suggesting antibody effector function is required. **(FIG. 50B).** Tumors were also harvested on day 7 post inoculation, processed, and stained with antibodies for FACS analysis, indicating that HCC1534 tumor growth inhibition was associated with increased NK cell numbers (**FIG. 50C**) and increased Granzyme B expression (**FIG. 50D**)

[0537] Next, $1x10^4$ B16 cells engineered to express MICA (B16-MICA002) upon Doxicycline (dox) induction were plated per well with 1ug/mL dox and 5ug/mL antibody for 24 hours. Cells were then harvested and stained for MICA surface expression. 1D5 treatment was found to stabilize MICA surface expression in B16-MICA002 in a dox-dependent manner **(FIG. 51A).** The anti-tumor effect of 1D5 treatment in xenograft tumor models propagated from B16-MICA002 cells was then analyzed. As shown in **FIG. 51B**, tumor volume by caliper measurement indicated modest prevention efficacy with anti-MIC 1D5 IgG2a treatment and anti-PD-L1 when administered as single agents. However, a robust additive effect in the group receiving both anti-PDL1 and anti-MIC 1D5 IgG2a was observed. Tumor growth was not impacted by treatment with anti-MIC 1D5 IgG1, suggesting that ADCC may be an important mechanism.

Conclusion

[0538] Anti-MIC antibody,1D5, displayed cleavage inhibition *in vitro*, demonostrated broad activity against multiple alleles, and did not interfere with MIC-NKG2D interactions. Additonally, the crystal structure of the 1D5-MICA*008 complex revealed overlap of the 1D5 epitope with MICA cleavage sites.

[0539] Anti-tumor efficacy was observed with preventative antibody dosing of 1D5 in a mouse xenograft model, HCC1534, and was associated with an increased number of NK cells and increased granzyme B expression in NK cells.

[0540] Finally, in a B16.F10 MICA002 inducible over-expression model, 1D5 had both a single agent effect and robust additive effect when combined with anti-PDL1.

Example 13: Comparison of Epitopes Using Different Mapping Methods

[0541] This Example summarizes the epitopes determined for anti-MIC antbodies 1D5, 13A9, and 6E1.1.12 using various epitope mapping methods as shown in **Table 18**.

Table 18: Epitope Comparison.

Antibody	Glyco-Engineering	Alanine Scanning	X-Ray Crystallography	FPOP
1D5	**G243**: R240, Q241, D242, V244, S245, R279, T281; **R279**: R240, Q241, D242, G243, E276, E277, N278, T281	R240, Q241, V244, S245, H248, D249, T250, E276, R279, Y283, E285, S287, H290, T292	R240, Q241, D242, G243, V244, S245, L246, S247, D249, T250, R279, Y283, H290, S291, T292, P294	N234, I235, I236, L237, T238, W239, R240; V268, A269, T270, R271, I272, C273, R274, G275, E276, E277, Q278, R279, F280
13A9	'back & top': V205, P206, M208, T212, G219, T222, T224, R226, S228, Y231, P232, Q241, D242, G243, T250, D255-G262, Y264, Q265, W267, R271, G275, E277, R279, G288, N289, H290	N.D	R240, D242, G243, V244, E277, Q278, R279, F280, T281, Y283, E285, G288, N289, H290, S291, T292, P294, V295, P296, S297	N.D
6E1.1.12	'back and top': V205, P206, M208, T212, G219, T222, T224, R226, S228, Y231, P232, Q241, D242, T250, D255- G262, Y264, Q265, W267, R271, G275, E277, G288, N289, H290	N.D	T224, R226, R233, W253, G254, D255, V256, L257, P258, D259, G260, N261, Q265, T266, W267	N.D
15F11	**G243**: R240, Q241, D242, V244, S245, R279, T281; **R279**: R240, Q241, D242,	N.D	N.D	N.D

	G243, E276, E277, N278, T281			
18G3	**G243**: R240, Q241, D242, V244, S245, R279, T281	N.D	N.D	N.D
12H10	**G243**: R240, Q241, D242, V244, S245, R279, T281	N.D	N.D	N.D

Note: N.D. = not determined.

Example 14: MICA Shedding Inhibition and Interference using Anti-MICA/B Humanized Variants

[0542] This Example describes the testing of humanized anti-MIC antibodies for MIC shedding inhibition and interference in HEK293 and CHO cell lines.

Results

[0543] For variant samples, hIgG2a HEK293-derived material was used, which contained residual soluble MIC (sMICA at greater quantities than sMICB). This interference caused an over-recovery of sMIC in the samples, leading to decreased apparent shedding inhibition (**Table 19**). The sMIC co-diluted out with the antibody; therefore, less antibody showed less interference. Due to varying concentrations of antibodies, the amount of sMIC in samples varied with each antibody. Complicating the interference, there may have been artificial under-recovery of sMIC due to antibody interference that was observed regardless of purification method (CHO vs HEK293). As a result, reported data is from 0.625ug/mL of MICA samples and 2.5ug/mL for MICB, both for inhibition and interference; at these respective concentrations, many samples diluted out the majority of positive sMIC-related interference, but antibody effect was still observed. It should be noted that the observed antibody effect may not have reflected maximum potential inhibition.

[0544] Previous data have shown there to be some negative signal interference from antibodies on HCC1534 (MICA*004 and MICB*005) and much less on MEL-JUSO (MICA*008). Due to these two types of conflicting signal interference, some positive signal (MIC) and negative signal (Ab) interference on HCC1534 MICA*004, a predominantly positive signal (MIC) interference on MEL-JUSO MICA*008, and a predominantly negative signal (Ab) interference on HCC1534 MICB*005 were expected. For control antibodies, mIgG2a CHO-derived material was used; thus, the only significant interference observed was negative, possibly leading to more potent results than the true level of biological activity. Due to assay variation, absolute amounts of sMIC may have varied between the inhibition and interference assay.

[0545] There was insufficient sample in the following antibodies: 1D5v2, 1D5v15, 1D5v28, 13A9v1, and 15F11v1. Therefore, a previous antibody preparation was used. Due to automation errors, the accuracy of the aforementioned samples could not be guaranteed. 1D5v14 was able to be re-diluted, but the new sample preparation was slightly lower in concentration than intended. The original and possibly inaccurate sample dilution of 1D5v14 was also analyzed (**Table 19**).

[0546] Some interference samples were missing due to cold wells on an assay plate from clogged washer pins, notably HCC1534 A004: 13A9 v1; MEL-JUSO A008: 1D5v10, and 13A9v1; and HCC1534 B005: 1D5v3, 1D5v22, and 15F11v3. Thus, a 'ND' value was assigned since results could not be determined for each of these conditions (**Table 19**).

[0547] As shown in **Table 20**, positive cutoff was defined as the percent (%) inhibition determined by 3 times the standard deviation of all of the non-treated samples on the relevant plate. The average was determined using 3 assay plates.

*MICA*004 shedding Inhibition in HCC1534 cells*

[0548] All 1D5 variants (except for 1D5v28, which may have been compromised) showed inhibition activity approximately equivalent to the non-variant antibody (~50-60%), with some variance, and only minor interference (**Table 19**). In general, 13A9 variants showed minimal inhibition. 15F11 variants showed activity approximately similar to non-variant antibody (~50%) except for potentially compromised sample 15F11v1, and 15F11v4 and 15F11v6, which showed significant positive sMIC interference, which would have masked activity.

*MICA*008 shedding Inhibition in MEL-JUSO cells*

[0549] MEL-JUSO typically showed very little antibody interference with CHO samples. Thus, most interference was expected to be attributed to sMIC in these samples, and indeed the sMIC-increased effect is greater than on other cell lines.

[0550] All 1D5 variants, except for 1D5v28, which may have been compromised, showed inhibition activity approximately equivalent to the non-variant antibody (~60-70%), with some variance, and almost no antibody inhibition interference (but much more sMIC interference from the HEK293 material vs other cell lines and alleles) (**Table 19**). In general, 13A9 variants showed little to no inhibition. 15F11 variants showed activity approximately similar to non-variant antibody (~60%) except for potentially compromised sample 15F11v1, and 15F11v4 and 15F11v6, which showed significant positive sMIC interference, which would have masked activity.

*MICB*005 shedding Inhibition in HCC1534 cells*

[0551] Due to the higher-than expected variation on the interference plates, the positive cutoff was higher than the inhibition cutoff. All 1D5 variants (except for 1D5v28, which might have been compromised) showed some inhibition activity (~40-50%) approximately 10% lower than the non-

variant antibody(~60%), with some variance, and varying degrees of negative signal (Ab) interference, likely attributed to the higher than expected variation on that assay (**Table 19**). In general, 13A9 variants showed significant inhibition approximately equivalent to non-variant 13A9, except for 13A9v1, which was possibly compromised. 15F11 variants showed activity approximately similar to non-variant antibody and 1D5 (~40%) except for potentially compromised sample 15F11v1, and 15F11v4 and 15F11v6, which showed significant positive sMIC interference, which would have masked activity.

Table 19: Percent Inhibition and Reduction due to Interference

Clone	Cell Line	Fc	Conc. (mg/ml)	% Mono-mer	HCC1534 (MICA*004)		MEL-JUSO (MICA*008)		HCC1534 (MICA*005)	
					% Inhib-ition	% reduction due to interfer-ence	% Inhib-ition	% reduction due to interfer-ence	% Inhib-ition	% reduct ion due to interf-erence
13A9	293	Hu IgG1	0.45	67.52	21%	0%	-7%	-33%	61%	28%
15F11	293	Hu IgG1	1.06	96.2	54%	13%	50%	-8%	43%	16%
1D5v1	293	Hu IgG1	0.92	96.59	54%	5%	52%	-20%	41%	3%
1D5v2	293	Hu IgG1	0.83	96.62	^38%	^15%	^33%	^7%	^26%	^15%
1D5v3	293	Hu IgG1	0.69	95.07	56%	6%	50%	-16%	38%	ND
1D5v4	293	Hu IgG1	0.57	95.39	46%	1%	43%	-22%	34%	15%
1D5v5	293	Hu IgG1	0.26	95.56	39%	-39%	36%	-92%	32%	-5%
1D5v6	293	Hu IgG1	0.22	93.48	47%	-8%	51%	-47%	37%	4%
1D5v7	293	Hu IgG1	0.31	92.59	48%	-2%	51%	-33%	42%	9%
1D5v8	293	Hu IgG1	0.88	91.42	42%	0%	52%	-22%	35%	20%
1D5v9	293	Hu IgG1	0.96	98.35	53%	7%	49%	-8%	41%	9%
1D5v10	293	Hu IgG1	1.67	96.58	62%	7%	58%	NA	43%	17%
1D5v11	293	Hu IgG1	0.83	96.63	52%	1%	56%	-14%	36%	8%
1D5v12	293	Hu IgG1	0.51	96.59	50%	-5%	44%	-18%	27%	1%
1D5v13	293	Hu IgG1	0.49	95.48	49%	-4%	51%	-17%	43%	1%
1D5v14	293	Hu IgG1	0.46	96.07	^49%	^8%	^63%	^-17%	^43%	^11%
1D5v14	**293**	**Hu IgG1**	**0.46**	**96.07**	**^^55%**	**^^7%**	**^^58%**	**^^-3%**	**^^31%**	**^^23%**
1D5v15	293	Hu IgG1	1.29	96.16	^45%	^14%	^48%	^13%	^39%	^13%
1D5v16	293	Hu IgG1	0.81	94.66	50%	-2%	51%	-20%	51%	24%

Clone	Cell Line	Fc	Conc. (mg/ml)	% Mono-mer	HCC1534 (MICA*004)		MEL-JUSO (MICA*008)		HCC1534 (MICA*005)	
					% Inhib-ition	% reduction due to interfer-ence	% Inhib-ition	% reduction due to interfer-ence	% Inhib-ition	% reduction due to interf-erence
1D5v17	293	Hu IgG1	2.02	98.79	52%	12%	68%	2%	50%	21%
1D5v18	293	Hu IgG1	0.83	99.74	47%	6%	64%	-23%	41%	22%
1D5v19	293	Hu IgG1	0.04	98.81	44%	4%	57%	-27%	40%	34%
1D5v20	293	Hu IgG1	0.92	95.58	46%	6%	56%	-20%	39%	28%
1D5v21	293	Hu IgG1	2.38	98.89	55%	16%	62%	13%	25%	28%
1D5v22	293	Hu IgG1	1.77	97.68	46%	7%	57%	10%	34%	***
1D5v23	293	Hu IgG1	0.76	94.35	50%	13%	63%	-7%	38%	31%
1D5v24	293	Hu IgG1	2.48	89.01	50%	20%	65%	17%	43%	28%
1D5v25	293	Hu IgG1	0.78	94.04	46%	8%	62%	-15%	28%	30%
1D5v26	293	Hu IgG1	0.79	92.31	37%	0%	50%	-19%	42%	33%
1D5v27	293	Hu IgG1	4.1	ND	53%	11%	69%	3%	-8%	13%
1D5v28	293	Hu IgG1	4.9	ND	^3%	^-2%	^0%	^9%	^36%	^30%
1D5v29	293	Hu IgG1	2.4	ND	40%	12%	57%	-18%	-8%	23%
13A9v1	293	Hu IgG1	3.08	97.25	^12%	ND	^10%	ND	^43%	^36%
13A9v2	293	Hu IgG1	1.65	90.96	10%	0%	12%	-7%	60%	33%
13A9v3	293	Hu IgG1	0.83	99.51	25%	1%	2%	-19%	42%	0%
13A9v4	293	Hu IgG1	0.2	99.12	-29%	-85%	-31%	-199%	63%	35%
13A9v5	293	Hu IgG1	1.41	91.8	9%	3%	4%	0%	63%	37%
13A9v6	293	Hu IgG1	1.62	99.25	16%	4%	8%	-4%	6%	16%
15F11v1	293	Hu IgG1	2.18	98.07	^3%	^3%	^4%	^7%	^43%	^26%
15F11v2	293	Hu IgG1	1.74	95.51	43%	7%	62%	-15%	35%	20%
15F11v3	293	Hu IgG1	3.97	99.42	39%	13%	50%	0%	-49%	ND
15F11v4	293	Hu IgG1	0.18	98.47	-34%	-91%	-19%	-383%	44%	9%
15F11v5	293	Hu IgG1	2.18	96.59	46%	10%	62%	-6%	-35%	-126%
15F11v6	293	Hu IgG1	0.2	98.55	-14%	-91%	-8%	-300%	59%	33%
1D5	293	Hu IgG1	2.65	96.96	52%	25%	69%	6%	49%	22%

Clone	Cell Line	Fc	Conc. (mg/ml)	% Mono-mer	HCC1534 (MICA*004)		MEL-JUSO (MICA*008)		HCC1534 (MICA*005)	
					% Inhib-ition	% reduction due to interfer-ence	% Inhib-ition	% reduction due to interfer-ence	% Inhib-ition	% reduction due to interf-erence
6E1	293	Hu IgG1	0.8	98.65	34%	13%	31%	-9%	58%	34%
1D5	CHO	m IgG2a	6.3	ND	59%	34%	74%	18%	76%	38%
13A9	CHO	m IgG2a	6	ND	34%	32%	29%	9%	60%	24%
15F11	CHO	m IgG2a	4	ND	60%	28%	69%	9%	57%	18%
6E1	CHO	m IgG2a	4.3	ND	40%	20%	36%	9%	47%	13%

Note: ND = not determined. ^ = Out of sample on rerun; robot normalized sample loaded from an earlier run and may refer to correct antibody dilution, incorrect antibody dilution, or no antibody added. ^^ = Diluted to slightly lower than intended concentration due to low volume. Shown in gray is a second data point collected for 1D5v14.

Table 20: Inhibtion and Interference Cutoff Values.

	Positive Cutoff HCC1534 MICA*004	Positive Cutoff HCC1534 MICB*005
Inhibition Avg (3 plates)	**20%**	**21%**
Interference Avg (3 plates)	**19%**	**32%**

Note: Positive cutoff was defined as inhibition within 3 standard devations below non-treated samples.

[0552] 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.

SEQUENCES

Hypervariable Region Sequences

Ab	HVR H1	HVR H2	HVR H3	HVR L1	HVR L2	HVR L3
3C9.10	GSGVN (SEQ ID NO: 1)	MIWGDGNTDYNSALKS (SEQ ID NO: 2)	GAYYGKRWYFDV (SEQ ID NO: 3)	SASQGISNYLN (SEQ ID NO: 4)	YTSSLHS (SEQ ID NO: 5)	QQYSKLPPT (SEQ ID NO: 6)
7D4.6	SDYAWN (SEQ ID NO: 17)	YINYSGTTNYNPSLKS (SEQ ID NO:18)	YRYDGAWFPY (SEQ ID NO: 19)	RASGNIHNYLA (SEQ ID NO: 20)	NAIMLAD (SEQ ID NO: 21)	QHFWSFPLT (SEQ ID NO: 22)
6F8.7	NDYYWN (SEQ ID NO: 33)	FISFGGSNNYNPSLKN (SEQ ID NO: 34)	YDGRGAWFAY (SEQ ID NO: 35)	RASGNIHNYLA (SEQ ID NO: 36)	DAITLAD (SEQ ID NO: 37)	QHFWSFPLT (SEQ ID NO: 38)
32D2	TFGMN (SEQ ID NO: 49)	YINSGSNTIYYADTVKG (SEQ ID NO: 50)	WEPVTGGFSY (SEQ ID NO: 51)	TASSSISSSYLH (SEQ ID NO: 52)	TTSNLAS (SEQ ID NO: 53)	HQHHRSPFT (SEQ ID NO: 54)
3E11	KYNIY (SEQ ID NO: 65)	YIDPYTGGTISNQKFTG (SEQ ID NO:66)	PGSYWYFGV (SEQ ID NO: 67)	RSSQSIVYTNGNTNLE (SEQ ID NO: 68)	KVSNRFS (SEQ ID NO: 69)	FQASYVPFT (SEQ ID NO: 70)
9C9.5.6	GQGVN (SEQ ID NO: 81)	MIWGDGSTDYNSALKS (SEQ ID NO: 82)	GAHYGKRWYFDV (SEQ ID NO: 83)	SASQNINNYLN (SEQ ID NO: 84)	YTSSLPS (SEQ ID NO: 85)	QQYSKLPPT (SEQ ID NO: 86)
1E6.1.3	GSGVN (SEQ ID NO: 97)	MIWGDGNTDYNSALKS (SEQ ID NO: 98)	GAHYGKRWYFDV (SEQ ID NO: 99)	SASQGINNYLN (SEQ ID NO: 100)	YTSTLPS (SEQ ID NO: 101)	QQYSKLPPT (SEQ ID NO: 102)
7A3.1.9	GSGVN (SEQ ID NO: 113)	MIWGDGNTDYNSALKS (SEQ ID NO: 114)	GAHYGKRWYFDV (SEQ ID NO: 115)	SASQGINNYLN (SEQ ID NO: 116)	YTSSLPS (SEQ ID NO: 117)	QQYSKLPPT (SEQ ID NO: 118)
6E12.5	DYYMY (SEQ ID NO: 129)	TISDGITYTYYSDSVRG (SEQ ID NO: 130)	GGGSTARGALDF (SEQ ID NO: 131)	HASQNIHVWLS (SEQ ID NO: 132)	GASHLHT (SEQ ID NO: 133)	LQGQSYPLT (SEQ ID NO: 134)
20G11	TFGIH (SEQ ID NO: 145)	YISYDSRTIYYADTVKG (SEQ ID NO: 146)	WAYEGGVNYFDN (SEQ ID NO: 147)	TATSGVSSSYLH (SEQ ID NO: 148)	SSSNLAS (SEQ ID NO: 149)	HQFHRSPLT (SEQ ID NO: 150)
6E1.1.12	DNYIS (SEQ ID NO: 161)	WIYAGTGGSSYNQKFRD (SEQ ID NO: 162)	HDYYGTSGAWFAY (SEQ ID NO: 163)	RSSQHIVHSNENTYLE (SEQ ID NO: 164)	KVSNRFS (SEQ ID NO: 165)	FQGSHVPWT (SEQ ID NO: 166)
2E5.2.3	DNYIS (SEQ ID NO: 177)	WIYAGTGGTSYNQKFTA (SEQ ID NO: 178)	HDYYGTSGAWFAY (SEQ ID NO: 179)	RSSQNIVHINGNTYLE (SEQ ID NO: 180)	KVSNRFS (SEQ ID NO: 181)	FQGSHVPWT (SEQ ID NO: 182)

Framework Sequences

Light Chain Framework Sequences

Ab	FR-L1	FR-L2	FR-L3	FR-L4
3C9.10	DIQMTQTTSSLSASLGDRVTISC (SEQ ID: 7)	WYQQKPDGTVKLLIY (SEQ ID: 8)	GVPSRFSGSGSGTDYS LTISNLEPEDIATYYC (SEQ ID: 9)	FGGGTKVEIK (SEQ ID: 10)
7D4.6	DIQMTQSPASLSASVGETVTITC (SEQ ID: 23)	WYQQKQGKSPQLLVY (SEQ ID: 24)	GVPSRFSASGSGTQYS LKINSLQPEDFGSYYC (SEQ ID: 25)	FGAGTKVEIK (SEQ ID: 26)
6F8.7	DIQMTQSPASLSASVGETVTITC (SEQ ID: 39)	WYQQKQGKSPQLLVY (SEQ ID: 40)	GVPSRFSGSGSGTQYS LKINSLQPEDFGNYYC (SEQ ID: 41)	FGAGTKVEIK (SEQ ID: 42)
32D2	QIVLTQSPAFKSSSLGERVTMTC (SEQ ID: 55)	WYQQKPGSSPKLWIY (SEQ ID: 56)	GVPARFSGSGSGTSYS LTISTMEAEDAATYYC (SEQ ID: 57)	FGSGTKVEIK (SEQ ID: 58)
3E11	DILMTQTPLSLPVSLGDQASISC (SEQ ID: 71)	WYLQKPGQSPKLLIY (SEQ ID: 72)	GVPDRFSGSGSGTDFT LKISRVEAEDLGVYYC (SEQ ID: 73)	FGSGTKVEIK (SEQ ID: 74)
9C9.5.6	DIQMTQTTSSLSASLGDRVTISC (SEQ ID: 87)	WYQQKPHGTVKLLIY (SEQ ID: 88)	GVPSRFSGSGSGTDYS LTISNLEPEDIASYYC (SEQ ID: 89)	FGGGTKVEIK (SEQ ID: 90)
1E6.1.3	DIQMTQTTFSLSASLGDRVTISC (SEQ ID: 103)	WYQQRPDGTVKLLIY (SEQ ID: 104)	GVPSRFSGSGSGTDYS LTISNLEPEDIASYYC (SEQ ID: 105)	FGGGTKVEIK (SEQ ID: 106)
7A3.1.9	DIQMTQTTSSLSASLGDRVTISC (SEQ ID: 119)	WYQQKPDGTVKLLIY (SEQ ID: 120)	GVPSRFSGSGSGTDYS LTISNLEPEDIATYYC (SEQ ID: 121)	FGGGTKVEIK (SEQ ID: 122)
6E12.5	DIQMNQSPSSLSASLGDTITITC (SEQ ID: 135)	WYQQKPGNIPKLLIY (SEQ ID: 136)	GVPSRFSGRGSGTGFT LTISSLQPEDIATYYC (SEQ ID: 137)	FGSGTKVEIK (SEQ ID: 138)
20G11	QIVLTQSPAIMSASLGERVTMTC (SEQ ID: 151)	WYQQKPGSSPKLWIY (SEQ ID: 152)	GVPARFSGSGSGTSYS LTIGSMEAEDAATYYC (SEQ ID: 153)	FGTGTKVEIK (SEQ ID: 154)
6E1.1.12	DVLMTQTPLSLPVSLGDQASISC (SEQ ID: 167)	WYLQKPGQSPKLLIY (SEQ ID: 168)	GVPDRFSGSGSGTDFT LKISRVEAEDLGVYYC (SEQ ID: 169)	FGGGTKVEIK (SEQ ID: 170)
2E5.2.3	DVLMTQTPLSLPVSLGDQASLSC (SEQ ID: 183)	WYLQKPGQSPKLLIY (SEQ ID: 184)	GVPDRFSGSGSGTDFT LKISRVEAEDLGVYYC (SEQ ID: 185)	FGGGTKVEIK (SEQ ID: 186)

Heavy Chain Framework Sequences

Ab	FR-H1	FR-H2	FR-H3	FR-H4
3C9.10	QVQLKESGPGLVAPSQSLSITC TVSGFSLT (SEQ ID: 11)	WVRQPPGKGLEWLG (SEQ ID: 12)	RLSISKDNSKSQIFLKMN SLQTDDTARYYCAR (SEQ ID: 13)	WGAGTTVTVSS (SEQ ID: 14)
7D4.6	DVQLQESGPGLVKPSQSLSLTC TVTGYSIT (SEQ ID: 27)	WIRQFPRNKLEWMG (SEQ ID: 28)	RISITRDTSKNQFFLQLIS VTTEDTATYYCSY (SEQ ID: 29)	WGQGTLVTVSA (SEQ ID: 30)
6F8.7	DVQLQGSGPGLVKPSQSLSLTC SVTGYSIT (SEQ ID: 43)	WIRQFPGNKLEWMG (SEQ ID: 44)	RISITRDTSKNQFFLKLSS VTTEDTATYYCAR (SEQ ID: 45)	WAQGTLVTVSA (SEQ ID: 46)
32D2	DVHLVESGGGLVQPGGSRKLS CAASGFTFN (SEQ ID: 59)	WVRQAPEKGLEWVA (SEQ ID: 60)	RFTISRDNPKNTLFLQMT SLRSEDTAMYYCTR (SEQ ID: 61)	WGQGTLVTVSA (SEQ ID: 62)

Ab	FR-H1	FR-H2	FR-H3	FR-H4
3E11	EIQLQQSGPELVKPGASVKVSC TASGYAFT (SEQ ID: 75)	WVKQSHGKSLEWIG (SEQ ID: 76)	RATLTVDKSSSTAYLHLN SLTSEDSAVYYCAR (SEQ ID: 77)	WGAGTTVTVSS (SEQ ID: 78)
9C9.5.6	QVQLKESGPGLVAPSQSLSITC TVSGFSLN (SEQ ID: 91)	WVRQPPGKGLEWLG (SEQ ID: 92)	RLSISKDNSRSQVFLKMN SLQTDDTARYYCAR (SEQ ID: 93)	WGAGTTVTVSS (SEQ ID: 94)
1E6.1.3	QVQLKESGPGLVAPSQSLSITC TVSGFSLT (SEQ ID: 107)	WVRQPPGQGLEWLG (SEQ ID: 108)	RLSISKDNSKSQVFLKMN SLQTDDTARYYCAR (SEQ ID: 109)	WGAGTTVTVSS (SEQ ID: 110)
7A3.1.9	QVQLKESGPGLVAPSQSLSITC TVSGFALT (SEQ ID: 123)	WVRQPPGKGLEWLG (SEQ ID: 124)	RLSISKDNSKSQIFLKMN SLQTDDTARYYCAR (SEQ ID: 125)	WGAGTTVTVSS (SEQ ID: 126)
6E12.5	EVQLVESGGGLVKPGGSLKLS CAASGFTFS (SEQ ID: 139)	WVRQTPEKRLEWVA (SEQ ID: 140)	RFTISRDNAENTLYLQMS SLKSEDTAMYYCSK (SEQ ID: 141)	WGQGTSVTVSS (SEQ ID: 142)
20G11	DVQLVESGGGLVQPGGSRKLS CAASGFTFS (SEQ ID: 155)	WVRQAPEKGLEWVA (SEQ ID: 156)	RFTISRDNPKNTLFLQMT SLRSEDTAMYYCAR (SEQ ID: 157)	WGQGTTLTVSS (SEQ ID: 158)
6E1.1.12	QGQMQQSGAELVKPGASVKL SCKTSGFTFS (SEQ ID: 171)	WLKQKPGQSLEWIA (SEQ ID: 172)	KAQLTVDTSSRTAYMQL SSLTTEDSAIYYCAR (SEQ ID: 173)	WGRGTLVTVSA (SEQ ID: 174)
2E5.2.3	QGQMQQSGAELVKPGASVKL SCKTSGFTFS (SEQ ID: 187)	WLKQKPGQSLEWIA (SEQ ID: 188)	KAQLTVDTSSSTAYMQF SSLTTEDSAIYYCAR (SEQ ID: 189)	WGQGTLVTVSA (SEQ ID: 190)

Variable Domain Sequences

Ab	VH	VL
3C9.10	QVQLKESGPGLVAPSQSLSITCTVSGFSLT GSGVNWVRQPPGKGLEWLGMIWGDGNT DYNSALKSRLSISKDNSKSQIFLKMNSLQ TDDTARYYCARGAYYGKRWYFDVWGA GTTVTVSS (SEQ ID NO: 15)	DIQMTQTTSSLSASLGDRVTISCSASQGIS NYLNWYQQKPDGTVKLLIYYTSSLHSGV PSRFSGSGSGTDYSLTISNLEPEDIATYYC QQYSKLPPTFGGGTKVEIK (SEQ ID NO: 16)
7D4.6	DVQLQESGPGLVKPSQSLSLTCTVTGYSIT SDYAWNWIRQFPRNKLEWMGYINYSGTT NYNPSLKSRISITRDTSKNQFFLQLISVTTE DTATYYCSYYRYDGAWFPYWGQGTLVT VSA (SEQ ID NO: 31)	DIQMTQSPASLSASVGETVTITCRASGNIH NYLAWYQQKQGKSPQLLVYNAIMLADG VPSRFSASGSGTQYSLKINSLQPEDFGSYY CQHFWSFPLTFGAGTKVEIK (SEQ ID NO: 32)
6F8.7	DVQLQGSGPGLVKPSQSLSLTCSVTGYSI TNDYYWNWIRQFPGNKLEWMGFISFGGS NNYNPSLKNRISITRDTSKNQFFLKLSSVT TEDTATYYCARYDGRGAWFAYWAQGTL VTVSA (SEQ ID NO: 47)	DIQMTQSPASLSASVGETVTITCRASGNIH NYLAWYQQKQGKSPQLLVYDAITLADG VPSRFSGSGSGTQYSLKINSLQPEDFGNY YCQHFWSFPLTFGAGTKVEIK (SEQ ID NO: 48)

Ab	VH	VL
32D2	DVHLVESGGGLVQPGGSRKLSCAASGFT FNTFGMNWVRQAPEKGLEWVAYINSGS NTIYYADTVKGRFTISRDNPKNTLFLQMT SLRSEDTAMYYCTRWEPVTGGFSYWGQ GTLVTVSA (SEQ ID NO: 63)	QIVLTQSPAFKSSSLGERVTMTCTASSSISS SYLHWYQQKPGSSPKLWIYTTSNLASGV PARFSGSGSGTSYSLTISTMEAEDAATYY CHQHHRSPFTFGSGTKVEIK (SEQ ID NO: 64)
3E11	EIQLQQSGPELVKPGASVKVSCTASGYAF TKYNIYWVVKQSHGKSLEWIGYIDPYTGG TISNQKFTGRATLTVDKSSSTAYLHLNSL TSEDSAVYYCARPGSYWYFGVWGAGTT VTVSS (SEQ ID NO: 79)	DILMTQTPLSLPVSLGDQASISCRSSQSIV YTNGNTNLEWYLQKPGQSPKLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDL GVYYCFQASYVPFTFGSGTKVEIK (SEQ ID NO: 80)
9C9.5.6	QVQLKESGPGLVAPSQSLSITCTVSGFSLN GQGVNWVRQPPGKGLEWLGMIWGDGST DYNSALKSRLSISKDNSRSQVFLKMNSLQ TDDTARYYCARGAHYGKRWYFDVWGA GTTVTVSS (SEQ ID NO: 95)	DIQMTQTTSSLSASLGDRVTISCSASQNIN NYLNWYQQKPHGTVKLLIYYTSSLPSGV PSRFSGSGSGTDYSLTISNLEPEDIASYYC QQYSKLPPTFGGGTKVEIK (SEQ ID NO: 96)
1E6.1.3	QVQLKESGPGLVAPSQSLSITCTVSGFSLT GSGVNWVRQPPGQGLEWLGMIWGDGNT DYNSALKSRLSISKDNSKSQVFLKMNSLQ TDDTARYYCARGAHYGKRWYFDVWGA GTTVTVSS (SEQ ID NO: 111)	DIQMTQTTFSLSASLGDRVTISCSASQGIN NYLNWYQQRPDGTVKLLIYYTSTLPSGV PSRFSGSGSGTDYSLTISNLEPEDIASYYC QQYSKLPPTFGGGTKVEIK (SEQ ID NO: 112)
7A3.1.9	QVQLKESGPGLVAPSQSLSITCTVSGFALT GSGVNWVRQPPGKGLEWLGMIWGDGNT DYNSALKSRLSISKDNSKSQIFLKMNSLQ TDDTARYYCARGAHYGKRWYFDVWGA GTTVTVSS (SEQ ID NO: 127)	DIQMTQTTSSLSASLGDRVTISCSASQGIN NYLNWYQQKPDGTVKLLIYYTSSLPSGV PSRFSGSGSGTDYSLTISNLEPEDIATYYC QQYSKLPPTFGGGTKVEIK (SEQ ID NO: 128)
6E12.5	EVQLVESGGGLVKPGGSLKLSCAASGFTF SDYYMYWVRQTPEKRLEWVATISDGITY TYYSDSVRGRFTISRDNAENTLYLQMSSL KSEDTAMYYCSKGGGSTARGALDFWGQ GTSVTVSS (SEQ ID NO: 143)	DIQMNQSPSSLSASLGDTITITCHASQNIH VWLSWYQQKPGNIPKLLIYGASHLHTGV PSRFSGRGSGTGFTLTISSLQPEDIATYYCL QGQSYPLTFGSGTKVEIK (SEQ ID NO: 144)
6E1.1.12	QGQMQQSGAELVKPGASVKLSCKTSGFT FSDNYISWLKQKPGQSLEWIAWIYAGTG GSSYNQKFRDKAQLTVDTSSRTAYMQLS SLTTEDSAIYYCARHDYYGTSGAWFAYW GRGTLVTVSA (SEQ ID NO: 159)	DVLMTQTPLSLPVSLGDQASISCRSSQHIV HSNENTYLEWYLQKPGQSPKLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDL GVYYCFQGSHVPWTFGGGTKVEIK (SEQ ID NO: 160)

Ab	VH	VL
2E5.2.3	QGQMQQSGAELVKPGASVKLSCKTSGFT FSDNYISWLKQKPGQSLEWIAWIYAGTG GTSYNQKFTAKAQLTVDTSSSTAYMQFS SLTTEDSAIYYCARHDYYGTSGAWFAYW GQGTLVTVSA (SEQ ID NO: 175)	DVLMTQTPLSLPVSLGDQASLSCRSSQNI VHINGNTYLEWYLQKPGQSPKLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDL GVYYCFQGSHVPWTFGGGTKVEIK (SEQ ID NO: 176)
20G11	DVQLVESGGGLVQPGGSRKLSCAASGFT FSTFGIHWVRQAPEKGLEWVAYISYDSRT IYYADTVKGRFTISRDNPKNTLFLQMTSL RSEDTAMYYCARWAYEGGVNYFDNWG QGTTLTVSS (SEQ ID NO: 191)	QIVLTQSPAIMSASLGERVTMTCTATSGV SSSYLHWYQQKPGSSPKLWIYSSSNLASG VPARFSGSGSGTSYSLTIGSMEAEDAATY YCHQFHRSPLTFGTGTKVEIK (SEQ ID NO: 192)

[0553] Hypervariable region sequences, heavy chain and light chain framework sequences, and variable domain sequences for humanized variants of 1D5, 13A9, and 15F11 as well as for mouse chimeras 1D5, 13A9, 15F11, 18G3, and 12H10 are shown in the following tables (SEQ ID NO: 209 to SEQ ID NO: 440).

Hypervariable Region Sequences

Ab	HVR H1	HVR H2	HVR H3	HVR L1	HVR L2	HVR L3
1D5 1D5v1 1D5v2 1D5v3 1D5v4 1D5v5 1D5v6 1D5v7 1D5v8 1D5v9 1D5v10 1D5v11 1D5v12 1D5v13 1D5v14 1D5v15 1D5v16 1D5v17 1D5v18 1D5v19 1D5v20 1D5v21 1D5v22 1D5v23 1D5v24 1D5v25 1D5v26	SQNIY (SEQ ID NO: 209)	YIEPYNVVPMY NPKFKG (SEQ ID NO: 210)	SGSSNFDY (SEQ ID NO: 211)	SASSSISSHYLH (SEQ ID NO: 212)	RTSNLASG (SEQ ID NO: 213)	QQGSSLPLT (SEQ ID NO: 214)
1D5v27	SQNIY (SEQ ID NO: 209)	YIEPYNVVPAY NPKFKG (SEQ ID NO: 215)	SGSSNFDY (SEQ ID NO: 211)	SASSSISSHYLH (SEQ ID NO: 212)	RTSNLASG (SEQ ID NO: 213)	QQGSSLPLT (SEQ ID NO: 214)
1D5v28	SQNIY (SEQ ID NO: 209)	YIEPYNVVPLY NPKFKG (SEQ ID NO: 216)	SGSSNFDY (SEQ ID NO: 211)	SASSSISSHYLH (SEQ ID NO: 212)	RTSNLASG (SEQ ID NO: 213)	QQGSSLPLT (SEQ ID NO: 214)

Ab	HVR H1	HVR H2	HVR H3	HVR L1	HVR L2	HVR L3
1D5v29	SQNIY (SEQ ID NO: 209)	YIEPYNVVPVY NPKFKG (SEQ ID NO: 217)	SGSSNFDY (SEQ ID NO: 211)	SASSSISSHYLH (SEQ ID NO: 212)	RTSNLASG (SEQ ID NO: 213)	QQGSSLPLT (SEQ ID NO: 214)
13A9 13A9v1 13A9v2 13A9v3 13A9v4 13A9v5 13A9v6	NYLIE (SEQ ID NO: 218)	AINPGSGATNY NEKFKD (SEQ ID NO: 219)	FLGNYFDN (SEQ ID NO: 220)	RASGNIHSYLA (SEQ ID NO: 221)	YAETLADG (SEQ ID NO: 222)	QQFWTTPYT (SEQ ID NO: 223)
15F11 15F11v1 15F11v2 15F11v3 15F11v4 15F11v5 15F11v6	SNNIY (SEQ ID NO: 224)	YIDPYIGRIIY NQQFKD (SEQ ID NO: 225)	SGERSNFDY (SEQ ID NO: 226)	SASSSISSNYLH (SEQ ID NO: 227)	RTSNLASG (SEQ ID NO: 228)	QQGGSLPLT (SEQ ID NO: 229)
6E1.1.12	DNYIS (SEQ ID NO: 230)	WIYAGTGGSSY NQKFRD (SEQ ID NO: 231)	HDYYGTSGAW FAY (SEQ ID NO: 232)	RSSQHIVHSNENT YLE (SEQ ID NO: 233)	KVSNRFS (SEQ ID NO: 234)	FQGSHVPWT (SEQ ID NO: 235)
18G3	GDYAWN (SEQ ID NO: 236)	YIGYTGSTTY NPSLKS (SEQ ID NO: 237)	WRNWAMDY (SEQ ID NO: 238)	RANQDISHYLN (SEQ ID NO: 239)	YTSRIHSG (SEQ ID NO: 240)	QQGNTPPTT (SEQ ID NO: 241)
12H10	SNYAWN (SEQ ID NO: 242)	YISSSGITKS NPSLKS (SEQ ID NO: 243)	WSNWSFDV (SEQ ID NO: 244)	RASQDIHNYFN (SEQ ID NO: 245)	YTSRFHSG (SEQ ID NO: 246)	QQGNSLPPT (SEQ ID NO: 247)

Framework Sequences

Light Chain Framework Sequences

Ab	FR-L1	FR-L2	FR-L3	FR-L4
1D5	EIILTQSPTTMAASPGEKITITC (SEQ ID NO: 248)	WYQQKSGFSPKLLIY (SEQ ID NO: 249)	VPARFSGSGSGTSYSLT IGTMEAEDVATYYC (SEQ ID NO: 250)	FGAGTKVEIK (SEQ ID NO: 251)
1D5v1 1D5v2 1D5v3 1D5v4	EIVLTQSPDFQSVTPKEKVTITC (SEQ ID NO: 252)	WYQQKPDQSPKLLIY (SEQ ID NO:253)	VPSRFSGSGSGTDYTLT INSLEAEDAATYYC (SEQ ID NO: 254)	FGQGTKVEIK (SEQ ID NO: 255)
1D5v5 1D5v6 1D5v7 1D5v8	EIVLTQSPDFQSVTPKEKVTITC (SEQ ID NO: 252)	WYQQKPDQSPKLLIK (SEQ ID NO: 256)	VPSRFSGSGSGTDFTLT INSLEAEDAATYYC (SEQ ID NO: 257)	FGQGTKVEIK (SEQ ID NO: 255)
1D5v9 1D5v10 1D5v11 1D5v12	DIQLTQSPSSLSASVGDRVTITC (SEQ ID NO: 258)	WYQQKPGKSPKLLIY (SEQ ID NO: 259)	VPSRFSGSGSGTDYTLT ISSLQPEDFATYYC (SEQ ID NO: 260)	FGQGTKVEIK (SEQ ID NO: 255)
1D5v13 1D5v14 1D5v15 1D5v16	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 261)	WYQQKPGKAPKLLIY (SEQ ID NO: 262)	VPSRFSGSGSGTDFTLT ISSLQPEDFATYYC (SEQ ID NO: 263)	FGQGTKVEIK (SEQ ID NO: 255)
1D5v17	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 261)	WYQQKPGKSPKLLIY (SEQ ID NO: 264)	VPSRFSGSGSGTDYTLT ISSLQPEDFATYYC (SEQ ID NO: 265)	FGQGTKVEIK (SEQ ID NO: 255)
1D5v18	DIQLTQSPSSLSASVGDRVTITC (SEQ ID NO: 266)	WYQQKPGKSPKLLIY (SEQ ID NO: 264)	VPSRFSGSGSGTDFTLT ISSLQPEDFATYYC (SEQ ID NO: 267)	FGQGTKVEIK (SEQ ID NO: 255)
1D5v19 1D5v20	DIQLTQSPSSLSASVGDRVTITC (SEQ ID NO: 266)	WYQQKPGKSPKLLIY (SEQ ID NO: 264)	VPSRFSGSGSGTDYTLT ISSLQPEDFATYYC	FGQGTKVEIK (SEQ ID NO:

Ab	FR-L1	FR-L2	FR-L3	FR-L4
1D5v21 1D5v22 1D5v23 1D5v24 1D5v25 1D5v26 1D5v27 1D5v28 1D5v29			(SEQ ID NO: 265)	255)
13A9	DIQMTQSPASLSASVGETVTITC (SEQ ID NO: 268)	WYQQKQGKSPQLLVY (SEQ ID NO: 269)	VPSRFSGRGSGTQYSL KINSLQPEDFGSYFC (SEQ ID NO: 270)	FGGGTKVEIK (SEQ ID NO: 271)
13A9v1 13A9v2 13A9v5	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 272)	WYQQKPGKAPKLLIY (SEQ ID NO: 273)	VPSRFSGSGSGTDFTLT ISSLQPEDFATYYC (SEQ ID NO: 274)	FGQGTKVEIK (SEQ ID NO: 255)
13A9v3 13A9v4 13A9v6	DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 272)	WYQQKPGKSPKLLVY (SEQ ID NO: 275)	VPSRFSGSGSGTDYTLT ISSLQPEDFATYFC (SEQ ID NO: 276)	FGQGTKVEIK (SEQ ID NO: 255)
15F11	EIVLTQSPTAMAASPGEKITITC (SEQ ID NO: 277)	WYQQKPGFSPKLLIY (SEQ ID NO: 278)	VPARFSGSGSGTSYSLT IGPMEAEDVATYYC (SEQ ID NO: 279)	FGAGTKVEIK (SEQ ID NO: 251)
15F11v1 15F11v2 15F11v5	EIVLTQSPATLSLSPGERATLSC (SEQ ID NO: 280)	WYQQKPGQAPRLLIY (SEQ ID NO: 281)	IPARFSGSGSGTDFTLTI SSLEPEDFAVYYC (SEQ ID NO: 282)	FGQGTKVEIK (SEQ ID NO: 255)
15F11v3 15F11v4 15F11v6	EIVLTQSPATLSLSPGERATLSC (SEQ ID NO: 280)	WYQQKPGQSPRLLIY (SEQ ID NO: 283)	VPARFSGSGSGTDYTL TISSLEPEDFAVYYC (SEQ ID NO: 284)	FGQGTKVEIK (SEQ ID NO: 255)
6E1.1.12	DVLMTQTPLSLPVSLGDQASISC (SEQ ID NO: 285)	WYLQKPGQSPKLLIY (SEQ ID NO: 286)	GVPDRFSGSGSGTDFT LKISRVEAEDLGVYYC (SEQ ID NO: 287)	FGGGTKVEIK (SEQ ID NO: 271)
18G3	DIQMTQTPSSLSASLGDRVTISC (SEQ ID NO: 288)	WYQQKPDGAVKLLIY (SEQ ID NO: 289)	VPSRFSGSGSGTDYSLT IANLEQEDVATYFC (SEQ ID NO: 290)	FGGGTKVEIK (SEQ ID NO: 271)
12H10	DIQMTQTPSSLSVSLGDRVTINC (SEQ ID NO: 291)	WYQQKPDGTIKLLIY (SEQ ID NO: 292)	VPSRFSGSGSGTDYSLT ISNLEEEDIATYFC (SEQ ID NO: 293)	FGGGTKLEIK (SEQ ID NO: 294)

Heavy Chain Framework Sequences

Ab	FR-H1	FR-H2	FR-H3	FR-H4
1D5	EIQLQQSGPELVKPGASVKVSCK ASGYAFT (SEQ ID NO: 295)	WVKQSHGKSLEWIG (SEQ ID NO: 296)	KATLTVDKSSSSAYIHL NSLTSEDSAIYYCAR (SEQ ID NO: 297)	WGQGTTLTVSS (SEQ ID NO: 298)
1D5v1 1D5v5 1D5v9 1D5v13	EIQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 299)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RATLTVDKSTSTAYMEL RSLRSDDTAVYYCAR (SEQ ID NO: 301)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v2 1D5v6 1D5v10 1D5v14	EVQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 303)	WVRQAPGQGLEWMG (SEQ ID NO: 304)	RVTMTTDTSTSTAYME LRSLRSDDTAVYYCAR (SEQ ID NO: 305)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v3 1D5v7 1D5v11 1D5v15 1D5v17 1D5v18 1D5v27 1D5v28 1D5v29	EIQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 299)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RATLTVDKSTSTAYLEL SSLRSEDTAVYYCAR (SEQ ID NO: 306)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v4 1D5v8 1D5v12 1D5v16	EVQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 303)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RVTITRDTSTSTAYLELS SLRSEDTAVYYCAR (SEQ ID NO: 307)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v19	EVQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 303)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RATLTVDKSTSTAYLEL SSLRSEDTAVYYCAR (SEQ ID NO: 306)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v20	EIQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 299)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RVTLTVDKSTSTAYLEL SSLRSEDTAVYYCAR (SEQ ID NO: 308)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v21	EIQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 299)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RATITVDKSTSTAYLELS SLRSEDTAVYYCAR (SEQ ID NO: 309)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v22	EIQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 299)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RATLTRDKSTSTAYLEL SSLRSEDTAVYYCAR (SEQ ID NO: 310)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v23	EIQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 299)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RATLTVDTSTSTAYLEL SSLRSEDTAVYYCAR (SEQ ID NO: 311)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v24	EIQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 299)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RVTITVDKSTSTAYLELS SLRSEDTAVYYCAR (SEQ ID NO: 312)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v25	EVQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 303)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RVTITVDTSTSTAYLELS SLRSEDTAVYYCAR (SEQ ID NO: 313)	WGQGTLVTVSS (SEQ ID NO: 302)
1D5v26	EVQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 303)	WVRQAPGQGLEWIG (SEQ ID NO: 300)	RVTITRDKSTSTAYLELS SLRSEDTAVYYCAR (SEQ ID NO: 314)	WGQGTLVTVSS (SEQ ID NO: 302)
13A9	QVQLQQSGAELVRPGTSVKVSC KASGYAFT (SEQ ID NO: 315)	WVKQRPGQGLEWIG (SEQ ID NO: 316)	KARLTADKSSNTAYLQF SSLTSDDSAVYFCAR (SEQ ID NO: 317)	WGQGATLTVSS (SEQ ID NO: 318)
13A9v1 13A9v3	EVQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 319)	WVRQAPGQGLEWIG (SEQ ID NO: 320)	RVTITADTSTSTAYLELS SLRSEDTAVYYCAR (SEQ ID NO: 321)	WGQGTLVTVSS (SEQ ID NO: 302)
13A9v2 13A9v4	EVQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 319)	WVRQAPGQGLEWIG (SEQ ID NO: 320)	RATLTADKSTNTAYLEL SSLRSEDTAVYFCAR (SEQ ID NO: 322)	WGQGTLVTVSS (SEQ ID NO: 302)
13A9v5 13A9v6	EVQLVQSGAEVKKPGSSVKVSC KASGYAFT (SEQ ID NO: 323)	WVRQAPGQGLEWIG (SEQ ID NO: 320)	RATLTADKSTNTAYME LSSLRSEDTAVYFCAR (SEQ ID NO: 324)	WGQGTLVTVSS (SEQ ID NO: 302)
15F11	EIQLQQSGPELVKPGASVRVSCK PSGYAFT (SEQ ID NO: 325)	WVKQSRRKSLEWIG (SEQ ID NO: 326)	KATLTVDKSSSTAYMH LNSLTSEDSAVYYCSR (SEQ ID NO: 327)	WGQGTTLTVSS (SEQ ID NO: 328)

Ab	FR-H1	FR-H2	FR-H3	FR-H4
15F11v1 15F11v3	EVQLVQSGAEVKKPGASVKVSC KASGYAFT (SEQ ID NO: 329)	WVRQAPGQGLEWIG (SEQ ID NO: 320)	RVTITADTSTSTAYLELS SLRSEDTAVYYCSR (SEQ ID NO: 330)	WGQGTLVTVSS (SEQ ID NO: 302)
15F11v2 15F11v4	EIQLVQSGAEVKKPGASVKVSC KPSGYAFT (SEQ ID NO: 331)	WVRQAPGQGLEWIG (SEQ ID NO: 320)	RATLTVDKSTSTAYLEL SSLRSEDTAVYYCSR (SEQ ID NO: 332)	WGQGTLVTVSS (SEQ ID NO: 302)
15F11v5 15F11v6	EIQLVQSGAEVKKPGSSVKVSC KPSGYAFT (SEQ ID NO: 333)	WVRQAPGQGLEWIG (SEQ ID NO: 320)	RATLTVDKSTSTAYMEL SSLRSEDTAVYYCSR (SEQ ID NO: 334)	WGQGTLVTVSS (SEQ ID NO: 302)
6E1.1.12	QGQMQQSGAELVKPGASVKLS CKTSGFTFS (SEQ ID NO: 335)	WLKQKPGQSLEWIA (SEQ ID NO: 336)	KAQLTVDTSSRTAYMQ LSSLTTEDSAIYYCAR (SEQ ID NO: 337)	WGRGTLVTVSA (SEQ ID NO: 338)
18G3	VQLQESGPGLVKPSQSLSLTCNV TGYSIT (SEQ ID NO: 339)	WIRQFPGNKLEWIG (SEQ ID NO: 340)	RVSITRDTSKNQFFLQL NSVTPEDTATYYCAR (SEQ ID NO: 341)	WGLGTSVTVSS (SEQ ID NO: 342)
12H10	DVQLQESGPGLVKPSQPLSLTCT VTGYSIT (SEQ ID NO: 343)	WIRQFPGDKLEWMG (SEQ ID NO: 344)	RISITRDTSKNQFFLQLN SLTTEDTATYYCSR (SEQ ID NO: 345)	WGAGTTVTVSS (SEQ ID NO: 346)

Variable Domain Sequences

Ab	VH	VL
1D5	EIQLQQSGPELVKPGASVKVSCKASGYAFTSQNIY WVKQSHGKSLEWIGYIEPYNVVPMYNPKFKGKAT LTVDKSSSSAYIHLNSLTSEDSAIYYCARSGSSNFD YWGQGTTLTVSS (SEQ ID NO: 347)	EIILTQSPTTMAASPGEKITITCSASSSISSHYLHWYQ QKSGFSPKLLIYRTSNLASGVP ARFSGSGSGTSYSLTIGTMEAEDVATYYCQQGSSLP LTFGAGTKVEIK (SEQ ID NO: 348)
1D5v1	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYMELRSLRSDDTAVYYCARSGSSNF DYWGQGTLVTVSS (SEQ ID NO: 349)	EIVLTQSPDFQSVTPKEKVTITCSASSSISSHYLHWY QQKPDQSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTINSLEAEDAATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 350)
1D5v2	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWMGYIEPYNVVPMYNPKFKGRV TMTTDTSTSTAYMELRSLRSDDTAVYYCARSGSSN FDYWGQGTLVTVSS (SEQ ID NO: 351)	EIVLTQSPDFQSVTPKEKVTITCSASSSISSHYLHWY QQKPDQSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTINSLEAEDAATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 352)
1D5v3	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 353)	EIVLTQSPDFQSVTPKEKVTITCSASSSISSHYLHWY QQKPDQSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTINSLEAEDAATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 354)
1D5v4	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRVT ITRDTSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 355)	EIVLTQSPDFQSVTPKEKVTITCSASSSISSHYLHWY QQKPDQSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTINSLEAEDAATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 356)
1D5v5	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYMELRSLRSDDTAVYYCARSGSSNF DYWGQGTLVTVSS (SEQ ID NO: 357)	EIVLTQSPDFQSVTPKEKVTITCSASSSISSHYLHWY QQKPDQSPKLLIKRTSNLASGVPSRFSGSGSGTDFT LTINSLEAEDAATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 358)

Ab	VH	VL
1D5v6	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWMGYIEPYNVVPMYNPKFKGRV TMTTDTSTSTAYMELRSLRSDDTAVYYCARSGSSN FDYWGQGTLVTVSS (SEQ ID NO: 359)	EIVLTQSPDFQSVTPKEKVTITCSASSSISSHYLHWY QQKPDQSPKLLIKRTSNLASGVPSRFSGSGSGTDFT LTINSLEAEDAATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 360)
1D5v7	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 361)	EIVLTQSPDFQSVTPKEKVTITCSASSSISSHYLHWY QQKPDQSPKLLIKRTSNLASGVPSRFSGSGSGTDFT LTINSLEAEDAATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 362)
1D5v8	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRVT ITRDTSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 363)	EIVLTQSPDFQSVTPKEKVTITCSASSSISSHYLHWY QQKPDQSPKLLIKRTSNLASGVPSRFSGSGSGTDFT LTINSLEAEDAATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 364)
1D5v9	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYMELRSLRSDDTAVYYCARSGSSNF DYWGQGTLVTVSS (SEQ ID NO: 365)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 366)
1D5v10	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWMGYIEPYNVVPMYNPKFKGRV TMTTDTSTSTAYMELRSLRSDDTAVYYCARSGSSN FDYWGQGTLVTVSS (SEQ ID NO: 367)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 368)
1D5v11	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 369)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 370)
1D5v12	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRVT ITRDTSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 371)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 372)
1D5v13	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYMELRSLRSDDTAVYYCARSGSSNF DYWGQGTLVTVSS (SEQ ID NO: 373)	DIQMTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKAPKLLIYRTSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 374)
1D5v14	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWMGYIEPYNVVPMYNPKFKGRV TMTTDTSTSTAYMELRSLRSDDTAVYYCARSGSSN FDYWGQGTLVTVSS (SEQ ID NO: 375)	DIQMTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKAPKLLIYRTSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 376)
1D5v15	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 377)	DIQMTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKAPKLLIYRTSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 378)

Ab	VH	VL
1D5v16	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRVT ITRDTSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 379)	DIQMTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKAPKLLIYRTSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 380)
1D5v17	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 381)	DIQMTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 382)
1D5v18	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 383)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 384)
1D5v19	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 385)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 386)
1D5v20	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRVT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 387)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 388)
1D5v21	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT ITVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 389)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 390)
1D5v22	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTRDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 391)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 392)
1D5v23	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRAT LTVDTSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 393)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 394)
1D5v24	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRVT ITVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 395)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 396)

Ab	VH	VL
1D5v25	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRVT ITVDTSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 397)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 398)
1D5v26	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPMYNPKFKGRVT ITRDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 399)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 400)
1D5v27	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPAYNPKFKGRAT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 401)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 402)
1D5v28	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPLYNPKFKGRAT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 403)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 404)
1D5v29	EIQLVQSGAEVKKPGASVKVSCKASGYAFTSQNIY WVRQAPGQGLEWIGYIEPYNVVPVYNPKFKGRAT LTVDKSTSTAYLELSSLRSEDTAVYYCARSGSSNFD YWGQGTLVTVSS (SEQ ID NO: 405)	DIQLTQSPSSLSASVGDRVTITCSASSSISSHYLHWY QQKPGKSPKLLIYRTSNLASGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGSSLPLTFGQGTKVEIK (SEQ ID NO: 406)
13A9	QVQLQQSGAELVRPGTSVKVSCKASGYAFTNYLIE WVKQRPGQGLEWIGAINPGSGATNYNEKFKDKAR LTADKSSNTAYLQFSSLTSDDSAVYFCARFLGNYF DNWGQGATLTVSS (SEQ ID NO: 407)	DIQMTQSPASLSASVGETVTITCRASGNIHSYLAWY QQKQGKSPQLLVYYAETLADGVPSRFSGRGSGTQ YSLKINSLQPEDFGSYFCQQFWTTPYTFGGGTKVEI K (SEQ ID NO: 408)
13A9v1	EVQLVQSGAEVKKPGASVKVSCKASGYAFTNYLIE WVRQAPGQGLEWIGAINPGSGATNYNEKFKDRVTI TADTSTSTAYLELSSLRSEDTAVYYCARFLGNYFD NWGQGTLVTVSS (SEQ ID NO: 409)	DIQMTQSPSSLSASVGDRVTITCRASGNIHSYLAWY QQKPGKAPKLLIYYAETLADGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQFWTTPYTFGQGTKVEIK (SEQ ID NO: 410)
13A9v2	EVQLVQSGAEVKKPGASVKVSCKASGYAFTNYLIE WVRQAPGQGLEWIGAINPGSGATNYNEKFKDRAT LTADKSTNTAYLELSSLRSEDTAVYFCARFLGNYF DNWGQGTLVTVSS (SEQ ID NO: 411)	DIQMTQSPSSLSASVGDRVTITCRASGNIHSYLAWY QQKPGKAPKLLIYYAETLADGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQFWTTPYTFGQGTKVEIK (SEQ ID NO: 412)
13A9v3	EVQLVQSGAEVKKPGASVKVSCKASGYAFTNYLIE WVRQAPGQGLEWIGAINPGSGATNYNEKFKDRVTI TADTSTSTAYLELSSLRSEDTAVYYCARFLGNYFD NWGQGTLVTVSS (SEQ ID NO: 413)	DIQMTQSPSSLSASVGDRVTITCRASGNIHSYLAWY QQKPGKSPKLLVYYAETLADGVPSRFSGSGSGTDY TLTISSLQPEDFATYFCQQFWTTPYTFGQGTKVEIK (SEQ ID NO: 414)

Ab	VH	VL
13A9v4	EVQLVQSGAEVKKPGASVKVSCKASGYAFTNYLIE WVRQAPGQGLEWIGAINPGSGATNYNEKFKDRAT LTADKSTNTAYLELSSLRSEDTAVYFCARFLGNYF DNWGQGTLVTVSS (SEQ ID NO: 415)	DIQMTQSPSSLSASVGDRVTITCRASGNIHSYLAWY QQKPGKSPKLLVYYAETLADGVPSRFSGSGSGTDY TLTISSLQPEDFATYFCQQFWTTPYTFGQGTKVEIK (SEQ ID NO: 416)
13A9v5	EVQLVQSGAEVKKPGSSVKVSCKASGYAFTNYLIE WVRQAPGQGLEWIGAINPGSGATNYNEKFKDRAT LTADKSTNTAYMELSSLRSEDTAVYFCARFLGNYF DNWGQGTLVTVSS (SEQ ID NO: 417)	DIQMTQSPSSLSASVGDRVTITCRASGNIHSYLAWY QQKPGKAPKLLIYYAETLADGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQFWTTPYTFGQGTKVEIK (SEQ ID NO: 418)
13A9v6	EVQLVQSGAEVKKPGSSVKVSCKASGYAFTNYLIE WVRQAPGQGLEWIGAINPGSGATNYNEKFKDRAT LTADKSTNTAYMELSSLRSEDTAVYFCARFLGNYF DNWGQGTLVTVSS (SEQ ID NO: 419)	DIQMTQSPSSLSASVGDRVTITCRASGNIHSYLAWY QQKPGKSPKLLVYYAETLADGVPSRFSGSGSGTDY TLTISSLQPEDFATYFCQQFWTTPYTFGQGTKVEIK (SEQ ID NO: 420)
15F11	EIQLQQSGPELVKPGASVRVSCKPSGYAFTSNNIYW VKQSRRKSLEWIGYIDPYIGRIIYNQQFKDKATLTV DKSSSTAYMHLNSLTSEDSAVYYCSRSGERSNFDY WGQGTTLTVSS (SEQ ID NO: 421)	EIVLTQSPTAMAASPGEKITITCSASSSISSNYLHWY QQKPGFSPKLLIYRTSNLASGVP ARFSGSGSGTSYSLTIGPMEAEDVATYYCQQGGSL PLTFGAGTKVEIK (SEQ ID NO: 422)
15F11v1	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSNNIY WVRQAPGQGLEWIGYIDPYIGRIIYNQQFKDRVTIT ADTSTSTAYLELSSLRSEDTAVYYCSRSGERSNFDY WGQGTLVTVSS (SEQ ID NO: 423)	EIVLTQSPATLSLSPGERATLSCSASSSISSNYLHWY QQKPGQAPRLLIYRTSNLASGIP ARFSGSGSGTDFTLTISSLEPEDFAVYYCQQGGSLP LTFGQGTKVEIK (SEQ ID NO: 424)
15F11v2	EIQLVQSGAEVKKPGASVKVSCKPSGYAFTSNNIY WVRQAPGQGLEWIGYIDPYIGRIIYNQQFKDRATLT VDKSTSTAYLELSSLRSEDTAVYYCSRSGERSNFDY WGQGTLVTVSS (SEQ ID NO: 425)	EIVLTQSPATLSLSPGERATLSCSASSSISSNYLHWY QQKPGQAPRLLIYRTSNLASGIP ARFSGSGSGTDFTLTISSLEPEDFAVYYCQQGGSLP LTFGQGTKVEIK (SEQ ID NO: 426)
15F11v3	EVQLVQSGAEVKKPGASVKVSCKASGYAFTSNNIY WVRQAPGQGLEWIGYIDPYIGRIIYNQQFKDRVTIT ADTSTSTAYLELSSLRSEDTAVYYCSRSGERSNFDY WGQGTLVTVSS (SEQ ID NO: 427)	EIVLTQSPATLSLSPGERATLSCSASSSISSNYLHWY QQKPGQSPRLLIYRTSNLASGVPARFSGSGSGTDYT LTISSLEPEDFAVYYCQQGGSLPLTFGQGTKVEIK (SEQ ID NO: 428)
15F11v4	EIQLVQSGAEVKKPGASVKVSCKPSGYAFTSNNIY WVRQAPGQGLEWIGYIDPYIGRIIYNQQFKDRATLT VDKSTSTAYLELSSLRSEDTAVYYCSRSGERSNFDY WGQGTLVTVSS (SEQ ID NO: 429)	EIVLTQSPATLSLSPGERATLSCSASSSISSNYLHWY QQKPGQSPRLLIYRTSNLASGVPARFSGSGSGTDYT LTISSLEPEDFAVYYCQQGGSLPLTFGQGTKVEIK (SEQ ID NO: 430)
15F11v5	EIQLVQSGAEVKKPGSSVKVSCKPSGYAFTSNNIY WVRQAPGQGLEWIGYIDPYIGRIIYNQQFKDRATLT VDKSTSTAYMELSSLRSEDTAVYYCSRSGERSNFD YWGQGTLVTVSS (SEQ ID NO: 431)	EIVLTQSPATLSLSPGERATLSCSASSSISSNYLHWY QQKPGQAPRLLIYRTSNLASGIP ARFSGSGSGTDFTLTISSLEPEDFAVYYCQQGGSLP LTFGQGTKVEIK (SEQ ID NO: 432)

Ab	VH	VL
15F11v6	EIQLVQSGAEVKKPGSSVKVSCKPSGYAFTSNNIY WVRQAPGQGLEWIGYIDPYIGRIIYNQQFKDRATLT VDKSTSTAYMELSSLRSEDTAVYYCSRSGERSNFD YWGQGTLVTVSS (SEQ ID NO: 433)	EIVLTQSPATLSLSPGERATLSCSASSSISSNYLHWY QQKPGQSPRLLIYRTSNLASGVPARFSGSGSGTDYT LTISSLEPEDFAVYYCQQGGSLPLTFGQGTKVEIK (SEQ ID NO: 434)
6E1.1.12	QGQMQQSGAELVKPGASVKLSCKTSGFTFSDNYIS WLKQKPGQSLEWIAWIYAGTGGSSYNQKFRDKAQ LTVDTSSRTAYMQLSSLTTEDSAIYYCARHDYYGT SGAWFAYWGRGTLVTVSA (SEQ ID NO: 435)	DVLMTQTPLSLPVSLGDQASISCRSSQHIVHSNENT YLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSG SGTDFTLKISRVEAEDLGVYYCFQGSHVPWTFGGG TKVEIK (SEQ ID NO: 436)
18G3	VQLQESGPGLVKPSQSLSLTCNVTGYSITGDYAWN WIRQFPGNKLEWIGYIGYTGSTTYNPSLKSRVSITR DTSKNQFFLQLNSVTPEDTATYYCARWRNWAMD YWGLGTSVTVSS (SEQ ID NO: 437)	DIQMTQTPSSLSASLGDRVTISCRANQDISHYLNWY QQKPDGAVKLLIYYTSRIHSGVPSRFSGSGSGTDYS LTIANLEQEDVATYFCQQGNTPPTTFGGGTKVEIK (SEQ ID NO: 438)
12H10	DVQLQESGPGLVKPSQPLSLTCTVTGYSITSNYAW NWIRQFPGDKLEWMGYISSSGITKSNPSLKSRISITR DTSKNQFFLQLNSLTTEDTATYYCSRWSNWSFDV WGAGTTVTVSS (SEQ ID NO: 439)	DIQMTQTPSSLSVSLGDRVTINCRASQDIHNYFNW YQQKPDGTIKLLIYYTSRFHSGVPSRFSGSGSGTDY SLTISNLEEEDIATYFCQQGNSLPPTFGGGTKLEIK (SEQ ID NO: 440)

SEQ ID NOs: 193 through SEQ ID NO: 208

SEQ ID NO: 193
MICA *008 (with signal sequence)
MGLGPVFLLLAGIFPFAPPGAAAEPHSLRYNLTVLSWDGSVQSGFLAEVHLDGQPFLRYDRQ
KCRAKPQGQWAEDVLGNKTWDRETRDLTGNGKDLRMTLAHIKDQKEGLHSLQEIRVCEIHE
DNSTRSSQHFYYDGELFLSQNLETEEWTVPQSSRAQTLAMNVRNFLKEDAMKTKTHYHAMH
ADCLQELRRYLESGVVLRRTVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLS
HDTQQWGDVLPDGNGTYQTWVATRICRGEEQRFTCYMEHSGNHSTHPVPSGKVLVLQSHW
QTFHVSAVAAGCCYFCYYYFLCPLL

Sequences for Constructs in Examples

SEQ ID NO: 194
>MICA008.muIgG2a (a3 domain in bold)

GS**TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGN**
GTYQTWVATRICRGEEQRFTCYMEHSGNHSTHPVPSGNSRAQVTDKKIEPRGPTIKPCPPC
KCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQT
HREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPP
EEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKK
NWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

SEQ ID NO: 195
>MICA008.His (a3 domain in bold)

GS**TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGN**
GTYQTWVATRICRGEEQRFTCYMEHSGNHSTHPVPSGNSHHHHHHHH

SEQ ID NO: 196
>MICA002.His (a3 domain in bold)

GS**TVPPMVNVTRSEASEGNITVTCRASGFYPWNITLSWRQDGVSLSHDTQQWGDVLPDG**
N GTYQTWVATRICQGEEQRFTCYMEHSGNHSTHPVPSGNSHHHHHHHH

SEQ ID NO: 197
>MICA004.His (a3 domain in bold)

GS**VPPMVNVTRSEASEGNITVTCRASSFYPRNITLTWRQDGVSLSHDTQQWGDVLPDGN**
G TYQTWVATRICQGEEQRFTCYMEHSGNHSTHPVPSGNSHHHHHHHH

SEQ ID NO: 198

>MICB005.His (a3 domain in bold)

GS**TVPPMVNVTCSEVSEGNITVTCRASSFYPRNITLTWRQDGVSLSHNTQQWGDVLPDG**
N GTYQTWVATRIRQGEEQRFTCYMEHSGNHGTHPVPSGNSHHHHHHHH

SEQ ID NO: 199

>MICA.008.a3.T204-S297.mIgG2a.Wild-type

TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGNGTYQ
TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS

SEQ ID NO: 200

>MICA.008.a3.T204-S297.E215N.G243N.H248N.R279N.C-terminal insert of N298.G299.S300.mIgG2a.Glyco4 (Hypergylcosylated)

TVPPMVNVTRS**N**ASEGNITVTCRASSFYPRNIILTWRQD**N**VSLS**N**DTQQWGDVLPDGNGTYQ
TWVATRICRGEEQ**N**FTCYMEHSGNHSTHPVPS**NGS**

SEQ ID NO: 201

>MICA.008.a3.R202-S297.R202N mIgG2a.Glyco11

NRTVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGNGT
YQTWVATRICRGEEQRFTCYMEHSGNHSTHPVPS

SEQ ID NO: 202

>MICA.008.a3.T204-S297.E215N.mIgG2a.Glyco12

TVPPMVNVTRS**N**ASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGNGTYQ
TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS

SEQ ID NO: 203

>MICA.008.a3.T204-S297.I236T.mIgG2a.Glyco13

TVPPMVNVTRSEASEGNITVTCRASSFYPRNI**T**LTWRQDGVSLSHDTQQWGDVLPDGNGTYQ
TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS

SEQ ID NO: 204

>MICA.008.a3.T204-S297.G243N.mIgG2a.Glyco14

TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQD**N**VSLSHDTQQWGDVLPDGNGTYQ
TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS

SEQ ID NO: 205

>MICA.008.a3.T204-S297.H248N.mIgG2a.Glyco15

TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLS**N**DTQQWGDVLPDGNGTYQ
TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS

SEQ ID NO: 206

>MICA.008.a3.T204-S297.R279N.mIgG2a.Glyco16

TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGNGTYQ
TWVATRICRGEEQ**N**FTCYMEHSGNHSTHPVPS

SEQ ID NO: 207

>MICA.008.a3.T204-S297.C-terminal insert of N298.G299.S300.mIgG2a.Glyco17

TVPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSHDTQQWGDVLPDGNGTYQ
TWVATRICRGEEQRFTCYMEHSGNHSTHPVPS**NGS**

SEQ ID NO: 208

>MILL1-MICA chimera.murine IgG2a Fc fusion

GSTVPPMVTVTSRNYPVGRVTLTCRASSPYPRNITLVWLQDGKPVQQKTFRSGDVLPDGN
GTYQTWVSIRVLPGQEPQFSCNLRHGNHSIMQTAVGNSRAQVTDKKIEPRGPTIKPCPPC
KCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTA
QTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQ
VYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMY
SKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

CLAIMS

WHAT IS CLAIMED IS:

1. An antibody that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, and Arg279 of human MICA*008.

2. The antibody of claim 1, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residues Glu215, His248, and Arg279 of human MICA*008.

