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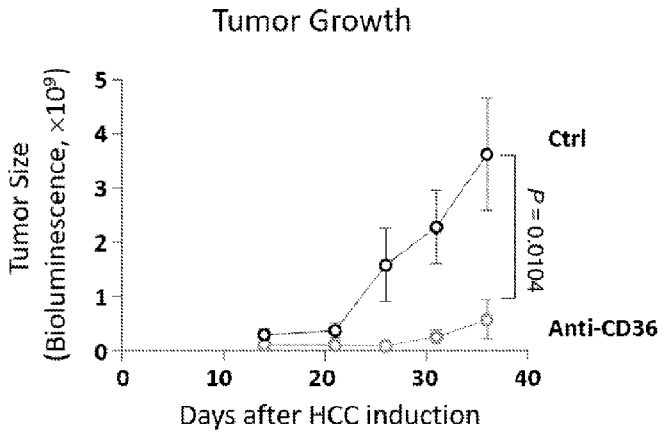
(54) **Title:** ANTI-CD36 ANTIBODIES AND USES THEREOF

FIG. 10A



(57) **Abstract:** The present disclosure provides antibodies which specifically bind to human CD36 protein (hCD36) and are capable of decreasing, inhibiting, and/or fully-blocking immune regulatory effects and/or ligand transport into cells mediated by CD36. Examples of CD36-mediated effects, among others, may include oxidized lipid transport into CD8 T cells potentially causing cell death and fatty acid transport in regulatory T cells increasing their survival and immune suppression in the tumor micro-environment (TME). The present disclosure also provides methods of using the antibodies (and compositions thereof) to treat various diseases and conditions responsive to decreasing, inhibiting and/or blocking CD36-dependent ligand transport into cells.

WO 2023/172863 A1

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ANTI-CD36 ANTIBODIES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority of U.S. Provisional Patent Application Number 63/317,160, filed March 7, 2022, the entirety of which is hereby incorporated by reference herein.

FIELD

[0002] The present disclosure relates to antibodies which bind to CD36 and methods of using such antibodies.

REFERENCE TO SEQUENCE LISTING

[0003] The official copy of the Sequence Listing is submitted concurrently with the specification as a WIPO Standard ST.26 formatted XML file with file name of "09793-005WO1.xml", a creation date of February 26, 2023, and a size of 75,330 bytes. The Sequence Listing filed via USPTO Patent Center is part of the specification and is incorporated in its entirety by reference herein.

BACKGROUND

[0004] Macrophages and other myeloid cells play a prominent role in creating as well as maintaining the immunosuppressive environment of tumors, and there are distinct microenvironments where tumor-associated macrophages (TAM) suppress immune response and promote other processes including angiogenesis, cancer cell motility and metastasis (Lewis & Pollard, 2006; Pollard, 2008; Wu et al., 2020). It was almost twenty years ago when, for example, vascular endothelial growth factor production by TAMs was proposed to play a role in lymphatic metastasis in several human cancers (e.g., Pepper et al., 2003), and 2006 when it was demonstrated that clodronate-liposome-mediated TAM depletion inhibits tumor growth in experimental models via mechanisms thought to include reduction of tumor angiogenesis (Zeisberger et al., 2006). What's more, macrophages not only promote tumor cell egress from a primary tumor but are also thought to facilitate seeding at distant metastatic sites (Joyce & Pollard, 2009; Psaila & Lyden, 2009).

[0005] While understanding of various pro-tumorigenic (often referred to as M2) and anti-tumorigenic M1 macrophages as well as other myeloid cells is still growing (e.g. see Wu et al., 2020), it has long been appreciated that high TAM number is linked to reduced patient survival (with a few notable exceptions) and is an independent prognostic factor in many cancers (Lewis & Pollard, 2006). Furthermore, myeloid cells referred to as myeloid-derived suppressor cells (MDSC) are found in peripheral blood and tumor tissues of cancer patients, and depletion or otherwise targeting of MDSC in mouse models results in improved immune responses and delayed tumor growth (Goedegebuure et al., 2011). MDSC are said to be somewhat heterogenous but are nonetheless described to fall into monocytic versus granulocytic subsets with distinct immunosuppressive mechanisms. Among other roles, tumor MDSC have been implicated in the recruitment and maintenance of immunosuppressive regulatory T cells, and to promote angiogenesis and metastasis (Goedegebuure et al., 2011; Oh et al., 2013). Like MDSC, as we now know

at least, a CD45-positive but CD14-negative subset of human peripheral blood cells was described by Barrett et al. (2007) as expressing CD36 and the immunosuppressive cytokine, interleukin-10.

[0006] CD36 is a transmembrane cell-surface protein that, among other names, is also known as platelet glycoprotein 4, fatty acid translocase (FAT) and scavenger receptor class B member 3 (SCARB3). CD36 binds and transports fatty acids into cells but also binds a number of other ligands such as apoptotic cells, low-density lipoprotein, phospholipids and oxidized forms of the same (Pepino et al., 2014; Wang & Li, 2019). CD36 also has a separate thrombospondin binding domain (Pepino et al., 2014; Wang & Li, 2019). Both of these two distinct functional events, thrombospondin versus fatty acid binding, can lead to various downstream signaling events that may differ depending on cell type and various other CD36 and/or ligand interacting partners (Pepino et al., 2014; Wang & Li, 2019). Fatty acid transport by CD36 is involved in metabolic 'wiring' of various cells if not required for cell differentiation state or survival. For example, fatty acid transport by CD36 has been shown to be required for M2 macrophage differentiation via a mechanism related to the level or state of endoplasmic reticulum stress (Oh et al., 2012).

[0007] CD36 has also been shown to be critical to M2 macrophage activation as measured by markers of immune-suppressive capacity such as PDL-2 expression (Huang et al., 2014). CD36-mediated lipid transport is also involved in the acquisition of immune-suppressive effectors during MDSC differentiation (Al-Khami et al., 2017). CD36-deficient mice have reduced numbers of tumor-resident MDSC in all (lung and colon) tumor models tested, when compared to tumor-bearing wild type mice, and this was reflected in tumors of wild type chimeric mice that were CD36-deficient in their bone marrow (Al-Khami et al., 2017). Most recently, TAMs were shown to be greatly reduced in melanoma and myeloma models in CD36-deficient mice, compared to such tumors of wild type mice, and that such TAM in wild type mice have elevated CD36 expression and lipid accumulation compared to normal macrophages (Su et al., 2020).

[0008] Given the importance of CD36-mediated fatty acid or other ligand uptake to M2 TAM and MDSC biology, it might be expected that blocking of CD36 ligand transport would reduce tumor growth, by resulting in reduced immunosuppressive capacity, angiogenesis and/or metastasis that would otherwise be promoted by M2 TAM and MDSC. Indeed, mouse tumor models showed that tumor growth (lung and colon) was greatly reduced in mice deficient in CD36, and that this was due to CD36 loss among bone marrow-derived cells (Al-Khami et al., 2017).

[0009] Besides myeloid cells, it has more recently been reported that CD36 is expressed on some tumor-resident regulatory T cells, and that blocking CD36 with an antibody that prevents CD36-mediated fatty acid transport results in reduced melanoma growth in experimental models (Wang et al., 2020). Furthermore, CD36-deficiency on mouse regulatory T cells alone was shown to be sufficient to cause reduced melanoma growth (Wang et al., 2020). Most recently, CD36 expression by tumor-resident CD8 T cells has been shown to impair their anti-tumor capacity, and to result in increased tumor CD8 T cell death by lipid peroxidation leading to ferroptosis (Ma et al., 2021). A previous report also described

CD36 on tumor-infiltrated CD8 T cells and its adverse consequences on such CD8 T cells (Xu et al., 2020) and this report was later published in a peer-reviewed format (Xu et al., 2021).

[0010] There are also many reports of a role for ligand transport by CD36 expressed on tumor cells. For example, CD36 has been implicated in breast cancer tamoxifen resistance (Liang et al., 2018), melanoma chemotherapy resistance (Alioa et al., 2019) and lung cancer HER2-targeted therapy resistance (Feng et al., 2019). As long ago as 1993, CD36 was linked to adriamycin-resistance in a screen of an adriamycin-resistant subline of the human myelogenous leukemia line, K562, albeit over-expression of CD36 in the adriamycin-sensitive parental cell line did not alone confer adriamycin resistance (Sugimoto et al., 1993). CD36 is also involved in increased glioblastoma (Hale et al., 2014) and leukemic (Ye et al., 2016) stem cell self-renewal, survival and/or proliferation. That is, CD36 appears to be involved in chemotherapy-resistance by leukemic stem cells in adipose tissue niches (Ye et al., 2016). Glioblastoma formation using patient-derived glioblastoma stem cells in a xenograft mouse model was greatly reduced when CD36 was 'knocked-down' in the stem cells by RNA interference, and proliferation of glioblastoma stem cells was increased in a CD36-dependent manner by exposure to oxidized low-density lipoprotein (Hale et al., 2014). CD36 was also shown to be an informative biomarker of brain malignancy and negatively correlated with patient prognosis (Hale et al., 2014).

[0011] More recently, CD36 was shown to be important to prostate cancer progression and tumor growth in mouse models and reducing CD36 expression in prostate cancer cells by RNA interference or otherwise blocking CD36-mediated lipid transport with antibodies caused reduced cell migration capacity and tumor growth in mouse models (Watt et al., 2019).

[0012] CD36 has also been proposed as a prognostic metastasis biomarker in various cancers (reviewed by Enciu et al., 2018) and has been shown to facilitate metastasis of oral squamous cell carcinoma (OSCC) cells, breast cancer cells and melanoma cells in mouse models (PCT/EP20 16/073208; Pascual et al., 2017). OSCC metastasis was seen after cell injection into an orthotopic site (the tongue) and this was reduced by an antibody that blocks fatty acid transport by CD36. Melanoma and breast cancer cells were injected into the blood of mice so that they could potentially colonize metastatic niches, and this metastasis was reduced when using such cells in which CD36 was 'knocked down' by RNA interference (PCT/EP20 16/073208; Pascual et al., 2017). As mentioned above, CD36 is also anticipated to be involved in intravasation of tumor cells from a primary tumor site into lymphatic and/or blood circulation in the first place, via its roles on immune system myeloid cells. Fatty acid transport by CD36 on liver cancer cells was also shown to cause increased epithelial-mesenchymal transition, and chemical inhibition of CD36-mediated fatty acid transport reduced this phenotype as well as liver cancer cell migration (Nath et al., 2015).

[0013] All of the above paints a picture in which fatty acid or other ligand transport by CD36 into cells is involved in metabolic adaptation or differentiation of various cell types but it should also be highlighted that CD36 signaling leads to more direct immunosuppressive effects. Some of these were mentioned above but it should be highlighted that it has been proposed that apoptotic cell binding via CD36 normally facilitates homeostatic anti-inflammatory processes, and apoptotic cell binding via CD36

is at least in part responsible for IL-10 production by macrophages (among other immunosuppressive effects on macrophages) subsequent to apoptotic cell binding (Chung et al., 2007). CD36-mediated ligand transport may also lead to immunosuppressive metabolites. That is, increased arachidonic acid production as a result of CD36 ligand-induced intracellular signaling provides substrate for production of prostaglandins (e.g. Kuda et al., 2011), some of which are known to have wide-ranging immune-modulatory effects (e.g. see Wang & Dubois, 2006; Mizuno et al., 2019). Certainly, immune-suppressive prostaglandin E2 is produced in many cancers by tumor cells as well as immune system cells, including myeloid cells and others (Wang & Dubois, 2006; Mizuno et al., 2019) such as inducible regulatory T cells (Whiteside & Jackson, 2013). What's more, mouse macrophages at least have been shown to produce prostaglandin E2 in a CD36-dependent manner, albeit this was not in a cancer model (Almeida et al., 2014).

[0014] Besides the many roles of CD36 in cancer, CD36 ligand transport apparently plays a significant pathological role in foam cell formation in atherosclerosis (e.g., Zhao et al., 2018), non-alcoholic fatty liver disease (e.g., Rada et al., 2020) and other conditions. Thus, there are several potential applications for antibodies that prevent apoptotic cell or other ligand binding/transport by CD36.

SUMMARY

[0015] The present disclosure provides anti-CD36 antibodies that specifically bind human CD36 with high affinity. The antibodies are capable of decreasing, inhibiting, and/or fully-blocking immune regulatory effects mediated by CD36, including CD36-mediated fatty acid transport (e.g., cellular uptake of oxidized low-density lipoprotein, or 'oxLDL').

[0016] In at least one embodiment, the present disclosure provides an anti-CD36 antibody comprising (i) a first light chain complementary determining region (CDR-L1), a second light chain complementary determining region (CDR-L2), and a third light chain complementary determining region (CDR-L3), and/or (ii) a first heavy chain complementary determining region (CDR-H1), a second heavy chain complementary determining region (CDR-H2), and a third heavy chain complementary determining region (CDR-H3), wherein:

(a) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, 21, 24, or 27;

(b) CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, 28, 31, 34, 37, 40 , or 43;

(c) CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5;

(d) CDR-L1 comprises an amino acid sequence selected from SEQ ID NO: 7;

(e) CDR-L2 comprises an amino acid sequence selected from SEQ ID NO: 8, 12, or 15; and

(f) CDR-L3 comprises an amino acid sequence selected from SEQ ID NO: 9, 13, or, 18.

[0017] In at least one embodiment of the anti-CD36 antibody of the present disclosure:

(a) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 8, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 9;

(b) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 12, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 13;

(c) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 13;

(d) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(e) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 21, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(f) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 24, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(g) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 27, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 28, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(h) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 31, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(i) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 34, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino

acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(j) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 37, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(k) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 40, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18; or

(l) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 24, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 43, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18.

[0018] In at least one embodiment, the present disclosure provides an anti-CD36 antibody comprising: (i) a first heavy chain complementary determining region (CDR-H1), a second heavy chain complementary determining region (CDR-H2), and a third heavy chain complementary determining region (CDR-H3), wherein the CDR-H1, CDR-H2, and CDR-H3 sequences are from a VH region having an amino acid sequence selected from SEQ ID NO: 2, 20, 23, 26, 30, 33, 36, 39, and 42; and (ii) a first light chain complementary determining region (CDR-L1), a second light chain complementary determining region (CDR-L2), and a third light chain complementary determining region (CDR-L3), wherein the CDR-L1, CDR-L2, and CDR-L3 sequences are from a VL region having an amino acid sequence selected from SEQ ID NO: 6, 11, 14, and 17; wherein the CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 are according to Kabat numbering.

[0019] In at least one embodiment of the anti-CD36 antibody:

(a) the VH amino acid sequence is SEQ ID NO: 2 and the VL amino acid sequence is SEQ ID NO: 6;

(b) the VH amino acid sequence is SEQ ID NO: 2 and the VL amino acid sequence is SEQ ID NO: 11:

(c) the VH amino acid sequence is SEQ ID NO: 2 and the VL amino acid sequence is SEQ ID NO: 14;

(d) the VH amino acid sequence is SEQ ID NO: 2 and the VL amino acid sequence is SEQ ID NO: 17;

(e) the VH amino acid sequence is SEQ ID NO: 20 and the VL amino acid sequence is SEQ ID NO: 17;

(f) the VH amino acid sequence is SEQ ID NO: 23 and the VL amino acid sequence is SEQ ID NO: 17;

(g) the VH amino acid sequence is SEQ ID NO: 26 and the VL amino acid sequence is SEQ ID NO: 17;

(h) the VH amino acid sequence is SEQ ID NO: 30 and the VL amino acid sequence is SEQ ID NO: 17;

(i) the VH amino acid sequence is SEQ ID NO: 33 and the VL amino acid sequence is SEQ ID NO: 17;

(j) the VH amino acid sequence is SEQ ID NO: 36 and the VL amino acid sequence is SEQ ID NO: 17;

(k) the VH amino acid sequence is SEQ ID NO: 39 and the VL amino acid sequence is SEQ ID NO: 17; or

(l) the VH amino acid sequence is SEQ ID NO: 42 and the VL amino acid sequence is SEQ ID NO: 17.

[0020] In at least one embodiment of the anti-CD36 antibody of the present disclosure, the antibody comprises a heavy chain variable domain (V_H) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 2, 20, 23, 26, 30, 33, 36, 39, or 42; and/or a light chain variable domain (V_L) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 6, 11, 14, or 17.

[0021] In at least one embodiment of the anti-CD36 antibody of the present disclosure:

(a) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 2 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 6;

(b) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 2 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 11:

(c) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 2 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 14;

(d) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 2 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17;

(e) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 20 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17;

(f) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 23 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17;

(g) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 26 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17;

(h) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 30 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17;

(i) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 33 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17;

(j) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 36 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17;

(k) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 39 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17; or

(l) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 42 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17.

[0022] In at least one embodiment of the anti-CD36 antibody of the present disclosure, the antibody comprises a heavy chain (HC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 44, 48, 50, 51, 52, 53, 54, 55, 56, or 57, and/or a light chain (LC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 45, 46, 47, or 49.

[0023] In at least one embodiment of the anti-CD36 antibody of the present disclosure, the antibody comprises:

(a) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 44 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 45;

(b) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 44 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 46;

(c) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 44 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 47;

(d) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 44, and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;

(e) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 48, and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;

(f) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 50 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;

(g) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 51 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;

(h) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 52 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;

(i) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 53 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;

(j) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 54 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;

(k) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 55 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;

(l) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 56 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49; or

(m) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 57 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49.

[0024] In at least one embodiment of the anti-CD36 antibody of the present disclosure:

(a) the antibody binds to human CD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a hCD36 polypeptide of SEQ ID NO: 58 or 59;

(b) the antibody binds to mouse CD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a mCD36 polypeptide of SEQ ID NO: 60 or 61;

(c) the antibody binds to rhesus CD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a rhesus CD36 polypeptide of SEQ ID NO: 62 or 63;

(d) the antibody inhibits CD36-dependent oxidized LDL uptake in F293 cells that overexpress surface human CD36 by at least 65%, at least 75%, at least 85%, at least 95%, or at least 100%; optionally, wherein at an oxLDL concentration of 1-10 μg/mL the antibody has an IC_{50} of 5 nM or less, 1 nM or less, 0.5 nM or less, or 0.1 nM or less;

(e) the antibody inhibits CD36-dependent oxidized LDL uptake in U937 cells by at least 65%, at least 75%, at least 85%, at least 95%, or at least 100%; optionally, wherein at an oxLDL concentration of 1-10 μg/mL the antibody has an IC_{50} of 5 nM or less, 1 nM or less, 0.5 nM or less, or 0.1 nM or less; and/or

(f) the antibody inhibits CD36-dependent oxidized LDL uptake in mouse CD45+ TILs by at least 65%, at least 75%, at least 85%, at least 95%, or at least 100%; optionally, wherein at an oxLDL concentration of 1-10 μg/mL the antibody has an IC_{50} of 5 nM or less, 1 nM or less, 0.5 nM or less, or 0.1 nM or less.

[0025] The present disclosure also provides embodiments of the anti-CD36 antibodies disclosed herein, including embodiments wherein: (i) the antibody is a human, humanized, or chimeric antibody; (ii) the antibody comprises a fusion to a protein; optionally, a fusion to an immunostimulatory cytokine, such as IL-2, IL-7, IL-10, IL-12, IL-15, IL-21, or IFN-α; (iii) the antibody is a full length antibody of class IgG, optionally, wherein the class IgG antibody has an isotype selected from IgG1, IgG2, IgG3, and IgG4; (iv) the antibody comprises an Fc region variant, optionally an Fc region variant that alters effector function and/or a variant that alters antibody half-life; (v) the antibody is an antibody fragment, optionally selected from the group consisting of $F(ab')_2$, Fab', Fab, Fv, single domain antibody (VHH), and scFv; (vi) the antibody comprises an immunoconjugate, optionally, wherein the immunoconjugate comprises a therapeutic agent for treatment of a CD36-mediated disease or condition; or (vii) the antibody is a multi-specific antibody, optionally a bispecific antibody.

[0026] In at least one embodiment the present disclosure provides an isolated polynucleotide or a vector comprising a polynucleotide, wherein the polynucleotide sequence encodes an anti-CD36 antibody of the present disclosure or a polypeptide chain of an anti-CD36 antibody of the present disclosure. In at least one embodiment, the isolated polynucleotide or vector comprises a sequence that encodes a polypeptide of an anti-CD36 antibody of the present disclosure. In at least one embodiment of the isolated

polynucleotide or vector, the encoded polypeptide chain having an amino acid sequence comprising:

(a) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, 21, 24, or 27; CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, 28, 31, 34, 37, 40 , or 43; and CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5;

(b) CDR-L1 comprises an amino acid sequence selected from SEQ ID NO: 7; CDR-L2 comprises an amino acid sequence selected from SEQ ID NO: 8, 12, or 15; and CDR-L3 comprises an amino acid sequence selected from SEQ ID NO: 9, 13, or, 18;

(c) a heavy chain variable domain (V_H) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 2, 20, 23, 26, 30, 33, 36, 39, or 42;

(d) a light chain variable domain (V_L) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 6, 11, 14, or 17;

(e) a heavy chain (HC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 44, 48, 50, 51, 52, 53, 54, 55, 56, or 57; and/or

(f) a light chain (LC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 45, 46, 47, or 49.

[0027] In at least one embodiment the present disclosure also provides an isolated host cell comprising a polynucleotide or vector encoding an anti-CD36 antibody, or a polypeptide chain of an anti-CD36 antibody of the present disclosure. In at least one embodiment, the present disclosure also provides a method of producing an anti-CD36 antibody of present disclosure comprising culturing a host cell comprising a polynucleotide or vector encoding an anti-CD36 antibody so that an antibody is produced.

[0028] In at least one embodiment, the present disclosure provides a pharmaceutical composition comprising an anti-CD36 antibody of the present disclosure and a pharmaceutically acceptable carrier; optionally, wherein the composition further comprises a chemotherapeutic agent, and/or an antibody comprising a specificity for an immune checkpoint molecule.

[0029] In at least one embodiment, the present disclosure provides a method of treating a CD36-mediated disease in a subject, the method comprising administering to the subject a therapeutically effective amount of an anti-CD36 antibody of the present disclosure, or administering to the subject a therapeutically effective amount of a pharmaceutical composition of the present disclosure; optionally, wherein the disease is cancer; optionally, wherein the cancer is selected from colon cancer, pancreatic cancer, ovarian cancer, HCC, renal cancer, breast cancer, lung cancer, gastric cancer, melanoma, head and neck cancer, and oral cancer.

[0030] In at least one embodiment, the present disclosure provides a method for treating cancer in a subject, comprising administering to the subject a CD36 antagonist and a chemotherapeutic agent, and/or an antibody comprising a specificity for an immune checkpoint molecule; optionally, wherein the CD36 antagonist comprises an anti-CD36 antibody, a shRNA, a siRNA, a miRNA, a small molecule inhibitor of CD36, or a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] A better understanding of the novel features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings (also "Figure" and "FIG." herein), of which:

[0032] **FIG. 1A, FIG. 1B, FIG. 1C,** and **FIG. 1D** depict ELISA results for exemplary anti-CD36 antibodies of the present disclosure (12P109, A8A) in full-length human IgG1 format binding to either recombinant human CD36.ECD (**FIG. 1A, FIG. 1C**) or mouse CD36.ECD (**FIG. 1B, FIG. 1D**). The ELISA was carried out as described in Example 1.

[0033] **FIG. 2A**, **FIG. 2B**, **FIG. 2C**, **FIG. 2D**, **FIG. 2E**, and **FIG. 2F** depict ELISA results for exemplary anti-CD36 antibodies of the present disclosure (12P109, and 117 variants) binding to recombinant human CD36.ECD. The ELISA was carried out as described in Example 1.

[0034] **FIG. 3A**, **FIG. 3B, FIG. 3C FIG. 3D** and **FIG. 3E** depict plots showing results from SEC-UPLC analysis of anti-CD36 antibodies (12P109, A8A, and 117 variants) as described in Example 1.

[0035] **FIG. 4A**, **FIG. 4B**, **FIG. 4C**, **FIG. 4D**, **FIG. 4E**, and **FIG. 4F** depict plots showing results from cell surface binding analysis by flow cytometry of exemplary anti-CD36 antibodies of the present disclosure (117 variants) to stable F293 cells overexpressing human CD36 ("hCD36") (**FIG. 4A** and **FIG. 4B**), rhesus CD36 (**FIG. 4C** and **FIG. 4D**), or murine CD36 ("mCD36") (**FIG. 4E** and **FIG. 4F**) as described in Example 2.

[0036] **FIG. 5A** and **FIG. 5B** depict plots of extracted flow cytometry data showing the activity of anti-CD36 antibodies of the present disclosure in blocking oxLDL binding (**FIG. 5A**) and oxLDL uptake (**FIG. 5B**) by U937 cells as described in Example 3.

[0037] **FIG. 6A**, **FIG. 6B**, and **FIG. 6C** depict plots extracted from flow cytometry data illustrating the oxLDL uptake blocking activity exhibited by the anti-CD36 antibodies (12P109, and 117 variants) in F293/hCD36 or F293/mCD36 cells, as described in Example 3.

[0038] **FIG. 7A**, **FIG. 7B**, **FIG. 7C**, **FIG. 7D,** and **FIG. 7E** depict plots of data showing the ability of the anti-CD36 antibodies of the present disclosure (12P109, A8A, and 117 variants) to inhibit oxLDL uptake in TILs from colon cancer (CT26, MC38), liver cancer (BNL 1MEA.7R.1), lung cancer (LL2), and melanoma (B16F10) syngeneic mouse models as described in Example 4.

[0039] **FIG. 8A, FIG. 8B, FIG. 8C, FIG. 8D, FIG. 8E,** and **FIG. 8F** depict plots of data illustrating the ability of the anti-CD36 antibodies of the present disclosure (12P109, and 117 variants) to inhibit M2 macrophage polarization (**FIG. 8A** and **FIG. 8B**) and oxLDL-induced M2 macrophage activation (**FIG. 8C, FIG. 8D, FIG. 8E** and **FIG. 8F**), as described in Example 5.

[0040] **FIG. 9** depicts tumor growth curve results measured by bioluminescence in the $MYC^{OE}/p53^{KO}$ HCC model mice following treatment with the anti-CD36 antibody 117_30DA as described in Example 6.

[0041] **FIG. 10A, FIG. 10B,** and **FIG. 10C** depict results measured in β-$catenin^{OE}$/ MYC^{OE} HCC model mice following treatment with the anti-CD36 antibody 117_DA57E as described in Example 6. **FIG. 10A** shows the tumor growth curve results measured by bioluminescence. **FIG. 10B** shows a plot of

endpoint liver weight results. **FIG. 10C** shows a plot of plasma ALT (alanine transaminase) activity analysis results.

DETAILED DESCRIPTION

[0042] The present disclosure provides antibodies, including humanized antibodies, that specifically bind CD36 with high affinity and thereby inhibit, decrease, and/or fully block the function of CD36 as a cell surface protein involved in immune regulation, particularly the function of CD36-mediated fatty-acid and/or oxidized lipid transport (e.g., oxLDL uptake) in various roles of CD36 such as those detailed in the Background section, including but not limited to the roles of CD36 in regulating the functions of and maintenance or survival (or death, as the case may be) of tumor cells, tumor-associated macrophages (TAMs), MDSCs, regulatory T cells and CD8 T cells.

[0043] Accordingly, it is contemplated that any of the compositions or formulations comprising an anti-CD36 antibody of the present disclosure can be used as therapeutics for treatment of diseases mediated by the function of CD36 in fatty acid transport, such as cancer. Further, it is contemplated that the anti-CD36 antibody of the present disclosure can be used as a therapeutic in combination with other therapeutics such as cellular therapies, cytokines and other biologics or drugs that modify the tumor micro-environment and/or increase immune response, antibody-drug conjugates, antibodies that modulate immune cells, and/or antibodies that target immune checkpoint molecules including, but not limited to, PD1, PD-L1, LAG3, CTLA-4, A2AR, TIM-3, BTLA, CD276, CD328, VTCN1, IDO, KIR, NOX2, VISTA, OX40, CD27, CD28, CD40, CD122, CD137, GITR, ICOS.

[0044] **Overview of Terminology and Techniques**

[0045] For the descriptions herein and the appended claims, the singular forms “a”, and “an” include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to “a protein” includes more than one protein, and reference to “a compound” refers to more than one compound. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation. The use of “comprise,” “comprises,” “comprising” “include,” “includes,” and “including” are interchangeable and not intended to be limiting. It is to be further understood that where descriptions of various embodiments use the term “comprising,” those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language “consisting essentially of” or “consisting of.”

[0046] Where a range of values is provided, unless the context clearly dictates otherwise, it is understood that each intervening integer of the value, and each tenth of each intervening integer of the value, unless the context clearly dictates otherwise, between the upper and lower limit of that range, and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range.

Where the stated range includes one or both of the limits, ranges excluding (i) either or (ii) both of those included limits are also included in the invention. For example, "1 to 50," includes "2 to 25," "5 to 20," "25 to 50," "1 to 10," etc.

[0047] Generally, the nomenclature used herein and the techniques and procedures described herein include those that are well understood and commonly employed by those of ordinary skill in the art, such as the common techniques and methodologies described in Sambrook et al., Molecular Cloning-A Laboratory Manual (2nd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989 (hereinafter "Sambrook"); Current Protocols in Molecular Biology, F. M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (supplemented through 2011) (hereinafter "Ausubel"); Antibody Engineering, Vols. 1 and 2, R. Kontermann and S. Dubel, eds., Springer-Verlag, Berlin and Heidelberg (2010); Monoclonal Antibodies: Methods and Protocols, V. Ossipow and N. Fischer, eds., 2nd Ed., Humana Press (2014); Therapeutic Antibodies: From Bench to Clinic, Z. An, ed., J. Wiley & Sons, Hoboken, N.J. (2009); and Phage Display, Tim Clackson and Henry B. Lowman, eds., Oxford University Press, United Kingdom (2004).

[0048] All publications, patents, patent applications, and other documents referenced in this disclosure are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference herein for all purposes.

[0049] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present invention pertains. It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. For purposes of interpreting this disclosure, the following description of terms will apply and, where appropriate, a term used in the singular form will also include the plural form and vice versa.

[0050] "CD36," as used herein, refers to the CD36 protein, and as used herein encompasses the CD36 proteins of human, cynomolgus monkey, mouse, and any isoforms of these proteins. Amino acid sequences of various exemplary CD36 proteins are known in the art and are provided in Table 1 below and the attached Sequence Listing.

[0051] "CD36 mediated condition" or "CD36 mediated disease," as used herein, encompasses any medical condition associated with the specific binding of ligands to the cell surface protein CD36. For example, specific binding by CD36 of lipids and/or fatty acids acts to regulate or increase the immuno-suppressive capacity of TAMs and regulatory T cells in the tumor microenvironment. Accordingly, CD36 mediated diseases can include, but are not limited to, any disease or condition mediated by and/or responsive to antagonists or inhibitors of CD36, including but not limited to cancers.

[0052] "Antibody," as used herein, refers to a molecule comprising one or more polypeptide chains that specifically binds to, or is immunologically reactive with, a particular antigen. Exemplary antibodies of the present disclosure include monoclonal antibodies, polyclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, antibody fusions (e.g., fusion proteins), multispecific

antibodies (*e.g.*, bispecific antibodies), monovalent antibodies (e.g., single-arm antibodies), multivalent antibodies, antigen-binding fragments (e.g., Fab', $F(ab')_2$, Fab, Fv, rIgG, and scFv fragments), and synthetic antibodies (or antibody mimetics).

[0053] "Anti-CD36 antibody" or "antibody that binds CD36" refers to an antibody that binds CD36 with sufficient affinity such that the antibody is useful as a therapeutic and/or diagnostic agent for targeting CD36. In some embodiments, the extent of binding of an anti-CD36 specific antibody to an unrelated, non-CD36 antigen is less than about 20%, less than about 15%, less than about 10%, or less than about 5% of the binding of the antibody to CD36 as measured by, e.g., radioimmunoassay (RIA) or surface plasmon resonance (SPR). In some embodiments, an anti-CD36 antibody of the present disclosure has a dissociation constant (K_D) of < 1 μM, < 100 nM, < 10 nM, < 1 nM, < 0.1 nM, < 0.01 nM, or < 1 pM (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). Amino acid sequences of exemplary CD36 proteins of the present disclosure are provided in Table 2 below and the attached Sequence Listing.

[0054] "Full-length antibody," "intact antibody," or "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.

[0055] "Antibody fusion" refers to an antibody that is covalently conjugated (or fused) to a polypeptide or protein, typically via a linker to a terminus of the antibody's light chain (LC) or heavy chain (HC). Exemplary antibody fusions contemplated by the present disclosure can include an anti-CD36 antibody fused via a linker to a protein that is a T-cell activating or immunostimulatory cytokine, such as IL-2, IL-7, IL-10, IL-12, IL-15, IL-21, or IFN-α.

[0056] "Antibody fragment" refers to a portion of a full-length antibody which is capable of binding the same antigen as the full-length antibody. Examples of antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; monovalent, or single-armed antibodies; single-chain antibody molecules (*e.g.*, scFv); and multispecific antibodies formed from antibody fragments.

[0057] "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 are further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[0058] "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 (V_H and V_L, 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). A single V_H or V_L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds the antigen to screen a

library of complementary V_L or V_H domains, respectively (see, *e.g.*, Portolano *et al.*, J. Immunol. 150:880-887 (1993); Clarkson et al., Nature 352:624-628 (1991)).

[0059] "Hypervariable region" or "HVR," as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native antibodies comprise four chains with six HVRs; three in the heavy chain variable domain, V_H (HVR-H1, HVR-H2, HVR-H3), and three in the light chain variable domain, V_L (HVR-L1, HVR-L2, HVR-L3). The HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs). A number of hypervariable region delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. "Contact" hypervariable regions are based on an analysis of the available complex crystal structures. Residue ranges for hypervariable regions defined under these systems are noted in the table below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B[1] H31-H35[2]	H26-H35B[1] H26-H35[2]	H26-H32[1] H26-H32[2]	H30-H35B[1] H30-H35[2]
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[1] Kabat numbering
[2] Chothia numbering

[0060] In addition to the systems described above, HVRs and CDRs can be identified using the international ImMunoGeneTics information system, referred to as IMGT/V-Quest, described in Brochet, X. et al., Nucl. Acids Res. 36, W503-508 (2008). PMID: 18503082; and available for use online at www.imgt.org/IMGT_vquest/input. IMGT/V-Quest analyzes alignments to closest germline V gene variable region nucleotide sequences using IMGT unique numbering to identify HVRs and CDRs.

[0061] Hypervariable regions (HVRs), as used herein, may include extended or alternative hypervariable regions as follows: 27-32, 27-36, 24-34, or 24-38 (HVR-L1); 50-52, 54-56, 50-56 or 54-60 (HVR-L2); 89-97 or 93-101 (HVR-L3); 26-33, 26-35, or 31-35 (HVR-H1); 51-58, 50-61, or 50-66 (H2); and 97-110, 97-112, 99-110, or 99-112 (H3) in the V_H domain. The variable domain residues are numbered according to Kabat et al., *supra*, for each of these definitions.

[0062] "Complementarity determining region," or "CDR," as used herein, refers to the regions within the HVRs of the variable domain which have the highest sequence variability and/or are involved in antigen recognition. Generally, native antibodies comprise four chains with six CDRs; three in the heavy chain variable domains, V_H (CDR-H1, CDR-H2, CDR-H3), and three in the light chain variable domains, V_L (CDR-L1, CDR-L2, CDR-L3). Exemplary CDRs occur at variable domain amino acid residue positions: 24-34, 27-32, 27-36, 24-38 (CDR-L1); 50-56, 50-52, 54-56, or 54-60 (CDR-L2); 89-97, or 93-101 (CDR-L3); 31-35, or 26-33 (CDR-H1), 50-66, or 51-58 (CDR-H2); and 99-112, 99-110, 97-112, or 97-110 (CDR-H3).

[0063] "Framework" or "FR" refers to variable domain residues other than the HVR residues. The FRs of a variable domain generally consist of four domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in V_H (or V_L): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0064] Unless otherwise indicated, the positions of residues in the HVRs, CDRs, FRs, and other residues in the variable domain are numbered herein according to Kabat *et al.*, *supra*.

[0065] "Native antibody" refers to a naturally occurring immunoglobulin molecule. 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 (V_H), 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 (V_L), 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.

[0066] "Monoclonal antibody" as used herein refers to an antibody obtained from a substantially homogeneous population of antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies (*e.g.*, variant antibodies contain mutations that occur naturally or arise during production of a monoclonal antibody, and generally are 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 term "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 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.

[0067] "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.

[0068] "Humanized antibody" refers to a chimeric antibody comprising amino acid sequences from non-human CDRs and amino acid sequences 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 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.

[0069] "Human antibody" refers to an antibody which possesses an amino acid sequence corresponding 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.

[0070] "Human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin V_L or V_H framework sequences. Generally, the selection of human immunoglobulin V_L or V_H 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 V_L, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the V_H, the subgroup is subgroup III as in Kabat et al., supra.

[0071] "Acceptor human framework" as used herein is a framework comprising the amino acid sequence of a light chain variable domain (V_L) framework or a heavy chain variable domain (V_H) framework derived from a human immunoglobulin framework or a human consensus framework. 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 V_L acceptor human framework is identical in sequence to the V_L human immunoglobulin framework sequence or human consensus framework sequence.

[0072] "Fc region," refers to a dimer complex comprising the C-terminal polypeptide sequences of an immunoglobulin heavy chain, wherein a C-terminal polypeptide sequence is that which is obtainable by papain digestion of an intact antibody. The Fc region may comprise native or variant Fc sequences. Although the boundaries of the Fc sequence of an immunoglobulin heavy chain may vary, the human IgG heavy chain Fc sequence is usually defined to stretch from an amino acid residue at about position Cys226, or from about position Pro230, to the carboxyl-terminus of the Fc sequence. However, the C-terminal lysine (Lys447) of the Fc sequence may or may not be present. The Fc sequence of an

immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain.

[0073] "Fc receptor" or "FcR," refers to a receptor that binds to the Fc region of an antibody. In some embodiments, an FcR is a native human FcR. In some embodiments, an FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of those receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain, (see, *e.g.,* Daeron, Annu. Rev. Immunol. 15:203-234 (1997)). FcR, as used herein, also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al, J. Immunol. 1 17:587 (1976) and Kim et al, J. Immunol. 24:249 (1994)) and regulation of homeostasis of immunoglobulins. FcRs are reviewed, for example, in Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991); Capel et al, Immunomethods 4:25-34 (1994); and de Haas et al, J. Lab. Clin. Med. 126:330-41 (1995).

[0074] "Multispecific antibody" is an antibody having at least two different binding sites, each site with a different binding specificity. A multispecific antibody can be a full-length antibody or an antibody fragment, and the different binding sites may bind each to a different antigen or the different binding sites may bind to two different epitopes of the same antigen.

[0075] "Fv fragment" refers to an antibody fragment which contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in tight association, which can be covalent in nature, for example in scFv. It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs or a subset thereof confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although usually at a lower affinity than the entire binding site.

[0076] "Fab fragment' refers to an antibody fragment that contains a variable and constant domain of the light chain and a variable domain and the first constant domain (CH1) of the heavy chain. "$F(ab')_2$ fragments" comprise a pair of Fab fragments which are generally covalently linked near their carboxy termini by hinge cysteines between them. Other chemical couplings of antibody fragments also are known in the art.

[0077] "Antigen binding arm," as used herein, refers to a component of an antibody that has an ability to specifically bind a target molecule of interest. Typically, the antigen binding arm is a complex of immunoglobulin polypeptide sequences, *e.g.,* CDR and/or variable domain sequences of an immunoglobulin light and heavy chain.

[0078] "Single-chain Fv" or "scFv" refer to antibody fragments comprising the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, an Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired antigen binding structure.

[0079] "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). "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 equilibrium dissociation constant (K_D). 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.

[0080] "Binds specifically" or "specific binding" refers to binding of an antibody to an antigen with an affinity value of no more than about 1×10^{-7} M. In some embodiments, an antibody may have a secondary affinity for an antigen other than the antigen to which it binds specifically, where "secondary affinity" will generally refer to binding of an antibody to a secondary antigen with an affinity value of more than about 10 nM as described elsewhere herein. Where an antibody may have a secondary affinity for a secondary antigen, such an antibody will nevertheless bind specifically to the primary antigen.

[0081] "Isolated antibody" refers to an antibody 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 methods (*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.

[0082] "Effector function" refer to a biological activity attributed to the Fc region of an antibody, which varies with the antibody isotype. Examples of antibody effector functions include: Clq 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.

[0083] "Immunoconjugate" refers to an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0084] "Treatment," "treat" or "treating" refers to clinical intervention in an attempt to alter the natural course of a disorder in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desired results of treatment can include, but are not limited to, preventing occurrence or recurrence of the disorder, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disorder, preventing metastasis, decreasing the rate of progression, amelioration or palliation of a disease state, and remission or improved prognosis. For example, treatment can include administration of a therapeutically effective amount of pharmaceutical formulation comprising an anti-CD36 antibody to a subject to delay development or slow progression of

a disease or condition mediated by CD36 and/or its binding to ligands, or a disease or condition in which CD36 may play a role in the pathogenesis and/or progression.

[0085] "Pharmaceutical formulation" refers to a preparation in a form that allows the biological activity of the active ingredient(s) to be effective, and which contain no additional components which are toxic to the subjects to which the formulation is administered. A pharmaceutical formulation may include one or more active agents. For example, a pharmaceutical formulation may include an anti-CD36 antibody as the sole active agent of the formulation or may include an anti-CD36 antibody and one or more additional active agents, such as an inhibitor of an immune checkpoint molecule.

[0086] "Sole active agent," as used herein, refers an active agent in a pharmaceutical formulation that is the only active agent present in that formulation that provides, or would be expected to provide, the relevant pharmacological effect to treat the subject for the condition being treated. A pharmaceutical formulation comprising a sole active agent does not exclude the presence of one or more non-active agents, such as e.g., a pharmaceutically acceptable carrier, in the formulation. A "non-active agent" is an agent that would not be expected to provide, or otherwise significantly contribute to, the relevant pharmacological effect intended to treat the subject for the condition.

[0087] "Pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to the subject to whom it is administered. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0088] "Immune checkpoint molecule," as used herein, refers to a molecule that functions to regulate an immune system pathway and thereby prevent it from attacking cells unnecessarily. Many immune checkpoint molecules, both inhibitory and co-stimulatory, are targets for immunotherapy (e.g., with blocking antibodies to block immune inhibition or with agonists to promote immune stimulation) in the treatment of cancer and viral infections. Exemplary immune checkpoint molecules targeted for cancer immunotherapy include, but are not limited to, PD1, PD-L1, LAG3, CTLA-4, A2AR, TIM-3, BTLA, CD276, CD328, VTCN1, IDO, KIR, NOX2, VISTA, OX40, CD27, CD28, CD40, CD122, CD137, GITR, ICOS.

[0089] "Therapeutically effective amount" refers to the amount of an active ingredient or agent (*e.g.*, a pharmaceutical formulation) to achieve a desired therapeutic or prophylactic result, e.g., to treat or prevent a disease, disorder, or condition in a subject. In the case of a CD36 mediated disease or condition, the therapeutically effective amount of the therapeutic agent is an amount that reduces, prevents, inhibits, and/or relieves to some extent one or more of the symptoms associated with the disease, disorder, or condition. For cancer therapy, efficacy *in vivo* can, for example, be measured by assessing the growth of a primary tumor, occurrence and/or growth of secondary tumor(s), occurrence and/or number of metastases, duration, severity, and/or recurrence of symptoms, the response rate (RR), duration of response, and/or quality of life.

[0090] "Concurrently," as used herein, refers to administration of two or more therapeutic agents, where at least part of the administration overlaps in time. Accordingly, concurrent administration includes a

dosing regimen when the administration of one or more agent(s) continues after discontinuing the administration of one or more other agent(s).

[0091] "Individual" or "subject" refers to a mammal, including but 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).

[0092] **Detailed Description of Various Embodiments**

[0093] **I. CD36**

[0094] CD36 is a multifunctional transmembrane glycoprotein that acts as a cell surface receptor for a broad range of ligands. Generally, CD36 has two distinct binding domains for binding of thrombospondin versus other ligands of a lipidic nature, such as oxidized low-density lipoprotein (oxLDL), anionic phospholipids, long-chain fatty acids and bacterial diacylated lipopeptides. The cellular responses mediated by CD36 binding of these ligands are believed to include fatty acid metabolism, dietary fat processing, angiogenesis, and inflammatory response. CD36 acts as a coreceptor for the TLR4:TLR6 heterodimer, and thereby promotes inflammation in monocytes/macrophages. It is believed that CD36, upon binding a ligand, such as oxidized LDL ("oxLDL"), interacts with the TLR4:TLR6 heterodimer, and the complex is internalized thereby triggering an inflammatory response that leads to NF-kappa-B-dependent production of CXCL1, CXCL2 and CCL9 cytokines, via MyD88 signaling pathway, and CCL5 cytokine, via TICAM1 signaling pathway, as well as IL1B secretion, through the priming and activation of the NLRP3 inflammasome. Other CD36-interacting co-receptors have also been described.

[0095] The sequence and annotation of human CD36 (also referred to herein as "hCD36") can be found at UniProt entry P16671, and the full-length 472 amino acid sequence of Isoform 1 is set forth herein as SEQ ID NO: 58. The sequence and annotation of murine CD36 (also referred to herein as "mCD36") can be found at UniProt entry Q08857, and the full-length 472 amino acid sequence is set forth herein as SEQ ID NO:60. Table 1 below provides a summary description of the sequences of the human and mouse CD36 polypeptides used in the present disclosure, and their sequence identifiers. The sequences also are included in the accompanying Sequence Listing.

[0096] **TABLE 1:** Human and mouse CD36 polypeptides

Name	Sequence	SEQ ID NO:
hCD36 (isoform 1) UniProt P16671	MGCDRNCGLIAGAVIGAVLAVFGGILMPVGDLLIQKTIKKQVVLEEGTIAF KNWVKTGTEVYRQFWIFDVQNPQEVMMNSSNIQVKQRGPYTYRVRFLAKEN VTQDAEDNTVSFLQPNGAIFEPSLSVGTEADNFTVLNLAVAAASHIYQNQF VQMILNSLINKSKSSMFQVRTLRELLWGYRDPFLSLVPYPVTTTVGLFYPY NNTADGVYKVFNGKDNISKVAIIDTYKGKRNLSYWESHCDMINGTDAASFP PFVEKSQVLQFFSSDICRSIYAVFESDVNLKGIPVYRFVLPSKAFASPVEN PDNYCFCTEKIISKNCTSYGVLDISKCKEGRPVYISLPHFLYASPDVSEPI DGLNPNEEEHRTYLDIEPITGFTLQFAKRLQVNLLVKPSEKIQVLKNLKRN YIVPILWLNETGTIGDEKANMFRSQVTGKINLLGLIEMILLSVGVVMFVAF MISYCACRSKTIK	58

hCD36.ECD	GDLLIQKTIKKQVVLEEGTIAFKNWVKTGTEVYRQFWIFDVQNPQEVMMNS SNIQVKQRGPYTYRVRFLAKENVTQDAEDNTVSFLQPNGAIFEPSLSVGTE ADNFTVLNLAVAAASHIYQNQFVQMILNSLINKSKSSMFQVRTLRELLWGY RDPFLSLVPYPVTTTVGLFYPYNNTADGVYKVFNGKDNISKVAIIDTYKGK RNLSYWESHCDMINGTDAASFPPFVEKSQVLQFFSSDICRSIYAVFESDVN LKGIPVYRFVLPSKAFASPVENPDNYCFCTEKIISKNCTSYGVLDISKCKE GRPVYISLPHFLYASPDVSEPIDGLNPNEEEHRTYLDIEPITGFTLQFAKR LQVNLLVKPSEKIQVLKNLKRNYIVPILWLNETGTIGDEKANMFRSQVTGK IN	59
mCD36 UniProt Q08857	MGCDRNCGLIAGAVIGAVLAVFGGILMPVGDMLIEKTIKREVVLEEGTTAF KNWVKTGTTVYRQFWIFDVQNPDDVAKNSSKIKVKQRGPYTYRVRYLAKEN ITQDPEDHTVSFVQPNGAIFEPSLSVGTEDDNFTVLNLAVAAAPHIYQNSF VQVVLNSLIKKSKSSMFQTRSLKELLWGYKDPFLSLVPYPISTTVGVFYPY NDTVDGVYKVFNGKDNISKVAIIESYKGKRNLSYWPSYCDMINGTDAASFP PFVEKSRTLRFFSSDICRSIYAVFGSEIDLKGIPVYRFVLPANAFASPLQN PDNHCFCTEKVISNNCTSYGVLDIGKCKEGKPVYISLPHFLHASPDVSEPI EGLHPNEDEHRTYLDVEPITGFTLQFAKRLQVNILVKPARKIEALKNLKRP YIVPILWLNETGTIGDEKAEMFKTQVTGKIKLLGMVEMALLGIGVVMFVAF MISYCACKSKNGK	60
mCD36.ECD	GDMLIEKTIKREVVLEEGTTAFKNWVKTGTTVYRQFWIFDVQNPDDVAKNS SKIKVKQRGPYTYRVRYLAKENITQDPEDHTVSFVQPNGAIFEPSLSVGTE DDNFTVLNLAVAAAPHIYQNSFVQVVLNSLIKKSKSSMFQTRSLKELLWGY KDPFLSLVPYPISTTVGVFYPYNDTVDGVYKVFNGKDNISKVAIIESYKGK RNLSYWPSYCDMINGTDAASFPPFVEKSRTLRFFSSDICRSIYAVFGSEID LKGIPVYRFVLPANAFASPLQNPDNHCFCTEKVISNNCTSYGVLDIGKCKE GKPVYISLPHFLHASPDVSEPIEGLHPNEDEHRTYLDVEPITGFTLQFAKR LQVNILVKPARKIEALKNLKRPYIVPILWLNETGTIGDEKAEM FKTQVTGKIK	61
Rhesus CD36 NP_001028085. 1	MGCDRNCGLITGAVIGAVLAVFGGILMPVGDMLIQKTIKKEVVLEEGTIAF KNWVKTGTEIYRQFWIFDVQNPQEVMMNSSNIQVKQRGPYTYRVRFLAKEN ITQDPKDNTVSFLQPNGAIFEPSLSVGTEADNFTVLNLAVAAASHIYPNPF VQVVLNSLINKSKSSMFQVRTLRELLWGYTDPFLSLVPYPVSTRVGMFYPY NNTADGVYKVFNGKDSISKVAIIDTYKGKRNLSYWESYCDMINGTDAASFP PFVEKSQVLQFFSSDICRSIYAVFESDVNLKGIPVYRFVLPSKAFASPVQN PDNHCFCTEKIISKNCTSYGVLDISKCKEGKPVYISLPHFLYASPDVSETI DGLNPNEEEHRTYLDIEPITGFTLQFAKRLQVNLLVKPSNKIQVLKRLKRN YIVPILWLNETGTIGDEKAKMFRSQVTGKINLLGLIEMILLSVGVVMFVAF MISYCACRSKTIK	62
Rhesus CD36.ECD	GDMLIQKTIKKEVVLEEGTIAFKNWVKTGTEIYRQFWIFDVQNPQEVMMNS SNIQVKQRGPYTYRVRFLAKENITQDPKDNTVSFLQPNGAIFEPSLSVGTE ADNFTVLNLAVAAASHIYPNPFVQVVLNSLINKSKSSMFQVRTLRELLWGY TDPFLSLVPYPVSTRVGMFYPYNNTADGVYKVFNGKDSISKVAIIDTYKGK RNLSYWESYCDMINGTDAASFPPFVEKSQVLQFFSSDICRSIYAVFESDVN LKGIPVYRFVLPSKAFASPVQNPDNHCFCTEKIISKNCTSYGVLDISKCKE GKPVYISLPHFLYASPDVSETIDGLNPNEEEHRTYLDIEPITGFTLQFAKR LQVNLLVKPSNKIQVLKRLKRNYIVPILWLNETGTIGDEKAKMFRSQVTGK IN	63

[0097] II. Anti-CD36 Antibodies

[0098] In some embodiments, the present disclosure provides structures of anti-CD36 antibodies in terms of the amino acid and encoding nucleotide sequences of the various well-known immunoglobulin features (e.g., CDRs, FRs, V_H, V_L domains, and full-length heavy and light chains). Table 2 below provides a summary description of the anti-CD36 antibodies of the presented disclosure as generated and functionally characterized as described in the Examples. The relevant sequences and sequence identifiers

for each of the antibodies are provided in Table 2 and also included in the accompanying Sequence Listing.

[0099] TABLE 2: Anti-CD36 antibody sequences

Description	Sequence	SEQ ID NO:
12P109 – scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGCAACTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCTACCTCCACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGTACTACACCTTGCCGTTCACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCACCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTTACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	1
12P109 - V_H A8A - V_H A8A -N52T- V_H 117 - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	2
12P109 – CDR-H1 A8A – CDR-H1 A8A-N52T – CDR-H1 117 - CDR-H1 117_57D - CDR-H1 117_57E - CDR-H1 117_57DE - CDR-H1 117_57EE - CDR-H1	AASGFTISSFGIH	3
12P109 - CDR-H2 A8A - CDR-H2 A8A-N52T - CDR-H2 117 - CDR-H2 117_30AA - CDR-H2 117_30DA - CDR-H2	WIAPYGGYTY	4
12P109 – CDR-H3 A8A – CDR-H3 A8A-N52T – CDR-H3 117 - CDR-H3 117_30AA - CDR-H3 117_30DA - CDR-H3 117_30DE - CDR-H3 117_57D - CDR-H3 117_57E - CDR-H3 117_57DE - CDR-H3 117_57EE - CDR-H3 117_DA57E - CDR-H3	ARSFFGYFDY	5

12P109 – V_L	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISTSTSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQYYTLPFTFGQGTKVEIKR	6
12P109 – CDR-L1 A8A – CDR-L1 A8A-N52T – CDR-L1 117 - CDR-L1 117_30AA - CDR-L1 117_30DA - CDR-L1 117_30DE - CDR-L1 117_57D - CDR-L1 117_57E - CDR-L1 117_57DE - CDR-L1 117_57EE - CDR-L1 117_DA57E - CDR-L1	RASQDVSNWVA	7
12P109 – CDR-L2	STSTSLYS	8
12P109 - CDR-L3	YYTLPFTF	9
A8A – scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCCAACAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACCTTCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGTGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTTACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	10
A8A – V_L	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYANSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNLPLTFGQGTKVEIKR	11
A8A – CDR-L2	SYANSLYS	12
A8A - CDR-L3 A8A-N52T - CDR-L3	HSNLPLTF	13
A8A-N52T – V_L	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYA**T**SLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNLPLTFGQGTKVEIKR	14
A8A-N52T – CDR-L2 117 - CDR-L2 117_30AA - CDR-L2 117_30DA - CDR-L2 117_30DE - CDR-L2 117_57D - CDR-L2 117_57E - CDR-L2 117_57DE - CDR-L2 117_57EE - CDR-L2 117_DA57E - CDR-L2	SYA**T**SLYS	15
117- scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC	16

	AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTTACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	
117 - V_L 117_30AA - V_L 117_30DA - V_L 117_30DE - V_L 117_57D - V_L 117_57E - V_L 117_57DE - V_L 117_57EE - V_L 117_DA57E - V_L	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYATSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNAPLTFGQGTKVEIKR	17
117 - CDR-L3 117_30AA - CDR-L3 117_30DA - CDR-L3 117_30DE - CDR-L3 117_57D - CDR-L3 117_57E - CDR-L3 117_57DE - CDR-L3 117_57EE - CDR-L3 117_DA57E - CDR-L3	HSNAPLTF	18
117_30AA (HC - DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTGC CGCCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTTACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	19
117_30AA - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTIAAFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	20
117_30AA - CDR-H1	AASGFTIAAFGIH	21
117_30DA- scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA	22

	GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTGA CGCCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTTACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	
117_30DA - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTIDAFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	23
117_30DA - CDR-H1 117_ DA57E - CDR-H1	AASGFTIDAFGIH	24
117_30DE- V_H (DNA)	GAAGTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTG GCGGCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCAT TGACAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAG GGGCTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGAAA CATACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGC GGACACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTG CGTGCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTT TCGGGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGT GAGCTCG	25
117_30DE - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTIDSFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	26
117_30DE - CDR-H1	AASGFTIDSFGIH	27
117_30DE - CDR-H2	WIAPYGGETY	28
117_57D- scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	29
117_57D - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGDTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	30
117_57D - CDR-H2	WIAPYGGDTY	31

117_57E- scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGAAACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	32
117_57E - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	33
117_57E - CDR-H2	WIAPYGGETY	34
117_57DE- V_H (DNA)	GAAGTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTG GCGGCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCAT TAGCAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAG GGGCTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGACG AATACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGC GGACACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTG CGTGCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTT TCGGGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGT GAGCTCG	35
117_57DE - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGDEYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	36
117_57DE - CDR-H2	WIAPYGGDEY	37
117_57EE- V_H (DNA)	GAAGTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTG GCGGCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCAT TAGCAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAG GGGCTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGAAG AATACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGC GGACACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTG CGTGCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTT TCGGGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGT GAGCTCG	38
117_57EE - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGEEYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	39
117_57EE - CDR-H2	WIAPYGGEEY	40
117_ DA57E- scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG	41

	ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGAAACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	
117_ DA57E - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTIDAFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	42
117_ DA57E - CDR-H2	WIAPYGGETY	43
12P109 – HC A8A – HC A8A-N52T – HC 117 – HC (IgG1-N297A)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla psskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkv epkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpe vtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqyastyr vvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqpr epqvytlppsreemtknqvsltclvkgfypsdiavewesngqp ennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhe alhnhytqkslslspg	44
12P109 - LC	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISTSTSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQYYTLPFTFGQGTKVEIKRtvaapsvfifppsdeqlksgt asvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdst yslsstltlskadyekhkvyacevthqglsspvtksfnrgec	45
A8A - LC	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYANSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNLPLTFGQGTKVEIKRtvaapsvfifppsdeqlksgt asvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdst yslsstltlskadyekhkvyacevthqglsspvtksfnrgec	46
A8A-N52T - LC	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYA**T**SLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNLPLTFGQGTKVEIKRtvaapsvfifppsdeqlksgt asvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdst yslsstltlskadyekhkvyacevthqglsspvtksfnrgec	47
117 – HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	48
117 – LC 117_30AA - LC 117_30DA - LC 117_30DE - LC 117_57D - LC 117_57E - LC	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYATSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNAPLTFGQGTKVEIKRtvaapsvfifppsdeqlksgt asvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdst yslsstltlskadyekhkvyacevthqglsspvtksfnrgec	49

117_57DE - LC 117_57EE - LC 117_DA57E - LC		
117_30AA – HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTIAAFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkcykckvsnkglpssicktiskakgqprcpq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	50
117_30DA – HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTIDAFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	51
117_30DE – HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTIDSFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	52
117_57D- HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGDTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstscstaalgclvkdyfpcpvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqcdpcvqfnwyvdgvcvhnaktkprccqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	53
117_57E- HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn	54

	ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	
117_57DE- HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGDEYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	55
117_57EE- HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGEEYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstscstaalgclvkdyfpcpvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	56
117_ DA57E- HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTIDAFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	57

[0100] 1. Anti-CD36 Antibody Binding Affinity and Functional Characteristics

[0101] In some embodiments, the anti-CD36 antibodies provided herein have an equilibrium dissociation constant (K_D) for binding to CD36 of < 100 nM, < 10 nM, < 1 nM, < 0.1 nM, < 0.01 nM, or < 0.001 nM (*e.g.*, 10^{-8} M or less, from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M).

