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(71) Applicant(s)
Eureka Therapeutics, Inc.

(72) Inventor(s)
Zhang, Pengbo;Xu, Yiyang;Morales, Javier;Nakano, Yoko;Liu, Hong;Xiang, Jingyi;Acker, Timothy

(74) Agent / Attorney
WRAYS PTY LTD, L7 863 Hay St, Perth, WA, 6000, AU

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(71) Applicant: **EUREKA THERAPEUTICS, INC.** [US/US]; 5858 Horton Street, Suite 170, Emeryville, California 94608 (US).

(72) Inventors: **ZHANG, Pengbo**; 4659 Rousillon Avenue, Fremont, California 94555 (US). **XU, Yiyang**; 7652 Chestnut Way, Pleasanton, California 94588 (US). **MORALES, Javier**; 1778 Bird Avenue, San Jose, California 95125 (US). **NAKANO, Yoko**; 10944 San Pablo Avenue, Apt. 723, El Cerrito, California 94530 (US). **LIU, Hong**; 5573 Woodview Drive, El Sobrante, California 94803 (US). **XIANG, Jingyi**; 2170 San Luis Road, Walnut Creek, California 94597 (US). **ACKER, Timothy**; 1434 Funston Avenue, San Francisco, California 94122 (US).

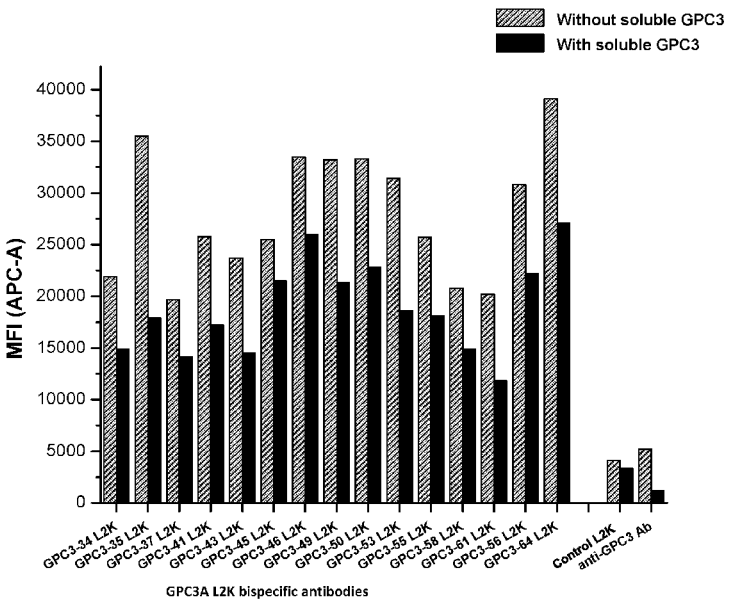
(74) Agent: **QI, Yan**; Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, California 94304-1018 (US).

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FIG. 6



(57) Abstract: The present application provides constructs comprising an antibody moiety specifically recognizing Glypican 3 (GPC3), such as a cell surface-bound GPC3. Also provided are methods of making and using these constructs.

[Continued on next page]

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CONSTRUCTS SPECIFICALLY RECOGNIZING GLYPICAN 3 AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is an Australian national phase of International patent application No. PCT/US2018/029221 (publication No. WO 2018/200586) filed on April 24, 2018, which claims priority to U.S. Provisional Application No. 62/490,586, filed on April 26, 2017, the contents of which are all hereby incorporated by reference in their entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 750042001240SEQLIST.txt, date recorded: April 24, 2018, size: 395 KB).

FIELD OF THE INVENTION

[0003] This invention pertains to antibody constructs that specifically recognize Glypican 3 (GPC3), methods of making, and uses thereof including treating and diagnosing diseases.

BACKGROUND OF THE INVENTION

[0004] Glypican 3 (GPC3, also known as SGB, DGSX, MXR7, SDYS, SGBS, OCI-5, SGBS1, GTR2-2) is a cell surface protein that is overexpressed in multiple cancer types, including many solid tumors, such as hepatocellular carcinoma (HCC), melanoma (Nakatsura T *et al.*, *Clin Cancer Res.* 2004), lung squamous cell carcinoma (Yu X *et al.*, Genet Mol Res. 2015), ovarian carcinoma (Stadlmann S *et al.*, *Int J Gynecol Pathol.* 2007), yolk sac tumor, choriocarcinoma (Zynger DL *et al.*, *Am J Surg Pathol.* 2006), Wilms' tumor, and liposarcoma (Baumhoer D et al., *Am J Clin Pathol.* 2008). There remains a medical need of developing therapies against various cancers including those overexpressing GPC3.

[0005] The GPC3 gene is located on the X chromosome and encodes a 70 kDa precursor protein of 580 amino acids (aa). This precursor protein can be cleaved by Furin between Arg^{358} and Cys^{359} and generate a 40 kDa N-terminal subunit and a 30 kDa C-terminal subunit linked by disulfide bonds. The mature GPC3 is attached to the cell surface by a glycosylphosphatidylinositol (GPI) anchor with two heparin sulfate (HS) chains on the C-terminal region close to the cell membrane. As a member of the heparin sulfate proteoglycan family, GPC3 is an oncofetal protein that is widely expressed in human embryos, and regulates morphogenesis or growth through interaction with signaling factors, such as Wnt, hedgehog signaling, *etc*. GPC3 is not expressed in normal adult livers; however, during hepatic

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carcinogenesis, GPC3 has been reported to be reactivated in HCC patients. Yamauchi N *et al.* examined GPC3 expression in normal, non-neoplastic, and neoplastic liver tissues, and found that GPC3 was present in 84% (47/56) of HCC patients, but absent in normal adult liver, liver cirrhosis or hepatitis (Yamauchi N *et al.*, *Modern Pathology* 2005). Similar results were reported by other groups (Nakatsura T *et al.*, *Biochem Biophys Res Commun.* 2003; Baumhoer D *et al.*, *Am J Clin Pathol.* 2008; Shirakawa H *et al.*, *Cancer Sci.* 2009; Wang L *et al.*, *Hepatobiliary Pancreat Dis Int.* 2015). Studies also showed that GPC3 could be released from cell surface to extracellular environment in different forms in HCC patients, but not in healthy donors. Therefore, GPC3 is currently used as a serum diagnostic marker for HCC (Hippo Y *et al.*, *Cancer Res.* 2004; Capurro M *et al.*, *Gastroenterology* 2003; Haruyama Y and Kataoka H, *World J Gastroenterol.* 2016).

[0006] Hepatocellular carcinoma (HCC) is the most common type of liver cancer, accounting for approximately 75% of all liver cancers. Liver cancer is the fifth most common cancer and the second most common cause of death from cancer in the world. Liver cancer incidence has more than tripled since 1980, and liver cancer death rates have increased by almost 3% per year since 2000 (https://www.cancer.org/cancer/liver-cancer/about/what-is-key-statistics.html). Prognosis for liver cancer is very poor with an overall ratio of mortality to incidence of 0.95. Currently liver cancer treatment is mainly limited to chemotherapy or surgery. However, the effect of chemotherapy is often limited because liver cells express ATP binding cassette (ABC) transporters which can export a large range of commonly used chemotherapeutic agents. Another option, surgery, is only available in early staged cancers, in which the 5-year survival rate is 31%. If cancer is diagnosed in the late stages (5-year survival rate: 3-11%), the only approved chemotherapy treatment is the tyrosine kinase inhibitor, sorafenib. This treatment increases the survival rate by only 2-3 months (Fleming BD and Ho M, *Toxins* 2016). Therefore, the development of a novel method to treat liver cancer is desired.

[0007] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application

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BRIEF SUMMARY OF THE INVENTION

[0008] The present application in one aspect provides anti-GPC3 constructs (such as isolated anti-GPC3 constructs) specifically recognizing a cell surface-bound GPC3 (referred to herein as a "native format GPC3," or "native GPC3 (nGPC3)"). In some embodiments, the constructs (referred to herein as "anti-GPC3 constructs") comprise an antibody moiety (referred to herein as an "anti-GPC3 antibody moiety") specifically recognizing native format GPC3.

[Continued on Page 3]

surface-bound GPC3. In some embodiments, the antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the K_d of the anti-GPC3 construct to the cell surface-bound GPC3 is about 0.1 nM to about 2 nM (*e.g.*, about 0.1 nM to about 1 nM, or about 0.1 nM to about 1.5 nM). In some embodiments, the IC50 of a soluble GPC3 to compete binding between the anti-GPC3 construct and the cell surface-bound GPC3 is about 1 μg/ml to about 100 μg/ml (*e.g.*, about 1 μg/ml to about 10 μg/ml, or about 2 μg/ml to about 5 μg/ml). In some embodiments, the cell surface-bound GPC3 comprises the amino acid sequence of SEQ ID NO: 460 or SEQ ID NO: 462. In some embodiments, the cell surface-bound GPC3 is GPC3 expressed on HepG2 cells. In some embodiments, the soluble GPC3 comprises the amino acid sequence of SEQ ID NO: 461 or SEQ ID NO: 463.

[0010] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460.

[0011] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461.

[0012] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462.

[0013] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463.

[0014] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464.

[0015] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain.

[0016] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety does not specifically bind to the same or substantially the same GPC3 epitope competitively with GC33. In some embodiments, the antibody moiety does not bind to an epitope within the amino acid sequence of SEQ ID NO: 536. In some embodiments, the antibody moiety does not bind to a fragment comprising the amino acid sequence of SEQ ID NO: 536.

[0017] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293.

[0018] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, GPC3 is expressed on the surface of a cancer cell. In some embodiments, the cancer cell is a liver cancer cell, such as hepatocellular carcinoma (HCC).

[0019] The present application in another aspect provides an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, wherein the antibody moiety comprises: i) a heavy chain variable domain (V_H) comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 amino acid substitutions; and ii) a light chain variable domain (V_L) comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 amino acid substitution. In some embodiments, the antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2

comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286.

[0020] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety comprises: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, or a variant thereof having at least about 95% sequence identify to any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, or a variant thereof having at least about 95% sequence identity to any one of SEQ ID NOs: 358-388. In some embodiments, the antibody moiety comprises: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388.

[0021] The present application in another aspect provides an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, wherein the antibody moiety comprises the HC-CDRs of V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337 and LC-CDRs of V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388 of an isolated anti-GPC3 construct.

[0022] The present application in another aspect provides an isolated anti-GPC3 construct comprising an antibody moiety specifically recognizing GPC3, wherein the antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino

acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306.

[0023] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety comprises: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, or a variant thereof having at least about 95% sequence identify to any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, or a variant thereof having at least about 95% sequence identity to any one of SEQ ID NOs: 389-408. In some embodiments, the antibody moiety comprises: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408.

[0024] The present application in another aspect provides an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an antibody moiety specifically recognizing GPC3, wherein the antibody moiety comprises the HC-CDRs of V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357 and LC-CDRs of V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408 of an isolated anti-GPC3 construct.

[0025] The present application in another aspect provides an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an antibody moiety that specifically binds to GPC3 competitively with the isolated anti-GPC3 construct of any one of the anti-GPC3 constructs described above.

[0026] The present application in another aspect provides an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with the isolated anti-GPC3 construct of any one of the anti-GPC3 constructs described above.

[0027] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety specifically recognizing GPC3 is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

[0028] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety specifically recognizing GPC3 is a full-length antibody, a Fab, a Fab', a F(ab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antibody moiety specifically recognizing GPC3 is an scFv. In some embodiments, the antibody moiety specifically recognizing GPC3 is a Fab or Fab'.

[0029] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the antibody moiety specifically recognizing GPC3 is fused to an Fc fragment optionally via a linker. In some embodiments, the Fc fragment is an IgG1 Fc fragment.

[0030] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the isolated anti-GPC3 construct is a full-length antibody.

[0031] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the isolated anti-GPC3 construct is monospecific.

[0032] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the isolated anti-GPC3 construct is multispecific, such as bispecific. In some embodiments, the isolated anti-GPC3 construct is a tandem scFv, a diabody (Db), a single chain diabody (scDb), a dual-affinity retargeting (DART) antibody, a F(ab')2, a dual variable domain (DVD) antibody, a knob-into-hole (KiH) antibody, a dock and lock (DNL) antibody, a chemically cross-linked antibody, a heteromultimeric antibody, or a heteroconjugate antibody. In some embodiments, the isolated anti-GPC3 construct is a tandem scFv comprising two scFvs linked by a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the isolated anti-GPC3 construct further comprises a second antibody moiety specifically recognizing a second antigen. In some embodiments, the second antigen is an antigen on the surface of a T cell, such as cytotoxic T cell, a helper T cell, or a natural killer T cell. In some embodiments, the second antigen is an antigen on the surface of a B cell, a natural killer cell, a dendritic cell, a macrophage, a monocyte, or a neutrophil. In some embodiments, the second antigen is selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM. In some embodiments, the second antigen is CD3ε. In some embodiments, the isolated anti-GPC3 construct is a tandem scFv comprising an N-terminal scFv specifically recognizing GPC3 and a C-terminal scFv specifically recognizing CD3ε. In some embodiments, the expression of the anti-GPC3 construct is induced by the activation of an engineered T cell. In some embodiments, the engineered T cell is a T cell comprising a chimeric antigen receptor (CAR). In some embodiments, the engineered T cell is a T cell comprising a chimeric antibody-T cell receptor (TCR) construct (caTCR).

[0033] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the isolated anti-GPC3 construct is a chimeric antigen

receptor (CAR) comprising: (a) an extracellular domain comprising the antibody moiety; (b) a transmembrane domain; and (c) an intracellular signaling domain. In some embodiments, the intracellular signaling domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0034] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the isolated anti-GPC3 construct is a chimeric antibody-T cell receptor (TCR) construct (caTCR) comprising: (a) an extracellular domain comprising the antibody moiety; and (b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and a second TCRD comprising a second TCR-TM, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule. In some embodiments, the first TCR-TM is derived from one of the transmembrane domains of a first naturally occurring TCR and the second TCR-TM is derived from the other transmembrane domain of the first naturally occurring TCR. In some embodiments, at least one of the TCR-TMs is non-naturally occurring. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the first naturally occurring T cell receptor transmembrane domains. In some embodiments, the first naturally occurring TCR is a γ/δ TCR. In some embodiments, the first naturally occurring TCR is an α/β TCR. In some embodiments, the TCR-associated signaling molecule is selected from the group consisting of CD3δε, CD3γε, and ζζ (also known as CD3ζ or CD3ζζ).

[0035] In some embodiments according to any of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) described above, the isolated anti-GPC3 construct is an immunoconjugate comprising the antibody moiety and an effector molecule. In some embodiments, the effector molecule is a therapeutic agent selected from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid. In some embodiments, therapeutic agent is a drug or a toxin. In some embodiments, the effector molecule is a label.

[0036] The present application in another aspect provides an isolated nucleic acid encoding the polypeptide components of any one of the isolated anti-GPC3 constructs described above.

[0037] The present application in another aspect provides a vector comprising an isolated nucleic acid encoding the polypeptide components of any one of the isolated anti-GPC3 constructs described above.

[0038] The present application in another aspect provides an isolated host cell comprising any one of the anti-GPC3 constructs described above.

[0039] The present application in another aspect provides an isolated host cell comprising an isolated nucleic acid encoding the polypeptide components of any one of the isolated anti-GPC3 constructs described above.

[0040] The present application in another aspect provides an isolated host cell comprising a vector comprising an isolated nucleic acid encoding the polypeptide components of any one of the isolated anti-GPC3 constructs described above.

[0041] The present application in another aspect provides an effector cell expressing any one of the isolated anti-GPC3 construct described above. In some embodiments, the effector cell is a T cell.

[0042] The present application in another aspect provides a pharmaceutical composition comprising any one of the anti-GPC3 construct (such as isolated anti-GPC3 construct) described above, and a pharmaceutically acceptable carrier.

[0043] Also provided are kits comprising any one of the isolated anti-GPC3 constructs described above, an isolated nucleic acid encoding the polypeptide components of any one of the isolated anti-GPC3 constructs described above, a vector comprising an nucleic acid encoding the polypeptide components of any one of the isolated anti-GPC3 constructs described above, any one of the isolated cell described above, or any one of the effector cell described above.

[0044] The present application in another aspect provides a method of detecting GPC3 in a sample, comprising contacting the sample with any one of the isolated anti-GPC3 immunoconjugate described above comprising an antibody moiety and a label, and detecting the presence of the label. In some embodiments, the sample comprises cells with cell surface-bound GPC3. In some embodiments, the sample comprises soluble GPC3.

[0045] The present application in another aspect provides a method of treating an individual having a GPC3-positive disease, comprising administering to the individual: a) an effective amount of any one of the pharmaceutical compositions described above; or b) an effective amount of any one of the effector cells described above. In some embodiments, the administration is via intravenous or intratumoral route. In some embodiments, the administration is to an injection site distal to a first disease site. In some embodiments, the method of treating an individual having a GPC3-positive disease further comprising administering to the individual an additional therapy. In some embodiments, the GPC3-positive disease is cancer, such as HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor,

gastric carcinoma, or liposarcoma. In some embodiments the cancer is HCC, such as metastatic HCC.

[0046] The present application in another aspect provides a method of diagnosing an individual having a GPC3-positive disease, comprising: a) administering an effective amount of any one of the isolated anti-GPC3 immunocon ugates described above comprising an antibody moiety and a label; and b) determining the level of the label in the individual, wherein a level of the label above a threshold level indicates that the individual has the GPC3-positive disease. In some embodiments, the GPC3-positive disease is cancer, such as HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments the cancer is HCC, such as metastatic HCC.

[0047] The present application in another aspect provides a method of diagnosing an individual having an GPC3-positive disease, comprising: a) contacting a sample derived from the individual with any one of the isolated anti-GPC3 immunocon ugates described above comprising an antibody moiety and a label; and b) determining the number of cells bound with the isolated anti-GPC3 construct in the sample, wherein a value for the number of cells bound with the isolated anti-GPC3 construct above a threshold level indicates that the individual has the GPC3-positive disease. In some embodiments, the GPC3-positive disease is cancer, such as HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments the cancer is HCC, such as metastatic HCC.

[0048] Also provided are methods of making any of the constructs described herein, articles of manufacture, and kits that are suitable for the methods described herein.

[a] In some embodiments there is provided an isolated anti-GPC3 construct according to any aspect embodiment or example of the invention comprising an anti-GPC3 antibody moiety specifically recognizing GPC3, wherein the anti-GPC3 antibody moiety comprises:

I. i) a heavy chain variable domain ($_H$) comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a light chain variable domain ($_L$) comprising a light chain complementarity

determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; or

II. i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; or

III. i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; or

I . i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 52, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 103; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 154, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 205, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 256; or

. i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 11, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 113; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 164, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 215, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 266; or

I. i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 12, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 63, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 114; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID

NO: 165, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 216, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 267; or

II. i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 95, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 146; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 197, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 248, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 299.

[ ] In some embodiments there is provided an isolated anti-GPC3 construct according to any aspect embodiment or example of the invention, wherein the anti-GPC3 antibody moiety comprises:

I. i) a $_H$ comprising the amino acid sequence of SEQ ID NO: 309; and ii) a $_L$ comprising the amino acid sequence of SEQ ID NO: 360; or

II. i) a $_H$ comprising the amino acid sequence of SEQ ID NO: 327; and ii) a $_L$ comprising the amino acid sequence of SEQ ID NO: 378; or

III. i) a $_H$ comprising the amino acid sequence of SEQ ID NO: 330; and ii) a $_L$ comprising the amino acid sequence of SEQ ID NO: 381; or

I . i) a $_H$ comprising the amino acid sequence of SEQ ID NO: 307; and ii) a $_L$ comprising the amino acid sequence of SEQ ID NO: 358; or

. i) a $_H$ comprising the amino acid sequence of SEQ ID NO: 317; and ii) a $_L$ comprising the amino acid sequence of SEQ ID NO: 368; or

I. i) a $_H$ comprising the amino acid sequence of SEQ ID NO: 318; and ii) a $_L$ comprising the amino acid sequence of SEQ ID NO: 369; or

II. i) a $_H$ comprising the amino acid sequence of SEQ ID NO: 350; and ii) a $_L$ comprising the amino acid sequence of SEQ ID NO: 401.

[ ] In some embodiments there is provided an isolated anti-GPC3 construct according to any aspect embodiment or example of the invention, wherein:

(i) the anti-GPC3 antibody moiety is a full-length antibody, a Fab, a Fab', a F(ab')$_2$, an Fv, or a single chain Fv (scFv);

(ii) the anti-GPC3 antibody moiety is fused to an Fc fragment optionally via a linker; and/or

(iii) the isolated anti-GPC3 construct is multi-specific.

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[d] In some embodiments there is provided an isolated anti-GPC3 construct according to any aspect embodiment or example of the invention, wherein the isolated anti-GPC3 construct is a tandem scFv comprising two scFvs linked by a peptide linker, wherein the anti-GPC3 antibody moiety is the first scFv, and wherein the second scFv is a second antibody moiety specifically recognizing a second antigen.

[e] In some embodiments there is provided an isolated anti-GPC3 construct according to any aspect embodiment or example of the invention, wherein the second antigen is selected from the group consisting of CD3 , CD3 , CD3 , CD3 , CD28, OX40, GITR, CD137, CD27, CD40L, and H EM, preferably wherein the second antigen is CD3 .

[ ] In some embodiments there is provided an isolated anti-GPC3 construct according to any aspect embodiment or example of the invention, wherein the isolated anti-GPC3 construct is a chimeric antigen receptor (CAR) comprising:

(a) an extracellular domain comprising the anti-GPC3 antibody moiety;

(b) a transmembrane domain; and

(c) an intracellular signaling domain.

[g] For example, wherein (i) the intracellular signaling domain comprises (a) a CD3 intracellular signaling sequence and (b) a CD28 or CD30 intracellular signaling sequence; and/or (ii) wherein the CAR comprises the amino acid sequence of any one of SEQ ID NOs: 491 and 516-521.

[ ] In some embodiments there is provided an isolated anti-GPC3 construct according to any aspect embodiment or example of the invention, wherein the isolated anti-GPC3 construct is a chimeric antibody-T cell receptor (TCR) construct (caTCR) comprising:

(a) an extracellular domain comprising the anti-GPC3 antibody moiety; and

(b) a TCR module (TCRM), wherein the TCRM comprises a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and a second TCRD comprising a second TCR-TM, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule.

[i] In some embodiments there is provided an isolated anti-GPC3 construct according to any aspect embodiment or example of the invention, wherein the isolated anti-GPC3 construct is a chimeric co-stimulatory receptor (CSR) comprising:

(a) a ligand-binding module comprising the anti-GPC3 antibody moiety;

(b) a transmembrane module; and

(c) a co-stimulatory immune cell signaling module.

[ ] In some embodiments there is provided an isolated anti-GPC3 construct according to any aspect embodiment or example of the invention, wherein the isolated anti-GPC3 construct is an immunocon ugate comprising the anti-GPC3 antibody moiety and an effector molecule, for example (i) wherein the effector molecule is a therapeutic agent selected from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid, or (ii) wherein the effector molecule is a label.

[ ] In some embodiments there is provided an isolated nucleic acid or a vector encoding the polypeptide components of the isolated anti-GPC3 construct according to any aspect embodiment or example of the invention.

[ ] In some embodiments there is provided an isolated host cell expressing the anti-GPC3 construct, or comprising the isolated nucleic acid or the vector according to any aspect, embodiment or example of the invention.

[ ] In some embodiments there is provided an effector cell expressing the isolated anti-GPC3 construct according to any aspect, embodiment or example of the invention or comprising the isolated nucleic acid or the vector according to any aspect, embodiment or example of the invention. for example, said effector cell may be a T cell, or a T cell is selected is from the group consisting of a cytotoxic T cell, a helper T cell, and a natural killer T cell.

[**n**] In some embodiments the caTCR of the effector T cell of the invention described herein comprises:

(a) an extracellular domain, wherein the extracellular domain comprises:

(i) an anti-GPC3 antibody moiety; or

(ii) an anti-AMC antibody moiety that specifically binds to a complex comprising an alpha-fetoprotein (AFP) peptide and a ma or histocompatibility complex (MHC) class I protein (an AFP/MHC class I complex, or AMC); and

(b) a TCR module (TCRM), wherein the TCRM comprises a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and a second TCRD comprising a second TCR-TM, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule.

[**o**] For example, the TCR-associated signaling module of the effector T cell of the invention described herein is selected from the group consisting of CD3 , CD3 , and .

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[ ] For example, the extracellular domain of the caTCR of the effector T cell of the invention described herein comprises an anti-GPC3 antibody moiety, and wherein:

(i) the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity; and/or

(ii) the IC_{50} of a soluble GPC3 to compete binding between the anti-GPC3 antibody moiety and a cell surface-bound GPC3 is about 1 g/mL to about 100 g/mL.

[ ] For example, the effector T cell of the invention described herein expressing the caTCR further comprises a CSR, and wherein the CSR comprises:

(a) a ligand-binding module comprising an antibody moiety;

(b) a transmembrane module; and

(c) a co-stimulatory immune cell signaling module.

[**r]** In one example, the antibody moiety of the CSR is an anti-GPC3 antibody moiety.

[ ] In some embodiments or examples of the invention there is provided an effector cell, wherein:

(I) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 309, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 360; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 309, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 360; or

(II) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 309, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 360; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 327, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 378; or

(III) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 309, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 360; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 330, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 381; or

(I) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 327, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 378; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 309, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 360; or

( ) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 327, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 378; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 327, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 378; or

(I) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 327, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 378; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID

NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a $_{L}$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a $_{H}$ comprising the amino acid sequence of SEQ ID NO: 330, and a $_{L}$ comprising the amino acid sequence of SEQ ID NO: 381; or

(II) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_{H}$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a $_{L}$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a $_{H}$ comprising the amino acid sequence of SEQ ID NO: 330, and a $_{L}$ comprising the amino acid sequence of SEQ ID NO: 381; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_{H}$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a $_{L}$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a $_{H}$ comprising the amino acid sequence of SEQ ID NO: 309, and a $_{L}$ comprising the amino acid sequence of SEQ ID NO: 360; or

(III) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_{H}$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a $_{L}$ comprising an LC-CDR1 comprising the amino acid sequence

of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 330, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 381; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 327, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 378; or

(I) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 330, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 381; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a $_H$ comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a $_L$ comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ

ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a $_H$ comprising the amino acid sequence of SEQ ID NO: 330, and a $_L$ comprising the amino acid sequence of SEQ ID NO: 381; or

(X) the caTCR is the anti-GPC3 caTCR according to any aspect, embodiment or example of the invention described herein.

[ta] In some embodiments there is provided pharmaceutical composition comprising the isolated anti-GPC3 construct according to any aspect, embodiment or example described herein or the effector cell according to any aspect, embodiment or example described herein, and a pharmaceutically acceptable carrier.

[t] In some embodiments there is provided a method of treating an individual having a GPC3-positive disease, comprising administering to the individual an effective amount of the isolated anti-GPC3 construct of any aspect, embodiment or example described herein or the effector cell according to any aspect, embodiment or example described herein, or the pharmaceutical composition of any aspect, embodiment or example described herein.

[u] In some embodiments, there is provided a use of the isolated anti-GPC3 construct according to any aspect, embodiment or example described herein, or the effector cell according to any aspect, embodiment or example described herein, or the pharmaceutical composition of any aspect, embodiment or example described herein in treating an individual having an GPC3-positive disease.

[ ] In some embodiments, there is provided a use of the isolated anti-GPC3 construct according to any aspect, embodiment or example described herein or the effector cell according to any aspect, embodiment or example described herein or the pharmaceutical composition of any aspect, embodiment or example desribed herein in the manufacture of a medicament for treating an individual having an GPC3-positive disease.

[ ] In some embodiments there is provided a use of the isolated anti-GPC3 construct comprising a label according to the invention in the preparation of a diagnostic agent for diagnosing a GPC3-postive disease in an individual, wherein the diagnostic agent is adapted to be administrable to the individual, and wherein a level of the label in the individual above a threshold level indicates that the individual has the GPC3-positive disease.

[ ] For example, the GPC3-positive disease may be cancer, or the GPC3-positive cancer is selected from the group consisting of HCC, melanoma, lung squamous cell carcinoma, ovarian

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carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, and liposarcoma.

[ ] In some embodiments there is provided a method of producing an isolated anti-GPC3 construct, comprising:

(a) culturing a host cell comprising the isolated nucleic acid or the vector of any aspect, embodiment or example of the invention described herein, or the isolated host cell of any aspect, embodiment or example of the invention desceibed herein, under a condition effective to express the anti-GPC3 construct; and

(b) obtaining the expressed anti-GPC3 construct from said host cell.

[ ] Throughout this specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

BRIEF DESCRIPTION OF THE DRA ING S

[0049] FIGs. 1A-1G show FACS analysis of the binding of 14 exemplary GPC3A phage clones to GPC3 HepG2 cells. The binding of helper phages to the GPC3 HepG2 cells was used as a control.

[0050] FIGs. 2A-2E show FACS analysis of the binding of 5 exemplary GPC3A phage clones to GPC3 HepG2 cell line and HepG2 GPC3-knockout cell lines (HepG2-GPC3-KO-2 and HepG2-GPC3-KO-3). Helper phages were used as a negative control.

[Continued on Page]

[0051] FIGs. 3A-3D show FACS analysis of the binding of 4 exemplary GPC3B phage clones to $GPC3^+$ HepG2 cell line and HepG2-GPC3-KO-2 cell line. Helper phages were used as a negative control.

[0052] FIGs. 4A-4B show FACS analysis of the binding of exemplary GPC3A L2K bispecific antibodies (anti-GPC3×CD3 di-scFvs derived from GPC3A screen) to SK-Hep1-GPC3 cells and GPC3-negative SK-Hep1 cells. The y-axis shows median fluorescence intensity (MFI).

[0053] FIGs. 5A-5B show FACS analysis of the binding of exemplary GPC3A L2K bispecific antibodies (anti-GPC3×CD3 di-scFvs derived from GPC3A screen) to GPC3+ HepG2 cells. The binding of a control L2K bispecific antibody to GPC3+ HepG2 cells was used as a negative control.

[0054] FIG. 6 shows FACS analysis of the binding of GPC3A L2K bispecific antibodies to GPC3+ HepG2 cells in a competition assay provided with or without soluble GPC3 antigen. A negative control L2K bispecific antibody and a commercial anti-GPC3 (1G12) antibody were used for comparison purposes.

[0055] FIG. 7A shows T cell-mediated target cell killing by GPC3A L2K bispecific antibodies, tested on GPC3+ HepG2 cells, GPC3-negative SK-Hep1 cells, SK-Hep1-GPC3 cells, HepG2-GPC3-KO cells (#2 and #11). FIG. 7B shows T cell-mediated target cell killing by GPC3B L2K bispecific antibodies, tested on GPC3+ HepG2 cells, GPC3-negative SK-Hep1 cells, SK-Hep1-GPC3 cells, and HepG2-GPC3-KO-2 cells.

[0056] FIG. 8 shows FACS analysis of HepG2 GPC3-knockout cell lines using a commercial mouse anti-human GPC3 antibody (1G12), demonstrating successful generation of HepG2-GPC3-KO cell lines.

[0057] FIG. 9A shows FACS analysis of the binding of anti-human GPC3 (hGPC3) monospecific IgG antibodies with mouse constant domain/Fc region (mIgG1) to GPC3-positive HepG2 cells. FIG. 9B shows FACS analysis of the binding of anti-hGPC3 mIgG1 to GPC3-negative SK-Hep1 cells.

[0058] FIG. 10 shows the FACS fluorescence intensity curves indicating competition for HepG2-binding of GPC3 L2K antibodies (GPC3B-87 L2K or GC33 L2K) by soluble GPC3.

[0059] FIG. 11 shows the FACS fluorescence intensity curves indicating the binding affinity of GPC3 LK2 clones towards HepG2 cells.

[0060] FIG. 12 shows the ELISA analysis of reactivity of GPC3 mIgG1 clones to rat GPC3 and human GPC3 (hGPC3).

[0061] FIG. 13 shows the GPC3 epitope binning analysis of GC33-mIgG, GPC3A-37 mIgG and GPC3A-55 mIgG using a linear format of epitope binning.

[0062] FIG. 14 shows the GPC3 epitope binning analysis of GC33-mIgG, GPC3A-37 mIgG and GPC3A-55 mIgG using a sandwich format of epitope binning.

[0063] FIGs. 15A and 15B show the plotted data from single-cycle kinetics analysis of binding between GPC3A L2K clones and GPC3 antigen.

[0064] FIG. 16 shows the LDH cytotoxicity analysis in GPC3-positive HepG2 cells, GPC3-negative SK-Hep1 cells, SK-Hep1-GPC3 cells, and HepG2-GPC3-KO cells treated by GPC3A or GPC3B CAR-T cells and GC33 CAR-T cells.

[0065] FIG. 17A shows the LDH cytotoxicity analysis in GPC3+ HepG2 cells, GPC3-negative SK-Hep1 cells, SK-Hep1-GPC3 cells, and HepG2-GPC3-KO cells treated by GPC3A or GPC3B CAR-T cells and GC33 CAR-T cells. FIG. 17B shows the LDH cytotoxicity analysis in GPC3-positive JHH5 cells, GPC3-negative A-498 cells, and GPC3-negative PANC-1 cells treated by GPC3A or GPC3B CAR-T cells and GC33 CAR-T cells.

[0066] FIG. 18 shows percent specific lysis from the killing of cancer cell lines HepG2 ($AFP^+/GPC3^+$) and HepG2-GPC3-KO ($AFP^+/GPC3^-$), mediated by T cells transduced with either anti-AFP158/HLA-A*2:01 caTCR alone or anti-AFP158/HLA-A*2:01 caTCR + anti-CD3/anti-GPC3 BsAb at the indicated percent caTCR positivity (5% to 40%).

[0067] FIG. 19 shows the concentration of cytokines (IL-2, IFN-γ, and TNF-α) found in the supernatant after *in vitro* killing of cancer cell lines HepG2 and HepG2-GPC3-KO, mediated by T cells transduced with either anti-AFP158/HLA-A*2:01 caTCR alone or anti-AFP158/HLA-A*2:01 caTCR + anti-CD3/anti-GPC3 BsAb at the indicated percent caTCR positivity (5% to 40%).

[0068] FIG. 20 shows the potentiation of target-specific cancer cell line killing mediated by anti-CD3/anti-GPC3 BsAbs released from activated anti-AFP158/HLA-A*2:01 caTCR + anti-CD3/anti-GPC3 BsAb T cells. The indicated transduced T cells and target cells were incubated together and separated from the indicated target cells and mock T cells by a membrane permeable to the BsAb, but not the T cells.

[0069] FIG. 21 shows percent specific lysis from the killing of cancer cell lines HepG2 ($AFP^+/GPC3^+$) and HepG2-GPC3.ko ($AFP^+/GPC3^-$), mediated by T cells transduced with anti-AFP158/HLA-A*2:01 caTCR-1-0 alone, anti-GPC3 CSR alone, or anti-AFP158/HLA-A*2:01 caTCR (1-0 and 1-TM5) + anti-GPC3 CSR.

[0070] FIG. 22 shows the concentration of cytokines (IL-2, GM-CSF, IFN-γ, and TNF-α) found in the supernatant after *in vitro* killing of cancer cell lines HepG2 and HepG2-GPC3-KO, mediated by T cells transduced with anti-AFP158/HLA-A*2:01 caTCR-1-0 alone, anti-GPC3 CSR alone, or anti-AFP158/HLA-A*2:01 caTCR (1-0 and 1-TM5) + anti-GPC3 CSR.

[0071] FIG. 23 shows the degranulation activity (as determined by CD107a expression) in T cells transduced with anti-AFP158/HLA-A*2:01 caTCR-1-0 alone, anti-GPC3 CSR alone, or anti-AFP158/HLA-A*2:01 caTCR (1-0 and 1-TM5) + anti-GPC3 CSR following stimulation with cancer cell line HepG2.

[0072] FIG. 24 shows the proliferation (as determined by CFSE dye dilution) of T cells transduced with anti-AFP158/HLA-A*2:01 caTCR-1-0 alone, anti-GPC3 CSR alone, or anti-AFP158/HLA-A*2:01 caTCR (1-0 and 1-TM5) + anti-GPC3 CSR following stimulation with cancer cell line HepG2.

[0073] FIG. 25 shows the tumor growth in a subcutaneous mouse model of HepG2 with mock treatment or with a single intratumoral injection of T cells transduced with an anti-AFP CAR, or an anti-AFP CAR in combination with an anti-GPC3 CSR.

DETAILED DESCRIPTION OF THE INVENTION

[0074] The present application in one aspect provides constructs (referred to herein as "anti-GPC3 constructs") (such as isolated anti-GPC3 construct) that comprise an antibody moiety (referred to herein as an "anti-GPC3 antibody moiety") specifically recognizing a cell surface-bound GPC3 (referred to herein as a "native format GPC3," or "native GPC3 (nGPC3)"). These anti-GPC3 constructs specifically recognize a cell surface-bound GPC3, as opposed to a non-cell surface bound GPC3 (referred to herein as a "soluble GPC3 (sGPC3)," or "non-native GPC3"), such as circulating GPC3 protein or free GPC3 peptides in the serum.

[0075] When armed as anti-CD3 bispecific antibodies or present in a chimeric antigen receptor (CAR) expressed by a T cell, the anti-GPC3 antibody moiety specifically redirected human T cells to kill GPC3-expressing target cells (such as GPC3-expressing cancer cells). This strategy provides a significant technical advantage over using antibodies directed against any format of GPC3 (especially soluble GPC3), because T cell targeting to tumor site can become a lot more efficient and precise, resulting in effective T cell-mediated cancer cell killing without harming normal tissues. Furthermore, when fused to a detectable moiety, the anti-GPC3 antibody moiety allows for diagnosis and prognosis of GPC3-positive diseases or disorders with high sensitivity to changes in the number and distribution of cells expressing GPC3 on the cell

surface (such as GPC3-positive tumor cells), a potentially more relevant measure of disease progression than circulating GPC3 levels.

[0076] The present application also provides anti-GPC3 constructs (such as isolated anti-GPC3 constructs) comprising an antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3). These constructs can also be armed as anti-CD3 bispecific antibodies or present in a CAR expressed by a T cell. GPC3 can be expressed on cell surface or released from cell surface to extracellular environment in different forms in cancer patients (such as HCC patients). Thus, these anti-GPC3 constructs can also be fused to a detectable moiety and used for diagnosis and prognosis purposes.

[0077] Using phage display technology, we generated multiple monoclonal antibodies that are specific and show high affinity against cell surface-bound human GPC3. Flow cytometry and T-cell mediated cytotoxicity assays demonstrated that these antibodies recognize GPC3-expressing cancer cell lines, in a native format GPC3-restricted manner. When armed as anti-CD3 bispecific antibodies or CAR-T cells, the antibodies re-directed human T cells to kill GPC3-positive target cancer cells. The data presented herein demonstrate that the anti-GPC3 constructs comprising an antibody moiety specifically recognizing cell surface-bound GPC3 described herein can be effective therapeutic agents for cancer indications, such as solid tumor indications (*e.g.*, HCC).

[0078] Using phage display technology, we also generated multiple monoclonal antibodies that are specific and high affinity against human GPC3 (*e.g.*, nGPC3 and/or sGPC3). Flow cytometry and T-cell mediated cytotoxicity assays demonstrated that these antibodies specifically recognize GPC3.

[0079] The present application thus provides constructs (such as isolated constructs) comprising an antibody moiety specifically recognizing GPC3, such as a cell surface-bound GPC3. The construct can be, for example, anti-GPC3 scFv, anti-GPC3 Fc fusion protein, full-length anti-GPC3 antibodies, multi-specific (such as bispecific) anti-GPC3 molecules (e.g., tandem di-scFv bispecific T cell engager), anti-GPC3 chimeric antigen receptors (CARs), anti-GPC3 chimeric antibody-T cell receptors (caTCRs), and anti-GPC3 immunoconjugates.

[0080] In another aspect, there are provided nucleic acids encoding the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) or the anti-GPC3 antibody moiety portion of the constructs (such as those specifically recognizing cell surface-bound GPC3).

[0081] In another aspect, there are provided compositions (such as pharmaceutical compositions) comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an antibody moiety specifically recognizing GPC3 (such as a cell surface-bound

GPC3). The composition can be a pharmaceutical composition comprising an anti-GPC3 construct (such as a cell surface-bound GPC3) or an effector cell expressing or associated with the anti-GPC3 construct (for example a T cell expressing an anti-GPC3 CAR, a CAR specifically recognizing a cell surface-bound GPC3).

[0082] Also provided are methods of making and using the anti-GPC3 constructs (such as isolated anti-GPC3 constructs, or cells expressing or associated with the anti-GPC3 constructs) for treatment or diagnostic purposes, as well as kits and articles of manufacture useful for such methods.

Definitions

[0083] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results, including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), preventing or delaying the spread (*e.g.*, metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing or improving the quality of life, increasing weight gain, and/or prolonging survival. Also encompassed by "treatment" is a reduction of pathological consequence of cancer (such as, for example, tumor volume). The methods of the invention contemplate any one or more of these aspects of treatment.

[0084] "Activation" as used herein in relation to T cells, refers to the state of a T cell that has been sufficiently stimulated to induce detectable cellular proliferation. Activation can also be associated with induced cytokine production, and detectable effector functions.

[0085] The term "antibody moiety" includes full-length antibodies and antigen-binding fragments thereof. A full-length antibody comprises two heavy chains and two light chains. The variable regions of the light and heavy chains are responsible for antigen binding. The variable regions in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain (LC) CDRs including LC-CDR1, LC-CDR2, and LC-CDR3, heavy chain (HC) CDRs including HC-CDR1, HC-CDR2, and HC-CDR3). CDR boundaries for the antibodies and antigen-binding fragments disclosed herein may be defined or identified by the conventions of Kabat, Chothia, or Al-Lazikani (Al-Lazikani 1997; Chothia

1985; Chothia 1987; Chothia 1989; Kabat 1987; Kabat 1991). The three CDRs of the heavy or light chains are interposed between flanking stretches known as framework regions (FRs), which are more highly conserved than the CDRs and form a scaffold to support the hypervariable loops. The constant regions of the heavy and light chains are not involved in antigen binding, but exhibit various effector functions. Antibodies are assigned to classes based on the amino acid sequence of the constant region of their heavy chain. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of α, δ, ε, γ, and μ heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as lgG1 (γ1 heavy chain), lgG2 (γ2 heavy chain), lgG3 (γ3 heavy chain), lgG4 (γ4 heavy chain), lgA1 (α1 heavy chain), or lgA2 (α2 heavy chain).

[0086] The term "antigen-binding fragment" as used herein refers to an antibody fragment including, for example, a diabody, a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)2, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain Fv (scFv), an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment (*e.g.*, a parent scFv) binds. In some embodiments, an antigen-binding fragment may comprise one or more CDRs from a particular human antibody grafted to a framework region from one or more different human antibodies.

[0087] The term "epitope" as used herein refers to the specific group of atoms or amino acids on an antigen to which an antibody or antibody moiety binds. Two antibodies or antibody moieties may bind the same epitope within an antigen if they exhibit competitive binding for the antigen.

[0088] As used herein, a first antibody moiety "competes" for binding to a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) with a second antibody moiety when the first antibody moiety inhibits target GPC3 binding of the second antibody moiety by at least about 50% (such as at least about any of 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) in the presence of an equimolar concentration of the first antibody moiety, or *vice versa*. A high throughput process for "binning" antibodies based upon their cross-competition is described in PCT Publication No. WO 03/48731.

[0089] As use herein, the term "specifically binds," "specifically recognizing," or "is specific for" refers to measurable and reproducible interactions, such as binding between a target and an antibody or antibody moiety, that is determinative of the presence of the target in the presence of a heterogeneous population of molecules, including biological molecules. For example, an antibody or antibody moiety that specifically recognizes a target (which can be an epitope) is an antibody or antibody moiety that binds this target with greater affinity, avidity, more readily, and/or with greater duration than its bindings to other targets. In some embodiments, an antibody or antibody moiety that specifically recognizes an antigen reacts with one or more antigenic determinants of the antigen (such as native format GPC3) with a binding affinity that is at least about 10 times its binding affinity for other targets (such as soluble GPC3).

[0090] An "isolated" anti-GPC3 construct as used herein refers to an anti-GPC3 construct that (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, (3) is expressed by a cell from a different species, or, (4) does not occur in nature.

[0091] The term "isolated nucleic acid" as used herein is intended to mean a nucleic acid of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated nucleic acid" (1) is not associated with all or a portion of a polynucleotide in which the "isolated nucleic acid" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

[0092] As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252:6609-6616 (1977); Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991); Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987); Al-Lazikani B. *et al.*, *J. Mol. Biol.*, 273: 927-948 (1997); MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996); Abhinandan and Martin, *Mol. Immunol.,* 45: 3832-3839 (2008); Lefranc M.P. *et al.*, *Dev. Comp. Immunol.*, 27: 55-77 (2003); and Honegger and Plückthun, *J. Mol. Biol.*, 309:657-670 (2001), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. CDR prediction algorithms and interfaces are known in the art, including, for example, Abhinandan and Martin, *Mol. Immunol.,* 45: 3832-3839 (2008); Ehrenmann F. *et al.*,

Nucleic Acids Res., 38: D301-D307 (2010); and Adolf-Bryfogle J. *et al.*, *Nucleic Acids Res.*, 43: D432-D438 (2015). The contents of the references cited in this paragraph are incorporated herein by reference in their entireties for use in the present invention and for possible inclusion in one or more claims herein.

TABLE 1: CDR DEFINITIONS

	Kabat[1]	Chothia[2]	MacCallum[3]	IMGT[4]	AHo[5]
V_H CDR1	31-35	26-32	30-35	27-38	25-40
V_H CDR2	50-65	53-55	47-58	56-65	58-77
V_H CDR3	95-102	96-101	93-101	105-117	109-137
V_L CDR1	24-34	26-32	30-36	27-38	25-40
V_L CDR2	50-56	50-52	46-55	56-65	58-77
V_L CDR3	89-97	91-96	89-96	105-117	109-137

[1]Residue numbering follows the nomenclature of Kabat *et al.*, *supra*

[2]Residue numbering follows the nomenclature of Chothia *et al.*, *supra*

[3]Residue numbering follows the nomenclature of MacCallum *et al.*, *supra*

[4]Residue numbering follows the nomenclature of Lefranc *et al.*, *supra*

[5]Residue numbering follows the nomenclature of Honegger and Plückthun, *supra*

[0093] The term "chimeric antibodies" refer to antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit a biological activity of this invention (*see* U.S. Patent No. 4,816,567; and Morrison *et al.*, Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)).

[0094] The term "semi-synthetic" in reference to an antibody or antibody moiety means that the antibody or antibody moiety has one or more naturally occurring sequences and one or more non-naturally occurring (*i.e.*, synthetic) sequences.

[0095] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the heavy and light chain) that contribute the amino acid residues for antigen binding and 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 at a lower affinity than the entire binding site.

[0096] "Single-chain Fv," also abbreviated as "sFv" or "scFv," are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. In some embodiments, the scFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, *see* Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0097] The term "diabodies" refers to small antibody fragments prepared by constructing scFv fragments (see preceding paragraph) typically with short linkers (such as about 5 to about 10 residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, *i.e.*, fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" scFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

[0098] "Humanized" forms of non-human (*e.g.*, rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (HVR) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired antibody specificity, affinity, and capability. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, *see* Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

[0099] "Percent (%) amino acid sequence identity" or "homology" with respect to the polypeptide and antibody sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the polypeptide being compared, after aligning the sequences considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, Megalign (DNASTAR), or MUSCLE software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program MUSCLE (Edgar, R.C., *Nucleic Acids Research* 32(5):1792-1797, 2004; Edgar, R.C., *BMC Bioinformatics* 5(1):113, 2004).

[0100] The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. In some embodiments, an FcR of this invention is one that binds an IgG antibody (a γ receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these 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* review M. in Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). The term includes allotypes, such as FcγRIIIA allotypes: FcγRIIIA-Phe158, FcγRIIIA-Val158, FcγRIIA-R131 and/or FcγRIIA-H131. FcRs are reviewed 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). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al., J. Immunol.* 117:587 (1976) and Kim *et al., J. Immunol.* 24:249 (1994)).

[0101] The term "FcRn" refers to the neonatal Fc receptor (FcRn). FcRn is structurally similar to major histocompatibility complex (MHC) and consists of an α-chain noncovalently bound to β2-microglobulin. The multiple functions of the neonatal Fc receptor FcRn are

reviewed in Ghetie and Ward (2000) *Annu. Rev. Immunol.* 18, 739-766. FcRn plays a role in the passive delivery of immunoglobulin IgGs from mother to young and the regulation of serum IgG levels. FcRn can act as a salvage receptor, binding and transporting pinocytosed IgGs in intact form both within and across cells, and rescuing them from a default degradative pathway.

[0102] The "CH1 domain" of a human IgG Fc region (also referred to as "C1" of "H1" domain) usually extends from about amino acid 118 to about amino acid 215 (EU numbering system).

[0103] "Hinge region" is generally defined as stretching from Glu216 to Pro230 of human IgG1 (Burton, *Molec. Immunol.*22:161-206 (1985)). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds in the same positions.

[0104] The "CH2 domain" of a human IgG Fc region (also referred to as "C2" of "H2" domain) usually extends from about amino acid 231 to about amino acid 340. The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It has been speculated that the carbohydrate may provide a substitute for the domain-domain pairing and help stabilize the CH2 domain. Burton, *Molec Immunol.* 22:161-206 (1985).

[0105] The "CH3 domain" (also referred to as "C2" or "H3" domain) comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (*i.e.* from about amino acid residue 341 to the C-terminal end of an antibody sequence, typically at amino acid residue 446 or 447 of an IgG).

[0106] A "functional Fc fragment" possesses an "effector function" of a native sequence Fc region. Exemplary "effector functions" include C1q binding; 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; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.* an antibody variable domain) and can be assessed using various assays known in the art.

[0107] An antibody with a variant IgG Fc with "altered" FcR binding affinity or ADCC activity is one which has either enhanced or diminished FcR binding activity (*e.g.*, FcγR or FcRn) and/or ADCC activity compared to a parent polypeptide or to a polypeptide comprising a native sequence Fc region. The variant Fc which "exhibits increased binding" to an FcR binds at least one FcR with higher affinity (*e.g.*, lower apparent K_d or IC_{50} value) than the parent polypeptide or a native sequence IgG Fc. According to some embodiments, the improvement in binding

compared to a parent polypeptide is about 3 fold, such as about any of 5, 10, 25, 50, 60, 100, 150, 200, or up to 500 fold, or about 25% to 1000% improvement in binding. The polypeptide variant which "exhibits decreased binding" to an FcR, binds at least one FcR with lower affinity (*e.g.*, higher apparent K_d or higher IC_{50} value) than a parent polypeptide. The decrease in binding compared to a parent polypeptide may be about 40% or more decrease in binding.

[0108] "Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound to Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.*, Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are absolutely required for such killing. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. 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. PNAS (USA)* 95:652-656 (1998).

[0109] The polypeptide comprising a variant Fc region which "exhibits increased ADCC" or mediates ADCC in the presence of human effector cells more effectively than a polypeptide having wild type IgG Fc or a parent polypeptide is one which *in vitro* or *in vivo* is substantially more effective at mediating ADCC, when the amounts of polypeptide with variant Fc region and the polypeptide with wild type Fc region (or the parent polypeptide) in the assay are essentially the same. Generally, such variants will be identified using any *in vitro* ADCC assay known in the art, such as assays or methods for determining ADCC activity, *e.g.*, in an animal model *etc.* In some embodiments, the variant is from about 5 fold to about 100 fold, *e.g.* from about 25 to about 50 fold, more effective at mediating ADCC than the wild type Fc (or parent polypeptide) .

[0110] "Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.* as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996), may be performed. Polypeptide variants with altered Fc region amino acid sequences and increased or

decreased C1q binding capability are described in US patent No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. *See* also, Idusogie *et al. J. Immunol.* 164: 4178-4184 (2000).

[0111] Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

[0112] The term "operably linked" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

[0113] "Homologous" refers to the sequence similarity or sequence identity between two polypeptides or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared times 100. For example, if 6 of 10 of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

[0114] An "effective amount" of an anti-GPC3 construct or composition as disclosed herein, is an amount sufficient to carry out a specifically stated purpose. An "effective amount" can be determined empirically and by known methods relating to the stated purpose.

[0115] The term "therapeutically effective amount" refers to an amount of an anti-GPC3 construct or composition as disclosed herein, effective to "treat" a disease or disorder in an individual. In the case of cancer, therapeutically effective amount of the anti-GPC3 construct or composition as disclosed herein can reduce the number of cancer cells; reduce the tumor size or

weight; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the anti-GPC3 construct or composition as disclosed herein can prevent growth and/or kill existing cancer cells, it can be cytostatic and/or cytotoxic. In some embodiments, therapeutically effective amount is a growth inhibitory amount. In some embodiments, therapeutically effective amount is an amount that extends the survival of a patient. In some embodiments, therapeutically effective amount is an amount that improves progression free survival of a patient.

[0116] As used herein, by "pharmaceutically acceptable" or "pharmacologically compatible" is meant a material that is not biologically or otherwise undesirable, *e.g.*, the material may be incorporated into a pharmaceutical composition administered to a patient without causing any significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. Pharmaceutically acceptable carriers or excipients have preferably met the required standards of toxicological and manufacturing testing and/or are included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug administration.

[0117] The term "label" when used herein refers to a detectable compound or composition which can be conjugated directly or indirectly to the anti-GPC3 antibody moiety. The label may be detectable by itself (*e.g.*, radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

[0118] It is understood that embodiments of the invention described herein include "consisting" and/or "consisting essentially of" embodiments.

[0119] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[0120] As used herein, reference to "not" a value or parameter generally means and describes "other than" a value or parameter. For example, the method is not used to treat cancer of type X means the method is used to treat cancer of types other than X.

[0121] As used herein and in the appended claims, the singular forms "a," "or," and "the" include plural referents unless the context clearly dictates otherwise.

Anti-GPC3 constructs

[0122] In one aspect, the present invention provides GPC3-specific constructs (such as isolated anti-GPC3 constructs) that comprise an antibody moiety that specifically binds to GPC3. The specificity of the anti-GPC3 construct derives from an anti-GPC3 antibody moiety, such as a full-length antibody or antigen-binding fragment thereof, which specifically binds to GPC3. In some embodiments, reference to a moiety (such as an antibody moiety) that specifically binds to GPC3 means that the moiety binds to the GPC3 with an affinity that is at least about 10 times (including for example at least about any of 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) its binding affinity for non-target. In some embodiments, the non-target is an antigen that is not GPC3. In some embodiments, for an anti-nGPC3 antibody moiety, the non-target is a soluble GPC3. In some embodiments, for an anti-sGPC3 antibody moiety, the non-target is a cell surface-bound GPC3. Binding affinity can be determined by methods known in the art, such as ELISA, fluorescence activated cell sorting (FACS) analysis, or radioimmunoprecipitation assay (RIA). K_d can be determined by methods known in the art, such as surface plasmon resonance (SPR) assay utilizing, for example, BIACORE™ instruments, or kinetic exclusion assay (KinExA) utilizing, for example, Sapidyne instruments.

[0123] Contemplated anti-GPC3 constructs include, for example, anti-GPC3 scFv, anti-GPC3 Fc fusion protein, full-length anti-GPC3 antibodies, multi-specific (such as bispecific) anti-GPC3 molecules (e.g., tandem di-scFv bispecific T cell engager), anti-GPC3 chimeric antigen receptors (CARs), anti-GPC3 chimeric antibody-T cell receptors (caTCRs), and anti-GPC3 immunoconjugates.

[0124] The different aspects are discussed in various sections below in further detail.

[0125] Although embodiments employing anti-GPC3 constructs comprising an anti-GPC3 antibody moiety that contain human sequences (i.e., human heavy and light chain variable region sequences comprising human CDR sequences) are extensively discussed herein, the present invention also provides non-human anti-GPC3 constructs. In some embodiments, non-human antibody agents comprise human CDR sequences from an antibody agent as described herein and non-human framework sequences. Non-human framework sequences include, in some embodiments, any sequence that can be used for generating synthetic heavy and/or light chain variable regions using one or more human CDR sequences as described herein, including, e.g., mammals, e.g., mouse, rat, rabbit, pig, bovine (e.g., cow, bull, buffalo), deer, sheep, goat, chicken, cat, dog, ferret, primate (e.g., marmoset, rhesus monkey), etc. In some embodiments, a provided antibody agent includes an antibody agent generated by grafting one or more human CDR sequences as described herein onto a non-human framework sequence (e.g., a mouse or

chicken framework sequence). In many embodiments, provided antibody agents are human antibody agents (e.g., a human monoclonal antibody or fragment thereof, human antigen-binding protein or polypeptide, human multi-specific binding agent [e.g., a human bi-specific antibody], a human polypeptide having one or more structural components of a human immunoglobulin polypeptide).

Anti-GPC3 antibody moiety

[0126] The anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprises an anti-GPC3 antibody moiety that specifically recognizes GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 (referred to herein as "anti-nGPC3 antibody moiety"). In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to nGPC3 is higher than that to an sGPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the cell expressing GPC3 on its surface is HepG2, Hep3B, Huh7, JHH-7, or 293. In some embodiments, the cell presents on its surface abnormally high levels of GPC3. In some embodiments, the cell is a cancer cell. In some embodiments, the cancer cell is in a solid tumor (such as liver cancer, e.g. HCC). In some embodiments, the cancer cell is a metastatic cancer cell (such as metastatic HCC). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3 (referred to herein as "anti-sGPC3 antibody moiety"). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both nGPC3 and sGPC3.

Glypican 3 (GPC3)

[0127] Glypican 3 (GPC3) is an oncofetal antigen belonging to the heparin sulfate proteoglycan family existing on the surface of cells, which is involved in cell signaling at the cellular-extracellular matrix interface. GPC3 is expressed in fetal liver and placenta during development and is down-regulated or silenced in normal adult tissues. This developmental stage- and tissue-specific expression manner suggests that GPC3 may be involved in morphogenesis.

[0128] The *GPC3* gene encodes a 70 kDa precursor protein of 580 amino acids. Upon translocation into the endoplasmic reticulum, the N-terminal signal peptide (SS; residues 1–24) and the C-terminal glycosylphosphatidylinositol (GPI) anchor addition signal (a predicted cleavage site: S^{560}) are removed and the latter is replaced with a GPI anchor. The GPC3 precursor protein can be cleaved by Furin between Arg^{358} and Ser^{359} to generate a 40 kDa N-terminal subunit ($Q^{25} – R^{358}$) and a 30 kDa C-terminal subunit (starting from S^{359}) linked by

disulfide bonds. The C-terminus of GPC3 links to the membrane through the GPI anchor and is post-translationally modified with two O-linked heparin sulfate (HS) side chains close to the cell surface. Based on the primary amino acid sequence, the N-terminal subunit, the C-terminal subunit and the two HS glycan chains form the three functional domains of GPC3. The N-terminal and C-terminal subunits form the core protein of GPC3.

[0129] The negatively-charged HS chains on GPC3 are functionally important glycosaminoglycans. They can bind positively-charged growth factors, such as HGFs, fibroblast growth factors (FGFs), Wnts, Hedgehog and bone morphogenetic proteins. Thus, the HS chains might serve as "docking sites" for these growth factors, depending on cellular context.

[0130] The N-terminal subunit of GPC3 is an N-terminal peptide of GPC3 and of about 40 kDa, which is found in the soluble form of the GPC3 core protein. In some embodiments, the N-terminal subunit is a peptide of an amino acid sequence comprising from Met^{1} to Arg^{358}. In some embodiments, the N-terminal subunit is a peptide of an amino acid sequence comprising from Gln^{25} to Arg^{358}. In accordance with the invention, fragments of such N-terminal peptide may also be employed, referred to herein as GPC3 N-terminal fragment. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope existing on the N-terminal subunit of the GPC3 protein. In some embodiments, the antibody moiety specifically recognizing the N-terminal subunit (or fragment thereof) of the GPC3 protein described herein specifically binds to the soluble format of GPC3. In some embodiments, the antibody moiety specifically recognizing the N-terminal subunit (or fragment thereof) of the GPC3 protein specifically binds to the native format of GPC3. In some embodiments, the antibody moiety specifically recognizing the N-terminal subunit (or fragment thereof) of the GPC3 protein can bind to both nGPC3 and sGPC3.

[0131] The C-terminal subunit of GPC3 is a C-terminal peptide of GPC3 and of about 30 kDa. Based on the cleavage site mentioned above, in some embodiments, the C-terminal subunit is a peptide of an amino acid sequence comprising from Ser^{359} to His^{580}. In some embodiments, the C-terminal subunit is a peptide of an amino acid sequence comprising from Ser^{359} to Ser^{560}. In accordance with the invention, fragments of such C-terminal peptide may also be employed, referred to herein as GPC3 C-terminal fragment. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope existing on the C-terminal subunit of the GPC3 protein. In some embodiments, the antibody moiety specifically recognizing the C-terminal subunit (or fragment thereof) of the GPC3 protein described herein specifically binds to the soluble format of GPC3. In some embodiments, the antibody moiety specifically recognizing the C-terminal subunit (or fragment thereof) of the GPC3 protein specifically binds to the native format of

GPC3. In some embodiments, the antibody moiety specifically recognizing the C-terminal subunit (or fragment thereof) of the GPC3 protein can bind to both nGPC3 and sGPC3.

[0132] The anti-GPC3 constructs described herein do not bind to the same epitope on GPC3 as known antibodies in the art, such as GC33 (Ishiguro et al., Cancer Res 2008; 68:9832-9838) (*e.g.*, SEQ ID NO: 510). In some embodiments, the anti-GPC3 antibody moiety does not specifically bind to the same or substantially the same GPC3 epitope competitively with GC33. In some embodiments, the anti-GPC3 antibody moiety does not bind to an epitope within the amino acid sequence of SEQ ID NO: 536. In some embodiments, the anti-GPC3 antibody moiety does not bind to a fragment comprising the amino acid sequence of SEQ ID NO: 536.

[0133] GPC3 has been reported to be expressed in various cancers and, in particular, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, Wilms' tumor, and liposarcoma. Thus, GPC3 may potentially serve as a therapeutic target for both antibody- and cell-based immunotherapy. Particularly, given that GPC3 is highly expressed in HCC, and is expressed in more than 70% of HCC tumors but not normal liver tissue, GPC3 can be a promising candidate for liver cancer therapy. For some patients with GPC3-positive cancers, soluble format of GPC3 can be detected in the blood. Thus, soluble and native formats of GPC3 can both serve as useful biomarkers for cancer diagnosis, such as HCC diagnosis. It was found that GPC3-positive HCC patients have a significantly lower 5-year survival rate than GPC3-negative HCC patients. GPC3 expression is therefore correlated with poor prognosis in HCC.

[0134] GPC3 cell surface localization is crucial for cell growth and Wnt activation in HCC. GPC3 binds to Wnt through its core protein. It was hypothesized that GPC3 stimulates Wnt signaling by facilitating and/or stabilizing the interaction of Wnt with Frizzled (Fz), its signaling receptor. Interestingly, it was noted that approximately 50% of HCC patients have secreted GPC3 in the sera. However, it is unclear what form of GPC3 is present in the circulating blood of cancer patients. The extracellular lipase, Notum, may be responsible for cleaving GPC3 from tumor cells into the extracellular environment.

[0135] In some embodiments, the anti-GPC3 antibody moiety described herein (against nGPC3 and/or sGPC3) specifically recognizes an epitope within human GPC3. The complete amino acid sequence of an exemplary human GPC3 has UniProt No. P51654 (SEQ ID NO: 460). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within amino acids 1-560 of

SEQ ID NO: 460 (SEQ ID NO: 461). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within amino acids 25-580 of SEQ ID NO: 460 (SEQ ID NO: 462). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within amino acids 25-560 of SEQ ID NO: 460 (SEQ ID NO: 463). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within amino acids 25-358 of SEQ ID NO: 460 (SEQ ID NO: 464). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within GPC3 carrying heparin sulfate side chain(s). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-GPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460) In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within a recombinant human GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the cell surface-bound GPC3 comprises (and in some embodiments consists of or consists essentially of) the amino acid sequence of SEQ ID NO: 460 or SEQ ID NO: 462. In some embodiments, the soluble GPC3 is circulating GPC3 protein or free GPC3 peptides in the serum. In some embodiments, the soluble GPC3 is a GPC3 protein or peptide present in a

solution. In some embodiments, the soluble GPC3 comprises (and in some embodiments consists of or consists essentially of) the amino acid sequence of SEQ ID NO: 461 or SEQ ID NO: 463. In some embodiments, the anti-GPC3 antibody moiety may cross-react with GPC3 from species other than human. In some embodiments, the anti-GPC3 antibody moiety may be completely specific for one or more human GPC3 proteins and may not exhibit species or other types of non-human cross-reactivity.

[0136] In some embodiments, the anti-GPC3 antibody moiety cross-reacts with at least one allelic variant of the GPC3 protein (or fragments thereof). In some embodiments, the allelic variant has up to about 30 (such as about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30) amino acid substitutions (such as a conservative substitution) when compared to the naturally occurring GPC3 (or fragments thereof). In some embodiments, the anti-GPC3 antibody moiety does not cross-react with any allelic variant of the GPC3 protein (or fragments thereof).

[0137] In some embodiments, the anti-GPC3 antibody moiety cross-reacts with at least one interspecies variant of the GPC3 protein. In some embodiments, for example, the GPC3 protein (or fragments thereof) is human GPC3 and the interspecies variant of the GPC3 protein (or fragments thereof) is a mouse or rat variant thereof. In some embodiments, the anti-GPC3 antibody moiety does not cross-react with any interspecies variant of the GPC3 protein.

[0138] In some embodiments, the anti-GPC3 antibody moiety specifically recognizes GPC3 expressed on the cell surface of HepG2, Hep3B, Huh7, JHH-7, or 293 cells. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes GPC3 expressed on the cell surface of a cancer cell (such as solid tumor). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes GPC3 expressed on the cell surface of liver cancer (such as HCC), melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, Wilms' tumor, hepatoblastoma, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma.

Binding affinity

[0139] Binding affinity can be indicated by K_d, K_{off}, K_{on}, or K_a. The term "K_{off}", as used herein, is intended to refer to the off-rate constant for dissociation of an antibody moiety from the antibody moiety/antigen complex, as determined from a kinetic selection set up. The term "K_{on}", as used herein, is intended to refer to the on-rate constant for association of an antibody moiety to the antigen to form the antibody moiety/antigen complex. The term equilibrium dissociation constant "K_d", as used herein, refers to the dissociation constant of a particular antibody moiety-antigen interaction, and describes the concentration of antigen

required to occupy one half of all of the antibody-binding domains present in a solution of antibody molecules at equilibrium, and is equal to K_{off}/K_{on}. The measurement of K_d presupposes that all binding agents are in solution. In the case where the antibody moiety is tethered to a cell wall, *e.g.*, in a yeast expression system, the corresponding equilibrium rate constant is expressed as EC_{50}, which gives a good approximation of K_d. The affinity constant, K_a, is the inverse of the dissociation constant, K_d.

[0140] The dissociation constant (K_d) is used as an indicator showing affinity of antibody moieties to antigens. For example, easy analysis is possible by the Scatchard method using antibodies marked with a variety of marker agents, as well as by using BIACORE™ (made by Amersham Biosciences), analysis of biomolecular interactions by surface plasmon resonance, according to the user's manual and attached kit. The K_d value that can be derived using these methods is expressed in units of M (Mols). An antibody moiety that specifically binds to a target may have a K_d of, for example, $\leq 10^{-7}$ M, $\leq 10^{-8}$ M, $\leq 10^{-9}$ M, $\leq 10^{-10}$ M, $\leq 10^{-11}$ M, $\leq 10^{-12}$ M, or $\leq 10^{-13}$ M.

[0141] Binding specificity of the antibody moiety can be determined experimentally by methods known in the art. Such methods comprise, but are not limited to, Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-, BIACORE™ -tests and peptide scans. In some embodiments, the binding affinity of the anti-GPC3 antibody moiety is measured by testing the binding affinity of the anti-GPC3 antibody moiety to cells expressing GPC3 on the surface (*e.g.*, HepG2 cells).

[0142] In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 (*e.g.*, the binding affinity of the antibody moiety to the cell surface-bound GPC3 is higher than that to a soluble GPC3). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-nGPC3 antibody moiety specifically binds to a cell surface-bound GPC3 in a competitive binding assay with soluble GPC3 antigen, using *e.g.*, flow cytometry. In some embodiments, the anti-nGPC3 antibody moiety specifically binds to a cell surface-bound GPC3 when the anti-nGPC3 antibody moiety has been pre-incubated with soluble GPC3 antigen in a competitive binding assay. For example, in some embodiments, the anti-GPC3 construct comprising an anti-nGPC3 antibody moiety can be pre-incubated with various concentrations of soluble GPC3 antigen (*e.g.*, a recombinant GPC3 fragment), then $GPC3^+$ cells (*e.g.*, HepG2 cells) can be added to the antibody/antigen mixture and incubated, cell surface-bound anti-GPC3 construct can then be detected: the binding of the soluble GPC3 pre-incubated anti-GPC3 construct to GPC3+ cells

may not show significant differences when compared to that of anti-GPC3 construct to GPC3+ cells without soluble GPC3 pre-incubation, demonstrating that the anti-GPC3 antibody moiety can specifically recognize a cell surface-bound GPC3, or that the binding affinity of the anti-nGPC3 antibody moiety to the cell surface-bound GPC3 is higher than that to a soluble GPC3, or that the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity.

[0143] In some embodiments, the anti-GPC3 antibody moiety specifically binds to a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) with a K_d of about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). Thus in some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3, the K_d of the binding between the anti-sGPC3 antibody moiety and sGPC3, or the K_d of the binding between the anti-GPC3 antibody moiety and GPC3 (any format), is about 10^{-7} M to about 10^{-13} M, about 1×10^{-7} M to about 5×10^{-13} M, about 10^{-7} M to about 10^{-12} M, about 10^{-7} M to about 10^{-11} M, about 10^{-7} M to about 10^{-10} M, about 10^{-7} M to about 10^{-9} M, about 10^{-8} M to about 10^{-13} M, about 1×10^{-8} M to about 5×10^{-13} M, about 10^{-8} M to about 10^{-12} M, about 10^{-8} M to about 10^{-11} M, about 10^{-8} M to about 10^{-10} M, about 10^{-8} M to about 10^{-9} M, about 5×10^{-9} M to about 1×10^{-13} M, about 5×10^{-9} M to about 1×10^{-12} M, about 5×10^{-9} M to about 1×10^{-11} M, about 5×10^{-9} M to about 1×10^{-10} M, about 10^{-9} M to about 10^{-13} M, about 10^{-9} M to about 10^{-12} M, about 10^{-9} M to about 10^{-11} M, about 10^{-9} M to about 10^{-10} M, about 5×10^{-10} M to about 1×10^{-13} M, about 5×10^{-10} M to about 1×10^{-12} M, about 5×10^{-10} M to about 1×10^{-11} M, about 10^{-10} M to about10^{-13} M, about 1×10^{-10} M to about 5×10^{-13} M, about 1×10^{-10} M to about 1×10^{-12} M, about 1×10^{-10} M to about 5×10^{-12} M, about 1×10^{-10} M to about 1×10^{-11} M, about 10^{-11} M to about 10^{-13} M, about 1×10^{-11} M to about 5×10^{-13} M, about 10^{-11} M to about 10^{-12} M, or about 10^{-12} M to about 10^{-13} M. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M.

[0144] In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target is more than the K_d of the binding between the anti-GPC3 antibody moiety and the target, and is herein referred to in some embodiments as the binding affinity of the anti-GPC3 antibody moiety to the target (*e.g.*, cell surface-bound GPC3) is higher than that to a non-target (*e.g.*, soluble GPC3). In some embodiments, the non-target is an antigen that is not GPC3. In some embodiments, for an anti-nGPC3 antibody moiety, the non-target is a soluble GPC3. In some embodiments, for an anti-sGPC3 antibody moiety, the non-target is a cell surface-bound GPC3. For example, the K_d of the binding between the anti-nGPC3 antibody moiety and a

soluble GPC3 can be at least about 10 times, such as about 10-100 times, about 100-1000 times, about 10^3-10^4 times, about 10^4-10^5 times, about 10^5-10^6 times, about 10^6-10^7 times, about 10^7-10^8 times, about 10^8-10^9 times, about 10^9-10^{10} times, about 10^{10}-10^{11} times, or about 10^{11}-10^{12} times of the K_d of the binding between the anti-nGPC3 antibody moiety and a cell surface-bound GPC3. Also for example, the K_d of the binding between the anti-sGPC3 antibody moiety and a cell surface-bound GPC3 can be at least about 10 times, such as about 10-100 times, about 100-1000 times, about 10^3-10^4 times, about 10^4-10^5 times, about 10^5-10^6 times, about 10^6-10^7 times, about 10^7-10^8 times, about 10^8-10^9 times, about 10^9-10^{10} times, about 10^{10}-10^{11} times, or about 10^{11}-10^{12} times of the K_d of the binding between the anti-sGPC3 antibody moiety and a soluble GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety (against nGPC3 and/or sGPC3) and a non-GPC3 target can be at least about 10 times, such as about 10-100 times, about 100-1000 times, about 10^3-10^4 times, about 10^4-10^5 times, about 10^5-10^6 times, about 10^6-10^7 times, about 10^7-10^8 times, about 10^8-10^9 times, about 10^9-10^{10} times, about 10^{10}-10^{11} times, or about 10^{11}-10^{12} times of the K_d of the binding between the anti-GPC3 antibody moiety and a target GPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and a soluble GPC3 is at least about 10 times of the K_d of the binding between the anti-nGPC3 antibody moiety and a cell surface-bound GPC3.