3. The antibody of claim 1, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residues Gly243 and Arg279 of human MICA*008.

4. The antibody of claim 1, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residues His248 and Arg279 of human MICA*008.

5. The antibody of claim 1, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residue His248 of human MICA*008.

6. The antibody of claim 1, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residues Arg279 of human MICA*008.

7. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1;
a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2;
a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3;
a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4;
a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5; and
a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6.

8. The antibody of claim 7, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 7;
a FR-L2 comprising the amino acid sequence of SEQ ID NO: 8;
a FR-L3 comprising the amino acid sequence of SEQ ID NO: 9; and
a FR-L4 comprising the amino acid sequence of SEQ ID NO: 10.

9. The antibody of claim 7 or 8, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 11,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 12;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 13; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 14.

10. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 15; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

11. The antibody of claim 10, comprising a VH sequence of SEQ ID NO: 15.

12. The antibody of claim 10, comprising a VL sequence of SEQ ID NO: 16.

13. An antibody comprising a VH sequence of SEQ ID NO: 15 and a VL sequence of SEQ ID NO: 16.

14. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 19;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 20;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 21; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 22.

15. The antibody of claim 14, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 23;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 24;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 25; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 26.

16. The antibody of claim 14 or 15, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 27,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 28;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 29; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 30.

17. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 31; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 32; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

18. The antibody of claim 17, comprising a VH sequence of SEQ ID NO: 31.

19. The antibody of claim 17, comprising a VL sequence of SEQ ID NO: 32.

20. An antibody comprising a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 32.

21. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 33;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 34;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 35;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 36;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 37; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 38.

22. The antibody of claim 21, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 39;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 40;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 41; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 42.

23. The antibody of claim 21 or 22, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 43,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 44;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 45; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 46.

24. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 47; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 48; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

25. The antibody of claim 24, comprising a VH sequence of SEQ ID NO: 47.

26. The antibody of claim 24, comprising a VL sequence of SEQ ID NO: 48.

27. An antibody comprising a VH sequence of SEQ ID NO: 47 and a VL sequence of SEQ ID NO: 48.

28. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 49;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 50;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 51;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 52;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 53; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 54.

29. The antibody of claim 28, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 55;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 56;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 57; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 58.

30. The antibody of claim 28 or 29, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 59,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 60;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 61; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 62.

31. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 63; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 64; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

32. The antibody of claim 31, comprising a VH sequence of SEQ ID NO: 63.

33. The antibody of claim 31, comprising a VL sequence of SEQ ID NO: 64.

34. An antibody comprising a VH sequence of SEQ ID NO: 63 and a VL sequence of SEQ ID NO: 64.

35. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 65;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 66;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 67;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 68;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 69; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 70.

36. The antibody of claim 35, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 71;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 72;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 73; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 74.

37. The antibody of claim 35 or 36, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 75,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 76;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 77; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 78.

38. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 79; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 80; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

39. The antibody of claim 38, comprising a VH sequence of SEQ ID NO: 79.

40. The antibody of claim 38, comprising a VL sequence of SEQ ID NO: 80.

41. An antibody comprising a VH sequence of SEQ ID NO: 79 and a VL sequence of SEQ ID NO: 80.

42. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 81;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 82;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 83;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 84;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 85; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 86.

43. The antibody of claim 42, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 87;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 88;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 89; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 90.

44. The antibody of claim 42 or 43, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 91,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 92;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 93; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 94.

45. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 95; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 96; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

46. The antibody of claim 45, comprising a VH sequence of SEQ ID NO: 95.

47. The antibody of claim 45, comprising a VL sequence of SEQ ID NO: 96.

48. An antibody comprising a VH sequence of SEQ ID NO: 95 and a VL sequence of SEQ ID NO: 96.

49. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 97;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 98;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 99;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 100;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 101; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 102.

50. The antibody of claim 49, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 103;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 104;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 105; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 106.

51. The antibody of claim 49 or 50, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 107,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 108;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 109; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 110.

52. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 111; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 112; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

53. The antibody of claim 52, comprising a VH sequence of SEQ ID NO: 111.

54. The antibody of claim 52, comprising a VL sequence of SEQ ID NO: 112.

55. An antibody comprising a VH sequence of SEQ ID NO: 111 and a VL sequence of SEQ ID NO: 112.

56. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 113;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 114;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 115;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 116;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 117; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 118.

57. The antibody of claim 56, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 119;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 120;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 121; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 122.

58. The antibody of claim 56 or 57, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 123,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 124;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 125; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 126.

59. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 127; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 128; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

60. The antibody of claim 59, comprising a VH sequence of SEQ ID NO: 127.

61. The antibody of claim 59, comprising a VL sequence of SEQ ID NO: 128.

62. An antibody comprising a VH sequence of SEQ ID NO: 127 and a VL sequence of SEQ ID NO: 128.

63. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 129;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 130;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 131;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 132;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 133; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 134.

64. The antibody of claim 63, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 135;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 136;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 137; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 138.

65. The antibody of claim 63 or 64, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 139,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 140;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 141; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 142.

66. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 143; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 144; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

67. The antibody of claim 66, comprising a VH sequence of SEQ ID NO: 143.

68. The antibody of claim 66, comprising a VL sequence of SEQ ID NO: 144.

69. An antibody comprising a VH sequence of SEQ ID NO: 143 and a VL sequence of SEQ ID NO: 144.

70. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 145;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 146;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 147;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 148;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 149; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 150.

71. The antibody of claim 70, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 151;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 152;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 153; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 154.

72. The antibody of claim 70 or 71, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 155,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 156;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 157; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 158.

73. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 159; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 160; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

74. The antibody of claim 73, comprising a VH sequence of SEQ ID NO: 159.

75. The antibody of claim 73, comprising a VL sequence of SEQ ID NO: 160.

76. An antibody comprising a VH sequence of SEQ ID NO: 159 and a VL sequence of SEQ ID NO: 160.

77. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 161;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 162;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 163;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 164;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 165; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 166.

78. The antibody of claim 77, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 167;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 168;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 169; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 170.

79. The antibody of claim 77 or 78, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 171,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 172;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 173; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 174.

80. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 175; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 176; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

81. The antibody of claim 80, comprising a VH sequence of SEQ ID NO: 175.

82. The antibody of claim 80, comprising a VL sequence of SEQ ID NO: 176.

83. An antibody comprising a VH sequence of SEQ ID NO: 175 and a VL sequence of SEQ ID NO: 176.

84. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 177;

a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 178;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 179;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 180;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 181; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 182.

85. The antibody of claim 84, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 183;

a FR-L2 comprising the amino acid sequence of SEQ ID NO: 184;

a FR-L3 comprising the amino acid sequence of SEQ ID NO: 185; and

a FR-L4 comprising the amino acid sequence of SEQ ID NO: 186.

86. The antibody of claim 84 or 85, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 187,

a FR-H2 comprising the amino acid sequence of SEQ ID NO: 188;

a FR-H3 comprising the amino acid sequence of SEQ ID NO: 189; and

a FR-H4 comprising the amino acid sequence of SEQ ID NO: 190.

87. The antibody of claim 1, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 191; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 192; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

88. The antibody of claim 87, comprising a VH sequence of SEQ ID NO: 191.

89. The antibody of claim 87, comprising a VL sequence of SEQ ID NO: 192.

90. An antibody comprising a VH sequence of SEQ ID NO: 191 and a VL sequence of SEQ ID NO: 192.

91. The antibody of any one of the preceding claims, wherein the antibody binds to human MICA with a Kd of about 100 nM or lower.

92. The antibody of any one of the preceding claims, wherein the antibody is capable of binding to human MICA*002, human MICA*004 and human MICB*005.

93. The antibody of any one of the preceding claims, wherein the antibody is capable of binding to the extracellular domain of human MICA*008, human MICA*002, human MICA*004, human MICA*008 and human MICB*005.

94. The antibody of any one of the preceding claims, wherein the antibody is capable of binding to the alpha3 domain of human MICA*008, human MICA*002, human MICA*004, and human MICB*005.

95. The antibody of any one of the preceding claims, wherein the antibody is monoclonal.

96. The antibody of any one of the preceding claims, wherein the antibody is human, humanized, or chimeric.

97. The antibody of any one of the preceding claims, wherein at least a portion of the framework sequence is a human consensus framework sequence.

98. The antibody of any one of the preceding claims, wherein the antibody is a full-length antibody.

99. The antibody of any one of the preceding claims, wherein the antibody is an antibody fragment that binds human MICA*008.

100. The antibody of claim 99, wherein the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, single chain variable fragment (scFv), and $(Fab')_2$ fragments.

101. The antibody of any one of the preceding claims, wherein the antibody is an IgG class antibody.

102. The antibody of claim 101, wherein the IgG class antibody is an IgG1 subclass antibody.

103. An isolated nucleic acid encoding the antibody of any one of the preceding claims.

104. A vector comprising the nucleic acid of claim 103.

105. A host cell comprising the vector of claim 104.

106. The host cell of claim 105, wherein the host cell is prokaryotic.

107. The host cell of claim 106, wherein the host cell is *Escherichia coli.*

108. The host cell of claim 105, wherein the host cell is eukaryotic.

109. The host cell of claim 108, wherein the host cell is a 293 cell, a CHO cell, a yeast cell, or a plant cell.

110. A method of producing the antibody of any one of claims 1 to 102, the method comprising culturing the host cell of any of claims 105- 109 in a culture medium.

111. The method of claim 110, wherein the method further comprises recovering the antibody from the host cell or culture medium.

112. An immunoconjugate comprising the antibody of any one of claims 1 to 102 and a cytotoxic agent.

113. A composition comprising the antibody of any one of claims 1 to 102.

114. The composition of claim 113, further comprising a pharmaceutically acceptable carrier, excipient, or diluent.

115. The composition of claim 114, wherein the composition is a pharmaceutical composition.

116. The composition of any one of claims 113 - 115, wherein the composition further comprises a PD-1 axis binding antagonist or an additional therapeutic agent.

117. The antibody of any one of claims 1 to 102, for use in reducing shedding of MIC.

118. The antibody of any one of claims 1 to 102, for use in reducing levels of soluble MIC.

119. The antibody of any of claims 1 to 102, for use in reducing shedding of MIC and reducing levels of soluble MIC.

120. The antibody of any one of claims 1 to 102 for use as a medicament.

121. The antibody of any one of claims 1 to 102 for use in treating or delaying progression of a cancer in a subject in need thereof.

122. The antibody of claim 121, wherein the cancer is selected from the group consisting of epithelial cancer, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, Merkel cell cancer, and other hematologic malignancies.

123. The antibody of any one of claims 1 to 102 for use in treating or delaying progression of an immune related disease in a subject in need thereof.

124. The antibody of claim 123, wherein the immune related disease is associated with a NKG2D ligand.

125. The antibody of claim 124, wherein the NKG2D ligand is MIC.

126. The antibody of any one of claims 123 - 125, wherein the immune related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity.

127. The antibody of any one of claims 1 to 102 for use in increasing, enhancing, or stimulating an immune response or function in a subject in need thereof.

128. Use of the antibody of any one of claims 1 to 102 in the manufacture of a medicament for reducing shedding of MIC.

129. Use of the antibody of any one of claims 1 to 102 in the manufacture of a medicament for reducing levels of soluble MIC.

130. Use of the antibody of any one of claims 1 to 102 in the manufacture of a medicament for reducing shedding of MIC and levels of soluble MIC.

131. Use of the antibody of any one of claims 1 to 102 in the manufacture of a medicament for treating or delaying progression of a cancer in a subject in need thereof.

132. The use of claim 131, wherein the cancer is selected from the group consisting of epithelial cancer, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, Merkel cell cancer, and other hematologic malignancies.

133. Use of the antibody of any one of claims 1 to 102 in the manufacture of a medicament for treating or delaying progression of an immune related disease in a subject in need thereof.

134. The use of claim 133, wherein the immune related disease is associated with a NKG2D ligand.

135. The use of claim 134, wherein the NKG2D ligand is MIC.

136. The use of any one of claims 133 - 135, wherein the immune related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity.

137. Use of the antibody of any one of claims 1 to 102 in the manufacture of a medicament for increasing, enhancing, or stimulating an immune response or function in a subject in need thereof.

138. A method of reducing shedding of MIC in an individual comprising administering to the individual an effective amount of the antibody of any one of claims 1 to 102 to reduce shedding of MIC.

139. A method of reducing levels of soluble MIC in an individual comprising administering to the individual an effective amount of the antibody of any one of claims 1 to 102 to reduce levels of soluble MIC.

140. A method of reducing shedding of MIC and reducing levels of soluble MIC in an individual comprising administering to the individual an effective amount of the antibody of any one of claims 1 to 102 to reduce shedding of MIC and levels of soluble MIC.

141. A method for treating or delaying progression of a cancer in a subject, the method comprising administering to the subject an effective amount of the antibody of any one of claims 1 to 102, thereby treating or delaying the progression of the cancer in the subject.

142. The method of claim 141, wherein the cancer is selected from the group consisting of epithelial cancer, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, Merkel cell cancer, and other hematologic malignancies.

143. A method for treating or delaying progression of an immune related disease in a subject, the method comprising administering to the subject an effective amount of the antibody of any one of claims 1 to 102, thereby treating or delaying the progression of the immune related disease in the subject.

144. The method of claim 143, wherein the immune related disease is associated with a NKG2D ligand.

145. The method of claim 144, wherein the NKG2D ligand is MIC.

146. The method of any one of claims 143 - 145, wherein the immune related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity.

147. A method of increasing, enhancing, or stimulating an immune response or function in a subject, the comprising administering to the subject an effective amount of the antibody of any one of claims 1 to 102, thereby of increasing, enhancing, or stimulating an immune response or function in the subject.

148. The method of any one of claims 138 - 147, further comprising administering to the subject a PD-1 axis binding antagonist.

149. The method of claim 148, wherein the PD-1 axis binding antagonist is administered prior to or subsequent to the administration of the antibody.

150. The method of claim 148, wherein the PD-1 axis binding antagonist is administered concurrently with the antibody.

151. The method of any one of claims 148- 150, wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist, and a PD-L2 binding antagonist.

152. The method of claim 151, wherein the PD-1 axis binding antagonist is a PD-1 binding antagonist.

153. The method of claim 152, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners.

154. The method of claim 153, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1.

155. The method of claim 153, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2.

156. The method of claim 153, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2.

157. The method of any one of claims 152-156, wherein the PD-1 binding antagonist is an anti-PD-1 antibody.

158. The method of claim 153, wherein the PD-1 binding antagonist is selected from the group consisting of MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, BGB-108, and BGB-A317.

159. The method of claim 151, wherein the PD-1 axis binding antagonist is a PD-L1 binding antagonist.

160. The method of claim 159, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1.

161. The method of claim 159, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1.

162. The method of claim 159, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1.

163. The method of claim 159-162, wherein the PD-L1 binding antagonist is an anti-PD-L1 antibody.

164. The method of claim 163, wherein the anti-PD-L1 antibody is selected from the group consisting of: MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab).

165. The method of claim 164, wherein the antibody is MPDL3280A.

166. The method of claim 151, wherein the PD-1 axis binding antagonist is a PD-L2 binding antagonist.

167. The method of claim 166, wherein the PD-L2 binding antagonist is an anti-PD-L2 antibody.

168. The method of claim 166, wherein the PD-L2 binding antagonist is an immunoadhesin.

169. The method of any one of claims 138 to 168, further comprising administering to the subject an agent that decreases or inhibits one or more additional inhibitory co-stimulatory receptors.

170. The method of claim 169, wherein the one or more additional inhibitory co-stimulatory receptor is selected from the group consisting of PD-1, CTLA-4, LAG3, TIM3, BTLA, VISTA, B7H4, and MM.

171. The method of any one of claims 138- 170, further comprising administering to the subject an additional therapeutic agent.

172. The method of claim 171, wherein the additional therapeutic agent is a chemotherapeutic agent.

173. The method of any one of claims 138- 172, wherein the antibody is administered parenterally, intrapulmonarily, intranasally, intramuscularly, intravenously, intraarterially, intraperitoneally, or subcutaneously.

174. The method of any one of claims 138-173, wherein the subject is a human.

175. A kit comprising the antibody of any one of claims 1 to 102 and a package insert comprising instructions for using the antibody for treating or delaying progression of a cancer in a subject.

176. A kit comprising the antibody of any one of claims 1 to 102 and a package insert comprising instructions for using the antibody for treating or delaying progression of an immune related disease in a subject.

177. A kit comprising the antibody of any one of claims 1 to 102 and a package insert comprising instructions for increasing, enhancing, or stimulating an immune response or function in a subject.

178. The kit of any one of claims 175- 177, wherein the subject is a human.

179. A method of mapping an epitope of an antibody comprising substituting an unglycosylated amino acid of a polypeptide to generate a glycosylated polypeptide comprising a substituted glycosylated amino acid;determining whether the antibody binds to the glycosylated polypeptide; and identifying at least one of the unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid as part of the epitope if binding of the antibody to the glycosylated polypeptide is reduced compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

180. A method of mapping an epitope of an antibody comprising substituting an unglycosylated amino acid of a polypeptide to generate a glycosylated polypeptide comprising a substituted glycosylated amino acid; and determining whether the antibody binds to the glycosylated polypeptide, wherein at least one of the unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid is identified as part of the epitope if binding of the antibody to the glycosylated polypeptide is reduced compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

181. A method of mapping an epitope of an antibody comprising identifying at least one of an unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid as part of the epitope if binding of the antibody to a glycosylated polypeptide comprising a substituted glycosylated amino acid is reduced compared to binding of the antibody to a polypeptide without the substituted glycosylated amino acid, wherein the glycosylated polypeptide is generated by substituting an unglycosylated amino acid of the polypeptide.

182. The method of any of claims 179 - 181, wherein substituting an unglycosylated amino acid of a polypeptide comprises introducing a glycosylation site in the polypeptide.

183. The method of any of claims 179 - 182, wherein the epitope is a conformational epitope.

184. The method of any of claims 179 - 183, wherein the unglycosylated amino acid is on the surface of the polypeptide without the substituted glycosylated amino acid.

185. The method of any of claims 179 - 184, wherein the substituted glycosylated amino acid comprises an N-linked glycan.

186. The method of any of claims 179- 185, wherein the polypeptide comprises a Fc domain.

187. The method of any of claims 179 - 186, wherein binding of antibody to the glycosylated polypeptide is detected by ELISA.

188. The method of any of claims 179 - 187, wherein binding of the antibody to the glycosylated polypeptide is reduced by at least 50% compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

189. A method for binning antibodies comprising mapping the epitope of a first antibody by the method of any of claims 179 - 188; mapping the epitope of a second antibody by the method of any of claims 179 - 188; and determining that the first antibody and the second antibody are in the same bin if they have the same epitope.

190. A method for identifying the contact amino acids of an antibody comprising substituting an unglycosylated amino acid of a polypeptide to generate a glycosylated polypeptide comprising a substituted glycosylated amino acid; determining whether the antibody binds to the glycosylated polypeptide; and identifying at least one of the unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid as one of the contact amino acids of the antibody if binding of the antibody to the glycosylated polypeptide is reduced compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

191. A method for identifying the contact amino acids of an antibody comprising substituting an unglycosylated amino acid of a polypeptide to generate a glycosylated polypeptide comprising a substituted glycosylated amino acid; and determining whether the antibody binds to the glycosylated polypeptide, wherein at least one of the unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid is identified as one of the contact amino acids of the antibody if binding of the antibody to the glycosylated polypeptide is reduced compared to binding of the antibody to the polypeptide without the substituted glycosylated amino acid.

192. A method for identifying the contact amino acids of an antibody comprising identifying at least one of an unglycosylated amino acid or surface-exposed amino acids within 5 Angstroms of the unglycosylated amino acid as one of the contact amino acids of the antibody if binding of the antibody to a glycosylated polypeptide comprising a substituted glycosylated amino acid is reduced compared to binding of the antibody to a polypeptide without the substituted glycosylated amino acid, wherein the glycosylated polypeptide is generated by substituting an unglycosylated amino acid of the polypeptide.

193. An antibody any one of claims 1 to 102, wherein the epitope is mapped by the method of any one of claims 179 - 188.

194. An antibody any one of claims 1 to 102, wherein the contact amino acid residues are identified by the method of any one of claims 190 - 192.

195. An antibody that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of Glu215, Gly243, His248, Arg279, Arg213, Ser214, Ala216, Ser217, Asn220, Arg271, Arg240, Gln241, Asp242, Val244, Ser245, Thr281, Ser247, Asp249, Thr250, Trp253, Glu276, Glu277, and Gln278 of human MICA*008

196. The antibody of claim 195, wherein the antibody binds to an epitope on human MICA*008 comprising a first amino acid residue, a second amino acid residue, and a third amino acid residue; wherein the first amino acid residue is selected from the group consisting of Glu215, Arg213, Ser214, Ala216, Ser217, Asn220, and Arg271 of human MICA*008; the second amino acid residue is selected from the group consisting of His248, Ser247, Asp249,

Thr250, and Trp253 of human MICA*008; and the third amino acid residue is selected from the group consisting of Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and Thr281 of human MICA*008.

197. The antibody of claim 195, wherein the antibody binds to an epitope on human MICA*008 comprising a first amino acid residue and a second amino acid residue; wherein the first amino acid residue is selected from the group consisting of Gly243, Arg240, Gln241, Asp242, Val244, Ser245, Arg279, and Thr281 of human MICA*008; and the second amino acid residue is selected from the group consisting of Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and Thr281 of human MICA*008.

198. The antibody of claim 195, wherein the antibody binds to an epitope on human MICA*008 comprising a first amino acid residue and a second amino acid residue; wherein the first amino acid residue is selected from the group consisting of His248, Ser247, Asp249, Thr250, or Trp253 of human MICA*008; and the second amino acid residue is selected from the group consisting of Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and Thr281 of human MICA*008 of human MICA*008.

199. The antibody of claim 195, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residues selected from the group consisting of His248, Ser247, Asp249, Thr250, and Trp253 of human MICA*008.

200. The antibody of claim 195, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residues selected from the group consisting of Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and Thr281 of human MICA*008.

201. The method of any of claims 179 - 182, wherein the epitope is a linear epitope.

202. An antibody that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residue Gly243 of human MICA*008.

203. The antibody of claim 202, wherein the antibody binds to an epitope on human MICA*008 comprising amino acid residues Gly243 and Arg279 of human MICA*008.

204. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 209;

a HVR-H2 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 210, SEQ ID NO: 215, SEQ ID NO: 216, and SEQ ID NO: 217;

a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 211;

a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 212;

a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 213; and

a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 214.

205. The antibody of claim 204, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 248, SEQ ID NO: 252, SEQ ID NO: 258, SEQ ID NO: 261, and SEQ ID NO: 266;
a FR-L2 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 249, SEQ ID NO: 253, SEQ ID NO: 256, SEQ ID NO: 259, SEQ ID NO: 262, and SEQ ID NO: 264;
a FR-L3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 250, SEQ ID NO: 254, SEQ ID NO: 257, SEQ ID NO: 260, SEQ ID NO: 263, SEQ ID NO: 265, and SEQ ID NO: 267; and
a FR-L4 comprising the amino acid sequence of SEQ ID NO: 251 or SEQ ID NO: 255.

206. The antibody of claim 204 or 205, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 295, SEQ ID NO: 299, and SEQ ID NO: 303;
a FR-H2 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 296, SEQ ID NO: 300, and SEQ ID NO: 304;
a FR-H3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 297, SEQ ID NO: 301, SEQ ID NO: 305, SEQ ID NO: 306, SEQ ID NO: 307, SEQ ID NO: 308, SEQ ID NO: 309; SEQ ID NO: 310, SEQ ID NO: 311, SEQ ID NO: 312, SEQ ID NO: 313, and SEQ ID NO: 314; and
a FR-H4 comprising the amino acid sequence of SEQ ID NO: 298 or SEQ ID NO: 302.

207. The antibody of claim 202, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 369; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 370; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

208. The antibody of claim 207, comprising a VH sequence of SEQ ID NO: 369.

209. The antibody of claim 207, comprising a VL sequence of SEQ ID NO: 370.

210. An antibody comprising a VH sequence of SEQ ID NO: 369 and a VL sequence of SEQ ID NO: 370.

211. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 218;
a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 219;
a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 220;
a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 221;
a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 222; and
a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 223.

212. The antibody of claim 211, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 268 or SEQ ID NO: 272;
a FR-L2 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 269, SEQ ID NO: 273, and SEQ ID NO: 275;
a FR-L3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 270, SEQ ID NO: 274, and SEQ ID NO: 276; and
a FR-L4 comprising the amino acid sequence of SEQ ID NO: 255 or SEQ ID NO: 271.

213. The antibody of claim 211 or 212, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 315, SEQ ID NO: 319, and SEQ ID NO: 323;
a FR-H2 comprising the amino acid sequence of SEQ ID NO: 316 or SEQ ID NO: 320;
a FR-H3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 317, SEQ ID NO: 321, SEQ ID NO: 322, and SEQ ID NO: 324; and
a FR-H4 comprising the amino acid sequence of SEQ ID NO: 302 or SEQ ID NO: 318.

214. The antibody of claim 202, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 415; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 416; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

215. The antibody of claim 214, comprising a VH sequence of SEQ ID NO: 415.

216. The antibody of claim 214, comprising a VL sequence of SEQ ID NO: 416.

217. An antibody comprising a VH sequence of SEQ ID NO: 415 and a VL sequence of SEQ ID NO: 416.

218. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 224;
a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 225;
a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 226;
a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 227;
a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 228; and
a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 229.

219. The antibody of claim 218, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 277 of SEQ ID NO: 280;
a FR-L2 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 278, SEQ ID NO: 281, and SEQ ID NO: 283;
a FR-L3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 279, SEQ ID NO: 282, and SEQ ID NO: 284; and
a FR-L4 comprising the amino acid sequence of SEQ ID NO: 251 or SEQ ID NO: 255.

220. The antibody of claim 218 or 219, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 325, SEQ ID NO: 329, SEQ ID NO: 331, and SEQ ID NO: 333;
a FR-H2 comprising the amino acid sequence of SEQ ID NO: 320 or SEQ ID NO: 326;
a FR-H3 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 327, SEQ ID NO: 330, SEQ ID NO: 332, and SEQ ID NO: 334; and
a FR-H4 comprising the amino acid sequence of SEQ ID NO: 302 or SEQ ID NO: 328.

.

221. The antibody of claim 202, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 429; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 430; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

222. The antibody of claim 221, comprising a VH sequence of SEQ ID NO: 429.

223. The antibody of claim 221, comprising a VL sequence of SEQ ID NO: 430.

224. An antibody comprising a VH sequence of SEQ ID NO: 429 and a VL sequence of SEQ ID NO: 430.

225. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 236;
a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 237;
a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 238;
a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 239;
a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 240; and
a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 241.

226. The antibody of claim 225, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 288;
a FR-L2 comprising the amino acid sequence of SEQ ID NO: 289;
a FR-L3 comprising the amino acid sequence of SEQ ID NO: 290; and
a FR-L4 comprising the amino acid sequence of SEQ ID NO: 271.

227. The antibody of claim 225 or 226, wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 339,
a FR-H2 comprising the amino acid sequence of SEQ ID NO: 340;
a FR-H3 comprising the amino acid sequence of SEQ ID NO: 341; and
a FR-H4 comprising the amino acid sequence of SEQ ID NO: 342.

228. The antibody of claim 202, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 437; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 438; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

229. The antibody of claim 228, comprising a VH sequence of SEQ ID NO: 437.

230. The antibody of claim 228, comprising a VL sequence of SEQ ID NO: 438.

231. An antibody comprising a VH sequence of SEQ ID NO: 437 and a VL sequence of SEQ ID NO: 438.

232. An antibody that specifically binds to human MICA*008, wherein the antibody comprises the following six hypervariable regions (HVRs):

a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 242;
a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 243;
a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 244;
a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 245;
a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 246; and
a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 247.

233. The antibody of claim 232, wherein the antibody further comprises the following light chain variable region framework regions (FRs):

a FR-L1 comprising the amino acid sequence of SEQ ID NO: 291;
a FR-L2 comprising the amino acid sequence of SEQ ID NO: 292;
a FR-L3 comprising the amino acid sequence of SEQ ID NO: 293; and
a FR-L4 comprising the amino acid sequence of SEQ ID NO: 294.

234. The antibody of claim 232 or 233,wherein the antibody further comprises the following heavy chain variable region FRs:

a FR-H1 comprising the amino acid sequence of SEQ ID NO: 343,
a FR-H2 comprising the amino acid sequence of SEQ ID NO: 344;
a FR-H3 comprising the amino acid sequence of SEQ ID NO: 345; and
a FR-H4 comprising the amino acid sequence of SEQ ID NO: 346.

235. The antibody of claim 202, comprising **(a)** a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 439; **(b)** a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 440; or **(c)** a VH sequence as in **(a)** and a VL sequence as in **(b).**

236. The antibody of claim 235, comprising a VH sequence of SEQ ID NO: 439.

237. The antibody of claim 235, comprising a VL sequence of SEQ ID NO: 440.

238. An antibody comprising a VH sequence of SEQ ID NO: 439 and a VL sequence of SEQ ID NO: 440.

239. The antibody of any one of claims 202 to 238, wherein the antibody binds to human MICA with a Kd of about 100 nM or lower.

240. The antibody of any one of claims 202 to 239, wherein the antibody is capable of binding to human MICA*002, human MICA*004 and human MICB*005.

241. The antibody of any one of claims 202 to 240, wherein the antibody is capable of binding to the extracellular domain of human MICA*008, human MICA*002, human MICA*004, human MICA*008 and human MICB*005.

242. The antibody of any one of claims 202 to 241, wherein the antibody is capable of binding to the alpha3 domain of human MICA*008, human MICA*002, human MICA*004, and human MICB*005.

243. The antibody of any one of claims 202 to 242, wherein the antibody is monoclonal.

244. The antibody of any one of claims 202 to 243, wherein the antibody is human, humanized, or chimeric.

245. The antibody of any one of claims 202 to 244, wherein at least a portion of the framework sequence is a human consensus framework sequence.

246. The antibody of any one of claims 202 to 245, wherein the antibody is a full-length antibody.

247. The antibody of any one of claims 202 to 245, wherein the antibody is an antibody fragment that binds human MICA*008.

248. The antibody of claim 247, wherein the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, single chain variable fragment (scFv), and $(Fab')_2$ fragments.

249. The antibody of any one of claims 202 to 248, wherein the antibody is an IgG class antibody.

250. The antibody of claim 249, wherein the IgG class antibody is an IgG1 subclass antibody.

251. An isolated nucleic acid encoding the antibody of any one of claims 202 to 250.

252. A vector comprising the nucleic acid of claim 251.

253. A host cell comprising the vector of claim 252.

254. The host cell of claim 253, wherein the host cell is prokaryotic.

255. The host cell of claim 254, wherein the host cell is *Escherichia coli.*

256. The host cell of claim 253, wherein the host cell is eukaryotic.

257. The host cell of claim 256, wherein the host cell is a 293 cell, a CHO cell, a yeast cell, or a plant cell.

258. A method of producing the antibody of any one of claims 202 to 250, the method comprising culturing the host cell of any of claims 253 to 257 in a culture medium.

259. The method of claim 258, wherein the method further comprises recovering the antibody from the host cell or culture medium.

260. An immunoconjugate comprising the antibody of any one of claims 202 to 250 and a cytotoxic agent.

261. A composition comprising the antibody of any one of claims 202 to 250.

262. The composition of claim 261, further comprising a pharmaceutically acceptable carrier, excipient, or diluent.

263. The composition of claim 262, wherein the composition is a pharmaceutical composition.

264. The composition of any one of claims 261 to 263, wherein the composition further comprises a PD-1 axis binding antagonist or an additional therapeutic agent.

265. The antibody of any one of claims 202 to 250, for use in reducing shedding of MIC.

266. The antibody of any one of claims 202 to 250, for use in reducing levels of soluble MIC.

267. The antibody of any of claims 202 to 250, for use in reducing shedding of MIC and reducing levels of soluble MIC.

268. The antibody of any one of claims 202 to 250 for use as a medicament.

269. The antibody of any one of claims 202 to 250 for use in treating or delaying progression of a cancer in a subject in need thereof.

270. The antibody of claim 269, wherein the cancer is selected from the group consisting of epithelial cancer, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, Merkel cell cancer, and other hematologic malignancies.

271. The antibody of any one of claims 202 to 250 for use in treating or delaying progression of an immune related disease in a subject in need thereof.

272. The antibody of claim 271, wherein the immune related disease is associated with a NKG2D ligand.

273. The antibody of claim 272, wherein the NKG2D ligand is MIC.

274. The antibody of any one of claims 271 to 273, wherein the immune related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity.

275. The antibody of any one of claims 202 to 250 for use in increasing, enhancing, or stimulating an immune response or function in a subject in need thereof.

276. Use of the antibody of any one of claims 202 to 250 in the manufacture of a medicament for reducing shedding of MIC.

277. Use of the antibody of any one of claims 202 to 250 in the manufacture of a medicament for reducing levels of soluble MIC.

278. Use of the antibody of any one of claims 202 to 250 in the manufacture of a medicament for reducing shedding of MIC and levels of soluble MIC.

279. Use of the antibody of any one of claims 202 to 250 in the manufacture of a medicament for treating or delaying progression of a cancer in a subject in need thereof.

280. The use of claim 279, wherein the cancer is selected from the group consisting of epithelial cancer, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, Merkel cell cancer, and other hematologic malignancies.

281. Use of the antibody of any one of claims 202 to 250 in the manufacture of a medicament for treating or delaying progression of an immune related disease in a subject in need thereof.

282. The use of claim 281, wherein the immune related disease is associated with a NKG2D ligand.

283. The use of claim 282, wherein the NKG2D ligand is MIC.

284. The use of any one of claims 281 to 283, wherein the immune related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity.

285. Use of the antibody of any one of claims 202 to 250 in the manufacture of a medicament for increasing, enhancing, or stimulating an immune response or function in a subject in need thereof.

286. A method of reducing shedding of MIC in an individual comprising administering to the individual an effective amount of the antibody of any one of claims 202 to 250 to reduce shedding of MIC.

287. A method of reducing levels of soluble MIC in an individual comprising administering to the individual an effective amount of the antibody of any one of claims 202 to 250 to reduce levels of soluble MIC.

288. A method of reducing shedding of MIC and reducing levels of soluble MIC in an individual comprising administering to the individual an effective amount of the antibody of any one of claims 202 to 250 to reduce shedding of MIC and levels of soluble MIC.

289. A method for treating or delaying progression of a cancer in a subject, the method comprising administering to the subject an effective amount of the antibody of any one of claims 202 to 250, thereby treating or delaying the progression of the cancer in the subject.

290. The method of claim 289, wherein the cancer is selected from the group consisting of epithelial cancer, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, Merkel cell cancer, and other hematologic malignancies.

291. A method for treating or delaying progression of an immune related disease in a subject, the method comprising administering to the subject an effective amount of the antibody of any one of claims 202 to 250, thereby treating or delaying the progression of the immune related disease in the subject.

292. The method of claim 291, wherein the immune related disease is associated with a NKG2D ligand.

293. The method of claim 292, wherein the NKG2D ligand is MIC.

294. The method of any one of claims 291 to 293, wherein the immune related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity.

295. A method of increasing, enhancing, or stimulating an immune response or function in a subject, the comprising administering to the subject an effective amount of the antibody of any one of claims 202 to 250, thereby of increasing, enhancing, or stimulating an immune response or function in the subject.

296. The method of any one of claims 286 to 295, further comprising administering to the subject a PD-1 axis binding antagonist.

297. The method of claim 296, wherein the PD-1 axis binding antagonist is administered prior to or subsequent to the administration of the antibody.

298. The method of claim 296, wherein the PD-1 axis binding antagonist is administered concurrently with the antibody.

299. The method of any one of claims 296 to 298, wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist, and a PD-L2 binding antagonist.

300. The method of claim 299, wherein the PD-1 axis binding antagonist is a PD-1 binding antagonist.

301. The method of claim 300, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners.

302. The method of claim 301, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1.

303. The method of claim 301, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2.

304. The method of claim 301, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2.

305. The method of any one of claims 300 to 304, wherein the PD-1 binding antagonist is an anti-PD-1 antibody.

306. The method of claim 301, wherein the PD-1 binding antagonist is selected from the group consisting of MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, BGB-108, and BGB-A317.

307. The method of claim 299, wherein the PD-1 axis binding antagonist is a PD-L1 binding antagonist.

308. The method of claim 307, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1.

309. The method of claim 307, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1.

310. The method of claim 307, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1.

311. The method of claim 307 to 310, wherein the PD-L1 binding antagonist is an anti-PD-L1 antibody.

312. The method of claim 311, wherein the anti-PD-L1 antibody is selected from the group consisting of: MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab).

313. The method of claim 312, wherein the antibody is MPDL3280A.

314. The method of claim 299, wherein the PD-1 axis binding antagonist is a PD-L2 binding antagonist.

315. The method of claim 314, wherein the PD-L2 binding antagonist is an anti-PD-L2 antibody.

316. The method of claim 314, wherein the PD-L2 binding antagonist is an immunoadhesin.

317. The method of any one of claims 286 to 316, further comprising administering to the subject an agent that decreases or inhibits one or more additional inhibitory co-stimulatory receptors.

318. The method of claim 317, wherein the one or more additional inhibitory co-stimulatory receptor is selected from the group consisting of PD-1, CTLA-4, LAG3, TIM3, BTLA, VISTA, B7H4, and MM.

319. The method of any one of claims 286 to 318, further comprising administering to the subject an additional therapeutic agent.

320. The method of claim 319, wherein the additional therapeutic agent is a chemotherapeutic agent.

321. The method of any one of claims 286 to 320, wherein the antibody is administered parenterally, intrapulmonarily, intranasally, intramuscularly, intravenously, intraarterially, intraperitoneally, or subcutaneously.

322. The method of any one of claims 286 to 321, wherein the subject is a human.

323. A kit comprising the antibody of any one of claims 202 to 250 and a package insert comprising instructions for using the antibody for treating or delaying progression of a cancer in a subject.

324. A kit comprising the antibody of any one of claims 202 to 250 and a package insert comprising instructions for using the antibody for treating or delaying progression of an immune related disease in a subject.

325. A kit comprising the antibody of any one of claims 202 to 250 and a package insert comprising instructions for increasing, enhancing, or stimulating an immune response or function in a subject.

326. The kit of any one of claims 323 to 325, wherein the subject is a human.

327. An antibody that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of: Gly243, Arg240, Gln241, Asp242, Val244, Ser245, Arg 279, and Thr281 of human MICA*008.

328. The antibody of claim 327, wherein the antibody binds to an epitope on human MICA*008 comprising a first amino acid residue and a second amino acid residue, wherein the first residue is selected from the group consisting of: Gly243, Arg240, Gln241, Asp242, Val244, Ser245, Arg 279, and Thr281 of human MICA*008, and the second residue is selected from the group consisting of Arg 279, Arg279, Arg240, Gln241, Asp242, Gly243, Glu276, Glu277, Gln278, and Thr281 of human MICA*008.

329. An antibody that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of: Arg240, Gln241, Val244, Ser245, His248, Glu276, Arg279,Tyr283, Glu285, His290, and Thr292 of human MICA*008.

330. An antibody that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of: Arg240, Gln241, Asp242, Gly243, Val244, Ser245, Leu246, Ser247, Asp249, Thr250, Arg279, Tyr283, His290, Ser291, Thr292, and Pro294 of human MICA*008.

331. An antibody that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of: Arg240, Asp242, Gly243, Val244, Glu277, Gln278, Arg279, Phe280, Thr281, Tyr283, Glu285, Gly288, Asn289, His290, Ser291, Thr292, Pro294, Val295, Pro296, and Ser297 of human MICA*008.

332. An antibody that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of: Asn234, Ile235, Ile236, Leu237, Thr238, Trp239, and Arg240 of human MICA*008.

333. An antibody that specifically binds to human MICA*008, wherein the antibody binds to an epitope on human MICA*008 comprising one or more amino acid residues selected from the group consisting of: Val268, Ala269, Thr270, Arg271, Ile272, Cys273, Arg274, Gly275, Glu276, Glu277, Gln278, Arg279, and Phe280 of human MICA*008.

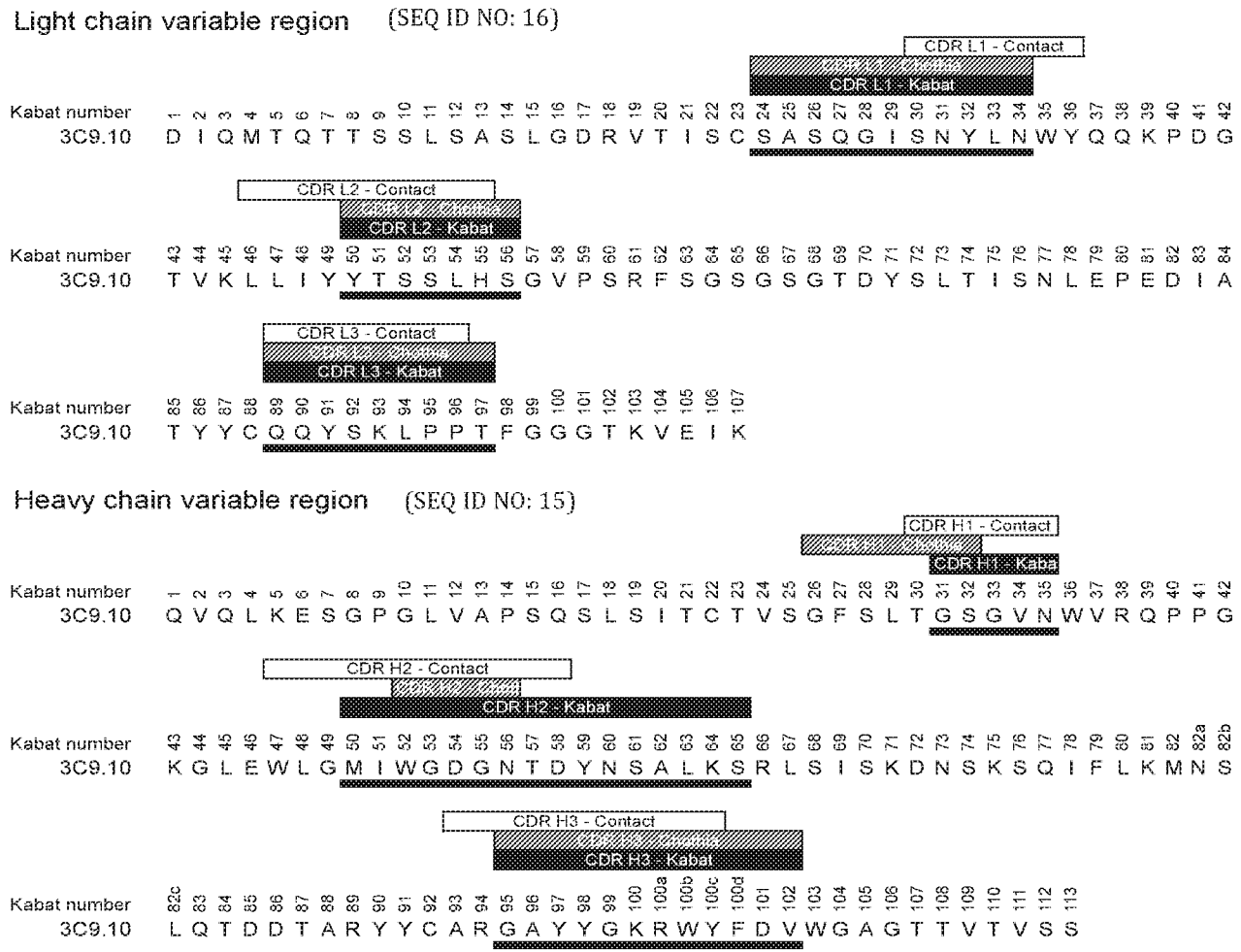
334. The antibody of any one of claims 1 to 102 and/or 202 to 250, wherein the antibody is a bispecific antibody.

335. The antibody of claim 101 or 249, wherein the IgG class antibody is an IgG2 subclass antibody.

336. The antibody of claim 101 or 249 , wherein the IgG class antibody is an IgG4 subclass antibody.

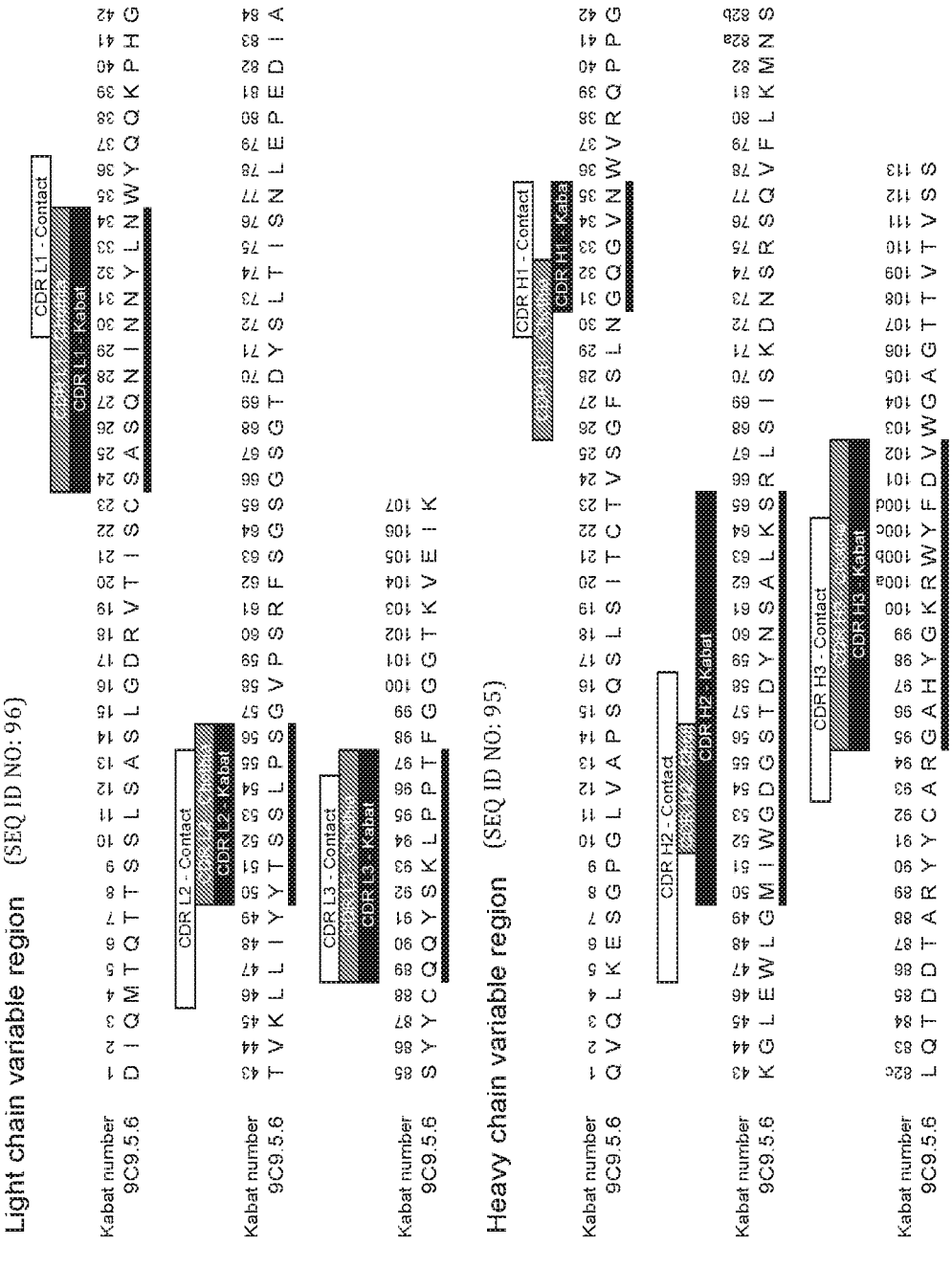
FIG. 1A: 3C9.10 Hypervariable Regions

CDR sequences according to Kabat definition are underlined



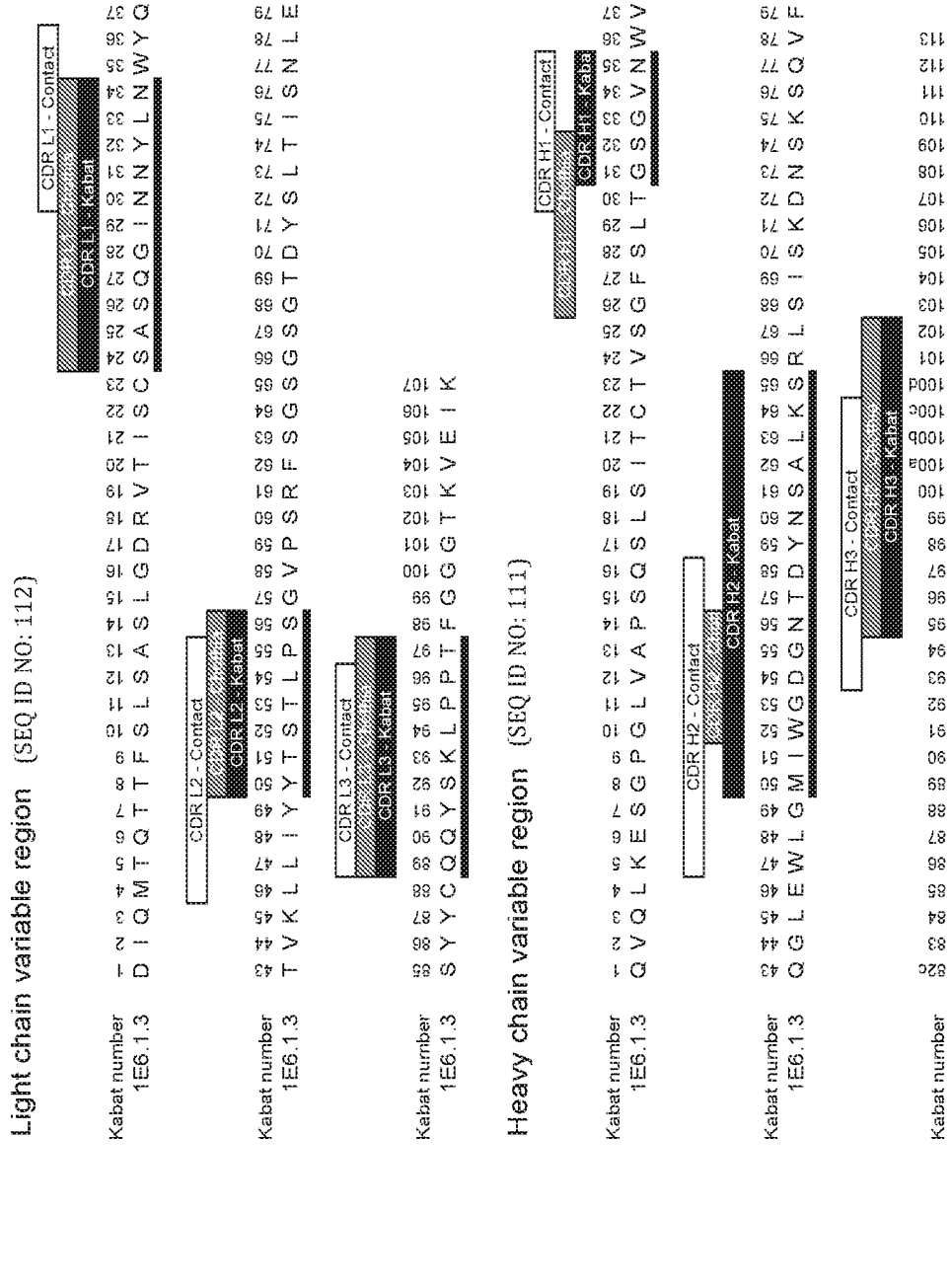
Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
3C9.10	SASQGISNYLN (SEQ ID NO: 4)	YTSSLHS (SEQ ID NO: 5)	QQYSKLPPT (SEQ ID NO: 6)	GSGVN (SEQ ID NO: 1)	MIWGDGNTDYNSALKS (SEQ ID NO: 2)	GAYYGKRWYFDV (SEQ ID NO: 3)

FIG. 1B: 9C9.5.6 Hypervariable Regions



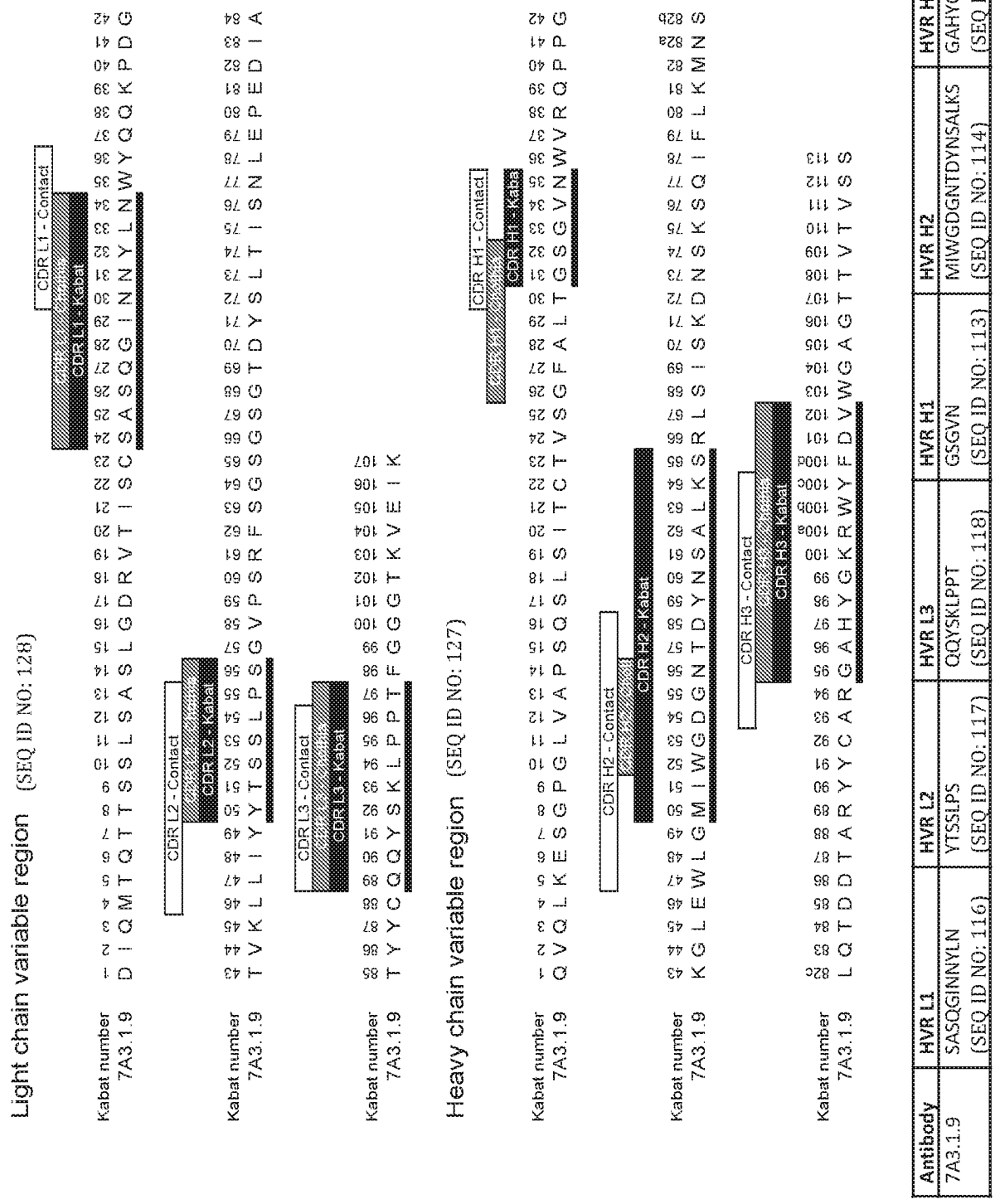
Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
9C9.5.6	SASQNINNYLN (SEQ ID NO: 84)	YTSSLPS (SEQ ID NO: 85)	QQYSKLPPT (SEQ ID NO: 86)	GQGVN (SEQ ID NO: 81)	MIWGDGSTDYNSALKS (SEQ ID NO: 82)	GAHYGKRWYFDV (SEQ ID NO: 83)

FIG. 1C: 1E6.1.3 Hypervariable Regions



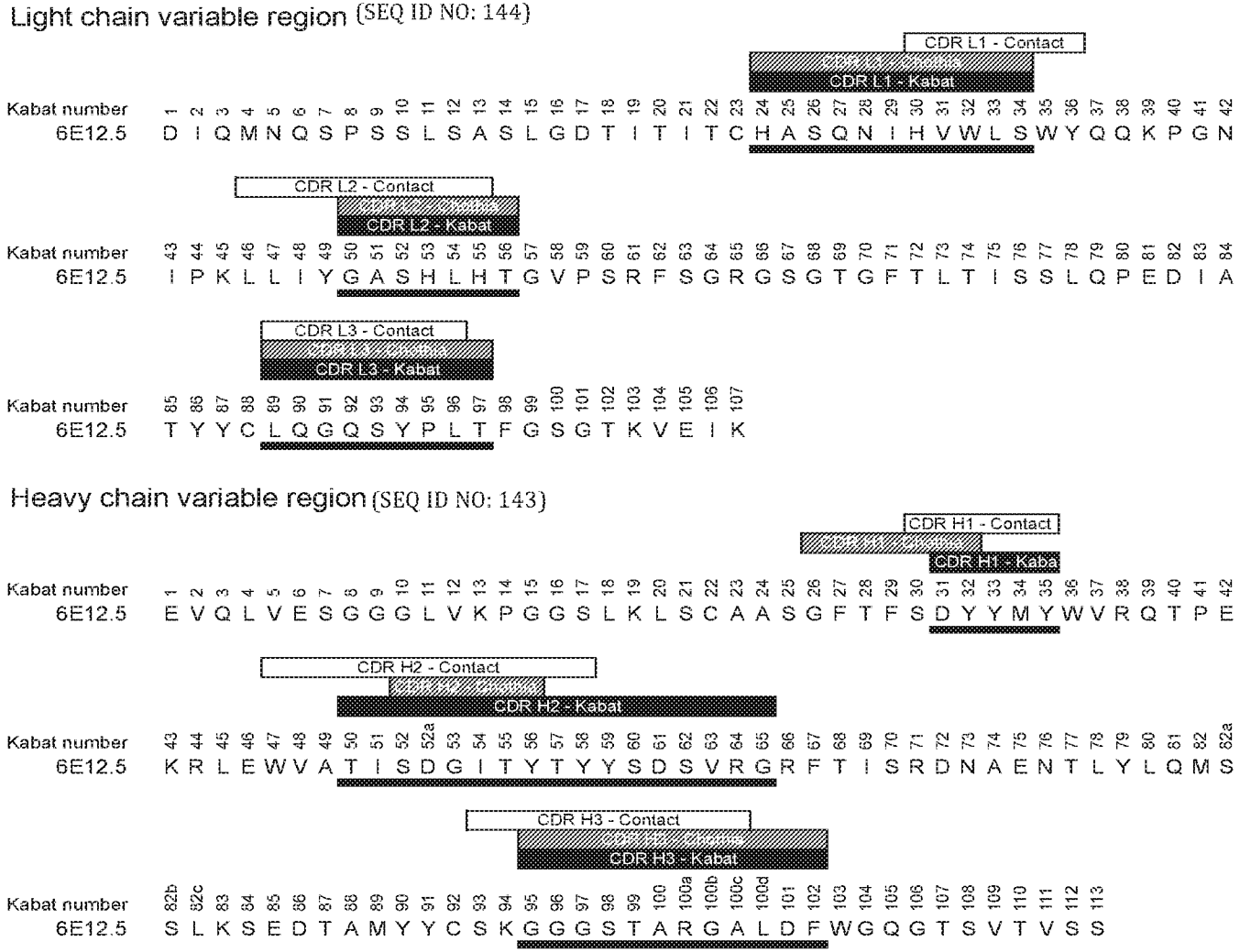
Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
1E6.1.3	SASQGINNYLN (SEQ ID NO: 100)	YTSTLPS (SEQ ID NO: 101)	QQYSKLPPT (SEQ ID NO: 102)	GSGVN (SEQ ID NO: 97)	MIWGDGNTDYNSALKS (SEQ ID NO: 98)	GAHYGKRWYFDV (SEQ ID NO: 99)