[0102] It is contemplated that the various anti-CD36 antibodies generated as disclosed herein include antibodies capable of high-affinity binding to hCD36, mCD36, rhesus CD36, both hCD36 and mCD36, and/or hCD36, mCD36, and rhesus CD36. More specifically, in some embodiments, the anti-CD36 antibodies of the present disclosure bind to hCD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less, or 1 x 10^{-11} M or less. In some embodiments, the binding affinity is measured as the equilibrium dissociation constant (K_D) for binding to the hCD36 polypeptide of SEQ ID NO: 58 or SEQ ID NO: 59. In some embodiments, the anti-CD36 antibodies of the present disclosure bind to mCD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less, or 1 x 10^{-11} M or less. In some embodiments, the binding affinity is measured as the equilibrium dissociation constant (K_D) for binding to the mCD36 polypeptide of SEQ ID NO: 60 or SEQ ID NO: 61. In some

embodiments, the anti-CD36 antibodies of the present disclosure bind to rhesus CD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less, or 1 x 10^{-11} M or less. In some embodiments, the binding affinity is measured as the equilibrium dissociation constant (K_D) for binding to the rhesus CD36 polypeptide of SEQ ID NO: 62 or SEQ ID NO: 63. In some embodiments, the anti-CD36 antibodies of the present disclosure bind to both hCD36 and mCD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less, or 1 x 10^{-11} M or less. In some embodiments, the anti-CD36 antibodies of the present disclosure bind to both hCD36 and rhesus CD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less, or 1 x 10^{-11} M or less.

[0103] Generally, binding affinity of a ligand to its receptor can be determined using any of a variety of assays and expressed in terms of a variety of quantitative values. Specific CD36 binding assays useful in determining affinity of the antibodies are disclosed in the Examples herein. Additionally, antigen binding assays are known in the art and can be used herein including without limitation any direct or competitive binding assays using techniques such as western blots, radioimmunoassays, enzyme-linked immunoabsorbent assay (ELISA), "sandwich" immunoassays, surface plasmon resonance based assay (such as the BIAcore assay as described in WO2005/012359), immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, flow cytometric and fluorescence activated cell sorting (FACS) assays, and the like.

[0104] Accordingly, in some embodiments, the binding affinity is expressed as K_D values and reflects intrinsic binding affinity (e.g., with minimized avidity effects). The anti-CD36 antibodies of the present disclosure exhibit strong binding affinities for the hCD36 polypeptide of SEQ ID NO: 58, for example, exhibiting K_D values of between 10 nM and 1 pM. Accordingly, anti-CD36 antibodies of the present disclosure may compete with antibodies having lower affinity for the same or overlapping epitopes of CD36.

[0105] In some embodiments, the anti-CD36 antibodies provided herein decrease, inhibit, and/or fully-block ligand binding to CD36, and immune regulation and/or immune signaling mediated by ligand binding to CD36, including the maintenance of TAMs in the tumor microenvironment. The ability of the antibodies to inhibit these immune regulatory and immune signaling pathways mediated by ligand binding to CD36 can be assayed *in vitro* using known cell-based assays including those assays described in the Examples of the present disclosure.

[0106] Accordingly, in some embodiments, the CD36 antibodies of the present disclosure are characterized by one or more of following functional properties based on the ability to decrease, inhibit, and/or fully-block intracellular signaling by CD36-mediated pathways.

[0107] In at least one embodiment, the anti-CD36 antibody binds to human CD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a hCD36 polypeptide of SEQ ID NO: 58 or 59.

[0108] In at least one embodiment of the anti-CD36 antibody, the antibody binds to mouse CD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less; optionally, wherein the

binding affinity is measured by equilibrium dissociation constant (K_D) to a mCD36 polypeptide of SEQ ID NO: 60 or 61.

[0109] In at least one embodiment of the anti-CD36 antibody, the antibody binds to rhesus CD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a rhesus CD36 polypeptide of SEQ ID NO: 62 or 62.

[0110] In at least one embodiment of the anti-CD36 antibody, the antibody inhibits CD36-dependent oxidized LDL uptake in F293 cells that overexpress surface human CD36 by at least 65%, at least 75%, at least 85%, at least 95%, or at least 100%; optionally, wherein at an oxLDL concentration of 1-10 μg/mL the antibody has an IC_{50} of 5 nM or less, 1 nM or less, 0.5 nM or less, or 0.1 nM or less.

[0111] In at least one embodiment of the anti-CD36 antibody, the antibody inhibits CD36-dependent oxidized LDL uptake in U937 cells by at least 65%, at least 75%, at least 85%, at least 95%, or at least 100%; optionally, wherein at an oxLDL concentration of 1-10 μg/mL the antibody has an IC_{50} of 5 nM or less, 1 nM or less, 0.5 nM or less, or 0.1 nM or less.

[0112] In at least one embodiment of the anti-CD36 antibody, the antibody inhibits CD36-dependent oxidized LDL uptake in mouse CD45+ TILs by at least 65%, at least 75%, at least 85%, at least 95%, or at least 100%; optionally, wherein at an oxLDL concentration of 1-10 μg/mL the antibody has an IC_{50} of 5 nM or less, 1 nM or less, 0.5 nM or less, or 0.1 nM or less.

[0113] 2. Anti-CD36 Antibody Fragments

[0114] In some embodiments, the anti-CD36 antibody of the present disclosure can be an antibody fragment. Antibody fragments useful with the binding determinants the present disclosure include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, scFv fragments, monovalent, single domain antibody, one-armed or single-arm antibody, and other fragments described herein and known in the art. Accordingly, in some embodiments of the anti-CD36 antibodies of the present disclosure, the antibody is an antibody fragment selected from the group consisting of $F(ab')_2$, Fab', Fab, Fv, single domain antibody (VHH), single-arm antibody, and scFv.

[0115] For a review of various antibody fragments, see e.g., Hudson et al. Nat. Med. 9: 129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO93/16185; and U.S. Pat. Nos. 5,571,894 and 5,587,458. For a description of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half- life, see U.S. Pat. No. 5,869,046. Other monovalent antibody forms are described in, e.g., WO2007/048037, WO2008/145137, WO2008/145138, and WO2007/059782. Monovalent, single-armed antibodies are described, e.g., in WO2005/063816. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific (see e.g., EP0404097; WO93/01161; Hudson et al., Nat. Med. 9: 129-134 (2003); and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993)).

[0116] In some embodiments, the antibody fragments are single-domain antibodies which comprise 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 some embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., US Pat. No. 6,248,516).

[0117] 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.

[0118] 3. Chimeric, Humanized, and Human Anti-CD36 Antibodies

[0119] In some embodiments, the anti-CD36 antibody of the present disclosure can be a chimeric antibody. (See e.g., chimeric antibodies as described in U.S. Pat. No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81: 6851-6855 (1984)). In one embodiment, 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 some embodiments, a chimeric antibody is a "class switched: antibody in which the class or subclass has been changed from that of the parent antibody. It is contemplated that chimeric antibodies can include antigen-binding fragments thereof.

[0120] In some embodiments, the anti-CD36 antibody of the present disclosure 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, 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 CDR residues are derived) to restore or improve antibody specificity or affinity.

[0121] Humanized antibodies and methods of making them are reviewed in, e.g., Almagro and Fransson, Front. Biosci. 13: 1619-1633 (2008), and are further described in, e.g., Riechmann et al., Nature 332:323-329 (1988); Queen et al., Proc. Natl. Acad. Sci. USA 86: 10029-10033 (1989); US Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al, Methods 36: 25-34 (2005) (describing SDR (a-HVR) 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).

[0122] 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)).

[0123] In some embodiments, the anti-CD36 antibody of the present disclosure can be 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). 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., XENOMOUSE™ technology in U.S. Pat. Nos. 6,075,181 and 6,150,584; HUMAB® technology in U.S. Pat. No. 5,770,429; K-M MOUSE® technology in U.S. Pat. No. 7,041,870; and VELOCIMOUSE® technology in U.S. Pat. Appl. Pub. No. US 2007/0061900). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0124] 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. Pat. No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines). 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).

[0125] 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.

[0126] 4. Library-Derived Variants of Anti-CD36 Antibodies

[0127] In at least one embodiment, improved variants of anti-CD36 antibodies may be isolated by screening combinatorial libraries for antibodies with the desired improved functional characteristic, such as binding affinity or cross-reactivity. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for variant antibodies possessing the improved binding characteristics. Other methods for producing such library-derived antibodies can be

found in e.g. , Hoogenboom *et al.*, Methods in Molecular Biology 178: 1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001); McCafferty et al., Nature 348: 552-554 (1990); Clackson et al., Nature 352: 624-628 (1991); Marks et al., J. Mol. Biol. 222: 581-597 (1992); Marks and Bradbury, m 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).

[0128] 5. Multispecific Antibodies and Antibody Fusions

[0129] In at least one embodiment, it is contemplated that an anti-CD36 antibody of the present disclosure can be a multispecific antibody, *e.g.*, a bispecific antibody. In some embodiments, the multispecific antibody has at least two different binding sites, each with a binding specificity for a different antigen, at least one of which specifically binds CD36. In at least one embodiment, it is contemplated that the multispecific antibody is a bispecific antibody comprising a specificity for CD36 and a specificity for another antigen that mediates immune regulation, immune signaling, and/or is expressed on a cancer or tumor cell. For example, the other specificity could be for an immune checkpoint molecule, such as PD1, LAG3, CTLA-4, A2AR, TIM-3, BTLA, CD276, CD328, VTCN1, IDO, KIR, NOX2, VISTA, OX40, CD27, CD28, CD40, CD122, CD137, GITR, or ICOS.

[0130] 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 e.g., Milstein and Cuello, Nature 305: 537 (1983), WO 93/08829, and Traunecker et al., EMBO J. 10: 3655 (1991)). "Knob-in-hole" engineering can also be used to generate bispecific antibodies useful with the anti-CD36 antibodies of the present disclosure. Techniques for knob-in-hole engineering are known in the art and described in e.g., U.S. Patent No. 5,731,168.

[0131] Multispecific antibodies can also be made by engineering "electrostatic steering" effects that favor formation of Fc-heterodimeric antibody molecules rather than homodimers (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Pat. No. 4,676,980, and Brennan et al., Science 229: 81 (1985)); using leucine zippers to produce bispecific 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)); using single-chain Fv (scFv) dimers (see, e.g. Gruber et al., J. Immunol. 152: 5368 (1994)); or tri-specific antibodies (see e.g., Tutt et al., J. Immunol. 147: 60 (1991).

[0132] In at least one embodiment, the anti-CD36 antibodies provided herein can comprise an antibody fusion with a protein. Methods for preparation and use of antibody fusions or fusion proteins are well known in the art. Typically, the antibody is covalently conjugated (or fused) to the protein, typically via a linker polypeptide. The conjugation can occur via the terminus of the antibody's light chain (LC) or heavy chain (HC). Antibody fusions can also be prepared with antibody fragments. In one exemplary embodiment of an antibody fusion contemplated by the present disclosure, the fusion includes a full length anti-CD36 antibody fused via a linker at a light or heavy chain terminus to a T-cell activating or immunostimulatory cytokine. The cytokine can include, but is not limited to, IL-2, IL-7, IL-10, IL-12,

IL-15, IL-21, or IFN-α. Such an anti-CD36 antibody fusion can block activity mediated by CD36 signaling and provide an immunostimulatory cytokine effect. The ability of such anti-CD36 antibody fusions to provide immunostimulatory cytokine effects can be assayed *in vitro* using known cell-based assays associated with the cytokine.

[0133] 6. Variants of Anti-CD36 Antibodies

[0134] In some embodiments, variants of the anti-CD36 antibodies of the present disclosure are contemplated having improved characteristics such as binding affinity and/or other biological properties of the antibody. Variants can 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 characteristic of CD36 antigen binding.

[0135] A. Substitution, Insertion, and Deletion Variants

[0136] In some embodiments, anti-CD36 antibody variants having one or more amino acid substitutions in addition to those described herein are provided. Sites for mutagenesis can include the CDRs, HVRs and FRs. Typical "conservative" amino acid substitutions and/or substitutions based on common side-chain class or properties are well-known in the art and can be used in the embodiments of the present disclosure. The present disclosure also contemplates variants based on non-conservative amino acid substitutions in which a member of one of amino acid side chain class is exchanged for an amino acid from another class. Amino acid side chains are typically grouped according to the following classes or common properties: (1) hydrophobic: Met, Ala, Val, Leu, Ile, Norleucine; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) chain orientation influencing: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. Techniques are well-known in the art for amino acid substitution into an antibody and subsequent screening for desired function, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

[0137] Amino acid substitution variants can also include variants having one or more substitutions in hypervariable regions of a parent antibody. Generally, the resulting variant(s) selected for further study will have modifications of certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will retain certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, using phage display-based affinity maturation techniques. 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).

[0138] One useful method for identifying residues or regions of an antibody that may be targeted for mutagenesis is "alanine scanning mutagenesis" (see e.g., Cunningham and Wells, Science 244: 1081-1085 (1989)). 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., Ala 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 can be determined. 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.

[0139] Amino acid sequence insertions which can be prepared include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intra-sequence 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 can include a fusion of the N- or C-terminus of the antibody to an enzyme or a polypeptide which increases antibody serum half-life.

[0140] Other residue substitutions can be made in HVRs to improve antibody affinity. Such alterations may be made in "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)) with the resulting variant V_H or V_L being tested for binding affinity. In one embodiment, affinity maturation can be carried out by constructing and reselecting from secondary libraries (see e.g., in Hoogenboom et al., Methods in Molecular Biology 178: 1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) 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. HVR-H3 and HVR-L3 in particular are often targeted. Generally, substitutions, insertions, or deletions can be made 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 be outside of HVR "hotspots."

[0141] In some embodiments, it is contemplated that the anti-CD36 antibody described herein can be substituted at specific non-HVR positions with cysteine residues so as to create reactive thiol groups. Such engineered "thioMAbs" can be used to conjugate the antibody to e.g., drug moieties or linker-drug moieties and thereby create immunoconjugates, as described elsewhere herein. Cysteine engineered antibodies can be generated as described in e.g., U.S. Pat. No. 7,521,541. In some embodiments, any one or more of the following antibody residues can 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.

[0142] B. Glycosylation Variants

[0143] In some embodiments, the anti-CD36 antibody of the present disclosure is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody can be carried out by altering the amino acid sequence such that one or more glycosylation sites is created or removed. In embodiments where the antibody comprises an Fc region, the

carbohydrate attached to the Fc region can be altered. Typically, native antibodies produced by mammalian cells comprise a branched, biantennary oligosaccharide attached by an N-linkage to the asparagine at about position 297 ("N297") 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, such as mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as, a fucose attached to a GlcNAc in the "stem" of the bi-antennary oligosaccharide structure. In some embodiments, the modifications of the oligosaccharide of an Fc region of an antibody can create a variant with certain improved properties.

[0144] In some embodiments, the anti-CD36 antibody of the present disclosure can be a variant comprising 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 about 1% to about 80%, from about 1% to about 65%, from about 5% to about 65%, or from about 20% to about 40%. The amount of fucose can be determined by calculating the average amount of fucose within the sugar chain attached to residue N297, relative to the sum of all glyco-structures attached at N297 (*e.g.,* complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry (see e.g., WO 2008/077546).

[0145] In some embodiments, the fucosylation variants can provide improved ADCC function of the variant antibody. See, e.g., US Patent Publication Nos. US 2003/0157108, or US 2004/0093621. Examples of "defucosylated" or "fucose-deficient" antibodies and associated methods for preparing them are disclosed in e.g., US2003/0157108; US2003/0115614; US2002/0164328; US2004/0093621; US2004/0132140; US2004/0110704; US2004/0110282; US2004/0109865; WO2000/61739; WO2001/29246; WO2003/085119; WO2003/084570; WO2005/035586; WO2005/035778; WO2005/053742; WO2002/031140; Okazaki et al., J. Mol. Biol. 336: 1239-1249 (2004); Yamane-Ohnuki et al., Biotech. Bioeng. 87: 614 (2004). Cell lines useful for producing defucosylated antibodies include Led 3 CHO cells deficient in protein fucosylation (see e.g., Ripka et al., Arch. Biochem. Biophys. 249: 533-545 (1986); US2003/0157108, and WO2004/056312), 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).

[0146] C. Fc Region Variants

[0147] In some embodiments, an anti-CD36 antibody of the present disclosure can comprise one or more amino acid modifications in the Fc region (i.e., 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 substitution at one or more amino acid residue positions. A wide range of Fc region variants known in the art that are useful with the anti-CD36 antibodies of the present disclosure are described below.

[0148] In some embodiments, the anti-CD36 antibody is an Fc region variant which has altered effector function. In some embodiments, the antibody with altered effector function possesses some (but not all of) the effector functions, decreased effector function, or none of the effector functions (e.g., effectorless) of the parent antibody. Effectorless Fc region variants are more desirable for certain applications where effector function (such as ADCC) is unnecessary or deleterious, and/or *in vivo* half-life of the antibody is

important. Fc region variant antibodies having reduced effector or effectorless function can result from amino acid substitution at one or more of the following Fc region positions: 238, 265, 269, 270, 297, 327 and 329. (see, e.g., U.S. Patent No. 6,737,056). Such Fc region variants can include amino acid substitutions at two or more of positions 265, 269, 270, 297 and 327. Such Fc region variants can also include substitutions of both residues 265 and 297 to alanine (see e.g., US Pat. No. 7,332,581).

[0149] Some Fc region variants are capable of providing improved or diminished binding to FcRs (see e.g., U.S. Pat. No. 6,737,056; WO 2004/056312; and Shields et al., J. Biol. Chem. 9(2): 6591- 6604 (2001)). Some Fc region variants capable of providing improved ADCC comprise one or more amino acid substitutions at e.g., positions 298, 333, and/or 334 of the Fc region (based on EU numbering). Fc region variants having altered (i.e., either improved or diminished) Clq binding and/or Complement Dependent Cytotoxicity (CDC), as described in e.g., US Pat. No. 6,194,551, WO99/51642, and Idusogie et al., J. Immunol. 164: 4178- 4184 (2000).

[0150] Some Fc region variants are capable of providing increased half-lives and improved binding to the neonatal Fc receptor (FcRn) are disclosed in e.g., US2005/0014934A1 (Hinton et al.). Such Fc region variants comprise amino acid substitutions at one or more of positions: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424, and 434. Other Fc region variants with increased half-lives include the set of YTE mutations at positions 252, 254, and 256 (i.e., M252Y/S254T/T256E) described in e.g., US 7658921B2 (Dall'Acqua et al.). Additional examples of Fc region variants can be found in e.g., U.S. Pat. Nos. 5,648,260 and 5,624,821; and WO94/29351.

[0151] Generally, *in vitro* and/or *in vivo* cytotoxicity assays can be carried out to confirm the reduction/depletion of CDC and/or ADCC activities in an Fc region variant. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity) but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells express FcγRIII only, whereas monocytes express FcγRI, FcγRII, and FcγRIII. Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see, e.g. Hellstrom, et al., Proc. Natl. Acad. Sci. USA 83: 7059-7063 (1986)) and Hellstrom, et al., Proc. Natl. 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 assay methods may be employed (see, for example, ACTI™ nonradioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox96® 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 an animal model such as that disclosed in Clynes et al. Proc. Natl. Acad. Sci. USA 95: 652-656 (1998). Clq binding assays may also be carried out to confirm that the antibody is unable to bind Clq and hence lacks CDC activity. See, e.g., Clq and C3c binding ELISA in WO2006/029879 and WO2005/100402. To assess complement activation, a CDC assay may be performed (see, e.g., 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 be performed using methods known in the art (see, e.g., Petkova et al., Intl. Immunol. 18(12): 1759-1769 (2006)).

[0152] D. Non-protein Antibody Derivatives - Immunoconjugates

[0153] In some embodiments, the anti-CD36 antibody of the present disclosure may be further modified (i.e., derivatized) with non-proteinaceous moieties. Non-proteinaceous moieties suitable for derivatization of the antibody include, but are not limited to, water soluble polymers, such as: polyethylene glycol (PEG), copolymers of ethylene glycol and propylene glycol, carboxy-methylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1, 3, 6-trioxane, ethylene/maleic anhydride copolymer, poly-amino acid homo-polymers or random co-polymers, and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homo-polymers, polypropylene oxide/ethylene oxide co-polymers, polyoxy-ethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. In some embodiments, modification of the antibody can be carried out using methoxy-polyethylene glycol propionaldehyde. The polymers 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 is 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, e.g., whether the antibody derivative will be used in a therapy under defined conditions.

[0154] In some embodiments, the anti-CD36 antibody of the present disclosure can also be an immunoconjugate, wherein the immunoconjugate comprises an anti-CD36 antibody conjugated to one or more cytotoxic agents. Suitable cytotoxic agents contemplated by the present disclosure include chemotherapeutic agents, drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes. In some embodiments, the immunoconjugate is an antibody-drug conjugate (ADC) in which an anti-CD36 antibody, as described herein, is conjugated to one or more drugs. In some embodiments, an immunoconjugate of the present disclosure comprises an anti-CD36 antibody as described herein conjugated to a drug or therapeutic agent for the treatment of a CD36-mediated disease or condition.

[0155] In some embodiments, an anti-CD36 antibody as described herein can be conjugated to an enzymatically active toxin or a 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, *Momordica charantia* inhibitor, curcin, crotin, *Sapaonaria officinalis* inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0156] In some embodiments, an immunoconjugate of the present disclosure comprises an anti-CD36 antibody as described herein conjugated to a radioactive isotope (*i.e.*, a radioconjugate). A variety of radioactive isotopes are available for the production of such radioconjugates. Examples include ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P, ^{212}Pb, and radioactive isotopes of Lu. In some embodiments, the immunoconjugate may comprise a radioisotope for scintigraphic detection, or a spin label for NMR

detection or MRI. Suitable radioisotopes or spin labels can include, as ^{123}I, ^{131}I, ^{111}In, ^{13}C, ^{19}F, ^{15}N, ^{17}O, various isotopes of Gd, Mn, and Fe.

[0157] Immunoconjugates of an anti-CD36 antibody and a cytotoxic agent, can be made using a variety of well-known bifunctional reagents and chemistries suitable for conjugating to proteins. Such reagents include but are not limited to: N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (e.g., dimethyl adipimidate HQ), active esters (e.g., disuccinimidyl suberate), aldehydes (e.g., glutaraldehyde), bis-azido compounds (e.g., bis-(p-azidobenzoyl)-hexanediamine), bis-diazonium derivatives (e.g., bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (e.g., toluene-2,6-diisocyanate), and bis-active fluorine compounds (e.g., 1,5-difluoro-2,4-dinitrobenzene). Reagents for preparing immunoconjugates of the present disclosure can also include commercially available "cross-linking" reagents such as: 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) (see e.g. , Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

[0158] III. Recombinant Methods and Compositions

[0159] The anti-CD36 antibody of the present disclosure can be produced using recombinant methods and materials well-known in the art of antibody production. In some embodiments, the present disclosure provides an isolated polynucleotide encoding an anti-CD36 antibody of the present disclosure, or a fragment, or a domain of an anti-CD36 antibody. For example, the isolated polynucleotide can encode an amino acid sequence comprising CDR or HVRs disclosed herein, an amino acid sequence comprising the V_L domain and/or the V_H domain of the antibody, or an amino acid sequence comprising a complete light chain and/or heavy chain of an anti-CD36 antibody. In at least one embodiment, an isolated polynucleotide can encode an amino acid sequence comprising CDR-H1, CDR-H2, and CDR-H3 sequences, or an amino acid sequence comprising CDR-L1, CDR-L2, and CDR-L3 sequences of any of the anti-CD36 antibodies disclosure herein. Similarly, it is contemplated that an isolated polynucleotide can encode an amino acid sequence comprising a V_L domain or the V_H domain, or a complete heavy chain (HC) or light chain (LC) of an anti-CD36 antibody of the present disclosure.

[0160] In some embodiments, the present disclosure also provides vectors (e.g., expression vectors) comprising a polynucleotide sequence (as described above) that encodes an anti-CD36 antibody of the present disclosure or a fragment, or a domain of an anti-CD36 antibody. Such vector constructs comprising polynucleotides for the recombinant production of antibodies are well-known in the art. Further, in some embodiments, a host cell comprising a polynucleotide or vector with a sequence encoding an anti-CD36 antibody, or a fragment, or a domain of an anti-CD36 antibody of the present disclosure are provided. In at least one embodiment, the host cell is a cell that has been transformed with a vector comprising a polynucleotide sequence that encodes an amino acid sequence comprising the V_L domain of the antibody and/or an amino acid sequence comprising the V_H domain of an anti-CD36

antibody of the present disclosure. In another embodiment, the host cell has been transformed with a first vector comprising a polynucleotide sequence that encodes an amino acid sequence comprising the V_L of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_H of the antibody.

[0161] In some embodiments of the recombinant methods, the host cell used is a eukaryotic cell, such as a Chinese Hamster Ovary (CHO) cell, or a lymphoid cell (e.g., Y0, NS0, Sp20). In one embodiment, a method of making an anti-CD36 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0162] Briefly, recombinant production of an anti-CD36 antibody is carried out by isolating a nucleic acid encoding an antibody (e.g., as described herein) and inserting this nucleic acid into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acids are readily isolated and sequenced using conventional procedures well-known in the art (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the desired antibody). Suitable host cells and culturing methods for cloning or expressing the antibody-encoding vectors are well-known in the art and include prokaryotic or eukaryotic cells. Typically, after expression, the antibody may be isolated from cell paste in a soluble fraction and further purified. 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 e.g., Gerngross, Nat. Biotech. 22: 1409-1414 (2004), and Li et al., Nat. Biotech. 24: 210-215 (2006)).

[0163] Suitable host cells for the expression of glycosylated anti-CD36 antibodies of the present disclosure can also be 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. Plant cell cultures can also be utilized as hosts (see, e.g., U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548, and 7,125,978.

[0164] Examples of mammalian host cell lines useful for the production of the anti-CD36 antibodies of the present disclosure include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells (see e.g., Urlaub et al., Proc. Natl. Acad. Sci. USA 77: 4216 (1980)); myeloma cell lines such as Y0, NS0 and Sp2/0; 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); TR1 cells (see e.g., in Mather et

al., Annals N. Y. Acad. Sci. 383: 44-68 (1982) and US 6,235,498); Medical Research Council 5 (MRC 5) cells (such as e.g., those available from ATCC and also referred to as CCL-171); and Foreskin 4 (FS-4) cells (see e.g., in Vilcek et al. Ann. N. Y. Acad. Sci. 284: 703-710 (1977), Gardner & Vilcek. J. Gen. Virol. 44: 161-168 (1979), and Pang et al. Proc. Natl. Acad. Sci. U.S.A. 77: 5341-5345 (1980)). For a general review of useful 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).

[0165] IV. Pharmaceutical Compositions and Formulations of Anti-CD36 Antibodies

[0166] The present disclosure also provides pharmaceutical compositions and pharmaceutical formulations comprising an anti-CD36 antibody. In some embodiments, the present disclosure provides a pharmaceutical formulation comprising an anti-CD36 antibody as described herein and a pharmaceutically acceptable carrier. In some embodiments, the anti-CD36 antibody is the sole active agent of the pharmaceutical composition. Such pharmaceutical formulations can be prepared by mixing an anti-CD36 antibody, having the desired degree of purity, with one or more pharmaceutically acceptable carriers. Typically, such antibody formulations can be prepared as an aqueous solution (see e.g., US Pat. No. 6,171,586, and WO2006/044908) or as a lyophilized formulation (see e.g., US Pat. No. 6,267,958).

[0167] Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed. A wide range of such pharmaceutically acceptable carriers are well-known in the art (see e.g., Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)). Exemplary pharmaceutically acceptable carriers useful in the formulations of the present disclosure can 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).

[0168] Pharmaceutically acceptable carriers useful in the formulations of the present disclosure can also include interstitial drug dispersion agents, such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP) (see e.g., US Pat. Publ. Nos. 2005/0260186 and 2006/0104968), such as human soluble PH-20 hyaluronidase glycoproteins (e.g., rHuPH20 or HYLENEX®, Baxter International, Inc.).

[0169] It is also contemplated that the formulations disclosed herein may contain active ingredients in addition to the anti-CD36, as necessary for the particular indication being treated in the subject to whom

the formulation is administered. Preferably, any additional active ingredient has activity complementary to that of the anti-CD36 antibody activity and the activities do not adversely affect each other.

[0170] In some embodiments, the pharmaceutical composition comprises the anti-CD36 antibody and an additional active agent for cancer treatment such as an immune checkpoint inhibitor. Checkpoint inhibitors useful in such embodiments include, but are not limited to, a second antibody comprising a specificity for an antigen that is an immune checkpoint molecule. In some embodiments, the second antibody comprises a specificity for an immune checkpoint molecule selected from PD1, PD-L1, LAG3, CTLA-4, A2AR, TIM-3, BTLA, CD276, CD328, VTCN1, IDO, KIR, NOX2, VISTA, OX40, CD27, CD28, CD40, CD122, CD137, GITR, ICOS. In at least one embodiment, the pharmaceutical composition comprising an anti-CD36 antibody and an additional active agent, wherein the additional active agent is an antibody comprising a specificity for the immune checkpoint molecule PD1. Exemplary antibodies comprising a specificity for PD1 that are useful in the pharmaceutical composition embodiments disclosed herein include, but are not limited to, dostarlimab, pembrolizumab, nivolumab, and pidilizumab.

[0171] It is also contemplated that in some embodiments the present disclosure provides pharmaceutical composition or formulation for use in therapy, wherein the composition further comprises a T-cell activating cytokine or an immunostimulatory cytokine. Such cytokines are well known in the art of immunotherapy, and include, but are not limited to, IL-2, IL-7, IL-10, IL-12, IL-15, IL-21, and IFN-α. In at least one embodiment, the immunostimulatory cytokine can be provided in the composition as a fusion of the anti-CD36 antibody.

[0172] 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).

[0173] In some embodiments, the formulation can be a sustained-release preparation of the antibody and/or other active ingredients. 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.

[0174] Typically, the formulations of the present disclosure to be administered to a subject are sterile. Sterile formulations may be readily prepared using well-known techniques, e.g., by filtration through sterile filtration membranes.

[0175] V. Uses and Methods of Treatment

[0176] It is contemplated that any of the compositions or formulations comprising an anti-CD36 antibody of the present disclosure can be used for any methods or uses, such as in therapeutic methods that utilize their ability to specifically bind to CD36 and thereby inhibit, decrease, and/or fully block the

function of CD36 as a cell surface protein involved in immune regulation or signaling, particularly the function of CD36 regulating uptake of lipoproteins, fatty acids, and other ligands involved in the survival and maintenance or survival (or death, as the case may be) of tumor cells, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), regulatory T cells and CD8 T cells. Regulatory T cells, for example, are a major cellular component of tumor microenvironment (TME), and contribute significantly to tumor growth and progression while CD8 T cells help to control tumor growth by killing tumor cells. Inhibition of CD36 binding can deplete regulatory T cells while at the same time increasing CD8 T cell survival and function, thereby inducing an increase in anti-tumor T-cell response.

[0177] There are a range of diseases, disorders, and conditions that can potentially be treated by inhibiting, decreasing, and/or fully blocking the immune regulatory and/or immune signaling activity of CD36, particularly, the effect of CD36 on TAMs. Diseases, disorders, and conditions include, but are not limited to, cancers, including but not limited to colon cancer, pancreatic cancer, ovarian cancer, HCC, renal cancer, breast cancer, lung cancer, gastric cancer, melanoma, head and neck cancer, or oral cancer. It is contemplated that any of the compositions or formulations comprising an anti-CD36 antibody of the present disclosure can be used for a method or use for the treatment of any of the above-listed cancers. In some embodiments, the cancer is selected from colon cancer, pancreatic cancer, ovarian cancer, HCC, renal cancer, breast cancer, lung cancer, gastric cancer, melanoma, head and neck cancer, or oral cancer. In some embodiments, the present disclosure provides a method of treating cancer in a subject, the method comprising administering to the subject in need thereof a therapeutically effective amount of an anti-CD36 antibody of the present disclosure or administering to a subject a therapeutically effective amount of a pharmaceutical composition comprising an anti-CD36 antibody of the present disclosure and a pharmaceutically acceptable carrier.

[0178] As disclosed herein, including in the Examples below, the anti-CD36 antibodies of the present disclosure have the ability to decrease, inhibit, and/or block binding of ligands to CD36, and thereby alter the immune signaling pathways mediated by CD36. Accordingly, in some embodiments, the present disclosure provides a method of treating a CD36-mediated disease or condition in a subject, the method comprising administering to the subject a therapeutically effective amount of an anti-CD36 antibody of the present disclosure or administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising an anti-CD36 antibody of the present disclosure and a pharmaceutically acceptable carrier. Similarly, in some embodiments, the present disclosure provides a method of treating a disease mediated by binding to CD36 expressed on cells in a subject, the method comprising administering to the subject, the method comprising administering to the subject a therapeutically effective amount of an anti-CD36 antibody of the present disclosure or administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising an anti-CD36 antibody of the present disclosure and a pharmaceutically acceptable carrier.

[0179] Administration of the anti-CD36 antibody, composition, or pharmaceutical formulation in accordance with the method of treatment provides an antibody-induced therapeutic effect that protects the subject from and/or treats the progression of a CD36-mediated disease in a subject. In some

embodiments, the method of treatment can further comprise administration of one or more additional therapeutic agents or treatments known to those of skill in the art to prevent and/or treat the CD36-mediated disease or condition. Such methods comprising administration of one or more additional agents can 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 composition or formulation can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent.

[0180] In some embodiments of the methods of treatment of the present disclosure, the anti-CD36 antibody or pharmaceutical formulation comprising an anti-CD36 antibody is administered to a subject by any mode of administration that delivers the agent systemically, or to a desired target tissue. Systemic administration generally refers to any mode of administration of the antibody into a subject at a site other than directly into the desired target site, tissue, or organ, such that the antibody or formulation thereof enters the subject's circulatory system and, thus, is subject to metabolism and other like processes.

[0181] Accordingly, modes of administration useful in the methods of treatment of the present disclosure can include, but are not limited to, injection, infusion, instillation, and inhalation. Administration by injection can include intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal injection and infusion.

[0182] In some embodiments, a pharmaceutical formulation of the anti-CD36 antibody is formulated such that the antibody is protected from inactivation in the gut. Accordingly, the method of treatments can comprise oral administration of the formulation.

[0183] In some embodiments, use of the compositions or formulations comprising an anti-CD36 antibody of the present disclosure as a medicament are also provided. Additionally, in some embodiments, the present disclosure also provides for the use of a composition or a formulation comprising an anti-CD36 antibody in the manufacture or preparation of a medicament, particularly a medicament for treating, preventing or inhibiting a CD36-mediated disease. In a further embodiment, the medicament is for use in a method for treating, preventing or inhibiting a CD36-mediated disease comprising administering to an individual having a CD36-mediated disease an effective amount of the medicament. In certain embodiments, the medicament further comprises an effective amount of at least one additional therapeutic agent, or treatment.

[0184] In at least one embodiment, it is contemplated that the additional therapeutic agents or treatments that can be used in such medicaments with anti-CD36 antibodies of the present disclosure can include but are not limited to therapeutic antibodies comprising a specificity for an immune checkpoint molecule such as PD1, PD-L1, LAG3, CTLA-4, A2AR, TIM-3, BTLA, CD276, CD328, VTCN1, IDO, KIR, NOX2, VISTA, OX40, CD27, CD28, CD40, CD122, CD137, GITR, ICOS. Exemplary antibodies comprising a specificity for an immune checkpoint molecule include, but are not limited to an anti-PD1 antibody selected from dostarlimab, pembrolizumab, nivolumab, and pidilizumab.

[0185] In a further embodiment, the medicament is for use in treating, inhibiting or preventing a CD36-mediated disease, such as a cancer, in a subject comprising administering to the subject an amount effective of the medicament to treat, inhibit or prevent the CD36-mediated disease.

[0186] The appropriate dosage of the anti-CD36 antibody contained in the compositions and formulations of the present disclosure (when used alone or in combination with one or more other additional therapeutic agents) will depend on the specific disease or condition being treated, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, the previous therapy administered to the patient, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The anti-CD36 antibody included in the compositions and formulations described herein, can be suitably administered to the patient at one time, or over a series of treatments. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0187] Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg of anti-CD36 antibody in a formulation of the present disclosure is an initial candidate dosage for administration to a human subject, whether, for example, by one or more separate administrations, or by continuous infusion. Generally, the administered dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. In some embodiments, 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 a patient.

[0188] Dosage administration can be maintained over several days or longer, depending on the condition of the subject, for example, administration can continue until the CD36-mediated disease is sufficiently treated, as determined by methods known in the art. In some embodiments, an initial higher loading dose may be administered, followed by one or more lower doses. However, other dosage regimens may be useful. The progress of the therapeutic effect of dosage administration can be monitored by conventional techniques and assays.

[0189] Accordingly, in some embodiments of the methods of the present disclosure, the administration of the anti-CD36 antibody comprises a daily dosage from about 1 mg/kg to about 100 mg/kg. In some embodiments, the dosage of anti-CD36 antibody comprises a daily dosage of at least about 1 mg/kg, at least about 5 mg/kg, at least about 10 mg/kg, at least about 20 mg/kg, or at least about 30 mg/kg.

EXAMPLES

[0190] Various features and embodiments of the disclosure are illustrated in the following representative examples, which are intended to be illustrative, and not limiting. Those skilled in the art will readily appreciate that the specific examples are only illustrative of the invention as described more fully in the claims which follow thereafter. Every embodiment and feature described in the application should be understood to be interchangeable and combinable with every embodiment contained within.

Example 1: Anti-CD36 Antibody Generation and CD36 Binding Analysis

[0191] This example illustrates the use of phage-displayed antibody library technology to generate exemplary anti-CD36 antibodies of the present disclosure that specifically bind to human and mouse CD36.

[0192] A. Selection of anti-CD36 scFv binders from phage-displayed antibody libraries

[0193] The panning procedure is briefly described below. First, human CD36.ECD antigen (5 μg per well, Sino Biological) in PBS buffer (pH 7.4) was coated onto 96-well plate (NUNC Maxisorb immunoplate) wells overnight at 4 °C and then blocked with 5% skim milk in PBST [0.1% (v/v) Tween 20] for 1 h. After blocking, 100 μL concentrated phage library (1013 cfu/mL in PBS buffer) was mixed with 100 μL blocking buffer, and then added to each well for 1 h under gentle shaking. The plate was washed 12 times with PBST and 3 times with PBS. The bound phages were eluted with 100 μL of 0.1 M HCl/glycine (pH 2.2) per well and then immediately neutralized with 20 μL of 1 M Tris-base buffer (pH 9.0). The eluted phages were mixed with 1 mL of *E. coli* ER2738 (A600 nm = 0.6) for 30 min at 37 °C; uninfected bacteria were eliminated by adding ampicillin. After ampicillin treatment for 30 minutes, the bacterial culture was infected with 100 μL M13KO7 helper phage (~1011 CFU total) at 37 °C for 1 h and then added to 50 mL of 2X YT medium containing kanamycin 50 μg/mL and ampicillin 100 μg/mL overnight at 37 °C with vigorous shaking. The rescued phage library was precipitated with 20% polyethylene glycol/NaCl and resuspended in PBS. The concentrated phage solution was used for the next round of panning.

[0194] After 3~4 rounds of selection-amplification cycle, single colonies were randomly selected into deep 96 well culture plate (plate A; secreted scFv); each well contained 950 μL 2YT (100 μg/mL ampicillin). After 3 h incubation at 37 °C with shaking, 50 μL of bacterial culture was transferred to the corresponding well of a fresh deep 96-well plate (plate B; phage form); each well contained 0.8 mL 2YT with 100 μg/mL ampicillin. In the meantime, 50 μL M13KO7 (~5 × 1010 CFU total) was added to each well of plate B. After 1 h incubation, 100 μL 2YT containing IPTG (10 mM) was added to each well of plate A; 100 μL 2YT containing kanamycin (500 μg/mL) was added to each well of plate B. After overnight incubation at 37 °C with vigorous shaking, the cultures were centrifuged at 3000 g for 10 min at 4 °C. The plate B was stored for further sequencing determination. For secreted scFv culture plate (plate A), 100 μL culture medium and 100 μL 5% PBST milk was added to a corresponding well of three 96-well plates pre-coated with protein L (0.1 μg/well), human CD36 (0.5~1 μg/well) and bovine serum albumin (BSA) (2 μg/well), respectively and blocked with 5% PBST milk. After 1 h incubation at room temperature, the plates were washed three times with PBST. 100 μL Protein A-HRP (Thermo Scientific) was added to each well of Protein L-coated immunoplate; 100 μL anti-E-tag-HRP (ICL Inc.) was added to each well of human CD36 antigen-coated and BSA-coated plates. After 1 h incubation, the plates were washed three times with PBST buffer and twice with PBS, developed for 3 min with 3,3',5,5'-tetramethyl-benzidine peroxidase substrate (Kirkegaard & Perry Laboratories), quenched with 1.0 M HCl and read spectrophotometrically at 450 nm.

[0195] Positive clones were selected by the following criteria: ELISA OD450 > 0.2 for the human CD36 antigen-coated well (antigen binding positive); OD450 < 0.05 for BSA-coated well (non-specific binding negative); OD450 > 0.5 for the Protein L-coated well (soluble scFv binding to both Protein L and Protein A to ensure proper folding in solution), and then subjected to DNA sequencing. The polynucleotide sequence of scFv of the exemplary anti-CD36 antibody 12P109 (SEQ ID NO: 1) obtained from phage display panning is provided in Table 2 and the accompanying Sequence Listing. Further panning of a phage display library composed of "shuffled" LC sequences derived from different anti-CD36 antibodies and the HC sequence of 12P109 provided the scFv of the exemplary anti-CD36 antibody A8A (SEQ ID NO: 10), also listed in Table 2 and the accompanying Sequence Listing. Standard PCR-based mutagenesis was carried out on the CDRs of the A8A antibody V_L domain (SEQ ID NO: 11) were further subjected to provide the exemplary anti-CD36 antibody VL domain of A8A-N52T (SEQ ID NO: 14), also listed in Table 2 and the accompanying Sequence Listing.

[0196] B. Generation of anti-CD36 antibodies in full-length IgG format

[0197] scFv reformatting and cloning: The CD36 binding determinants of the scFvs selected from phage display panning were reformatted into full-length IgG antibodies by cloning the V_H and V_L domains of the fragments into the human IgG1-N297A heavy chain vector and the human kappa light chain vector using the restriction sites MluI/NheI and BsiWI/DraIII, respectively. V_H and V_L domains were amplified using the following forward and reverse oligonucleotide primer pairs: (1) PhageLib_VL_Fw: 5'-AATCACgATgTgATATTCAAATgACCCAgAgCCCgAgC-3' (SEQ ID NO: 64), (2) PhageLib_VL_Rv: 5'-AATCgTACgTTTgATTTCCACTTTggTgCCTTg-3' (SEQ ID NO: 65), (3) PhageLib_VH_Fw: 5'-AATACgCgTgTCCTgTCCgAAgTgCAgCTggTggAATCg-3' (SEQ ID NO: 66), and (4) PhageLib_VH_Rv: 5'-AATgCTAgCCgAgCTCACggTAACAAg-3'(SEQ ID NO: 67).

[0198] Expression and purification of full-length antibodies: The vectors cloned with the reformatted anti-CD36 antibody genes were transiently expressed in ExpiCHO-S cells (Thermo Scientific). During exponential growth phase, ExpiCHO-S cells were diluted to a final density of 6×10^6 cells/mL with ExpiCHO Expression Medium. ExpiFectamine CHO/plasmid DNA complexes were prepared using cold reagents according to the manual, incubated at room temperature for 1–5 minutes, and then slowly added to cells. One day after transfection, the culture was supplemented with ExpiFectamine CHO Enhancer and incubated for another 7 days. Transfected cell supernatants were centrifuged and subsequently filtered through a 0.22 μm filter. Antibodies were purified from the transfected cell supernatants with Protein A beads (Cytiva, 17127903). Antibody loaded columns were washed with 50 column volumes of PBS, and then eluted with 10 beads volume of 0.1 M Glycine (pH 3) directly into 1/10 volume of 1M Tris buffer (pH 9.0). The eluent was buffer-exchanged and concentrated with PBS, pH7.4 containing 0.1M Arginine. The quality of purified anti-CD36 antibodies was determined using SDS-PAGE in the presence and absence of a reducing agent.

[0199] Results: Examination of SDS-PAGE images showed that the expression and purification resulted in purified full-length anti-CD36 antibodies.

[0200] C. Optimization of anti-CD36 antibodies

[0201] To further improve the druggability of the anti-CD36 antibody A8A, two phage display libraries composed of mutations on N52, H91 and L94 were used for panning. The scFv clone 117 selected from phage display panning was reformatted into full-length IgG antibodies by cloning the V_H and V_L domains of the fragments into the human IgG1-N297A heavy chain vector and the human kappa light chain vector using the restriction sites MluI/NheI and BsiWI/DraIII, respectively. V_H and V_L domains were amplified using the following forward and reverse oligonucleotide primer pairs: (1) PhageLib_VL_Fw: 5'-AATCACgATgTgATATTCAAATgACCCAgAgCCCgAgC-3' (SEQ ID NO: 64), (2) PhageLib_VL_Rv: 5'-AATCgTACgTTTgATTTCCACTTTggTgCCTTg-3' (SEQ ID NO: 65), (3) PhageLib_VH_Fw: 5'-AATACgCgTgTCCTgTCCgAAgTgCAgCTggTggAATCg-3' (SEQ ID NO: 66), and (4) PhageLib_VH_Rv: 5'-AATgCTAgCCgAgCTCACggTAACAAg-3'(SEQ ID NO: 67).

[0202] The 117 antibody shares the same CDR-H1, H2, H3, and CDR-L1 sequences as A8A, and has a N52T mutation in CDR-L2, and a L94A mutation in CDR-L3. The Fab fragment of 117 antibody was also constructed into human IgG4-S228P/F234A/L235A heavy chain vector using the restriction sites NheI and BamHI. CDRs of the VH and VL domains of the 117 antibody were subjected to further PCR-based mutagenesis to generate a series of variants as summarized in Table 3 below.

[0203] TABLE 3

	V_H								**V_L**
	CDR-H1				**CDR-H2**			**CDR-H3**	**CDR-L3**
	T28	**S30**	**S31**	**F32**	**Y54**	**Y57**	**T58**	**F101**	**S92**
117 Variant Name	**Single Mutation Variants**								
117_28A 117_28D	A D								
117_30A 117_30D		A D							
117_31A 117_31D			A D						
117_32A 117_32D				A D					
117_54E					E				
117_57A 117_57D 117_57E 117_57R 117_57T						A D E R T			
117_58E							E		
117_101A 117_101D 117_101H 117_101K								A D H K	

117_101S 117_101Y								S Y	
117_92A 117_92E									A E
	Double Mutations								
117_30AA		A	A						
117_30AT		A	T						
117_30DA		D	A						
117_30DT		D	T						
117_30DE		D				E			
117_30FA		F	A						
117_30FT		F	T						
117_31AD			A	D					
117_31AT			A	T					
117_57DE						D	E		
117_57EE						E	E		
	Triple Mutations								
117_AA57D		A	A			D			
117_AA57E		A	A			E			
117_DA57D		D	A			D			
117_DA57E		D	A			E			

[0204] The 117 antibody and variants were expressed and purified as full-length antibodies as described above. Examination of SDS-PAGE images showed that the expression and purification resulted in purified full-length anti-CD36 antibodies. Amino acid sequences of the CDRs, VH, VL, HC, LC regions of the 117 antibody and the exemplary variants 117_30AA, 117_30DA, 117_57D, 117_57E, and 117_DA57E, also listed in Table 2 and the accompanying Sequence Listing.

[0205] D. ELISA of CD36-specific binding by anti-CD36 antibodies

[0206] ELISA materials and methods: Recombinant His-tagged human CD36.ECD protein or His-tagged mouse CD36.ECD protein (both from Sino Biological) was immobilized on 96 well microtiter plate at a concentration of 1 μg/mL in Coating Solution (SeraCare) overnight at 4°C. The wells were washed with wash solution (0.05% Tween20 in PBS) and blocked with 1% BSA in PBS. Serial dilutions of anti-CD36 antibodies were added to wells. After incubation at 37°C for 1 h, the wells were washed with wash solution. Peroxidase-conjugated Goat anti-human kappa Light chain antibody (Sigma) was applied to each well at 37°C for 1 h incubation. After washing, the wells were developed with TMB substrate for 5-10 min at room temperature and then stopped with 1 N HCl. Thereafter absorbance was measured at 450 nm and 650 nm. EC50 values were calculated through GraphPad Prism7.

[0207] Results: ELISA data plots showing binding to either human CD36.ECD or mouse CD36.ECD are depicted in **FIG. 1A** and **FIG. 1B**, respectively, for the full-length IgG anti-CD36 antibody 12P109, and additional positive clones, 12P102, 12P103, 12P104, 12P105, 12P106, 12P107, 12P110 and 12P212. Binding curves for the antibodies labeled 12P109, 12P103, and 12P212 showed the highest affinity for human CD36 (hCD36, see **FIG. 1A**). 12P109 also showed high binding affinity for mouse CD36 (mCD36, see **FIG. 1B**). The EC50 values calculated from the ELISA data are shown in Table 4 below.

[0208] TABLE 4

	EC_{50} (nM)	
mAb	**hCD36**	**mCD36**
12P102	>1000	NA
12P103	0.222	5.987
12P104	0.688	9.182
12P105	3.907	>400
12P106	3.87	6.81
12P107	2.246	11.87
12P109	0.213	0.137
12P110	74.62	0.508
12P212	4.217	>500

[0209] ELISA data plots showing binding of anti-CD36 antibodies to recombinant human CD36.ECD or mouse CD36.ECD coated on ELISA plates are depicted in **FIG. 1C** and **1D**, respectively. This employed full-length human IgG1-format anti-CD36 antibodies 12P109 and A8A, and the commercial mouse IgA antibody, D2712 (clone CRF D-2712, BD Biosciences, US) that specifically binds CD36. EC50 values derived from the curves of **FIG. 1C** and **FIG. 1D** are shown in Table 5 below.

[0210] TABLE 5

	EC_{50} (nM)	
mAb	**hCD36**	**mCD36**
12P109	43.24	9.45
A8A	5.17	0.94
D2712	>500	0.93

[0211] ELISA data plots showing binding to human CD36.ECD are depicted in **FIG. 2A**, **FIG. 2B**, **FIG. 2C**, **FIG. 2D**, **FIG. 2E**, and **FIG. 2F**, for the full-length IgG anti-CD36 antibodies, 12P109, 117, and the various 117 variants listed in Table 3. The competitor anti-CD36 antibody ONA, which is derived from clone "ONA-0-v1" as disclosed in WO2021176424A1 in hIgG4-S228P-FALA format. The EC50 values calculated from the ELISA data of **FIGS. 2A-2F** are shown in Table 7 below.

[0212] TABLE 7

mAb	**hCD36 EC_{50} (nM)**	**mAb**	**hCD36 EC_{50} (nM)**
FIG. 2A		**FIG. 2D**	
12P109	1.53	117	0.571
117	0.0475	117_30DA	0.0803
117_57D	0.0529	117_DA57D	0.0490
117_57E	0.0492	117_DA57E	0.0539

117_57R	0.0695	117_101A	0.0751
117_57A	1.52	117_101K	1.46
117_57T	NA	ONA	0.167
117_58E	0.422		
FIG. 2B		**FIG. 2E**	
117	0.102	117	0.0932
117_28A	0.129	117_101D	NA
117_28D	0.0735	117_101H	1.60
117_30A	0.0964	117_101Y	0.355
117_30D	0.141	117_101S	0.929
117_31A	0.0991		
117_31D	0.0375		
117_32D	0.0276		
FIG. 2C		**FIG. 2F**	
117	0.571	117	0.124
117_30AA	0.145	117_57E	0.206
117_30AT	0.247	117_DA57E	0.190
117_30DA	0.137	117_AA57D	0.192
117_30DT	0.103	117_AA57E	0.251
117_30FA	0.202		
117_30FT	0.254		

[0213] F. SEC-UPLC analysis

[0214] Extended retention time (RT) of an antibody as determined by SEC-UPLC analysis may be associated with non-specific hydrophobic interactions that create a risk for development of the antibody for therapeutic uses. Accordingly, the anti-CD36 antibodies, 12P109, A8A, 117, and the 117 variants, as well as an IgG standard ("STD", BEH200 SEC Protein Standard Mix) and control monoclonal antibody ("ctl Ab") were analyzed by SEC-UPLC for increased RT indicating formation of protein aggregates.

[0215] Materials and methods: After protein A purification, the anti-CD36 antibodies, 12P109, A8A, 117, and the 117 variants were analyzed for protein aggregates and retention time by standard Size Exclusion UPLC (SEC-UPLC). 3 ug of antibody was applied to an UPLC (Waters ARC UPLC) and separation was accomplished on a gel filtration column (Waters, XBridge BEH450 SEC 4.6 x 150) using a mobile phase of PBS. The antibody peak was monitoring UV absorbance at 280 and the peak area was determined using Empower software.

[0216] Results: Plots of SEC-UPLC results are shown in **FIG. 3A, FIG. 3B**, **FIG. 3C**, **FIG. 3D**, and **FIG. 3E** along with determined RT values. The control antibody exhibited a RT value of 7.243 min, whereas the anti-CD36 antibodies, 12P109 and 117 both exhibited relatively increased RT values. The increased RT values of 117, however, was decreased significantly by the mutations present in the variants, 117_30DA, 117_31AD, 117_57D, 117_57E, 117_57EE, 117_101D, and 117_DA57E. The RT values of anti-CD36 antibodies as determined in SEC-UPLC experiments are provided in Table 8 below. In addition, some aggregates were found in 117_DA57E by UPLC analysis. Aggregates may be related to the fact that 117_DA57E is less stable to acidic elution conditions. R409K mutation in IgG4 has been shown to increase CH3 domain interaction strength and reduce tendency to aggregate at low pH. The SEC-UPLC analysis showed that R409K addition to 117_DA57E reduced aggregation caused by acidic condition.