[0145] In some embodiments, the anti-GPC3 antibody moiety binds to a non-target with a K_d of about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the non-target is an antigen that is not GPC3. In some embodiments, for an anti-nGPC3 antibody moiety, the non-target is a soluble GPC3. In some embodiments, for an anti-sGPC3 antibody moiety, the non-target is a cell surface-bound GPC3. Thus in some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-GPC3 target, the K_d of the binding between the anti-nGPC3 antibody moiety and a soluble GPC3, or K_d of the binding between the anti-sGPC3 antibody moiety and a cell surface-bound GPC3, is about 10^{-1} M to about 10^{-6} M, about 1×10^{-1} M to about 5×10^{-6} M, about 10^{-1} M to about 10^{-5} M, about 1×10^{-1} M to about 5×10^{-5} M, about 10^{-1} M to about 10^{-4} M, about 1×10^{-1} M to about 5×10^{-4} M, about 10^{-1} M to about 10^{-3} M, about 1×10^{-1} M to about 5×10^{-3} M, about 10^{-1} M to about 10^{-2} M, about 10^{-2} M to about 10^{-6} M, about 1×10^{-2} M to about 5×10^{-6} M, about 10^{-2} M to about 10^{-5} M, about 1×10^{-2} M to about 5×10^{-5} M, about 10^{-2} M to about 10^{-4} M, about 1×10^{-2} M to about 5×10^{-4} M, about 10^{-2} M to about 10^{-3} M, about 10^{-3} M to about 10^{-6} M, about 1×10^{-3} M to about 5×10^{-6} M, about 10^{-3} M to about 10^{-5} M, about 1×10^{-3} M to about 5×10^{-5} M, about 10^{-3} M to about 10^{-4} M, about 10^{-4} M to about 10^{-6} M, about 1×10^{-4} M to about

5×10^{-6} M, about 10^{-4} M to about 10^{-5} M, or about 10^{-5} M to about 10^{-6} M. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and a soluble GPC3 is about 10^{-1} M to about 10^{-6} M. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizing a cell surface-bound GPC3 does not bind to a soluble GPC3.

[0146] In some embodiments, when refering to that the anti-GPC3 antibody moiety specifically recognizes a target GPC3 (*e.g.*, cell surface-bound GPC3) at a high binding affinity and binds to a non-target (*e.g.*, soluble GPC3) at a low binding affinity, the anti-GPC3 antibody moiety will bind to the target GPC3 (*e.g.*, cell surface-bound GPC3) with a K_d of about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M), and will bind to the non-target (*e.g.*, soluble GPC3) with a K_d of about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M).

[0147] In some embodiments, when referring to that the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 (*e.g.*, the binding affinity of the antibody moiety to the cell surface-bound GPC3 is higher than that to a soluble GPC3), or when referring to that the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity while binds to a soluble GPC3 at a low binding affinity, the binding affinity of the anti-GPC3 antibody moiety is compared to a control anti-GPC3 antibody, such as the monoclonal antibody GC33. In some embodiments, the K_d of the binding between GC33 (*e.g.*, SEQ ID NO: 510) and a cell surface-bound GPC3 can be at least about 2 times, such as about 2 times, about 3 times, about 4 times, about 5 times, about 6 times, about 7 times, about 8 times, about 9 times, about 10 times, about 10-100 times, about 100-1000 times, about 10^3-10^4 times, about 10^4-10^5 times, about 10^5-10^6 times, about 10^6-10^7 times, about 10^7-10^8 times, about 10^8-10^9 times, about 10^9-10^{10} times, about 10^{10}-10^{11} times, or about 10^{11}-10^{12} times of the K_d of the binding between the anti-nGPC3 antibody moiety described herein and a cell surface-bound GPC3. In some embodiments, the K_d of the anti-GPC3 construct to the cell surface-bound GPC3 is about 0.1 nM to about 2 nM, such as about 0.1 nM to about 0.5 nM, about 0.5 nM to about 1 nM, or about 1.5 to about 2 nM. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety described herein and a soluble GPC3 can be at least about 2 times, such as about 2 times, about 3 times, about 4 times, about 5 times, about 6 times, about 7 times, about 8 times, about 9 times, about 10 times, about 10-100 times, about 100-1000 times, about 10^3-10^4 times, about 10^4-10^5 times, about 10^5-10^6 times, about 10^6-10^7 times, about 10^7-10^8 times, about 10^8-10^9 times, about 10^9-10^{10} times, about 10^{10}-10^{11} times, or about 10^{11}-10^{12} times of the K_d of

the binding between GC33 (*e.g.*, SEQ ID NO: 510) and a soluble GPC3. In some embodiments, the K_d of the anti-GPC3 construct to the cell surface-bound GPC3 is about 10 nM to about 100 nM, such as about 10 nM to about 20 nM, about 20 nM to about 40 nM, about 40 nM to about 80 nM or about 80 nM to about 100 nM. In some embodiments, the K_d of the binding between a control anti-GPC3 antibody and a cell surface-bound GPC3 can be at least about 2 times, such as about 2 times, about 3 times, about 4 times, about 5 times, about 6 times, about 7 times, about 8 times, about 9 times, about 10 times, about 10-100 times, about 100-1000 times, about 10^3-10^4 times, about 10^4-10^5 times, about 10^5-10^6 times, about 10^6-10^7 times, about 10^7-10^8 times, about 10^8-10^9 times, about 10^9-10^{10} times, about 10^{10}-10^{11} times, or about 10^{11}-10^{12} times of the K_d of the binding between the anti-nGPC3 antibody moiety described herein and a cell surface-bound GPC3, wherein the control antibody comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 503, or a variant thereof having at least about 95% sequence identify to SEQ ID NO: 503; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 504, or a variant thereof having at least about 95% sequence identity to SEQ ID NO: 504. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety described herein and a soluble GPC3 can be at least about 2 times, such as about 2 times, about 3 times, about 4 times, about 5 times, about 6 times, about 7 times, about 8 times, about 9 times, about 10 times, about 10-100 times, about 100-1000 times, about 10^3-10^4 times, about 10^4-10^5 times, about 10^5-10^6 times, about 10^6-10^7 times, about 10^7-10^8 times, about 10^8-10^9 times, about 10^9-10^{10} times, about 10^{10}-10^{11} times, or about 10^{11}-10^{12} times of the K_d of the binding between a control anti-GPC3 antibody and a soluble GPC3, wherein the control antibody comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 503, or a variant thereof having at least about 95% sequence identify to SEQ ID NO: 503; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 504, or a variant thereof having at least about 95% sequence identity to SEQ ID NO: 504. In some embodiments, the K_d of the binding between a control anti-GPC3 antibody and a cell surface-bound GPC3 can be at least about 2 times, such as about 2 times, about 3 times, about 4 times, about 5 times, about 6 times, about 7 times, about 8 times, about 9 times, about 10 times, about 10-100 times, about 100-1000 times, about 10^3-10^4 times, about 10^4-10^5 times, about 10^5-10^6 times, about 10^6-10^7 times, about 10^7-10^8 times, about 10^8-10^9 times, about 10^9-10^{10} times, about 10^{10}-10^{11} times, or about 10^{11}-10^{12} times of the K_d of the binding between the anti-nGPC3 antibody moiety described herein and a cell surface-bound GPC3, wherein the control antibody comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 497, an HC-CDR2 comprising the amino acid sequence of SEQ ID

NO: 498, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 499; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 500, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 501, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 502. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety described herein and a soluble GPC3 can be at least about 2 times, such as about 2 times, about 3 times, about 4 times, about 5 times, about 6 times, about 7 times, about 8 times, about 9 times, about 10 times, about 10-100 times, about 100-1000 times, about 10^3-10^4 times, about 10^4-10^5 times, about 10^5-10^6 times, about 10^6-10^7 times, about 10^7-10^8 times, about 10^8-10^9 times, about 10^9-10^{10} times, about 10^{10}-10^{11} times, or about 10^{11}-10^{12} times of the K_d of the binding between a control anti-GPC3 antibody and a soluble GPC3, wherein the control antibody comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 497, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 498, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 499; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 500, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 501, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 502.

[0148] In some embodiments, the IC50 of a soluble GPC3 to compete binding between the anti-GPC3 construct and a cell surface-bound GPC3 is about 1 μg/ml to about 100 μg/ml, such as about 1 μg/ml to about 5 μg/ml, 1 μg/ml to about 10 μg/ml, about 10 μg/ml to about 20 μg/ml, about 20 μg/ml to about 50 μg/ml, or 50 μg/ml to about 100 μg/ml. In some embodiments, the IC50 of a soluble GPC3 to compete binding between the anti-GPC3 construct and a cell surface-bound GPC3 is at least about any one of 2x, 3x, 4x, 5x, 10x, 20x, 50x, 100x, or more than the IC50 of a soluble GPC3 to compete binding between a control anti-GPC3 antibody (such as GC33) and the cell surface bound GPC3.

Anti-GPC3 antibody moiety format

[0149] The anti-GPC3 antibody moiety (against *e.g.*, nGPC3 and/or sGPC3) described herein can be of any antibody or antigen-binding fragment format.

[0150] In some embodiments, the anti-GPC3 antibody moiety (against *e.g.*, nGPC3 and/or sGPC3) is a full-length antibody or immunoglobulin derivatives. In some embodiments, the anti-GPC3 antibody moiety is an antigen-binding fragment, for example an antigen-binding fragment selected from the group consisting of a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), or a single-chain Fv (scFv). In some embodiments, the anti-GPC3

antibody moiety is an scFv. In some embodiments, the anti-GPC3 antibody moiety is a Fab or Fab'. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

[0151] In some embodiments, the anti-GPC3 antibody moiety (against nGPC3 and/or sGPC3) is a semi-synthetic antibody moiety comprising fully human sequences and one or more synthetic regions. In some embodiments, the anti-GPC3 antibody moiety is a semi-synthetic antibody moiety comprising a fully human light chain variable domain and a semi-synthetic heavy chain variable domain comprising fully human FR1, HC-CDR1, FR2, HC-CDR2, FR3, and FR4 regions and a synthetic HC-CDR3. In some embodiments, the semi-synthetic heavy chain variable domain comprises a fully synthetic HC-CDR3 having a sequence from about 5 to about 25 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) amino acids in length. In some embodiments, the semi-synthetic heavy chain variable domain or the synthetic HC-CDR3 is obtained from a semi-synthetic library (such as a semi-synthetic human library) comprising fully synthetic HC-CDR3s having a sequence from about 5 to about 25 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) amino acids in length, wherein each amino acid in the sequence is randomly selected from the standard human amino acids, minus cysteine. In some embodiments, the synthetic HC-CDR3 is from about 5 to about 19 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19) amino acids in length. In some embodiments, the anti-GPC3 antibody moiety is a semi-synthetic antibody moiety comprising human CDRs and non-human framework sequences. Non-human framework sequences include, in some embodiments, any sequence that can be used for generating synthetic heavy and/or light chain variable regions using one or more human CDR sequences as described herein, including, e.g., mammals, such as mouse, rat, rabbit, pig, bovine (e.g., cow, bull, buffalo), deer, sheep, goat, chicken, cat, dog, ferret, primate (e.g., marmoset, rhesus monkey), *etc*. In some embodiments, the anti-GPC3 antibody moiety is generated by grafting one or more human CDR sequences as described herein onto a non-human framework sequence (e.g., a mouse or chicken framework sequence).

Anti-GPC3 antibody moiety sequences

[0152] The anti-GPC3 antibody moieties in some embodiments comprise specific sequences or certain variants of such sequences. In some embodiments, the amino acid substitutions in the variant sequences do not substantially reduce the ability of the anti-nGPC3 antibody moiety to specifically recognize a cell surface-bound GPC3, the ability of the anti-sGPC3 antibody moiety to specifically recognize soluble GPC3, or the ability of the anti-GPC3 antibody moiety to

specifically recognize a target GPC3 (*e.g.*, nGPC3 and/or sGPC3). For example, alterations that do not substantially reduce target GPC3 (*e.g.*, nGPC3 and/or sGPC3) binding affinity may be made. Alterations that substantially improve target GPC3 binding affinity or affect some other property, such as specificity and/or cross-reactivity with related variants of the target GPC3, are also contemplated.

[0153] Exemplary antibody sequences are shown in Tables 6-9. The exemplary CDR sequences in Tables 6 and 8 are predicted using the IgBLAST algorithm. *See*, for example, Ye J. *et al.* Nucleic Acids Research, 41:W34-W40 (2013), the disclosure of which is incorporated herein by reference in its entirety. Those skilled in the art will recognize that many algorithms are known for prediction of CDR positions in antibody heavy chain and light chain variable regions, and antibody agents comprising CDRs from antibodies described herein, but based on prediction algorithms other than IgBLAST, are within the scope of this invention.

[0154] The exemplary antibody heavy chain and light chain variable region sequences in Tables 7 and 9 are delimited according to the INTERNATIONAL IMMUNOGENETICS INFORMATION SYSTEM® (IMGT). *See*, for example, Lefranc, M.-P. et al., Nucleic Acids Res., 43:D413-422 (2015), the disclosure of which is incorporated herein by reference in its entirety. Those skilled in the art will recognize that antibody agents comprising V_H or V_L sequences from antibodies described herein, but based on algorithms other than IMGT, are within the scope of this invention.

Anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3

[0155] In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 (anti-nGPC3 antibody moiety). In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity.

[0156] In some embodiments, the anti-nGPC3 antibody moiety comprises i) a heavy chain variable domain (V_H) comprising an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a light chain variable domain (V_L) comprising an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0157] In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286.

[0158] In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0159] In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286.

[0160] In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-

CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences.

[0161] In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, wherein the amino acid substitutions are in HC-CDR1 or HC-CDR2; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, wherein the amino acid substitutions are in LC-CDR1 or LC-CDR2.

[0162] In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286.

[0163] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, the amino acid sequence of any one of SEQ ID NOs: 52-82, and the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, the amino acid sequence of any one of SEQ ID NOs: 205-235, and the amino acid sequence of any one of SEQ ID NOs: 256-286.

[0164] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising one, two or three CDRs of any one of SEQ ID NOs: 307-337, and b) a V_L

comprising one, two or three CDRs of any one of SEQ ID NOs: 358-388. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising HC-CDR1, HC-CDR2 and HC-CDR3 of the heavy chain variable domain of any one of SEQ ID NOs: 307-337, and b) a VL comprising LC-CDR1, LC-CDR2 and LC-CDR3 of the light chain variable domain of CDRs of any one of SEQ ID NOs: 358-388.

[0165] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 307-337; and b) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 358-388.

[0166] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and b) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388.

[0167] In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388.

[0168] The heavy and light chain variable domains can be combined in various pair-wise combinations to generate a number of anti-nGPC3 antibody moieties. Exemplary anti-nGPC3 antibodies are provided in Tables 6 and 7.

TABLE 6 anti-GPC3 antibody moiety specifically recognizing cell surface-bound GPC3 CDR sequences

Clone No.	SEQ ID NO:	HCDR1	SEQ ID NO:	HCDR2	SEQ ID NO:	HCDR3	SEQ ID NO:	LCDR1	SEQ ID NO:	LCDR2	SEQ ID NO:	LCDR3
GPC3A-034	1	GGSFSGYY	52	INHSGST	103	ARGYGGRFDY	154	SSNIGSNN	205	SNH	256	AAWDDSLDGYL
GPC3A-035	2	GYTFTGYY	53	INPNSGGT	104	ARSWTSGFDY	155	SSNIGSNY	206	KNF	257	AAWDDALSGYV
GPC3A-037	3	GFTFSSYA	54	IYSGGSST	105	ARTSYLNHGDY	156	RSNIGSDY	207	GDN	258	GTWDYTLNGVV
GPC3A-038	4	GYTFTSYY	55	INPSGGST	106	ARKVTGYDS	157	NIGSKS	208	YDS	259	QVWDSSSDHVV
GPC3A-039	5	GFTFSSYA	56	IGTGGGT	107	ARYGRKSIDA	158	NIGSKS	209	YDS	260	QVWDSSSDHWV
GPC3A-040	6	GYTFTGYY	57	INPNSGGT	108	ARRGYYGYDS	159	SSNIGSNY	210	SNN	261	AAWDDSLSGYV
GPC3A-041	7	GYTFTGYY	58	INPNSGGT	109	ARSGKYYGDK	160	SSNIGSNY	211	KNF	262	AAWDDALSGYV
GPC3A-042	8	GYSFTGYY	59	MNPRSGGT	110	ARSSYYWADS	161	SSDIGSNS	212	STQ	263	ATWDDSLNGYV
GPC3A-043	9	GYTFTDYY	60	VDPEDGET	111	ARELRDVAYYPWGVEDF	162	SSNIGTNY	213	RNN	264	AVWDDSLSGVV
GPC3A-044	10	GGSFSGYY	61	INHSGST	112	ARYYVPYLSD	163	NIGYKG	214	DDS	265	QVWDSSSDHVV

Clone No.	SEQ ID NO:	HCDR1	SEQ ID NO:	HCDR2	SEQ ID NO:	HCDR3	SEQ ID NO:	LCDR1	SEQ ID NO:	LCDR2	SEQ ID NO:	LCDR3
GPC3A-045	11	GFTFSDYY	62	ISSSGSTI	113	ARASDLYGD	164	TSNIGTNT	215	SNN	266	AAWDDSLNGVV
GPC3A-046	12	GYRFSNYG	63	ISGSNGNT	114	ARGNRRYYSPIIDP	165	SSNFGSNT	216	SNT	267	AAWDDSLTGVV
GPC3A-047	13	GYTFTGYY	64	INPNSGGT	115	ARSDYGSLYDK	166	RSNIASND	217	KKN	268	AAWDDNLSGYV
GPC3A-048	14	GFTFSSYA	65	ISYDGSNK	116	ARSSFVATDY	167	NIGSKS	218	YDS	269	QVWDSSSDRGV
GPC3A-049	15	GYTFTGYY	66	INPNSGGT	117	ARHGGIGSMRSFDQ	168	SSNIGSNY	219	RNN	270	AAWDDSLSG
GPC3A-050	16	GYSFTGYY	67	MNPRSGGT	118	ARSGYRWLDV	169	SSNIGSNT	220	SNN	271	AAWDDSLNGPV
GPC3A-051	17	GGAFSSYA	68	IIPIFGTA	119	ARMLYLSGRYYWDS	170	SSNIGAGYD	221	GNS	272	QSYDSSLSGYV
GPC3A-052	18	GYTFTGYY	69	INPNSGGT	120	ARSHSSGYDK	171	SSNIGSNY	222	RNN	273	AAWDDSLSGYV
GPC3A-053	19	GGSISSSSYY	70	IYYSGST	121	ARWWSGSYDT	172	SSNIGSNY	223	GNS	274	QSYDSSLSGSNV
GPC3A-054	20	GYTFTSYG	71	ISAYNGNT	122	ARIPMYSGSSDY	173	SSNIGSNY	224	RNN	275	AAWDDSLSGYV
GPC3A-055	21	GYTFTSYY	72	INPSGGST	123	ARWHGGPYDY	174	NIGSKS	225	YDS	276	QVWDSSSDHYV
GPC3A-056	22	GYSFNDYY	73	INPNNGDT	124	ARFSTHNWWWPTYDY	175	QSISSY	226	AAS	277	QQSYSTPIT
GPC3A-057	23	GFTFSSYA	74	ISGSGGST	125	ARYNYMSSGFYDR	176	NIGSKS	227	YDS	278	QVWDSSSDHVV
GPC3A-058	24	GYTFASHG	75	ISPYTGNT	126	ARGKRTLASCFDY	177	NIGSKS	228	DDS	279	QVWDSSSDHV
GPC3A-059	25	GYTFTRYG	76	ISAYSDKT	127	ARSRWSYMDV	178	NIGSKS	229	YDS	280	QVWDSSSDHV
GPC3A-060	26	GYTFNSYA	77	ISAYNGNT	128	AREGYGSWAMDQ	179	NIGSES	230	DDD	281	QTWDSSTAI
GPC3A-061	27	GYTFTSYG	78	ISAYNGNT	129	ARKGSSQFDQ	180	NIGSKS	231	YDS	282	QVWDSSSDHYV
GPC3A-062	28	GGTFSSYA	79	IIPKIGTA	130	ARMYMDMGWGWGYWDW	181	SSNIGAGYD	232	GNS	283	QSYDSSLSGSYV
GPC3A-063	29	GYTFTSYY	80	INPSGGSA	131	ARDRLASDAFDI	182	WSNIGSYT	233	GNN	284	AAWDDNLNGVV
GPC3A-064	30	GYTFTIYG	81	ISPYNDNT	132	ARMGVGWGYAQDS	183	NIGSKS	234	DDT	285	QVWDRSSAHWV
GPC3A-067	31	GGTFSSYA	82	IIPIFGIT	133	ARGAEMSDY	184	NIGSKS	235	YDS	286	QVWDSSSDHVV

TABLE 7 anti-GPC3 antibody moiety specifically recognizing cell surface-bound GPC3

V_H/V_L sequences

(CDR sequences are underlined)

Clone No.	SEQ ID NO:	V_H	SEQ ID NO:	V_L
GPC3A-034	307	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGY YWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKS RVTISVDTSKNQFSLELSSVTAADTAVYYCARGY GGRFDYWGQGTLVTVSS	358	QPVLTQPPSASGTPGQRVTISCSGSSSNIGS NNVIWYQQLPGAAPKLLIYSNHRRPSGVPD RFSGSRSGTSASLAISGLQSEDEADYYCAA WDDSLDGYLFGTGTKVTVLG
GPC3A-035	308	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGY YMHWVRQAPGQGLEWMGWINPNSGGTNYAQ KFQGRVTMTRDTSISTAYMELSRLRSDDTAVYY CARSWTSGFDYWGQGTLVTVSS	359	QAVLTQPPSASQTPGQMVTISCSGTSSNIG SNYVFWYQQLPGTAPKLLIYKNFQRPSGVP GRFSGSKSGTAASLAISGLRSEDEADYFCA AWDDALSGYVFGAGTKVTVLG
GPC3A-037	309	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSVIYSGGSSTYYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA RTSYLNHGDYWGQGTLVTVSS	360	QSVLTQPPSVSAAPGQRVTISCSGTRSNIGS DYVSWYQHLPGTAPKLLVYGDNLRPSGIPD RFSASKSGTSATLGITGLQTGDEADYYCGT WDYTLNGVVFGGGTKLTVLG
GPC3A-038	310	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSY YMHWVRQAPGQGLEWMGIINPSGGSTSYAQKF QGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCA RKVTGYDSWGQGTLVTVSS	361	QSVLTQPPSVSVAPGKTARITCGGNNIGSKS VHWYQQKPGQAPVLVIYYDSDRPSGIPERF SGSNSGNTATLTISRVEAGDEADYYCQVWD SSSDHVVFGGGTKLTVLG

Clone No.	SEQ ID NO:	V_H	SEQ ID NO:	V_L
GPC3A-039	311	QVQLVQSGGGLVHPGGSLRLSCAGSGFTFSSYAMHWVRQAPGKGLEWVSAIGTGGGTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARYGRKSIDAWGQGTLVTVSS	362	SYVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTKLTVLG
GPC3A-040	312	QVQLVQSGAEVKKPGASVTVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARRGYYGYDSWGQGTLVTVSS	363	LPVLTQPPSASGTPGQRVTISCSGSSSNIGSNYVYWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSGYVFGTGTKVTVLG
GPC3A-041	313	EVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAMYYCARSGKYYGDKWGQGTLVTVSS	364	QAVLTQPPSASQTPGQMVTISCSGTSSNIGSNYVFWYQQLPGTAPKLLIYKNFQRPSGVPGRFSGSKSGTAASLAISGLRSEDEADYFCAAWDDALSGYVFGAGTKVTVLG
GPC3A-042	314	QVQLQQSGAEVKKPGASVKVSCKASGYSFTGYYVYWMRQAPGKGLEWMGWMNPRSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLTSDDTAVYYCARSSYYWADSWGQGTLVTVSS	365	SYVLTQPPSASGTPGQRVTISCFGSSSDIGSNSVFWYQQLPGAAPKLLIYSTQYRPSGVPDRFSGSKSGTSASLAISGLQSEDEAEYHCATWDDSLNGYVFGSGTKVTVLG
GPC3A-043	315	EVQLVQSGAEVKKPGATVKVSCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTITADTSTDTAYMELSSLRSEDTAVYYCARELRDVAYYPWGVEDFWGQGTLVTVSS	366	QSVLTQPPSASGTPGQRVTISCSGSSSNIGTNYVYWYQQLPGTAPKLIIYRNNQRPSGVPDRFSGSESGTSASLAISGLRSEDEADYYCAVWDDSLSGVVFGGGTKLTVLG
GPC3A-044	316	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARYYVPYLSDWGQGTLVTVSS	367	SYVLTQPPSVSVAPGKTARITCGGDNIGYKGVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVLG
GPC3A-045	317	QMQLVQSGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARASDLYGDWGQGTLVTVSS	368	QSVLTQPPSVSGTPGQRVIISCPGSTSNIGTNTVNWYQQFPGTAPKLLIYSNNQRPSGVPDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGVVFGGGTKLTVLG
GPC3A-046	318	QVQLVQSGAEVKKPGASVTVSCKASGYRFSNYGVSWVRQAPGQGLEWMGWISGSNGNTNYAQKFLGRVTMTTDTSTTTAYMELSSLRSDDTAVYYCARGNRRYYSPIIDPWGQGTLVTVSS	369	QAVLTQPPSVSGTPGQRVTISCSGSSSNFGSNTVHWYQQVPGTAPKLLIFSNTQRPSEIPDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLTGVVFGGGTKLTVLG
GPC3A-047	319	QVQLVQSGAEVKKPGASVKVSCKAPGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARSDYGSLYDKWGQGTLVTVSS	370	LPVLTQPPSASGTPGQRVTISCSGSRSNIASNDVYWYQQLPGTAPKRLIYKKNQRPSGVPDRFSASKSGTSASLAISGLRSEDEADYYCAAWDDNLSGYVFGTGTKVTVLG
GPC3A-048	320	EVQLVESGGGEVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSSFVATDYWGQGTLVTVSS	371	QPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADFYCQVWDSSSDRGVFGGGTKLTVLG
GPC3A-049	321	QVQLVQSGAEVKEPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARHGGIGSMRSFDQWGQGTLVTVSS	372	QAVLTQPSSASGTPGQRVTISCSGGSSNIGSNYVYWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSGWVFGGRTKLTVLG
GPC3A-050	322	QITLKESGAEVKKPGASVKVSCKASGYSFTGYYVYWMRQAPGKGLEWMGWMNPRSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLTSDDTATYYCARSGYRWLDVWGQGTLVTVSS	373	QAVLTQPSSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGTGTKVTVLG
GPC3A-051	323	QVQLVQSGAEVKKPGSSVKVSCKASGGAFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARMLYLSGRYYWDSWGQGTLVTVSS	374	QAVLTQPSSASGAPGQRVTISCTGGSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGYVFGTGTKVTVLG
GPC3A-052	324	EVQLVESGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARSHSSGYDKWGQGTLVTVSS	375	QAVLTQPSSASGTPGQRVTISCSGSSSNIGSNYVYWYQQLSGTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSGYVFGTGTKVTVLG

Clone No.	SEQ ID NO:	V_H	SEQ ID NO:	V_L
GPC3A-053	325	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSY YWGWIRQPPGKGLEWIGSIYYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSSVTAADTAVYYCARW WSGSYDTWGQGTLVTVSS	376	QSVLTQPPSASAAPGQRVTISCSGTSSNIGS NYVWWYQQLPGAAPRLLIYGNSNRPSGVP DRFSGSKSGTSASLAITGLQAEDEADYYCQ SYDSSLSGSNVFGTGTKVTVLG
GPC3A-054	326	QMQLVQSGAEVKKPGASVKVSCKASGYTFTSY GISWVRQAPGQGLEWMGWISAYNGNTNYAQKL QGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCA RIPMYSGSSDYWGQGTLVTVSS	377	SYELTQPPSASGTPGQRVTISCSGSSSNIGS NYVYWYQQLPGTAPKLLIYRNNQRPSGVPD RFSGSKSGTSASLAISGLRSEDEADYYCAA WDDSLSGYVFGTGTKVTVLG
GPC3A-055	327	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSY YMHWVRQAPGQGLEWMGIINPSGGSTSYAQKF QGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCA RWHGGPYDYWGQGTLVTVSS	378	QPVLTQPPSVSVAPGKTARITCGGNNIGSKS VHWYQQKPGQAPVLVIYYDSDRPSGIPERF SGSNSGNTATLTISRVEAGDEADYYCQVWD SSSDHYVFGTGTKVTVLG
GPC3A-056	328	QVQLQQSGAEVKKFGASVKVSCKASGYSFNDY YIHWVRQAPGQGLEWMGWINPNNGDTKYEKK WQGRVTMTRDTSITTAYMELSSLRSDDTAVYYC ARFSTHNWWWPTYDYWGQGTLVTVSS	379	DIQLTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYS TPITFGQGTRLEIKR
GPC3A-057	329	EVQLVESGGGLIQPGGSLRLSCAASGFTFSSYA MSWVRQAPGKGLEWVSAISGSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR YNYMSSGFYDRWGQGTLVTVSS	380	EIVLTQSPSVSVAPGKTARITCGGNNIGSKS VHWYQQKPGQAPVLVIYYDSDRPSGIPERF SGSNSGNTATLTISRVEAGDEADYYCQVWD SSSDHVVFGGGTKVEIKR
GPC3A-058	330	QVQLVQSGADVRKPGASVKVSCKASGYTFASH GISWVRQAPGQGLEWLGWISPYTGNTNYAQKF QGRVTMATDTSTSTAYMELRSLRSDDTAIYYCA RGKRTLASCFDYWGQGTLVTVSS	381	QSVLTQPPSVSVAPGKTARITCGGNNIGSKS VHWYQQKPGQAPVLVVYDDSDRPSGIPER FSGSNSGNTATLTISRVEAGDEADYYCQVW DSSSDHVFGTGTKVTVLG
GPC3A-059	331	QVQLVQSGAEVKKPGASVTVSCKASGYTFTRY GITWVRQAPGQGLEWMGWISAYSDKTNYAQKL QGRVTMTTDISTNTAYMELSSLRSEDTAVYYCA RSRWSYMDVWGQGTLVTVSS	382	SYVLTQPPSVSVAPGKTARITCGGNNIGSKS VYWYQQKPGQAPVLVIYYDSDRPSGIPERF SGSNSGNTATLTISRVEAGDEADYYCQVWD SSSDHVFGTGTKVTVLG
GPC3A-060	332	QVQLVQSGGEVKKPGASVKVSCKASGYTFNSY AISWVRQAPGQGLEWMGWISAYNGNTNYAQKL QGRVTMTTDTSTNTAFMELRSLRSDDTAVYYCA REGYGSWAMDQWGQGTLVTVSS	383	SYVLTQPPSVSVAPGKTARLTCGGNNIGSE SVHWYQQKPGQAPLLVVYDDDDRPSGIPE RFSGSNSEDTATLTISGTQALDEAEYYCQT WDSSTAIFGTGTKLTVLG
GPC3A-061	333	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSY GISWVRQTPGQGLEWMGWISAYNGNTNYAQKL QGRVTMTTDTSTSTAYMELSSLRSEDTAVYYCA RKGSSQFDQWGQGTLVTVSS	384	QPVLTQPPSVSVAPGKTARITCGGNNIGSKS VHWYQQKPGQAPVLVIYYDSDRPSGIPERF SGSNSGNTATLTISRVEAGDEADYYCQVWD SSSDHYVFGTGTKVTVLG
GPC3A-062	334	QMQLVQSGSELKKPGASVKVSCKASGGTFSSY AISWVRQAPGQGLEWMGGIIPKIGTANYAQKFQ GRVTITADESTSTAYMELSSLRSEDTAMYYCAR MYMDMGWGWGYWDWWGQGTLVTVSS	385	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGA GYDVHWYQQLPGTAPKLLIYGNSNWPSGV PDRFSGSKSGTSASLAITGLRAEDEADYYC QSYDSSLSGSYVFGTGTKVTVLG
GPC3A-063	335	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSY YMHWVRQAPGQGLEWMGIINPSGGSASYAQKF QGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCA RDRLASDAFDIWGQGTMVTVSS	386	QTVVTQPPSASGTPGQRVTISCSGSWSNIG SYTVNWYQHLPGTAPKLLISGNNQRPSGVP GRFSGSKSGTSASLAISGLQSEDEADYHCA AWDDNLNGVVFGGGTKLTVLG
GPC3A-064	336	QMQLVQSGAEVKKPGASVKVSCKASGYTFTIYG ISWVRQAPGQGLEWMGWISPYNDNTIYAQKVQ GRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR MGVGWGYAQDSWGQGTLVTVSS	387	LPVLTQPPSLSVAPGKTARLTCGGNNIGSKS VHWYHQKPGQAPVLVVYDDTDRPSGIPERF SGSNSGNTAALTISRVEVGDEADYYCQVWD RSSAHWVFGGGTKLTVLG
GPC3A-067	337	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSY AISWVRQAPGQGLEWMGRIIPIFGITNYAQKFQG RVTITADKSTSTAYMELSSLRSEDTAVYYCARGA EMSDYWGQGTLVTVSS	388	QSVLTQPPSVSVAPGKTARITCGGNNIGSKS VHWYQQKPGQAPVLVIYYDSDRPSGIPERF SGSNSGNTATLTISRVEAGDEADYYCQVWD SSSDHVVFGGGTKLTVLG

[0169] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 52, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 103, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 154, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 205, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 256, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 52, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 103; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 154, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 205, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 256; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 52, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 103; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 154, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 205, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 256.

[0170] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 53, or a variant thereof comprising

up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 104, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 206, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 257, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 53, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 104; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 155, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 206, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 257; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 53, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 104; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 155, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 206, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 257.

[0171] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, or a variant thereof

comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258.

[0172] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 55, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 106, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 157, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 208, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 259, or a variant thereof comprising up to

about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 106; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 157, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 208, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 259; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 106; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 157, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 208, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 259.

[0173] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 5, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 56, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 107, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 158, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 209, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 260, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 5, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 56, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 107; or

a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 158, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 209, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 260; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 5, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 56, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 107; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 158, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 209, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 260.

[0174] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 6, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 108, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 159, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 210, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 261, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 6, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 57, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 108; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 159, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 210, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 261;

or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 6, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 57, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 108; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 159, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 210, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 261.

[0175] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 58, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 109, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 160, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 211, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 262, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 58, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 109; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 160, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 211, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 262; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H

comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 58, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 109; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 160, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 211, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 262.

[0176] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 59, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 110, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 161, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 212, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 263, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 8, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 59, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 110; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 161, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 212, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 263; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 8, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 59, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 110; and ii) a V_L comprising an LC-CDR1 comprising the

amino acid sequence of SEQ ID NO: 161, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 212, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 263.

[0177] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 9, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 60, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 111, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 213, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 264, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 9, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 60, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 111; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 162, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 213, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 264; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 9, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 60, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 111; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 162, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 213, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 264.

[0178] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 10, or a variant thereof

comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 112, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 163, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 214, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 265, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 10, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 61, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 112; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 163, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 214, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 265; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 10, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 61, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 112; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 163, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 214, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 265.

[0179] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 11, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 113, or a variant thereof comprising up to

about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 164, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 215, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 266, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 11, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 113; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 164, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 215, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 266; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 11, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 113; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 164, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 215, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 266.

[0180] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 12, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 114, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 165, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 216, or a variant thereof comprising

up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 267, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 12, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 63, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 114; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 165, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 216, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 267; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 12, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 63, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 114; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 165, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 216, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 267.

[0181] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 13, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 166, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 217, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 268, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the

amino acid sequence of SEQ ID NO: 13, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 64, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 115; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 166, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 217, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 268; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 13, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 64, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 115; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 166, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 217, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 268.

[0182] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 14, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 116, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 167, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 218, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 269, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 14, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 65, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 116; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the

amino acid sequence of SEQ ID NO: 167, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 218, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 269; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 14, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 65, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 116; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 167, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 218, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 269.

[0183] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 66, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 117, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 168, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 219, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 270, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 15, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 66, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 117; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 168, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 219, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 270; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are

in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 15, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 66, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 117; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 168, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 219, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 270.

[0184] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 16, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 118, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 220, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 271, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 16, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 67, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 118; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 169, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 220, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 271; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 16, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 67, and an HC-CDR3 comprising the

amino acid sequence of SEQ ID NO: 118; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 169, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 220, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 271.

[0185] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 17, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 68, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 119, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 170, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 221, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 272, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 17, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 68, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 119; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 170, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 221, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 272; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 17, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 68, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 119; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 170, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 221, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 272.

[0186] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 18, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 69, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 120, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 171, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 222, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 273, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 18, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 120; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 171, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 222, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 273; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 18, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 120; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 171, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 222, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 273.

[0187] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof comprising

up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 121, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 172, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 223, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 274, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 19, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 70, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 121; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 172, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 223, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 274; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 19, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 70, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 121; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 172, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 223, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 274.

[0188] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 20, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 122, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 173, or a variant thereof

comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 224, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 275, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 20, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 71, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 122; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 173, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 224, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 275; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 20, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 71, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 122; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 173, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 224, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 275.

[0189] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276, or a variant thereof comprising up to

about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276.

[0190] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 73, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 124, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 175, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 226, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 277, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 22, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 73, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 124; or

a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 175, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 226, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 277; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 22, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 73, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 124; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 175, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 226, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 277.

[0191] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 23, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 74, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 125, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 176, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 227, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 278, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 23, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 74, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 125; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 176, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 227, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 278;

or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 23, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 74, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 125; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 176, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 227, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 278.

[0192] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H

comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279.

[0193] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 25, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 76, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 127, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 178, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 229, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 280, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 25, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 127; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 178, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 229, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 280; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 25, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 127; and ii) a V_L comprising an LC-CDR1 comprising the

amino acid sequence of SEQ ID NO: 178, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 229, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 280.

[0194] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 26, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 77, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 128, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 179, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 230, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 281, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 26, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 77, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 128; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 179, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 230, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 281; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 26, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 77, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 128; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 179, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 230, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 281.

[0195] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 27, or a variant thereof

comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 129, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 180, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 231, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 282, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 27, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 78, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 129; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 180, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 231, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 282; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 27, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 78, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 129; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 180, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 231, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 282.

[0196] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 28, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 79, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 130, or a variant thereof comprising up to

about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 181, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 232, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 283, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 28, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 79, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 130; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 181, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 232, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 283; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 28, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 79, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 130; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 181, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 232, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 283.

[0197] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 80, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 131, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 182, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 233, or a variant thereof comprising

up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 284, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 29, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 80, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 131; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 182, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 233, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 284; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 29, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 80, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 131; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 182, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 233, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 284.

[0198] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 30, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 81, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 132, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 183, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 234, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 285, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the

amino acid sequence of SEQ ID NO: 30, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 81, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 132; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 183, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 234, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 285; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 30, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 81, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 132; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 183, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 234, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 285.

[0199] In some embodiments, the anti-nGPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-nGPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 31, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 82, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 133; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the

amino acid sequence of SEQ ID NO: 184, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 235, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 286; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-nGPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 31, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 82, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 184, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 235, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 286.

[0200] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 307, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 307; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 358, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 358. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 307; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 358. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 307, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 358.

[0201] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 308, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 308; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 359, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 359. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 308; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 359. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 308, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 359.

[0202] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 309, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 309; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 360, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 360. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 309; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 360. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 309, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 360.

[0203] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 310, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 310; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 361, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 361. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 310; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 361. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 310, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 361.

[0204] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 311, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 311; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 362, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 362. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 311; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 362. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 311, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 362.

[0205] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 312, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 312; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 363, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 363. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 312; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 363. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 312, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 363.

[0206] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 313, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 313; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 364, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 364. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 313; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 364. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 313, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 364.

[0207] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 314, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 314; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 365, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 365. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 314; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 365. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 314, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 365.

[0208] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 315, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 315; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 366, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 366. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 315; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 366. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 315, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 366.

[0209] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 316, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 316; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 367, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 367. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 316; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 367. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 316, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 367.

[0210] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 317, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 317; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 368, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 368. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 317; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 368. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 317, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 368.

[0211] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 318, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 318; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 369, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 369. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 318; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 369. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 318, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 369.

[0212] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 319, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 319; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 370, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 370. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 319; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 370. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 319, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 370.

[0213] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 320, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 320; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 371, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 371. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 320; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 371. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 320, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 371.

[0214] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 321, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 321; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 372, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 372. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 321; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 372. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 321, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 372.

[0215] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 322, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 322; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 373, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 373. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 322; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 373. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 322, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 373.

[0216] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 323, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 323; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 374, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 374. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 323; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 374. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 323, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 374.

[0217] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 324, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 324; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 375, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 375. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 324; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 375. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 324, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 375.

[0218] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 325, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 325; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 376, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 376. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 325; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 376. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 325, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 376.

[0219] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 326, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 326; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 377, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 377. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 326; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 377. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 326, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 377.

[0220] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 327, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 327; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 378, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 378. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 327; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 378. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 327, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 378.

[0221] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 328, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 328; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 379, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 379. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 328; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 379. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 328, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 379.

[0222] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 329, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 329; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 380, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 380. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 329; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 380. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 329, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 380.

[0223] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 330, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 330; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 381, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 381. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 330; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 381. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 330, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 381.

[0224] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 331, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 331; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 382, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 382. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 331; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 382. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 331, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 382.

[0225] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 332, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 332; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 383, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 383. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 332; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 383. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 332, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 383.

[0226] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 333, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 333; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 384, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 384. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 333; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 384. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 333, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 384.

[0227] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 334, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 334; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 385, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 385. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 334; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 385. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 334, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 385.

[0228] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 335, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 335; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 386, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 386. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 335; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 386. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 335, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 386.

[0229] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 336, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 336; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 387, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 387. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 336; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 387. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 336, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 387.

[0230] In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 337, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 337; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 388, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 388. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 337; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 388. In some embodiments, the anti-nGPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 337, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 388.

[0231] In some embodiments, the anti-nGPC3 antibody moiety competes for binding to a cell surface-bound GPC3 with a second anti-nGPC3 antibody moiety according to any of the anti-nGPC3 antibody moieties described herein. In some embodiments, the anti-nGPC3 antibody moiety binds to the same, or substantially the same, epitope as the second anti-nGPC3 antibody moiety. In some embodiments, binding of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 inhibits the binding of a second anti-nGPC3 antibody moiety to the same cell surface-bound GPC3 by at least about 70% (such as by at least about any of 75%, 80%, 85%, 90%, 95%, 98% or 99%), or *vice versa*. In some embodiments, the anti-nGPC3 antibody moiety and the second anti-nGPC3 antibody moiety cross-compete for binding to the cell surface-bound GPC3, *i.e.*, each of the anti-nGPC3 antibody moieties competes with the other for binding to the cell surface-bound GPC3.

[0232] Anti-GPC3 antibody moiety specifically recognizing GPC3

[0233] In some embodiments, the anti-GPC3 antibody moiety specifically recognizes GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3.

[0234] In some embodiments, the anti-GPC3 antibody moiety comprises i) a heavy chain variable domain (V_H) comprising an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a light chain variable domain (V_L) comprising an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0235] In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306.

[0236] In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to

about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[0237] In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306.

[0238] In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences.

[0239] In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, wherein the amino acid substitutions are in HC-CDR1 or HC-CDR2; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-

CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, wherein the amino acid substitutions are in LC-CDR1 or LC-CDR2.

[0240] In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306.

[0241] In some embodiments, the anti-GPC3 antibody moiety comprises: a) V_H comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, the amino acid sequence of any one of SEQ ID NOs: 83-102, and the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, the amino acid sequence of any one of SEQ ID NOs: 236-255, and the amino acid sequence of any one of SEQ ID NOs: 287-306.

[0242] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising one, two or three CDRs of any one of SEQ ID NOs: 338-357, and b) a V_L comprising one, two or three CDRs of any one of SEQ ID NOs: 389-408. In some embodiments, the anti-nGPC3 antibody moiety comprises: a) a V_H comprising HC-CDR1, HC-CDR2 and HC-CDR3 of the heavy chain variable domain of any one of SEQ ID NOs: 389-408, and b) a VL comprising LC-CDR1, LC-CDR2 and LC-CDR3 of the light chain variable domain of CDRs of any one of SEQ ID NOs: 358-388.

[0243] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 338-357; and b) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 389-408.

[0244] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and b) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408.

[0245] In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408.

[0246] The heavy and light chain variable domains can be combined in various pair-wise combinations to generate a number of anti-GPC3 antibody moieties. Exemplary anti-GPC3 antibody moieties are provided in Tables 8 and 9.

TABLE 8 anti-GPC3 antibody moiety CDR sequences

Clone No.	SEQ ID NO:	HCDR1	SEQ ID NO:	HCDR2	SEQ ID NO:	HCDR3	SEQ ID NO:	LCDR1	SEQ ID NO:	LCDR2	SEQ ID NO:	LCDR3
GPC3B-54	32	GYTFIDYY	83	IIPIFGTA	134	ARERRYSSSPSDH	185	NSNIGSDT	236	RDN	287	TTWDDSLNGLV
GPC3B-60	33	GYTFTSYY	84	INPSGGST	135	ARSLYSQPYIDG	186	NLENKF	237	EDN	288	QTWDSPTGLFV
GPC3B-63	34	GYTFTSYY	85	INPSGGST	136	ARSLHAMRWSQTMDS	187	QSLLHSNGYNY	238	LGS	289	MQALQTPPT
GPC3B-66	35	GYTFIGQY	86	INPMTGVT	137	ARFSSGYSRDT	188	NIGSKS	239	YDS	290	QVWDSSSDHLYV
GPC3B-68	36	GGTFSSYA	87	IIPILGIA	138	ARYGYEGHDT	189	SSNIGNNY	240	DNI	291	GTWDSSLSAGV
GPC3B-71	37	GFTFSRYT	88	ISSSGSYI	139	ARQGHMWYVPVDA	190	NIGSKS	241	YDS	292	QVWDSSSDHYV
GPC3B-76	38	GYTFTDYY	89	INPNSGGT	140	ARNYD	191	SSNIGNNY	242	DNN	293	GTWDSSLSAGV
GPC3B-78	39	GYTFTSYY	90	INPSGGST	141	ARGYSSFFDS	192	KLGDKY	243	QDN	294	QTWDRSTYV
GPC3B-80	40	GGTLSRFA	91	IIPIFRTA	142	ARMSKYYGSYSSYDE	193	SSNIGSNT	244	SNN	295	AAWDDSLNG
GPC3B-81	41	GYTFTGYY	92	INPNSGGT	143	ARGLWDS	194	NIGSKS	245	YDS	296	QVWDSSSDLLYV
GPC3B-82	42	GYSFTSYY	93	INPSGGST	144	ARYPVYMETSDFDS	195	SSNIGAGFD	246	DNN	297	QSFDSSLSGWV
GPC3B-85	43	GYTFTSYA	94	INTNTGNP	145	ARSSLYWMGSKWSRQTDM	196	SSNIGSNT	247	SNN	298	AAWDDSLNGYV
GPC3B-87	44	GGTFGSYA	95	IIPVLGRT	146	ARTNDS	197	QSLLHSNGYNY	248	LGS	299	MQALQTPWT
GPC3B-92	45	GYTFSNYY	96	INPSGGTT	147	ARPSMWTSSMGDV	198	TLAKRY	249	RDT	300	QSADNSRTFV
GPC3B-93	46	GYTFTSYY	97	INPSGGST	148	ARYTALKPRGIYSVDS	199	SGSIASNY	250	EDN	301	QSYDSSNWV
GPC3B-110	47	GGTFTTYS	98	IIPTFGTT	149	ARYYWRGGSGQGSVTSDY	200	SSNIGSNT	251	SSN	302	AAWDDSLNGPV
GPC3B-113	48	GYTLTELS	99	FDPEDGET	150	ARYSGDY	201	SSNIGSNS	252	SNN	303	AAWDDSLNGVL
GPC3B-115	49	GYTFTAYY	100	INANTGGT	151	ARISGYHSSGWDY	202	NIGSKS	253	DDS	304	QVWDSSSDPLYV
GPC3B-119	50	GYTFTSYA	101	INTNTGNP	152	ARGYYGKYDK	203	SSNIGNNY	254	DNN	305	ATWDNSLSALI
GPC3B-125	51	GYTFTSYA	102	INTNTGNP	153	ARQSHDE	204	SSDVGGYNY	255	EVS	306	SSYTSSTTVI

TABLE 9 anti-GPC3 antibody moiety V_H/V_L sequences

(CDR sequences are underlined)

Clone No.	SEQ ID NO:	VH	SEQ ID NO:	VL
GPC3B-54	338	EVQLVQSGAEVKKPGASVKLSCKTSGYTFIDYYVY WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVT ITADKSTSTAYMELSSLGSEDTAVYYCARERRYSS SPSDHWGQGTLVTVSS	389	QSVLTQPPSASGTPGQRVTISCSGSNSNI GSDTVNWYQQLPGTAPKLLIYRDNQRPS GVPDRFSGSKSGTSASLAISGLQSEDEA DYYCTTWDDSLNGLVFGGGTKVTVLG
GPC3B-60	339	EVQLVESGAEVKKPGASVKVSCKASGYTFTSYYM HWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGR VTMTRDTSTSTVYMELSSLRSEDTAVYYCARSLY SQPYIDGWSQGTLVTVSS	390	SYVLTQPPSVSVSPGQTATIACSGDNLEN KFVYWYHQKPGQSPVLVMYEDNKRPSGI PERFSGSNSGNTAALTISGAQPMDEADY YCQTWDSPTGLFVFGTGTKVTVLG
GPC3B-63	340	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYM HWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGR VTMTRDTSTSTVYMELSSLRSEDTAVYYCARSLH AMRWSQTMDSWGQGTLVTVSS	391	EIVLTQSPLSLPVTPGEPASISCRSSQSLL HSNGYNYLDWYLQKPGQSPQLLIYLGSN RASGVPDRFSGSGSGTDFTLKISRVEAE DVGVYYCMQALQTPPTFGQGTKVEIKR
GPC3B-66	341	QVQLVQSGAEVKRPGASVKVSCKASGYTFIGQYL HWVRQAPGQGLEWMGRINPMTGVTNYAPKFQG RVTMTRDTSISTGYMEISRLRSDDTAVYYCARFSS GYSRDTWGQGTLVTVSS	392	QAVLTQPPSVSVAPGKTASITCGGNNIGS KSVHWYQQKPGQAPVLVIYYDSDRPSGI PERFSGSNSGNTATLTISRVEAGDEADYY CQVWDSSSDHLYVFGTGTKVTVLG
GPC3B-68	342	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAI SWVRQAPGQGLEWMGRIIPILGIANYAQKFQGRV TITADKSTSTAYMELSSLRSEDTAVYYCARYGYEG HDTWGQGTLVTVSS	393	QSVLTQPPSVSAAPGQKVTISCSGSSSNI GNNYVSWYQQLPGTAPKLLIYDNIKRPSG IPDRFSGSKSGTSATLGITGLQTGDEADY YCGTWDSSLSAGVFGGGTKLTVLG
GPC3B-71	343	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYTM NWVRQAPGKGLEWVSSISSSGSYIYYADSVKGRF TISRDNAKNSLFLQMDSLRAEDTAVYYCARQGHM WYVPVDAWGQGTLVTVSS	394	SYVLTQPPSVSVAPGKTARVTCGGNNIG SKSVHWYQQKPGQAPVLVMYYDSDRPS GIPERFSGSNSGNTATLTISSVEAGDEAD YYCQVWDSSSDHYVFGTGTKVTVLG
GPC3B-76	344	QMQLVQSGAEVKEPGASVKVSCKASGYTFTDYYI HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQG RVTMTRDTSISTAYMELSRLRSDDTAVYYCARNY DWGQGTLVTVSS	395	QSVVTQPPSVSAAPGQKVTISCSGSSSNI GNNYVSWYQQLPGTAPKLLIYDNNKRPS GIPDRFSGSKSGTSATLGITGLQTGDEAA YYCGTWDSSLSAGVFGTGTKVTVLG
GPC3B-78	345	QMQLVQSGAEVKKPGASVKVSCKASGYTFTSYY MHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQG RVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGY SSFFDSWGQGTLVTVSS	396	LPVLTQPPSVSVSPGQTASITCSGDKLGD KYAYWYQQKPGQSPVLVIYQDNKRPSGI PERFSGSNSGNTATLTISGTQAMDAADY YCQTWDRSTYVFGTGTKVTVLG
GPC3B-80	346	QVQLVQSGAEVKKPGSSVKVSCKASGGTLSRFAI SWVRQAPGQGLEWMGGIIPIFRTANYAQKFQGRV TITADESTSTAYMELSSLRSEDTAVYYCARMSKYY GSYSSYDEWGQGTLVTVSS	397	QAVLTQPPSASGTPGQRVTISCSGSSSNI GSNTVNWYQQLPGTAPKLLIYSNNQRPS GVPDRFSGSKSGTSAYLAISGLQSEDEA DYYCAAWDDSLNGWGVFGGGTKLTVLG
GPC3B-81	347	EVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYM HWVRQAPGQGLEWMGRINPNSGGTNYAQKFQG RVTMTRDTSISTAYMELSRLRSDDTAVYYCARGL WDSWGQGTLVTVSS	398	SYELTQPPSVSVAPGKTARITCGGNNIGS KSVHWYQQKPGQAPVLVIYYDSDRPSGI PERFSGSNSGNTATLTISRVEAGDEADYY CQVWDSSSDLLYVFGTGTKVTVLG
GPC3B-82	348	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYYM HWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGR VTMTRDTSTSTVYMEMSSLRSEDTAVYYCARYPV YMETSDFDSWGSRYSGDRLL	399	QSVLTQPPSVSGAPGQRVTISCTGSSSNI GAGFDVHWYQQLPGTAPKLLIYDNNNRP SGVPDRFSGSKSDTSASLAITGLQAEDEA DYYCQSFDSSLSGWVFGGGTKLTVLG
GPC3B-85	349	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAM NWVRQAPGQGLEWMGWINTNTGNPTYAQGFTG RFVFSLDTSVSTAYLQISSLKAEDTAVYYCARSSLY WMGSKWSRQTDMWGQGTLVTVSS	400	QAVLTQPPSASGTPGQRVTISCSGSSSNI GSNTVNWYQQLPGTAPKLLIYSNNQRPS GVPDRFSGSKSGTSASLAISGLQSEDEA DYYCAAWDDSLNGYVFGTGTKVTVLG
GPC3B-87	350	EVQLVQSGAEVRKPGSSVKVSCQASGGTFGSYAI SWVRQAPGQGLEWMGRIIPVLGRTKYAQKFQGR VTVTADTSTSTVYMELTSLTSEDTAVYYCARTNDS WGQGTLVTVSS	401	DVVMTQSPLSLPVTPGEPASVSCRSSQS LLHSNGYNYLDWYLQKPGQSPQLLIYLGS NRASGVPDRFSGSGSGTDFTLKISRVEA EDVGVYYCMQALQTPWTFGQGTKVEIKR

Clone No.	SEQ ID NO:	VH	SEQ ID NO:	VL
GPC3B-92	351	EVQLVQSGAEVKKPGASVKVSCKASGYTFSNYYMHWVRQAPGQGLEWMGIINPSGGTTTYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARPSMWTSSMGDVWGQGTLVTVSS	402	QSVLTQPPSVSVSPGQTARITCSGETLAKRYAHWYQQKPGQAPVLLIYRDTERPSGIPERFSGSSSGTTITLTITGVQAEDEADYYCQSADNSRTFVFGPGTKVTVLG
GPC3B-93	352	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTRYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYTALKPRGIYSVDSWGQGTLVTVSS	403	NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSSNWVFGGGTKLTVLG
GPC3B-110	353	EVQLVQSGAEVKKPGSSVKVSCQASGGTFTTYSINWVRQAPGQGLEWMGGIIPTFGTTNYAQNFQDRVTISADESTNTAYMELTSLRSEDTAVYYCARYYWRGGSGQGSVTSDYWGQGTLVTVSS	404	QSVLTQPPSASGTPGQRVTVSCSGSSSNIGSNTVNWYQQLPGTAPKLLLYSSNQRPSGVPDRFSGSRSGTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGGGTKLTVLG
GPC3B-113	354	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCARYSGDYWGQGTLVTVSS	405	SYELTQPPSASGTPGQRVTISCSGSSSNIGSNSVSWYQHLPGVAPKLLIYSNNQRPSGVPDRFSGSKTGTSASLAISGLQSEDEGDYYCAAWDDSLNGVLFGGGTKLTVLG
GPC3B-115	355	QVQLVQSGAEVKRPGATIKVSCKTSGYTFTAYYTHWVRQAPGQGLEWVGRINANTGGTDYAPKFRDRVIMTRDTSISTAYMELGRLTSEDTAVYYCARISGYHSSGWDYWGQGTLVTVSS	406	SYELTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDPLYVFGTGTKVTVLG
GPC3B-119	356	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTNTGNPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARGYYGKYDKWGQGTLVTVSS	407	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQIPGTAPKLLIYDNNKRPSGIPDRFSGSRSGTSATLGITGLQTGDEAHYYCATWDNSLSALIFGGGTKVTVLG
GPC3B-125	357	QMQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTNTGNPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARQSHDEWGQGTLVTVSS	408	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWFQQHPGKAPKLIIYEVSNRPSGVSDRFSGSKSGSTASLTISGLQAEDEANYYCSSYTSSTTVIFGGGTKLTVLG

[0247] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 83, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 134, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 185, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 236, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 287, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an HC-CDR2 comprising the amino acid sequence of

SEQ ID NO: 83, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 134; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 185, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 236, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 287; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 134; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 185, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 236, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 287.

[0248] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 33, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 84, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 135, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 186, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 237, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 288, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 33, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 84, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 135; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 186, an LC-CDR2 comprising the amino acid sequence of

SEQ ID NO: 237, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 288; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 33, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 84, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 135; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 186, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 237, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 288.

[0249] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 34, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 136, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 187, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 238, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 289, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 34, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 85, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 136; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 187, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 238, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 289; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1

or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 34, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 85, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 136; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 187, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 238, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 289.

[0250] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 86, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 137, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 188, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 239, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 290, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 35, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 86, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 137; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 188, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 239, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 290; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 35, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 86, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 137; and ii) a V_L comprising an LC-CDR1 comprising the

amino acid sequence of SEQ ID NO: 188, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 239, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 290.

[0251] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 87, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 138, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 189, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 240, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 291, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 36, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 87, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 138; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 189, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 240, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 291; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 36, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 87, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 138; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 189, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 240, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 291.

[0252] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof

comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 88, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 139, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 190, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 241, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 292, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 37, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 88, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 139; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 190, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 241, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 292; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 37, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 88, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 139; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 190, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 241, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 292.

[0253] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 38, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 89, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 140, or a variant thereof comprising up to

about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 191, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 242, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 293, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 38, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 89, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 140; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 191, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 242, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 293; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 38, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 89, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 140; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 191, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 242, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 293.

[0254] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 90, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 141, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 192, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 243, or a variant thereof comprising

up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 294, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 39, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 141; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 192, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 243, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 294; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 39, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 141; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 192, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 243, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 294.

[0255] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 91, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 142, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 193, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 244, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 295, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the

amino acid sequence of SEQ ID NO: 40, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 91, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 142; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 193, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 244, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 295; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 40, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 91, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 142; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 193, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 244, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 295.

[0256] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 143, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 194, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 245, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 296, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 92, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 143; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the

amino acid sequence of SEQ ID NO: 194, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 245, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 296; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 92, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 143; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 194, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 245, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 296.

[0257] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 93, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 144, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 195, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 246, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 297, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 42, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 93, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 144; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 195, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 246, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 297; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are

in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 42, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 93, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 144; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 195, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 246, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 297.

[0258] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 94, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 145, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 196, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 247, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 298, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 43, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 94, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 145; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 196, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 247, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 298; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 43, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 94, and an HC-CDR3 comprising the

amino acid sequence of SEQ ID NO: 145; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 196, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 247, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 298.

[0259] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 95, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 146, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 197, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 248, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 299, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 95, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 146; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 197, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 248, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 299; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 95, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 146; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 197, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 248, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 299.

[0260] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 45, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 96, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 147, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 198, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 249, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 300, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 45, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 96, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 147; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 198, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 249, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 300; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 45, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 96, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 147; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 198, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 249, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 300.

[0261] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 97, or a variant thereof comprising

up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 148, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 199, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 250, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 301, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 46, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 97, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 148; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 199, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 250, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 301; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 46, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 97, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 148; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 199, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 250, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 301.

[0262] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 47, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 98, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 149, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 200, or a variant thereof

comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 251, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 302, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 47, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 98, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 149; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 200, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 251, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 302; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 47, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 98, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 149; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 200, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 251, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 302.

[0263] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 48, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 99, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 150, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 201, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 252, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 303, or a variant thereof comprising up to

about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 48, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 99, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 150; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 201, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 252, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 303; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 48, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 99, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 150; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 201, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 252, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 303.

[0264] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 49, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 100, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 151, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 202, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 253, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 304, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 49, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 100, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 151;

or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 202, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 253, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 304; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 49, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 100, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 151; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 202, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 253, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 304.

[0265] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 101, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 152, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 203, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 254, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 305, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 50, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 101, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 152; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 203, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 254, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 305;

or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 50, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 101, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 152; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 203, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 254, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 305.

[0266] In some embodiments, the anti-GPC3 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the anti-GPC3 antibody moiety comprises: (1) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 51, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 102, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 153; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 204, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 255, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 306; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, the anti-GPC3 antibody moiety comprises i) a V_H

comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 51, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 102, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 204, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 255, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 306.

[0267] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 338, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 338; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 389, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 389. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 338; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 389. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 338, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 389.

[0268] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 339, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 339; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 390, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 390. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 339; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 390. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 339, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 390.

[0269] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 340, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 340; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 391, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 391.

In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 340; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 391. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 340, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 391.

[0270] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 341, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 341; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 392, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 392. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 341; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 392. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 341, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 392.

[0271] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 342, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 342; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 393, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 393. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 342; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 393. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 342, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 393.

[0272] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 343, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 343; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 394, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 394.

In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 343; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 394. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 343, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 394.

[0273] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 344, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 344; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 395, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 395. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 344; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 395. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 344, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 395.

[0274] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 345, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 345; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 396, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 396. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 345; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 396. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 345, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 396.

[0275] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 346, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 346; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 397, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 397.

In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 346; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 397. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 346, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 397.

[0276] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 347, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 347; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 398, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 398. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 347; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 398. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 347, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 398.

[0277] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 348, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 348; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 399, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 399. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 348; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 399. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 348, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 399.

[0278] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 349, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 349; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 400, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 400.

In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 349; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 400. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 349, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 400.

[0279] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 350, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 350; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 401, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 401. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 350; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 401. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 350, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 401.

[0280] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 351, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 351; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 402, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 402. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 351; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 402. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 351, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 402.

[0281] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 352, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 352; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 403, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 403.

In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 352; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 403. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 352, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 403.

[0282] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 353, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 353; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 404, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 404. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 353; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 404. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 353, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 404.

[0283] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 354, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 354; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 405, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 405. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 354; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 405. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 354, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 405.

[0284] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 355, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 355; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 406, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 406.

In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 355; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 406. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 355, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 406.

[0285] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 356, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 356; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 407, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 407. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 356; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 407. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 356, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 407.

[0286] In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 357, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 357; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 408, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 408. In some embodiments, the anti-GPC3 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 357; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 408. In some embodiments, the anti-GPC3 antibody moiety comprises the HC-CDRs of a V_H comprising the amino acid sequence of SEQ ID NO: 357, and the LC-CDRs of a V_L comprising the amino acid sequence of SEQ ID NO: 408.

[0287] In some embodiments, the anti-GPC3 antibody moiety competes for binding to a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) with a second anti-GPC3 antibody moiety according to any of the anti-GPC3 antibody moieties described herein. In some embodiments, the anti-GPC3 antibody moiety binds to the same, or substantially the same, epitope as the second anti-GPC3 antibody moiety. In some embodiments, binding of the anti-GPC3 antibody moiety to the target GPC3 inhibits binding of the second anti-GPC3 antibody moiety to the target GPC3 by at

least about 70% (such as by at least about any of 75%, 80%, 85%, 90%, 95%, 98% or 99%), or *vice versa*. In some embodiments, the anti-GPC3 antibody moiety and the second anti-GPC3 antibody moiety cross-compete for binding to the target GPC3, *i.e.*, each of the anti-GPC3 antibody moieties competes with the other for binding to the target GPC3.

[0288] In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, wherein the anti-nGPC3 antibody moiety can be any one of the anti-nGPC3 antibody moieties described herein. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), wherein the anti-GPC3 antibody moiety can be any one of the anti-GPC3 antibody moieties described herein.

[0289] For example, in some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3 (referred to herein as "anti-nGPC3 antibody moiety"; *e.g.*, the binding affinity of the antibody moiety to the cell surface-bound GPC3 is higher than that to a soluble GPC3, or the antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In

some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the anti-GPC3 construct is a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-GPC3 construct is a Fab or a Fab'. In some embodiments, the anti-GPC3 construct is an scFv. In some embodiments, the anti-GPC3 construct comprising an anti-GPC3 antibody moiety fused to an Fc fragment (*e.g.*, IgG1 Fc fragment) optionally via a linker. In some embodiments, the anti-GPC3 construct is a full-length antibody (*e.g.*, monoclonal antibody). In some embodiments, the anti-GPC3 construct is a multi-specific (such as bispecific) molecule, such as tandem di-scFv anti-GPC3 T cell engager (tandem di-scFv anti-GPC3×CD3ε). In some embodiments, the anti-GPC3 construct is a CAR. In some embodiments, the anti-GPC3 construct is a caTCR. In some embodiments, the anti-GPC3 construct is an immunoconjugate. In some embodiments, the anti-GPC3 construct is monospecific. In some embodiments, the anti-GPC3 construct is multispecific (*e.g.*, bispecific). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to

about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

[0290] In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs:

256-286; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 358-388. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially

the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the anti-GPC3 construct is a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-GPC3 construct is a Fab or a Fab'. In some embodiments, the anti-GPC3 construct is an scFv. In some embodiments, the anti-GPC3 construct comprising an anti-GPC3 antibody moiety fused to an Fc fragment (*e.g.*, IgG1 Fc fragment) optionally via a linker. In some embodiments, the anti-GPC3 construct is a full-length antibody (*e.g.*, monoclonal antibody). In some embodiments, the anti-GPC3 construct is a multi-specific (such as bispecific) molecule, such as tandem di-scFv anti-GPC3 T cell engager (tandem di-scFv anti-GPC3×CD3ε). In some embodiments, the anti-GPC3 construct is a CAR. In some embodiments, the anti-GPC3 construct is a caTCR. In some embodiments, the anti-GPC3 construct is an immunoconjugate. In some embodiments, the anti-GPC3 construct is monospecific. In some embodiments, the anti-GPC3 construct is multispecific (*e.g.*, bispecific). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

[0291] In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3). In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5

(such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the amino acid substitutions are in HC-CDR1 or HC-CDR2. In some embodiments, the amino acid substitutions are in LC-CDR1 or LC-CDR2. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing GPC3, comprising: i) a V_H comprising the amino acid sequence

of any one of SEQ ID NOs: 338-357, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, or a variant thereof having at least about 80% (including for example at least about any of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 389-408. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety specifically recognizing GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that competes for binding to GPC3 (*e.g.*, nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. n some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and

293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the anti-GPC3 construct is a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-GPC3 construct is a Fab or a Fab'. In some embodiments, the anti-GPC3 construct is an scFv. In some embodiments, the anti-GPC3 construct comprising an anti-GPC3 antibody moiety fused to an Fc fragment (*e.g.*, IgG1 Fc fragment) optionally via a linker. In some embodiments, the anti-GPC3 construct is a full-length antibody (*e.g.*, monoclonal antibody). In some embodiments, the anti-GPC3 construct is a multi-specific (such as bispecific) molecule, such as tandem di-scFv anti-GPC3 T cell engager (tandem di-scFv anti-GPC3×CD3ε). In some embodiments, the anti-GPC3 construct is a CAR. In some embodiments, the anti-GPC3 construct is a caTCR. In some embodiments, the anti-GPC3 construct is an immunoconjugate. In some embodiments, the anti-GPC3 construct is monospecific. In some embodiments, the anti-GPC3 construct is multispecific (*e.g.*, bispecific). In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3 antibody moiety and the target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

Anti-GPC3 scFv

[0292] The anti-GPC3 constructs (such as isolated anti-GPC3 constructs) in some embodiments are scFvs (hereinafter referred to as "anti-GPC3 scFv") comprising an anti-GPC3 antibody moiety described herein (targeting *e.g.*, nGPC3 and/or sGPC3). In some embodiments, the anti-GPC3 scFv specifically recognizes a cell surface-bound GPC3. The anti-GPC3-scFv can comprise any one of the anti-GPC3 antibody moieties described herein (*see* "anti-GPC3 antibody moiety" section). The anti-GPC3 scFv (targeting *e.g.*, nGPC3 and/or sGPC3) can have the configuration of (from N-terminus to C-terminus) V_L(GPC3)-L-V_H(GPC3), or V_H(GPC3)-L-V_L(GPC3), wherein L is a linker (such as peptide linker). In some embodiments, the anti-GPC3

scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. Exemplary anti-GPC3 scFv sequences are shown in Tables 10-11.