FIG. 1D: 7A3.1.9 Hypervariable Regions



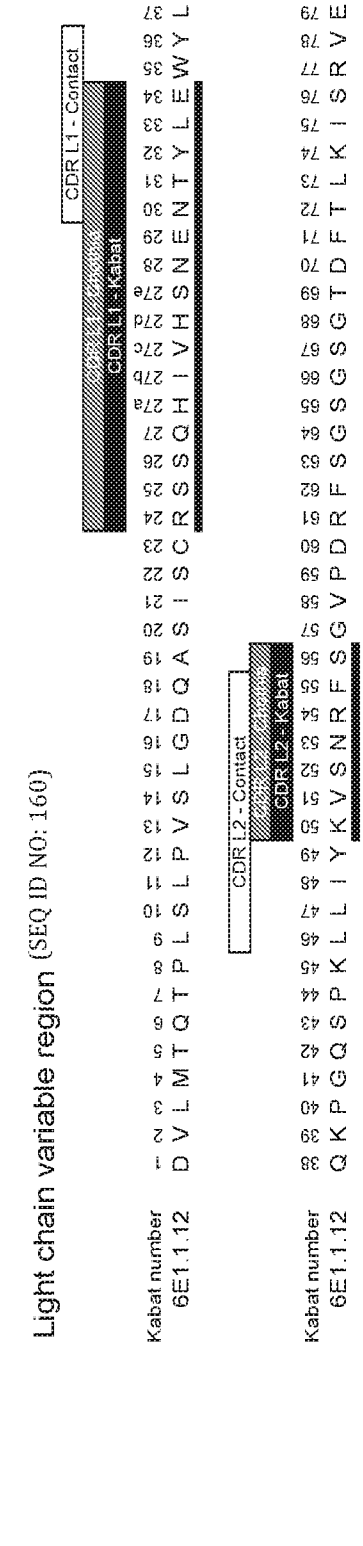
Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
7A3.1.9	SASQGINNYLN (SEQ ID NO: 116)	YTSSLPS (SEQ ID NO: 117)	QQYSKLPPT (SEQ ID NO: 118)	GSGVN (SEQ ID NO: 113)	MIWGDGNTDYNSALKS (SEQ ID NO: 114)	GAHYGKRWYFDV (SEQ ID NO: 115)

FIG. 1E: 6E12.5 Hypervariable Regions



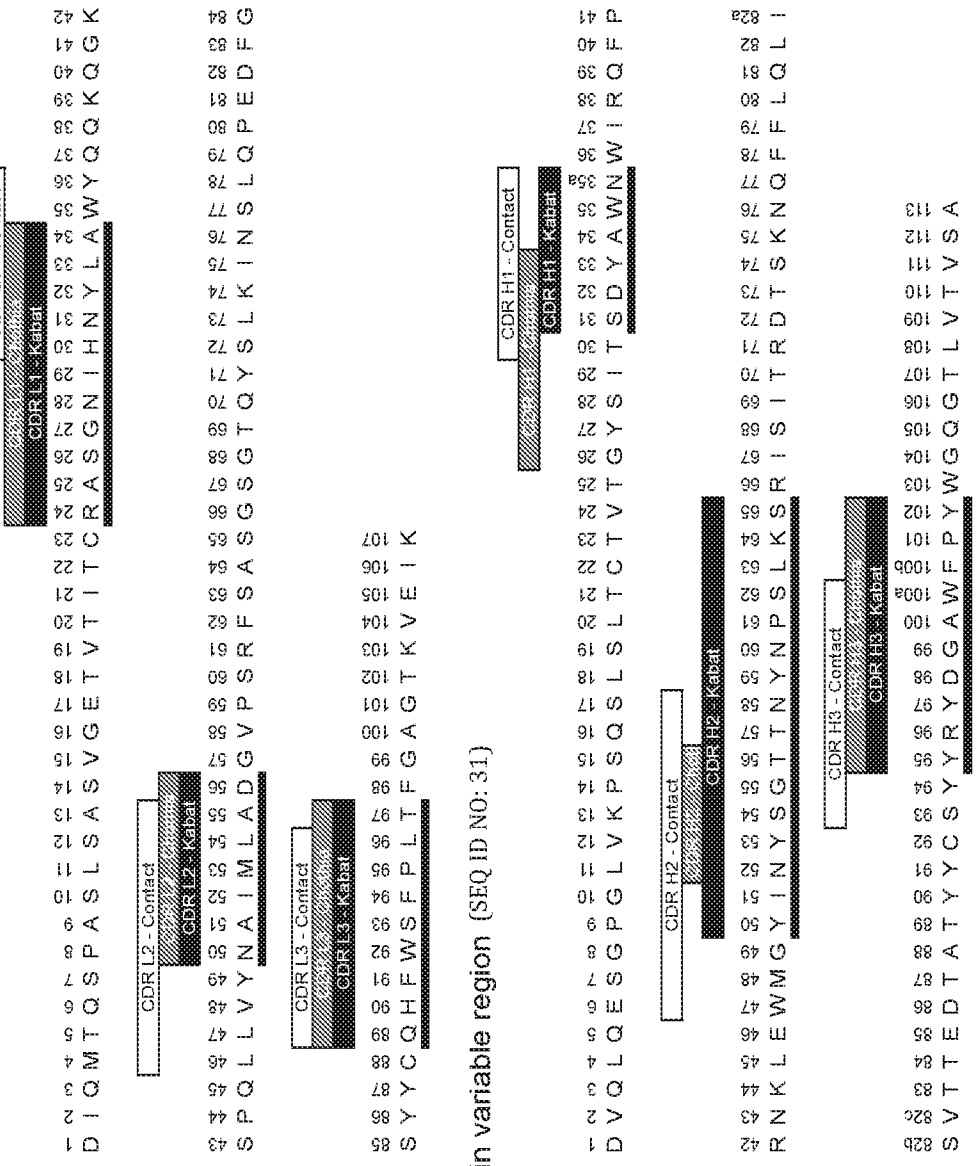
Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
6E12.5	HASQNIHVWLS (SEQ ID NO: 132)	GASHLHT (SEQ ID NO: 133)	LQGQSYPLT (SEQ ID NO: 134)	DYYMY (SEQ ID NO: 129)	TISDGITYTYYSDSVRG (SEQ ID NO: 130)	GGGSTARGALDF (SEQ ID NO: 131)

FIG. 1F: 6E1.1.12 Hypervariable Regions



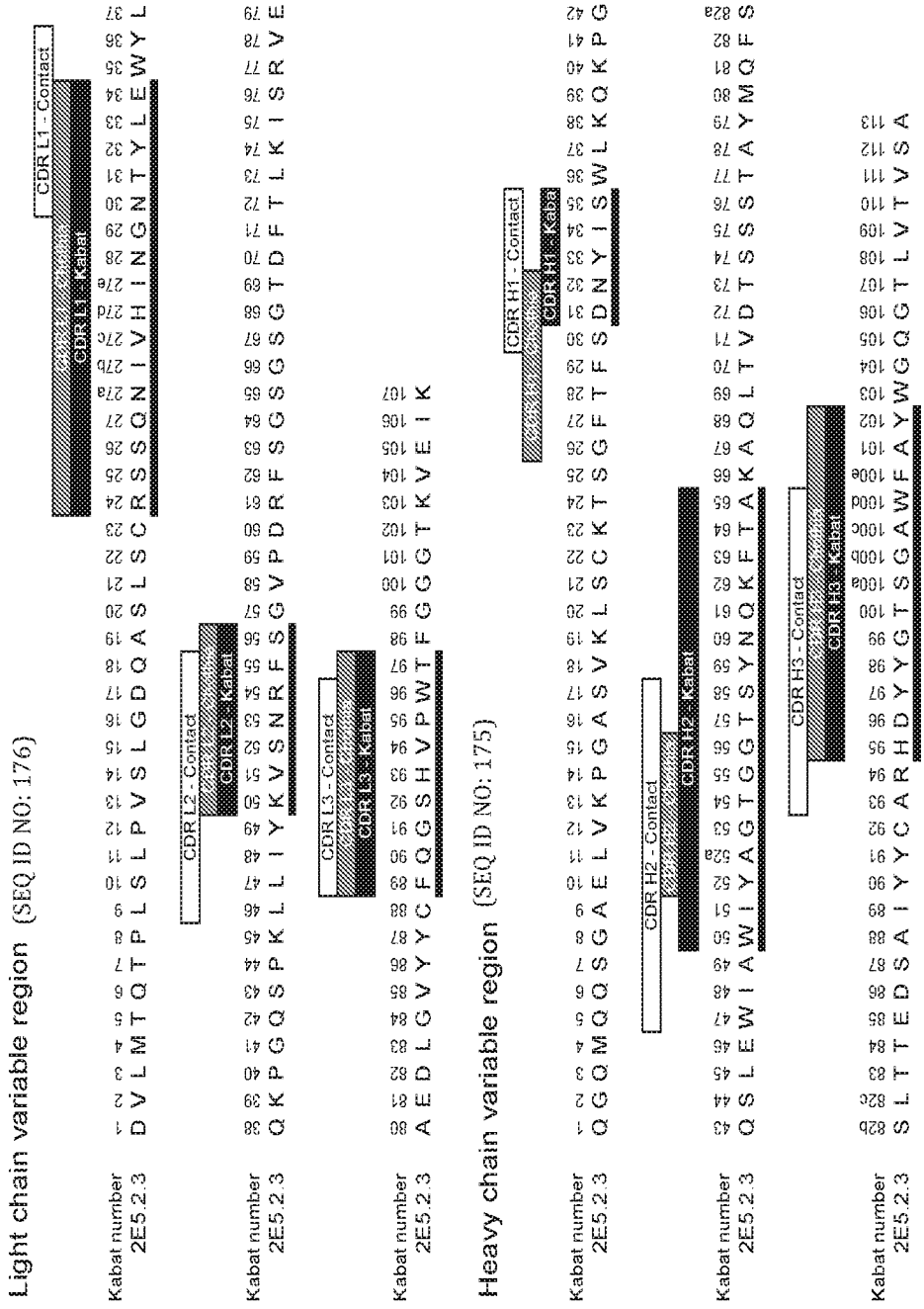
Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
6E1.1.12	RSSQHIVHSNENTYLE (SEQ ID NO: 164)	KVSNRFS (SEQ ID NO: 165)	FQGSHVPWT (SEQ ID NO: 166)	DNYIS (SEQ ID NO: 161)	WIYAGTGGSSYNQKFRD (SEQ ID NO: 162)	HDYYGTSGAWFAY (SEQ ID NO: 163)

FIG. 1G: 7D4.6 Hypervariable Regions



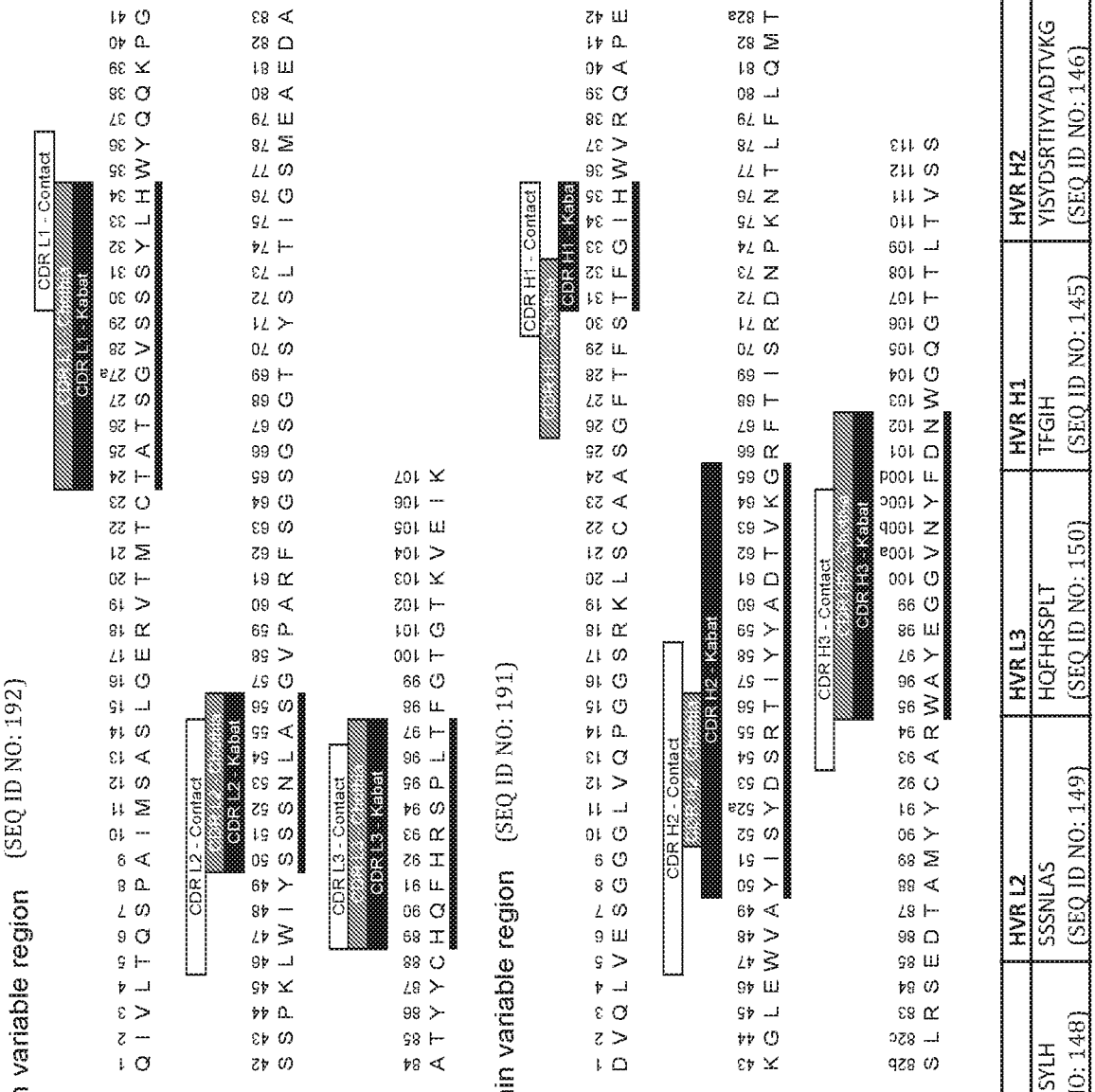
Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
7D4.6	RASGNIHNYLA (SEQ ID NO: 20)	NAIMLAD (SEQ ID NO: 21)	QHFWSFPLT (SEQ ID NO: 22)	SDYAWN (SEQ ID NO: 17)	YINYSGTTNYNPSLKS (SEQ ID NO: 18)	YRYDGAWFPY (SEQ ID NO: 19)

FIG. 1H: 2E5.2.3 Hypervariable Regions



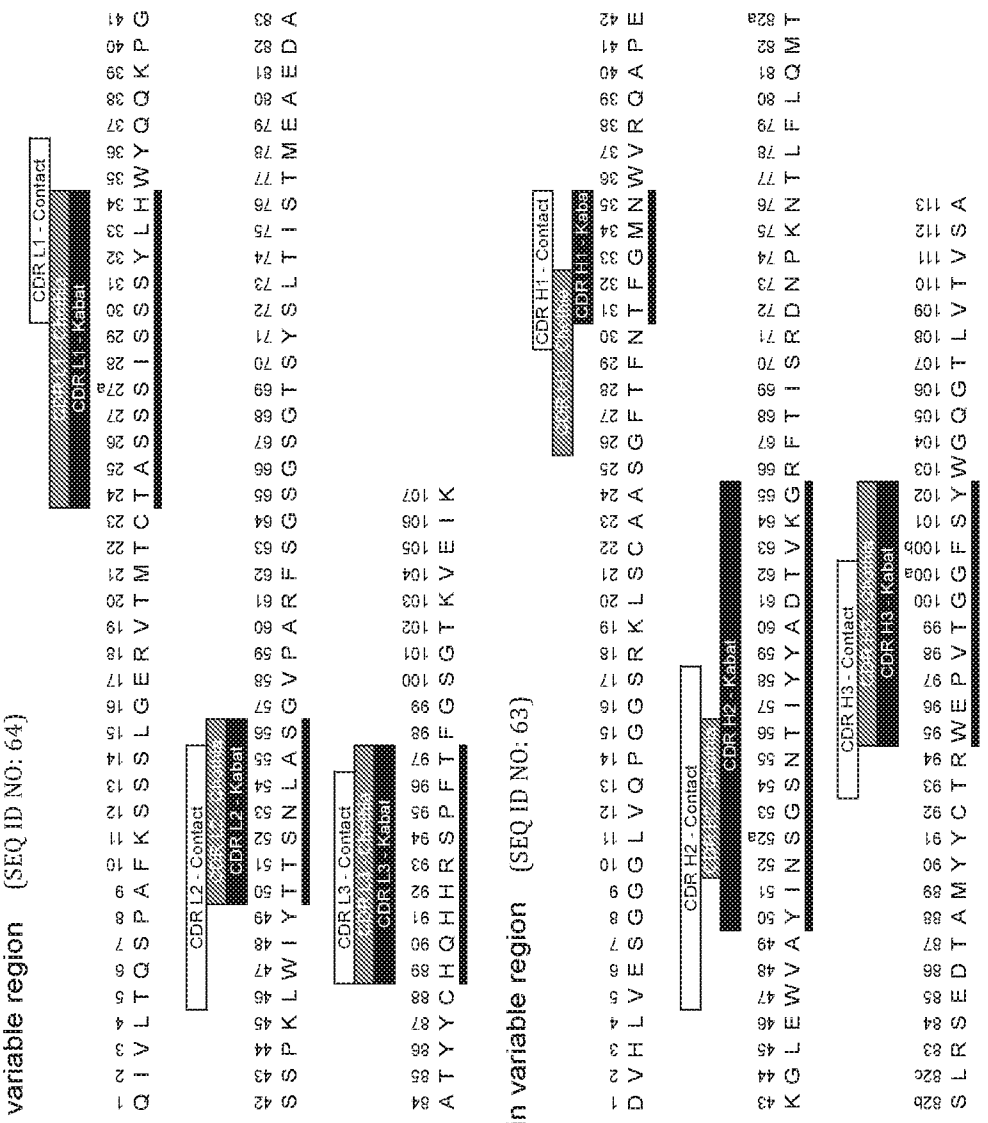
Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
2E5.2.3	RSSQNIVHINGNTYLE (SEQ ID NO: 180)	KVSNRFS (SEQ ID NO: 181)	FQGSHVPWT (SEQ ID NO: 182)	DNYIS (SEQ ID NO: 177)	WIYAGTGGTSYNQKFTA (SEQ ID NO: 178)	HDYYGTSGAWFAY (SEQ ID NO: 179)

FIG. 1-I: 20G11 Hypervariable Regions



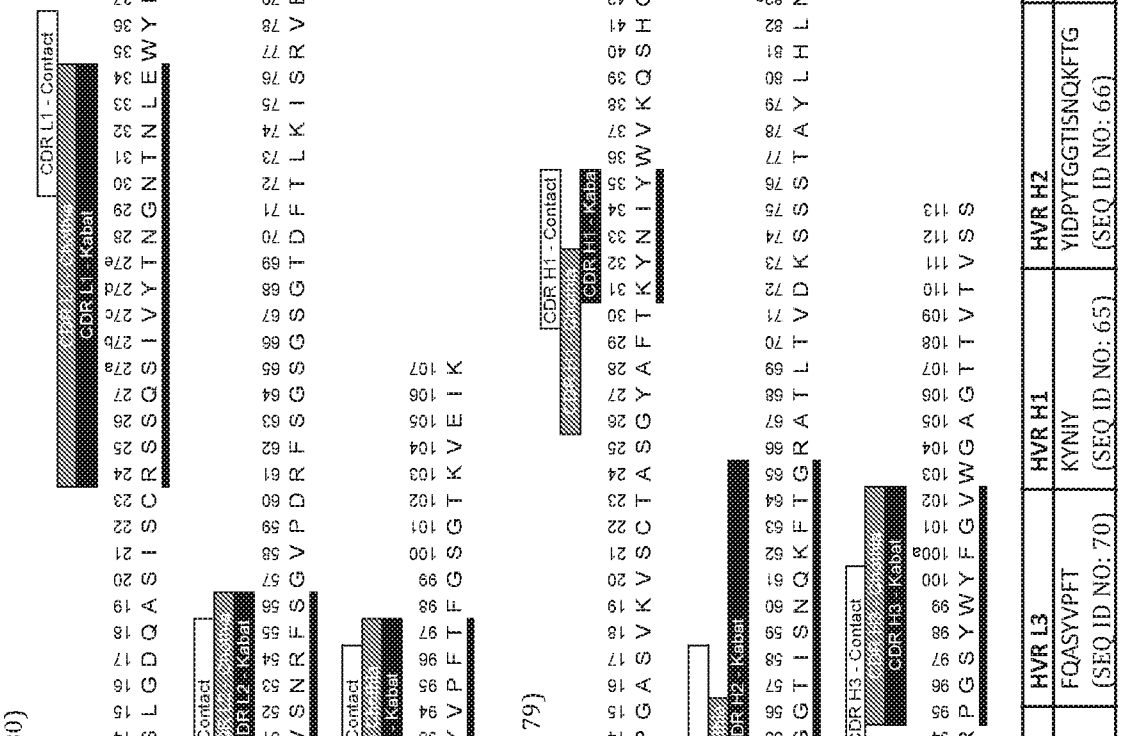
Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
20G11	TATSGVSSSYLH (SEQ ID NO: 148)	SSSNLAS (SEQ ID NO: 149)	HQFHRSPLT (SEQ ID NO: 150)	TFGIH (SEQ ID NO: 145)	YISYDSRTIYYADTVKG (SEQ ID NO: 146)	WAYEGGVNYFDN (SEQ ID NO: 147)

FIG. 1-J: 32D2 Hypervariable Regions



Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
32D2	TASSISSSYLH (SEQ ID NO: 52)	TTSNLAS (SEQ ID NO: 53)	HQHHRSPFT (SEQ ID NO: 54)	TFGMN (SEQ ID NO: 49)	YINSGSNTIYYADTVKG (SEQ ID NO: 50)	WEPVTGGFSY (SEQ ID NO: 51)

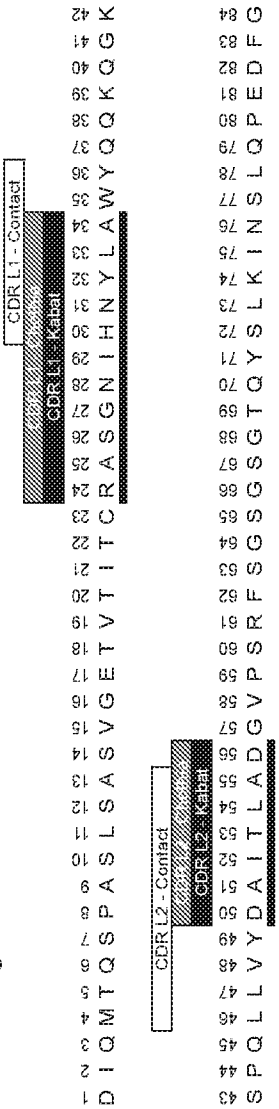
FIG. 1K: 3E11 Hypervariable Regions



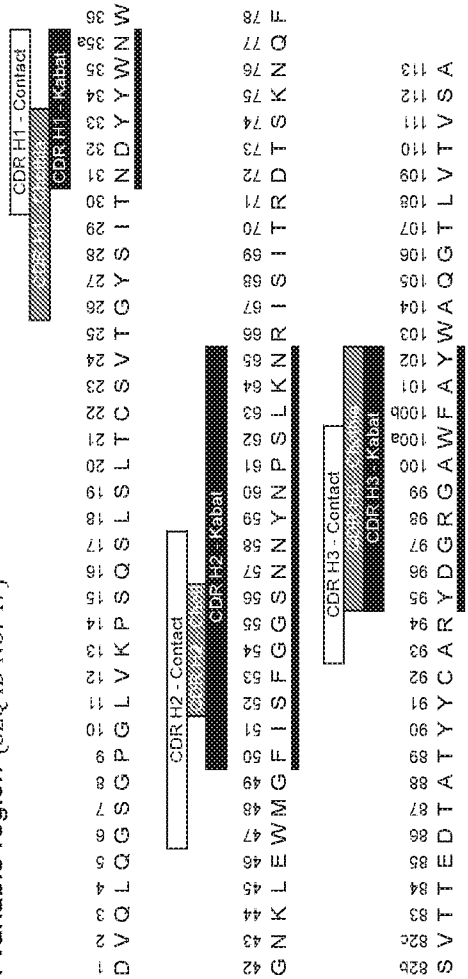
Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
3E11	RSSQSIVYTNGNTNLE (SEQ ID NO: 68)	KVSNRFS (SEQ ID NO: 69)	FQASYVPFT (SEQ ID NO: 70)	KYNIY (SEQ ID NO: 65)	YIDPYTGGTISNQKFTG (SEQ ID NO: 66)	PGSYWYFGV (SEQ ID NO: 67)

FIG. 1L: 6F8.7 Hypervariable Regions

Light chain variable region (SEQ ID NO: 48)



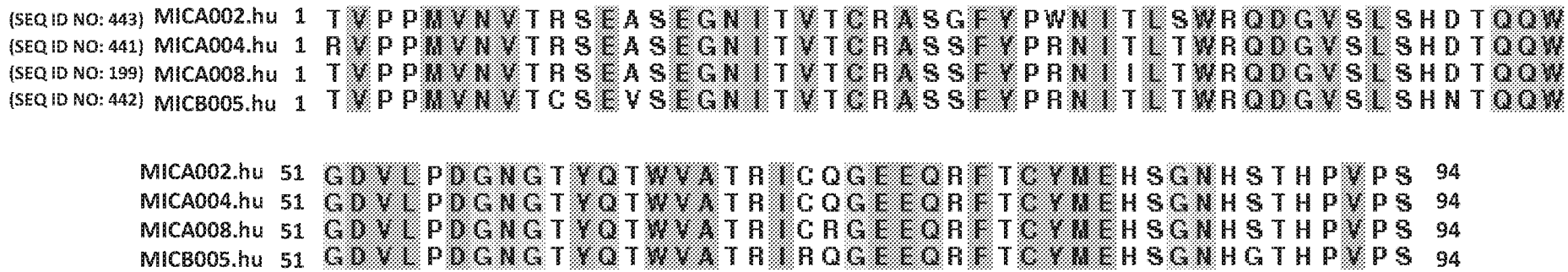
Heavy chain variable region (SEQ ID NO: 47)



Antibody	HVR L1	HVR L2	HVR L3	HVR H1	HVR H2	HVR H3
6F8.7	RASGNIHNYLA (SEQ ID NO: 36)	DAITLAD (SEQ ID NO: 37)	QHFWSFPLT (SEQ ID NO: 38)	NDYYWN (SEQ ID NO: 33)	FISFGGSNNYNPSLKN (SEQ ID NO: 34)	YDGRGAWFAY (SEQ ID NO: 35)

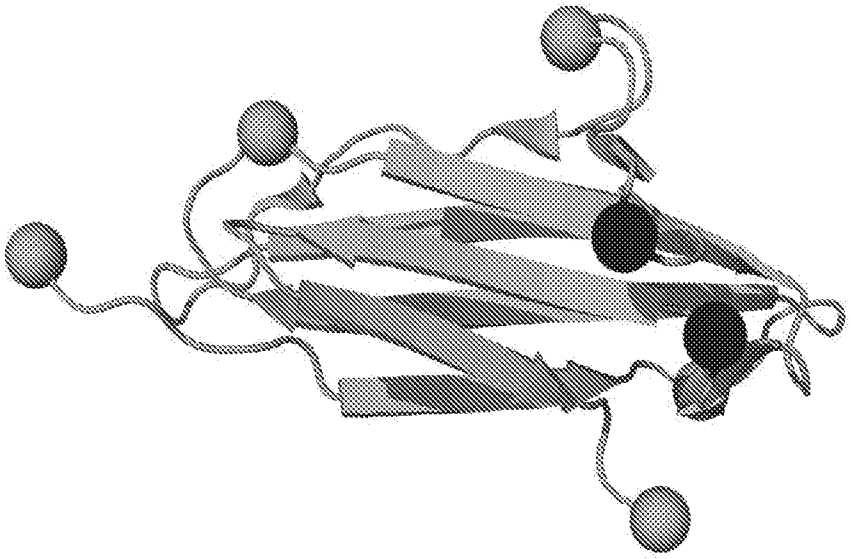
FIG. 2

MICA/B a3 domain alignment, % identity, % similarity



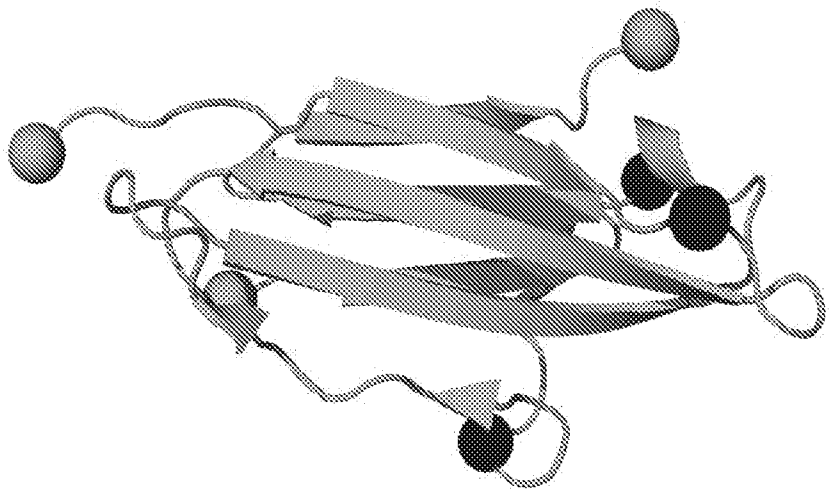
Identity Percentage				
	MICA002.hu	MICA004.hu	MICA008.hu	MICB005.hu
MICA002.hu	100.0	95.74	94.68	91.48
MICA004.hu		100.0	96.8	93.61
MICA008.hu			100.0	92.55
MICB005.hu				100.0
Similarity Percentage				
	MICA002.hu	MICA004.hu	MICA008.hu	MICB005.hu
MICA002.hu	100.0	97.05	96.43	91.38
MICA004.hu		100.0	97.26	92.01
MICA008.hu			100.0	91.59
MICB005.hu				100.0

FIG. 3B



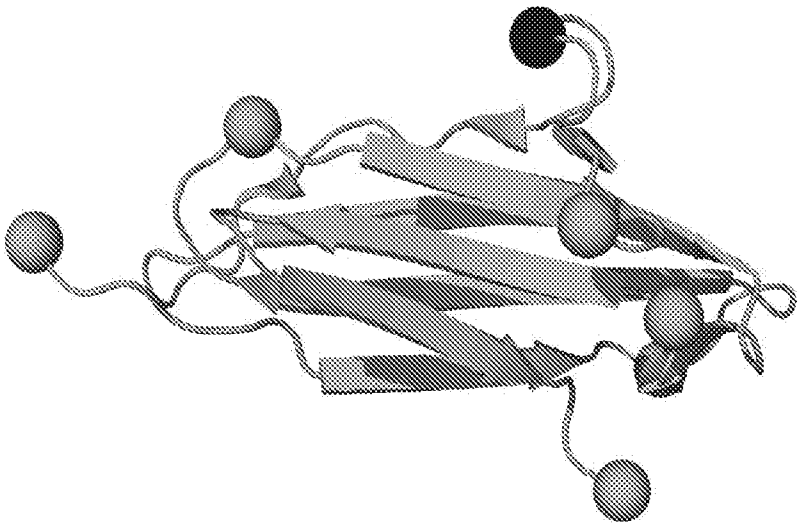
Bin 2 on MICA

FIG. 3A



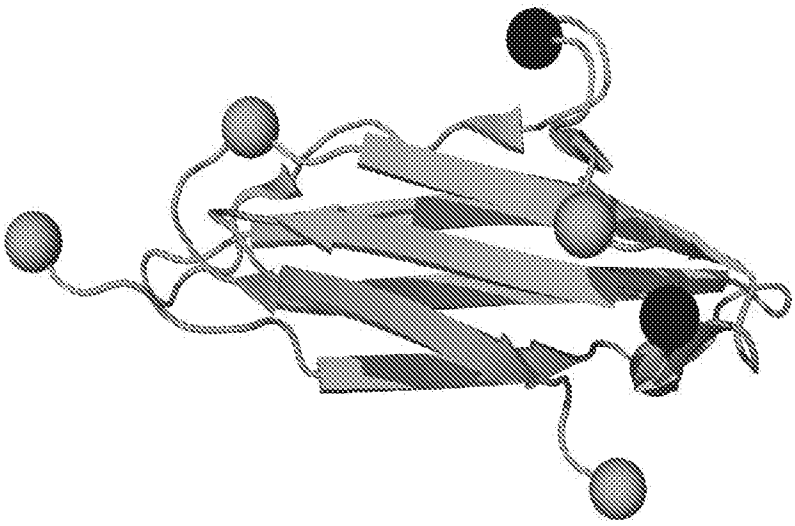
Bin 1 on MICA

FIG. 3D



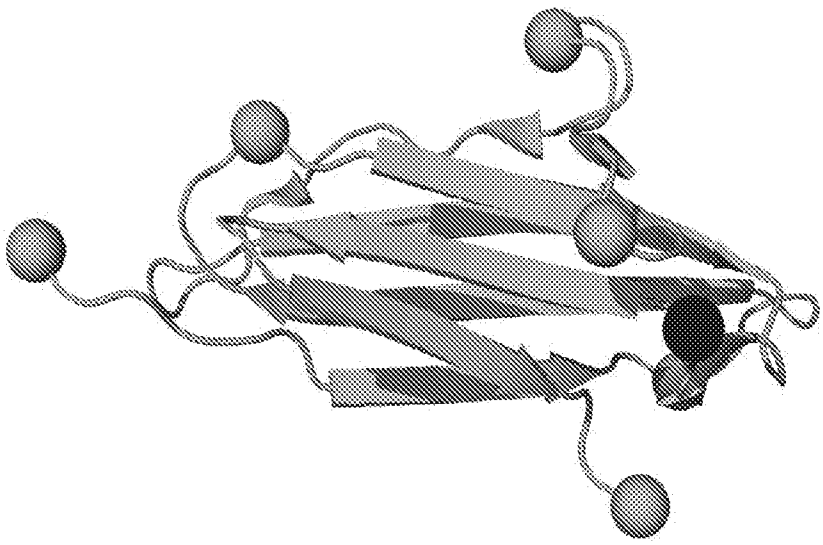
Bin 4 on MICA

FIG. 3C



Bin 3 on MICA

FIG. 3E



Bin 5 on MICA

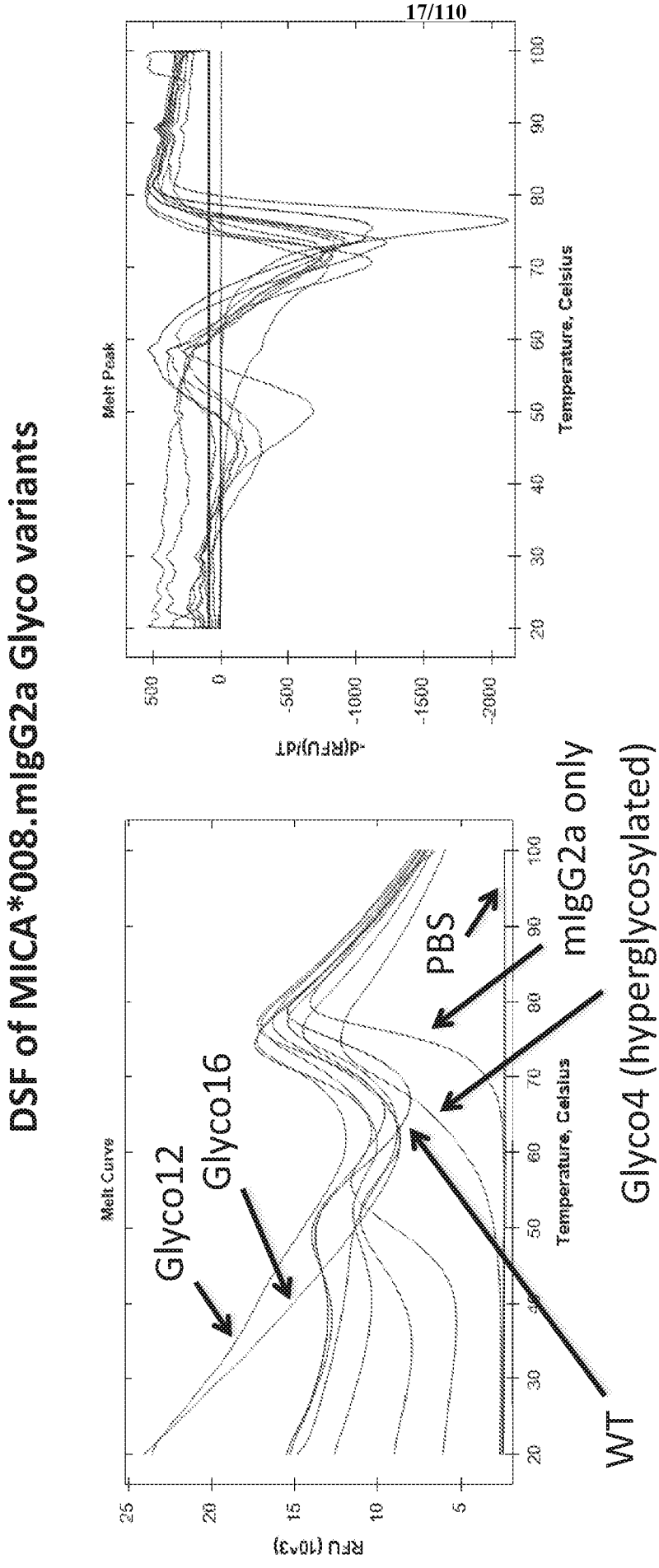

DSF of MICA*008.mIgG2a Glyco variants
Melt Curve
RFU (10^3)
Temperature, Celsius
Glyco12
Glyco16
PBS
mIgG2a only
Glyco4 (hyperglycosylated)
WT
Melt Peak
-d(RFU)/dT
Temperature, Celsius

FIG. 4A

FIG. 4B

FIG. 5

T_m values from DSF for Glyco variants of MICA*008.mIgG2a

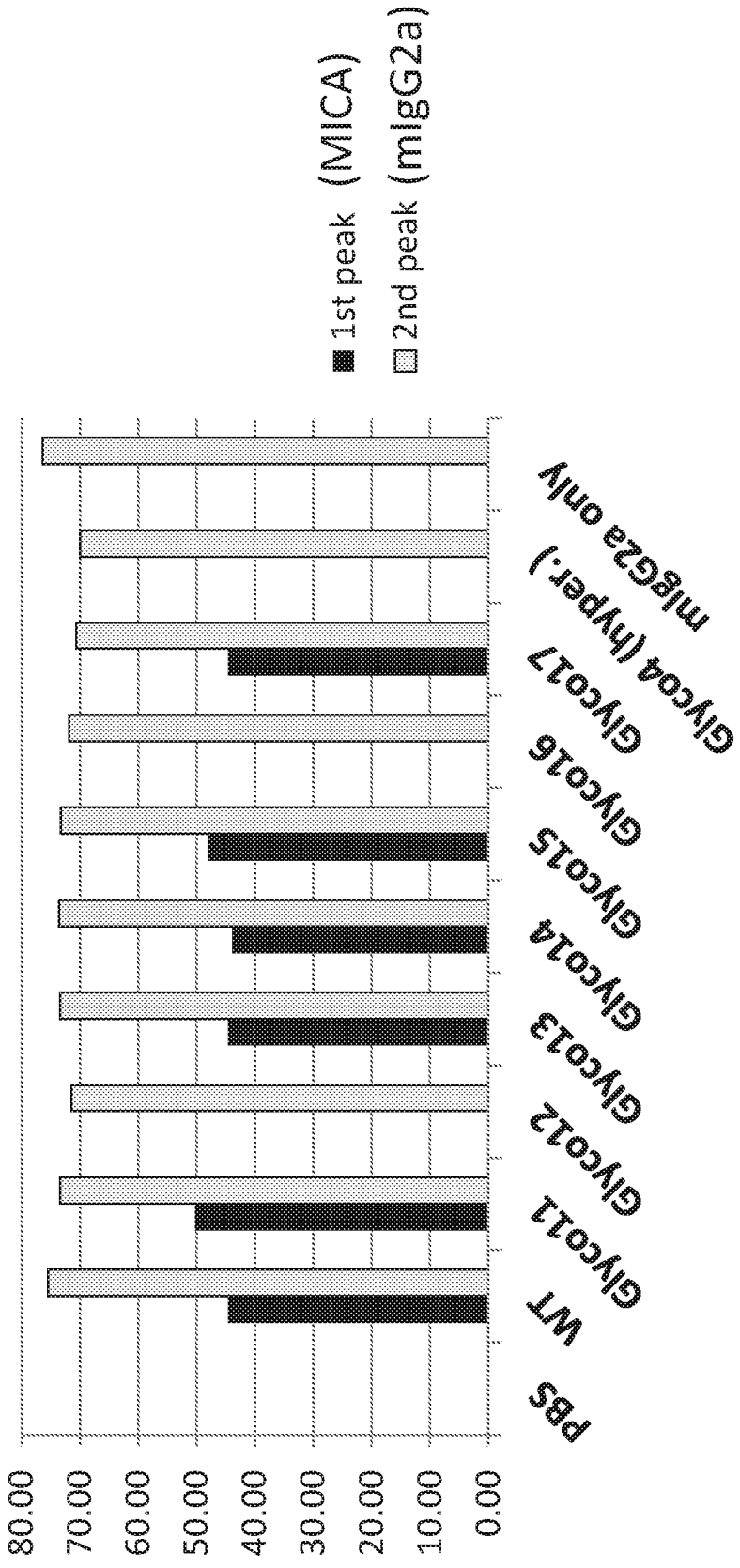
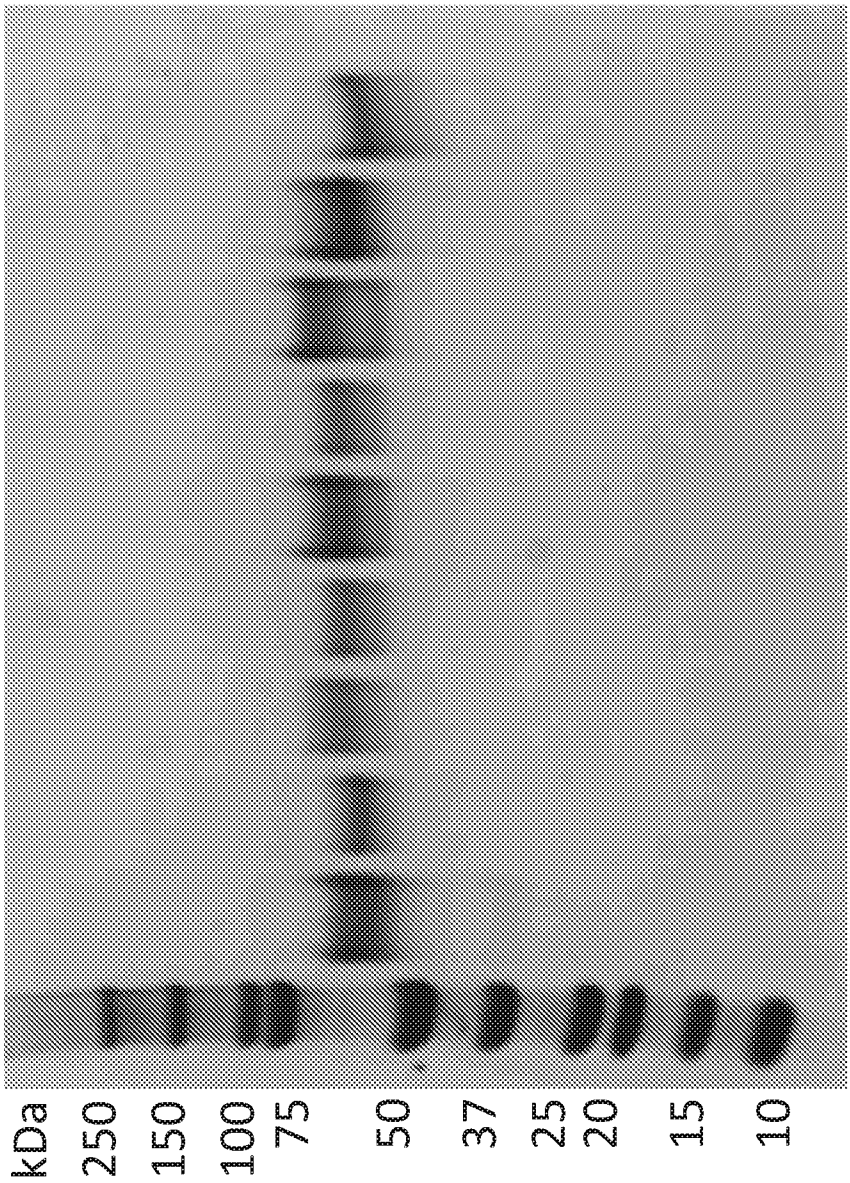


FIG. 6 Gel of Glyco variants



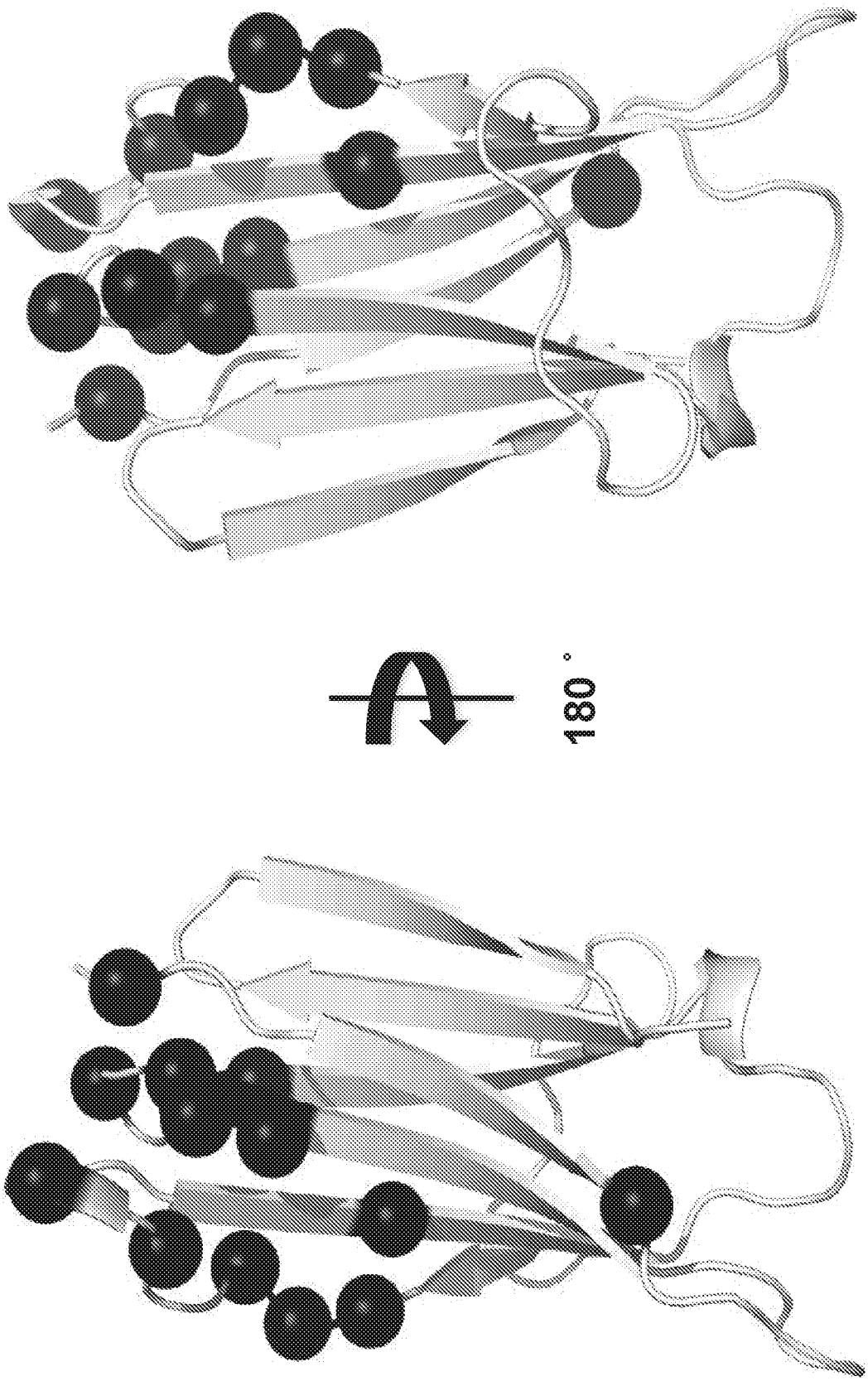


FIG. 7A: MICA *008 residues grafted on MILL

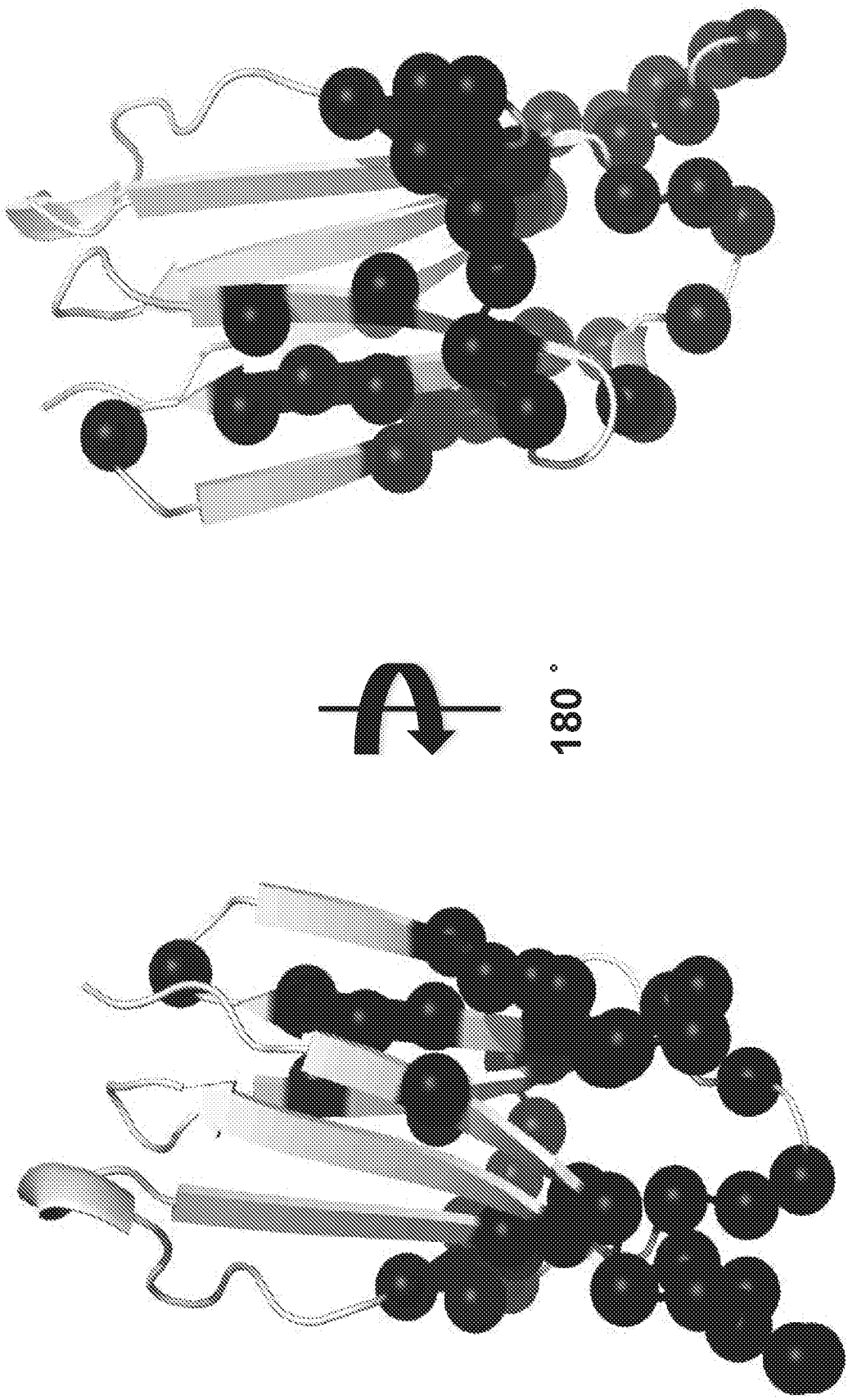
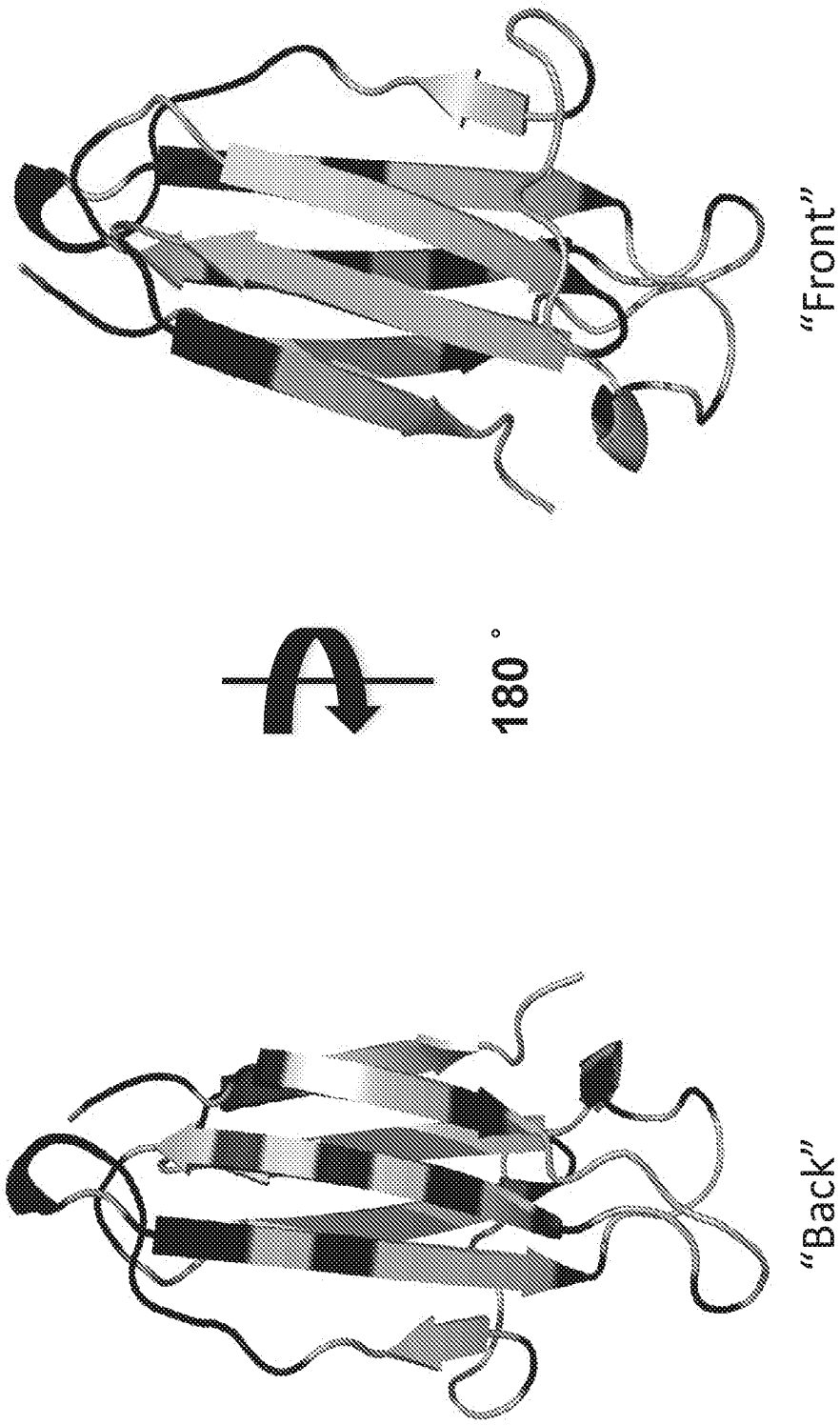


FIG. 7B: Non-MICA *008 residues in MILL chimera

FIG. 8



6E1.1.12 (Final Bin 6) and 2E5.2.3 (Final Bin 7)
on MILL1-MICA chimera

FIG. 9

	6F8	7D4	2E5.2.3	1D5	% monomer
WT	1	1	1	1	91.57
I236A	1.4	1.2	0.9	0.01	92.49
T238A	0.1	0.1	1.3	0.4	96
R240A	4.5	1499	1	15500	93.8
Q241A	1.1	0.4	1.1	9830	97.6
V244A	5.5	8.6	1	10400	97.6
S245A	1.2	1.6	1	13	93.96
S247A	0.9	0.8	1.1	0.2	94.87
H248A	0.6	0.6	1	16	92.05
D249A	0.8	0.7	1	3.5	86.64
T250A	0.5	0.6	1.2	8.8	91.61
R274A	1	0.9	1.1	3	94
G275A	4.6	3.1	1	0.03	98.9
E276A	4.3	0.7	1.2	22	86.14
E277A	2.7	1.3	1.1	1.9	81.58
Q278A	0.5	4.9	1	0.7	92.32
R279A	4.5	5.1	0.8	3950	95.2
Y283A	1.7	3.2	0.2	6260	92.7
E285A	3.9	7.9	0.9	1060	83.78
S287A	1.1	1.3	1	4.9	90.34
G288A	1	0.8	0.6	1	95.3
N289A	1.2	3.5	1.1	0.2	89.74
H290A	8.4	8.8	0.8	14400	90.37
S291A	2.7	2.7	1.1	1.1	83.21
T292A	1.7	2.1	1.3	3040	80.56
P294A	0.9	1	1	1	89.16
P296A	1	0.7	0.6	1.9	92.3
S297A	1	1.1	1.2	0.05	89.24

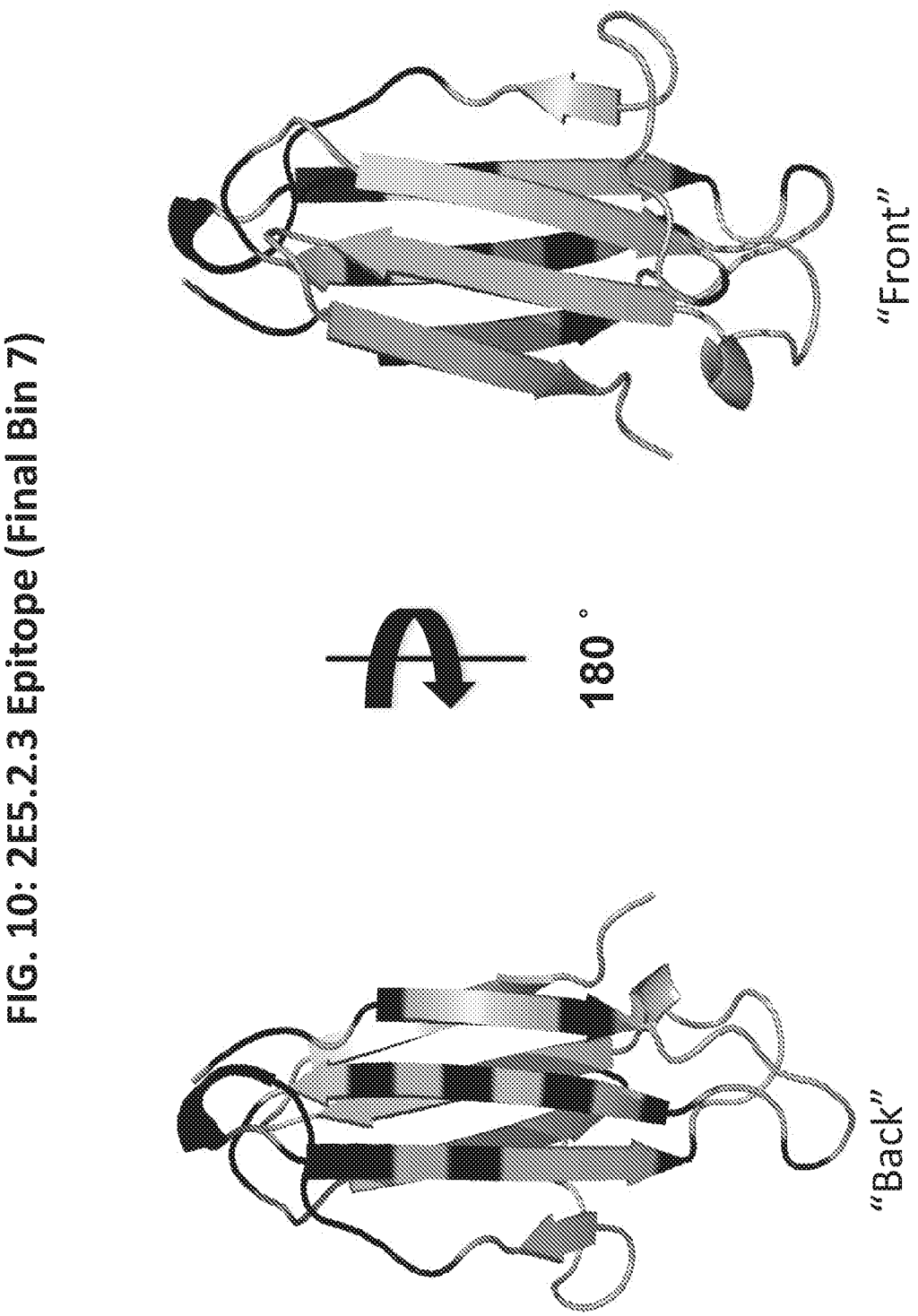
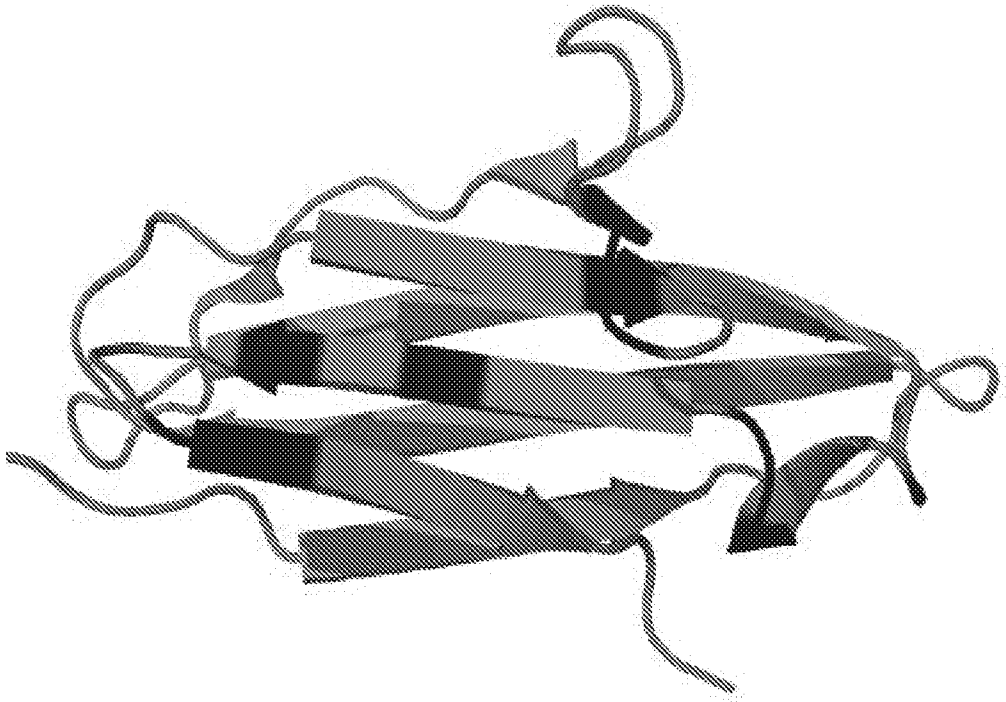