[0217] TABLE 8

mAb	Retention time	mAb	Retention time
FIG. 3A		**FIG. 3C**	
STD IgG	6.81 min	STD IgG	6.81 min
Ctl Ab	7.24 min	Ctl Ab	7.12 min
12P109	8.98 min	12P109	9.24 min
A8A	10.82 min	117	9.65 min
117_28A	9.40 min	117_57D	7.62 min
117_30A	9.49 min	117_57E	7.60 min
117_31A	9.43 min	117_57A	8.47 min
117_32A	7.99 min	117_57R	8.87 min
117_28D	8.26 min	117_57T	8.17 min
117_31D	7.93 min	117_58E	8.41 min
FIG. 3B		**FIG. 3D**	
STD IgG	6.81 min	STD IgG	6.87 min
117	8.93 min	Ctl Ab	7.19 min
117_30DA	7.97 min	12P109	10.64 min
117_30DT	8.02 min	117	11.46 min
117_30FA	13.22 min	117_DA57E	7.42 min
117_30FT	13.56 min	117_30DE	7.41 min
117_31AD	7.58 min	117_AA57E	9.53 min
117_31AT	8.22 min		
FIG. 3E			
ONA	7.21 min		
117_57EE	7.41 min		
117_DA57E	7.22 min		
117_DA57E (R409K)	7.21 min		

[0218] G. BLI analysis of CD36 binding kinetics

[0219] BLI assay materials and method: Kinetic rate constants, ka and kd, for binding of the anti-CD36 antibodies to human CD36 were measured by Bio-Layer Interferometry (BLI) (ForteBio Octet RED96). The BLI assay was performed using AHC (Anti-hIgG Fc Capture) biosensors (ForteBio) to capture each anti-CD36 antibody (5 μg/mL) to acquire a 0.5 nm shift and then the biosensors were dipped into varying concentrations (i.e., 0, 1.5625, 3.125, 6.25, 4.94, 12.5, 25, 50 and 100 nM) of recombinant His-tagged human CD36.ECD protein (Sino Biological) in running buffer containing 0.1% BSA, 0.1% Tween-20, 250 mM NaCl in PBS. Rate constants were calculated by curve fitting analyses (1:1 Langmuir model) of binding response with a 2.5-minute association and 5-minute dissociation interaction time.

[0220] Results: The determined dissociation constant, KD, and kinetic rate constants, ka, and kd, for binding to human CD36 of the anti-CD36 antibodies, 12P109, A8A, 117, and various 117 variants (in either IgG1 and IgG4 format) as determined in separate BLI analysis experiment are provided in Table 10 below.

[0221] TABLE 10

	hCD36			
	KD (M)	**Ka (1/Ms)**	**Kd (1/s)**	**R2**
A8A (IgG1)	3.42E-09	1.35E+06	4.62E-03	0.972

117 (IgG1)	4.79E-09	4.45E+05	2.13E-03	0.9916
117_31A (IgG1)	4.05E-09	4.49E+05	1.82E-03	0.9931
117_31A (IgG4)	4.32E-09	4.75E+05	2.05E-03	0.9928
117_30AA (IgG4)	5.74E-09	4.91E+05	2.82E-03	0.9964
12P109 (IgG1)	1. 85E-08	5.22E+05	9.63E-03	0.9538
117 (IgG1)	6.72E-09	3.50E+05	2.35E-03	0.9739
117_30DA (IgG4)	3.91E-09	3.39E+05	1.33E-03	0.9859
117_54E (IgG1)	4.16E-09	9.78E+05	4.07E-03	0.8375
117_57D (IgG1)	4.69E-09	3.73E+05	1.75E-03	0.9795
117_57E (IgG1)	4.93E-09	3.70E+05	1.82E-03	0.9785
117 (IgG4)	6.49E-09	3.86E+05	2.50E-03	0.9829
117_57D (IgG4)	4.96E-09	3.36E+05	1.67E-03	0.9864
117_57E (IgG4)	5.28E-09	3.25E+05	1.71E-03	0.986
117_DA57D (IgG4)	2.64E-09	3.17E+05	8.38E-04	0.9938
117_DA57E (IgG4)	2.52E-09	3.19E+05	8.03E-04	0.994
117_57EE (IgG1)	4.27E-09	3.64E+05	1.55E-03	0.9891
117_30DE (IgG4)	3.09E-09	3.17E+05	9.81E-04	0.9919

Example 2: Binding of Cell Surface Expressed CD36 by Anti-CD36 Antibodies

[0222] This example illustrates the preparation of stable F293 cell lines that overexpress hCD36 or mCD36 on the cell surface, and studies to determine binding affinity of exemplary anti-CD36 antibodies of the present disclosure to cell surface expressed human, rhesus, or mouse CD36.

[0223] A. Generation of CD36-overexpressing stable F293 cell lines

[0224] Materials and methods: The gene segment encoding full-length human CD36-Flag (Sino Biological), rhesus CD36-His (Sino Biological), and mouse CD36-His (Sino Biological) were sub-clone into pcDNA3.4 topo vector using XbaI/HindIII. Freestyle 293-F cells (Thermo Scientific) were transfected with the plasmid encoding CD36 by polyethylenimine (PEI) method and selected with Geneticin (Thermo Scientific) to establish CD36 stable cell pool. The expression of human CD36 was verified by surface staining of anti-CD36 (clone 5-271, Biolegend) or by intracellular staining of anti-FLAG. The expression of rhesus CD36 was verified by surface staining of anti-CD36 clone 117) or by intracellular staining of anti-His. The expression of mouse CD36 was verified by surface staining of anti-CD36 antibody D2712 (clone CRF D-2712; BD Biosciences, US) or by intracellular staining of anti-His. To enrich CD36-expressing stable cell lines, hCD36, rhesus CD36, or mCD36-overexpressing F293 cell pool were stained with anti-CD36 (clone 5-271, Biolegend), anti-CD36 clone 117, or anti-CD36 D2712

(clone CRF D-2712, BD Biosciences), respectively. The CD36-high cell populations were sorted by FACSAria IIIu (BD).

[0225] Results: Analysis of the FACS data confirmed that the stable F293 cells F293/hCD36, F293/rhesusCD36, and F293/mCD36 overexpress surface CD36 that is capable of binding by an anti-CD36 antibody.

[0226] B. Cell surface CD36 binding activity of anti-CD36 antibodies

[0227] Materials and methods: CD36-high expressing F293 cells generated as described in part A above were incubated with a series dilution of anti-CD36 antibodies, 117, and several variants of 117 (see listed in Table 3) at 4°C for 30 min. After washing with FACS buffer (2%FBS in PBS), the cells were stained with anti-human IgG-Alexa Fluor 647 and analyzed by Attune NxT Flow Cytometer (Thermo Scientific).

[0228] Results: Analysis of the flow cytometry data expressed as geometric mean (MFI) confirmed that the anti-CD36 antibodies specifically bind to cells that surface expressed hCD36 and mCD36.

[0229] Data plots showing the surface binding to F293 cells expressing human, rhesus, or mouse CD36 are depicted in **FIG. 4A, FIG. 4B**, **FIG. 4C**, **FIG. 4D,** and **FIG. 4E,** for the full-length IgG anti-CD36 antibody, 117, and the several 117 variants. EC_{50} values derived from the curves of **FIGS 4A-4E.** are shown in Table 11 below.

[0230] TABLE 11

	EC_{50} (µg/mL)		
mAb	**hCD36**	**rhesus CD36**	**mCD36**
117	0.180	0.886	0.460
117_57D	0.111	0.397	0.309
117_57E	0.110	0.419	0.331
117_30DE	0.107	0.403	0.342
117_57EE	0.093	0.407	0.319
117_DA57D	0.089	0.416	0.271
117_DA57E	0.109	0.563	0.425
117	0.123	0.582	0.361
117_30DA	0.157	1.041	0.488
117_57E	0.091	0.362	0.279
117_57DE	0.129	0.529	0.447
117_DA57E	0.0968	0.671	0.375

Example 3: Oxidized LDL Uptake Blocking Activity of Anti-CD36 Antibodies

[0231] This example illustrates studies to determine ability of the exemplary anti-CD36 antibodies of the present disclosure to block oxidized LDL uptake by CD36 expressing cells.

[0232] A. Inhibition of oxidized LDL binding and uptake in U937 cells by anti-CD36 antibodies

[0233] CD36-high expressing U937 cells were pre-incubated with control IgG or anti-CD36 antibodies at 4°C for 30 min. To measure the binding or uptake of oxLDL, Dil-oxLDL (5-10 µg/mL) (Cat. No. 770232-9; Kalen Biomedical), a purified human LDL that has been oxidized with copper (II) sulfate and labeled with "Dil" (1,1'-dioctadecyl- 3,3,3',3'-tetramethylindocarbocyanine perchlorate), was added in

serum-free medium and incubated at 4°C for 2 h or at 37°C for 5 min, respectively. After washing with PBS, the cells were analyzed by Attune NxT Flow Cytometer (Thermo Scientific).

[0234] The binding of the anti-CD36 antibodies of the present disclosure to U937 cells was observed to result in a characteristic shift demonstrating endogenous expression by U937 of surface CD36. Analysis of the extracted U937 flow cytometry data confirmed a reduction of oxLDL binding **FIG. 5A**) and oxLDL uptake (**FIG. 5B**) by the U937 cells relative to the IgG isotype control when incubated with an anti-CD36 antibodies of the present disclosure, 12P103, 12P110, or 12P109.

[0235] B. Inhibition of oxidized LDL uptake in F293/CD36 cells by anti-CD36 antibodies

[0236] F293/hCD36 cells were pre-incubated with control IgG or anti-CD36 antibodies at 4°C for 30 min. To measure the binding or uptake of oxLDL, Dil-oxLDL (5 μg/mL) (Kalen Biomedical) was added in serum-free medium and incubated at 4°C for 2 h to measure "oxLDL binding," or at 37°C for 5 min to measure "oxLDL uptake", respectively. After washing with PBS, the cell preparations were analyzed by Attune NxT Flow Cytometer (Thermo Scientific).

[0237] Results: As shown by the plots of flow cytometry data depicted in **FIG. 6A**, **FIG. 6B** and **FIG. 6C**, the presence of an anti-CD36 antibodies 12P109, 117, 117_30DA, 117_57D, 117_57E, 117_30DE, 117_57EE, 117_DA57D, or 117_DA57E, effectively blocked oxidized LDL uptake by the F293 cells that overexpress human CD36 on their surface. Additionally, as shown by the plots in **FIG. 6C,** the presence of an anti-CD36 antibodies 12P109, 117_30DA, 117_57D, or 117_57E, effectively blocked ox LDL uptake by the F293 cells that overexpress mouse CD36 on their surface. IC_{50} values for inhibition of oxLDL uptake by the F293/hCD36 or F293/mCD36 cells determined for these anti-CD36 antibodies are shown in Table 13 below.

[0238] TABLE 13

mAb	oxLDL uptake IC50 (nM)	
	F293/hCD36	**F293/mCD36**
12P109	0.401	0.860
117_30DA	0.691	1.07
117_57D	0.349	0.783
117_57E	0.419	0.796
117	0.702	n.d.
117_30DE	0.299	n.d.
117_57EE	0.348	n.d.
117_DA57D	0.332	n.d.
117_DA57E	0.306	n.d.

Example 4: Activity of Anti-CD36 Antibodies in Blocking oxLDL Uptake by TILs

[0239] This example illustrates studies anti-CD36 antibodies in blocking oxidized LDL uptake in mouse tumor-infiltrating lymphocyte cells (TILs).

[0240] Materials and methods: The murine colon carcinoma cell line CT-26 (2×10^5 cells, ATCC number: CRL-2638) or murine liver cancer cell line BNL 1MEA.7R.1 (5×10^6 cells, ATCC number: TIB-75) were subcutaneously injected into the right flank of BALB/c mice. The murine colon carcinoma cell line MC38 (1×10^6 cells, Kerafast #ENH204-FP), murine melanoma cell line B16-F10 (1×10^6 cells, BCRC #60031), or murine lung cancer cell line LL/2 (2×10^5 cells, BCRC #60050) were subcutaneously injected into the right flank of C57BL/6 mice. The CT-26 tumors were harvested after 4 weeks. The B16-F10, BNL 1MEA.7R.1, MC38, and LL/2 tumors were harvested after 2 weeks. Tumor cell suspensions were prepared from solid tumors using mouse Tumor Dissociation Kit (Miltenyi Biotec) according to manufacturers' instructions. CD45+ TILs were isolated from the tumor cell suspensions using CD45 microbeads (Miltenyi Biotec).

[0241] Isolated CD45+ TILs were pre-incubated with control IgG or anti-CD36 antibodies (5-10 μg/mL) at 4°C for 30 min. Dil-oxLDL (5-10 μg/mL) was added in RPMI medium containing 1% fatty acid-free BSA and incubated at 37°C for 15 min. After washing with PBS, the cells were analyzed by Attune NxT Flow Cytometer (Thermo Scientific). The binding and uptake of oxLDL were analyzed by flow cytometry and expressed as percentage (%) positive cells based on a histogram gate placed on negative control cells.

[0242] Data represent mean ± SD. *$p < 0.05$, **$p < 0.005$, one-way ANOVA.

[0243] Results: As shown by the plot of results depicted in **FIG. 7A**, the anti-CD36 antibodies of the present disclosure that bind specifically to hCD36 and mCD36, 12P109 and A8A, provided approximately 35% inhibition of oxLDL uptake in CD45+ TILs isolated from CT26 tumors injected into BALB/c mice. The level of inhibition was equivalent to that observed for the commercial antibody, D2712, that specifically binds mouse CD36.

[0244] As shown by the plot of results depicted in **FIG. 7B**, **FIG. 7C**, **FIG. 7D** and **FIG. 7E**, the anti-CD36 antibodies 117_57E, 117_30DA, 117_57DE, and 117_DA57E, showed inhibitory activity to oxLDL uptake in CD45+ TIL from B16-F10, BNL 1MEA.7R.1, MC38, and LL/2 tumors.

Example 5: M2 Macrophage Polarization and Activation Blocking Activity

[0245] This study was carried out to determine the ability of the anti-CD36 antibodies of the present disclosure inhibit M2 macrophage polarization and activation.

[0246] Materials and methods: To generate monocyte derived macrophages, human CD14+ monocytes were isolated from PBMC and cultured at 2×10^6 cells/ml in RPMI1640 supplemented with 10% FBS and 20 ng/ml CSF1 for 6 days. Then adherent macrophages were collected by EDTA detachment and seeded in a 24-well plate for further polarization. For M0 macrophages, cells were culture in culture medium for 2 days. To polarize M2 macrophages, macrophages were culture in medium containing 50 ng/mL IL-4 and 50 ng/mL IL-13 for another 2 days. 10 μg/mL control IgG or anti-CD36 antibodies were added during M2 macrophage polarization. For oxLDL activation, macrophages were pre-incubated with control IgG or anti-CD36 antibodies for 10 min and then cultured in M2 medium containing 10 μg/mL oxLDL (Kalen Biomedical) for another 2 days. To examine M2 macrophage activation, macrophages

were harvested and stained with antibodies against CD206, CD301 and PDL2. The level of surface markers was analyzed by Attune NxT Flow Cytometer (Thermo Scientific).

[0247] Results: As shown by the plots of data depicted in **FIG. 8A** and **FIG. 8B**, the anti-CD36 antibody, 117_30AA exhibited very strong inhibition of M2 macrophage polarization in both CD206+/CD301+ (**FIG. 8A**) and the CD206+/PDL2+ (**FIG. 8B**) double-positive cell populations. As shown by the plots of data depicted in **FIG. 8C**, **FIG. 8D, FIG. 8E** and **FIG. 8F**, the anti-CD36 antibodies, 117_30DA, 117_57D, 117_57E, and 117_DA57E exhibited very strong inhibition of oxLDL-induced M2 macrophage activation in both the CD206+/CD301+ (**FIG. 8C**, **FIG. 8D**) and the CD206+/PDL2+ (**FIG. 8E**, **FIG. 8F**) double-positive cell populations. As shown by the plots of data depicted in **FIG. 8D** and **FIG. 8F**, the anti-CD36 antibodies, 117_30DA, and 117_DA57E exhibited very strong inhibition of oxLDL-induced M2 macrophage activation compared to the IgG control and the competitor anti-CD36 antibody "ONA", which is Fab clone "ONA-0-v1" as disclosed in WO2021176424A1 with hIgG4-S228P-FALA Fc region.

Example 6: Activity of Anti-CD36 Antibodies in a Mouse Hepatocellular Carcinoma Model

[0248] This example illustrates a study of anti-CD36 antibody activity in suppressing tumor growth in two genetically induced mouse models of hepatocellular carcinoma (HCC): (1) HCC induced by the Sleeping Beauty transposon (SB100x) system-mediated transgenes of MYC-luc-ova overexpression and p53 knockout; and (2) HCC induced by the Sleeping Beauty transposon (SB100x) system-mediated transgenes of MYC-luc-ova and β-catenin ^{N90}.

[0249] Materials and methods

[0250] 6-week old mice are restrained, and hydrodynamic delivery of endotoxin-free plasmid DNA is conducted through the lateral tail vein injection in a volume equivalent to 10% of the body weight within 5-7 seconds. For the MYC^{OE}/p53^{KO} HCC model, pT3-Myc-luc-ova plasmid (Addgene #129776), p53 gRNA plasmid (Addgene #59910), and SB100x (transposase-containing plasmid; Addgene #34879) were injected into the mice. For the β-catenin/Myc (β-catenin^{OE}/ MYC^{OE}) HCC model, pT3-bcatenin (Addgene #31785), pT3-Myc-luc-ova plasmid, and SB100x were injected into each mouse. β-catenin-driven liver tumor represents a more aggressive HCC with cold tumor phenotype.

[0251] To monitor tumor growth, mice were injected with luciferin (150 mg/Kg), and the bioluminescence activity was analyzed in the IVIS imaging system. Mice with continued tumor growth were randomly grouped and administered the anti-CD36 antibody (10mg/kg) or PBS (control) through intraperitoneal injection as described below.

[0252] After 2 weeks, MYC^{OE}/p53^{KO} HCC model mice were intraperitoneally injected with 4 doses of anti-CD36 antibody (10mg/kg; clone: 117_30DA) or control antibody every 3 days, and the bioluminescence imaging of mice was measured in IVIS system every 6 days (n =5 per group).

[0253] After 3 weeks, β-catenin^{OE}/ MYC^{OE} HCC model mice were intraperitoneally injected with anti-CD36 antibody (10 mg/mL, clone: 117_DA57E) or control antibody (n=6 per group), and the bioluminescence imaging of mice was measured in IVIS system every 5 days.

[0254] In the β-catenin^{OE}/ MYC^{OE} HCC model, tumors exhibit aggressive progress and tumor-bearing mice usually die 38 days after hydrodynamic injection. Accordingly, tumors were harvested and weighed 35-38 days after hydrodynamic tail vein injection.

[0255] Results

[0256] **FIG. 9** shows the tumor growth curves measured by bioluminescence in the MYC^{OE}/p53^{KO} HCC model mice following treatment with the anti-CD36 antibody 117_30DA. Starting at 14 days after HCC induction and continuing out to 38 days, tumor growth was observed to be significantly suppressed in the treated mice versus controls. *P*-value =0.0114, t-test, two-tail.

[0257] **FIG. 10A** shows the tumor growth curves measured by bioluminescence in the β-catenin^{OE}/ MYC^{OE} HCC model mice following treatment with the anti-CD36 antibody 117_DA57E. Starting 21 days after HCC induction and continuing out to 36 days, tumor growth was observed to be significantly suppressed in the treated mice versus controls. *P*-value =0.0104, t-test, two-tail. **FIG. 10B** shows that the endpoint liver weights were significantly reduced in the anti-CD36 treated mice. Additionally, as shown in **FIG. 10C** plasma ALT (alanine transaminase) activity analysis indicated that the anti-CD36 treatment also alleviated liver damage caused by cancer development.

[0258] While the foregoing disclosure of the present invention has been described in some detail by way of example and illustration for purposes of clarity and understanding, this disclosure including the examples, descriptions, and embodiments described herein are for illustrative purposes, are intended to be exemplary, and should not be construed as limiting the present disclosure. It will be clear to one skilled in the art that various modifications or changes to the examples, descriptions, and embodiments described herein can be made and are to be included within the spirit and purview of this disclosure and the appended claims. Further, one of skill in the art will recognize a number of equivalent methods and procedure to those described herein. All such equivalents are to be understood to be within the scope of the present disclosure and are covered by the appended claims.

[0259] Additional embodiments of the invention are set forth in the following claims.

[0260] The disclosures of all publications, patent applications, patents, or other documents mentioned herein are expressly incorporated by reference in their entirety for all purposes to the same extent as if each such individual publication, patent, patent application or other document were individually specifically indicated to be incorporated by reference herein in its entirety for all purposes and were set forth in its entirety herein. In case of conflict, the present specification, including specified terms, will control.

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CLAIMS

What is claimed is:

1. An anti-CD36 antibody comprising (i) a first heavy chain complementary determining region (CDR-H1), a second heavy chain complementary determining region (CDR-H2), and a third heavy chain complementary determining region (CDR-H3), and (ii) a first light chain complementary determining region (CDR-L1), a second light chain complementary determining region (CDR-L2), and a third light chain complementary determining region (CDR-L3), wherein:
   (a) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 24, 3, 21, or 27;
   (b) CDR-H2 comprises the amino acid sequence of SEQ ID NO: 43, 4, 28, 31, 34, 37, or 40;
   (c) CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5;
   (d) CDR-L1 comprises an amino acid sequence selected from SEQ ID NO: 7;
   (e) CDR-L2 comprises an amino acid sequence selected from SEQ ID NO: 15, 8, or 12; and
   (f) CDR-L3 comprises an amino acid sequence selected from SEQ ID NO: 18, 9, or 13.

2. The antibody of claim 1, wherein:
   (a) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 24, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 43, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;
   (b) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 8, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 9;
   (c) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 12, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 13;
   (d) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 13;
   (e) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino

acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(f) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 21, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(g) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 24, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(h) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 27, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 28, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(i) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 31, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(j) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 34, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18;

(k) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 37, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18; or

(l) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, CDR-H2 comprises the amino acid sequence of SEQ ID NO: 40, CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5, CDR-L1 comprises the amino acid sequence of SEQ ID NO: 7, CDR-L2 comprises the amino acid sequence of SEQ ID NO: 15, and CDR-L3 comprises the amino acid sequence of SEQ ID NO: 18.

3. An anti-CD36 antibody comprising:

(i) a first heavy chain complementary determining region (CDR-H1), a second heavy chain complementary determining region (CDR-H2), and a third heavy chain complementary determining region (CDR-H3), wherein the CDR-H1, CDR-H2, and CDR-H3 sequences are from a VH region having an amino acid sequence selected from SEQ ID NO: 42, 2, 20, 23, 26, 30, 33, 36, and 39;

(ii) a first light chain complementary determining region (CDR-L1), a second light chain complementary determining region (CDR-L2), and a third light chain complementary determining region (CDR-L3), wherein the CDR-L1, CDR-L2, and CDR-L3 sequences are from a VL region having an amino acid sequence selected from SEQ ID NO: 17, 6, 11, and 14, and

wherein the CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 are according to Kabat numbering.

4. The antibody of claim 3, wherein:
   (a) the VH amino acid sequence is SEQ ID NO: 42 and the VL amino acid sequence is SEQ ID NO: 17;
   (b) the VH amino acid sequence is SEQ ID NO: 2 and the VL amino acid sequence is SEQ ID NO: 6;
   (c) the VH amino acid sequence is SEQ ID NO: 2 and the VL amino acid sequence is SEQ ID NO: 11:
   (d) the VH amino acid sequence is SEQ ID NO: 2 and the VL amino acid sequence is SEQ ID NO: 14;
   (e) the VH amino acid sequence is SEQ ID NO: 2 and the VL amino acid sequence is SEQ ID NO: 17;
   (f) the VH amino acid sequence is SEQ ID NO: 20 and the VL amino acid sequence is SEQ ID NO: 17;
   (g) the VH amino acid sequence is SEQ ID NO: 23 and the VL amino acid sequence is SEQ ID NO: 17;
   (h) the VH amino acid sequence is SEQ ID NO: 26 and the VL amino acid sequence is SEQ ID NO: 17;
   (i) the VH amino acid sequence is SEQ ID NO: 30 and the VL amino acid sequence is SEQ ID NO: 17;
   (j) the VH amino acid sequence is SEQ ID NO: 33 and the VL amino acid sequence is SEQ ID NO: 17;
   (k) the VH amino acid sequence is SEQ ID NO: 36 and the VL amino acid sequence is SEQ ID NO: 17; or
   (l) the VH amino acid sequence is SEQ ID NO: 39 and the VL amino acid sequence is SEQ ID NO: 17.

5. The antibody of any one of claims 1-4, wherein the antibody comprises a heavy chain variable domain (V_H) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID

NO: 42, 2, 20, 23, 26, 30, 33, 36, or 39; and a light chain variable domain (V_L) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 17, 6, 11, or 14.

6. The antibody of any one of claims 1-4, wherein:
   (a) the antibody comprises a V_H amino acid sequence having at least 90% identity to SEQ ID NO: 42 and a V_L amino acid sequence having at least 90% identity to SEQ ID NO: 17;
   (b) the antibody comprises a V_H amino acid sequence having at least 90% identity to SEQ ID NO: 2 and a V_L amino acid sequence having at least 90% identity to SEQ ID NO: 6;
   (c) the antibody comprises a V_H amino acid sequence having at least 90% identity to SEQ ID NO: 2 and a V_L amino acid sequence having at least 90% identity to SEQ ID NO: 11:
   (d) the antibody comprises a V_H amino acid sequence having at least 90% identity to SEQ ID NO: 2 and a V_L amino acid sequence having at least 90% identity to SEQ ID NO: 14;
   (e) the antibody comprises a V_H amino acid sequence having at least 90% identity to SEQ ID NO: 2 and a V_L amino acid sequence having at least 90% identity to SEQ ID NO: 17;
   (f) the antibody comprises a V_H amino acid sequence having at least 90% identity to SEQ ID NO: 20 and a V_L amino acid sequence having at least 90% identity to SEQ ID NO: 17;
   (g) the antibody comprises a V_H amino acid sequence having at least 90% identity to SEQ ID NO: 23 and a V_L amino acid sequence having at least 90% identity to SEQ ID NO: 17;
   (h) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 26 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17;
   (i) the antibody comprises a V_H amino acid sequence having at least 90% identity to SEQ ID NO: 30 and a V_L amino acid sequence having at least 90% identity to SEQ ID NO: 17;
   (j) the antibody comprises a V_H amino acid sequence having at least 90% identity to SEQ ID NO: 33 and a V_L amino acid sequence having at least 90% identity to SEQ ID NO: 17;
   (k) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 36 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17; or
   (l) the antibody comprises a VH amino acid sequence having at least 90% identity to SEQ ID NO: 39 and a VL amino acid sequence having at least 90% identity to SEQ ID NO: 17.

7. The antibody of any one of claims 1-4, wherein the antibody comprises a heavy chain (HC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 57, 44, 48, 50, 51, 52, 53, 54, 55, or 56, and/or a light chain (LC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 49, 45, 46, or 47.

8. The antibody of any one of claims 1-4, wherein the antibody comprises:
   (a) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 57 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;
   (b) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 44 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 45;

(c) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 44 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 46;
(d) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 44 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 47;
(e) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 44, and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;
(f) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 48, and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;
(g) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 50 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;
(h) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 51 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;
(i) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 52 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;
(j) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 53 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;
(k) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 54 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49;
(l) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 55 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49; or
(m) an HC amino acid sequence having at least 90% identity to SEQ ID NO: 56 and an LC amino acid sequence having at least 90% identity to SEQ ID NO: 49.

9. The antibody of any one of claim 1-4, wherein the antibody:
(a) is an antibody fragment, optionally selected from the group consisting of $F(ab')_2$, Fab', Fab, Fv, single domain antibody (VHH), and scFv;
(b) comprises a fusion to a protein; optionally, wherein the protein is an immunostimulatory cytokine selected from IL-2, IL-7, IL-10, IL-12, IL-15, IL-21, or IFN-α;
(c) is a human, humanized, or chimeric antibody;
(d) is a full length antibody of class IgG, optionally, wherein the class IgG antibody has an isotype selected from IgG1, IgG2, IgG3, and IgG4;
(e) comprises an Fc region variant, optionally an Fc region variant that alters effector function and/or a variant that alters antibody half-life;
(f) comprises an immunoconjugate, optionally, wherein the immunoconjugate comprises a therapeutic agent for treatment of a CD36-mediated disease or condition; and/or
(g) is a multi-specific antibody; optionally a bispecific antibody.

10. The antibody of any one of claims 1-9, wherein:
(a) the antibody binds to human CD36 with a binding affinity of 1×10^{-8} M or less, 1×10^{-9} M or

less, 1 x 10^{-10} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a hCD36 polypeptide of SEQ ID NO: 58 or 59;

(b) the antibody binds to mouse CD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a mCD36 polypeptide of SEQ ID NO: 60 or 61;

(c) the antibody binds to rhesus CD36 with a binding affinity of 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a rhesus CD36 polypeptide of SEQ ID NO: 62 or 63;

(d) the antibody inhibits CD36-dependent oxidized LDL uptake in F293 cells that overexpress surface human CD36 by at least 65%, at least 75%, at least 85%, at least 95%, or at least 100%; optionally, wherein at an oxLDL concentration of 1-10 μg/mL the antibody has an IC_{50} of 5 nM or less, 1 nM or less, 0.5 nM or less, or 0.1 nM or less;

(e) the antibody inhibits CD36-dependent oxidized LDL uptake in U937 cells by at least 65%, at least 75%, at least 85%, at least 95%, or at least 100%; optionally, wherein at an oxLDL concentration of 1-10 μg/mL the antibody has an IC_{50} of 5 nM or less, 1 nM or less, 0.5 nM or less, or 0.1 nM or less; and/or

(f) the antibody inhibits CD36-dependent oxidized LDL uptake in mouse CD45+ TILs by at least 65%, at least 75%, at least 85%, at least 95%, or at least 100%; optionally, wherein at an oxLDL concentration of 1-10 μg/mL the antibody has an IC_{50} of 5 nM or less, 1 nM or less, 0.5 nM or less, or 0.1 nM or less.

11. An isolated polynucleotide or vector comprising a sequence that encodes a polypeptide of an anti-CD36 antibody of any one of claims 1-10.

12. The isolated polynucleotide or vector of claim 11, wherein the polypeptide comprises an amino acid sequence comprising:

(a) CDR-H1 comprises the amino acid sequence of SEQ ID NO: 3, 21, 24, or 27; CDR-H2 comprises the amino acid sequence of SEQ ID NO: 4, 28, 31, 34, 37, 40 , or 43; and CDR-H3 comprises the amino acid sequence of SEQ ID NO: 5;

(b) CDR-L1 comprises an amino acid sequence selected from SEQ ID NO: 7; CDR-L2 comprises an amino acid sequence selected from SEQ ID NO: 8, 12, or 15; and CDR-L3 comprises an amino acid sequence selected from SEQ ID NO: 9, 13, or, 18;

(c) a heavy chain variable domain (V_H) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 2, 20, 23, 26, 30, 33, 36, 39, or 42;

(d) a light chain variable domain (V_L) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 6, 11, 14, or 17;

(e) a heavy chain (HC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 44, 48, 50, 51, 52, 53, 54, 55, 56, or 57; and/or

(f) a light chain (LC) amino acid sequence having at least 90% identity to a sequence selected

from SEQ ID NO: 45, 46, 47, or 49.

13. An isolated host cell comprising the polynucleotide or vector of any one of claims 11-12; optionally, wherein the host cell is selected from a Chinese hamster ovary (CHO) cell, a myeloma cell (e.g.,Y0, NS0, Sp2/0), a monkey kidney cell (COS-7), a human embryonic kidney line (293), a baby hamster kidney cell (BHK), a mouse Sertoli cell (e.g., TM4), an African green monkey kidney cell (VERO-76), a human cervical carcinoma cell (HELA), a canine kidney cell, a human lung cell (W138), a human liver cell (Hep G2), a mouse mammary tumor cell, a TR1 cell, a Medical Research Council 5 (MRC 5) cell, and a FS4 cell.

14. A method of producing an antibody comprising culturing the host cell of claim 13 so that an antibody is produced.

15. A pharmaceutical composition comprising an antibody of any one of claims 1-10 and a pharmaceutically acceptable carrier.

16. The composition of claim 15, wherein the composition further comprises a chemotherapeutic agent.

17. The composition of claim 15, wherein the composition further comprises an antibody comprising a specificity for an immune checkpoint molecule; optionally, wherein the immune checkpoint molecule is selected from PD1, PD-L1, LAG3, CTLA-4, A2AR, TIM-3, BTLA, CD276, CD328, VTCN1, IDO, KIR, NOX2, VISTA, OX40, CD27, CD28, CD40, CD122, CD137, GITR, ICOS.

18. The composition of claim 15, wherein the composition further comprises an immunostimulatory cytokine; optionally, wherein the immunostimulatory cytokine is selected from IL-2, IL-7, IL-10, IL-12, IL-15, IL-21, and IFN-α.

19. A method of treating a CD36-mediated disease in a subject, the method comprising administering to the subject a therapeutically effective amount of an antibody of any one of claims 1-10, or administering to the subject a therapeutically effective amount of a pharmaceutical composition of any one of claims 15-18; optionally, wherein the disease is cancer; optionally, wherein the cancer is colon cancer, pancreatic cancer, ovarian cancer, HCC, renal cancer, breast cancer, lung cancer, gastric cancer, melanoma, head and neck cancer, or oral cancer.

20. The method of claim 19, wherein the cancer is colon cancer, pancreatic cancer, ovarian cancer, HCC, renal cancer, breast cancer, lung cancer, gastric cancer, melanoma, head and neck cancer, or oral cancer.

FIG. 1A

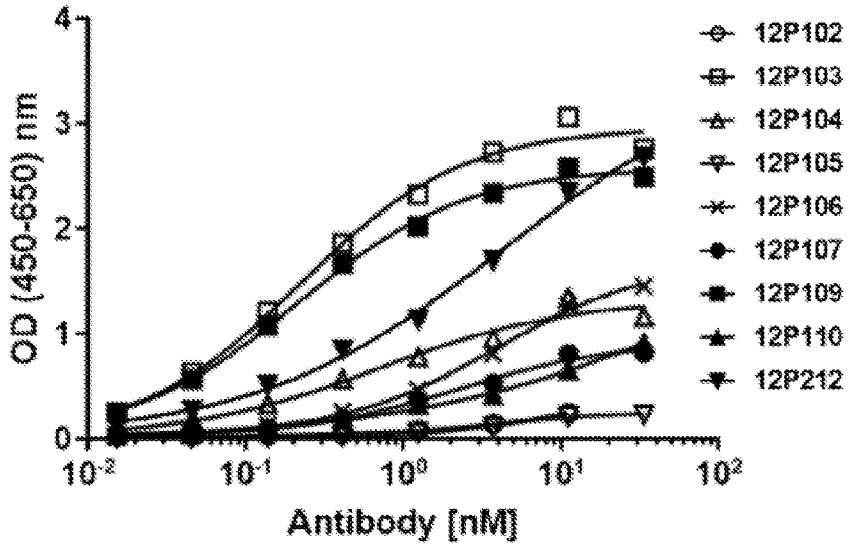

OD (450-650) nm
Antibody [nM]
12P102
12P103
12P104
12P105
12P106
12P107
12P109
12P110
12P212

FIG. 1B

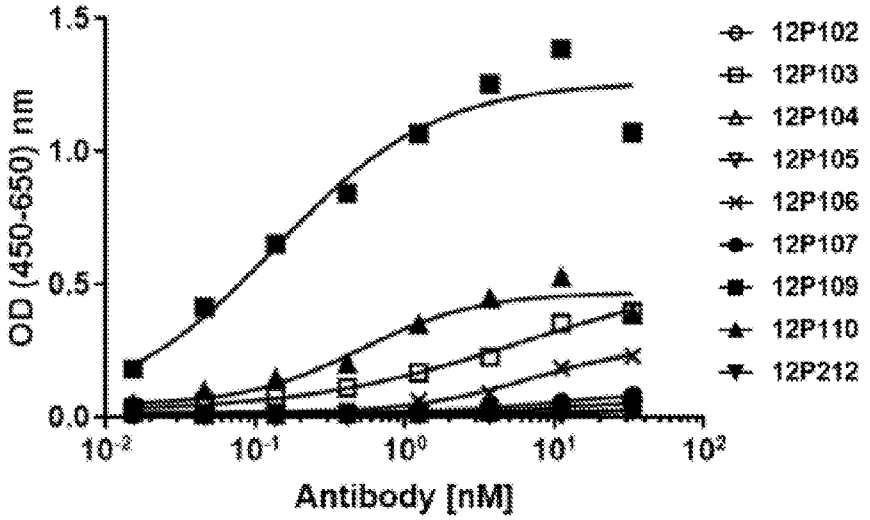

OD (450-650) nm
Antibody [nM]
12P102
12P103
12P104
12P105
12P106
12P107
12P109
12P110
12P212

FIG. 1C

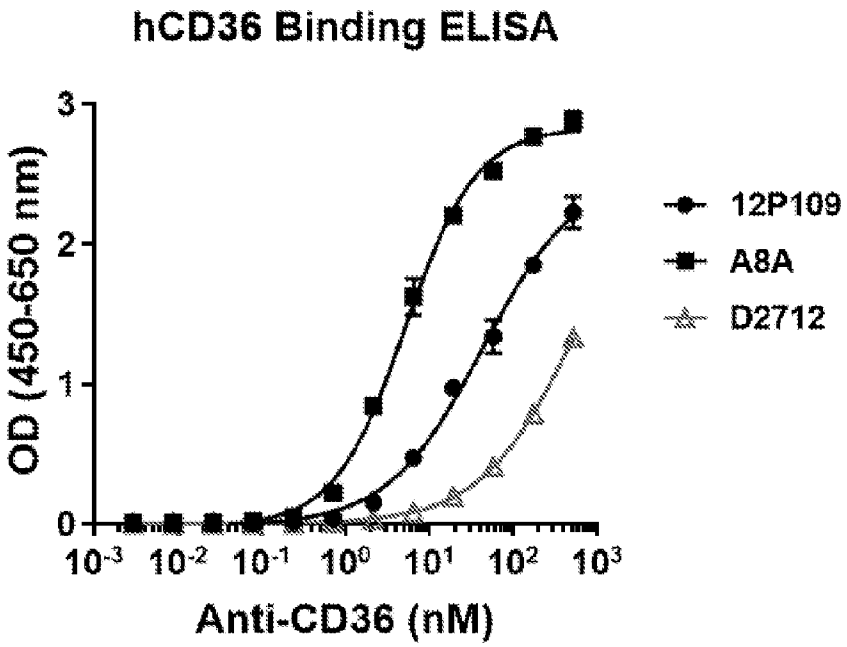

hCD36 Binding ELISA
OD (450-650 nm)
0
1
2
3
10-3 10-2 10-1 100 101 102 103
Anti-CD36 (nM)
12P109
A8A
D2712

FIG. 1D

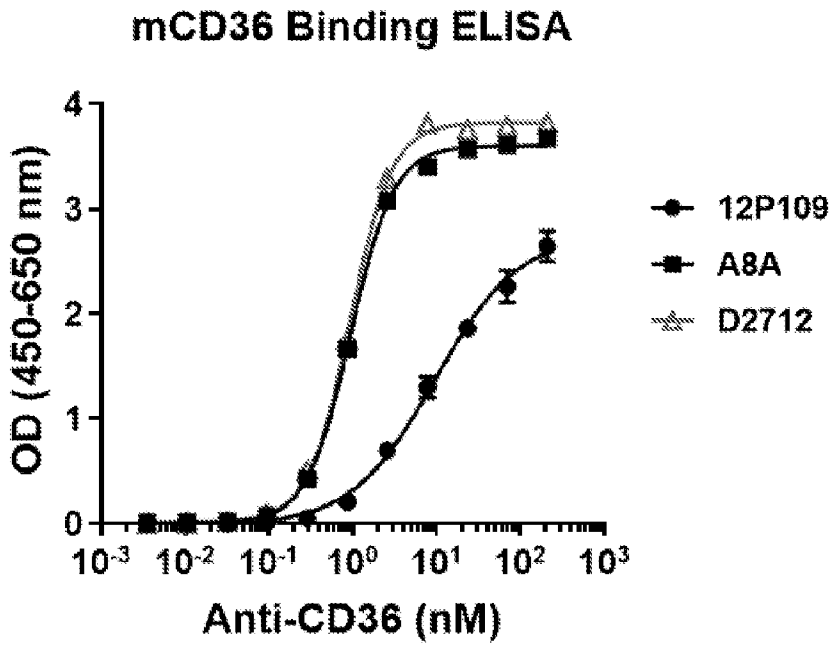

mCD36 Binding ELISA
OD (450-650 nm)
0
1
2
3
4
10-3 10-2 10-1 100 101 102 103
Anti-CD36 (nM)
12P109
A8A
D2712

FIG. 2A

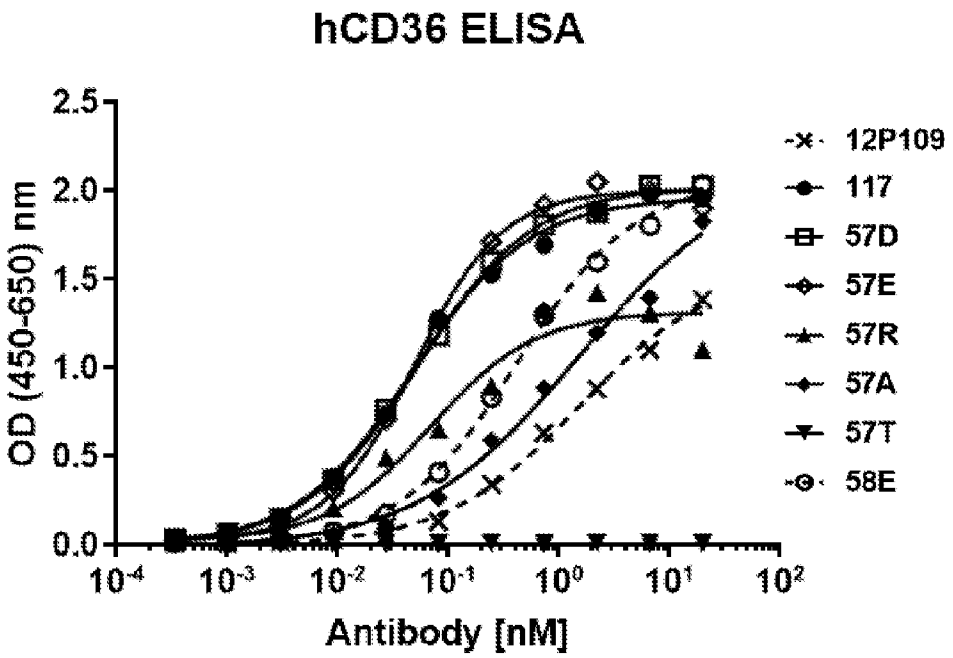

hCD36 ELISA
OD (450-650) nm
2.5
2.0
1.5
1.0
0.5
0.0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
Antibody [nM]
12P109
117
57D
57E
57R
57A
57T
58E

FIG. 2B

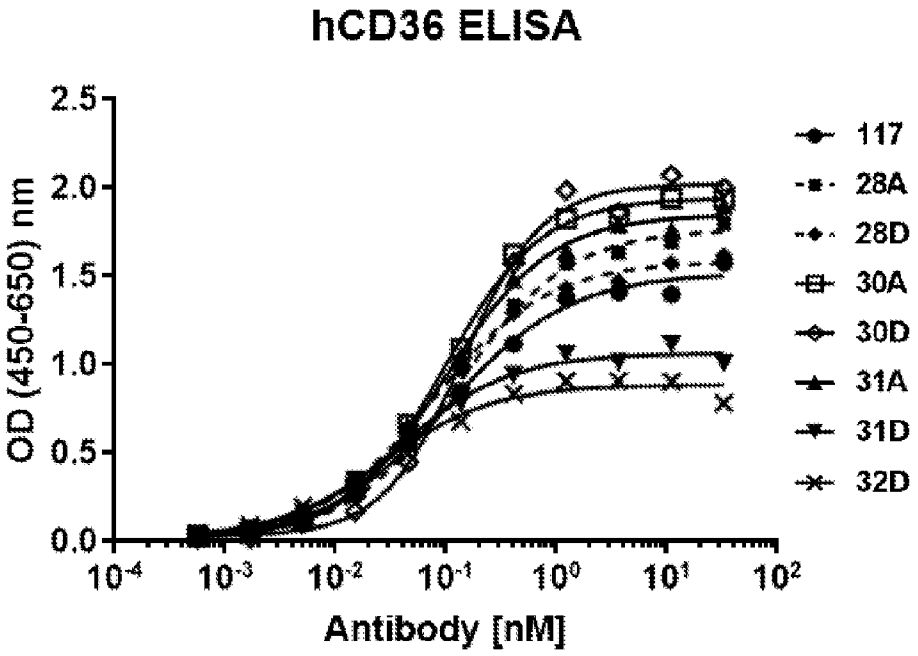

hCD36 ELISA
OD (450-650) nm
2.5
2.0
1.5
1.0
0.5
0.0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
Antibody [nM]
117
28A
28D
30A
30D
31A
31D
32D

FIG. 2C

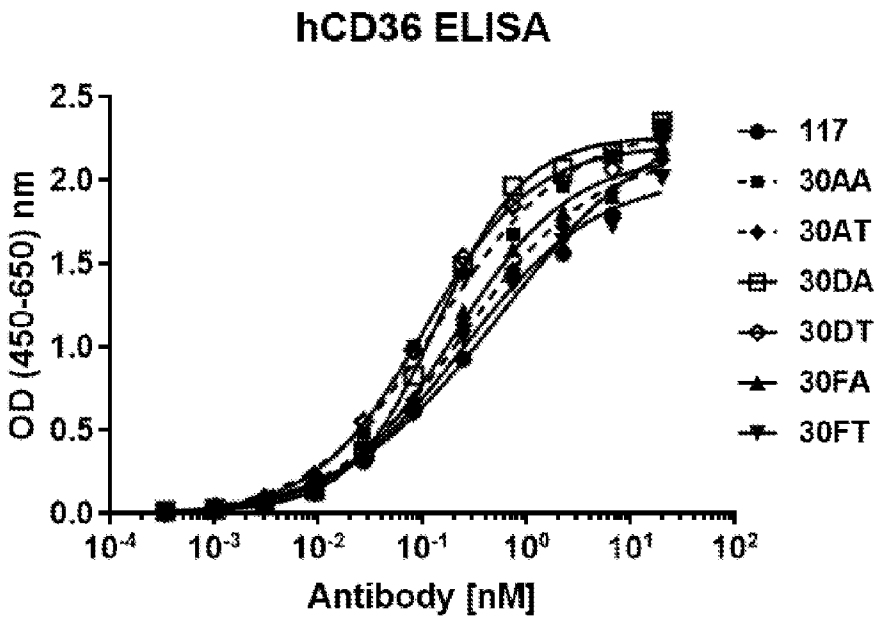

hCD36 ELISA
OD (450-650) nm
2.5
2.0
1.5
1.0
0.5
0.0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
Antibody [nM]
117
30AA
30AT
30DA
30DT
30FA
30FT

FIG. 2D

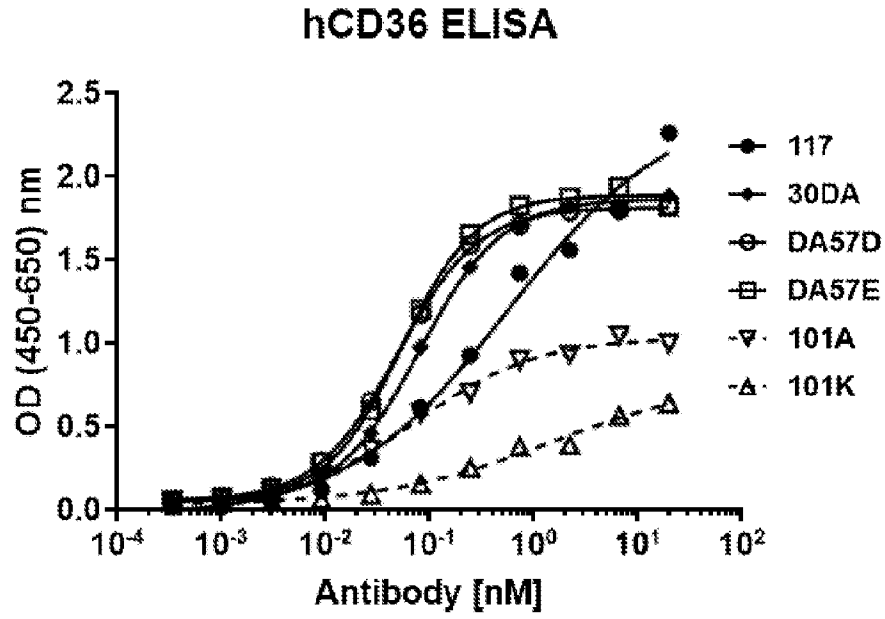

hCD36 ELISA
OD (450-650) nm
2.5
2.0
1.5
1.0
0.5
0.0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
Antibody [nM]
117
30DA
DA57D
DA57E
101A
101K

FIG. 2E

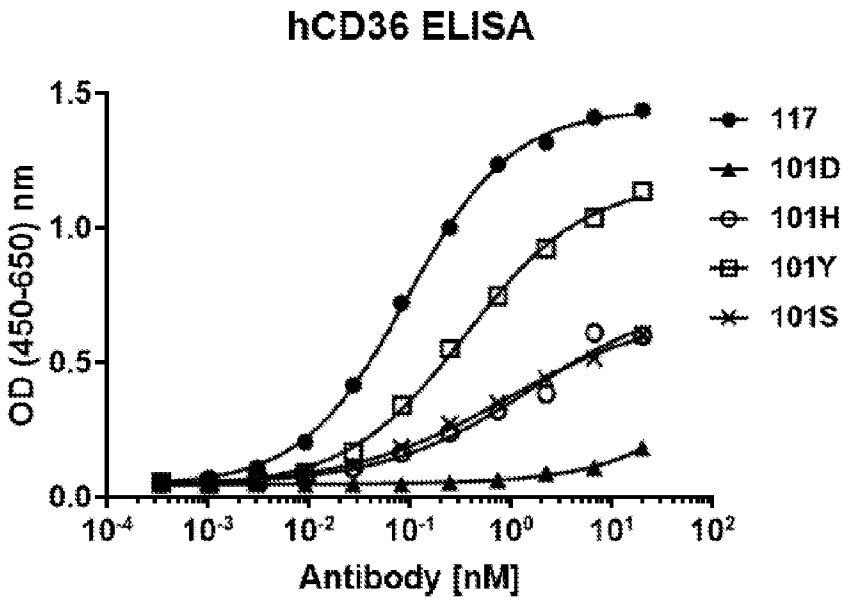

hCD36 ELISA
OD (450-650) nm
1.5
1.0
0.5
0.0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
Antibody [nM]
117
101D
101H
101Y
101S

FIG. 2F

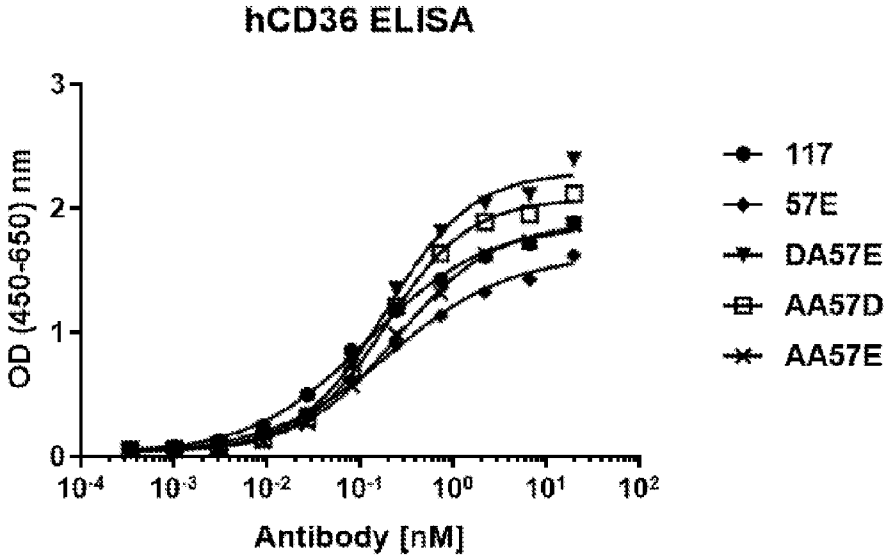

hCD36 ELISA
OD (450-650) nm
3
2
1
0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
Antibody [nM]
117
57E
DA57E
AA57D
AA57E

FIG. 3A

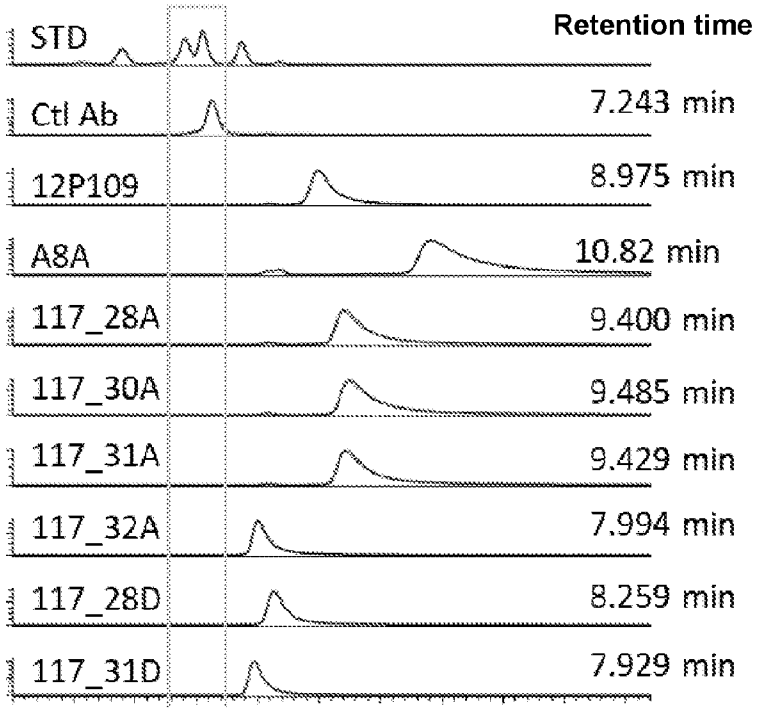

Retention time
STD
Ctl Ab
7.243 min
12P109
8.975 min
A8A
10.82 min
117_28A
9.400 min
117_30A
9.485 min
117_31A
9.429 min
117_32A
7.994 min
117_28D
8.259 min
117_31D
7.929 min

FIG. 3B

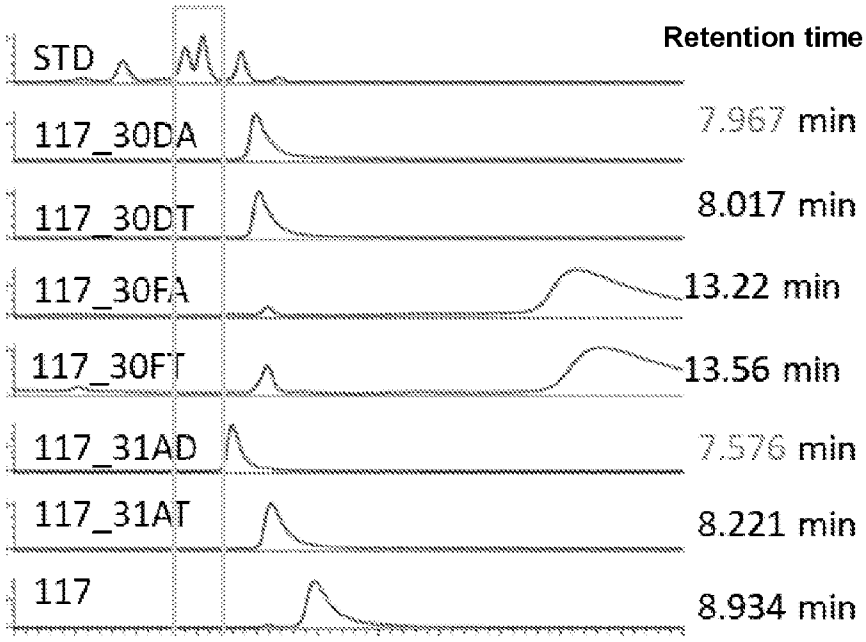

Retention time
STD
117_30DA
7.967 min
117_30DT
8.017 min
117_30FA
13.22 min
117_30FT
13.56 min
117_31AD
7.576 min
117_31AT
8.221 min
117
8.934 min

FIG. 3C

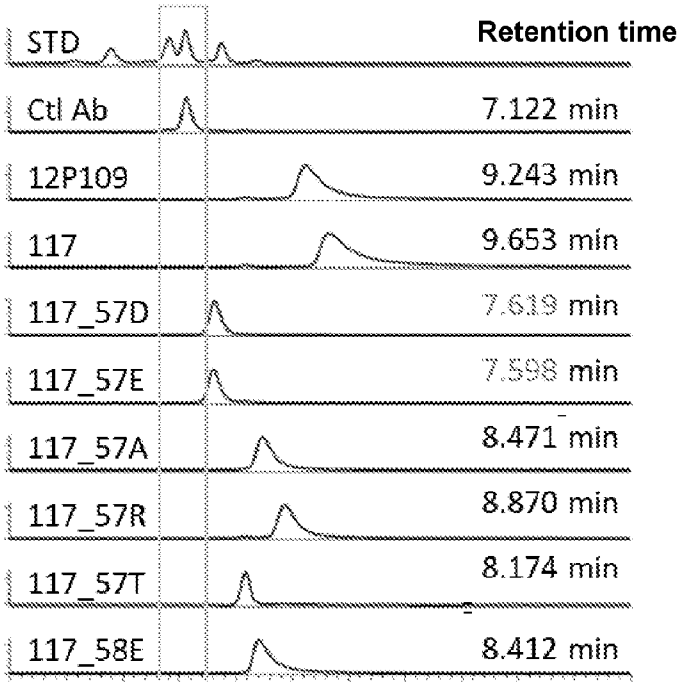

Retention time
STD
Ctl Ab
7.122 min
12P109
9.243 min
117
9.653 min
117_57D
7.619 min
117_57E
7.598 min
117_57A
8.471 min
117_57R
8.870 min
117_57T
8.174 min
117_58E
8.412 min