TABLE 10 anti-GPC3 antibody moiety specifically recognizing cell surface-bound GPC3 scFv sequences

(CDR sequences are underlined; linker sequences are **bolded**)

Clone No.	SEQ ID NO:	scFv
GPC3A-034	**409**	QPVLTQPPSASGTPGQRVTISCSGSSSNIGSNNVIWYQQLPGAAPKLLIYSNHRRPSGVPDRFSGSRS GTSASLAISGLQSEDEADYYCAAWDDSLDGYLFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QV QLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTIS VDTSKNQFSLELSSVTAADTAVYYCARGYGGRFDYWGQGTLVTVSS
GPC3A-035	**410**	QAVLTQPPSASQTPGQMVTISCSGTSSNIGSNYVFWYQQLPGTAPKLLIYKNFQRPSGVPGRFSGSKS GTAASLAISGLRSEDEADYFCAAWDDALSGYVFGAGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QV QLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARSWTSGFDYWGQGTLVTVSS
GPC3A-037	**411**	QSVLTQPPSVSAAPGQRVTISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSGIPDRFSASKS GTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QV QLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSVIYSGGSSTYYADSVKGRFT ISRDNSKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDYWGQGTLVTVSS
GPC3A-038	**412**	QSVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGN TATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQL VQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTM TRDTSTSTVYMELSSLRSEDTAVYYCARKVTGYDSWGQGTLVTVSS
GPC3A-039	**413**	SYVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGN TATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQ LVQSGGGLVHPGGSLRLSCAGSGFTFSSYAMHWVRQAPGKGLEWVSAIGTGGGTYYADSVKGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCARYGRKSIDAWGQGTLVTVSS
GPC3A-040	**414**	LPVLTQPPSASGTPGQRVTISCSGSSSNIGSNYVYWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDDSLSGYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QV QLVQSGAEVKKPGASVTVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARRGYYGYDSWGQGTLVTVSS
GPC3A-041	**415**	QAVLTQPPSASQTPGQMVTISCSGTSSNIGSNYVFWYQQLPGTAPKLLIYKNFQRPSGVPGRFSGSKS GTAASLAISGLRSEDEADYFCAAWDDALSGYVFGAGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**EV QLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAMYYCARSGKYYGDKWGQGTLVTVSS
GPC3A-042	**416**	SYVLTQPPSASGTPGQRVTISCFGSSSDIGSNSVFWYQQLPGAAPKLLIYSTQYRPSGVPDRFSGSKS GTSASLAISGLQSEDEAEYHCATWDDSLNGYVFGSGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QV QLQQSGAEVKKPGASVKVSCKASGYSFTGYYVYWMRQAPGKGLEWMGWMNPRSGGTNYAQKFQG RVTMTRDTSISTAYMELSRLTSDDTAVYYCARSSYYWADSWGQGTLVTVSS
GPC3A-043	**417**	QSVLTQPPSASGTPGQRVTISCSGSSSNIGTNYVYWYQQLPGTAPKLIIYRNNQRPSGVPDRFSGSES GTSASLAISGLRSEDEADYYCAVWDDSLSGVVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**EV QLVQSGAEVKKPGATVKVSCKVSGYTFTDYYMHWVQQAPGKGLEWMGLVDPEDGETIYAEKFQGRV TITADTSTDTAYMELSSLRSEDTAVYYCARELRDVAYYPWGVEDFWGQGTLVTVSS
GPC3A-044	**418**	SYVLTQPPSVSVAPGKTARITCGGDNIGYKGVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGN TATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQL QQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTISVD TSKNQFSLKLSSVTAADTAVYYCARYYVPYLSDWGQGTLVTVSS
GPC3A-045	**419**	QSVLTQPPSVSGTPGQRVIISCPGSTSNIGTNTVNWYQQFPGTAPKLLIYSNNQRPSGVPDRFSGSKSG TSASLAISGLQSEDEADYYCAAWDDSLNGVVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QM QLVQSGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCARASDLYGDWGQGTLVTVSS
GPC3A-046	**420**	QAVLTQPPSVSGTPGQRVTISCSGSSSNFGSNTVHWYQQVPGTAPKLLIFSNTQRPSEIPDRFSGSKS GTSASLAISGLQSEDEADYYCAAWDDSLTGVVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QV QLVQSGAEVKKPGASVTVSCKASGYRFSNYGVSWVRQAPGQGLEWMGWISGSNGNTNYAQKFLGR VTMTTDTSTTTAYMELSSLRSDDTAVYYCARGNRRYYSPIIDPWGQGTLVTVSS

Clone No.	SEQ ID NO:	scFv
GPC3A-047	**421**	LPVLTQPPSASGTPGQRVTISCSGSRSNIASNDVYWYQQLPGTAPKRLIYKKNQRPSGVPDRFSASKS GTSASLAISGLRSEDEADYYCAAWDDNLSGYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QV QLVQSGAEVKKPGASVKVSCKAPGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARSDYGSLYDKWGQGTLVTVSS
GPC3A-048	**422**	QPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGN TATLTISRVEAGDEADFYCQVWDSSSDRGVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**EVQL VESGGGEVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARSSFVATDYWGQGTLVTVSS
GPC3A-049	**423**	QAVLTQPSSASGTPGQRVTISCSGGSSNIGSNYVYWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDDSLSGWVFGGRTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QV QLVQSGAEVKEPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARHGGIGSMRSFDQWGQGTLVTVSS
GPC3A-050	**424**	QAVLTQPSSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKS GTSASLAISGLQSEDEADYYCAAWDDSLNGPVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QI TLKESGAEVKKPGASVKVSCKASGYSFTGYYVYWMRQAPGKGLEWMGWMNPRSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLTSDDTATYYCARSGYRWLDVWGQGTLVTVSS
GPC3A-051	**425**	QAVLTQPSSASGAPGQRVTISCTGGSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSK SGTSASLAITGLQAEDEADYYCQSYDSSLSGYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**Q VQLVQSGAEVKKPGSSVKVSCKASGGAFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVT ITADESTSTAYMELSSLRSEDTAVYYCARMLYLSGRYYWDSWGQGTLVTVSS
GPC3A-052	**426**	QAVLTQPSSASGTPGQRVTISCSGSSSNIGSNYVYWYQQLSGTAPKLLIYRNNQRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDDSLSGYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**EV QLVESGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARSHSSGYDKWGQGTLVTVSS
GPC3A-053	**427**	QSVLTQPPSASAAPGQRVTISCSGTSSNIGSNYVWWYQQLPGAAPRLLIYGNSNRPSGVPDRFSGSKS GTSASLAITGLQAEDEADYYCQSYDSSLSGSNVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**Q LQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGSIYYSGSTYYNPSLKSRVTI SVDTSKNQFSLKLSSVTAADTAVYYCARWWSGSYDTWGQGTLVTVSS
GPC3A-054	**428**	SYELTQPPSASGTPGQRVTISCSGSSSNIGSNYVYWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDDSLSGYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QM QLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRV TMTTDTSTSTAYMELRSLRSDDTAVYYCARIPMYSGSSDYWGQGTLVTVSS
GPC3A-055	**429**	QPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGN TATLTISRVEAGDEADYYCQVWDSSSDHYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQL VQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTM TRDTSTSTVYMELSSLRSEDTAVYYCARWHGGPYDYWGQGTLVTVSS
GPC3A-056	**430**	DIQLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGT DFTLTISSLQPEDFATYYCQQSYSTPITFGQGTRLEIKR**SRGGGGSGGGGSGGGGSLEMA**QVQLQQS GAEVKKFGASVKVSCKASGYSFNDYYIHWVRQAPGQGLEWMGWINPNNGDTKYEKKWQGRVTMTR DTSITTAYMELSSLRSDDTAVYYCARFSTHNWWWPTYDYWGQGTLVTVSS
GPC3A-057	**431**	EIVLTQSPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNT ATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKVEIKR**SRGGGGSGGGGSGGGGSLEMA**EVQLV ESGGGLIQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCARYNYMSSGFYDRWGQGTLVTVSS
GPC3A-058	**432**	QSVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGN TATLTISRVEAGDEADYYCQVWDSSSDHVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQLV QSGADVRKPGASVKVSCKASGYTFASHGISWVRQAPGQGLEWLGWISPYTGNTNYAQKFQGRVTMA TDTSTSTAYMELRSLRSDDTAIYYCARGKRTLASCFDYWGQGTLVTVSS
GPC3A-059	**433**	SYVLTQPPSVSVAPGKTARITCGGNNIGSKSVYWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNT ATLTISRVEAGDEADYYCQVWDSSSDHVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQLVQ SGAEVKKPGASVTVSCKASGYTFTRYGITWVRQAPGQGLEWMGWISAYSDKTNYAQKLQGRVTMTTD ISTNTAYMELSSLRSEDTAVYYCARSRWSYMDVWGQGTLVTVSS
GPC3A-060	**434**	SYVLTQPPSVSVAPGKTARLTCGGNNIGSESVHWYQQKPGQAPLLVVYDDDDRPSGIPERFSGSNSE DTATLTISGTQALDEAEYYCQTWDSSTAIFGTGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQLVQ SGGEVKKPGASVKVSCKASGYTFNSYAISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTT DTSTNTAFMELRSLRSDDTAVYYCAREGYGSWAMDQWGQGTLVTVSS

Clone No.	SEQ ID NO:	scFv
GPC3A-061	435	QPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGN TATLTISRVEAGDEADYYCQVWDSSSDHYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQL VQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQTPGQGLEWMGWISAYNGNTNYAQKLQGRVTM TTDTSTSTAYMELSSLRSEDTAVYYCARKGSSQFDQWGQGTLVTVSS
GPC3A-062	436	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNWPSGVPDRFSGSK SGTSASLAITGLRAEDEADYYCQSYDSSLSGSYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA** QMQLVQSGSELKKPGASVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPKIGTANYAQKFQGR VTITADESTSTAYMELSSLRSEDTAMYYCARMYMDMGWGWGYWDWWGQGTLVTVSS
GPC3A-063	437	QTVVTQPPSASGTPGQRVTISCSGSWSNIGSYTVNWYQHLPGTAPKLLISGNNQRPSGVPGRFSGSKS GTSASLAISGLQSEDEADYHCAAWDDNLNGVVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QV QLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSASYAQKFQGRV TMTRDTSTSTVYMELSSLRSEDTAVYYCARDRLASDAFDIWGQGTMVTVSS
GPC3A-064	438	LPVLTQPPSLSVAPGKTARLTCGGNNIGSKSVHWYHQKPGQAPVLVVYDDTDRPSGIPERFSGSNSGN TAALTISRVEVGDEADYYCQVWDRSSAHWVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QMQ LVQSGAEVKKPGASVKVSCKASGYTFTIYGISWVRQAPGQGLEWMGWISPYNDNTIYAQKVQGRVTM TTDTSTSTAYMELRSLRSDDTAVYYCARMGVGWGYAQDSWGQGTLVTVSS
GPC3A-067	439	QSVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGN TATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**EVQL VQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPIFGITNYAQKFQGRVTITAD KSTSTAYMELSSLRSEDTAVYYCARGAEMSDYWGQGTLVTVSS

TABLE 11 anti-GPC3 antibody moiety scFv sequences

(CDR sequences are underlined; linker sequences are **bolded**)

Clone No.	SEQ ID NO:	scFv
GPC3B-54	440	QSVLTQPPSASGTPGQRVTISCSGSNSNIGSDTVNWYQQLPGTAPKLLIYRDNQRPSGVPDRFSGSKSGT SASLAISGLQSEDEADYYCTTWDDSLNGLVFGGGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**EVQLVQ SGAEVKKPGASVKLSCKTSGYTFIDYYVYWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTS TAYMELSSLGSEDTAVYYCARERRYSSSPSDHWGQGTLVTVSS
GPC3B-60	441	SYVLTQPPSVSVSPGQTATIACSGDNLENKFVYWYHQKPGQSPVLVMYEDNKRPSGIPERFSGSNSGNT AALTISGAQPMDEADYYCQTWDSPTGLFVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**EVQLVES GAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTS TSTVYMELSSLRSEDTAVYYCARSLYSQPYIDGWSQGTLVTVSS
GPC3B-63	442	EIVLTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSG SGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGQGTKVEIKR**SRGGGGSGGGGSGGGGSLEMA**QVQLV QSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRD TSTSTVYMELSSLRSEDTAVYYCARSLHAMRWSQTMDSWGQGTLVTVSS
GPC3B-66	443	QAVLTQPPSVSVAPGKTASITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDHLYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQLVQS GAEVKRPGASVKVSCKASGYTFIGQYLHWVRQAPGQGLEWMGRINPMTGVTNYAPKFQGRVTMTRDTSI STGYMEISRLRSDDTAVYYCARFSSGYSRDTWGQGTLVTVSS
GPC3B-68	444	QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNIKRPSGIPDRFSGSKSGTS ATLGITGLQTGDEADYYCGTWDSSLSAGVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQLVQ SGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGIANYAQKFQGRVTITADKSTS TAYMELSSLRSEDTAVYYCARYGYEGHDTWGQGTLVTVSS
GPC3B-71	445	SYVLTQPPSVSVAPGKTARVTCGGNNIGSKSVHWYQQKPGQAPVLVMYYDSDRPSGIPERFSGSNSGNT ATLTISSVEAGDEADYYCQVWDSSSDHYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**EVQLVES GGGLVKPGGSLRLSCAASGFTFSRYTMNWVRQAPGKGLEWVSSISSSGSYIYYADSVKGRFTISRDNAKN SLFLQMDSLRAEDTAVYYCARQGHMWYVPVDAWGQGTLVTVSS
GPC3B-76	446	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSKSGT SATLGITGLQTGDEAAYYCGTWDSSLSAGVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QMQLV QSGAEVKEPGASVKVSCKASGYTFTDYYIHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTR DTSISTAYMELSRLRSDDTAVYYCARNYDWGQGTLVTVSS

Clone No.	SEQ ID NO:	scFv
GPC3B-78	**447**	LPVLTQPPSVSVSPGQTASITCSGDKLGDKYAYWYQQKPGQSPVLVIYQDNKRPSGIPERFSGSNSGNTA TLTISGTQAMDAADYYCQTWDRSTYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QMQLVQSGA EVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTS TVYMELSSLRSEDTAVYYCARGYSSFFDSWGQGTLVTVSS
GPC3B-80	**448**	QAVLTQPPSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKSGT SAYLAISGLQSEDEADYYCAAWDDSLNGWGVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQL VQSGAEVKKPGSSVKVSCKASGGTLSRFAISWVRQAPGQGLEWMGGIIPIFRTANYAQKFQGRVTITADE STSTAYMELSSLRSEDTAVYYCARMSKYYGSYSSYDEWGQGTLVTVSS
GPC3B-81	**449**	SYELTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDLLYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**EVQLVQS GAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGRINPNSGGTNYAQKFQGRVTMTRDT SISTAYMELSRLRSDDTAVYYCARGLWDSWGQGTLVTVSS
GPC3B-82	**450**	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGFDVHWYQQLPGTAPKLLIYDNNNRPSGVPDRFSGSKSD TSASLAITGLQAEDEADYYCQSFDSSLSGWVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**EVQLV QSGAEVKKPGESLKISCKGSGYSFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRD TSTSTVYMEMSSLRSEDTAVYYCARYPVYMETSDFDSWGSRYSGDRLL
GPC3B-85	**451**	QAVLTQPPSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKSGT SASLAISGLQSEDEADYYCAAWDDSLNGYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQLV QSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTNTGNPTYAQGFTGRFVFSLD TSVSTAYLQISSLKAEDTAVYYCARSSLYWMGSKWSRQTDMWGQGTLVTVSS
GPC3B-87	**452**	DVVMTQSPLSLPVTPGEPASVSCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSG SGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKR**SRGGGGSGGGGSGGGGSLEMA**EVQ LVQSGAEVRKPGSSVKVSCQASGGTFGSYAISWVRQAPGQGLEWMGRIIPVLGRTKYAQKFQGRVTVTA DTSTSTVYMELTSLTSEDTAVYYCARTNDSWGQGTLVTVSS
GPC3B-92	**453**	QSVLTQPPSVSVSPGQTARITCSGETLAKRYAHWYQQKPGQAPVLLIYRDTERPSGIPERFSGSSSGTTIT LTITGVQAEDEADYYCQSADNSRTFVFGPGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**EVQLVQSGAE VKKPGASVKVSCKASGYTFSNYYMHWVRQAPGQGLEWMGIINPSGGTTTYAQKFQGRVTMTRDTSTST VYMELSSLRSEDTAVYYCARPSMWTSSMGDVWGQGTLVTVSS
GPC3B-93	**454**	NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVPDRFSGSIDSS SNSASLTISGLKTEDEADYYCQSYDSSNWVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**EVQLVQ SGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTRYAQKFQGRVTMTRDT STSTVYMELSSLRSEDTAVYYCARYTALKPRGIYSVDSWGQGTLVTVSS
GPC3B-110	**455**	QSVLTQPPSASGTPGQRVTVSCSGSSSNIGSNTVNWYQQLPGTAPKLLLYSSNQRPSGVPDRFSGSRSG TSASLAISGLQSEDEADYYCAAWDDSLNGPVFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**EVQLV QSGAEVKKPGSSVKVSCQASGGTFTTYSINWVRQAPGQGLEWMGGIIPTFGTTNYAQNFQDRVTISADES TNTAYMELTSLRSEDTAVYYCARYYWRGGSGQGSVTSDYWGQGTLVTVSS
GPC3B-113	**456**	SYELTQPPSASGTPGQRVTISCSGSSSNIGSNSVSWYQHLPGVAPKLLIYSNNQRPSGVPDRFSGSKTGT SASLAISGLQSEDEGDYYCAAWDDSLNGVLFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQLV QSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTED TSTDTAYMELSSLRSEDTAVYYCARYSGDYWGQGTLVTVSS
GPC3B-115	**457**	SYELTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTA TLTISRVEAGDEADYYCQVWDSSSDPLYVFGTGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQLVQS GAEVKRPGATIKVSCKTSGYTFTAYYTHWVRQAPGQGLEWVGRINANTGGTDYAPKFRDRVIMTRDTSIS TAYMELGRLTSEDTAVYYCARISGYHSSGWDYWGQGTLVTVSS
GPC3B-119	**458**	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQIPGTAPKLLIYDNNKRPSGIPDRFSGSRSGTS ATLGITGLQTGDEAHYYCATWDNSLSALIFGGGTKVTVLG**SRGGGGSGGGGSGGGGSLEMA**QVQLVQS GSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTNTGNPTYAQGFTGRFVFSLDTS VSTAYLQISSLKAEDTAVYYCARGYYGKYDKWGQGTLVTVSS
GPC3B-125	**459**	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWFQQHPGKAPKLIIYEVSNRPSGVSDRFSGSKSG STASLTISGLQAEDEANYYCSSYTSSTTVIFGGGTKLTVLG**SRGGGGSGGGGSGGGGSLEMA**QMQLVQS GSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTNTGNPTYAQGFTGRFVFSLDTS VSTAYLQISSLKAEDTAVYYCARQSHDEWGQGTLVTVSS

Linkers

[0293] *See* "Linkers" subsection under the "Anti-GPC3 Fc fusion protein" section below for all applicable linkers that can be used in the anti-GPC3 scFv described herein. In some embodiments, the linker comprises the amino acid sequence of SRGGGGSGGGGSGGGGSLEMA (SEQ ID NO: 473). In some embodiments, the linker is or comprises a $(GGGGS)_n$ sequence (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the linker comprises the amino acid sequence of GEGTSTGSGGSGGSGGAD (SEQ ID NO: 490).

[0294] In some embodiments, there is provided an anti-GPC3 construct (*e.g.*, isolated anti-GPC3 construct) comprising an scFv specifically recognizing a cell surface-bound GPC3 (referred to herein as "anti-nGPC3 scFv"; *e.g.*, the binding affinity of the anti-nGPC3 scFv to the cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity). In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some

embodiments, the anti-nGPC3-scFv specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3-scFv can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460). In some embodiments, the anti-nGPC3-scFv specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3-scFv (*e.g.*, the binding affinity of the anti-nGPC3-scFv to the cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3-scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3-scFv (*e.g.*, the binding affinity of the anti-nGPC3-scFv to the cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3-scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3-scFv and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3-scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the V_H of the anti-nGPC3-scFv is N-terminal to the V_L of the anti-nGPC3-scFv. In some embodiments, the V_H of the anti-nGPC3-scFv is C-terminal to

the V_L of the anti-nGPC3-scFv. In some embodiments, the linker between V_H and V_L of the anti-nGPC3-scFv comprises the amino acid sequence of SRGGGGSGGGGSGGGGSLEMA (SEQ ID NO: 473). In some embodiments, the linker between V_H and V_L of the anti-nGPC3-scFv is or comprises a $(GGGGS)_n$ sequence (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker between V_H and V_L of the anti-nGPC3-scFv comprises the amino acid sequence of GEGTSTGSGGSGGSGGAD (SEQ ID NO: 490). In some embodiments, the anti-nGPC3 scFv further comprises a tag (*e.g.*, a peptide tag for purification purpose). In some embodiments, the tag is N-terminal to the anti-nGPC3 scFv. In some embodiments, the tag is C-terminal to the anti-nGPC3 scFv. In some embodiments, the tag comprises a His-tag and an HA-tag. In some embodiments, the tag comprises the amino acid sequence of TSGQAGQHHHHHHGAYPYDVPDYAS (SEQ ID NO: 477).

[0295] In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing a cell surface-bound GPC3. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid

sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing a cell surface-bound GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3-scFv that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3-scFv that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3-scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the V_H of the anti-nGPC3-scFv is N-terminal to the V_L of the anti-nGPC3-scFv. In some embodiments, the V_H of the anti-nGPC3-scFv is C-terminal to the V_L of the anti-nGPC3-scFv. In some embodiments, the linker between V_H and V_L of the anti-nGPC3-

scFv comprises the amino acid sequence of SRGGGGSGGGGSGGGGSLEMA (SEQ ID NO: 473). In some embodiments, the linker between V_H and V_L of the anti-nGPC3-scFv is or comprises a $(GGGGS)_n$ sequence (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker between V_H and V_L of the anti-nGPC3-scFv comprises the amino acid sequence of GEGTSTGSGGSGGSGGAD (SEQ ID NO: 490). In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing a cell surface-bound GPC3, wherein the anti-nGPC3 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 409-439. In some embodiments, the anti-nGPC3 scFv further comprises a tag (*e.g.*, a peptide tag for purification purpose). In some embodiments, the tag is N-terminal to the anti-nGPC3 scFv. In some embodiments, the tag is C-terminal to the anti-nGPC3 scFv. In some embodiments, the tag comprises a His-tag and an HA-tag. In some embodiments, the tag comprises the amino acid sequence of TSGQAGQHHHHHHGAYPYDVPDYAS (SEQ ID NO: 477).

[0296] In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3). In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1

comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv that competes for binding to GPC3 (*e.g.*, nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein. In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein. In some embodiments, the anti-GPC3-scFv specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-GPC3-scFv to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-GPC3-scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3-scFv specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-GPC3-scFv to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3-scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3-scFv specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3-scFv specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some

embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the K_d of the binding between the anti-GPC3-scFv and a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-GPC3-scFv and a non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3-scFv and the target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3-scFv and a non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-GPC3-scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the V_H of the anti-GPC3-scFv is N-terminal to the V_L of the anti-GPC3-scFv. In some embodiments, the V_H of the anti-GPC3-scFv is C-terminal to the V_L of the anti-GPC3-scFv. In some embodiments, the linker between V_H and V_L of the anti-GPC3-scFv comprises the amino acid sequence of SRGGGGSGGGGSGGGGSLEMA (SEQ ID NO: 473). In some embodiments, the linker between V_H and V_L of the anti-GPC3-scFv is or comprises a $(GGGGS)_n$ sequence (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker between V_H and V_L of the anti-GPC3-scFv comprises the amino acid sequence of GEGTSTGSGGSGGSGGAD (SEQ ID NO: 490). In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3-scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), wherein the anti-GPC3 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 440-459. In some embodiments, the anti-GPC3-scFv further comprises a tag (*e.g.*, a peptide tag for purification purpose). In some embodiments, the tag is N-terminal to the anti-GPC3-scFv. In some embodiments, the tag is C-terminal to the anti-GPC3-scFv. In some embodiments, the tag comprises a His-tag and an HA-tag. In some embodiments, the tag comprises the amino acid sequence of TSGQAGQHHHHHHGAYPYDVPDYAS (SEQ ID NO: 477).

Anti-GPC3 Fc fusion protein

[0297] The anti-GPC3 constructs (such as isolated anti-GPC3 constructs) in some embodiments are Fc fusion proteins (hereinafter referred to as "anti-GPC3-Fc fusion protein") comprising an anti-GPC3 antibody moiety described herein fused to an Fc fragment (such as IgG1 Fc fragment). In some embodiments, the anti-GPC3 antibody moiety is fused to an Fc fragment via a linker (such as peptide linker). In some embodiments, the anti-GPC3-Fc fusion

protein comprises an antibody comprising an Fc fragment. In some embodiments, the anti-GPC3-Fc fusion protein is a full-length antibody. Any of the anti-GPC3 antibody moieties described in the "anti-GPC3 antibody moiety section" can be employed in the anti-GPC3 Fc fusion protein.

Fc fragment

[0298] The term "Fc region," "Fc domain" or "Fc" refers to a C-terminal non-antigen binding region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native Fc regions and variant Fc regions. In some embodiments, a human IgG heavy chain Fc region extends from Cys226 to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present, without affecting the structure or stability of the Fc region. Unless otherwise specified herein, numbering of amino acid residues in the IgG or Fc region is according to the EU numbering system for antibodies, also called the EU index, as described in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

[0299] In some embodiments, the Fc fragment comprises an immunoglobulin IgG heavy chain constant region comprising a hinge region (starting at Cys226), an IgG CH2 domain and CH3 domain. The term "hinge region" or "hinge sequence" as used herein refers to the amino acid sequence located between the linker and the CH2 domain. In some embodiments, the fusion protein comprises an Fc fragment comprising a hinge region. In some embodiments, the hinge region comprises the amino acid sequence CPPCP (SEQ ID NO: 478), a sequence found in the native IgG1 hinge region, to facilitate dimerization. In some embodiments, the Fc fragment of the fusion protein starts at the hinge region and extends to the C-terminus of the IgG heavy chain. In some embodiments, the fusion protein comprises an Fc fragment that does not comprise the hinge region.

[0300] In some embodiments, the fusion protein comprises an Fc fragment selected from the group consisting of Fc fragments from IgG, IgA, IgD, IgE, IgM, and combinations and hybrids thereof. In some embodiments, the Fc fragment is derived from a human IgG. In some embodiments, the Fc fragment comprises the Fc region of human IgG1, IgG2, IgG3, IgG4, or a combination or hybrid IgG. In some embodiments, the Fc fragment is an IgG1 Fc fragment. In some embodiments, the Fc fragment comprises the CH2 and CH3 domains of IgG1. In some embodiments, the Fc fragment is an IgG4 Fc fragment. In some embodiments, the Fc fragment comprises the CH2 and CH3 domains of IgG4. IgG4 Fc is known to exhibit less effector activity

than IgG1 Fc, and thus may be desirable for some applications. In some embodiments, the Fc fragment is derived from of a mouse immunoglobulin.

[0301] In some embodiments, the IgG CH2 domain starts at Ala231. In some embodiments, the CH3 domain starts at Gly341. It is understood that the C-terminus Lys residue of human IgG can be optionally absent. It is also understood that conservative amino acid substitutions of the Fc region without affecting the desired structure and/or stability of Fc is contemplated within the scope of the invention.

[0302] Additionally, anti-GPC3-Fc fusion proteins comprising any of the Fc variants described below, or combinations thereof, are contemplated. In some embodiments, the Fc fragment comprises sequence that has been altered or otherwise changed so that it has enhanced antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) effector function.

[0303] In some embodiments, each chain of the Fc fragment is fused to the same entity. In some embodiments, the anti-GPC3-Fc fusion protein comprises two identical anti-GPC3 antibody moieties described herein (specifically recognizing *e.g.*, nGPC3 and/or sGPC3), each fused with one chain of the Fc fragment. In some embodiments, the two chains of the Fc fragment are identical. In some embodiments, the anti-GPC3-Fc fusion protein (including anti-GPC3-Fc fusion proteins comprising an antibody) comprising the Fc fragment is a homodimer.

[0304] In some embodiments, each chain of the Fc fragment is fused to a different entity. In some embodiments, the fusion protein comprises two different anti-GPC3 antibody moieties, each fused to one chain of the Fc fragment. In some embodiments, the two anti-GPC3 antibody moieties are different but both specifically recognize nGPC3. In some embodiments, the two anti-GPC3 antibody moieties are different but both specifically recognize sGPC3. In some embodiments, one anti-GPC3 antibody moiety specifically recognizes nGPC3, and the other anti-GPC3 antibody moiety specifically recognizes sGPC3. In some embodiments, the anti-GPC3-Fc fusion protein is monovalent, *i.e.*, only one anti-GPC3 antibody moiety is fused to one chain of the Fc fragment, and the second chain of the Fc fragment is not fused to an anti-GPC3 antibody moiety. In some embodiments, the anti-GPC3-Fc fusion protein (including anti-GPC3-Fc fusion proteins comprising an antibody) comprising the Fc fragment is a heterodimer.

[0305] Heterodimerization of non-identical polypeptides in the anti-GPC3-Fc fusion protein can be facilitated by methods known in the art, including without limitation, heterodimerization by the knob-into-hole technology. The structure and assembly method of the knob-into-hole technology can be found in, e.g., US5,821,333, US7,642,228, US 201 1/0287009 and

PCT/US2012/059810, hereby incorporated by reference in their entireties. This technology was developed by introducing a "knob" (or a protuberance) by replacing a small amino acid residue with a large one in the CH3 domain of one Fc and introducing a "hole" (or a cavity) in the CH3 domain of the other Fc by replacing one or more large amino acid residues with smaller ones. In some embodiments, one chain of the Fc fragment in the fusion protein comprises a knob, and the second chain of the Fc fragment comprises a hole.

[0306] The preferred residues for the formation of a knob are generally naturally occurring amino acid residues and are preferably selected from arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W). Most preferred are tryptophan and tyrosine. In one embodiment, the original residue for the formation of the knob has a small side chain volume, such as alanine, asparagine, aspartic acid, glycine, serine, threonine or valine. Exemplary amino acid substitutions in the CH3 domain for forming the knob include without limitation the T366W, T366Y or F405W substitution.

[0307] The preferred residues for the formation of a hole are usually naturally occurring amino acid residues and are preferably selected from alanine (A), serine (S), threonine (T) and valine (V). In one embodiment, the original residue for the formation of the hole has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan. Exemplary amino acid substitutions in the CH3 domain for generating the hole include without limitation the T366S, L368A, F405A, Y407A, Y407T and Y407V substitutions. In certain embodiments, the knob comprises T366W substitution, and the hole comprises the T366S/L368A/Y 407V substitutions. It is understood that other modifications to the Fc region known in the art that facilitate heterodimerization are also contemplated and encompassed by the instant application.

[0308] Other anti-GPC3 Fc fusion protein variants (such as variants of isolated anti-GPC3-Fc fusion protein, *e.g.*, a full-length anti-GPC3 antibody variant) comprising any of the variants described herein (*e.g.*, Fc variants, effector function variants, glycosylation variants, cysteine engineered variants), or combinations thereof, are contemplated. *See* "anti-GPC3 variants" section for all applicable variations for the anti-GPC3 Fc fusion protein (*e.g.*, full-length anti-GPC3 antibody).

Linkers

[0309] In some embodiments, the anti-GPC3-Fc fusion proteins described herein comprise an anti-GPC3 antibody moiety described herein fused to an Fc fragment via a linker.

[0310] The length, the degree of flexibility and/or other properties of the linker used in the anti-GPC3-Fc fusion proteins may have some influence on properties, including but not limited

to the affinity, specificity or avidity for the anti-GPC3 antibody moiety, and/or one or more particular antigens or epitopes of the anti-GPC3-Fc fusion protein (such as an antibody comprising an Fc fragment and an anti-GPC3 antibody moiety). For example, longer linkers may be selected to ensure that two adjacent antibody moieties do not sterically interfere with one another. In some embodiments, a linker (such as peptide linker) comprises flexible residues (such as glycine and serine) so that the adjacent antibody moieties are free to move relative to each other. For example, a glycine-serine doublet can be a suitable peptide linker. In some embodiments, the linker is a non-peptide linker. In some embodiments, the linker is a peptide linker. In some embodiments, the linker is a non-cleavable linker. In some embodiments, the linker is a cleavable linker.

[0311] Other linker considerations include the effect on physical or pharmacokinetic properties of the resulting anti-GPC3-Fc fusion protein, such as solubility, lipophilicity, hydrophilicity, hydrophobicity, stability (more or less stable as well as planned degradation), rigidity, flexibility, immunogenicity, modulation of antibody moiety binding, the ability to be incorporated into a micelle or liposome, and the like.

Non-peptide linkers

[0312] Any one or all of the linkers described herein can be accomplished by any chemical reaction that will bind the two molecules so long as the components or fragments retain their respective activities, *i.e.* binding to target GPC3 (*e.g.*, nGPC3 and/or sGPC3), binding to FcR, or ADCC/CDC. This linkage can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. In some embodiments, the binding is covalent binding. Covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as an Fc fragment to the anti-GPC3 antibody moiety of the present invention. For example, representative coupling agents can include organic compounds such as thioesters, carbodiimides, succinimide esters, diisocyanates, glutaraldehyde, diazobenzenes and hexamethylene diamines. This listing is not intended to be exhaustive of the various classes of coupling agents known in the art but, rather, is exemplary of the more common coupling agents (*see* Killen and Lindstrom, Jour. Immun. 133:1335-2549 (1984); Jansen *et al.*, Immunological Reviews 62:185-216 (1982); and Vitetta *et al.*, Science 238:1098 (1987)).

[0313] Linkers that can be applied in the present application are described in the literature (*see*, for example, Ramakrishnan, S. *et al.*, Cancer Res. 44:201-208 (1984) describing use of

MBS (M-maleimidobenzoyl-N-hydroxysuccinimide ester)). In some embodiments, non-peptide linkers used herein include: (i) EDC (1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride; (ii) SMPT (4-succinimidyloxycarbonyl-alpha-methyl-alpha-(2-pridyl-dithio)-toluene (Pierce Chem. Co., Cat. (21558G); (iii) SPDP (succinimidyl-6 [3-(2-pyridyldithio) propionamido]hexanoate (Pierce Chem. Co., Cat #21651G); (iv) Sulfo-LC-SPDP (sulfosuccinimidyl 6 [3-(2-pyridyldithio)-propianamide] hexanoate (Pierce Chem. Co. Cat. #2165-G); and (v) sulfo-NHS (N-hydroxysulfo-succinimide: Pierce Chem. Co., Cat. #24510) conjugated to EDC.

[0314] The linkers described above contain components that have different attributes, thus leading to anti-GPC3-Fc fusion proteins with differing physio-chemical properties. For example, sulfo-NHS esters of alkyl carboxylates are more stable than sulfo-NHS esters of aromatic carboxylates. NHS-ester containing linkers are less soluble than sulfo-NHS esters. Further, the linker SMPT contains a sterically hindered disulfide bond, and can form fusion protein with increased stability. Disulfide linkages, are in general, less stable than other linkages because the disulfide linkage is cleaved *in vitro*, resulting in less fusion protein available. Sulfo-NHS, in particular, can enhance the stability of carbodimide couplings. Carbodimide couplings (such as EDC) when used in conjunction with sulfo-NHS, forms esters that are more resistant to hydrolysis than the carbodimide coupling reaction alone.

Peptide linkers

[0315] Any one or all of the linkers described herein can be peptide linkers. The peptide linker may have a naturally occurring sequence, or a non-naturally occurring sequence. For example, a sequence derived from the hinge region of heavy chain only antibodies may be used as the linker. *See,* for example, WO1996/34103. In some embodiments, the peptide linker comprises the amino acid sequence of CPPCP (SEQ ID NO: 478), a sequence found in the native IgG1 hinge region.

[0316] The peptide linker can be of any suitable length. In some embodiments, the peptide linker is at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids (aa) long. In some embodiments, the peptide linker is no more than about any of 100, 75, 50, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 or fewer amino acids long. In some embodiments, the length of the peptide linker is any of about 1 aa to about 10 aa, about 1 aa to about 20 aa, about 1 aa to about 30 aa, about 5 aa to about 15 aa, about 10 aa

to about 25 aa, about 5 aa to about 30 aa, about 10 aa to about 30 aa, about 30 aa to about 50 aa, about 50 aa to about 100 aa, or about 1 aa to about 100 aa.

[0317] An essential technical feature of such peptide linker is that said peptide linker does not comprise any polymerization activity. The characteristics of a peptide linker, which comprise the absence of the promotion of secondary structures, are known in the art and described, *e.g.*, in Dall'Acqua *et al.* (Biochem. (1998) 37, 9266-9273), Cheadle *et al.* (Mol Immunol (1992) 29, 21-30) and Raag and Whitlow (FASEB (1995) 9(1), 73-80). A particularly preferred amino acid in context of the "peptide linker" is Gly. Furthermore, peptide linkers that also do not promote any secondary structures are preferred. The linkage of the molecules to each other can be provided by, *e.g.*, genetic engineering. Methods for preparing fused and operatively linked antibody constructs and expressing them in mammalian cells or bacteria are well-known in the art (*e.g.* WO 99/54440, Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N. Y. 1989 and 1994 or Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y., 2001).

[0318] In some embodiments, the peptide linker is a stable linker, which is not cleavable by protease, such as by Matrix metalloproteinases (MMPs).

[0319] In some embodiments, the peptide linker tends not to adopt a rigid three-dimensional structure, but rather provide flexibility to a polypeptide (e.g., first and/or second components), such as providing flexibility between the anti-GPC3 antibody moiety and the Fc fragment. In some embodiments, the peptide linker is a flexible linker. Exemplary flexible linkers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$ (SEQ ID NO: 469), $(GSGGS)_n$ (SEQ ID NO: 470), $(GGGGS)_n$ (SEQ ID NO: 471), and $(GGGS)_n$ (SEQ ID NO: 472), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (*see* Scheraga, Rev. Computational Chem. 11 173-142 (1992)). The ordinarily skilled artisan will recognize that design of an anti-GPC3-Fc fusion protein can include linkers that are all or partially flexible, such that the linker can include a flexible linker portion as well as one or more portions that confer less flexible structure to provide a desired fusion protein structure.

[0320] In some embodiments, the anti-GPC3 antibody moiety and the Fc fragment are linked together by a linker of sufficient length to enable the anti-GPC3-Fc fusion protein to fold in such

a way as to permit binding to target GPC3 (*e.g.*, nGPC3 and/or sGPC3), as well as to FcR. In some embodiments, the linker comprises the amino acid sequence of SRGGGGSGGGGSGGGGSLEMA (SEQ ID NO: 473). In some embodiments, the linker is or comprises a $(GGGGS)_n$ sequence (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the linker comprises the amino acid sequence of GEGTSTGSGGSGGSGGAD (SEQ ID NO: 490).

[0321] Natural linkers adopt various conformations in secondary structure, such as helical, β-strand, coil/bend and turns, to exert their functions. Linkers in an α-helix structure might serve as rigid spacers to effectively separate protein domains, thus reducing their unfavorable interactions. Non-helical linkers with Pro-rich sequence could increase the linker rigidity and function in reducing inter-domain interference. In some embodiments, the anti-GPC3 antibody moiety (specifically recognizing *e.g.*, nGPC3 and/or sGPC3) and the Fc fragment (or an antibody comprising an Fc fragment) is linked together by an α-helical linker with an amino acid sequence of $A(EAAAK)_4A$ (SEQ ID NO: 475).

Anti-GPC3-Fc fusion protein sequences

[0322] In some embodiments, the anti-GPC3 construct (such as an isolated anti-GPC3 construct) is an anti-GPC3-Fc fusion protein comprising an anti-GPC3 antibody moiety fused to an Fc fragment (such as IgG1 Fc fragment) optionally via a linker (such as peptide linker). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the anti-GPC3 antibody moiety (targeting *e.g.*, nGPC3 and/or sGPC3) is a Fab, a Fab', an Fc, or an scFv. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-GPC3-Fc fusion protein is a full-length antibody. In some embodiments, the full-length anti-GPC3 antibody is a monoclonal antibody. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-

GPC3 antibody) is monospecific. In some embodiments, the anti-GPC3-Fc fusion protein is monovalent. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is multivalent. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is multispecific. In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of CPPCP (SEQ ID NO: 478).

[0323] Thus, for example, in some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising: a) an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3 (*e.g.*, the binding affinity of the anti-GPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity); and b) an Fc fragment. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope

spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising: a) an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein (*e.g.*, the binding affinity of the anti-GPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity); and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein (*e.g.*, the binding affinity of the anti-GPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity); and b) an Fc fragment. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the anti-nGPC3 antibody moiety and the Fc fragment are connected via a linker (such as peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of CPPCP (SEQ ID NO: 478). In some embodiments, the Fc fragment comprises an IgG1 Fc sequence. In some embodiments, the Fc fragment comprises a human IgG1 Fc sequence. In some embodiments, the Fc fragment comprises a mouse IgG1 Fc sequence. In some embodiments, the anti-GPC3-Fc fusion protein is a full-length antibody. In some embodiments, the full-length anti-GPC3 antibody is a monoclonal antibody. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is monospecific. In some embodiments, the anti-GPC3-Fc fusion protein is monovalent. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is

multivalent. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is multispecific. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M).

[0324] In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising: a) an anti-nGPC3 antibody moiety comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising: a) an anti-nGPC3 antibody moiety comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one

of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286; and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising: a) an anti-nGPC3 antibody moiety comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388; and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising: a) an anti-nGPC3 antibody moiety comprising: i) the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388; and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising a) an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein; and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising a) an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) an Fc fragment. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3 antibody moiety and the Fc fragment are connected via a linker (such as peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of CPPCP (SEQ ID NO: 478). In some embodiments, the Fc fragment comprises an IgG1 Fc sequence. In some embodiments, the Fc fragment comprises a human IgG1 Fc sequence. In some embodiments, the Fc fragment comprises a mouse IgG1 Fc sequence. In some embodiments, the anti-GPC3-Fc fusion protein is a full-length antibody. In some embodiments, the full-length anti-GPC3 antibody is a monoclonal antibody. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is monospecific. In some

embodiments, the anti-GPC3-Fc fusion protein is monovalent. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is multivalent. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is multispecific. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

[0325] In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising an anti-nGPC3 antibody moiety competes for binding to a cell surface-bound GPC3 with a second anti-GPC3-Fc fusion protein (such as a second full-length anti-GPC3 antibody) comprising an anti-nGPC3 antibody moiety according to any of the anti-GPC3-Fc fusion proteins (such as a full-length anti-GPC3 antibody) described herein. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising an anti-nGPC3 antibody moiety binds to the same, or substantially the same, epitope as the second anti-GPC3-Fc fusion protein (such as a second full-length anti-GPC3 antibody) comprising an anti-nGPC3 antibody moiety. In some embodiments, binding of the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising an anti-nGPC3 antibody moiety to the cell surface-bound GPC3 inhibits binding of the second anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising an anti-nGPC3 antibody moiety to the same cell surface-bound GPC3 by at least about 70% (such as by at least about any of 75%, 80%, 85%, 90%, 95%, 98% or 99%), or *vice versa*. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising an anti-nGPC3 antibody moiety and the second anti-GPC3-Fc fusion protein (such as a second full-length anti-GPC3 antibody) comprising an anti-nGPC3 antibody moiety cross-compete for binding to the cell surface-bound GPC3, *i.e.*, each of the anti-GPC3-Fc fusion proteins (such as full-length anti-GPC3 antibodies) competes with the other for binding to the cell surface-bound GPC3.

[0326] In some embodiments, there is provided an anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions,

and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising: a) an anti-GPC3 antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306; and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising: a) an anti-GPC3 antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408; and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising: a) an anti-GPC3 antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408; and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising a) an anti-GPC3 antibody moiety that competes for binding to GPC3 (*e.g.*, nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein; and b) an Fc fragment. In some embodiments, there is provided an anti-GPC3-Fc fusion protein comprising a) an anti-GPC3 antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) an Fc fragment. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3.

In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and target GPC3 (*e.g.*, nGPC3 and/or sGPC3) is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3 antibody moiety and target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-GPC3 antibody moiety and the Fc fragment are connected via a linker (such as peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of CPPCP (SEQ ID NO: 478). In some embodiments, the Fc fragment comprises an IgG1 Fc sequence. In some embodiments, the Fc fragment comprises a human IgG1 Fc sequence. In some embodiments, the Fc fragment comprises a mouse IgG1 Fc sequence. In some embodiments, the anti-GPC3-Fc fusion protein is a full-length antibody. In some embodiments, the full-length anti-GPC3 antibody is a monoclonal antibody. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is monospecific. In some embodiments, the anti-GPC3-Fc fusion protein is monovalent. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is multivalent. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) is multispecific. In some embodiments, the anti-GPC3 antibody moiety is a Fab or a Fab'. In some

embodiments, the anti-GPC3 antibody moiety is an scFv. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

[0327] In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising an anti-GPC3 antibody moiety competes for binding to a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) with a second anti-GPC3-Fc fusion protein (such as a second full-length anti-GPC3 antibody) comprising an anti-GPC3 antibody moiety according to any of the anti-GPC3-Fc fusion proteins (such as a full-length anti-GPC3 antibody) described herein. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising an anti-GPC3 antibody moiety binds to the same, or substantially the same, epitope as the second anti-GPC3-Fc fusion protein (such as a second full-length anti-GPC3 antibody) comprising an anti-GPC3 antibody moiety. In some embodiments, binding of the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising an anti-GPC3 antibody moiety to the target GPC3 (*e.g.*, nGPC3 and/or sGPC3) inhibits binding of the second anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising an anti-GPC3 antibody moiety to the target GPC3 by at least about 70% (such as by at least about any of 75%, 80%, 85%, 90%, 95%, 98% or 99%), or *vice versa*. In some embodiments, the anti-GPC3-Fc fusion protein (such as a full-length anti-GPC3 antibody) comprising an anti-GPC3 antibody moiety and the second anti-GPC3-Fc fusion protein (such as a second full-length anti-GPC3 antibody) comprising an anti-GPC3 antibody moiety cross-compete for binding to the target GPC3 (*e.g.*, nGPC3 and/or sGPC3), *i.e.*, each of the anti-GPC3-Fc fusion proteins (such as full-length anti-GPC3 antibodies) competes with the other for binding to the target GPC3.

[0328] In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3 (*e.g.*, the binding affinity of the anti-GPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity); and b) an Fc fragment. In some embodiments, the Fc fragment comprises an IgG1 Fc sequence (*e.g.*, human or mouse IgG1 Fc sequence). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462.

In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided a full-length anti-GPC3-Fc fusion protein comprising: a) an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein (*e.g.*, the binding affinity of the anti-GPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity); and b) an Fc fragment. In some embodiments, there is provided a full-length anti-GPC3-Fc fusion protein comprising a) an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-GPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same,

or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) an Fc fragment. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the full-length anti-GPC3 antibody is non-naturally occurring. In some embodiments, the full-length anti-GPC3 antibody is monoclonal. In some embodiments, the full-length anti-GPC3 antibody is monospecific. In some embodiments, the full-length anti-GPC3 antibody is multispecific (*e.g.*, bispecific). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the full-length anti-GPC3 antibody comprises a mouse IgG1 heavy cahin constant domain (C_H) comprising the amino acid sequence of SEQ ID NO: 507. In some embodiments, the full-length anti-GPC3 antibody comprises a mouse λ light cahin constant domain (C_L) comprising the amino acid sequence of SEQ ID NO: 508. For example, in some embodiments, the full-length anti-GPC3 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 505, and a light chain comprising the amino acid sequence of SEQ ID NO: 506. In some embodiments, the heavy and/or light chain of the full-length anti-GPC3 antibody optionally comprises an N-terminal signal peptide. In some embodiments, the signal peptide comprises the amino acid seqeuence of SEQ ID NO: 509.

[0329] In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid

substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and b) an Fc fragment. In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286; and b) an Fc fragment. In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388. In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388. In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3 competitively with any of the anti-GPC3 constructs described herein; and an Fc fragment. In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially

the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and an Fc fragment. In some embodiments, the Fc fragment comprises an IgG1 Fc sequence (*e.g.*, human or mouse IgG1 Fc sequence). In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the full-length anti-GPC3 antibody is non-naturally occurring. In some embodiments, the full-length anti-GPC3 antibody is monoclonal. In some embodiments, the full-length anti-GPC3 antibody is monospecific. In some embodiments, the full-length anti-GPC3 antibody is multispecific (*e.g.*, bispecific). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the full-length anti-GPC3 antibody comprises a mouse IgG1 heavy cahin constant domain (C_H) comprising the amino acid sequence of SEQ ID NO: 507. In some embodiments, the full-length anti-GPC3 antibody comprises a mouse λ light cahin constant domain (C_L) comprising the amino acid sequence of SEQ ID NO: 508. For example, in some embodiments, the full-length anti-GPC3 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 505, and a light chain comprising the amino acid sequence of SEQ ID NO: 506. In some embodiments, the heavy and/or light chain of the full-length anti-GPC3 antibody optionally comprises an N-terminal signal peptide. In some embodiments, the signal peptide comprises the amino acid seqeuence of SEQ ID NO: 509.

[0330] In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a

variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and b) an Fc fragment. In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306; and b) an Fc fragment. In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408. In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408. In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an isolated full-length anti-GPC3 antibody) comprising: a) an anti-GPC3 antibody moiety that specifically binds to a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) competitively with any of the anti-GPC3 constructs described herein; and an Fc fragment. In some embodiments, there is provided a full-length anti-GPC3 antibody (such as an

isolated full-length anti-GPC3 antibody) comprising an anti-GPC3 antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and an Fc fragment. In some embodiments, the Fc fragment comprises an IgG1 Fc sequence (e.g., human or mouse IgG1 Fc sequence). In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the full-length anti-GPC3 antibody is monoclonal. In some embodiments, the full-length anti-GPC3 antibody is monospecific. In some embodiments, the full-length anti-GPC3 antibody is multispecific (*e.g.*, bispecific). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3 antibody moiety and the target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the full-length anti-GPC3 antibody comprises a mouse IgG1

heavy cahin constant domain (C_H) comprising the amino acid sequence of SEQ ID NO: 507. In some embodiments, the full-length anti-GPC3 antibody comprises a mouse λ light cahin constant domain (C_L) comprising the amino acid sequence of SEQ ID NO: 508. In some embodiments, the heavy and/or light chain of the full-length anti-GPC3 antibody optionally comprises an N-terminal signal peptide. In some embodiments, the signal peptide comprises the amino acid seqeuence of SEQ ID NO: 509.

Multi-specific anti-GPC3 molecules

[0331] The anti-GPC3 constructs (such as isolated anti-GPC3 constructs) in some embodiments comprise a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising an anti-GPC3 antibody moiety according to any one of the anti-GPC3 antibody moieties described herein, and a second binding moiety (such as a second antibody moiety) specifically recognizing a second antigen. In some embodiments, the multi-specific anti-GPC3 molecule comprises an anti-GPC3 antibody moiety and a second antibody moiety specifically recognizing a second antigen. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3.

[0332] Multi-specific molecules are molecules that have binding specificities for at least two different antigens or epitopes *(e.g.,* bispecific antibodies have binding specificities for two antigens or epitopes). Multi-specific molecules with more than two valencies and/or specificities are also contemplated. For example, trispecific antibodies can be prepared (Tutt *et al. J. Immunol.* 147: 60 (1991)). It is to be appreciated that one of skill in the art could select appropriate features of individual multi-specific molecules described herein to combine with one another to form a multi-specific anti-GPC3 molecule of the invention.

[0333] Thus, for example, in some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing a target GPC3 (*e.g.*, nGPC3 and/or sGPC3), and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen.

In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the second binding moiety specifically recognizes the same format of GPC3 (*e.g.*, nGPC3 and/or sGPC3) as the anti-GPC3 antibody moiety. In some embodiments, the second binding moiety specifically recognizes a different GPC3 epitope compared to the anti-GPC3 antibody moiety. In some embodiments, the second binding moiety specifically recognizes a different format of GPC3 compared to the anti-GPC3 antibody moiety. For example, in some embodiments, the second binding moiety specifically recognizes an sGPC3 (or both sGPC3 and nGPC3), while the anti-GPC3 antibody moiety specifically recognizes nGPC3 (with no or little binding to sGPC3). In some embodiments, the second binding moiety specifically recognizes a second antigen that is not GPC3. In some embodiments, the second binding moiety specifically recognizes a second antigen on the surface of a cell, such as a cytotoxic cell. In some embodiments, the second binding moiety specifically binds to an antigen on the surface of a lymphocyte, such as a T cell, a B cell, a natural killer (NK) cell, a neutrophil, a monocyte, a macrophage, or a dendritic cell. In some embodiments, the second binding moiety specifically binds to an effector T cell, such as a cytotoxic T cell (also known as cytotoxic T lymphocyte (CTL) or T killer cell) or natural killer T (NKT) cell. In some embodiments, the second binding moiety specifically binds to a second antigen on the surface of an effector cell, including for example CD3γ, CD3δ, CD3ε, CD3ζ, CD27, CD28, CD16a, CD40L, CD56, CD68, CD137, OX40, GITR, HVEM and GDS2D. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-GPC3 antibody moiety is a full-length antibody, a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the second binding moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the multi-specific anti-GPC3 molecule further comprises at least one (such as at least about any of 2, 3, 4, 5, or more) additional antibody moieties.

[0334] In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing a cell surface-bound GPC3 (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to the cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically

recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising: a) an anti-nGPC3 antibody moiety (*e.g.*, scFv; *e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to the cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising: a) an anti-nGPC3 antibody moiety (*e.g.*, scFv; *e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to the cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the first and/or the second antibody moieties are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the first and/or the second antibody moieties are full-length antibody, a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the first and/or the second antibody moiety is scFv. In some embodiments, the first and the second antibody moieties are connected by a linker (*e.g.*, SEQ ID NO: 474). In some embodiments, the first anti-GPC3 antibody moiety is N-terminal to the second antibody

moiety. In some embodiments, the effector cell is T cell (*e.g.*, cytotoxic T cell, helper T cell, or natural killer T cell), B cell, NK cell, dendritic cell, macrophage, monocyte, or a neutrophil. In some embodiments, the second antigen is CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, or HVEM.

[0335] In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing a

cell surface-bound GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing a cell surface-bound GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing a cell surface-bound GPC3, wherein the anti-nGPC3 antibody moiety competes for binding to the cell surface-bound GPC3 with a second anti-nGPC3 antibody moiety according to any of the anti-nGPC3 antibody moieties described herein; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising: a) an anti-nGPC3 antibody moiety (*e.g.*, scFv) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the first and/or the second antibody moieties are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the first and/or the second antibody moieties are full-length antibody, a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the first and/or the second antibody moiety is scFv. In some embodiments, the first and the second antibody moieties are connected

by a linker (*e.g.*, SEQ ID NO: 474). In some embodiments, the first anti-GPC3 antibody moiety is N-terminal to the second antibody moiety. In some embodiments, the effector cell is T cell (*e.g.*, cytotoxic T cell, helper T cell, or natural killer T cell), B cell, NK cell, dendritic cell, macrophage, monocyte, or a neutrophil. In some embodiments, the second antigen is CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, or HVEM.

[0336] In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3

molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing GPC3, wherein the anti-GPC3 antibody moiety competes for binding to the target GPC3 (*e.g.*, nGPC3 and/or sGPC3) with a second anti-GPC3 antibody moiety according to any of the anti-GPC3 antibody moieties described herein; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising: a) an anti-GPC3 antibody moiety (*e.g.*, scFv) that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) a second binding moiety (such as a second antibody moiety, *e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cell). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In

some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and target GPC3 (*e.g.*, gGPC3 and/or sGPC3) is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3 antibody moiety and the target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the first and/or the second antibody moieties are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the first and/or the second antibody moieties are full-length antibody, a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the first and/or the second antibody moiety is scFv. In some embodiments, the first and the second antibody moieties are connected by a linker (*e.g.*, SEQ ID NO: 474). In some embodiments, the first anti-GPC3 antibody moiety is N-terminal to the second antibody moiety. In some embodiments, the effector cell is T cell (*e.g.*, cytotoxic T cell, helper T cell, or natural killer T cell), B cell, NK cell, dendritic cell, macrophage, monocyte, or a neutrophil. In some embodiments, the second antigen is CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, or HVEM.

[0337] In some embodiments, the multi-specific anti-GPC3 molecule is, for example, a diabody (Db), a single-chain diabody (scDb), a tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), a circular dimeric scDb (CD-scDb), a di-diabody, a tandem scFv, a tandem di-scFv (*e.g.*, a bispecific T cell engager), a tandem tri-scFv, a tri(a)body, a bispecific Fab2, a di-miniantibody, a tetrabody, an scFv-Fc-scFv fusion, a dual-affinity retargeting (DART) antibody, a dual variable domain (DVD) antibody, an IgG-scFab, an scFab-ds-scFv, an Fv2-Fc, an IgG-scFv fusion, a dock and lock (DNL) antibody, a knob-into-hole (KiH) antibody (bispecific IgG prepared by the KiH technology), a DuoBody (bispecific IgG prepared by the Duobody technology), a heteromultimeric antibody, or a heteroconjugate antibody. In some embodiments, the multi-specific anti-GPC3 molecule is a tandem scFv (*e.g.*, a tandem di-scFv, such as a bispecific T cell engager).

[0338] ***Second antigen***

[0339] In some embodiments, the anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprises a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising an anti-GPC3 antibody moiety (such as an anti-nGPC3 antibody moiety) and a second antibody moiety

(e.g. scFv) specifically recognizing a second antigen. In some embodiments, the second antigen is also GPC3 but comprises a different epitope compared to that recognized by the anti-GPC3 antibody moiety. In some embodiments, the second antigen is not GPC3. In some embodiments, the second antigen is a tumor antigen. In some embodiments, the second antigen is a cell surface molecule. In some embodiments, the second antigen is a cell surface molecule on an effector cell.

[0340] Exemplary tumor antigens that can be recognized by the second antibody moiety described herein include, but are not limited to, alpha fetoprotein (AFP), CA15-3, CA27-29, CA19-9, CA-125, calretinin, carcinoembryonic antigen, CD34, CD99, CD117, chromogranin, cytokeratin, desmin, epithelial membrane protein (EMA), Factor VIII, CD31 FL1, glial fibrillary acidic protein (GFAP), gross cystic disease fluid protein (GCDFP-15), HMB-45, human chorionic gonadotropin (hCG), inhibin, keratin, CD45, a lymphocyte marker, MART-1 (Melan-A), Myo Dl, muscle-specific actin (MSA), neurofilament, neuron-specific enolase (NSE), placental alkaline phosphatase (PLAP), prostate-specific antigen, S100 protein, smooth muscle actin (SMA), synaptophysin, thyroglobulin, thyroid transcription factor- 1, tumor M2-PK, and vimentin.

[0341] In some embodiments, the multi-specific (*e.g.*, bispecific) anti-GPC3 molecules described herein can be engineered to facilitate killing (*e.g.*, cytotoxic lysis or phagocytosis) of tumor cells by directing (or recruiting) an effector cell (such as a cytotoxic T cell) to a tumor site. In some embodiments, tumor cytotoxicitiy can be tested using an LDH Cytotoxicity Assay. In some embodiments, the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule can effectively direct an effector cell (*e.g.*, T cell, NK cell, CAR-T cell, caTCR-T cell) to a target cell in an immunosuppressive environment, such as an immunosuppressive tumor environment.

[0342] Exemplary effector cells include without limitation a T cell, a B cell, a natural killer (NK) cell, a dendritic cell (DC), a macrophage, a monocyte, a neutrophil, a natural killer T (NKT) cell, an antibody-dependent cytotoxic cell, a chimeric antigen receptor (CAR) effector cell (e.g., CAR-T), a chimeric antibody-T cell receptor (TCR) construct (caTCR) effector cell (*see* caTCR section below), or the like. In some embodiments, the effector cell is a T cell (e.g., a cytotoxic T cell, a helper T cell, or an NKT cell). In some embodiments, the effector cell is a cytotoxic T cell. In some embodiments, the effector cell is allogenic. In some embodiments, the effector cell is autologous.

[0343] A cell surface molecule of the present invention is a molecule found on the external cell wall or plasma membrane of a specific cell type or a limited number of cell types. Examples of cell surface molecules include, but are not limited to, membrane proteins such as

receptors, transporters, ion channels, proton pumps, and G protein-coupled receptors; extracellular matrix molecules such as adhesion molecules (*e.g.*, integrins, cadherins, selectins, or NCAMS); *see*, *e.g.*, U.S. Pat. No. 7,556,928, which is incorporated herein by reference in its entirety. Cell surface molecules on an effector cell include but not limited to CD3 (*e.g.*, CD3γ, CD3δ, CD3ε, or CD3ζ), CD4, CD5, CD7, CD8, CD13, CD14, CD16, CD27 (TNFRSF7), CD28, CD31, CD38, CD40L (TNFSF5 or CD154), CD56, CD64, CD68, CD89, CD94, CD137 (TNFRSF9 or 4-1BB), CD278, NKp46, NKp30, NKG2D, MAC-l/MAC-3, IL-2Ra, OX40 (TNFRSF4 or CD134), GITR, HVEM (TNFRSF14 or CD270), Ly49, or an invariant TCR.

[0344] The skilled artisan will recognize that immune cells have different cell surface molecules. For example, CD3 is a cell surface molecule on T cells, whereas CD16, NKG2D, or NKp30 are cell surface molecules on NK cells, and CD3 or an invariant TCR are the cell surface molecules on NKT-cells. In some embodiments, *e.g.*, wherein the effector cell is a T cell, the activation molecule is one or more of CD3 (*e.g.*, CD3γ, CD3δ or CD3ε), CD27, CD28, CD40, CD134, CD137, and CD278. In some embodiments, *e.g.*, wherein the effector cell is a NK cell, the cell surface molecule is CD16, NKG2D, or NKp30; or wherein the effector cell is a NKT-cell, the cell surface molecule is CD3 or an invariant TCR.

[0345] In some embodiments, the second antibody moiety binds specifically to CD3. CD3 is an antigen expressed by T cells and comprises three different polypeptide chains (ε, δ and γ chains). The three CD3 polypeptide chains associate with the T cell receptor (TCR) and the ζ-chain to form the TCR complex, which has the function of activating signaling cascades in T cells. Currently, many therapeutic strategies target the TCR signal transduction to treat diseases using anti-human CD3 monoclonal antibodies. The skilled artisan will recognize that the TCR complex is an octomeric complex of variable TCR α and β chains with three dimeric signaling modules CD3δ/ε, CD3γ/ε and CD3ζ/ζ or ζ/η. Although in some embodiments the second antibody moiety (such as an scFv) described herein binds to CD3ε, targeting other CD3 molecules, especially CD3ζ, or the TCR α and β chains, is also encompassed in the disclosure.

[0346] In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (e.g. scFv) specifically recognizing a target GPC3 (*e.g.*, nGPC3 and/or sGPC3), and b) a second antibody moiety (e.g. scFv) that binds specifically to CD3. In some embodiments, the second antibody moiety specifically binds to CD3ε. In some embodiments, the second antibody moiety specifically binds to an agonistic epitope of CD3ε. The term "agonistic epitope," as used herein, means (a) an epitope that, upon binding of the multi-specific molecule, optionally upon binding of several multi-specific

molecules on the same cell, allows said multi-specific molecules to activate TCR signaling and induce T cell activation, and/or (b) an epitope that is solely composed of amino acid residues of the epsilon chain of CD3 and is accessible for binding by the multi-specific molecule, when presented in its natural context on T cells (*i.e.* surrounded by the TCR, the CD3γ chain, etc.), and/or (c) an epitope that, upon binding of the multi-specific molecule, does not lead to stabilization of the spatial position of CD3ε relative to CD3γ.

[0347] In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (e.g. scFv) specifically recognizing a target GPC3 (*e.g.*, nGPC3 and/or sGPC3), and b) a second antibody moiety (e.g. scFv) that binds specifically to an antigen on the surface of an effector cell, including for example CD3γ, CD3δ, CD3ε, CD3ζ, CD28, CD16a, CD56, CD68, and GDS2D.

[0348] In some embodiments, there is provided a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprising a) an anti-GPC3 antibody moiety (e.g. scFv) specifically recognizing a target GPC3 (*e.g.*, nGPC3 and/or sGPC3), and b) a second antibody moiety (e.g. scFv) that binds specifically to a component of the complement system, such as C1q. C1q is a subunit of the C1 enzyme complex that activates the serum complement system.

[0349] In some embodiments, the second antibody moiety specifically binds to an Fc receptor. In some embodiments, the second antibody moiety specifically binds to an Fcγ receptor (FcγR). The FcγR may be an FcγRIII present on the surface of NK cells or one of FcγRI, FcγRIIA, FcγRIIBI, FcγRIIB2, and FcγRIIIB present on the surface of macrophages, monocytes, neutrophils and/or dendritic cells. In some embodiments, the second antibody moiety is an Fc region or functional fragment thereof. A "functional fragment" as used in this context refers to a fragment of an antibody Fc region that is still capable of binding to an FcR, in particular to an FcγR, with sufficient specificity and affinity to allow an FcγR bearing effector cell, in particular a macrophage, a monocyte, a neutrophil and/or a dendritic cell, to kill the target cell by cytotoxic lysis or phagocytosis. A functional Fc fragment is capable of competitively inhibiting the binding of the original, full-length Fc portion to an FcR such as the activating FcγRI. In some embodiments, a functional Fc fragment retains at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of its affinity to an activating FcγR. In some embodiments, the Fc region or functional fragment thereof is an enhanced Fc region or functional fragment thereof. The term "enhanced Fc region", as used herein, refers to an Fc region that is modified to enhance Fc receptor-mediated effector-functions, in particular antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-mediated phagocytosis. This

can be achieved as known in the art, for example by altering the Fc region in a way that leads to an increased affinity for an activating receptor (*e.g.* FcγRIIIA (CD16A) expressed on NK cells) and/or a decreased binding to an inhibitory receptor (*e.g.* FcγRIIB1/B2 (CD32B)). In yet other embodiments, the second antibody moiety is an antibody or antigen-binding fragment thereof that specifically binds to an FcR, in particular to an FcγR, with sufficient specificity and affinity to allow an FcγR bearing effector cell, in particular a macrophage, a monocyte, a neutrophil and/or a dendritic cell, to kill the target cell by cytotoxic lysis or phagocytosis.

[0350] In some embodiments, the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule allows killing of GPC3+ cells (such as HCC cells) and/or can effectively redirect CTLs to lyse cells expressing GPC3 on the target cell surface. In some embodiments, the multi-specific anti-GPC3 molecule of the present invention shows an *in vitro* EC_{50} ranging from 10 to 500 ng/ml, and is able to induce redirected lysis of about 50% of the target cells through CTLs at a ratio of CTLs to target cells of from about 1:1 to about 50:1 (such as from about 1:1 to about 15:1, or from about 2:1 to about 10:1).

[0351] In some embodiments, the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule is capable of cross-linking a stimulated or unstimulated CTL and the target cell (such as $GPC3^+$ cells, e.g. HCC cell) in such a way that the target cell is lysed. This offers the advantage that no generation of target-specific T cell clones or common antigen presentation by dendritic cells is required for the multi-specific anti-GPC3 molecule to exert its desired activity. In some embodiments, the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule of the present invention is capable of redirecting CTLs to lyse the target cells (such as $GPC3^+$ cells, e.g. HCC cell) in the absence of other activating signals. In some embodiments, the second antibody moiety of the multi-specific anti-GPC3 molecule specifically binds to CD3 (*e.g.*, specifically binds to CD3ε), and signaling through CD28 and/or IL-2 is not required for redirecting CTLs to lyse the target cells.

[0352] Methods for measuring the preference of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule to simultaneously bind to two antigens (*e.g.*, antigens on two different cells) are within the normal capabilities of a person skilled in the art. For example, when the second binding moiety (*e.g.*, second antibody moiety) specifically binds to CD3, the multi-specific anti-GPC3 molecule may be contacted with a mixture of $CD3^+/GPC3^-$ cells and $CD3^-/GPC3^+$ cells. The number of multi-specific anti-GPC3 molecule-positive single cells and the number of cells cross-linked by multi-specific anti-GPC3 molecules may then be assessed by microscopy or fluorescence-activated cell sorting (FACS) as known in the art.

[0353] In some embodiments, the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule comprises a) an anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing a target GPC3 (*e.g.*, nGPC3 and/or sGPC3), and b) a second antibody moiety (*e.g.*, scFv) that binds specifically to CD3ε on a T cell (herein after referred to as "anti-CD3 antibody moiety"). In some embodiments, anti-CD3 antibody moiety (*e.g.*, scFv) comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 479, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 480, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 481; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 482, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 483, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 484. In some embodiments, anti-CD3 antibody moiety (*e.g.*, scFv) comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 485; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 486. In some embodiments, anti-CD3 antibody moiety (*e.g.*, scFv) comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 485; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 486. In some embodiments, the anti-CD3 antibody moiety is an scFv. In some embodiments, the anti-CD3-scFv comprises the amino acid sequence of SEQ ID NO: 487.

Linkers

[0354] In some embodiments, the anti-GPC3 antibody moiety (*e.g.*, scFv) specifically recognizing a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) and the second antibody moiety (*e.g.*, scFv) specifically recognizing a second antigen (*e.g.*, CD3 on T cells) are connected by a linker (such as a peptide linker). *See* "Linkers" subsection under the "Anti-GPC3 Fc fusion protein" section for all applicable linkers that can be used in the multi-specific anti-GPC3 molecules described herein. In some embodiments, the linker comprises the amino acid sequence of SRGGGGSGGGGSGGGGSLEMA (SEQ ID NO: 473). In some embodiments, the linker is or comprises a $(GGGGS)_n$ sequence (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the linker comprises the amino acid sequence of GEGTSTGSGGSGGSGGAD (SEQ ID NO: 490).

Tandem scFv

[0355] The multi-specific anti-GPC3 molecule in some embodiments is a tandem scFv comprising a first scFv comprising an anti-GPC3 antibody moiety specifically recognizing GPC3 (referred to herein as "anti-GPC3 scFv"; recognizing nGPC3 and/or sGPC3) and a second

scFv specifically recognizing a second antigen (also referred to herein as a "tandem scFv multi-specific anti-GPC3 antibody"). In some embodiments, the tandem scFv multi-specific anti-GPC3 antibody further comprises at least one (such as at least about any of 2, 3, 4, 5, or more) additional scFv.

[0356] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3, and b) a second scFv specifically recognizing a second antigen (*e.g.*, CD3 on T cell), wherein the tandem scFv multi-specific anti-GPC3 antibody is a tandem di-scFv or a tandem tri-scFv. In some embodiments, the tandem scFv multi-specific anti-GPC3 antibody is a tandem di-scFv. In some embodiments, the tandem scFv multi-specific anti-GPC3 antibody is a bispecific T-cell engager. In some embodiments, the first anti-GPC3 scFv specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the first anti-nGPC3 scFv to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the first anti-nGPC3 scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the first anti-GPC3 scFv specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the first anti-sGPC3 scFv to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the first anti-GPC3 scFv specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the second scFv specifically binds to a different GPC3 epitope. In some embodiments, the second scFv specifically recognizes a second antigen that is not GPC3. In some embodiments, the second scFv specifically recognizes a second antigen on the surface of a cell, such as a cytotoxic cell. In some embodiments, the second scFv specifically binds to an antigen on the surface of a lymphocyte, such as a T cell (*e.g.*, CTL, helper T cell, or NKT), a B cell, a natural killer (NK) cell, a neutrophil, a monocyte, a macrophage, or a dendritic cell. In some embodiments, the second scFv specifically binds to an effector T cell, such as a CTL or NKT cell. In some embodiments, the second scFv specifically binds to a second antigen on the surface of an effector cell, including for example CD3γ, CD3δ, CD3ε, CD3ζ, CD27, CD28, CD16a, CD40L, CD56, CD68, CD137, OX40, GITR, HVEM and GDS2D. In some embodiments, the first anti-GPC3 scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the second scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, both the first and second scFvs are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the tandem scFv multi-specific anti-GPC3

antibody further comprises at least one (such as at least about any of 2, 3, 4, 5, or more) additional scFv. In some embodiments, the first anti-GPC3 scFv and the second scFv are connected by a linker (*e.g.*, peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the first anti-GPC3 scFv is N-terminal to the second scFv. In some embodiments, the first anti-GPC3 scFv is C-terminal to the second scFv. In some embodiments, the tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody further comprises a tag (*e.g.*, a peptide tag for purification purpose). In some embodiments, the tag is N-terminal to the tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody. In some embodiments, the tag is C-terminal to the tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody. In some embodiments, the tag comprises the amino acid sequence of HHHHHH (SEQ ID NO: 476).

[0357] In some embodiments, the tandem scFv multi-specific anti-GPC3 antibody is a tandem di-scFv comprising two scFvs (referred to herein as "tandem di-scFv bispecific anti-GPC3 antibody"). The tandem di-scFv bispecific anti-GPC3 antibody can have V_H and V_L assembled in any configurations, such as the configurations listed below (from N-terminus to C-terminus), wherein X is the second antigen specifically bound by the second scFv, L1, L2, and L3 are optional linkers (such as peptide linkers). *See* "Linkers" subsection under the "Anti-GPC3 Fc fusion protein" section for all applicable linkers. In some embodiments, the linker (L1, L2, or L3) comprises the amino acid sequence of SRGGGGSGGGGSGGGGSLEMA (SEQ ID NO: 473). In some embodiments, the linker (L1, L2, or L3) is or comprises a $(GGGGS)_n$ sequence (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker (L1, L2, or L3) comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the linker (L1, L2, or L3) comprises the amino acid sequence of GEGTSTGSGGSGGSGGAD (SEQ ID NO: 490).

[0358] In some embodiments, the multi-specific anti-GPC3 antibody is a tandem di-scFv having one of the following structures:

[0359] V_L(GPC3)-L1-V_H(GPC3)-L2-V_L(X)-L3-V_H(X);

[0360] V_L(GPC3)-L1-V_H(GPC3)-L2-V_H(X)-L3-V_L(X);

[0361] V_H(GPC3)-L1-V_L(GPC3)-L2-V_L(X)-L3-V_H(X);

[0362] V_H(GPC3)-L1-V_L(GPC3)-L2-V_H(X)-L3-V_L(X);

[0363] V_L(X)-L1-V_H(X)-L2-V_L(GPC3)-L3-V_H(GPC3);

[0364] V_L(X)-L1-V_H(X)-L2-V_H(GPC3)-L3-V_L(GPC3);

[0365] V_H(X)-L1-V_L(X)-L2-V_L(GPC3)-L3-V_H(GPC3);

[0366] V_H(X)-L1-V_L(X)-L2-V_H(GPC3)-L3-V_L(GPC3);

[0367] V_L(GPC3)-L1-V_H(X)-L2-V_L(X)-L3-V_H(GPC3);

[0368] V_L(GPC3)-L1-V_L(X)-L2-V_H(X)-L3-V_H(GPC3);

[0369] V_H(GPC3)-L1-V_H(X)-L2-V_L(X)-L3-V_L(GPC3);

[0370] V_H(GPC3)-L1-V_L(X)-L2-V_H(X)-L3-V_L(GPC3);

[0371] V_L(X)-L1-V_H(GPC3)-L2-V_L(GPC3)-L3-V_H(X);

[0372] V_L(X)-L1-V_L(GPC3)-L2-V_H(GPC3)-L3-V_H(X);

[0373] V_H(X)-L1-V_H(GPC3)-L2-V_L(GPC3)-L3-V_L(X); or

[0374] V_H(X)-L1-V_L(GPC3)-L2-V_H(GPC3)-L3-V_L(X).