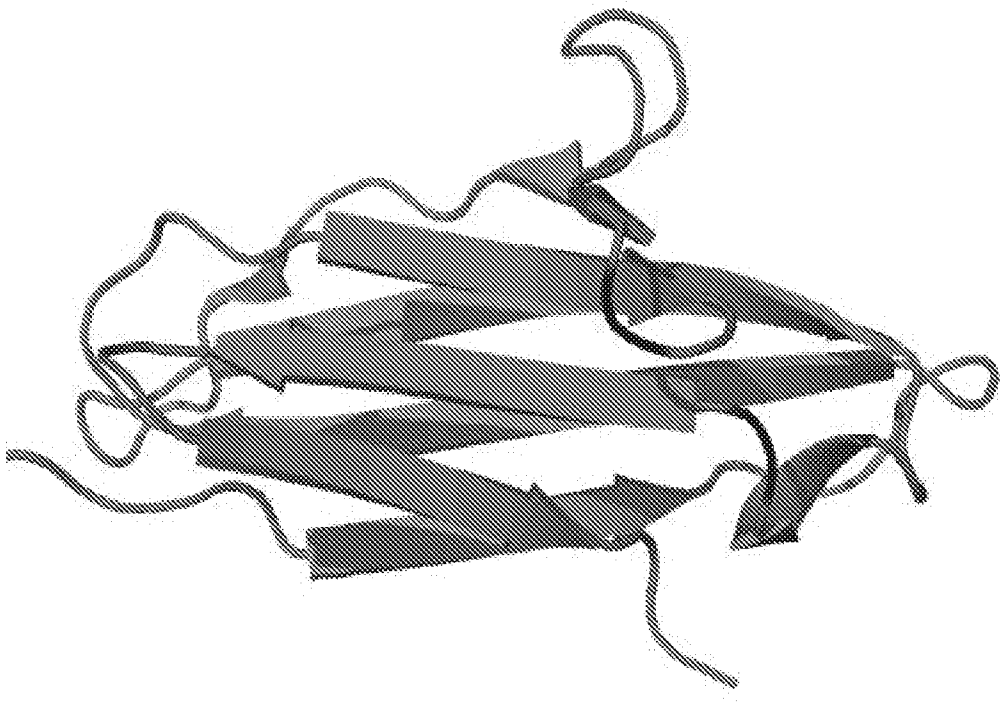
FIG. 10: 2E5.2.3 Epitope (Final Bin 7)

FIG. 11A: 6F8 Epitope

FIG. 11B: 7D4 Epitope



Epitope by Ala scan



Epitope by Glyco mapping

FIG. 12

Overview of Sequence Identifiers (SEQ ID NOs:) for Anti-MICA Antibody Sequences

Ab	HVR-H1	HVR-H2	HVR-H3	HVR-L1	HVR-L2	HVR-L3	FR-L1	FR-L2	FR-L3	FR-L4	FR-H1	FR-H2	FR-H3	FR-H4	VH	VL
3C9.10	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
7D4.6	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
6F8.7	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
32D2	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
3E11	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
9C9.5.6	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
1E6.1.3	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112
7A3.1.9	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128
6E12.5	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144
20G11	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160
6E1.1.12	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176
2E5.2.3	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192

FIG. 13A

Inhibition

HCC1534 (MICA*004)								
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
13A9 ug/mL \/								
10	52%	53%	55%	55%	57%	57%	59%	58%
2.5	54%	57%	57%	53%	50%	53%	51%	47%
0.625	60%	58%	59%	57%	52%	42%	38%	34%
0.15625	58%	62%	62%	59%	49%	36%	31%	14%
0.0390625	60%	60%	62%	59%	50%	30%	15%	20%
0.009765625	58%	66%	64%	63%	50%	32%	12%	14%
0.002441406	58%	64%	61%	61%	50%	34%	16%	7%
None	58%	61%	62%	63%	50%	36%	15%	-5%
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	72%	72%	75%	72%	68%	62%	63%	50%
2.5	69%	72%	73%	68%	64%	57%	54%	43%
0.625	66%	70%	69%	64%	59%	52%	40%	38%
0.15625	63%	65%	68%	66%	50%	45%	29%	25%
0.0390625	61%	61%	63%	61%	47%	25%	19%	10%
0.009765625	63%	61%	60%	56%	42%	23%	9%	4%
0.002441406	58%	59%	58%	55%	40%	19%	-4%	-9%
None	58%	56%	56%	48%	40%	13%	-5%	-17%
13A9 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	66%	61%	59%	57%	55%	53%	48%	50%
2.5	62%	60%	58%	53%	52%	50%	52%	52%
0.625	61%	58%	51%	54%	50%	41%	42%	39%
0.15625	62%	53%	46%	36%	37%	29%	31%	23%
0.0390625	54%	51%	43%	35%	31%	22%	20%	15%
0.009765625	57%	52%	40%	32%	24%	15%	12%	11%
0.002441406	66%	54%	38%	30%	20%	17%	15%	-1%
None	52%	47%	27%	26%	21%	14%	6%	-4%

FIG. 13B

Inhibition

MEL-JUSO (MICA*008)								
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
13A9 ug/mL \/								
10	65%	66%	62%	55%	51%	45%	44%	38%
2.5	70%	70%	70%	66%	55%	48%	46%	39%
0.625	69%	75%	73%	73%	62%	45%	40%	27%
0.15625	72%	76%	73%	70%	60%	39%	28%	19%
0.0390625	70%	74%	79%	75%	67%	41%	24%	10%
0.009765625	72%	75%	78%	74%	65%	45%	28%	11%
0.002441406	72%	78%	79%	75%	65%	45%	26%	8%
None	72%	77%	76%	76%	71%	48%	28%	11%
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	81%	82%	84%	82%	75%	64%	57%	49%
2.5	79%	81%	82%	79%	71%	59%	52%	44%
0.625	76%	79%	80%	77%	68%	52%	45%	36%
0.15625	76%	78%	76%	75%	61%	42%	31%	22%
0.0390625	70%	70%	77%	72%	57%	36%	19%	8%
0.009765625	72%	74%	74%	71%	54%	30%	12%	6%
0.002441406	72%	73%	73%	70%	52%	25%	6%	-6%
None	70%	72%	68%	64%	44%	17%	-1%	-21%
13A9 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	40%	29%	27%	32%	31%	32%	32%	29%
2.5	37%	36%	31%	37%	38%	36%	40%	35%
0.625	40%	41%	34%	33%	28%	31%	30%	29%
0.15625	39%	39%	29%	22%	17%	23%	24%	24%
0.0390625	37%	42%	27%	24%	13%	9%	14%	15%
0.009765625	40%	36%	26%	11%	12%	16%	15%	13%
0.002441406	41%	38%	28%	14%	13%	19%	12%	10%
None	35%	38%	26%	17%	16%	19%	15%	16%

FIG. 13C

Inhibition

HCC1534 (MICB*005)								
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
13A9 ug/mL \/								
10	53%	58%	68%	73%	73%	74%	75%	75%
2.5	47%	57%	63%	69%	70%	74%	76%	77%
0.625	53%	53%	58%	61%	65%	68%	70%	68%
0.15625	52%	53%	52%	50%	51%	53%	56%	54%
0.0390625	52%	53%	52%	46%	40%	33%	39%	39%
0.009765625	52%	53%	55%	48%	38%	31%	31%	27%
0.002441406	52%	57%	53%	46%	38%	32%	25%	13%
None	48%	51%	51%	48%	40%	29%	15%	7%

1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	78%	77%	78%	76%	71%	67%	63%	57%
2.5	76%	77%	78%	73%	70%	64%	60%	52%
0.625	72%	75%	74%	71%	65%	60%	54%	46%
0.15625	68%	69%	71%	67%	57%	48%	43%	35%
0.0390625	60%	60%	60%	58%	39%	33%	26%	15%
0.009765625	56%	57%	54%	49%	34%	21%	11%	-1%
0.002441406	56%	51%	52%	48%	36%	17%	-3%	-11%
None	53%	51%	52%	43%	25%	7%	-9%	-15%

13A9 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	78%	70%	68%	65%	61%	51%	43%	42%
2.5	75%	75%	71%	65%	64%	58%	50%	48%
0.625	78%	76%	71%	68%	63%	58%	51%	42%
0.15625	77%	75%	69%	59%	53%	41%	40%	32%
0.0390625	73%	75%	68%	56%	44%	34%	25%	23%
0.009765625	74%	75%	67%	53%	41%	29%	31%	10%
0.002441406	75%	74%	68%	56%	39%	25%	19%	2%
None	72%	74%	65%	54%	39%	27%	9%	2%

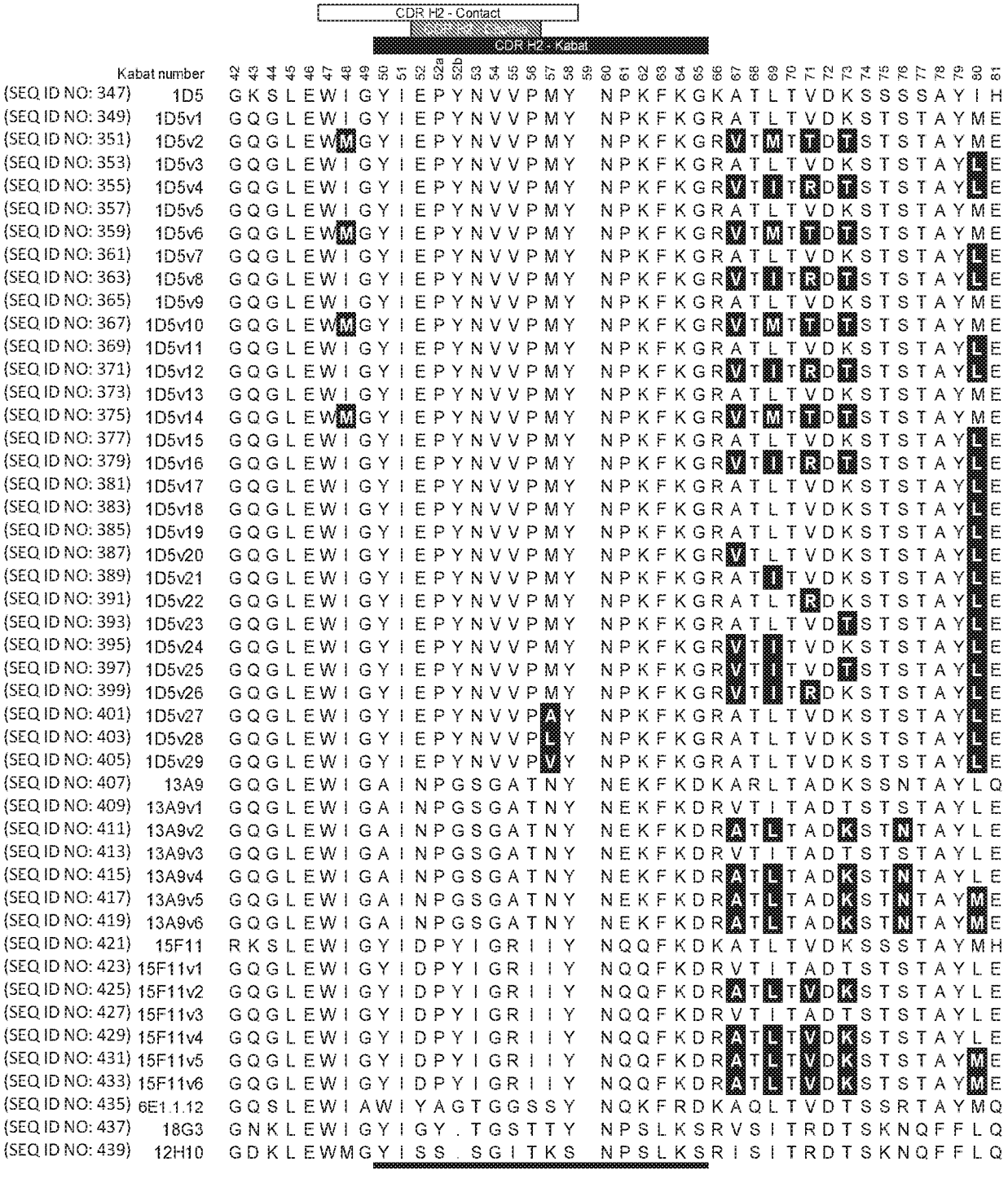
FIG. 14A

CDR sequences according to Kabat definition are underlined

Heavy chain variable region

	Kabat number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	35a	36	37	38	39	40	41
																														CDR H1 - Contact													
																																CDR H1 - Kabat									
(SEQ ID NO: 347)	1D5	E	I	Q	L	Q	Q	S	G	P	E	L	V	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	K	Q	S	H
(SEQ ID NO: 349)	1D5v1	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 351)	1D5v2	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 353)	1D5v3	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 355)	1D5v4	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 357)	1D5v5	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 359)	1D5v6	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 361)	1D5v7	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 363)	1D5v8	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 365)	1D5v9	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 367)	1D5v10	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 369)	1D5v11	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 371)	1D5v12	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 373)	1D5v13	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 375)	1D5v14	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 377)	1D5v15	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 379)	1D5v16	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 381)	1D5v17	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 383)	1D5v18	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 385)	1D5v19	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 387)	1D5v20	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 389)	1D5v21	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 391)	1D5v22	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 393)	1D5v23	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 395)	1D5v24	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 397)	1D5v25	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 399)	1D5v26	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 401)	1D5v27	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 403)	1D5v28	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 405)	1D5v29	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	Q	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 407)	13A9	Q	V	Q	L	Q	Q	S	G	A	E	L	V	R	P	G	T	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	N	Y	L	I	E	.	W	V	K	Q	R	P
(SEQ ID NO: 409)	13A9v1	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	N	Y	L	I	E	.	W	V	R	Q	A	P
(SEQ ID NO: 411)	13A9v2	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	N	Y	L	I	E	.	W	V	R	Q	A	P
(SEQ ID NO: 413)	13A9v3	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	N	Y	L	I	E	.	W	V	R	Q	A	P
(SEQ ID NO: 415)	13A9v4	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	N	Y	L	I	E	.	W	V	R	Q	A	P
(SEQ ID NO: 417)	13A9v5	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	N	Y	L	I	E	.	W	V	R	Q	A	P
(SEQ ID NO: 419)	13A9v6	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	N	Y	L	I	E	.	W	V	R	Q	A	P
(SEQ ID NO: 421)	15F11	E	I	Q	L	Q	Q	S	G	P	E	L	V	K	P	G	A	S	V	R	V	S	C	K	P	S	G	Y	A	F	T	S	N	N	I	Y	.	W	V	K	Q	S	R
(SEQ ID NO: 423)	15F11v1	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	N	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 425)	15F11v2	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	P	S	G	Y	A	F	T	S	N	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 427)	15F11v3	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	A	F	T	S	N	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 429)	15F11v4	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	P	S	G	Y	A	F	T	S	N	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 431)	15F11v5	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V	S	C	K	P	S	G	Y	A	F	T	S	N	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 433)	15F11v6	E	I	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V	S	C	K	P	S	G	Y	A	F	T	S	N	N	I	Y	.	W	V	R	Q	A	P
(SEQ ID NO: 435)	6E1.1.12	Q	G	Q	M	Q	Q	S	G	A	E	L	V	K	P	G	A	S	V	K	L	S	C	K	T	S	G	F	T	F	S	D	N	Y	I	S	.	W	L	K	Q	K	P
(SEQ ID NO: 437)	18G3		V	Q	L	Q	E	S	G	P	G	L	V	K	P	S	Q	S	L	S	L	T	C	N	V	T	G	Y	S	I	T	G	D	Y	A	W	N	W	I	R	Q	F	P
(SEQ ID NO: 439)	12H10	D	V	Q	L	Q	E	S	G	P	G	L	V	K	P	S	Q	P	L	S	L	T	C	T	V	T	G	Y	S	I	T	S	N	Y	A	W	N	W	I	R	Q	F	P

FIG. 14B


CDR H2 - Contact
CDR H2 - Kabat
Kabat number
42 43 44 45 46 47 48 49 50 51 52 52a 52b 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81
(SEQ ID NO: 347) 1D5 G K S L E W I G Y I E P Y N V V P M Y N P K F K G K A T L T V D K S S S S S A Y I H
(SEQ ID NO: 349) 1D5v1 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y M E
(SEQ ID NO: 351) 1D5v2 G Q G L E W M G Y I E P Y N V V P M Y N P K F K G R V T M T T D T S T S T A Y M E
(SEQ ID NO: 353) 1D5v3 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y L E
(SEQ ID NO: 355) 1D5v4 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R V T I T R D T S T S T A Y L E
(SEQ ID NO: 357) 1D5v5 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y M E
(SEQ ID NO: 359) 1D5v6 G Q G L E W M G Y I E P Y N V V P M Y N P K F K G R V T M T T D T S T S T A Y M E
(SEQ ID NO: 361) 1D5v7 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y L E
(SEQ ID NO: 363) 1D5v8 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R V T I T R D T S T S T A Y L E
(SEQ ID NO: 365) 1D5v9 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y M E
(SEQ ID NO: 367) 1D5v10 G Q G L E W M G Y I E P Y N V V P M Y N P K F K G R V T M T T D T S T S T A Y M E
(SEQ ID NO: 369) 1D5v11 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y L E
(SEQ ID NO: 371) 1D5v12 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R V T I T R D T S T S T A Y L E
(SEQ ID NO: 373) 1D5v13 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y M E
(SEQ ID NO: 375) 1D5v14 G Q G L E W M G Y I E P Y N V V P M Y N P K F K G R V T M T T D T S T S T A Y M E
(SEQ ID NO: 377) 1D5v15 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y L E
(SEQ ID NO: 379) 1D5v16 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R V T I T R D T S T S T A Y L E
(SEQ ID NO: 381) 1D5v17 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y L E
(SEQ ID NO: 383) 1D5v18 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y L E
(SEQ ID NO: 385) 1D5v19 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D K S T S T A Y L E
(SEQ ID NO: 387) 1D5v20 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R V T L T V D K S T S T A Y L E
(SEQ ID NO: 389) 1D5v21 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T I T V D K S T S T A Y L E
(SEQ ID NO: 391) 1D5v22 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T R D K S T S T A Y L E
(SEQ ID NO: 393) 1D5v23 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R A T L T V D T S T S T A Y L E
(SEQ ID NO: 395) 1D5v24 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R V T I T V D K S T S T A Y L E
(SEQ ID NO: 397) 1D5v25 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R V T I T V D T S T S T A Y L E
(SEQ ID NO: 399) 1D5v26 G Q G L E W I G Y I E P Y N V V P M Y N P K F K G R V T I T R D K S T S T A Y L E
(SEQ ID NO: 401) 1D5v27 G Q G L E W I G Y I E P Y N V V P A Y N P K F K G R A T L T V D K S T S T A Y L E
(SEQ ID NO: 403) 1D5v28 G Q G L E W I G Y I E P Y N V V P L Y N P K F K G R A T L T V D K S T S T A Y L E
(SEQ ID NO: 405) 1D5v29 G Q G L E W I G Y I E P Y N V V P V Y N P K F K G R A T L T V D K S T S T A Y L E
(SEQ ID NO: 407) 13A9 G Q G L E W I G A I N P G S G A T N Y N E K F K D K A R L T A D K S S N T A Y L Q
(SEQ ID NO: 409) 13A9v1 G Q G L E W I G A I N P G S G A T N Y N E K F K D R V T I T A D T S T S T A Y L E
(SEQ ID NO: 411) 13A9v2 G Q G L E W I G A I N P G S G A T N Y N E K F K D R A T L T A D K S T N T A Y L E
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(SEQ ID NO: 427) 15F11v3 G Q G L E W I G Y I D P Y I G R I I Y N Q Q F K D R V T I T A D T S T S T A Y L E
(SEQ ID NO: 429) 15F11v4 G Q G L E W I G Y I D P Y I G R I I Y N Q Q F K D R A T L T V D K S T S T A Y L E
(SEQ ID NO: 431) 15F11v5 G Q G L E W I G Y I D P Y I G R I I Y N Q Q F K D R A T L T V D K S T S T A Y M E
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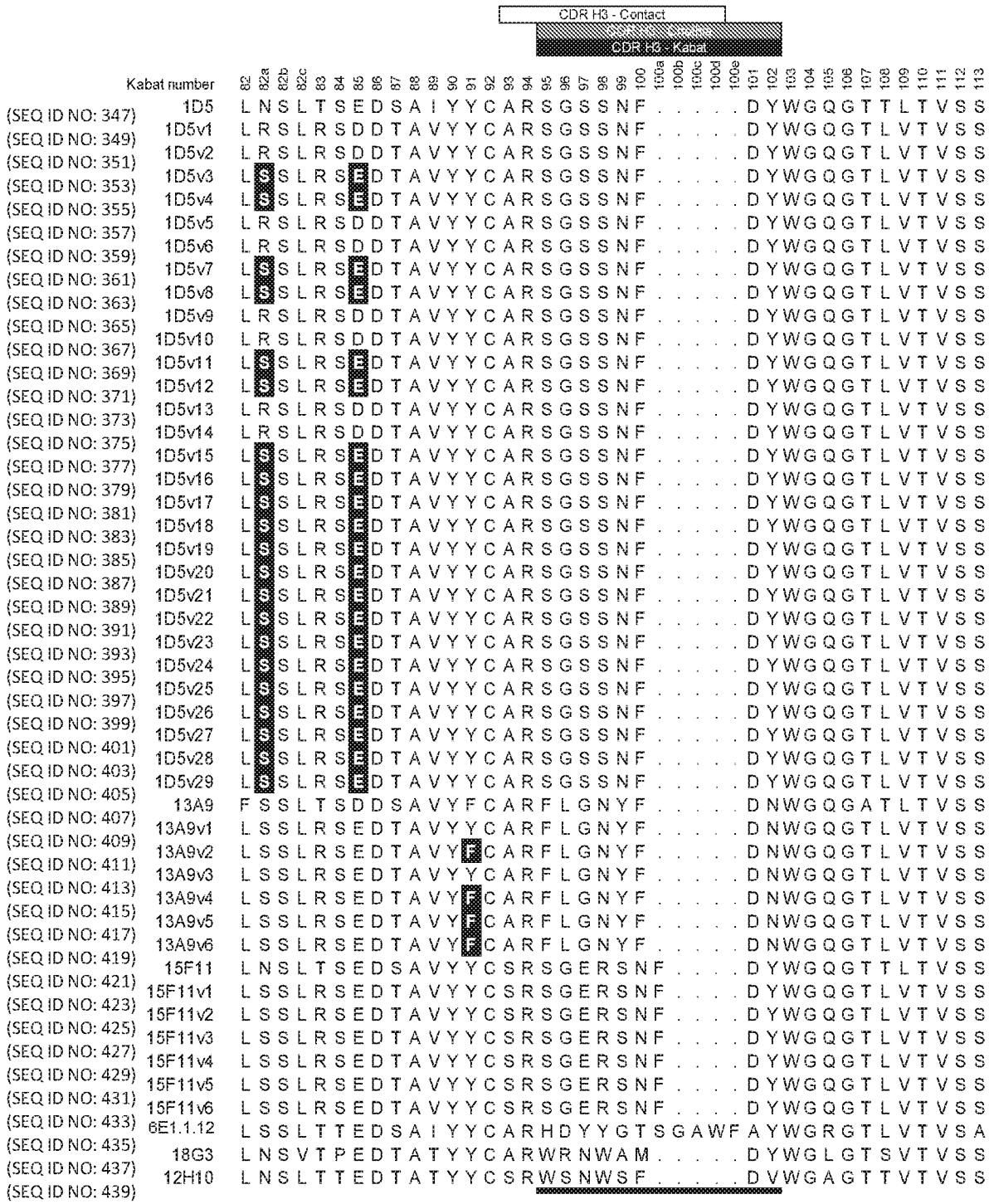
FIG. 14C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(SEQ ID NO: 371) 1D5v12 L S S L R S E D T A V Y Y C A R S G S S N F . . . . . D Y W G Q G T L V T V S S
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(SEQ ID NO: 407) 13A9 F S S L T S D D S A V Y F C A R F L G N Y F . . . . . D N W G Q G A T L T V S S
(SEQ ID NO: 409) 13A9v1 L S S L R S E D T A V Y Y C A R F L G N Y F . . . . . D N W G Q G T L V T V S S
(SEQ ID NO: 411) 13A9v2 L S S L R S E D T A V Y F C A R F L G N Y F . . . . . D N W G Q G T L V T V S S
(SEQ ID NO: 413) 13A9v3 L S S L R S E D T A V Y Y C A R F L G N Y F . . . . . D N W G Q G T L V T V S S
(SEQ ID NO: 415) 13A9v4 L S S L R S E D T A V Y F C A R F L G N Y F . . . . . D N W G Q G T L V T V S S
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(SEQ ID NO: 439) 12H10 L N S L T T E D T A T Y Y C S R W S N W S F . . . . . D V W G A G T T V T V S S

FIG. 14D

CDR sequences according to Kabat definition are underlined

Light chain variable region

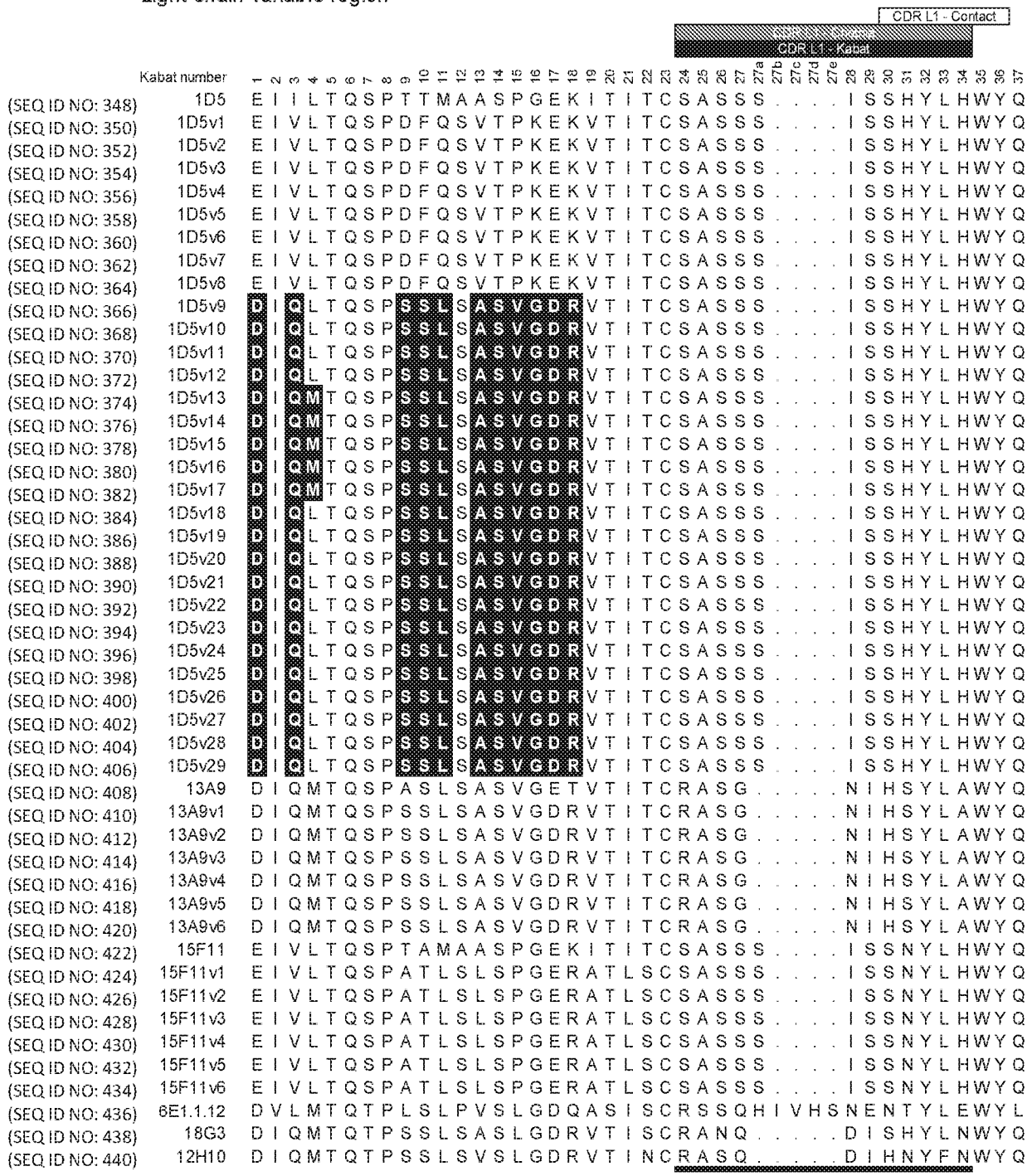
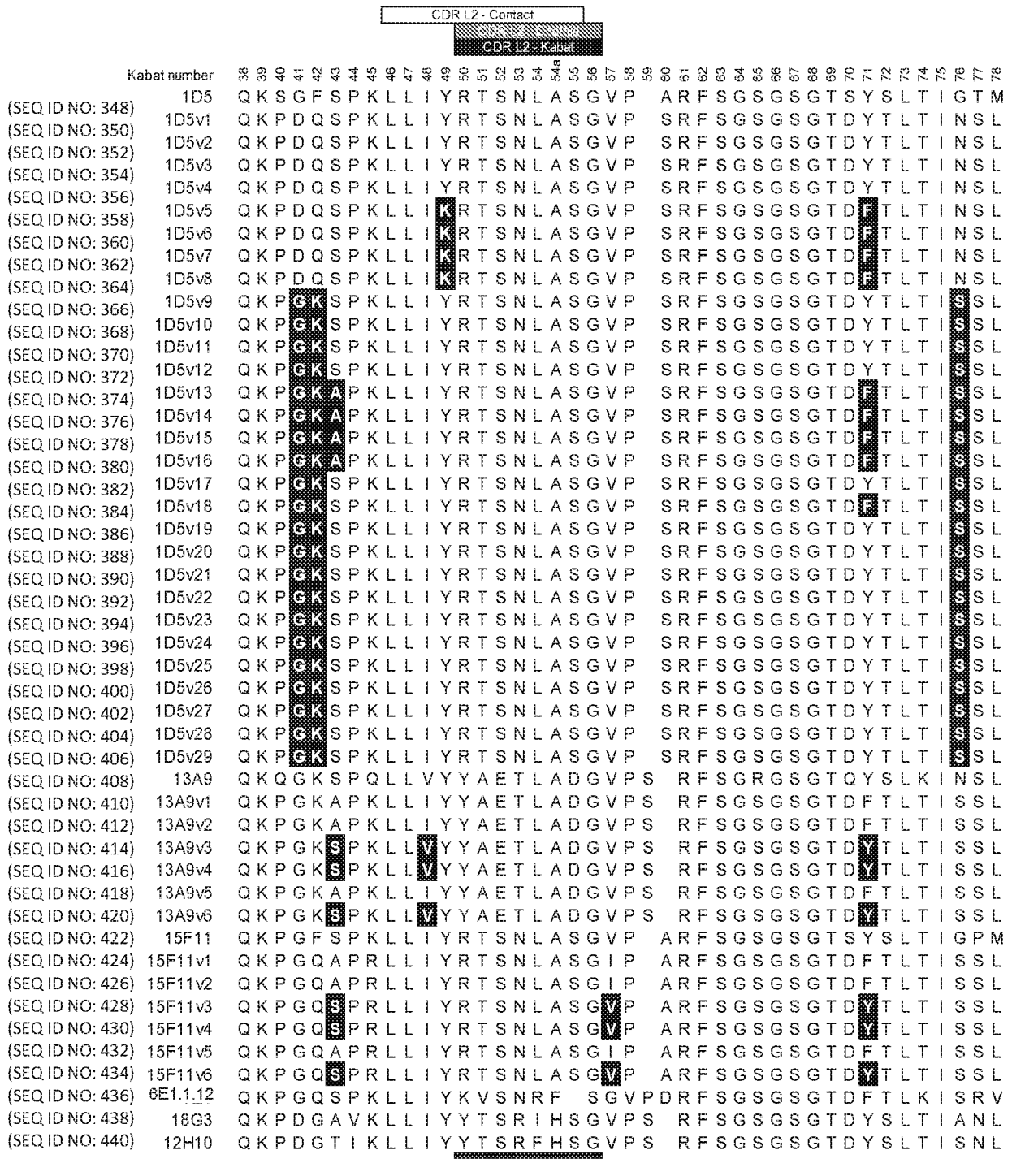


FIG. 14E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(SEQ ID NO: 352) 1D5v2 Q K P D Q S P K L L I Y R T S N L A S G V P S R F S G S G S G S G T D Y T L T I N S L
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(SEQ ID NO: 360) 1D5v6 Q K P D Q S P K L L I K R T S N L A S G V P S R F S G S G S G S G T D F T L T I N S L
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(SEQ ID NO: 364) 1D5v8 Q K P D Q S P K L L I K R T S N L A S G V P S R F S G S G S G S G T D F T L T I N S L
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(SEQ ID NO: 374) 1D5v13 Q K P G K A P K L L I Y R T S N L A S G V P S R F S G S G S G S G T D F T L T I S S L
(SEQ ID NO: 376) 1D5v14 Q K P G K A P K L L I Y R T S N L A S G V P S R F S G S G S G S G T D F T L T I S S L
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(SEQ ID NO: 386) 1D5v19 Q K P G K S P K L L I Y R T S N L A S G V P S R F S G S G S G S G T D Y T L T I S S L
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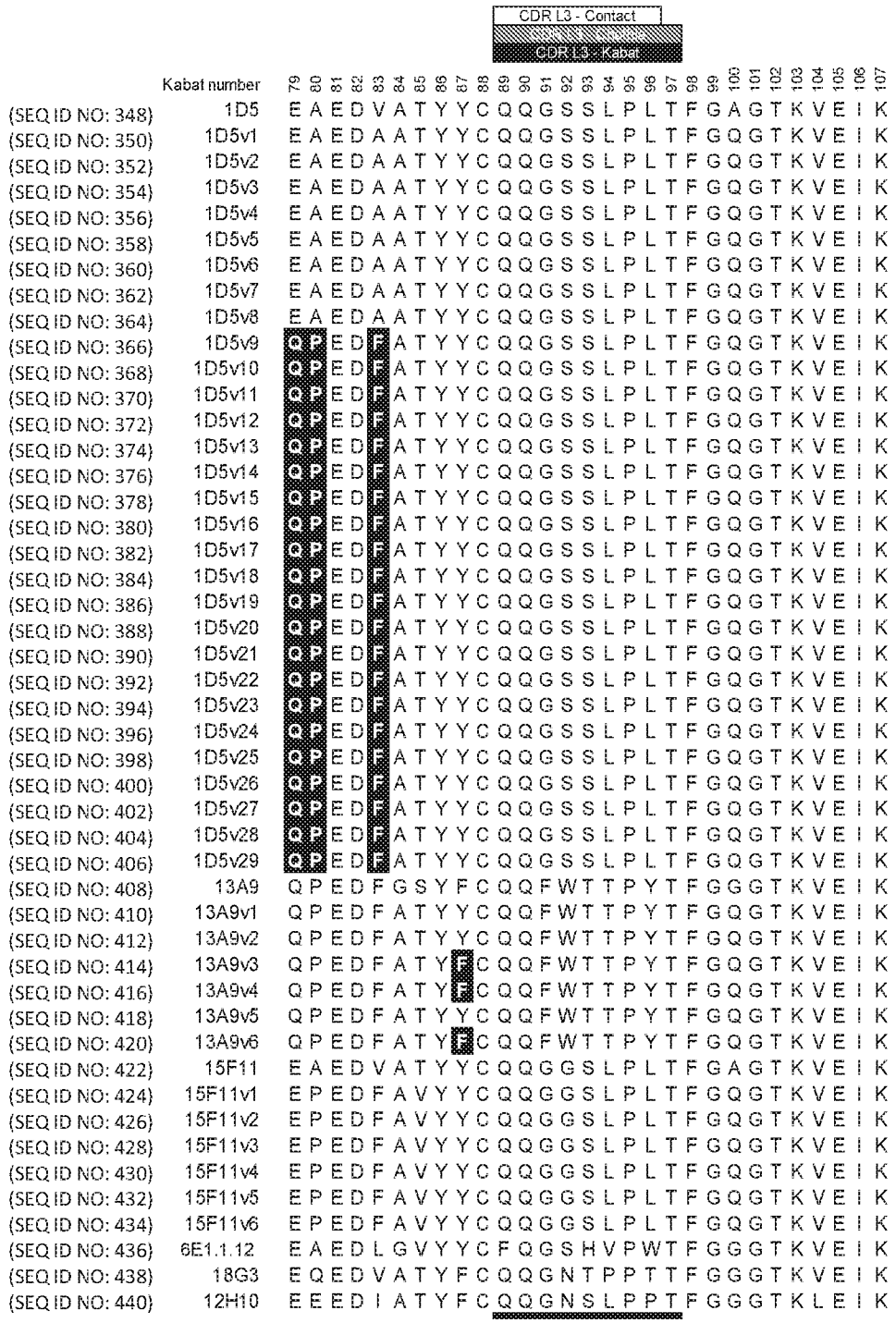
FIG. 14F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(SEQ ID NO: 404) 1D5v28 Q P E D F A T Y Y C Q Q G S S L P L T F G Q G T K V E I K
(SEQ ID NO: 406) 1D5v29 Q P E D F A T Y Y C Q Q G S S L P L T F G Q G T K V E I K
(SEQ ID NO: 408) 13A9 Q P E D F G S Y F C Q Q F W T T P Y T F G G G T K V E I K
(SEQ ID NO: 410) 13A9v1 Q P E D F A T Y Y C Q Q F W T T P Y T F G Q G T K V E I K
(SEQ ID NO: 412) 13A9v2 Q P E D F A T Y Y C Q Q F W T T P Y T F G Q G T K V E I K
(SEQ ID NO: 414) 13A9v3 Q P E D F A T Y E C Q Q F W T T P Y T F G Q G T K V E I K
(SEQ ID NO: 416) 13A9v4 Q P E D F A T Y E C Q Q F W T T P Y T F G Q G T K V E I K
(SEQ ID NO: 418) 13A9v5 Q P E D F A T Y Y C Q Q F W T T P Y T F G Q G T K V E I K
(SEQ ID NO: 420) 13A9v6 Q P E D F A T Y E C Q Q F W T T P Y T F G Q G T K V E I K
(SEQ ID NO: 422) 15F11 E A E D V A T Y Y C Q Q G G S L P L T F G A G T K V E I K
(SEQ ID NO: 424) 15F11v1 E P E D F A V Y Y C Q Q G G S L P L T F G Q G T K V E I K
(SEQ ID NO: 426) 15F11v2 E P E D F A V Y Y C Q Q G G S L P L T F G Q G T K V E I K
(SEQ ID NO: 428) 15F11v3 E P E D F A V Y Y C Q Q G G S L P L T F G Q G T K V E I K
(SEQ ID NO: 430) 15F11v4 E P E D F A V Y Y C Q Q G G S L P L T F G Q G T K V E I K
(SEQ ID NO: 432) 15F11v5 E P E D F A V Y Y C Q Q G G S L P L T F G Q G T K V E I K
(SEQ ID NO: 434) 15F11v6 E P E D F A V Y Y C Q Q G G S L P L T F G Q G T K V E I K
(SEQ ID NO: 436) 6E1.1.12 E A E D L G V Y Y C F Q G S H V P W T F G G G T K V E I K
(SEQ ID NO: 438) 18G3 E Q E D V A T Y F C Q Q G N T P P T T F G G G T K V E I K
(SEQ ID NO: 440) 12H10 E E E D I A T Y F C Q Q G N S L P P T F G G G T K L E I K

FIG. 15A

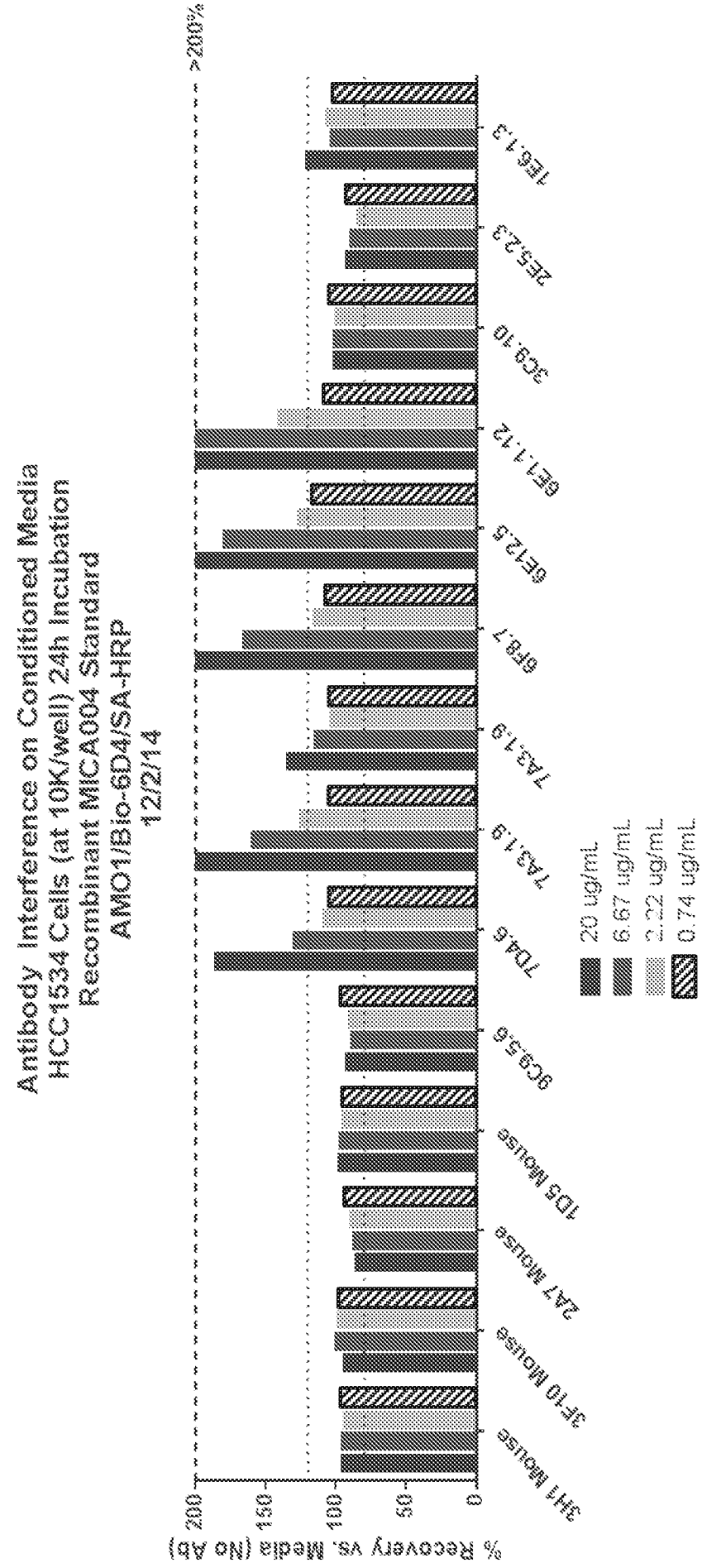

Antibody Interference on Conditioned Media
HCC1534 Cells (at 10K/well) 24h Incubation
Recombinant MICA004 Standard
AMO1/Bio-6D4/SA-HRP
12/2/14
% Recovery vs. Media (No Ab)
200
150
100
50
0
>200%
20 ug/mL
6.67 ug/mL
2.22 ug/mL
0.74 ug/mL
3H1 Mouse
3F10 Mouse
2A7 Mouse
1D5 Mouse
9C9.5.6
7D4.6
7A3.1.9
7A3.1.9
6F8.7
6E12.5
6E1.1.12
3C9.10
2E5.2.3
1E6.1.3

FIG. 15B

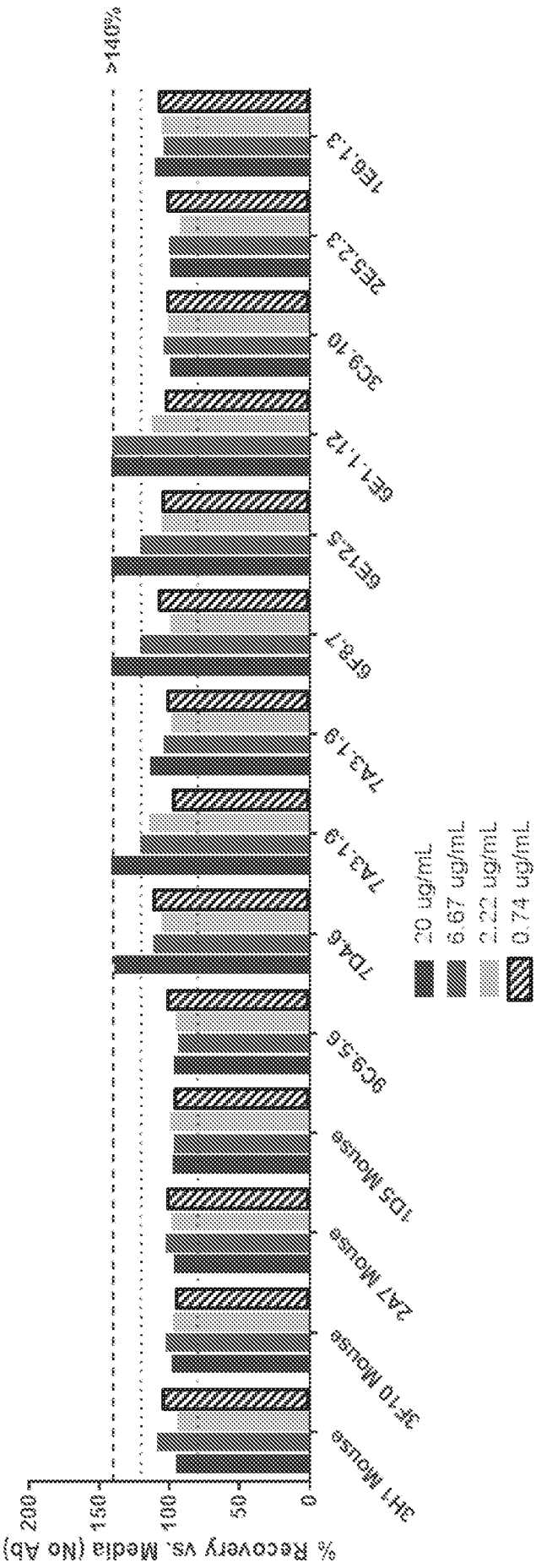

Antibody Interference on Conditioned Media
MEL-JUSO Cells (at 20K/well) 24h Incubation
Recombinant MICA008 Standard
AMO1/Bio-6D4/SA-HRP
% Recovery vs. Media (No Ab)
200
150
100
50
0
>140%
3H1 Mouse
3F10 Mouse
2A7 Mouse
1D5 Mouse
9C9.5.6
7D4.6
7A3.1.9
7A3.1.9
7A3.1.9
6F6.1.7
6E12.5
6E1.1.12
3C9.10
2E5.2.3
1E6.1.3
20 ug/mL
6.67 ug/mL
2.22 ug/mL
0.74 ug/mL

FIG. 16A

Interference

HCC1534 (MICA*004) (AMO1/8C5)								
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
13A9 ug/mL \/								
10	27%	28%	29%	33%	31%	27%	22%	23%
2.5	26%	31%	34%	36%	33%	31%	28%	22%
0.625	18%	30%	30%	33%	36%	30%	26%	23%
0.15625	27%	29%	34%	37%	34%	26%	27%	25%
0.0390625	24%	31%	33%	36%	34%	32%	23%	19%
0.009765625	22%	28%	31%	34%	35%	29%	25%	14%
0.002441406	21%	29%	30%	34%	32%	30%	18%	13%
None	22%	27%	31%	32%	29%	28%	17%	9%
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	34%	36%	45%	42%	38%	31%	26%	13%
2.5	32%	33%	36%	37%	36%	33%	21%	14%
0.625	25%	35%	31%	34%	31%	25%	22%	6%
0.15625	23%	24%	29%	30%	24%	15%	12%	0%
0.0390625	23%	17%	19%	20%	18%	14%	6%	-7%
0.009765625	10%	17%	16%	16%	15%	3%	-8%	-8%
0.002441406	12%	21%	17%	24%	13%	8%	-4%	-4%
None	9%	15%	14%	12%	7%	6%	-12%	-13%
13A9 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	34%	39%	30%	30%	26%	16%	13%	22%
2.5	31%	35%	42%	33%	21%	18%	29%	19%
0.625	27%	35%	29%	27%	24%	19%	20%	13%
0.15625	24%	20%	28%	19%	19%	13%	10%	14%
0.0390625	18%	21%	25%	24%	17%	14%	12%	11%
0.009765625	17%	30%	21%	25%	21%	17%	16%	8%
0.002441406	11%	20%	18%	24%	22%	27%	15%	12%
None	19%	20%	23%	19%	17%	13%	10%	7%

FIG. 16B

Interference

MEL-JUSO (MICA*008) (AMO1/8C5)								
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
13A9 ug/mL \/								
10	-8%	-1%	-4%	4%	15%	17%	12%	20%
2.5	2%	-3%	14%	10%	11%	20%	24%	15%
0.625	-2%	9%	11%	8%	15%	20%	20%	14%
0.15625	4%	3%	18%	15%	9%	8%	15%	12%
0.0390625	0%	7%	8%	11%	14%	11%	15%	10%
0.009765625	5%	12%	8%	13%	15%	16%	14%	10%
0.002441406	-1%	10%	7%	7%	16%	21%	12%	11%
None	14%	11%	8%	7%	13%	14%	14%	6%
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	11%	17%	N/A	14%	13%	15%	12%	8%
2.5	13%	13%	6%	14%	15%	19%	16%	9%
0.625	7%	12%	15%	14%	16%	17%	20%	6%
0.15625	14%	6%	11%	12%	8%	7%	11%	1%
0.0390625	14%	9%	3%	13%	8%	5%	3%	6%
0.009765625	-2%	9%	1%	3%	19%	6%	1%	6%
0.002441406	-10%	14%	20%	22%	28%	11%	10%	9%
None	12%	32%	7%	4%	4%	9%	-10%	-6%
13A9 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	13%	-3%	15%	7%	5%	9%	8%	6%
2.5	6%	3%	7%	4%	9%	15%	13%	7%
0.625	13%	12%	10%	16%	21%	19%	13%	9%
0.15625	4%	11%	10%	19%	13%	16%	10%	11%
0.0390625	6%	18%	16%	17%	25%	22%	15%	7%
0.009765625	2%	9%	17%	21%	20%	21%	20%	13%
0.002441406	1%	15%	19%	20%	24%	24%	15%	11%
None	11%	14%	17%	19%	20%	23%	14%	8%

FIG. 16C

Interference

HCC1534 (MICB*005) (236511/7E3)								
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
13A9 ug/mL \/								
10	18%	28%	18%	33%	35%	38%	31%	36%
2.5	20%	18%	34%	32%	36%	41%	46%	28%
0.625	13%	30%	25%	35%	38%	33%	46%	36%
0.15625	26%	25%	33%	35%	33%	33%	37%	41%
0.0390625	31%	33%	32%	26%	34%	29%	36%	35%
0.009765625	29%	30%	18%	34%	32%	39%	32%	23%
0.002441406	15%	31%	35%	31%	27%	31%	25%	22%
None	25%	28%	33%	34%	31%	25%	24%	15%
1D5 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	41%	38%	29%	49%	43%	33%	31%	24%
2.5	44%	43%	40%	43%	44%	41%	29%	14%
0.625	39%	43%	39%	42%	43%	24%	35%	25%
0.15625	35%	37%	36%	36%	30%	15%	17%	5%
0.0390625	31%	31%	12%	36%	14%	12%	4%	7%
0.009765625	28%	17%	27%	27%	27%	13%	-1%	-2%
0.002441406	8%	20%	24%	24%	30%	13%	8%	-2%
None	14%	16%	17%	5%	11%	1%	-8%	-14%
13A9 ug/mL>	10	2.5	0.625	0.15625	0.039063	0.009766	0.002441	None
6E1 ug/mL \/								
10	31%	31%	31%	37%	36%	27%	25%	28%
2.5	32%	23%	26%	32%	33%	36%	32%	22%
0.625	25%	28%	29%	28%	30%	26%	28%	20%
0.15625	29%	25%	24%	30%	30%	24%	22%	19%
0.0390625	27%	31%	31%	26%	31%	19%	22%	10%
0.009765625	18%	23%	28%	33%	29%	26%	19%	13%
0.002441406	23%	26%	27%	28%	29%	17%	15%	7%
None	21%	28%	30%	33%	31%	23%	15%	8%

FIG. 17

Clone ID	MICA*008 kD (nM)
12H10	1.9
18G3	2.6
1D5	0.5
1G3	0.75
20G11	3.8
2E5.2.3	1.8
32D2	4
3E11	2.5
6E1.1.12	6.8
6F8.7	8.4
7D4.6	10
HZ.10A2	4.7
HZ.10B4	4.6
HZ.10G11	0.57
HZ.10G12	6
HZ.11H3	4.2
HZ.12G2	1.2
HZ.13A9	3.1
HZ.13G6	0.62
HZ.14C1	4.9
HZ.15D2	1.6
HZ.15E12	1.5
HZ.15F11	2.1
HZ.15G6	1.3
HZ.16E2	3.2
HZ.16F11	4.8
HZ.18B6	1.7
HZ.19E8	0.89
HZ.19H3	3.6
HZ.20B2	4.8
HZ.2C11	1.7
HZ.2D10	1
HZ.2H4	0.47
HZ.2H7	1.7
HZ.3B1	1.6
HZ.3B10	0.52
HZ.3B4	2.1
HZ.3C9	3.9
HZ.3E8	1.8
HZ.3F2	2.5
HZ.3F7	1.8
HZ.3G4	1.9
HZ.3H5	1.8
HZ.4H6	2.4
HZ.5C5	3.5
HZ.6A11	2.8
HZ.6A7	1.3
HZ.6G2	2.1
HZ.7A6	4.6
HZ.7A9	4.4
HZ.7G11	3.5
HZ.9H10	6.2

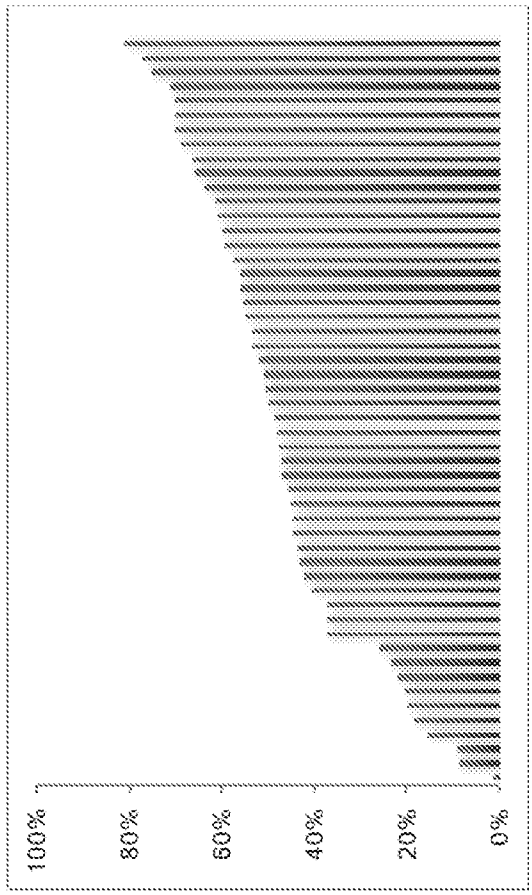


FIG. 18A

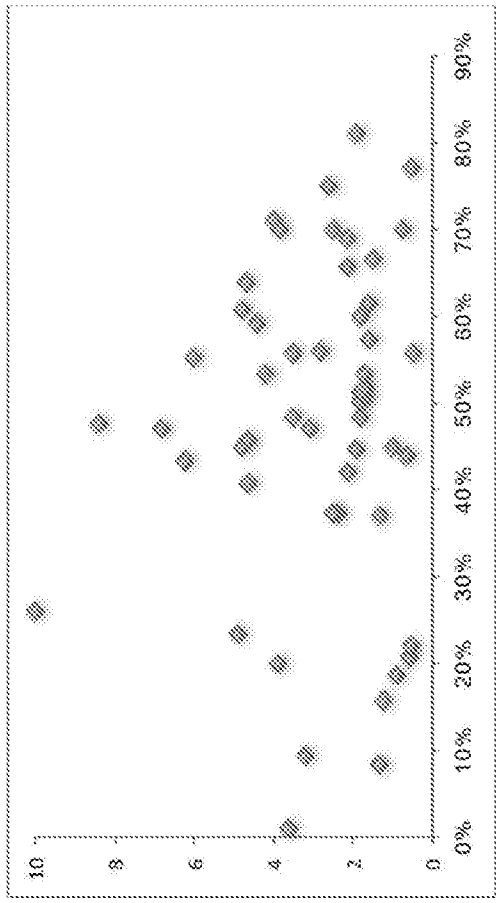


FIG. 18B

FIG. 19A

Clone ID	Bin 1 (1D5-like)	Bin2	Bin 3 (6E1-like)
12H10			
18G3			
1D5			
1G3			
20G11			
32D2			
3E11			
6F8.7			
HZ.10G11			
HZ.10G12			
HZ.12G2			
HZ.13A9			
HZ.15E12			
HZ.15F11			
HZ.18B6			
HZ.19E8			
HZ.20B2			
HZ.2C11			
HZ.2D10			
HZ.2H7			
HZ.3B1			
HZ.3B10			
HZ.3E8			
HZ.3F2			
HZ.3F7			
HZ.3G4			
HZ.3H5			
HZ.5C5			
HZ.6A11			
HZ.6G2			
HZ.7A6			
HZ.7G11			
HZ.9H10			
7D4.6			
HZ.10B4			
HZ.3B4			
HZ.4H6			
2E5.2.3			
6E1.1.12			
HZ.10A2			
HZ.11H3			
HZ.13G6			
HZ.14C1			
HZ.15D2			
HZ.15G6			
HZ.16E2			
HZ.16F11			
HZ.19H3			
HZ.2H4			
HZ.3C9			
HZ.6A7			
HZ.7A9			

FIG. 19B

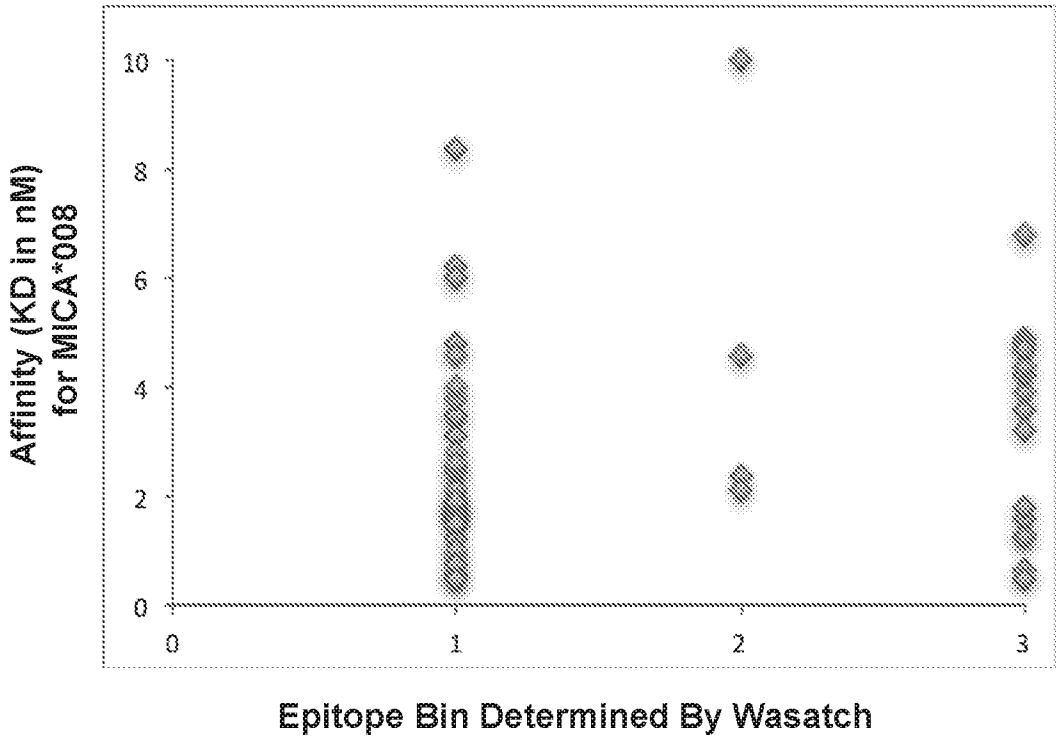


FIG. 19C

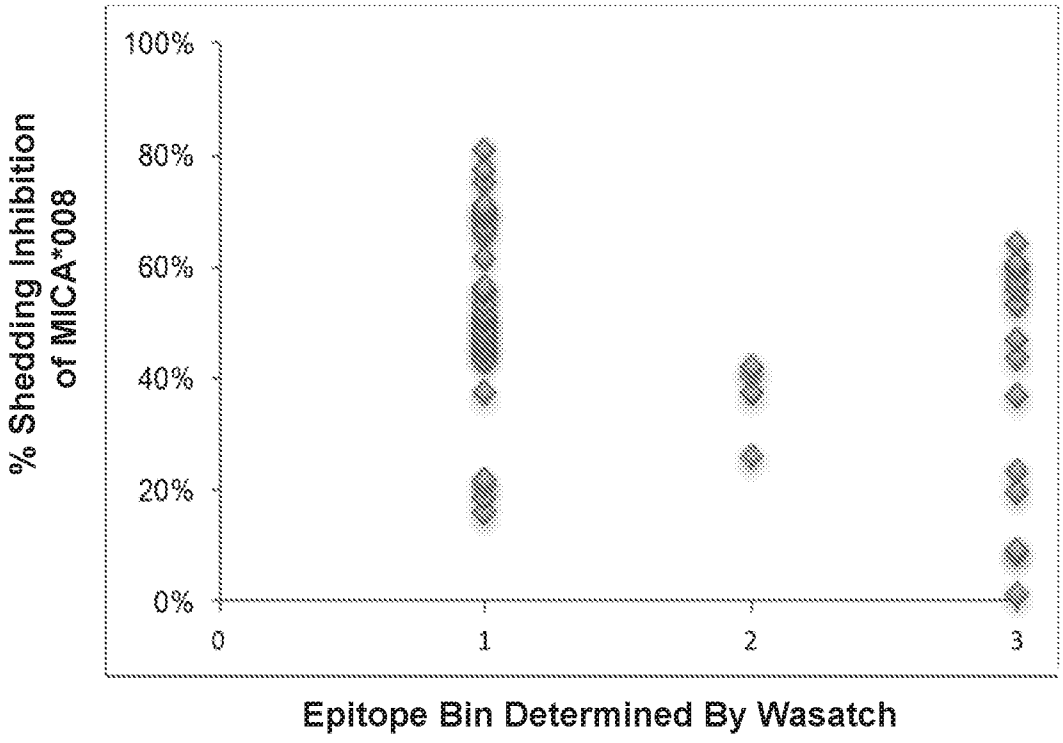


FIG. 20

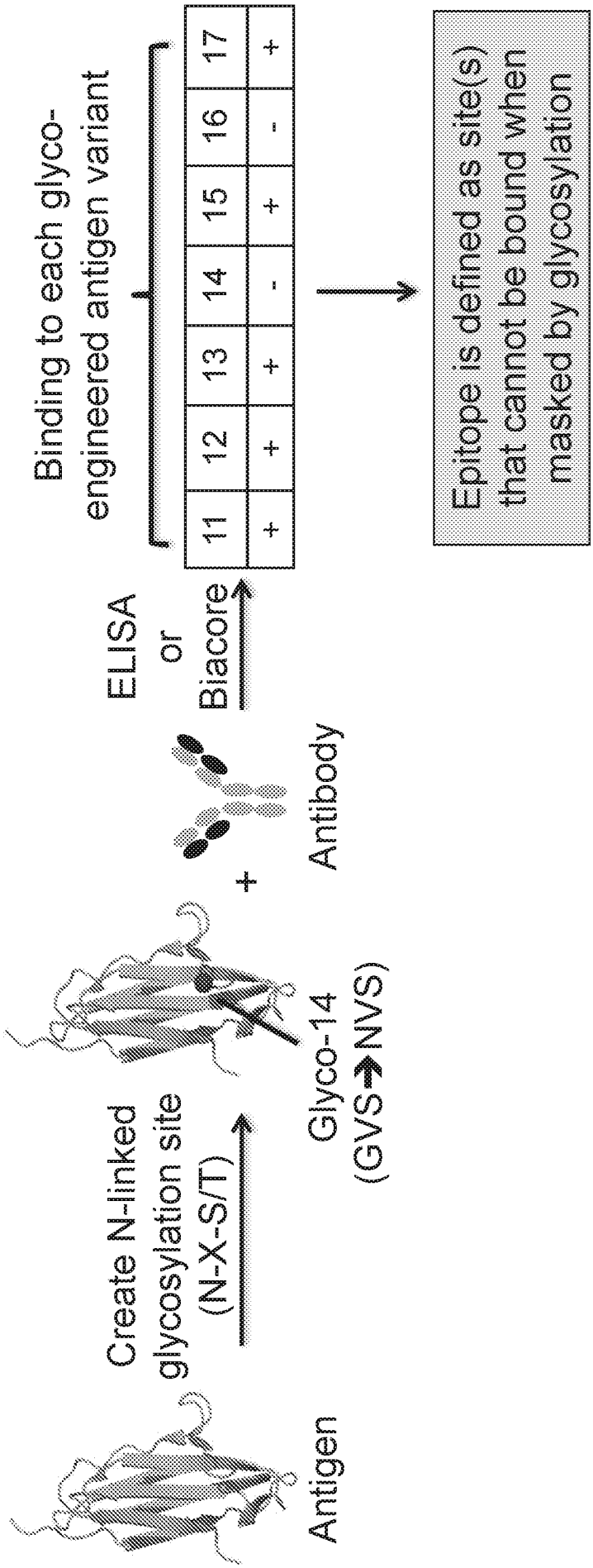
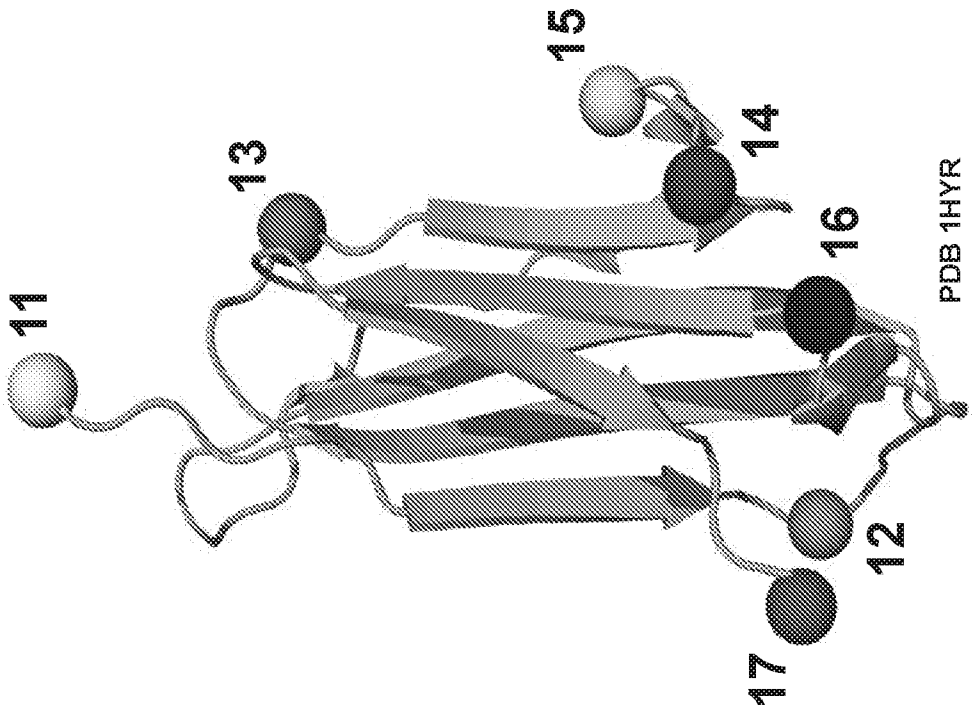