FIG. 3D

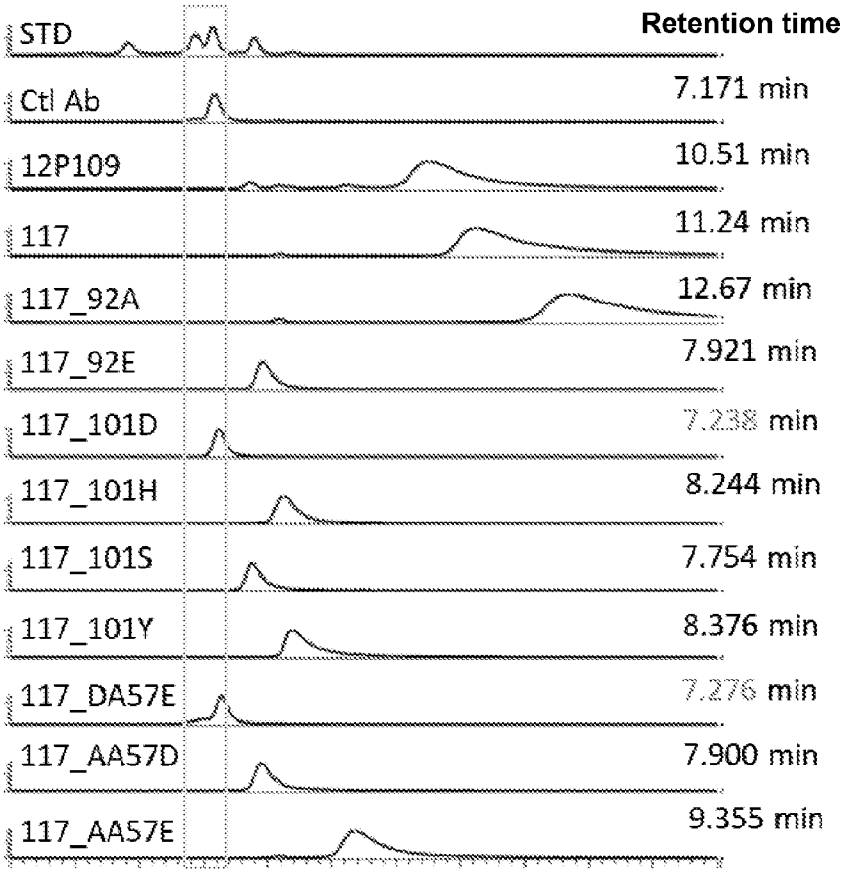

Retention time
STD
Ctl Ab
7.171 min
12P109
10.51 min
117
11.24 min
117_92A
12.67 min
117_92E
7.921 min
117_101D
7.238 min
117_101H
8.244 min
117_101S
7.754 min
117_101Y
8.376 min
117_DA57E
7.276 min
117_AA57D
7.900 min
117_AA57E
9.355 min

FIG. 4A

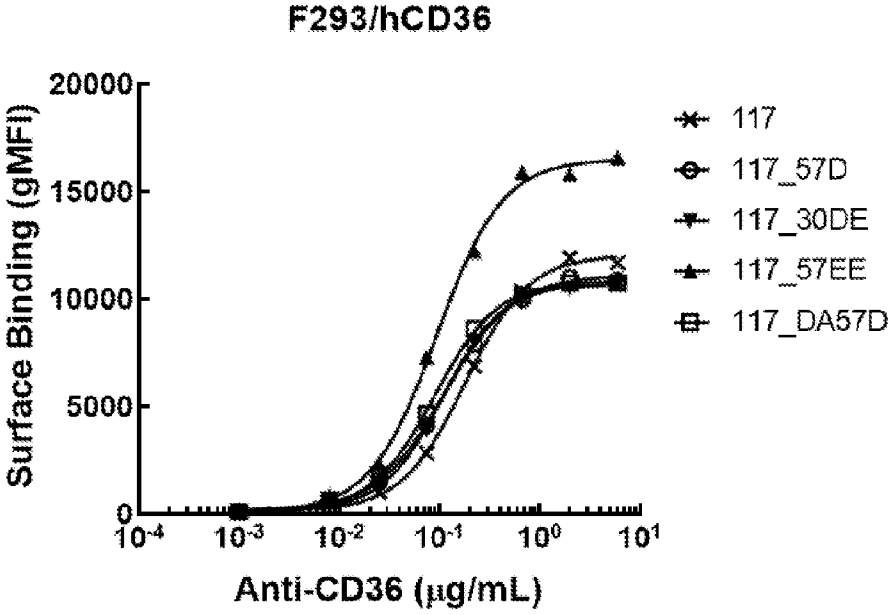

F293/hCD36
Surface Binding (gMFI)
20000
15000
10000
5000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
Anti-CD36 (μg/mL)
117
117_57D
117_30DE
117_57EE
117_DA57D

FIG. 4B

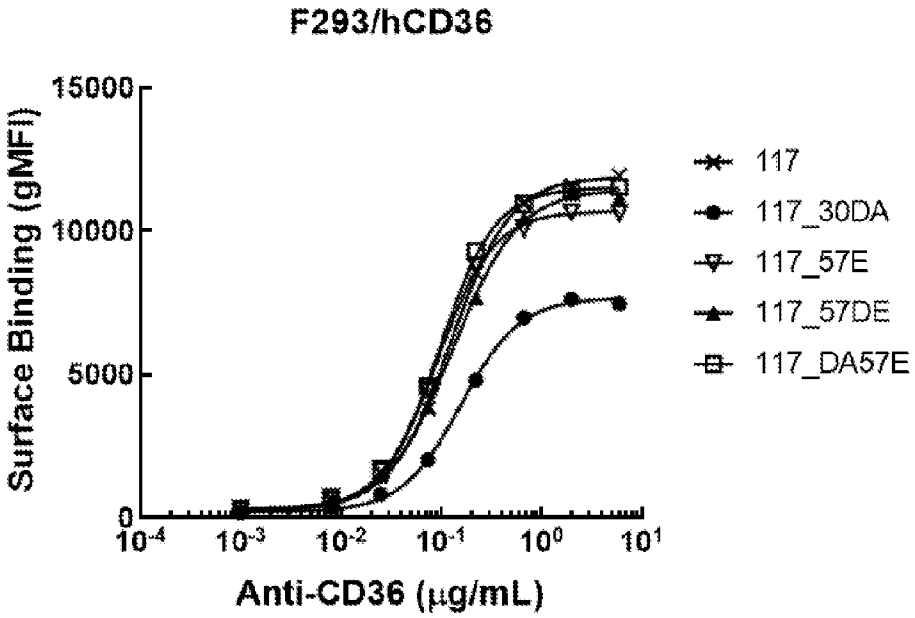

F293/hCD36
Surface Binding (gMFI)
15000
10000
5000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
Anti-CD36 (μg/mL)
117
117_30DA
117_57E
117_57DE
117_DA57E

FIG. 4C

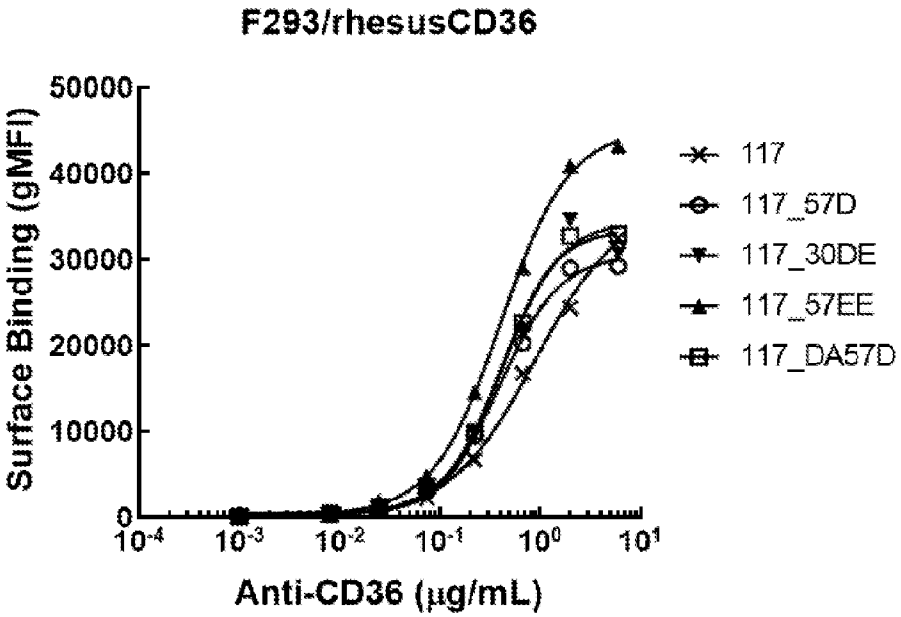

F293/rhesusCD36
Surface Binding (gMFI)
50000
40000
30000
20000
10000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
Anti-CD36 (μg/mL)
117
117_57D
117_30DE
117_57EE
117_DA57D

FIG. 4D

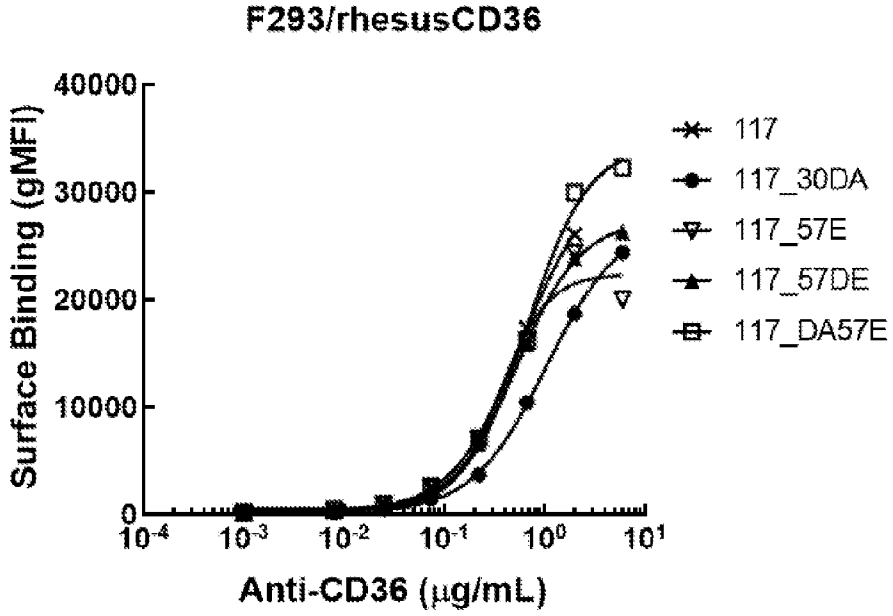

F293/rhesusCD36
Surface Binding (gMFI)
40000
30000
20000
10000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
Anti-CD36 (μg/mL)
117
117_30DA
117_57E
117_57DE
117_DA57E

FIG. 4E

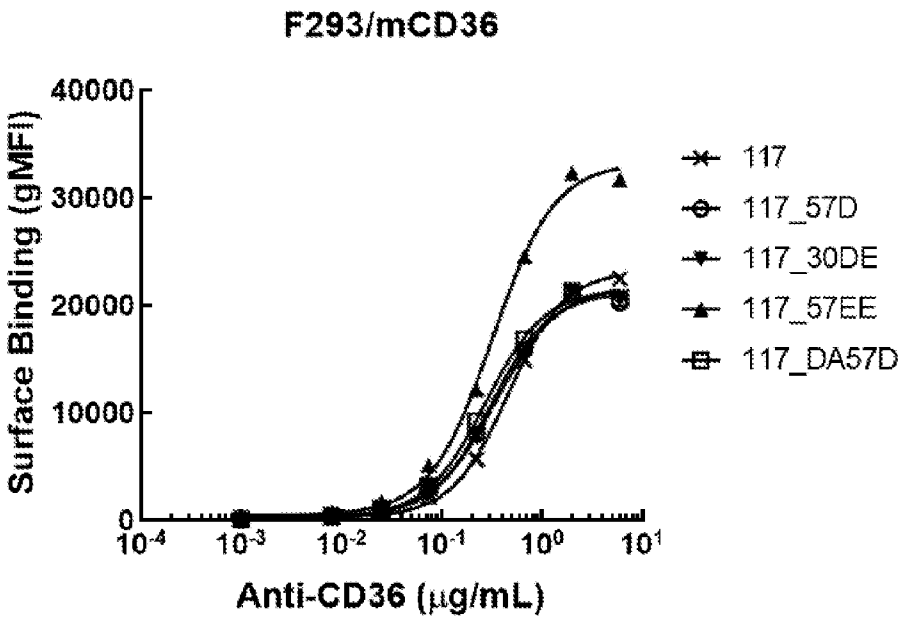

F293/mCD36
Surface Binding (gMFI)
40000
30000
20000
10000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
Anti-CD36 (μg/mL)
117
117_57D
117_30DE
117_57EE
117_DA57D

FIG. 4F

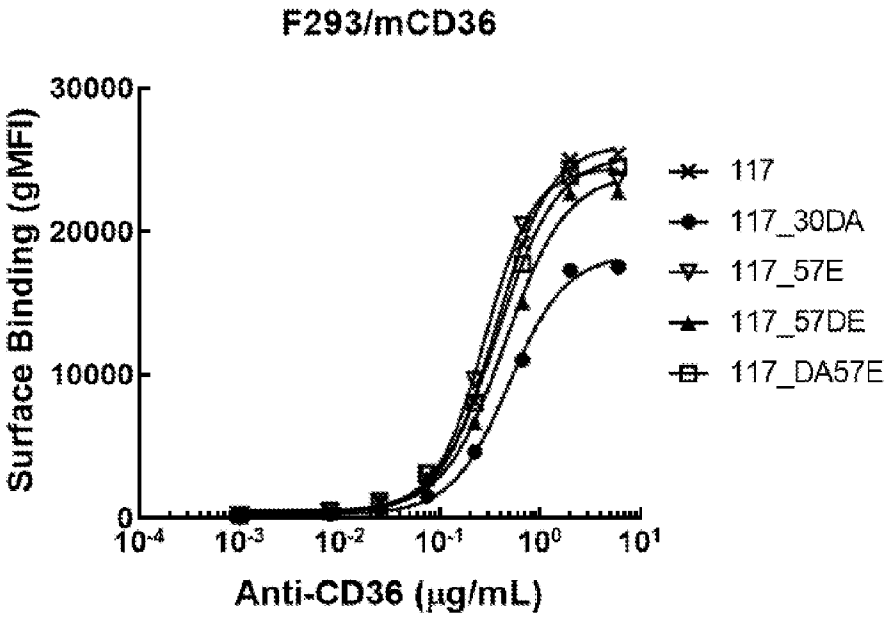

F293/mCD36
Surface Binding (gMFI)
30000
20000
10000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
Anti-CD36 (μg/mL)
117
117_30DA
117_57E
117_57DE
117_DA57E

FIG. 5A

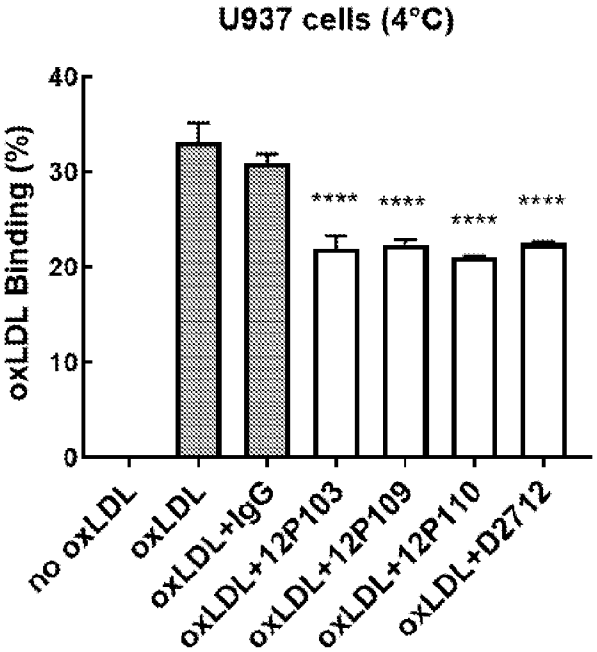

U937 cells (4°C)
oxLDL Binding (%)
40
30
20
10
0
****
****
****
****
no oxLDL
oxLDL
oxLDL+IgG
oxLDL+12P103
oxLDL+12P109
oxLDL+12P110
oxLDL+D2712

FIG. 5B

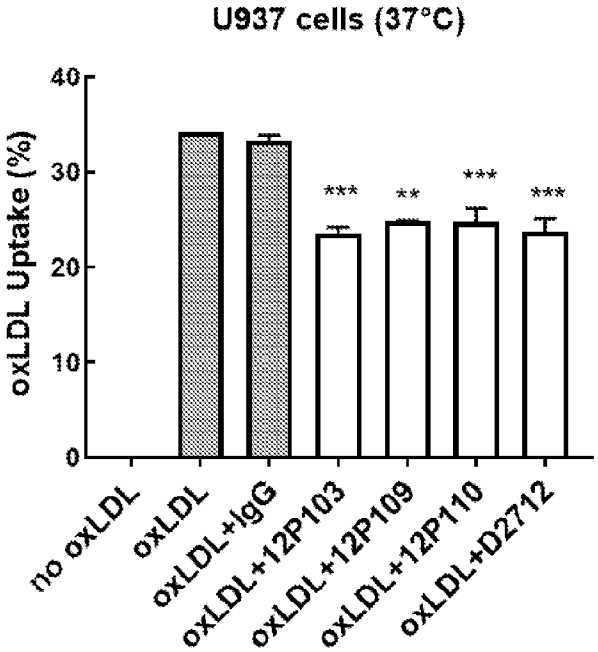

U937 cells (37°C)
oxLDL Uptake (%)
40
30
20
10
0
***
**
***
***
no oxLDL
oxLDL
oxLDL+IgG
oxLDL+12P103
oxLDL+12P109
oxLDL+12P110
oxLDL+D2712

FIG. 6A

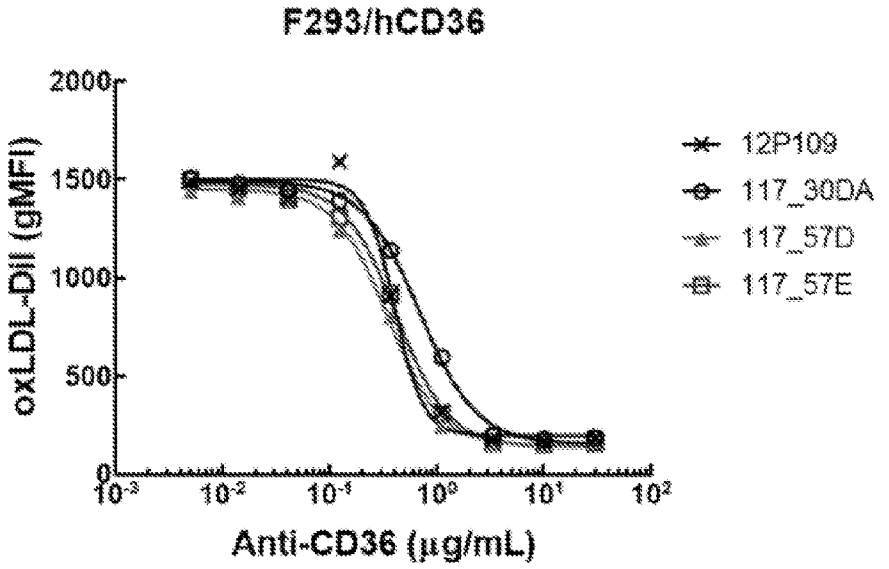

F293/hCD36
oxLDL-DiI (gMFI)
2000
1500
1000
500
0
10-3
10-2
10-1
100
101
102
Anti-CD36 (μg/mL)
12P109
117_30DA
117_57D
117_57E

FIG. 6B

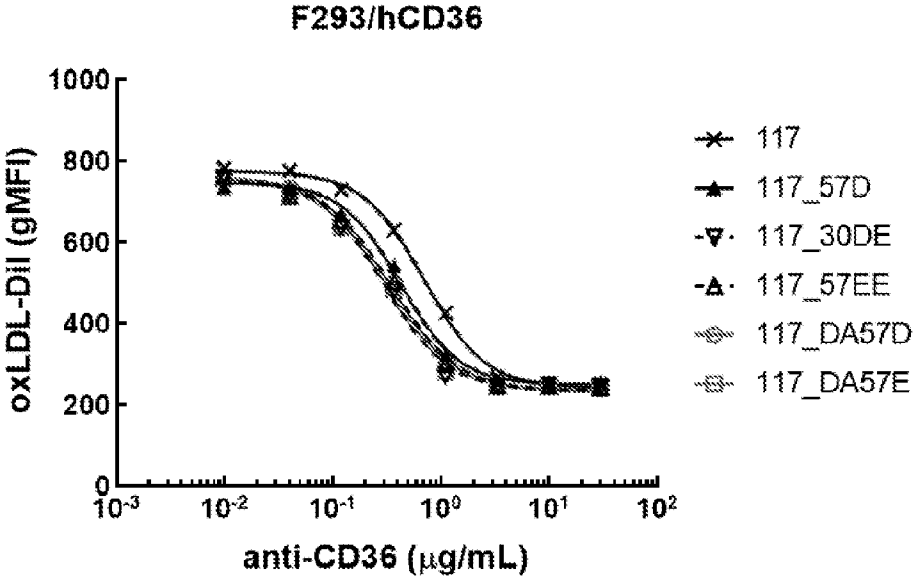

F293/hCD36
oxLDL-DiI (gMFI)
1000
800
600
400
200
0
10-3
10-2
10-1
100
101
102
anti-CD36 (μg/mL)
117
117_57D
117_30DE
117_57EE
117_DA57D
117_DA57E

FIG. 6C

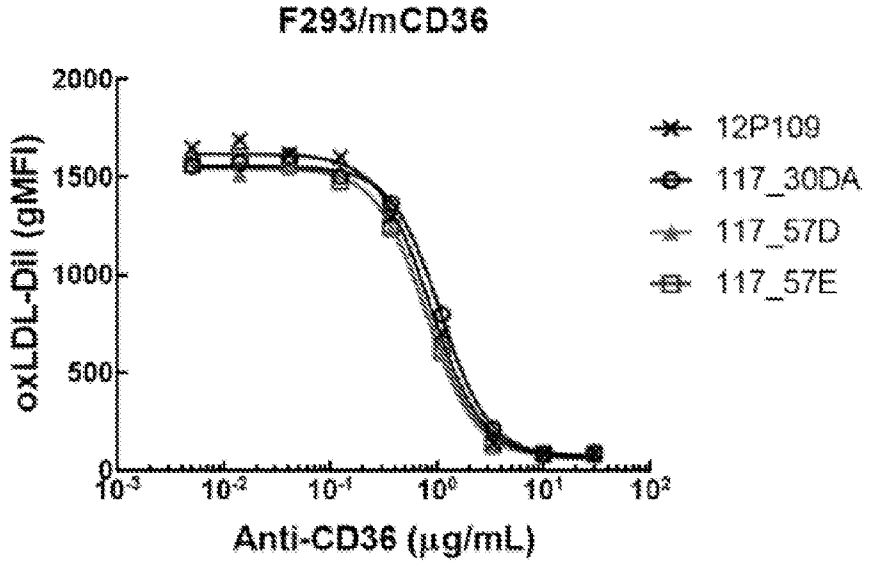

F293/mCD36
oxLDL-DiI (gMFI)
2000
1500
1000
500
0
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
Anti-CD36 (μg/mL)
12P109
117_30DA
117_57D
117_57E

FIG. 7A

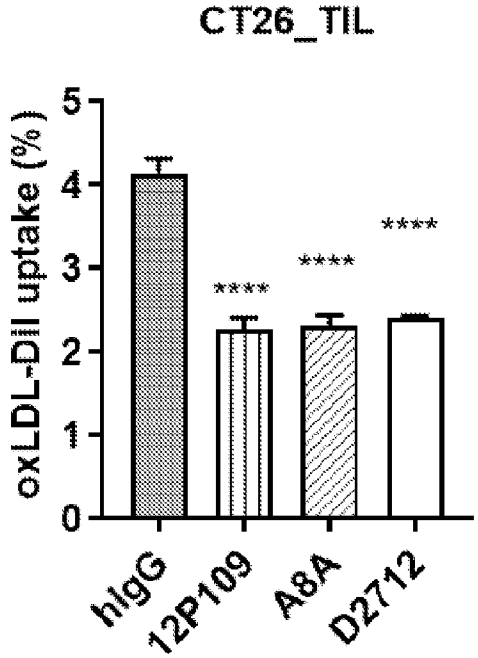

CT26_TIL
oxLDL-DiI uptake (%)
5
4
3
2
1
0
****
****
****
hIgG
12P109
A8A
D2712

FIG. 7B

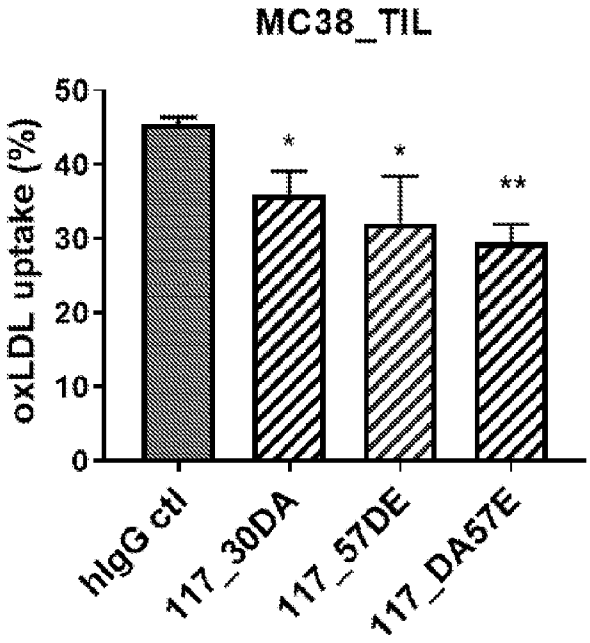

MC38_TIL
oxLDL uptake (%)
50
40
30
20
10
0
*
*
**
hIgG ctl
117_30DA
117_57DE
117_DA57E

FIG. 7C

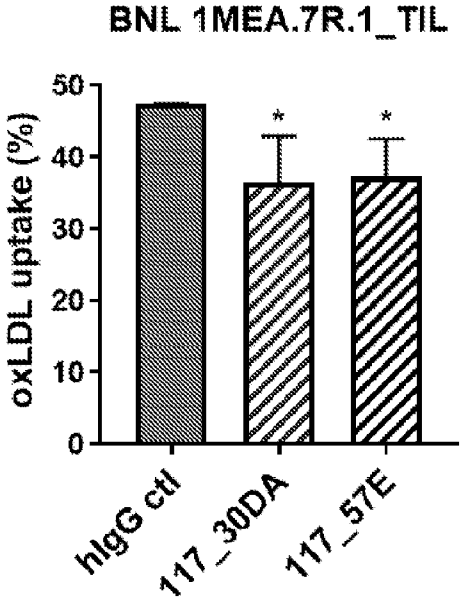

BNL 1MEA.7R.1_TIL
oxLDL uptake (%)
50
40
30
20
10
0
*
*
hIgG ctl
117_30DA
117_57E

FIG. 7D

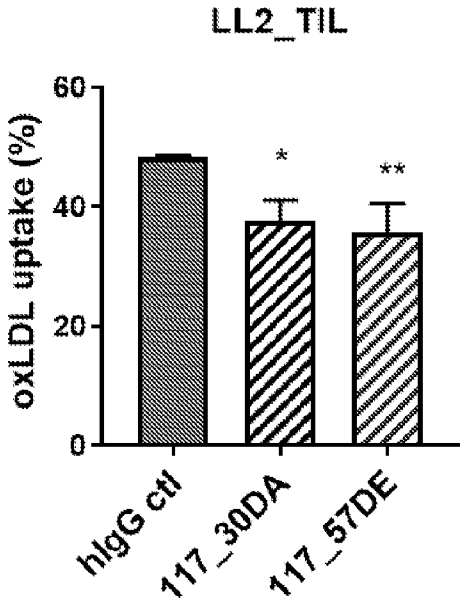

LL2_TIL
oxLDL uptake (%)
60
40
20
0
*
**
hIgG ctl
117_30DA
117_57DE

FIG. 7E

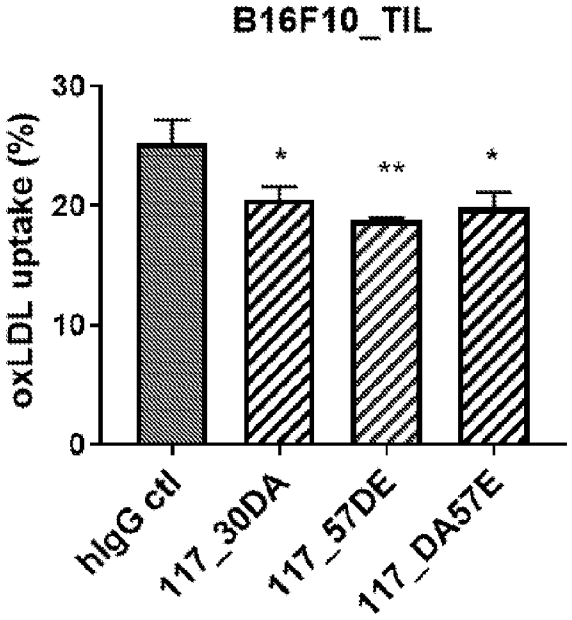

B16F10_TIL
oxLDL uptake (%)
30
20
10
0
*
**
*
hIgG ctl
117_30DA
117_57DE
117_DA57E

FIG. 8A

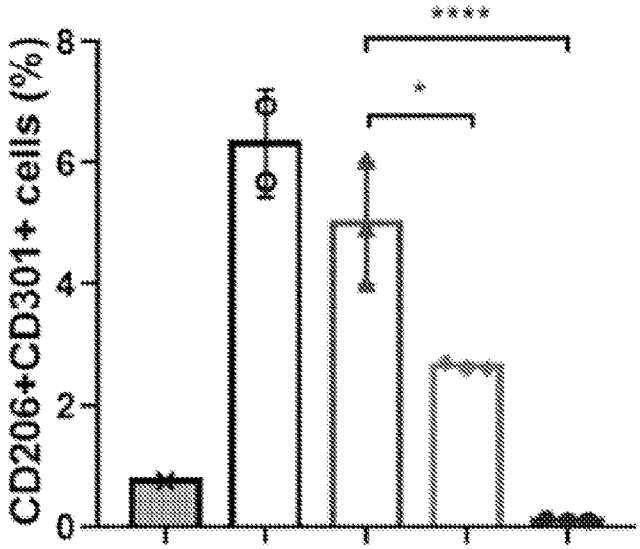

CD206+CD301+ cells (%)
0
2
4
6
8
****
*
M0
M2
M2
M2
M2
Ab
-
-
IgG
12P109
117_30AA

FIG. 8B

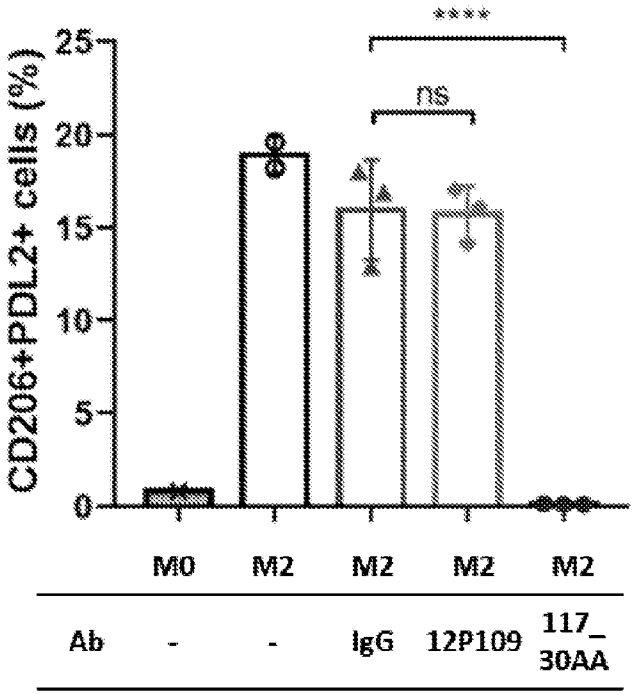

CD206+PDL2+ cells (%)
0
5
10
15
20
25
****
ns
M0
M2
M2
M2
M2
Ab
-
-
IgG
12P109
117_30AA

FIG. 8C

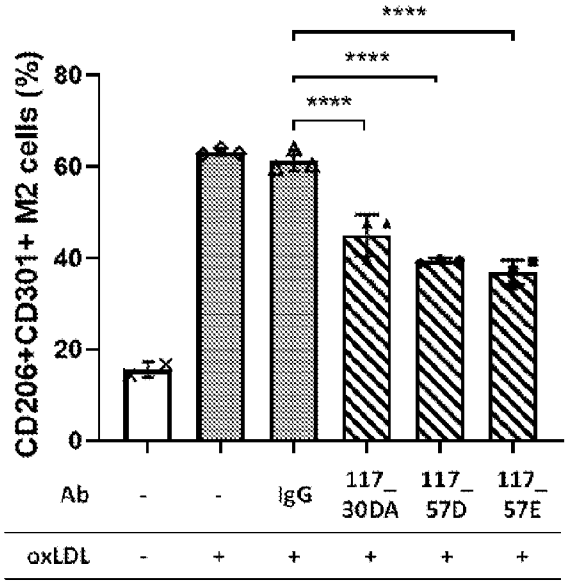

****
****
****
CD206+CD301+ M2 cells (%)
80
60
40
20
0
Ab - - IgG 117_ 30DA 117_ 57D 117_ 57E
oxLDL - + + + + +

FIG. 8D

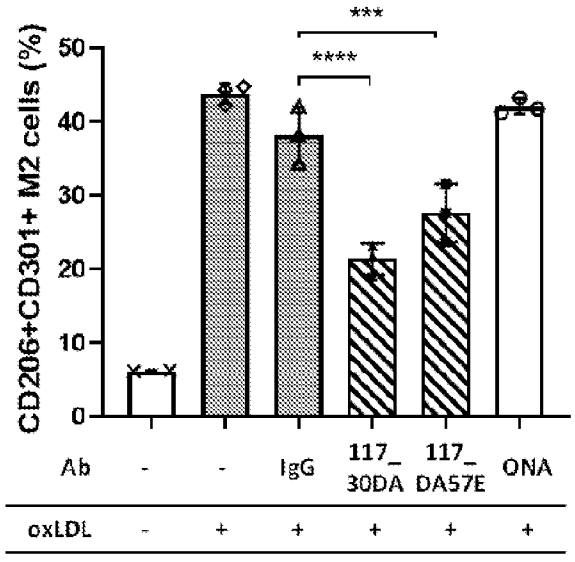

***
****
CD206+CD301+ M2 cells (%)
50
40
30
20
10
0
Ab - - IgG 117_ 30DA 117_ DA57E ONA
oxLDL - + + + + +

FIG. 8E

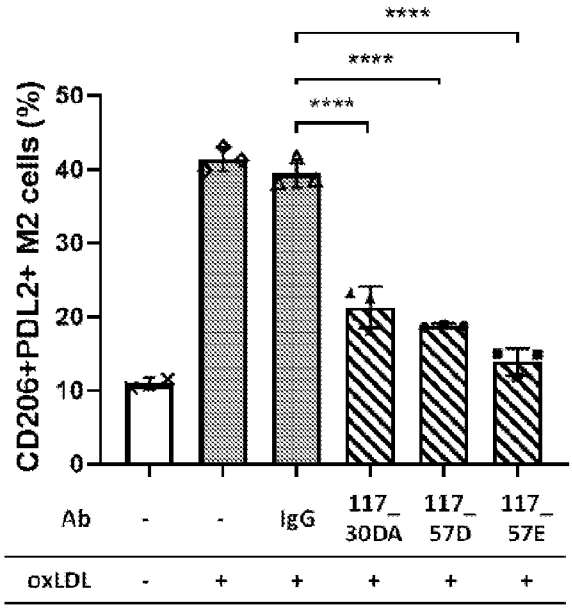

****
****
****
CD206+PDL2+ M2 cells (%)
50
40
30
20
10
0
Ab - - IgG 117_ 30DA 117_ 57D 117_ 57E
oxLDL - + + + + +

FIG. 8F

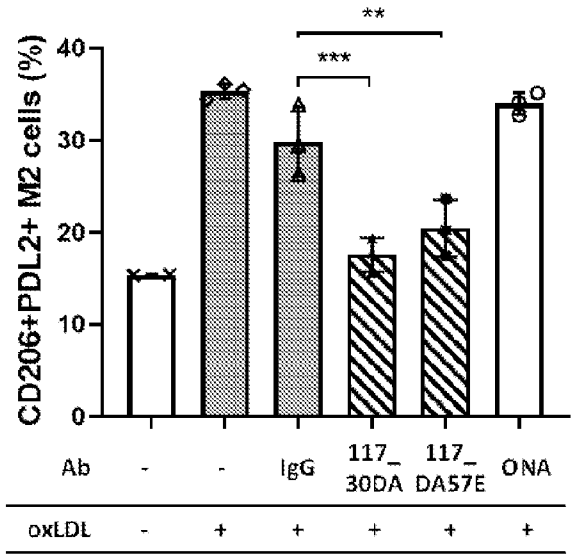

**
***
CD206+PDL2+ M2 cells (%)
40
30
20
10
0
Ab - - IgG 117_ 30DA 117_ DA57E ONA
oxLDL - + + + + +

FIG. 9

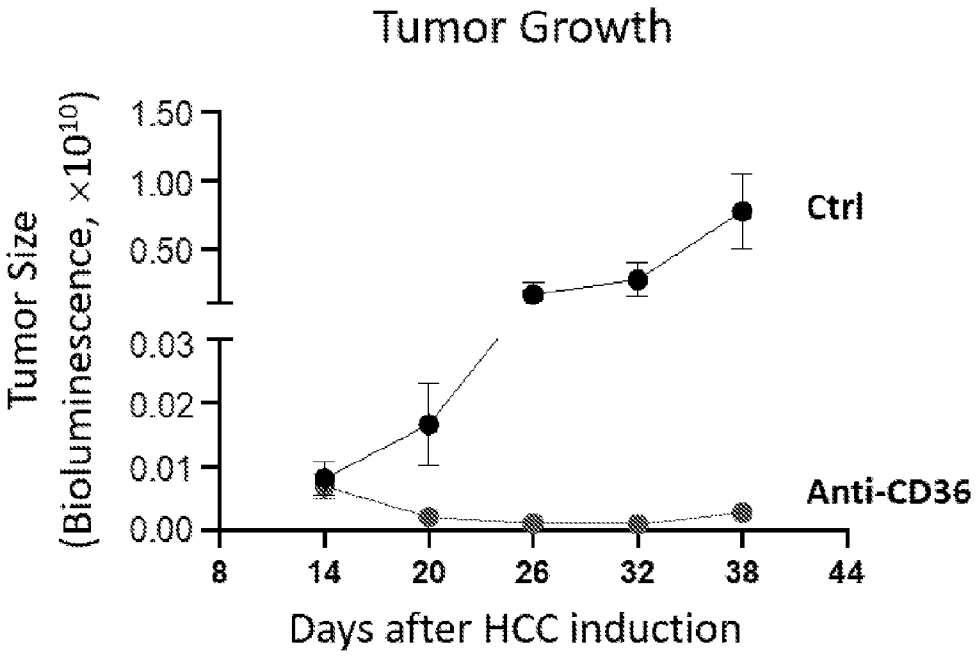

Tumor Growth
Tumor Size
(Bioluminescence, ×10^10)
1.50
1.00
0.50
0.03
0.02
0.01
0.00
Ctrl
Anti-CD36
8
14
20
26
32
38
44
Days after HCC induction

FIG. 10A

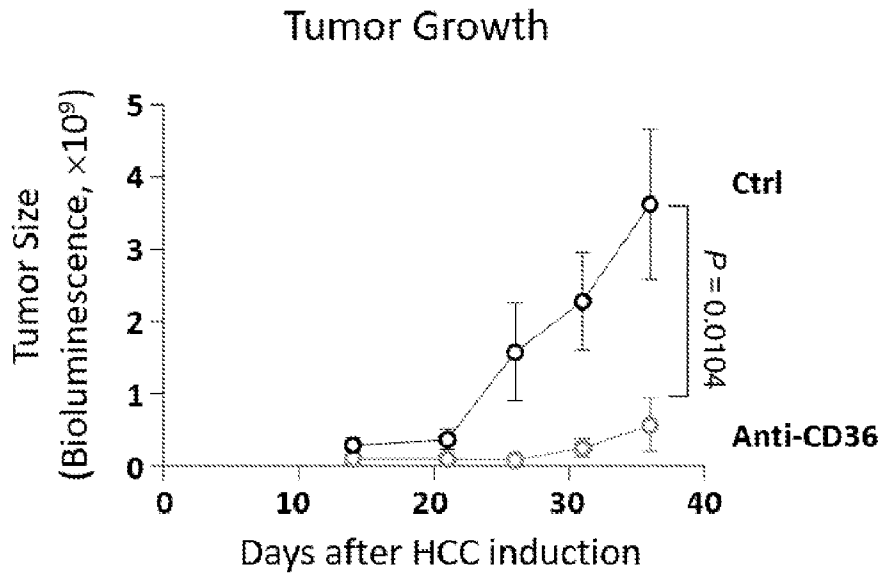


FIG. 10B

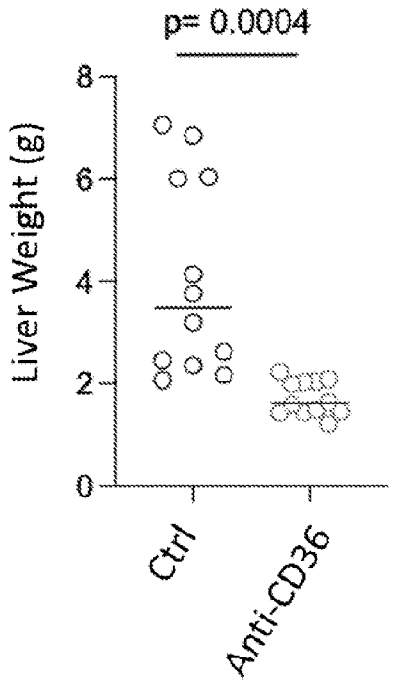
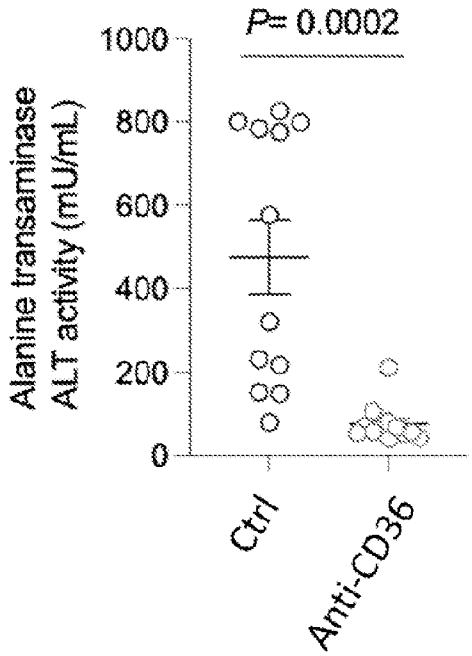


FIG. 10C



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US23/63766

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C07K 16/18; A61P 35/04; C07K 16/28 (2023.01)
ADD.
CPC - INV. C07K 16/18; C07K 16/2896; A61K 39/001129; A61P 35/04; C07K 16/28
ADD. A61K 2039/505

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2021/0341478 A1 (ANTAIMMU BIOMED CO. LTD.) 04 November 2021; [0017], [0019], [0024]	1-9
A	US 2020/0157519 A1 (IMMUNWORK INC.) 21 May 2020; [0019]	1-9
A	US 2006/0147451 A1 (KIRCHHOFER DANIEL K) 06 July 2006; [0032]	1-2, 5/1-2, 6/1-2, 7/1-2, 8/1-2, 9/1-2
A	US 2018/0371107 A1 (GENENTECH INC.) 27 December 2018; [0029]	3-4, 5/3-4, 6/3-4, 7/3-4, 8/3-4, 9/3-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 June 2023 (05.06.2023)	JUL 10 2023
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US23/63766

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed.

   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US23/63766

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10-20
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(19)国家知识产权局

(12)发明专利申请

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(51)Int.Cl.
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A61P 35/04(2006.01)
C07K 16/28(2006.01)

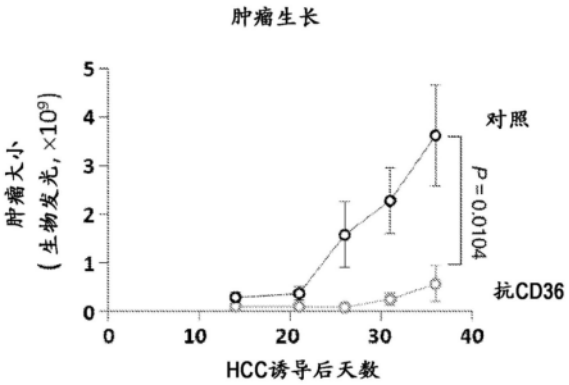
权利要求书6页 说明书58页
序列表（电子公布） 附图20页

(54)发明名称

抗CD36抗体及其用途

(57)摘要

本公开提供了抗体,所述抗体特异性结合人CD36蛋白(hCD36)并且能够降低、抑制和/或完全阻断通过CD36介导的免疫调节作用和/或配体转运到细胞中。除此之外,CD36介导作用的示例可包括氧化脂质转运到CD8T细胞中可能造成细胞死亡,以及脂肪酸转运到调节性T细胞中增加其在肿瘤微环境(TME)中的存活率和免疫抑制。本公开还提供了使用所述抗体(及其组合物)治疗对降低、抑制和/或阻断CD36依赖性配体转运到细胞中有反应的各种疾病和病况的方法。



CN 119095872 A

1.一种抗CD36抗体,其包含(i)第一重链互补决定区(CDR-H1)、第二重链互补决定区(CDR-H2)和第三重链互补决定区(CDR-H3),以及(ii)第一轻链互补决定区(CDR-L1)、第二轻链互补决定区(CDR-L2)和第三轻链互补决定区(CDR-L3),其中:

(a)CDR-H1包含SEQ ID NO:24、3、21或27的氨基酸序列;

(b)CDR-H2包含SEQ ID NO:43、4、28、31、34、37或40的氨基酸序列;

(c)CDR-H3包含SEQ ID NO:5的氨基酸序列;

(d)CDR-L1包含选自SEQ ID NO:7的氨基酸序列;

(e)CDR-L2包含选自SEQ ID NO:15、8或12的氨基酸序列;以及

(f)CDR-L3包含选自SEQ ID NO:18、9或13的氨基酸序列。

2.根据权利要求1所述的抗体,其中:

(a)CDR-H1包含SEQ ID NO:24的氨基酸序列,CDR-H2包含SEQ ID NO:43的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

(b)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:8的氨基酸序列,以及CDR-L3包含SEQ ID NO:9的氨基酸序列;

(c)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:12的氨基酸序列,以及CDR-L3包含SEQ ID NO:13的氨基酸序列;

(d)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:13的氨基酸序列;

(e)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

(f)CDR-H1包含SEQ ID NO:21的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

(g)CDR-H1包含SEQ ID NO:24的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

(h)CDR-H1包含SEQ ID NO:27的氨基酸序列,CDR-H2包含SEQ ID NO:28的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

(i)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:31的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

(j)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:34的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包

含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

(k)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:37的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;或

(l)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:40的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列。

3.一种抗CD36抗体,其包含:

(i)第一重链互补决定区(CDR-H1)、第二重链互补决定区(CDR-H2)和第三重链互补决定区(CDR-H3),其中所述CDR-H1、CDR-H2和CDR-H3的序列来自VH区域,所述VH区域具有选自SEQ ID NO:42、2、20、23、26、30、33、36和39的氨基酸序列;

(ii)第一轻链互补决定区(CDR-L1)、第二轻链互补决定区(CDR-L2)和第三轻链互补决定区(CDR-L3),其中所述CDR-L1、CDR-L2和CDR-L3的序列来自VL区域,所述VL区域具有选自SEQ ID NO:17、6、11和14的氨基酸序列,以及

其中所述CDR-L1、CDR-L2、CDR-L3、CDR-H1、CDR-H2和CDR-H3按照Kabat编号。

4.根据权利要求3所述的抗体,其中:

(a)VH氨基酸序列为SEQ ID NO:42并且VL氨基酸序列为SEQ ID NO:17;

(b)VH氨基酸序列为SEQ ID NO:2并且VL氨基酸序列为SEQ ID NO:6;

(c)VH氨基酸序列为SEQ ID NO:2并且VL氨基酸序列为SEQ ID NO:11;

(d)VH氨基酸序列为SEQ ID NO:2并且VL氨基酸序列为SEQ ID NO:14;

(e)VH氨基酸序列为SEQ ID NO:2并且VL氨基酸序列为SEQ ID NO:17;

(f)VH氨基酸序列为SEQ ID NO:20并且VL氨基酸序列为SEQ ID NO:17;

(g)VH氨基酸序列为SEQ ID NO:23并且VL氨基酸序列为SEQ ID NO:17;

(h)VH氨基酸序列为SEQ ID NO:26并且VL氨基酸序列为SEQ ID NO:17;

(i)VH氨基酸序列为SEQ ID NO:30并且VL氨基酸序列为SEQ ID NO:17;

(j)VH氨基酸序列为SEQ ID NO:33并且VL氨基酸序列为SEQ ID NO:17;

(k)VH氨基酸序列为SEQ ID NO:36并且VL氨基酸序列为SEQ ID NO:17;或

(l)VH氨基酸序列为SEQ ID NO:39并且VL氨基酸序列为SEQ ID NO:17。

5.根据权利要求1-4中任一项所述的抗体,其中所述抗体包含与选自SEQ ID NO:42、2、20、23、26、30、33、36或39的序列具有至少90%同一性的重链可变结构域(V_H)氨基酸序列;以及与选自SEQ ID NO:17、6、11或14的序列具有至少90%同一性的轻链可变结构域(V_L)氨基酸序列。

6.根据权利要求1-4中任一项所述的抗体,其中:

(a)所述抗体包含与SEQ ID NO:42具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90%同一性的V_L氨基酸序列;

(b)所述抗体包含与SEQ ID NO:2具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:6具有至少90%同一性的V_L氨基酸序列;

(c)所述抗体包含与SEQ ID NO:2具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:11具有至少90%同一性的V_L氨基酸序列;

(d)所述抗体包含与SEQ ID NO:2具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:14具有至少90％同一性的V_L氨基酸序列;

(e)所述抗体包含与SEQ ID NO:2具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列;

(f)所述抗体包含与SEQ ID NO:20具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列;

(g)所述抗体包含与SEQ ID NO:23具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列;

(h)所述抗体包含与SEQ ID NO:26具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列;

(i)所述抗体包含与SEQ ID NO:30具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列;

(j)所述抗体包含与SEQ ID NO:33具有至少90％同一性的VH氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列;

(k)所述抗体包含与SEQ ID NO:36具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列;或

(l)所述抗体包含与SEQ ID NO:39具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列。

7.根据权利要求1-4中任一项所述的抗体,其中所述抗体包含与选自SEQ ID NO:57、44、48、50、51、52、53、54、55或56的序列具有至少90％同一性的重链(HC)氨基酸序列,和/或与选自SEQ ID NO:49、45、46或47的序列具有至少90％同一性的轻链(LC)氨基酸序列。

8.根据权利要求1-4中任一项所述的抗体,其中所述抗体包含:

(a)与SEQ ID NO:57具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

(b)与SEQ ID NO:44具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:45具有至少90％同一性的LC氨基酸序列;

(c)与SEQ ID NO:44具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:46具有至少90％同一性的LC氨基酸序列;

(d)与SEQ ID NO:44具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:47具有至少90％同一性的LC氨基酸序列;

(e)与SEQ ID NO:44具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

(f)与SEQ ID NO:48具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

(g)与SEQ ID NO:50具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

(h)与SEQ ID NO:51具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

(i)与SEQ ID NO:52具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:49具有至

少90％同一性的LC氨基酸序列；

(j)与SEQ ID NO:53具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列；

(k)与SEQ ID NO:54具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列；

(l)与SEQ ID NO:55具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列；或

(m)与SEQ ID NO:56具有至少90％同一性的HC氨基酸序列,和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列。

9.根据权利要求1-4中任一项所述的抗体,其中所述抗体：

(a)为抗体片段,任选地选自由$F(ab')_2$、Fab'、Fab、Fv、单域抗体(VHH)和scFv组成的组；

(b)包含与蛋白质的融合物；任选地,其中所述蛋白质是选自IL-2、IL-7、IL-10、IL-12、IL-15、IL-21或IFN-α的免疫刺激细胞因子；

(c)为人抗体、人源化抗体或嵌合抗体；

(d)为IgG类的全长抗体,任选地,其中所述IgG类抗体具有选自IgG1、IgG2、IgG3和IgG4的同种型；

(e)包含Fc区变体,任选地,包含改变效应子功能的Fc区变体和/或改变抗体半衰期的变体；

(f)包含免疫缀合物,任选地,其中所述免疫缀合物包含用于治疗CD36介导的疾病或病况的治疗剂；和/或

(g)为多特异性抗体；任选地,为双特异性抗体。

10.根据权利要求1-9中任一项所述的抗体,其中：

(a)所述抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低的结合亲和力与人CD36结合；任选地,其中所述结合亲和力通过与SEQ ID NO:58或59的hCD36多肽的平衡解离常数(K_D)来测量；

(b)所述抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低的结合亲和力与小鼠CD36结合；任选地,其中所述结合亲和力通过与SEQ ID NO:60或61的mCD36多肽的平衡解离常数(K_D)来测量；

(c)所述抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低的结合亲和力与恒河猴CD36结合；任选地,其中所述结合亲和力通过与SEQ ID NO:62或63的恒河猴CD36多肽的平衡解离常数(K_D)来测量；

(d)所述抗体在过表达表面人CD36的F293细胞中抑制CD36依赖性氧化LDL的摄取至少65％、至少75％、至少85％、至少95％或至少100％；任选地,其中在1-10μg/mL的oxLDL浓度下,所述抗体具有5nM或更低、1nM或更低、0.5nM或更低、或者0.1nM或更低的IC_{50}；

(e)所述抗体在U937细胞中抑制CD36依赖性氧化LDL的摄取至少65％、至少75％、至少85％、至少95％或至少100％；任选地,其中在1-10μg/mL的oxLDL浓度下,所述抗体具有5nM或更低、1nM或更低、0.5nM或更低、或者0.1nM或更低的IC_{50}；和/或

(f)所述抗体在小鼠CD45+TIL中抑制CD36依赖性氧化LDL的摄取至少65％、至少75％、至少85％、至少95％或至少100％；任选地,其中在1-10μg/mL的oxLDL浓度下,所述抗体具有

5nM或更低、1nM或更低、0.5nM或更低、或者0.1nM或更低的IC_{50}。

11.一种分离的多核苷酸或载体,其包含编码根据权利要求1-10中任一项所述的抗CD36抗体的多肽的序列。

12.根据权利要求11所述的分离的多核苷酸或载体,其中所述多肽包含氨基酸序列,所述氨基酸序列包含:

(a)CDR-H1包含SEQ ID NO:3、21、24或27的氨基酸序列;CDR-H2包含SEQ ID NO:4、28、31、34、37、40或43的氨基酸序列;以及CDR-H3包含SEQ ID NO:5的氨基酸序列;

(b)CDR-L1包含选自SEQ ID NO:7的氨基酸序列;CDR-L2包含选自SEQ ID NO:8、12或15的氨基酸序列;以及CDR-L3包含选自SEQ ID NO:9、13或18的氨基酸序列;

(c)与选自SEQ ID NO:2、20、23、26、30、33、36、39或42的序列具有至少90%同一性的重链可变结构域(V_H)氨基酸序列;

(d)与选自SEQ ID NO:6、11、14或17的序列具有至少90%同一性的轻链可变结构域(V_L)氨基酸序列;

(e)与选自SEQ ID NO:44、48、50、51、52、53、54、55、56或57的序列具有至少90%同一性的重链(HC)氨基酸序列;和/或

(f)与选自SEQ ID NO:45、46、47或49的序列具有至少90%同一性的轻链(LC)氨基酸序列。

13.一种分离的宿主细胞,其包含根据权利要求11-12中任一项所述的多核苷酸或载体;任选地,其中所述宿主细胞选自中国仓鼠卵巢(CHO)细胞、骨髓瘤细胞(例如,Y0、NS0、Sp2/0)、猴肾细胞(COS-7)、人胚胎肾细胞系(293)、幼仓鼠肾细胞(BHK)、小鼠塞托利细胞(例如,TM4)、非洲绿猴肾细胞(VERO-76)、人宫颈癌细胞(HELA)、犬肾细胞、人肺细胞(W138)、人肝细胞(Hep G2)、小鼠乳腺肿瘤细胞、TR1细胞、医学研究委员会5(MRC 5)细胞和FS4细胞。