[0375] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first scFv specifically recognizing a cell surface-bound GPC3 (*e.g.*, the binding affinity of the first anti-nGPC3 scFv to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the first anti-nGPC3 scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some

embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the first anti-nGPC3 scFv can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first anti-nGPC3 scFv (*e.g.*, the binding affinity of the first anti-nGPC3 scFv to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the first anti-nGPC3 scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first anti-nGPC3 scFv (*e.g.*, the binding affinity of the first anti-nGPC3 scFv to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the first anti-nGPC3 scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the first anti-nGPC3 scFv and the second scFv are connected by a linker (*e.g.*, peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the first anti-nGPC3 scFv is N-terminal to the second scFv. In some

embodiments, the first anti-nGPC3 scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the second scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, both the first and second scFvs are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3-scFv and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the second scFv specifically binds to an antigen on the surface of a lymphocyte, such as a T cell (*e.g.*, CTL, helper T cell, or NKT), a B cell, a natural killer (NK) cell, a neutrophil, a monocyte, a macrophage, or a dendritic cell. In some embodiments, the second scFv specifically binds to an effector T cell, such as a CTL or NKT cell. In some embodiments, the second scFv specifically binds to a second antigen on the surface of an effector cell, including for example CD3γ, CD3δ, CD3ε, CD3ζ, CD27, CD28, CD16a, CD40L, CD56, CD68, CD137, OX40, GITR, HVEM and GDS2D.

[0376] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first scFv specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid

substitutions, and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first scFv specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first scFv specifically recognizing a cell surface-bound GPC3, comprising: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first scFv specifically recognizing a cell surface-bound GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388; and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first anti-nGPC3 scFv that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first anti-nGPC3 scFv that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M).

In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3-scFv and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the first anti-nGPC3 scFv and the second scFv are connected by a linker (*e.g.*, peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the first anti-nGPC3 scFv is N-terminal to the second scFv. In some embodiments, the first anti-nGPC3 scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the second scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, both the first and second scFvs are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the second scFv specifically binds to an antigen on the surface of a lymphocyte, such as a T cell (*e.g.*, CTL, helper T cell, or NKT), a B cell, a natural killer (NK) cell, a neutrophil, a monocyte, a macrophage, or a dendritic cell. In some embodiments, the second scFv specifically binds to an effector T cell, such as a CTL or NKT cell. In some embodiments, the second scFv specifically binds to a second antigen on the surface of an effector cell, including for example CD3γ, CD3δ, CD3ε, CD3ζ, CD27, CD28, CD16a, CD40L, CD56, CD68, CD137, OX40, GITR, HVEM and GDS2D.

[0377] In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs:

236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3, comprising: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408; and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first anti-GPC3 scFv that competes for binding to GPC3 with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments, there is provided a tandem scFv multi-specific (*e.g.*, bispecific) anti-GPC3 antibody comprising a) a first anti-GPC3 scFv that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing a second antigen (such as a second antigen on an effector cell, *e.g.*, CD3 on T cell). In some embodiments,

the first anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the first anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the first anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the first anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the first anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the first anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the first anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the first anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the K_d of the binding between the first anti-GPC3-scFv and a target GPC3 (e.g., nGPC3 and/or sGPC3) is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the first anti-GPC3-scFv and a non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3-scFv and the target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3-scFv and a non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the first anti-GPC3 scFv and the second scFv are connected by a linker (*e.g.*, peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the first anti-GPC3 scFv is N-terminal to the second scFv. In some embodiments, the first anti-GPC3 scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the second scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, both the first and second scFvs are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the second scFv specifically binds to an antigen on the surface of a lymphocyte, such as a T cell (*e.g.*, CTL, helper T cell, or NKT), a B cell, a natural killer (NK) cell, a neutrophil, a monocyte, a macrophage, or a dendritic cell. In some embodiments, the second scFv specifically binds to an effector T cell, such as a CTL or NKT cell. In some

embodiments, the second scFv specifically binds to a second antigen on the surface of an effector cell, including for example CD3γ, CD3δ, CD3ε, CD3ζ, CD27, CD28, CD16a, CD40L, CD56, CD68, CD137, OX40, GITR, HVEM and GDS2D.

[0378] In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3, and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, the tandem di-scFv bispecific anti-GPC3 antibody is a bispecific T-cell engager. In some embodiments, the first anti-GPC3 scFv specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the first anti-nGPC3 scFv to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the first anti-nGPC3 scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the first anti-GPC3 scFv specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the first anti-sGPC3 scFv to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the first anti-GPC3 scFv specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the first anti-GPC3 scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the second scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, both the first and second scFvs are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the first anti-GPC3 scFv and the second scFv are connected by a linker (*e.g.*, peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the first anti-GPC3 scFv is N-terminal to the second anti-CD3-scFv. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 479, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 480, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 481; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 482, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 483, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 484. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 485; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 486. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 485; and ii) a V_L

comprising the amino acid sequence of SEQ ID NO: 486. In some embodiments, the anti-CD3-scFv comprises the amino acid sequence of SEQ ID NO: 487.

[0379] In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing a cell surface-bound GPC3 (*e.g.*, the binding affinity of the first anti-nGPC3 scFv to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the first anti-nGPC3 scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the first anti-nGPC3 scFv can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460). In some embodiments, the first anti-nGPC3 scFv specifically recognizes an epitope within the

C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising: a) a first anti-nGPC3-scFv (*e.g.*, the binding affinity of the first anti-nGPC3 scFv to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the first anti-nGPC3 scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising: a) a first anti-nGPC3-scFv (*e.g.*, the binding affinity of the first anti-nGPC3 scFv to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the first anti-nGPC3 scFv specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the first anti-nGPC3 scFv and the second scFv are connected by a linker (*e.g.*, peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the first anti-nGPC3 scFv is N-terminal to the second scFv. In some embodiments, the first anti-nGPC3 scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the second scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, both the first and second scFvs are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3-scFv and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In

some embodiments, anti-CD3-scFv comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 479, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 480, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 481; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 482, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 483, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 484. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 485; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 486. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 485; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 486. In some embodiments, the anti-CD3-scFv comprises the amino acid sequence of SEQ ID NO: 487. In some embodiments, the tandem di-scFv bispecific anti-GPC3 antibody further comprises a tag (*e.g.*, a peptide tag for purification purpose). In some embodiments, the tag is N-terminal to the tandem di-scFv bispecific anti-GPC3 antibody. In some embodiments, the tag is C-terminal to the tandem di-scFv bispecific anti-GPC3 antibody. In some embodiments, the tag comprises the amino acid sequence of HHHHHH (SEQ ID NO: 476).

[0380] In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv

specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing a cell surface-bound GPC3, comprising: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing a cell surface-bound GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388; and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising: a) a first anti-nGPC3-scFv that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising: a) a first anti-nGPC3-scFv that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3-scFv and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3-scFv and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the first anti-nGPC3 scFv and the second scFv are connected by

a linker (*e.g.*, peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the first anti-nGPC3 scFv is N-terminal to the second scFv. In some embodiments, the first anti-nGPC3 scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the second scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, both the first and second scFvs are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-nGPC3 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 409-439. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 479, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 480, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 481; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 482, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 483, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 484. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 485; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 486. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 485; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 486. In some embodiments, the anti-CD3-scFv comprises the amino acid sequence of SEQ ID NO: 487. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing a cell surface-bound GPC3; and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell, wherein the tandem di-scFv bispecific anti-GPC3 antibody comprises the amino acid sequence of any one of SEQ ID NOs: 488 and 511-515. In some embodiments, the tandem di-scFv bispecific anti-GPC3 antibody further comprises a tag (*e.g.*, a peptide tag for purification purpose). In some embodiments, the tag is N-terminal to the tandem di-scFv bispecific anti-GPC3 antibody. In some embodiments, the tag is C-terminal to the tandem di-scFv bispecific anti-GPC3 antibody. In some embodiments, the tag comprises the amino acid sequence of HHHHHH (SEQ ID NO: 476).

[0381] In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3,

4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, wherein the amino acid substitutions are in HC-CDR1 or HC-CDR2; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408; and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising: a) a first anti-GPC3-scFv

that competes for binding to target GPC3 (*e.g.*, nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising: a) a first anti-GPC3-scFv that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein; and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell. In some embodiments, the first anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the first anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the first anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the first anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the first anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the first anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the K_d of the binding between the anti-GPC3-scFv and target GPC3 (*e.g.*, nGPC3 and/or sGPC3) is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-GPC3-scFv and an non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3-scFv and target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the first anti-GPC3 scFv and the second ani-CD3-scFv are connected by a linker (*e.g.*, peptide linker). In some embodiments, the linker comprises the amino acid sequence of $(GGGGS)_n$ (SEQ ID NO: 471), wherein n is equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, the linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474). In some embodiments, the first anti-GPC3 scFv is N-terminal to the second scFv. In some embodiments, the first anti-GPC3 scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the second scFv is

chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, both the first and second scFvs are chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-GPC3 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 440-459. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 479, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 480, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 481; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 482, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 483, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 484. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 485; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 486. In some embodiments, anti-CD3-scFv comprises: i) a V_H comprising the amino acid sequence of SEQ ID NO: 485; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 486. In some embodiments, the anti-CD3-scFv comprises the amino acid sequence of SEQ ID NO: 487. In some embodiments, there is provided a tandem di-scFv bispecific anti-GPC3 antibody comprising a) a first scFv specifically recognizing GPC3 (e.g., nGPC3 and/or sGPC3); and b) a second scFv specifically recognizing CD3ε on the cell surface of a T cell, wherein the tandem di-scFv bispecific anti-GPC3 antibody comprises the amino acid sequence exemplified by SEQ ID NO: 489. In some embodiments, the tandem di-scFv bispecific anti-GPC3 antibody further comprises a tag (*e.g.*, a peptide tag for purification purpose). In some embodiments, the tag is N-terminal to the tandem di-scFv bispecific anti-GPC3 antibody. In some embodiments, the tag is C-terminal to the tandem di-scFv bispecific anti-GPC3 antibody. In some embodiments, the tag comprises the amino acid sequence of HHHHHH (SEQ ID NO: 476).

Inducible expression

[0382] In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is inducible. In some embodiments, an effector cell (*e.g.*, T cell, CAR-T cell, caTCR T cell) comprises a nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter, including any inducible promoter described herein (*e.g.*, see "Nucleic Acids" section). In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) in the effector cell (*e.g.*, T cell, CAR-T cell, caTCR T cell) is

inducible upon signaling through a signaling receptor on the effector cell (*e.g.*, TCR, CAR, caTCR). In some such embodiments, a CAR-T cell comprises a nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) operably linked to a promoter or regulatory element responsive to signaling through the CAR. In some such embodiments, a caTCR-T cell comprises a nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) operably linked to a promoter or regulatory element responsive to signaling through the caTCR. In some embodiments, the nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is operably linked to a nuclear-factor of the activated T-cell (NFAT)-derived promoter. In some embodiments, the NFAT-derived promoter is an NFAT-derived minimal promoter (*see* for example Durand, D. *et. al.*, *Molec. Cell. Biol.* 8, 1715-1724 (1988); Clipstone, NA, Crabtree, GR. *Nature. 1992* 357(6380): 695-7; Chmielewski, M., et al. *Cancer research* 71.17 (2011): 5697-5706; and Zhang, L., et al. *Molecular therapy* 19.4 (2011): 751-759). The NFAT family of transcription factors are important regulators of T cell activation. In some embodiments, the nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is operably linked to an IL-2 promoter.

[0383] In some embodiments, there is provided an engineered immune cell (such as a T cell) expressing on its surface a dimeric caTCR and expressing or capable of expressing a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific antibody) according to any one of the multispecific anti-GPC3 molecule described herein, wherein the engineered immune cell comprises: a) a first caTCR nucleic acid sequence encoding a first caTCR polypeptide chain of the caTCR; b) a second caTCR nucleic acid sequence encoding a second caTCR polypeptide chain of the caTCR; and c) a nucleic acid sequence encoding the multispecific anti-GPC3 molecule, wherein the caTCR localizes to the surface of the immune cell and the multispecific anti-GPC3 molecule is capable of being secreted from the immune cell. In some embodiments, the first caTCR nucleic acid sequence is contained in a first vector (such as a viral vector, e.g., a lentiviral vector), the second caTCR nucleic acid sequence is contained in a second vector (such as a viral vector, e.g., a lentiviral vector), and the nucleic acid sequence encoding the multispecific anti-GPC3 molecule is contained in a third vector (such as a viral vector, e.g., a lentiviral vector). In some embodiments, some or all of the first and second caTCR nucleic acid sequences and the nucleic acid sequence encoding the multispecific anti-GPC3

molecule are contained in the same vector (such as a viral vector, e.g., a lentiviral vector). In some embodiments, each of the first and second caTCR nucleic acid sequences and the nucleic acid sequence encoding the multispecific anti-GPC3 molecule are, individually, operably linked to a promoter. In some embodiments, some or all of the nucleic acid sequences are under the control of a single promoter. In some embodiments, some or all of the promoters have the same sequence. In some embodiments, some or all of the promoters have different sequences. In some embodiments, some or all of the promoters are inducible. In some embodiments, the nucleic acid sequences encoding the caTCR are under the control of one or more constitutive promoters, such as a EF1-alpha promoter (*e.g.*, a EF1-alpha promoter comprising the nucleic acid sequence of SEQ ID NO: 527). In some embodiments, the nucleic acid sequence encoding the multispecific anti-GPC3 molecule is under the control of an inducible promoter. In some embodiments, the inducible promoter is inducible upon activation of the immune cell. In some embodiments, the inducible promoter is an NFAT-derived promoter, *e.g.*, an NFAT-derived promoter comprising the nucleic acid sequence of SEQ ID NO: 524 or 526. In some embodiments, some or all of the vectors are viral vectors (such as lentiviral vectors). In some embodiments, the immune cell does not express the TCR subunits from which the TCR-TMs of the caTCR are derived. For example, in some embodiments, the immune cell is an αβ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR δ and γ chains, or the immune cell is a γδ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR α and β chains. In some embodiments, the immune cell is modified to block or decrease the expression of one or both of its endogenous TCR subunits. For example, in some embodiments, the immune cell is an αβ T cell modified to block or decrease the expression of the TCR α and/or β chains, or the immune cell is a γδ T cell modified to block or decrease the expression of the TCR γ and/or δ chains. In some embodiments, the immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell. In some embodiments, some or all of the vectors are viral vectors (such as lentiviral vectors) integrated into the host genome of the immune cell. In some embodiments, the caTCR specifically recognizes an AFP/MHC class I complex (*e.g.*, AFP158/HLA-A*02:01). In some embodiments, the caTCR comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 522 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 523. In some embodiments, the multispecific anti-GPC3 molecule comprises the amino acid sequence of SEQ ID NO: 511.

Chimeric Antigen Receptor (CAR) and CAR effector cells

[0384] The anti-GPC3 construct in some embodiments is a CAR comprising an anti-GPC3 antibody moiety (also referred to herein as an "anti-GPC3 CAR"). Any one of the anti-GPC3 antibody moieties described herein can be employed in the anti-GPC3 CAR. Also provided is a CAR effector cell (*e.g.*, T cell) comprising a CAR comprising an anti-GPC3 antibody moiety (also referred to herein as an "anti-GPC3 CAR effector cell", *e.g.*, "anti-GPC3 CAR T cell"). In some embodiments, the anti-GPC3 CAR comprises an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 CAR comprises an anti-GPC3 antibody moiety specifically recognizing a soluble GPC3.

[0385] The anti-GPC3 CAR comprises a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), and b) an intracellular signaling domain. A transmembrane domain may be present between the extracellular domain and the intracellular domain.

[0386] Between the extracellular domain and the transmembrane domain of the anti-GPC3 CAR, or between the intracellular domain and the transmembrane domain of the anti-GPC3 CAR, there may be a spacer domain. The spacer domain can be any oligo- or polypeptide that functions to link the transmembrane domain to the extracellular domain or the intracellular domain in the polypeptide chain. A spacer domain may comprise up to about 300 amino acids, including for example about 10 to about 100, or about 25 to about 50 amino acids.

[0387] The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this invention may be derived from (*i.e.* comprise at least the transmembrane region(s) of) the α, β, δ, or γ chain of the T-cell receptor, CD28, CD3ε, CD3ζ, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, or CD154. In some embodiments, the transmembrane domain may be synthetic, in which case it may comprise predominantly hydrophobic residues such as leucine and valine. In some embodiments, a triplet of phenylalanine, tryptophan and valine may be found at each end of a synthetic transmembrane domain. In some embodiments, a short oligo- or polypeptide linker, having a length of, for example, between about 2 and about 10

(such as about any of 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acids in length may form the linkage between the transmembrane domain and the intracellular signaling domain of the anti-GPC3 CAR. In some embodiments, the linker is a glycine-serine doublet.

[0388] In some embodiments, the transmembrane domain that is naturally associated with one of the sequences in the intracellular domain of the anti-GPC3 CAR is used (*e.g.*, if an anti-GPC3 CAR intracellular domain comprises a CD28 co-stimulatory sequence, the transmembrane domain of the anti-GPC3 CAR is derived from the CD28 transmembrane domain). In some embodiments, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0389] The intracellular signaling domain of the anti-GPC3 CAR is responsible for activation of at least one of the normal effector functions of the immune cell in which the anti-GPC3 CAR has been placed in. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term "intracellular signaling sequence" is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

[0390] Examples of intracellular signaling domains for use in the anti-GPC3 CAR of the invention include the cytoplasmic sequences of the TCR and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

[0391] It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or co-stimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of intracellular signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (co-stimulatory signaling sequences).

[0392] Primary signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary signaling sequences that act in a stimulatory

manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. The anti-GPC3 CAR constructs in some embodiments comprise one or more ITAMs.

[0393] Examples of ITAM containing primary signaling sequences that are of particular use in the invention include those derived from TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, and CD66d.

[0394] In some embodiments, the anti-GPC3 CAR comprises a primary signaling sequence derived from CD3ζ. For example, the intracellular signaling domain of the CAR can comprise the CD3ζ intracellular signaling sequence by itself or combined with any other desired intracellular signaling sequence(s) useful in the context of the anti-GPC3 CAR of the invention. For example, the intracellular domain of the anti-GPC3 CAR can comprise a CD3ζ intracellular signaling sequence and a costimulatory signaling sequence. The costimulatory signaling sequence can be a portion of the intracellular domain of a costimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like.

[0395] In some embodiments, the intracellular signaling domain of the anti-GPC3 CAR comprises the intracellular signaling sequence of CD3ζ and the intracellular signaling sequence of CD28. In some embodiments, the intracellular signaling domain of the anti-GPC3 CAR comprises the intracellular signaling sequence of CD3ζ and the intracellular signaling sequence of 4-1BB. In some embodiments, the intracellular signaling domain of the anti-GPC3 CAR comprises the intracellular signaling sequence of CD3ζ and the intracellular signaling sequences of CD28 and 4-1BB.

[0396] Thus, for example, in some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some

embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher

than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-GPC3 CAR comprises an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) described herein fused to the N-terminus of SEQ ID NO: 494. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, humanized, or semi-synthetic. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M).

[0397] In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid

sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR

comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, the anti-GPC3 CAR comprises an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 described herein fused to the N-terminus of SEQ ID NO: 494. For example, in some embodiments, the anti-GPC3 CAR comprises the amino acid seuqnece of SEQ ID NO: 491. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, humanized, or semi-synthetic.

[0398] In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds GPC3 (*e.g.*, nGPC3 and/or sGPC3), b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody

moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-GPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-GPC3 antibody moiety is an scFv. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, humanized, or semi-synthetic.

[0399] In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5)

amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that competes for binding to a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain. In some

embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the intracellular signaling domain is capable of activating an immune cell. In some embodiments, the intracellular signaling domain comprises a primary signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the primary signaling sequence comprises a CD3ζ intracellular signaling sequence. In some embodiments, the co-stimulatory signaling sequence comprises a CD28 intracellular signaling sequence. In some embodiments, the intracellular domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-GPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-GPC3 antibody moiety is an scFv. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, humanized, or semi-synthetic. In some embodiments, the anti-GPC3 CAR comprises an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3) described herein fused to the N-terminus of SEQ ID NO: 494. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3 antibody moiety and the target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M).

[0400] In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody

moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-GPC3 CAR comprises an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 described herein fused to the N-terminus of SEQ ID NO: 494. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-nGPC3

antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv.

[0401] In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence

of any one of SEQ ID NOs: 358-388, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-GPC3 CAR comprises an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 described herein fused to the N-terminus of SEQ ID NO: 494. For example, in some embodiments, the anti-GPC3 CAR comprises the amino acid sequence of SEQ ID NO: 491. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, humanized, or semi-synthetic.

[0402] In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, b) a transmembrane domain, and c) an intracellular signaling domain. In some embodiments, there is provided an

anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that competes for binding to a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the anti-GPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-GPC3 antibody moiety is an scFv. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, humanized, or semi-synthetic. In some embodiments, the anti-GPC3 CAR comprises an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3) described herein fused to the N-

terminus of SEQ ID NO: 494. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3 antibody moiety and the target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M).

[0403] Also provided herein are effector cells (such as lymphocytes, *e.g.*, T cells) expressing an anti-GPC3 CAR.

[0404] Also provided is a method of producing an effector cell expressing an anti-GPC3 CAR, the method comprising introducing a vector comprising a nucleic acid encoding the anti-GPC3 CAR into the effector cell. In some embodiments, introducing the vector into the effector cell comprises transducing the effector cell with the vector. In some embodiments, introducing the vector into the effector cell comprises transfecting the effector cell with the vector. Transduction or transfection of the vector into the effector cell can be carried about using any method known in the art.

[0405] In some embodiments, there is provided an anti-GPC3 CAR effector cell (such as lymphocytes, *e.g.*, T cells) comprising a nucleic acid sequence encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) described herein operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) in the anti-GPC3 CAR effector cell is inducible upon signaling through the anti-GPC3 CAR. In some embodiments, the nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is operably linked to an NFAT-derived promoter. In some embodiments, the NFAT-derived promoter is an NFAT-derived minimal promoter. In some embodiments, the nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is operably linked to an IL-2 promoter.

Chimeric antibody-T cell receptor (TCR) construct (caTCR) and caTCR effector cells

[0406] The anti-GPC3 construct in some embodiments is a chimeric antibody-T cell receptor construct (caTCR) comprising an anti-GPC3 antibody moiety (also referred to herein as an "anti-

GPC3 caTCR"). Exemplary caTCRs are provided in PCT/US2016/058305, incorporated herein by reference. Any of the anti-GPC3 antibody moieties (targeting *e.g.*, nGPC3 and/or sGPC3) described herein can be employed in the anti-GPC3 caTCR. The anti-GPC3 caTCR can specifically bind to GPC3 and is capable of recruiting at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ). Also provided is a caTCR effector cell (*e.g.*, T cell) comprising a caTCR comprising an anti-GPC3 antibody moiety (also referred to herein as an "anti-GPC3 caTCR effector cell", *e.g.*, "anti-GPC3 caTCR T cell"). In some embodiments, the anti-GPC3 caTCR comprises an anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-GPC3 caTCR comprises an anti-GPC3 antibody moiety specifically recognizing a soluble GPC3.

[0407] In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3) described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, the anti-GPC3 caTCR comprises naturally occurring TCR domains. In some embodiments, the anti-GPC3 caTCR comprises at least one non-naturally occurring TCR domain. The anti-GPC3 caTCR comprises an antigen-binding module comprising an anti-GPC3 antibody moiety that provides the antigen specificity and a TCRM that allows for CD3 recruitment and signaling. The antigen-binding module is not a naturally occurring T cell receptor antigen-binding moiety. In some embodiments, the antigen-binding module is linked to the N-terminus of a polypeptide chain in the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the anti-GPC3 antibody moiety is a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-GPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-GPC3 antibody moiety is an scFv. The TCRM comprises a transmembrane module derived from the transmembrane domains of one or more TCRs (TCR-TMs), such as an αβ and/or γδ TCR, and optionally further

comprises one or both of the connecting peptides or fragments thereof of a TCR and/or one or more TCR intracellular domains or fragments thereof. In some embodiments, the TCRM comprises two polypeptide chains, each polypeptide chain comprising, from N-terminus to C-terminus, a connecting peptide, a transmembrane domain, and optionally a TCR intracellular domain. In some embodiments, the TCRM comprises one or more non-naturally occurring TCR domains. For example, in some embodiments, the TCRM comprises one or two non-naturally occurring TCR transmembrane domains. A non-naturally occurring TCR domain may be a corresponding domain of a naturally occurring TCR modified by substitution of one or more amino acids, and/or by replacement of a portion of the corresponding domain with a portion of an analogous domain from another TCR. The anti-GPC3 caTCR may comprise a first polypeptide chain and a second polypeptide chain, wherein the first and second polypeptide chains together form the antigen-binding module and the TCRM. In some embodiments, the first and second polypeptide chains are separate polypeptide chains, and the caTCR is a multimer, such as a dimer. In some embodiments, the first and second polypeptide chains are covalently linked, such as by a peptide linkage, or by another chemical linkage, such as a disulfide linkage. In some embodiments, the first polypeptide chain and the second polypeptide chain are linked by at least one disulfide bond. In some embodiments, the anti-GPC3 caTCR further comprises one or more T cell co-stimulatory signaling sequences. The one or more co-stimulatory signaling sequences can be, individually, all or a portion of the intracellular domain of a co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the one or more co-stimulatory signaling sequences are between the first TCR-TM and the first TCR intracellular domain and/or between the second TCR-TM and the second TCR intracellular domain. In some embodiments, the one or more co-stimulatory signaling sequences are C-terminal to the first TCRD and/or the second TCRD. In some embodiments, the anti-GPC3 caTCR lacks a T cell co-stimulatory signaling sequence. In some embodiments, the anti-GPC3 caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the anti-GPC3 caTCR. In some embodiments, the stabilization module is located between the antigen-binding module and the TCRM. In some embodiments, the anti-GPC3 caTCR further comprises a spacer module between any two caTCR modules or domains. In some embodiments, the spacer module comprises one or more peptide linkers connecting two

caTCR modules or domains. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, there is provided an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising V_H and C_H1 antibody domains and a first T cell receptor domain (TCRD) comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising V_L and C_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H and C_H1 domains of the first antigen-binding domain and the V_L and C_L domains of the second antigen-binding domain form an antigen-binding module that specifically binds to GPC3, and wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the antigen-binding module comprises a disulfide bond between a residue in the C_H1 domain and a residue in the C_L domain. In some embodiments, there is provided an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising a V_H antibody domain and a first TCRD comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a V_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H domain of the first antigen-binding domain and the V_L domain of the second antigen-binding domain form an antigen-binding module that specifically binds to GPC3, wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In

some embodiments, the TCR-TM comprises the amino acid sequence of SEQ ID NO: 495. In some embodiments, the TCR-TM comprises the amino acid sequence of SEQ ID NO: 496.

[0408] In some embodiments, the TCRM described herein comprises a) a first T cell receptor domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and b) a second TCRD comprising a second TCR-TM, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule. In some embodiments, both of the TCR-TMs are naturally occurring. In some embodiments, at least one of the TCR-TMs is non-naturally occurring. In some embodiments, both of the TCR-TMs are non-naturally occurring. In some embodiments, the first TCR-TM is derived from one of the transmembrane domains of a T cell receptor (such as an αβ TCR or a γδ TCR) and the second TCR-TM is derived from the other transmembrane domain of the T cell receptor. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the transmembrane domains of the T cell receptor. Recruitment of TCR-associated signaling molecules can be determined by methods known in the art, such as FACS analysis for TCR-CD3 complex surface expression or co-immunoprecipitation of CD3 subunits with the caTCR.

[0409] In some embodiments, the antigen-binding module comprises a first antigen-binding domain comprising a V_H antibody domain (such as any of the V_H antibody domain described herein for anti-GPC3 antibody moieties) and a second antigen-binding domain comprising a V_L antibody domain (such as any of the V_L antibody domain described herein for anti-GPC3 antibody moieties). In some embodiments, the V_H antibody domain and V_L antibody domain CDRs are derived from the same antibody moiety. In some embodiments, some of the V_H antibody domain and V_L antibody domain CDRs are derived from different antibody moieties. In some embodiments, the V_H antibody domain and/or V_L antibody domain are human, humanized, chimeric, semi-synthetic, or fully synthetic.

[0410] In some embodiments, the caTCR comprises an antigen-binding module described herein linked to a TCRM described herein, optionally including a stabilization module. For example, in some embodiments, the caTCR comprises the antigen-binding module linked to the N-terminus of one or both of the TCRDs. In some embodiments, the caTCR comprises a stabilization module between a TCRM and an antigen-binding module. In some embodiments, the caTCR further comprises a spacer module between any two caTCR modules or domains. In some embodiments, the spacer module comprises one or more peptide linkers between about 5 to about 70 (such as about any of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or 70, including

any ranges between these values) amino acids in length. In some embodiments, the caTCR further comprises one or more accessory intracellular domains. In some embodiments, the one or more accessory intracellular domains are carboxy-terminal to the first and/or second TCRD. In some embodiments, the one or more accessory intracellular domains are between the first TCR-TM and the first TCR intracellular domain and/or between the second TCR-TM and the second TCR intracellular domain. In some embodiments, the one or more accessory intracellular domains comprise, individually, a TCR co-stimulatory domain. In some embodiments, the TCR co-stimulatory domain comprises all or a portion of the intracellular domain of an immune co-stimulatory molecule (such as CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like). In some embodiments, the TCR co-stimulatory domain comprises all or a portion of the amino acid sequence of any one of SEQ ID NOs: 51-56, or a variant thereof.

[0411] In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing a cell surface-bound GPC3 (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising V_H and C_H1 antibody domains and a first T cell receptor domain (TCRD) comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising V_L and C_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H and C_H1 domains of the first antigen-binding domain and the V_L and C_L domains of the second antigen-binding domain form an antigen-binding module that specifically binds to cell surface bound-GPC3 (*e.g.*,

the binding affinity of the V_H and V_L to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the V_H and V_L specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), and wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the antigen-binding module comprises a disulfide bond between a residue in the C_H1 domain and a residue in the C_L domain. In some embodiments, there is provided an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising a V_H antibody domain and a first TCRD comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a V_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H domain of the first antigen-binding domain and the V_L domain of the second antigen-binding domain form an antigen-binding module that specifically binds to cell surface bound-GPC3 (*e.g.*, the binding affinity of the V_H and V_L to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the V_H and V_L specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically

recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains

of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the anti-nGPC3 antibody moiety is human, humanized, chimeric, or synthetic. In some embodiments, the anti-nGPC3 antibody moiety is linked to the N-terminus of the first and/or second TCRDs. In some embodiments, the first naturally occurring TCR is a γ/δ TCR. In some embodiments, the first naturally occurring TCR is an α/β TCR. In some embodiments, at least one of the TCR-TMs is non-naturally occurring. In some embodiments, the first and second TCR-TMs are non-naturally occurring. In some embodiments, the first TCR-TM comprises up to 5 (such as any of 1, 2, 3, 4, or 5) amino acid substitutions compared to the transmembrane domain from which it is derived and/or the second TCR-TM comprises up to 5 (such as any of 1, 2, 3, 4, or 5) amino acid substitutions compared to the transmembrane domain from which it is derived. In some embodiments, the TCRM comprises a transmembrane module derived from the transmembrane domains of one or more TCRs (TCR-TMs), such as an αβ and/or γδ TCR, and optionally further comprises one or both of the connecting peptides or fragments thereof of a TCR and/or one or more TCR intracellular domains or fragments thereof. In some embodiments, the TCRM comprises two polypeptide chains, each polypeptide chain comprising, from amino terminus to carboxy terminus, a connecting peptide, a transmembrane

domain, and optionally a TCR intracellular domain. In some embodiments, anti-GPC3 caTCR may comprise a first polypeptide chain and a second polypeptide chain, wherein the first and second polypeptide chains together form the antigen-binding module and the TCRM. In some embodiments, the first and second polypeptide chains are separate polypeptide chains, and the caTCR is a multimer, such as a dimer. In some embodiments, the first and second polypeptide chains are covalently linked, such as by a peptide linkage, or by another chemical linkage, such as a disulfide linkage. In some embodiments, the first polypeptide chain and the second polypeptide chain are linked by at least one disulfide bond. In some embodiments, the caTCR further comprises one or more T cell co-stimulatory signaling sequences. The one or more co-stimulatory signaling sequences can be, individually, all or a portion of the intracellular domain of a co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the one or more co-stimulatory signaling sequences are between the first TCR-TM and the first TCR intracellular domain and/or between the second TCR-TM and the second TCR intracellular domain. In some embodiments, the one or more co-stimulatory signaling sequences are carboxy-terminal to the first TCRD and/or the second TCRD. In some embodiments, the caTCR lacks a T cell co-stimulatory signaling sequence. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the stabilization module is located between the antigen-binding module and the TCRM. In some embodiments, the stabilization module comprises a fist stabilization domain comprising a C_H1 antibody domain or variant thereof and a second stabilization domain comprising a C_L antibody domain or variant thereof. In some embodiments, the caTCR further comprises a spacer module between any two caTCR modules or domains. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the anti-GPC3 caTCR is a heteromultimer, such as a heterodimer. For example, in some embodiments, the anti-GPC3 caTCR is a heterodimer

comprising a first polypeptide chain comprising the first TCRD and a second polypeptide chain comprising the second TCRD, wherein the antibody moiety is linked to the first and/or second polypeptide chains. In some embodiments, the TCRM comprises one or more non-naturally occurring TCR domains. In some embodiments, the TCR-TM comprises the amino acid sequence of SEQ ID NO: 495. In some embodiments, the TCR-TM comprises the amino acid sequence of SEQ ID NO: 496.

[0412] In some embodiments, there is provided an anti-GPC3 caTCR comprising a) a an antigen-binding module comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a

second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any

one of SEQ ID NOs: 358-388, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337 and C_H1 antibody domains and a first T cell receptor domain (TCRD) comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388 and C_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR

subunit, wherein the V_H and C_H1 domains of the first antigen-binding domain and the V_L and C_L domains of the second antigen-binding domain form an antigen-binding module that specifically binds to cell surface bound-GPC3, and wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the antigen-binding module comprises a disulfide bond between a residue in the C_H1 domain and a residue in the C_L domain. In some embodiments, there is provided an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising a V_H antibody domain comprising the amino acid sequence of any one of SEQ ID NOs: 307-337 and a first TCRD comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a V_L antibody domains comprising the amino acid sequence of any one of SEQ ID NOs: 358-388 and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H domain of the first antigen-binding domain and the V_L domain of the second antigen-binding domain form an antigen-binding module that specifically binds to cell surface bound-GPC3, wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the anti-GPC3 caTCR comprises two polypeptide chains, comprising amino acid sequence of SEQ ID NO: 492 and SEQ ID NO: 493. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the anti-nGPC3 antibody moiety is human, humanized, chimeric, or synthetic. In some embodiments, the anti-nGPC3 antibody moiety is linked to the N-terminus of the first and/or second TCRDs. In some embodiments, the first naturally occurring TCR is a γ/δ TCR. In some embodiments, the first naturally occurring TCR is an α/β TCR. In some embodiments, at least one of the TCR-TMs is non-naturally occurring. In some embodiments, the first and second TCR-TMs are non-naturally occurring. In

some embodiments, the first TCR-TM comprises up to 5 (such as any of 1, 2, 3, 4, or 5) amino acid substitutions compared to the transmembrane domain from which it is derived and/or the second TCR-TM comprises up to 5 (such as any of 1, 2, 3, 4, or 5) amino acid substitutions compared to the transmembrane domain from which it is derived. In some embodiments, the TCRM comprises a transmembrane module derived from the transmembrane domains of one or more TCRs (TCR-TMs), such as an αβ and/or γδ TCR, and optionally further comprises one or both of the connecting peptides or fragments thereof of a TCR and/or one or more TCR intracellular domains or fragments thereof. In some embodiments, the TCRM comprises two polypeptide chains, each polypeptide chain comprising, from amino terminus to carboxy terminus, a connecting peptide, a transmembrane domain, and optionally a TCR intracellular domain. In some embodiments, anti-GPC3 caTCR may comprise a first polypeptide chain and a second polypeptide chain, wherein the first and second polypeptide chains together form the antigen-binding module and the TCRM. In some embodiments, the first and second polypeptide chains are separate polypeptide chains, and the caTCR is a multimer, such as a dimer. In some embodiments, the first and second polypeptide chains are covalently linked, such as by a peptide linkage, or by another chemical linkage, such as a disulfide linkage. In some embodiments, the first polypeptide chain and the second polypeptide chain are linked by at least one disulfide bond. In some embodiments, the caTCR further comprises one or more T cell co-stimulatory signaling sequences. The one or more co-stimulatory signaling sequences can be, individually, all or a portion of the intracellular domain of a co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the one or more co-stimulatory signaling sequences are between the first TCR-TM and the first TCR intracellular domain and/or between the second TCR-TM and the second TCR intracellular domain. In some embodiments, the one or more co-stimulatory signaling sequences are carboxy-terminal to the first TCRD and/or the second TCRD. In some embodiments, the caTCR lacks a T cell co-stimulatory signaling sequence. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the stabilization module is located between the antigen-binding module and the TCRM. In some embodiments, the stabilization module comprises a fist stabilization domain comprising a C_H1 antibody domain or variant thereof and a second stabilization domain comprising a C_L antibody

domain or variant thereof. In some embodiments, the caTCR further comprises a spacer module between any two caTCR modules or domains. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the anti-GPC3 caTCR is a heteromultimer, such as a heterodimer. For example, in some embodiments, the anti-GPC3 caTCR is a heterodimer comprising a first polypeptide chain comprising the first TCRD and a second polypeptide chain comprising the second TCRD, wherein the antibody moiety is linked to the first and/or second polypeptide chains. In some embodiments, the TCRM comprises one or more non-naturally occurring TCR domains. In some embodiments, the TCR-TM comprises the amino acid sequence of SEQ ID NO: 495. In some embodiments, the TCR-TM comprises the amino acid sequence of SEQ ID NO: 496.

[0413] In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), and b) a TCRM comprising first and second TCR-TMs derived from the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM is capable of recruiting at least one TCR-associated signaling molecule. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising

the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, and b) a TCRM comprising first and second TCR-TMs derived from the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM is capable of recruiting at least one TCR-associated signaling molecule. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an antibody moiety specifically recognizing (*e.g.*, nGPC3 and/or sGPC3), comprising HC-CDRs of a V_H comprising

the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an ani-GPC3 antibody moiety that competes for binding to GPC3 (*e.g.*, nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an ani-GPC3 antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, and b) aa T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357 and C_H1 antibody domains and a first T cell receptor domain (TCRD) comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising V_L comprising the amino acid sequence of any one

of SEQ ID NOs: 389-408 and C_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H and C_H1 domains of the first antigen-binding domain and the V_L and C_L domains of the second antigen-binding domain form an antigen-binding module that specifically binds to GPC3 (e.g., nGPC3 and/or sGPC3), and wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the antigen-binding module comprises a disulfide bond between a residue in the C_H1 domain and a residue in the C_L domain. In some embodiments, there is provided an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising a V_H antibody domain comprising the amino acid sequence of any one of SEQ ID NOs: 338-357 and a first TCRD comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a V_L antibody domains comprising the amino acid sequence of any one of SEQ ID NOs: 389-408 and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H domain of the first antigen-binding domain and the V_L domain of the second antigen-binding domain form an antigen-binding module that specifically binds to GPC3 (e.g., nGPC3 and/or sGPC3), wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the anti-GPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-GPC3 antibody moiety is an scFv. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) is about 10^{-7} M

to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3 antibody moiety and the target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the anti-nGPC3 antibody moiety is human, humanized, chimeric, or synthetic. In some embodiments, the anti-nGPC3 antibody moiety is linked to the N-terminus of the first and/or second TCRDs. In some embodiments, the first naturally occurring TCR is a γ/δ TCR. In some embodiments, the first naturally occurring TCR is an α/β TCR. In some embodiments, at least one of the TCR-TMs is non-naturally occurring. In some embodiments, the first and second TCR-TMs are non-naturally occurring. In some embodiments, the first TCR-TM comprises up to 5 (such as any of 1, 2, 3, 4, or 5) amino acid substitutions compared to the transmembrane domain from which it is derived and/or the second TCR-TM comprises up to 5 (such as any of 1, 2, 3, 4, or 5) amino acid substitutions compared to the transmembrane domain from which it is derived. In some embodiments, the TCRM comprises a transmembrane module derived from the transmembrane domains of one or more TCRs (TCR-TMs), such as an αβ and/or γδ TCR, and optionally further comprises one or both of the connecting peptides or fragments thereof of a TCR and/or one or more TCR intracellular domains or fragments thereof. In some embodiments, the TCRM comprises two polypeptide chains, each polypeptide chain comprising, from amino terminus to carboxy terminus, a connecting peptide, a transmembrane domain, and optionally a TCR intracellular domain. In some embodiments, anti-GPC3 caTCR may comprise a first polypeptide chain and a second polypeptide chain, wherein the first and second polypeptide chains together form the antigen-binding module and the TCRM. In some embodiments, the first and second polypeptide chains are separate polypeptide chains, and the caTCR is a multimer, such as a dimer. In some embodiments, the first and second polypeptide chains are covalently linked, such as by a peptide linkage, or by another chemical linkage, such as a disulfide linkage. In some embodiments, the first polypeptide chain and the second polypeptide chain are linked by at least one disulfide bond. In some embodiments, the caTCR further comprises one or more T cell co-

stimulatory signaling sequences. The one or more co-stimulatory signaling sequences can be, individually, all or a portion of the intracellular domain of a co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the one or more co-stimulatory signaling sequences are between the first TCR-TM and the first TCR intracellular domain and/or between the second TCR-TM and the second TCR intracellular domain. In some embodiments, the one or more co-stimulatory signaling sequences are carboxy-terminal to the first TCRD and/or the second TCRD. In some embodiments, the caTCR lacks a T cell co-stimulatory signaling sequence. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the stabilization module is located between the antigen-binding module and the TCRM. In some embodiments, the stabilization module comprises a fist stabilization domain comprising a C_H1 antibody domain or variant thereof and a second stabilization domain comprising a C_L antibody domain or variant thereof. In some embodiments, the caTCR further comprises a spacer module between any two caTCR modules or domains. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the anti-GPC3 caTCR is a heteromultimer, such as a heterodimer. For example, in some embodiments, the anti-GPC3 caTCR is a heterodimer comprising a first polypeptide chain comprising the first TCRD and a second polypeptide chain comprising the second TCRD, wherein the antibody moiety is linked to the first and/or second polypeptide chains. In some embodiments, the TCRM comprises one or more non-naturally occurring TCR domains. In some embodiments, the TCR-TM comprises the amino acid sequence of SEQ ID NO: 495. In some embodiments, the TCR-TM comprises the amino acid sequence of SEQ ID NO: 496.

[0414] Also provided herein are effector cells (such as lymphocytes, *e.g.*, T cells) expressing an anti-GPC3 caTCR.

[0415] Also provided is a method of producing an effector cell expressing an anti-GPC3 caTCR, the method comprising introducing a vector comprising a nucleic acid encoding the anti-GPC3 caTCR into the effector cell. In some embodiments, introducing the vector into the effector cell comprises transducing the effector cell with the vector. In some embodiments, introducing the vector into the effector cell comprises transfecting the effector cell with the vector. Transduction or transfection of the vector into the effector cell can be carried about using any method known in the art.

[0416] In some embodiments, there is provided an anti-GPC3 caTCR effector cell (such as lymphocytes, *e.g.*, T cells) comprising a nucleic acid sequence encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) described herein operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) in the anti-GPC3 caTCR effector cell is inducible upon signaling through the anti-GPC3 caTCR. In some embodiments, the nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is operably linked to an NFAT-derived promoter. In some embodiments, the NFAT-derived promoter is an NFAT-derived minimal promoter. In some embodiments, the nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is operably linked to an IL-2 promoter.

Chimeric co-stimulatory receptor (CSR) constructs

[0417] The anti-GPC3 construct in some embodiments is a chimeric co-stimulatory receptor (CSR) that specifically binds to GPC3 and is capable of stimulating an immune cell on the surface of which it is functionally expressed upon target ligand binding. The CSR comprises a ligand-binding module that provides the ligand-binding specificity, a transmembrane module, and a co-stimulatory immune cell signaling module that allows for stimulating the immune cell. The CSR lacks a functional primary immune cell signaling sequence. In some embodiments, the CSR lacks any primary immune cell signaling sequence. In some embodiments, the CSR comprises a single polypeptide chain comprising the ligand-binding module, transmembrane module, and co-stimulatory signaling module. In some embodiments, the CSR comprises a first polypeptide chain and a second polypeptide chain, wherein the first and second polypeptide chains together form the ligand-binding module, transmembrane module, and co-stimulatory signaling module. In some embodiments, the first and second polypeptide chains are separate polypeptide chains, and the CSR is a multimer, such as a dimer. In some embodiments, the first

and second polypeptide chains are covalently linked, such as by a peptide linkage, or by another chemical linkage, such as a disulfide linkage. In some embodiments, the first polypeptide chain and the second polypeptide chain are linked by at least one disulfide bond. In some embodiments, the expression of the CSR in the caTCR plus CSR immune cell is inducible. In some embodiments, the expression of the CSR in the caTCR plus CSR immune cell is inducible upon signaling through the caTCR.

[0418] In some embodiments, there is provided a CSR that specifically binds to GPC3, comprising a) an scFv comprising a VH domain having the amino acid sequence of SEQ ID NO: 309 and a VL domain having the amino acid sequence of SEQ ID NO: 360; and b) a fragment of CD28 comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 530. In some embodiments, the scFv comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 441. In some embodiments, the CSR comprises, from amino terminus to carboxy terminus, the scFv, a peptide linker having the amino acid sequence AAA, and the fragment of CD28. In some embodiments, the CSR comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 530.

[0419] In some embodiments, there is provided an immune cell (such as a T cell) comprising nucleic acid encoding a caTCR (such as a caTCR that specifically binds to an AFP/MHC class I molecule, *e.g.*, AFP158/HLA-A02) and an anti-GPC3 CSR according to any of the CSRs described herein, wherein the caTCR and CSR are expressed from the nucleic acid and localized to the immune cell surface. In some embodiments, the nucleic acid comprises a first caTCR nucleic acid sequence encoding a first caTCR polypeptide chain of the caTCR, a second caTCR nucleic acid sequence encoding a second caTCR polypeptide chain of the caTCR, and a CSR nucleic acid sequence encoding a CSR polypeptide chain of the CSR. In some embodiments, the first and second caTCR nucleic acid sequences and CSR nucleic acid sequence are each contained in different vectors. In some embodiments, some or all of the nucleic acid sequences are contained in the same vector. Vectors may be selected, for example, from the group consisting of mammalian expression vectors and viral vectors (such as those derived from retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses). In some embodiments, one or more of the vectors is integrated into the host genome of the immune cell. In some embodiments, the first and second caTCR nucleic acid sequences and CSR nucleic acid sequence are each under the control of different promoters. In some embodiments, some or all of the promoters have the same sequence. In some embodiments, some or all of the promoters have different sequences. In some embodiments, some or all of the nucleic acid sequences are under

the control of a single promoter. In some embodiments, some or all of the promoters are inducible. In some embodiments, the immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell.

Immunoconjugates

[0420] The anti-GPC3 constructs (such as isolated anti-GPC3 constructs) in some embodiments comprise an immunoconjugate comprising an anti-GPC3 antibody moiety according to any of the anti-GPC3 antibody moieties described herein attached to an effector molecule (also referred to herein as an "anti-GPC3 immunoconjugate"). In some embodiments the effector molecule is a therapeutic agent, such as drug, a toxin, a radioisotope, a protein, a peptide, a carbohydrate, a lipid, or a nucleic acid. In some embodiments the effector molecule is a cancer therapeutic agent, which is either cytotoxic, cytostatic or otherwise provides some therapeutic benefit. In some embodiments, therapeutic agents for use in accordance with the present invention may have a biological activity relevant to modulation of the immune system and/or enhancement of T-cell mediated cytotoxicity. In some embodiments, the effector molecule is a label, which can generate a detectable signal, either directly or indirectly. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the anti-GPC3 antibody moiety is a full-length antibody, a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

[0421] In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising an anti-GPC3 antibody moiety and a therapeutic agent (also referred to herein as an "antibody-drug conjugate", or "ADC"). In some embodiments, therapeutic agent is a toxin that is either cytotoxic, cytostatic or otherwise prevents or reduces the ability of the target cells to divide. The use of ADCs for the local delivery of cytotoxic or cytostatic agents, *i.e.*, drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos, *Anticancer Research* 19:605-614

(1999); Niculescu-Duvaz and Springer, *Adv. Drg. Del. Rev.* 26:151 -172 (1997); U.S. Patent No. 4,975,278) allows targeted delivery of the drug moiety to target cells, and intracellular accumulation therein, where systemic administration of these unconjugated therapeutic agents may result in unacceptable levels of toxicity to normal cells as well as the target cells sought to be eliminated (Baldwin *et al.*, *Lancet* (Mar. 15, 1986):603-605 (1986); Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, A. Pinchera *et al.* (eds.), pp. 475- 506). Maximal efficacy with minimal toxicity is sought thereby.

[0422] Therapeutic agents used in anti-GPC3 immunoconjugates include, for example, daunomycin, doxorubicin, methotrexate, and vindesine (Rowland *et al.*, *Cancer Immunol. Immunother.* 21:183-187 (1986)). Toxins used in anti-GPC3 immunoconjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler *et al.*, *J.Nat. Cancer Inst.* 92(19):1573-1581 (2000); Mandler *et al.*, *Bioorganic & Med. Chem. Letters* 10:1025- 1028 (2000); Mandler *et al.*, *Bioconjugate Chem.* 13:786-791 (2002)), maytansinoids (EP 1391213; Liu *et al.*, *Proc. Natl. Acad. Sci. USA* 93:8618-8623 (1996)), and calicheamicin (Lode *et al.*, *Cancer Res.* 58:2928 (1998); Hinman *et al.*, *Cancer Res.* 53:3336-3342 (1993)). The toxins may exert their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition. Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

[0423] Enzymatically active toxins and fragments thereof that can be used include, for example, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain,α-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. *See*, *e.g.*, WO 93/21232 published October 28, 1993.

[0424] In some embodiments, the anti-GPC3 immunoconjugate comprises an anti-GPC3 antibody moiety and one or more small molecule toxins, such as a calicheamicin, maytansinoids, dolastatins, aurostatins, a trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are also contemplated herein.

[0425] In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a therapeutic agent that has an intracellular activity. In some embodiments, the anti-GPC3

immunoconjugate is internalized and therapeutic agent is a cytotoxin that blocks the protein synthesis of the cell, therein leading to cell death. In some embodiments, therapeutic agent is a cytotoxin comprising a polypeptide having ribosome-inactivating activity including, for example, gelonin, bouganin, saporin, ricin, ricin A chain, bryodin, diphtheria toxin, restrictocin, Pseudomonas exotoxin A and variants thereof. In some embodiments, where therapeutic agent is a cytotoxin comprising a polypeptide having a ribosome-inactivating activity, the anti-GPC3 immunoconjugate must be internalized upon binding to the target cell in order for the protein to be cytotoxic to the cells.

[0426] In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a therapeutic agent that acts to disrupt DNA. In some embodiments, therapeutic agent that acts to disrupt DNA is, for example, selected from the group consisting of enediyne (*e.g.*, calicheamicin and esperamicin) and non-enediyne small molecule agents (*e.g.*, bleomycin, methidiumpropyl-EDTA-Fe(II)). Other cancer therapeutic agents useful in accordance with the present application include, without limitation, daunorubicin, doxorubicin, distamycin A, cisplatin, mitomycin C, ecteinascidins, duocarmycin/CC-1065, and bleomycin/pepleomycin.

[0427] The present invention further contemplates an anti-GPC3 immunoconjugate formed between the anti-GPC3 antibody moiety and a compound with nucleolytic activity (*e.g.*, a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

[0428] In some embodiments, the anti-GPC3 immunoconjugate comprises an agent that acts to disrupt tubulin. Such agents may include, for example, rhizoxin/maytansine, paclitaxel, vincristine and vinblastine, colchicine, auristatin dolastatin 10 MMAE, and peloruside A.

[0429] In some embodiments, the anti-GPC3 immunoconjugate comprises an alkylating agent including, for example, Asaley NSC 167780, AZQ NSC 182986, BCNU NSC 409962, Busulfan NSC 750, carboxyphthalatoplatinum NSC 271674, CBDCA NSC 241240, CCNU NSC 79037, CHIP NSC 256927, chlorambucil NSC 3088, chlorozotocin NSC 178248, cis-platinum NSC 119875, clomesone NSC 338947, cyanomorpholinodoxorubicin NSC 357704, cyclodisone NSC 348948, dianhydrogalactitol NSC 132313, fluorodopan NSC 73754, hepsulfam NSC 329680, hycanthone NSC 142982, melphalan NSC 8806, methyl CCNU NSC 95441 , mitomycin C NSC 26980, mitozolamide NSC 353451 , nitrogen mustard NSC 762, PCNU NSC 95466, piperazine NSC 344007, piperazinedione NSC 135758, pipobroman NSC 25154, porfiromycin NSC 56410, spirohydantoin mustard NSC 172112, teroxirone NSC 296934, tetraplatin NSC 363812, thio-tepa NSC 6396, triethylenemelamine NSC 9706, uracil nitrogen mustard NSC 34462, and Yoshi-864 NSC 102627.

[0430] In some embodiments, the cancer therapeutic agent portion of the anti-GPC3 immunoconjugate of the present application may comprise an antimitotic agent including, without limitation, allocolchicine NSC 406042, Halichondrin B NSC 609395, colchicine NSC 757, colchicine derivative NSC 33410, dolastatin 10 NSC 376128 (NG - auristatin derived), maytansine NSC 153858, rhizoxin NSC 332598, taxol NSC 125973, taxol derivative NSC 608832, thiocolchicine NSC 361792, trityl cysteine NSC 83265, vinblastine sulfate NSC 49842, and vincristine sulfate NSC 67574.

[0431] In some embodiments, the anti-GPC3 immunoconjugate comprises a topoisomerase I inhibitor including, without limitation, camptothecin NSC 94600, camptothecin, Na salt NSC 100880, aminocamptothecin NSC 603071 , camptothecin derivative NSC 95382, camptothecin derivative NSC 107124, camptothecin derivative NSC 643833, camptothecin derivative NSC 629971 , camptothecin derivative NSC 295500, camptothecin derivative NSC 249910, camptothecin derivative NSC 606985, camptothecin derivative NSC 374028, camptothecin derivative NSC 176323, camptothecin derivative NSC 295501 , camptothecin derivative NSC 606172, camptothecin derivative NSC 606173, camptothecin derivative NSC 610458, camptothecin derivative NSC 618939, camptothecin derivative NSC 610457, camptothecin derivative NSC 610459, camptothecin derivative NSC 606499, camptothecin derivative NSC 610456, camptothecin derivative NSC 364830, camptothecin derivative NSC 606497, and morpholinodoxorubicin NSC 354646.

[0432] In some embodiments, the anti-GPC3 immunoconjugate comprises a topoisomerase II inhibitor including, without limitation, doxorubicin NSC 123127, amonafide NSC 308847, m-AMSA NSC 249992, anthrapyrazole derivative NSC 355644, pyrazoloacridine NSC 366140, bisantrene HCL NSC 337766, daunorubicin NSC 82151 , deoxydoxorubicin NSC 267469, mitoxantrone NSC 301739, menogaril NSC 269148, N,N-dibenzyl daunomycin NSC 268242, oxanthrazole NSC 349174, rubidazone NSC 164011 , VM-26 NSC 122819, and VP-16 NSC 141540.

[0433] In some embodiments, the anti-GPC3 immunoconjugate comprises an RNA or DNA antimetabolite including, without limitation, L-alanosine NSC 153353, 5-azacytidine NSC 102816, 5-fluorouracil NSC 19893, acivicin NSC 163501 , aminopterin derivative NSC 132483, aminopterin derivative NSC 184692, aminopterin derivative NSC 134033, an antifol NSC 633713, an antifol NSC 623017, Baker's soluble antifol NSC 139105, dichlorallyl lawsone NSC 126771 , brequinar NSC 368390, ftorafur (pro-drug) NSC 148958, 5,6- dihydro-5-azacytidine NSC 264880, methotrexate NSC 740, methotrexate derivative NSC 174121 , N-

(phosphonoacetyl)-L-aspartate (PALA) NSC 224131 , pyrazofurin NSC 143095, trimetrexate NSC 352122, 3-HP NSC 95678, 2'-deoxy-5-fluorouridine NSC 27640, 5-HP NSC 107392,α-TGDR NSC 71851 , aphidicolin glycinate NSC 303812, ara-C NSC 63878, 5-aza-2'-deoxycytidine NSC 127716,β-TGDR NSC 71261 , cyclocytidine NSC 145668, guanazole NSC 1895, hydroxyurea NSC 32065, inosine glycodialdehyde NSC 118994, macbecin Il NSC 330500, pyrazoloimidazole NSC 51143, thioguanine NSC 752, and thiopurine NSC 755.

[0434] In some embodiments, the anti-GPC3 immunoconjugate comprises a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. 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.

[0435] In some embodiments, the anti-GPC3 antibody moiety can be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" *(e.g.,* avidin) that is conjugated to a cytotoxic agent *(e.g.,* a radionucleotide).

[0436] In some embodiments, an anti-GPC3 immunoconjugate may comprise an anti-GPC3 antibody moiety conjugated to a prodrug-activating enzyme. In some such embodiments, a prodrug-activating enzyme converts a prodrug (*e.g.*, a peptidyl chemotherapeutic agent, see WO 81/01145) to an active drug, such as an anti-cancer drug. Such anti-GPC3 immunoconjugates are useful, in some embodiments, in antibody-dependent enzyme-mediated prodrug therapy ("ADEPT"). Enzymes that may be conjugated to an antibody include, but are not limited to, alkaline phosphatases, which are useful for converting phosphate-containing prodrugs into free drugs; arylsulfatases, which are useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase, which is useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), which are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, which are useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase, which are useful for converting glycosylated prodrugs into free drugs; β-lactamase, which is useful for converting drugs derivatized with β -lactams into free drugs; and penicillin amidases, such as penicillin V amidase and penicillin G amidase, which are useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. In some embodiments,

enzymes may be covalently bound to antibody moieties by recombinant DNA techniques well known in the art. See, *e.g.*, Neuberger *et al.*, *Nature* 312:604-608 (1984).

[0437] In some embodiments, therapeutic portion of the anti-GPC3 immunoconjugates may be a nucleic acid. Nucleic acids that may be used include, but are not limited to, anti-sense RNA, genes or other polynucleotides, including nucleic acid analogs such as thioguanine and thiopurine.

[0438] The present application further provides anti-GPC3 immunoconjugates comprising an anti-GPC3 antibody moiety attached to an effector molecule, wherein the effector molecule is a label, which can generate a detectable signal, indirectly or directly. These anti-GPC3 immunoconjugates can be used for research or diagnostic applications, such as for the *in vivo* detection of cancer. The label is preferably capable of producing, either directly or indirectly, a detectable signal. For example, the label may be radio-opaque or a radioisotope, such as ^{3}H, actinium-225, astatine-211, bismuth-212, carbon-14, chromium-51, chlorine-36, cobalt-57, cobalt-58, copper-67, Europium-152, gallium-67, hydrogen-3, iodine-123, iodine-124, iodine-125, iodine-131, indium-111, iron-59, lead-212, lutetium-177, phosphorus-32, radium-223, radium-224, rhenium-186, rhenium-188, selenium-75, sulphur-35, technicium-99m, thorium-227, yttrium-90, or zirconium-89; a fluorescent (fluorophore) or chemiluminescent (chromophore) compound, such as fluorescein isothiocyanate, rhodamine, luciferin, Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy3, Cy5,6-FAM, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Renographin, ROX, TAMRA, TET, Tetramethylrhodamine, and/or Texas Red; an enzyme, such as alkaline phosphatase, β-galactosidase or horseradish peroxidase; an imaging agent; or a metal ion, *e.g.*, chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III), erbium (III), lanthanum (III), gold (III), lead (II), and/or bismuth (III). In some embodiments, the label is a radioactive atom for scintigraphic studies, for example ^{99}Tc or ^{123}I, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, *e.g.*, for PET imaging (WO 2011/056983).

[0439] In some embodiments, the anti-GPC3 immunoconjugate is detectable indirectly. For example, a secondary antibody that is specific for the anti-GPC3 immunoconjugate and contains a detectable label can be used to detect the anti-GPC3 immunoconjugate.

[0440] In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3 (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), and b) an effector molecule. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 25-580 of SEQ ID NO: 460 (SEQ ID NO: 463). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-

terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, and b) an effector molecule. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, and b) an effector molecule. In some embodiments, the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293. In some embodiments, the GPC3 is expressed on the surface of a cancer cell (such as liver cancer cell, *e.g.*, HCC). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the effector molecule is covalently attached to the anti-GPC3 antibody moiety. In some embodiments, the effector molecule is a therapeutic agent selected, for example, from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid. In some embodiments, the effector molecular is a cancer therapeutic agent. In some embodiments, the cancer therapeutic agent is a chemotherapeutic. In some embodiments, the cancer therapeutic agent is a highly radioactive atom selected, for example, from the group consisting of ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P, and ^{212}Pb. In some embodiments, the effector molecule

is a label that can generate a detectable signal, either directly or indirectly. In some embodiments, the label is a radioisotope selected, for example, from the group consisting of ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{123}I, ^{125}I, and ^{131}I. In some embodiments, the anti-nGPC3 antibody moiety is a full-length antibody, a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, humanized, or semi-synthetic.

[0441] In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and b) an effector molecule. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, and b) an effector molecule. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of

SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, and b) an effector molecule. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, and b) an effector molecule. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, and b) an effector molecule. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, and b) an effector molecule. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3 is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-nGPC3 antibody moiety and nGPC3. In some embodiments, the K_d of the binding between the anti-nGPC3 antibody moiety and an sGPC3 is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the effector molecule is covalently attached to the anti-GPC3 antibody moiety. In some embodiments, the effector molecule is a therapeutic agent selected, for example, from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid. In some embodiments, the effector molecular is a cancer therapeutic agent. In some embodiments, the cancer therapeutic agent is a chemotherapeutic. In some embodiments, the cancer therapeutic agent is a highly radioactive atom selected, for example, from the group consisting of ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P, and ^{212}Pb. In some embodiments, the effector molecule is a label that can generate a detectable signal, either directly or indirectly. In some embodiments, the label is a radioisotope selected, for example, from the group consisting of ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{123}I, ^{125}I, and ^{131}I. In some embodiments, the anti-nGPC3 antibody moiety is a full-length antibody, a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, humanized, or semi-synthetic.

[0442] In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and b) an effector molecule. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, and b) an effector molecule. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, and b) an effector molecule. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, and b) an effector molecule. In some embodiments, there is

provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that competes for binding to GPC3 (*e.g.*, nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein, and b) an effector molecule. In some embodiments, there is provided an anti-GPC3 immunoconjugate comprising a) an anti-GPC3 antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, and b) an effector molecule. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a target GPC3 (*e.g.*, nGPC3 and/or sGPC3) is about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target can be at least about 10 times (such as at least about 10, 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 times) of the K_d of the binding between the anti-GPC3 antibody moiety and the target GPC3. In some embodiments, the K_d of the binding between the anti-GPC3 antibody moiety and a non-target is about 10^{-1} M to about 10^{-6} M (such as about 10^{-1} M to about 10^{-6} M, about 10^{-1} M to about 10^{-5} M, or about 10^{-2} M to about 10^{-4} M). In some embodiments, the effector molecule is covalently attached to the anti-GPC3 antibody moiety. In some embodiments, the effector molecule is a therapeutic agent selected, for example, from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid. In some embodiments, the effector molecular is a cancer therapeutic agent. In some embodiments, the cancer therapeutic agent is a chemotherapeutic. In some embodiments, the cancer therapeutic agent is a highly radioactive atom selected, for example, from the group consisting of ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm,

^{212}Bi, ^{32}P, and ^{212}Pb. In some embodiments, the effector molecule is a label that can generate a detectable signal, either directly or indirectly. In some embodiments, the label is a radioisotope selected, for example, from the group consisting of ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{123}I, ^{125}I, and ^{131}I. In some embodiments, the anti-GPC3 antibody moiety is a full-length antibody, a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-GPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-GPC3 antibody moiety is an scFv. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, humanized, or semi-synthetic.

Nucleic Acids

[0443] Nucleic acid molecules encoding the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) or anti-GPC3 antibody moieties are also contemplated. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding a full-length anti-GPC3 antibody. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding an anti-GPC3 scFv. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding an anti-GPC3 Fc fusion protein. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding a multi-specific anti-GPC3 molecule (*e.g.*, a multi-specific anti-GPC3 antibody, a bispecific anti-GPC3 antibody, or a bispecific T-cell engager anti-GPC3 antibody), or polypeptide portion thereof. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding an anti-GPC3 CAR. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding an anti-GPC3 caTCR. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding an anti-GPC3 immunoconjugate, or polypeptide portion thereof. In some embodiments, the nucleic acid (or a set of nucleic acids) encoding the anti-GPC3 construct described herein may further comprises a nucleic acid sequence encoding a peptide tag (such as protein purification tag, *e.g.*, His-tag, HA tag).

[0444] Also contemplated here are isolated host cell comprising an anti-GPC3 construct, an isolated nucleic acid encoding the polypeptide components of the anti-GPC3 construct, or a vector comprising a nucleic acid encoding the polypeptide components of the anti-GPC3 construct described herein.

[0445] The present application also includes variants to these nucleic acid sequences. For example, the variants include nucleotide sequences that hybridize to the nucleic acid sequences encoding the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) or anti-GPC3 antibody moieties of the present application under at least moderately stringent hybridization conditions.

[0446] The present invention also provides vectors in which a nucleic acid of the present invention is inserted.

[0447] In brief summary, the expression of an anti-GPC3 construct (*e.g.*, anti-GPC3 CAR, anti-GPC3 caTCR) or polypeptide portion thereof by a natural or synthetic nucleic acid encoding the anti-GPC3 construct or polypeptide portion thereof can be achieved by inserting the nucleic acid into an appropriate expression vector, such that the nucleic acid is operably linked to 5' and 3' regulatory elements, including for example a promoter (*e.g.*, a lymphocyte-specific promoter) and a 3' untranslated region (UTR). The vectors can be suitable for replication and integration in eukaryotic host cells. Typical cloning and expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

[0448] The nucleic acids of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, *e.g.*, U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In some embodiments, the invention provides a gene therapy vector.

[0449] The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[0450] Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook *et al.* (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers (*see*, *e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[0451] A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of

the subject either *in vivo* or *ex vivo*. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In some embodiments, lentivirus vectors are used. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity.

[0452] Additional promoter elements, *e.g.*, enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline.

[0453] One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Factor-1α (EF-1α). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence to which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to, a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

[0454] As mentioned above, in some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is

inducible. In some embodiments, an effector cell (*e.g.*, T cell, CAR-T cell, caTCR T cell) comprises a nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter, including any inducible promoter described herein. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) in the effector cell (*e.g.*, T cell, CAR-T cell, caTCR T cell) is inducible upon signaling through a signaling receptor on the effector cell (*e.g.*, TCR, CAR, caTCR). In some embodiments, the nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is operably linked to an NFAT-derived promoter. In some embodiments, the NFAT-derived promoter is an NFAT-derived minimal promoter. In some embodiments, the nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is operably linked to an IL-2 promoter.

[0455] Inducible promoters

[0456] The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Exemplary inducible promoter systems for use in eukaryotic cells include, but are not limited to, hormone-regulated elements (e.g., *see* Mader, S. and White, J. H. (1993) Proc. Natl. Acad. Sci. USA 90:5603-5607), synthetic ligand-regulated elements (see, e.g., Spencer, D. M. et al 1993) Science 262: 1019-1024) and ionizing radiation-regulated elements (e.g., *see* Manome, Y. et al. (1993) Biochemistry 32: 10607-10613; Datta, R. et al. (1992) Proc. Natl. Acad. Sci. USA 89: 1014-10153). Further exemplary inducible promoter systems for use in in vitro or in vivo mammalian systems are reviewed in Gingrich et al. (1998) Annual Rev. Neurosci 21:377-405. In some embodiments, the inducible promoter system for use to express the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule is the Tet system. In some embodiments, the inducible promoter system for use to express the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule is the lac repressor system from *E. coli*. In some embodiments, the inducible promoter system for use to express the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule is NFAT-derived promoter.

[0457] An exemplary inducible promoter system for use in the present invention is the Tet system. Such systems are based on the Tet system described by Gossen et al. (1993). In an exemplary embodiment, a polynucleotide of interest is under the control of a promoter that comprises one or more Tet operator (TetO) sites. In the inactive state, Tet repressor (TetR) will

bind to the TetO sites and repress transcription from the promoter. In the active state, e.g., in the presence of an inducing agent such as tetracycline (Tc), anhydrotetracycline, doxycycline (Dox), or an active analog thereof, the inducing agent causes release of TetR from TetO, thereby allowing transcription to take place. Doxycycline is a member of the tetracycline family of antibiotics having the chemical name of 1-dimethylamino-2,4a,5,7,12-pentahydroxy-11-methyl-4,6-dioxo-1,4a,11,11a,12,12a-hexahydrotetracene-3-carboxamide.

[0458] In one embodiment, a TetR is codon-optimized for expression in mammalian cells, e.g., murine or human cells. Most amino acids are encoded by more than one codon due to the degeneracy of the genetic code, allowing for substantial variations in the nucleotide sequence of a given nucleic acid without any alteration in the amino acid sequence encoded by the nucleic acid. However, many organisms display differences in codon usage, also known as "codon bias" (i.e., bias for use of a particular codon(s) for a given amino acid). Codon bias often correlates with the presence of a predominant species of tRNA for a particular codon, which in turn increases efficiency of mRNA translation. Accordingly, a coding sequence derived from a particular organism (e.g., a prokaryote) may be tailored for improved expression in a different organism (e.g., a eukaryote) through codon optimization.

[0459] Other specific variations of the Tet system include the following "Tet-Off" and "Tet-On" systems. In the Tet-Off system, transcription is inactive in the presence of Tc or Dox. In that system, a tetracycline-controlled transactivator protein (tTA), which is composed of TetR fused to the strong transactivating domain of VP16 from Herpes simplex virus, regulates expression of a target nucleic acid that is under transcriptional control of a tetracycline-responsive promoter element (TRE). The TRE is made up of TetO sequence concatamers fused to a promoter (commonly the minimal promoter sequence derived from the human cytomegalovirus (hCMV) immediate-early promoter). In the absence of Tc or Dox, tTA binds to the TRE and activates transcription of the target gene. In the presence of Tc or Dox, tTA cannot bind to the TRE, and expression from the target gene remains inactive.

[0460] Conversely, in the Tet-On system, transcription is active in the presence of Tc or Dox. The Tet-On system is based on a reverse tetracycline-controlled transactivator, rtTA. Like tTA, rtTA is a fusion protein comprised of the TetR repressor and the VP16 transactivation domain. However, a four amino acid change in the TetR DNA binding moiety alters rtTA's binding characteristics such that it can only recognize the tetO sequences in the TRE of the target transgene in the presence of Dox. Thus, in the Tet-On system, transcription of the TRE-regulated target gene is stimulated by rtTA only in the presence of Dox.

[0461] Another inducible promoter system is the lac repressor system from *E. coli* (*See* Brown et al., Cell 49:603-612 (1987)). The lac repressor system functions by regulating transcription of a polynucleotide of interest operably linked to a promoter comprising the lac operator (lacO). The lac repressor (lacR) binds to LacO, thus preventing transcription of the polynucleotide of interest. Expression of the polynucleotide of interest is induced by a suitable inducing agent, e.g., isopropyl-β-D-thiogalactopyranoside (IPTG).

[0462] Another exemplary inducible promoter system for use in the present invention is the NFAT system. The NFAT family of transcription factors are important regulators of T cell activation. NFAT response elements are found, for example, in the IL-2 promoter (*see* for example Durand, D. *et. al.*, *Molec. Cell. Biol.* 8, 1715-1724 (1988); Clipstone, NA, Crabtree, GR. *Nature. 1992* 357(6380): 695-7; Chmielewski, M., et al. *Cancer research* 71.17 (2011): 5697-5706; and Zhang, L., et al. *Molecular therapy* 19.4 (2011): 751-759).

[0463] In order to assess the expression of a polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

[0464] Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, β-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tel *et al.*, 2000 *FEBS Letters* 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

[0465] In some embodiments, there is provided nucleic acid encoding an anti-GPC3 CAR and/or a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) according to any of the anti-GPC3 CARs and multi-specific (*e.g.*, bispecific) anti-GPC3 molecules described herein. In some embodiments, the nucleic acid comprises one or more nucleic acid sequences encoding all of the polypeptide chains of the anti-GPC3 CAR. In some embodiments, the nucleic acid comprises one or more nucleic acid sequences encoding all of the polypeptide chains of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule. In some embodiments, the nucleic acid comprises one or more nucleic acid sequences encoding all of the polypeptide chains of the anti-GPC3 CAR and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule. In some embodiments, each of the one or more nucleic acid sequences are contained in separate vectors. In some embodiments, at least some of the nucleic acid sequences are contained in the same vector. In some embodiments, all of the nucleic acid sequences are contained in the same vector. Vectors may be selected, for example, from the group consisting of mammalian expression vectors and viral vectors (such as those derived from retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses).