Antigen
Create N-linked glycosylation site (N-X-S/T)
Glyco-14 (GVS→NVS)
+
Antibody
ELISA or Biacore
Binding to each glyco-engineered antigen variant
11 12 13 14 15 16 17
+ + + - + - +
Epitope is defined as site(s) that cannot be bound when masked by glycosylation

FIG. 21A



MICA α3 domain shows Glyco11-Glyco17, the 7 distinct and individually engineered glycosites

FIG. 21B

	Sample								Tryptic Peptide	
	WT	11	12	13	14	15	16	17		
Site		N5							GSNR (sample 11)	(SEQ ID NO: 444)
% Fucosylation										
% Afucosylation		N/C								
Total Glycosylation										
Site	N9	N11	N5	N9	N9	N9	N5	N9	TVPPMVNVTR (all samples)	(SEQ ID NO: 445)
% Fucosylation	53%	64%	43%	65%	75%	64%	64%	75%		
% Afucosylation	20%	36%	57%	31%	25%	36%	36%	25%		
Total Glycosylation	73%	>99%	98%	96%	>99%	>99%	>99%	>99%		
Site	N15	N21	N14, N19	N19	N19	N19	N15	N19	SNASEGNITVTCR (sample 12)	(SEQ ID NO: 446)
% Fucosylation	24%	91%	57%	86%	82%	83%	54%	94%	SEASEGNITVTCR (all other samples)	(SEQ ID NO: 447)
% Afucosylation	15%	8%	43%	14%	18%	17%	43%	6%		
Total Glycosylation	38%	>99%	>99%	>99%	>99%	>99%	>99%	>99%		
Site				N93					NITLTNR (sample 13)	(SEQ ID NO: 448)
% Fucosylation				73%						
% Afucosylation				27%						
Total Glycosylation				>99%						
Site	N42	N52	N53	N60	N42, N60	N47, N60	N60	N60	QDGNSLSHTTQQWGDVLPDGNETYQTWVATR (sample 14)	(SEQ ID NO: 449)
% Fucosylation									QDGNSLSNTTQQWGDVLPDGNETYQTWVATR (sample 15)	(SEQ ID NO: 450)
% Afucosylation	N/C	N/C	N/C	N/C	N/C	N/C	N/C	N/C	QDGNSLSHTTQQWGDVLPDGNETYQTWVATR (all other samples)	(SEQ ID NO: 451)
Total Glycosylation										
Site	N68	N69	N83	N88	N68	N68	N78, N88	N88, N97	GEEQNFTCYMEHSGNHSTHPVPSGNKSR (sample 16)	(SEQ ID NO: 452)
% Fucosylation	49%	80%	73%	65%	77%	75%		77%	FTCYMEHSGNHSTHPVPSGNKSR (sample 17)	(SEQ ID NO: 453)
% Afucosylation	18%	17%	23%	22%	17%	19%	N/C	9%	FTCYMEHSGNHSTHPVPSGNKSR (all other samples)	(SEQ ID NO: 454)
Total Glycosylation	68%	97%	96%	87%	94%	94%		86%		
Site	N191	N193	N181	N191	N191	N191	N191	N194	EDYNSTLR (all samples)	(SEQ ID NO: 455)
% Fucosylation	59%	88%	94%	69%	84%	73%	84%	93%		
% Afucosylation	28%	12%	6%	26%	6%	25%	6%	7%		
Total Glycosylation	85%	>99%	>99%	95%	>99%	98%	>99%	>99%		

N/C = No coverage

■ = Glyco-variant site

FIG. 21C

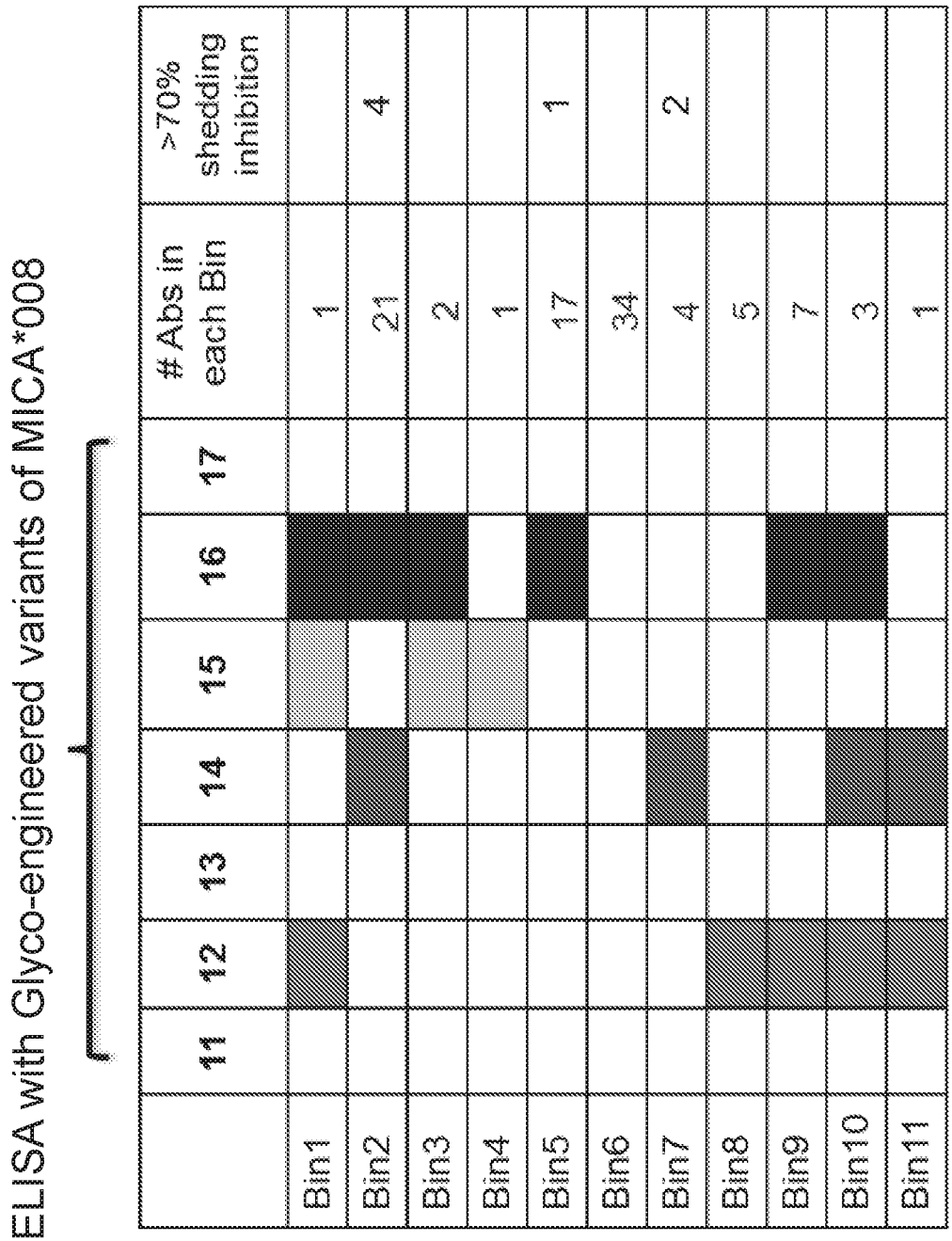
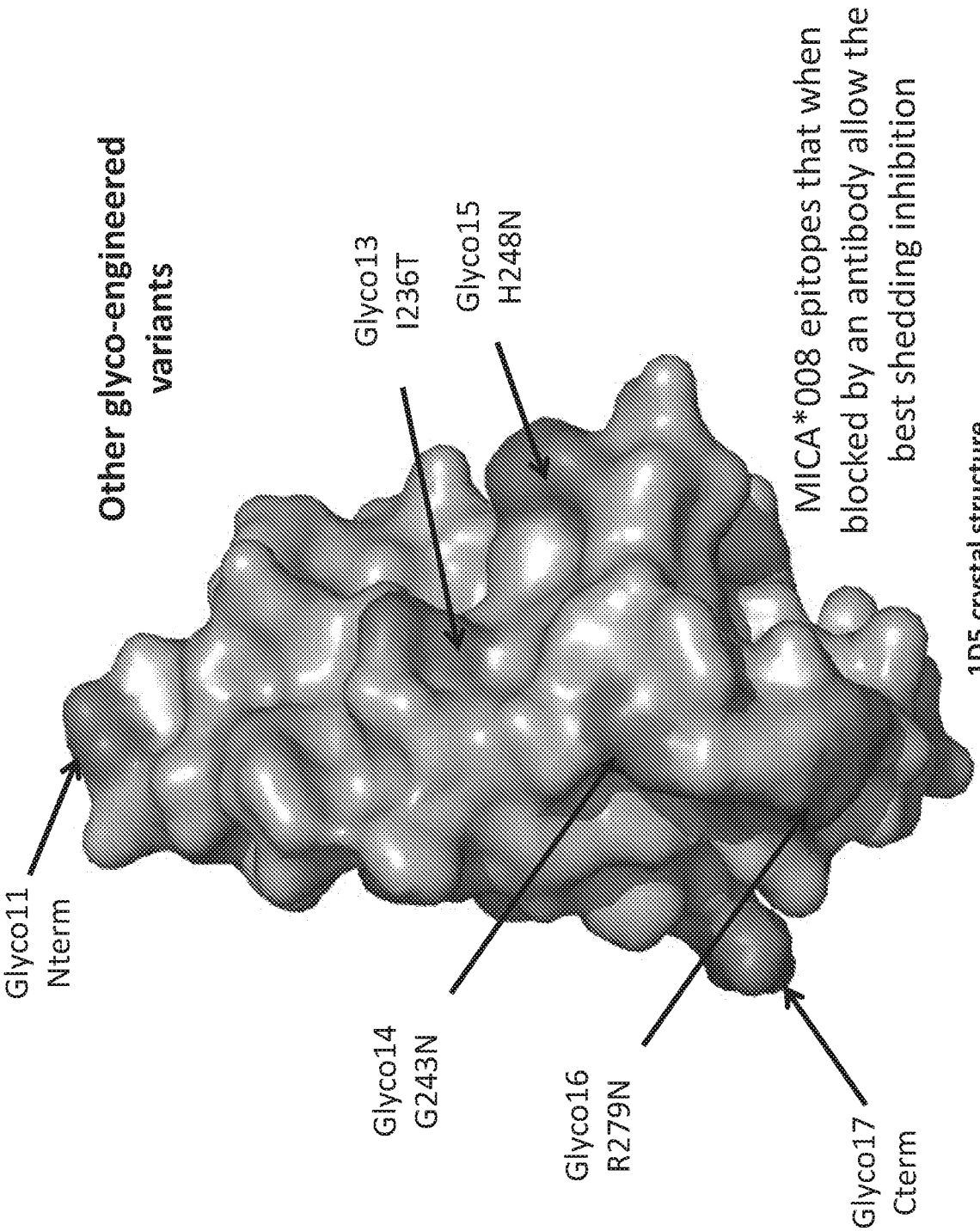

ELISA with Glyco-engineered variants of MICA*008
11
12
13
14
15
16
17
Abs in each Bin
>70% shedding inhibition
Bin1
1
Bin2
21
4
Bin3
2
Bin4
1
Bin5
17
1
Bin6
34
Bin7
4
2
Bin8
5
Bin9
7
Bin10
3
Bin11
1

FIG. 21D


Other glyco-engineered variants
Glyco11 Nterm
Glyco13 I236T
Glyco15 H248N
Glyco14 G243N
Glyco16 R279N
Glyco17 Cterm
MICA*008 epitopes that when blocked by an antibody allow the best shedding inhibition
1D5 crystal structure

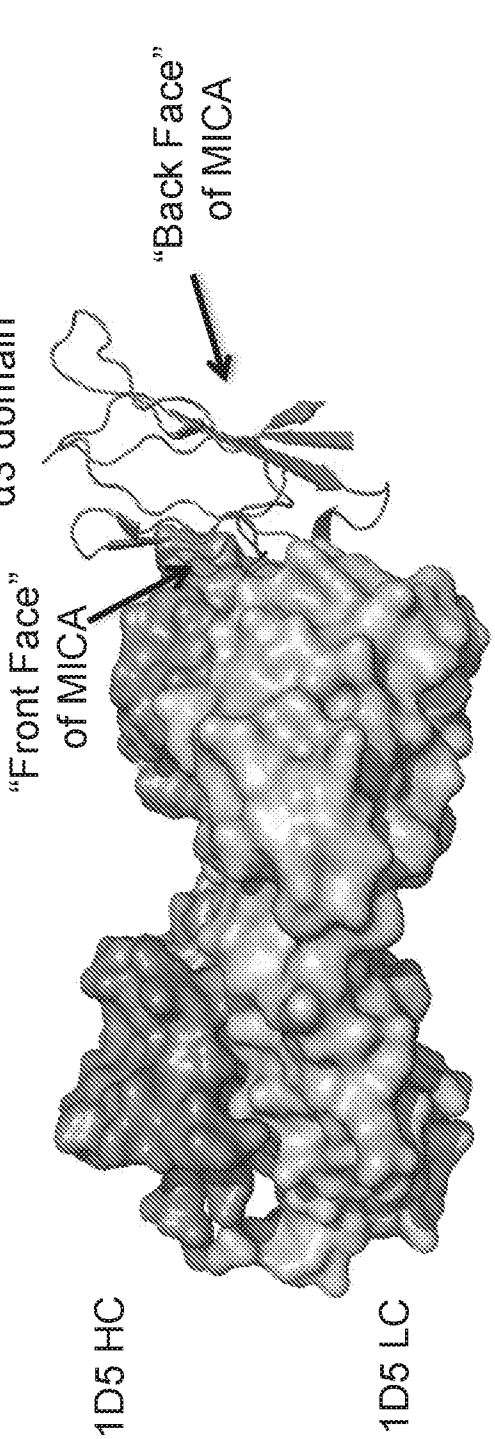


FIG. 22A

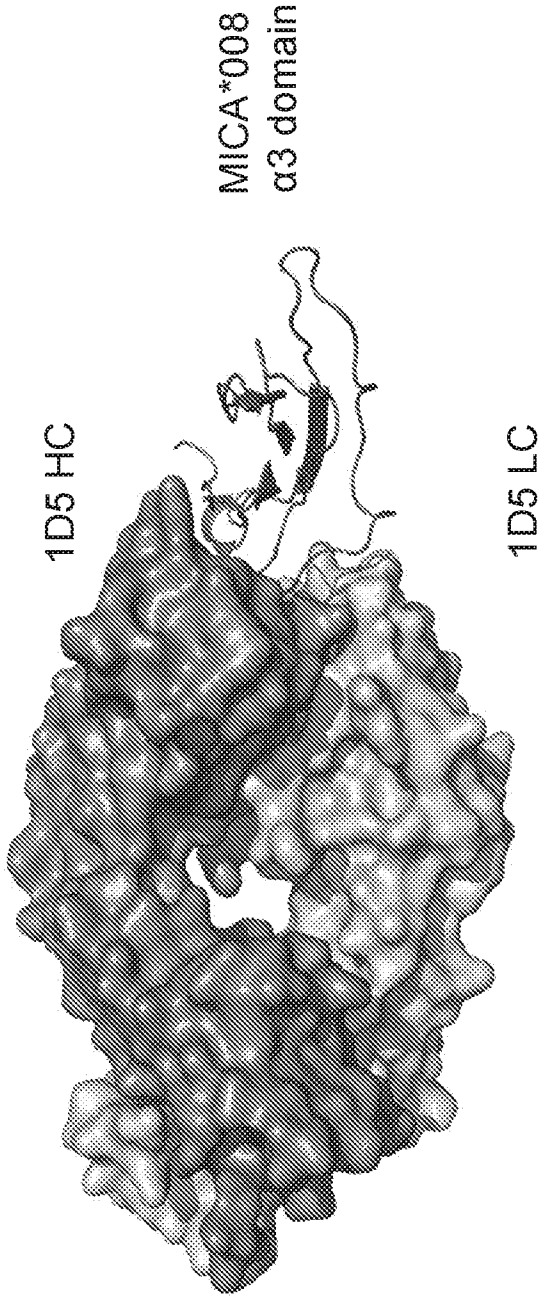


FIG. 22B

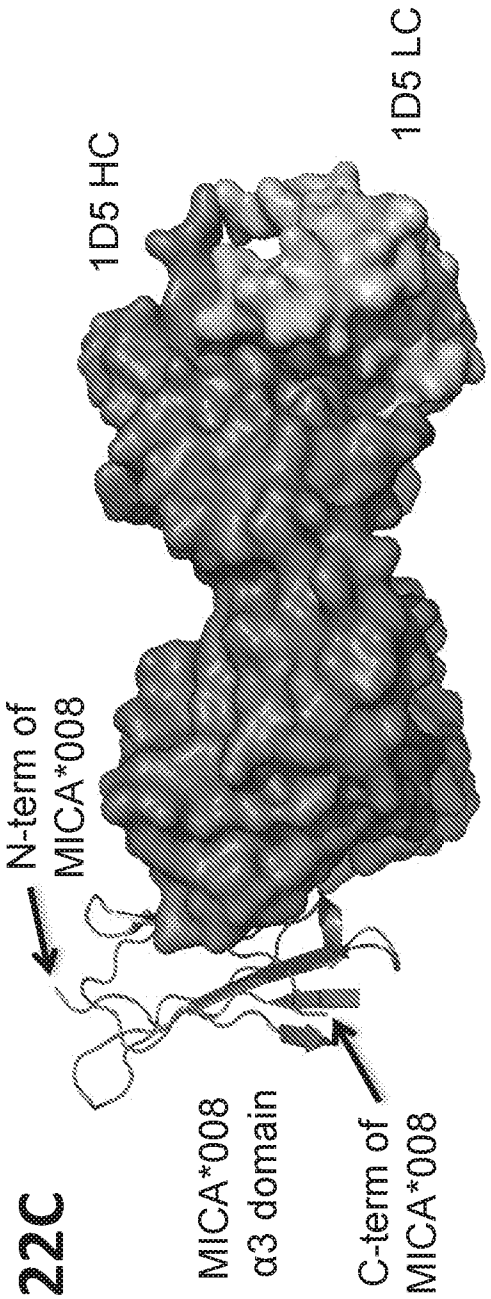


FIG. 22C

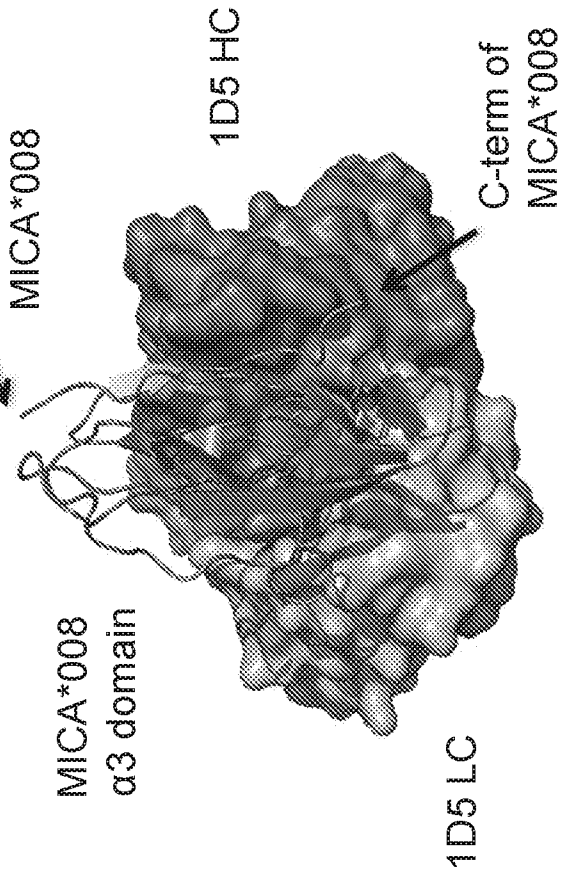


FIG. 22D

FIG. 23A

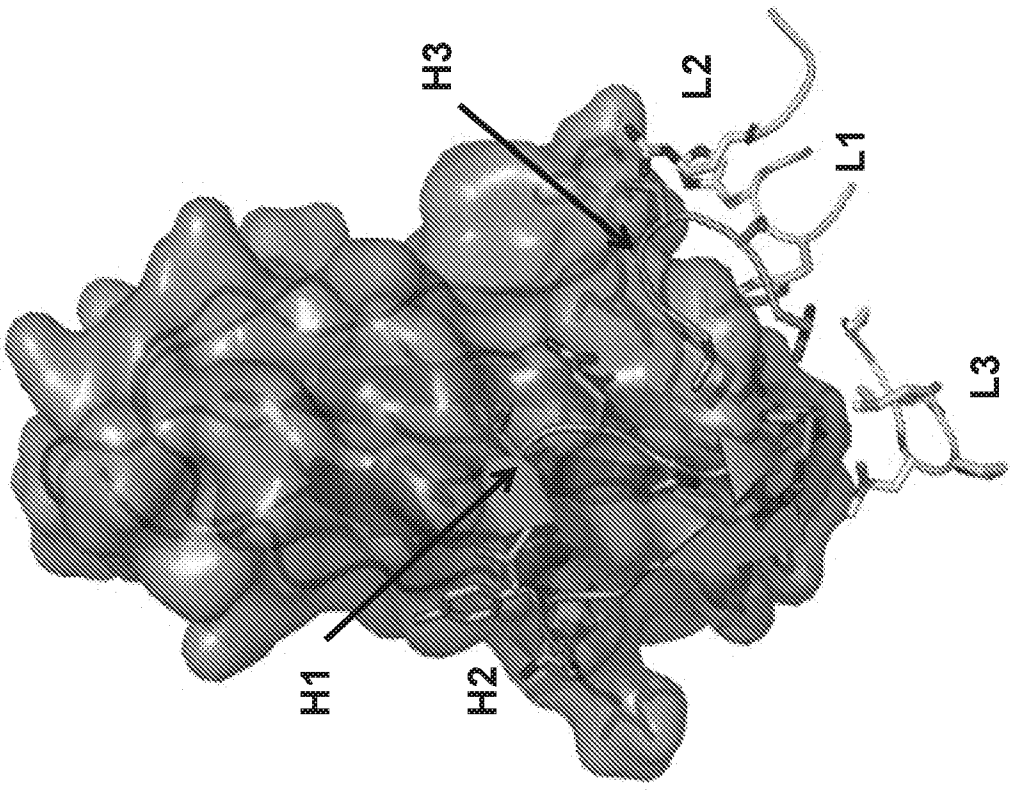


FIG. 23B

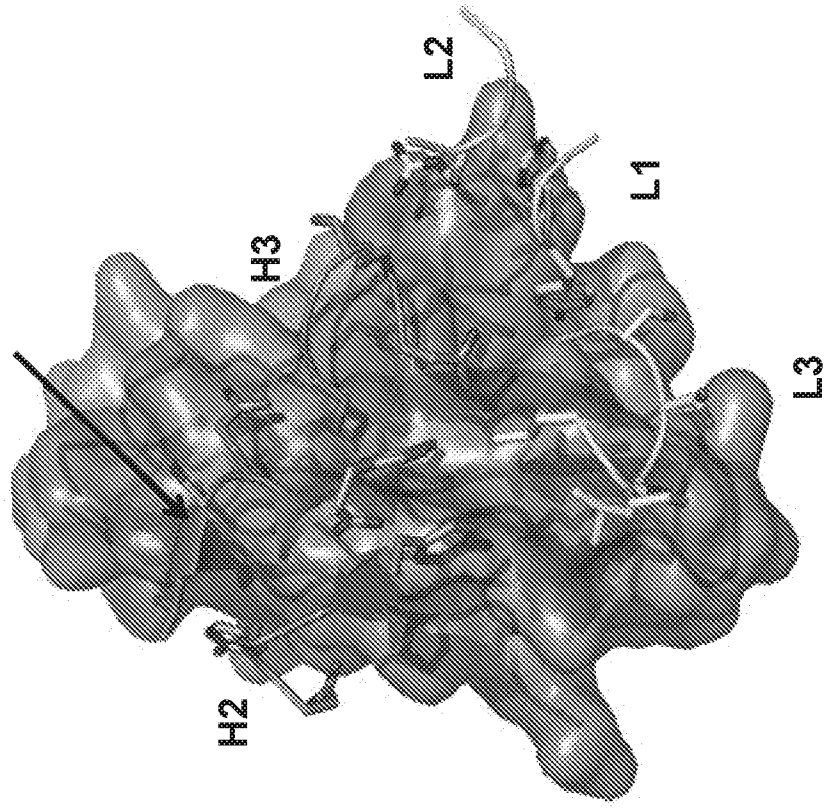


FIG. 24A

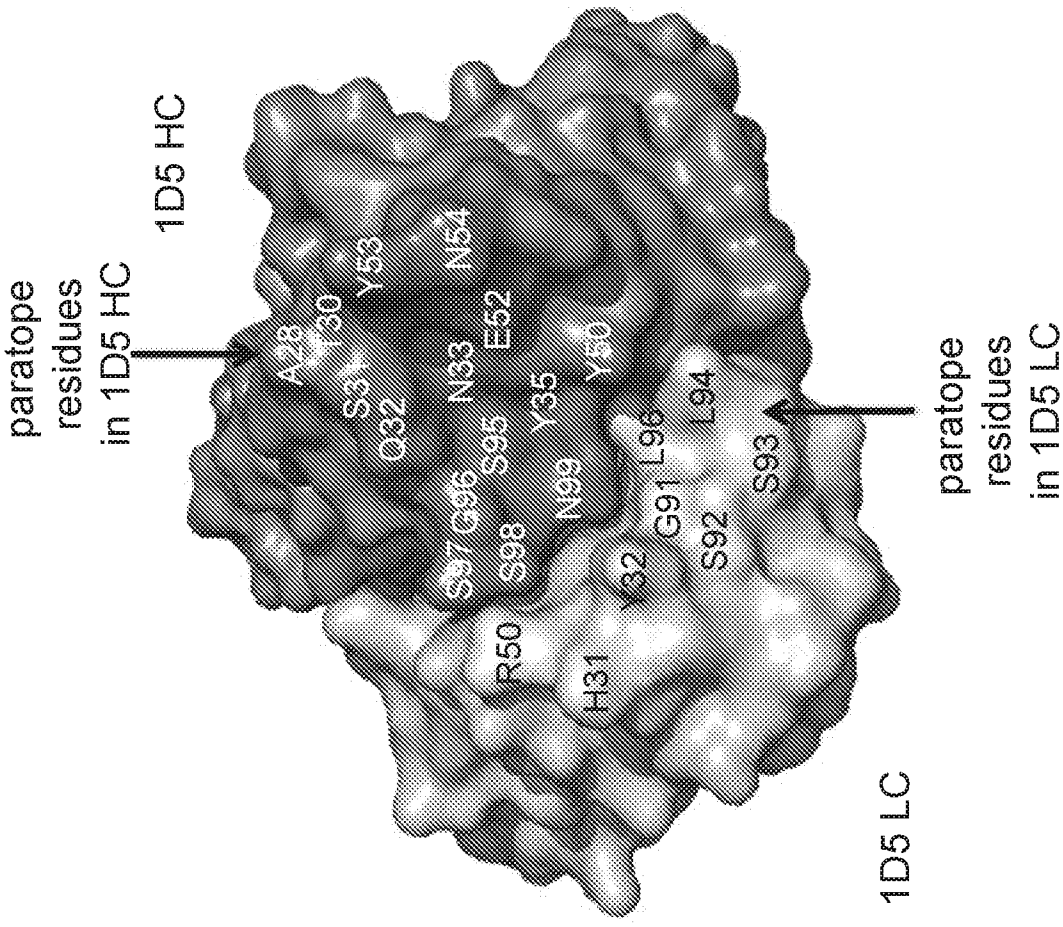


FIG. 24B

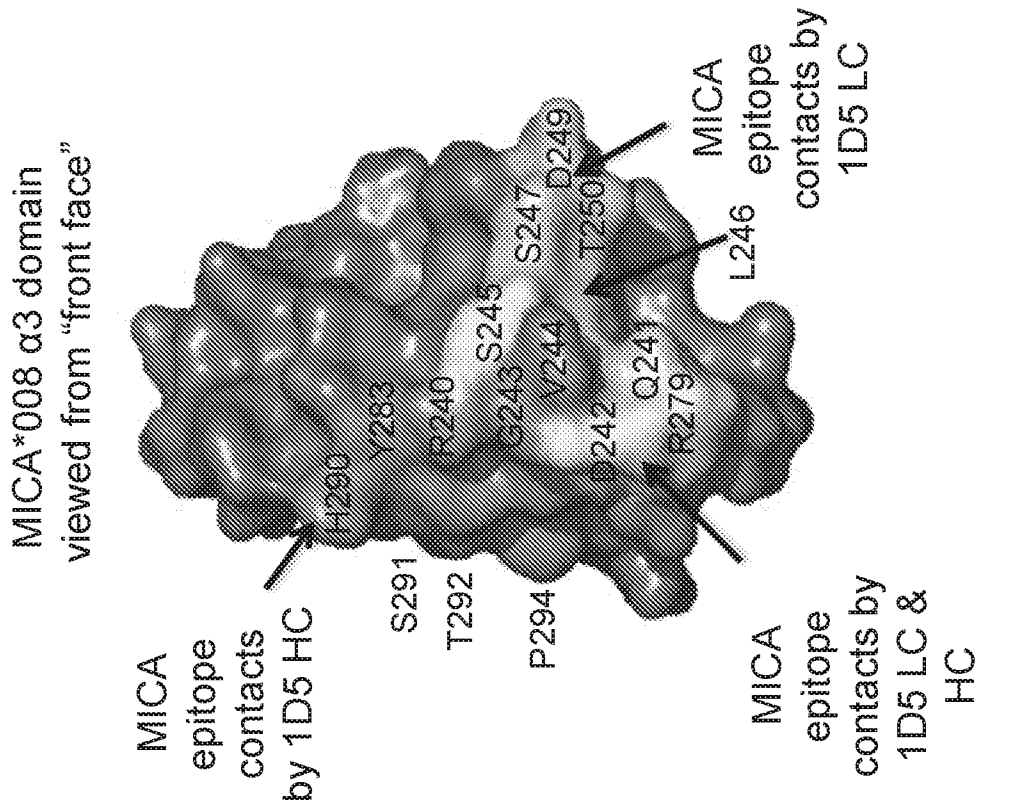


FIG. 24C

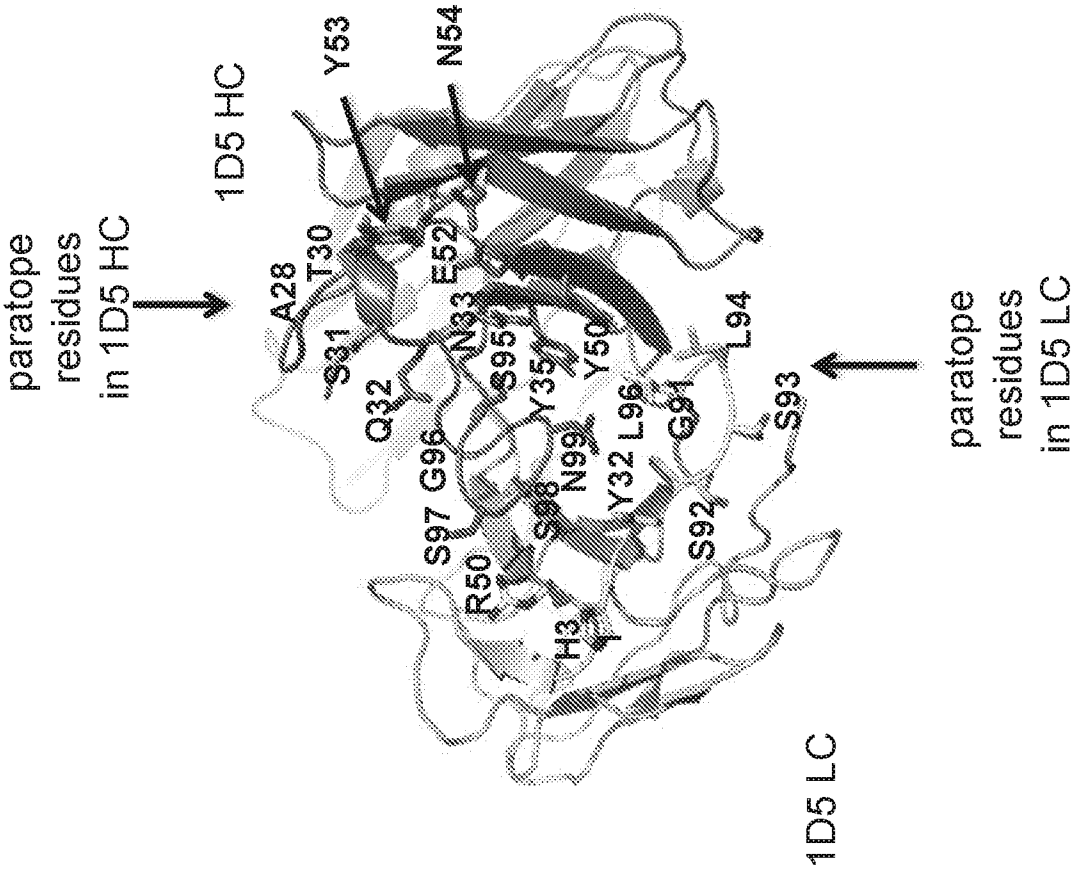


FIG. 24D

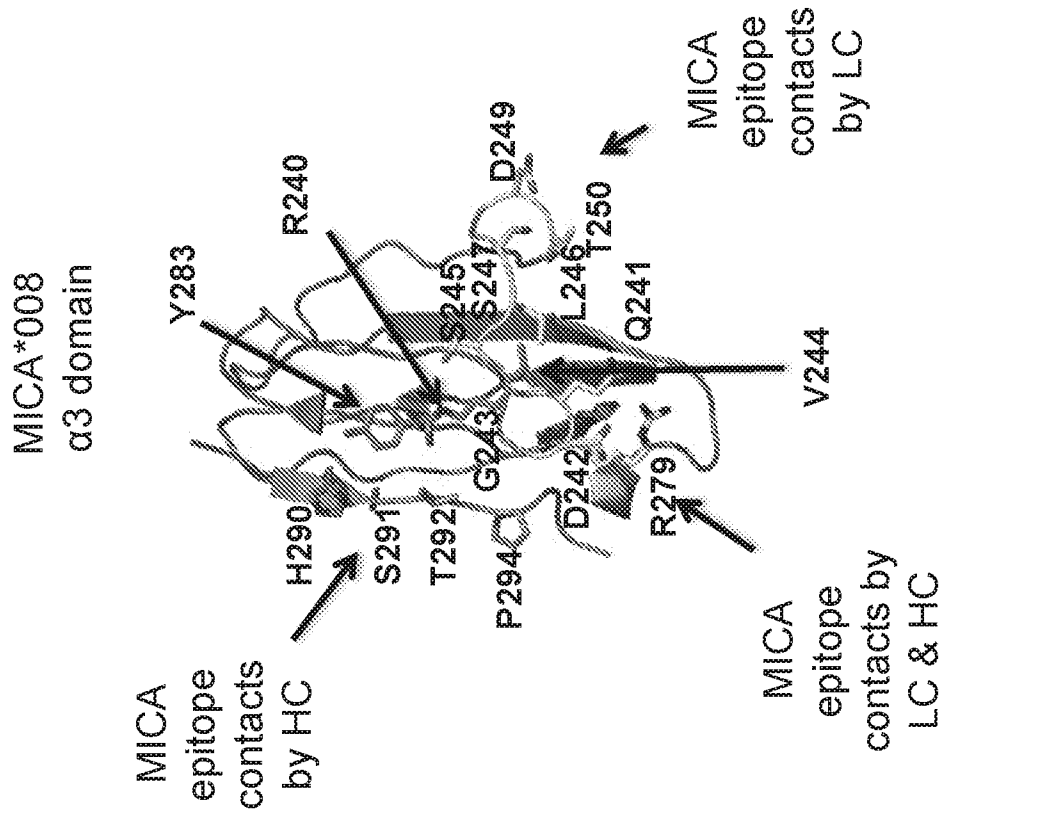


FIG. 25A

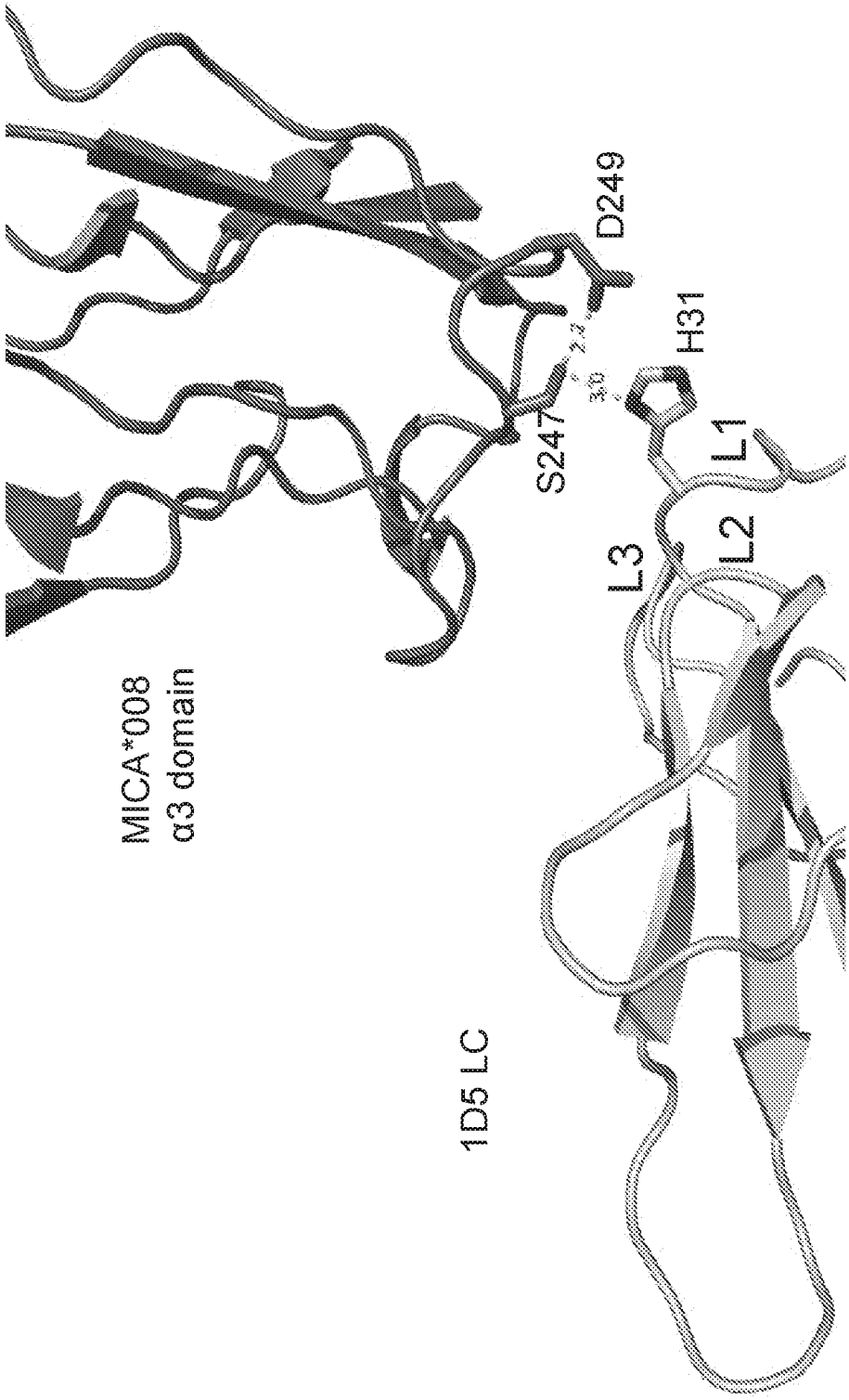


FIG. 25B

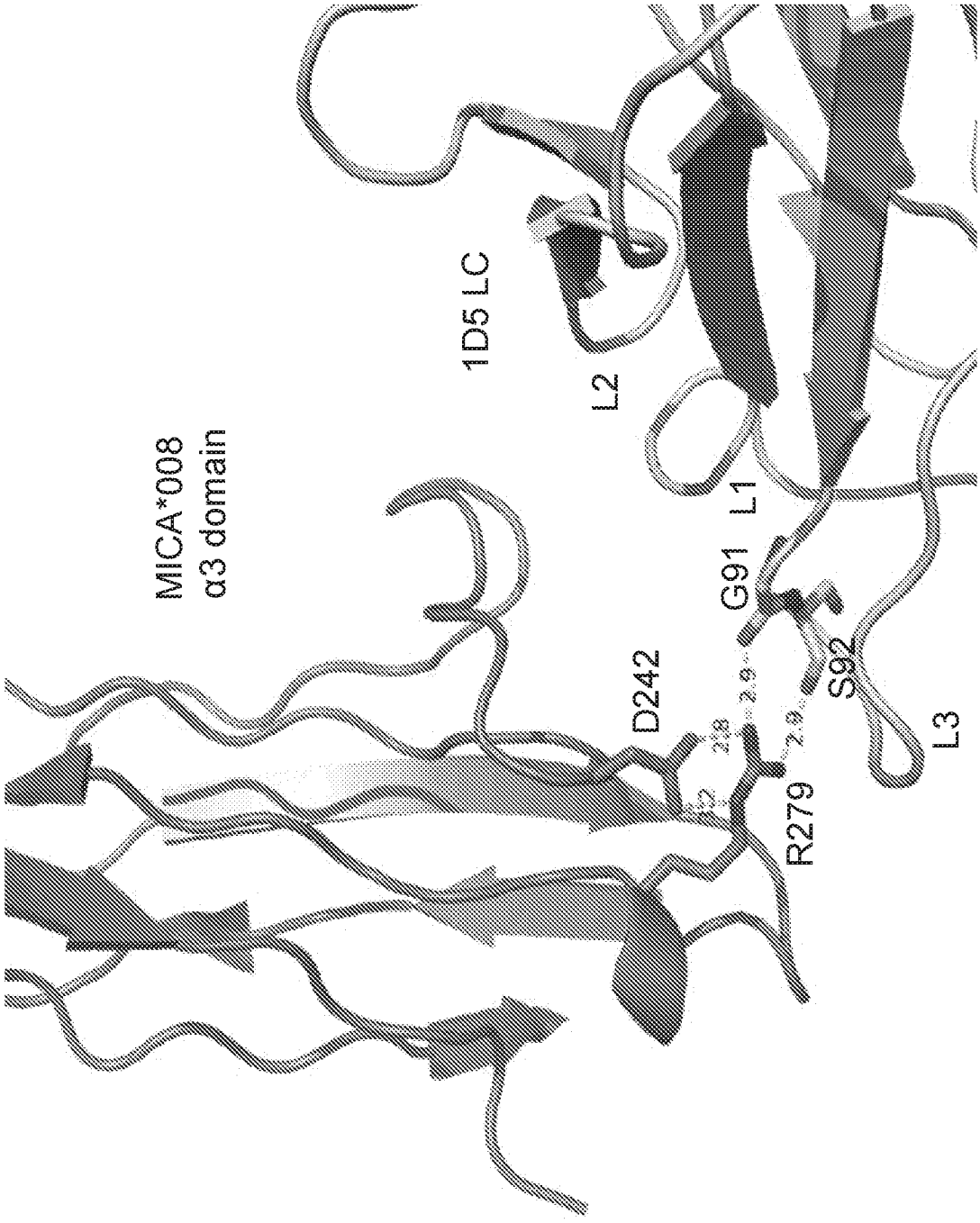


FIG. 25C

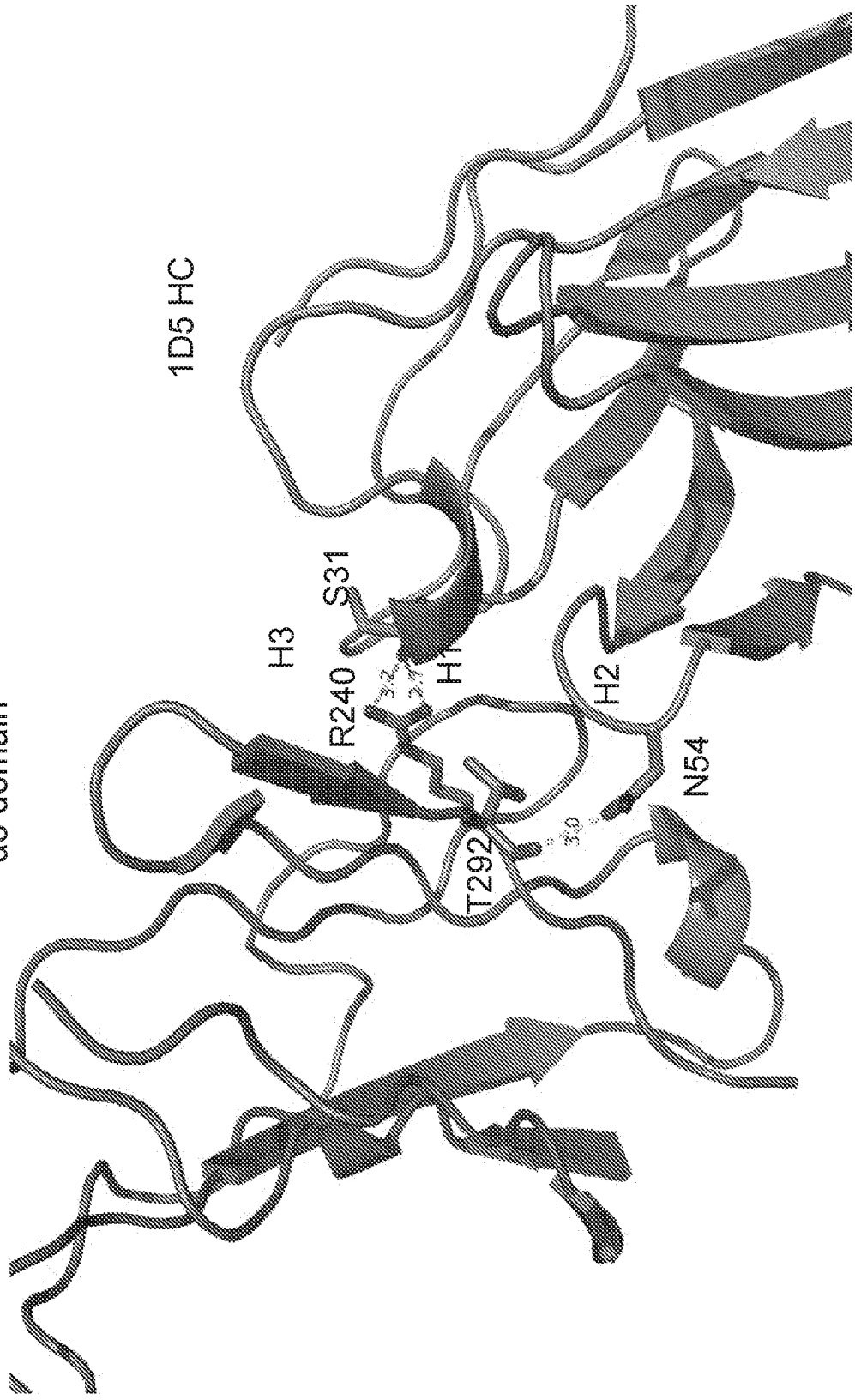


FIG. 25D

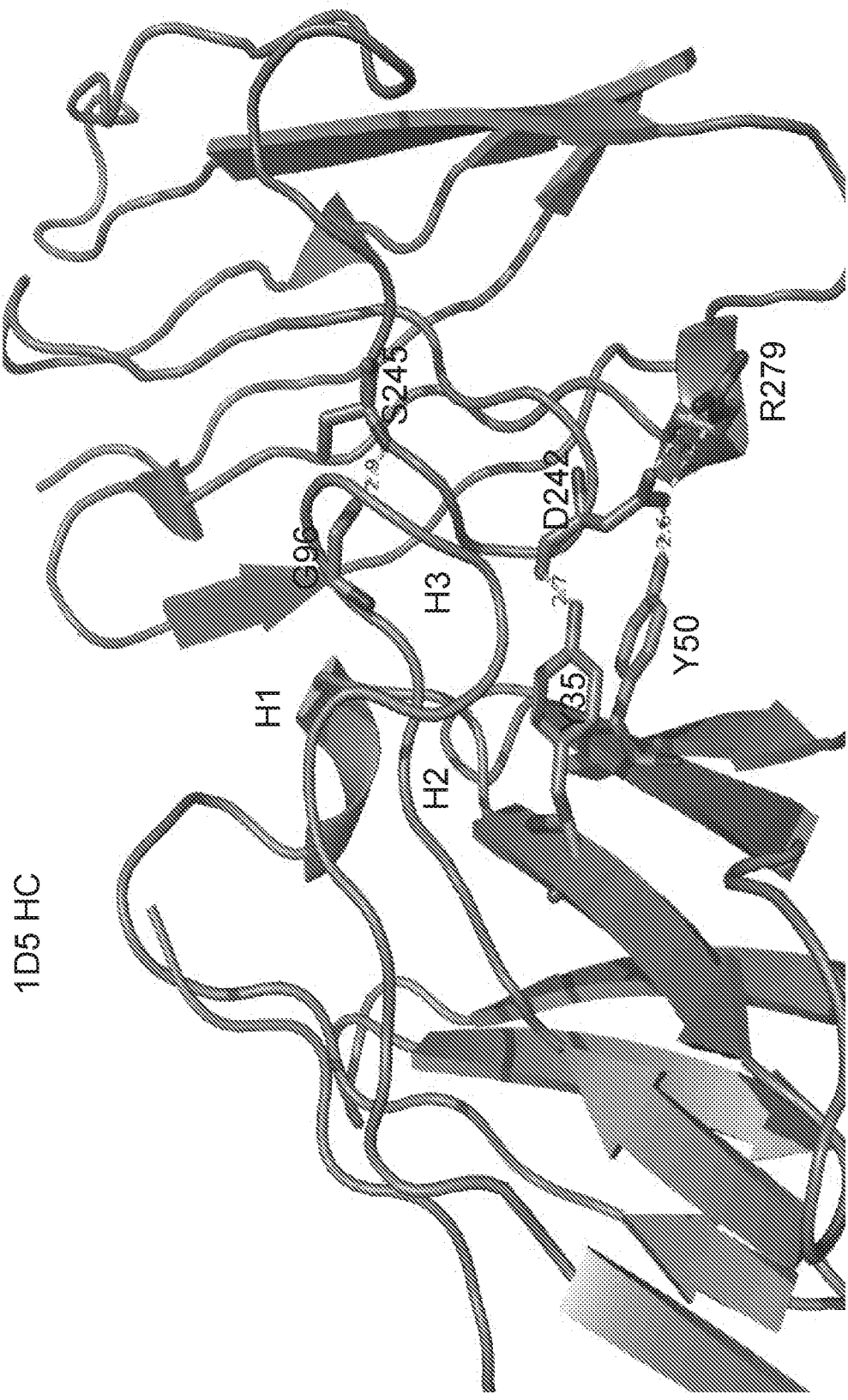


FIG. 26A

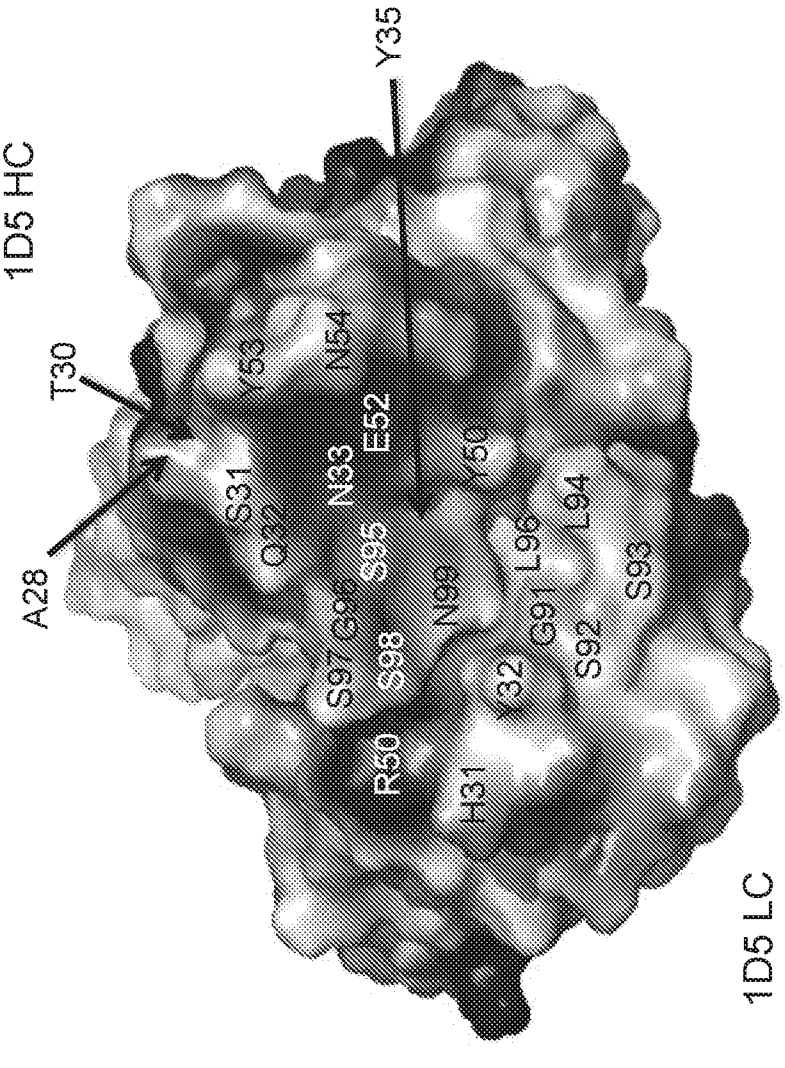


FIG. 26B

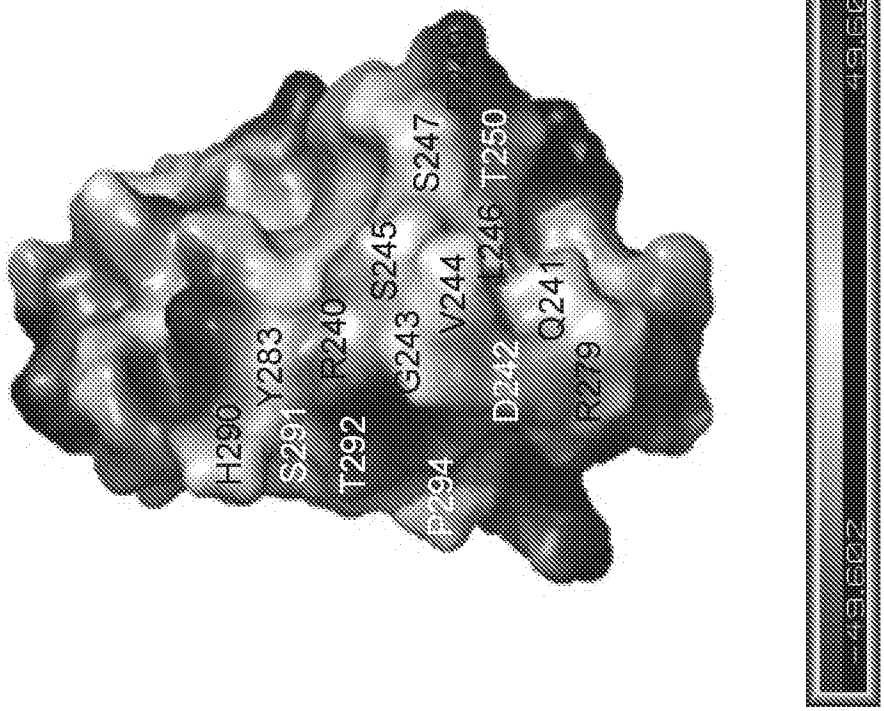


FIG. 27A

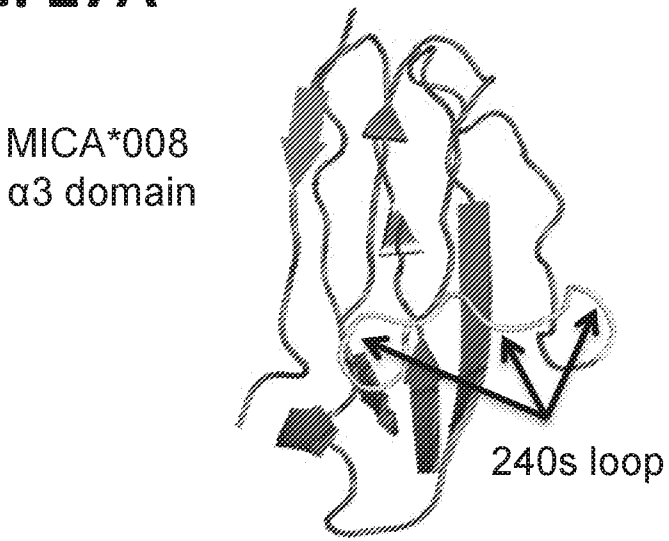


FIG. 27B

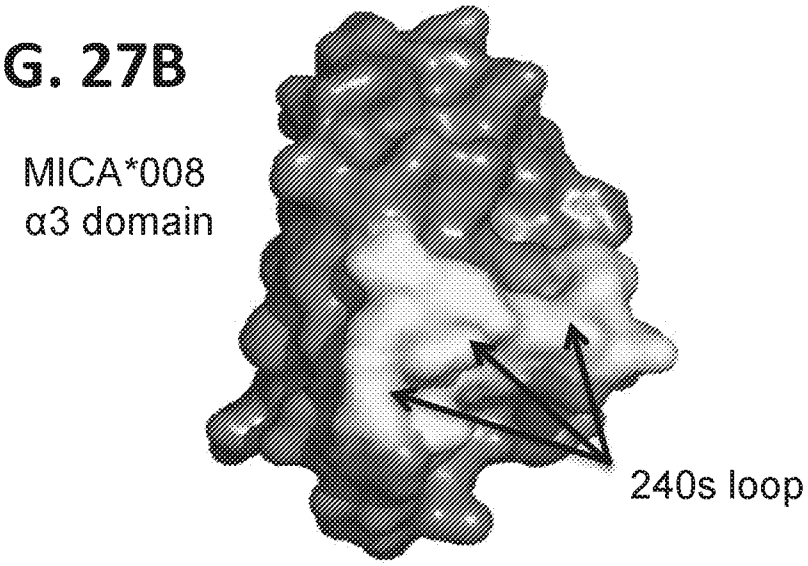


FIG. 27C

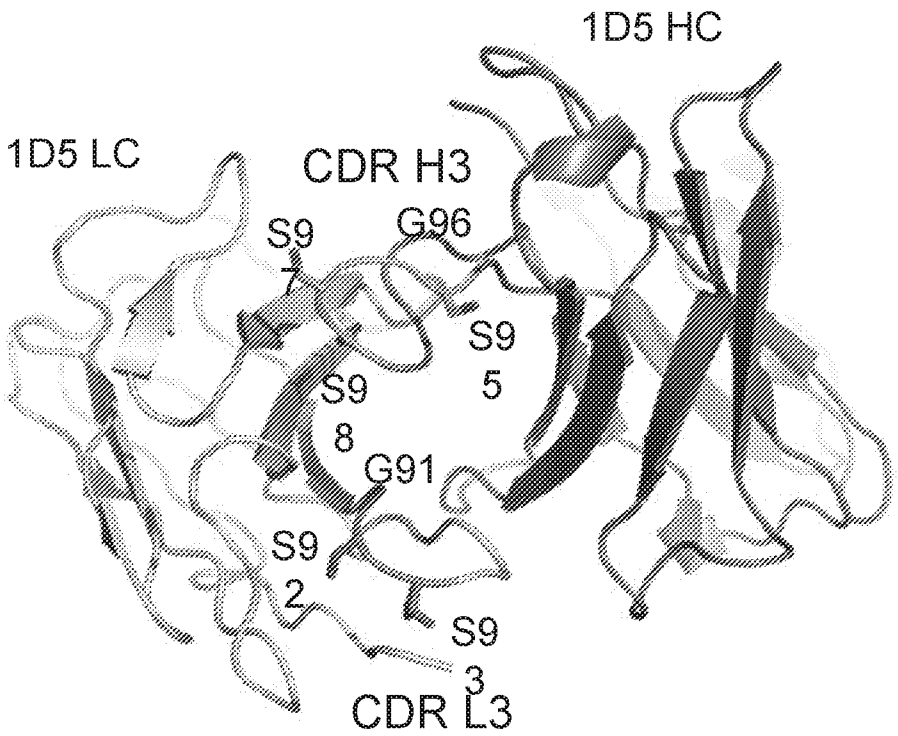


FIG. 27D

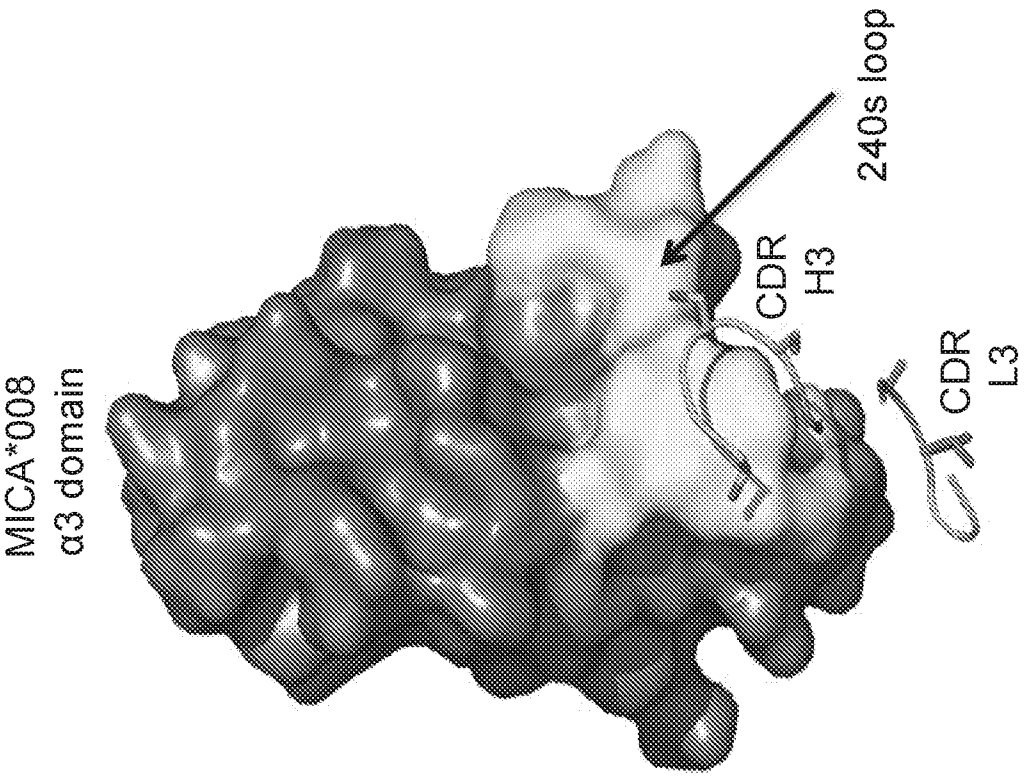


FIG. 27E

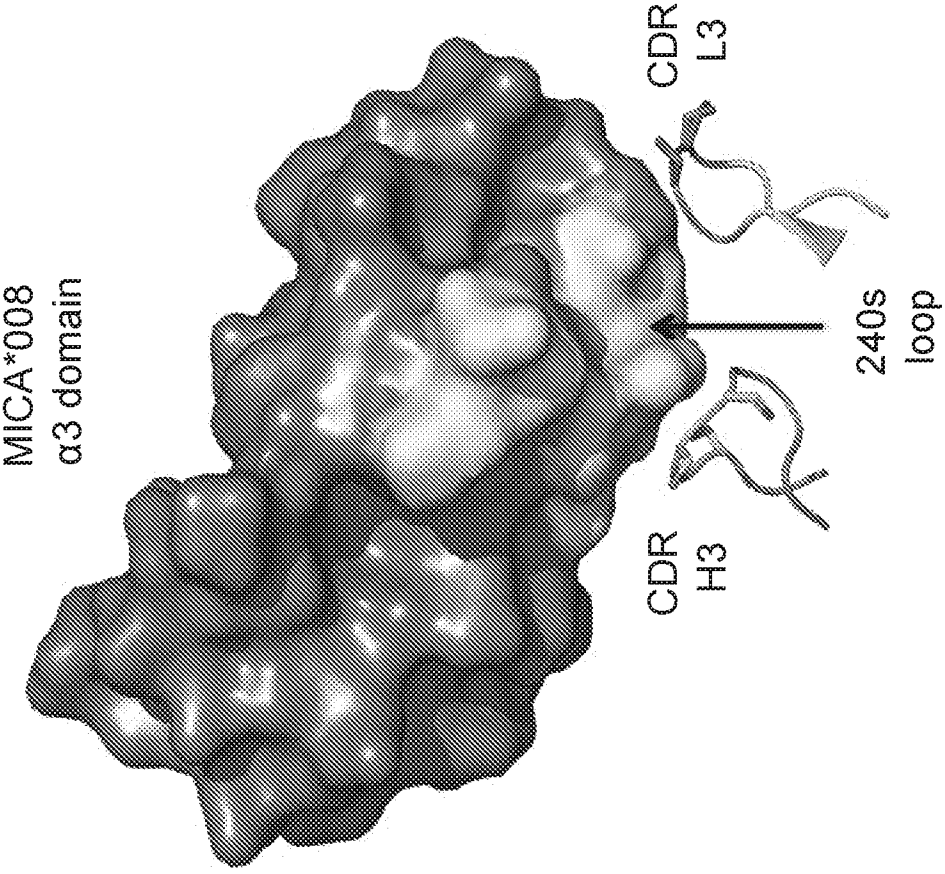


FIG. 27F

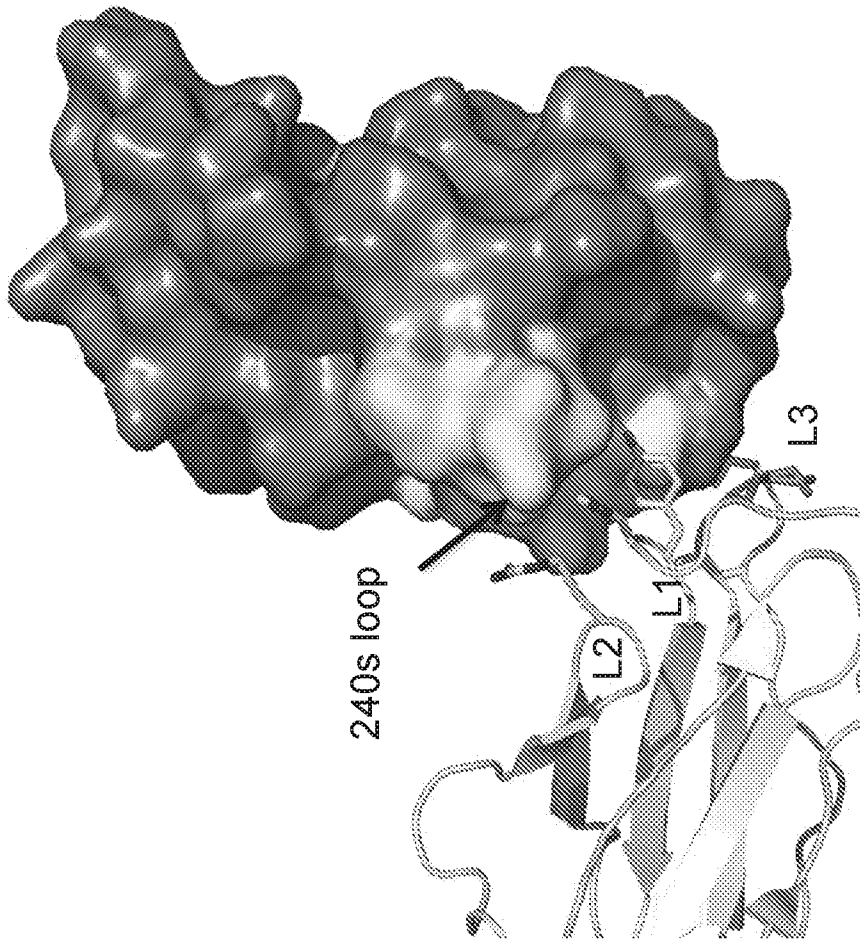
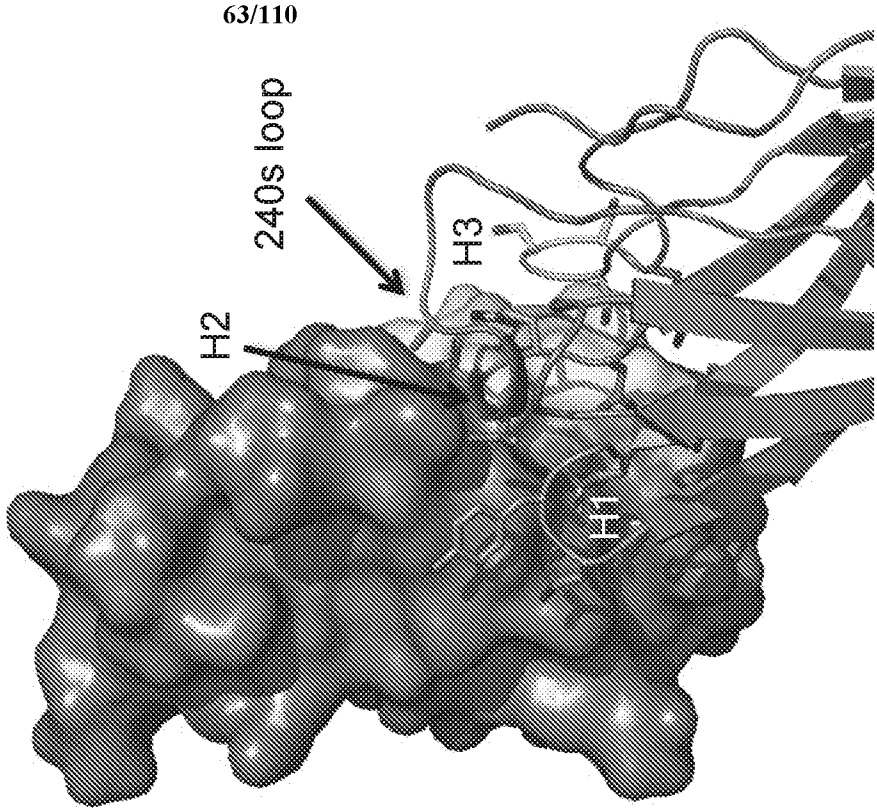