14.一种产生抗体的方法,其包括培养根据权利要求13所述的宿主细胞,从而产生抗体。

15.一种药物组合物,其包含根据权利要求1-10中任一项所述的抗体和药学上可接受的载体。

16.根据权利要求15所述的组合物,其中所述组合物还包含化学治疗剂。

17.根据权利要求15所述的组合物,其中所述组合物还包含对免疫检查点分子具有特异性的抗体;任选地,其中所述免疫检查点分子选自PD1、PD-L1、LAG3、CTLA-4、A2AR、TIM-3、BTLA、CD276、CD328、VTCN1、IDO、KIR、NOX2、VISTA、OX40、CD27、CD28、CD40、CD122、CD137、GITR、ICOS。

18.根据权利要求15所述的组合物,其中所述组合物还包含免疫刺激细胞因子;任选地,其中所述免疫刺激细胞因子选自IL-2、IL-7、IL-10、IL-12、IL-15、IL-21和IFN-α。

19.一种治疗受试者中CD36介导的疾病的方法,所述方法包括向所述受试者施用治疗有效量的根据权利要求1-10中任一项所述的抗体,或向所述受试者施用治疗有效量的根据权利要求15-18中任一项所述的药物组合物;任选地,其中所述疾病为癌症;任选地,其中所述癌症为结肠癌、胰腺癌、卵巢癌、HCC、肾癌、乳腺癌、肺癌、胃癌、黑色素瘤、头颈癌或口腔癌。

20.根据权利要求19所述的方法,其中所述癌症为结肠癌、胰腺癌、卵巢癌、HCC、肾癌、乳腺癌、肺癌、胃癌、黑色素瘤、头颈癌或口腔癌。

抗CD36抗体及其用途

[0001] 本申请要求2022年3月7日提交的美国临时专利申请号63/317,160的优先权,其全部内容通过引用并入本文。

技术领域

[0002] 本公开涉及与CD36结合的抗体以及使用此类抗体的方法。

[0003] 序列表的引用

[0004] 序列表的官方副本,以WIPO标准ST.26格式的XML文件与说明书同时提交,其文件名为“09793-005WO1.xml”,创建日期为2023年2月26日,以及大小为75,330字节。通过USPTO专利中心提交的序列表是说明书的一部分,并且其全部内容通过引用并入本文。

背景技术

[0005] 巨噬细胞和其他髓系细胞在创造以及维持肿瘤的免疫抑制环境中起着突出的作用,并且存在不同的微环境,其中肿瘤相关巨噬细胞(TAM)抑制免疫反应并促进其他过程,包括血管生成、癌细胞迁移和转移(Lewis&Pollard,2006;Pollard,2008;Wu等人,2020)。大约二十年前,例如,通过TAM产生的血管内皮生长因子被提出在几种人类癌症的淋巴转移中发挥作用(例如,Pepper等人,2003),以及2006年,证明氯膦酸盐脂质体介导的TAM耗尽经由被认为包括减少肿瘤血管生成的机制抑制实验模型中肿瘤生长(Zeisberger等人,2006)。此外,巨噬细胞不仅促进肿瘤细胞从原发性肿瘤中流出,还被认为有助于在远处转移部位接种(Joyce&Pollard,2009;Psaila&Lyden,2009)。

[0006] 虽然对各种促肿瘤发生(通常称为M2)和抗肿瘤发生M1巨噬细胞以及其他髓系细胞的了解仍在不断加深(例如,参见Wu等人,2020),但高TAM数与患者生存率降低有联系(只有少数显著的例外)并且是许多癌症中的独立预后因素这一点早已被认识到(Lewis&Pollard,2006)。此外,在癌症患者的外周血和肿瘤组织中发现了被称为髓系来源抑制细胞(MDSC)的髓系细胞,并且对小鼠模型中MDSC的耗尽或其他靶向方式会导致改善的免疫反应和延缓的肿瘤生长(Goedegebuure等人,2011)。MDSC据称具有一定的异质性,但仍然被描述为属于具有不同免疫抑制机制的单核细胞与粒细胞亚群。除其他作用外,肿瘤MDSC已参与了免疫抑制调节性T细胞的募集和维持,以及促进血管生成和转移(Goedegebuure等人,2011;Oh等人,2013)。与MDSC一样,至少正如我们现在所知,CD45阳性但CD14阴性的人外周血细胞亚群被Barrett等人(2007)描述为表达CD36和免疫抑制细胞因子白细胞介素-10。

[0007] CD36是一种跨膜细胞表面蛋白,除其他名称外,还被称为血小板糖蛋白4、脂肪酸转位酶(FAT)和清道夫受体B类成员3(SCARB3)。CD36结合并将脂肪酸转运到细胞中,但也结合大量其他配体,如凋亡细胞、低密度脂蛋白、磷脂及其氧化形式(Pepino等人,2014;Wang&Li,2019)。CD36还具有分离的血小板反应蛋白结合结构域(Pepino等人,2014;Wang&Li,2019)。这两种不同的功能事件,即血小板反应蛋白与脂肪酸结合,均可导致各种下游信号传导事件,这些事件可能因细胞类型和各种其他CD36和/或配体相互作用伴侣的不同而不同(Pepino等人,2014;Wang&Li,2019)。通过CD36的脂肪酸转运如果不是细胞分化状态或存

活所需的,则涉及各种细胞的代谢“布线”。例如,已证明通过CD36的脂肪酸转运是M2巨噬细胞分化所需的,所述M2巨噬细胞分化经由与内质网应激水平或状态相关的机制(Oh等人,2012)。

[0008] CD36还被证明对M2巨噬细胞活化至关重要,如通过免疫抑制能力标志物例如PDL-2表达来测量的(Huang等人,2014)。CD36介导的脂质转运还涉及MDSC分化期间免疫抑制效应物的获得(Al-Khami等人,2017)。当与荷瘤野生型小鼠相比时,CD36缺陷小鼠在测试的所有(肺和结肠)肿瘤模型中肿瘤驻留的MDSC的数量有所减少,并且这一点反映在野生型嵌合小鼠的肿瘤中,所述野生型嵌合小鼠在其骨髓中是CD36缺陷的(Al-Khami等人,2017)。最近已证明,与野生型小鼠的黑色素瘤和骨髓瘤相比,CD36缺陷小鼠的黑色素瘤和骨髓瘤模型中的TAM显着减少,并且与正常巨噬细胞相比,野生型小鼠的此类TAM具有升高的CD36表达和脂质积累(Su等人,2020)。

[0009] 鉴于CD36介导的脂肪酸或其他配体摄取对M2 TAM和MDSC生物学的重要性,可以预期阻断CD36配体转运会减少肿瘤生长,通过导致免疫抑制能力、血管产生和/或转移降低,而这些原本会被M2 TAM和MDSC促进。事实上,小鼠肿瘤模型表明,缺乏CD36的小鼠的肿瘤生长(肺和结肠)显著减少,并且这是由于骨髓衍生细胞中CD36丧失所致(Al-Khami等人,2017)。

[0010] 除了髓系细胞外,最近有报道称,CD36在某些肿瘤驻留调节性T细胞上被表达,并且用阻止CD36介导的脂肪酸转运的抗体阻断CD36会导致实验模型中的黑色素瘤生长的减少(Wang等人,2020)。此外,已证明仅小鼠调节性T细胞上的CD36缺陷就足以造成黑色素瘤生长的减少(Wang等人,2020)。最近,通过肿瘤驻留CD8 T细胞的CD36表达已被证明会削弱其抗肿瘤能力,并导致通过导致铁死亡的脂质过氧化所导致的肿瘤CD8 T细胞死亡的增加(Ma等人,2021)。先前的一份报告还描述了肿瘤浸润的CD8 T细胞上的CD36及其对此类CD8 T细胞的不良后果(Xu等人,2020),并且该报告后来以同行评议的格式发表(Xu等人,2021)。

[0011] 还有许多对于通过肿瘤细胞上表达的CD36的配体转运的作用的报道。例如,CD36涉及乳腺癌他莫昔芬耐药性(Liang等人,2018)、黑色素瘤化疗耐药性(Alioa等人,2019)和肺癌HER2靶向治疗耐药性(Feng等人,2019)。早在1993年,在对人髓性白血病系K562的阿霉素耐药亚系的筛选中发现,CD36与阿霉素耐药性有联系,尽管CD36在阿霉素敏感的亲本细胞系中的过表达本身并不会赋予阿霉素耐药性(Sugimoto等人,1993)。CD36还涉及胶质母细胞瘤(Hale等人,2014)和白血病(Ye等人,2016)干细胞自我更新、存活和/或增殖的增加。也就是说,CD36似乎涉及了脂肪组织微环境中通过白血病干细胞的化疗耐药性(Ye等人,2016)。当通过RNA干扰“敲低”干细胞中的CD36时,异种移植小鼠模型中使用患者来源的胶质母细胞瘤干细胞形成的胶质母细胞瘤显著减少,并且通过暴露于氧化低密度脂蛋白,胶质母细胞瘤干细胞的增殖以CD36依赖的方式增加(Hale等人,2014)。CD36还被证明是脑恶性肿瘤的信息性生物标志物,并且与患者预后呈负相关(Hale等人,2014)。

[0012] 最近,CD36被证明对小鼠模型中的前列腺癌进展和肿瘤生长很重要,并且通过RNA干扰或其他使用抗体阻断CD36介导的脂质转运的方式降低前列腺癌细胞中的CD36表达会造成小鼠模型中细胞迁移能力和肿瘤生长的降低(Watt等人,2019)。

[0013] CD36还被提出作为各种癌症的预后转移生物标志物(Enciu等人评议,2018),并且已被证明可促进小鼠模型中口腔鳞状细胞癌(OSCC)细胞、乳腺癌细胞和黑色素瘤细胞的转

移(PCT/EP2016/073208;Pascual等人,2017)。在细胞注射至原位部位(舌头)后观察到OSCC转移,并且通过阻断通过CD36的脂肪酸转运的抗体可减少OSCC转移。将黑色素瘤和乳腺癌细胞注射到小鼠的血液中,以便它们能够潜在定居在转移性微环境,并且当使用通过RNA干扰"敲除"CD36的此类细胞时,这种转移会减少(PCT/EP2016/073208;Pascual等人,2017)。如上所述,CD36还预期经由其在免疫系统髓系细胞中的作用,首先涉及肿瘤细胞从原发性肿瘤部位侵入淋巴和/或血液循环。在肝癌细胞上通过CD36的脂肪酸转运也被证明导致上皮-间质转变的增加,而CD36介导的脂肪酸转运的化学抑制降低了这种表型以及肝癌细胞迁移(Nath等人,2015)。

[0014] 以上所有内容都描绘出一幅图景,其中脂肪酸或其他配体通过CD36转运到细胞中涉及各种细胞类型的代谢适应或分化,但也应强调的是,CD36信号传导会导致更直接的免疫抑制作用。其中一些已在上文提及,但应强调的是,已被提出,经由CD36的凋亡细胞结合通常促进稳态抗炎过程,并且经由CD36的凋亡细胞结合至少部分导致在凋亡细胞结合后巨噬细胞产生IL-10(以及对巨噬细胞的其他免疫抑制作用)(Chung等人,2007)。CD36介导的配体转运也可能导致免疫抑制代谢物产生。也就是说,由于CD36配体诱导的细胞内信号传导导致花生四烯酸的产生的增加,为前列腺素的产生提供了底物(例如Kuda等人,2011),其中一些已知具有广泛的免疫调节作用(例如参见Wang和Dubois,2006;Mizuno等人,2019)。当然,免疫抑制性前列腺素E2在许多癌症中由肿瘤细胞以及免疫系统细胞产生,包括髓系细胞以及其他(Wang和Dubois,2006;Mizuno等人,2019),例如可诱导的调节性T细胞(Whiteside和Jackson,2013)。此外,至少已证明小鼠巨噬细胞以CD36依赖的方式产生前列腺素E2,尽管在癌症模型中并非如此(Almeida等人,2014)。

[0015] 除了CD36在癌症中的许多作用外,CD36配体转运显然在动脉粥样硬化(例如,Zhao等人,2018)、非酒精性脂肪肝疾病(例如,Rada等人,2020)和其他病况中的泡沫细胞形成中起着显著的病理作用。因此,可以阻止通过CD36的凋亡细胞或其他配体结合/转运的抗体有几种潜在的应用。

发明内容

[0016] 本公开提供了以高亲和力特异性地结合人CD36的抗CD36抗体。所述抗体能够降低、抑制和/或完全阻断通过CD36介导的免疫调节作用,包括CD36介导的脂肪酸转运(例如,氧化低密度脂蛋白或"oxLDL"的细胞摄取)。

[0017] 在至少一个实施方案中,本公开提供了一种抗CD36抗体,其包含(i)第一轻链互补决定区(CDR-L1)、第二轻链互补决定区(CDR-L2)和第三轻链互补决定区(CDR-L3),和/或(ii)第一重链互补决定区(CDR-H1)、第二重链互补决定区(CDR-H2)和第三重链互补决定区(CDR-H3),其中:

[0018] (a)CDR-H1包含SEQ ID NO:3、21、24或27的氨基酸序列;

[0019] (b)CDR-H2包含SEQ ID NO:4、28、31、34、37、40或43的氨基酸序列;

[0020] (c)CDR-H3包含SEQ ID NO:5的氨基酸序列;

[0021] (d)CDR-L1包含选自SEQ ID NO:7的氨基酸序列;

[0022] (e)CDR-L2包含选自SEQ ID NO:8、12或15的氨基酸序列;以及

[0023] (f)CDR-L3包含选自SEQ ID NO:9、13或18的氨基酸序列。

[0024] 在本公开的所述抗CD36抗体的至少一个实施方案中:

[0025] (a)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:8的氨基酸序列,以及CDR-L3包含SEQ ID NO:9的氨基酸序列;

[0026] (b)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:12的氨基酸序列,以及CDR-L3包含SEQ ID NO:13的氨基酸序列;

[0027] (c)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:13的氨基酸序列;

[0028] (d)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

[0029] (e)CDR-H1包含SEQ ID NO:21的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

[0030] (f)CDR-H1包含SEQ ID NO:24的氨基酸序列,CDR-H2包含SEQ ID NO:4的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

[0031] (g)CDR-H1包含SEQ ID NO:27的氨基酸序列,CDR-H2包含SEQ ID NO:28的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

[0032] (h)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:31的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

[0033] (i)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:34的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

[0034] (j)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:37的氨基酸序列,CDR-H3包含氨SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含氨SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;

[0035] (k)CDR-H1包含SEQ ID NO:3的氨基酸序列,CDR-H2包含SEQ ID NO:40的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列;或

[0036] (l)CDR-H1包含SEQ ID NO:24的氨基酸序列,CDR-H2包含SEQ ID NO:43的氨基酸序列,CDR-H3包含SEQ ID NO:5的氨基酸序列,CDR-L1包含SEQ ID NO:7的氨基酸序列,CDR-L2包含SEQ ID NO:15的氨基酸序列,以及CDR-L3包含SEQ ID NO:18的氨基酸序列。

[0037] 在至少一个实施方案中,本公开提供了一种抗CD36抗体,其包含:(i)第一重链互补决定区(CDR-H1)、第二重链互补决定区(CDR-H2)和第三重链互补决定区(CDR-H3),其中

所述CDR-H1、CDR-H2和CDR-H3的序列来自VH区域,所述VH区域具有选自SEQ ID NO:2、20、23、26、30、33、36、39和42的氨基酸序列;和(ii)第一轻链互补决定区(CDR-L1)、第二轻链互补决定区(CDR-L2)和第三轻链互补决定区(CDR-L3),其中所述CDR-L1、CDR-L2和CDR-L3的序列来自VL区域,所述VL区域具有选自SEQ ID NO:6、11、14和17的氨基酸序列;其中所述CDR-L1、CDR-L2、CDR-L3、CDR-H1、CDR-H2和CDR-H3按照Kabat编号。

[0038] 在所述抗CD36抗体的至少一个实施方案中:

[0039] (a)VH氨基酸序列为SEQ ID NO:2并且VL氨基酸序列为SEQ ID NO:6;

[0040] (b)VH氨基酸序列为SEQ ID NO:2并且VL氨基酸序列为SEQ ID NO:11;

[0041] (c)VH氨基酸序列为SEQ ID NO:2并且VL氨基酸序列为SEQ ID NO:14;

[0042] (d)VH氨基酸序列为SEQ ID NO:2并且VL氨基酸序列为SEQ ID NO:17;

[0043] (e)VH氨基酸序列为SEQ ID NO:20并且VL氨基酸序列为SEQ ID NO:17;

[0044] (f)VH氨基酸序列为SEQ ID NO:23并且VL氨基酸序列为SEQ ID NO:17;

[0045] (g)VH氨基酸序列为SEQ ID NO:26并且VL氨基酸序列为SEQ ID NO:17;

[0046] (h)VH氨基酸序列为SEQ ID NO:30并且VL氨基酸序列为SEQ ID NO:17;

[0047] (i)VH氨基酸序列为SEQ ID NO:33并且VL氨基酸序列为SEQ ID NO:17;

[0048] (j)VH氨基酸序列为SEQ ID NO:36并且VL氨基酸序列为SEQ ID NO:17;

[0049] (k)VH氨基酸序列为SEQ ID NO:39并且VL氨基酸序列为SEQ ID NO:17;或

[0050] (l)VH氨基酸序列为SEQ ID NO:42并且VL氨基酸序列为SEQ ID NO:17。

[0051] 在本公开的抗CD36抗体的至少一个实施方案中,所述抗体包含与选自SEQ ID NO:2、20、23、26、30、33、36、39或42的序列具有至少90%同一性的重链可变结构域(V_H)氨基酸序列;和/或与选自SEQ ID NO:6、11、14或17的序列具有至少90%同一性的轻链可变结构域(V_L)氨基酸序列。

[0052] 在本公开的所述抗CD36抗体的至少一个实施方案中:

[0053] (a)所述抗体包含与SEQ ID NO:2具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:6具有至少90%同一性的V_L氨基酸序列;

[0054] (b)所述抗体包含与SEQ ID NO:2具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:11具有至少90%同一性的V_L氨基酸序列;

[0055] (c)所述抗体包含与SEQ ID NO:2具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:14具有至少90%同一性的V_L氨基酸序列;

[0056] (d)所述抗体包含与SEQ ID NO:2具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90%同一性的V_L氨基酸序列;

[0057] (e)所述抗体包含与SEQ ID NO:20具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90%同一性的V_L氨基酸序列;

[0058] (f)所述抗体包含与SEQ ID NO:23具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90%同一性的V_L氨基酸序列;

[0059] (g)所述抗体包含与SEQ ID NO:26具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90%同一性的V_L氨基酸序列;

[0060] (h)所述抗体包含与SEQ ID NO:30具有至少90%同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90%同一性的V_L氨基酸序列;

[0061] (i)所述抗体包含与SEQ ID NO:33具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列;

[0062] (j)所述抗体包含与SEQ ID NO:36具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列;

[0063] (k)所述抗体包含与SEQ ID NO:39具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列;或

[0064] (l)所述抗体包含与SEQ ID NO:42具有至少90％同一性的V_H氨基酸序列和与SEQ ID NO:17具有至少90％同一性的V_L氨基酸序列。

[0065] 在本公开的所述抗CD36抗体的至少一个实施方案中,所述抗体包含与选自SEQ ID NO:44、48、50、51、52、53、54、55、56或57的序列具有至少90％同一性的重链(HC)氨基酸序列,和/或与选自SEQ ID NO:45、46、47或49的序列具有至少90％同一性的轻链(LC)氨基酸序列。

[0066] 在本公开的所述抗CD36抗体的至少一个实施方案中,所述抗体包含:

[0067] (a)与SEQ ID NO:44具有至少90％同一性的HC氨基酸序列和与SEQ ID NO:45具有至少90％同一性的LC氨基酸序列;

[0068] (b)与SEQ ID NO:44具有至少90％同一性的HC氨基酸序列和与SEQ ID NO:46具有至少90％同一性的LC氨基酸序列;

[0069] (c)与SEQ ID NO:44具有至少90％同一性的HC氨基酸序列和与SEQ ID NO:47具有至少90％同一性的LC氨基酸序列;

[0070] (d)与SEQ ID NO:44具有至少90％同一性的HC氨基酸序列,以及与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

[0071] (e)与SEQ ID NO:48具有至少90％同一性的HC氨基酸序列,以及与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

[0072] (f)与SEQ ID NO:50具有至少90％同一性的HC氨基酸序列以及与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

[0073] (g)与SEQ ID NO:51具有至少90％同一性的HC氨基酸序列以及与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

[0074] (h)与SEQ ID NO:52具有至少90％同一性的HC氨基酸序列和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

[0075] (i)与SEQ ID NO:53具有至少90％同一性的HC氨基酸序列和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

[0076] (j)与SEQ ID NO:54具有至少90％同一性的HC氨基酸序列和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

[0077] (k)与SEQ ID NO:55具有至少90％同一性的HC氨基酸序列和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;

[0078] (l)与SEQ ID NO:56具有至少90％同一性的HC氨基酸序列和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列;或

[0079] (m)与SEQ ID NO:57具有至少90％同一性的HC氨基酸序列和与SEQ ID NO:49具有至少90％同一性的LC氨基酸序列。

[0080] 在本公开的所述抗CD36抗体的至少一个实施方案中：

[0081] (a)所述抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低的结合亲和力与人CD36结合；任选地，其中所述结合亲和力通过与SEQ ID NO:58或59的hCD36多肽的平衡解离常数(K_D)来测量；

[0082] (b)所述抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低的结合亲和力与小鼠CD36结合；任选地，其中所述结合亲和力通过与SEQ ID NO:60或61的mCD36多肽的平衡解离常数(K_D)来测量；

[0083] (c)所述抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低的结合亲和力与恒河猴CD36结合；任选地，其中所述结合亲和力通过与SEQ ID NO:62或63的恒河猴CD36多肽的平衡解离常数(K_D)来测量；

[0084] (d)所述抗体在过表达表面人CD36的F293细胞中抑制CD36依赖性氧化LDL的摄取至少65％、至少75％、至少85％、至少95％或至少100％；任选地，其中在1-10μg/mL的oxLDL浓度下，所述抗体具有5nM或更低、1nM或更低、0.5nM或更低、或者0.1nM或更低的IC_{50}；

[0085] (e)所述抗体在U937细胞中抑制CD36依赖性氧化LDL的摄取至少65％、至少75％、至少85％、至少95％或至少100％；任选地，其中在1-10μg/mL的oxLDL浓度下，所述抗体具有5nM或更低、1nM或更低、0.5nM或更低、或者0.1nM或更低的IC_{50}；和/或

[0086] (f)所述抗体在小鼠CD45+TIL中抑制CD36依赖性氧化LDL的摄取至少65％、至少75％、至少85％、至少95％或至少100％；任选地，其中在1-10μg/mL的oxLDL浓度下，所述抗体具有5nM或更低、1nM或更低、0.5nM或更低、或者0.1nM或更低的IC_{50}。

[0087] 本公开还提供了本文所公开的所述抗CD36抗体的实施方案，其包括实施方案其中：(i)所述抗体为人抗体、人源化抗体或嵌合抗体；(ii)所述抗体包含与蛋白质的融合物；任选地，与免疫刺激细胞因子(例如IL-2、IL-7、IL-10、IL-12、IL-15、IL-21或IFN-α)的融合物；(iii)所述抗体为IgG类的全长抗体，任选地，其中IgG类抗体具有选自IgG1、IgG2、IgG3和IgG4的同种型；(iv)所述抗体包含Fc区变体，任选地，改变效应子功能的Fc区变体和/或改变抗体半衰期的变体；(v)所述抗体为抗体片段，任选地，选自由$F(ab')_2$、Fab'、Fab、Fv、单域抗体(VHH)和scFv组成的组；(vi)所述抗体包含免疫缀合物，任选地，其中所述免疫缀合物包含用于治疗CD36介导的疾病或病况的治疗剂；或(vii)所述抗体为多特异性抗体；任选地，为双特异性抗体。

[0088] 在至少一个实施方案中，本公开提供了分离的多核苷酸或包含多核苷酸的载体，其中所述多核苷酸的序列编码本公开的抗CD36抗体或本公开的抗CD36抗体的多肽链。在至少一个实施方案中，所述分离的多核苷酸或载体包含编码本公开的抗CD36抗体的多肽的序列。在所述分离的多核苷酸或载体的至少一个实施方案中，编码的多肽链具有包含以下的氨基酸序列：

[0089] (a)CDR-H1包含SEQ ID NO:3、21、24或27的氨基酸序列；CDR-H2包含SEQ ID NO:4、28、31、34、37、40或43的氨基酸序列；以及CDR-H3包含SEQ ID NO:5的氨基酸序列；

[0090] (b)CDR-L1包含选自SEQ ID NO:7的氨基酸序列；CDR-L2包含选自SEQ ID NO:8、12或15的氨基酸序列；以及CDR-L3包含选自SEQ ID NO:9、13或18的氨基酸序列；

[0091] (c)与选自SEQ ID NO:2、20、23、26、30、33、36、39或42的序列具有至少90％同一性的重链可变结构域(V_H)氨基酸序列；

[0092] (d)与选自SEQ ID NO:6、11、14或17的序列具有至少90％同一性的轻链可变结构域(V_L)氨基酸序列;

[0093] (e)与选自SEQ ID NO:44、48、50、51、52、53、54、55、56或57的序列具有至少90％同一性的重链(HC)氨基酸序列;和/或

[0094] (f)与选自SEQ ID NO:45、46、47或49的序列具有至少90％同一性的轻链(LC)氨基酸序列。

[0095] 在至少一个实施方案中,本公开还提供了一种分离的宿主细胞,其包含编码本公开的抗CD36抗体或抗CD36抗体的多肽链的多核苷酸或载体。在至少一个实施方案中,本公开还提供了一种产生本公开的抗CD36抗体的方法,其包括培养包含编码抗CD36抗体的多核苷酸或载体的宿主细胞,从而产生抗体。

[0096] 在至少一个实施方案中,本公开提供了一种药物组合物,其包含本公开的抗CD36抗体和药学上可接受的载体;任选地,其中所述组合物还包含化疗剂,和/或对免疫检查点分子具有特异性的抗体。

[0097] 在至少一个实施方案中,本公开提供了一种治疗受试者的CD36介导的疾病的方法,所述方法包括向所述受试者施用治疗有效量的本公开的抗CD36抗体,或向所述受试者施用治疗有效量的本公开的药物组合物;任选地,其中所述疾病为癌症;任选地,其中所述癌症选自结肠癌、胰腺癌、卵巢癌、HCC、肾癌、乳腺癌、肺癌、胃癌、黑色素瘤、头颈癌或口腔癌。

[0098] 在至少一个实施方案中,本公开提供了一种用于治疗受试者的癌症的方法,其包括向所述受试者施用CD36拮抗剂和化疗剂,和/或对免疫检查点分子包含特异性的抗体;任选地,其中所述CD36拮抗剂包含抗CD36抗体、shRNA、siRNA、miRNA、CD36的小分子抑制剂或其组合。

附图说明

[0099] 通过参考以下详细说明会获得对本公开的新颖的特征和优点的更好的理解,其中详细说明阐述了利用本公开的原理的说明性的实施方案,以及附图(本文中也称为“图”),其中:

[0100] 图1A、图1B、图1C和图1D描绘了本公开的示例性抗CD36抗体(12P109、A8A)以全长人IgG1形式与重组人CD36.ECD(图1A、图1C)或小鼠CD36.ECD(图1B、图1D)结合的ELISA结果。ELISA如实施例1所述进行。

[0101] 图2A、图2B、图2C、图2D、图2E和图2F描绘了本公开的示例性抗CD36抗体(12P109和117变体)与重组人CD36.ECD结合的ELISA结果。ELISA如实施例1所述进行。

[0102] 图3A、图3B、图3C、图3D和图3E描绘了显示来自如实施例1所述的抗CD36抗体(12P109、A8A和117变体)的SEC-UPLC分析的结果的图。

[0103] 图4A、图4B、图4C、图4D、图4E和图4F描绘了显示来自通过流式细胞术对本公开的示例性抗CD36抗体(117变体)与过表达人CD36(“hCD36”)(图4A和图4B)、恒河猴CD36(图4C和图4D)或小鼠CD36(“mCD36”)(图4E和图4F)的稳定F293细胞进行细胞表面结合分析的结果的图,如实施例2所述。

[0104] 图5A和图5B描绘了提取的流式细胞术数据的图,其显示了本公开的抗CD36抗体在

阻断U937细胞的oxLDL结合(图5A)和oxLDL摄取(图5B)中的活性,如实施例3所述。

[0105] 图6A、图6B和图6C描绘了从流式细胞术数据中提取的图,阐明了抗CD36抗体(12P109和117变体)在F293/hCD36或F293/mCD36细胞中表现出的oxLDL摄取阻断活性,如实施例3所述。

[0106] 图7A、图7B、图7C、图7D和图7E描绘了显示本公开的抗CD36抗体(12P109、A8A和117变体)在结肠癌(CT26、MC38)、肝癌(BNL 1MEA.7R.1)、肺癌(LL2)和黑色素瘤(B16F10)同源小鼠模型的TIL中抑制oxLDL摄取能力的数据的图,如实施例4所述。

[0107] 图8A、图8B、图8C、图8D、图8E和图8F描绘了阐明本公开的抗CD36抗体(12P109和117变体)抑制M2巨噬细胞极化(图8A和图8B)和oxLDL诱导的M2巨噬细胞活化(图8C、图8D、图8E和图8F)的能力的数据的图,如实施例5所述。

[0108] 图9描绘了在用如实施例6所述的抗CD36抗体117_30DA治疗后通过在$MYC^{OE}/p53^{KO}$ HCC模型小鼠中生物发光测量的肿瘤生长曲线结果。

[0109] 图10A、图10B和图10C描绘了在用如实施例6所述的抗CD36抗体117_DA57E治疗后在β-连环蛋白$^{OE}/MYC^{OE}$ HCC模型小鼠中测量的结果。图10A显示通过生物发光测量的肿瘤生长曲线结果。图10B显示终点肝重量结果图。图10C显示血浆ALT(丙氨酸转氨酶)活性分析结果图。

具体实施方式

[0110] 本公开提供了抗体,包括人源化抗体,其以高亲和力特异性地结合CD36,从而抑制、降低和/或完全阻断作为涉及免疫调节的细胞表面蛋白的CD36的功能,特别是在CD36的各种作用中CD36介导的脂肪酸和/或氧化脂质转运(例如,oxLDL摄取)的功能,所述CD36的各种作用例如在背景技术部分中详述的那些,其包括但不限于CD36在调节肿瘤细胞、肿瘤相关巨噬细胞(TAM)、MDSC、调节性T细胞和CD8 T细胞的功能以及维持或存活(或死亡,视情况而定)中的作用。

[0111] 因此,考虑包含本公开的抗CD36抗体的任何组合物或制剂可用作用于治疗由CD36在脂肪酸转运中的功能介导的疾病(例如癌症)的治疗剂。此外,考虑本公开的抗CD36抗体可用作与其他治疗剂组合的治疗剂,例如细胞疗法、细胞因子、和改变肿瘤微环境和/或增加免疫反应的其他生物制剂或药物、抗体-药物偶联物、调控免疫细胞的抗体和/或靶向免疫检查点分子的抗体,其包括但不限于PD1、PD-L1、LAG3、CTLA-4、A2AR、TIM-3、BTLA、CD276、CD328、VTCN1、IDO、KIR、NOX2、VISTA、OX40、CD27、CD28、CD40、CD122、CD137、GITR、ICOS。

[0112] 术语和技术概述

[0113] 对于本文的描述和所附权利要求,除非上下文另有明确说明,否则单数形式“一种”包括复数指代物。因此,例如,对“一种蛋白质”的指代包括超过一个蛋白质,并且对“一种化合物”的指代指超过一个化合物。进一步注意的是,可撰写权利要求书以排除任何可选的元素。因此,该声明旨在作为用于在记载权利要求元素时使用如“仅仅”、“只有”等此类排他性术语或使用“负面”限定的在先依据。“包含”、“包括”的使用是可互换的,并不旨在限制。还应理解,当各种实施方案的描述使用术语“包含”时,本领域技术人员应理解,在某些特定情况下,实施方案也可以使用“基本上由……组成”或“由……组成”的语言来替代性地描述。

[0114] 当提供数值范围时,除非上下文另有明确规定,否则应理解,该范围的上限和下限之间的数值的每个中间整数,和每个中间整数的十分之一,除非上下文另有明确规定,以及该陈述的范围内的任何其他陈述的或中间数值,均涵盖在本发明内。这些较小范围的上限和下限可独立地被包括在较小范围内,并且也涵盖在本发明内,但所述范围内的任何具体排除的限制除外。当所述范围包括限制的一者或两者时,排除那些被包括的限制中的(i)其中一个或(ii)两个的范围也被包括在本发明内。例如,"1至50"包括"2至25"、"5至20"、"25至50"、"1至10"等。

[0115] 通常,本文所使用的命名以及本文所述的技术和程序包括本领域的普通技术人员所充分理解和普遍运用的那些,例如下述的普遍的技术和方法学:Sambrook等人,分子克隆-实验室手册(第2版),第1-3卷,冷泉港实验室,纽约冷泉港,1989(以下简称"Sambrook");分子生物学现有实验方案,F.M.Ausubel等人编辑,Current Protocols,格林出版协会有限公司(Greene Publishing Associates,Inc.)和约翰威利父子公司(John Wiley&Sons,Inc.)之间的合资企业(2011年增刊)(以下简称"Ausubel");抗体工程,第1和第2卷,R.Kontermann和S.Dubel编辑,施普林格出版社(Springer-Verlag),柏林和海德堡(2010);单克隆抗体:方法和方案,V.Ossipow和N.Fischer编辑,第2版,人类出版社(Humana Press)(2014);治疗性抗体:从实验室到临床,Z.An编辑,约翰威利父子公司(J.Wiley&Sons),新泽西州霍博肯(2009);以及噬菌体展示,Tim Clackson和Henry B.Lowman编辑,牛津大学出版社,英国(2004)。

[0116] 本公开中引用的所有出版物、专利、专利申请和其他文件在此以引用的方式全文并入本文用于所有目的,其程度如同每个单独的出版物、专利、专利申请或其他文件被单独地指明为以引用的方式被并入本文用于所有目的。

[0117] 除非另有定义,否则本文使用的所有技术和科学术语均具有与本发明所属领域的普通技术人员普遍理解的相同的含义。应理解,本文使用的术语仅用于描述特定实施方案的目的,而非旨在限制。为了解释本公开的目的,以下术语描述会应用,并且在适当的时候,以单数形式使用的术语也将包括复数形式,反之亦然。

[0118] 如本文使用,"CD36"是指CD36蛋白,并且如本文所使用,其涵盖人、食蟹猴、小鼠的CD36蛋白以及这些蛋白的任何同种型。各种示例性CD36蛋白的氨基酸序列是本领域已知的,并在下表1和后附序列表中提供。

[0119] 本文使用的"CD36介导的病况"或"CD36介导的疾病"涵盖与配体与细胞表面蛋白CD36的特异性结合相关的任何医学病况。例如,CD36与脂质和/或脂肪酸的特异性结合起到调节或增加肿瘤微环境中TAM和调节性T细胞的免疫抑制能力的作用。因此,CD36介导的疾病可以包括但不限于由CD36的拮抗剂或抑制剂介导的和/或对CD36的拮抗剂或抑制剂有反应的任何疾病或病况,其包括但不限于癌症。

[0120] 本文使用的"抗体"是指包含一个或多个多肽链的分子,其特异性地结合特定抗原或与特定抗原具有免疫反应性。本公开的示例性抗体包括单克隆抗体、多克隆抗体、嵌合抗体、人源化抗体、人抗体、抗体融合物(例如融合蛋白)、多特异性抗体(例如双特异性抗体)、单价抗体(例如单臂抗体)、多价抗体、抗原结合片段(例如Fab'、$F(ab')_2$、Fab、Fv、rIgG和scFv片段)和合成抗体(或抗体模拟物)。

[0121] "抗CD36抗体"或"结合CD36的抗体"是指以足够的亲和力结合CD36的抗体,使得所

述抗体可用作用于靶向CD36的治疗剂和/或诊断剂。在一些实施方案中,抗CD36特异性抗体与不相关的非CD36抗原的结合程度小于所述抗体与CD36结合的约20%、约15%、约10%或约5%,如通过例如放射免疫测定法(RIA)或表面等离子体共振(SPR)测量的。在一些实施方案中,本公开的抗CD36抗体具有<1μM、<100nM、<10nM、<1nM、<0.1nM、<0.01nM或<1pM(例如,10^{-8}M或更小,例如,从10^{-8}M至10^{-13}M,例如,从10^{-9}M至10^{-13}M)的解离常数(K_D)。下表2和后附序列表中提供了本公开的示例性CD36蛋白的氨基酸序列。

[0122] "全长抗体"、"完整抗体"或"整个抗体"在本文中可互换使用,指具有与天然抗体结构基本相似的结构或具有含有如本文所定义的Fc区的重链的抗体。

[0123] "抗体融合物"是指与多肽或蛋白质共价缀合(或融合)的抗体,通常经由接头与抗体轻链(LC)或重链(HC)的末端结合。本公开考虑的示例性抗体融合物可以包括经由接头与蛋白质融合的抗CD36抗体,所述蛋白质是T细胞活化或免疫刺激细胞因子,例如IL-2、IL-7、IL-10、IL-12、IL-15、IL-21或IFN-α。

[0124] "抗体片段"是指全长抗体的一部分,其能够与全长抗体结合相同的抗原。抗体片段的示例包括但不限于Fv、Fab、Fab'、Fab'-SH、F(ab')$_2$;双抗体;线性抗体;单价或单臂抗体;单链抗体分子(例如scFv);以及由抗体片段形成的多特异性抗体。

[0125] 抗体的"类别"是指其重链所拥有的恒定结构域或恒定区的类型。抗体有五种主要的类别:IgA、IgD、IgE、IgG和IgM,并且这些之中的几种进一步分为亚类(同种型),例如IgG1、IgG2、IgG3、IgG4、IgA1和IgA2。对应于不同类的免疫球蛋白的重链恒定结构域被分别称为α、δ、ε、γ和μ。

[0126] "可变区"或"可变结构域"是指涉及抗体与抗原结合的抗体重链或轻链的结构域。天然抗体的重链和轻链的可变结构域(分别为V_H和V_L)通常具有相似的结构,每个结构域包含四个保守的框架区(FR)和三个高变区(HVR)(参见,例如,Kindt等人,Kuby Immunology,第6版,W.H.Freeman和同事,第91页)。单个V_H或V_L结构域可能足以赋予抗原结合特异性。此外,使用来自结合抗原的抗体的V_H或V_L结构域可分离结合特定抗原的抗体,以分别筛选互补V_L或V_H结构域的文库(参见,例如,Portolano等人,J.Immunol.150:880-887(1993);Clarkson等人,Nature 352:624-628(1991))。

[0127] 本文使用的"高变区"或"HVR"是指抗体可变结构域的每一个区域,其在序列中是高变和/或形成结构上定义的环("高变环")。通常,天然抗体包含具有六个HVR的四条链;三个在重链可变结构域中,V_H(HVR-H1、HVR-H2、HVR-H3),以及三个在轻链可变结构域中,V_L(HVR-L1、HVR-L2、HVR-L3)。所述HVR通常包含来自高变环和/或来自"互补决定区"(CDR)的氨基酸残基。大量高变区描述正在使用中,并涵盖在本文中。Kabat互补决定区(CDR)基于序列变异性并且是最常用的(Kabat等人,具有免疫学意义的蛋白质序列,第5版,公共卫生服务部,国立卫生研究院,马里兰州贝塞斯达(1991))。而Chothia指的是结构环的位置(Chothia和Lesk,J.Mol.Biol.196:901-917(1987))。AbM高变区代表Kabat CDR和Chothia结构环之间的折中,并通过牛津分子的AbM抗体建模软件使用。"Contact"高变区基于对可用复杂晶体结构的分析。在这些系统下定义的高变区的残基范围在下表中示出。

[0128]

环	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B[1] H31-H35[2]	H26-H35B[1] H26-H35[2]	H26-H32[1] H26-H32[2]	H30-H35B[1] H30-H35[2]
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0129]

环	Kabat	AbM	Chothia	Contact
[1] Kabat 编号 [2] Chothia 编号				

[0130] 除了上述系统之外,可以使用国际ImMunoGeneTics信息系统(称为IMGT/V-Quest)来识别HVR和CDR,该系统在Brochet,X.等人,Nucl.Acids Res.36,W503-508(2008)中被描述。PMID:18503082;并且可在www.imgt.org/IMGT_vquest/input线上使用。IMGT/V-Quest使用IMGT唯一编号分析与最接近的种系V基因可变区核苷酸序列的比对以识别HVR和CDR。

[0131] 如本文使用,高变区(HVR)可包括如下的扩展或替代的高变区域:27-32、27-36、24-34或24-38(HVR-L1);50-52、54-56、50-56或54-60(HVR-L2);89-97或93-101(HVR-L3);26-33、26-35或31-35(HVR-H1);51-58、50-61或50-66(H2);以及97-110、97-112、99-110或99-112(H3)在V_H结构域中。对于这些定义中的每一个,可变结构域残基均按照Kabat等人(上文)的方式进行编号。

[0132] 如本文使用,“互补决定区”或“CDR”是指可变结构域的HVR内具有最高序列变异性和/或涉及抗原识别的区域。通常,天然抗体包含具有六个HVR的四条链;三个在重链可变结构域中,V_H(CDR-H1、CDR-H2、CDR-H3),并且三个在轻链可变结构域中,V_L(CDR-L1、CDR-L2、CDR-L3)。示例性的CDR出现在可变结构域氨基酸残基位置:24-34、27-32、27-36、24-38(CDR-L1);50-56、50-52、54-56或54-60(CDR-L2);89-97或93-101(CDR-L3);31-35或26-33(CDR-H1),50-66或51-58(CDR-H2);以及99-112,99-110,97-112或97-110(CDR-H3)。

[0133] “框架”或“FR”是指除HVR残基之外的可变结构域残基。可变结构域的FR通常由四个结构域组成:FR1、FR2、FR3和FR4。因此,HVR和FR序列通常以以下顺序出现在V_H(或V_L)中:FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4。

[0134] 除非另有说明,本文中HVR、CDR、FR中的残基和可变结构域中的其他残基的位置在本文中按照Kabat等人(上文)进行编号。

[0135] “天然抗体”是指天然存在的免疫球蛋白分子。例如,天然IgG抗体是约150,000道尔顿的异四聚体糖蛋白,由两个相同的轻链和两个相同的重链组成,它们是以二硫键连接的。从N端到C端,每条重链具有可变区(V_H),也称为可变重结构域或重链可变结构域,随后

是三个恒定结构域(CH1、CH2和CH3)。类似地,从N端到C端,每条轻链具有可变区(V_L),也称为可变轻结构域或轻链可变结构域,随后是恒定轻(CL)结构域。基于其恒定结构域的氨基酸序列,抗体的轻链可被分配为两种类型中的一种,即kappa(κ)和lambda(λ)。

[0136] 如本文使用,"单克隆抗体"是指从基本上同质的抗体群中获得的抗体,即,包含所述群的单独的抗体是相同的和/或结合相同的表位,除了可能的变体抗体(例如,变体抗体含有自然发生或在单克隆抗体产生期间出现的突变,并且通常以少量存在)。与通常包含针对不同决定簇(表位)的不同抗体的多克隆抗体制备物相比,单克隆抗体制备的每个单克隆抗体针对抗原上的单个决定簇。因此,术语"单克隆"表示抗体的特性是从基本上同质的抗体群中获得的,并且不应解释为需要通过任何特定方法产生抗体。例如,所用的单克隆抗体可通过多种技术来制得,包括但不限于杂交瘤方法、重组DNA方法、噬菌体展示方法和利用含有全部或部分人免疫球蛋白基因座的转基因动物的方法、本文描述的制备单克隆抗体的此类方法和其他示例性方法。

[0137] "嵌合抗体"是指抗体中重链和/或轻链的一部分源自特定来源或种类,而重链和/或轻链的其余部分源自不同来源或种类的抗体。

[0138] "人源化抗体"是指包含来自非人CDR的氨基酸序列和来自人FR的氨基酸序列的嵌合抗体。在某些实施方案中,人源化抗体会包含至少一个(且通常为两个)可变结构域的基本上全部,其中所有或基本上所有的CDR均对应于非人抗体的那些CDR,并且所有或基本上所有的FR均对应于人抗体的那些FR。人源化抗体任选地可包含源自人抗体的抗体恒定区的至少一部分。抗体的"人源化形式",例如非人抗体,是指已经历人源化的抗体。

[0139] "人抗体"是指拥有与由人或人细胞产生的抗体或源自利用人抗体库或其他人抗体编码序列的非人来源的抗体的氨基酸序列相对应的氨基酸序列的抗体。人抗体的这一定义具体地排除了包含非人抗原结合残基的人源化抗体。

[0140] "人共识框架"是代表在人免疫球蛋白V_L或V_H框架序列的选择中最常出现的氨基酸残基的框架。通常,人免疫球蛋白V_L或V_H序列的选择来自可变结构域序列的亚组。通常,序列的亚组是Kabat等人,Sequences of Proteins of Immunological Interest,Fifth Edition,NIH Publication 91-3242,Bethesda MD(1991),vols.1-3中的亚组。在一个实施方案中,对于V_L,所述亚组是如Kabat等人(见上文)中的亚组kappa I。在一个实施方案中,对于V_H,所述亚组是如Kabat等人(见上文)中的亚组III。

[0141] 本文使用的"受体人框架"是包含源自人免疫球蛋白框架或人共识框架的轻链可变结构域(V_L)框架或重链可变结构域(V_H)框架的氨基酸序列的框架。"源自"人免疫球蛋白框架或人共识框架的受体人框架可包含与其相同的氨基酸序列,或者它可包含氨基酸序列变化。在一些实施方案中,氨基酸变化的数量为10个或更少、9个或更少、8个或更少、7个或更少、6个或更少、5个或更少、4个或更少、3个或更少、或2个或更少。在一些实施方案中,V_L受体人框架在序列上与V_L人免疫球蛋白框架序列或人共识框架序列相同。

[0142] "Fc区"是指包含免疫球蛋白重链的C-末端多肽序列的二聚体复合物,其中C-末端多肽序列是通过木瓜蛋白酶消化完整抗体才可获得的序列。Fc区可包含天然或变体Fc序列。尽管免疫球蛋白重链的Fc序列的边界可能有变化,但人IgG重链Fc序列通常被定义为从在约Cys226位置或从约Pro230位置的氨基酸残基延伸到Fc序列的羧基末端。然而,Fc序列的C-末端赖氨酸(Lys447)可能存在或可能不存在。免疫球蛋白的Fc序列通常包含两个恒定

结构域,CH2结构域和CH3结构域,并且任选地包含CH4结构域。

[0143] “Fc受体”或“FcR”是指与抗体的Fc区结合的受体。在一些实施方案中,FcR是天然人FcR。在一些实施方案中,FcR是结合IgG抗体的受体(γ受体),并且包括FcγRI、FcγRII和FcγRIII亚类的受体,其包括这些受体的等位基因变体和替代剪接形式。FcγRII受体包括FcγRIIA(“活化受体”)和FcγRIIB(“抑制受体”),其具有相似的氨基酸序列,区别主要在于其胞质域。活化受体FcγRIIA在其胞质域中含有基于免疫受体酪氨酸的活化基序(ITAM)。抑制受体FcγRIIB在其胞质域中含有基于免疫受体酪氨酸的抑制基序(ITIM)(参见例如Daeron,Annu.Rev.Immunol.15:203-234(1997))。如本文所用,FcR还包括新生儿受体FcRn,其负责将母体IgG转移到胎儿(Guyer等人,J.Immunol.117:587(1976)和Kim等人,J.Immunol.24:249(1994))和免疫球蛋白内稳态的调节。FcR在例如Ravetch和Kinet,Annu.Rev.Immunol.9:457-92(1991);Capel等人,Immunomethods 4:25-34(1994);以及de Haas等人,J.Lab.Clin.Med.126:330-41(1995)中被综述。

[0144] “多特异性抗体”是具有至少两个不同结合位点的抗体,每个位点具有不同的结合特异性。多特异性抗体可以是全长抗体或抗体片段,并且不同的结合位点可使每一个结合位点与不同的抗原结合,或不同的结合位点可与相同抗原的两个不同表位结合。

[0145] “Fv片段”是指含有完整抗原识别和结合位点的抗体片段。该区域由紧密结合的一个重链可变结构域和一个轻链可变结构域的二聚体组成,其性质可以是共价的,例如在scFv中。在这种构型中,每个可变结构域的三个CDR相互作用以在V_H-V_L二聚体的表面上定义抗原结合位点。总的来说,六个CDR或其子集赋予抗体抗原结合特异性。然而,即使是单个可变结构域(或仅包含三个特异性针对抗原的CDR的Fv的一半)也具有识别和结合抗原的能力,尽管通常以低于整个结合位点的亲和力结合。

[0146] “Fab片段”是指含有轻链的可变和恒定结构域以及重链的可变结构域和第一恒定结构域(CH1)的抗体片段。“$F(ab')_2$片段”包含一对Fab片段,其通常在它们的羧基末端附近通过它们之间的铰链半胱氨酸共价连接。抗体片段的其他化学偶联也是本领域已知的。

[0147] 如本文所用,“抗原结合臂”是指具有特异性地结合目标靶分子能力的抗体组分。通常,所述抗原结合臂是免疫球蛋白多肽序列的复合物,例如免疫球蛋白轻链和重链的CDR和/或可变结构域序列。

[0148] “单链Fv”或“scFv”是指包含抗体的V_H和V_L结构域的抗体片段,其中这些结构域存在于单个多肽链中。通常,Fv多肽还包含V_H和V_L结构域之间的多肽接头,其使得scFv能够形成所需的抗原结合结构。

[0149] “亲和力”是指分子(例如抗体)的单个结合位点与其结合伴侣(例如抗原)之间的非共价相互作用总和的强度。“结合亲和力”是指内在结合亲和力,其反映结合对(例如抗体和抗原)的成员之间的1:1相互作用。分子X对其伴侣Y的亲和力通常可以通过平衡解离常数(K_D)来表示。亲和力可以通过本领域已知的普遍方法(包括本文中描述的方法)来测量。下文描述了用于测量结合亲和力的具体说明性和示例性实施方案。

[0150] “特异性地结合”或“特异性结合”是指抗体与抗原以不超过约1×10^{-7}M的亲和力值结合。在一些实施方案中,抗体可能对其特异性地结合的抗原以外的抗原具有次级亲和力,其中“次级亲和力”通常会指抗体与次级抗原以超过约10nM的亲和力值结合,如本文中其他地方所述。当抗体可能对次级抗原具有次级亲和力时,此类抗体仍会特异性地结合到初级

抗原上。

[0151] “分离抗体”是指已从其天然环境的组分中分离出来的抗体。在一些实施方案中，抗体被纯化到大于95％或99％的纯度，如通过例如电泳(例如，SDS-PAGE、等电聚焦(IEF)、毛细管电泳)或色谱法(例如，离子交换或反相HPLC)来测定。对于用于评估抗体纯度的方法的综述，参见例如Flatman等人，J.Chromatogr.B 848:79-87。

[0152] “效应子功能”是指归因于抗体Fc区的生物活性，其随抗体同种型而变化。抗体效应子功能的示例包括:Clq结合和补体依赖性细胞毒性(CDC);Fc受体结合;抗体依赖性细胞介导的细胞毒性(ADCC);吞噬作用;细胞表面受体(例如B细胞受体)的下调;以及B细胞活化。

[0153] “免疫缀合物”是指与一种或多种异源分子(包括但不限于细胞毒性剂)缀合的抗体。

[0154] “治疗(treatment)”、“治疗(treat)”或“治疗(treating)”是指试图改变待治疗的个体的病症自然进程的临床干预，并且可以用于预防或在临床病理进程期间被施行。治疗的所需结果可以包括但不限于预防病症的发生或复发、缓解症状、减轻病症的任何直接或间接病理后果、预防转移、降低进展速度、改善或减轻疾病状态以及缓解或改进的预后。例如，治疗可以包括向受试者施用治疗有效量的包含抗CD36抗体的药物制剂，以延迟由CD36和/或其与配体的结合介导的疾病或病症或CD36在发病机制和/或进展中可能发挥作用的疾病或病症的发展或减缓其进展。

[0155] “药物制剂”是指一种以允许活性成分的生物活性有效的形式的制备物，并且其不含有对被施用所述制剂的受试者有毒的附加组分。药物制剂可包括一种或多种活性剂。例如，药物制剂可包括抗CD36抗体作为所述制剂的唯一活性剂，或可包括抗CD36抗体和一种或多种附加活性剂，例如免疫检查点分子的抑制剂。

[0156] 如本文所用，“唯一活性剂”是指药物制剂中的活性剂，该活性剂是该制剂中仅存的活性剂，其提供或会被预期提供相关药理作用以治疗受试者待治疗的病况。包含唯一活性剂的药物制剂不排除所述制剂中存在一种或多种非活性剂，例如药学上可接受的载体。“非活性剂”是不会被预期提供或以其他方式显著促进预计治疗所述受试者的所述病况的相关药理作用的药剂。

[0157] “药学上可接受的载体”是指药物制剂中除活性成分以外的成分，其对被施用的受试者是无毒的。药学上可接受的载体包括但不限于缓冲剂、赋形剂、稳定剂或防腐剂。

[0158] 如本文所用，“免疫检查点分子”是指具有调节免疫系统通路并进而阻止其不必要地攻击细胞的分子。许多免疫检查点分子(包括抑制性和共刺激性两者)在癌症和病毒感染的治疗中均为免疫疗法的靶点(例如，使用阻断抗体来阻断免疫抑制或使用激动剂来促进免疫刺激)。用于癌症免疫疗法的示例性免疫检查点分子包括但不限于PD1、PD-L1、LAG3、CTLA-4、A2AR、TIM-3、BTLA、CD276、CD328、VTCN1、IDO、KIR、NOX2、VISTA、OX40、CD27、CD28、CD40、CD122、CD137、GITR、ICOS。

[0159] “治疗有效量”是指达到所需的治疗或预防结果的活性成分或药剂(例如药物制剂)的量，例如治疗或预防受试者的疾病、病症或病况。在CD36介导的疾病或病况的情况下，治疗剂的治疗有效量是在一定程度上降低、预防、抑制和/或缓解与疾病、病症或病况相关的症状中的一种或多种的量。对于癌症的疗法，体内疗效可以例如通过评估原发性肿瘤的

生长、继发性肿瘤的发生和/或生长、转移的发生和/或数量、症状的持续时间、严重程度和/或复发、反应率(RR)、反应持续时间和/或生活质量来测量。

[0160] 如本文所使用的“同时”是指施用两种或多种治疗剂,其中所述施用的至少一部分在时间上重叠。因此,同时施用包括在中止一种或多种其他药剂的施用后当一种或多种药剂的施用继续时的给药方案。

[0161] “个体”或“受试者”是指哺乳动物,其包括但不限于家养动物(例如,牛、羊、猫、狗和马)、灵长类动物(例如,人和非人灵长类动物,如猴子)、兔子和啮齿动物(例如,小鼠和大鼠)。

[0162] 各种实施方案的详细描述

[0163] I.CD36

[0164] CD36是一种多功能跨膜糖蛋白,其作为广泛范围的配体的细胞表面受体。通常,CD36具有两个不同的结合结构域以用于血小板反应蛋白与其他脂质性质配体的结合,例如氧化低密度脂蛋白(oxLDL)、阴离子磷脂、长链脂肪酸和细菌二酰化脂肽。据信,由CD36与这些配体的结合介导的细胞反应包括脂肪酸代谢、饮食脂肪加工、血管生成和炎症反应。CD36作为TLR4:TLR6异二聚体的辅助受体,从而促进单核细胞/巨噬细胞中的炎症。据信,在结合配体(例如氧化LDL(“oxLDL”))后,CD36会与TLR4:TLR6异二聚体相互作用,并且复合物被内化,从而引发炎症反应,导致NF-kappa-B依赖性的经由MyD88信号传导通路的CXCL1、CXCL2和CCL9细胞因子的产生,和经由TICAM1信号传导通路的CCL5细胞因子的产生,以及通过NLRP3炎症小体的启动和活化的IL1B的分泌。其他与CD36相互作用的辅助受体也已被描述。

[0165] 人CD36(本文中也称为“hCD36”)的序列和注释可在UniProt entry P16671处找到,并且同种型1的全长472个氨基酸的序列在本文中如SEQ ID NO:58所示。小鼠CD36(本文中也称为“mCD36”)的序列和注释可在UniProt entry Q08857处找到,并且全长472个氨基酸的序列在本文中如SEQ ID NO:60所示。下表1提供了本公开中使用的人和小鼠CD36多肽的序列的概述及其序列标识符。这些序列也被包括在随附的序列表中。

[0166] 表1:人和小鼠CD36多肽

[0167]