[0466] In some embodiments, the anti-GPC3 CAR is a monomer comprising a single polypeptide chain (*e.g.*, anti-GPC3 scFv-CAR) and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule is a monomer comprising a single multi-specific (*e.g.*, bispecific) anti-GPC3 polypeptide chain (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager), and the nucleic acid comprises a first nucleic acid sequence encoding the anti-GPC3 CAR polypeptide chain, and a second nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 polypeptide chain. In some embodiments, the first nucleic acid sequence is contained in a first vector, and the second nucleic acid sequence is contained in a second vector. In some embodiments, the first and second nucleic acid sequences are contained in the same vector. In some embodiments, the first nucleic acid sequence is under the control of a first promoter, and the second nucleic acid sequence is under the control of a second promoter. In some embodiments, one or both of the first and second promoters have the same sequence. In some embodiments, one or both of the first and second promoters have different sequences. In some embodiments, one or both of the first and second nucleic acid sequences are expressed as a single transcript under the control of a single promoter in a multicistronic vector. *See* for example Kim, JH, *et al.*, PLoS One 6(4):e18556, 2011. In some embodiments, one or both of the promoters are inducible. In some embodiments, the promoter controlling the nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 polypeptide chain is inducible

(such as inducible through the activation of the anti-GPC3 CAR). In some embodiments, the inducible promoter is an NFAT-derived promoter.

[0467] In some embodiments, there is provided nucleic acid encoding an anti-GPC3 caTCR and/or a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) according to any of the anti-GPC3 caTCRs and multi-specific (*e.g.*, bispecific) anti-GPC3 molecules described herein. In some embodiments, the nucleic acid comprises one or more nucleic acid sequences encoding all of the polypeptide chains of the anti-GPC3 caTCR. In some embodiments, the nucleic acid comprises one or more nucleic acid sequences encoding all of the polypeptide chains of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule. In some embodiments, the nucleic acid comprises one or more nucleic acid sequences encoding all of the polypeptide chains of the anti-GPC3 caTCR and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule. In some embodiments, each of the one or more nucleic acid sequences are contained in separate vectors. In some embodiments, at least some of the nucleic acid sequences are contained in the same vector. In some embodiments, all of the nucleic acid sequences are contained in the same vector. Vectors may be selected, for example, from the group consisting of mammalian expression vectors and viral vectors (such as those derived from retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses).

[0468] In some embodiments, the anti-GPC3 caTCR is a dimer comprising a first caTCR polypeptide chain and a second caTCR polypeptide chain and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule is a monomer comprising a single multi-specific (*e.g.*, bispecific) anti-GPC3 polypeptide chain (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager), and the nucleic acid comprises a first nucleic acid sequence encoding the first caTCR polypeptide chain, a second nucleic acid encoding the second caTCR chain, and a third nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 polypeptide chain. In some embodiments, the first nucleic acid sequence is contained in a first vector, the second nucleic acid sequence is contained in a second vector, and the third nucleic acid sequence is contained in a third vector. In some embodiments, the first and second nucleic acid sequences are contained in a first vector, and the third nucleic acid sequence is contained in a second vector. In some embodiments, the first and third nucleic acid sequences are contained in a first vector, and the second nucleic acid sequence is contained in a second vector. In some embodiments, the second and third nucleic acid sequences are contained in a first vector, and the first nucleic acid sequence is contained in a second vector. In some embodiments, the first, the second, and the third nucleic acid sequences are contained in the same vector. In some embodiments, the first

nucleic acid sequence is under the control of a first promoter, the second nucleic acid sequence is under the control of a second promoter, and the third nucleic acid sequence is under the control of a third promoter. In some embodiments, some (such as all) of the first, second, and third promoters have the same sequence. In some embodiments, some (such as all) of the first, second, and third promoters have different sequences. In some embodiments, some (such as all) of the first, second, and third nucleic acid sequences are expressed as a single transcript under the control of a single promoter in a multicistronic vector. See for example Kim, JH, *et al.*, PLoS One 6(4):e18556, 2011. In some embodiments, one or more of the promoters are inducible. In some embodiments, the promoter controlling the nucleic acid sequence encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 polypeptide chain is inducible (such as inducible through the activation of the anti-GPC3 caTCR). In some embodiments, the inducible promoter is an NFAT-derived promoter.

[0469] Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, *e.g.*, mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

[0470] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook *et al.* (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York). In some embodiments, the introduction of a polynucleotide into a host cell is carried out by calcium phosphate transfection.

[0471] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method of inserting genes into mammalian, *e.g.*, human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus 1, adenoviruses and adeno-associated viruses, and the like. *See*, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[0472] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*e.g.*, an artificial membrane vesicle).

[0473] In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (*in vitro*, *ex vivo* or *in vivo*). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[0474] Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, *e.g.*, by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

Preparation of anti-GPC3 constructs and anti-GPC3 antibody moieties

[0475] In some embodiments, the anti-GPC3 construct or anti-GPC3 antibody moiety is a monoclonal antibody. In some embodiments, the anti-GPC3 construct is an anti-GPC3 CAR or anti-GPC3 caTCR which comprises an anti-GPC3 antibody moiety, the anti-GPC3 antibody moiety (*e.g.*, Fab, Fab', F(ab')2, Fv, or scFv) comprises sequences derived from a monoclonal antibody. In some embodiments, the anti-GPC3 antibody moiety comprises V_H and V_L domains, or variants thereof, from the monoclonal antibody. In some embodiments, the anti-GPC3 antibody moiety further comprises C_H1 and C_L domains, or variants thereof, from the

monoclonal antibody. Monoclonal antibodies can be prepared, *e.g.,* using hybridoma methods, such as those described by Kohler and Milstein, *Nature,* 256:495 (1975) and Sergeeva *et al.*, *Blood*, 117(16):4262-4272, using the phage display methods described herein and in the Examples below, or using recombinant DNA methods (*see*, *e.g.*, US Patent No. 4,816,567).

[0476] In a hybridoma method, a hamster, mouse, or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized *in vitro.* The immunizing agent can include a polypeptide or a fusion protein of the protein of interest, or a complex comprising at least two molecules, such as a complex comprising a peptide and an MHC protein. Generally, peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. *See, e.g.,* Goding, Monoclonal Antibodies: Principles and Practice (New York: Academic Press, 1986), pp. 59-103. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine, and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which prevents the growth of HGPRT-deficient cells.

[0477] In some embodiments, the immortalized cell lines fuse efficiently, support stable high-level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. In some embodiments, the immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies. Kozbor, *J. Immunol.,* 133:3001 (1984); Brodeur *et al.* Monoclonal Antibody Production Techniques and Applications (Marcel Dekker, Inc.: New York, 1987) pp. 51-63.

[0478] The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the polypeptide. The binding specificity

of monoclonal antibodies produced by the hybridoma cells can be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, *Anal. Biochem.,* 107:220 (1980).

[0479] After the desired hybridoma cells are identified, the clones can be sub cloned by limiting dilution procedures and grown by standard methods. Goding, *supra.* Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown *in vivo* as ascites in a mammal.

[0480] The monoclonal antibodies secreted by the sub clones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[0481] In some embodiments, according to any of the anti-GPC3 constructs described herein comprising an anti-GPC3 antibody moiety, the anti-GPC3 antibody moiety comprises sequences from a clone selected from an antibody moiety library (such as a phage library presenting scFv or Fab fragments). The clone may be identified by screening combinatorial libraries for antibody fragments with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom *et al.*, *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, N.J., 2001) and further described, *e.g.*, in McCafferty *et al.*, *Nature* 348:552-554; Clackson *et al.*, *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, N.J., 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).

[0482] In certain phage display methods, repertoires of V_H and V_L genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.*, *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as scFv fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naïve

repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths *et al.*, *EMBO J*, 12: 725-734 (1993). Finally, naïve libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0483] The anti-GPC3 constructs can be prepared using phage display to screen libraries for anti-GPC3 antibody moieties specific to the target GPC3 (*e.g.*, nGPC3 and/or sGPC3). The library can be a human scFv phage display library having a diversity of at least one x 10^9 (such as at least about any of 1×10^9, 2.5×10^9, 5×10^9, 7.5×10^9, 1×10^{10}, 2.5×10^{10}, 5×10^{10}, 7.5×10^{10}, or 1×10^{11}) unique human antibody fragments. In some embodiments, the library is a naïve human library constructed from DNA extracted from human PMBCs and spleens from healthy donors, encompassing all human heavy and light chain subfamilies. In some embodiments, the library is a naïve human library constructed from DNA extracted from PBMCs isolated from patients with various diseases, such as patients with autoimmune diseases, cancer patients, and patients with infectious diseases. In some embodiments, the library is a semi-synthetic human library, wherein heavy chain CDR3 is completely randomized, with all amino acids (with the exception of cysteine) equally likely to be present at any given position (*see, e.g.,* Hoet, R.M. *et al.*, *Nat. Biotechnol.* 23(3):344-348, 2005). In some embodiments, the heavy chain CDR3 of the semi-synthetic human library has a length from about 5 to about 24 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24) amino acids. In some embodiments, the library is a fully-synthetic phage display library. In some embodiments, the library is a non-human phage display library.

[0484] Phage clones that bind to the target GPC3 (*e.g.*, nGPC3 and/or sGPC3) with high affinity can be selected by iterative binding of phage to the target GPC3, which is bound to a solid support (such as, for example, beads for solution panning or mammalian cells for cell panning), followed by removal of non-bound phage and by elution of specifically bound phage. In an example of solution panning, GPC3 polypeptide can be biotinylated for immobilization to a solid support. The biotinylated GPC3 is mixed with the phage library and a solid support, such as streptavidin-conjugated DYNABEADS® M-280, and then GPC3-phage-bead complexes are

isolated. The bound phage clones are then eluted and used to infect an appropriate host cell, such as *E. coli* XL1-Blue, for expression and purification. In an example of cell panning, SK-Hep1 cells over-expressing GPC3 on cell surface are mixed with the phage library, after which the cells are collected and the bound clones are eluted and used to infect an appropriate host cell for expression and purification (all *see* Examples). The panning can be performed for multiple (such as about any of 2, 3, 4, 5, 6 or more) rounds with solution panning, cell panning, or a combination of both, to enrich for phage clones binding specifically to the target GPC3. Enriched phage clones can be tested for specific binding to the target GPC3 by any methods known in the art, including for example ELISA and FACS.

[0485] Monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.,* by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Hybridoma cells as described above or GPC3-specific phage clones of the invention can serve as a source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains and/or framework regions in place of the homologous non-human sequences (U.S. Patent No. 4,816,567; Morrison *et al., supra*) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

[0486] The antibodies can be monovalent antibodies. Methods for preparing monovalent antibodies are known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy-chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

[0487] *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using any method known in the art.

[0488] Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant-domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. In some embodiments, the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding is present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies, *see,* for example, Suresh *et al., Methods in Enzymology,* 121: 210 (1986).

Human and Humanized Antibodies

[0489] The anti-GPC3 constructs (*e.g.*, anti-GPC3 CAR or caTCR) or anti-GPC3 antibody moieties can be humanized antibodies or human antibodies. Humanized forms of non-human (*e.g.,* murine) antibody moieties are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$, scFv, or other antigen-binding subsequences of antibodies) that typically contain minimal sequence derived from non-human immunoglobulin. Humanized antibody moieties include human immunoglobulins, immunoglobulin chains, or fragments thereof (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibody moieties can also comprise residues that are found neither in the recipient antibody moiety nor in the imported CDR or framework sequences. In general, the humanized antibody moiety can comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin, and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. *See*, *e.g.*, Jones *et al.*, *Nature,* 321: 522-525 (1986); Riechmann *et al., Nature,* 332: 323-329 (1988); Presta, *Curr. Op. Struct. Biol.,* 2:593-596 (1992).

[0490] Generally, a humanized antibody moiety has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are

often referred to as "import" residues, which are typically taken from an "import" variable domain. According to some embodiments, humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature,* 321: 522-525 (1986); Riechmann *et al.*, *Nature,* 332: 323-327 (1988); Verhoeyen *et al.*, *Science,* 239: 1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody moiety. Accordingly, such "humanized" antibody moieties are antibody moieties (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibody moieties are typically human antibody moieties in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0491] As an alternative to humanization, human antibody moieties can be generated. For example, it is now possible to produce transgenic animals *(e.g.,* mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.,* Jakobovits *et al.*, *PNAS USA,* 90:2551 (1993); Jakobovits *et al., Nature,* 362:255-258 (1993); Bruggemann *et al.*, *Year in Immunol.,* 7:33 (1993); U.S. Patent Nos. 5,545,806, 5,569,825, 5,591,669; 5,545,807; and WO 97/17852. Alternatively, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.,* mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed that closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016, and Marks *et al., Bio/Technology,* 10: 779-783 (1992); Lonberg *et al., Nature,* 368: 856-859 (1994); Morrison, *Nature,* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnology,* 14: 845-851 (1996); Neuberger, *Nature Biotechnology,* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.,* 13: 65-93 (1995).

[0492] Human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275) or by using various techniques known in the art, including phage display libraries. Hoogenboom and Winter, *J. Mol. Biol.,* 227:381 (1991); Marks *et al., J. Mol. Biol.,* 222:581 (1991). The techniques of Cole *et al.* and Boerner *et al.* are also available for

the preparation of human monoclonal antibodies. Cole *et al., Monoclonal Antibodies and Cancer Therapy,* Alan R. Liss, p. 77 (1985) and Boerner *et al., J. Immunol.,* 147(1): 86-95 (1991).

[0493] *Multi-specific Antibodies*

[0494] In some embodiments, the anti-GPC3 construct (such as an isolated anti-GPC3 construct) is a multi-specific antibody (*e.g.*, tandem di-scFv bispecific anti-GPC3 antibody). Suitable methods for making multi-specific (*e.g.*, bispecific) antibodies are well known in the art. For example, the production of bispecific antibodies based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two pairs each have different specificities, and upon association result in a heterodimeric antibody (*see, e.g.,* Milstein and Cuello, *Nature,* 305: 537-539 (1983); WO 93/08829, and Traunecker *et al., EMBO J.* 10: 3655 (1991)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829 and in Traunecker *et al., EMBO,* 10: 3655-3659 (1991). Alternatively, the combining of heavy and light chains can be directed by taking advantage of species-restricted pairing (*see, e.g.*, Lindhofer *et al.*, *J. Immunol.*, 155:219-225 (1995)) and the pairing of heavy chains can be directed by use of "knob-into hole" engineering of CH3 domains (*see, e.g.,* U.S. Pat. No. 5,731,168; Ridgway *et al., Protein Eng.*, 9(7):617-621 (1996)). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (*see, e.g.,* WO 2009/089004A1). In yet another method, stable bispecific antibodies can be generated by controlled Fab-arm exchange, where two parental antibodies having distinct antigen specificity and matched point mutations in the CH3 domains are mixed in reducing condition to allow for separation, reassembly, and reoxidation to form highly pure bispecific antibodies. Labrigin *et al., Proc. Natl. Acad. Sci.*, 110(13):5145-5150 (2013). Such antibodies, comprising a mixture of heavy-chain/light-chain pairs, are also referred to herein as "heteromultimeric antibodies".

[0495] Antibodies or antigen-binding fragments thereof having different specificities can also be chemically cross-linked to generate multi-specific heteroconjugate antibodies. For example, two F(ab')2 molecules, each having specificity for a different antigen, can be chemically linked. Pullarkat *et al.*, *Trends Biotechnol.*, 48:9-21 (1999). Such antibodies have, for example, been proposed to target immune-system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection. WO 91/00360; WO 92/200373; EP 03089. It is contemplated that

the antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

[0496] In some embodiments, multi-specific antibodies can be prepared using recombinant DNA techniques. For example, a bispecific antibody can be engineered by fusing two scFvs, such as by fusing them through a peptide linker, resulting in a tandem scFv. One example of a tandem scFv is a bispecific T cell engager. Bispecific T cell engagers are made by linking an anti-CD3 scFv to an scFv specific for a surface antigen of a target cell, such as GPC3, resulting in the redirection of T cells to the target cells (*e.g.*, HCC cells). Mack *et al., Proc. Natl. Acad. Sci.*, 92:7021-7025 (1995); Brischwein *et al., Mol. Immunol.,* 43(8):1129-1143 (2006). By shortening the length of a peptide linker between two variable domains, they can be prevented from self-assembling and forced to pair with domains on a second polypeptide, resulting in a compact bispecific antibody called a diabody (Db). Holliger *et al.*, *Proc. Natl. Acad. Sci.*, 90:6444-6448 (1993). The two polypeptides of a Db each comprise a V_H connected to a V_L by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and VL domains of one polypeptide are forced to pair with the complementary V_L and V_H domains of another polypeptide, thereby forming two antigen-binding sites. In a modification of this format, the two polypeptides are linked by another peptide linker, resulting in a single chain diabody (scDb). In yet another modification of the Db format, dual-affinity retargeting (DART) bispecific antibodies can be generated by introducing a disulfide linkage between cysteine residues at the C-terminus of each polypeptide, optionally including domains prior to the C-terminal cysteine residues that drive assembly of the desired heterodimeric structure. Veri *et al., Arthritis Rheum.*, 62(7):1933-1943 (2010). Dual-variable-domain immunoglobulins (DVD-Ig™), in which the target-binding variable domains of two monoclonal antibodies are combined via naturally occurring linkers to yield a tetravalent, bispecific antibody, are also known in the art. Gu and Ghayur, *Methods Enzymol.*, 502:25-41 (2012). In yet another format, Dock and Lock (DNL), bispecific antibodies are prepared by taking advantage of the dimerization of a peptide (DDD2) derived from the regulatory subunit of human cAMP-dependent protein kinase (PKA) with a peptide (AD2) derived from the anchoring domains of human A kinase anchor proteins (AKAPs). Rossi *et al., Proc. Natl. Acad. Sci.*, 103:6841-6846 (2006).

[0497] Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al., J. Immunol.,* 148(5):1547-1553 (1992). This method can also be utilized for the production of antibody homodimers.

Anti-GPC3 variants

[0498] In some embodiments, amino acid sequence variants of the anti-GPC3 constructs (*e.g.*, anti-GPC3 full-length antibody) provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

[0499] In some embodiments, anti-GPC3 antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

[0500] Conservative substitutions are shown in Table 2 below.

TABLE 2: CONSERVATIVE SUBSTITITIONS

Original Residue	**Exemplary Substitutions**	**Preferred Substitutions**
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu

Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0501] Amino acids may be grouped into different classes according to common side-chain properties:

a. hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

b. neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

c. acidic: Asp, Glu;

d. basic: His, Lys, Arg;

e. residues that influence chain orientation: Gly, Pro;

f. aromatic: Trp, Tyr, Phe.

[0502] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0503] An exemplary substitutional variant is an affinity matured antibody moiety, which may be conveniently generated, *e.g.,* using phage display-based affinity maturation techniques. Briefly, one or more CDR residues are mutated and the variant antibody moieties displayed on phage and screened for a particular biological activity *(e.g.,* binding affinity). Alterations *(e.g.,* substitutions) may be made in HVRs, *e.g.,* to improve antibody moiety affinity. Such alterations may be made in HVR "hotspots," *i.e.,* residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see*, *e.g.,* Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or specificity determining residues (SDRs), with the resulting variant V_H or V_L being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.,* in Hoogenboom *et al.* in *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.,* ed., Human Press, Totowa, NJ, (2001).)

[0504] In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods *(e.g.,* error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody moiety variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues *(e.g.,* 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.,* using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0505] In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In some embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0506] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex can be determined to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0507] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.* for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Fc Region Variants

[0508] In some embodiments, one or more amino acid modifications may be introduced into the Fc region of a full-length anti-GPC3 antibody or anti-GPC3 Fc fusion protein provided herein, thereby generating an Fc region variant. In some embodiments, the Fc region variant has enhanced ADCC effector function, often related to binding to Fc receptors (FcRs). In some embodiments, the Fc region variant has decreased ADCC effector function. There are many examples of changes or mutations to Fc sequences that can alter effector function. For example, WO 00/42072 and Shields *et al. J Biol. Chem.* 9(2): 6591-6604 (2001) describe antibody variants with improved or diminished binding to FcRs. The contents of those publications are specifically incorporated herein by reference.

[0509] Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) is a mechanism of action of therapeutic antibodies against tumor cells. ADCC is a cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell (*e.g.*, a cancer cell), whose membrane-surface antigens have been bound by specific antibodies (*e.g.*, an anti-GPC3 antibody). The typical ADCC involves activation of NK cells by antibodies. An NK cell expresses CD16 which is an Fc receptor. This receptor recognizes, and binds to, the Fc portion of an antibody bound to the surface of a target cell. The most common Fc receptor on the surface of an NK cell is called CD16 or FcγRIII. Binding of the Fc receptor to the Fc region of an antibody results in NK cell activation, release of cytolytic granules and consequent target cell apoptosis. The contribution of ADCC to tumor cell killing can be measured with a specific test that uses NK-92 cells that have been transfected with a high-affinity FcR. Results are compared to wild-type NK-92 cells that do not express the FcR.

[0510] In some embodiments, the invention contemplates an anti-GPC3 construct variant (such as an isolated anti-GPC3 construct variant) comprising an Fc region that possesses some but not all effector functions, which makes it a desirable candidate for applications in which the half-life of the anti-GPC3 construct *in vivo* is important yet certain effector functions (such as CDC and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in*

vitro assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see, *e.g.* Hellstrom, I. *et al. Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I *et al.*, *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); U.S. Pat. No. 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™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96™ non-radioactive cytotoxicity assay (Promega, Madison, Wis.). 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. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M. S. *et al.*, *Blood* 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, *e.g.*, Petkova, S. B. *et al.*, *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0511] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[0512] Certain antibody variants with improved or diminished binding to FcRs are described. (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).)

[0513] In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, anti-GPC3-Fc fusion protein, a full-length anti-GPC3 antibody) variant comprising a variant Fc region comprising one or more amino acid substitutions which improve ADCC. In some embodiments, the variant Fc region comprises one or more amino acid substitutions which improve ADCC, wherein the substitutions are at positions 298, 333, and/or 334 of the variant Fc region (EU numbering of residues). In some embodiments, the anti-GPC3

construct (*e.g.*, full-length anti-GPC3 antibody) variant comprises the following amino acid substitution in its variant Fc region: S298A, E333A, and K334A.

[0514] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie *et al.*, *J. Immunol.* 164: 4178-4184 (2000).

[0515] In some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, anti-GPC3-Fc fusion protein, a full-length anti-GPC3 antibody) variant comprising a variant Fc region comprising one or more amino acid substitutions which increase half-life and/or improve binding to the neonatal Fc receptor (FcRn). Antibodies with increased half-lives and improved binding to FcRn are described in US2005/0014934A1 (Hinton *et al.*). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (U.S. Pat. No. 7,371,826).

[0516] See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

[0517] Anti-GPC3 constructs (such as isolated anti-GPC3 constructs, *e.g.*, anti-GPC3-Fc fusion protein, full-length anti-GPC3 antibodies) comprising any of the Fc variants described herein, or combinations thereof, are contemplated.

Glycosylation Variants

[0518] In some embodiments, an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, anti-GPC3-Fc fusion protein, a full-length anti-GPC3 antibody) provided herein is altered to increase or decrease the extent to which the anti-GPC3 construct is glycosylated. Addition or deletion of glycosylation sites to an anti-GPC3 construct may be conveniently accomplished by altering the amino acid sequence of the anti-GPC3 construct or polypeptide portion thereof such that one or more glycosylation sites is created or removed.

[0519] Where the anti-GPC3 construct comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, *e.g.*, Wright *et al.*, *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine

(GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an anti-GPC3 construct of the invention may be made in order to create anti-GPC3 construct variants with certain improved properties.

[0520] The N-glycans attached to the CH2 domain of Fc is heterogeneous. Antibodies or Fc fusion proteins generated in CHO cells are fucosylated by fucosyltransferase activity. *See* Shoji-Hosaka *et al.*, J. Biochem. 2006, 140:777- 83. Normally, a small percentage of naturally occurring afucosylated IgGs may be detected in human serum. N-glycosylation of the Fc is important for binding to FcγR; and afucosylation of the N-glycan increases Fc's binding capacity to FcγRIIIa. Increased FcγRIIIa binding can enhance ADCC, which can be advantageous in certain antibody therapeutic applications in which cytotoxicity is desirable.

[0521] In some embodiments, an enhanced effector function can be detrimental when Fc-mediated cytotoxicity is undesirable. In some embodiments, the Fc fragment or CH2 domain is not glycosylated. In some embodiments, the N-glycosylation site in the CH2 domain is mutated to prevent from glycosylation.

[0522] In some embodiments, anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3-Fc fusion protein, a full-length anti-GPC3 antibody) variants are provided comprising an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose, which may improve ADCC function. Specifically, anti-GPC3 constructs are contemplated herein that have reduced fusose relative to the amount of fucose on the same anti-GPC3 construct produced in a wild-type CHO cell. That is, they are characterized by having a lower amount of fucose than they would otherwise have if produced by native CHO cells (*e.g.*, a CHO cell that produce a native glycosylation pattern, such as, a CHO cell containing a native FUT8 gene). In some embodiments, the anti-GPC3 construct is one wherein less than about 50%, 40%, 30%, 20%, 10%, or 5% of the N-linked glycans thereon comprise fucose. For example, the amount of fucose in such an anti-GPC3 construct may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. In some embodiments, the anti-GPC3 construct is one wherein none of the N-linked glycans thereon comprise fucose, *i.e.*, wherein the anti-GPC3 construct is completely without fucose, or has no fucose or is afucosylated. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297

in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ±3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, *e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such asα-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).

[0523] Anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3-Fc fusion protein, a full-length anti-GPC3 antibody) variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the anti-GPC3 construct is bisected by GlcNAc. Such anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3-Fc fusion protein, a full-length anti-GPC3 antibody) variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet *et al.*); U.S. Pat. No. 6,602,684 (Umana *et al.*); US 2005/0123546 (Umana *et al.*), and Ferrara *et al.*, *Biotechnology and Bioengineering*, 93(5): 851-861 (2006). Anti-GPC3 construct (such as full-length anti-GPC3 antibody) variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such anti-GPC3 construct variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel *et al.*); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

[0524] In some embodiments, the anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3-Fc fusion protein, a full-length anti-GPC3 antibody) variants comprising an Fc region are capable of binding to an FcγRIII. In some embodiments, the anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3-Fc fusion protein,

a full-length anti-GPC3 antibody) variants comprising an Fc region have ADCC activity in the presence of human effector cells (*e.g.*, T cell) or have increased ADCC activity in the presence of human effector cells compared to the otherwise same anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3-Fc fusion protein, a full-length anti-GPC3 antibody) comprising a human wild-type IgG1Fc region.

Cysteine Engineered Variants

[0525] In some embodiments, it may be desirable to create cysteine engineered anti-GPC3 constructs (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3-Fc fusion protein, a full-length anti-GPC3 antibody) in which one or more amino acid residues are substituted with cysteine residues. In some embodiments, the substituted residues occur at accessible sites of the anti-GPC3 construct. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the anti-GPC3 construct and may be used to conjugate the anti-GPC3 construct to other moieties, such as drug moieties or linker-drug moieties, to create an anti-GPC3 immunoconjugate, as described further herein. Cysteine engineered anti-GPC3 constructs (such as full-length anti-GPC3 antibodies) may be generated as described, *e.g.*, in U.S. Pat. No. 7,521,541.

Derivatives

[0526] In some embodiments, an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3-Fc fusion protein, a full-length anti-GPC3 antibody) provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the anti-GPC3 construct include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the anti-GPC3 construct may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be

determined based on considerations including, but not limited to, the particular properties or functions of the anti-GPC3 construct to be improved, whether the anti-GPC3 construct derivative will be used in a therapy under defined conditions, etc.

[0527] In some embodiments, conjugates of an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, a full-length anti-GPC3 antibody) and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In some embodiments, the nonproteinaceous moiety is a carbon nanotube (Kam *et al.*, *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the anti-GPC3 construct-nonproteinaceous moiety are killed.

CAR Effector Cell and caTCR Effector Cell Preparation

[0528] The present invention in one aspect provides effector cells (such as lymphocytes, for example T cells) expressing an anti-GPC3 CAR. Exemplary methods of preparing effector cells (such as T cells) expressing the anti-GPC3 CARs (anti-GPC3 CAR effector cells, such as anti-GPC3 CAR T cells) are provided herein.

[0529] In some embodiments, an anti-GPC3 CAR effector cell (such as T cell) can be generated by introducing a vector (including for example a lentiviral vector) comprising an anti-GPC3 CAR (for example a CAR comprising an anti-GPC3 antibody moiety and CD28 and CD3ζ intracellular signaling sequences) into the effector cell (such as T cell). In some embodiments, the anti-GPC3 CAR effector cells (such as T cells) of the invention are able to replicate *in vivo*, resulting in long-term persistence that can lead to sustained control of a GPC3-positive disease (such as cancer, *e.g.*, HCC). In some embodiments, the anti-GPC3 CAR effector cell (such as T cell) further expresses a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule described herein, by introducing a vector (including for example a lentiviral vector) comprising a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) into the effector cell (such as T cell). In some embodiments, the nucleic acids encoding the anti-GPC3 CAR and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecules are contained in the same vector. In some embodiments, the nucleic acid encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule is under control of an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule is inducible through the activation of the anti-GPC3 CAR. In some embodiments, the inducible promoter is an NFAT-derived promoter.

[0530] In some embodiments, the anti-GPC3 CAR T cell expresses an anti-GPC3 CAR comprising an anti-GPC3 antibody moiety (also referred to herein as an "anti-GPC3 CAR T cell"). In some embodiments, the anti-GPC3 CAR T cell expresses an anti-GPC3 CAR comprising an extracellular domain comprising an anti-GPC3 antibody moiety and an intracellular domain comprising intracellular signaling sequences of CD3ζ and CD28. In some embodiments, the anti-GPC3 CAR T cell expresses an anti-GPC3 CAR comprising an anti-GPC3 antibody moiety, and a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) described herein. The anti-GPC3 CAR T cells of the invention can undergo robust *in vivo* T cell expansion and can establish GPC3-specific memory cells that persist at high levels for an extended amount of time in blood and bone marrow. In some embodiments, the anti-GPC3 CAR T cells of the invention infused into a patient can eliminate $GPC3^+$ cells, such as $GPC3^+$ cancer cells (e.g., HCC cells), *in vivo* in patients having an GPC3-positive disease.

[0531] The present invention in another aspect provides effector cells (such as lymphocytes, for example T cells) expressing an anti-GPC3 caTCR. Exemplary methods of preparing effector cells (such as T cells) expressing the anti-GPC3 caTCRs (anti-GPC3 caTCR effector cells, such as anti-GPC3 caTCR T cells) are provided herein.

[0532] In some embodiments, an anti-GPC3 caTCR effector cell (such as T cell) can be generated by introducing a vector (including for example a lentiviral vector) comprising an anti-GPC3 caTCR into the effector cell (such as T cell). In some embodiments, the anti-GPC3 caTCR effector cells (such as T cells) of the invention are able to replicate *in vivo*, resulting in long-term persistence that can lead to sustained control of an GPC3-positive disease (such as cancer, *e.g.*, HCC). In some embodiments, the anti-GPC3 caTCR effector cell (such as T cell) further expresses a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule described herein, by introducing a vector (including for example a lentiviral vector) comprising a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) into the effector cell (such as T cell). In some embodiments, the nucleic acids encoding the anti-GPC3 caTCR and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecules are contained in the same vector. In some embodiments, the nucleic acid encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule is under control of an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule is inducible through the activation of the anti-GPC3 caTCR. In some embodiments, the inducible promoter is an NFAT-derived promoter.

[0533] In some embodiments, the anti-GPC3 caTCR T cell expresses an anti-GPC3 caTCR comprising an anti-GPC3 antibody moiety (also referred to herein as an "anti-GPC3 caTCR T cell"). In some embodiments, the anti-GPC3 caTCR T cell expresses an anti-GPC3 caTCR comprising an extracellular domain comprising an anti-GPC3 antibody moiety and a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and a second TCRD comprising a second TCR-TM, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule. In some embodiments, the TCR-TM is derived from an αβTCR or a γδTCR. In some embodiments, the anti-GPC3 caTCR T cell expresses an anti-GPC3 caTCR comprising an anti-GPC3 antibody moiety, and a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) described herein. The anti-GPC3 caTCR T cells of the invention can undergo robust *in vivo* T cell expansion and can establish GPC3-specific memory cells that persist at high levels for an extended amount of time in blood and bone marrow. In some embodiments, the anti-GPC3 caTCR T cells of the invention infused into a patient can eliminate $GPC3^+$ cells, such as $GPC3^+$ cancer cells (e.g., HCC cells), *in vivo* in patients having an GPC3-positive disease.

[0534] In some embodiments, the invention relates to administering a genetically modified T cell expressing an anti-GPC3 CAR or a genetically modified T cell expressing an anti-GPC3 caTCR for the treatment of a patient having an GPC3-positive disease or at risk of having an GPC3-positive disease using lymphocyte infusion. In some embodiments, autologous lymphocyte infusion is used in the treatment. Autologous PBMCs are collected from a patient in need of treatment and T cells are activated and expanded using the methods described herein and known in the art and then infused back into the patient.

[0535] In some embodiments, the anti-GPC3 CAR T cell expresses the anti-GPC3 CAR with even cell surface distribution. In some embodiments, the anti-GPC3 caTCR T cell expresses the anti-GPC3 caTCR with even cell surface distribution. Even cell surface distribution can be characterized, for example, by staining patterns with continuous appearance and even thickness or signal intensity. For example, in some embodiments, a composition, such as a pharmaceutical composition, comprising anti-GPC3 CAR T cells (or anti-GPC3 caTCR T cells) comprises fewer than about 10 % (such as fewer than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 %) cells with aggregation of the anti-GPC3 CAR (or anti-GPC3 caTCR) on the cell surface. Aggregation can be characterized, for example, by staining patterns with uneven thickness or signal intensity, or discontinuous, lumpy, punctate, and/or uneven distribution patterns. In some embodiments, the

anti-GPC3 CAR T cell expresses the anti-GPC3 CAR with less than about 10 % (such as less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 %) aggregation of the anti-GPC3 CAR on the cell surface. In some embodiments, the anti-GPC3 caTCR T cell expresses the anti-GPC3 caTCR with less than about 10 % (such as less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 %) aggregation of the anti-GPC3 caTCR on the cell surface. In some embodiments, the anti-GPC3 CAR T cell has a low level of antigen-independent anti-GPC3 CAR activation. In some embodiments, the anti-GPC3 caTCR T cell has a low level of antigen-independent anti-GPC3 caTCR activation. In some embodiments, the anti-GPC3 CAR T cell or anti-GPC3 caTCR T cell has a low level of T cell exhaustion. T cell exhaustion naturally occurs during conditions of extended immune activation, such as with cancer or chronic infection, where T cells become dysfunctional. T cell exhaustion may be characterized by impaired effector function, prolonged expression of inhibitory receptors, and/or an altered transcriptional state compared to functional effector or memory T cells. Optimal clearance of tumor cells and infections is prevented by T cell exhaustion. T cell exhaustion of the anti-GPC3 CAR T cell or anti-GPC3 caTCR T cell can be characterized by any means known in the art, for example, by determining its functional and/or phenotypic profile (Wherry, E. J., *Nature immunology* 12(6): 492-499, 2011; Jiang, Y., *et al.*, *Cell death & disease* 6(6): e1792, 2015). For example, in some embodiments, the anti-GPC3 CAR T cell or anti-GPC3 caTCR T cell expresses low levels of one or more markers of T cell exhaustion, including, for example, PD-1, LAG-3, TIM-3, CTLA-4, BTLA, and TIGIT. In some embodiments, the anti-GPC3 CAR T cell or anti-GPC3 caTCR T cell maintains levels characteristic of non-exhausted T cells for IL-2 production, TNF-α production, IFN-γ production, and granzyme B production, and/or maintains *ex vivo* killing capacity in the presence of target cells, suggesting that the anti-GPC3 CAR T cell or the anti-GPC3 caTCR T cell is not undergoing self-activation and premature exhaustion.

[0536] Prior to expansion and genetic modification of the T cells, a source of T cells is obtained from a subject. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In some embodiments of the present invention, any number of T cell lines available in the art may be used. In some embodiments of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In some embodiments, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T

cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In some embodiments, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as Ca^{2+}-free, Mg^{2+}-free PBS, PlasmaLyte A, or other saline solutions with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[0537] In some embodiments, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as $CD3^+$, $CD28^+$, $CD4^+$, $CD8^+$, $CD45RA^+$, and $CD45RO^+$ T cells, can be further isolated by positive or negative selection techniques. For example, in some embodiments, T cells are isolated by incubation with anti-CD3/anti-CD28 (*i.e.*, 3×28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In some embodiments, the time period is about 30 minutes. In some embodiments, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In some embodiments, the time period is at least one, 2, 3, 4, 5, or 6 hours. In some embodiments, the time period is 10 to 24 hours. In some embodiments, the incubation time period is 24 hours. For isolation of T cells from patients with leukemia, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such as in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immune-compromised individuals. Further, use of longer incubation times can increase the efficiency of capture of $CD8^+$ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-

CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In some embodiments, it may be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process. "Unselected" cells can also be subjected to further rounds of selection.

[0538] Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD-14, CD20, CD11b, CD-16, HLA-DR, and CD8. In some embodiments, it may be desirable to enrich for or positively select for regulatory T cells which typically express $CD4^+$, $CD25^+$, CD62Lhi, $GITR^+$, and $FoxP3^+$. Alternatively, in some embodiments, T regulatory cells are depleted by anti-CD25 conjugated beads or other similar methods of selection.

[0539] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (*e.g.*, particles such as beads) can be varied. In some embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in some embodiments, a concentration of about 2 billion cells/ml is used. In some embodiments, a concentration of about 1 billion cells/ml is used. In some embodiments, greater than about 100 million cells/ml is used. In some embodiments, a concentration of cells of about any of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In some embodiments, a concentration of cells of about any of 75, 80, 85, 90, 95, or 100 million cells/ml is used. In some embodiments, a concentration of about 125 or about 150 million cells/ml is used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (*i.e.*, leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of $CD8^+$ T cells that normally have weaker CD28 expression.

[0540] In some embodiments of the present invention, T cells are obtained from a patient directly following treatment. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand *ex vivo*. Likewise, following *ex vivo* manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and *in vivo* expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in some embodiments, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

[0541] Whether prior to or after genetic modification of the T cells to express a desirable anti-GPC3 CAR or anti-GPC3 caTCR (and in some embodiments further co-expressing a multi-speicif (*e.g.*, bispecific) anti-GPC3 molecule), the T cells can be activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

[0542] Generally, the T cells of the invention are expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD3 antibody immobilized on a surface, or by contact with a protein kinase C activator (*e.g.*, bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either $CD4^+$ T cells or $CD8^+$ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besançon, France) can be used as can other methods commonly

known in the art (Berg *et al.*, *Transplant Proc.* 30(8):3975-3977, 1998; Haanen *et al.*, *J. Exp. Med.* 190(9):13191328, 1999; Garland *et al.*, *J. Immunol. Meth.* 227(1-2):53-63, 1999).

Immunoconjugate preparation

[0543] The anti-GPC3 immunoconjugates may be prepared using any methods known in the art. *See, e.g.,* WO 2009/067800, WO 2011/133886, and U.S. Patent Application Publication No. 2014322129, incorporated by reference herein in their entirety.

[0544] The anti-GPC3 antibody moiety of an anti-GPC3 immunoconjugate may be "attached to" the effector molecule by any means by which the anti-GPC3 antibody moiety can be associated with, or linked to, the effector molecule. For example, the anti-GPC3 antibody moiety of an anti-GPC3 immunoconjugate may be attached to the effector molecule by chemical or recombinant means. Chemical means for preparing fusions or conjugates are known in the art and can be used to prepare the anti-GPC3 immunoconjugate. The method used to conjugate the anti-GPC3 antibody moiety and effector molecule must be capable of joining the binding protein with the effector molecule without interfering with the ability of the binding protein to bind to the antigen on the target cell.

[0545] The anti-GPC3 antibody moiety of an anti-GPC3 immunoconjugate may be linked indirectly to the effector molecule. For example, the anti-GPC3 antibody moiety of an anti-GPC3 immunoconjugate may be directly linked to a liposome containing the effector molecule of one of several types. The effector molecule(s) and/or the anti-GPC3 antibody moiety may also be bound to a solid surface.

[0546] In some embodiments, the anti-GPC3 antibody moiety of an anti-GPC3 immunoconjugate and the effector molecule are both proteins and can be conjugated using techniques well known in the art. There are several hundred crosslinkers available that can conjugate two proteins. (*See* for example "Chemistry of Protein Conjugation and Crosslinking". 1991, Shans Wong, CRC Press, Ann Arbor). The crosslinker is generally chosen based on the reactive functional groups available or inserted on the anti-GPC3 antibody moiety and/or effector molecule. In addition, if there are no reactive groups, a photoactivatible crosslinker can be used. In certain instances, it may be desirable to include a spacer between the anti-GPC3 antibody moiety and the effector molecule. Crosslinking agents known to the art include the homobifunctional agents: glutaraldehyde, dimethyladipimidate and Bis(diazobenzidine) and the heterobifunctional agents: m Maleimidobenzoyl-N-Hydroxysuccinimide and Sulfo-m Maleimidobenzoyl-N-Hydroxysuccinimide.

[0547] In some embodiments, the anti-GPC3 antibody moiety of an anti-GPC3 immunoconjugate may be engineered with specific residues for chemical attachment of the effector molecule. Specific residues used for chemical attachment of molecule known to the art include lysine and cysteine. The crosslinker is chosen based on the reactive functional groups inserted on the anti-GPC3 antibody moiety, and available on the effector molecule.

[0548] An anti-GPC3 immunoconjugate may also be prepared using recombinant DNA techniques. In such a case a DNA sequence encoding the anti-GPC3 antibody moiety is fused to a DNA sequence encoding the effector molecule, resulting in a chimeric DNA molecule. The chimeric DNA sequence is transfected into a host cell that expresses the fusion protein. The fusion protein can be recovered from the cell culture and purified using techniques known in the art.

[0549] Examples of attaching an effector molecule, which is a label, to the binding protein include the methods described in Hunter, *et al.*, *Nature* 144:945 (1962); David, *et al.*, *Biochemistry* 13:1014 (1974); Pain, *et al.*, *J. Immunol. Meth.* 40:219 (1981); Nygren, J. *Histochem. and Cytochem.* 30:407 (1982); Wensel and Meares, *Radioimmunoimaging And Radioimmunotherapy*, Elsevier, N.Y. (1983); and Colcher *et al.*, "Use of Monoclonal Antibodies as Radiopharmaceuticals for the Localization of Human Carcinoma Xenografts in Athymic Mice", *Meth. Enzymol.*, 121:802-16 (1986).

[0550] The radio- or other labels may be incorporated in the immunoconjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as ^{99}Tc or ^{123}I, ^{186}Re, ^{188}Re and ^{111}In can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker *et al.*, *Biochem. Biophys. Res. Commun.* 80:49-57 (1978)) can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

[0551] Immunoconjugates of the anti-GPC3 antibody moiety and a cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1 - carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCI), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)- ethylenediamine), diisocyanates (such

as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1 ,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene tnaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, *e.g.*, WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari *et al.*, *Cancer Research* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[0552] The anti-GPC3 immunoconjugates of the invention expressly contemplate, but are not limited to, ADC prepared with cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (*e.g.*, from Pierce Biotechnology, Inc., Rockford, IL, U.S.A). *See* pages 467-498, 2003-2004 Applications Handbook and Catalog.

Pharmaceutical Compositions

[0553] Also provided herein are compositions (such as pharmaceutical compositions, also referred to herein as formulations) comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct), nucleic acid encoding the construct, vector comprising the nucleic acid encoding the construct, host cell comprising the nucleic acid or vector, or effector cell comprising the nucleic acid or vector. In some embodiments, the composition further comprises a cell (such as an effector cell, *e.g.*, a T cell) associated with the anti-GPC3 construct. In some embodiments, there is provided a pharmaceutical composition comprising an anti-GPC3 construct and optionally a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition further comprises a cell (such as an effector cell, *e.g.*, a T cell, CAR-T cell, caTCR-T cell) associated with the anti-GPC3 construct.

[0554] Suitable formulations of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) are obtained by mixing an anti-GPC3 construct having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers *(Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include 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 propylparaben; 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 olyvinylpyrrolidone; 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 TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Exemplary formulations are described in WO98/56418, expressly incorporated herein by reference. Lyophilized formulations adapted for subcutaneous administration are described in WO97/04801. Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the individual to be treated herein. Lipofectins or liposomes can be used to deliver the anti-GPC3 constructs of this invention into cells.

[0555] The formulation herein may also contain one or more active compounds in addition to the anti-GPC3 construct (such as an isolated anti-GPC3 construct) as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or a chemotherapeutic agent in addition to the anti-GPC3 construct. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of anti-GPC3 construct present in the formulation, the type of disease or disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein or about from 1 to 99% of the heretofore employed dosages.

[0556] The anti-GPC3 constructs (such as isolated anti-GPC3 constructs) may also 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). Sustained-release preparations may be prepared.

[0557] Sustained-release preparations of the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody (or fragment thereof), which matrices are in the form of shaped articles, *e.g.,* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D (-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydro gels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they can denature or aggregate as a result of exposure to moisture at 37 °C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization of anti-GPC3 constructs depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization can be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[0558] In some embodiments, the anti-GPC3 construct (such as an isolated anti-GPC3 construct) is formulated in a buffer comprising a citrate, NaCl, acetate, succinate, glycine, polysorbate 80 (Tween 80), or any combination of the foregoing. In some embodiments, the anti-GPC3 construct is formulated in a buffer comprising about 100 mM to about 150 mM glycine. In some embodiments, the anti-GPC3 construct is formulated in a buffer comprising about 50mM to about 100 mM NaCl. In some embodiments, the anti-GPC3 construct is formulated in a buffer comprising about 10mM to about 50 mM acetate. In some embodiments, the anti-GPC3 construct is formulated in a buffer comprising about 10mM to about 50 mM succinate. In some embodiments, the anti-GPC3 construct is formulated in a buffer comprising about 0.005% to about 0.02% polysorbate 80. In some embodiments, the anti-GPC3 construct is formulated in a buffer having a pH between about 5.1 and 5.6. In some embodiments, the anti-GPC3 construct is formulated in a buffer comprising 10 mM citrate, 100 mM NaCl, 100mM glycine, and 0.01% polysorbate 80, wherein the formulation is at pH 5.5.

[0559] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by, *e.g.*, filtration through sterile filtration membranes.

Methods for treatment using anti-GPC3 constructs

[0560] The anti-GPC3 constructs (such as isolated anti-GPC3 constructs) and/or compositions of the invention can be administered to individuals (*e.g.*, mammals such as humans) to treat a disease and/or disorder involving abnormally high GPC3 expression (also referred to herein as an "GPC3-positive" disease or disorder), including, for example, cancer (such as hepatocellular carcinoma (HCC)). The present application thus in some embodiments provides a method of treating an GPC3-positive disease (such as cancer) in an individual comprising administering to the individual an effective amount of a composition (such as a pharmaceutical composition) comprising an anti-GPC3 construct comprising an anti-GPC3 antibody moiety, such as any one of the anti-GPC3 constructs described herein. In some embodiments, the composition further comprises a cell (such as an effector cell, *e.g.*, T cell) associated with the anti-GPC3 construct (*e.g.*, anti-GPC3 CAR or anti-GPC3 caTCR). In some embodiments, there is provided a method of treating an GPC3-positive disease (such as cancer) in an individual comprising administering to the individual an effective amount of a composition (such as a pharmaceutical composition) comprising an anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager), wherein the expression of the anti-GPC3 construct is inducible upon signaling through a signaling receptor (e.g., TCR, CAR, caTCR) on an effector cell (*e.g.*, T cell). In some embodiments, there is provided a method of treating an GPC3-positive disease (such as cancer) in an individual comprising administering to the individual an effective amount of a composition (such as a pharmaceutical composition) comprising an effector cell (e.g., T cell, CAR-T cell, caTCR-T cell), wherein the effector cell induces expression of an anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) through the activation of the effector cell signaling receptor (e.g., TCR, CAR, caTCR). In some embodiments, the nucleic acid encoding the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is operably linked to an inducible promoter, such as a signaling receptor (e.g., TCR, CAR, caTCR)-activation dependent promoter. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the effector cell is an anti-GPC3 CAR-T cell. In some embodiments, the effector cell is an anti-GPC3 caTCR-T cell.

[0561] In some embodiments, the cancer is selected, for example, from the group consisting of HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor,

choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, and liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is hepatocellular carcinoma and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the individual is human.

[0562] For example, in some embodiments, there is provided a method of treating an individual having a GPC3-positive disease comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the anti-GPC3 construct is an scFv. In some embodiments, the anti-GPC3 construct is a full-length antibody. In some embodiments, the anti-GPC3 construct is a multi-specific molecule (*e.g.*, a bispecific T cell engager). In some embodiments, the anti-GPC3 construct is a CAR or caTCR. In some embodiments, the anti-GPC3 construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell, *e.g.*, T cell) associated with the anti-GPC3 construct. In some embodiments, the composition comprises an effector cell (e.g., T cell, CAR-T cell, caTCR-T cell), wherein the effector cell induces expression of an anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) through the activation of the effector cell signaling receptor (e.g., TCR, CAR, caTCR). In some embodiments, the nucleic acid encoding the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is operably linked to an inducible promoter, such as a signaling receptor (e.g., TCR, CAR, caTCR)-activation dependent promoter. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments,

the inducible promoter is an IL-2 promoter. In some embodiments, the effector cell is an anti-GPC3 CAR-T cell. In some embodiments, the effector cell is an anti-GPC3 caTCR-T cell. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the individual is human.

[0563] In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease (e.g., cancer) comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3 (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino

acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease (e.g., cancer) comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease (e.g., cancer) comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein. In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the anti-GPC3 construct is an scFv. In some embodiments, the anti-GPC3 construct is a full-length antibody. In some embodiments, the anti-GPC3 construct is a multi-specific molecule (*e.g.*, a bispecific T cell engager). In some embodiments, the anti-GPC3 construct is a CAR or caTCR. In some embodiments, the anti-GPC3 construct is an

immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell, *e.g.*, T cell, CAR-T cell, caTCR-T cell) associated with the anti-GPC3 construct. In some embodiments, the effector cell (e.g., T cell, CAR-T cell, caTCR-T cell) induces the expression of the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) through the activation of the effector cell signaling receptor (e.g., TCR, CAR, caTCR). In some embodiments, the expression of the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is under control of an inducible promoter, such as a signaling receptor (e.g., TCR, CAR, caTCR)-activation dependent promoter. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the effector cell is an anti-GPC3 CAR-T cell. In some embodiments, the effector cell is an anti-GPC3 caTCR-T cell. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the individual is human.

[0564] In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease (e.g., cancer) comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a

variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface-bound GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease (e.g., cancer) comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease (e.g., cancer) comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct)

comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein. In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the anti-GPC3 construct is an scFv. In some embodiments, the anti-GPC3 construct is a full-length antibody. In some embodiments, the anti-GPC3 construct is a multi-specific molecule (*e.g.*, a bispecific T cell engager). In some embodiments, the anti-GPC3 construct is a CAR or caTCR. In some embodiments, the anti-GPC3 construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell, *e.g.*, T cell, CAR-T cell, caTCR-T cell) associated with the anti-GPC3 construct. In some embodiments, the effector cell (e.g., T cell, CAR-T cell, caTCR-T cell) induces the expression of the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) through the activation of the effector cell signaling receptor (e.g., TCR, CAR, caTCR). In some embodiments, the expression of the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is under control of an inducible promoter, such as a signaling receptor (e.g., TCR, CAR, caTCR)-activation dependent promoter. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the effector cell is an anti-GPC3 CAR-T cell. In some embodiments, the effector cell is an anti-GPC3 caTCR-T cell. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the individual is human.

[0565] In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (e.g., nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of

any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (e.g., nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (e.g., nGPC3 and/or sGPC3), comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (e.g., nGPC3 and/or sGPC3), comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408. In some embodiments, there is provided a

method of treating an individual having a GPC3-positive disease (e.g., cancer) comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-GPC3 antibody moiety that competes for binding to GPC3 (e.g., nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein. In some embodiments, there is provided a method of treating an individual having a GPC3-positive disease (e.g., cancer) comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the anti-GPC3 construct is an scFv. In some embodiments, the anti-GPC3 construct is a full-length antibody. In some embodiments, the anti-GPC3 construct is a multi-specific molecule (*e.g.*, a bispecific T cell engager). In some embodiments, the anti-GPC3 construct is a CAR or caTCR. In some embodiments, the anti-GPC3 construct is an immunoconjugate. In some embodiments, the composition further comprises a cell (such as an effector cell, *e.g.*, T cell, CAR-T cell, caTCR-T cell) associated with the anti-GPC3 construct. In some embodiments, the effector cell (e.g., T cell, CAR-T cell, caTCR-T cell) induces the expression of the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) through the activation of the effector cell signaling receptor (e.g., TCR, CAR, caTCR). In some embodiments, the expression of the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is under control of an inducible promoter, such as a signaling receptor (e.g., TCR,

CAR, caTCR)-activation dependent promoter. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the effector cell is an anti-GPC3 CAR-T cell. In some embodiments, the effector cell is an anti-GPC3 caTCR-T cell. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the individual is human.

[0566] In some embodiments, there is provided a method of treating metastatic HCC in an individual comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) according to any of the embodiments described above. In some embodiments, the composition further comprises a cell (such as an effector cell, *e.g.*, T cell, CAR-T cell, caTCR-T cell) associated with the anti-GPC3 construct. In some embodiments, the individual is human.

[0567] In some embodiments, there is provided a method of inhibiting (such as preventing) metastasis of hepatocellular carcinoma in an individual comprising administering to the individual an effective amount of a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct) according to any of the embodiments described above. In some embodiments, the individual is human.

[0568] In some embodiments of any of the methods for treating a GPC3-positive disease described above, the anti-GPC3 construct (such as an isolated anti-GPC3 construct) is conjugated to a cell (such as an immune cell, *e.g.*, a T cell) prior to being administered to the individual. Thus, for example, there is provided a method of treating an GPC3-positive disease in an individual comprising a) conjugating any one of the anti-GPC3 constructs described herein to a cell (such as an immune cell, *e.g.*, a T cell) to form an anti-GPC3 construct/cell conjugate, and b) administering to the individual an effective amount of a composition comprising the anti-GPC3 construct/cell conjugate. In some embodiments, the cell is derived from the individual. In some embodiments, the cell is not derived from the individual. In some embodiments, the anti-GPC3 construct is conjugated to the cell by covalent linkage to a molecule on the surface of the

cell. In some embodiments, the anti-GPC3 construct is conjugated to the cell by non-covalent linkage to a molecule on the surface of the cell. In some embodiments, the anti-GPC3 construct is conjugated to the cell by insertion of a portion of the anti-GPC3 construct into the outer membrane of the cell. In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the anti-GPC3 construct is a full-length antibody. In some embodiments, the anti-GPC3 construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-GPC3 construct is a chimeric antigen receptor. In some embodiments, the anti-GPC3 construct is a caTCR. In some embodiments, the method of treating an individual having a GPC3-positive disease comprises administering to the individual an effective amount of anti-GPC3 CAR T cell. In some embodiments, the method of treating an individual having a GPC3-positive disease comprises administering to the individual an effective amount of anti-GPC3 caTCR T cell. In some embodiments, the anti-GPC3 construct is an immunoconjugate. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the individual is human.

[0569] In some embodiments of any of the methods for treating a GPC3-positive disease described above, the anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, anti-GPC3×CD3 bispecific T cell engager) is expressed upon activation of an effector cell (*e.g.*, a T cell, CAR-T cell, caTCR-T cell) through its signaling receptor (e.g., TCR, CAR, caTCR). In some embodiments, the effector cell comprises a nucleic acid sequence encoding the anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter, such as a signaling receptor (e.g., TCR, CAR, caTCR)-activation dependent promoter. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the effector cell is an anti-GPC3 CAR-T cell. In some embodiments, the effector cell is an anti-GPC3 caTCR-T cell. Thus, for example, there is provided a method of treating an GPC3-positive disease in an individual comprising a) introducing a nucleic acid encoding any one of the anti-GPC3 constructs described herein to an effector cell (*e.g.*, T cell, CAR-T cell, caTCR-T cell), wherein the nucleic

acid is operably linked to an inducible promoter (such as a signaling receptor (e.g., TCR, CAR, caTCR)-activation dependent promoter), and b) administering to the individual an effective amount of a composition comprising the effector cell. In some embodiments, the cell is derived from the individual. In some embodiments, the cell is not derived from the individual. In some embodiments, the anti-GPC3 construct is non-naturally occurring. In some embodiments, the anti-GPC3 construct is an scFv. In some embodiments, the anti-GPC3 construct is a full-length antibody. In some embodiments, the anti-GPC3 construct is a multi-specific (such as bispecific) molecule. In some embodiments, the anti-GPC3 construct is a chimeric antigen receptor. In some embodiments, the anti-GPC3 construct is a caTCR. In some embodiments, the method of treating an individual having a GPC3-positive disease comprises administering to the individual an effective amount of anti-GPC3 CAR T cell. In some embodiments, the method of treating an individual having a GPC3-positive disease comprises administering to the individual an effective amount of anti-GPC3 caTCR T cell. In some embodiments, the anti-GPC3 construct is an immunoconjugate. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the individual is human.

[0570] In some embodiments, the individual is a mammal (*e.g.*, human, non-human primate, rat, mouse, cow, horse, pig, sheep, goat, dog, cat, *etc.*). In some embodiments, the individual is a human. In some embodiments, the individual is a clinical patient, a clinical trial volunteer, an experimental animal, *etc.* In some embodiments, the individual is younger than about 60 years old (including for example younger than about any of 50, 40, 30, 25, 20, 15, or 10 years old). In some embodiments, the individual is older than about 60 years old (including for example older than about any of 70, 80, 90, or 100 years old). In some embodiments, the individual is diagnosed with or genetically prone to one or more of the diseases or disorders described herein (such as HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma). In some embodiments, the individual has one or more risk factors associated with one or more diseases or disorders described herein.

[0571] The present application in some embodiments provides a method of delivering an anti-GPC3 construct (such as any one of the anti-GPC3 constructs described herein, *e.g.*, an isolated anti-GPC3 construct) to a cell expressing GPC3 on its surface in an individual, or an individual having soluble GPC3 (*e.g.*, having circulating GPC3 protein or free GPC3 peptides in the serum), the method comprising administering to the individual a composition comprising the anti-GPC3 construct. In some embodiments, the anti-GPC3 construct to be delivered is associated with a cell (such as an effector cell, *e.g.*, a T cell). In some embodiments, the method comprises administering to the individual an anti-GPC3 CAR T cell. In some embodiments, the method comprises administering to the individual an anti-GPC3 caTCR T cell.

[0572] Many diagnostic methods for cancer (such as HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma) or any other disease exhibiting abnormal GPC3 expression and the clinical delineation of those diseases are known in the art. Such methods include, but are not limited to, *e.g.*, immunohistochemistry, PCR, and fluorescent in situ hybridization (FISH).

[0573] In some embodiments, the anti-GPC3 constructs (such as isolated anti-GPC3 constructs) and/or compositions of the invention are administered in combination with a second, third, or fourth agent (including, *e.g.,* an antineoplastic agent, a growth inhibitory agent, a cytotoxic agent, or a chemotherapeutic agent) to treat diseases or disorders involving abnormal GPC3 expression. In some embodiments, the agent includes, for example, IFN receptor agonists, Hsp90 inhibitors, enhancers of p53 expression, and chemotherapeutic agents. In some embodiments, the agent is an IFN receptor agonist including, for example, IFNγ, IFNβ, and IFNα. In some embodiments, the agent is an Hsp90 inhibitor including, for example, tanespimycin (17-AAG), alvespimycin (17-DMAG), retaspimycin (IPI-504), IPI-493, CNF2024/BIIB021, MPC-3100, Debio 0932 (CUDC-305), PU-H71, Ganetespib (STA-9090), NVP-AUY922 (VER-52269), HSP990, KW-2478, AT13387, SNX-5422, DS-2248, and XL888. In some embodiments, the agent is an enhancer of p53 expression including, for example, 5-fluorouracil and nutlin-3. In some embodiments, the agent is a chemotherapeutic agent including, for example, topotecan, etoposide, cisplatin, paclitaxel, and vinblastine.

[0574] Cancer treatments can be evaluated by, *e.g.,* tumor regression, tumor weight or size shrinkage, time to progression, duration of survival, progression free survival, overall response rate, duration of response, quality of life, protein expression and/or activity. Approaches to

determining efficacy of therapy can be employed, including for example, measurement of response through radiological imaging.

[0575] In some embodiments, the efficacy of treatment is measured as the percentage tumor growth inhibition (% TGI), calculated using the equation 100-(T/C × 100), where T is the mean relative tumor volume of the treated tumor, and C is the mean relative tumor volume of a non-treated tumor. In some embodiments, the %TGI is about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, or more than 95%.

[0576] *Dosing and Method of Administering the anti-GPC3 construct Compositions*

[0577] The dose of the anti-GPC3 construct (such as isolated anti-GPC3 construct or administered in the format of anti-GPC3 CAR T cell or anti-GPC3 caTCR T cell) compositions administered to an individual (such as a human) may vary with the particular composition, the mode of administration, and the type of disease being treated. In some embodiments, the amount of the composition (such as composition comprising isolated anti-GPC3 construct, anti-GPC3 CAR T cell, or anti-GPC3 caTCR T cell) is effective to result in an objective response (such as a partial response or a complete response). In some embodiments, the amount of the anti-GPC3 construct composition is sufficient to result in a complete response in the individual. In some embodiments, the amount of the anti-GPC3 construct composition is sufficient to result in a partial response in the individual. In some embodiments, the amount of the anti-GPC3 construct composition administered (for example when administered alone) is sufficient to produce an overall response rate of more than about any of 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 64%, 65%, 70%, 75%, 80%, 85%, or 90% among a population of individuals treated with the anti-GPC3 construct composition. Responses of an individual to the treatment of the methods described herein can be determined, for example, based on RECIST levels.

[0578] In some embodiments, the amount of the composition (such as composition comprising isolated anti-GPC3 construct, anti-GPC3 CAR T cell, or anti-GPC3 caTCR T cell) is sufficient to prolong progress-free survival of the individual. In some embodiments, the amount of the composition is sufficient to prolong overall survival of the individual. In some embodiments, the amount of the composition (for example when administered along) is sufficient to produce clinical benefit of more than about any of 50%, 60%, 70%, or 77% among a population of individuals treated with the anti-GPC3 construct composition.

[0579] In some embodiments, the amount of the composition (such as composition comprising isolated anti-GPC3 construct, anti-GPC3 CAR T cell, or anti-GPC3 caTCR T cell),

alone or in combination with a second, third, and/or fourth agent, is an amount sufficient to decrease the size of a tumor, decrease the number of cancer cells, or decrease the growth rate of a tumor by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100% compared to the corresponding tumor size, number of cancer cells, or tumor growth rate in the same subject prior to treatment or compared to the corresponding activity in other subjects not receiving the treatment. Standard methods can be used to measure the magnitude of this effect, such as *in vitro* assays with purified enzyme, cell-based assays, animal models, or human testing.

[0580] In some embodiments, the amount of the anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3 scFv, full-length anti-GPC3 antibody, multi-specific anti-GPC3 molecule, anti-GPC3 CAR, anti-GPC3 caTCR, or anti-GPC3 immunoconjugate) in the composition is below the level that induces a toxicological effect (*i.e.*, an effect above a clinically acceptable level of toxicity) or is at a level where a potential side effect can be controlled or tolerated when the composition is administered to the individual.

[0581] In some embodiments, the amount of the composition is close to a maximum tolerated dose (MTD) of the composition following the same dosing regimen. In some embodiments, the amount of the composition is more than about any of 80%, 90%, 95%, or 98% of the MTD.

[0582] In some embodiments, the amount of an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, full-length anti-GPC3 antibody, multi-specific anti-GPC3 molecule, or anti-GPC3 immunoconjugate) in the composition is included in a range of about 0.001 μg to about 1000 μg.

[0583] In some embodiments of any of the above aspects, the effective amount of an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, full-length anti-GPC3 antibody, multi-specific anti-GPC3 molecule, or anti-GPC3 immunoconjugate) in the composition is in the range of about 0.1 μg/kg to about 100 mg/kg of total body weight.

[0584] The anti-GPC3 construct compositions can be administered to an individual (such as human) via various routes, including, for example, intravenous, intra-arterial, intraperitoneal, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intraocular, intrathecal, transmucosal, and transdermal. In some embodiments, sustained continuous release formulation of the composition may be used. In some embodiments, the composition is administered intravenously. In some embodiments, the composition is administered intraportally. In some embodiments, the composition is administered intraarterially. In some embodiments, the composition is administered intraperitoneally. In some embodiments, the composition is administered intrahepatically. In some embodiments, the composition is

administered by hepatic arterial infusion. In some embodiments, the administration is to an injection site distal to a first disease site.

[0585] *Anti-GPC3 CAR Effector Cell Therapy and anti-GPC3 caTCR Effector Cell Therapy*

[0586] The present application also provides methods of using an anti-GPC3 CAR or anti-GPC3 caTCR to redirect the specificity of an effector cell (such as a primary T cell) to GPC3 (such as cell surface-bound GPC3 or soluble GPC3). Thus, the present invention also provides a method of stimulating an effector cell-mediated response (such as a T cell-mediated immune response) to a target cell population or tissue comprising GPC-expressing cells in a mammal, comprising the step of administering to the mammal an effector cell (such as a T cell) that expresses an anti-GPC3 CAR or anti-GPC3 caTCR. In some embodiments, the anti-GPC3 CAR effector cell or the anti-GPC3 caTCR effector cell further comprises a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) in the anti-GPC3 CAR effector cell or the anti-GPC3 caTCR effector cell is inducible upon signaling through the anti-GPC3 CAR or anti-GPC3 caTCR (respectively). In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter.

[0587] Anti-GPC3 CAR effector cells (such as T cells) expressing the anti-GPC3 CAR (optionally further expressing an anti-GPC3 bispecific T cell engager) can be infused to a recipient in need thereof. Anti-GPC3 caTCR effector cells (such as T cells) expressing the anti-GPC3 caTCR (optionally further expressing an anti-GPC3 bispecific T cell engager) can be infused to a recipient in need thereof. The infused cell is able to kill cells expressing GPC3 on its cell surface in the recipient. In some embodiments, unlike antibody therapies, anti-GPC3 CAR effector cells (such as T cells) or anti-GPC3 caTCR effector cells (such as T cells) are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained tumor control.

[0588] In some embodiments, the anti-GPC3 CAR effector cells are anti-GPC3 CAR T cells that can undergo robust *in vivo* T cell expansion and can persist for an extended amount of time. In some embodiments, the anti-GPC3 CAR T cells of the invention develop into specific memory T cells that can be reactivated to inhibit any additional tumor formation or growth.

[0589] In some embodiments, the anti-GPC3 caTCR effector cells are anti-GPC3 caTCR T cells that can undergo robust *in vivo* T cell expansion and can persist for an extended amount of

time. In some embodiments, the anti-GPC3 caTCR T cells of the invention develop into specific memory T cells that can be reactivated to inhibit any additional tumor formation or growth.

[0590] The anti-GPC3 CAR T cells or anti-GPC3 caTCR T cells of the invention may also serve as a type of vaccine for *ex vivo* immunization and/or *in vivo* therapy in a mammal. In some embodiments, the mammal is a human. In some embodiments, the anti-GPC3 CAR T cell or the anti-GPC3 caTCR T cell further comprises a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is inducible upon signaling through the anti-GPC3 CAR or anti-GPC3 caTCR. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter.

[0591] With respect to *ex vivo* immunization, at least one of the following occurs *in vitro* prior to administering the cell into a mammal: i) expansion of the cells, ii) introducing a nucleic acid encoding an anti-GPC3 CAR or anti-GPC3 caTCR to the cells, and/or iii) cryopreservation of the cells. In some embodiments, the cells are further *in vitro* manipulated by introducing a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter.

[0592] *Ex vivo* procedures are well known in the art and are discussed more fully below. Briefly, cells are isolated from a mammal (preferably a human) and genetically modified (*i.e.*, transduced or transfected *in vitro*) with a vector expressing an anti-GPC3 CAR or an anti-GPC3 caTCR disclosed herein. The anti-GPC3 CAR effector cell or anti-GPC3 caTCR effector cell can be administered to a mammalian recipient to provide a therapeutic benefit. The mammalian recipient may be a human and the anti-GPC3 CAR cell (or anti-GPC3 caTCR cell) can be autologous with respect to the recipient. Alternatively, the cells can be allogeneic, syngeneic or xenogeneic with respect to the recipient. In some embodiments, the anti-GPC3 CAR effector cell or the anti-GPC3 caTCR effector cell further comprises a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter, wherein the inducible promoter is responsive to signaling through the anti-GPC3 CAR or anti-GPC3 caTCR.

[0593] The procedure for *ex vivo* expansion of hematopoietic stem and progenitor cells is described in U.S. Pat. No. 5,199,942, incorporated herein by reference, can be applied to the cells of the present invention. Other suitable methods are known in the art; therefore the present invention is not limited to any particular method of *ex vivo* expansion of the cells. Briefly, *ex vivo* culture and expansion of T cells comprises: (1) collecting $CD34^+$ hematopoietic stem and progenitor cells from a mammal from peripheral blood harvest or bone marrow explants; and (2) expanding such cells *ex vivo*. In addition to the cellular growth factors described in U.S. Pat. No. 5,199,942, other factors such as flt3-L, IL-1, IL-3 and c-kit ligand, can be used for culturing and expansion of the cells.

[0594] In addition to using a cell-based vaccine in terms of *ex vivo* immunization, the present invention also provides compositions and methods for *in vivo* immunization to elicit an immune response directed against an antigen in a patient.

[0595] The anti-GPC3 CAR effector cells (such as T cells) or anti-GPC3 caTCR effector cells (such as T cells) of the present invention may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations. Briefly, pharmaceutical compositions of the present invention may comprise anti-GPC3 CAR effector cells (such as T cells) or anti-GPC3 caTCR effector cells (such as T cells), in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. In some embodiments, anti-GPC3 CAR effector cell (such as T cell) compositions or anti-GPC3 caTCR effector cell (such as T cell) compositions are formulated for intravenous administration.

[0596] The precise amount of the anti-GPC3 CAR effector cell (such as T cell) compositions or the anti-GPC3 caTCR effector cell (such as T cell) compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). In some embodiments, a pharmaceutical composition comprising the anti-GPC3 CAR effector cells (such as T cells) or the anti-GPC3 caTCR effector cells (such as T cells) is administered at a dosage of about 10^4 to about 10^9 cells/kg body weight, such any of about 10^4 to about 10^5, about 10^5 to about 10^6, about 10^6 to about 10^7, about 10^7 to about 10^8, or about 10^8 to about 10^9

cells/kg body weight, including all integer values within those ranges. Anti-GPC3 CAR effector cell (such as T cell) compositions or anti-GPC3 caTCR effector cell (such as T cell) compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, *e.g.*, Rosenberg *et al.*, New Eng. J. of Med. 319:1676, 1988). The optimal dosage and treatment regimen for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[0597] In some embodiments, it may be desired to administer activated anti-GPC3 CAR T cells or activated anti-GPC3 caTCR T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom according to the present invention, and reinfuse the patient with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In some embodiments, T cells can be activated from blood draws of from 10 cc to 400 cc. In some embodiments, T cells are activated from blood draws of 20 cc, 30 cc, 40 cc, 50 cc, 60 cc, 70 cc, 80 cc, 90 cc, or 100 cc.

[0598] The administration of the anti-GPC3 CAR effector cells (such as T cells) or anti-GPC3 caTCR effector cells (such as T cells) may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In some embodiments, the anti-GPC3 CAR effector cell (such as T cell) compositions or the anti-GPC3 caTCR effector cell (such as T cell) compositions of the present invention are administered to a patient by intradermal or subcutaneous injection. In some embodiments, the anti-GPC3 CAR effector cell (such as T cell) compositions or the anti-GPC3 caTCR effector cell (such as T cell) compositions of the present invention are administered by i.v. injection. The compositions of anti-GPC3 CAR effector cells (such as T cells) or anti-GPC3 caTCR effector cells (such as T cells) may be injected directly into a tumor, lymph node, or site of infection.