FIG. 27G



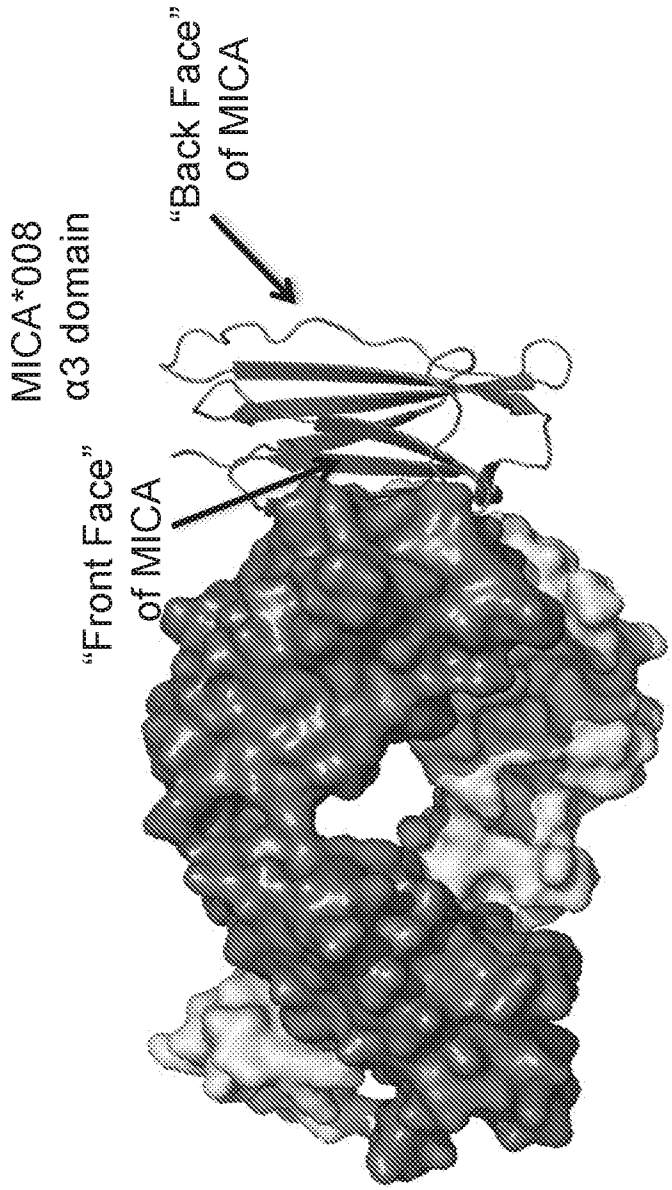


FIG. 28A

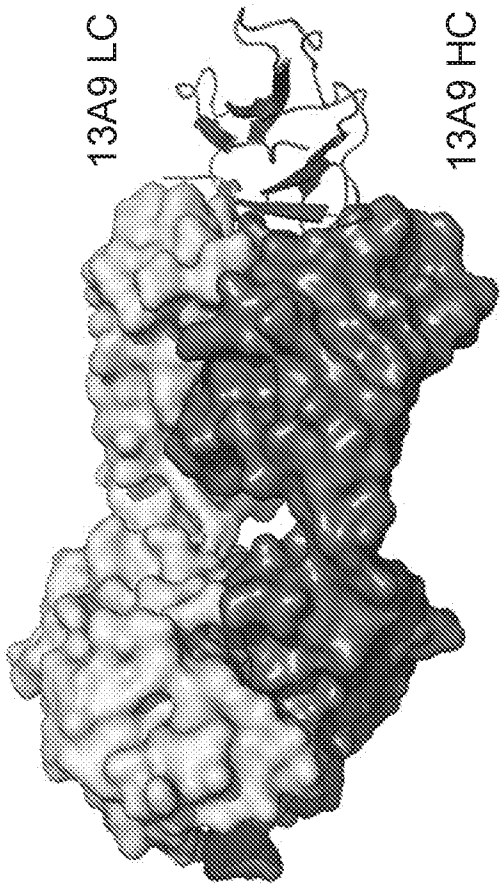


FIG. 28B

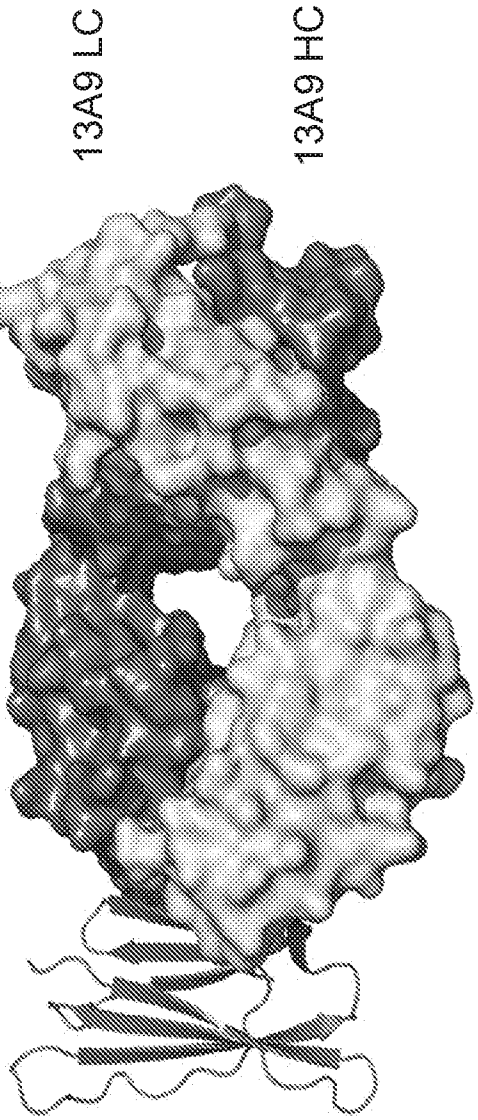


FIG. 28C

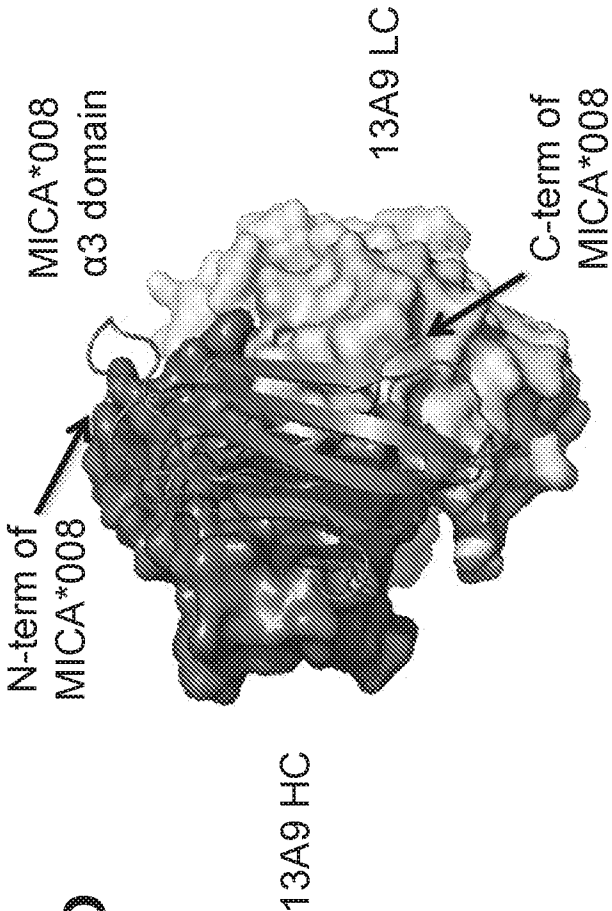


FIG. 28D

FIG. 29A

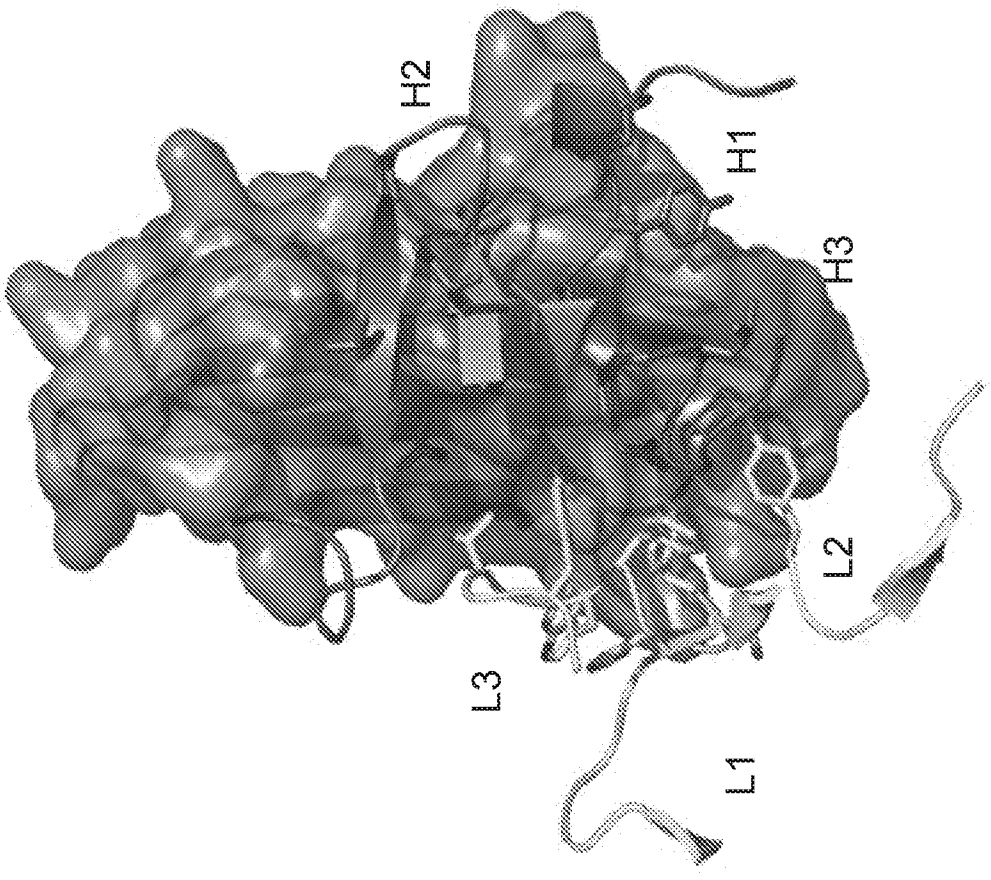


FIG. 29B

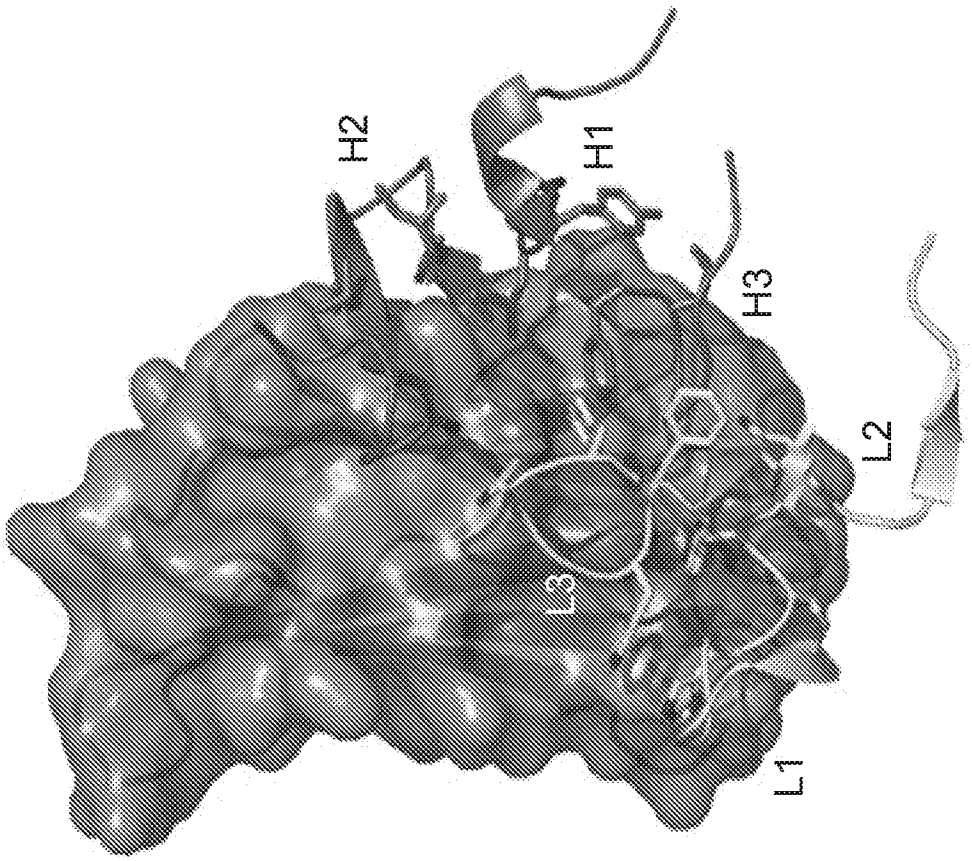


FIG. 30A

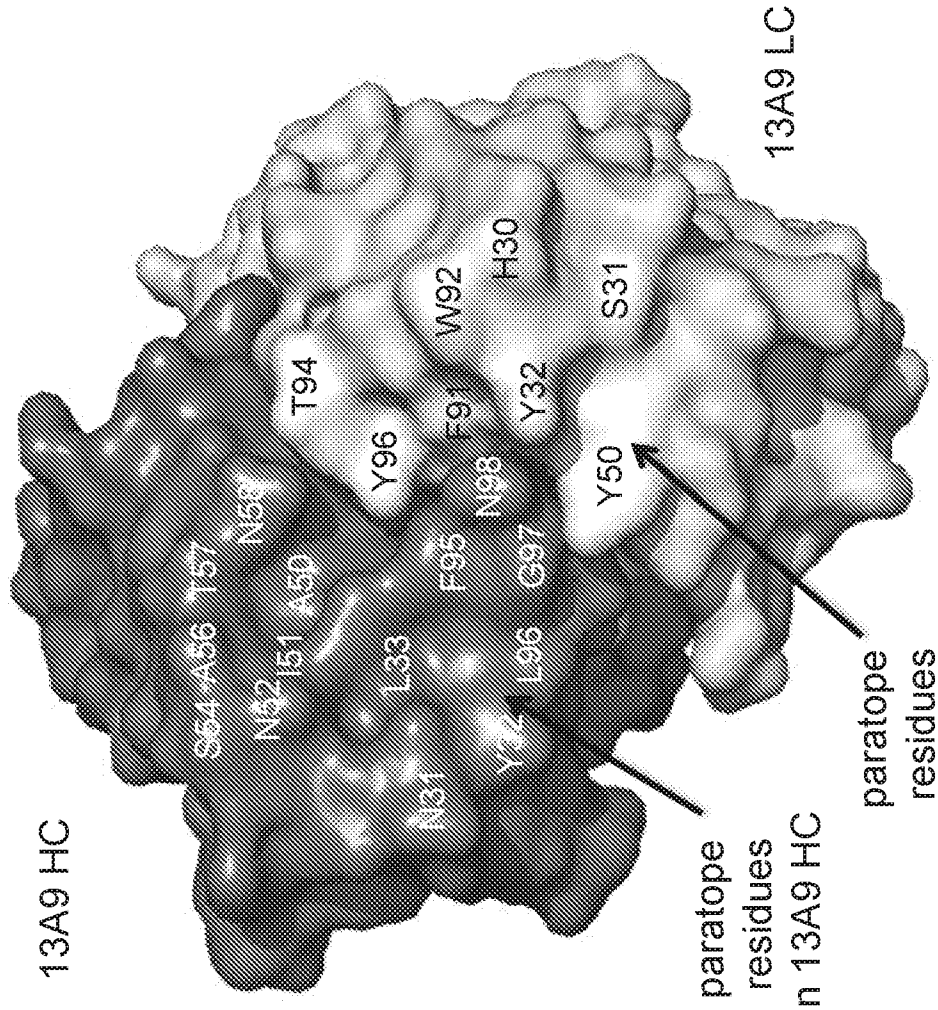


FIG. 30B

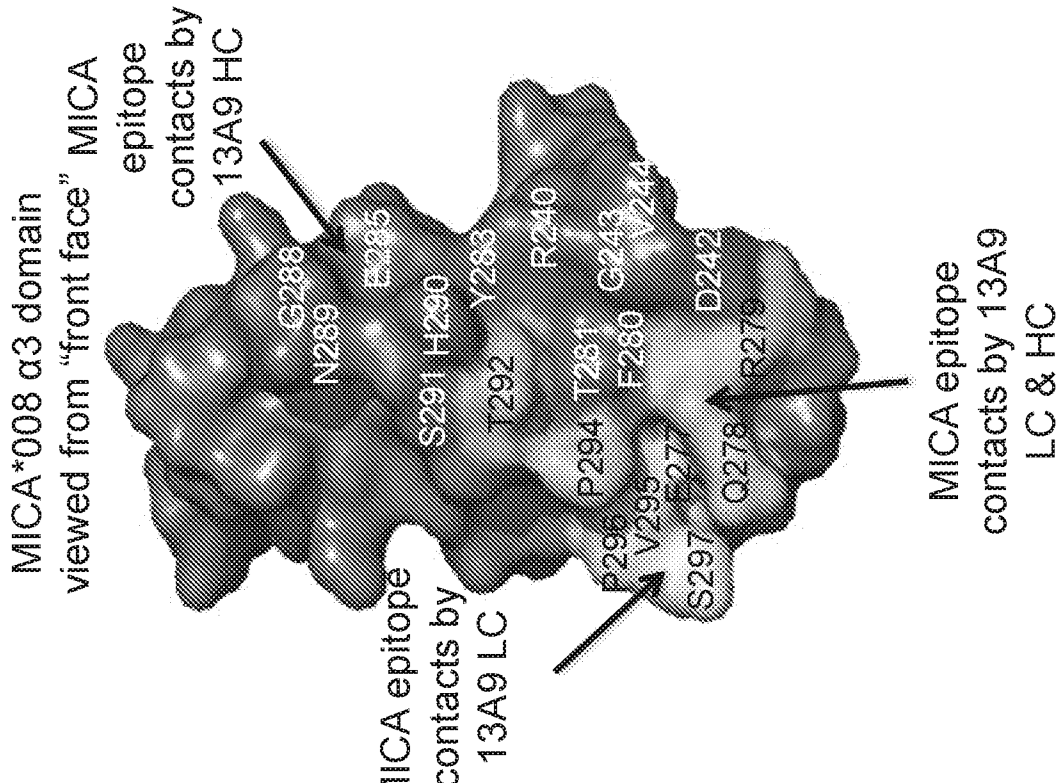


FIG. 30C

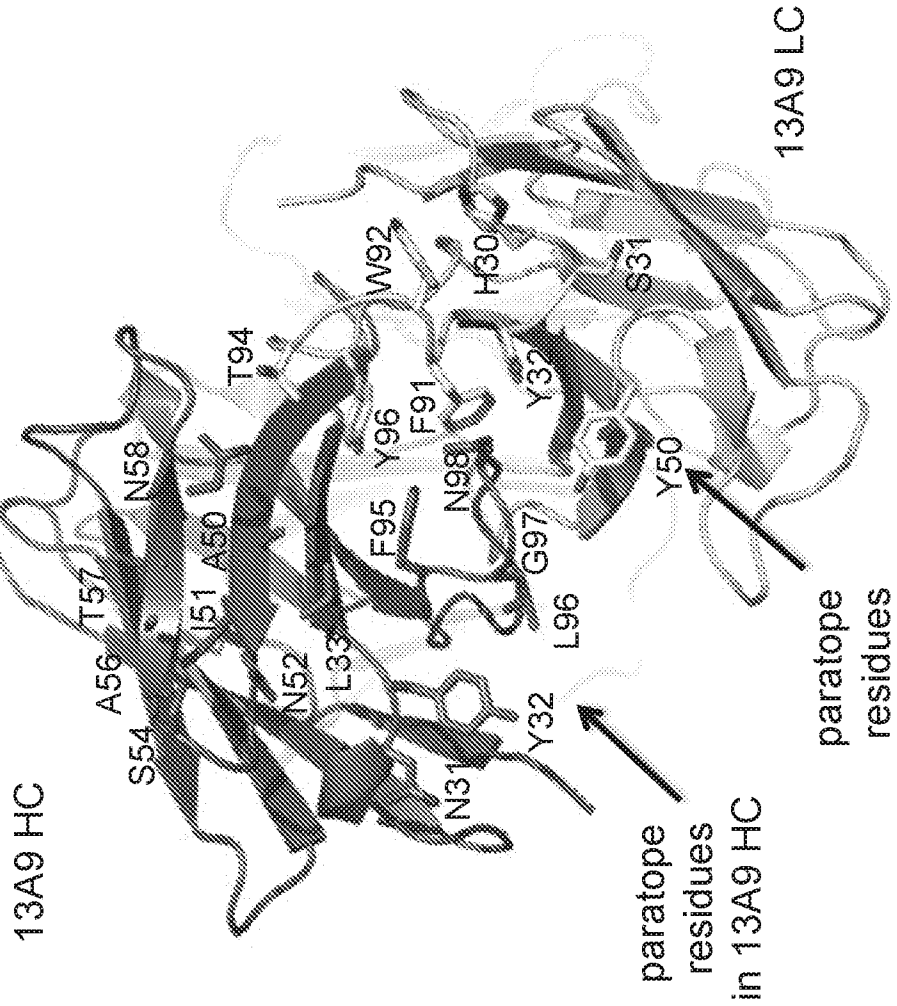


FIG. 30D

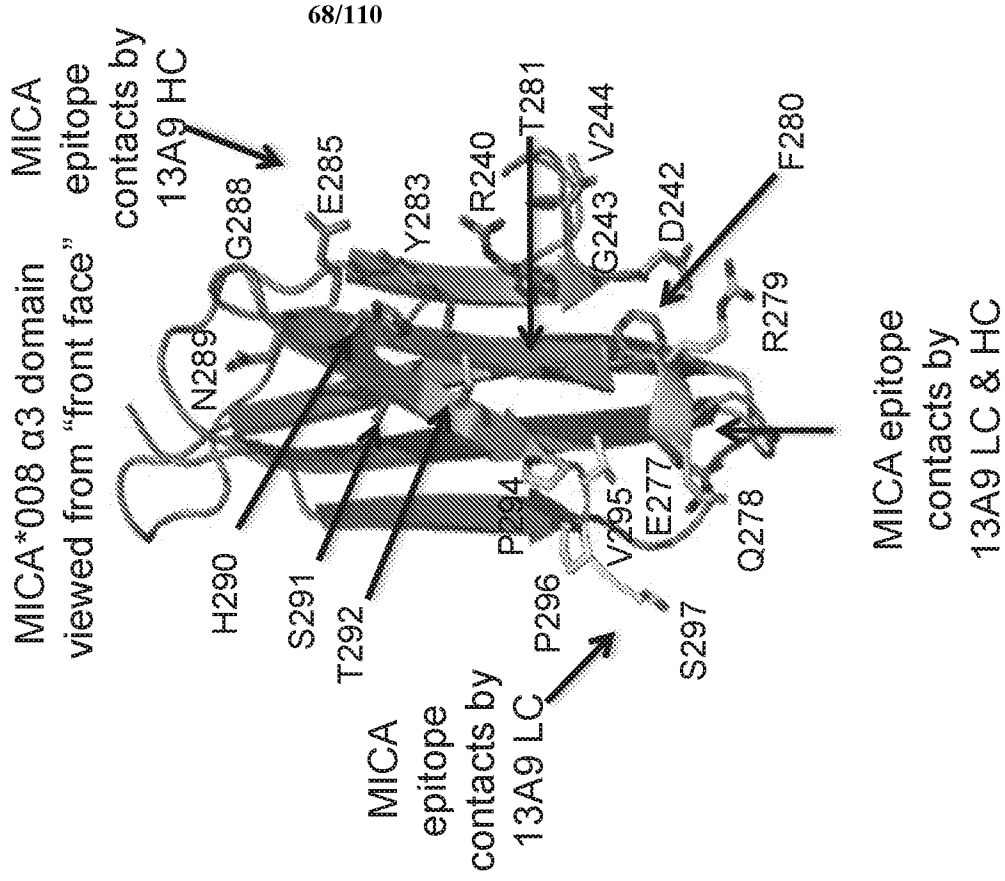
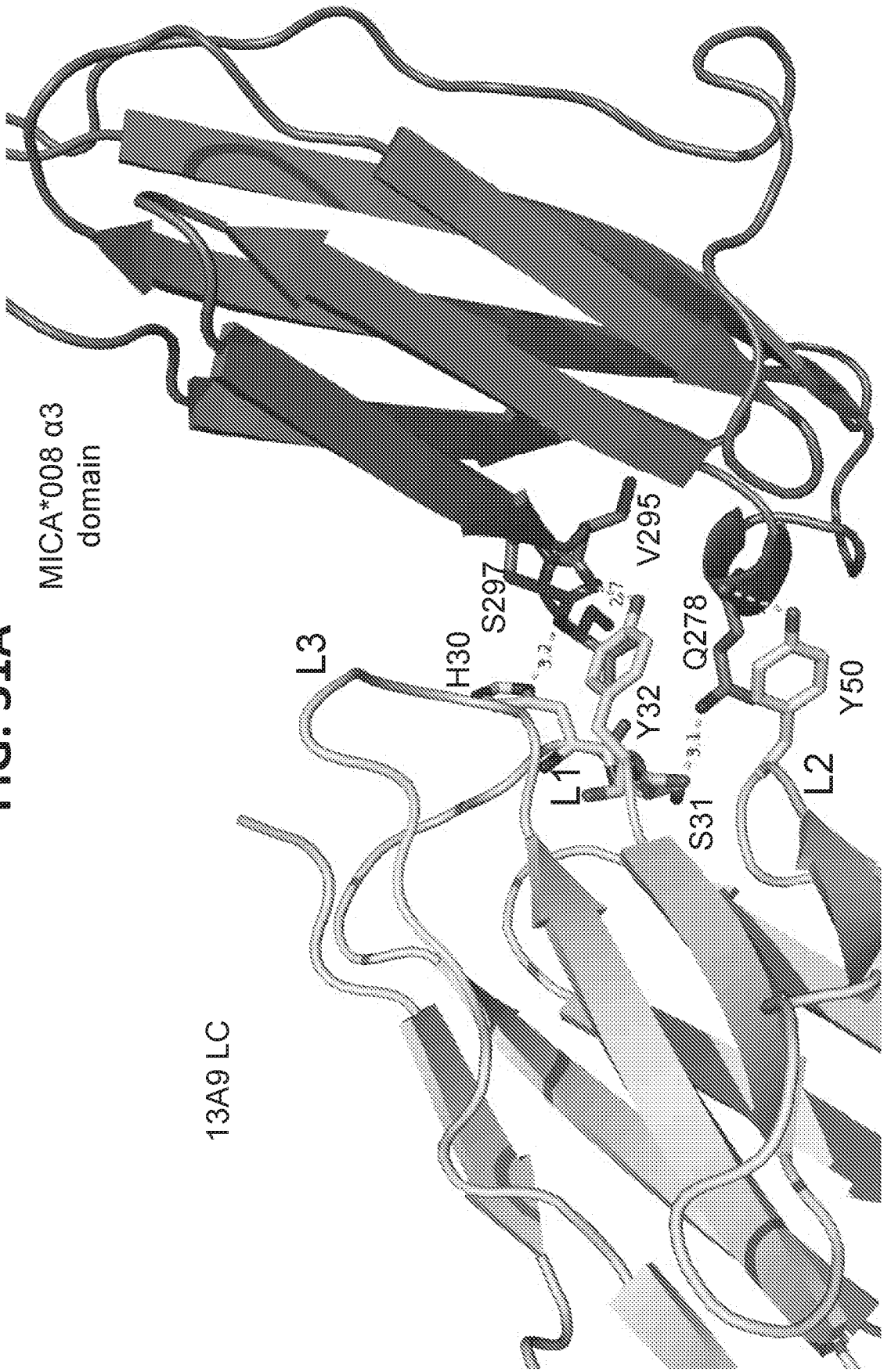


FIG. 31A


MICA*008 α3 domain
13A9 LC
L3
H30
S297
V295
Y32
L1
S31
Q278
L2
Y50

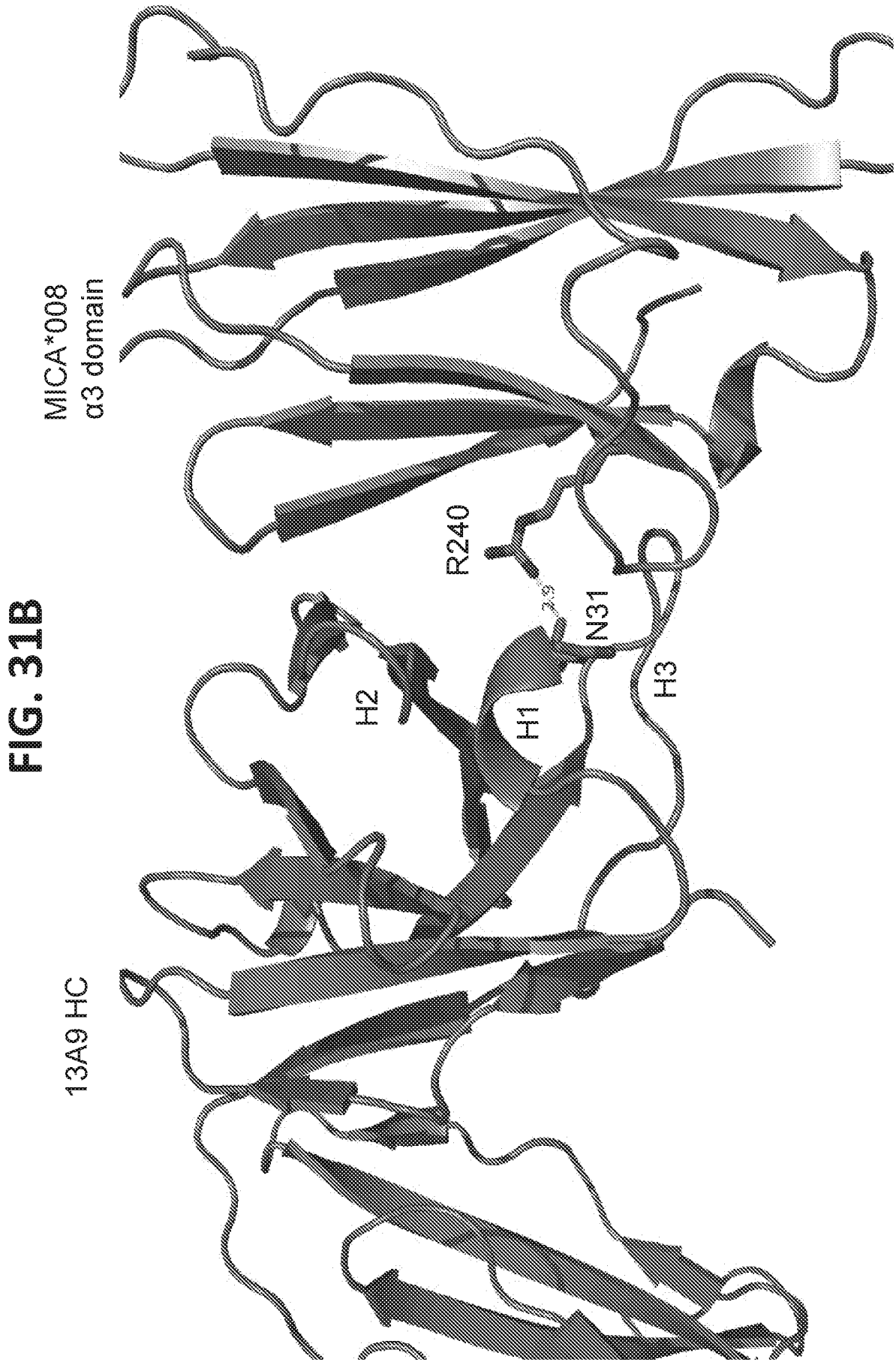


FIG. 31B

FIG. 31C

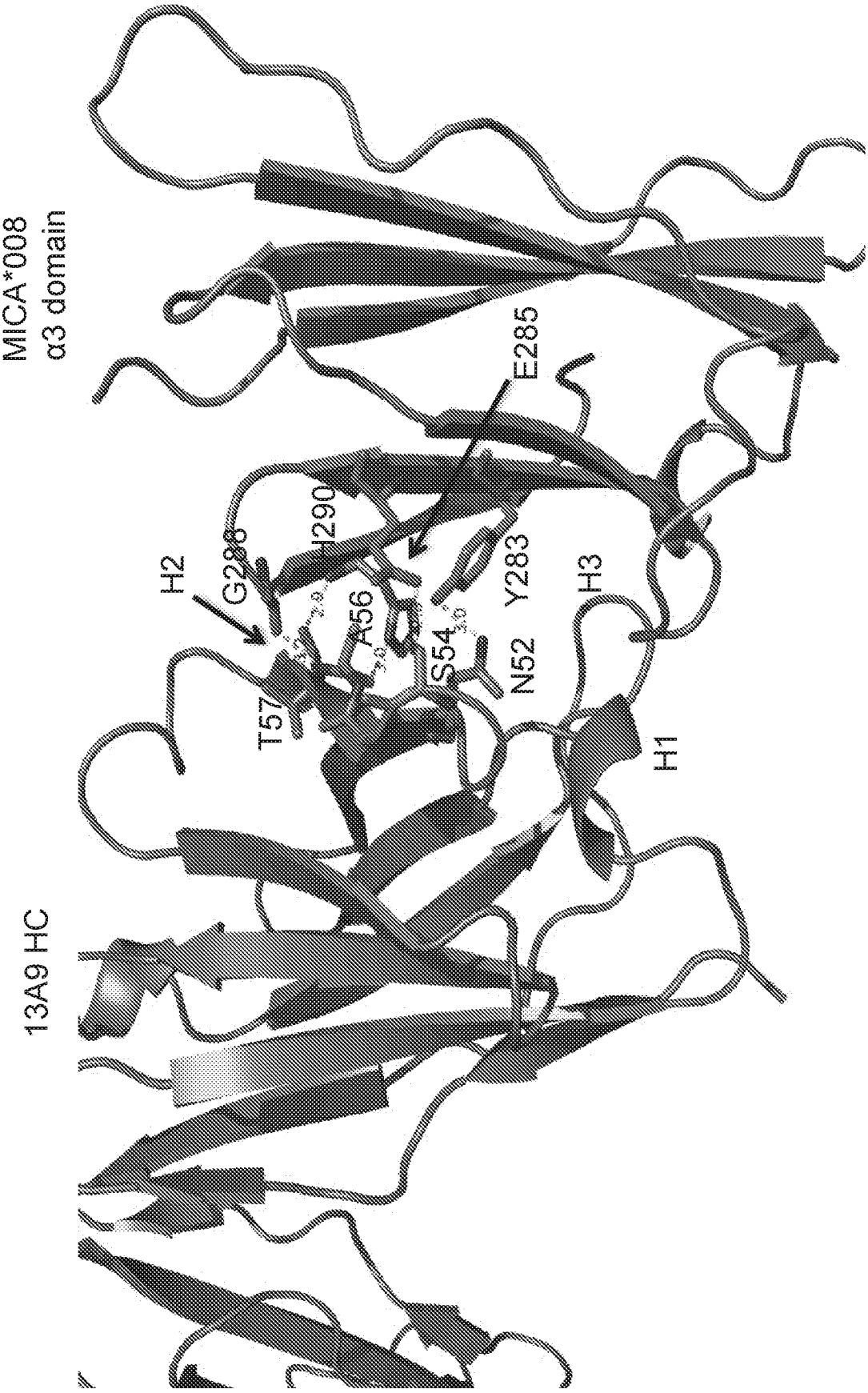

MICA*008
α3 domain
13A9 HC
H2
G288
H290
E285
T57
A56
S54
N52
Y283
H3
H1

FIG. 31D

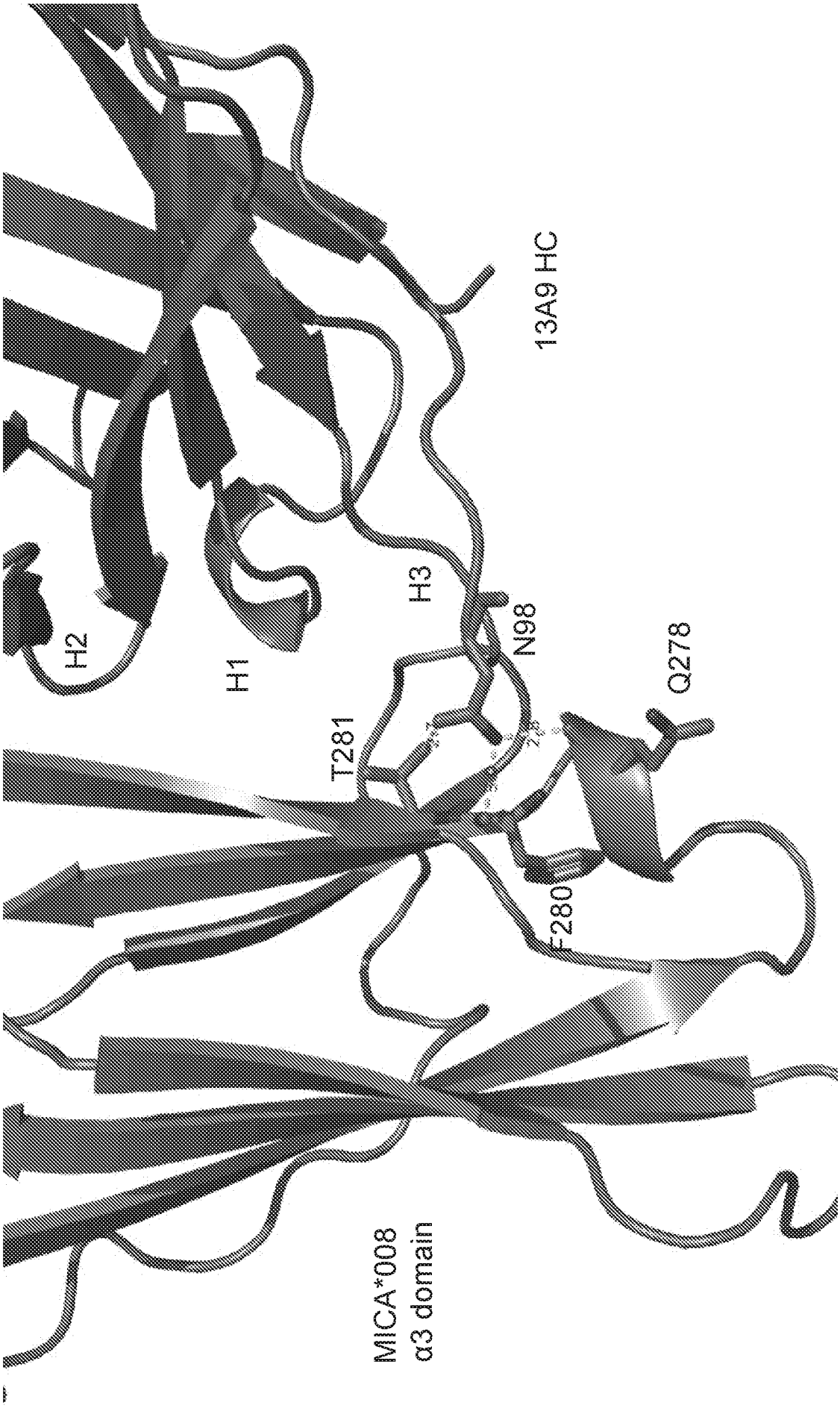


FIG. 32A

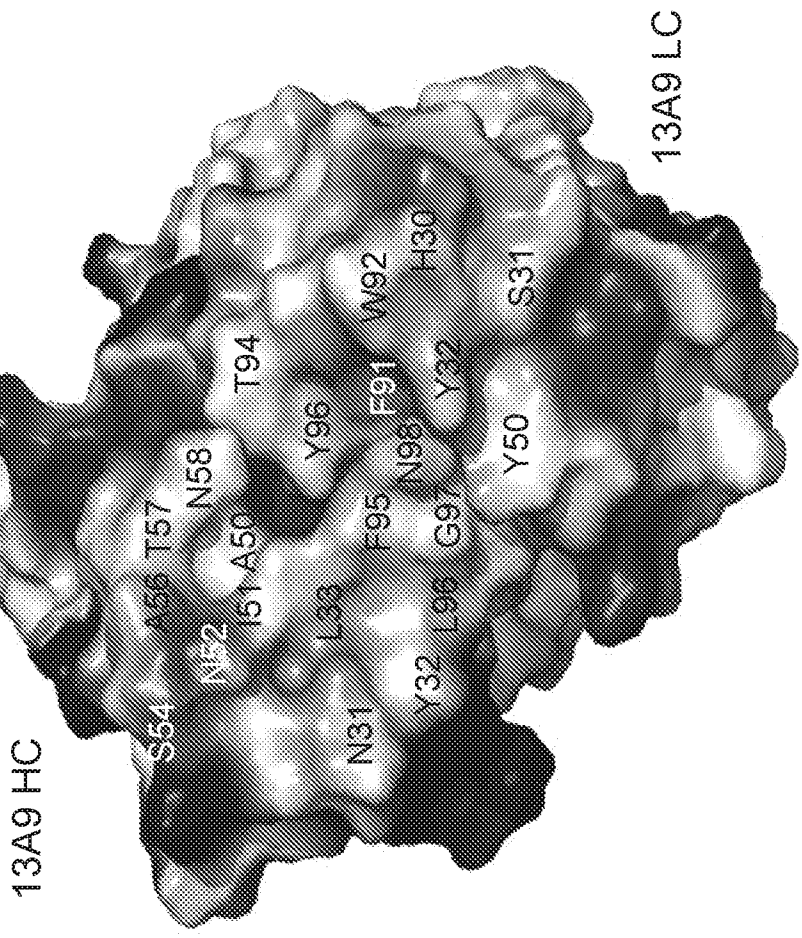


FIG. 32B

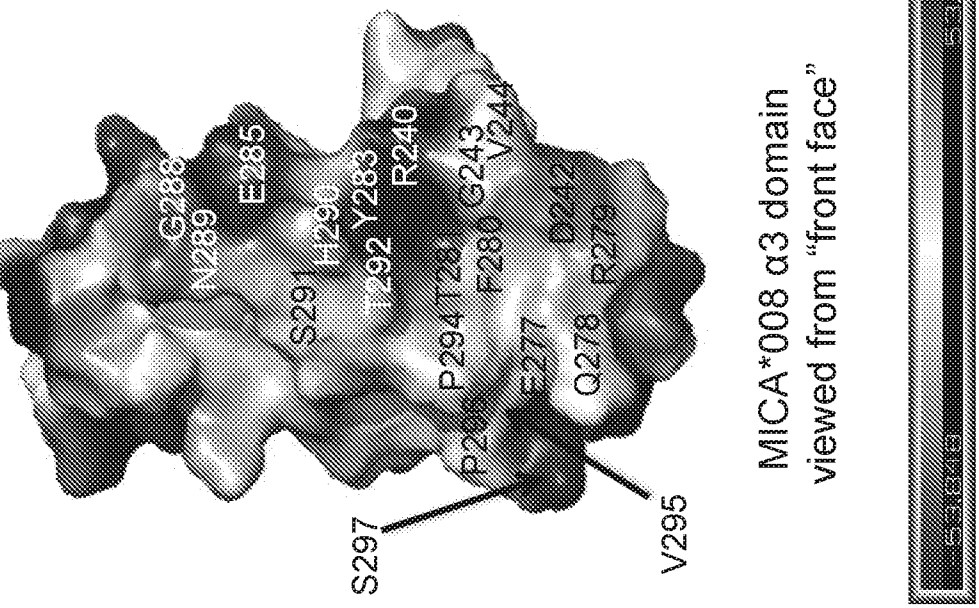


FIG. 33A

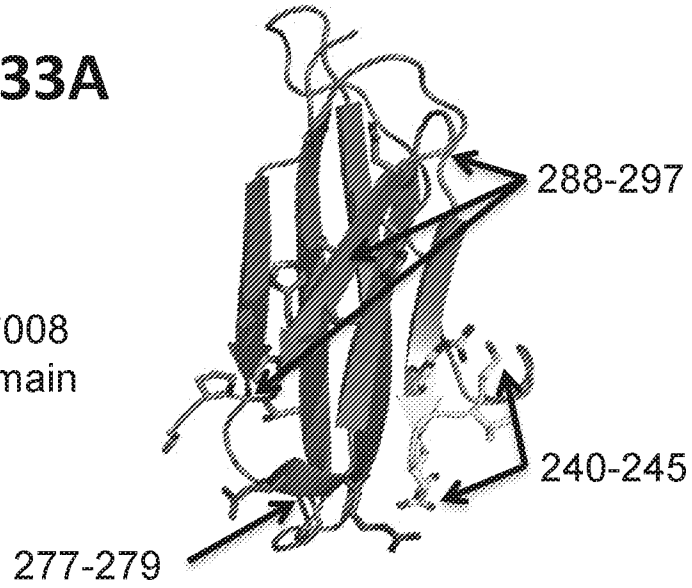


FIG. 33B

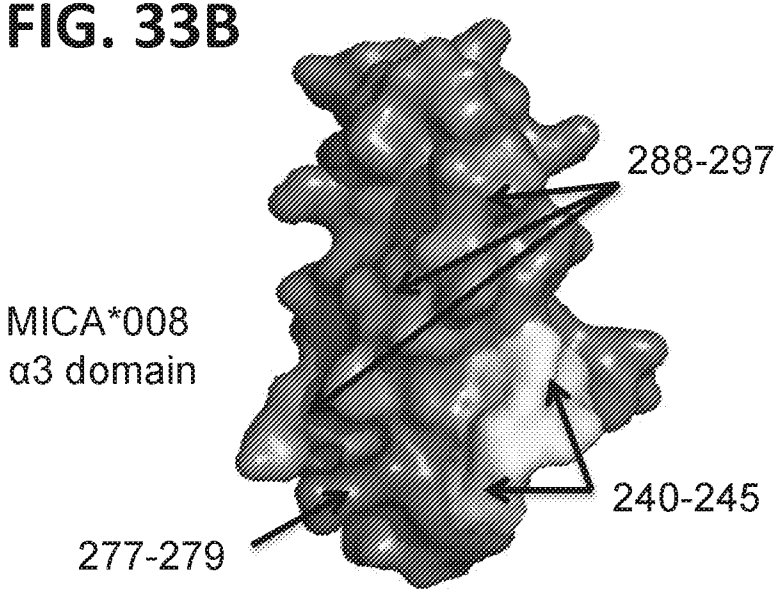


FIG. 33C

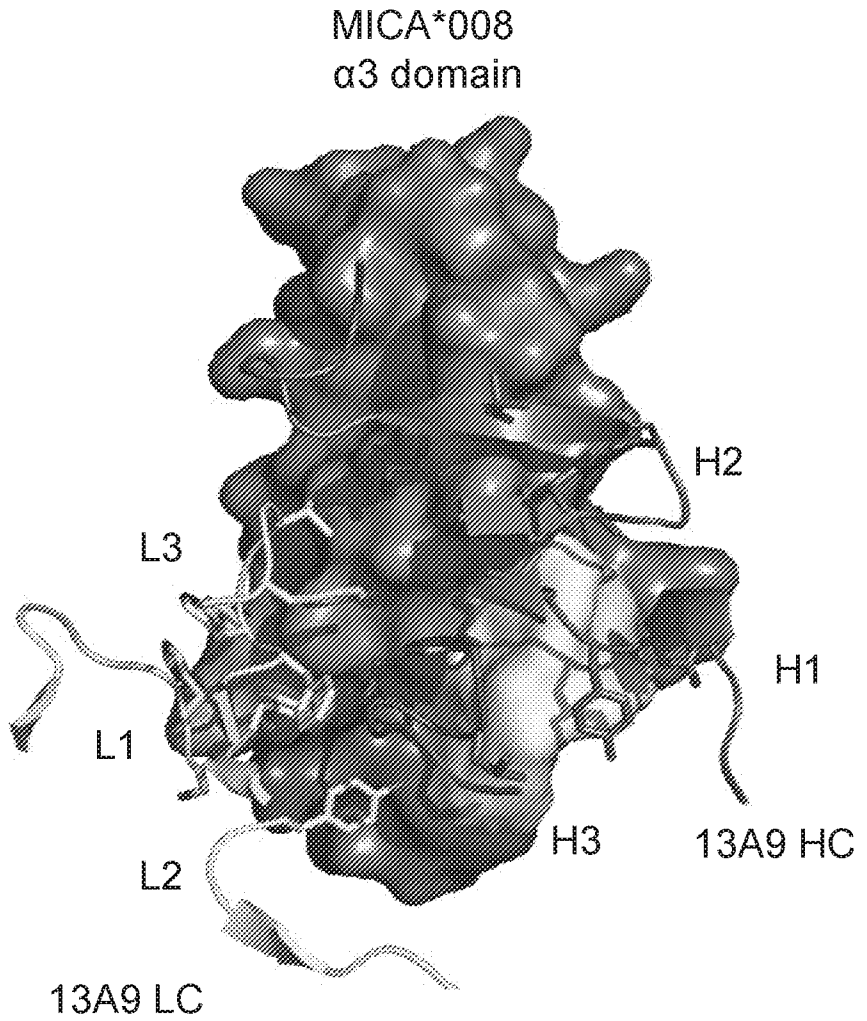


FIG. 33D

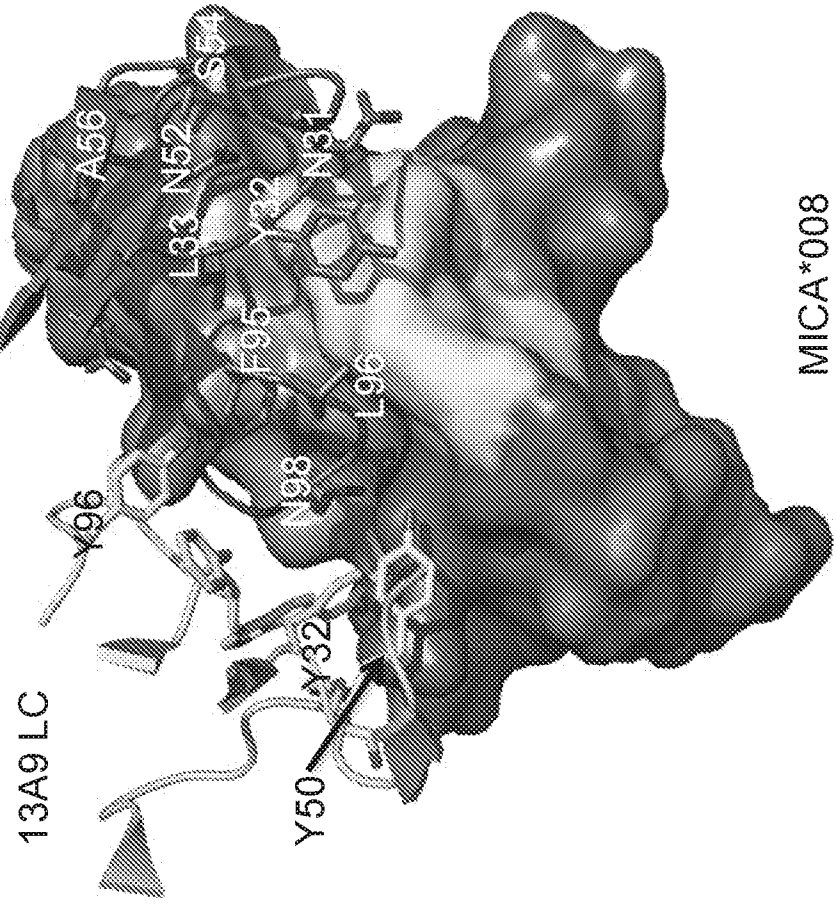
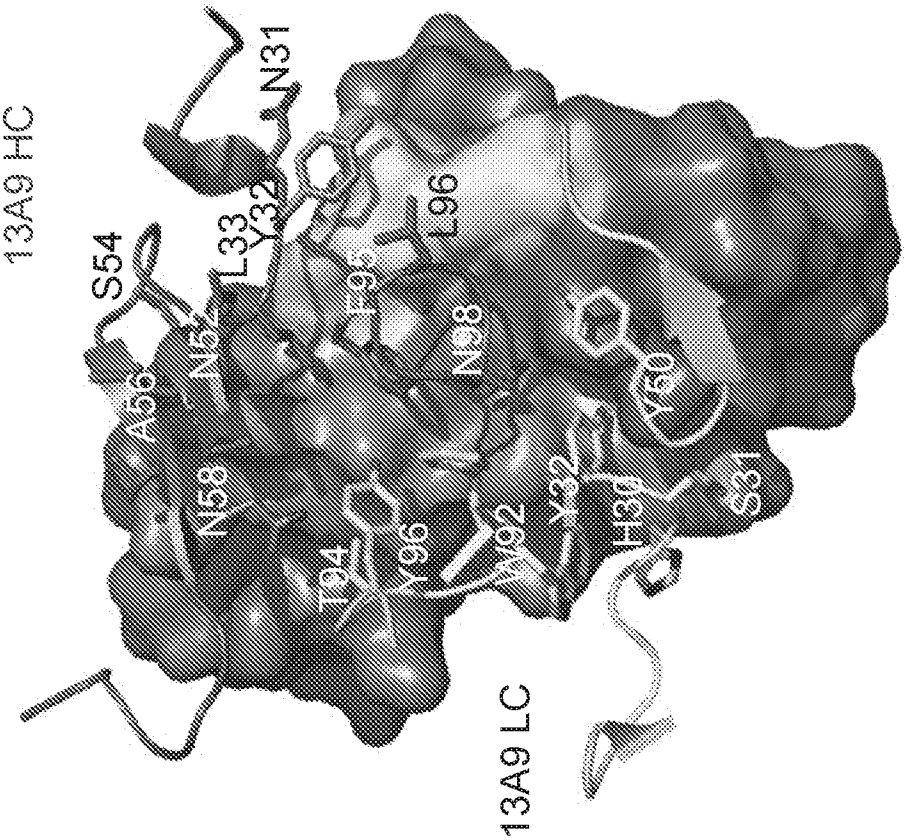