描述	序列	SEQ ID NO:
hCD36 (同种型1) UniProt P16671	MGCDRNCGLIAGAVIGAVLAVFGGILMPVGDLLIQKTIKKQVVLEEGTIAF KNWVKTGTEVYRQFWIFDVQNPQEVMMNSSNIQVKQRGPYTYRVRFLAKEN VTQDAEDNTVSFLQPNGAIFEPSLSVGTEADNFTVLNLAVAAASHIYQNQF VQMILNSLINKSKSSMFQVRTLRELLWGYRDPFLSLVPYPVTTTVGLFYPY NNTADGVYKVFNGKDNISKVAIIDTYKGKRNLSYWESHCDMINGTDAASFP PFVEKSQVLQFFSSDICRSIYAVFESDVNLKGIPVYRFVLPSKAFASPVEN	58

[0168]

	PDNYCFCTEKIISKNCTSYGVLDISKCKEGRPVYISLPHFLYASPDVSEPI DGLNPNEEEHRTYLDIEPITGFTLQFAKRLQVNLLVKPSEKIQVLKNLKRN YIVPILWLNETGTIGDEKANMFRSQVTGKINLLGLIEMILLSVGVVMFVAF MISYCACRSKTIK	
hCD36.ECD	GDLLIQKTIKKQVVLEEGTIAFKNWVKTGTEVYRQFWIFDVQNPQEVMMNS SNIQVKQRGPYTYRVRFLAKENVTQDAEDNTVSFLQPNGAIFEPSLSVGTE ADNFTVLNLAVAAASHIYQNQFVQMILNSLINKSKSSMFQVRTLRELLWGY RDPFLSLVPYPVTTTVGLFYPYNNTADGVYKVFNGKDNISKVAIIDTYKGK RNLSYWESHCDMINGTDAASFPPFVEKSQVLQFFSSDICRSIYAVFESDVN LKGIPVYRFVLPSKAFASPVENPDNYCFCTEKIISKNCTSYGVLDISKCKE GRPVYISLPHFLYASPDVSEPIDGLNPNEEEHRTYLDIEPITGFTLQFAKR LQVNLLVKPSEKIQVLKNLKRNYIVPILWLNETGTIGDEKANMFRSQVTGK IN	59
mCD36 UniProt Q08857	MGCDRNCGLIAGAVIGAVLAVFGGILMPVGDMLIEKTIKREVVLEEGTTAF KNWVKTGTTVYRQFWIFDVQNPDDVAKNSSKIKVKQRGPYTYRVRYLAKEN ITQDPEDHTVSFVQPNGAIFEPSLSVGTEDDNFTVLNLAVAAAPHIYQNSF VQVVLNSLIKKSKSSMFQTRSLKELLWGYKDPFLSLVPYPISTTVGVFYPY NDTVDGVYKVFNGKDNISKVAIIESYKGKRNLSYWPSYCDMINGTDAASFP PFVEKSRTLRFFSSDICRSIYAVFGSEIDLKGIPVYRFVLPANAFASPLQN PDNHCFCTEKVISNNCTSYGVLDIGKCKEGKPVYISLPHFLHASPDVSEPI EGLHPNEDEHRTYLDVEPITGFTLQFAKRLQVNILVKPARKIEALKNLKRP YIVPILWLNETGTIGDEKAEMFKTQVTGKIKLLGMVEMALLGIGVVMFVAF MISYCACKSKNGK	60
mCD36.ECD	GDMLIEKTIKREVVLEEGTTAFKNWVKTGTTVYRQFWIFDVQNPDDVAKNS SKIKVKQRGPYTYRVRYLAKENITQDPEDHTVSFVQPNGAIFEPSLSVGTE DDNFTVLNLAVAAAPHIYQNSFVQVVLNSLIKKSKSSMFQTRSLKELLWGY K_DPFLSLVPYPISTTVGVFYPYNDTVDGVYKVFNGK_DNISKVAIIESYKGK RNLSYWPSYCDMINGTDAASFPPFVEKSRTLRFFSSDICRSIYAVFGSEID LKGIPVYRFVLPANAFASPLQNPDNHCFCTEKVISNNCTSYGVLDIGKCKE GKPVYISLPHFLHASPDVSEPIEGLHPNEDEHRTYLDVEPITGFTLQFAKR LQVNILVKPARKIEALKNLKRPYIVPILWLNETGTIGDEKAEM FKTQVTGKIK	61
恒河猴 CD36 NP_001028085.1	MGCDRNCGLITGAVIGAVLAVFGGILMPVGDMLIQKTIKKEVVLEEGTIAF KNWVKTGTEIYRQFWIFDVQNPQEVMMNSSNIQVKQRGPYTYRVRFLAKEN ITQDPKDNTVSFLQPNGAIFEPSLSVGTEADNFTVLNLAVAAASHIYPNPF VQVVLNSLINKSKSSMFQVRTLRELLWGYTDPFLSLVPYPVSTRVGMFYPY NNTADGVYKVFNGKDSISKVAIIDTYKGKRNLSYWESYCDMINGTDAASFP PFVEKSQVLQFFSSDICRSIYAVFESDVNLKGIPVYRFVLPSKAFASPVQN PDNHCFCTEKIISKNCTSYGVLDISKCKEGKPVYISLPHFLYASPDVSETI	62

[0169]

	DGLNPNEEEHRTYLDIEPITGFTLQFAKRLQVNLLVKPSNKIQVLKRLKRN YIVPILWLNETGTIGDEKAKMFRSQVTGKINLLGLIEMILLSVGVVMFVAF MISYCACRSKTIK	
恒河猴 CD36.ECD	GDMLIQKTIKKEVVLEEGTIAFKNWVKTGTEIYRQFWIFDVQNPQEVMMNS SNIQVKQRGPYTYRVRFLAKENITQDPKDNTVSFLQPNGAIFEPSLSVGTE ADNFTVLNLAVAAASHIYPNPFVQVVLNSLINKSKSSMFQVRTLRELLWGY TDPFLSLVPYPVSTRVGMFYPYNNTADGVYKVFNGKDSISKVAIIDTYKGK RNLSYWESYCDMINGTDAASFPPFVEKSQVLQFFSSDICRSIYAVFESDVN LKGIPVYRFVLPSKAFASPVQNPDNHCFCTEKIISKNCTSYGVLDISKCKE GKPVYISLPHFLYASPDVSETIDGLNPNEEEHRTYLDIEPITGFTLQFAKR LQVNLLVKPSNKIQVLKRLKRNYIVPILWLNETGTIGDEKAKMFRSQVTGK IN	63

[0170] II.抗CD36抗体

[0171] 在一些实施方案中,本公开提供了抗CD36抗体在各种众所周知的免疫球蛋白特征(例如,CDR、FR、V_H、V_L结构域以及全长重链和轻链)的氨基酸和编码核苷酸序列方面的结构。下表2提供了如实施例中所述产生和功能表征的本公开的抗CD36抗体的概述。表2中提供了每个抗体的相关序列和序列标识符,并且还被包括在随附的序列表中。

[0172] 表2:抗CD36抗体序列

[0173]

描述	序列	SEQ ID NO:
12P109 – scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGCAACTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCTACCTCCACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGTACTACACCTTGCCGTTCACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCACCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTTACACAT	1

[0174]

	ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	
12P109 - V_H A8A - V_H A8A -N52T- V_H 117 - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	2
12P109 – CDR-H1 A8A – CDR-H1 A8A-N52T – CDR-H1 117 - CDR-H1 117_57D - CDR-H1 117_57E - CDR-H1 117_57DE - CDR-H1 117_57EE - CDR-H1	AASGFTISSFGIH	3
12P109 - CDR-H2 A8A - CDR-H2 A8A-N52T - CDR-H2 117 - CDR-H2 117_30AA - CDR-H2 117_30DA - CDR-H2	WIAPYGGYTY	4
12P109 – CDR-H3 A8A – CDR-H3 A8A-N52T – CDR-H3 117 - CDR-H3 117_30AA - CDR-H3 117_30DA - CDR-H3 117_30DE - CDR-H3 117_57D - CDR-H3 117_57E - CDR-H3 117_57DE - CDR-H3 117_57EE - CDR-H3 117_DA57E - CDR-H3	ARSFFGYFDY	5
12P109 – V_L	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISTSTSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQYYTLPFTFGQGTKVEIKR	6

[0175]

12P109 – CDR-L1 A8A – CDR-L1 A8A-N52T – CDR-L1 117 - CDR-L1 117_30AA - CDR-L1 117_30DA - CDR-L1 117_30DE - CDR-L1 117_57D - CDR-L1 117_57E - CDR-L1 117_57DE - CDR-L1 117_57EE - CDR-L1 117_DA57E - CDR-L1	RASQDVSNWVA	7
12P109 – CDR-L2	STSTSLYS	8
12P109 - CDR-L3	YYTLPFTF	9
A8A – scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCCAACAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACCTTCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGTGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTTACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	10
A8A – V_L	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYANSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNLPLTFGQGTKVEIKR	11
A8A – CDR-L2	SYANSLYS	12
A8A - CDR-L3 A8A-N52T - CDR-L3	HSNLPLTF	13

[0176]

A8A-N52T – V_L	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYA**T**SLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNLPLTFGQGTKVEIKR	14
A8A-N52T – CDR-L2 117 - CDR-L2 117_30AA - CDR-L2 117_30DA - CDR-L2 117_30DE - CDR-L2 117_57D - CDR-L2 117_57E - CDR-L2 117_57DE - CDR-L2 117_57EE - CDR-L2 117_DA57E - CDR-L2	SYA**T**SLYS	15
117- scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTTACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	16
117 - V_L 117_30AA - V_L 117_30DA - V_L 117_30DE - V_L 117_57D - V_L 117_57E - V_L 117_57DE - V_L	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYATSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNAPLTFGQGTKVEIKR	17

[0177]

117_57EE - V_L 117_DA57E - V_L		
117 - CDR-L3 117_30AA - CDR-L3 117_30DA - CDR-L3 117_30DE - CDR-L3 117_57D - CDR-L3 117_57E - CDR-L3 117_57DE - CDR-L3 117_57EE - CDR-L3 117_DA57E - CDR-L3	HSNAPLTF	18
117_30AA (HC - DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTGC CGCCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTTACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	19
117_30AA - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTIAAFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	20
117_30AA - CDR-H1	AASGFTIAAFGIH	21
117_30DA- scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG	22

[0178]

	ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTGA CGCCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTTACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	
117_30DA - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTIDAFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	23
117_30DA - CDR-H1 117_ DA57E - CDR-H1	AASGFTIDAFGIH	24
117_30DE- V_H (DNA)	GAAGTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTG GCGGCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCAT TGACAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAG GGGCTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGAAA CATACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGC GGACACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTG CGTGCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTT TCGGGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGT GAGCTCG	25
117_30DE - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTIDSFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	26
117_30DE - CDR-H1	AASGFTIDSFGIH	27
117_30DE - CDR-H2	WIAPYGGETY	28
117_57D- scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG	29

[0179]

	ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGACACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	
117_57D - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGDTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	30
117_57D - CDR-H2	WIAPYGGDTY	31
117_57E- scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGAAACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	32
117_57E - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	33
117_57E - CDR-H2	WIAPYGGETY	34
117_57DE- V_H (DNA)	GAAGTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTG GCGGCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCAT	35

[0180]

	TAGCAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAG GGGCTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGACG AATACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGC GGACACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTG CGTGCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTT TCGGGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGT GAGCTCG	
117_57DE - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGDEYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	36
117_57DE - CDR-H2	WIAPYGGDEY	37
117_57EE- V_H (DNA)	GAAGTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTG GCGGCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCAT TAGCAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAG GGGCTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGAAG AATACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGC GGACACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTG CGTGCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTT TCGGGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGT GAGCTCG	38
117_57EE - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGEEYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	39
117_57EE - CDR-H2	WIAPYGGEEY	40
117_ DA57E- scFv (DNA)	ATGGCCGATATTCAAATGACCCAGAGCCCGAGCAGCCTGAGCG CGAGCGTGGGAGATCGCGTGACCATTACCTGCCGTGCGAGCCA GGATGTTAGTAATTGGGTCGCATGGTATCAGCAGAAACCAGGC AAAGCGCCGAAACTTCTGATATCCTATGCTACTAGCCTGTATA GCGGCGTGCCGTCGCGTTTTTCGGGCAGTGGCAGCGGCACGGA CTTTACCCTGACGATATCTTCCTTACAACCGGAGGATTTTGCG ACCTACTACTGTCAACAGCACTCTAACGCGCCGCTGACCTTCG GTCAAGGCACCAAAGTGGAAATCAAACGCGGTGGTTCCTCTAG ATCTTCCTCCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGGAA GTGCAGCTGGTGGAATCGGGAGGCGGTCTGGTGCAACCTGGCG GCAGCCTTCGTCTGAGCTGTGCGGCGAGCGGGTTCACCATTAG CAGCTTTGGGATTCATTGGGTGCGTCAAGCTCCCGGCAAGGGG CTGGAGTGGGTCGCGTGGATTGCTCCCTACGGTGGTGAAACAT ACTATGCCGACAGCGTGAAAGGTCGCTTTACGATTAGTGCGGA	41

[0181]

	CACCAGCAAAAATACCGCGTACCTGCAGATGAATAGCCTGCGT GCGGAAGACACAGCGGTGTATTATTGCGCGCGTTCGTTTTTCG GGTACTTCGATTATTGGGGGCAGGGCACCCTTGTTACCGTGAG CTCGGCGTCAGCGGCCGCA	
117_ DA57E - V_H	EVQLVESGGGLVQPGGSLRLSCAASGFTIDAFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSS	42
117_ DA57E - CDR-H2	WIAPYGGETY	43
12P109 – HC A8A – HC A8A-N52T – HC 117 – HC (IgG1-N297A)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla psskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkv epkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpe vtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqyastyr vvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqpr epqvytlppsreemtknqvsltclvkgfypsdiavewesngqp ennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhe alhnhytqkslslspg	44
12P109 - LC	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISTSTSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQYYTLPFTFGQGTKVEIKRtvaapsvfifppsdeqlksgt asvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdst yslsstltlskadyekhkvyacevthqglsspvtksfnrgec	45
A8A - LC	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYANSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNLPLTFGQGTKVEIKRtvaapsvfifppsdeqlksgt asvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdst yslsstltlskadyekhkvyacevthqglsspvtksfnrgec	46
A8A-N52T - LC	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYA**T**SLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNLPLTFGQGTKVEIKRtvaapsvfifppsdeqlksgt asvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdst yslsstltlskadyekhkvyacevthqglsspvtksfnrgec	47
117 – HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv	48

[0182]

	eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	
117 – LC 117_30AA - LC 117_30DA - LC 117_30DE - LC 117_57D - LC 117_57E - LC 117_57DE - LC 117_57EE - LC 117_DA57E - LC	DIQMTQSPSSLSASVGDRVTITCRASQDVSNWVAWYQQKPGKA PKLLISYATSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQHSNAPLTFGQGTKVEIKRtvaapsvfifppsdeqlksgt asvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdst yslsstltlskadyekhkvyacevthqglsspvtksfnrgec	49
117_30AA – HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTIAAFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	50
117_30DA – HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTIDAFGIHWVRQAPGK GLEWVAWIAPYGGYTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	51
117_30DE – HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTIDSFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL	52

[0183]

	RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	
117_57D- HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGDTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	53
117_57E- HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	54
117_57DE- HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGDEYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq	55

[0184]

	vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	
117_57EE- HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTISSFGIHWVRQAPGK GLEWVAWIAPYGGEEYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	56
117_ DA57E- HC (IgG4-S228P- FALA)	EVQLVESGGGLVQPGGSLRLSCAASGFTIDAFGIHWVRQAPGK GLEWVAWIAPYGGETYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARSFFGYFDYWGQGTLVTVSSastkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpa vlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv eskygppcppcpapeaaggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq vytlppsqeemtknqvsltclvkgfypsdiavewesngqpenn ykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealh nhytqkslslslg	57

[0185] 1.抗CD36抗体结合亲和力和功能特性

[0186] 在一些实施方案中,本文提供的抗CD36抗体与CD36结合的平衡解离常数(K_D)为<100nM、<10nM、<1nM、<0.1nM、<0.01nM或<0.001nM(例如,10^{-8}M或更低,从10^{-8}M至10^{-13}M,例如,从10^{-9}M至10^{-13}M)。

[0187] 考虑到如本文所公开的产生的各种抗CD36抗体包括能够高亲和力结合hCD36、mCD36、恒河猴CD36、hCD36和mCD36两者和/或hCD36、mCD36和恒河猴CD36的抗体。更具体地说,在一些实施方案中,本公开的抗CD36抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低、或1×10^{-11}M或更低的结合亲和力与hCD36结合。在一些实施方案中,所述结合亲和力以与SEQ ID NO:58或SEQ ID NO:59的hCD36多肽结合的平衡解离常数(K_D)来测量。在一些实施方案中,本公开的抗CD36抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低、或1×10^{-11}M或更低的结合亲和力与mCD36结合。在一些实施方案中,所述结合亲和力以与SEQ ID NO:60或SEQ ID NO:61的mCD36多肽结合的平衡解离常数(K_D)来测量。在一些实施方案中,本公开的抗CD36抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低、或1×10^{-11}M或更低的结合亲和力与恒河猴CD36结合。在一些实施方案中,所述结合亲和力以与SEQ ID NO:62或SEQ ID NO:63的恒河猴CD36多肽结合的平衡解离常数(K_D)来测量。在一些实施方案中,本公开的抗CD36抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低、或

1×10^{-11}M或更低的结合亲和力与hCD36和mCD36两者结合。在一些实施方案中,本公开的抗CD36抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低、或1×10^{-11}M或更低的结合亲和力与hCD36和恒河猴CD36两者结合。

[0188] 通常,配体与其受体的结合亲和力可使用多种测定法中的任一种来确定,并以多种定量数值的方式表示。本文的实施例中公开了可用于确定抗体亲和力的特定的CD36结合测定法。此外,抗原结合测定法是本领域已知的,并且可以在本文中使用,其包括但不限于任何直接或竞争性的结合测定法,其使用技术例如蛋白质印迹、放射免疫测定法、酶联免疫吸附测定法(ELISA)、"夹心"免疫测定法、基于表面等离子体共振的测定法(例如,如WO2005/012359所述的BIAcore测定法)、免疫沉淀测定法、荧光免疫测定法、蛋白质A免疫测定法、流式细胞术和荧光激活细胞分选(FACS)测定法等。

[0189] 因此,在一些实施方案中,结合亲和力以K_D值表示,并反映内在结合亲和力(例如,具有最小化的亲和力效应)。本公开的抗CD36抗体对SEQ ID NO:58的hCD36多肽表现出强结合亲和力,例如,表现出10nM和1pM之间的K_D值。因此,本公开的抗CD36抗体可与对CD36的相同或重叠表位具有较低亲和力的抗体竞争。

[0190] 在一些实施方案中,本文提供的抗CD36抗体降低、抑制和/或完全阻断配体与CD36的结合,以及由配体与CD36结合介导的免疫调节和/或免疫信号传导,其包括在肿瘤微环境中维持TAM。可以使用已知的基于细胞的测定法(包括本公开的实施例中描述的测定法)在体外测定抗体抑制由配体与CD36结合介导的这些免疫调节和免疫信号传导通路的能力。

[0191] 因此,在一些实施方案中,本公开的CD36抗体通过以下功能性质中的一种或多种而表征,所述以下功能性质中的一种或多种基于由CD36介导的通路降低、抑制和/或完全阻断细胞内信号传导的能力。

[0192] 在至少一个实施方案中,抗CD36抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低的结合亲和力与人CD36结合;任选地,其中所述结合亲和力通过与SEQ ID NO:58或59的hCD36多肽的平衡解离常数(K_D)来测量。

[0193] 在抗CD36抗体的至少一个实施方案中,所述抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低的结合亲和力与小鼠CD36结合;任选地,其中所述结合亲和力通过与SEQ ID NO:60或61的mCD36多肽的平衡解离常数(K_D)来测量。

[0194] 在抗CD36抗体的至少一个实施方案中,所述抗体以1×10^{-8}M或更低、1×10^{-9}M或更低、1×10^{-10}M或更低的结合亲和力与恒河猴CD36结合;任选地,其中所述结合亲和力通过与SEQ ID NO:62或63的恒河猴CD36多肽的平衡解离常数(K_D)来测量。

[0195] 在抗CD36抗体的至少一个实施方案中,所述抗体抑制过表达表面人CD36的F293细胞中CD36依赖性氧化LDL的摄取至少65%、至少75%、至少85%、至少95%或至少100%;其中在1-10μg/mL的oxLDL浓度下,所述抗体具有5nM或更低、1nM或更低、0.5nM或更低、或者0.1nM或更低的IC_{50}。

[0196] 在抗CD36抗体的至少一个实施方案中,所述抗体抑制U937细胞中CD36依赖性氧化LDL的摄取至少65%、至少75%、至少85%、至少95%或至少100%;其中在1-10μg/mL的oxLDL浓度下,所述抗体具有5nM或更低、1nM或更低、0.5nM或更低、或者0.1nM或更低的IC_{50}。

[0197] 在抗CD36抗体的至少一个实施方案中,所述抗体抑制小鼠CD45+TIL中CD36依赖性氧化LDL的摄取至少65%、至少75%、至少85%、至少95%或至少100%;任选地,其中在1-10

μg/mL的oxLDL浓度下,所述抗体具有5nM或更低、1nM或更低、0.5nM或更低、或者0.1nM或更低的IC_{50}。

[0198] 2.抗-CD36抗体片段

[0199] 在一些实施方案中,本公开的抗-CD36抗体可以是抗体片段。可与本公开的结合决定簇一起使用的抗体片段包括但不限于Fab、Fab'、Fab'-SH、$F(ab')_2$、Fv、scFv片段、单价、单域抗体、一臂或单臂抗体以及本文所述和本领域已知的其他片段。因此,在本公开的抗-CD36抗体的一些实施方案中,所述抗体是选自由$F(ab')_2$、Fab'、Fab、Fv、单域抗体(VHH)、单臂抗体和scFv的组成的组的抗体片段。

[0200] 对于各种抗体片段的综述,参见例如Hudson等人Nat.Med.9:129-134(2003)。对于scFv片段的综述,参见例如Pluckthun的《单克隆抗体药理学》,第113卷,Rosenburg和Moore编辑,(纽约施普林格出版社),第269-315页(1994年);另参见WO93/16185;以及美国专利号5,571,894和5,587,458。对于包含补救受体结合表位残基且体内半衰期增加的Fab和$F(ab')_2$片段的描述,参见美国专利号5,869,046。其他单价抗体形式描述于例如WO2007/048037、WO2008/145137、WO2008/145138和WO2007/059782中。单价单臂抗体描述于例如WO2005/063816中。双抗体是具有两个抗原结合位点的抗体片段,其可以是二价的或双特异性的(参见例如EP0404097;WO93/01161;Hudson等人,Nat.Med.9:129-134(2003);和Hollinger等人,Proc.Natl.Acad.Sci.USA 90:6444-6448(1993))。

[0201] 在一些实施方案中,抗体片段是单域抗体,其包含抗体的重链可变结构域的全部或部分或轻链可变结构域的全部或部分。在一些实施方案中,单域抗体是人单域抗体(Domantis,Inc.,Waltham,MA;参见例如美国专利号6,248,516)。

[0202] 抗体片段可以通过各种技术制得,其包括但不限于完整抗体的蛋白水解消化以及通过重组宿主细胞(例如大肠杆菌或噬菌体)产生,如本文所述。

[0203] 3.嵌合、人源化和人抗CD36抗体

[0204] 在一些实施方案中,本公开的抗CD36抗体可以是嵌合抗体。(参见例如美国专利号4,816,567中所述的嵌合抗体;以及Morrison等人,Proc.Natl.Acad.Sci.USA 81:6851-6855(1984))。在一个实施方案中,嵌合抗体包含非人可变区(例如,源自小鼠、大鼠、仓鼠、兔子或非人灵长类动物(例如猴子)的可变区)和人恒定区。在一些实施方案中,嵌合抗体是"类转换"抗体,其中类或亚类已与亲本抗体的类或亚类有所改变。考虑嵌合抗体可以包括其抗原结合片段。

[0205] 在一些实施方案中,本公开的抗CD36抗体是人源化抗体。通常,非人抗体被人源化以降低对人的免疫原性,同时保留亲本非人抗体的特异性和亲和力。通常,人源化抗体包含一个或多个可变结构域,其中HVR、CDR(或其部分)源自非人抗体,并且FR(或其部分)源自人抗体序列。人源化抗体任选地还会包含至少一部分人恒定区。在一些实施方案中,人源化抗体中的一些FR残基被来自非人抗体(例如,CDR残基源自的抗体)的相应残基取代,以恢复或改进抗体特异性或亲和力。

[0206] 人源化抗体及其制备方法综述于例如Almagro和Fransson,Front.Biosci.13:1619-1633(2008),并且进一步描述于例如Riechmann等人,Nature 332:323-329(1988);Queen等人,Proc.Natl.Acad.Sci.USA 86:10029-10033(1989);美国专利号5,821,337、7,527,791、6,982,321和7,087,409;Kashmiri等人,Methods 36:25-34(2005)(描述SDR(a-

HVR)嫁接);Padlan,Mol.Immunol.28:489-498(1991)(描述“表面重塑”);Dall'Acqua等人,Methods 36:43-60(2005)(描述“FR改组”);以及Osbourn等人,Methods 36:61-68(2005)和Klimka等人,Br.J.Cancer,83:252-260(2000)(描述对FR改组的“指引选择”方法)。

[0207] 可用于人源化的人框架区包括但不限于:使用“最佳拟合”方法选择的框架区(参见例如Sims等人J.Immunol.151:2296(1993));源自轻链或重链可变区的特定亚组的人抗体的共识序列的框架区(参见例如Carter等人,Proc.Natl.Acad.Sci.USA 89:4285(1992);和Presta等人,J.Immunol.151:2623(1993));人成熟(体细胞突变的)框架区或人种系框架区(参见例如Almagro和Fransson,Front.Biosci.13:1619-1633(2008));以及源自筛选FR文库的框架区(参见例如Baca等人,J.Biol.Chem.272:10678-10684(1997)和Rosok等人,J.Biol.Chem.271:22611-22618(1996))。

[0208] 在一些实施方案中,本公开的抗CD36抗体可以是人抗体。人抗体可以使用本领域已知的各种技术来产生。人抗体通常描述于van Dijk和van de Winkel,Curr.Opin.Pharmacol.5:368-74(2001)和Lonberg,Curr.Opin.Immunol.20:450-459(2008)中。人抗体可通过向转基因动物施用免疫原来制备,所述转基因动物已被修饰以产生完整的人抗体或具有人可变区的完整抗体从而响应抗原攻击。此类动物通常含有人免疫球蛋白基因座的全部或部分,其取代内源性免疫球蛋白基因座,或存在于染色体外或被随机整合到动物的染色体中。在此类转基因小鼠中,内源性免疫球蛋白基因座通常已被灭活。对于从转基因动物获得人抗体的方法的综述,参见Lonberg,Nat.Biotech.23:1117-1125(2005)。另请参见例如美国专利号6,075,181和6,150,584中的XENOMOUSE™技术;美国专利号5,770,429中的HUMAB®技术;美国专利号7,041,870中的K-M MOUSE®技术;以及美国专利申请公开号US2007/0061900中的VELOCIMOUSE®技术。来自由此类动物产生的完整抗体的人可变区可被进一步修饰,例如通过与不同的人恒定区组合。

[0209] 人抗体也可以通过基于杂交瘤的方法制得。已经描述了用于产生人单克隆抗体的人骨髓瘤和小鼠-人异骨髓瘤细胞系。参见例如Kozbor J.Immunol,133:3001(1984);Brodeur等人,单克隆抗体生产技术和应用,第51-63页(Marcel Dekker,Inc.,New York,1987);和Boerner等人,J.Immunol.147:86(1991)。经由人B细胞杂交瘤技术产生的人抗体也在Li等人,Proc.Natl.Acad.Sci.USA 103:3557-3562(2006)中被描述。另外的方法包括例如美国专利号7,189,826(描述从杂交瘤细胞系产生单克隆人IgM抗体)中描述的方法。人杂交瘤技术(Trioma technology)也在Vollmers和Brandlein,Histology and Histopathology,20(3):927-937(2005)和Vollmers和Brandlein,Methods and Findings in Experimental and Clinical Pharmacology 27(3):185-91(2005)中被描述。

[0210] 人抗体也可通过分离选自人源噬菌体展示文库的Fv克隆可变结构域序列来产生。然后可将此类可变结构域序列与所需的人恒定域组合。下面描述了用于从抗体文库中选择人抗体的技术。

[0211] 4.抗CD36抗体的文库衍生变体

[0212] 在至少一个实施方案中,可通过筛选组合文库以寻找具有所需改进的功能特性(例如结合亲和力或交叉反应性)的抗体来分离抗CD36抗体的改进变体。例如,本领域已知多种方法用于产生噬菌体展示文库以及筛选此类文库以寻找具有改进的结合特性的变体抗体。用于产生此类文库衍生抗体的其他方法可参见例如,Hoogenboom等人,Methods in

Molecular Biology 178:1-37(O'Brien et al.,ed.,Human Press,Totowa,NJ,2001);McCafferty等人,Nature 348:552-554(1990);Clackson等人,Nature 352:624-628(1991);Marks等人,J.Mol.Biol.222:581-597(1992);Marks和Bradbury,m Methods in Molecular Biology 248:161-175(Lo,ed.,Human Press,Totowa,NJ,2003);Sidhu等人,J.Mol.Biol.338(2):299-310(2004);Lee等人,J.Mol.Biol.340(5):1073-1093(2004);Fellouse,Proc.Natl.Acad.Sci.USA 101(34):12467-12472(2004);以及Lee等人,J.Immunol.Methods 284(1-2):119-132(2004)。

[0213] 5.多特异性抗体和抗体融合物

[0214] 在至少一个实施方案中,考虑本公开的抗CD36抗体可以是多特异性抗体,例如双特异性抗体。在一些实施方案中,多特异性抗体具有至少两个不同结合位点,每个结合位点具有针对不同抗原的结合特异性,其中至少一个特异性地结合CD36。在至少一个实施方案中,考虑所述多特异性抗体是双特异性抗体,其包含针对CD36的特异性和针对介导免疫调节、免疫信号传导和/或在癌症或肿瘤细胞上表达的另一种抗原的特异性。例如,另一种特异性可以是针对免疫检查点分子,例如PD1、LAG3、CTLA-4、A2AR、TIM-3、BTLA、CD276、CD328、VTCN1、IDO、KIR、NOX2、VISTA、OX40、CD27、CD28、CD40、CD122、CD137、GITR或ICOS。

[0215] 用于制得多特异性抗体的技术包括但不限于具有不同特异性的两种免疫球蛋白重链-轻链对的重组共表达(参见例如Milstein和Cuello,Nature,305:537(1983),WO93/08829,以及Traunecker等人,EMBO J.10:3655(1991))。"旋钮入孔"工程也可用于产生与本公开的抗CD36抗体一起使用的双特异性抗体。用于旋钮入孔工程的技术是本领域已知的,并且描述于例如美国专利号5,731,168中。

[0216] 还可以通过工程"静电转向"效应制得多特异性抗体,其有利于形成Fc-异二聚体抗体分子而不是同二聚体(WO2009/089004A1);交联两个或多个抗体或片段(参见例如美国专利号4,676,980和Brennan等人,Science 229:81(1985));使用亮氨酸拉链产生双特异性抗体(参见例如Kostelny等人,J.Immunol.148(5):1547-1553(1992));使用"双抗体"技术用于制得双特异性抗体片段(参见,例如,Hollinger等人,Proc.Natl.Acad.Sci.USA 90:6444-6448(1993));使用单链Fv(scFv)二聚体(参见,例如,Gruber等人,J.Immunol.152:5368(1994));或三特异性抗体(参见,例如,Tutt等人,J.Immunol.147:60(1991))。

[0217] 在至少一个实施方案中,本文提供的抗CD36抗体可以包含与蛋白质的抗体融合物。用于制备和使用抗体融合物或融合蛋白的方法是本领域众所周知的。通常,抗体与蛋白质共价缀合(或融合),通常经由接头多肽。所述缀合可以经由所述抗体的轻链(LC)或重链(HC)的末端发生。抗体融合物也可以用抗体片段制备。在由本公开考虑的抗体融合物的一个示例性实施方案中,融合物包括全长抗CD36抗体,其经由位于轻链或重链末端的接头与T细胞活化或免疫刺激细胞因子融合。所述细胞因子可以包括但不限于IL-2、IL-7、IL-10、IL-12、IL-15、IL-21或IFN-α。此类抗CD36抗体融合物可以阻断由CD36信号传导介导的活性并提供免疫刺激细胞因子效应。可以使用与所述细胞因子相关的已知的基于细胞的测定法在体外测定此类抗CD36抗体融合物提供免疫刺激细胞因子效应的能力。

[0218] 6.抗CD36抗体的变体

[0219] 在一些实施方案中,本公开的抗CD36抗体的变体被考虑具有改进的特性,例如抗体的结合亲和力和/或其他生物学性质。可以通过将适当的修饰引入编码抗体的核苷酸序

列或通过肽合成来制备变体。此类修饰包括,例如,抗体的氨基酸序列内的缺失和/或向抗体的氨基酸序列中插入和/或抗体的氨基酸序列内的残基的取代。可以进行任何缺失、插入和取代的组合以得到最终构建体,前提是最终构建体拥有所需的CD36抗原结合特性。

[0220] A.取代、插入和缺失的变体

[0221] 在一些实施方案中,提供了具有除本文所述之外的一个或多个氨基酸取代的抗CD36抗体变体。用于诱变的位点可以包括CDR、HVR和FR。典型的"保守"氨基酸取代和/或基于普遍侧链类别或性质的取代在本领域中是众所周知的,并且可以用于本公开的实施方案中。本公开还考虑了基于非保守氨基酸取代的变体,其中氨基酸侧链类别之一的成员被交换为来自另一类别的氨基酸。氨基酸侧链通常根据以下类别或普遍性质分组:(1)疏水性:Met、Ala、Val、Leu、Ile、正亮氨酸;(2)中性亲水性:Cys、Ser、Thr、Asn、Gln;(3)酸性:Asp、Glu;(4)碱性:His、Lys、Arg;(5)影响链方向的:Gly、Pro;和(6)芳香族:Trp、Tyr、Phe。将氨基酸取代到抗体中并随后筛选所需功能(例如,保留/改进的抗原结合、降低的免疫原性或改进的ADCC或CDC)的技术是本领域众所周知的。

[0222] 氨基酸取代变体还可以包括在亲本抗体的高变区中具有一个或多个取代的变体。通常,选择用于进一步研究的所得变体会具有相对于亲本抗体的某些生物学性质的修改(例如,增加的亲和力、降低的免疫原性)和/或会保留亲本抗体的某些生物学性质。示例性取代变体是亲和力成熟的抗体,其可使用基于噬菌体展示的亲和力成熟技术方便地产生。简而言之,突变一个或多个HVR残基,并将变体抗体展示在噬菌体上并筛选出特定的生物活性(例如,结合亲和力)。

[0223] 一种用于鉴定可能被靶向用于诱变的抗体残基或区域的有用方法是"丙氨酸扫描诱变"(参见例如,Cunningham和Wells,Science244:1081-1085(1989))。在该方法中,识别残基或一组目标残基(例如,带电残基,如Arg、Asp、His、Lys和Glu)并通过中性或带负电的氨基酸(例如,Ala或多聚丙氨酸)将其取代以确定抗体与抗原的相互作用是否受到影响。可在被证实对初始取代具有功能敏感性的氨基酸位置处引入进一步的取代。或者,或另外,以识别抗体和抗原之间的接触点的抗原-抗体复合物的晶体结构可以被测定。可将此类接触残基和相邻残基作为用于取代的候选物进行靶向或消除。可筛选变体以确定它们是否含有所需的性质。

[0224] 可以制备的氨基酸序列插入包括长度范围从一个残基到含有一百个或更多个残基的多肽的氨基和/或羧基末端融合物,以及单个或多个氨基酸残基的序列内插入。末端插入的示例包括具有N末端甲硫氨酰残基的抗体。抗体分子的其他插入变体可以包括抗体的N-末端或C-末端与酶或多肽的融合物,这会增加抗体血清半衰期。

[0225] 可以在HVR中进行其他残基取代以提高抗体亲和力。此类改变可在"热点"中进行,即在体细胞成熟过程期间经历高频率突变的由密码子编码的残基(参见例如Chowdhury,Methods Mol.Biol.207:179-196(2008)),并对所得变体VH或VL测试结合亲和力。在一个实施方案中,亲和力成熟可以通过构建和从次级文库中重新选择来进行(参见例如,在Hoogenboom等人,Methods in Molecular Biology 178:1-37(O'Brien等人编辑,Human Press,Totowa,NJ,(2001))。引入多样性的另一种方法涉及HVR定向方法,其中几个HVR残基(例如,每次4-6个残基)被随机化。可特异性地鉴定涉及抗原结合的HVR残基,例如,使用丙氨酸扫描诱变或建模。通常特别靶向HVR-H3和HVR-L3。通常,可以在一个或多个HVR内进行

取代、插入或缺失,只要此类改变不会实质上降低抗体结合抗原的能力。例如,可在HVR中进行不实质上降低结合亲和力的保守改变(例如,如本文提供的保守取代)。此类改变可能在HVR“热点”之外。

[0226] 在一些实施方案中,考虑本文所述的抗CD36抗体可在特定的非HVR位置用半胱氨酸残基取代从而创造反应性硫醇基团。此类工程化的“硫代单克隆抗体”可用于将抗体与例如药物部分或接头-药物部分缀合进而创造出免疫缀合物,如本文其他地方所述。半胱氨酸工程化抗体可如在例如美国专利号7,521,541中所述来产生。在一些实施方案中,以下抗体残基中的任何一个或多个可用半胱氨酸取代:轻链的V205(Kabat编号);重链的A118(EU编号);和重链Fc区的S400(EU编号)。

[0227] B.糖基化变体

[0228] 在一些实施方案中,改变本公开的抗CD36抗体以增加或减少抗体被糖基化的程度。可以通过改变氨基酸序列来进行对抗体的糖基化位点的添加或缺失,从而创造或去除一个或多个糖基化位点。在抗体包含Fc区的实施方案中,可以改变附着于Fc区的碳水化合物。通常,由哺乳动物细胞产生的天然抗体包含通过N连接附着于Fc区CH2结构域的约第297位(“N297”)处的天冬酰胺的支链双触角寡糖(参见,例如,Wright等人,TIBTECH 15:26-32(1997))。寡糖可包括各种碳水化合物,例如甘露糖、N-乙酰葡萄糖胺(GlcNAc)、半乳糖和唾液酸,以及双触角寡糖结构的“茎”中附着于GlcNAc的岩藻糖。在一些实施方案中,抗体Fc区的寡糖修饰可以创造出具有某些改进的性质的变体。

[0229] 在一些实施方案中,本公开的抗CD36抗体可以是包含缺乏(直接或间接)附着于Fc区的岩藻糖的碳水化合物结构的变体。例如,此类抗体中的岩藻糖量可以是从约1%至约80%、从约1%至约65%、从约5%至约65%或从约20%至约40%。可以通过计算附着于残基N297的糖链内岩藻糖的平均量,相对于附着于N297的所有糖结构的总和(例如,复杂、混合和高甘露糖结构)来确定岩藻糖的量,如通过MALDI-TOF质谱法测量的(参见例如WO2008/077546)。

[0230] 在一些实施方案中,岩藻糖基化变体可以提供变体抗体的改进的ADCC功能。参见例如美国专利公开号US2003/0157108或US2004/0093621。“脱岩藻糖基化”或“岩藻糖缺乏”抗体的示例以及用于制备它们的相关方法公开于例如US2003/0157108;US2003/0115614;US2002/0164328;US2004/0093621;US2004/0132140;US2004/0110704;US2004/0110282;US2004/0109865;WO2000/61739;WO2001/29246;WO2003/085119;WO2003/084570;WO2005/035586;WO2005/035778;WO2005/053742;WO2002/031140;Okazaki等人,J.Mol.Biol.336:1239-1249(2004);Yamane-Ohnuki等人,Biotech.Bioeng.87:614(2004)。用于产生脱岩藻糖基化抗体的细胞系包括蛋白质岩藻糖基化缺乏的Led 3CHO细胞(参见例如Ripka等人,Arch.Biochem.Biophys.249:533-545(1986);US2003/0157108和WO2004/056312)和敲除细胞系,例如α-1,6-岩藻糖基转移酶基因FUT8敲除CHO细胞(参见例如Yamane-Ohnuki等人,Biotech.Bioeng.87:614(2004);Kanda,Y.等人,Biotechnol.Bioeng.94(4):680-688(2006)和WO2003/085107)。

[0231] C.Fc区变体

[0232] 在一些实施方案中,本公开的抗CD36抗体可在Fc区中包含一个或多个氨基酸修饰(即,Fc区变体)。Fc区变体可包含人Fc区序列(例如,人IgG1、IgG2、IgG3或IgG4 Fc区),其在

一个或多个氨基酸残基位置处包含氨基酸取代。下文描述了本领域已知的可用于本公开的抗CD36抗体的广泛的Fc区变体。

[0233] 在一些实施方案中,抗CD36抗体是具有改变的效应子功能的Fc区变体。在一些实施方案中,具有改变的效应子功能的抗体具有亲本抗体的一些(但不是全部)效应子功能、降低的效应子功能或没有效应子功能(例如,无效应子)。对于某些其中效应子功能(如ADCC)是不必要的或有害的,和/或抗体的体内半衰期很重要的应用,无效应子的Fc区变体是更需要的。具有降低的效应子或无效应子功能的Fc区变体抗体可由以下Fc区位置中的一处或多处的氨基酸取代引起:238、265、269、270、297、327和329。(参见例如美国专利号6,737,056)。此类Fc区变体可包括位于位置265、269、270、297和327中的两处或多处的氨基酸取代。此类Fc区变体还可包括将残基265和297二者都取代为丙氨酸(参见例如美国专利号7,332,581)。

[0234] 一些Fc区变体能够提供增强的或减弱的与FcR的结合(参见例如美国专利号6,737,056;WO2004/056312;和Shields等人,J.Biol.Chem.9(2):6591-6604(2001))。一些能够提供改进的ADCC的Fc区变体包含一个或多个氨基酸取代,位于例如Fc区的位置298、333和/或334(基于EU编号)。具有改变的(即增强的或减弱的)Clq结合和/或补体依赖性细胞毒性(CDC)的Fc区变体,如例如美国专利号6,194,551、WO99/51642和Idusogie等人,J.Immunol.164:4178-4184(2000)中所述。

[0235] 在例如US2005/0014934A1(Hinton等人)中公开了一些Fc区变体能够提供增加的半衰期和改善的与新生儿Fc受体(FcRn)的结合。此类Fc区变体在以下位置的一处或多处包含氨基酸取代:238、256、265、272、286、303、305、307、311、312、317、340、356、360、362、376、378、380、382、413、424和434。其他具有增加的半衰期的Fc区变体包括在例如US7658921B2(Dall'Acqua等人)中描述的位于位置252、254和256的YTE突变组(即M252Y/S254T/T256E)。Fc区变体的另外的示例可参见例如美国专利号5,648,260和5,624,821;和WO94/29351。

[0236] 通常地,可以进行体外和/或体内细胞毒性测定以证实Fc区变体中CDC和/或ADCC活性的降低/耗尽。例如,可以进行Fc受体(FcR)结合测定以确保抗体缺乏FcγR结合(因此可能缺乏ADCC活性)但保留FcRn结合能力。用于介导ADCC的主要细胞NK细胞仅表达FcγRIII,而单核细胞表达FcγRI、FcγRII和FcγRIII。评估目标分子的ADCC活性的体外测定的非限制性示例在美国专利号5,500,362(参见例如Hellstrom等人,Proc.Natl.Acad.Sci.USA 83:7059-7063(1986))和Hellstrom等人,Proc.Natl.Acad.Sci.USA 82:1499-1502(1985);5,821,337(参见Bruggemann,M.等人,J.Exp.Med.166:1351-1361(1987))中被描述。或者,可运用非放射性测定方法(参见,例如,用于流式细胞术的ACTI™非放射性细胞毒性测定(CellTechnology,Inc.Mountain View,CA);和CytoTox96®非放射性细胞毒性测定(Promega,Madison,WI)。用于此类测定法的有用的效应细胞包括外周血单核细胞(PBMC)和自然杀伤(NK)细胞。或者,或另外,可在体内评估目标分子的ADCC活性,例如在动物模型中(如在Clynes等人,Proc.Natl.Acad.Sci.USA 95:652-656(1998)中公开的动物模型)。还可进行Clq结合测定以证实抗体不能结合Clq并因此缺乏CDC活性。参见,例如,WO2006/029879和WO2005/100402中的Clq和C3c结合ELISA。为了评估补体活化,可进行CDC测定(参见,例如,Gazzano-Santoro等人,J.Immunol.Methods 202:163(1996);Cragg,M.S.等人,Blood 101:1045-

1052(2003);以及Cragg,M.S.和M.J.Glennie,Blood 103:2738-2743(2004))。FcRn结合和体内清除/半衰期测定可使用本领域已知方法进行(参见,例如,Petkova等人,Intl.Immunol.18(12):1759-1769(2006))。

[0237] D.非蛋白质抗体衍生物-免疫缀合物

[0238] 在一些实施方案中,本公开的抗CD36抗体可用非蛋白质部分进一步修饰(即衍生化)。适合于抗体衍生化的非蛋白质部分包括但不限于水溶性聚合物,例如:聚乙二醇(PEG)、乙二醇和丙二醇的共聚物、羧甲基纤维素、葡聚糖、聚乙烯醇、聚乙烯吡咯烷酮、聚-1,3-二氧戊环、聚-1,3,6-三氧杂环己烷、乙烯/马来酸酐共聚物、聚氨基酸均聚物或随机共聚物、以及葡聚糖或聚(n-乙烯基吡咯烷酮)聚乙二醇、丙二醇均聚物、聚环氧丙烷/环氧乙烷共聚物、聚氧乙基化多元醇(例如甘油)、聚乙烯醇及其混合物。在一些实施方案中,可以使用甲氧基聚乙二醇丙醛进行抗体的修饰。聚合物可以是任何分子量,并且可以是支链的或非支链的。附着于抗体的聚合物的数量可以变化,并且如果附着了多于一个的聚合物,它们可以是相同或不同的分子。通常,用于衍生化的聚合物的数量和/或类型可以基于包括但不限于抗体的特定性质或功能的考虑因素来确定,例如,抗体衍生物是否会在特定条件下用于疗法中。

[0239] 在一些实施方案中,本公开的抗CD36抗体也可以是免疫缀合物,其中所述免疫缀合物包含与一种或多种细胞毒性剂缀合的抗CD36抗体。由本公开考虑的合适的细胞毒性剂包括化疗剂、药物、生长抑制剂、毒素(例如,蛋白类毒素,细菌、真菌、植物或动物来源的酶活性毒素,或其片段)或放射性同位素。在一些实施方案中,所述免疫缀合物是抗体-药物缀合物(ADC),其中如本文所述的抗CD36抗体与一种或多种药物缀合。在一些实施方案中,本公开的免疫缀合物包含与用于治疗CD36介导的疾病或病况的药物或治疗剂缀合的如本文所述的抗CD36抗体。

[0240] 在一些实施方案中,如本文所述的抗CD36抗体可以与酶活性毒素或其片段缀合,其包括但不限于白喉毒素A链、白喉毒素的非结合活性片段、外毒素A链(来自铜绿假单胞菌)、蓖麻毒素A链、相思豆毒素A链、蒴莲毒素A链、α-八叠球菌素、油桐蛋白、香石竹毒素蛋白、美洲商陆蛋白(Phytolaca americana protein)、苦瓜抑制剂、麻疯树毒素、巴豆毒素、肥皂草抑制剂、白树毒素、丝林霉素(mitogellin)、局限曲霉素、酚霉素、依诺霉素和单端孢菌素。

[0241] 在一些实施方案中,本公开的免疫缀合物包含与放射性同位素缀合的如本文所述的抗CD36抗体(即放射性缀合物)。有多种放射性同位素可用于生产此类放射性缀合物。实例包括^{211}At、^{131}I、^{125}I、^{90}Y、^{186}Re、^{188}Re、^{153}Sm、^{212}Bi、^{32}P、^{212}Pb和Lu的放射性同位素。在一些实施方案中,免疫缀合物可包含用于闪烁显像检测的放射性同位素,或用于NMR检测或MRI的自旋标记。合适的放射性同位素或自旋标记可以包括,如^{123}I、^{131}I、^{111}In、^{13}C、^{19}F、^{15}N、^{17}O、Gd、Mn和Fe的各种同位素。

[0242] 可以使用多种众所周知的适合于与蛋白质缀合的双功能试剂和化学物质来制得抗CD36抗体和细胞毒剂的免疫缀合物。此类试剂包括但不限于:N-琥珀酰亚胺基-3-(2-吡啶基二硫代)丙酸酯(SPDP)、琥珀酰亚胺基-4-(N-马来酰亚胺基甲基)环己烷-1-羧酸酯(SMCC)、亚氨基硫烷(IT)、亚氨酸酯的双功能衍生物(例如,二甲基己二酰亚胺HQ)、活性酯(例如,二琥珀酰亚胺基辛二酸酯)、醛(例如,戊二醛)、双叠氮化合物(例如,双-(对叠氮苯

甲酰)-己二胺)、双重氮衍生物(例如,双-(对重氮苯甲酰)-乙二胺)、二异氰酸酯(例如,甲苯-2,6-二异氰酸酯)和双活性氟化合物(例如,1,5-二氟-2,4-二硝基苯)。用于制备本公开的免疫缀合物的试剂还可以包括市售的“交联”试剂,例如:BMPS、EMCS、GMBS、HBVS、LC-SMCC、MBS、MPBH、SBAP、SIA、SIAB、SMCC、SMPB、SMPH、磺基-EMCS、磺基-GMBS、磺基-KMUS、磺基-MBS、磺基-SIAB、磺基-SMCC和磺基-SMPB以及SVSB(琥珀酰亚胺基-(4-乙烯基砜)苯甲酸酯)(参见例如,Pierce Biotechnology,Inc.,Rockford,IL.,U.S.A)。

[0243] III.重组方法和组合物

[0244] 本公开的抗CD36抗体可以使用抗体生产领域中众所周知的重组方法和材料来生产。在一些实施方案中,本公开提供了编码本公开的抗CD36抗体或抗CD36抗体的片段或结构域的分离的多核苷酸。例如,分离的多核苷酸可以编码包含本文公开的CDR或HVR的氨基酸序列、包含抗体的V_L结构域和/或V_H结构域的氨基酸序列、或包含抗CD36抗体的完整轻链和/或重链的氨基酸序列。在至少一个实施方案中,分离的多核苷酸可以编码本文公开的任何抗CD36抗体的包含CDR-H1、CDR-H2和CDR-H3序列的氨基酸序列、或包含CDR-L1、CDR-L2和CDR-L3序列的氨基酸序列。类似地,考虑分离的多核苷酸可以编码包含本公开的抗CD36抗体的V_L结构域或V_H结构域或完整重链(HC)或轻链(LC)的氨基酸序列。

[0245] 在一些实施方案中,本公开还提供了载体(例如,表达载体),其包含编码本公开的抗CD36抗体或抗CD36抗体的片段或结构域的多核苷酸序列(如上所述)。包含用于抗体的重组产生的多核苷酸的此类载体构建体在本领域中是众所周知的。此外,在一些实施方案中,提供了包含具有编码本公开的抗CD36抗体或抗CD36抗体的片段或结构域的序列的多核苷酸或载体的宿主细胞。在至少一个实施方案中,宿主细胞是已用载体转化的细胞,所述载体包含编码包含抗体的V_L结构域的氨基酸序列和/或包含本公开的抗CD36抗体的V_H结构域的氨基酸序列的多核苷酸序列。在另一个实施方案中,宿主细胞已用第一载体和第二载体转化,所述第一载体包含编码包含抗体的V_L的氨基酸序列的多核苷酸序列,以及所述第二载体包含编码包含抗体的V_H的氨基酸序列的核酸。

[0246] 在重组方法的一些实施方案中,使用的宿主细胞是真核细胞,例如中国仓鼠卵巢(CHO)细胞或淋巴样细胞(例如,Y0、NS0、Sp20)。在一个实施方案中,提供了一种制备抗CD36抗体的方法,其中所述方法包括在适合于表达抗体的条件下培养包含编码抗体的核酸的宿主细胞(如上所述),并且任选地从宿主细胞(或宿主细胞培养基)中回收抗体。

[0247] 简而言之,抗CD36抗体的重组产生是通过分离编码抗体的核酸(例如,如本文所述)并将该核酸插入到一个或多个载体中用于在宿主细胞中进一步克隆和/或表达来进行的。使用本领域众所周知的常规程序(例如,通过使用能够特异性地结合编码所需抗体的重链和轻链的基因的寡核苷酸探针),可以容易地分离和测序此类核酸。用于克隆或表达编码抗体的载体的合适的宿主细胞和培养方法是本领域众所周知的,并且包括原核细胞或真核细胞。通常,在表达后,抗体可从细胞浆中以可溶级分被分离并进一步纯化。除了原核生物外,真核微生物如丝状真菌或酵母也是用于编码抗体的载体的合适的克隆或表达宿主,其包括其糖基化通路已被“人源化”的真菌和酵母菌株,这导致具有部分或完全人糖基化模式的抗体的产生(参见例如,Gerngross,Nat.Biotech.22:1409-1414(2004),和Li等人,Nat.Biotech.24:210-215(2006))。

[0248] 用于表达本公开的糖基化抗CD36抗体的合适的宿主细胞也可以源自多细胞生物

(无脊椎动物和脊椎动物)。无脊椎动物细胞的示例包括植物和昆虫细胞。已鉴定出许多杆状病毒株,它们可与昆虫细胞结合使用,特别是用于转染草地贪夜蛾细胞。植物细胞培养物也可用作宿主(参见例如美国专利号5,959,177、6,040,498、6,420,548和7,125,978)。

[0249] 可用于产生本公开的抗CD36抗体的哺乳动物宿主细胞系的示例包括中国仓鼠卵巢(CHO)细胞,其包括DHFR-CHO细胞(参见例如Urlaub等人,Proc.Natl.Acad.Sci.USA 77:4216(1980));骨髓瘤细胞系例如YO、NSO和Sp2/0;由SV40转化的猴肾CV1系(COS-7);人胚胎肾细胞系(293或293细胞,如例如在Graham等人,J.Gen.Virol.36:59(1977)中所述);幼仓鼠肾细胞(BHK);小鼠塞托利细胞(TM4细胞,如例如在Mather,Biol.Reprod.23:243-251(1980)中所述);猴肾细胞(CV1);非洲绿猴肾细胞(VERO-76);人宫颈癌细胞(HELA);犬肾细胞(MDCK);水牛大鼠肝细胞(BRL 3A);人肺细胞(W138);人肝细胞(Hep G2);小鼠乳腺肿瘤(MMT 060562);TR1细胞(参见例如Mather等人,Annals N.Y.Acad.Sci.383:44-68(1982)和US6,235,498);医学研究委员会5(MRC 5)细胞(例如可从ATCC获得并且也称为CCL-171的细胞);以及包皮4(FS-4)细胞(参见例如Vilcek等人,Ann.N.Y.Acad.Sci.284:703-710(1977),Gardner和Vilcek,J.Gen.Virol.44:161-168(1979),以及Pang等人,Proc.Natl.Acad.Sci.U.S.A.77:5341-5345(1980))。对于适用于抗体生产的有用哺乳动物宿主细胞系的一般综述,参见例如Yazaki和Wu,Methods in Molecular Biology,Vol.248(B.K.C.Lo,ed.,Humana Press,Totowa,NJ),pp.255-268(2003)。

[0250] IV.抗CD36抗体的药物组合物和制剂

[0251] 本公开还提供了包含抗CD36抗体的药物组合物和药物制剂。在一些实施方案中,本公开提供了包含如本文所述的抗CD36抗体和药学上可接受的载体的药物制剂。在一些实施方案中,抗CD36抗体是药物组合物的唯一活性剂。可以通过将具有所需纯度的抗CD36抗体与一种或多种药学上可接受的载体混合来制备此类药物制剂。通常,可以将此类抗体制剂制备为水溶液(参见例如美国专利号6,171,586和WO2006/044908)或为冻干制剂(参见例如美国专利号6,267,958)。

[0252] 药学上可接受的载体在所运用的剂量和浓度下通常对接受者无毒。广泛的此类药学上可接受的载体是本领域众所周知的(参见例如,Remington's Pharmaceutical Sciences 16th edition,Osol,A.Ed.(1980))。可用于本公开制剂的示例性的药学上可接受的载体可以包括但不限于:缓冲剂,例如磷酸盐、柠檬酸盐和其他有机酸;抗氧化剂,包括抗坏血酸和蛋氨酸;防腐剂(例如十八烷基二甲基苄基氯化铵;六甲双铵氯化物;苯扎氯铵;苄索氯铵;苯酚;丁醇或苯甲醇;烷基对羟基苯甲酸酯,例如甲基或丙基对羟基苯甲酸酯;儿茶酚;间苯二酚;环己醇;3-戊醇和间甲酚);低分子量(少于约10个残基)多肽;蛋白质,例如血清白蛋白、明胶或免疫球蛋白;亲水性聚合物,例如聚乙烯吡咯烷酮;氨基酸,例如甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、精氨酸或赖氨酸;单糖、二糖和其他碳水化合物,包括葡萄糖、甘露糖或糊精;螯合剂,例如EDTA;糖,例如蔗糖、甘露醇、海藻糖或山梨糖醇;成盐反离子,例如钠;金属复合物(例如Zn-蛋白质复合物);和/或非离子表面活性剂,例如聚乙二醇(PEG)。