[0599] Thus, for example, in some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or

the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 462. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an

effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-GPC3 CAR comprises an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 described herein fused to the N-terminus of SEQ ID NO: 494. In some embodiments, the effector cell (such as a T cell) expressing the anti-GPC3 CAR further comprises a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is inducible upon signaling through the anti-GPC3 CAR. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-nGPC3 antibody moiety is a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC,

melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is metastatic HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the administration comprises local injection an injection site distal to a site of the GPC3-positive disease (such as an GPC3-positive tumor). In some embodiments, the injection site is a first GPC3-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the injection site is a first GPC3-positive tumor and the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the individual is human.

[0600] In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, b) a transmembrane domain, and

c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3

CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-GPC3 CAR comprises an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 described herein fused to the N-terminus of SEQ ID NO: 494. For example, in some embodiments, the anti-GPC3 CAR comprises the amino acid sequence of any one of SEQ ID NOs: 491 and 516-521. In some embodiments, the effector cell (such as a T cell) expressing the anti-GPC3 CAR further comprises a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is inducible upon signaling through the anti-GPC3 CAR. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-nGPC3 antibody moiety is a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the

administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the administration comprises local injection an injection site distal to a site of the GPC3-positive disease (such as an GPC3-positive tumor). In some embodiments, the injection site is a first GPC3-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the injection site is a first GPC3-positive tumor and the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the individual is human.

[0601] In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or

sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that competes for binding to GPC3 (*e.g.*, nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, there is provided a method of treating an GPC3-positive disease in an

individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR comprising a) an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, b) a transmembrane domain, and c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence. In some embodiments, the anti-GPC3 CAR comprises an extracellular domain comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3) described herein fused to the N-terminus of SEQ ID NO: 494. In some embodiments, the effector cell (such as a T cell) expressing the anti-GPC3 CAR further comprises a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is inducible upon signaling through the anti-GPC3 CAR. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-GPC3 antibody moiety is a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-GPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-GPC3 antibody moiety is an scFv. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for

example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the administration comprises local injection an injection site distal to a site of the GPC3-positive disease (such as an GPC3-positive tumor). In some embodiments, the injection site is a first GPC3-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the injection site is a first GPC3-positive tumor and the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the individual is human.

[0602] In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3 (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the

amino acid sequence of SEQ ID NO: 462. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 463. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 1-358 of SEQ ID NO: 460 (SEQ ID NO: 468). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 464. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-560 of SEQ ID NO: 460 (SEQ ID NO: 465). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 359-580 of SEQ ID NO: 460 (SEQ ID NO: 466). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope spanning the Furin cleavage site at amino acids R^{358}/S^{359} of SEQ ID NO: 460. In some embodiments, the anti-nGPC3 antibody moiety can specifically bind to a full-length mature human GPC3 (e.g., amino acids 25-560 or 25-580 of SEQ ID NO: 460) but does not bind to an N-terminal fragment of human GPC3 (*e.g.*, amino acids 25-358 of SEQ ID NO: 460) or to a C-terminal fragment of human GPC3 (*e.g.*, amino acids 359-560 or 359-580 of SEQ ID NO: 460). In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a

naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-nGPC3 antibody moiety (*e.g.*, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity) that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising V_H and C_H1 antibody domains and a first T cell receptor domain (TCRD) comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising V_L and C_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H and C_H1 domains of the first antigen-binding domain and the V_L and C_L domains of the second antigen-binding domain form an antigen-binding module that specifically binds to cell surface bound-GPC3 (*e.g.*, the binding affinity of the V_H and V_L to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the V_H and V_L specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), and wherein the first TCRD and the second TCRD form a T cell receptor

module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the antigen-binding module comprises a disulfide bond between a residue in the C_H1 domain and a residue in the C_L domain. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising a V_H antibody domain and a first TCRD comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a V_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H domain of the first antigen-binding domain and the V_L domain of the second antigen-binding domain form an antigen-binding module that specifically binds to cell surface bound-GPC3 (*e.g.*, the binding affinity of the V_H and V_L to a cell surface-bound GPC3 is higher than that to a soluble GPC3, or the V_H and V_L specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity), wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the effector cell (such as a T cell) expressing the anti-GPC3 caTCR further comprises a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is inducible upon signaling through the anti-GPC3 caTCR. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the first TCR-TM is derived from one of the transmembrane domains of a naturally occurring T cell receptor (such as an αβ TCR or a γδ TCR). In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-nGPC3 antibody moiety is a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma,

or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the administration comprises local injection an injection site distal to a site of the GPC3-positive disease (such as an GPC3-positive tumor). In some embodiments, the injection site is a first GPC3-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the injection site is a first GPC3-positive tumor and the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the individual is human.

[0603] In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM

derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody

moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that specifically binds to a cell surface bound-GPC3, comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-nGPC3 antibody moiety that competes for binding to nGPC3 with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-nGPC3 antibody moiety that specifically binds to the same, or substantially the same, nGPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an

αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337 and C_H1 antibody domains and a first T cell receptor domain (TCRD) comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388 and C_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H and C_H1 domains of the first antigen-binding domain and the V_L and C_L domains of the second antigen-binding domain form an antigen-binding module that specifically binds to cell surface bound-GPC3, and wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the antigen-binding module comprises a disulfide bond between a residue in the C_H1 domain and a residue in the C_L domain. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising a V_H antibody domain comprising the amino acid sequence of any one of SEQ ID NOs: 307-337 and a first TCRD comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a V_L antibody domains comprising the amino acid sequence of any one of SEQ ID NOs: 358-388 and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H domain of the first antigen-binding domain and the V_L domain of the second antigen-binding domain form an antigen-binding module that specifically binds to cell surface bound-GPC3, wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the anti-GPC3 caTCR comprises two polypeptide chains, comprising amino acid sequences of SEQ ID NO:

492 and SEQ ID NO: 493. In some embodiments, the effector cell (such as a T cell) expressing the anti-GPC3 caTCR further comprises a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is inducible upon signaling through the anti-GPC3 caTCR. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the first TCR-TM is derived from one of the transmembrane domains of a naturally occurring T cell receptor (such as an αβ TCR or a γδ TCR). In some embodiments, the anti-nGPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-nGPC3 antibody moiety is a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-nGPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-nGPC3 antibody moiety is an scFv. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the administration comprises local injection an injection site distal to a site of the GPC3-positive disease (such as an GPC3-positive tumor). In some embodiments, the injection site is a first GPC3-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the injection site is a first GPC3-positive tumor and the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the individual is human.

[0604] In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that

specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-

TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease (*e.g.*, HCC) in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that specifically binds to GPC3 (*e.g.*, nGPC3 and/or sGPC3), comprising the HC-CDRs of a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, and the LC-CDRs of a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the

individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that competes for binding to GPC3 (*e.g.*, nGPC3 and/or sGPC3) with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising a) an antigen-binding module comprising an anti-GPC3 antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with any one of the anti-GPC3 constructs described herein, and b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) derived from one of the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR) and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), and wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357 and C_H1 antibody domains and a first T cell receptor domain (TCRD) comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408 and C_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H and C_H1 domains of the first antigen-binding domain and the V_L and C_L domains of the second antigen-binding domain form an antigen-binding

module that specifically binds to GPC3 (e.g., nGPC3 and/or sGPC3), and wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the antigen-binding module comprises a disulfide bond between a residue in the C_H1 domain and a residue in the C_L domain. In some embodiments, there is provided a method of treating an GPC3-positive disease in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising a V_H antibody domain comprising the amino acid sequence of any one of SEQ ID NOs: 338-357 and a first TCRD comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a V_L antibody domains comprising the amino acid sequence of any one of SEQ ID NOs: 389-408 and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H domain of the first antigen-binding domain and the V_L domain of the second antigen-binding domain form an antigen-binding module that specifically binds to GPC3 (e.g., nGPC3 and/or sGPC3), wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some embodiments, the effector cell (such as a T cell) expressing the anti-GPC3 caTCR further comprises a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is inducible upon signaling through the anti-GPC3 caTCR. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter. In some embodiments, the first TCR-TM is derived from one of the transmembrane domains of a naturally occurring T cell receptor (such as an αβ TCR or a γδ TCR). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a cell surface-bound GPC3. In some embodiments, the binding affinity of the anti-nGPC3 antibody moiety to a cell surface-bound GPC3 is higher than that to a soluble GPC3. In some embodiments, the anti-nGPC3 antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity. In some embodiments, the anti-GPC3 antibody moiety specifically recognizes a soluble GPC3. In some embodiments, the binding affinity of the anti-sGPC3 antibody moiety to a soluble GPC3 is

higher than that to a cell surface-bound GPC3. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within the amino acid sequence of SEQ ID NO: 461. In some embodiments, the anti-sGPC3 antibody moiety specifically recognizes an epitope within amino acids 510-560 of SEQ ID NO: 460 (SEQ ID NO: 467). In some embodiments, the anti-GPC3 antibody moiety specifically recognizes both cell surface-bound GPC3 and soluble GPC3. In some embodiments, the anti-GPC3 antibody moiety is chimeric, human, partially humanized, fully humanized, or semi-synthetic. In some embodiments, the anti-GPC3 antibody moiety is a Fab, a Fab', a F(ab')2, an Fv, or an scFv. In some embodiments, the anti-GPC3 antibody moiety is a Fab or a Fab'. In some embodiments, the anti-GPC3 antibody moiety is an scFv. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is HCC and the treating comprises preventing the spread of the cancer, *e.g.*, inhibiting (such as preventing) metastasis of the cancer. In some embodiments, the cancer is metastatic HCC. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the administration comprises local injection an injection site distal to a site of the GPC3-positive disease (such as an GPC3-positive tumor). In some embodiments, the injection site is a first GPC3-positive tumor (*i.e.*, the administration is via intratumoral route). In some embodiments, the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the injection site is a first GPC3-positive tumor and the site of the GPC3-positive disease is a second GPC3-positive tumor. In some embodiments, the individual is human.

[0605] In some embodiments, there is provided a method of treating metastatic HCC in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR or anti-GPC3 caTCR according to any of the embodiments described above. In some embodiments, the individual is human. In some embodiments, the effector cell (such as a T cell) expressing the anti-GPC3 CAR or anti-GPC3 caTCR further comprises a nucleic acid encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager)

is inducible upon signaling through the anti-GPC3 CAR or anti-GPC3 caTCR. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter.

[0606] In some embodiments, there is provided a method of inhibiting (such as preventing) metastasis of HCC in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR or anti-GPC3 caTCR according to any of the embodiments described above. In some embodiments, the individual is human. In some embodiments, the effector cell (such as a T cell) expressing the anti-GPC3 CAR or anti-GPC3 caTCR further comprises a nucleic acid encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) operably linked to an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 bispecific T cell engager) is inducible upon signaling through the anti-GPC3 CAR or anti-GPC3 caTCR. In some embodiments, the inducible promoter is an NFAT-derived promoter, such as an NFAT-derived minimal promoter. In some embodiments, the inducible promoter is an IL-2 promoter.

[0607] In some embodiments, there is provided a method of priming T cells in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 CAR according to any of the anti-GPC3 CARs described above. In some embodiments, there is provided a method of priming T cells in an individual comprising administering to the individual an effective amount of a composition comprising an effector cell (such as a T cell) expressing an anti-GPC3 caTCR according to any of the anti-GPC3 caTCRs described above. In some embodiments, the individual has an GPC3-positive disease. In some embodiments, the GPC3-positive disease is cancer. In some embodiments, the cancer is, for example, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the administration is via intravenous, intraperitoneal, or intratumoral route. In some embodiments, the administration is via intravenous route. In some embodiments, the administration is via intratumoral route. In some embodiments, the administration is to an injection site distal to a first disease site. In some embodiments, the individual is human.

[0608] In some embodiments, according to any of the methods described above, the method further comprising administering antigen presenting cells, or APCs, (such as monocytes or

monocyte-differentiated dendritic cells) to the individual. Dendritic cells can be generated ex vivo via culturing monocytes with specific cytokines (Palucka and Banchereau, *Nature Reviews Cancer 12:265-277, 2012*). In some embodiments, the APCs are administered simultaneously with the effector cell composition. In some embodiments, the APCs are administered concurrently with the effector cell composition. In some embodiments, the APCs are administered sequentially with the effector cell composition. In some embodiments, the APCs are administered via the same route as the effector cell composition. In some embodiments, the APCs are administered to the same site as the effector cell composition. In some embodiments, the effector cell composition comprises the APCs.

Cancers

[0609] The anti-GPC3 constructs (such as isolated anti-GPC3 constructs), anti-GPC3 CAR cells, and anti-GPC3 caTCR cells in some embodiments can be useful for treating cancer. Cancers that may be treated using any of the methods described herein include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may comprise solid tumors. Types of cancers to be treated with the anti-GPC3 constructs, anti-GPC3 CAR cells, and anti-GPC3 caTCR cells of the invention include, but are not limited to, carcinoma, blastoma, sarcoma, benign and malignant tumors, and malignancies *e.g.*, sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

[0610] Solid tumors are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumors can be benign or malignant. Different types of solid tumors are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers (*e.g.*, lung squamous cell carcinoma), ovarian cancer, yolk sac tumor (endodermal sinus tumor), prostate cancer, hepatocellular carcinoma (HCC), squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, melanoma, and CNS tumors (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as

glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyogioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, neuroblastoma, retinoblastoma and brain metastases).

[0611] In some embodiments, the cancer that can be treated by the present invention is HCC. GPC3 is highly expressed in HCC and HCC cell lines, *e.g.*, HepG2, Hep3B, HT17, HuH6, HuH7 and PLC/PRF/5. GPC3 expression is correlated with poor prognosis in HCC. In some embodiments, the HCC is early stage HCC, non-metastatic HCC, primary HCC, advanced HCC, locally advanced HCC, metastatic HCC, HCC in remission, or recurrent HCC. In some embodiments, the HCC is localized resectable (*i.e.*, tumors that are confined to a portion of the liver that allows for complete surgical removal), localized unresectable (*i.e.*, the localized tumors may be unresectable because crucial blood vessel structures are involved or because the liver is impaired), or unresectable (*i.e.*, the tumors involve all lobes of the liver and/or has spread to involve other organs (*e.g.*, lung, lymph nodes, bone). In some embodiments, the HCC is, according to TNM classifications, a stage I tumor (single tumor without vascular invasion), a stage II tumor (single tumor with vascular invasion, or multiple tumors, none greater than 5 cm), a stage III tumor (multiple tumors, any greater than 5 cm, or tumors involving major branch of portal or hepatic veins), a stage IV tumor (tumors with direct invasion of adjacent organs other than the gallbladder, or perforation of visceral peritoneum), N1 tumor (regional lymph node metastasis), or M1 tumor (distant metastasis). In some embodiments, the HCC is, according to AJCC (American Joint Commission on Cancer) staging criteria, stage T1, T2, T3, or T4 HCC. In some embodiments, the HCC is any one of liver cell carcinomas, fibrolamellar variants of HCC, and mixed hepatocellular cholangiocarcinomas.

[0612] In some embodiments, the GPC3-positive cancer is yolk sac tumor (YST, endodermal sinus tumor). In some embodiments, the GPC3-positive cancer is ovarian carcinoma. In some embodiments, the GPC3-positive cancer is choriocarcinoma. In some embodiments, the GPC3-positive cancer is testicular nonseminomatous germ cell tumor.

[0613] YST is the most common testicular tumor in children under 3 and is also known as infantile embryonal carcinoma. Unlike the pure form typical of infants, adult YSTs are often found in combination with other kinds of germ cell tumor, particularly teratoma and embryonal carcinoma. Although pure teratoma is usually benign, YST is malignant.

[0614] GPC3 is expressed in a less than 20% of ovarian carcinomas, but in more than 60% of ovarian clear-cell carcinomas, suggesting that GPC3 may represent a potential biomarker for

ovarian clear-cell carcinomas. GPC3 immunostaining may be beneficial in the diagnosis of YST and choriocarcinoma.

[0615] Choriocarcinoma is a malignant, trophoblastic cancer usually at placenta. Choriocarcinoma occurs rarely in primary locations other than the placenta; very rarely, it occurs in testicles. Pure choriocarcinoma of the adult testis is rare, but represents the most aggressive pathologic variant of germ cell tumors (GCTs) in adults. It is characterized with early hematogenous and lymphatic metastatic spread. Patients have very poor prognosis due to the early spread and inherent resistance to anticancer drugs.

[0616] GPC3 might be used as a tumor marker for the diagnosis of melanoma, especially in the early stages of the disease, since GPC3 mRNA and protein have been found in >80% of melanomas and melanocytic nevus.

[0617] Expression of GPC3 is also found in neuroblastoma, hepatoblastoma, Wilms' tumor cells, testicular nonseminomatous germ cell tumors, and a distinct group of gastric carcinomas called GPC3-GC. Thus, in some embodiments, the present invention can be used for treating or diagnosing these GPC3-positive diseases.

[0618] Cancer treatments can be evaluated by, *e.g.*, tumor regression, tumor weight or size shrinkage, time to progression, duration of survival, progression free survival, overall response rate, duration of response, quality of life, protein expression and/or activity. Approaches to determining efficacy of therapy can be employed, including for example, measurement of response through radiological imaging.

[0619] **Methods for Diagnosis, Detection and Imaging Using anti-GPC3 constructs**

[0620] Labeled anti-GPC3 antibody moieties and derivatives and analogs thereof (*e.g.*, anti-GPC3 immunoconjugates comprising a label), which specifically bind to an GPC3 (either cell surface-bound or soluble), can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or disorders associated with the expression, aberrant expression and/or activity of GPC3, including any of the diseases and disorders described above, such as cancer (*e.g.*, HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma). For example, the anti-GPC3 antibody moieties of the invention can be used in *in situ, in vivo, ex vivo,* and *in vitro* diagnostic assays or imaging assays.

[0621] Additional embodiments of the invention include methods of detecting GPC3 in a sample, comprising contacting the sample with an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, anti-GPC3 immunoconjugate comprising a label) described herein, and

detecting the presence of the label. In some embodiments, the sample comprises cells with cell surface-bound GPC3. In some embodiments, the sample comprises soluble GPC3.

[0622] Additional embodiments of the invention include methods of diagnosing a disease or disorder associated with expression or aberrant expression of GPC3 in an individual *(e.g.,* a mammal, such as a human). The methods comprise detecting cell surface-bound GPC3 or soluble GPC3 in the individual. In some embodiments, there is provided a method of diagnosing a disease or disorder associated with expression or aberrant expression of GPC3 in an individual (*e.g.,* a mammal, such as a human) comprising (a) administering an effective amount of a labeled anti-GPC3 antibody moiety (*e.g.*, anti-GPC3 immunoconjugate comprising a label) according to any of the embodiments described above to the individual; and (b) determining the level of the label in the individual, such that a level of the label above a threshold level indicates that the individual has the GPC3-positive disease or disorder. In some embodiments, the GPC3-positive disease or disorder is cancer. In some embodiments, the cancer is selected, for example, from the group consisting of HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, and liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is metastatic HCC. In some embodiments, the cancer is metastatic HCC, and the method further comprises determining the level of the label in the individual's blood. In some embodiments, there is provided a method of diagnosing HCC (*e.g.*, metastatic HCC) in an individual (*e.g.,* a mammal, such as a human) comprising (a) administering an effective amount of a labeled anti-GPC3 antibody moiety (*e.g.*, anti-GPC3 immunoconjugate comprising a label) according to any of the embodiments described above to the individual; and (b) determining the level of the label in the individual, such that a level of the label above a threshold level indicates that the individual has HCC (*e.g.*, metastatic HCC). The threshold level can be determined by various methods, including, for example, by detecting the label according to the method of diagnosing described above in a first set of individuals that have the disease or disorder and a second set of individuals that do not have the disease or disorder, and setting the threshold to a level that allows for discrimination between the first and second sets. In some embodiments, the threshold level is zero, and the method comprises determining the presence or absence of the label in the individual. In some embodiments, the method further comprises waiting for a time interval following the administering of step (a) to permit the labeled anti-GPC3 antibody moiety (*e.g.*, anti-GPC3 immunoconjugate comprising a label) to preferentially concentrate at sites in the individual where GPC3 is expressed (*e.g.*,

GPC3-positive tumor site, and for unbound labeled anti-GPC3 antibody moiety to be cleared). In some embodiments, the method further comprises subtracting a background level of the label. Background level can be determined by various methods, including, for example, by detecting the label in the individual prior to administration of the labeled anti-GPC3 antibody moiety (*e.g.*, anti-GPC3 immunoconjugate comprising a label), or by detecting the label according to the method of diagnosing described above in an individual that does not have the disease or disorder. In some embodiments, the individual is human.

[0623] In some embodiments, there is provided a method of diagnosing a disease or disorder associated with expression or aberrant expression of GPC3 in an individual (*e.g.,* a mammal, such as a human), comprising (a) contacting a labeled anti-GPC3 antibody moiety (*e.g.*, anti-GPC3 immunoconjugate comprising a label) according to any of the embodiments described above with a sample (such as whole blood or homogenized tissue) derived from the individual; and (b) determining the number of cells bound with the labeled anti-GPC3 antibody moiety in the sample, such that a value for the number of cells bound with the labeled anti-GPC3 antibody moiety above a threshold level indicates that the individual has the disease or disorder. In some embodiments, the disease or disorder is cancer. In some embodiments, the cancer is selected, for example, from the group consisting of HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, and liposarcoma. In some embodiments, the cancer is HCC. In some embodiments, the cancer is metastatic HCC, and the sample is a blood sample (such as whole blood). In some embodiments, the cancer is metastatic HCC. In some embodiments, there is provided a method of diagnosing HCC (*e.g.*, metastatic HCC) in an individual (*e.g.,* a mammal, such as a human), comprising (a) contacting a labeled anti-GPC3 antibody moiety according to any of the embodiments described above with a sample (such as whole blood) derived from the individual; and (b) determining the number of cells bound with the labeled anti-GPC3 antibody moiety in the sample, such that a value for the number of cells bound with the labeled anti-GPC3 antibody moiety above a threshold level indicates that the individual has HCC (*e.g.*, metastatic HCC). The threshold level can be determined by various methods, including, for example, by determining the number of cells bound with the labeled anti-GPC3 antibody moiety according to the method of diagnosing described above in a first set of individuals that have the disease or disorder and a second set of individuals that do not have the disease or disorder, and setting the threshold to a level that allows for discrimination between the first and second sets. In some embodiments, the threshold

level is zero, and the method comprises determining the presence or absence of cells bound with the labeled anti-GPC3 antibody moiety in the sample. In some embodiments, the method further comprises subtracting a background level of the number of cells bound with the labeled anti-GPC3 antibody moiety. Background level can be determined by various methods, including, for example, by determining the number of cells bound with the labeled anti-GPC3 antibody moiety in the individual prior to administration of the labeled anti-GPC3 antibody moiety, or by determining the number of cells bound with the labeled anti-GPC3 antibody moiety according to the method of diagnosing described above in an individual that does not have the disease or disorder. In some embodiments, the individual is human.

[0624] Anti-GPC3 antibody moieties of the invention can be used to assay levels of GPC3 in a biological sample using methods known to those of skill in the art. Suitable antibody labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), samarium (^{153}Sm), lutetium (^{177}Lu), gadolinium (^{159}Gd), promethium (^{149}Pm), lanthanum (^{140}La), ytterbium (^{175}Yb) , holmium (^{166}Ho), yttrium (^{90}Y), scandium (^{47}Sc), rhenium (^{186}Re, ^{188}Re), praseodymium (^{142}Pr), rhodium (^{105}Rh), and ruthenium (^{97}Ru); luminol; fluorescent labels, such as fluorescein and rhodamine; and biotin.

[0625] Techniques known in the art may be applied to labeled anti-GPC3 antibody moieties (*e.g.*, anti-GPC3 immunoconjugate comprising a label) of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see *e.g.,* U.S. Pat. Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003). Aside from the above assays, various *in vivo* and *ex vivo* assays are available to the skilled practitioner. For example, one can expose cells within the body of the subject to an anti-GPC3 antibody moiety which is optionally labeled with a detectable label, *e.g.,* a radioactive isotope, and binding of the anti-GPC3 antibody moiety to the cells can be evaluated, *e.g.,* by external scanning for radioactivity or by analyzing a sample (*e.g.*, a biopsy or other biological sample) derived from a subject previously exposed to the anti-GPC3 antibody moiety.

Articles of Manufacture and Kits

[0626] In some embodiments of the invention, there is provided an article of manufacture containing materials useful for the treatment of an GPC3-positive disease such as cancer (for

example HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma), for delivering an anti-GPC3 construct (such as an isolated anti-GPC3 construct) to a cell expressing GPC3 on its surface, or for isolation or detection of cells expressing GPC3 on cell surface, or for isolation or detection of soluble GPC3 in an individual. The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. Generally, the container holds a composition which is effective for treating a disease or disorder described herein and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-GPC3 construct of the invention. The label or package insert indicates that the composition is used for treating the particular condition. The label or package insert will further comprise instructions for administering the anti-GPC3 construct composition to the patient. Articles of manufacture and kits comprising combinatorial therapies described herein are also contemplated.

[0627] Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. In some embodiments, the package insert indicates that the composition is used for treating cancer (such as HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, or liposarcoma).

[0628] Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0629] Kits are also provided that are useful for various purposes, *e.g.*, for treatment of an GPC3-positive disease or disorder described herein, for delivering an anti-GPC3 construct (such as an isolated anti-GPC3 construct) to a cell expressing GPC3 on its surface, or for isolation or detection of cells expressing GPC3 on cell surface, or for isolation or detection of soluble GPC3 in an individual, optionally in combination with the articles of manufacture. Kits of the invention

include one or more containers comprising an anti-GPC3 construct composition (or unit dosage form and/or article of manufacture), and in some embodiments, further comprise another agent (such as the agents described herein) and/or instructions for use in accordance with any of the methods described herein. The kit may further comprise a description of selection of individuals suitable for treatment. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk) are also acceptable.

[0630] For example, in some embodiments, the kit comprises a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3 scFv, a full-length anti-GPC3 antibody, a multi-specific anti-GPC3 molecule (such as a bispecific anti-GPC3 antibody), or an anti-GPC3 immunoconjugate). In some embodiments, the kit comprises a) a composition comprising an anti-GPC3 construct, and b) an effective amount of at least one other agent, wherein the other agent enhances the effect (*e.g.*, treatment effect, detecting effect) of the anti-GPC3 construct. In some embodiments, the kit comprises a) a composition comprising an anti-GPC3 construct, and b) instructions for administering the anti-GPC3 construct composition to an individual for treatment of an GPC3-positive disease, such as HCC. In some embodiments, the kit comprises a) a composition comprising an anti-GPC3 construct, b) an effective amount of at least one other agent, wherein the other agent enhances the effect (*e.g.*, treatment effect, detecting effect) of the anti-GPC3 construct, and c) instructions for administering the anti-GPC3 construct composition and the other agent(s) to an individual for treatment of an GPC3-positive disease, such as HCC. The anti-GPC3 construct and the other agent(s) can be present in separate containers or in a single container. For example, the kit may comprise one distinct composition or two or more compositions wherein one composition comprises an anti-GPC3 construct and another composition comprises another agent.

[0631] In some embodiments, the kit comprises a) a composition comprising an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3 scFv, a full-length anti-GPC3 antibody, a multi-specific anti-GPC3 molecule (such as a bispecific anti-GPC3 antibody), or an anti-GPC3 immunoconjugate), and b) instructions for combining the anti-GPC3 construct with cells (such as effector cells, *e.g.*, immune cells, derived from an individual, CAR-T cell, or caTCR-T cell) to form a composition comprising anti-GPC3 construct/cell conjugates and administering the anti-GPC3 construct/cell conjugate composition to the individual for treatment of an GPC3-positive disease (such as HCC). In some embodiments, the kit comprises a) a

composition comprising an anti-GPC3 construct, and b) a cell (such as a cytotoxic cell, *e.g.*, cytotoxic T cell, CAR-T cell, or caTCR-T cell). In some embodiments, the kit comprises a) a composition comprising an anti-GPC3 construct, b) a cell (such as a cytotoxic cell, *e.g.*, cytotoxic T cell, CAR-T cell, or caTCR-T cell), and c) instructions for combining the anti-GPC3 construct with the cell to form a composition comprising anti-GPC3 construct/cell conjugates and administering the anti-GPC3 construct/cell conjugate composition to an individual for the treatment of an GPC3-positive disease, such as HCC. In some embodiments, the kit comprises a composition comprising an anti-GPC3 construct in association with a cell (such as a cytotoxic cell, *e.g.*, cytotoxic T cell, CAR-T cell, or caTCR-T cell). In some embodiments, the kit comprises a) a composition comprising an anti-GPC3 construct in association with a cell (such as a cytotoxic cell, *e.g.*, cytotoxic T cell, CAR-T cell, or caTCR-T cell), and b) instructions for administering the composition to an individual for the treatment of an GPC3-positive disease, such as HCC. In some embodiments, the association is by conjugation of the anti-GPC3 construct to a molecule on the surface of the cell. In some embodiments, the association is by insertion of a portion of the anti-GPC3 construct (*e.g.*, anti-GPC3 CAR, anti-GPC3 caTCR) into the outer membrane of the cell.

[0632] In some embodiments, the kit comprises a nucleic acid (or set of nucleic acids) encoding an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3 scFv, a full-length anti-GPC3 antibody, a multi-specific anti-GPC3 molecule (such as a bispecific anti-GPC3 antibody), an anti-GPC3 CAR, an anti-GPC3caTCR, or an anti-GPC3 immunoconjugate) or polypeptide portions thereof. In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding an anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof, and b) a host cell (such as an effector cell, *e.g.*, T cell, CAR-T cell, or caTCR-T cell) for expressing the nucleic acid (or set of nucleic acids). In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding an anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof, and b) instructions for i) expressing the anti-GPC3 construct in a host cell (such as an effector cell, *e.g.*, a T cell, CAR-T cell, or caTCR-T cell), ii) preparing a composition comprising the anti-GPC3 construct or the host cell expressing the anti-GPC3 construct, and iii) administering the composition comprising the anti-GPC3 construct or the host cell expressing the anti-GPC3 construct to an individual for the treatment of an GPC3-positive disease, such as HCC. In some embodiments, the host cell is derived from the individual. In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding an anti-

GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof, b) a host cell (such as an effector cell, *e.g.*, T cell, CAR-T cell, or caTCR-T cell) for expressing the nucleic acid (or set of nucleic acids), and c) instructions for i) expressing the anti-GPC3 construct in the host cell, ii) preparing a composition comprising the anti-GPC3 construct or the host cell expressing the anti-GPC3 construct, and iii) administering the composition comprising the anti-GPC3 construct or the host cell expressing the anti-GPC3 construct to an individual for the treatment of an GPC3-positive disease, such as HCC. In some embodiments, the expression of the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof is inducible. In some embodiments, the nucleic acid (or set of nucleic acids) encoding an anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof are under control of inducible promoter(s). In some embodiments, the expression of the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof in the effector cell (*e.g.*, T cell, CAR-T cell, caTCR T cell) is inducible upon signaling through a signaling receptor on the effector cell (*e.g.*, TCR, CAR, caTCR). For example, in some embodiments, the nucleic acid (or set of nucleic acids) encoding the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof are under control of inducible promoter(s) responsive to signaling through CAR on the CAR-T cell, upon introducing the nucleic acid (or set of nucleic acids) encoding the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof into the CAR-T cell, and introducing the composition comprising the anti-GPC3 construct or the host cell expressing the anti-GPC3 construct to an individual carrying GPC3-positive disease according to the kit instructions. In some embodiments, the nucleic acid (or set of nucleic acids) encoding the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof are under control of inducible promoter(s) responsive to signaling through caTCR on an caTCR-T cell, upon introducing the nucleic acid (or set of nucleic acids) encoding the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof into the caTCR-T cell, and introducing the composition comprising the anti-GPC3 construct or the host cell expressing the anti-GPC3 construct to an individual carrying GPC3-positive disease according to the kit instructions. In some embodiments, the nucleic acid (or set of nucleic acids) encoding the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof is operably linked to an NFAT-derived promoter. In some embodiments, the NFAT-derived promoter is an NFAT-derived minimal promoter. In some embodiments, the nucleic acid

(or set of nucleic acids) encoding the anti-GPC3 construct (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) or polypeptide portions thereof is operably linked to an IL-2 promoter.

[0633] In some embodiments, the kit comprises a nucleic acid encoding an anti-GPC3 CAR. In some embodiments, the kit comprises a vector comprising a nucleic acid encoding an anti-GPC3 CAR. In some embodiments, the kit comprises a) a vector comprising a nucleic acid encoding an anti-GPC3 CAR, and b) instructions for i) introducing the vector into effector cells, such as T cells derived from an individual, ii) preparing a composition comprising the anti-GPC3 CAR effector cells, and iii) administering the anti-GPC3 CAR effector cell composition to the individual for treatment of an GPC3-positive disease, such as HCC.

[0634] In some embodiments, the kit comprises a first nucleic acid encoding an anti-GPC3 CAR, and a second nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager). In some embodiments, the kit comprises a first vector comprising a nucleic acid encoding an anti-GPC3 CAR, and a second vector comprising a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager). In some embodiments, the kit comprises a vector comprising a first nucleic acid encoding an anti-GPC3 CAR, and a second nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager). In some embodiments, the kit comprises a) a first vector comprising a nucleic acid encoding an anti-GPC3 CAR, and a second vector comprising a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager), and b) instructions for i) introducing the vectors into effector cells, such as T cells derived from an individual, ii) preparing a composition comprising the anti-GPC3 CAR effector cells and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule, and iii) administering the anti-GPC3 CAR effector cell and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule composition to the individual for treatment of an GPC3-positive disease, such as HCC. In some embodiments, the kit comprises a) a vector comprising a first nucleic acid encoding an anti-GPC3 CAR, and a second nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager), and b) instructions for i) introducing the vector into effector cells, such as T cells derived from an individual, ii) preparing a composition comprising the anti-GPC3 CAR effector cells and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule, and iii) administering the anti-GPC3 CAR effector cell and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule composition to the individual for treatment of an GPC3-positive disease, such as HCC. In some embodiments, the

nucleic acid encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is under control of an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is inducible through the signaling of the anti-GPC3-CAR (e.g., in a GPC3-positive disease environment). In some embodiments, the nucleic acid encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is under control of an NFAT-derived promoter. In some embodiments, the NFAT-derived promoter is an NFAT-derived minimal promoter. In some embodiments, the nucleic acid encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is under control of an IL-2 promoter.

[0635] In some embodiments, the kit comprises a nucleic acid encoding an anti-GPC3 caTCR. In some embodiments, the kit comprises a vector comprising a nucleic acid encoding an anti-GPC3 caTCR. In some embodiments, the kit comprises a) a vector comprising a nucleic acid encoding an anti-GPC3 caTCR, and b) instructions for i) introducing the vector into effector cells, such as T cells derived from an individual, ii) preparing a composition comprising the anti-GPC3 caTCR effector cells, and iii) administering the anti-GPC3 caTCR effector cell composition to the individual for treatment of an GPC3-positive disease, such as HCC.

[0636] In some embodiments, the kit comprises a first nucleic acid encoding an anti-GPC3 caTCR, and a second nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager). In some embodiments, the kit comprises a first vector comprising a nucleic acid encoding an anti-GPC3 caTCR, and a second vector comprising a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager). In some embodiments, the kit comprises a vector comprising a first nucleic acid encoding an anti-GPC3 caTCR, and a second nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager). In some embodiments, the kit comprises a) a first vector comprising a nucleic acid encoding an anti-GPC3 caTCR, and a second vector comprising a nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager), and b) instructions for i) introducing the vectors into effector cells, such as T cells derived from an individual, ii) preparing a composition comprising the anti-GPC3 caTCR effector cells and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule, and iii) administering the anti-GPC3 caTCR effector cell and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule composition to the individual for treatment of an GPC3-

positive disease, such as HCC. In some embodiments, the kit comprises a) a vector comprising a first nucleic acid encoding an anti-GPC3 caTCR, and a second nucleic acid encoding a multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager), and b) instructions for i) introducing the vector into effector cells, such as T cells derived from an individual, ii) preparing a composition comprising the anti-GPC3 caTCR effector cells and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule, and iii) administering the anti-GPC3 caTCR effector cell and the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule composition to the individual for treatment of an GPC3-positive disease, such as HCC. In some embodiments, the nucleic acid encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is under control of an inducible promoter. In some embodiments, the expression of the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is inducible through the signaling of the anti-GPC3 caTCR (e.g., in a GPC3-positive disease environment). In some embodiments, the nucleic acid encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is under control of an NFAT-derived promoter. In some embodiments, the NFAT-derived promoter is an NFAT-derived minimal promoter. In some embodiments, the nucleic acid encoding the multi-specific (*e.g.*, bispecific) anti-GPC3 molecule (*e.g.*, anti-GPC3×CD3 tandem di-scFv T cell engager) is under control of an IL-2 promoter.

[0637] The kits of the invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, and the like.

[0638] The instructions relating to the use of the anti-GPC3 construct compositions generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. For example, kits may be provided that contain sufficient dosages of an anti-GPC3 construct (such as an isolated anti-GPC3 construct, *e.g.*, an anti-GPC3 scFv, a full-length anti-GPC3 antibody, a multi-specific anti-GPC3 molecule (such as a bispecific anti-GPC3 antibody), an anti-GPC3 CAR, an anti-GPC3 caTCR, or an anti-GPC3 immunoconjugate) as disclosed herein to provide effective treatment of an individual for an extended period, such as any of a week, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8

weeks, 3 months, 4 months, 5 months, 7 months, 8 months, 9 months, or more. Kits may also include multiple unit doses of the anti-GPC3 construct and pharmaceutical compositions and instructions for use and packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies.

[0639] Those skilled in the art will recognize that several embodiments are possible within the scope and spirit of this invention. The invention will now be described in greater detail by reference to the following non-limiting examples. The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXEMPLARY EMBODIMENTS

[0640] Embodiment 1. An isolated anti-Glypican 3 (GPC3) construct comprising an antibody moiety specifically recognizing a cell surface-bound GPC3.

[0641] Embodiment 2. The isolated anti-GPC3 construct of embodiment 1, wherein the antibody moiety specifically recognizes a cell surface-bound GPC3 at a high binding affinity and binds to a soluble GPC3 at a low binding affinity.

[0642] Embodiment 3. The isolated anti-GPC3 construct of embodiment 2, wherein the K_d of the anti-GPC3 construct to the cell surface-bound GPC3 is about 0.1 nM to about 2 nM.

[0643] Embodiment 4. The isolated anti-GPC3 construct of embodiment 2 or 3, wherein the IC50 of a soluble GPC3 to compete binding between the anti-GPC3 construct and the cell surface-bound GPC3 is about 1 μg/ml to about 100 μg/ml.

[0644] Embodiment 5. The isolated anti-GPC3 construct of embodiment 1, wherein the antibody moiety specifically recognizes a cell surface-bound GPC3 at a low binding affinity, and binds to a soluble GPC3 at a high binding affinity.

[0645] Embodiment 6. The isolated anti-GPC3 construct of embodiment 5, wherein the K_d of the anti-GPC3 construct to the cell surface-bound GPC3 is about 10 nM to about 100 nM.

[0646] Embodiment 7. The isolated anti-GPC3 construct of embodiment 5 or 6, wherein the IC50 of a soluble GPC3 to compete binding between the anti-GPC3 construct and the cell surface-bound GPC3 is about 0.01 μg/ml to about 1 μg/ml.

[0647] Embodiment 8. The isolated anti-GPC3 construct of any one of embodiments 3-7, wherein the cell surface-bound GPC3 is GPC3 expressed on HepG2 cells.

[0648] Embodiment 9. The isolated anti-GPC3 construct of any one of embodiments 1-8, wherein the cell surface-bound GPC3 comprises the amino acid sequence of SEQ ID NO: 460 or SEQ ID NO: 462.

[0649] Embodiment 10. The isolated anti-GPC3 construct of any one of embodiments 1-9, wherein the soluble GPC3 comprises the amino acid sequence of SEQ ID NO: 461 or SEQ ID NO: 463.

[0650] Embodiment 11. The isolated anti-GPC3 construct of any one of embodiments 1-10, wherein the antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of SEQ ID NO: 460.

[0651] Embodiment 12. The isolated anti-GPC3 construct of embodiment 11, wherein the antibody moiety specifically recognizes an epitope within amino acids 1-560 of SEQ ID NO: 460 (SEQ ID NO: 461).

[0652] Embodiment 13. The isolated anti-GPC3 construct of embodiment 11, wherein the antibody moiety specifically recognizes an epitope within amino acids 25-580 of SEQ ID NO: 460 (SEQ ID NO: 462).

[0653] Embodiment 14. The isolated anti-GPC3 construct of embodiment 11 or 12, wherein the antibody moiety specifically recognizes an epitope within amino acids 25-560 of SEQ ID NO: 460 (SEQ ID NO: 463).

[0654] Embodiment 15. The isolated anti-GPC3 construct of any one of embodiments 1-15, wherein the antibody moiety specifically recognizes an epitope within the N-terminal fragment of GPC3.

[0655] Embodiment 16. The isolated anti-GPC3 construct of embodiment 15, wherein the antibody moiety specifically recognizes an epitope within amino acids 25-358 of SEQ ID NO: 460 (SEQ ID NO: 464).

[0656] Embodiment 17. The isolated anti-GPC3 construct of any one of embodiments 1-15, wherein the antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3.

[0657] Embodiment 18. The isolated anti-GPC3 construct of embodiment17, wherein the antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain.

[0658] Embodiment 19. The isolated anti-GPC3 construct of any one of embodiments 1-18, wherein the antibody moiety does not specifically bind to the same or substantially the same GPC3 epitope competitively with GC33.

[0659] Embodiment 20. The isolated anti-GPC3 construct of embodiment 19, wherein the antibody moiety does not bind to an epitope within amino acids 524-565 of SEQ ID NO: 460 (SEQ ID NO: 536).

[0660] Embodiment 21. The isolated anti-GPC3 construct of embodiment 19 or 20, wherein the antibody moiety does not bind to a fragment comprising amino acids 524-565 of SEQ ID NO: 460 (SEQ ID NO: 536).

[0661] Embodiment 22. The isolated anti-GPC3 construct of any one of embodiments 1-21, wherein the GPC3 is expressed on the surface of a cell selected from the group consisting of HepG2, Hep3B, Huh7, JHH-7, and 293.

[0662] Embodiment 23. The isolated anti-GPC3 construct of any one of embodiments 1-22, wherein the GPC3 is expressed on the surface of a cancer cell.

[0663] Embodiment 24. The isolated anti-GPC3 construct of embodiment 23, wherein the cancer cell is a liver cancer cell.

[0664] Embodiment 25. The isolated anti-GPC3 construct of embodiment 24, wherein the cancer cell is hepatocellular carcinoma (HCC).

[0665] Embodiment 26. An isolated anti-GPC3 construct comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, wherein the antibody moiety comprises: i) a heavy chain variable domain (V_H) comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, or a variant thereof comprising up to about 5 amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, or a variant thereof comprising up to about 5 amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133, or a variant thereof comprising up to about 5 amino acid substitutions; and ii) a light chain variable domain (V_L) comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, or a variant thereof comprising up to about 5 amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 205-235, or a variant thereof comprising up to about 3 amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286, or a variant thereof comprising up to about 5 amino acid substitution.

[0666] Embodiment 27. The isolated anti-GPC3 construct of embodiment 26, wherein the antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 52-82, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, an LC-CDR2 comprising the amino acid

sequence of any one of SEQ ID NOs: 205-235, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 256-286.

[0667] Embodiment 28. The isolated anti-GPC3 construct of embodiment 26 or 27, wherein the antibody moiety comprises: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337, or a variant thereof having at least about 95% sequence identify to any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388, or a variant thereof having at least about 95% sequence identity to any one of SEQ ID NOs: 358-388.

[0668] Embodiment 29. The isolated anti-GPC3 construct of embodiment 28, wherein the antibody moiety comprises: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 358-388.

[0669] Embodiment 30. An isolated anti-GPC3 construct comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, wherein the antibody moiety comprises the HC-CDRs of V_H comprising the amino acid sequence of any one of SEQ ID NOs: 307-337; and LC-CDRs of V_L comprising the amino acid sequence of any one of SEQ ID Nos: 358-388.

[0670] Embodiment 31. An isolated anti-GPC3 construct comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, wherein the antibody moiety comprises: i) a VH comprising the amino acid sequence of any one of SEQ ID NOs: 1-31, the amino acid sequence of any one of SEQ ID NOs: 52-82, and the amino acid sequence of any one of SEQ ID NOs: 103-133; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 154-184, the amino acid sequence of any one of SEQ ID NOs: 205-235, and the amino acid sequence of any one of SEQ ID NOs: 256-286.

[0671] Embodiment 32. An isolated anti-GPC3 construct comprising an antibody moiety specifically recognizing GPC3, wherein the antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, or a variant thereof comprising up to about 5 amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, or a variant thereof comprising up to about 5 amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153, or a variant thereof comprising up to about 5 amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, or a variant thereof comprising up to about 5 amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, or a variant

thereof comprising up to about 3 amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306, or a variant thereof comprising up to about 5 amino acid substitutions.

[0672] Embodiment 33. The isolated anti-GPC3 construct of embodiment 32, wherein the antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 83-102, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 236-255, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 287-306.

[0673] Embodiment 34. The isolated anti-GPC3 construct of embodiment 32 or 33, wherein the antibody moiety comprises: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357, or a variant thereof having at least about 95% sequence identify to any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408, or a variant thereof having at least about 95% sequence identity to any one of SEQ ID NOs: 389-408.

[0674] Embodiment 35. The isolated anti-GPC3 construct of embodiment 34, wherein the antibody moiety comprises: i) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408.

[0675] Embodiment 36. An isolated anti-GPC3 construct comprising an antibody moiety specifically recognizing GPC3, wherein the antibody moiety comprises the HC-CDRs of V_H comprising the amino acid sequence of any one of SEQ ID NOs: 338-357; and LC-CDRs of V_L comprising the amino acid sequence of any one of SEQ ID NOs: 389-408.

[0676] Embodiment 37. An isolated anti-GPC3 construct comprising an antibody moiety specifically recognizing a cell surface-bound GPC3, wherein the antibody moiety comprises: i) a VH comprising the amino acid sequence of any one of SEQ ID NOs: 32-51, the amino acid sequence of any one of SEQ ID NOs: 83-102, and the amino acid sequence of any one of SEQ ID NOs: 134-153; and ii) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 185-204, the amino acid sequence of any one of SEQ ID NOs: 236-255, and the amino acid sequence of any one of SEQ ID NOs: 287-306.

[0677] Embodiment 38. An isolated anti-GPC3 construct comprising an antibody moiety that specifically binds to GPC3 competitively with the isolated anti-GPC3 construct of any one of embodiments 1-37.

[0678] Embodiment 39. An isolated anti-GPC3 construct comprising an antibody moiety that specifically binds to the same, or substantially the same, GPC3 epitope competitively with the isolated anti-GPC3 construct of any one of embodiments 1-37.

[0679] Embodiment 40. The isolated anti-GPC3 construct of any one of embodiments 1-39, wherein the antibody moiety specifically recognizing GPC3 is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

[0680] Embodiment 41. The isolated anti-GPC3 construct of any one of embodiments 1-40, wherein the antibody moiety specifically recognizing GPC3 is a full-length antibody, a Fab, a Fab', a F(ab')2, an Fv, or a single chain Fv (scFv).

[0681] Embodiment 42. The isolated anti-GPC3 construct of embodiment 41, wherein the antibody moiety specifically recognizing GPC3 is an scFv.

[0682] Embodiment 43. The isolated anti-GPC3 construct of embodiment 41, wherein the antibody moiety specifically recognizing GPC3 is a Fab or Fab'.

[0683] Embodiment 44. The isolated anti-GPC3 construct of any one of embodiments 1-43, wherein the antibody moiety specifically recognizing GPC3 is fused to an Fc fragment optionally via a linker.

[0684] Embodiment 45. The isolated anti-GPC3 construct of embodiment 44, wherein the Fc fragment is an IgG1 Fc fragment.

[0685] Embodiment 46. The isolated anti-GPC3 construct of any one of embodiments 1-40 and 44-45, wherein the isolated anti-GPC3 construct is a full-length antibody.

[0686] Embodiment 47. The isolated anti-GPC3 construct of any one of embodiments 1-46, wherein the isolated anti-GPC3 construct is monospecific.

[0687] Embodiment 48. The isolated anti-GPC3 construct of any one of embodiments 1-46, wherein the isolated anti-GPC3 construct is multispecific.

[0688] Embodiment 49. The isolated anti-GPC3 construct of embodiment 48, wherein the isolated anti-GPC3 construct is bispecific.

[0689] Embodiment 50. The isolated anti-GPC3 construct of embodiment 48 or 49, wherein the isolated anti-GPC3 construct is a tandem scFv, a diabody (Db), a single chain diabody (scDb), a dual-affinity retargeting (DART) antibody, a F(ab')2, a dual variable domain (DVD)

antibody, a knob-into-hole (KiH) antibody, a dock and lock (DNL) antibody, a chemically cross-linked antibody, a heteromultimeric antibody, or a heteroconjugate antibody.

[0690] Embodiment 51. The isolated anti-GPC3 construct of embodiment 50, wherein the isolated anti-GPC3 construct is a tandem scFv comprising two scFvs linked by a peptide linker.

[0691] Embodiment 52. The isolated anti-GPC3 construct of embodiment 51, wherein the peptide linker comprises the amino acid sequence of TSGGGGS (SEQ ID NO: 474).

[0692] Embodiment 53. The isolated anti-GPC3 construct of any one of embodiments 48-52, wherein the isolated anti-GPC3 construct further comprises a second antibody moiety specifically recognizing a second antigen.

[0693] Embodiment 54. The isolated anti-GPC3 construct of embodiment 53, wherein the second antigen is an antigen on the surface of a T cell.

[0694] Embodiment 55. The isolated anti-GPC3 construct of embodiment 54, wherein the second antigen is selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM.

[0695] Embodiment 56. The isolated anti-GPC3 construct of embodiment 55, wherein the second antigen is CD3ε.

[0696] Embodiment 57. The isolated anti-GPC3 construct of embodiment 56, wherein the isolated anti-GPC3 construct is a tandem scFv comprising an N-terminal scFv specifically recognizing GPC3 and a C-terminal scFv specifically recognizing CD3ε.

[0697] Embodiment 58. The isolated anti-GPC3 construct of embodiment 53, wherein the second antigen is an antigen on the surface of a B cell, a natural killer cell, a dendritic cell, a macrophage, a monocyte, or a neutrophil.

[0698] Embodiment 59. The isolated anti-GPC3 construct of any one of embodiments 1-43, wherein the isolated anti-GPC3 construct is a chimeric antigen receptor (CAR) comprising: (a) an extracellular domain comprising the antibody moiety; (b) a transmembrane domain; and (c) an intracellular signaling domain.

[0699] Embodiment 60. The isolated anti-GPC3 construct of embodiment 59, wherein the intracellular signaling domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.

[0700] Embodiment 61. The isolated anti-GPC3 construct of any one of embodiments 1-43, wherein the isolated anti-GPC3 construct is a chimeric antibody-T cell receptor (TCR) construct (caTCR) comprising: (a) an extracellular domain comprising the antibody moiety; and (b) a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR

transmembrane domain (TCR-TM) and a second TCRD comprising a second TCR-TM, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule.

[0701] Embodiment 62. The isolated anti-GPC3 construct of embodiment 61, wherein the first TCR-TM is derived from one of the transmembrane domains of a first naturally occurring TCR and the second TCR-TM is derived from the other transmembrane domain of the first naturally occurring TCR.

[0702] Embodiment 63. The isolated anti-GPC3 construct of embodiment 61, wherein the at least one of the TCR-TMs is non-naturally occurring.

[0703] Embodiment 64. The isolated anti-GPC3 construct of embodiment 63, wherein the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the first naturally occurring T cell receptor transmembrane domains.

[0704] Embodiment 65. The isolated anti-GPC3 construct of embodiment 62, wherein the first naturally occurring TCR is a γ/δ TCR.

[0705] Embodiment 66. The isolated anti-GPC3 construct of embodiment 62, wherein the first naturally occurring TCR is an α/β TCR.

[0706] Embodiment 67. The isolated anti-GPC3 construct of any one of embodiments 61-66, wherein the TCR-associated signaling molecule is selected from the group consisting of CD3δε, CD3γε, and ζζ.

[0707] Embodiment 68. The isolated anti-GPC3 construct of any one of embodiments 1-58, wherein the isolated anti-GPC3 construct is an immunoconjugate comprising the antibody moiety and an effector molecule.

[0708] Embodiment 69. The isolated anti-GPC3 construct of embodiment 68, wherein the effector molecule is a therapeutic agent selected from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid.

[0709] Embodiment 70. The isolated anti-GPC3 construct of embodiment 69, wherein therapeutic agent is a drug or a toxin.

[0710] Embodiment 71. The isolated anti-GPC3 construct of embodiment 68, wherein the effector molecule is a label.

[0711] Embodiment 72. An isolated nucleic acid encoding the polypeptide components of the isolated anti-GPC3 construct of any one of embodiments 1-71.

[0712] Embodiment 73. A vector comprising the isolated nucleic acid of embodiment 72.

[0713] Embodiment 74. An isolated host cell comprising the anti-GPC3 construct of any one of embodiments 1-71, isolated nucleic acid of embodiment 72, or the vector of 73.

[0714] Embodiment 75. An effector cell expressing the isolated anti-GPC3 construct of any one of embodiments 53-67.

[0715] Embodiment 76. The effector cell of embodiment 75, wherein the effector cell is a T cell.

[0716] Embodiment 77. The effector cell of embodiment 76, wherein the T cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, and a natural killer T cell.

[0717] Embodiment 78. The effector cell of any one of embodiments 75-77, wherein the expression of the anti-GPC3 construct is induced by activation of the effector cell.

[0718] Embodiment 79. The effector cell of any one of embodiments 75-78, wherein the effector cell further comprises a chimeric antibody-T cell receptor (TCR) construct (caTCR).

[0719] Embodiment 80. A pharmaceutical composition comprising the isolated anti-GPC3 construct of any one of embodiments1-71 or the effector cell of any one of embodiments 75-79, and a pharmaceutically acceptable carrier.

[0720] Embodiment 81. A kit comprising the isolated anti-GPC3 construct of any one of embodiments 1-71, the isolated nucleic acid of embodiment 72, the vector of embodiment 73, the isolated host cell of embodiment 74, the effector cell of any one of embodiments 75-79, or the pharmaceutical composition of embodiment 80.

[0721] Embodiment 82. A method of detecting GPC3 in a sample, comprising contacting the sample with the isolated anti-GPC3 construct of embodiment 71 and detecting the presence of the label.

[0722] Embodiment 83. The method of embodiment 82, wherein the sample comprises cells with cell surface-bound GPC3.

[0723] Embodiment 84. The method of embodiment 83, wherein the sample comprises soluble GPC3.

[0724] Embodiment 85. A method of treating an individual having a GPC3-positive disease, comprising administering to the individual an effective amount of the pharmaceutical composition of embodiment 80.

[0725] Embodiment 86. The method of embodiment 85, wherein the administration is via intravenous or intratumoral route.

[0726] Embodiment 87. The method of embodiment 85, wherein the administration is to an injection site distal to a first disease site.

[0727] Embodiment 88. The method of any one of embodiments 85-87, further comprising administering to the individual an additional therapy.

[0728] Embodiment 89. A method of diagnosing an individual having a GPC3-positive disease, comprising: a) administering an effective amount of the isolated anti-GPC3 construct of embodiment 71 to the individual; and b) determining the level of the label in the individual, wherein a level of the label above a threshold level indicates that the individual has the GPC3-positive disease.

[0729] Embodiment 90. A method of diagnosing an individual having an GPC3-positive disease, comprising: a) contacting a sample derived from the individual with the isolated anti-GPC3 construct of embodiment 71; and b) determining the number of cells bound with the isolated anti-GPC3 construct in the sample, wherein a value for the number of cells bound with the isolated anti-GPC3 construct above a threshold level indicates that the individual has the GPC3-positive disease.

[0730] Embodiment 91. The method of any one of embodiments 85-90, wherein the GPC3-positive disease is cancer.

[0731] Embodiment 92. The method of embodiment 91, wherein the cancer is selected from the group consisting of HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, and liposarcoma.

[0732] Embodiment 93. The method of embodiment 92, wherein the cancer is HCC.

[0733] Embodiment 94. The method of embodiment 93, wherein the cancer is metastatic HCC.

[0734] Embodiment 95. A method of producing an isolated anti-GPC3 construct, comprising: (a) culturing a host cell comprising the isolated nucleic acid of embodiment 72 or the vector of embodiment 73, or the isolated host cell of embodiment 74 under conditions effective to express the anti-GPC3 construct; and (b) obtaining the expressed anti-GPC3 construct from said host cell.

[0735] Embodiment 96. The method of embodiment 95, wherein step (a) further comprises producing a host cell comprising the isolated nucleic acid or the vector.

EXAMPLES

Materials and Methods

[0736] Cell lines HepG2 and SK-Hep1 were obtained from the American Type Culture Collection. HepG2 is human hepatocellular carcinoma cell line (ATCC HB-8065). SK-Hep1 is a

liver endothelial adenocarcinoma cell line (ATCC HTB-52). Cell lines were cultured in RPMI 1640 or DMEM supplemented with 10% FBS at 37°C/5% CO_2.

[0737] FITC-labeled anti-His antibody was purchased from Thermo Fisher Scientific (Cat MA1-81891) for detection of bispecific anti-GPC3 T cell engager antibody described herein, which bears a 6×His tag at the C-terminus.

[0738] GC33 is a monoclonal antibody derived from mice immunized with a human C-terminal GPC3 fragment consisting of amino acids 524-565 (Ishiguro et al., Cancer Res 2008; 68:9832-9838). The GC33 antibody used in FIG. 7B was purchased from Roche Diagnostics (Cat. 790-4564).

Generation of recombinant human GPC3 fragments

[0739] The recombinant human $GPC3_{(560)}$ protein comprises amino acids 1-560 of the full-length human GPC3 precursor protein (SEQ ID NO: 461), which comprises the extracellular domain of human GPC3 lacking the GPI anchor site, and further fused at its C-terminus a 6×His tag. The recombinant human $GPC3_{(560)}$ protein was expressed from the lentiviral vector pCDH EF1-MCS (System Biosciences).

[0740] Biotinylated $GPC3_{(560)}$ (bt-$GPC3_{(560)}$) was prepared according to standard protocols (Altman, J.D. & Davis, M.M., *Current Protocols in Immunology* 17.3.1-17.3.33, 2003). Briefly, DNA encoding the BirA substrate peptide (BSP) was ligated to the C-terminus of the recombinant human $GPC3_{(560)}$ protein (SEQ ID NO: 461) and cloned into vector pET-27b, transformed into *E.coli* BL21 cells, then isolated as inclusion bodies from bacterial culture. $GPC3_{(560)}$-BSP protein was concentrated by ultrafiltration and further purified through size-exclusion chromatography. $GPC3_{(560)}$-BSP peptide was then biotinylated via BirA-mediated enzymatic reaction and subsequently purified by high-resolution anion-exchange chromatography.

[0741] The GPC3-51mer fragment represents amino acids 510-560 of the full-length human GPC3 precursor protein (SEQ ID NO: 467), this peptide and the biotinylated form of the peptide was synthesized by Elim Biopharmaceuticals.

Generation of SK-Hep1-GPC3 cells

[0742] SK-Hep1 cells over-expressing GPC3 protein were made by Eureka Therapeutics using full-length human GPC3 (SEQ ID NO: 460) carried on a lentivirus vector (pCDH EF1-MCS, System Biosciences), referred to herein as "SK-Hep1-GPC3".

Generation of HepG2 GPC3 knockout (HepG2-GPC3-KO) cells

[0743] HepG2 GPC3 knock out cell lines HepG2-GPC3-KO-2, KO-3, and KO-11were generated using a commercial CRISPR-Cas9 vector as described (lentiCRISPR-V2, OriGene; Sanjana *et al.*, Nat Methods. 2014; 11:783-784). GPC3-specific guide RNA (gRNA) sequences were cloned into the lentiCRISPR-V2 backbone and the vector was used to transform Stb13 bacteria. Positive clones were sequenced and transfected into HEK 293FT cells to create GPC3-CRISPR lentivirus. HepG2 cells were infected and cultured under selection for 2 weeks and measured for GPC3 expression by FACS analysis.

FACS analysis

[0744] FACS was performed using a commercial mouse anti-human GPC3 antibody (1G12, Santa Cruz Biotech sc-65443), which binds to the C-terminal portion of GPC3. 50µl of IG12 antibody was diluted 50, 100 and 200 times in PBS plus 5% FBS and incubated for one hour at 4°C with GPC3+ HepG2 cells. The cells were washed in PBS and a PE-conjugated mouse anti-IgG secondary antibody (Vector Labs #EI-2007) was added at a 1:200 dilution in PBS with 5% FBS and incubated for another 30 minutes at 4°C in the dark. The cells were washed twice with PBS and fixed.

[0745] FACS analysis results are summarized in FIG. 8. All 13 HepG2-GPC3-KO cell lines generated were negative for anti-hGPC3 antibody (1G12) binding, demonstrating successful knockout of GPC3 expression in HepG2 cells. Sequencing analysis further confirmed that HepG2-GPC3-KO cell lines KO#2, KO#3, and KO#11 were GPC3 negative. Of the 13 HepG2-GPC3-KO cell lines, HepG2-GPC3-KO-2, HepG2-GPC3-KO-3, and HepG2-GPC3-KO-11 cell lines were used for further study.

Panning of phage display libraries

[0746] Eureka Therapeutics E-ALPHA™ phage library (human scFv phage library) was screened by panning for GPC3 reactive phage clones. Isolation and FACS analysis of phage clones were performed with the following reagents: mouse anti-M13 mAb (Thermo Fisher Scientific (#MA1-12900), PE-anti-mouse IgG (Vector Labs (#EI-2007) and fixation buffer (Biolegend #420801). Control mouse anti-GPC3 antibody (Clone 1G12) was purchased from Santa Cruz Biotech (#SC-65443).

ELISA panning

[0747] ELISA panning was performed in 96-well plates coated with streptavidin, blocked and coated with biotinylated GPC3 peptide target (bt- $GPC3_{(560)}$ or bt-GPC3-51mer), then incubated

with the phage library (with human scFv expressed on the phage capsid). After washing away unbound phages, the bound phages were eluted, amplified and re-panned over the course of several rounds of selection. ELISA-panned clones were then screened for binding to GPC3-expressing HepG2 cells.

Bead panning

[0748] Bead panning was performed on DYNABEADS® coated with streptavidin and bt-GPC3-51mer peptide. The phage library was added for incubation. After washing away unbound phages, the bead-bound phages were eluted with trypsin and put through several rounds of bead-GPC3-51mer incubation, washing and elution. Positive phage clones were further tested for binding to $GPC3^+$ HepG2 cells and $GPC3^-$ HepG2-GPC3-KO cells.

Cell panning

[0749] Cell panning was performed by incubating phage clones with GPC3 expressing SK-Hep1-GPC3 cells in suspension. Unbound phages were removed by washing. Cells with bound phages were incubated with mouse anti-M13 antibody followed by PE-conjugated anti-mouse secondary antibody, then confirmed by flow cytometry. The phage clones selected against SK-Hep1-GPC3 cells were further screened against $GPC3^+$ HepG2 cells and $GPC3^-$ HepG2-GPC3-KO cells.

Example 1. Generation and selection of human antibody constructs specific for human GPC3

[0750] This example demonstrates the production of human antibody constructs specific for human GPC3 (hGPC3). In particular, this example demonstrates the production of human scFvs that specifically bind to human GPC3 in native format (cell surface-bound GPC3). Human antibody constructs described herein were developed using naïve or semi-synthetic human antibody libraries generated from normal donors and/or autoimmune disease donors and were selected based on high specificity for human GPC3 via panning against cell surface-bound human GPC3 in its native conformation. Thus, these human anti-GPC3 antibody constructs (*e.g.*, anti-GPC3 scFv) provide a valuable source of antibody components for the construction of, *e.g.*, full-length IgG, multi-specific anti-GPC3 antibody molecules, anti-GPC3 CARs, or anti-GPC3 caTCRs.

[0751] An exemplary outline for the development of anti-human GPC3 constructs is set forth in Table 3. The process started with identification of human GPC3-specific and biologically active antibody constructs from Eureka Therapeutics E-ALPHA™ phage library.

TABLE 3

Stage	Methodology
Primary panning with E-ALPHA™ phage library	Cell Panning, ELISA Panning FACS screening of phage clones
Clone Characterization	Binding to HepG2 cells Target cancer cell killing Human liver cancer cell binding Screen against GPC3 knockout cell line
Creation of bispecific antibodies	Cloning of hGPC3-specific phage clones and anti-CD3ε scFvs into an expression vector

[0752] A collection of human scFv antibody phage display libraries (diversity = 10×10^{10}) constructed at Eureka Therapeutics (trademarked as E-ALPHA™ phage libraries) was used for the selection of human antibody constructs (*e.g.*, scFv) specific for human GPC3. E-ALPHA™ phage libraries included naïve libraries comprising fully naïve human heavy and light chain repertoires, and semi-synthetic libraries containing fully naïve human light chain repertoires and semi-synthetic heavy chain with completely randomized heavy chain CDR3 regions. The naïve antibody repertoires were cloned from PBMCs and spleens of healthy donors or from PBMCs of autoimmune disease (such as systemic lupus erythematosus and rheumatoid arthritis) donors.

Panning strategies and phage library screening against cell surface-bound GPC3 (GPC3A)

Panning against GPC3+ cells

[0753] The E-APLPHA™ scFv phage libraries were screened (panned) against human GPC3 by co-incubation with recombinant GPC3-expressing SK-Hep1 cells (SK-Hep1-GPC3), then confirmed by binding to GPC3+ HepG2 cells, both with flow cytometry (see Methods). 0.2 million SK-Hep1-GPC3 cells were co-incubated with the phage library (about 1×10^{7} pfu) for 1 hours at 4°C. The cell/phage mixture was washed in PBST, incubated with mouse anti-M13 antibody for 1 hour at 4°C, washed again in PBST, then incubated for 30 minutes in the dark at 4°C with PE-conjugated anti-mouse IgG, and subjected to FACS analysis.

[0754] The 36 positive clones from the initial SK-Hep1-GPC3 screen were further validated for cell surface hGPC3 binding against the GPC3+ HepG2 cell line using the same procedure. 33 phage clones demonstrated specific binding to $GPC3^{+}$ HepG2 cells (Table 4) using FACS analysis. FACS was performed as described in Methods. Briefly, a commercial mouse anti-human GPC3 antibody (1G12, Santa Cruz Biotech sc-65443) was diluted and incubated for one hour at 4°C with GPC3+ HepG2 cells. The cells were washed in PBS and a PE-conjugated mouse anti-IgG secondary antibody (Vector Labs #EI-2007) was added and incubated for

another 30 minutes at 4°C in the dark. Then cells were washed with PBS and fixed. FACS analysis of 14 exemplary GPC3$^+$ HepG2 bound clones is shown in FIGs. 1A-1G, demonstrating strong cell surface-bound GPC3 binding. Helper phages were also used for incubation with GPC3+ HepG2 cells, serving as a control.

[0755] The phage clones from the GPC3A screen were further tested for binding to GPC3 knockout cell lines HepG2-GPC3-KO-2 and HepG2-GPC3-KO-3 made by Eureka Therapeutics (*see* "Generation of HepG2 GPC3 knockout (HepG2-GPC3-KO) cells" in Methods). Of the 33 clones identified by binding to the GPC3$^+$ HepG2 cell line, five clones (GPC3A-34, 37, 38, 58 and 62) showed little or no binding to HepG2-GPC3-KO cell lines (HepG2-GPC3-KO-2 and HepG2-GPC3-KO-3), demonstrating their high specificity for GPC3 positive cell binding. FACS analysis is exemplified in FIGs. 2A-2F: GPC3A-39 (FIG. 2A), GPC3A-46 (FIG. 2B), GPC3A-56 (FIG. 2C), and GPC3A-64 (FIG. 2E) showed significant binding to GPC3$^+$ HepG2 cell line, and relatively low levels of binding to HepG2-GPC3-KO cell lines; while GPC3A-58 (FIG. 2D) demonstrated high level of binding to GPC3$^+$ HepG2 cell line and nearly no binding to HepG2-GPC3-KO cell lines. A few clones showed high levels of binding to both GPC3$^+$ HepG2 and HepG2-GPC3-KO cell lines (data not shown).

[0756] Binding of GPC3A phage clones to soluble GPC3 was tested by ELISA. Three forms of soluble biotinylated GPC3 protein ($GPC3_{(560)}$, R&D Systems GPC3 protein [Cat. 2119-GP-0505], and GPC3-51mer) were used to coat 96 well ELISA plates as described (*see* Method). All clones were able to bind $GPC3_{(560)}$ and GPC3 (R&D Systems), with clones GPC3A-37,-42,-43 and -47 displaying the least binding to $GPC3_{(560)}$ and no binding to GPC3-51mer (data not shown).

TABLE 4

	Cell panning SK-Hep1-GPC3 cells (GPC3A)	Cell/ELISA panning GPC3-51mer SK-Hep1-GPC3 cells (GPC3B)
No. of clones (FACS screening)	540	368
Cell-surface GPC3 binding unique clones	33	14
GPC3 knockout cell binding clones	5	4
Clones converted to bispecific antibodies	20	14

Target cell killing by bispecific antibodies	16	1

Panning strategies and phage library screening using a 51mer GPC3 fragment (GPC3B)

[0757] On the other hand, three types of phage library panning were implemented using a biotinylated C-terminal 51mer hGPC3 fragment (bt-GPC3-51mer, *see* Methods). This screening strategy was named GPC3B and outlined in Table 4.

Plate panning

[0758] A plate panning screen was performed using a biotinylated GPC3-51mer fragment (*see* Methods) in a volume of 100µl at 2ug/ml in PBS and added to a Streptavidin coated 96-well ELISA plate, allowing for incubation at 4°C overnight. The plate was blocked with 5% non-fat dried milk (NFDM) in PBS for 2 hours at room temperature. After two additional washes, 100µl of phage library (with a titer of 2×10^{12} pfu/ml) was added into individual wells. 138 phage clones were tested. Plates were incubated for 2 hours at 4°C and washed with PBSTB 6 times. The wells were eluted by trypsin (100µl at 1mg/ml) for 10 minutes at room temperature. The eluted phages were recovered according to standard methods. Three rounds of plate panning were conducted to enrich for specific phage binding against the hGPC3-51mer fragment. Using this screen, of the 138 isolated phage clones, 99 were positive by ELISA and 53 unique clones were identified.

Plate plus cell panning

[0759] A second panning screen was performed on 138 isolated phage clones with two rounds of plate panning followed by one round of cell panning, using the SK-Hep1 cell line for subtraction panning and the SK-Hep1-GPC3 cell line for positive panning. In the third round of cell panning, about 4×10^7 SK-Hep1 cells were used for subtraction panning of each phage library by co-incubation for 2 hours at room temperature. About 1×10^7 SK-Hep1-GPC3 cells were used for positive panning of each phage library, also by co-incubation for 2 hours at 4°C. The cell/phage mixture was washed 4 times in 10% FBS in PBS and twice with PBS only. Specific bound phages were eluted with trypsin (1ml at 1mg/ml) for 10 minutes at room temperature. The eluted phages were rescued using standard techniques. Using this panning method, of 138 clones, a further 14 unique clones were isolated.

Bead panning

[0760] A bead panning screen was also performed for additional 92 isolated phage clones. 50µl DYNABEADS® were washed 2 times in PBST and resuspended in 500µl of phage library (with titer of 2×10^{12} pfu/ml). The phage/beads mixture was incubated for 2 hours at room

temperature to deplete phages showing non-specific binding to streptavidin. Another 50µl of DYNABEADS® were prepared using the same washing procedure and resuspended in 500µl bt-GPC3-51mer fragment solution at 2.4µg/ml in 2.5% PBSM, allowed for incubation for 15 minutes at room temperature, then washed twice. The phage library was aspirated and mixed with the bt-GPC3-51mer fragment/bead mixture, then incubated for 2 hours at 4°C and washed with PBSM 5-10 times. The bound phages were eluted using trypsin (400µl at 1mg/ml) for 10 minutes at room temperature and rescued using standard techniques. Three rounds of bead panning were conducted to enrich for GPC3-specific phage binding against the hGPC3-51mer fragment. A further 62 unique clones were isolated using the bead panning method.