FIG. 33E



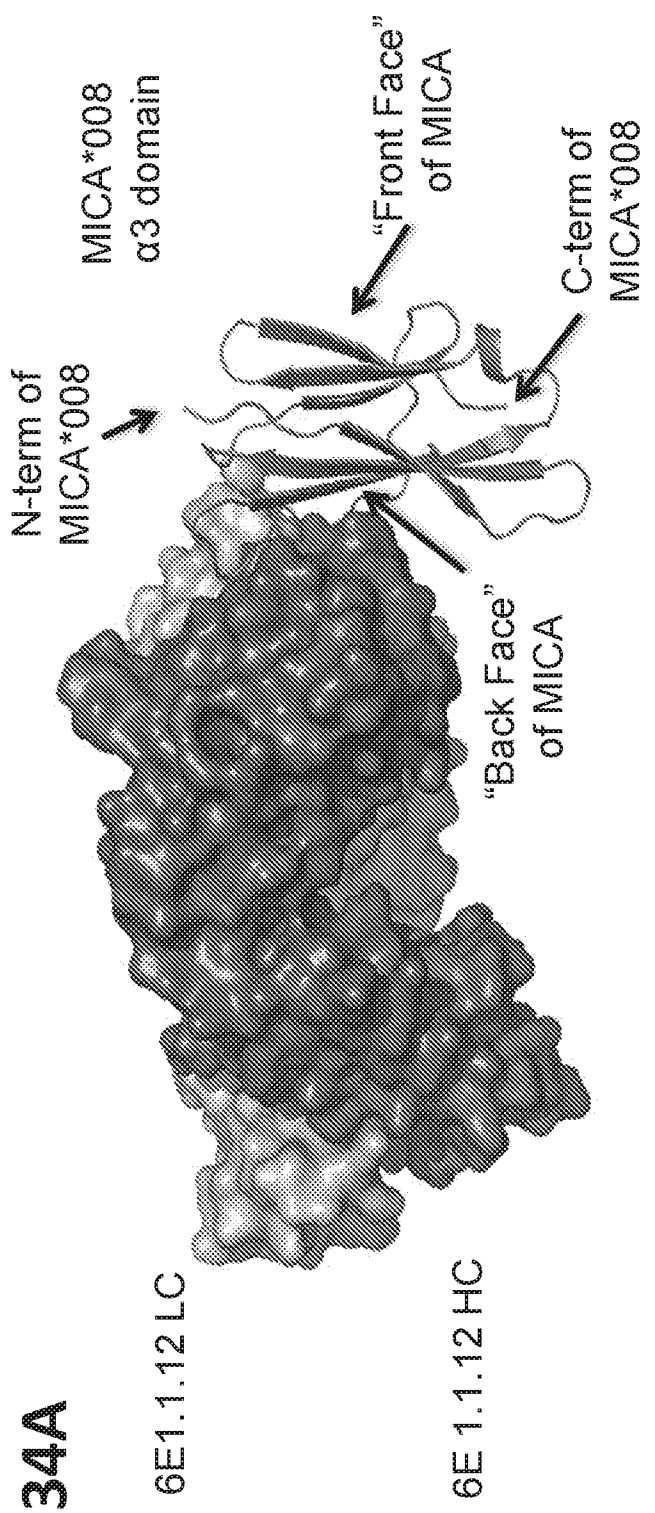


FIG. 34A

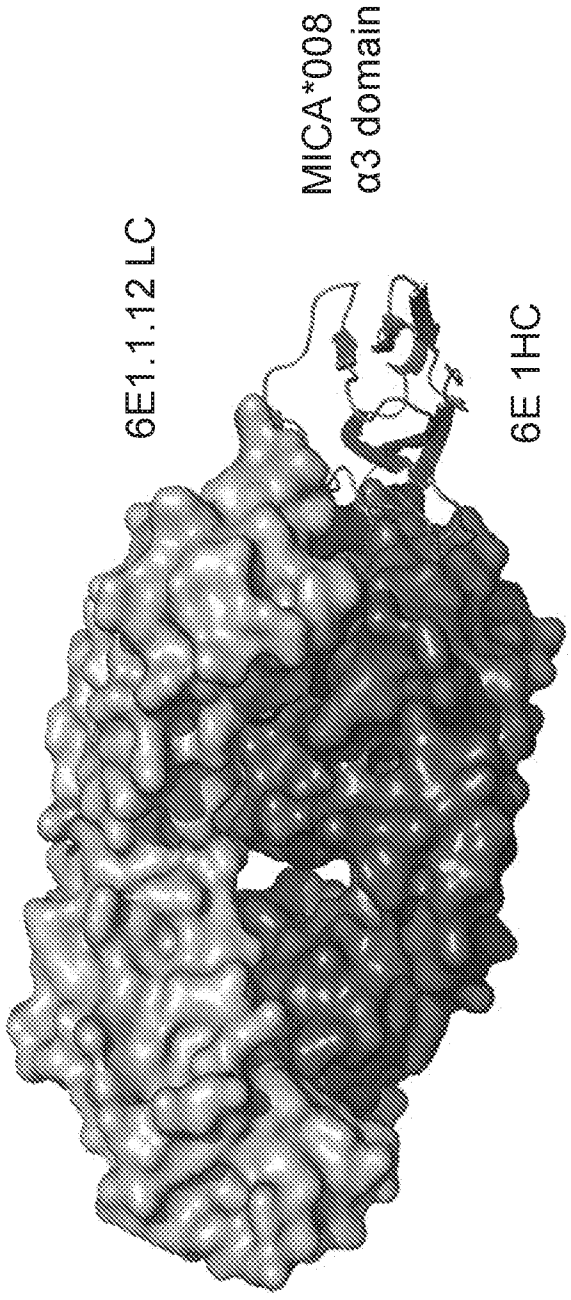


FIG. 34B

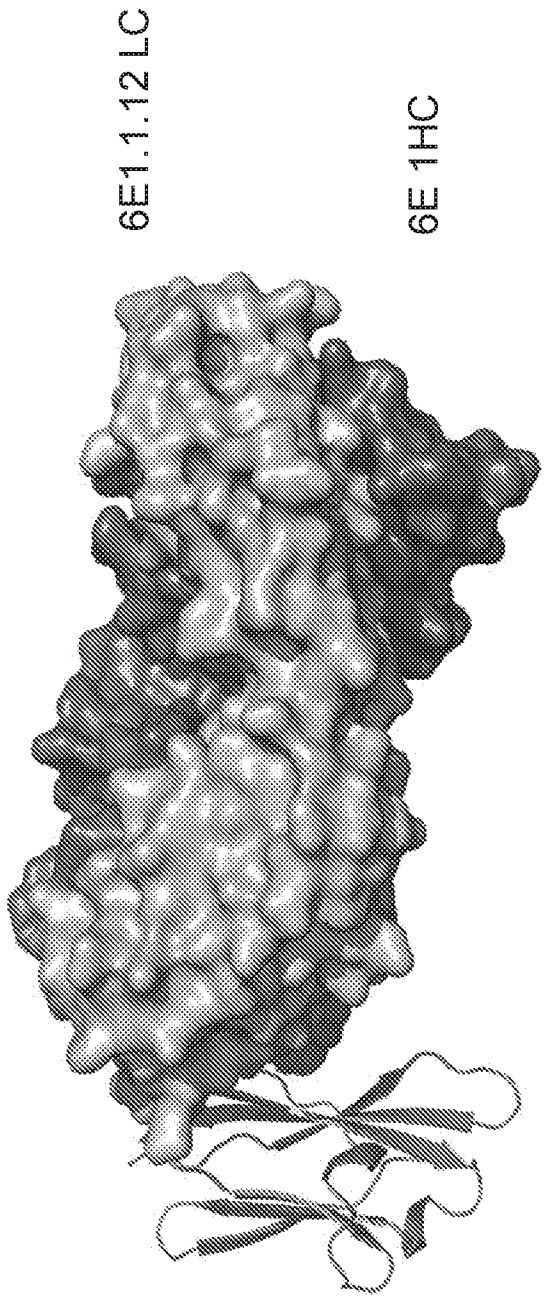


FIG. 34C

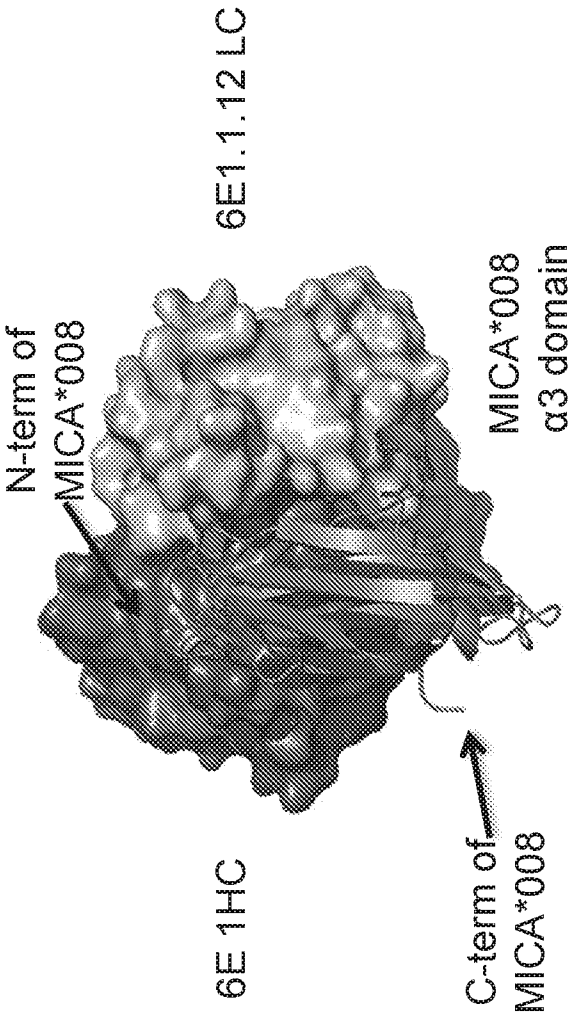


FIG. 34D

FIG. 35A

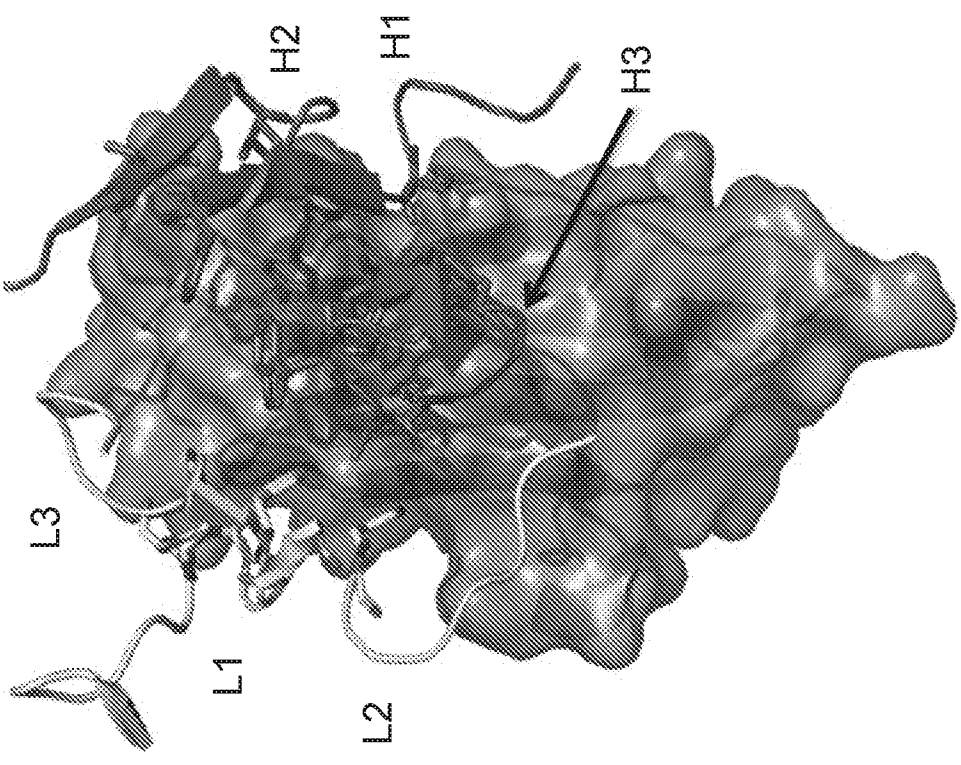


FIG. 35B

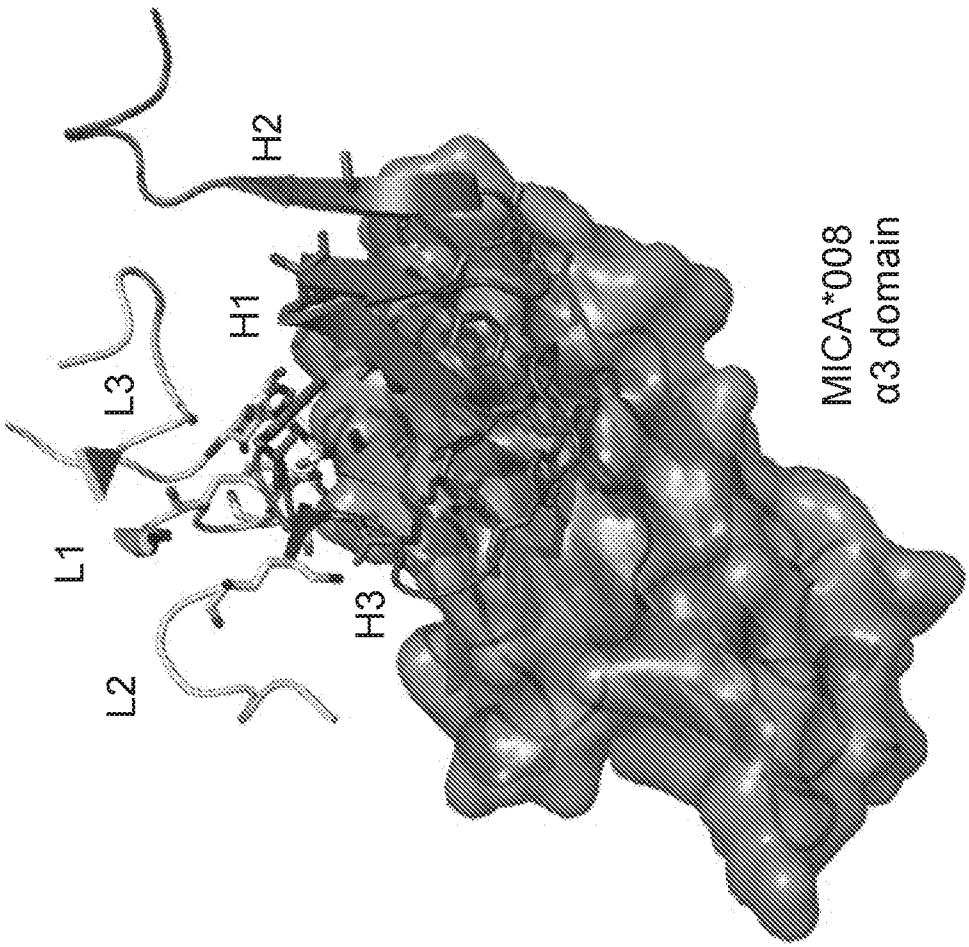


FIG. 36A

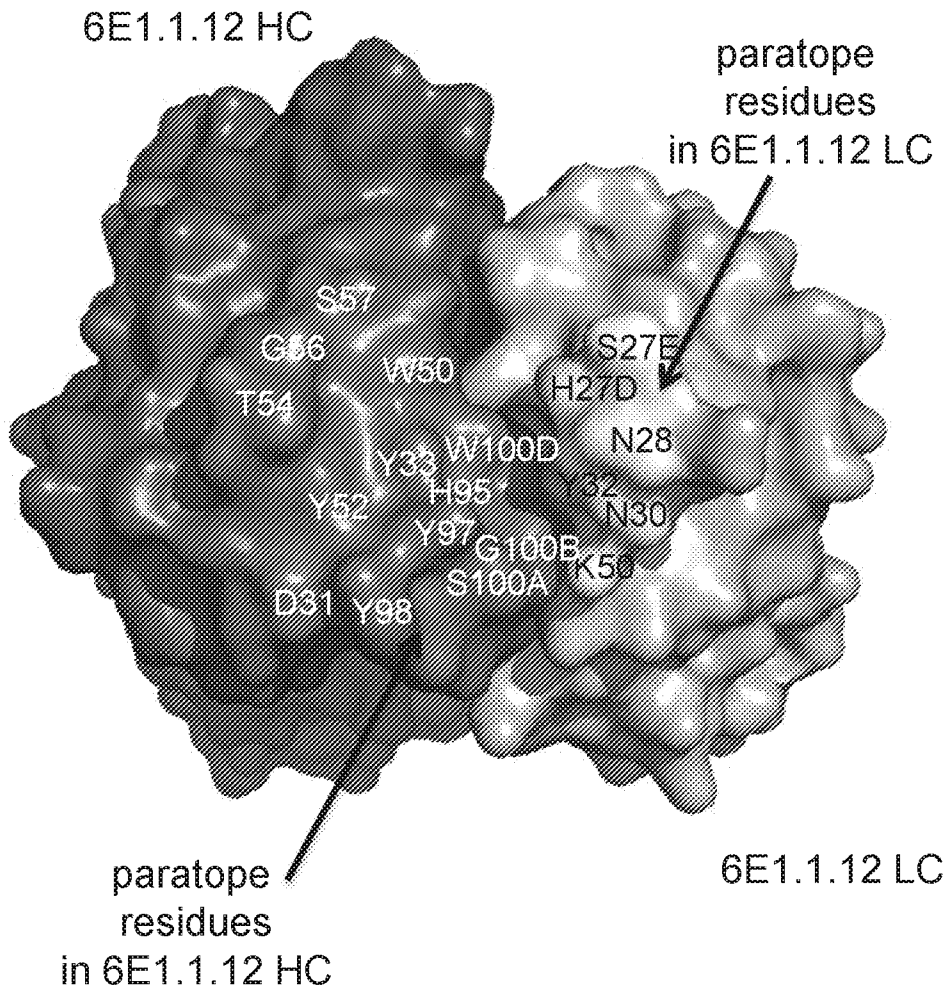


FIG. 36B

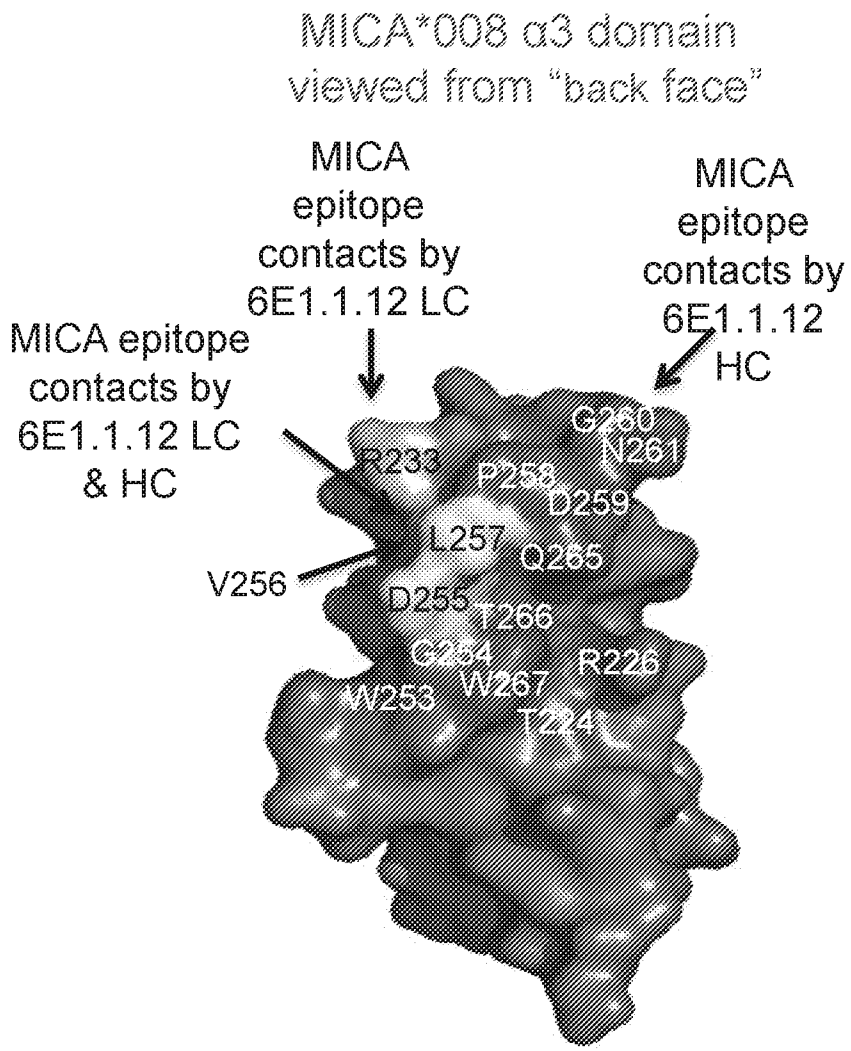


FIG. 36C

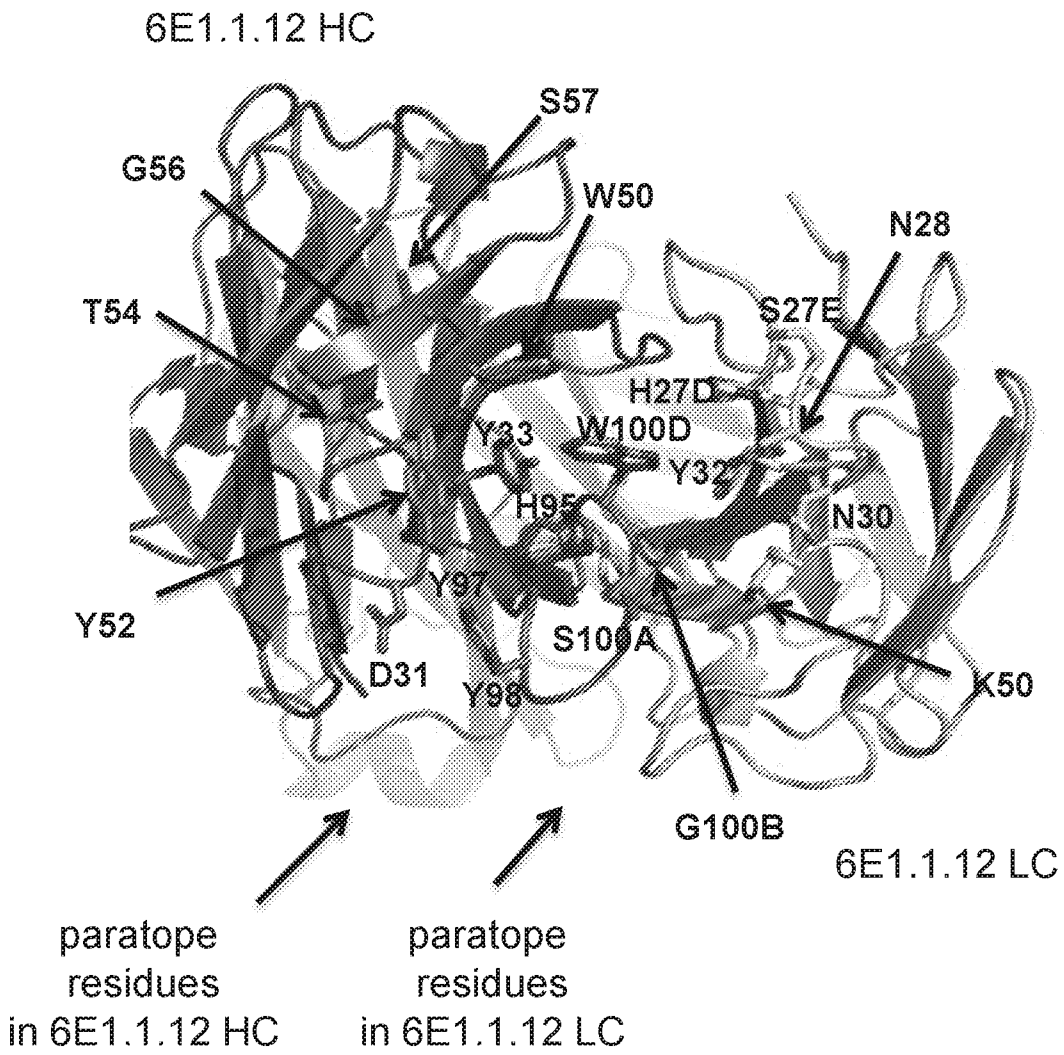


FIG. 36D

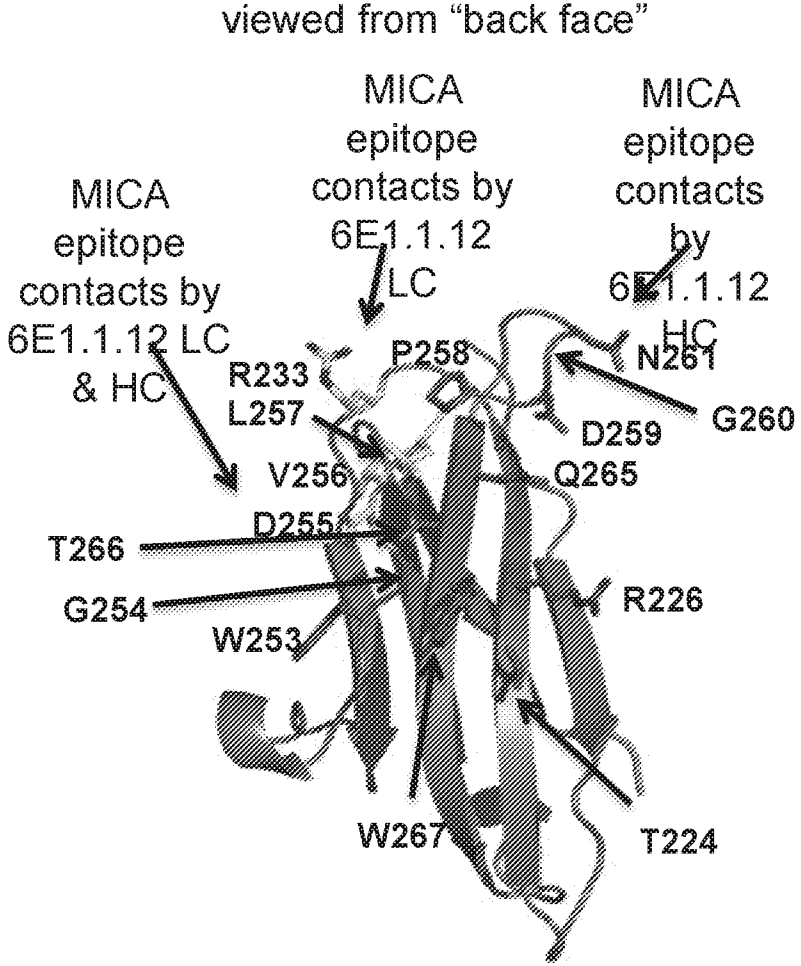


FIG. 37A

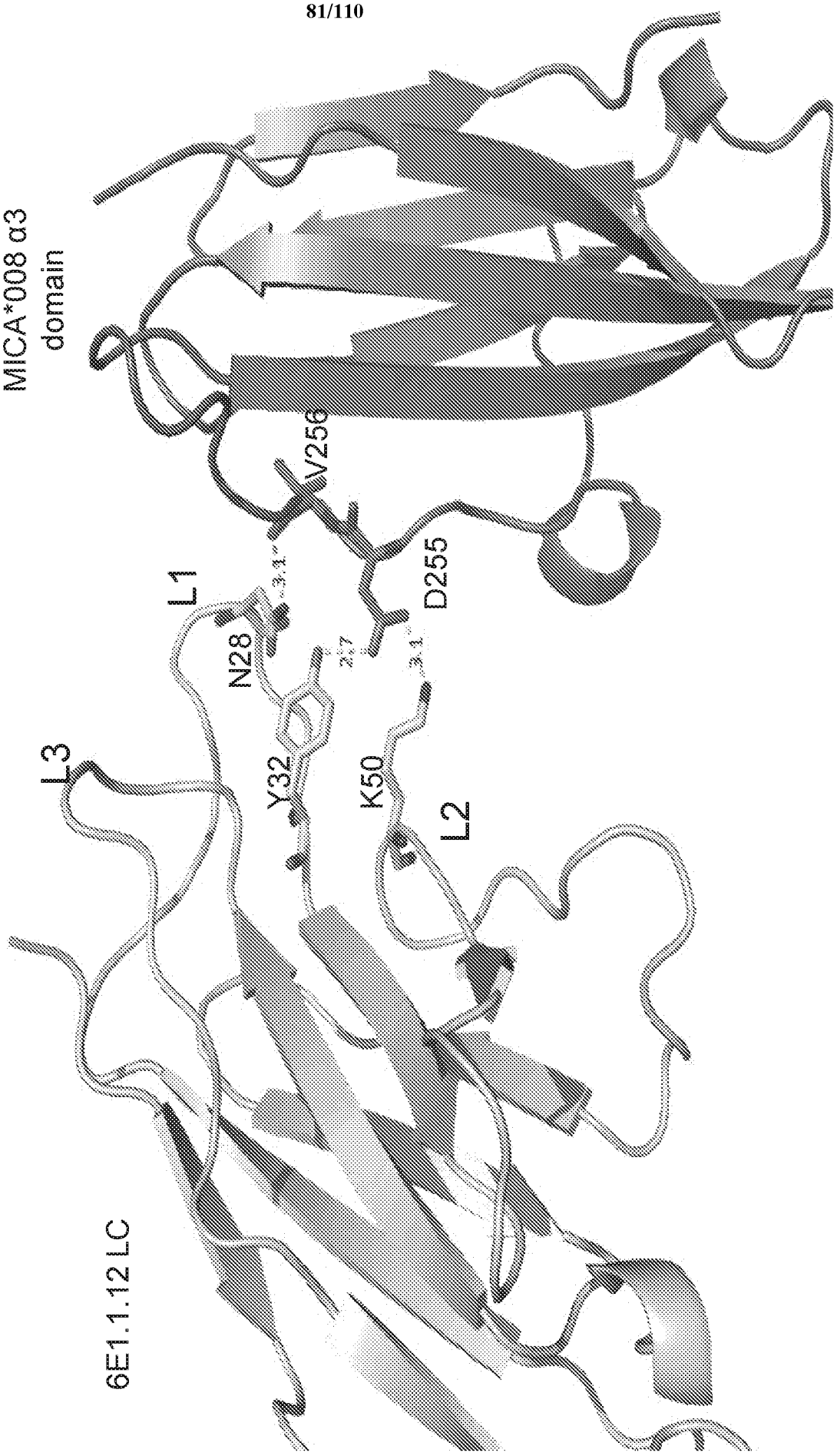

MICA*008 α3 domain
V256
D255
L1
N28
3.1
2.7
3.1
Y32
K50
L3
L2
6E1.1.12 LC

FIG. 37B

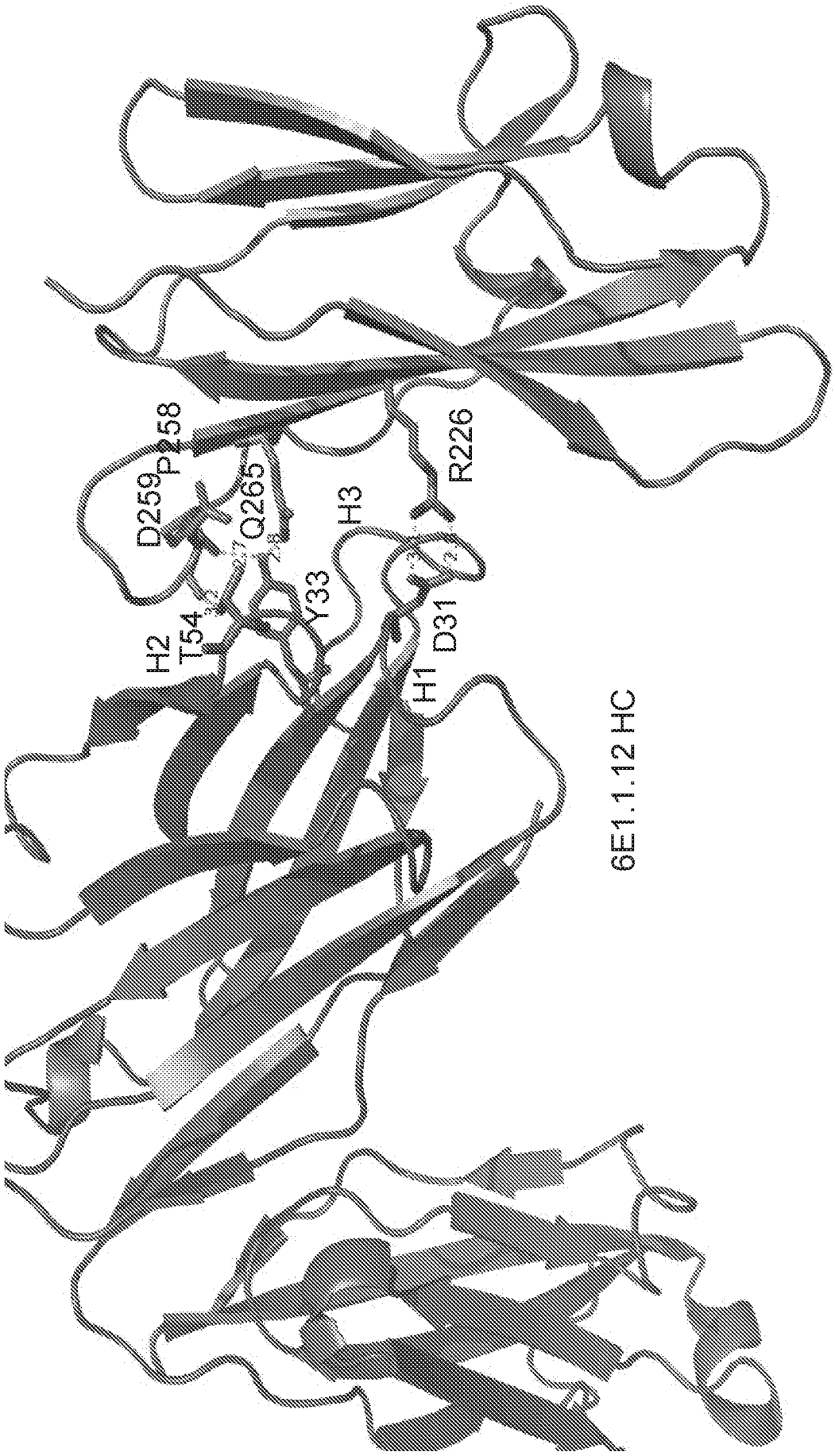

MICA*008 α3 domain
6E1.1.12 HC
D259
P258
Q265
H3
R226
H2
T54
Y33
D31
H1

FIG. 37C

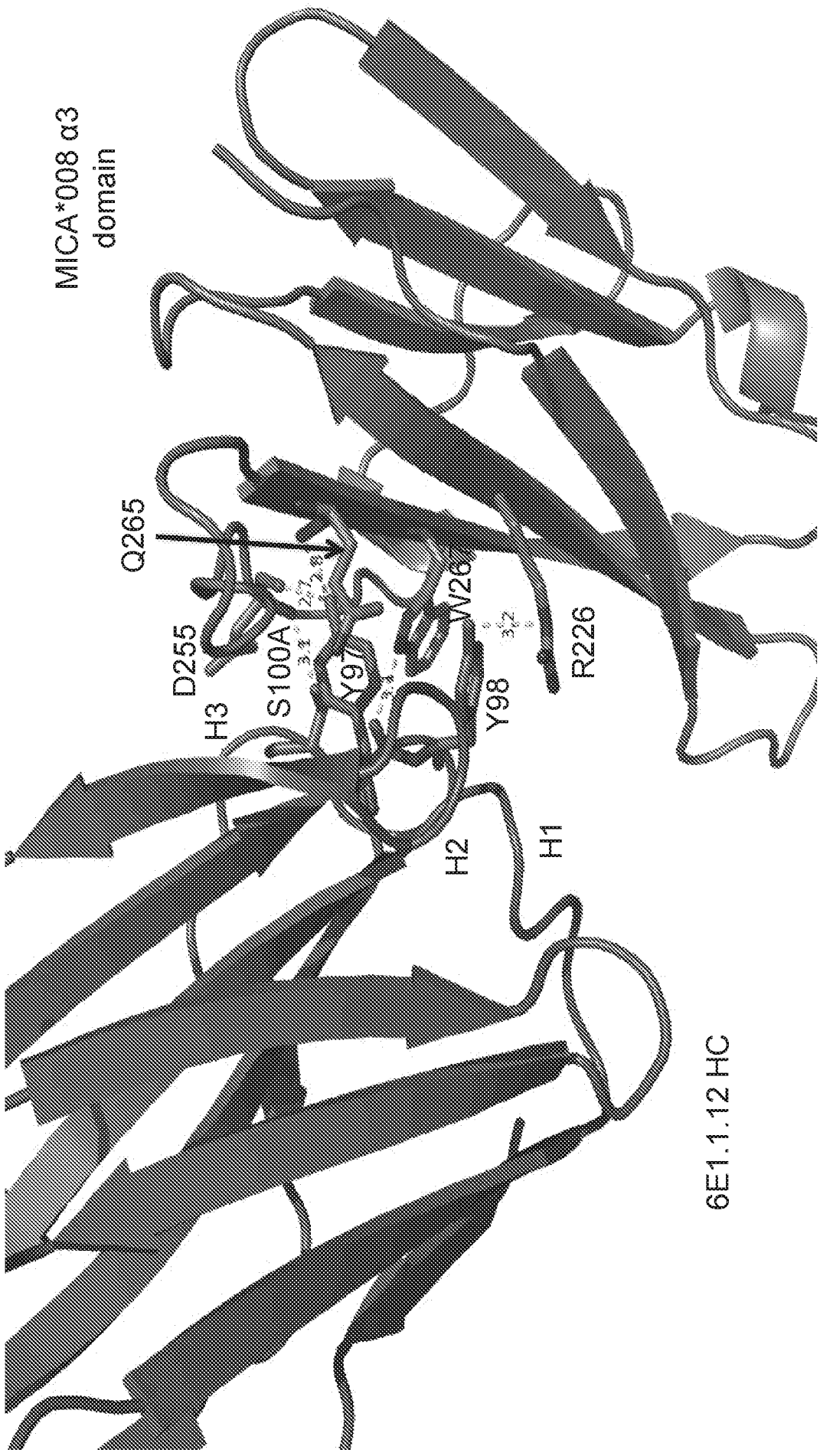

MICA*008 α3 domain
Q265
D255
S100A
Y97
W26
R226
Y98
H3
H2
H1
6E1.1.12 HC

FIG. 38A

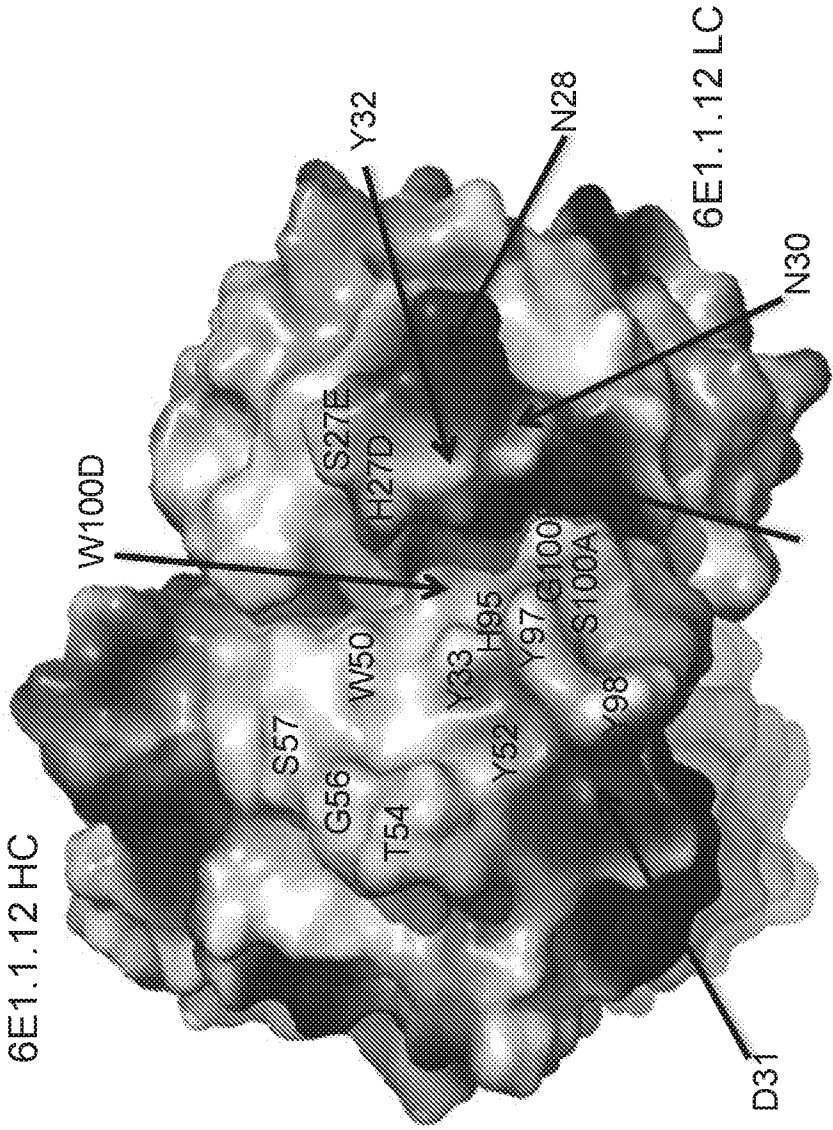


FIG. 38B

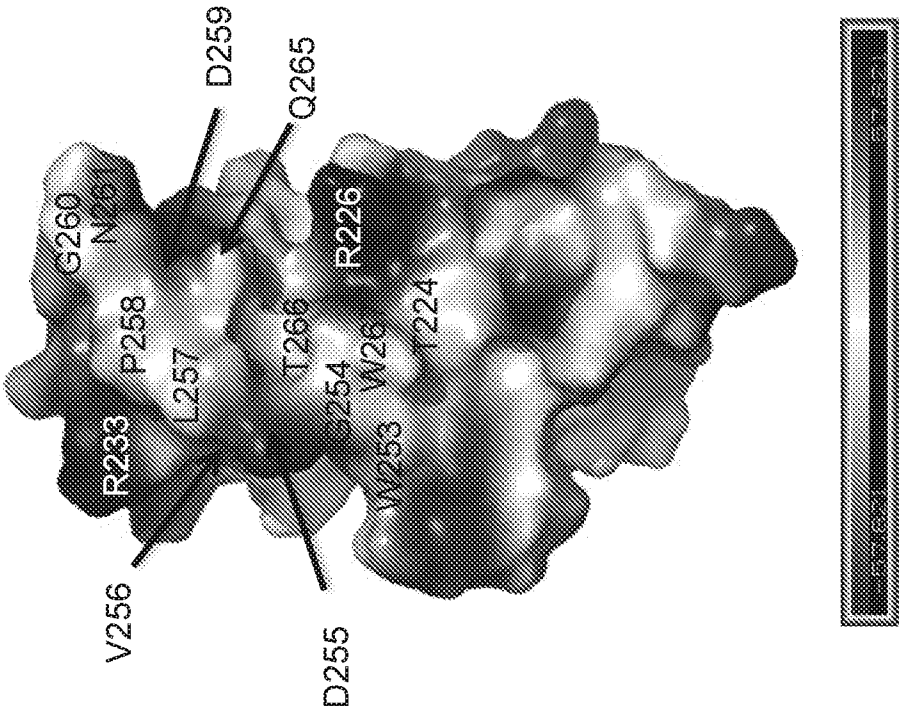


FIG. 39B

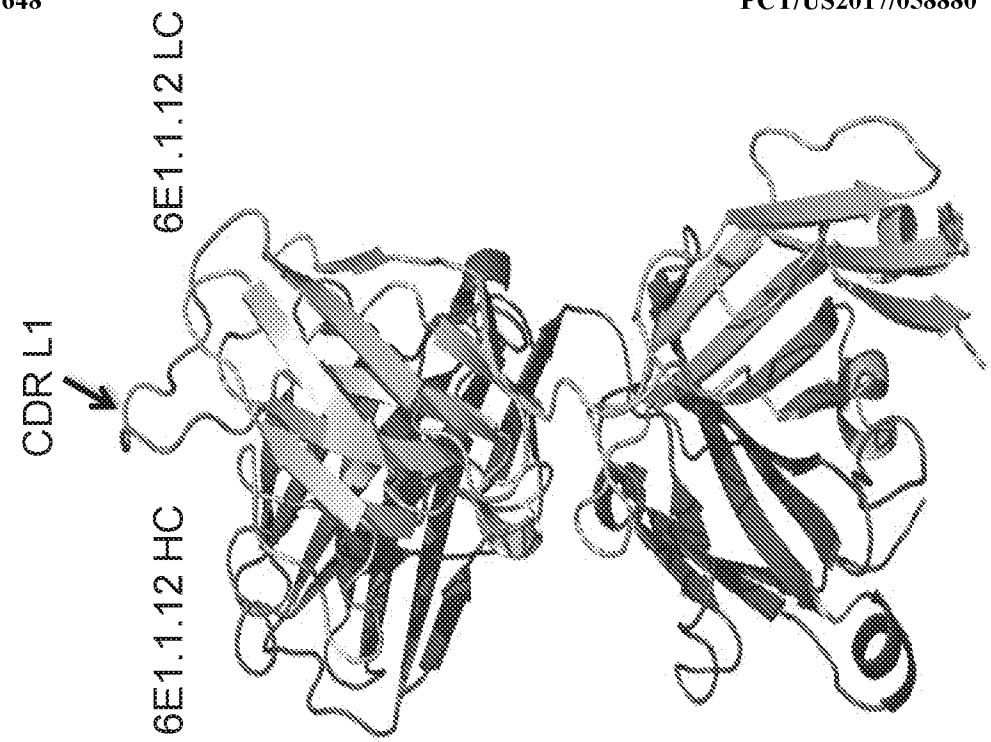


FIG. 39A

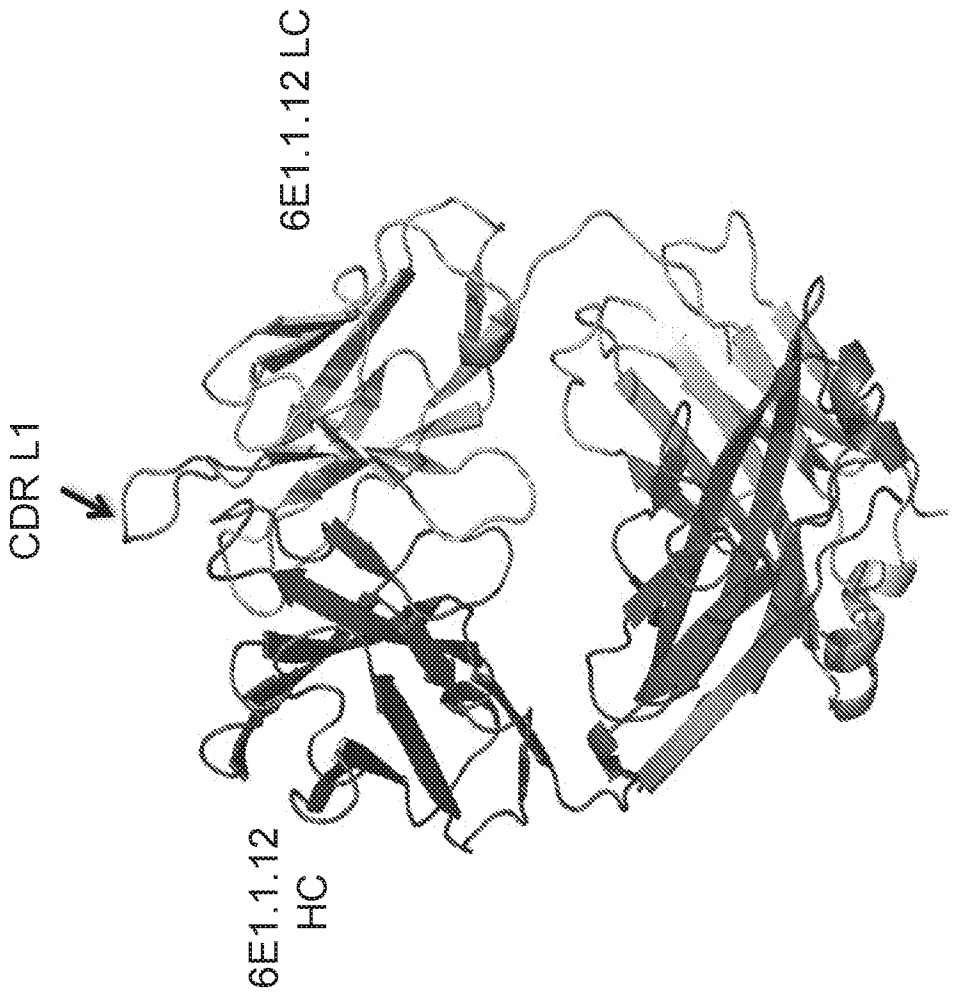


FIG. 39C

MICA*008 α3 domain viewed from "back face"

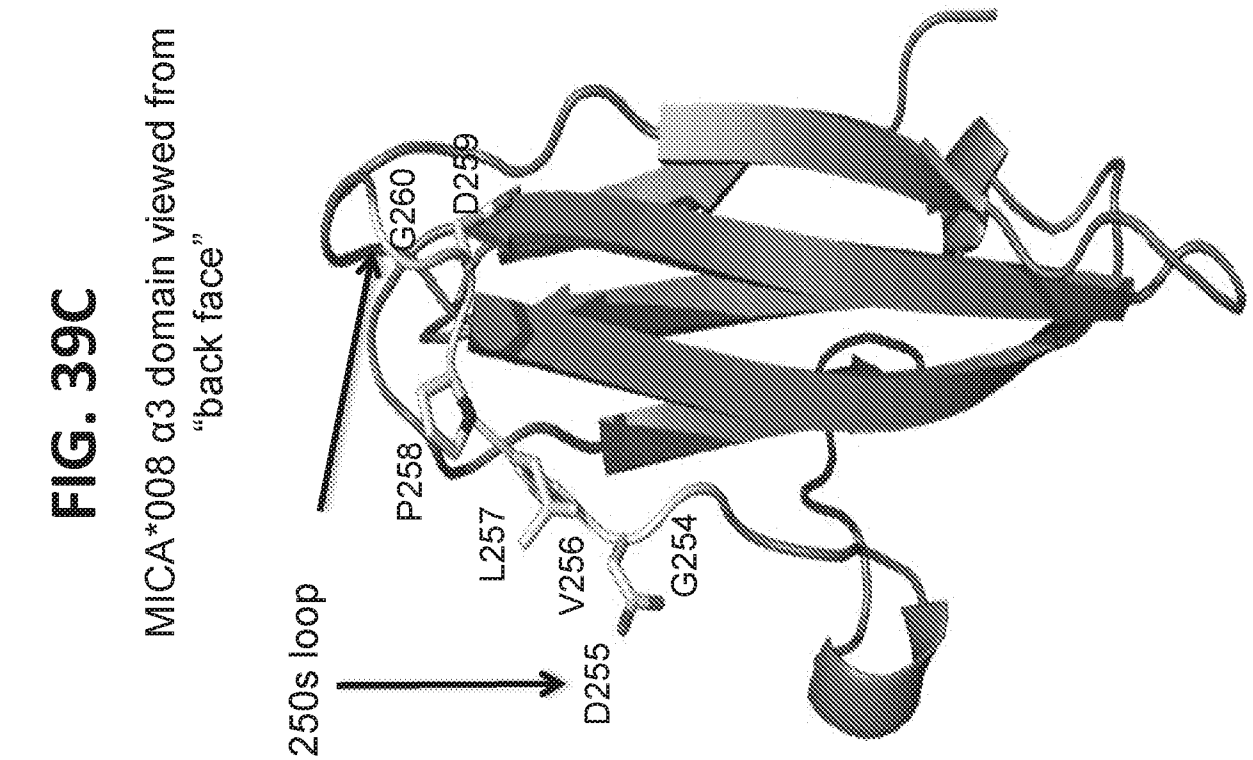


FIG. 39D

MICA*008 α3 domain viewed from "back face"

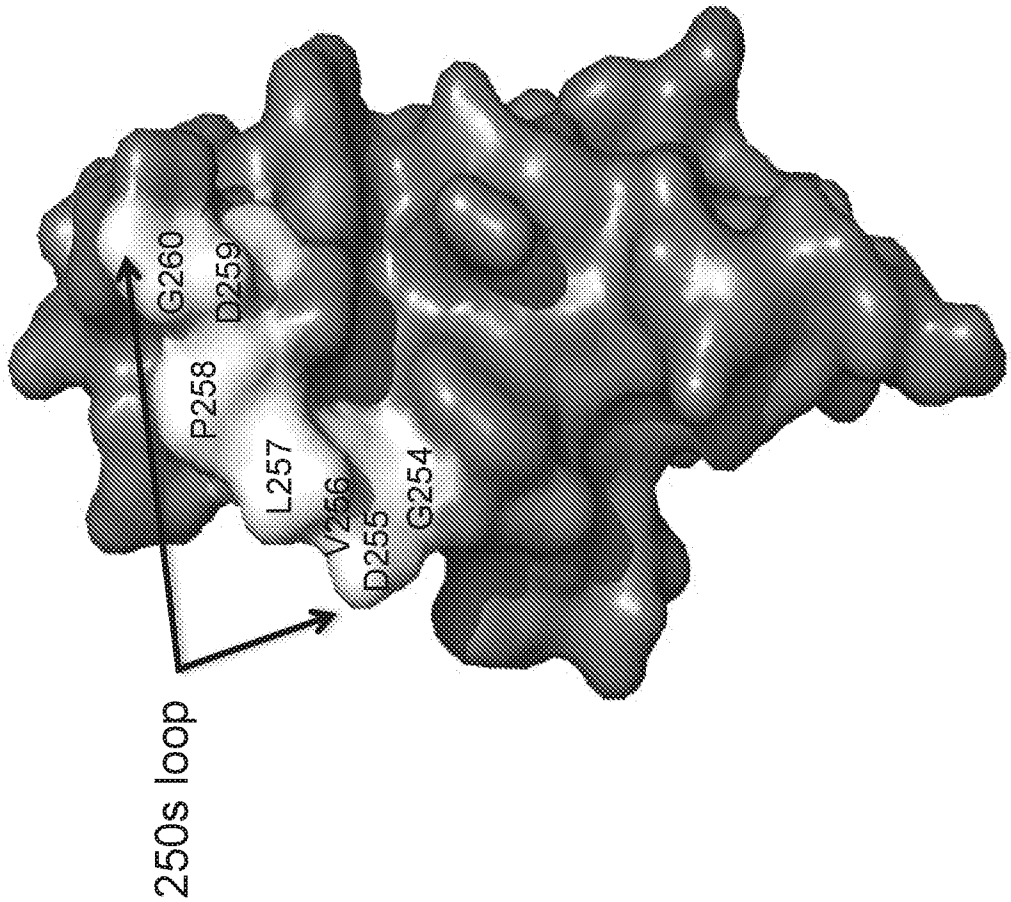


FIG. 39E

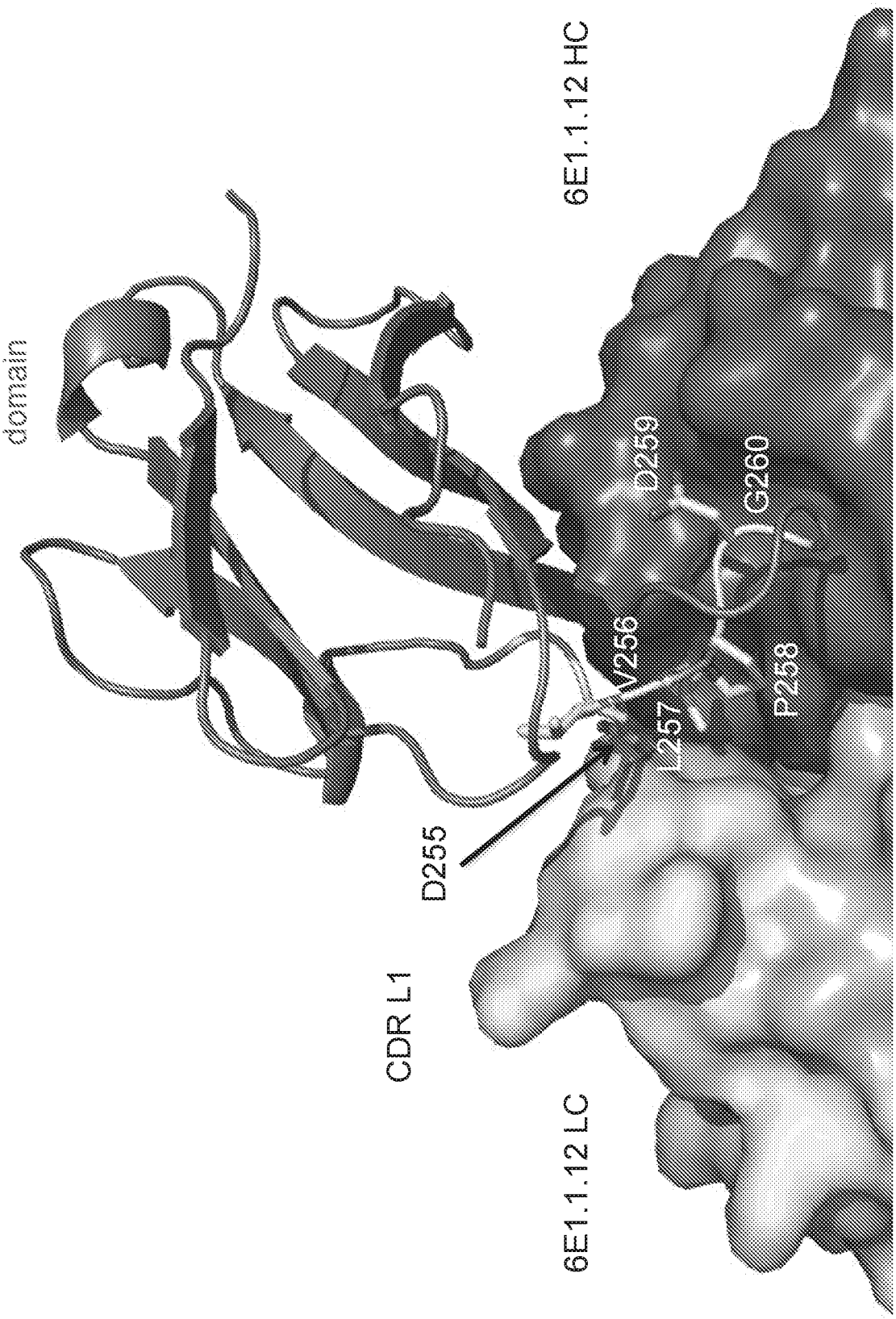

MICA*008 α3 domain
6E1.1.12 HC
6E1.1.12 LC
CDR L1
D255
V256
L257
P258
D259
G260

FIG. 39F

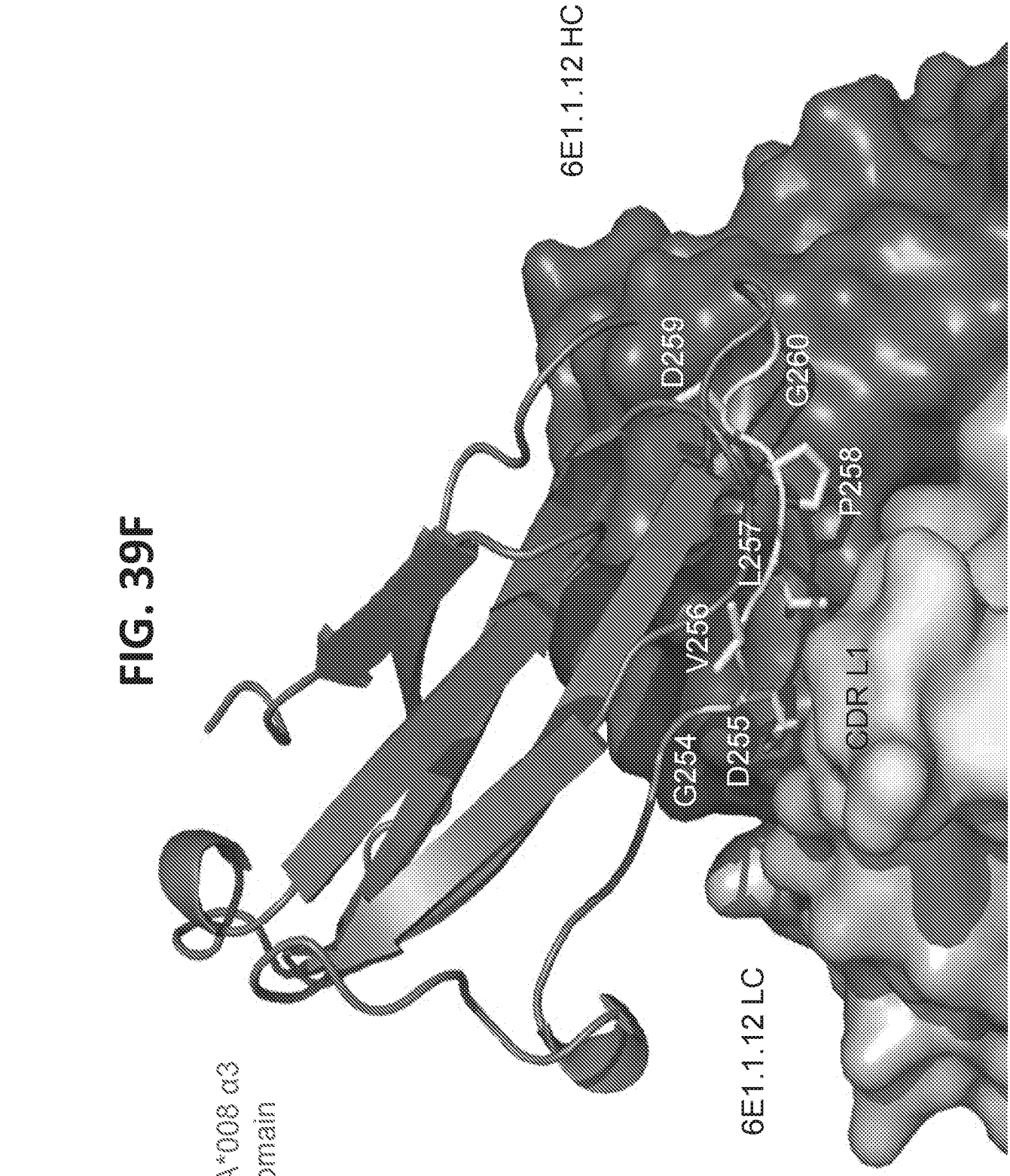

MICA*008 α3 domain
6E1.1.12 LC
6E1.1.12 HC
G254
D255
V256
L257
P258
D259
G260
CDR L1

FIG. 39G

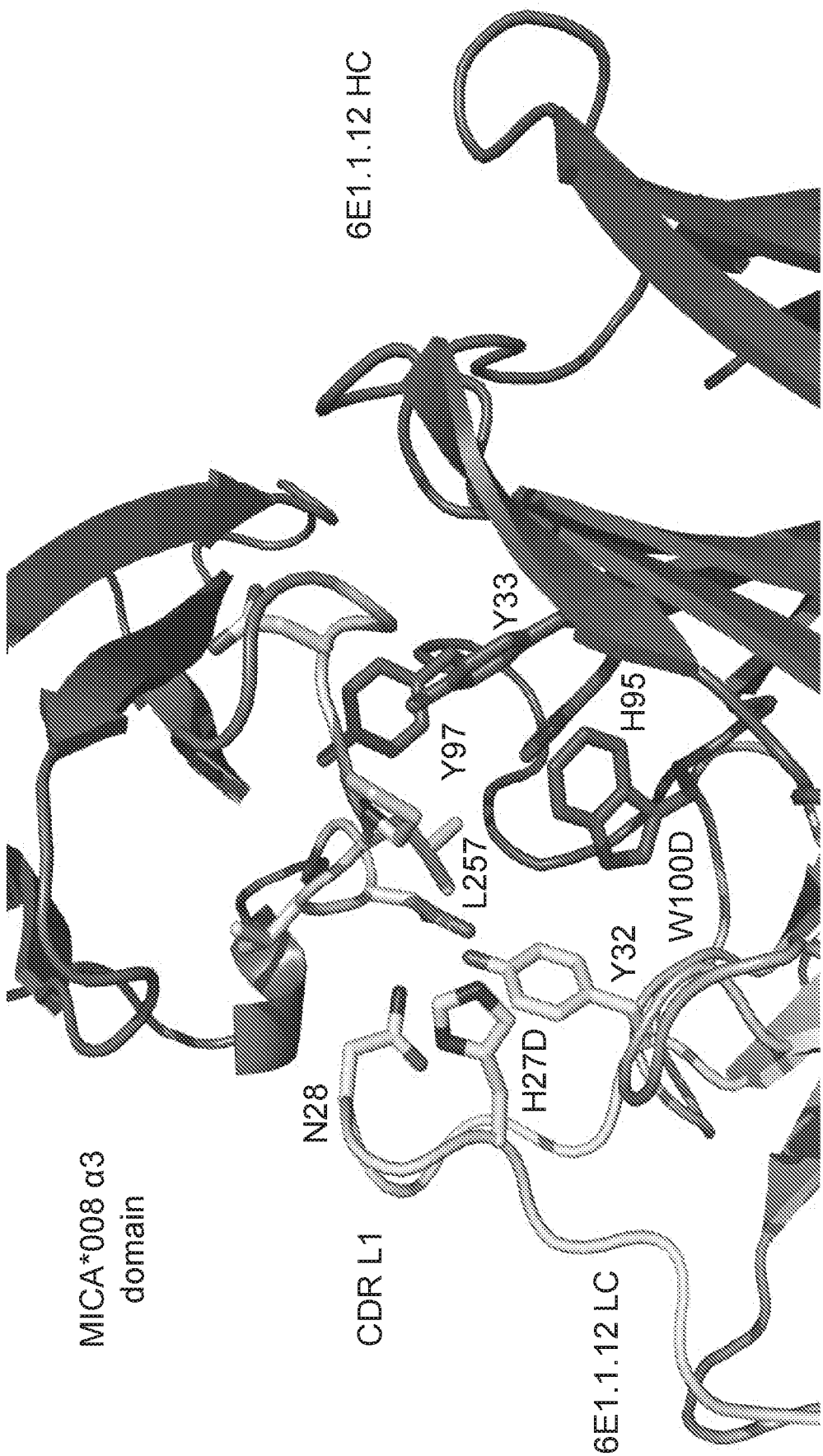

6E1.1.12 HC
MICA*008 α3 domain
CDR L1
6E1.1.12 LC
N28
H27D
Y32
L257
Y97
Y33
H95
W100D

FIG. 39H

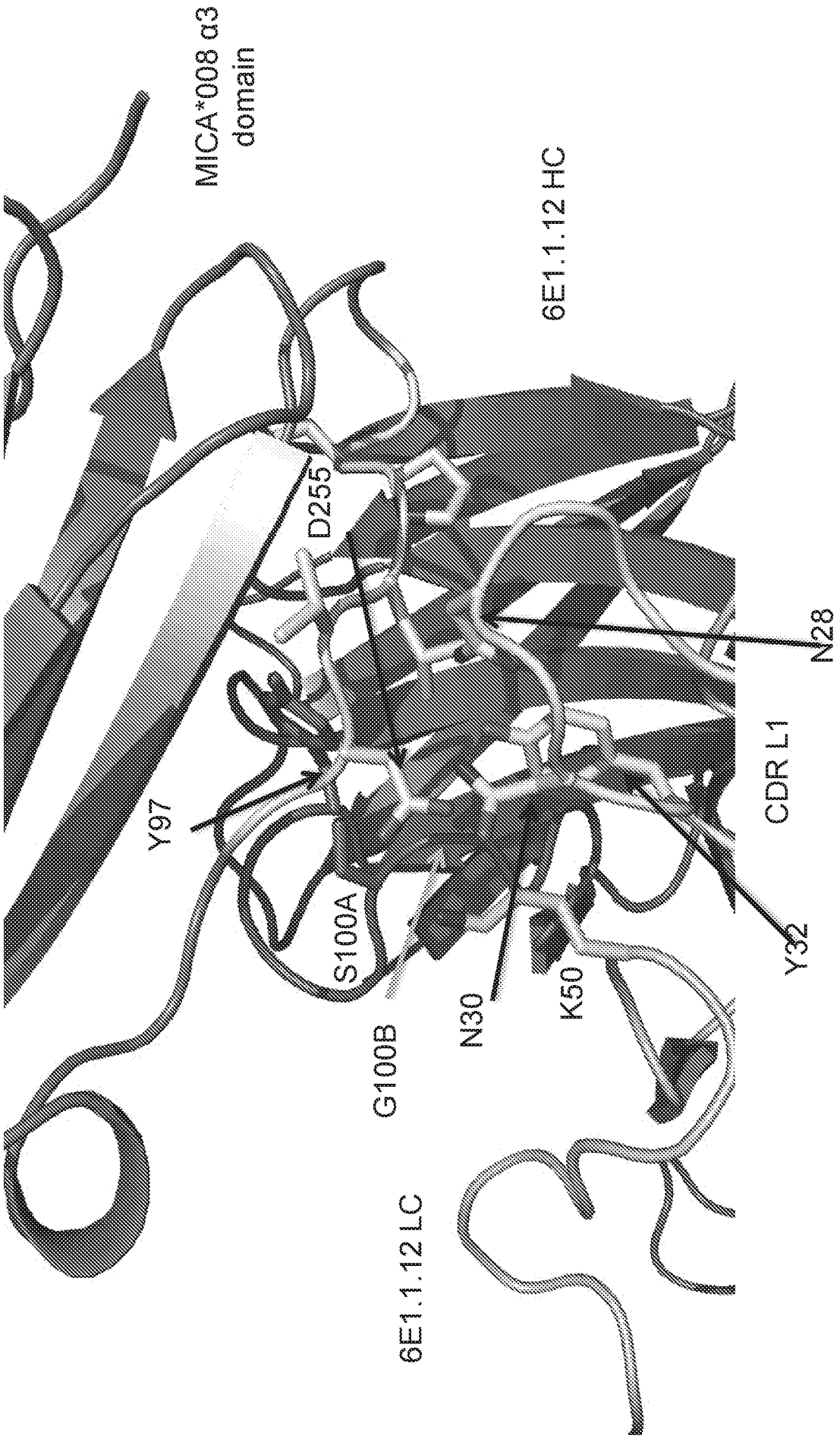

MICA*008 α3 domain
6E1.1.12 HC
D255
Y97
S100A
G100B
N30
K50
6E1.1.12 LC
CDR L1
N28
Y32

FIG. 40A

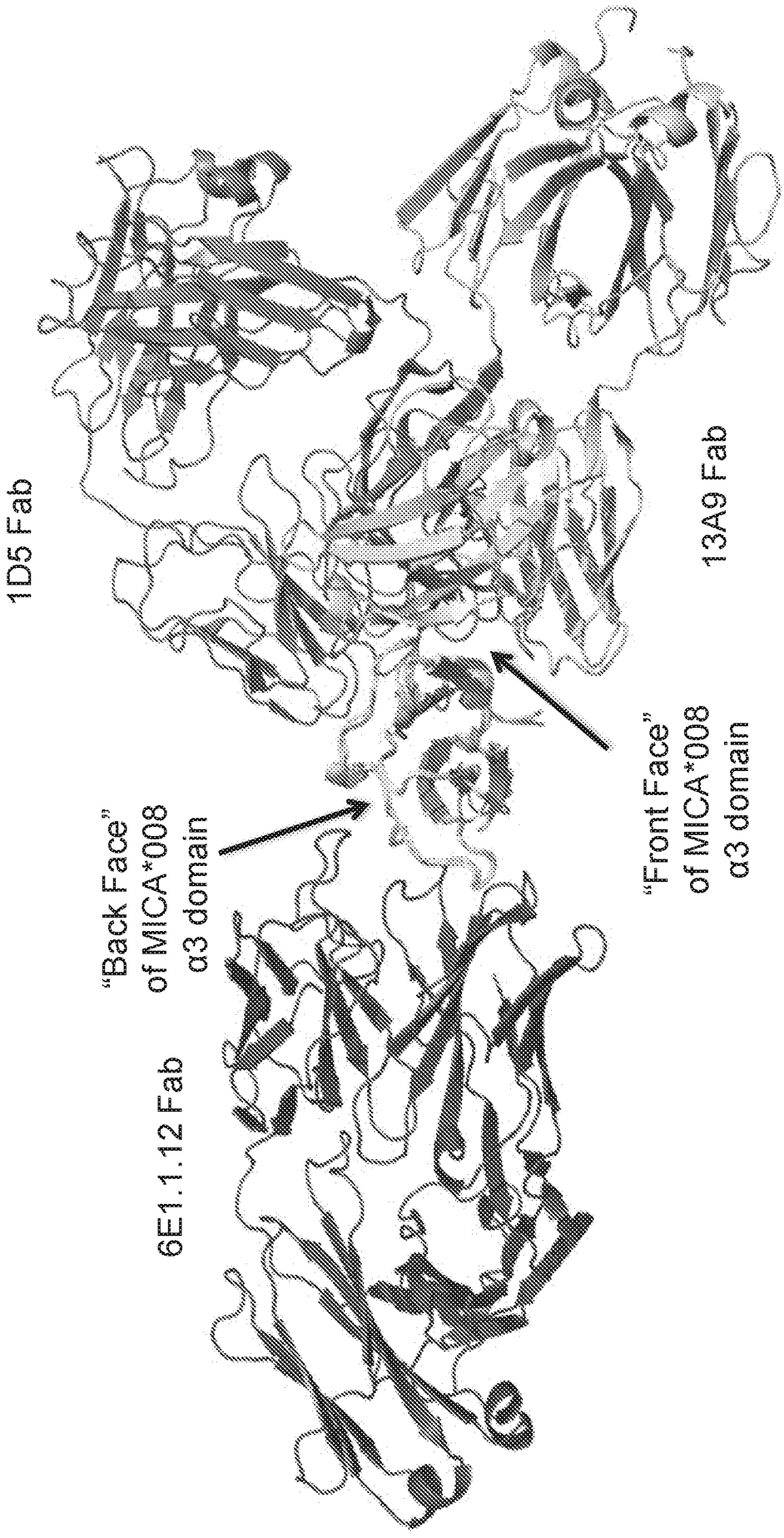

1D5 Fab
13A9 Fab
"Back Face" of MICA*008 α3 domain
"Front Face" of MICA*008 α3 domain
6E1.1.12 Fab

FIG. 40B

FIG. 40C

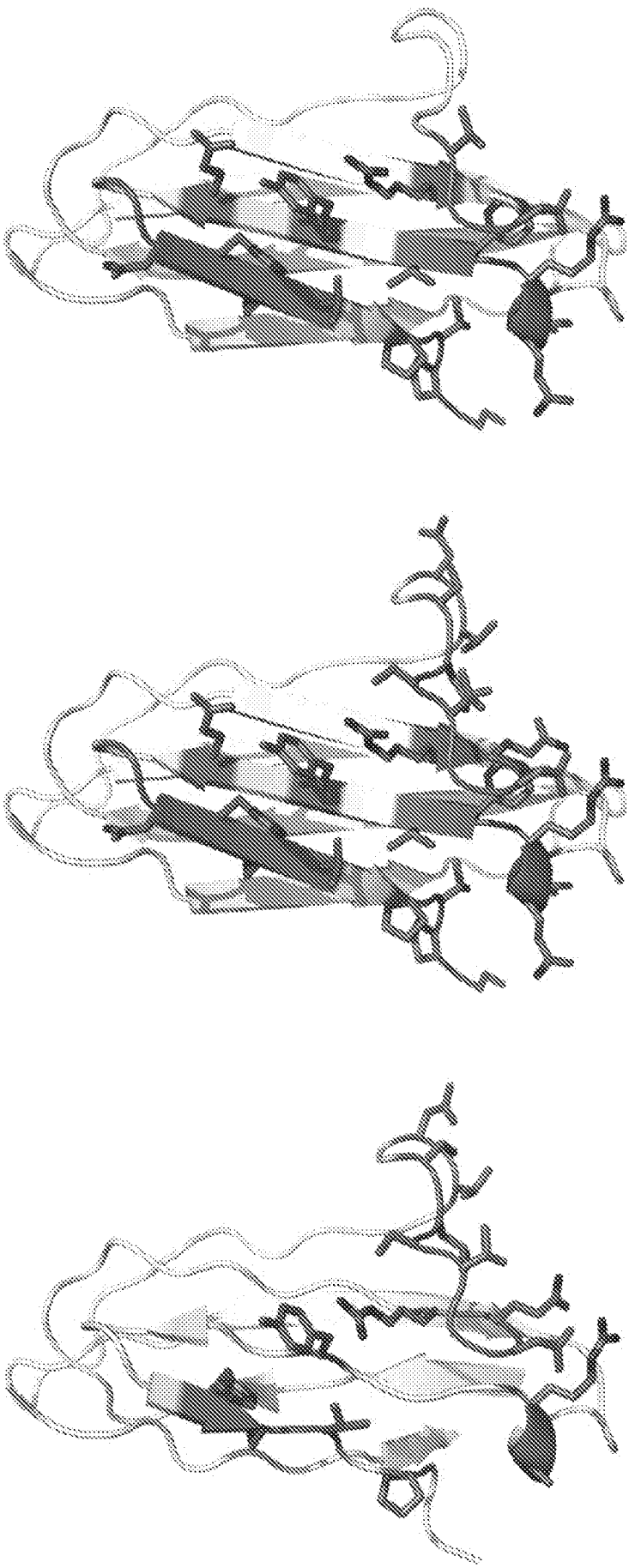


FIG. 41A

204 211 221 231 241
TVPPMVN VTRSEASEGN ITVTCRASGF YPWNITLSWR QDGVSLSHDT

251 261 271 281 291
QQWGDVLPDG NGTYQTWVAT RICQGEEQRF TCYMEHSGNH STHPVPSGKV

311
LVLQSHWQTF HVS (SEQ ID NO:456)

⬇ C1R-MICA*001 cleavage site
Waldhauer, I. et al. (2008) Cancer Res 68: 6368.