[0253] 可用于本公开的制剂中的药学上可接受的载体还可以包括间质药物分散剂,例如可溶的中性活性透明质酸酶糖蛋白(sHASEGP)(参见例如美国专利公开号2005/0260186和2006/0104968),例如人可溶PH-20透明质酸酶糖蛋白(例如rHuPH20或HYLENEX®,

Baxter International,Inc.)。

[0254] 还考虑到本文公开的制剂除了抗CD36之外还可含有活性成分,这对于被施用制剂的受试者中被治疗的特定的适应症是必要的。优选地,任何另外的活性成分具有与抗CD36抗体活性互补的活性,并且这些活性不会相互产生不利影响。

[0255] 在一些实施方案中,药物组合物包含抗CD36抗体和用于癌症治疗的附加活性剂,例如免疫检查点抑制剂。可用于此类实施方案的检查点抑制剂包括但不限于包含对作为免疫检查点分子的抗原的特异性的第二抗体。在一些实施方案中,第二抗体包含对选自PD1、PD-L1、LAG3、CTLA-4、A2AR、TIM-3、BTLA、CD276、CD328、VTCN1、IDO、KIR、NOX2、VISTA、OX40、CD27、CD28、CD40、CD122、CD137、GITR、ICOS的免疫检查点分子的特异性。在至少一个实施方案中,药物组合物包含抗CD36抗体和其他活性剂,其中所述其他活性剂是包含对免疫检查点分子PD1具有特异性的抗体。可用于本文公开的药物组合物实施方案的具有对PD1包含特异性的示例性抗体包括但不限于多司他利单抗、派姆单抗、纳武单抗和匹地利珠单抗。

[0256] 还考虑到,在一些实施方案中,本公开提供了用于疗法中的药物组合物或制剂,其中所述组合物还包含T细胞活化细胞因子或免疫刺激细胞因子。此类细胞因子在免疫疗法领域是众所周知的,其包括但不限于IL-2、IL-7、IL-10、IL-12、IL-15、IL-21和IFN-α。在至少一个实施方案中,免疫刺激细胞因子可以作为抗CD36抗体的融合物被提供在组合物中。

[0257] 活性成分可被包封在例如通过凝聚技术或通过界面聚合制备的微胶囊中,例如,分别在胶体药物递送系统(例如,脂质体、白蛋白微球、微乳剂、纳米颗粒和纳米胶囊)或在大乳剂中的羟甲基纤维素或明胶微胶囊中和聚(甲基丙烯酸甲酯)微胶囊中。此类技术公开于Remington's Pharmaceutical Sciences 16th edition,Osol,A.Ed.(1980)。

[0258] 在一些实施方案中,制剂可以是抗体和/或其他活性成分的缓释制备物。缓释制备物的合适示例包括含有所述抗体的固体疏水聚合物的半透性基质,该基质为成形物品的形式,例如薄膜或微胶囊。

[0259] 通常,待施用于受试者的本公开的制剂是无菌的。无菌制剂可使用众所周知的技术容易地制备,例如通过无菌过滤膜过滤。

[0260] V.用途和治疗方法

[0261] 考虑包含本公开的抗CD36抗体的任何组合物或制剂可用于任何方法或用途,例如在利用其特异性地结合CD36的能力的治疗方法中,从而抑制、降低和/或完全阻断CD36作为涉及免疫调节或信号传导的细胞表面蛋白的功能,特别是CD36调节脂蛋白、脂肪酸和其他配体的摄取的功能,所述其他配体涉及肿瘤细胞、肿瘤相关巨噬细胞(TAM)、髓系衍生抑制细胞(MDSC)、调节性T细胞和CD8 T细胞的存活和维持或存活(或死亡,视情况而定)。例如,调节性T细胞是肿瘤微环境(TME)的主要细胞组分,并且对肿瘤生长和进展有显著贡献,而CD8 T细胞通过杀死肿瘤细胞来帮助控制肿瘤生长。抑制CD36结合可耗尽调节性T细胞,同时增加CD8 T细胞存活和功能,从而诱导抗肿瘤T细胞反应的增加。

[0262] 有一系列疾病、病症和病况可通过抑制、降低和/或完全阻断CD36的免疫调节和/或免疫信号传导活性(特别是CD36对TAM的影响)被潜在治疗。疾病、病症和病况包括但不限于癌症,所述癌症包括但不限于结肠癌、胰腺癌、卵巢癌、HCC、肾癌、乳腺癌、肺癌、胃癌、黑色素瘤、头颈癌或口腔癌。考虑包含本公开的抗CD36抗体的任何组合物或制剂可用于治疗任何上文列出的癌症的方法或用途。在一些实施方案中,所述癌症选自结肠癌、胰腺癌、卵

巢癌、HCC、肾癌、乳腺癌、肺癌、胃癌、黑色素瘤、头颈癌或口腔癌。在一些实施方案中,本公开提供了一种治疗受试者癌症的方法,所述方法包括向有需要的受试者施用治疗有效量的本公开的抗CD36抗体或向受试者施用治疗有效量的包含本公开的抗CD36抗体和药学上可接受的载体的药物组合物。

[0263] 如本文所公开的,包括在以下实施例中,本公开的抗CD36抗体具有降低、抑制和/或阻断配体与CD36结合的能力,从而改变由CD36介导的免疫信号传导通路。因此,在一些实施方案中,本公开提供了一种治疗受试者中CD36介导的疾病或病况的方法,所述方法包括向受试者施用治疗有效量的本公开的抗CD36抗体或向有需要的受试者施用治疗有效量的包含本公开的抗CD36抗体和药学上可接受的载体的药物组合物。类似地,在一些实施方案中,本公开提供了一种治疗受试者中由与细胞上表达的CD36结合介导的疾病的方法,所述方法包括向受试者施用治疗有效量的本公开的抗CD36抗体或向有需要的受试者施用治疗有效量的包含本公开的抗CD36抗体和药学上可接受的载体的药物组合物。

[0264] 根据治疗方法施用抗CD36抗体、组合物或药物制剂提供抗体诱导的治疗效果,其保护受试者免受受试者中CD36介导的疾病的进展和/或治疗受试者中CD36介导的疾病的进展。在一些实施方案中,治疗方法可以进一步包括施用对本领域技术人员已知的一种或多种另外的治疗剂或治疗以预防和/或治疗CD36介导的疾病或病况。包括施用一种或多种另外的药剂的此类方法可以涵盖组合施用(其中两种或多种治疗剂被包括在相同或分开的制剂中)和分开施用,在这种情况下,抗体组合物或制剂的施用可以在附加治疗剂施用之前、同时和/或之后进行。

[0265] 在本公开的治疗方法的一些实施方案中,抗CD36抗体或包含抗CD36抗体的药物制剂通过将药剂全身递送或递送至所需靶组织的任何施用方式施用于受试者。全身施用通常是指将抗体施用于受试者的位点而非直接施用于所需的靶位点、组织或器官的任何方式,使得抗体或其制剂进入受试者的循环系统,从而经历代谢和其他类似过程。

[0266] 因此,可用于本公开的治疗方法的施用方式可以包括但不限于注射、输注、滴注和吸入。通过注射施用可以包括静脉内、肌肉内、动脉内、鞘内、心室内、囊内、眶内、心内、皮内、腹膜内、气管内、皮下、表皮下、关节内、囊下、蛛网膜下腔、椎管内、脑内脊髓和胸骨内注射以及输注。

[0267] 在一些实施方案中,抗CD36抗体的药物制剂被配制为使得抗体在肠道中免于失活。因此,治疗方法可以包括口服施用所述制剂。

[0268] 在一些实施方案中,还提供了包含本公开的抗CD36抗体的组合物或制剂作为药物的用途。此外,在一些实施方案中,本公开还提供了包含抗CD36抗体的组合物或制剂在制造或制备药物中的用途,特别是用于治疗、预防或抑制CD36介导的疾病的药物。在进一步的实施方案中,所述药物是用于治疗、预防或抑制CD36介导的疾病的方法,所述方法包括向患有CD36介导的疾病的个体施用有效量的所述药物。在某些实施方案中,所述药物还包含有效量的至少一种附加治疗剂或治疗。

[0269] 在至少一个实施方案中,考虑可以与本公开的抗CD36抗体一起用于此类药物中的附加治疗剂或治疗可以包括但不限于包含对免疫检查点分子的特异性的治疗性抗体,例如PD1、PD-L1、LAG3、CTLA-4、A2AR、TIM-3、BTLA、CD276、CD328、VTCN1、IDO、KIR、NOX2、VISTA、OX40、CD27、CD28、CD40、CD122、CD137、GITR、ICOS。包含对免疫检查点分子具有特异性的示

例性抗体包括但不限于选自多司他利单抗、派姆单抗、纳武单抗和匹地利珠单抗的抗PD1抗体。

[0270] 在进一步的实施方案中,所述药物是用于在受试者中治疗、抑制或预防CD36介导的疾病,例如癌症,所述方法包括向所述受试者施用有效量的药物以治疗、抑制或预防CD36介导的疾病。

[0271] 本公开的组合物和制剂中含有的抗CD36抗体的适当剂量(当单独使用或与一种或多种其他另外的治疗剂组合使用时)将取决于所治疗的特定疾病或病况、疾病的严重程度和进程、施用抗体是用于预防目的还是治疗目的、施用于患者的先前的疗法、患者的临床病史和对抗体的反应以及主治医生的判断。本文所述组合物和制剂中包含的抗CD36抗体可以一次或在一系列治疗中适当地施用于患者。本文考虑了各种给药方案,其包括但不限于在不同时间点的单次或多次施用、推注施用和脉冲输注。

[0272] 取决于疾病的类型和严重程度,本公开的制剂中约1μg/kg至15mg/kg的抗CD36抗体是用于施用于人类受试者的初始候选剂量,无论是例如通过一次或多次分开施用,还是通过连续输注。通常,抗体的施用剂量会在从约0.05mg/kg至约10mg/kg的范围内。在一些实施方案中,可向患者施用约0.5mg/kg、2.0mg/kg、4.0mg/kg或10mg/kg(或其任何组合)的一个或多个剂量。

[0273] 可以维持剂量施用持续数天或更长时间,这取决于受试者的状况,例如,施用可以持续直至CD36介导的疾病得到充分治疗,如通过本领域已知的方法所确定的。在一些实施方案中,可施用初始较高的负荷剂量,随后施用一个或多个较低的剂量。然而,其他剂量方案可能是有用的。可以通过常规技术和测定法来监测剂量施用的治疗效果的进展。

[0274] 因此,在本公开的方法的一些实施方案中,抗CD36抗体的施用包含从约1mg/kg至约100mg/kg的日剂量。在一些实施方案中,抗CD36抗体的剂量包含至少约1mg/kg、至少约5mg/kg、至少约10mg/kg、至少约20mg/kg或至少约30mg/kg的日剂量。

[0275] 实施例

[0276] 本公开的各种特征和实施方案在以下代表性实施例中进行了说明,这些实施例旨在说明性而非限制性。本领域技术人员会容易地理解,具体实施例仅用于阐述本发明,如随后的权利要求书中更全面地描述的。本申请中描述的每个实施方案和特征应理解为可与其中含有的每个实施方案互换和组合。

[0277] 实施例1:抗CD36抗体产生和CD36结合分析

[0278] 本实施例阐述了使用噬菌体展示抗体库技术产生特异性结合人类和小鼠CD36的本公开的示例性抗CD36抗体。

[0279] A.从噬菌体展示抗体库中选择抗CD36 scFv结合物

[0280] 下面简要描述筛选程序。首先,将PBS缓冲液(pH 7.4)中的人类CD36.ECD抗原(每孔5μg,Sino Biological)在4℃下包被到96孔板(NUNC Maxisorb免疫板)的孔上过夜,然后用5%脱脂牛奶的PBST[0.1%(v/v)Tween 20]封闭1小时。封闭后,将100μL浓缩的噬菌体文库(PBS缓冲液中1013cfu/mL)与100μL封闭缓冲液混合,然后在轻轻振荡下加入到每个孔中持续1小时。将板用PBST洗涤12次,并用PBS洗涤3次。使用100μL的0.1M HCl/甘氨酸(pH 2.2)/孔洗脱结合的噬菌体,然后立即用20μL的1M Tris-碱缓冲液(pH 9.0)中和。将洗脱的噬菌体与1mL大肠杆菌ER2738(A600 nm=0.6)在37℃下混合30分钟;通过加入氨苄西林清

除未感染的细菌。在氨苄西林处理30分钟后,在37℃下用100μL M13KO7辅助噬菌体(总计~1011CFU)感染细菌培养物1小时,然后在37℃下将其加入到50mL的含有卡那霉素50μg/mL和氨苄西林100μg/mL的2X YT培养基中剧烈振荡过夜。用20%聚乙二醇/NaCl沉淀获救的噬菌体文库,并在PBS中重悬。浓缩的噬菌体溶液用于下一轮筛选。

[0281] 在3~4轮选择-扩增循环后,将单个菌落随机选入深孔96孔培养板(板A;分泌型scFv);每孔含有950μL 2YT(100μg/mL氨苄西林)。在37℃下振荡孵育3小时后,将50μL细菌培养物转移至新鲜的深孔96孔板(板B;噬菌体形式)的相应孔中;每孔含有具有100μg/mL氨苄西林的0.8mL 2YT。同时,向板B的每个孔中加入50μL M13KO7(总计~5×1010CFU)。孵育1小时后,向板A的每个孔中加入100μL含有IPTG(10mM)的2YT;向板B的每个孔中加入100μL含有卡那霉素(500μg/mL)的2YT。在37℃下剧烈振荡孵育过夜后,将培养物在4℃下3000g离心10分钟。将板B保存以用于进一步测序测定。对于分泌型scFv培养板(板A),向三块分别预包被有蛋白质L(0.1μg/孔)、人CD36(0.5~1μg/孔)和牛血清白蛋白(BSA)(2μg/孔)的96孔板的相应孔中加入100μL培养基和100μL 5% PBST牛奶,用5%PBST牛奶封闭。在室温下孵育1小时后,用PBST洗涤板3次。将100μL蛋白质A-HRP(Thermo Scientific)加入到蛋白质L包被的免疫板的每个孔中;将100μL抗E标签HRP(ICL Inc.)加入到人CD36抗原包被和BSA包被的板的每个孔中。孵育1小时后,将板用PBST缓冲液洗涤三次,并用PBS洗涤两次,用3,3',5,5'-四甲基联苯胺过氧化物酶底物(Kirkegaard&Perry Laboratories)显影3分钟,用1.0M HCl淬灭并在450nm处进行分光光度读数。

[0282] 阳性克隆按以下标准选择:对人CD36抗原包被的孔(抗原结合阳性),ELISA OD450>0.2;对BSA包被的孔(非特异性结合阴性),OD450<0.05;对蛋白质L包被的孔(可溶性scFv与蛋白质L和蛋白质A二者结合以确保在溶液中正确折叠),OD450>0.5,然后进行DNA测序。从噬菌体展示筛选中获得的示例性抗CD36抗体12P109(SEQ ID NO:1)的scFv的多核苷酸序列在表2和随附的序列表中提供。进一步筛选由源自不同抗CD36抗体的"改组"LC序列和12P109的HC序列组成的噬菌体展示文库,提供了示例性抗CD36抗体A8A(SEQ ID NO:10)的scFv,其也列于表2和随附的序列表中。对A8A抗体V_L结构域(SEQ ID NO:11)的CDR进一步进行标准的基于PCR的诱变以提供A8A-N52T的示例性抗CD36抗体VL结构域(SEQ ID NO:14),其也列于表2和随附的序列表中。B.全长IgG形式的抗CD36抗体的产生

[0283] scFv重新格式化和克隆:将从噬菌体展示筛选中选择的scFv的CD36结合决定簇通过使用限制酶切位点MluI/NheI和BsiWI/DraIII分别将片段的V_H和V_L结构域克隆到人IgG1-N297A重链载体和人kappa轻链载体中来重新格式化为全长IgG抗体。使用以下正向和反向寡核苷酸引物对来扩增V_H和V_L结构域:(1)PhageLib_VL_Fw:5'-AATCACgATgTgATATTCAAATgACCCAgAgCCCgAgC-3'(SEQ ID NO:64),(2)PhageLib_VL_Rv:5'-AATCgTACgTTTgATTTCCACTTTggTgCCTTg-3'(SEQ ID NO:65),(3)PhageLib_VH_Fw:5'-AATACgCgTgTCCTgTCCgAAgTgCAgCTggTggAATCg-3'(SEQ ID NO:66),以及(4)PhageLib_VH_Rv:5'-AATgCTAgCCgAgCTCACggTAACAAg-3'(SEQ ID NO:67)。

[0284] 全长抗体的表达和纯化:使用重新格式化的抗CD36抗体基因克隆的载体在ExpiCHO-S细胞(Thermo Scientific)中瞬时表达。在指数生长阶段,用ExpiCHO表达培养基将ExpiCHO-S细胞稀释至最终密度为6×10^6细胞/mL。根据手册使用冷试剂制备ExpiFectamine CHO/质粒DNA复合物,在室温下孵育1-5分钟,然后缓慢加入到细胞中。转染

后一天,向培养物中补充ExpiFectamine CHO增强剂并再孵育7天。将转染的细胞上清液离心,随后通过0.22μm过滤器过滤。用蛋白质A珠(Cytiva,17127903)从转染的细胞上清液中纯化抗体。用50倍柱体积的PBS洗涤抗体负载柱,然后用10倍珠体积的0.1M甘氨酸(pH 3)直接洗脱到1/10体积的1M Tris缓冲液(pH 9.0)中。用含有0.1M精氨酸的PBS(pH 7.4)对洗脱液进行缓冲液交换和浓缩。在有和没有还原剂的情况下使用SDS-PAGE测定纯化的抗CD36抗体的质量。

[0285] 结果:对SDS-PAGE图像的检查显示表达和纯化得到了纯化的全长抗CD36抗体。

[0286] C.抗CD36抗体的优化

[0287] 为进一步提高抗CD36抗体A8A的成药性,将由N52、H91和L94的突变组成的两个噬菌体展示文库用于筛选。将从噬菌体展示筛选中选择的scFv克隆117通过使用限制酶切位点MluI/NheI和BsiWI/DraIII分别将片段的V_H和V_L结构域克隆到人IgG1-N297A重链载体和人kappa轻链载体中来重新格式化为全长IgG抗体。使用以下正向和反向寡核苷酸引物对来扩增V_H和V_L结构域:(1)PhageLib_VL_Fw:5'-AATCACgATgTgATATTCAAATgACCCAgAgCCCgAgC-3'(SEQ ID NO:64),(2)PhageLib_VL_Rv:5'-AATCgTACgTTTgATTTCCACTTTggTgCCTTg-3'(SEQ ID NO:65)(3)PhageLib_VH_Fw:5'-AATACgCgTgTCCTgTCCgAAgTgCAgCTggTggAATCg-3'(SEQ ID NO:66),以及(4)PhageLib_VH_Rv:5'-AATgCTAgCCgAgCTCACggTAACAAg-3'(SEQ ID NO:67)。

[0288] 117抗体的CDR-H1、H2、H3和CDR-L1序列与A8A的CDR-H1、H2、H3和CDR-L1序列相同,并且在CDR-L2中存在N52T突变,在CDR-L3中存在L94A突变。使用限制酶切位点NheI和BamHI将117抗体的Fab片段也构建到人IgG4-S228P/F234A/L235A重链载体中。对117抗体的VH和VL结构域的CDR进行进一步的基于PCR的诱变,以产生一系列变体,如下表3中所总结的。

[0289] 表3

[0290]

	V_H								V_L
	CDR-H1				CDR-H2			CDR-H3	CDR-L3
	T28	S30	S31	F32	Y54	Y57	T58	F101	S92

[0291]

117 变体名称	单突变变体								
117_28A 117_28D	A D								
117_30A 117_30D		A D							
117_31A 117_31D			A D						
117_32A 117_32D				A D					
117_54E					E				
117_57A 117_57D 117_57E 117_57R 117_57T						A D E R T			
117_58E							E		
117_101A 117_101D 117_101H 117_101K 117_101S 117_101Y								A D H K S Y	
117_92A 117_92E									A E
	双突变								
117_30AA		A	A						
117_30AT		A	T						
117_30DA		D	A						
117_30DT		D	T						
117_30DE		D				E			
117_30FA		F	A						

[0292]

117_30FT		F	T						
117_31AD			A	D					
117_31AT			A	T					
117_57DE						D	E		
117_57EE						E	E		
	三突变								
117_AA57D		A	A			D			
117_AA57E		A	A			E			
117_DA57D		D	A			D			
117_DA57E		D	A			E			

[0293] 如上所述,117抗体和变体被表达和纯化为全长抗体。对SDS-PAGE图像的检查显示表达和纯化得到了纯化的全长抗CD36抗体。117抗体和示例性变体117_30AA、117_30DA、117_57D、117_57E和117_DA57E的CDR、VH、VL、HC、LC区域的氨基酸序列也列于表2和随附的序列表中。

[0294] D.抗CD36抗体对CD36特异性结合的ELISA

[0295] ELISA材料和方法:将重组的His标记的人类CD36.ECD蛋白或His标记的小鼠CD36.ECD蛋白(均来自Sino Biological)以在包被溶液(SeraCare)中1μg/mL的浓度固定在96孔微量滴定板上,在4℃下过夜。用洗涤液(0.05%Tween20的PBS溶液)洗涤孔,并用1%BSA的PBS溶液封闭。将抗CD36抗体的连续稀释液加入到孔中。在37℃下孵育1小时后,用洗涤液洗涤孔。将过氧化物酶缀合的山羊抗人kappa轻链抗体(Sigma)应用至每个孔,在37℃下孵育1小时。洗涤后,在室温下用TMB底物使孔显色5-10分钟,然后用1N HCl终止。之后在450nm和650nm处测量吸光度。通过GraphPad Prism7计算EC50值。

[0296] 结果:图1A和图1B分别描绘了显示全长IgG抗CD36抗体12P109和另外的阳性克隆12P102、12P103、12P104、12P105、12P106、12P107、12P110和12P212与人CD36.ECD或小鼠CD36.ECD结合的ELISA数据图。标记有12P109、12P103和12P212的抗体的结合曲线显示出对人CD36(hCD36,参见图1A)的最高的亲和力。12P109还显示出对小鼠CD36(mCD36,参见图1B)的高结合亲和力。由ELISA数据计算出的EC50值如下表4所示。

[0297] 表4

[0298]

mAb	EC_{50} (nM)	
	hCD36	mCD36
12P102	>1000	NA
12P103	0.222	5.987
12P104	0.688	9.182
12P105	3.907	>400
12P106	3.87	6.81
12P107	2.246	11.87
12P109	0.213	0.137
12P110	74.62	0.508
12P212	4.217	>500

[0299] 图1C和1D分别描绘了显示抗CD36抗体与包被在ELISA板上的重组人CD36.ECD或小鼠CD36.ECD结合的ELISA数据图,其应用了特异性结合CD36的全长人IgG1形式的抗CD36抗体12P109和A8A,和商业小鼠IgA抗体D2712(克隆CRF D-2712,BD Biosciences,US)。来自图1C和图1D的曲线的EC50值示于下表5中。

[0300] 表5

[0301]

mAb	EC_{50} (nM)	
	hCD36	mCD36
12P109	43.24	9.45
A8A	5.17	0.94
D2712	>500	0.93

[0302] 图2A、图2B、图2C、图2D、图2E和图2F描绘了显示全长IgG抗CD36抗体12P109、117和表3中列出的各个117变体与人CD36.ECD的结合的ELISA数据图。竞争物抗CD36抗体ONA,其源自如WO2021176424A1中公开的hIgG4-S228P-FALA形式的克隆“ONA-0-v1”。由图2A-2F的ELISA数据计算出的EC50值示于下表7中。

[0303] 表7

[0304]

mAb	hCD36 EC_{50} (nM)	mAb	hCD36 EC_{50} (nM)
图 2A		图 2D	
12P109	1.53	117	0.571
117	0.0475	117_30DA	0.0803
117_57D	0.0529	117_DA57D	0.0490
117_57E	0.0492	117_DA57E	0.0539
117_57R	0.0695	117_101A	0.0751
117_57A	1.52	117_101K	1.46
117_57T	NA	ONA	0.167
117_58E	0.422		
图 2B		图 2E	
117	0.102	117	0.0932
117_28A	0.129	117_101D	NA
117_28D	0.0735	117_101H	1.60
117_30A	0.0964	117_101Y	0.355
117_30D	0.141	117_101S	0.929
117_31A	0.0991		
117_31D	0.0375		
117_32D	0.0276		
图 2C		图 2F	
117	0.571	117	0.124
117_30AA	0.145	117_57E	0.206
117_30AT	0.247	117_DA57E	0.190
117_30DA	0.137	117_AA57D	0.192
117_30DT	0.103	117_AA57E	0.251
117_30FA	0.202		
117_30FT	0.254		

[0305] F.SEC-UPLC分析

[0306] 如通过SEC-UPLC分析测定的抗体的延长保留时间(RT)可能与非特异性疏水相互作用有关,这会为用于治疗用途的所述抗体的开发创造风险。因此,通过SEC-UPLC分析抗CD36抗体12P109、A8A、117和117变体以及IgG标准(“STD”,BEH200 SEC蛋白质标准混合物)和对照单克隆抗体(“ctl Ab”)的RT增加,其表明蛋白质聚集体的形成。

[0307] 材料和方法:在蛋白质A纯化后,通过标准尺寸排阻UPLC(SEC-UPLC)分析抗CD36抗体12P109、A8A、117和117变体的蛋白质聚集体和保留时间。将3μg抗体应用于UPLC(Waters ARC UPLC),并在凝胶过滤柱(Waters,Xbridge BEH450 SEC 4.6×150)上使用PBS流动相完成分离。在280处监测抗体峰紫外吸光度,并使用Empower软件确定峰面积。

[0308] 结果:SEC-UPLC结果的图示于图3A、图3B、图3C、图3D和图3E中,一并示出了测定的RT值。对照抗体表现出7.243分钟的RT值,而抗CD36抗体12P109和117均表现出相对增加的RT值。然而,117的增加的RT值由于变体117_30DA、117_31AD、117_57D、117_57E、117_57EE、117_101D和117_DA57E中存在的突变而显著降低。如在SEC-UPLC实验中测定的抗CD36抗体的RT值提供于下表8中。此外,通过UPLC分析在117_DA57E中发现了一些聚集体。聚集体可能与117_DA57E对酸性洗脱条件不太稳定有关。IgG4中的R409K突变已被证明会增加CH3结构域相互作用强度并降低在低pH下聚集的趋势。SEC-UPLC分析表明,将R409K加入到117_DA57E中会减少由酸性条件造成的聚集。

[0309] 表8

[0310]

mAb	保留时间	mAb	保留时间
图 3A		图 3C	
STD IgG	6.81 min	STD IgG	6.81 min
Ctl Ab	7.24 min	Ctl Ab	7.12 min
12P109	8.98 min	12P109	9.24 min
A8A	10.82 min	117	9.65 min
117_28A	9.40 min	117_57D	7.62 min
117_30A	9.49 min	117_57E	7.60 min
117_31A	9.43 min	117_57A	8.47 min
117_32A	7.99 min	117_57R	8.87 min
117_28D	8.26 min	117_57T	8.17 min

[0311]

117_31D	7.93 min	117_58E	8.41 min
图 3B		图 3D	
STD IgG	6.81 min	STD IgG	6.87 min
117	8.93 min	Ctl Ab	7.19 min
117_30DA	7.97 min	12P109	10.64 min
117_30DT	8.02 min	117	11.46 min
117_30FA	13.22 min	117_DA57E	7.42 min
117_30FT	13.56 min	117_30DE	7.41 min
117_31AD	7.58 min	117_AA57E	9.53 min
117_31AT	8.22 min		
图 3E			
ONA	7.21 min		
117_57EE	7.41 min		
117_DA57E	7.22 min		
117_DA57E (R409K)	7.21 min		

[0312] G.CD36结合动力学的BLI分析

[0313] BLI试验的材料和方法:通过生物层干涉法(BLI)(ForteBio Octet RED96)测量抗CD36抗体与人CD36结合的动力学速率常数ka和kd。使用AHC(抗hIgG Fc捕获)生物传感器(ForteBio)进行BLI测定,以捕获每种抗CD36抗体(5μg/mL)以获取0.5nm的偏移,然后将生物传感器浸入变化的浓度(即0、1.5625、3.125、6.25、4.94、12.5、25、50和100nM)的重组His标记的人CD36.ECD蛋白(Sino Biological)在运行缓冲液中,所述运行缓冲液包含在PBS中的0.1% BSA、0.1% Tween-20、250mM NaCl。通过结合反应的曲线拟合分析(1:1Langmuir模型)计算出速率常数,为2.5分钟的结合相互作用时间和5分钟的解离相互作用时间。

[0314] 结果:下表10提供了如在单独的BLI分析实验中测定的抗CD36抗体12P109、A8A、117和各种117变体(以IgG1或IgG4格式)与人CD36结合的测定的解离常数KD和动力学速率常数ka和kd。

[0315] 表10

[0316]

	hCD36			
	KD	**Ka**	kd	**R2**

[0317]

	(M)	(1/Ms)	(1/s)	
A8A (IgG1)	3.42E-09	1.35E+06	4.62E-03	0.972
117 (IgG1)	4.79E-09	4.45E+05	2.13E-03	0.9916
117_31A (IgG1)	4.05E-09	4.49E+05	1.82E-03	0.9931
117_31A (IgG4)	4.32E-09	4.75E+05	2.05E-03	0.9928
117_30AA (IgG4)	5.74E-09	4.91E+05	2.82E-03	0.9964
12P109 (IgG1)	1. 85E-08	5.22E+05	9.63E-03	0.9538
117 (IgG1)	6.72E-09	3.50E+05	2.35E-03	0.9739
117_30DA (IgG4)	3.91E-09	3.39E+05	1.33E-03	0.9859
117_54E (IgG1)	4.16E-09	9.78E+05	4.07E-03	0.8375
117_57D (IgG1)	4.69E-09	3.73E+05	1.75E-03	0.9795
117_57E (IgG1)	4.93E-09	3.70E+05	1.82E-03	0.9785
117 (IgG4)	6.49E-09	3.86E+05	2.50E-03	0.9829
117_57D (IgG4)	4.96E-09	3.36E+05	1.67E-03	0.9864
117_57E (IgG4)	5.28E-09	3.25E+05	1.71E-03	0.986
117_DA57D (IgG4)	2.64E-09	3.17E+05	8.38E-04	0.9938
117_DA57E (IgG4)	2.52E-09	3.19E+05	8.03E-04	0.994
117_57EE (IgG1)	4.27E-09	3.64E+05	1.55E-03	0.9891
117_30DE (IgG4)	3.09E-09	3.17E+05	9.81E-04	0.9919

[0318] 实施例2:由抗CD36抗体与细胞表面表达的CD36的结合

[0319] 本实施例阐述了在细胞表面过表达hCD36或mCD36的稳定F293细胞系的制备,以及测定本公开的示例性抗CD36抗体与细胞表面表达的人、恒河猴或小鼠CD36的结合亲和力的研究。

[0320] A.过表达CD36的稳定F293细胞系的产生

[0321] 材料和方法:使用XbaI/HindIII将编码全长人CD36-Flag(Sino Biological)、恒河猴CD36-His(Sino Biological)和小鼠CD36-His(Sino Biological)的基因片段亚克隆到pcDNA3.4拓扑载体中。将Freestyle 293-F细胞(Thermo Scientific)通过聚乙烯亚胺(PEI)方法用编码CD36的质粒转染并用遗传霉素(Thermo Scientific)选择以建立CD36稳定细胞池。人CD36的表达通过抗CD36(克隆5-271,Biolegend)的表面染色或通过抗FLAG的

细胞内染色来验证。恒河猴CD36的表达通过抗CD36克隆117的表面染色或通过抗His的细胞内染色来验证。小鼠CD36的表达通过抗CD36抗体D2712(克隆CRF D-2712;BD Biosciences,US)的表面染色或通过抗His的细胞内染色来验证。为了富集表达CD36的稳定细胞系,分别用抗CD36(克隆5-271,Biolegend)、抗CD36克隆117或抗CD36 D2712(克隆CRF D-2712,BD Biosciences)对过表达hCD36、恒河猴CD36或mCD36的F293细胞池进行染色。通过FACSAria IIIu(BD)对高CD36的细胞群进行挑选。

[0322] 结果:对FACS数据的分析证实,稳定的F293细胞F293/hCD36、F293/恒河猴CD36和F293/mCD36过表达能够与抗CD36抗体结合的表面CD36。

[0323] B.抗CD36抗体的细胞表面CD36结合活性

[0324] 材料和方法:如上文A部分所描述产生的高表达CD36的F293细胞与抗CD36抗体117和117的几种变体(参见表3所列)的一系列稀释物在4℃下孵育30分钟。用FACS缓冲液(2%FBS的PBS溶液)洗涤后,用抗人IgG-Alexa Fluor 647对细胞进行染色,并通过Attune NxT流式细胞仪(Thermo Scientific)分析。

[0325] 结果:以几何平均值(MFI)表示的流式细胞术数据的分析证实,抗CD36抗体特异性地结合表面表达hCD36和mCD36的细胞。

[0326] 图4A、图4B、图4C、图4D和图4E中描绘了显示全长IgG抗CD36抗体117和几种117变体与表达人、恒河猴或小鼠CD36的F293细胞的表面结合的数据图。来自图4A-4E曲线的EC_{50}值示于下表11中。

[0327] 表11

[0328]

mAb	EC_{50} (μg/mL)		
	hCD36	rhesus CD36	mCD36
117	0.180	0.886	0.460
117_57D	0.111	0.397	0.309
117_57E	0.110	0.419	0.331
117_30DE	0.107	0.403	0.342
117_57EE	0.093	0.407	0.319
117_DA57D	0.089	0.416	0.271
117_DA57E	0.109	0.563	0.425
117	0.123	0.582	0.361
117_30DA	0.157	1.041	0.488
117_57E	0.091	0.362	0.279
117_57DE	0.129	0.529	0.447
117_DA57E	0.0968	0.671	0.375

[0329] 实施例3:抗CD36抗体的氧化LDL的摄取阻断活性

[0330] 本实施例阐述了测定本公开的示例性抗CD36抗体阻断表达CD36的细胞的氧化LDL摄取的能力的研究。

[0331] A.通过抗CD36抗体对U937细胞中氧化LDL结合和摄取的抑制

[0332] 将高表达CD36的U937细胞与对照IgG或抗CD36抗体在4℃下预孵育30分钟。为了测量oxLDL的结合或摄取,将Dil-oxLDL(5-10μg/mL)(货号770232-9;Kalen Biomedical)(一种纯化的人LDL,其已被硫酸铜(II)氧化并用“Dil”(1,1’-双十八烷基-3,3,3’,3’-四甲基吲哚菁高氯酸盐)标记)加入无血清培养基中,并且分别在4℃下孵育2小时或在37℃下孵育5分钟。用PBS洗涤后,通过Attune NxT流式细胞仪(Thermo Scientific)分析细胞。

[0333] 观察到本公开的抗CD36抗体与U937细胞的结合导致特征性偏移,证实了U937的表面CD36的内源性表达。对提取的U937流式细胞术数据的分析证实,当与本公开的抗CD36抗体12P103、12P110或12P109孵育时,相比于IgG同种型对照,U937细胞的oxLDL结合(图5A)和oxLDL摄取(图5B)有所减少。

[0334] B.通过抗CD36抗体对F293/CD36细胞中氧化LDL的摄取的抑制F293/hCD36细胞与对照IgG或抗CD36抗体在4℃下预孵育30分钟。为了测量oxLDL的结合或摄取,将Dil-oxLDL(5μg/mL)(Kalen Biomedical)加入无血清培养基中,并分别在4℃下孵育2小时以测量“oxLDL结合”,或在37℃下孵育5分钟以测量“oxLDL摄取”。用PBS洗涤后,用Attune NxT流式细胞仪(Thermo Scientific)分析细胞制备物。

[0335] 结果:如图6A、图6B和图6C中描绘的流式细胞术数据图所示,抗CD36抗体12P109、117、117_30DA、117_57D、117_57E、117_30DE、117_57EE、117_DA57D或117_DA57E的存在有效地阻断了在其表面上过表达人CD36的F293细胞的氧化LDL摄取。此外,如图6C中的图所示,抗CD36抗体12P109、117_30DA、117_57D或117_57E的存在有效地阻断了在其表面上过表达小鼠CD36的F293细胞的oxLDL摄取。针对确定抑制F293/hCD36或F293/mCD36细胞的oxLDL摄取的这些抗CD36抗体的IC_{50}值示于下表13中。

[0336] 表13

[0337]

mAb	oxLDL 摄取 IC50 (nM)	
	F293/hCD36	F293/mCD36
12P109	0.401	0.860
117_30DA	0.691	1.07
117_57D	0.349	0.783
117_57E	0.419	0.796
117	0.702	n.d.
117_30DE	0.299	n.d.
117_57EE	0.348	n.d.
117_DA57D	0.332	n.d.
117_DA57E	0.306	n.d.

[0338] 实施例4:抗CD36抗体在阻断TIL的oxLDL摄取中的活性

[0339] 本实施例阐述了抗CD36抗体在小鼠肿瘤浸润淋巴细胞(TIL)中阻断氧化LDL的摄取的研究。

[0340] 材料和方法:将小鼠结肠癌细胞系CT-26(2×10^5个细胞,ATCC编号:CRL-2638)或小鼠肝癌细胞系BNL 1MEA.7R.1(5×10^6个细胞,ATCC编号:TIB-75)皮下注射至BALB/c小鼠右侧腹侧。将小鼠结肠癌细胞系MC38(1×10^6个细胞,Kerafast#ENH204-FP)、小鼠黑色素瘤细胞系B16-F10(1×10^6个细胞,BCRC#60031)或小鼠肺癌细胞系LL/2(2×10^5个细胞,BCRC#60050)皮下注射至C57BL/6小鼠右侧腹侧。4周后收获CT-26肿瘤。2周后收获B16-F10、BNL 1MEA.7R.1、MC38和LL/2肿瘤。根据制造商的说明书,使用小鼠肿瘤分离试剂盒(Miltenyi Biotec)从实体肿瘤中制备肿瘤细胞悬浮液。使用CD45微珠(Miltenyi Biotec)从肿瘤细胞悬浮液中分离CD45+TIL。

[0341] 分离的CD45+TIL与对照IgG或抗CD36抗体(5-10μg/mL)在4℃下预孵育30分钟。将Dil-oxLDL(5-10μg/mL)加入含有1%无脂肪酸BSA的RPMI培养基中,并在37℃下孵育15分钟。用PBS洗涤后,通过Attune NxT流式细胞仪(Thermo Scientific)分析细胞。通过流式细胞术分析oxLDL的结合和摄取,并基于置于阴性对照细胞上的直方图门控以百分比(%)阳性细胞表示。

[0342] 使用平均值±SD表示数据。*p<0.05,**p<0.005,单向方差分析。

[0343] 结果:如图7A中描绘的结果图所示,本公开的特异性地结合hCD36和mCD36的抗CD36抗体12P109和A8A对分离自注射到BALB/c小鼠的CT26肿瘤的CD45+TIL中的oxLDL摄取提供了约35%的抑制。抑制水平相当于在特异性地结合小鼠CD36的市售抗体D2712观察到的抑制水平。

[0344] 如图7B、图7C、图7D和图7E中描绘的结果图所示,抗CD36抗体117_57E、117_30DA、117_57DE和117_DA57E显示出对来自B16-F10、BNL 1MEA.7R.1、MC38和LL/2肿瘤的CD45+TIL中oxLDL摄取的抑制活性。

[0345] 实施例5:M2巨噬细胞极化和活化阻断活性

[0346] 进行本研究以测定本公开的抗CD36抗体抑制M2巨噬细胞极化和活化的能力。

[0347] 材料和方法:为了产生单核细胞衍生的巨噬细胞,从PBMC分离人CD14+单核细胞,并在补充有10% FBS和20ng/ml CSF1的RPMI1640中以2×10^6细胞/ml培养6天。然后通过EDTA分离收集粘附的巨噬细胞并接种在24孔板中以进一步极化。对于M0巨噬细胞,将细胞在培养基中培养2天。为了极化M2巨噬细胞,将巨噬细胞在含有50ng/mL IL-4和50ng/mL IL-13的培养基中再培养2天。在M2巨噬细胞极化期间加入10μg/mL对照IgG或抗CD36抗体。对于oxLDL活化,将巨噬细胞与对照IgG或抗CD36抗体预孵育10分钟,然后在含有10μg/mL oxLDL(Kalen Biomedical)的M2培养基中再培养2天。为了检测M2巨噬细胞活化,收获巨噬细胞并用抗CD206、CD301和PDL2的抗体染色。通过Attune NxT流式细胞仪(Thermo Scientific)分析表面标志物的水平。

[0348] 结果:如图8A和图8B中描绘的数据图所示,抗CD36抗体117_30AA在CD206+/CD301+(图8A)和CD206+/PDL2+(图8B)双阳性细胞群中均表现出对M2巨噬细胞极化的非常强的抑制。如图8C、图8D、图8E和图8F中描绘的数据图所示,抗CD36抗体117_30DA、117_57D、117_57E和117_DA57E在CD206+/CD301+(图8C、图8D)和CD206+/PDL2+(图8E、图8F)双阳性细胞群中均表现出对oxLDL诱导的M2巨噬细胞活化的非常强的抑制。如图8D和图8F中描绘的数据图所示,与IgG对照和竞争物抗CD36抗体"ONA"相比,抗CD36抗体117_30DA和117_DA57E表现出对oxLDL诱导的M2巨噬细胞活化的非常强的抑制,所述"ONA"是如WO2021176424A1中公开

的具有hIgG4-S228P-FALA Fc区的Fab克隆“ONA-0-v1”。

[0349] 实施例6:抗CD36抗体在小鼠肝细胞癌模型中的活性

[0350] 本实施例阐述了抗CD36抗体在两种基因诱导的肝细胞癌(HCC)的小鼠模型中抑制肿瘤生长的活性的研究:(1)由Sleeping Beauty转座子(SB100x)系统介导的MYC-luc-ova过表达和p53敲除的转基因诱导的HCC;和(2)由Sleeping Beauty转座子(SB100x)系统介导的MYC-luc-ova和β-连环蛋白^{N90}的转基因诱导的HCC。

[0351] 材料和方法

[0352] 将6周龄小鼠束缚,在5-7秒内,通过相当于体重10%的体积的侧尾静脉注射进行无内毒素质粒DNA的流体动力学递送。对于MYC^{OE}/p53^{KO} HCC模型,将pT3-Myc-luc-ova质粒(Addgene#129776)、p53 gRNA质粒(Addgene#59910)和SB100x(含转座酶的质粒;Addgene#34879)注射到小鼠体内。对于β-连环蛋白/Myc(β-连环蛋白^{OE}/MYC^{OE})HCC模型,将pT3-bcatenin(Addgene#31785)、pT3-Myc-luc-ova质粒和SB100x注射到每只小鼠体内。β-连环蛋白驱动的肝肿瘤代表具有冷肿瘤表型的更具侵袭性的HCC。

[0353] 为了监测肿瘤生长,将荧光素(150mg/Kg)注射到小鼠体内,并在IVIS成像系统中分析生物发光活性。将具有着持续肿瘤生长的小鼠随机分组,并通过如下所述的腹腔注射施用抗CD36抗体(10mg/kg)或PBS(对照)。

[0354] 2周后,MYC^{OE}/p53^{KO} HCC模型小鼠每3天腹腔注射4剂量的抗CD36抗体(10mg/kg;克隆:117_30DA)或对照抗体,并且每6天在IVIS系统中测量小鼠的生物发光成像(每组n=5)。

[0355] 3周后,β-连环蛋白^{OE}/MYC^{OE} HCC模型小鼠腹腔注射抗CD36抗体(10mg/mL,克隆:117_DA57E)或对照抗体(每组n=6),并且每5天在IVIS系统中测量小鼠的生物发光成像。

[0356] 在β-连环蛋白^{OE}/MYC^{OE} HCC模型中,肿瘤表现出侵袭性进展,并且荷瘤小鼠通常在流体动力注射后38天死亡。因此,在流体动力尾静脉注射后35-38天收获并称重肿瘤。

[0357] 结果

[0358] 图9显示在用抗CD36抗体117_30DA治疗后,在MYC^{OE}/p53^{KO} HCC模型小鼠中通过生物发光测量的肿瘤生长曲线。从HCC诱导后14天开始,一直持续到38天,与对照组作比较,观察到接受治疗的小鼠中的肿瘤生长受到显著抑制。P值=0.0114,t检验,双尾。

[0359] 图10A显示在用抗CD36抗体117_DA57E治疗后,在β-连环蛋白^{OE}/MYC^{OE} HCC模型小鼠中通过生物发光测量的肿瘤生长曲线。从HCC诱导后21天开始,一直持续到36天,与对照组作比较,观察到接受治疗的小鼠中的肿瘤生长受到显著抑制。P值=0.0104,t检验,双尾。图10B显示了在接受抗CD36治疗的小鼠中,终点肝重量显著降低。此外,如图10C所示,血浆ALT(丙氨酸转氨酶)活性分析表明,抗CD36治疗也减轻了由癌症发展造成的肝损伤。

[0360] 尽管为了清楚和理解的目的,已经通过实施例和说明的方式对本发明的上述公开进行了详细描述,但包括本文所述实施例、描述和实施方案的本公开用于说明目的,旨在作为示例,不应被解释为限制本公开。本领域技术人员会清楚,可以对本文所述实施例、描述和实施方案作各种修改或改变,并且这些修改或改变应包含在本公开和所附权利要求的精神和范围内。此外,本领域技术人员会认识到大量与本文所述方法和程序等效的方法和程序。所有此类等效物应被理解为在本公开的范围内,并由所附权利要求覆盖。

[0361] 本发明的其他实施方案在以下权利要求中阐述。

[0362] 本文提及的所有出版物、专利申请、专利或其他文件的公开内容均明确地以引用

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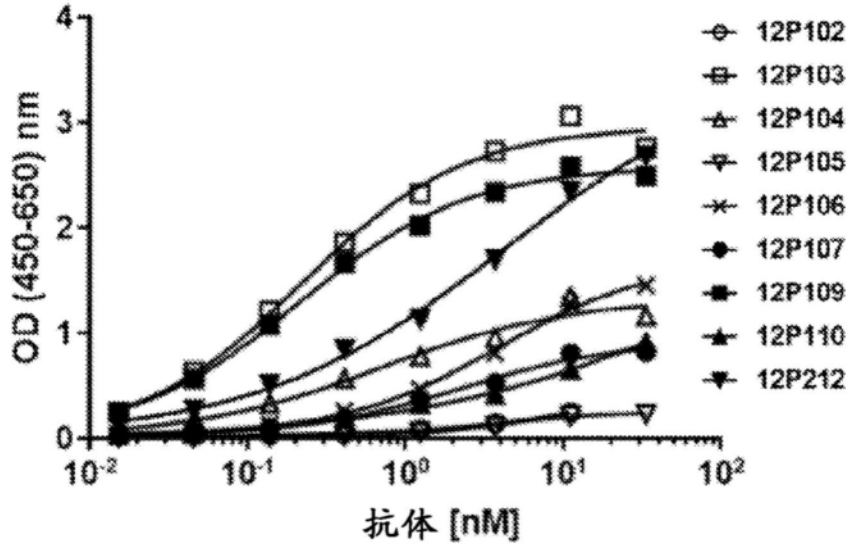
OD (450-650) nm
抗体 [nM]
12P102
12P103
12P104
12P105
12P106
12P107
12P109
12P110
12P212

图1A

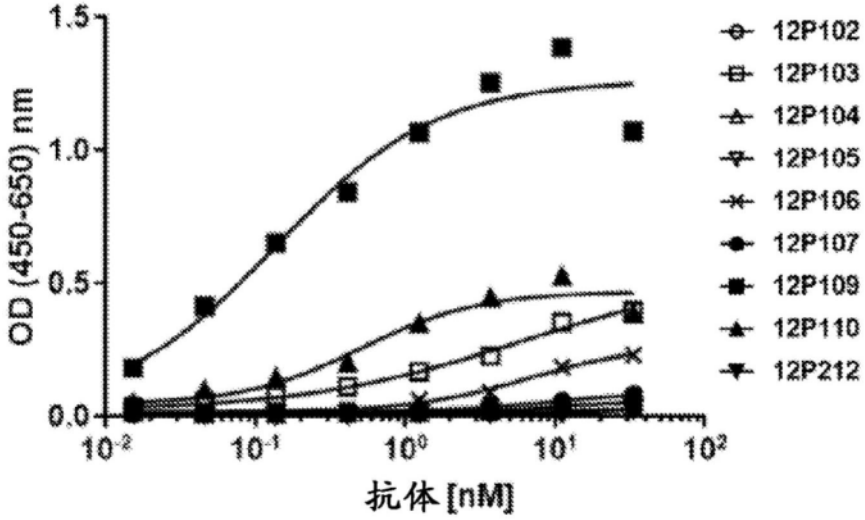
OD (450-650) nm
抗体 [nM]
12P102
12P103
12P104
12P105
12P106
12P107
12P109
12P110
12P212

图1B

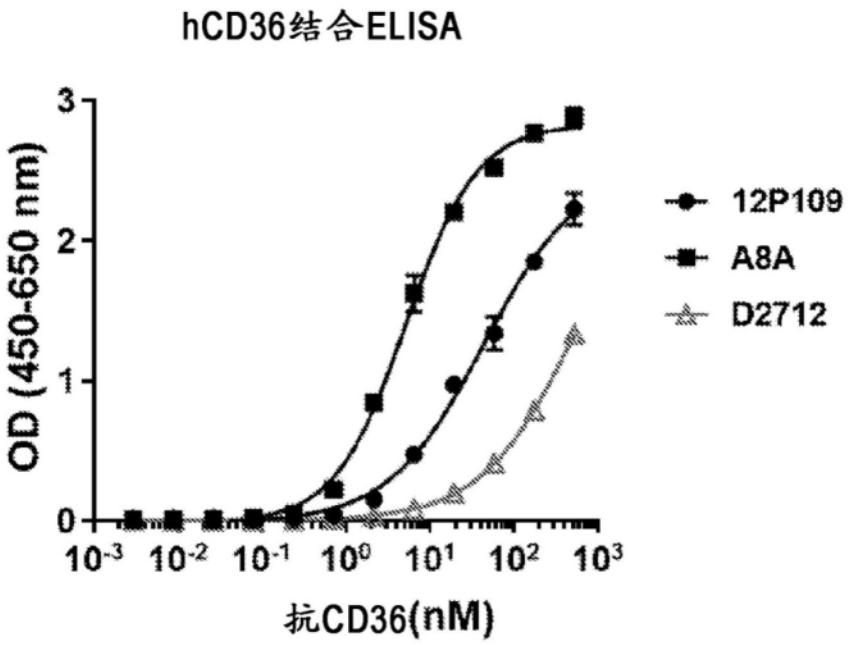
hCD36结合ELISA
OD (450-650 nm)
3
2
1
0
10-3 10-2 10-1 100 101 102 103
抗CD36(nM)
12P109
A8A
D2712

图1C

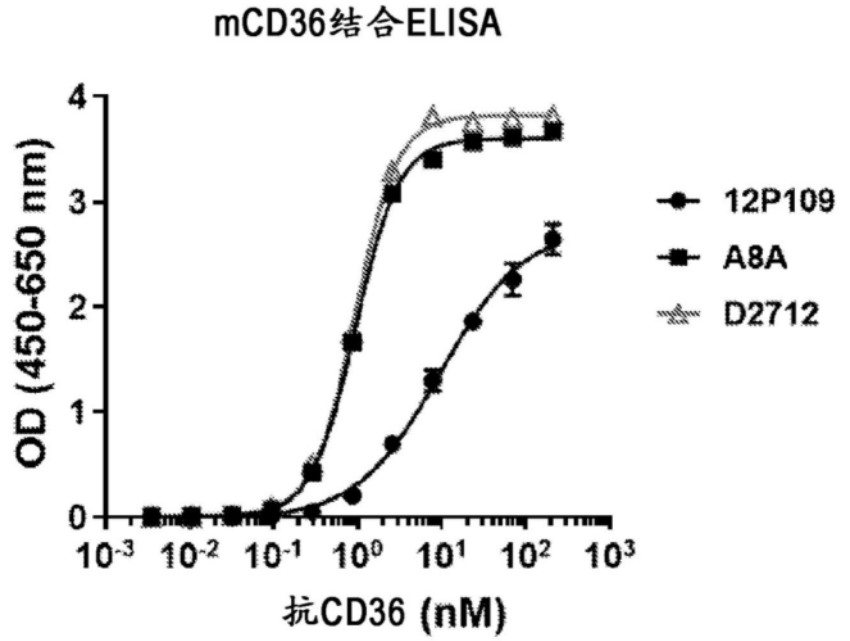
mCD36结合ELISA
OD (450-650 nm)
4
3
2
1
0
10-3 10-2 10-1 100 101 102 103
抗CD36 (nM)
12P109
A8A
D2712

图1D

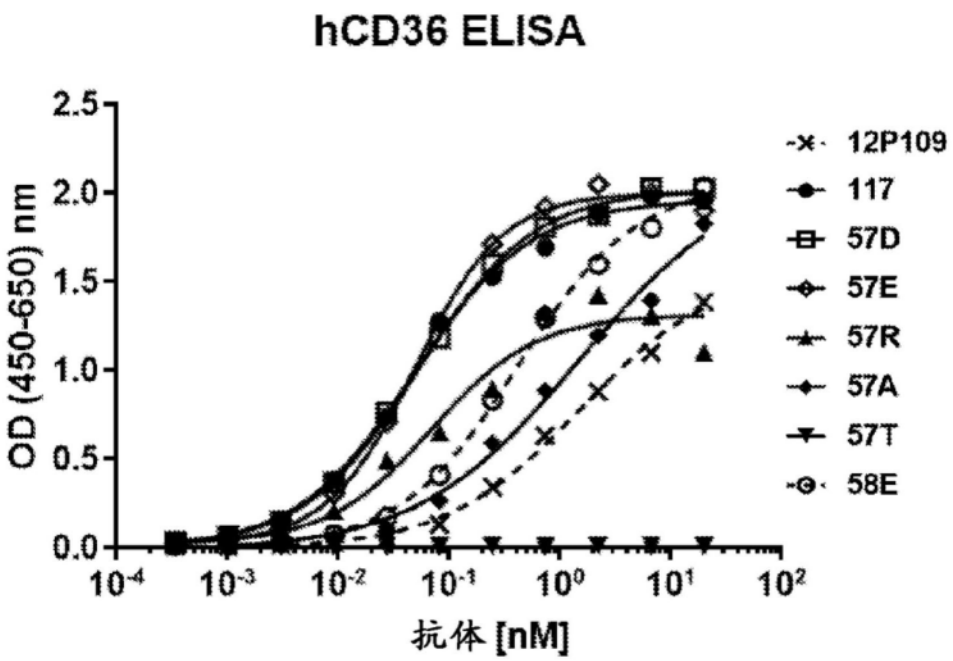
hCD36 ELISA
OD (450-650) nm
2.5
2.0
1.5
1.0
0.5
0.0
10-4
10-3
10-2
10-1
100
101
102
抗体 [nM]
12P109
117
57D
57E
57R
57A
57T
58E

图2A

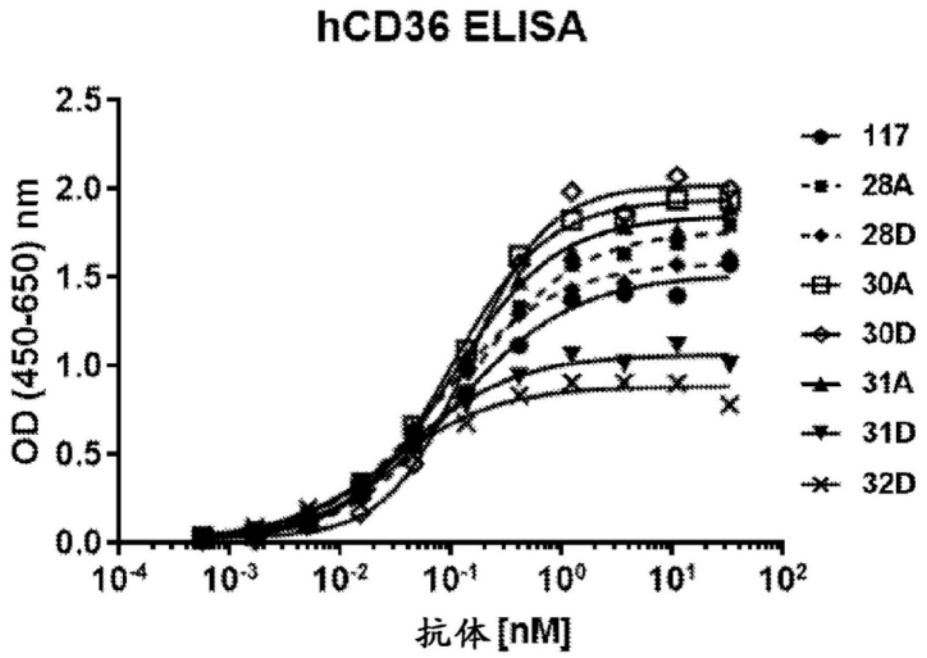
hCD36 ELISA
OD (450-650) nm
2.5
2.0
1.5
1.0
0.5
0.0
10-4
10-3
10-2
10-1
100
101
102
抗体 [nM]
117
28A
28D
30A
30D
31A
31D
32D