[0761] Of all these 129 unique clones identified from the GPC3B screen, 20 were tested for their ability to bind GPC3+ HepG2 cells using flow cytometry. For positive cell panning, 0.2 million GPC3+ HepG2 cells were suspended in 50µl of 5% FBS in PBS and 0.05% NaN_3. For subtraction panning, 0.2 million HepG2-GPC3 knockout cells (HepG2-GPC3-KO-2) were suspended in 50µl of 5% FBS in PBS and 0.05% NaN_3. GPC3B phages and negative control phages (helper phages) were added at about 1.0×10^{11} pfu/mL in PBS and incubated at 4°C for 1 hour. The mixture was washed in PBST, and a 200-fold dilution of mouse anti-M13 antibody (see Methods) was added in a volume of 100µl in 5% FBS (in PBS) and 0.05% NaN_3 and incubated for another hour at 4°C. The mixture was washed again in PBS and secondary PE-anti-mouse IgG antibody was added at a 200-fold dilution in 100µl of 5% FBS in PBS and 0.05% NaN_3 and incubated in the dark at 4°C for another hour. The mixture was washed twice in PBS and fixed in fixation buffer at a three-fold dilution in a volume of 150µl. Of the 20 clones tested, none of them showed binding to HepG2-GPC3-KO-2 cells. As exemplified in FIGs. 3A-3D, GPC3B phage clones #81, #87 and #115 showed strong binding to GPC3+ HepG2 cells, while GPC3B-80 clone showed weak binding, but none of them showed any binding to HepG2-GPC3-KO-2 cells.

[0762] These 20 clones from GPC3B screen were further tested for their binding to soluble biotinylated $GPC3_{(560)}$ and GPC3-51mer using an ELISA assay (*see* Methods). 16 out of the 20 clones showed strong binding to bt-GPC3-51mer and even stronger binding to soluble bt-$GPC3_{(560)}$. 3 clones showed strong binding to bt-GPC3-51mer but no binding to soluble bt-$GPC3_{(560)}$. 1 clone failed to bind either soluble protein (data not shown).

Example2. Anti-GPC3 bispecific antibody constructs

Generation of bispecific antibody constructs using human anti-GPC3 antibodies

[0763] This example demonstrates the construction of multi-specific anti-GPC3 molecules using human scFv fragments specific for human GPC3. In particular, this example demonstrates the construction of bispecific antibodies having a first antibody moiety (*e.g.*, scFv) that binds human GPC3 in native format (cell-surface expressed) and a second antibody moiety (*e.g.*, scFv) that binds CD3 on T cells. These multi-specific anti-GPC3 molecules (*e.g.*, tandem di-scFv anti-GPC3 T cell engager) described herein can be used for directing T cells to kill target cells that express human GPC3.

[0764] Bispecific antibodies (referred to as "L2K") were generated using scFv sequences of the human GPC3-specific phage clones showing specific cell surface-GPC3 binding and little or no GPC3-KO cell binding (from both GPC3A and GPC3B screenings). The bispecific antibodies were constructed using a single-chain format comprising the V_L-V_H scFv sequence of a human GPC3-specific phage clone at the N-terminus and an anti-human CD3ε mouse monoclonal scFv at the C-terminus (anti-GPC3×CD3 di-scFv; *e.g.*, see Brischwein, K. *et al.*, *Mol. Immunol.* 43:1129-1143, 2006). Exemplary anti-GPC3×CD3 di-scFv antibodies derived from GPC3A-34, GPC3B-87, GPC3A-37, GPC3A-45, GPC3A-46, GPC3A-55, and GPC3A-58 antibody clones comprise the amino acid sequences of SEQ ID NOs: 488, 489 and 511-515 respectively.

[0765] DNA fragments encoding the anti-human GPC3 scFv and the anti-human CD3ε scFv were synthesized by Genewiz or Genscript and subcloned into a mammalian expression vector pQD-T (Eureka Therapeutics, Inc.) using standard recombinant DNA technology. A hexahistidine tag was inserted at the C-terminus for purification and detection. HEK293 cells were transfected with the bispecific antibody expression vector and cultured for seven days for bispecific anti-GPC3 antibody production. Bispecific antibodies were purified from HEK293 cell supernatants using HisTrap HP column (GE healthcare) by FPLC AKTA system or His GraviTrap columns (GE healthcare) based on the cell culture volume. HEK293 cell culture supernatant was clarified and loaded onto the column with low imidazole concentration (20 mM), and then an isocratic high imidazole concentration elution buffer (500 mM) was used to elute bound bispecific anti-GPC3 antibodies. Molecular weights of purified human GPC3 bispecific antibodies were measured under non-reducing conditions by gel electrophoresis. A band (~98kD) corresponding to the bispecific anti-GPC3 antibodies (anti-GPC3 scFv-anti-CD3 scFv) was observed as the major species on the gel (data not shown).

[0766] Taken together, this example demonstrates the successful construction of bispecific anti-GPC3 molecules having a first antibody moiety that is specific for human GPC3 expressed on the cell surface (*i.e.*, native format) and a second antibody moiety that is specific for CD3. Further characterization of such bispecific molecules is described below. 20 unique clones from GPC3A screen and 14 unique clones from GPC3B screen were selected for generating bispecific anti-GPC3 antibodies and further characterization (Table 4).

Anti-GPC3 bispecific antibodies (GPC3A L2K) binding to human hepatocellular carcinoma cell lines

[0767] HCC cell lines expressing GPC3 were tested for anti-GPC3 bispecific antibody binding by flow cytometry, using human GPC3+ HepG2 cells, GPC3-negative SK-Hep1cells, and SK-Hep1-GPC3 (GPC3 over-expressing) cells in two experiments.

[0768] A control-L2K bispecific antibody was similarly generated as above by fusing a non-GPC3 binding scFv with an anti-human CD3ε scFv, with a hexahistidine tag inserted at the C-terminus for purification and detection. This control-L2K antibody was used as a negative GPC3 binding control.

[0769] GPC3A L2K bispecific antibodies or control-L2K bispecific antibody were incubated with GPC3-negative SK-Hep1 cells and (separately) SK-Hep1-GPC3 cells for 1 hour at 4° C, at a final concentration of 5 μg/ml in 5% FBS in PBS with 0.05% NaN_3. Cells were then washed once with PBS. APC-conjugated anti-His tag mouse antibody (R&D Systems) was added to the washed cells in a volume of 100μl, at 20-fold dilution of 5% FBS in PBS and 0.05% NAN_3 and incubated for 1 hour at 4°C in the dark. The cells were then washed twice with PBS. The cells were fixed in 3-fold dilution of fixation buffer (BioLegend) in PBS in a volume of 150μl. As shown in FIGs. 4A and 4B, GPC3A L2K bispecific antibodies failed to bind to GPC3-negative SK-Hep1 cells and bound well to GPC3 over-expressing SK-Hep1-GPC3 cells.

[0770] GPC3A L2K bispecific antibodies or control-L2K bispecific antibody were incubated with GPC3+ HepG2 cells for 1 hour at a final concentration of 5 μg/ml in 5% FBS in PBS with and 0.05% NaN_3 at 4° C, then washed once with PBS. APC-conjugated anti-His tag mouse antibody (R&D Systems) was added in a volume of 100μl, a 20-fold dilution of 5% FBS in PBS and 0.05% NaN_3 and incubated for 1 hour at 4 ° C in the dark, then washed twice with PBS. The cells were fixed in 3-fold dilution of fixation buffer (BioLegend) in PBS in a volume of 150μl. FIGs. 5A-5B demonstrate significant binding of GPC3A L2K bispecific antibodies to GPC3+ HepG2 cells, as compared to the binding between the control-L2K antibody and GPC3+ HepG2 cells.

Anti-GPC3 bispecific antibodies (GPC3B L2K) binding to human hepatocellular carcinoma cell lines

[0771] Anti-GPC3×CD3 di-scFv antibodies derived from B screen (GPC3B L2K bispecific antibodies) are similarly tested by flow cytometry, using human GPC3+ HepG2 cells, HepG2-GPC3-KO cells, GPC3-negative SK-Hep1cells, and SK-Hep1-GPC3 (GPC3 over-expressing) cells in two experiments.

[0772] GPC3B L2K bispecific antibodies or control-L2K bispecific antibody are incubated with GPC3-negative SK-Hep1 cells and (separately) SK-Hep1-GPC3 cells for 1 hour at 4° C, at a final concentration of 5 μg/ml in 5% FBS in PBS with 0.05% NaN_3. Cells are then washed once with PBS. APC-conjugated anti-His tag mouse antibody (R&D Systems) is added to the washed cells in a volume of 100μl, at 20-fold dilution of 5% FBS in PBS and 0.05% NaN_3 and incubated for 1 hour at 4°C in the dark. The cells are then washed twice with PBS. The cells are fixed in 3-fold dilution of fixation buffer (BioLegend) in PBS in a volume of 150μl. FACS analysis is performed to compare the binding of GPC3B L2K bispecific antibodies to GPC3-negative SK-Hep1 cells and GPC3 over-expressing SK-Hep1-GPC3 cells. GPC3B L2K bispecific antibodies demonstrating cell surface-bound human GPC3 specificity are expected to show significant binding to SK-Hep1-GPC3 cells, and little or no binding to GPC3-negative SK-Hep1 cells.

[0773] GPC3B L2K bispecific antibodies and control L2K bispecific antibody are also tested for binding to GPC3+ HepG2 cells. The two sets of antibodies are incubated with GPC3+ HepG2 cells for 1 hour at a final concentration of 5 μg/ml in 5% FBS in PBS with and 0.05% NaN_3 at 4° C, then washed once with PBS. APC-conjugated anti-His tag mouse antibody (R&D Systems) is added in a volume of 100μl, a 20-fold dilution of 5% FBS in PBS and 0.05% NaN_3 and incubated for 1 hour at 4 ° C in the dark, then washed twice with PBS. The cells are fixed in 3-fold dilution of fixation buffer (BioLegend) in PBS in a volume of 150μl. FACS analysis is performed to compare the binding of GPC3B L2K bispecific antibodies and control L2K bispecific antibodies to GPC3+ HepG2 cells. GPC3B L2K bispecific antibodies demonstrating cell surface-bound human GPC3 specificity are expected to show significant binding to GPC3+ HepG2 cells, while little or no binding to these cells are expected to see for control L2K bispecific antibodies.

[0774] GPC3B L2K bispecific antibodies and control-L2K bispecific antibody are also incubated with HepG2-GPC3-KO cells for 1 hour at 4°C, at a final concentration of 5 μg/ml in 5% FBS in PBS with 0.05% NaN_3. Cells are then be washed once with PBS. APC-conjugated anti-

His tag mouse antibody (R&D Systems) is added to the washed cells in a volume of 100µl, at 20-fold dilution of 5% FBS in PBS and 0.05% NAN_3 and incubated for 1 hour at 4°C in the dark. The cells are then washed twice with PBS. The cells are fixed in 3-fold dilution of fixation buffer (BioLegend) in PBS in a volume of 150µl. FACS analysis is performed to compare the binding of GPC3B L2K bispecific antibodies and control IgG1 isotype antibodies to HepG2-GPC3-KO cells. GPC3B L2K bispecific antibodies demonstrating cell surface-bound human GPC3 specificity are expected to show little or no binding to HepG2-GPC3-KO cells, similar to IgG1 isotype control antibodies.

Example 3. Characterization of human GPC3 bispecific antibodies

Competition for binding to human liver cell lines with soluble GPC3

[0775] Antibodies raised against cell surface-bound GPC3 were tested for their ability to bind GPC3+ HepG2 cells in a competitive binding assay (IC50) using the recombinant biotinylated GPC3(560) protein (see Methods). Anti-GPC3 L2K antibodies (derived from the GPC3A screen) or control-L2K bispecific antibody were premixed with various concentrations of the soluble bt-GPC3(560) protein and incubated at 4°C for one hour; HepG2 cells were then added and the mixture was incubated for another hour at 4°C. APC-conjugated anti-His tag antibody (R&D Systems, #IC050A) was added at a 20-fold dilution to detect L2K antibody binding (L2K antibodies carry a C-terminal His tag). Streptavidin-PE (Vector Labs EI-2007) was also added at a 200-fold dilution and incubated for 30 minutes at 4°C in the dark to monitor the soluble bt-GPC3(560) protein. Mouse anti-GPC3 antibody positive control (1G12, Santa Cruz #sc-65443) was treated with or without soluble bt-GPC3(560) protein under the same conditions using PE-conjugated anti-mouse IgG (H+L). Table 5 shows the IC50values for the six GPC3A L2K antibodies tested. All GPC3 L2Ks showed much higher IC50values than the anti-GPC3 positive control antibody (1G12), with GPC3A-37 and GPC3A-46 showing the highest values, i.e. least affected by the presence of soluble GPC3. As can be seen from FIG. 6, the median fluorescence intensity (MFI) of all GPC3A L2K bispecific antibodies demonstrated significantly stronger binding to GPC3 present on HepG2 cells than both the control-L2K bispecific antibody and the anti-GPC3 positive control antibody (1G12). Moreover, most GPC3A L2K bispecific antibodies demonstrated much higher specificity for cell surface-bound GPC3 than for soluble bt-GPC3(560) protein in the competition assay, such as GPC3A-37 and GPC3A-46 clones.

TABLE 5

GPC3×CD3 Bispecific antibody clone	IC_{50} (µg/ml)

GPC3A-34 L2K	2.4
GPC3A-37 L2K	4.6
GPC3A-45 L2K	1.2
GPC3A-46 L2K	4.1
GPC3A-55 L2K	2.7
GPC3A-58 L2K	2.4
NC16 L2K negative control	-
Anti-GPC3 positive control Ab	0.25

[0776] Similarly, the clone GPC3B-87 L2K was tested for its ability to bind to GPC3+ HepG2 cells in a competitive binding assay (IC_{50}) using the recombinant biotinylated $GPC3_{(560)}$ protein as described above. GC33 L2K (GC33 antibody converted to an L2K) and an anti-CD19 L2K were used as controls. Briefly, the GPC3B-87 L2K bispecific antibody or control-L2K bispecific antibodies were premixed with various concentrations of the soluble bt-$GPC3_{(560)}$ protein and incubated at 4°C for one hour; HepG2 cells were added and the mixture incubated for another hour at 4°C. APC-conjugated anti-His tag antibody (R&D Systems, #IC050A) was then added at a 20-fold dilution to detect L2K antibody binding (L2K antibodies carry a C-terminal His tag). Streptavidin-PE (Vector Labs EI-2007) was also added at a 200-fold dilution and incubated for 30 minutes at 4°C in the dark to monitor the soluble bt-$GPC3_{(560)}$ protein. Mouse anti-GPC3 antibody positive control (1G12, Santa Cruz #sc-65443) was treated with or without soluble bt-$GPC3_{(560)}$ protein under the same conditions using PE-conjugated anti-mouse IgG (H+L). FACS analysis was performed.

[0777] As shown in FIG. 10 and Table 12, both GPC3B-87 L2K (IC_{50} 0.12 μg/ml) and GC33 L2K (IC_{50} 0.86 μg/ml) showed much lower IC_{50} than the GPC3A L2Ks tested (IC_{50} between 1.2 and 4.6 μg/ml). This suggests that binding of GPC3B-87 L2K and GC33 L2K to cell surface GPC3 can be more easily competed off by soluble GPC3 than binding of GPC3A L2Ks to cell surface GPC3.

TABLE 12

GPC3×CD3 Bispecific antibody clone	IC_{50} (μg/ml)
GPC3B-87 L2K	0.12
anti-CD19 L2K negative control	-
GC33 L2K	0.86

Binding affinity of GPC3A and GPC3B L2K clones to HepG2 cells

[0778] HCC cell lines expressing GPC3 were tested for anti-GPC3 bispecific antibody (GPC3 L2K clones) binding by flow cytometry, using human GPC3+ HepG2 cells.

[0779] A control-L2K bispecific antibody was generated by fusing a non-GPC3 binding scFv with an anti-human CD3ε scFv, with a hexahistidine tag inserted at the C-terminus for purification and detection. This control-L2K antibody, designated ET-901 L2K, was used as a

negative control for GPC3 binding. The reference anti-GPC3 bispecific antibody was also similarly generated using the scFv of GC33, and was designated GC33 L2K.

[0780] GPC3A L2K clones (GPC3A-34 L2K, GPC3A-37 L2K, GPC3A-45 L2K, GPC3A-46 L2K, GPC3A-55 L2K, and GPC3A-58 L2K), GPC3B L2K clone (GPC3B-87 L2K), reference GC33 L2K bispecific antibody, or negative control anti-CD19 L2K were incubated with GPC3 positive HepG2 cells for 1 hour at a final concentration of 5 μg/ml in 5% FBS in PBS and 0.05% NaN_3 at 4° C, then washed once with PBS. APC-conjugated anti-His tag mouse antibody (R&D Systems) was added in a volume of 100μl, a 20-fold dilution of 5% FBS in PBS and 0.05% NaN_3 and incubated for 1 hour at 4 ° C in the dark, then washed twice with PBS. The cells were fixed in 3-fold dilution of fixation buffer (BioLegend) in PBS in a volume of 150μl. As a separate control, HepG2 cells were also incubated with APC-conjugated anti-His tag mouse antibody (R&D Systems) in the absence of any L2K bispecific antibody. FIG. 11 demonstrated significant binding of GPC3A L2K clones to GPC3 positive HepG2 cells.

[0781] Specifically, 6 GPC3A L2K bispecific antibody clones (GPC3A-34 L2K, GPC3A-37 L2K, GPC3A-45 L2K, GPC3A-46 L2K, GPC3A-55 L2K, GPC3A-58 L2K) and 1 GPC3B L2K bispecific antibody clone (GPC3B-87 L2K) were assayed by FACS to determine their binding affinity to GPC3+ HepG2 cells, along with reference anti-GPC3 GC33 L2K. The 6 GPC3A L2K clones showed EC_{50} between 0.045 – 0.12 μg/ml, with apparent K_d values between 0.86 -2.4 nM. The GPC3B L2K clone (GPC3B-87 L2K) showed much weaker binding, with EC_{50} at 4.3 μg/ml, and an apparent K_d of 84 nM. GC33 L2K showed an EC_{50} of 0.14 μg/ml, with an apparent K_d of 2.7 nM. Compared to GC33 L2K (K_d = 2.7 nM), all GPC3A L2K clones showed higher binding affinity, especially GPC3A-37 L2K (K_d = 0.92 nM), GPC3A-46 L2K (K_d = 1.0 nM) and GPC3A-55 L2K (K_d = 0.86 nM) (*See* Table 13 and FIG. 11).

TABLE 13

GPC3×CD3 Bispecific antibody clone	EC_{50} (μg/ml)	Apparent K_d (nM)
GPC3A-34 L2K	0.11	2.0
GPC3A-37 L2K	0.048	0.92
GPC3A-45 L2K	0.12	2.4
GPC3A-46 L2K	0.050	1.0
GPC3A-55 L2K	0.045	0.86
GPC3A-58 L2K	0.12	2.4
GPC3B-87 L2K	4.3	81
Anti-GPC3 positive control Ab	0.14	2.7

T cell-mediated cytotoxicity assay

[0782] Tumor cytotoxicity was assayed using an LDH Cytotoxicity Assay (Promega). Human T cells (AllCells) or Ficoll-purified cells from whole blood (Blood Centers of the Pacific) were activated and expanded with CD3/CD28 DYNABEADS® (Invitrogen) according to manufacturer's specifications. Activated T cells were cultured and maintained in RPMI1640 medium with 10% FBS plus 100 U/mL IL-2 and used at day 7-14 post-activation. T cells were >99% $CD3^+$ by FACS analysis. Activated T cells and target cells were co-cultured at a 5:1 ratio with bispecific antibodies (0.2 μg/ml; GPC3A L2K or GPC3B L2K) for 16 hours. Cytotoxicity was determined by measuring LDH activity in culture supernatants.

[0783] As shown in FIG. 7A and FIG. 7B, bispecific anti-GPC3×CD3 antibodies, derived from both GPC3A and GPC3B screens effectively mediated killing of cancer cells in a human GPC3-specific manner. A monoclonal antibody GC33, which was derived from mice immunized with a C-terminal human GPC3 fragment (see Methods), was also tested for GPC3-specific killing (FIG. 7B), The results show that GC33's cancer cell killing capability was not GPC3 specific, because GC33 mediated killing of GPC3 knockout cell line HepG-GPC3-KO-2 as well as GPC3-positive cell lines HepG2 and SK-Hep1-GPC3. On the other hand, GPC3A L2K bispecific antibodies #43, #53, #58 and #61 (among others; FIG. 7A) and GPC3B L2K bispecific antibody #87 (FIG. 7B) mediated robust killing of GPC3-positive cells (HepG2 andSK-Hep1-GPC3), while having no significant effect on GPC3-negative cell lines (SK-Hep1 and HepG2-GPC3-KO cells).

Example 4. Construction of mouse anti-hGPC3 monospecific IgG antibodies

[0784] Full-length mouse anti-human GPC3 (hGPC3) monospecific IgG antibodies were constructed using heavy chain and light chain variable region sequences of GPC3A-39, 46, 51, 56, 58 and 64 (SEQ ID NOs: 311 and 362, 318 and 369, 323 and 374, 328 and 379, 330 and 381, 336 and 387, for V_H and V_L respectively) to fuse to mouse IgG1 constant region and Fc region sequences using a cloning plasmid expressing the Fc region of murine IgG1 heavy chain and a plasmid expressing the IgG light chain. The resulting antibody clones were named anti-GPC3 mIgG1 clones A-39, -46, -51, -56, -58, and -64. The sequence of an exemplary anti-GPC3 mIgG1 A-39 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 505, and a light chain comprising the amino acid sequence of SEQ ID NO: 506. A full-length GPC3B monospecific IgG antibody and a full-length GC33 antibody are constructed in the same fashion and are named anti-GPC3 mIgG1clone B-87, and anti-GPC3 mIgG1-GC33, respectively, using heavy chain and light chain variable regions of the GPC3B clone 87 (SEQ ID NOs: 350 and 401)

and heavy chain and light chain variable regions of the anti-GPC3 antibody GC33 (SEQ ID NOs: 503 and 504).

[0785] In brief, full-length mouse IgG1 of the selected phage clones were produced in HEK293 and Chinese hamster ovary (CHO) cell lines, as described (Tomimatsu, K. et al., Biosci. Biotechnol. Biochem. 73(7):1465-1469, 2009) (data not shown). The GPC3 clone variable regions were subcloned into mammalian expression vectors with mouse IgG1 heavy chain and light chain constant regions. Molecular weight of the purified full-length IgG antibodies was measured under both reducing and non-reducing conditions by electrophoresis to determine protein purity, which was calculated to be in excess of 95% (data not shown).

[0786] Anti-GPC3 mIgG1 antibodies were tested for the binding towards GPC3-positive HepG2 and GPC3-negative SK-Hep1 cells by flow cytometry. 10 μg/mL of constructed antibody was added to cells on ice for 1 hour. After washing, PE conjugated anti-mouse IgG (H and L) (Vector Labs#EI-2007) was added (30 minutes at 4°C in the dark and washed twice) to detect antibody binding. As can be seen from FIG. 9A, all anti-GPC3 mIgGs were found to bind to GPC3-positive HepG2 cells, while the secondary antibody and a control antibody alone (control mIgG1) did not bind to the same cells. None of the anti-GPC3 mIgGs or control mIgG1 displayed binding to SK-Hep1 cells (FIG. 9B). The same FACS analysis is performed with the anti-GPC3 mIgG1clone B-87 and mIgG-GC33 antibodies.

Reactivity of GPC3A mIgG1 antibodies against rat GPC3 by ELISA

[0787] GPC3-specific and negative control (ET-901) mouse chimeric IgG1 were tested for binding towards rat GPC3 peptide in an ELISA assay. Antibodies were tested at 3X serial dilution, starting from 100 ng/mL, for a total of 8 concentrations. Biotinylated rat GPC3 was coated onto streptavidin plates in a volume of 100μL at 2ug/ml in PBS and added to a Streptavidin coated 96-well ELISA plate, allowing for incubation at 4°C overnight. The plate was blocked with 5% non-fat dried milk (NFDM) in PBS for 2 hours at room temperature. Each of the GPC3A mIgG1 clones (A-34, -46, -53, -58, -61), as well as the negative control ET-901 mIgG1, constituted at 5μg/mL in PBST buffer, was respectively incubated with separate rat bt-GPC3-coated wells for 1 hour at room temperature. The plates were washed with PBST, and incubated with anti-mouse Fc (Bethyl Labs #A90-131P, 1:2000 dilution) for 30 minutes at room temperature. After washing with PBST, the reaction was developed with 1-STEP™ Ultra TMB-ELISA Substrate Solution (Thermofisher Scientific #34028) and stopped with 2N sulfuric acid. 4 out of 5 GPC3A mIgG1 clones (A-34, -46, -58, -61) reacted with rat bt-GPC3, as compared to negative control anti-CD19 mIgG1 (FIG. 12). GPC3A-53 mIgG1 clone did not react with rat bt-

GPC3. In an accompanying experiment, all GPC3A mIgG1 clones tested (A-34, -46, -53, -58, -61) reacted with human GPC3 (FIG. 12).

Example 5. Binding profile determination of anti-hGPC3 monospecific and bispecific antibodies to cell-surface and soluble GPC3

[0788] This example demonstrates GPC3 binding profile for selected monospecific and bispecific antibodies. In particular, this example describes the epitope-binding site characterization of selected anti-hGPC3 monospecific mIgG1 clones (as described in Example 4). On the other hand, this example also describes the binding profile of selected anti-hGPC3 monospecific mIgG1 clones and human anti-hGPC3 anti-CD3 bispecific antibodies (GPC3A L2K and GPC3B L2K, as described in Example 2) of the present invention, along with clone GC33 mIgG1 and bispecific GC33-L2K, to soluble recombinant human GPC3 (i.e., human $GPC3_{(560)}$, residues 1-560aa of hGPC3) or GPC3 antigen on cancer cell surface, such as GPC3-positive HepG2 and SK-Hep1-GPC3 cells.

Epitope binding site characterization for GPC3A mIgG clones

[0789] GPC3 epitope binning was carried out to determine if distinct epitopes are recognized by reference GC33-mIgG1 and the anti-hGPC3 monospecific mIgG1 clones GPC3A-37 mIgG and GPC3A-55 mIgG. A first pair of mIgG protein and GPC3 was allowed to react for the first mIgG to occupy its binding epitope on GPC3 before addition of a second distinct mIgG in order to determine whether the two mIgG proteins bind to different epitopes. Briefly, binding affinity was determined and binding parameters were calculated using a 1:1 binding site, partial fit model. After the first pairing of a mIgG protein and GPC3 (where one entity of the pair is immobilized to a streptavidin-biosensor), a spike in signal detected by the biosensor reflects binding between the first mIgG and GPC3, and after washing, the first pair of mIgG/GPC3 remains bound to the streptavidin-coated plate. Subsequently, upon adding a second mIgG to the immobilized pair of GPC3/first mIgG, a further spike in signal reflects binding between the second mIgG and the immobilized GPC3, indicating the second mIgG reacted with the unbound epitopes left open on GPC3 after reaction with the first mIgG. Therefore, a spike in signal upon addition of the second mIgG demonstrates that the first and second mIgGs bind to distinct epitopes. On the contrary, the absence of spikes in signal upon addition of the second mIgG may demonstrate that the first and second mIgGs bind to the same epitope.

[0790] In a linear format of GPC3 epitope binning, 5 μg/mL biotinylated GPC3 was loaded onto a streptavidin biosensor. After washing off excess antigens, mIgG proteins were

sequentially added, where the first mIgG was added at 50 μg/mL, followed by the second mIgG at 100 μg/mL. Specifically, GC33-mIgG1 was first added at 50 μg/mL, triggering a spike in signal, thereby indicating specific binding of GC33-mIgG1 to a GPC3 epitope (FIG. 13). Subsequently, either GPC3A-37 mIgG or GPC3A-55 mIgG was added at 100 μg/mL. In both cases, a second spike was recorded upon the addition of either GPC3A-37 mIgG or A-55 mIgG (FIG. 13), indicating that GPC3A-37 mIgG and GC33-mIgG1 bind to distinct epitopes, and that GPC3A-55 mIgG and GC33-mIgG1 also bind to distinct epitopes.

[0791] In an additional experiment, GPC3A-37 mIgG was first added at 50 μg/mL, which triggered a spike in signal, thereby indicating specific binding of GPC3A-37 mIgG to a GPC3 epitope. Subsequently, GPC3A-55 mIgG was added at 100 μg/mL, which triggered a second spike, indicating that GPC3A-37 mIgG and GPC3A-55 mIgG bound to distinct epitopes (FIG. 13). Combining the above results, GC33 mIgG1, GPC3A-37 mIgG and GPC3A-55 mIgG each binds to a distinct epitope on GPC3.

[0792] In a sandwich format of GPC3 epitope binning, a mIgG protein was biotinylated and immobilized on Streptavidin biosensors, at 5 μg/mL. After washing off excess mIgGs, GPC3 was added to the immobilized antibody at 10 μg/mL and stayed bound to the immobilized mIgG. After washing, a second mIgG was then added at10 μg/mL and binding was monitored.

[0793] Specifically, each of GC33-mIgG, GPC3A-37 mIgG and GPC3A-55 mIgG protein (as the first mIgG1) was biotinylated and immobilized respectively on Streptavidin biosensors at 5 μg/mL. After washing off excess mIgGs, 10 μg/mL of GPC3 was added to the immobilized antibody, which triggered a spike for each of the three tested mIgG's, thereby indicating specific binding of each of GC33-mIgG, GPC3A-37 mIgG and GPC3A-55 mIgG to a GPC3 epitope. After washing off excess GPC3 antigens, 10 μg/mL of a second mIgG (different from the first mIgG) was added and binding was monitored. Similar to the linear format of epitope binning, the subsequent addition of a second, different mIgG triggered a further spike in signal (FIG. 14), therefore indicating that GC33-mIgG1, GPC3A-37 mIgG and GPC3A-55 mIgG each binds to a distinct epitope on GPC3.

<u>GPC3-L2K binding to soluble recombinant human GPC3 protein</u>

[0794] The binding properties of anti-hGPC3 anti-hGPC3 L2K bispecific antibodies (GPC3A L2K derived from clones GPC3A-34, GPC3A-37, GPC3A-45, GPC3A-46, GPC3A-55, GPC3A-58) to biotinylated recombinant $GPC3_{(560)}$ (bt-$GPC3_{(560)}$) was measured by surface plasmon resonance technology using a BIACORE™ X100 instrument (GE Healthcare) and a Biotin CAPture Kit (GE Healthcare, Cat# 28-9202-33) in single-cycle kinetics mode according to the

manufacturer's instructions. All of the proteins used in the assay were diluted in HBS-E buffer prior to use. Briefly, 5-10 µg/ml of bt-GPC3$_{(560)}$ was immobilized onto a sensor chip pre-functionalized with CAPture Reagent and injected onto the flow cell. Affinity of the L2K bispecific antibodies for immobilized antigen was measured by injection of the various bispecific antibodies at 0.3-5 mg/ml with a 90 second association phase followed by a 90 second dissociation phase at a flow rate of 30 µl/min. At the end of each run, the surface of the sensor chip was regenerated using the reagents provided in the Biotin CAPture kit. Binding constants were determined by nonlinear regression analysis using a 1:1 binding model (BIACORE™ X-100 evaluation software, FIGs. 15A and 15B). The association-on-rate-k_{on}, affinity constant-k_a, dissociation constant-k_d, and equilibrium dissociation constant- K_d were then calculated for each L2K clone (Table 14).

TABLE 14

GPC3×CD3 Bispecific antibody clone	k_a (1/Ms)	k_d (1/s)	K_d (nM)
GPC3A-34 L2K	4.00E+05	0.001398	3.5
GPC3A-37 L2K	5.67E+05	0.002886	5.1
GPC3A-45 L2K	1.28E+05	0.000455	3.5
GPC3A-46 L2K	3.99E+05	0.001289	3.2
GPC3A-55 L2K	7.05E+05	0.001606	2.3
GPC3A-58 L2K	3.68E+05	0.00188	5.1

Binding to HepG2 (GPC3-positive) cells

[0795] HepG2 cells are tested for anti-hGPC3 mIgG1 or BsAb binding with dose dependency. Briefly, HepG2 cells are incubated with anti-hGPC3 mIgG1 or BsAb at 10, 3.3, 1.1, 0.37, 0.12, 0.041, 0.014 or 0 µg/ml on ice for 1 hour. After a round of brief washing with PBS buffer, BsAbs are detected with the secondary antibody APC-labeled anti-His tag antibody. mIgG1 antibodies are detected with a PE-labeled anti-mouse secondary antibody. The anti-GPC3 mIgG1 or BsAbs are evaluated for their affinity towards HepG2 cells by EC50 of the dose dependence curve (MFI vs. BsAb concentration). Furthermore, apparent K_d is determined based on EC50 value. The EC_{50} or apparent K_d ratios of mIgG1 clones of the invention against clone GC33 mIgG1 are calculated. The EC_{50} or apparent K_d ratios of BsAb clones of the invention against clone GC33 BsAb are also calculated.

Example 6. Construction and Characterization of anti-GPC3 chimeric antigen receptors

[0796] Anti-GPC3 CARs were constructed using a lentiviral CAR expression vector. Each of the scFvs derived from anti-GPC3 clones (GPC3A-34, GPC3A-37, GPC3A-45, GPC3A-46, GPC3A-55, GPC3A-58 and GPC3B-87; referred herein as "GPC3A-CAR" or "GPC3B-CAR")

were grafted onto the CAR expression vector. An scFv derived from GC33 was also used to construct a GC33 CAR. Each CAR construct comprises an intracellular signaling domain of CD28 and an intracellular signaling domain of CD3ζ. Exemplary CARs derived from GPC3A-34, GPC3A-37, GPC3A-45, GPC3A-46, GPC3A-55, and GPC3A-58 antibody clones comprise the amino acid sequences of SEQ ID NOs: 491 and 516-521 respectively.

[0797] Human primary T-cells were activated by stimulation with CD3/CD28 beads (DYNABEADS®, Invitrogen) in the presence of interleukin-7 and interleukin-15 for one day. Concentrated lentiviruses were applied to T-cells in Retronectin (Takara) coated 6-well plates for 72 hours, at an MOI of 6. Transduction efficiencies are shown in Table 15.

TABLE 15

T cells	Transduction efficiency (CAR positive %)
GC33-CAR	95.9
GPC3A-CAR-34	66.8
GPC3A-CAR-37	85.9
GPC3A-CAR-45	80.7
GPC3A-CAR-46	91.9
GPC3A-CAR-55	76.7
GPC3A-CAR-58	95.5
GPC3A-CAR-87	94.1
Mock	0.67

[0798] Functional assessment of the transduced CAR T cells (GPC3A, GPC3B or GC33 CAR-T cells) was performed using a LDH Cytotoxicity Assay. CAR-T cells and target cells (HepG2, HepG2-GPC3-KO, SK-Hep1, or SK-Hep1-GPC3) were mixed at an effector-to-target ratio of 2:1, in which 100,000 target cells were seeded per test well and treated with 200,000 CAR-T cells (infected CAR T cells were normalized to be 67% CAR-positive). The LDH cytotoxicity assay was performed after overnight incubation.

[0799] A panel of seven GPC3A or GPC3B CARs, as well as the GC33 CAR were transduced into T cells and tested against the target cell lines HepG2, HepG2-GPC3-KO, SK-Hep1, and SK-Hep1-GPC3. T cells transduced with the CAR constructs, GPC3A-CAR-37, GPC3A-CAR-45, GPC3A-CAR-46, GPC3A-CAR-55, GPC3A-CAR-58, GPC3B-CAR-87) specifically killed GPC3 positive cell lines such as HepG2 and SK-Hep1-GPC3 (FIG. 16). GPC3-negative cells such as wild-type SK-Hep1 or HepG2-GPC3-KO, however, were poorly recognized by the same CAR T cells. In these LDH cytotoxicity experiments, CAR-Ts comprising GPC3A-CAR-37, GPC3A-CAR-55, and GPC3A-CAR-58 CAR displayed high efficiency in killing GPC3-positive target cells but low non-specific killing of GPC3 negative cells (FIG. 16).

[0800] In a second batch of LDH cytotoxicity assays, CAR-T cells and target cells (HepG2, HepG2-GPC3-KO, SK-Hep1, SK-Hep1-GPC3, JHH-5, A498 or PANC-1) were mixed at an effector-to-target ratio of 2:1, in which 100,000 cells were seeded per test well and treated with 200,000 anti-GPC3 CAR positive T cells (infected CAR T cells were normalized to be 37.1% positive for CAR). Transduction efficiencies of the CAR-T cells are shown in Table 16. The LDH assay was performed after overnight incubation.

TABLE 16

T cells	Transduction efficiency (CAR positive %)
GC33-CAR	74.4
GPC3A-CAR-34	51.2
GPC3A-CAR-37	69.2
GPC3A-CAR-45	58.8
GPC3A-CAR-46	60.3
GPC3A-CAR-55	37.1
GPC3A-CAR-58	78
GPC3A-CAR-87	74.8
Mock	0.67

[0801] As shown in FIGs. 17A and 17B, T cells transduced with the CAR constructs, GPC3A-CAR-37, GPC3A-CAR-45, GPC3A-CAR-46, GPC3A-CAR-55, and GPC3B-CAR-87 specifically killed GPC3 positive cell lines HepG2, SK-Hep1-GPC3, and JHH-5. GPC-negative target cells such as A-498, PANC-1, wild-type SK-Hep1 cells and HepG2-GPC3-KO, however, were poorly recognized by the same CAR-T cells (FIG.s 16A and 16B). These experiments demonstrate therapeutic benefit in employing the anti-GPC3 GPC3A or GPC3B CAR-Ts in specifically targeting GPC3 expressing tumor cells without significant effects on GPC3 negative cells.

Example 7. Construction and characterization of T cells transduced with anti-AFP caTCR-1 and anti-CD3/anti-GPC3 bispecific antibody

[0802] Primary T cells were transduced with a lentivirus encoding either anti-AFP caTCR (SEQ ID NOs: 522 and 523) or anti-AFP caTCR + anti-GPC3/anti-CD3 BsAb (SEQ ID NO: 511). Measurements of the percentages of caTCR-positive cells were determined to assess transduction efficiency. The anti-AFP caTCR was under the control of the EF1-alpha promoter (SEQ ID NO: 527) and the anti-GPC3/anti-CD3 BsAb was under the control of an NFAT-derived promoter (SEQ ID NO: 526) comprising 6 NFAT response elements (SEQ ID NO: 524) and a minimal TA promoter (SEQ ID NO: 525). T-cells were matched at the indicated receptor positive percentages by mixing with mock T-cells. Two cell lines were used: HEPG2 (AFP+/GPC3+) and HepG2-GPC3-KO (AFP+/GPC3-) at an effector-to-target ratio of 2.5:1.

Specific T-cell lysis was measured after 16hr incubation using the Cytox 96 Non-radioactive Cytotoxicity Assay (Promega).

[0803] *In vitro* killing data shows that the expression of the anti-GPC3/anti-CD3 BsAb in T-cells transduced with the anti-AFP caTCR + anti-GPC3/anti-CD3 BsAb increased the potency of the transduced T-cells compared to T cell transduced only with the anti-AFP caTCR in a GPC3-dependent manner, throughout a range of T-cell doses (FIG. 18). Reactions that demonstrated an increase in cytotoxic potency also showed increases in the amounts of cytokines (IFNγ, TNFα, and IL-2) released (FIG. 19). Thus, the induced expression of the anti-GPC3/anti-CD3 BsAb increased the potency and cytokine production of T-cells.

[0804] To directly measure the cytotoxicity of antigen-induced anti-GPC3/anti-CD3 BsAbs apart from the anti-AFP caTCR T-cell toxicity, we used transwells with BsAb permeable membranes. 2.5 x 10^5 SK-HEP1-GPC3 ($GPC3^+$) or SK-HEP1 ($GPC3^-$) tumor cells and 4 x 10^6 receptor-negative "mock" T-cells were seeded together in the lower chamber. 2.5 x 10^5 anti-AFP caTCR transfected T-cells along with 2.5 x 10^5 corresponding target cells SK-HEP1-MG (AFP^+) or SK-HEP1 (AFP^-) were seeded together in the upper chamber. When anti-AFP caTCR + anti-GPC3/anti-CD3 BsAb T-cells were stimulated with AFP positive (SK-Hep1-MG) tumor cells, the secreted anti-GPC3/anti-CD3 BsAbs passed through the transwell membrane and were able to stimulate the lysis of GPC3-positive SK-Hep1-GPC3 tumor cells (FIG. 20). These results demonstrate that the anti-AFP caTCR + anti-GPC3/anti-CD3 BsAb T-cells were able to secrete specific fully functional anti-GPC3/anti-CD3 BsAbs in response to caTCR receptor engagement, and that therapeutic effects of such BsAbs can be used in an additive fashion to caTCR therapy.

Example 8. Construction and characterization of T cells transduced with anti-AFP caTCR-1 and anti-GPC3 chimeric stimulatory receptor

[0805] A nucleic acid fragment encoding the anti-AFP binding moiety (SEQ ID NOs: 528 and 529) was used to generate caTCR-1 constructs (caTCR-1-0 or caTCR-1-TM5). A nucleic acid fragment encoding the anti-GPC3 binding moiety (SEQ ID NOs: 309 and 360) was used to generate a CSR (*i.e.*, CSR1) comprising CD28 transmembrane and intracellular signaling sequences (SEQ ID NO: 530).

In vitro killing

[0806] HEPG2 cells (human liver cancer cells expressing AFP and GPC3) and HEPG2-GPC3-KO cells were used as target cells for T-cell stimulation at an effector-to-target ratio of 2.5:1. Specific T-cell lysis was measured after 16hr incubation using the Cytox 96 Non-radioactive Cytotoxicity Assay (Promega).

[0807] Expression of anti-GPC3-CSR (SEQ ID NO: 531) with either anti-AFP-caTCR-1-0 (SEQ ID NOs: 532, 533) or anti-AFP-caTCR-1-TM5 (SEQ ID NOs: 534, 535) resulted in fully functional cytotoxic T-cells capable of lysing HEPG2 cells *in vitro* (FIG. 21). T cells expressing only the caTCR-1-0 had much less specific killing (about 15%) than those expressing both caTCR-1 (caTCR-1-0 or caTCR-1–TM5) and the CSR (between about 55% to about 65%). By contrast, specific killing was reduced to about 10% for T cells expressing both the caTCR and CSR when using HEPG2-GPC3-KO target cells (FIG. 21), indicating that engagement of the CSR with its target ligand is responsible for the increased cytotoxicity.

Cytokine secretion

[0808] The concentration of cytokines released into the supernatant of the *in vitro* killing experiments was measured with a Bioplex200 (Luminex) using the Bio-plex Pro Human Cytokine 8-plex kit (BioRad). The CSR-positive and caTCR-positive T-cells released more cytotoxic cytokines than T-cells expressing ca-TCR-1-0 alone for the HEPG2 target cells expressing both the caTCR target antigen and the CSR target ligand (FIG. 22). By contrast, there we little or no difference between the T cells expressing ca-TCR-1-0 alone or both a caTCR and the CSR for the HEPG2-GPC3-KO target cells lacking the CSR target ligand (FIG. 22).

Intracellular cytokine expression

[0809] T-cells were stimulated with target cells (HEPG2) at an E:T ratio 1:2 in the presence of secretion inhibitor brefeldin A (BFA) for 4 hours. T-cells were permeabilized and cytokine specific antibodies were used to detect cytokines expressed in response to tumor stimulation. The percent of cytokine-positive cells was determined using flow cytometry. The CSR-positive and caTCR-positive T-cells expressed more intracellular cytokines than T-cells expressing ca-TCR-1-0 alone (Table 17).

Table 17

Intracellular TNFα Expression in CD8+ T-cells		
% Positive	HEPG2	T-cell Alone
CSR	0.3	0.2
caTCR-1-0	14.7	0.1
caTCR-1-0+CSR	17.0	0.1
caTCR-1-TM5+CSR	15.7	0.1

Intracellular IL-2 Expression in CD4+ T-cells

% Positive	HEPG2	T-cell Alone
CSR	0.1	0.04
caTCR-1-0	8.2	0.11
caTCR-1-0+CSR	9.8	0.05
caTCR-1-TM5+CSR	11.2	0.03

Intracellular IFNγ Expression in CD8+ T-cells		
% Positive	HEPG2	T-cell Alone
CSR	0.2	0.08
caTCR-1-0	3.1	0.2
caTCR-1-0+CSR	4.5	0.1
caTCR-1-TM5+CSR	5.1	0.06

[0810] Taken together, the results indicate that the addition of the CSR increases the sensitivity and responsiveness of caTCR plus CSR T-cells having a different caTCR target antigen and CSR target ligand. The increased quantity of cytokines expressed in and released from these CSR-caTCR double positive T cells provides further evidence that the co-stimulation of both caTCR-1 and CSR raises the cytotoxic potential of the T-cells.

Degranulation

[0811] T cells were mixed with fluorescently-conjugated anti-CD107a and stimulated with HEPG2 target cells at an E:T ratio of 1:2 in the presence of the endocytosis inhibitor monensin for 4 hours. Engagement of the CSR on caTCR T-cells increased T-cell degranulation, further demonstrating that the CSR makes therapeutic T-cells more reactive towards the intended tumor cells (FIG. 23).

Proliferation

[0812] T-cells were labeled with the intracellular dye CFSE, and dye dilution and number of CFSE-positive cells remaining at the indicated day was measured.

[0813] Respective T-cells were serum starved overnight and labeled with CFSE using CellTrace CFSE (Thermo Fisher C34554). 100,000 T-cells were incubated at an E:T ratio of 2:1 and flow cytometry was used to observe serial dilution of the CFSE dye as the T-cells divide at the indicated day. The total number of T-cells was counted by FACs.

[0814] CFSE dilution increased with CSR stimulation, indicating these T-cells had a higher proliferation potential (FIG. 24). Importantly there is also an increase in the cell number,

meaning that the cells not only proliferate better but their persistence is also maintained (Table 18).

Table 18

# of T-Cells Persisting After Engagement with HEPG2			
	Day3	**Day5**	**Day7**
CSR	7,147	4,519	3,055
caTCR1-0	5,674	4,362	3,372
caTCR1-0 +CSR	31,422	18,689	8,833
caTCR-1-TM5 +CSR	28,874	21,978	9,471

[0815] The results show that we were able to simultaneously stimulate both CSR and caTCR with ligand positive tumor cells, and that the co-stimulation of CSR and caTCR enhanced the cytotoxicity, proliferation potential, and persistence of caTCR T-cells. These are all characteristics that will increase therapeutic potential of caTCR-based therapies using adoptive transfer.

Example 9: *In vivo* efficacy study of T cells transduced with anti-AFP caTCR-1 and anti-GPC3 CSR

[0816] The *in vivo* anti-tumor activity of T cells expressing both anti-AFP caTCR-1 and anti-GPC3 CSR-1 (*see*, Example 8 for construct information) was tested in an established human $AFP^{+}/HLA\text{-}A2^{+}$ Hep G2 liver cancer xenograft model. Hep G2 cells were implanted subcutaneously (s.c.) over the right flank of SCID-Beige mice. When tumors reached ~100mm^3, mice were intratumorally (i.t.) injected with either (1) 5×10^6 un-transduced donor-matched (Mock) T cells, (2) 2×10^6 T cells expressing an anti-AFP CAR comprising the same anti-AFP binding moiety (SEQ ID NOs: 528 and 529), or (3) 2×10^6 T cells expressing both anti-AFP caTCR-1 and anti-GPC3 CSR-1 (n=6 mice/group). Health effects resulting from the T cell infusions in mice were assessed by monitoring their general appearance, body weight, and other clinical signs of adverse response (including hypothermia, labored respiration, and hind-limb paralysis/weakness).

[0817] As shown in FIG. 25, both anti-AFP CAR T cell treatment and anti-AFP caTCR-1/anti-GPC3 CSR-1 T cell treatment resulted in profound and significant (****$P<0.0001$; Dunnett's multiple comparison test) tumor growth inhibition. All anti-AFP CAR-T treated and anti-AFP caTCR-1/anti-GPC3 CSR-1 T cell-treated mice demonstrated normal gait, posture, and activity/responsiveness for the duration of the study. In addition, anti-AFP CAR-T treated and anti-AFP caTCR-1/anti-GPC3 CSR-1 T cell-treated mice did not lose body weight during the

study. Overall, the lack of observable abnormal parameters in treated mice demonstrates the safety of the anti-AFP caTCR-1/anti-GPC3 CSR-1 T cell therapy.

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CLAIMS

1. An isolated anti-GPC3 construct comprising an anti-GPC3 antibody moiety specifically recognizing GPC3, wherein the anti-GPC3 antibody moiety comprises:

I. i) a heavy chain variable domain (V_H) comprising a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a light chain variable domain (V_L) comprising a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; or

II. i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; or

III. i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; or

IV. i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 52, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 103; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 154, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 205, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 256; or

V. i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 11, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 62,

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and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 113; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 164, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 215, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 266; or

VI. i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 12, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 63, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 114; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 165, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 216, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 267; or

VII. i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 95, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 146; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 197, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 248, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 299.

2. The isolated anti-GPC3 construct of claim 1, wherein the anti-GPC3 antibody moiety comprises:

I. i) a V_H comprising the amino acid sequence of SEQ ID NO: 309; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 360; or

II. i) a V_H comprising the amino acid sequence of SEQ ID NO: 327; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 378; or

III. i) a V_H comprising the amino acid sequence of SEQ ID NO: 330; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 381; or

IV. i) a V_H comprising the amino acid sequence of SEQ ID NO: 307; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 358; or

V. i) a V_H comprising the amino acid sequence of SEQ ID NO: 317; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 368; or

VI. i) a V_H comprising the amino acid sequence of SEQ ID NO: 318; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 369; or

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VII. i) a V_H comprising the amino acid sequence of SEQ ID NO: 350; and ii) a V_L comprising the amino acid sequence of SEQ ID NO: 401.

3. The isolated anti-GPC3 construct of claim 1 or claim 2, wherein:

(i) the anti-GPC3 antibody moiety is a full-length antibody, a Fab, a Fab', a $F(ab')_2$, an Fv, or a single chain Fv (scFv);

(ii) the anti-GPC3 antibody moiety is fused to an Fc fragment optionally via a linker; and/or

(iii) the isolated anti-GPC3 construct is multi-specific.

4. The isolated anti-GPC3 construct of any one of claims 1 to 3, wherein the isolated anti-GPC3 construct is a chimeric antigen receptor (CAR) comprising:

(a) an extracellular domain comprising the anti-GPC3 antibody moiety;

(b) a transmembrane domain; and

(c) an intracellular signaling domain.

5. The isolated anti-GPC3 construct of claim 4, wherein

(i) the intracellular signaling domain comprises (a) a CD3ζ intracellular signaling sequence and (b) a CD28 or CD30 intracellular signaling sequence and/or

(ii) wherein the CAR comprises the amino acid sequence of any one of SEQ ID NOs: 491 and 516-521.

6. The isolated anti-GPC3 construct of any one of claims 1 to 3, wherein the isolated anti-GPC3 construct is a chimeric antibody-T cell receptor (TCR) construct (caTCR) comprising:

(a) an extracellular domain comprising the anti-GPC3 antibody moiety; and

(b) a TCR module (TCRM), wherein the TCRM comprises a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and a second TCRD comprising a second TCR-TM, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule.

7. The isolated anti-GPC3 construct of claim 6, wherein:

(1) (i) the first TCR-TM is derived from a TCRγ transmembrane domain, and the second TCR-TM is derived from a TCRδ transmembrane domain; or

(ii) the first TCR-TM is derived from a TCRδ transmembrane domain, and the second TCR-TM is derived from a TCRγ transmembrane domain; or

(2) (i) the first TCR-TM is derived from a TCRα transmembrane domain, and the second TCR-TM is derived from a TCRβ transmembrane domain; or

(ii) the first TCR-TM is derived from a TCRβ transmembrane domain, and the second TCR-TM is derived from a TCRα transmembrane domain.

8. The isolated anti-GPC3 construct of claim 6 or 7, wherein:

(i) the caTCR does not comprise a functional primary immune cell signaling sequence; and/or

(ii) the TCR-associated signaling module that the TCRM facilitates recruitment of is selected from the group consisting of CD3δε, CD3γε, and ζζ.

9. The isolated anti-GPC3 construct of any one of claims 1 to 3, wherein the isolated anti-GPC3 construct is a chimeric co-stimulatory receptor (CSR) comprising:

(a) a ligand-binding module comprising the anti-GPC3 antibody moiety;

(b) a transmembrane module; and

(c) a co-stimulatory immune cell signaling module.

10. The isolated anti-GPC3 construct of claim 9, wherein:

(i) the CSR does not comprise a functional primary immune cell signaling sequence;

(ii) the co-stimulatory immune cell signaling module of the CSR comprises a portion of the intracellular domain of a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, LIGHT, KG2C, B7-H3, and a ligand that specifically binds with CD83; and/or

(iii) the CSR comprises the amino acid sequence of SEQ ID NO: 531.

11. The isolated anti-GPC3 construct of any one of claims 1 to 3, wherein the isolated anti-GPC3 construct is an immunoconjugate comprising the anti-GPC3 antibody moiety and an effector molecule, (i) wherein the effector molecule is a therapeutic agent selected from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid, or (ii) wherein the effector molecule is a label.

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12. An isolated nucleic acid or a vector encoding the polypeptide components of the isolated anti-GPC3 construct of any one of claims 1 to 11.

13. An isolated host cell expressing the anti-GPC3 construct of any one of claims 1 to 11, or comprising the isolated nucleic acid or the vector of claim 12.

14. An effector cell expressing the isolated anti-GPC3 construct of any one of claims 1 to 11 or comprising the isolated nucleic acid or the vector of claim 12.

15. An effector cell expressing the CSR of claim 9 or claim 10, wherein the effector cell further expresses a caTCR.

16. The effector cell of claim 15, wherein the caTCR comprises:

(a) an extracellular domain, wherein the extracellular domain comprises:

(i) an anti-GPC3 antibody moiety; or

(ii) an anti-AMC antibody moiety that specifically binds to a complex comprising an alpha-fetoprotein (AFP) peptide and a major histocompatibility complex (MHC) class I protein (an AFP/MHC class I complex, or AMC); and

(b) a TCR module (TCRM), wherein the TCRM comprises a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and a second TCRD comprising a second TCR-TM, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule.

17. An effector cell expressing the caTCR of any one of claims 6 to 8, wherein the effector cell further comprises a CSR, and wherein the CSR comprises:

(a) a ligand-binding module comprising an antibody moiety, optionally wherein the antibody moiety is an anti-GPC3 antibody moiety;

(b) a transmembrane module; and

(c) a co-stimulatory immune cell signaling module.

18. The effector cell of claim 16 or 17, wherein:

(I) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID

NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 309, and a V_L comprising the amino acid sequence of SEQ ID NO: 360; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 309, and a V_L comprising the amino acid sequence of SEQ ID NO: 360; or

(II) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 309, and a V_L comprising the amino acid sequence of SEQ ID NO: 360; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO:

123; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 327, and a V_L comprising the amino acid sequence of SEQ ID NO: 378; or

(III) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 309, and a V_L comprising the amino acid sequence of SEQ ID NO: 360; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 330, and a V_L comprising the amino acid sequence of SEQ ID NO: 381; or

(IV) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of

SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 327, and a V_L comprising the amino acid sequence of SEQ ID NO: 378; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 309, and a V_L comprising the amino acid sequence of SEQ ID NO: 360; or

(V) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 327, and a V_L comprising the amino acid sequence of SEQ ID NO: 378; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID

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NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 327, and a V_L comprising the amino acid sequence of SEQ ID NO: 378; or

(VI) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 327, and a V_L comprising the amino acid sequence of SEQ ID NO: 378; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 330, and a V_L comprising the amino acid sequence of SEQ ID NO: 381; or

(VII) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID

NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 330, and a V_L comprising the amino acid sequence of SEQ ID NO: 381; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 54, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 105; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 156, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 258; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 309, and a V_L comprising the amino acid sequence of SEQ ID NO: 360; or

(VIII) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 330, and a V_L comprising the amino acid sequence of SEQ ID NO: 381; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 21, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 123; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 174, an LC-CDR2 comprising the amino acid sequence of SEQ ID

NO: 225, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 276; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 327, and a V_L comprising the amino acid sequence of SEQ ID NO: 378; or

(VIV) (1) the caTCR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 330, and a V_L comprising the amino acid sequence of SEQ ID NO: 381; and

(2) the CSR comprises an anti-GPC3 antibody moiety comprising:

(a) i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 24, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 75, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 126; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 177, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 228, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 279; and/or

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 330, and a V_L comprising the amino acid sequence of SEQ ID NO: 381; or

(X) the caTCR is the anti-GPC3 caTCR of any one of claims 6 to 8.

19. The effector cell of any one of claims 14 to 18, wherein the effector cell is a T cell, optionally wherein the T cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, and a natural killer T cell.

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20. A pharmaceutical composition comprising the isolated anti-GPC3 construct of any one of claims 1 to 11 or the effector cell of any one of claims 14 to 19, and a pharmaceutically acceptable carrier.

21. A method of treating an individual having a GPC3-positive disease, comprising administering to the individual an effective amount of the isolated anti-GPC3 construct of any one of claims 1 to 11 or the effector cell of any one of claims 14 to 19 or the pharmaceutical composition of claim 20.

22. Use of the isolated anti-GPC3 construct of any one of claims 1 to 11 or the effector cell of any one of claims 14 to 19 or the pharmaceutical composition of claim 20, (i) in treating an individual having an GPC3-positive disease or (ii) in the manufacture of a medicament for treating an individual having an GPC3-positive disease.

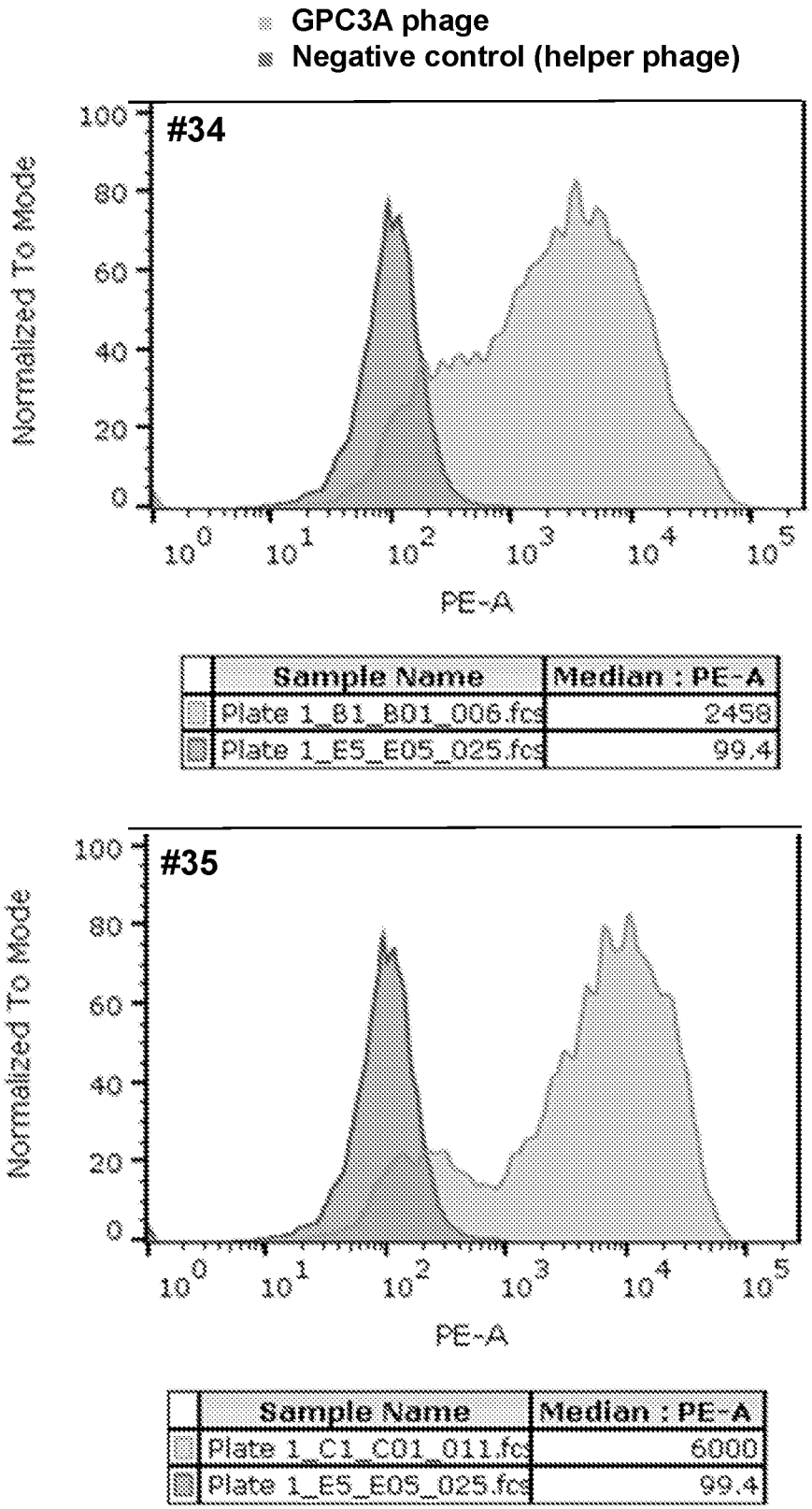
23. The method of claim 21, or the use of claim 22, wherein the GPC3-positive disease is a GPC3-positive cancer, optionally wherein the GPC3-positive cancer is selected from the group consisting of HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, and liposarcoma.

24. A method of producing an isolated anti-GPC3 construct, comprising:

(a) culturing a host cell comprising the isolated nucleic acid or the vector of claim 12, or the isolated host cell of claim 13, under a condition effective to express the anti-GPC3 construct; and

(b) obtaining the expressed anti-GPC3 construct from said host cell.

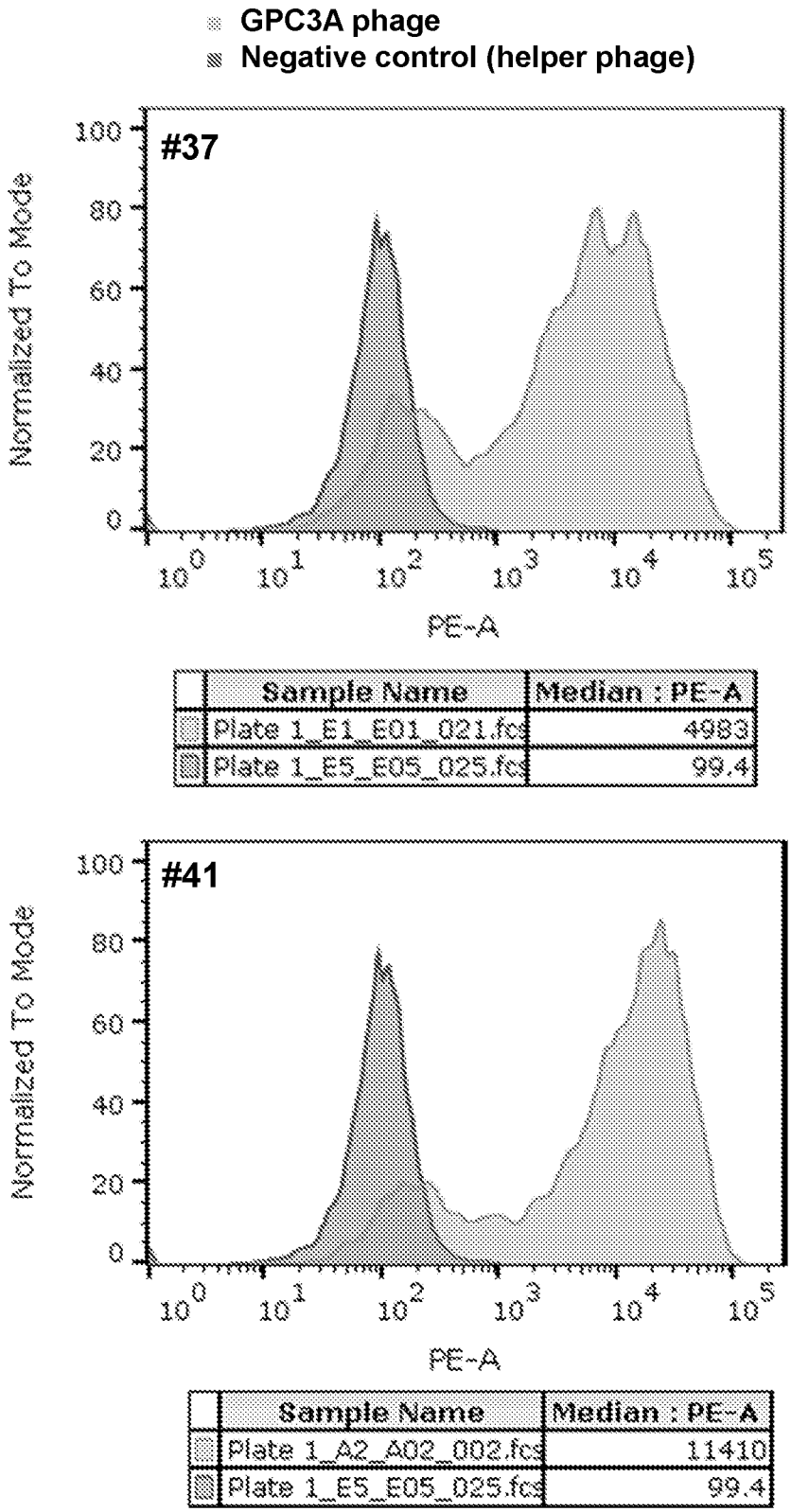
FIG. 1A



	Sample Name	Median : PE-A
	Plate 1_B1_B01_006.fcs	2458
	Plate 1_E5_E05_025.fcs	99.4

	Sample Name	Median : PE-A
	Plate 1_C1_C01_011.fcs	6000
	Plate 1_E5_E05_025.fcs	99.4

FIG. 1B



Sample Name	Median : PE-A
Plate 1_E1_E01_021.fcs	4983
Plate 1_E5_E05_025.fcs	99.4

Sample Name	Median : PE-A
Plate 1_A2_A02_002.fcs	11410
Plate 1_E5_E05_025.fcs	99.4

FIG. 1C

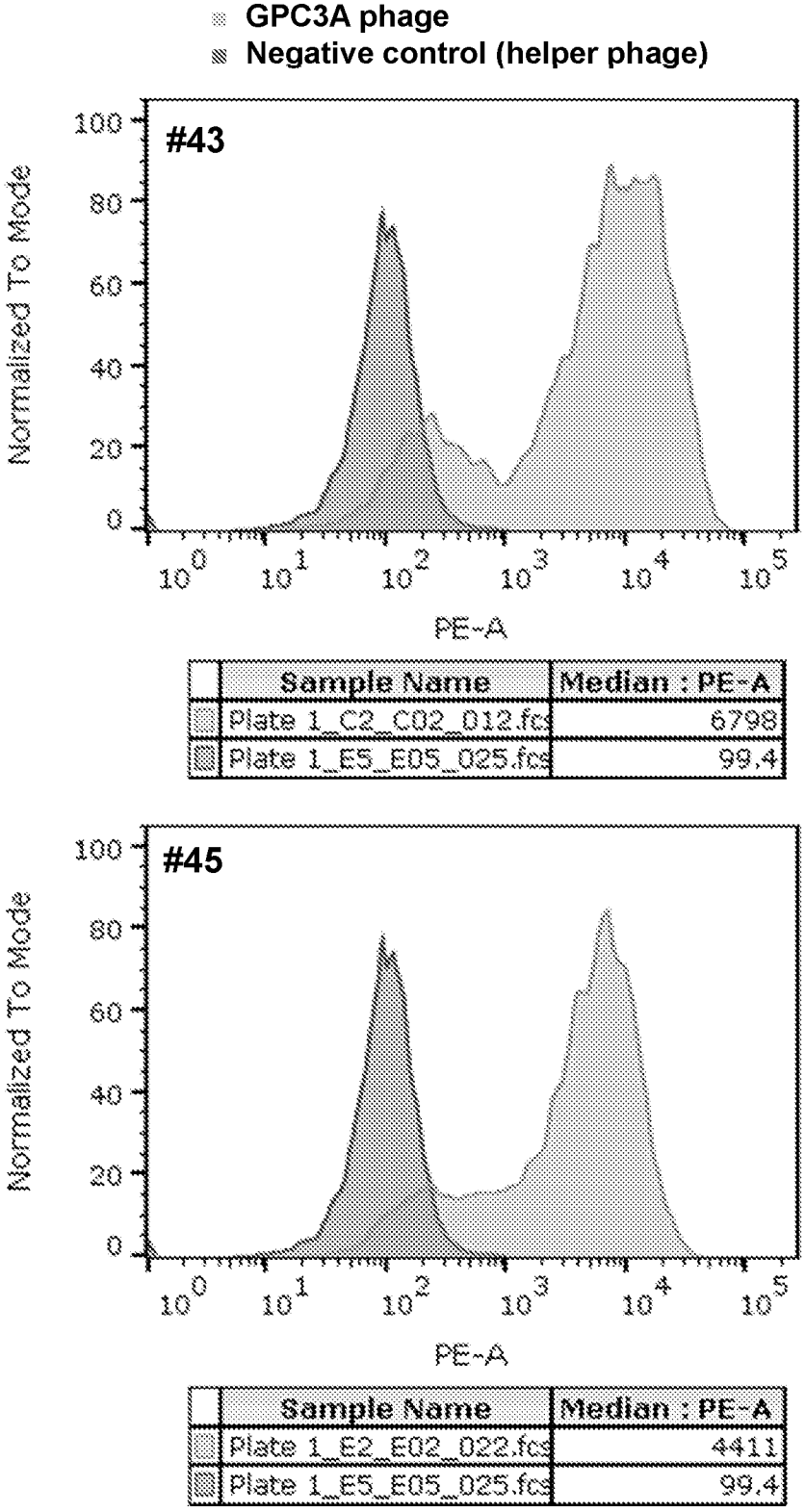

GPC3A phage
Negative control (helper phage)
#43
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_C2_C02_012.fcs
6798
Plate 1_E5_E05_025.fcs
99.4
#45
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_E2_E02_022.fcs
4411
Plate 1_E5_E05_025.fcs
99.4

FIG. 1D

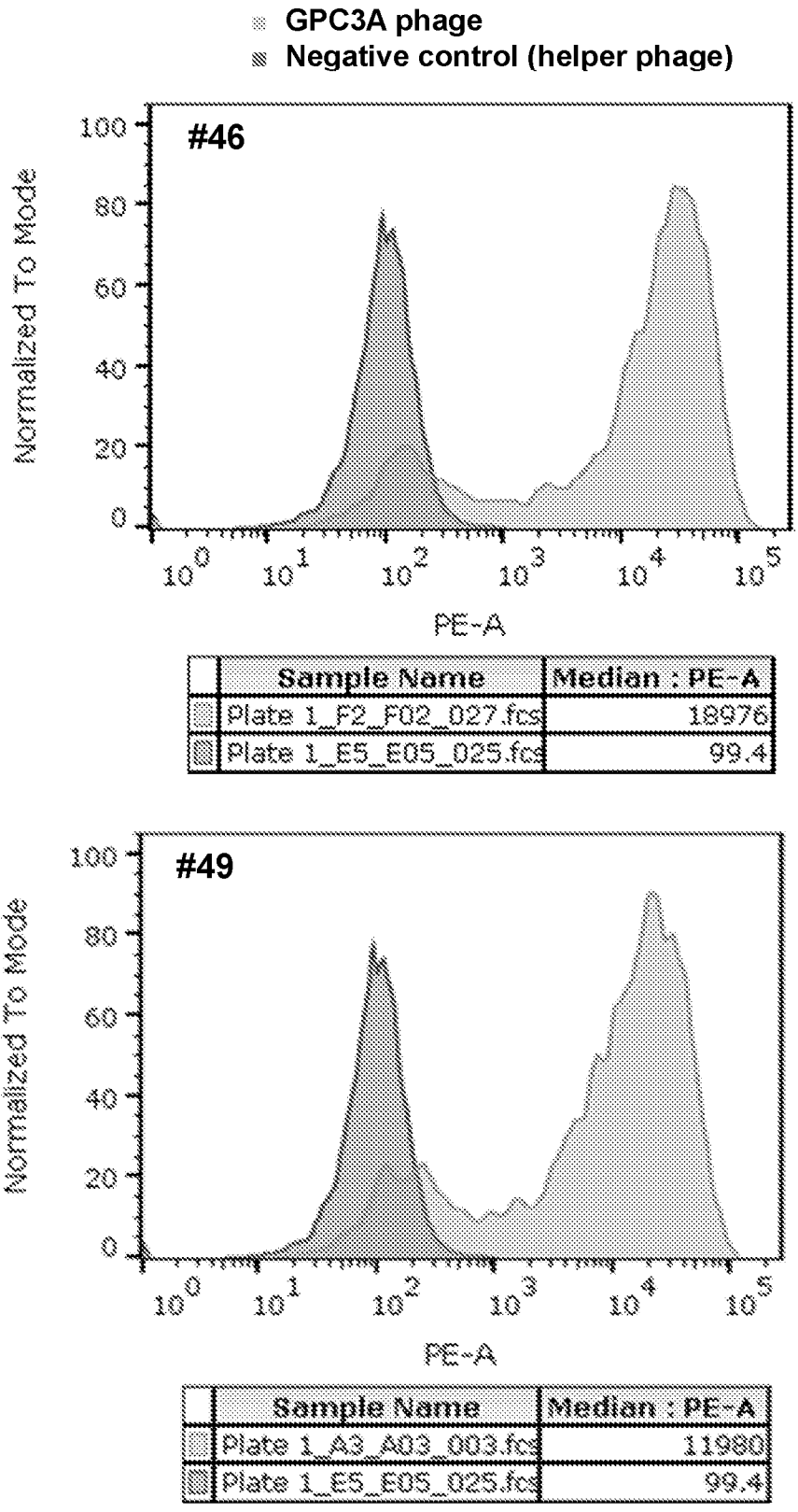

GPC3A phage
Negative control (helper phage)
#46
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_F2_F02_027.fcs
18976
Plate 1_E5_E05_025.fcs
99.4
#49
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_A3_A03_003.fcs
11980
Plate 1_E5_E05_025.fcs
99.4