⬆ TRAMP C2-MICA*001 cleavage site
Wang, X. et al. (2009) BBRC 387: 476.

α3 Domain of MICA*001

Stalk of MICA*001

Front face view
MICA*008
α3 domain

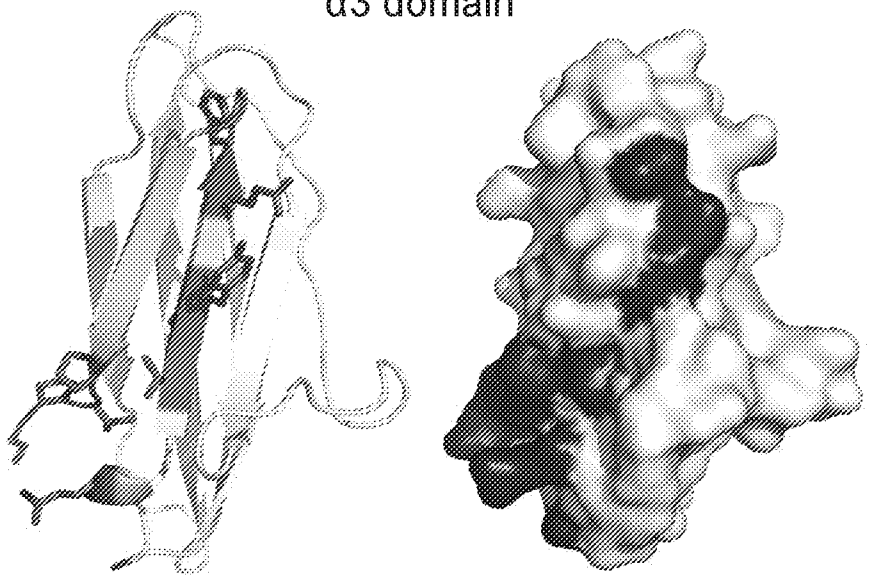


FIG. 41B

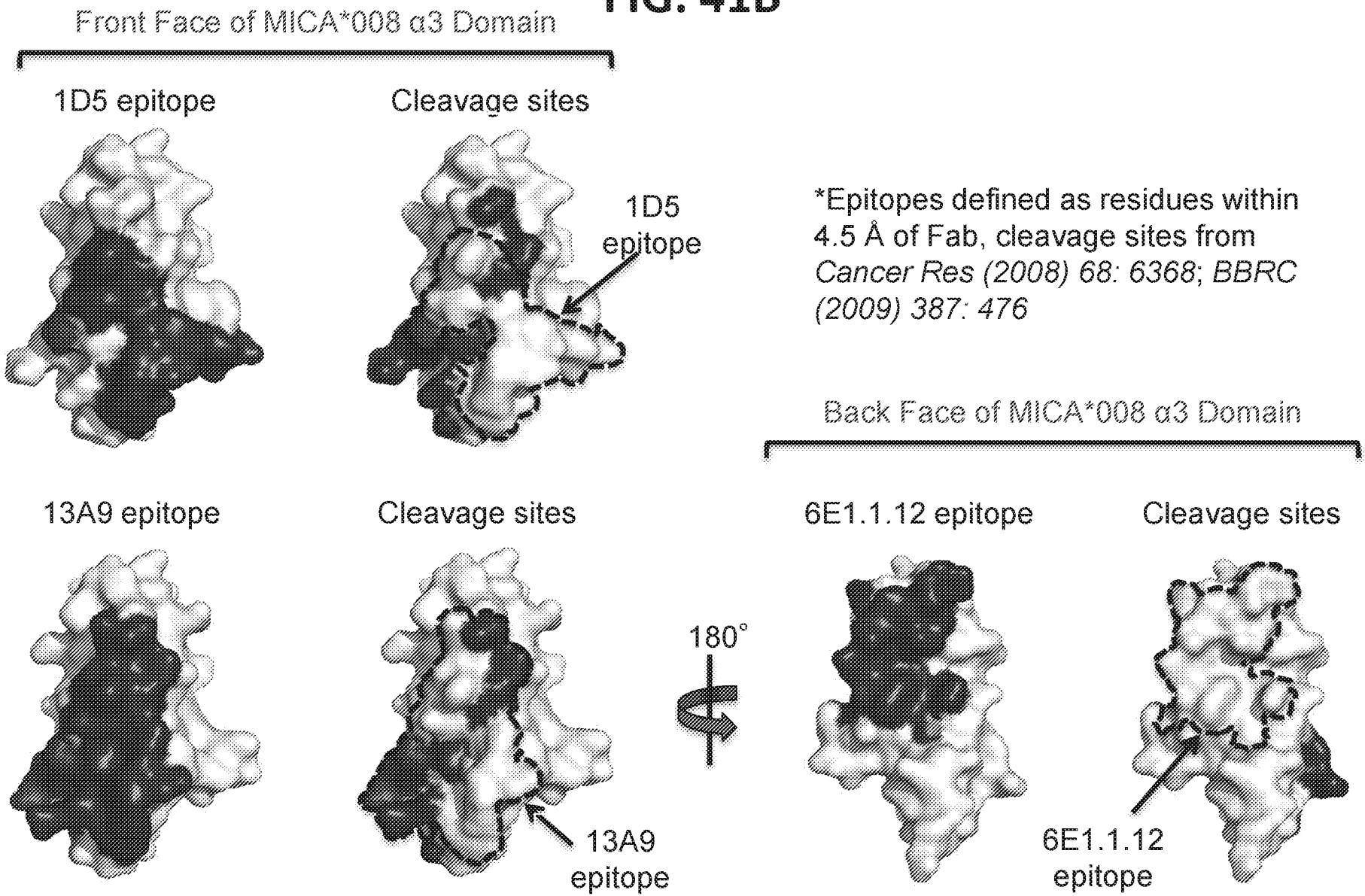

Front Face of MICA*008 α3 Domain
1D5 epitope
Cleavage sites
1D5 epitope
*Epitopes defined as residues within 4.5 Å of Fab, cleavage sites from Cancer Res (2008) 68: 6368; BBRC (2009) 387: 476
Back Face of MICA*008 α3 Domain
13A9 epitope
Cleavage sites
6E1.1.12 epitope
Cleavage sites
180°
13A9 epitope
6E1.1.12 epitope

FIG. 42

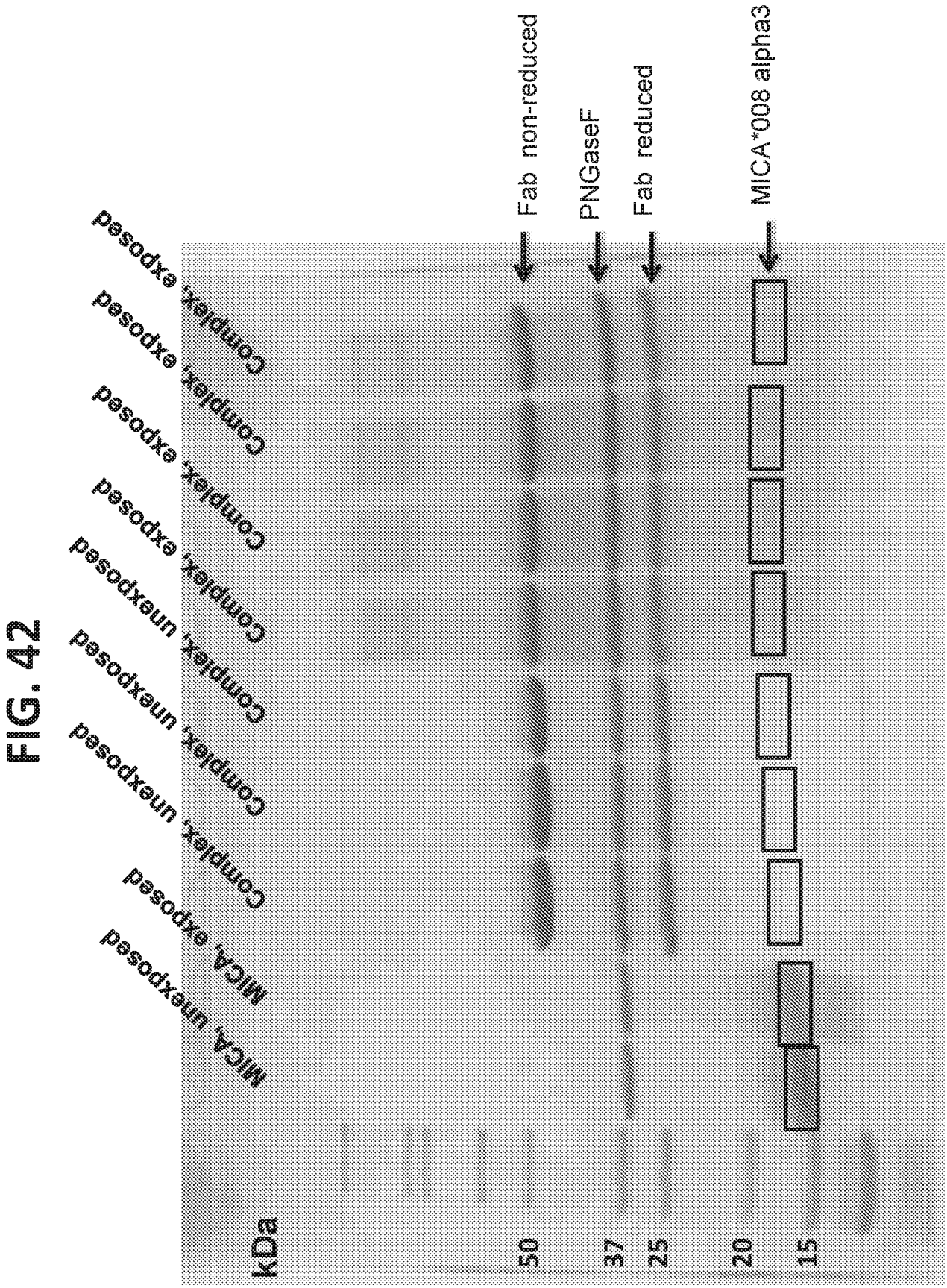

Fab non-reduced
PNGaseF
Fab reduced
MICA*008 alpha3
kDa
50
37
25
20
15
MICA, unexposed
MICA, exposed
Complex, unexposed
Complex, unexposed
Complex, unexposed
Complex, unexposed
Complex, exposed
Complex, exposed
Complex, exposed
Complex, exposed

FIG. 43A

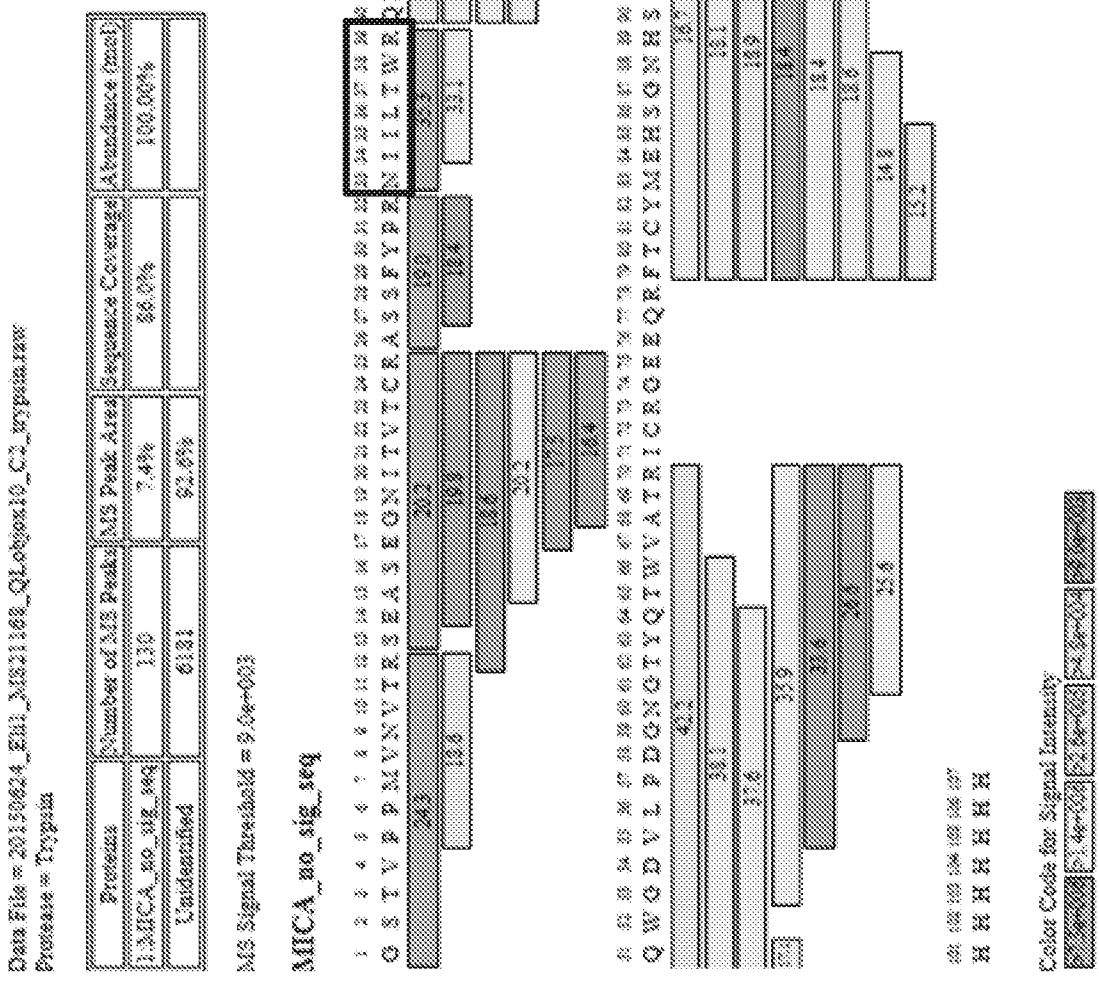

Data File = 20170824_EM1_XS211161_QLoQpx10_C2_trypsin.raw
Protease = Trypsin
Protein
Number of MS Peaks
MS Peak Area
Sequence Coverage
Abundance (max)
1.MCA_ao_sig_seq
130
7.4%
86.0%
100.00%
Unidentified
6181
92.6%
MS Signal Threshold = 9.0e+003
MCA_ao_sig_seq
Color Code for Signal Intensity

FIG. 43B

Data File = 20150624_Eli1_MS21168_QLebjon10_C2_chymo.raw
Protease = Chymotrypsin

Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
1:MICA_no_sig_seq	82	3.2%	57.9%	100.00%
Unidentified	2932	96.8%		

MS Signal Threshold = 7.7e+003

MICA_no_sig_seq

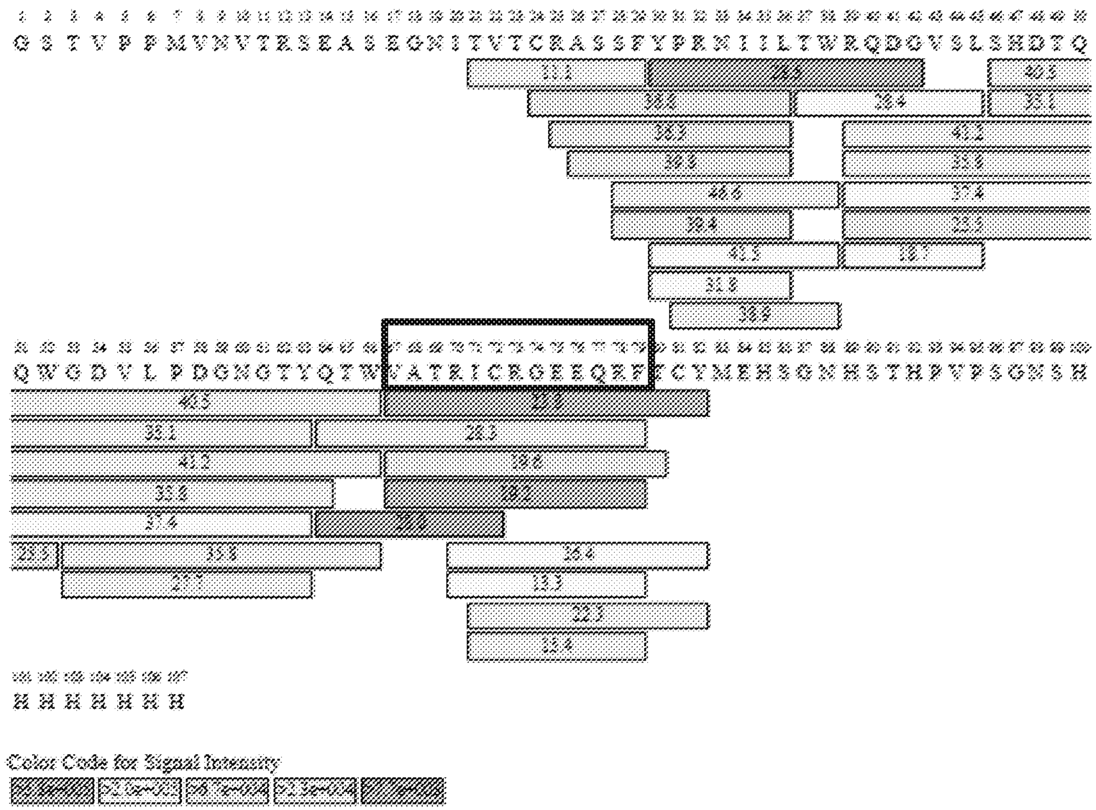


FIG. 44

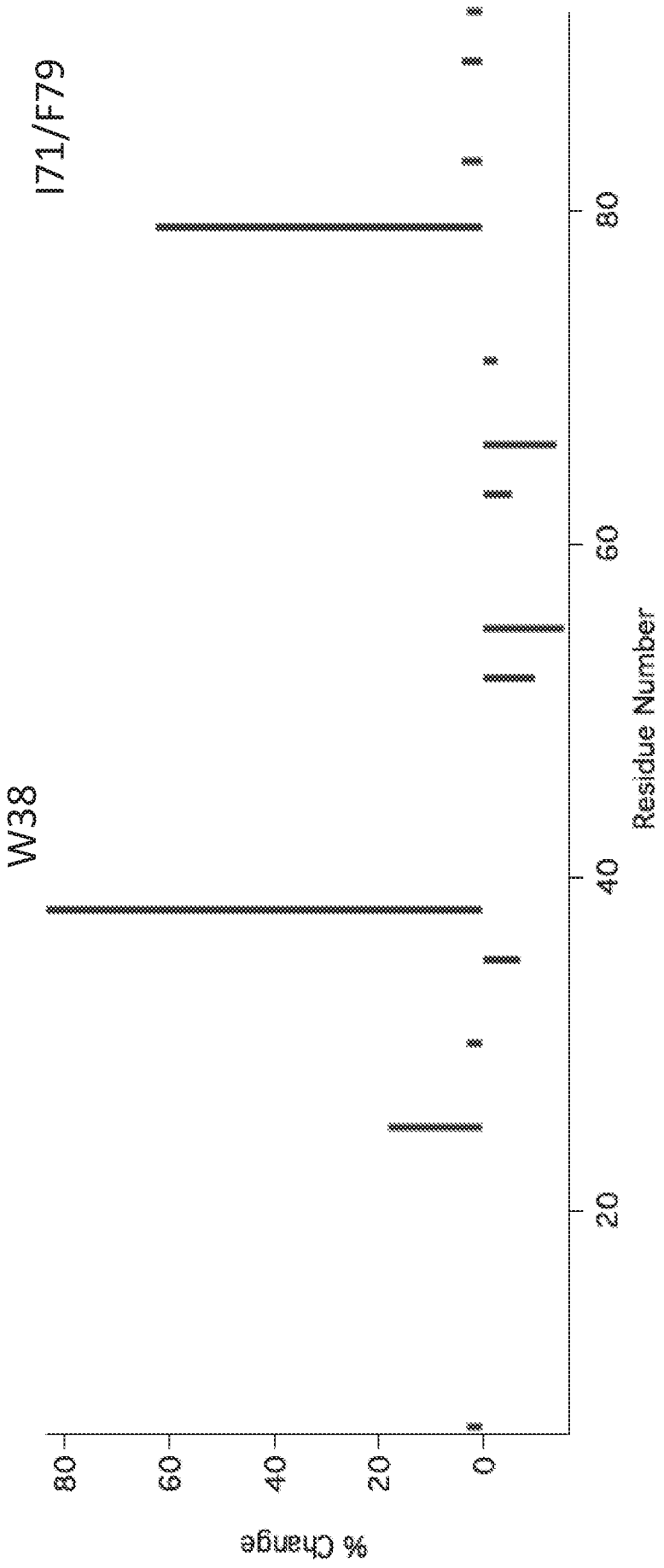


FIG. 45

1D5 epitope from FPOP

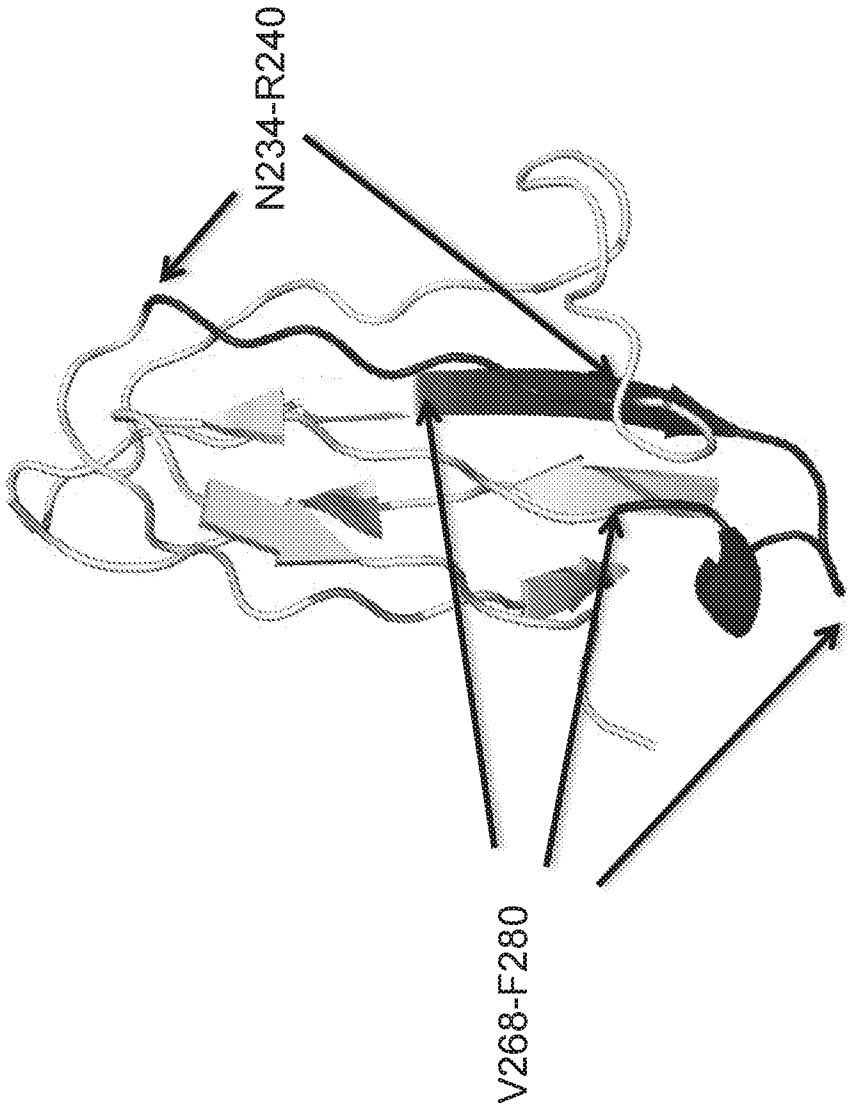
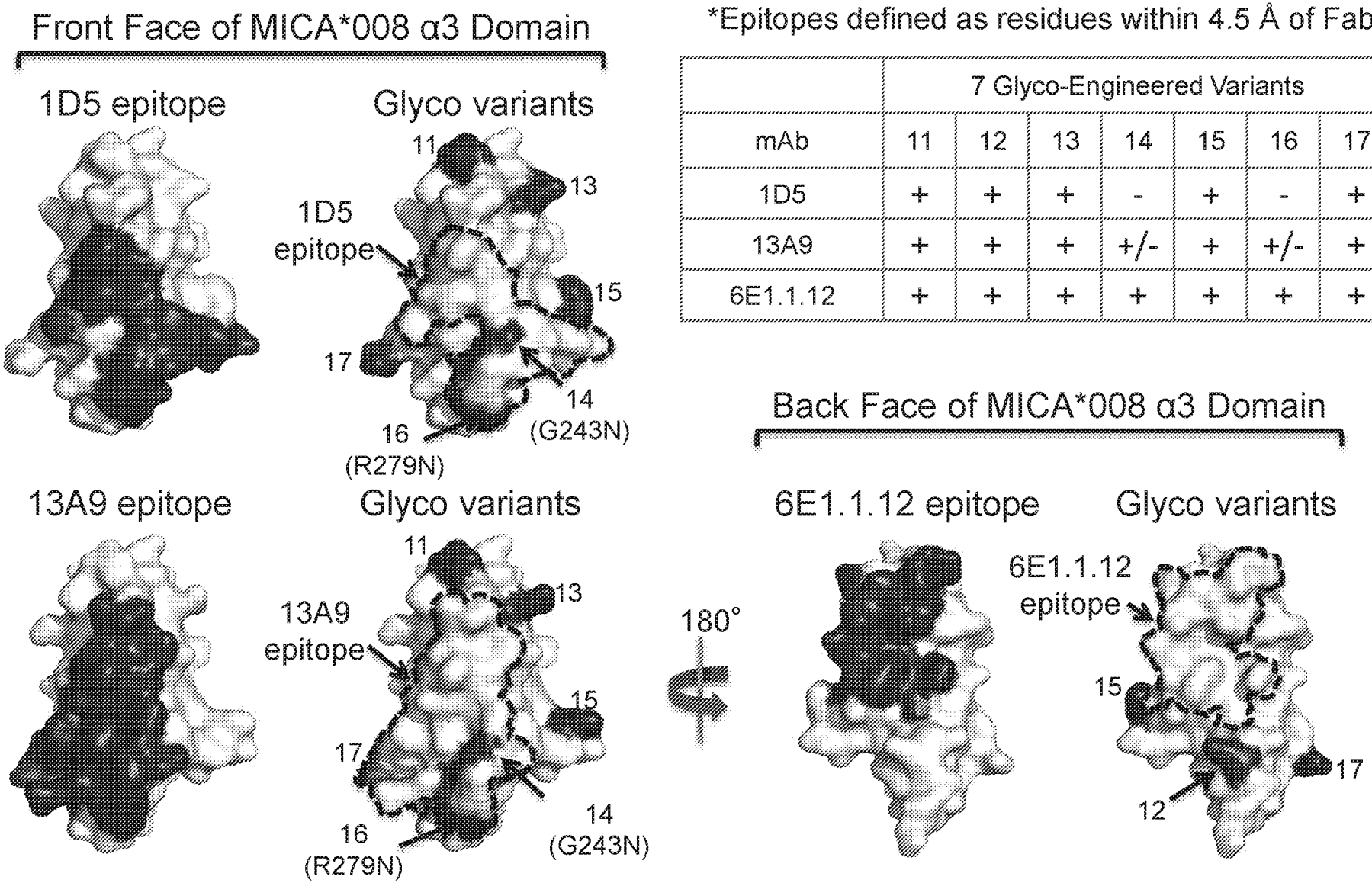


FIG. 46A



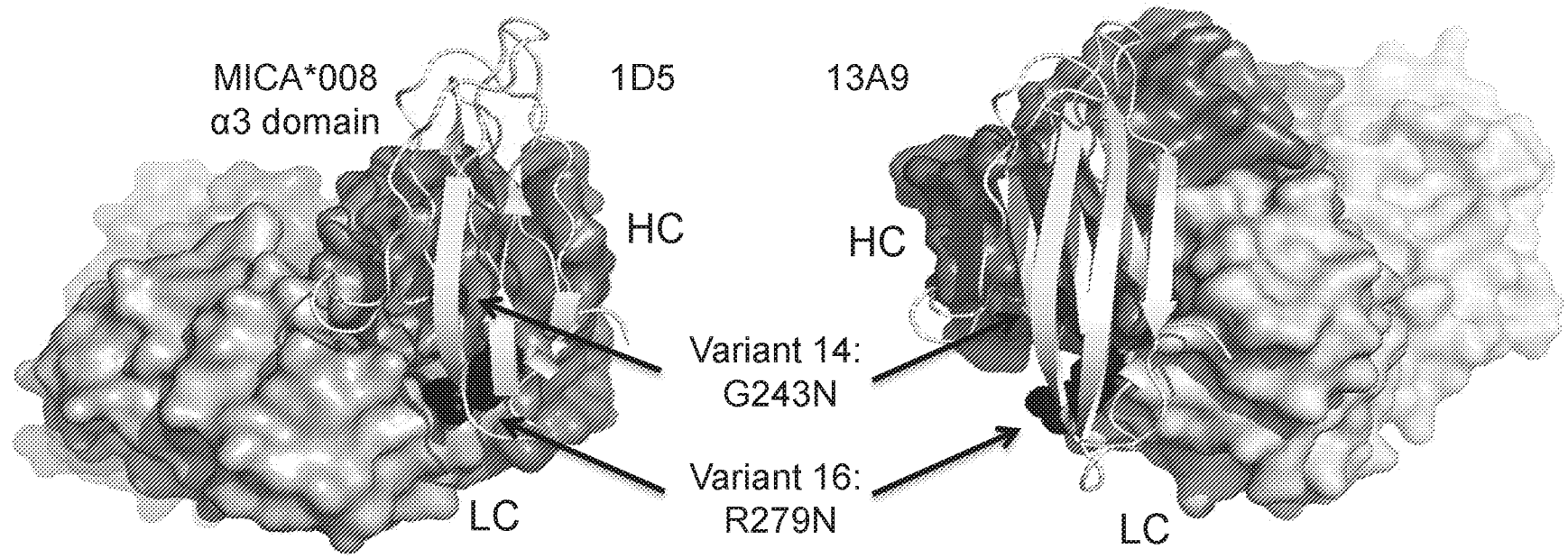
*Epitopes defined as residues within 4.5 Å of Fab

	7 Glyco-Engineered Variants						
mAb	11	12	13	14	15	16	17
1D5	+	+	+	-	+	-	+
13A9	+	+	+	+/-	+	+/-	+
6E1.1.12	+	+	+	+	+	+	+

WO 2018/081648 PCT/US2017/058880

FIG. 46B

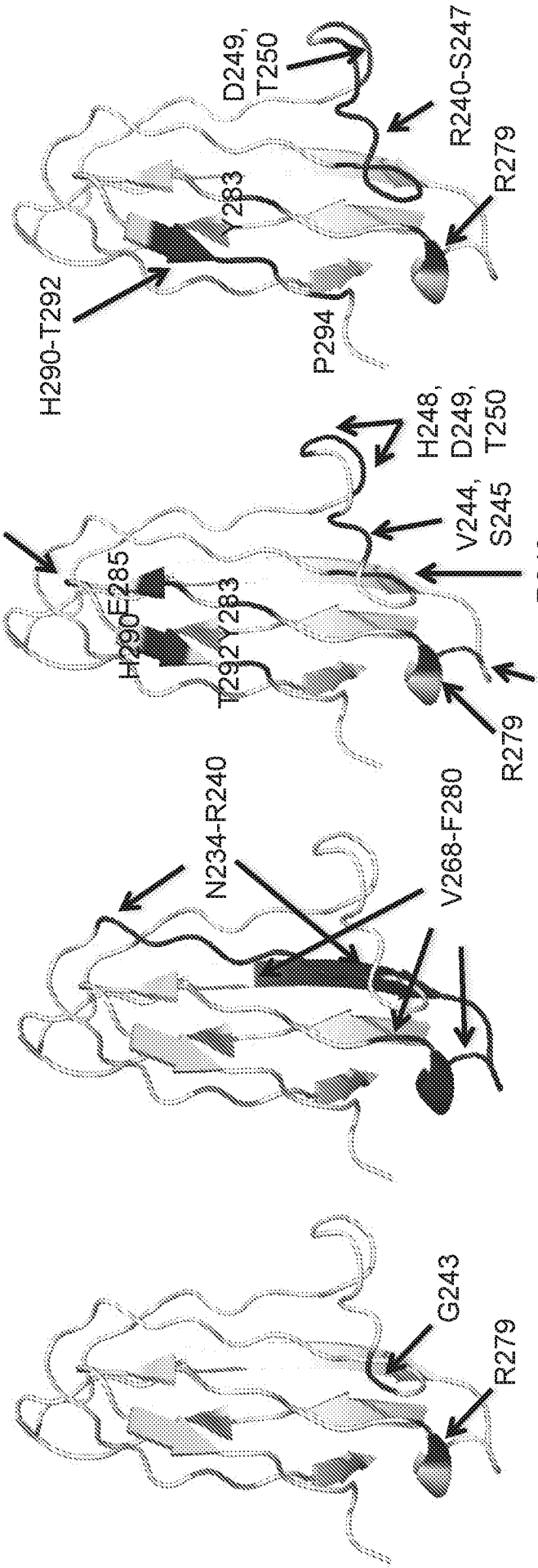
	7 Glyco-Engineered Variants						
mAb	11	12	13	14	15	16	17
1D5	+	+	+	-	+	-	+
13A9	+	+	+	+/-	+	+/-	+



Variants 14 and 16 are at *center* of 1D5 epitope

Variants 14 and 16 are at *periphery* of 13A9 epitope

FIG. 46C



*Binding to mutants D242A, G243A, L246A, T281A, H293A could not be assessed due to misfolded MICA*008 protein.

FIG. 47A

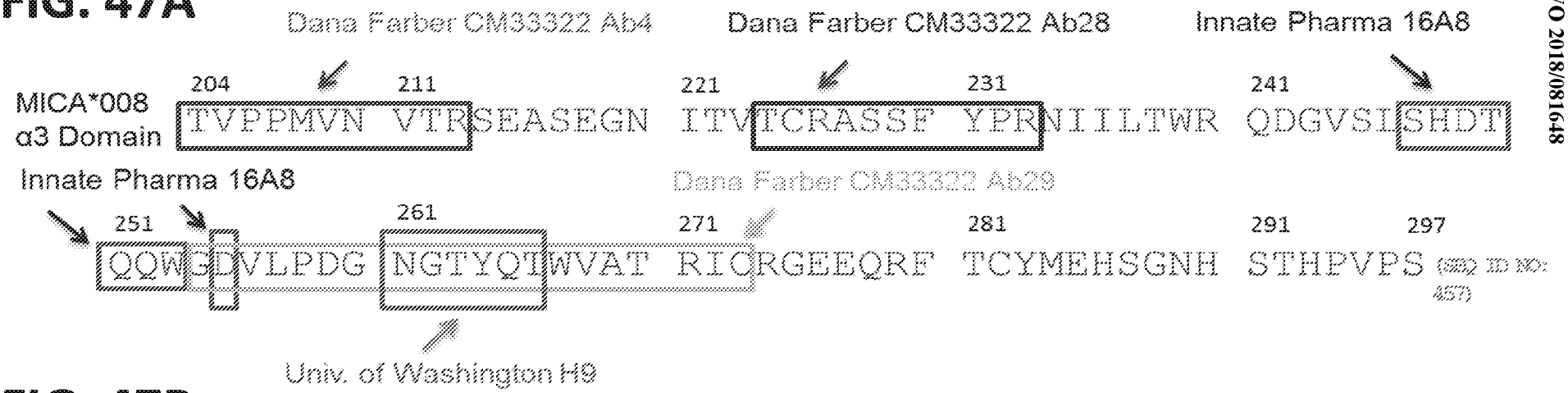


FIG. 47B

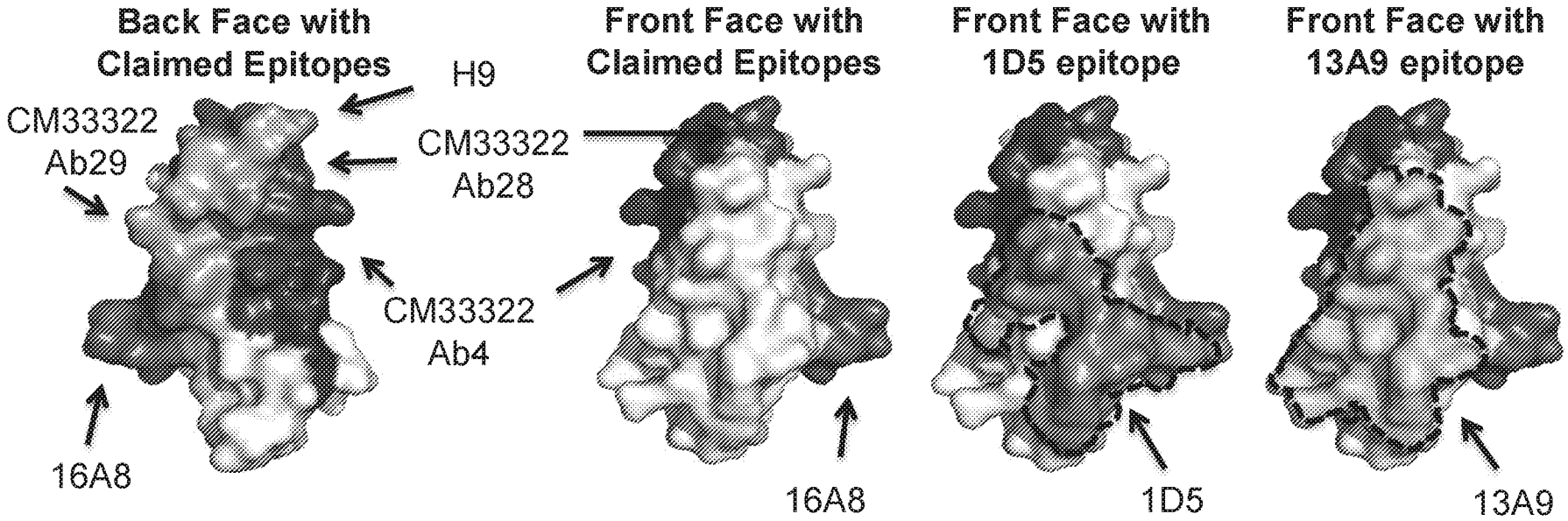
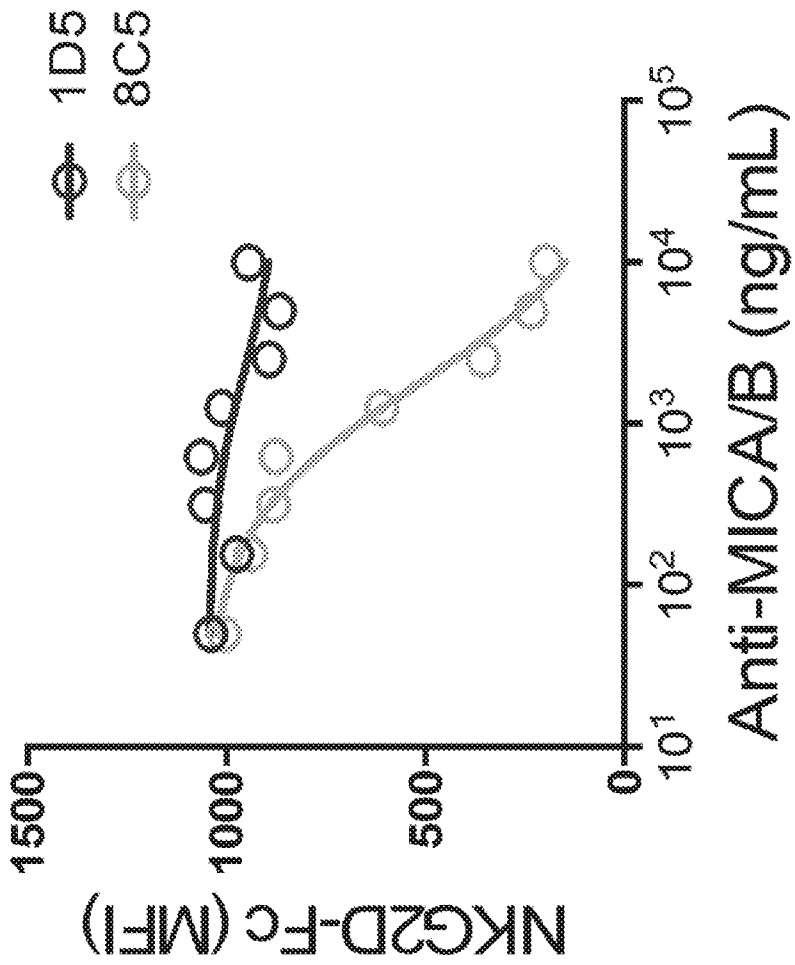


FIG. 48


1D5
8C5
NKG2D-Fc (MFI)
1500
1000
500
0
10^1
10^2
10^3
10^4
10^5
Anti-MICA/B (ng/mL)

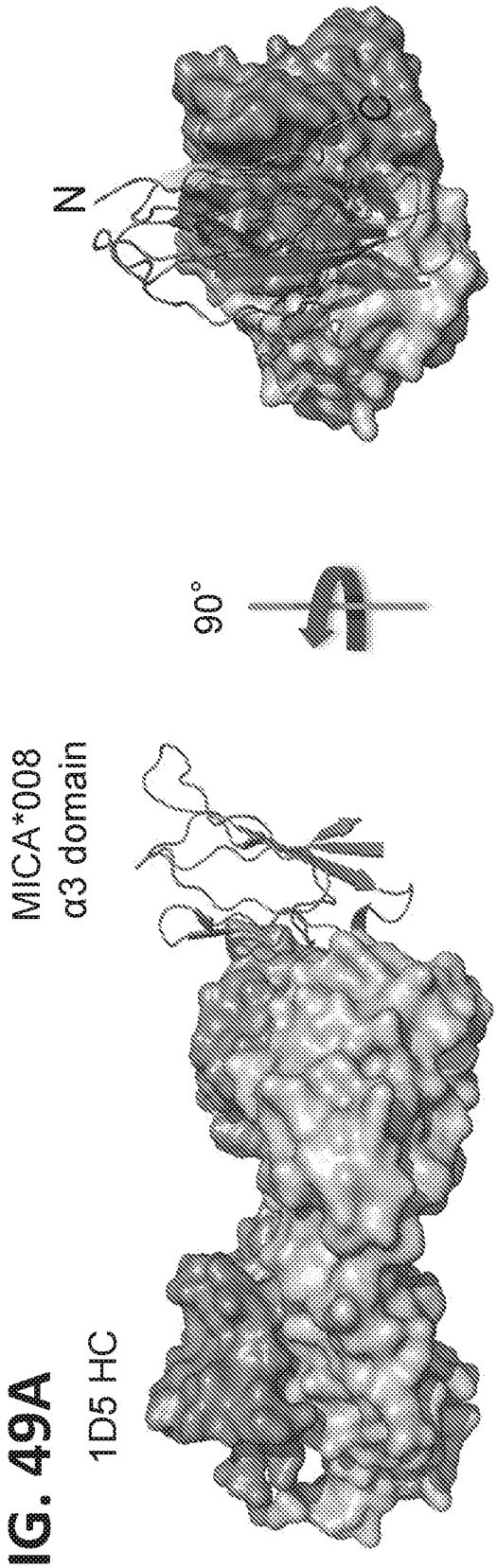


FIG. 49A

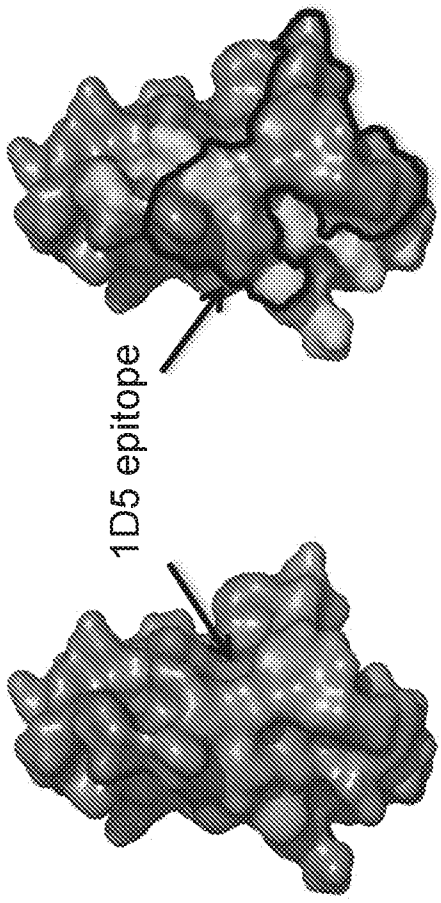


FIG. 49B

FIG. 50A

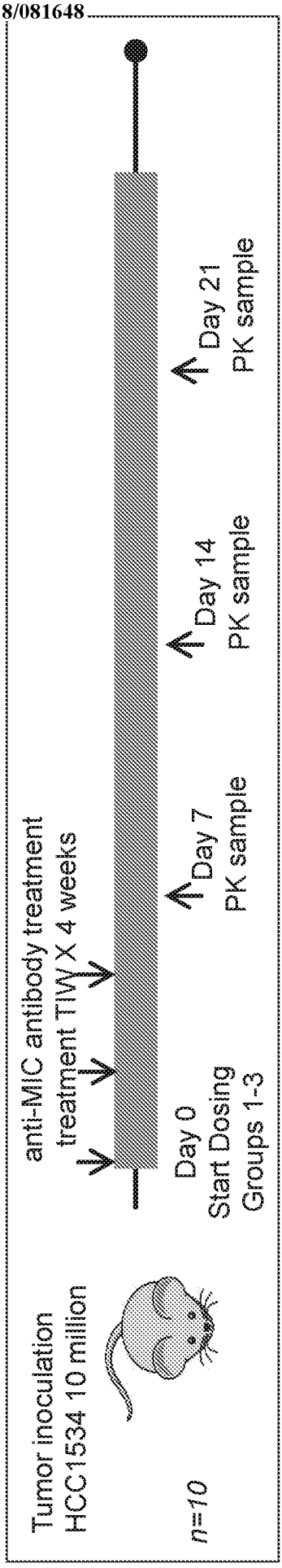


FIG. 50B

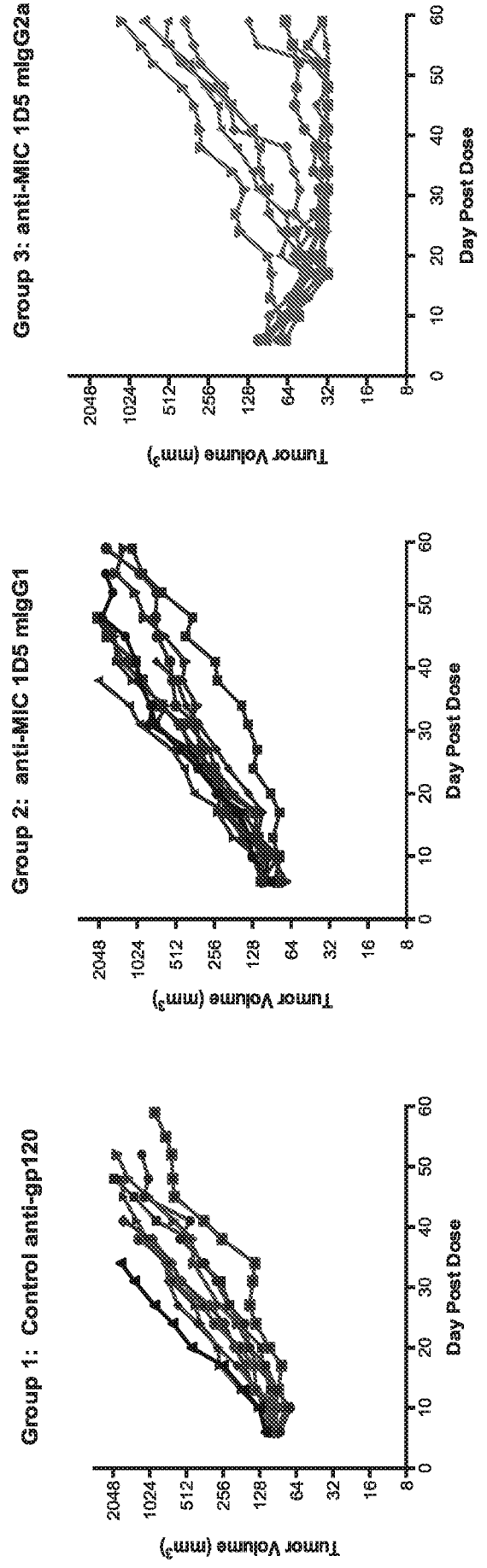


FIG. 50C

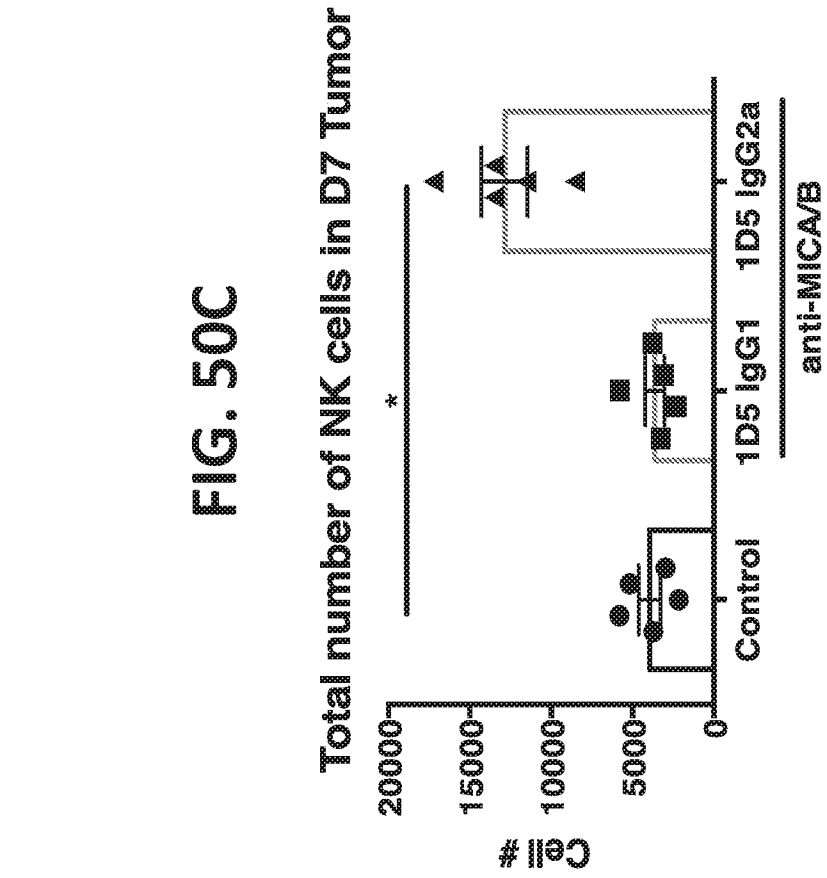


FIG. 50D

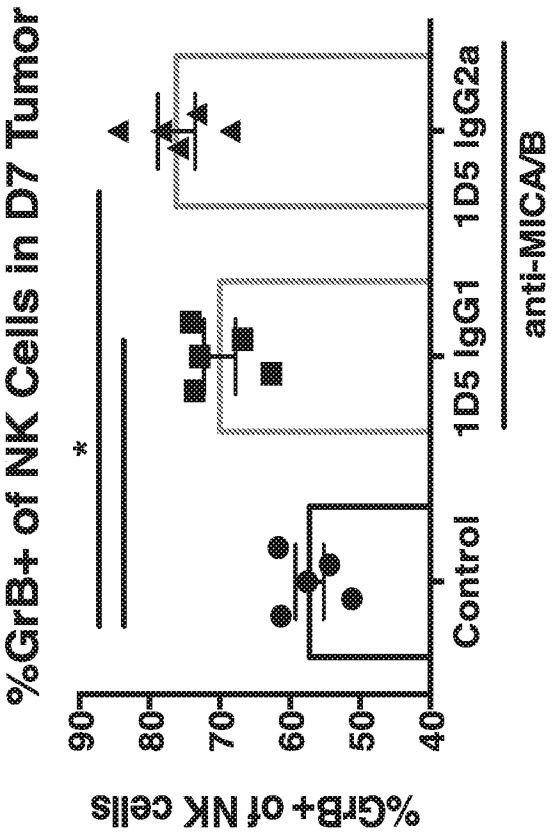
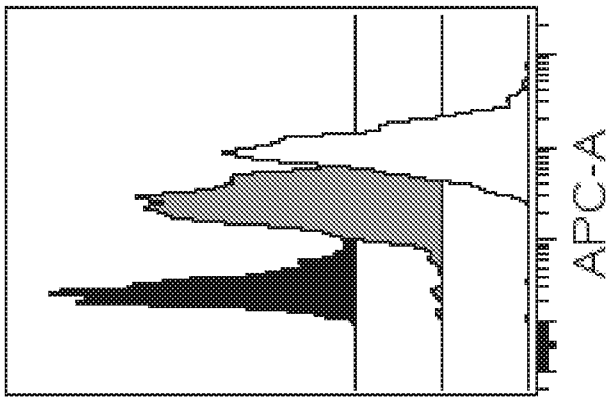
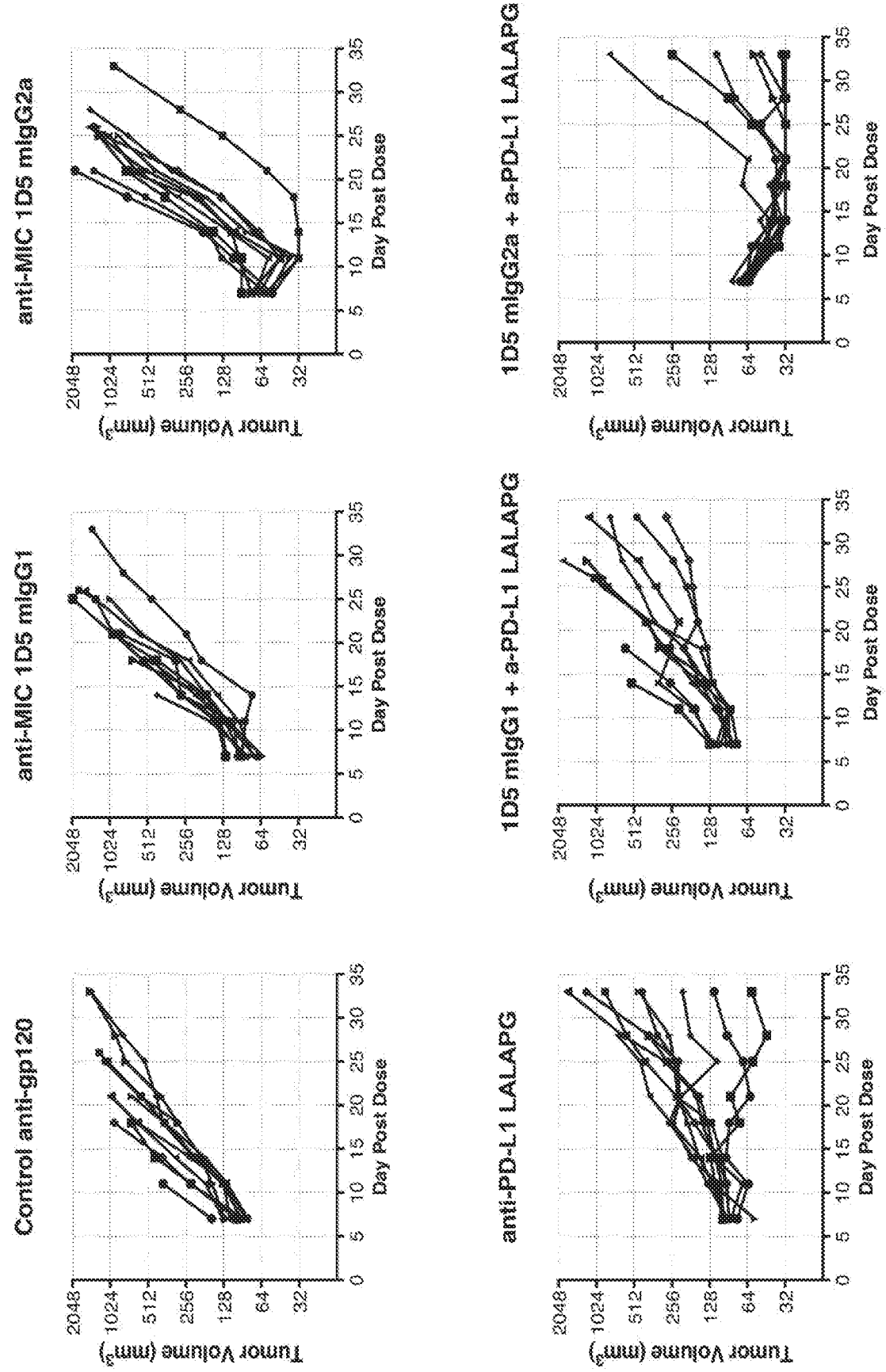


FIG. 51A



B16 FL14	MICA-AF647 MFI
-Dox Rag	517.0
+Dox Rag	3313
+Dox 1D5	9604

FIG. 51B


Control anti-gp120
anti-MIC 1D5 migG1
anti-MIC 1D5 migG2a
anti-PD-L1 LALAPG
1D5 migG1 + a-PD-L1 LALAPG
1D5 migG2a + a-PD-L1 LALAPG
Tumor Volume (mm³)
Day Post Dose
2048
1024
512
256
128
64
32
0
5
10
15
20
25
30
35