图2B

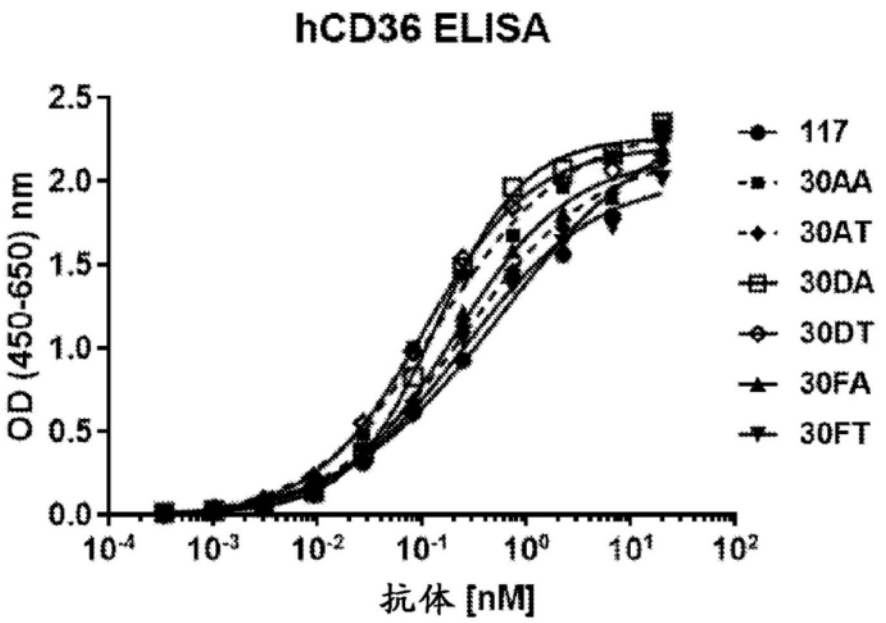
hCD36 ELISA
OD (450-650) nm
2.5
2.0
1.5
1.0
0.5
0.0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
抗体 [nM]
117
30AA
30AT
30DA
30DT
30FA
30FT

图2C

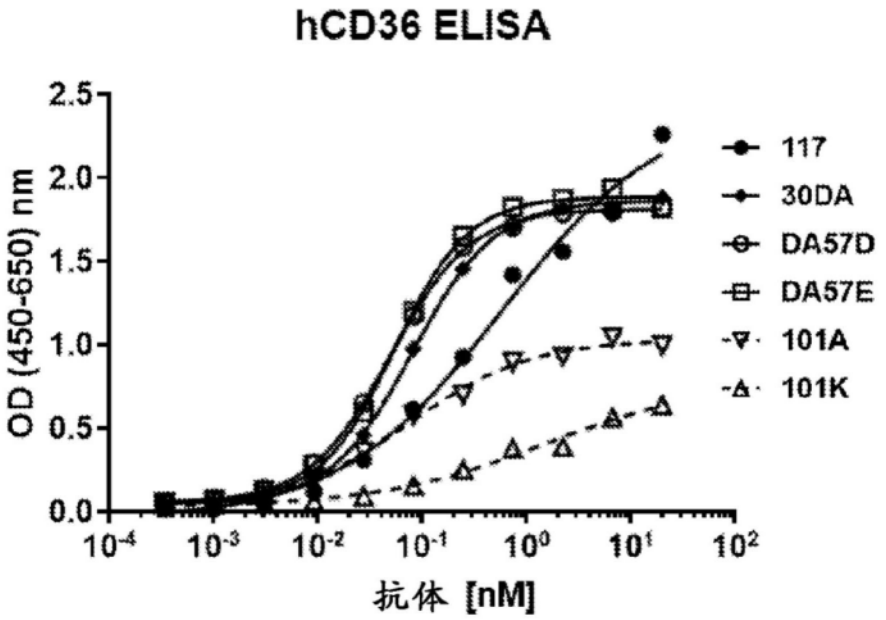
hCD36 ELISA
OD (450-650) nm
2.5
2.0
1.5
1.0
0.5
0.0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
抗体 [nM]
117
30DA
DA57D
DA57E
101A
101K

图2D

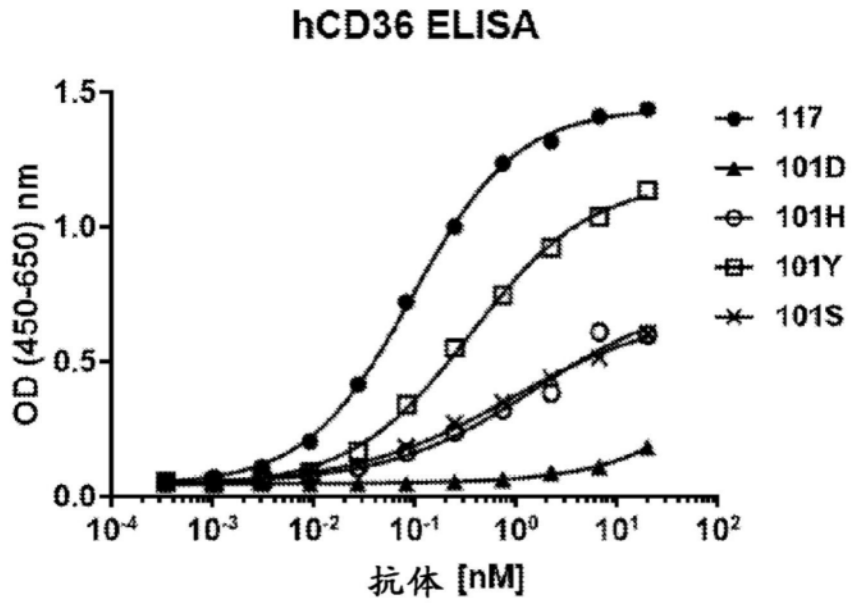
hCD36 ELISA
OD (450-650) nm
1.5
1.0
0.5
0.0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
抗体 [nM]
117
101D
101H
101Y
101S

图2E

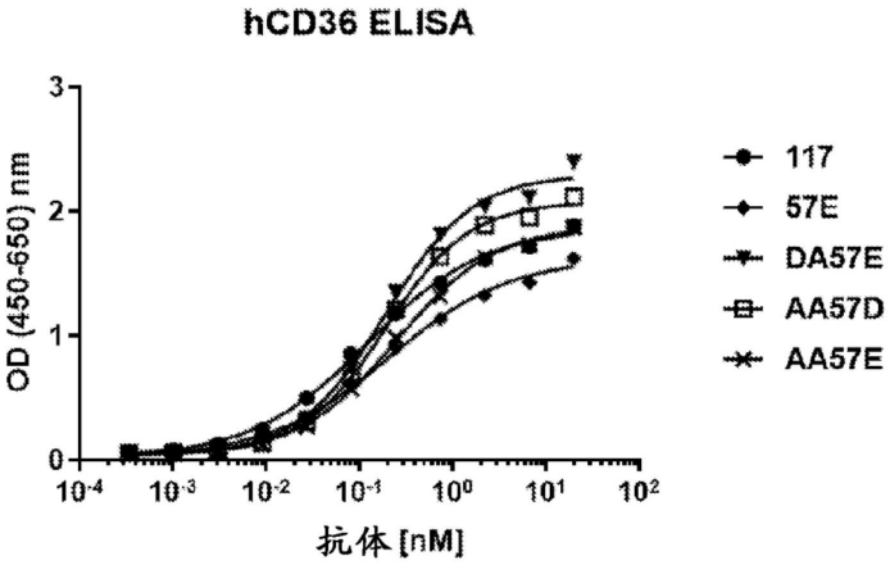
hCD36 ELISA
OD (450-650) nm
3
2
1
0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
抗体 [nM]
117
57E
DA57E
AA57D
AA57E

图2F

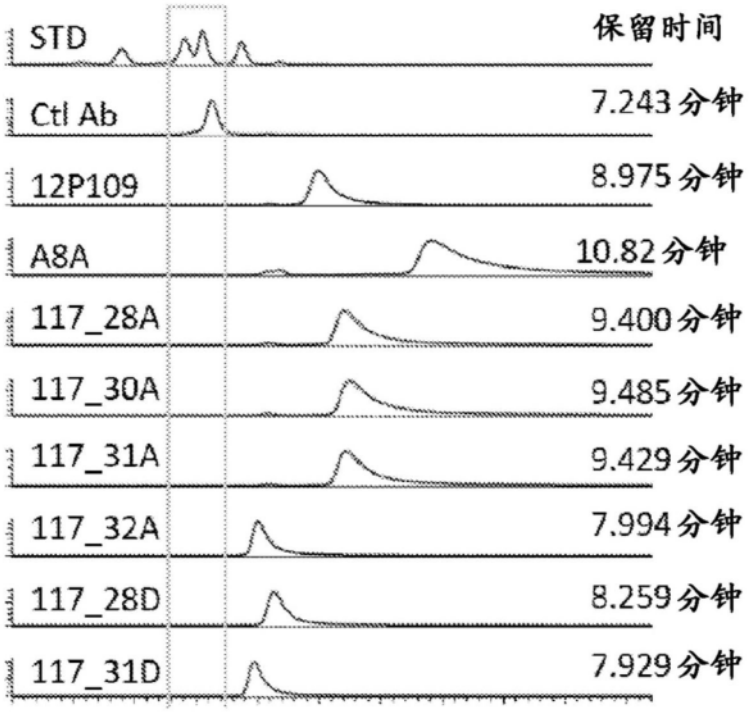
保留时间
STD
Ctl Ab
7.243 分钟
12P109
8.975 分钟
A8A
10.82 分钟
117_28A
9.400 分钟
117_30A
9.485 分钟
117_31A
9.429 分钟
117_32A
7.994 分钟
117_28D
8.259 分钟
117_31D
7.929 分钟

图3A

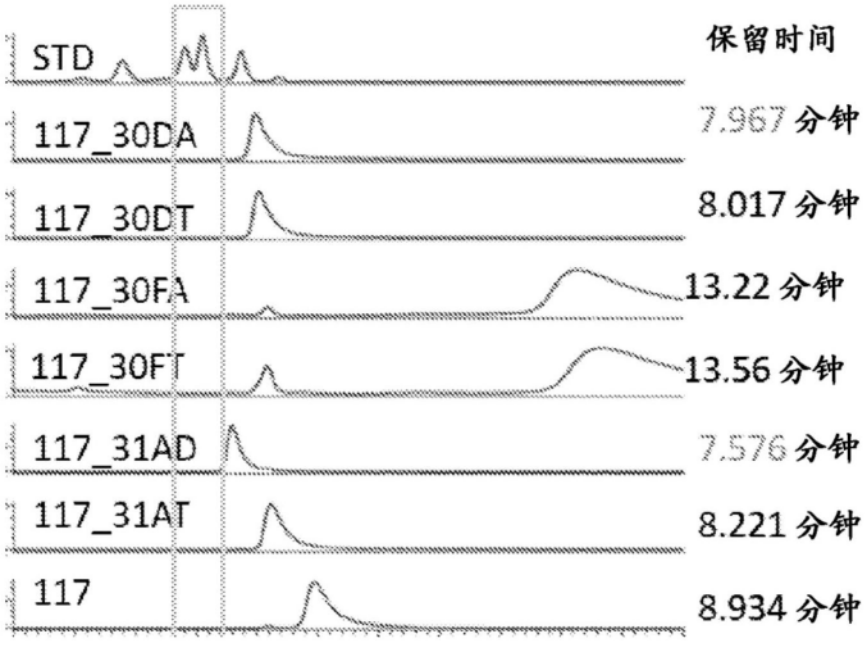
保留时间
STD
117_30DA
7.967 分钟
117_30DT
8.017 分钟
117_30FA
13.22 分钟
117_30FT
13.56 分钟
117_31AD
7.576 分钟
117_31AT
8.221 分钟
117
8.934 分钟

图3B

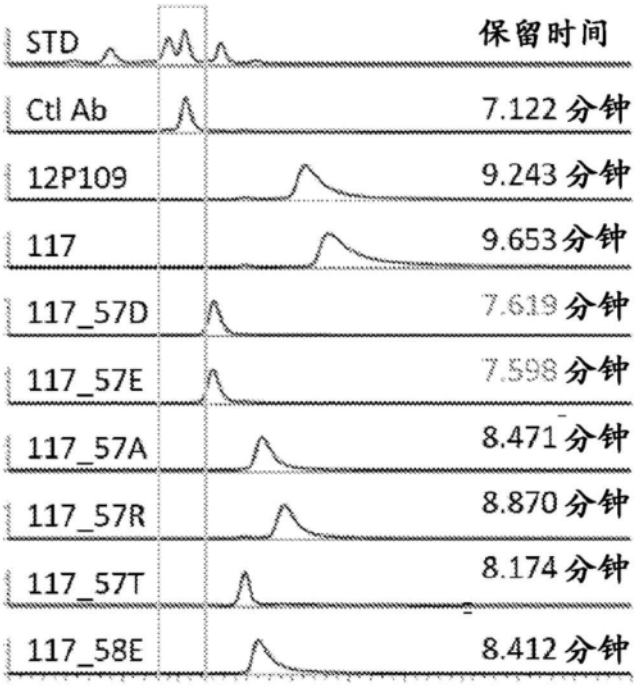
STD
保留时间
Ctl Ab
7.122 分钟
12P109
9.243 分钟
117
9.653分钟
117_57D
7.619 分钟
117_57E
7.598 分钟
117_57A
8.471 分钟
117_57R
8.870 分钟
117_57T
8.174 分钟
117_58E
8.412 分钟

图3C

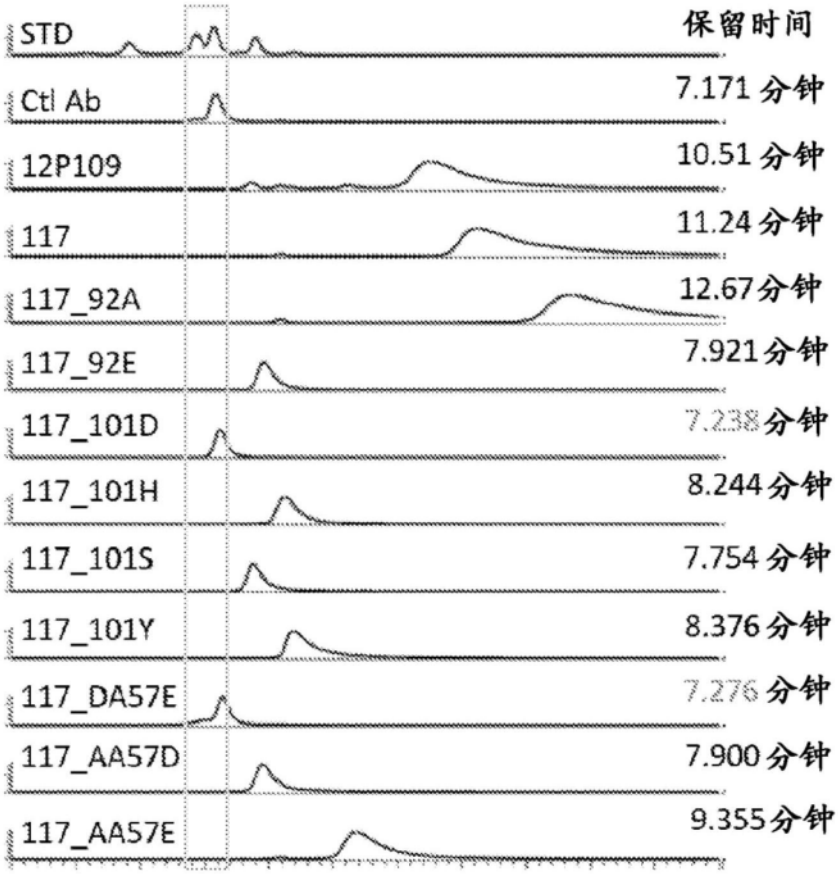
STD
保留时间
Ctl Ab
7.171 分钟
12P109
10.51 分钟
117
11.24 分钟
117_92A
12.67分钟
117_92E
7.921分钟
117_101D
7.238分钟
117_101H
8.244分钟
117_101S
7.754 分钟
117_101Y
8.376分钟
117_DA57E
7.276 分钟
117_AA57D
7.900分钟
117_AA57E
9.355分钟

图3D

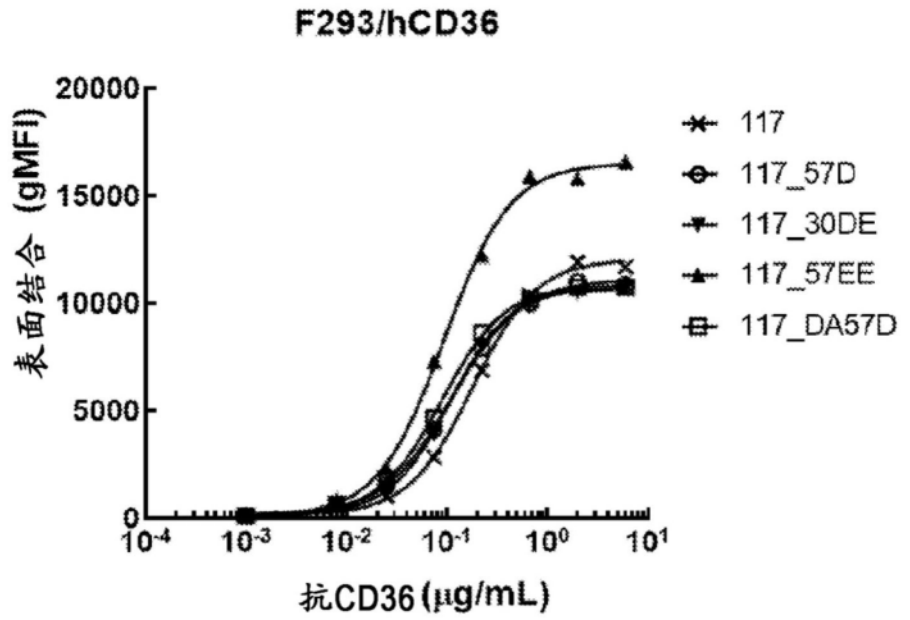
F293/hCD36
表面结合 (gMFI)
20000
15000
10000
5000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
抗CD36 (μg/mL)
117
117_57D
117_30DE
117_57EE
117_DA57D

图4A

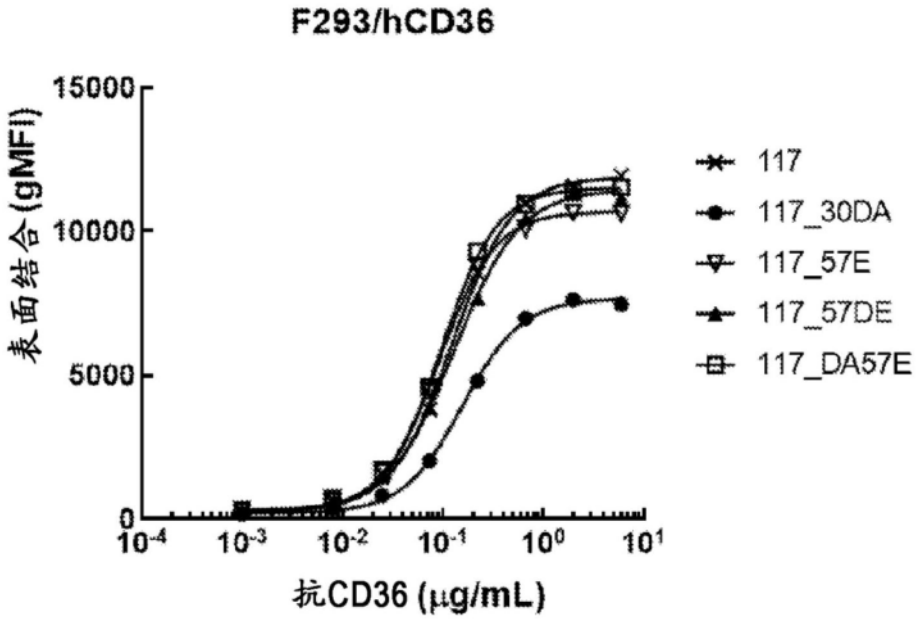
F293/hCD36
表面结合 (gMFI)
15000
10000
5000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
抗CD36 (μg/mL)
117
117_30DA
117_57E
117_57DE
117_DA57E

图4B

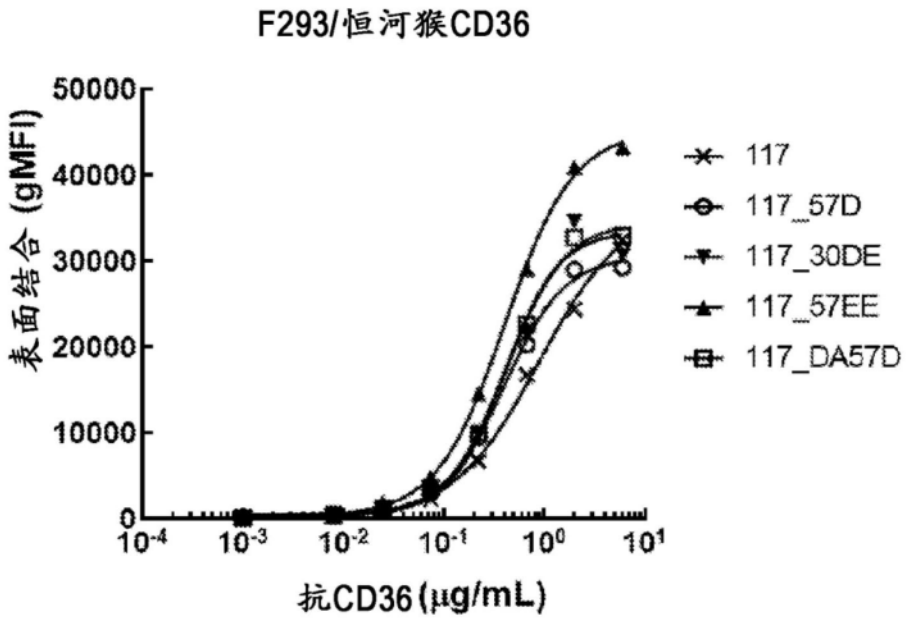
F293/恒河猴CD36
表面结合 (gMFI)
50000
40000
30000
20000
10000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
抗CD36 (μg/mL)
117
117_57D
117_30DE
117_57EE
117_DA57D

图4C

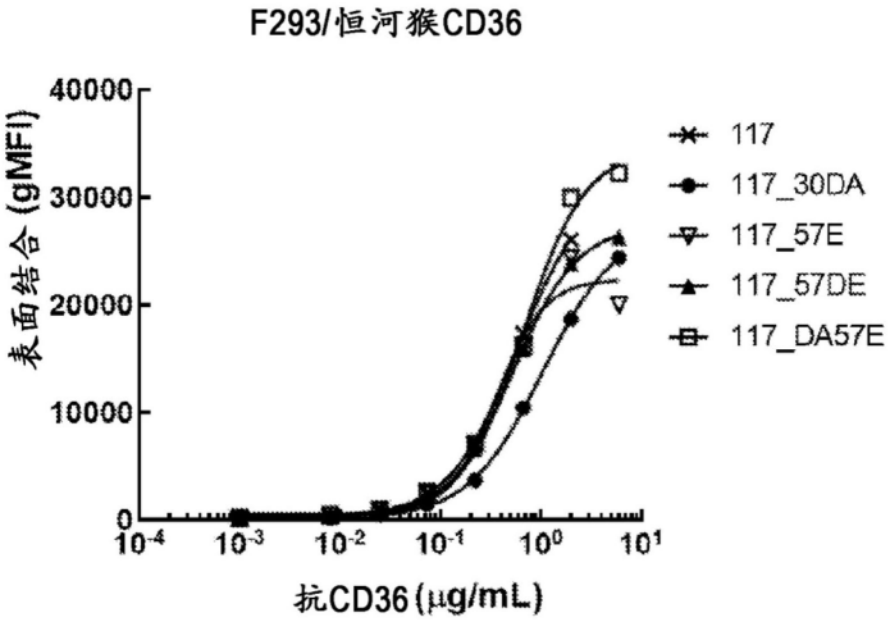
F293/恒河猴CD36
表面结合 (gMFI)
40000
30000
20000
10000
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
抗CD36 (μg/mL)
117
117_30DA
117_57E
117_57DE
117_DA57E

图4D

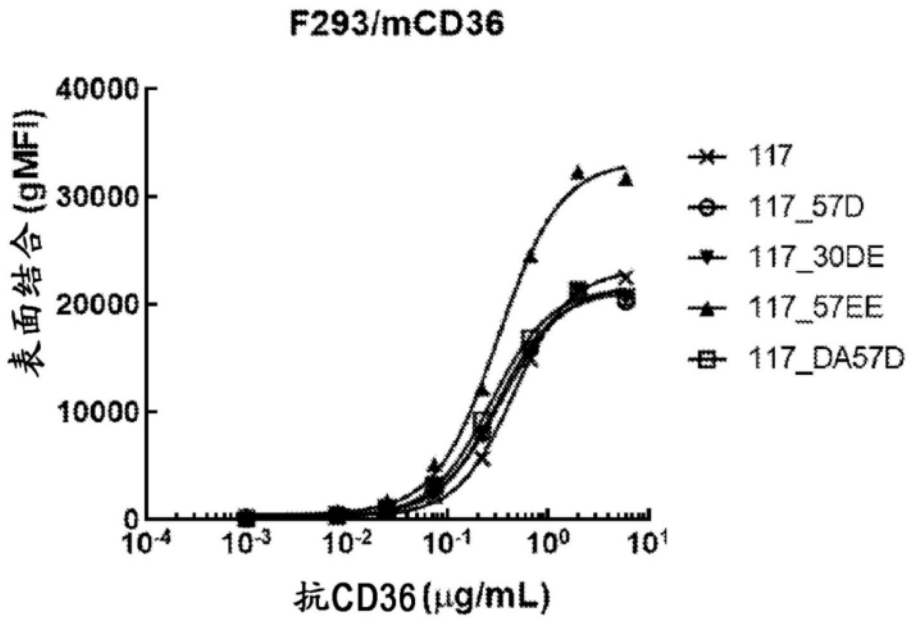
F293/mCD36
表面结合(gMFI)
40000
30000
20000
10000
0
10-4
10-3
10-2
10-1
100
101
抗CD36(μg/mL)
117
117_57D
117_30DE
117_57EE
117_DA57D

图4E

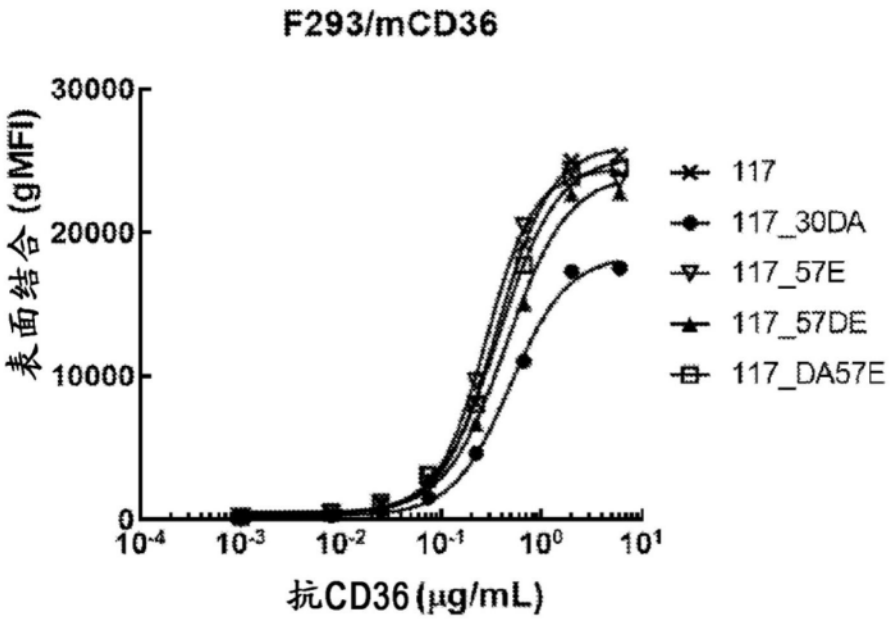
F293/mCD36
表面结合(gMFI)
30000
20000
10000
0
10-4
10-3
10-2
10-1
100
101
抗CD36(μg/mL)
117
117_30DA
117_57E
117_57DE
117_DA57E

图4F

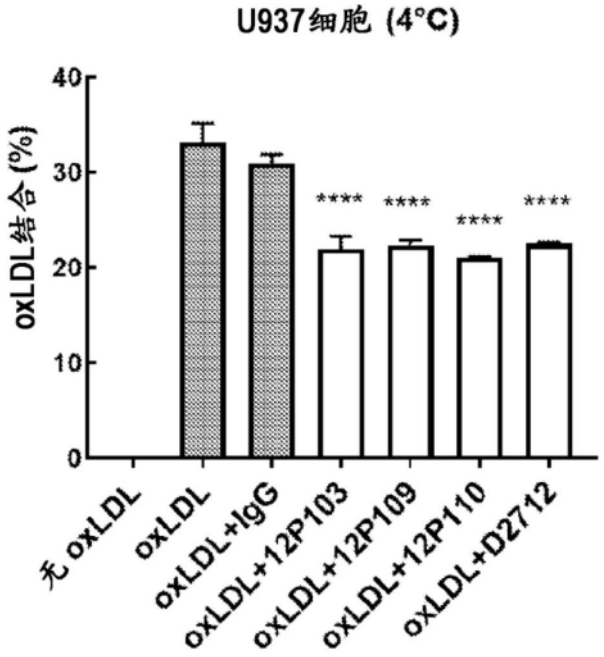
U937细胞 (4°C)
oxLDL结合 (%)
40
30
20
10
0
****
****
****
****
无 oxLDL
oxLDL
oxLDL+IgG
oxLDL+12P103
oxLDL+12P109
oxLDL+12P110
oxLDL+D2712

图5A

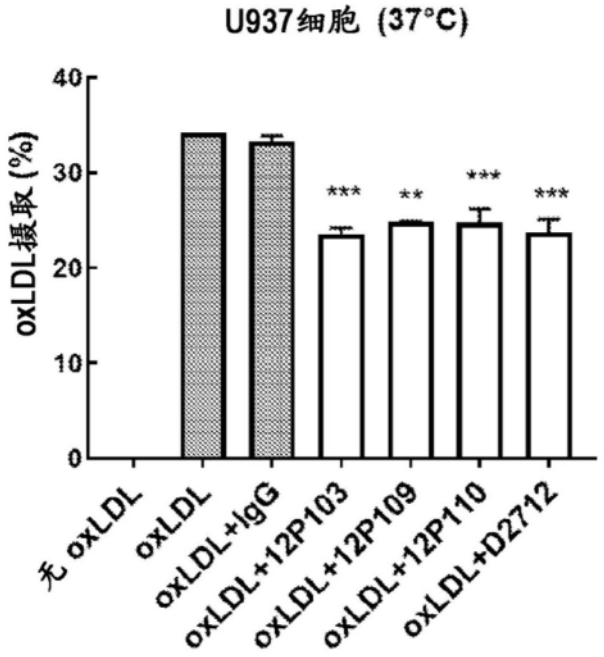
U937细胞 (37°C)
oxLDL摄取 (%)
40
30
20
10
0
***
**
***
***
无 oxLDL
oxLDL
oxLDL+IgG
oxLDL+12P103
oxLDL+12P109
oxLDL+12P110
oxLDL+D2712

图5B

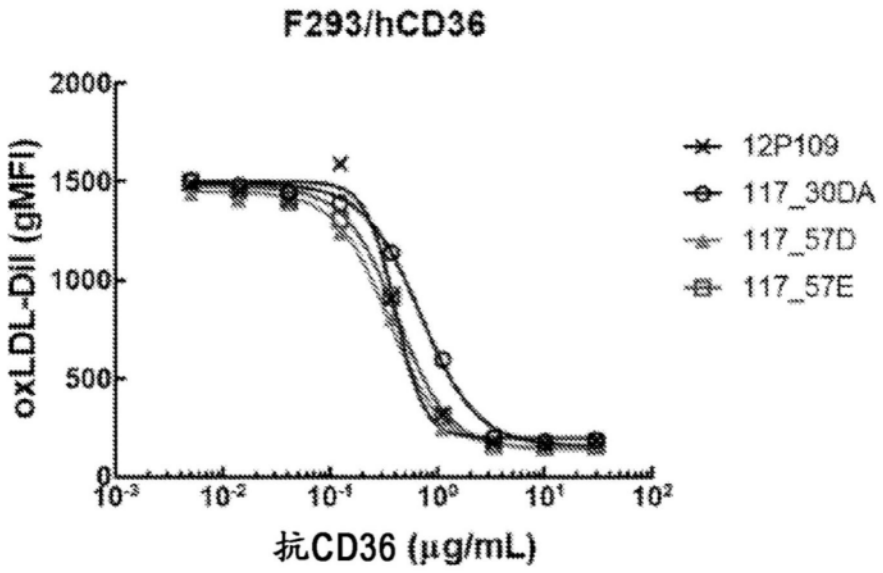
F293/hCD36
oxLDL-DiI (gMFI)
2000
1500
1000
500
0
10-3
10-2
10-1
100
101
102
抗CD36 (μg/mL)
12P109
117_30DA
117_57D
117_57E

图6A

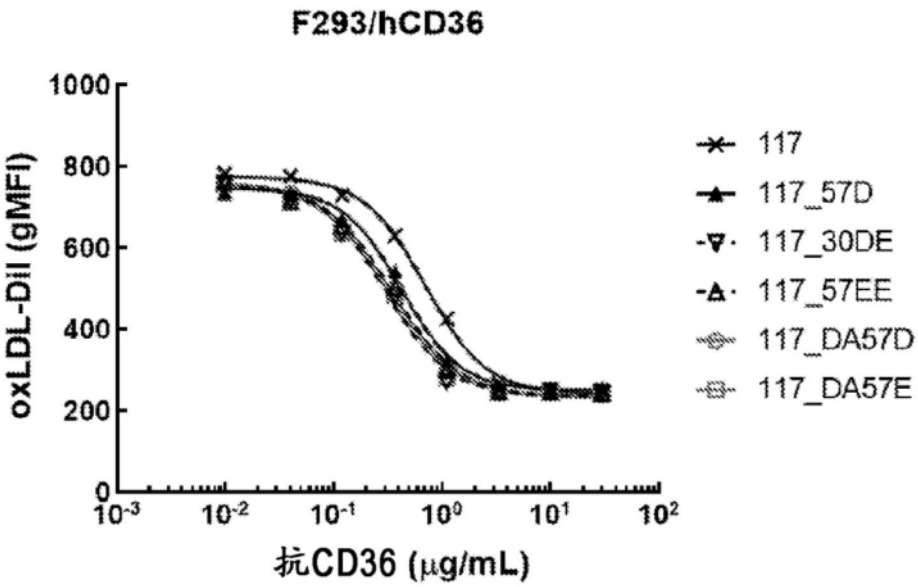
F293/hCD36
oxLDL-DiI (gMFI)
1000
800
600
400
200
0
10-3
10-2
10-1
100
101
102
抗CD36 (μg/mL)
117
117_57D
117_30DE
117_57EE
117_DA57D
117_DA57E

图6B

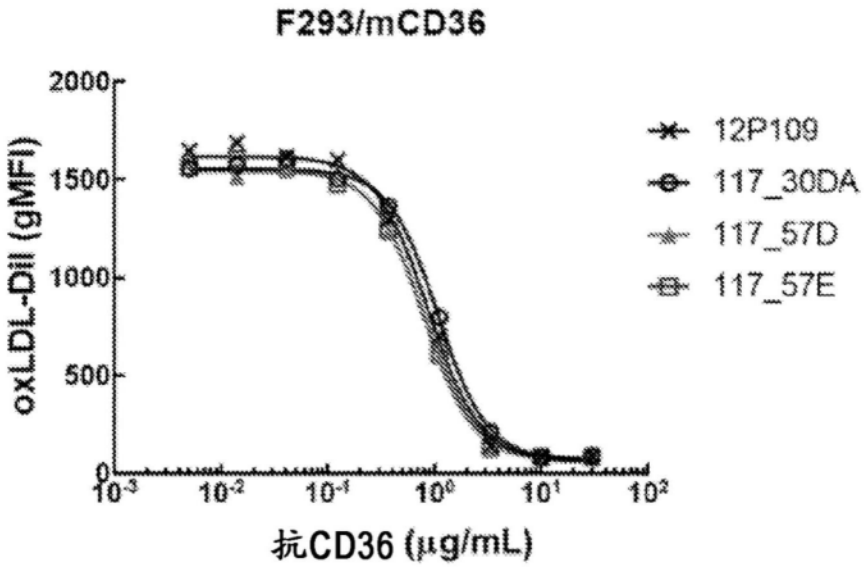
F293/mCD36
oxLDL-DiI (gMFI)
2000
1500
1000
500
0
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
抗CD36 (μg/mL)
12P109
117_30DA
117_57D
117_57E

图6C

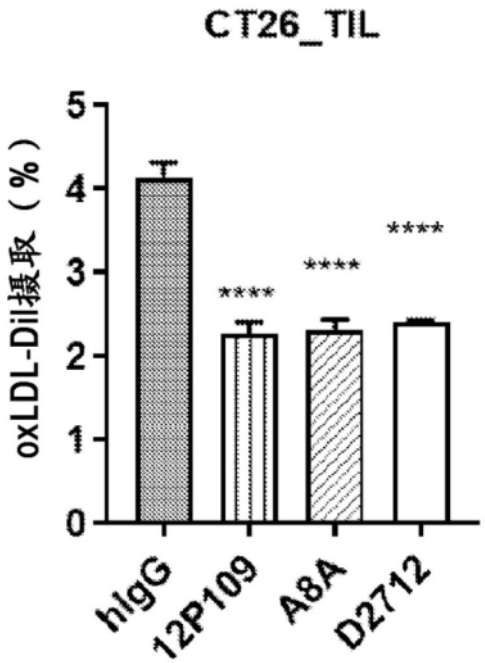
CT26_TIL
oxLDL-DiI摄取（%）
5
4
3
2
1
0
****
****
****
hIgG
12P109
A8A
D2712

图7A

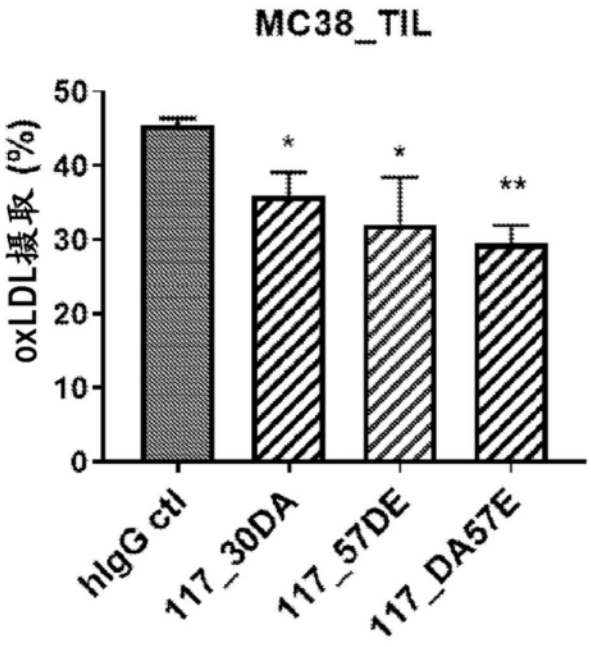
MC38_TIL
oxLDL摄取 (%)
50
40
30
20
10
0
*
*
**
hIgG ctl
117_30DA
117_57DE
117_DA57E

图7B

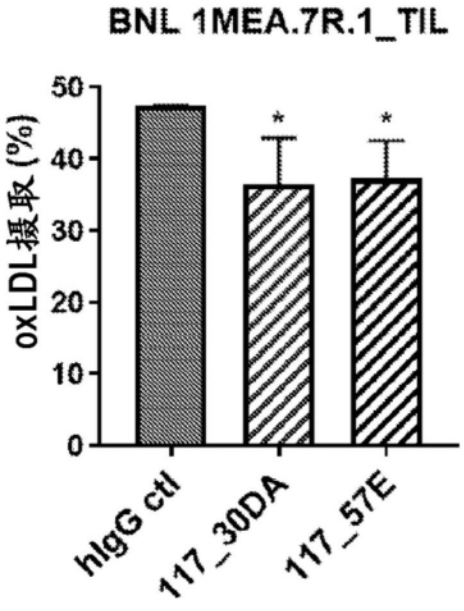
BNL 1MEA.7R.1_TIL
oxLDL摄取 (%)
50
40
30
20
10
0
*
*
hIgG ctl
117_30DA
117_57E

图7C

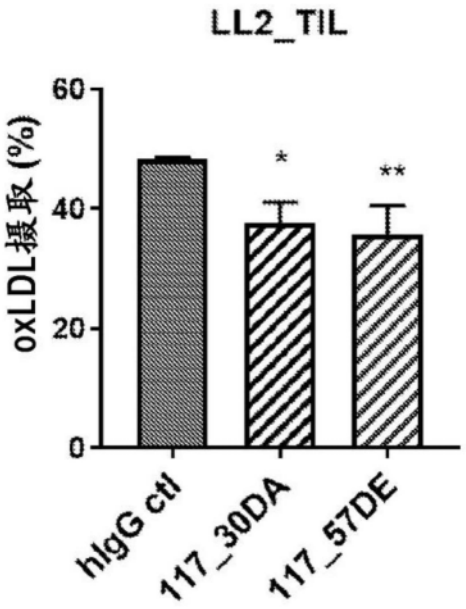
LL2_TIL
oxLDL摄取 (%)
60
40
20
0
*
**
hIgG ctl
117_30DA
117_57DE

图7D

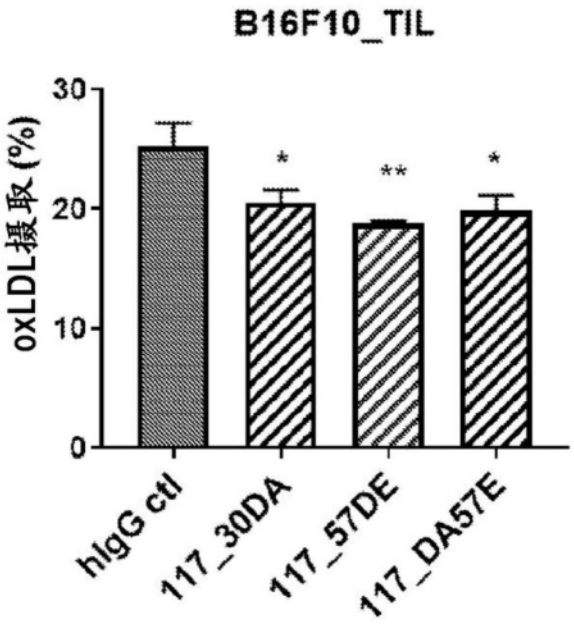
B16F10_TIL
oxLDL摄取 (%)
30
20
10
0
*
**
*
hIgG ctl
117_30DA
117_57DE
117_DA57E

图7E

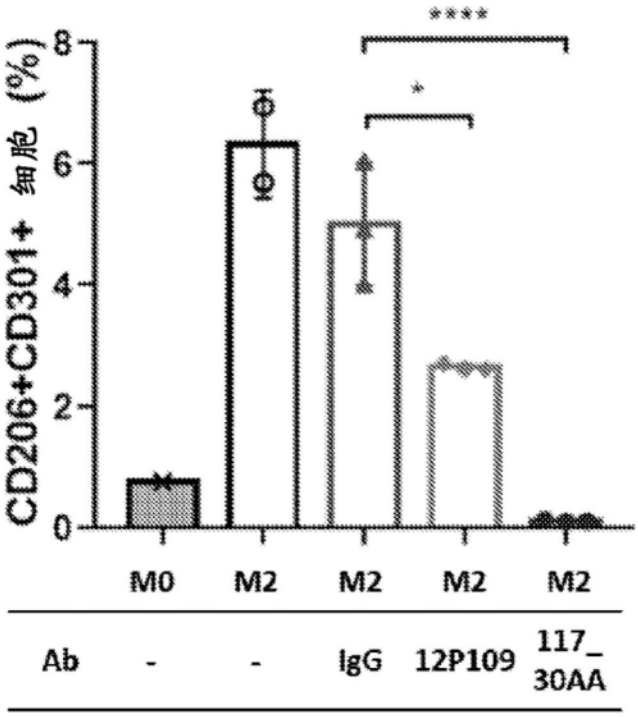
CD206+CD301+ 细胞 (%)
8
6
4
2
0
****
*
M0
M2
M2
M2
M2
Ab
-
-
IgG
12P109
117_30AA

图8A

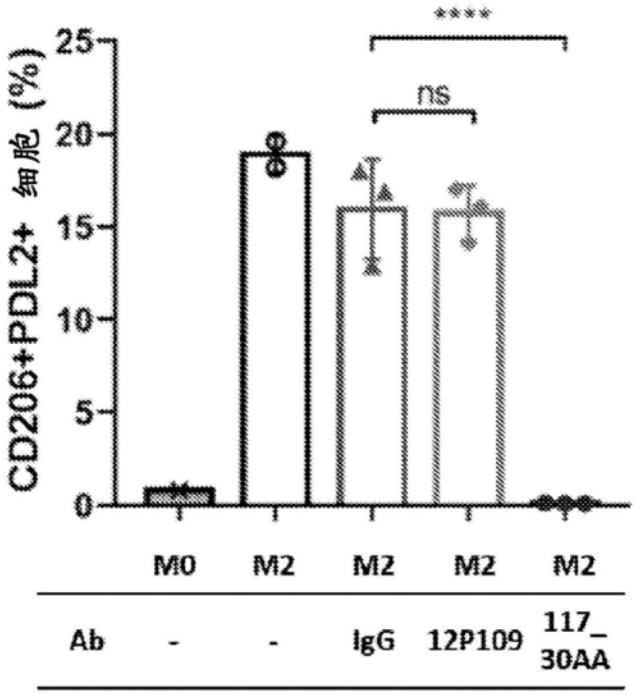
CD206+PDL2+ 细胞 (%)
25
20
15
10
5
0
****
ns
M0
M2
M2
M2
M2
Ab
-
-
IgG
12P109
117_30AA

图8B

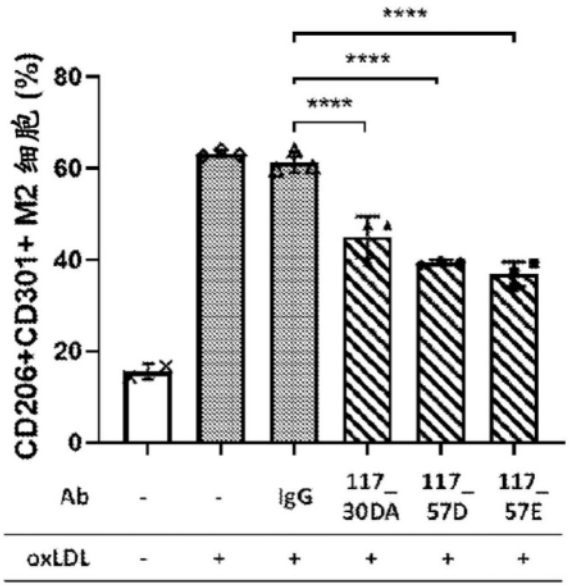
CD206+CD301+ M2 细胞 (%)
80
60
40
20
0
****
****
****
Ab - - IgG 117_ 30DA 117_ 57D 117_ 57E
oxLDL - + + + + +

图8C

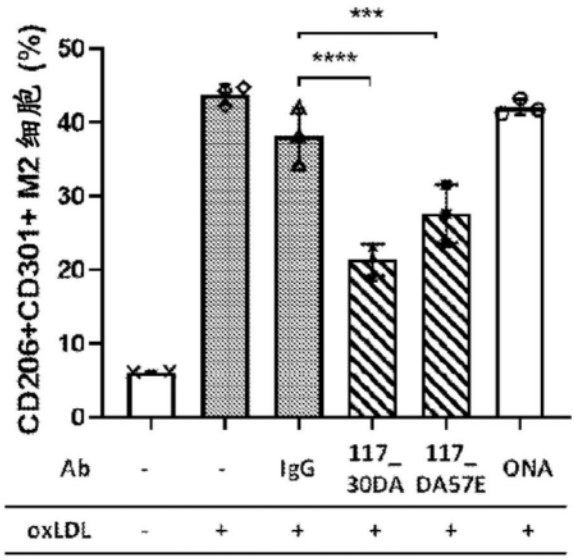
CD206+CD301+ M2 细胞 (%)
50
40
30
20
10
0
***
****
Ab - - IgG 117_ 30DA 117_ DA57E ONA
oxLDL - + + + + +

图8D

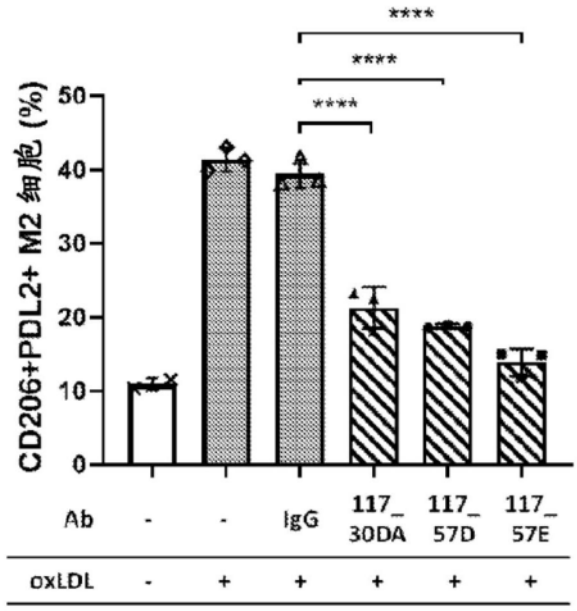
****
****
****
CD206+PDL2+ M2 细胞 (%)
50
40
30
20
10
0
Ab - - IgG 117_ 30DA 117_ 57D 117_ 57E
oxLDL - + + + + +

图8E

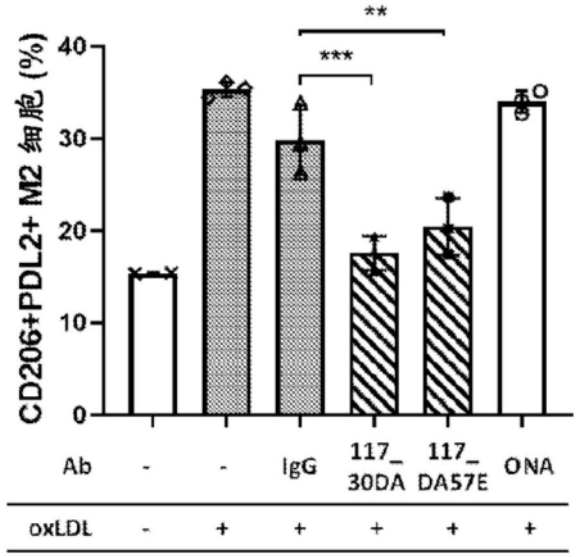
**
***
CD206+PDL2+ M2 细胞 (%)
40
30
20
10
0
Ab - - IgG 117_ 30DA 117_ DA57E ONA
oxLDL - + + + + +

图8F

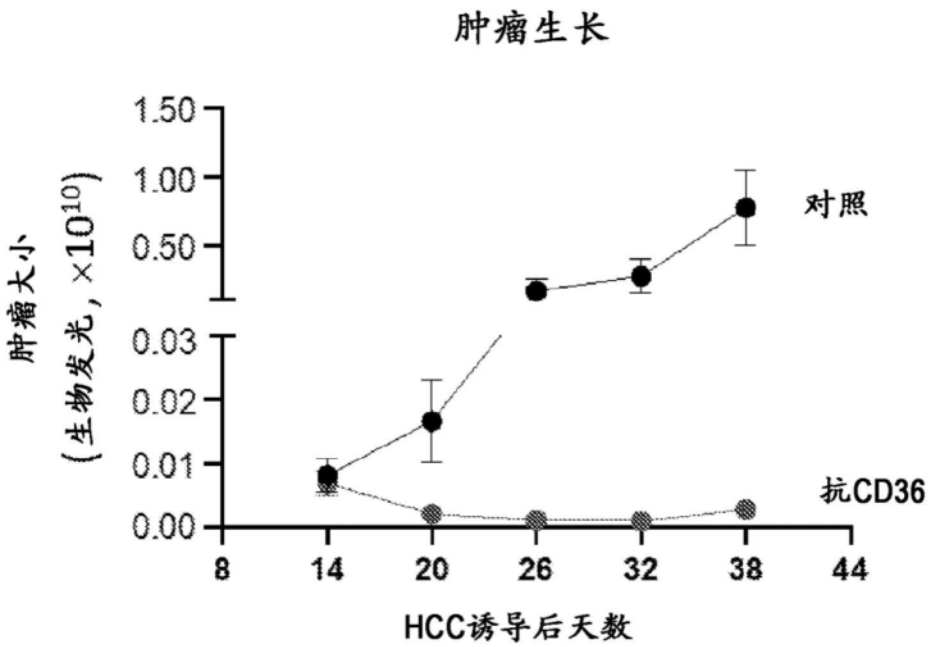
肿瘤生长
肿瘤大小
(生物发光, ×10^10)
1.50
1.00
0.50
0.03
0.02
0.01
0.00
8
14
20
26
32
38
44
HCC诱导后天数
对照
抗CD36

图9

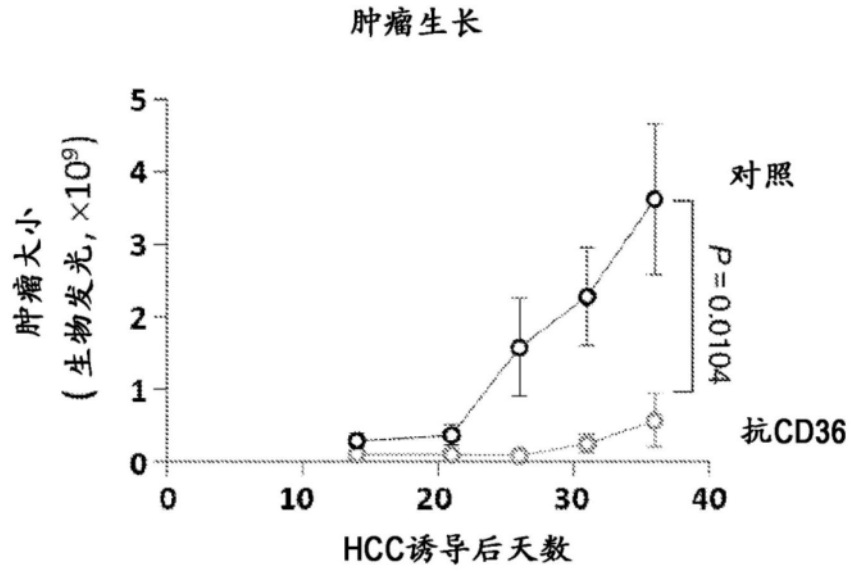
肿瘤生长
肿瘤大小
(生物发光, ×10^9)
5
4
3
2
1
0
0
10
20
30
40
HCC诱导后天数
对照
抗CD36
P = 0.0104

图10A

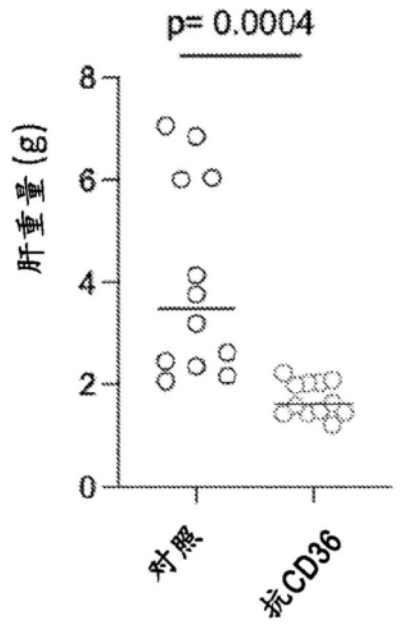
p= 0.0004
肝重量(g)
8
6
4
2
0
对照
抗CD36

图10B

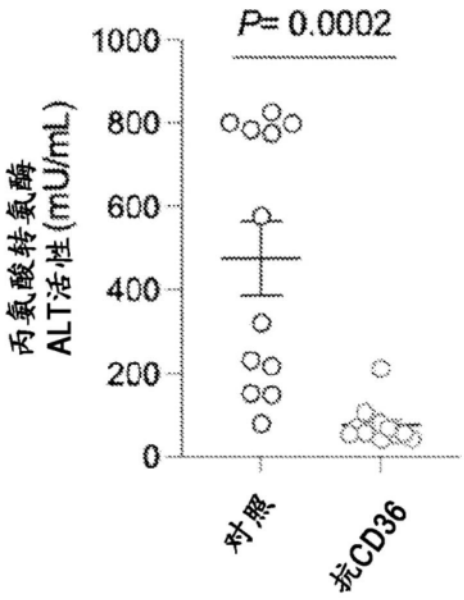
P= 0.0002
丙氨酸转氨酶
ALT活性(mU/mL)
1000
800
600
400
200
0
对照
抗CD36

图10C