FIG. 1E

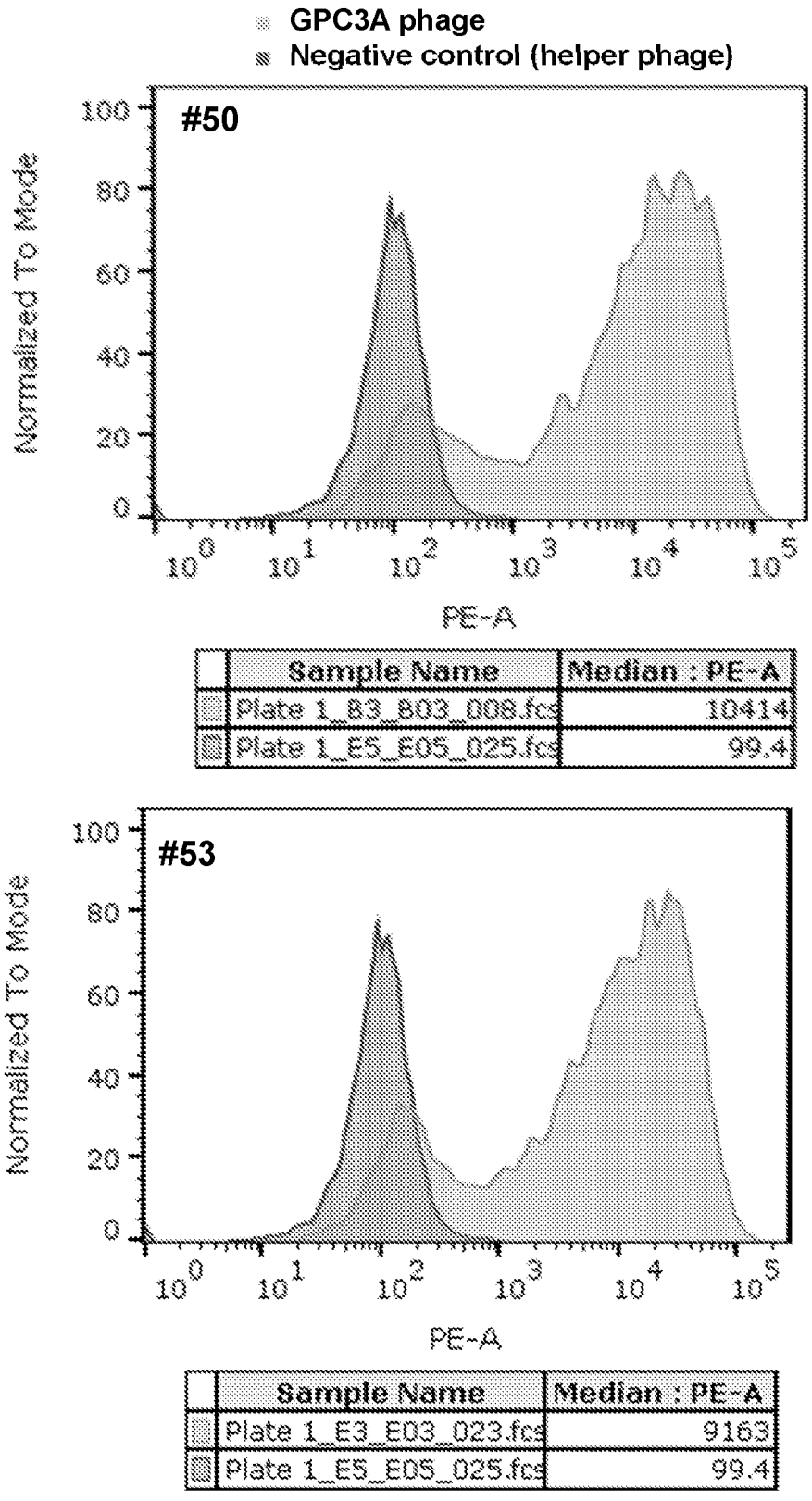
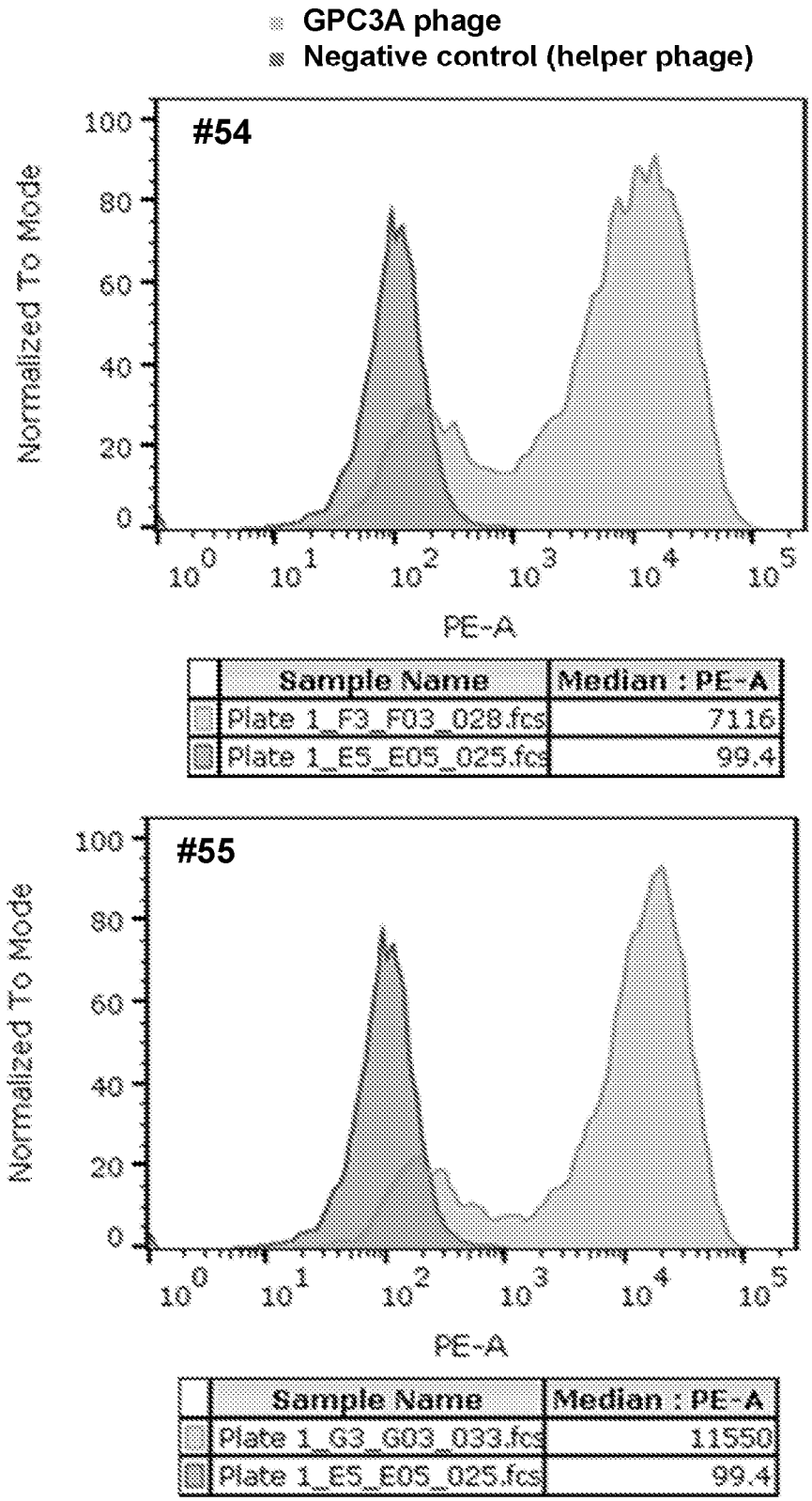

GPC3A phage
Negative control (helper phage)
#50
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_B3_B03_008.fcs
10414
Plate 1_E5_E05_025.fcs
99.4
#53
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_E3_E03_023.fcs
9163
Plate 1_E5_E05_025.fcs
99.4

FIG. 1F



	Sample Name	Median : PE-A
	Plate 1_F3_F03_028.fcs	7116
	Plate 1_E5_E05_025.fcs	99.4

	Sample Name	Median : PE-A
	Plate 1_G3_G03_033.fcs	11550
	Plate 1_E5_E05_025.fcs	99.4

FIG. 1G

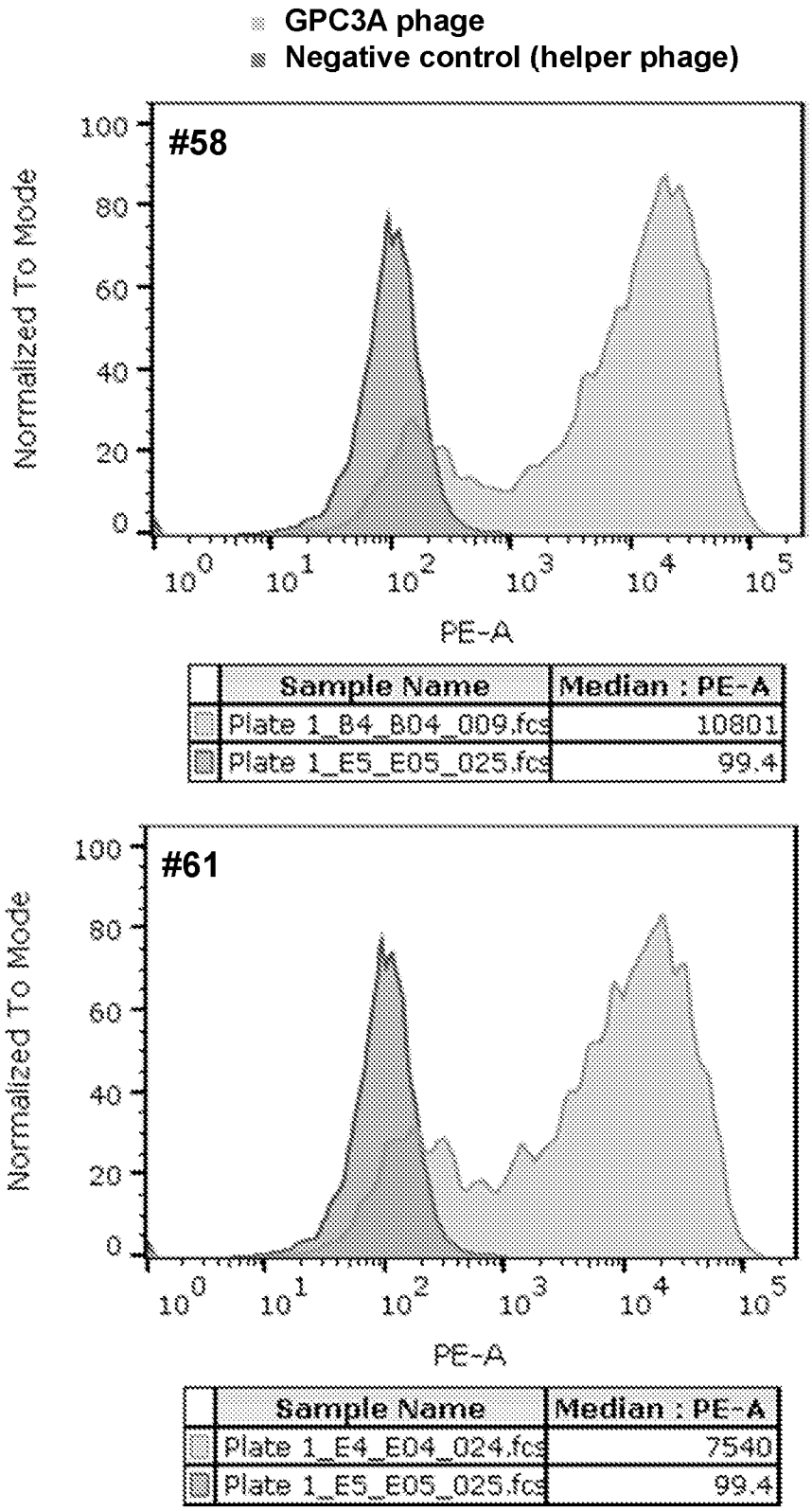

GPC3A phage
Negative control (helper phage)
#58
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_B4_B04_009.fcs
10801
Plate 1_E5_E05_025.fcs
99.4
#61
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_E4_E04_024.fcs
7540
Plate 1_E5_E05_025.fcs
99.4

FIG. 2A

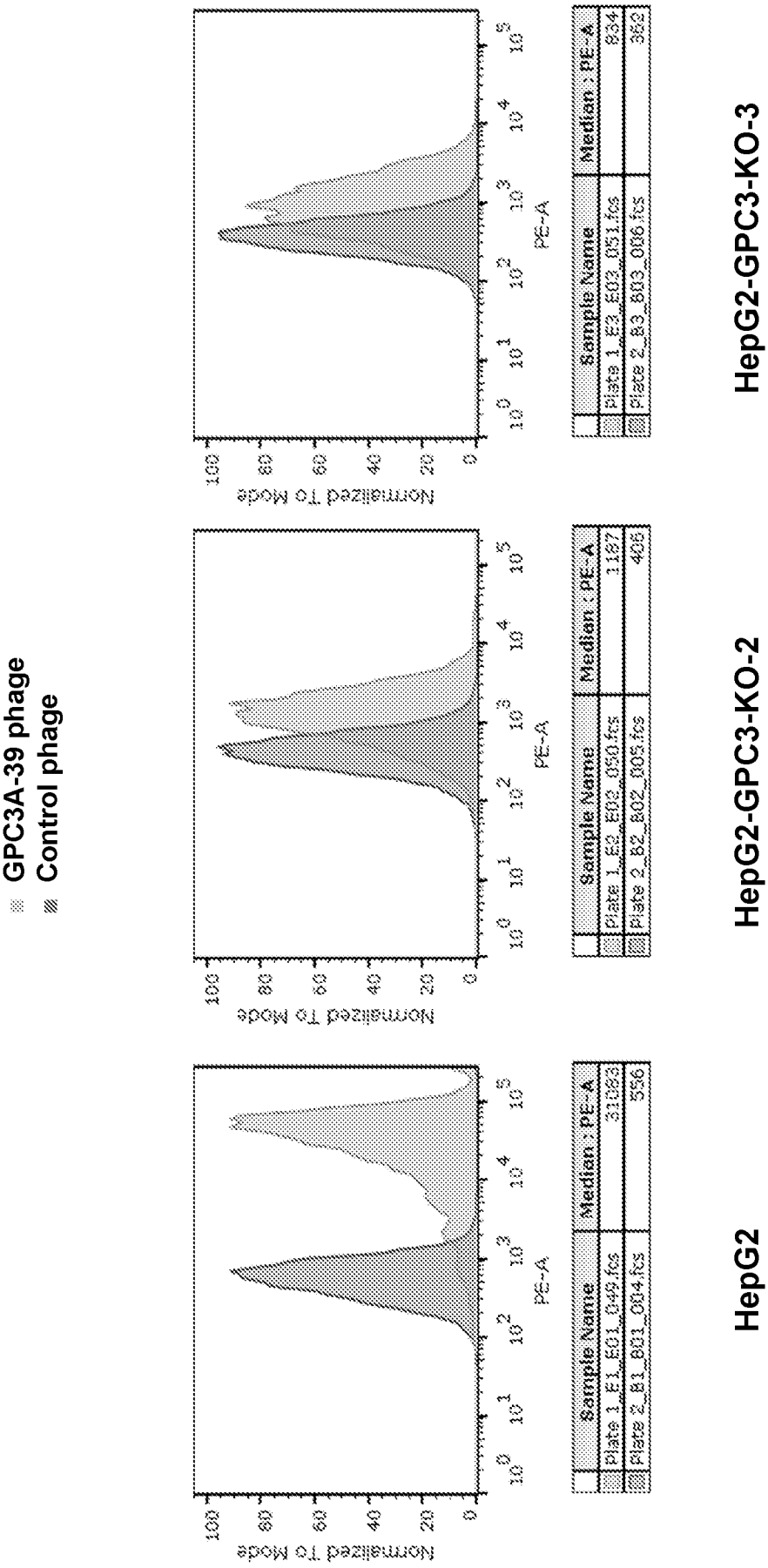

GPC3A-39 phage
Control phage
Normalized To Mode
PE-A
HepG2
HepG2-GPC3-KO-2
HepG2-GPC3-KO-3
Sample Name | Median : PE-A
Plate 1_E1_E01_049.fcs | 31083
Plate 2_B1_B01_004.fcs | 556
Plate 1_E2_E02_050.fcs | 1187
Plate 2_B2_B02_005.fcs | 406
Plate 1_E3_E03_051.fcs | 834
Plate 2_B3_B03_006.fcs | 362

FIG. 2B

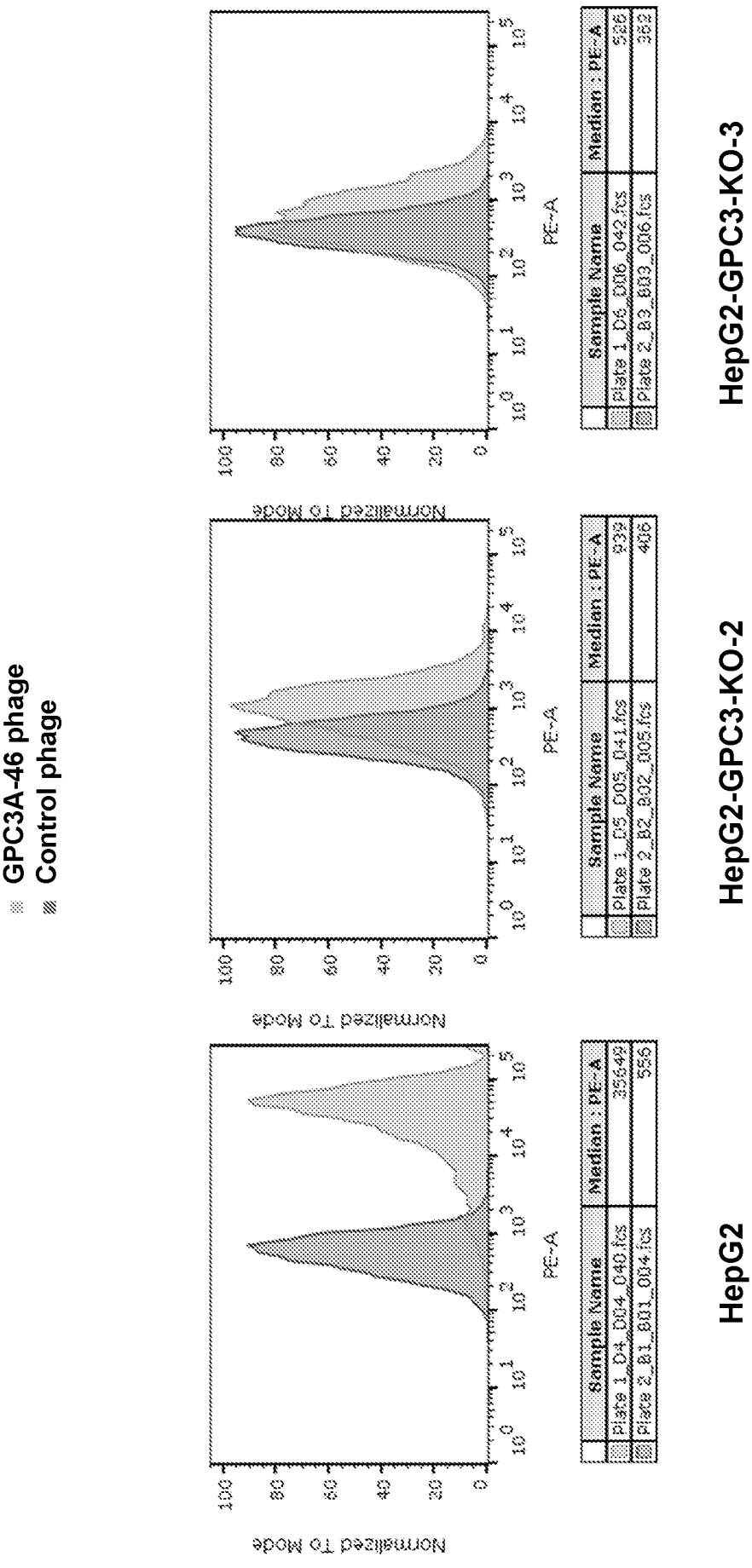

GPC3A-46 phage
Control phage
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_D4_D04_040.fcs
35649
Plate 2_B1_B01_014.fcs
556
HepG2
Plate 1_D5_D05_041.fcs
939
Plate 2_B2_B02_005.fcs
406
HepG2-GPC3-KO-2
Plate 1_D6_D06_042.fcs
826
Plate 2_B3_B03_006.fcs
362
HepG2-GPC3-KO-3

FIG. 2C

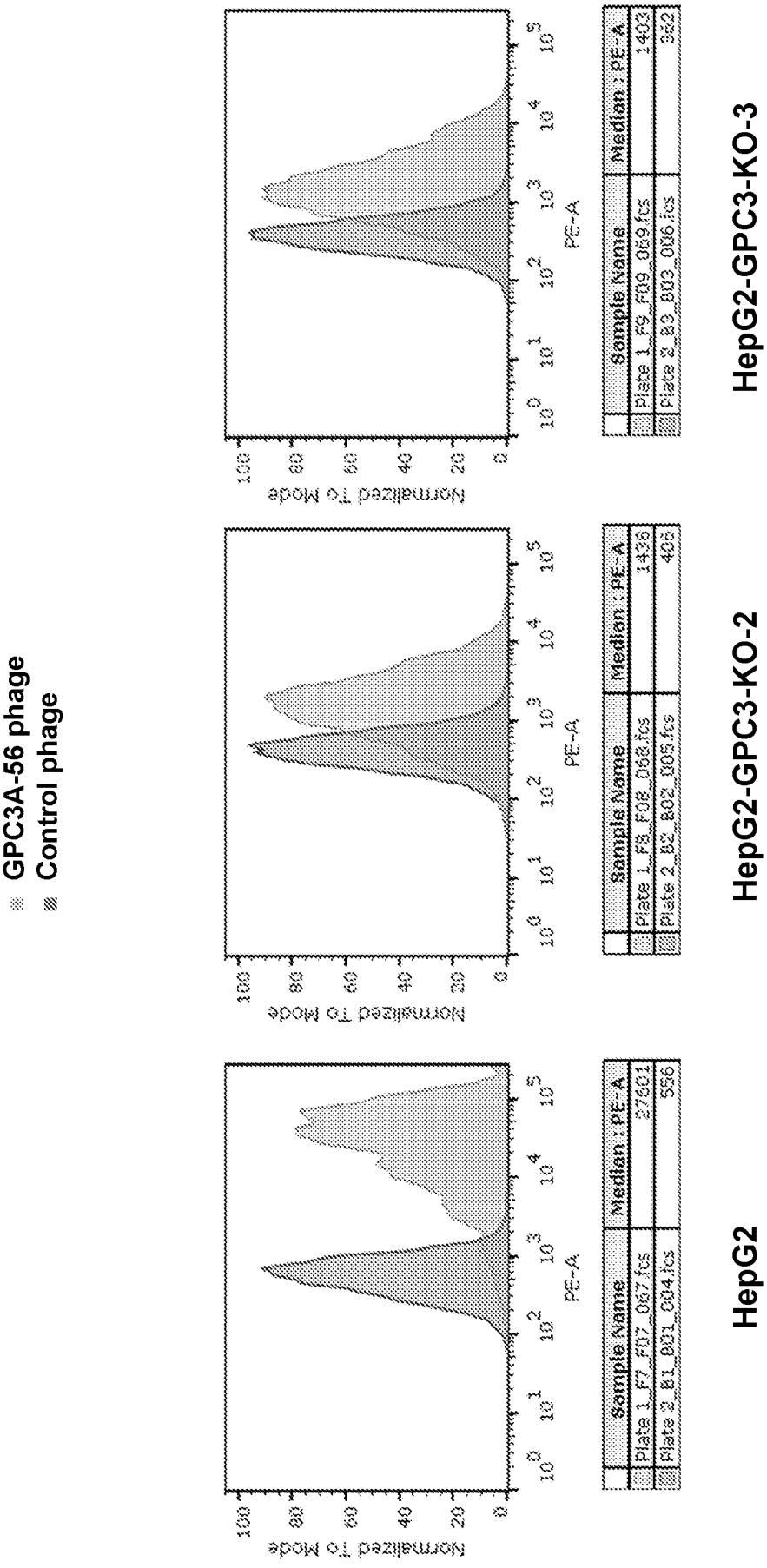

GPC3A-56 phage
Control phage
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_F7_F07_067.fcs
27601
Plate 2_B1_B01_004.fcs
556
HepG2
Plate 1_F8_F08_068.fcs
1438
Plate 2_B2_B02_005.fcs
405
HepG2-GPC3-KO-2
Plate 1_F9_F09_069.fcs
1403
Plate 2_B3_B03_006.fcs
362
HepG2-GPC3-KO-3

FIG. 2D

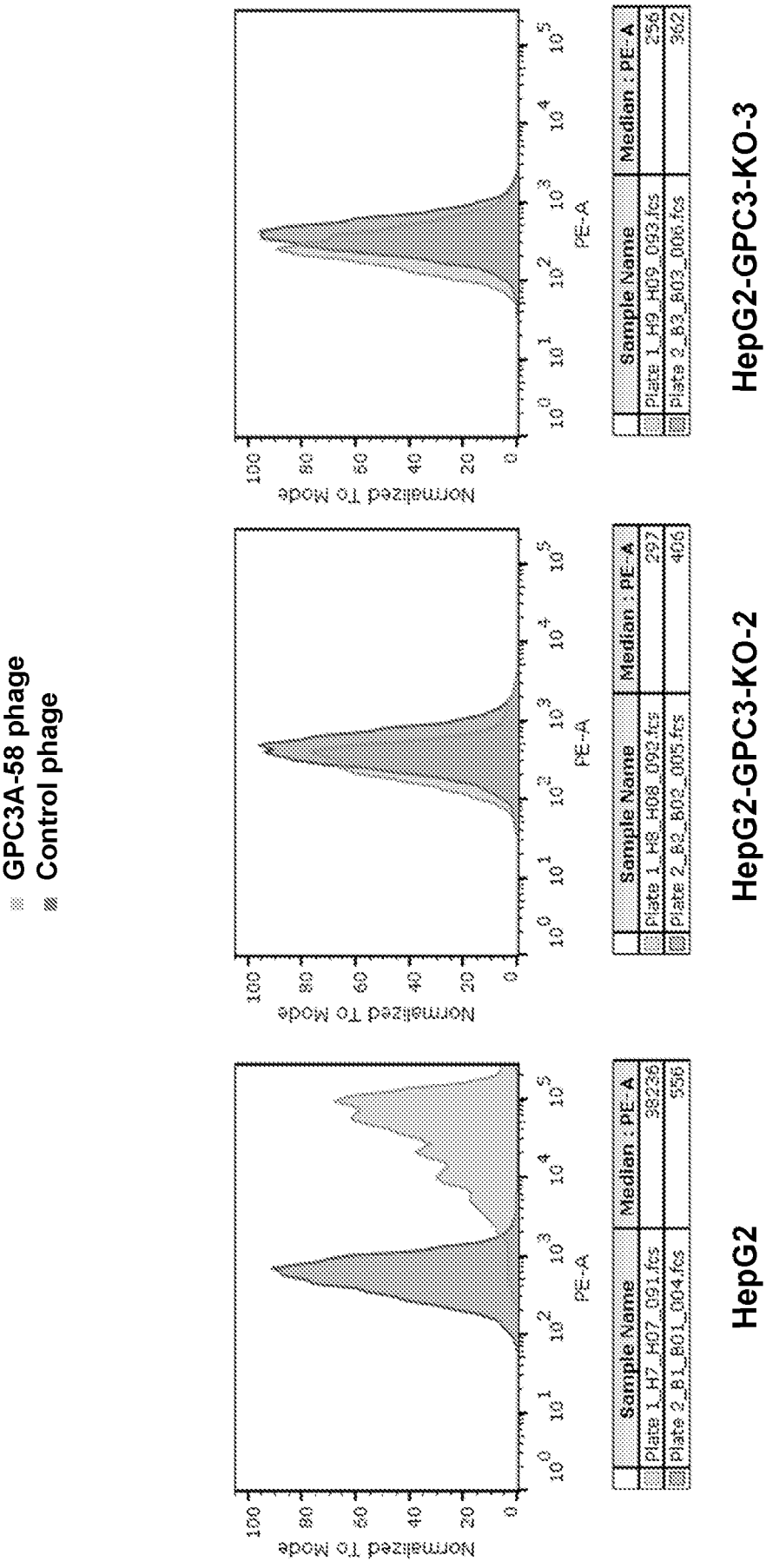

GPC3A-58 phage
Control phage
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_H7_H07_091.fcs
38236
Plate 2_B1_B01_004.fcs
556
HepG2
Plate 1_H8_H08_092.fcs
297
Plate 2_B2_B02_005.fcs
406
HepG2-GPC3-KO-2
Plate 1_H9_H09_093.fcs
256
Plate 2_B3_B03_006.fcs
362
HepG2-GPC3-KO-3

FIG. 2E

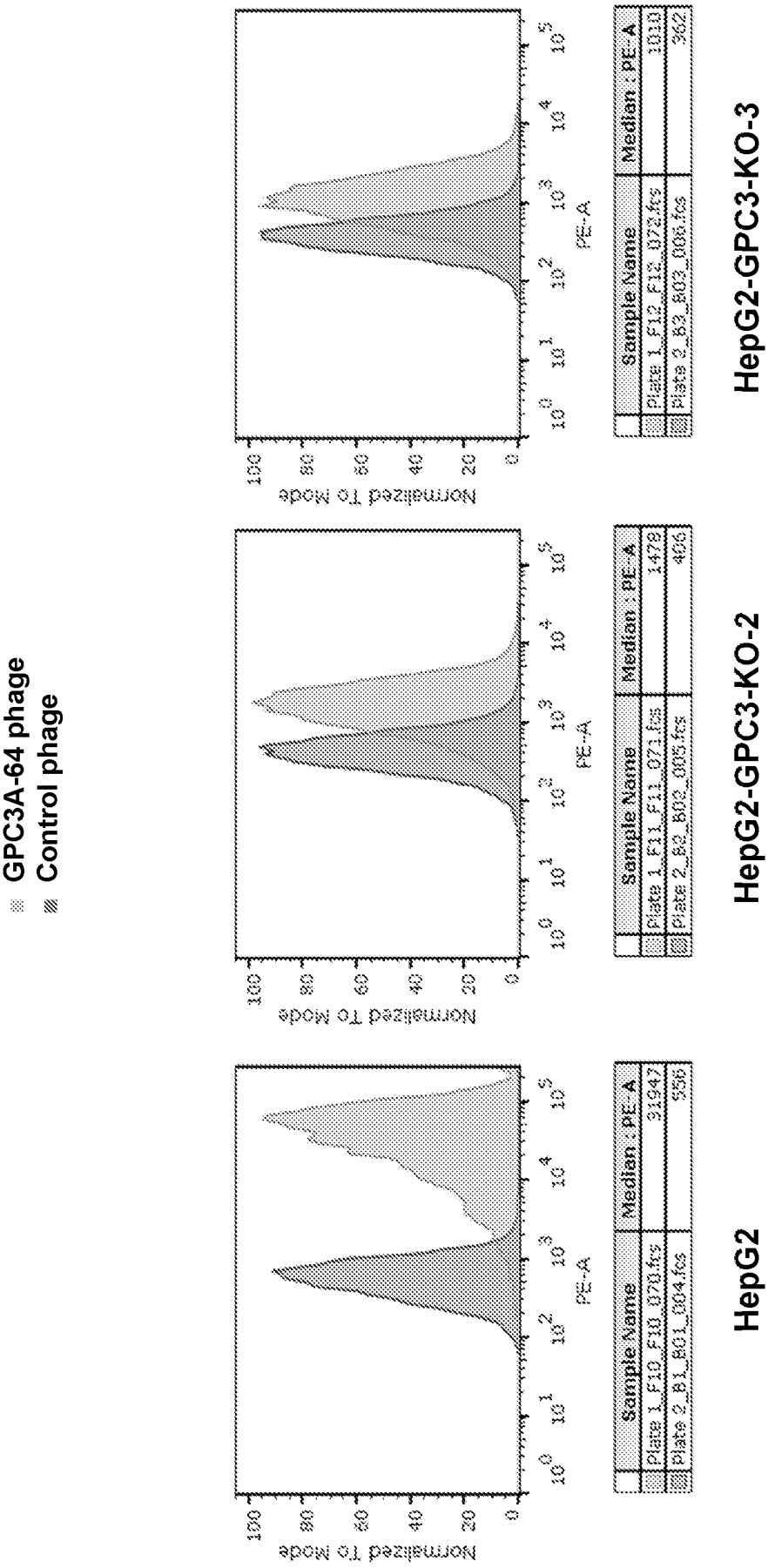
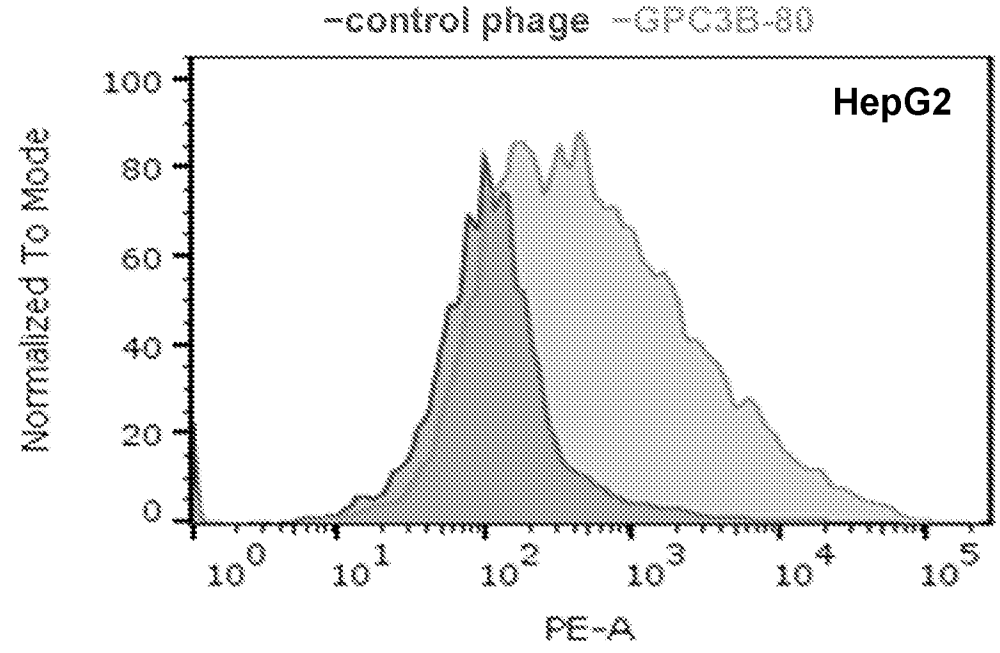
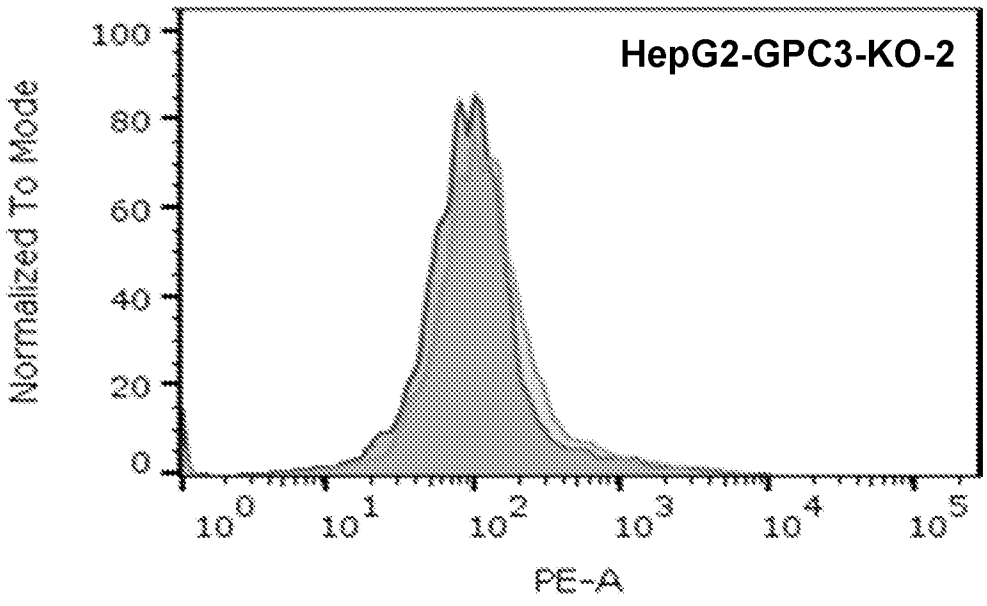

GPC3A-64 phage
Control phage
Normalized To Mode
PE-A
HepG2
HepG2-GPC3-KO-2
HepG2-GPC3-KO-3
Sample Name
Median : PE-A
Plate 1_F10_F10_070.fcs 31947
Plate 2_B1_B01_004.fcs 556
Plate 1_F11_F11_071.fcs 1478
Plate 2_B2_B02_005.fcs 406
Plate 1_F12_F12_072.fcs 1010
Plate 2_B3_B03_006.fcs 362

FIG. 3A



Sample Name	Median : PE-A
Plate 1_A3_A03_003.fcs	446
Plate 1_F5_F05_041.fcs	106



Sample Name	Median : PE-A
Plate 1_A4_A04_004.fcs	112
Plate 1_F6_F06_042.fcs	92.4

FIG. 3B

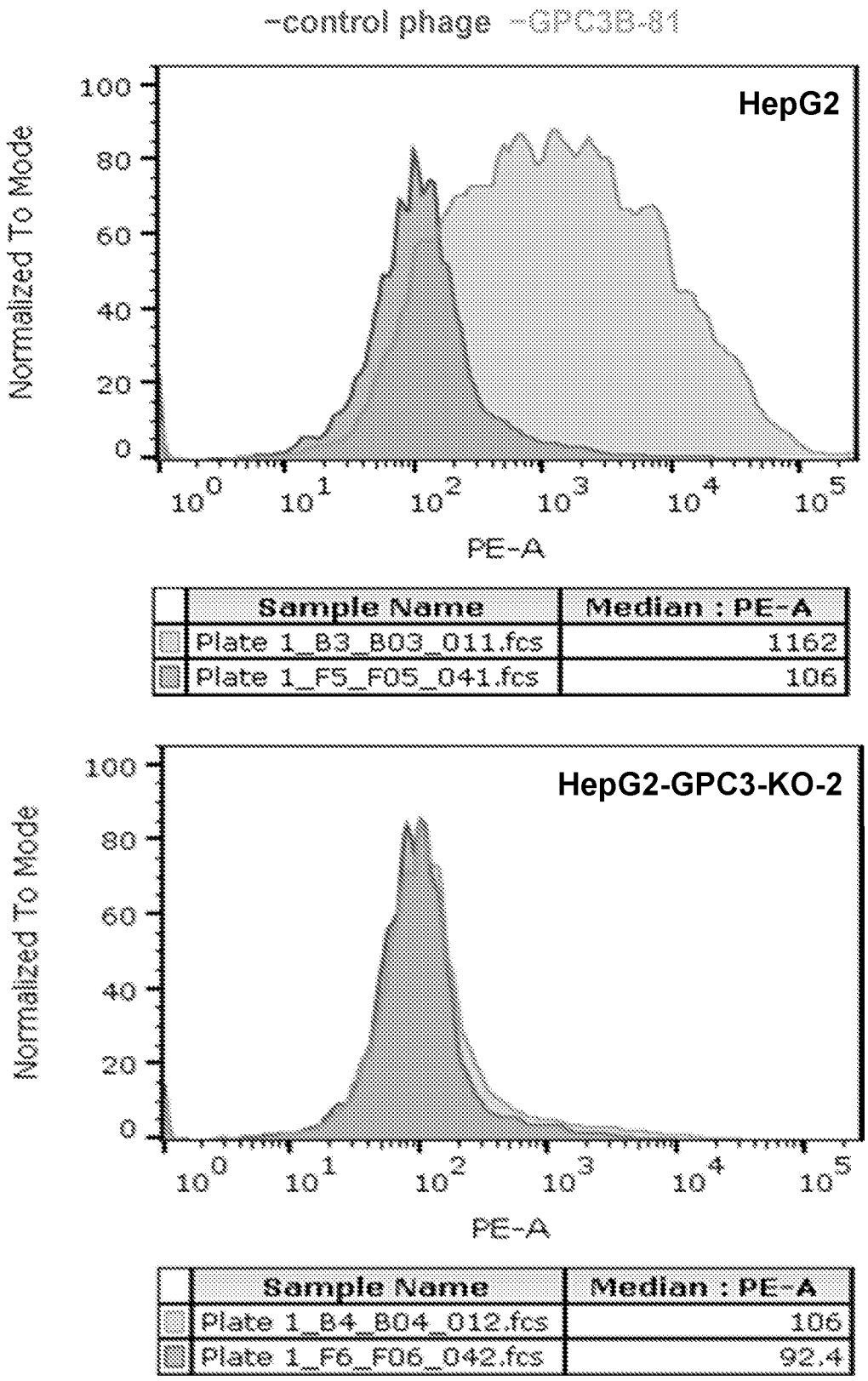

-control phage -GPC3B-81
HepG2
Normalized To Mode
100
80
60
40
20
0
10^0 10^1 10^2 10^3 10^4 10^5
PE-A
Sample Name
Median : PE-A
Plate 1_B3_B03_011.fcs
1162
Plate 1_F5_F05_041.fcs
106
HepG2-GPC3-KO-2
Normalized To Mode
100
80
60
40
20
0
10^0 10^1 10^2 10^3 10^4 10^5
PE-A
Sample Name
Median : PE-A
Plate 1_B4_B04_012.fcs
106
Plate 1_F6_F06_042.fcs
92.4

FIG. 3C

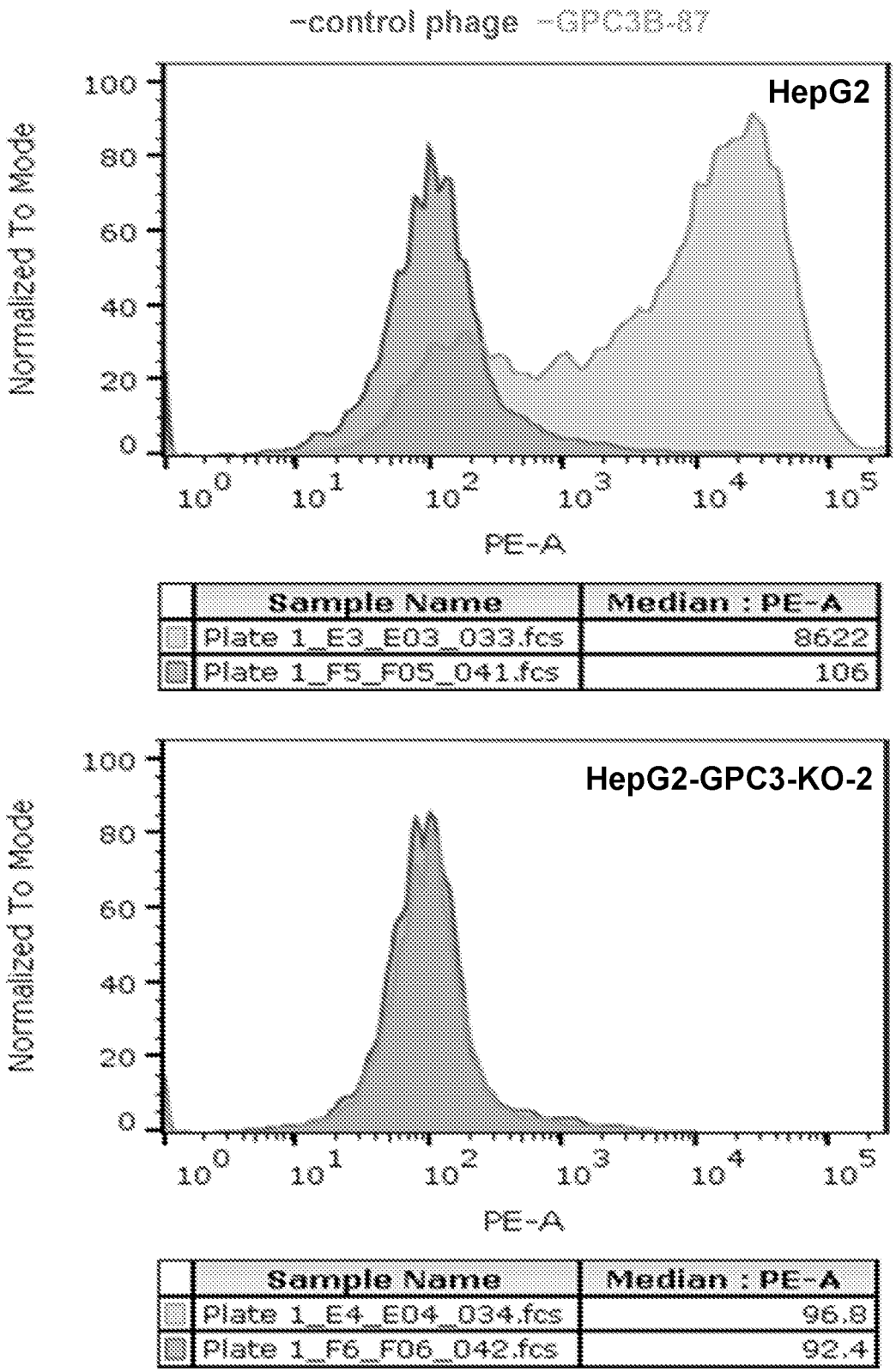
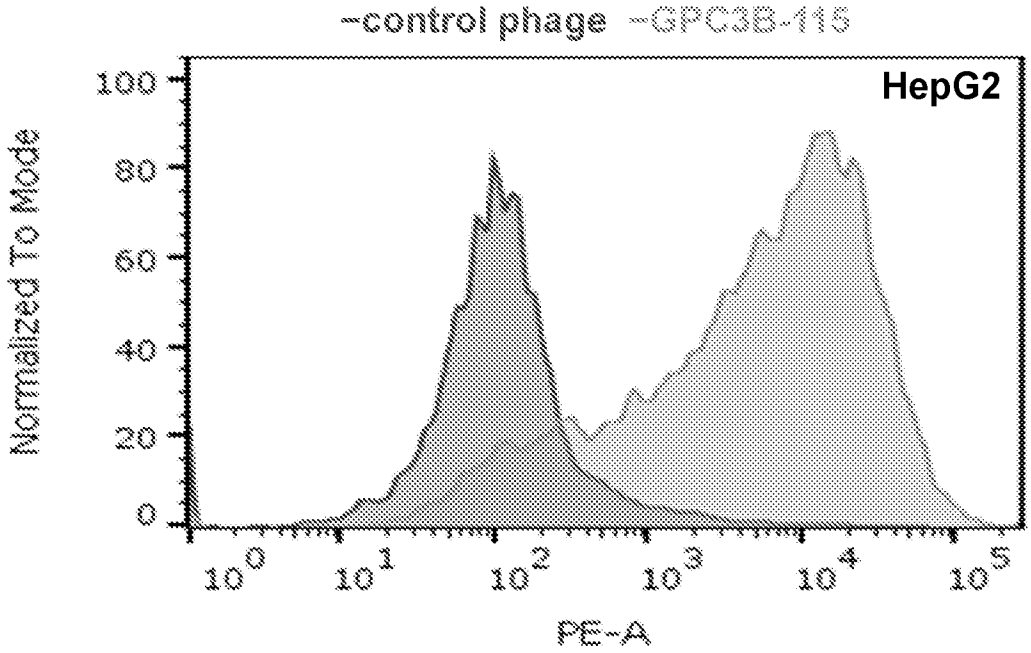
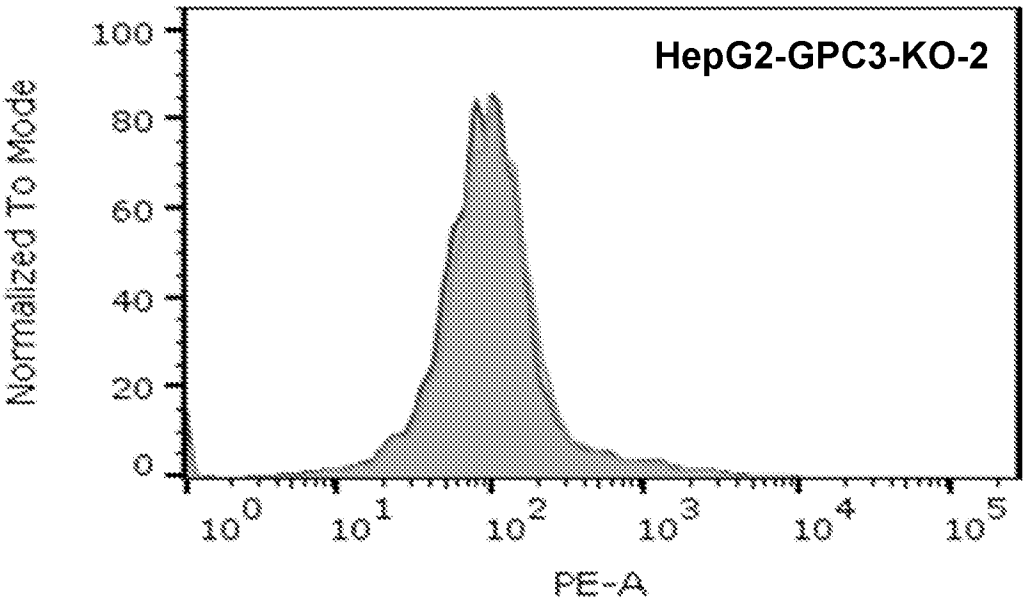

-control phage -GPC3B-87
HepG2
Normalized To Mode
100
80
60
40
20
0
10^0 10^1 10^2 10^3 10^4 10^5
PE-A
Sample Name
Median : PE-A
Plate 1_E3_E03_033.fcs
8622
Plate 1_F5_F05_041.fcs
106
HepG2-GPC3-KO-2
Normalized To Mode
100
80
60
40
20
0
10^0 10^1 10^2 10^3 10^4 10^5
PE-A
Sample Name
Median : PE-A
Plate 1_E4_E04_034.fcs
96.8
Plate 1_F6_F06_042.fcs
92.4

FIG. 3D



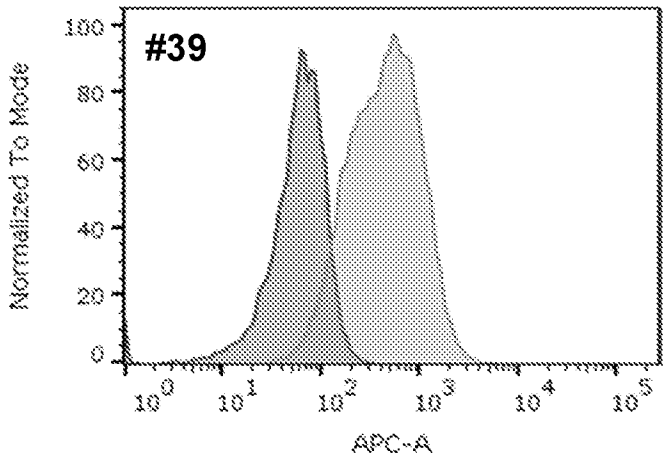
	Sample Name	Median : PE-A
	Plate 1_B5_B05_013.fcs	6319
	Plate 1_F5_F05_041.fcs	106



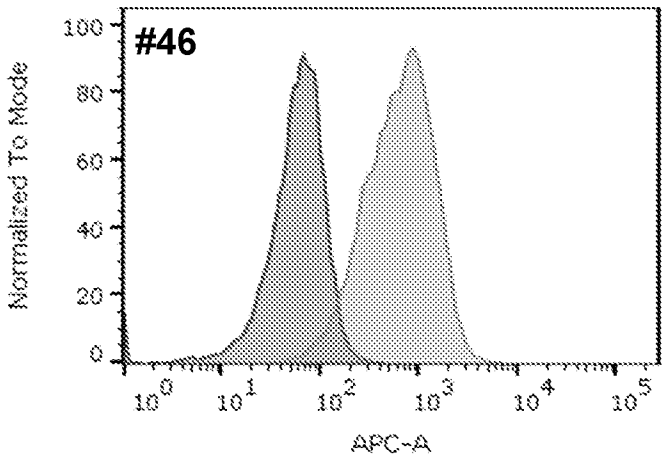
	Sample Name	Median : PE-A
	Plate 1_B6_B06_014.fcs	98.2
	Plate 1_F6_F06_042.fcs	92.4

FIG. 4A

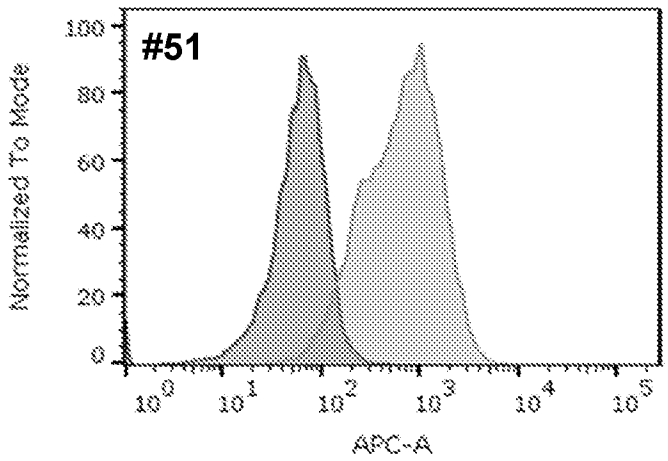
-SK-Hep1
-SK-Hep1-GPC3



Sample Name	Median : APC-A
Plate 1_Hep1-GPC3_A2_A02_002.fc	445
Plate 1_Hep1-GPC3_A1_A01_001.fc	61.6



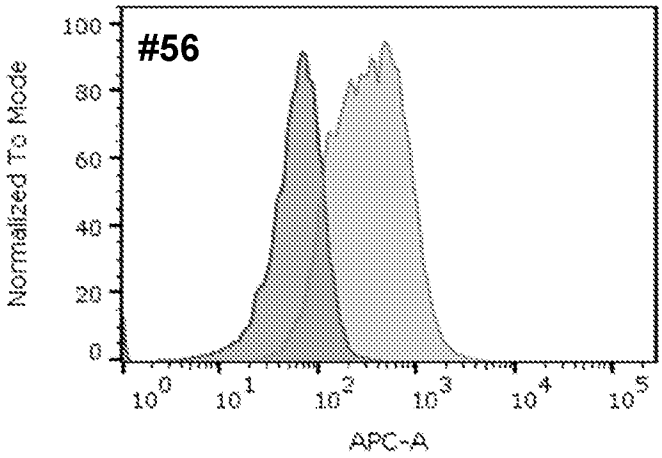
Sample Name	Median : APC-A
Plate 1_Hep1-GPC3_B2_B02_006.fc	657
Plate 1_Hep1-GPC3_B1_B01_005.fc	61.6



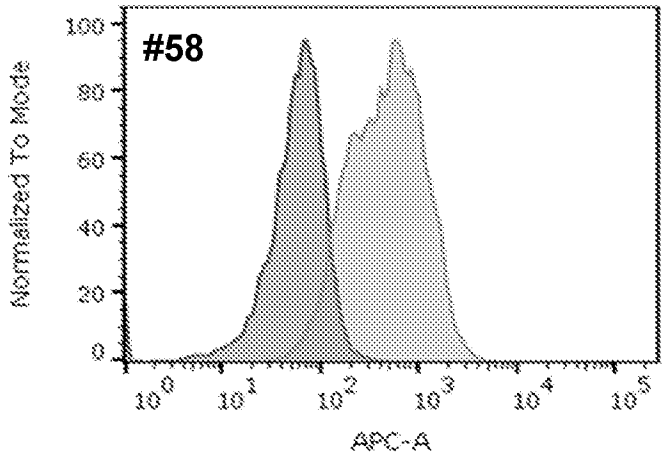
Sample Name	Median : APC-A
Plate 1_Hep1-GPC3_C2_C02_010.fc	677
Plate 1_Hep1-GPC3_C1_C01_009.fc	61.6

FIG. 4B

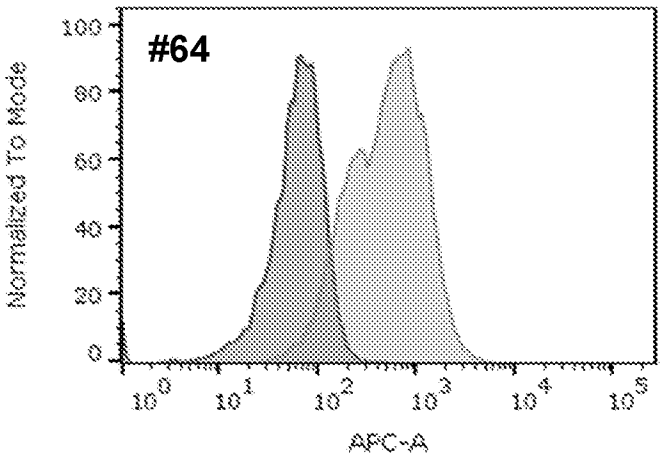
~SK-Hep1
~SK-Hep1-GPC3



Sample Name	Median : APC-A
Plate 1_Hep1-GPC3_D2_D02_014.fc	338
Plate 1_Hep1-GPC3_D1_D01_013.fc	62.9



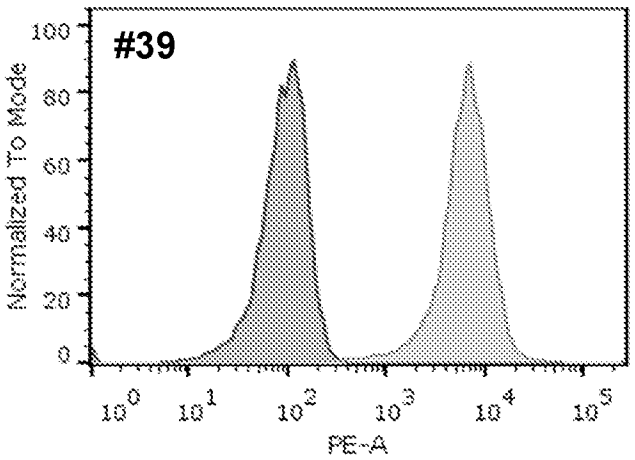
Sample Name	Median : APC-A
Plate 1_Hep1-GPC3_E2_E02_016.fc	478
Plate 1_Hep1-GPC3_E1_E01_015.fc	60.4



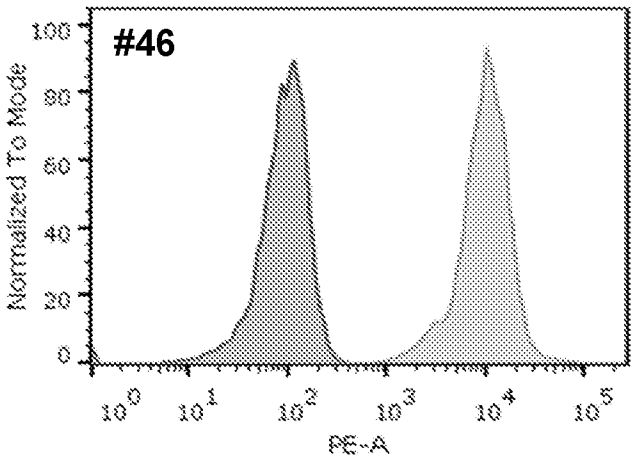
Sample Name	Median : APC-A
Plate 1_Hep1-GPC3_F2_F02_018.fc	525
Plate 1_Hep1-GPC3_F1_F01_017.fc	64.2

FIG. 5A

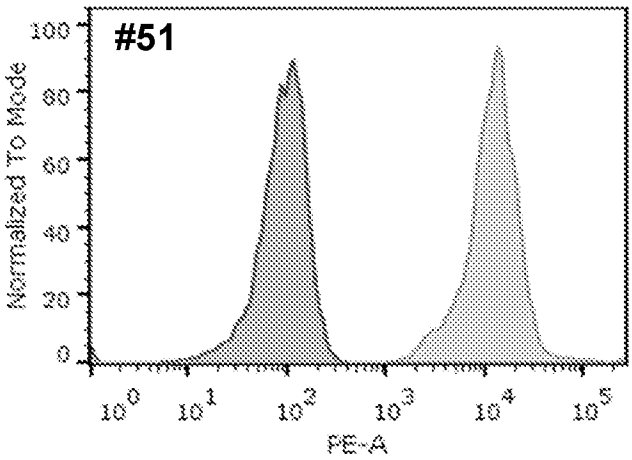
~control L2K
~GPC3A L2K



Sample Name	Median : PE-A
HepG2_A2_A02_001.fcs	6377
HepG2_G2_G02_009.fcs	93.8



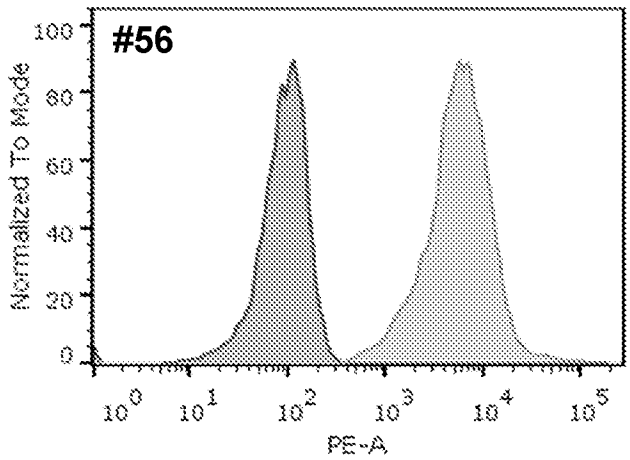
Sample Name	Median : PE-A
HepG2_B2_B02_003.fcs	9852
HepG2_G2_G02_009.fcs	93.8



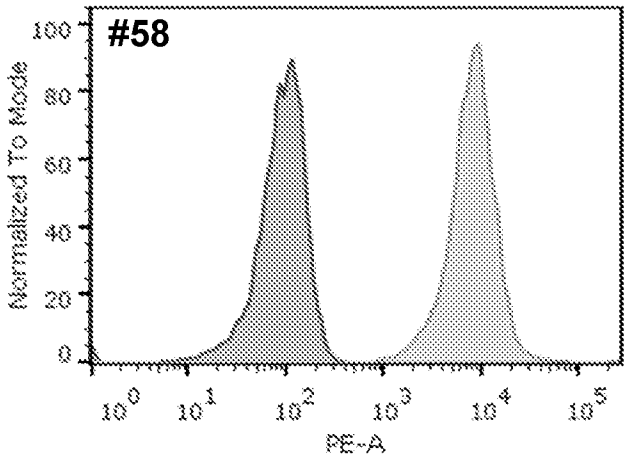
Sample Name	Median : PE-A
HepG2_C2_C02_005.fcs	12156
HepG2_G2_G02_009.fcs	93.8

FIG. 5B

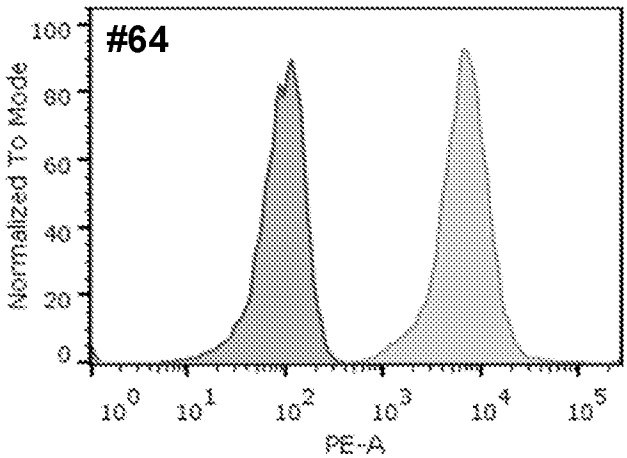
-control L2K
-GPC3A L2K



Sample Name	Median : PE-A
HepG2_D2_D02_006.fcs	5629
HepG2_G2_G02_009.fcs	93.8



Sample Name	Median : PE-A
HepG2_E2_E02_007.fcs	8027
HepG2_G2_G02_009.fcs	93.8



Sample Name	Median : PE-A
HepG2_F2_F02_008.fcs	6602
HepG2_G2_G02_009.fcs	93.8

FIG. 6

Competition for Hep2 cell binding with and without soluble GPC3

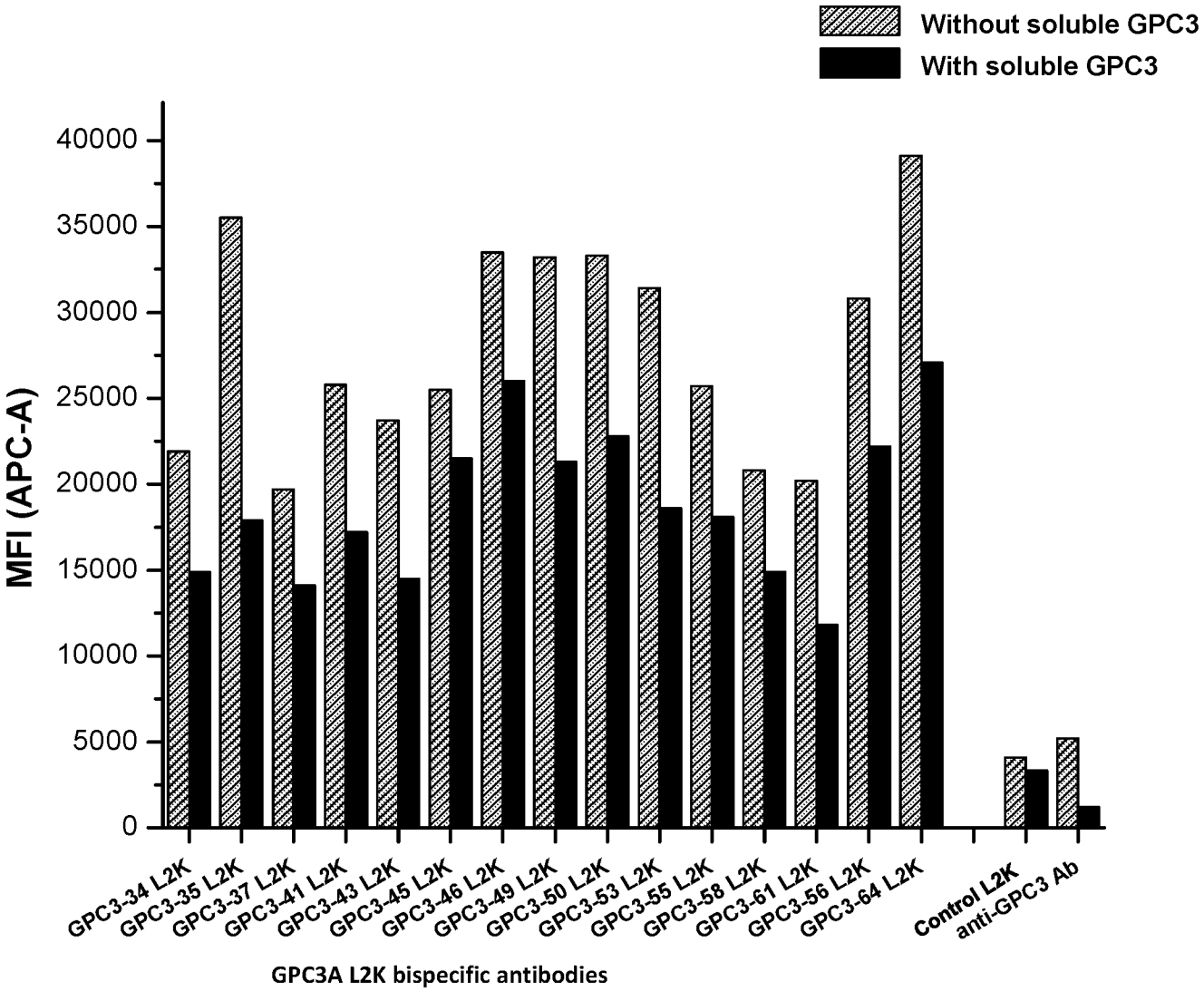


FIG. 7A

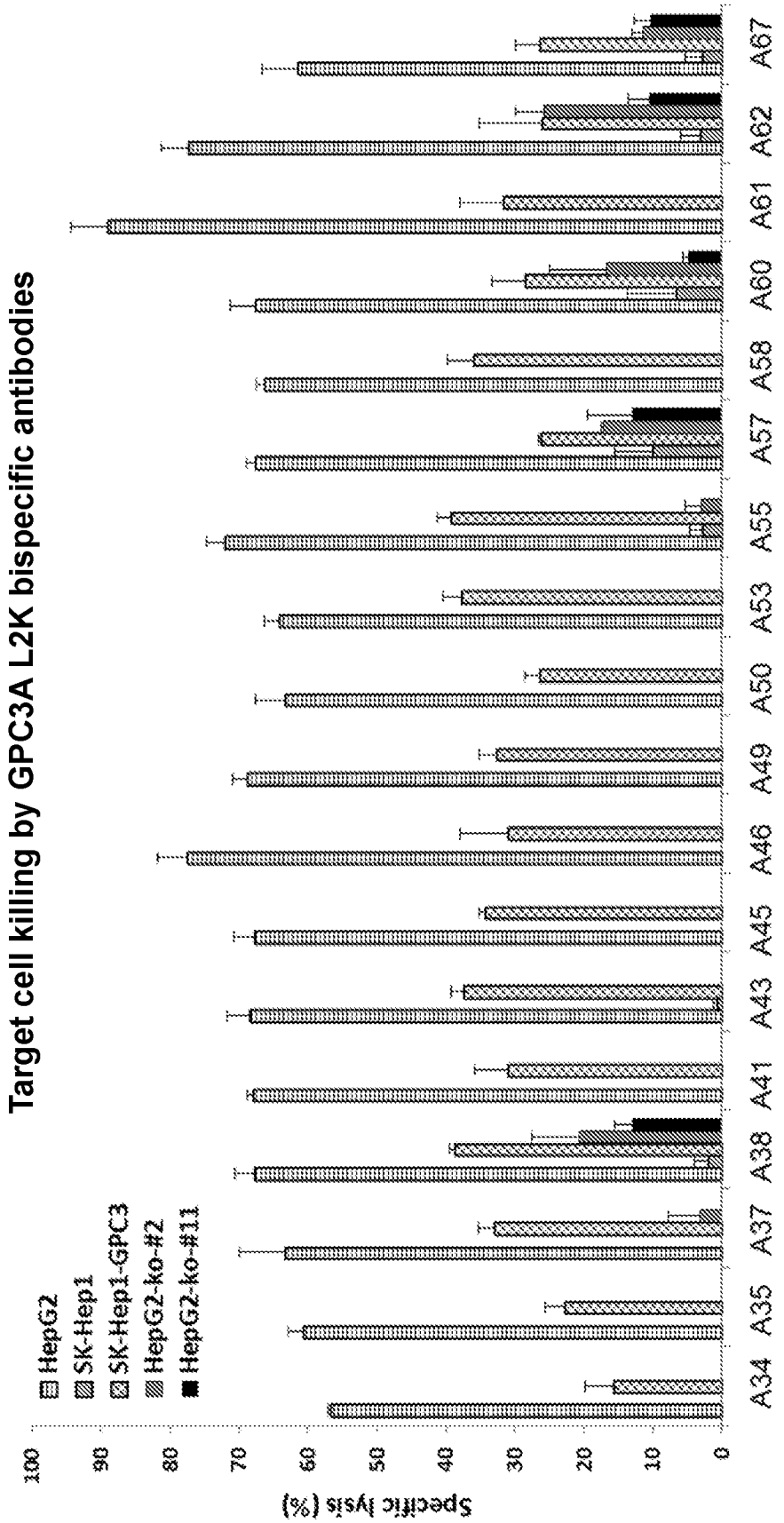

Target cell killing by GPC3A L2K bispecific antibodies
Specific lysis (%)
100
90
80
70
60
50
40
30
20
10
0
HepG2
SK-Hep1
SK-Hep1-GPC3
HepG2-ko-#2
HepG2-ko-#11
A34
A35
A37
A38
A41
A43
A45
A46
A49
A50
A53
A55
A57
A58
A60
A61
A62
A67

FIG. 7B

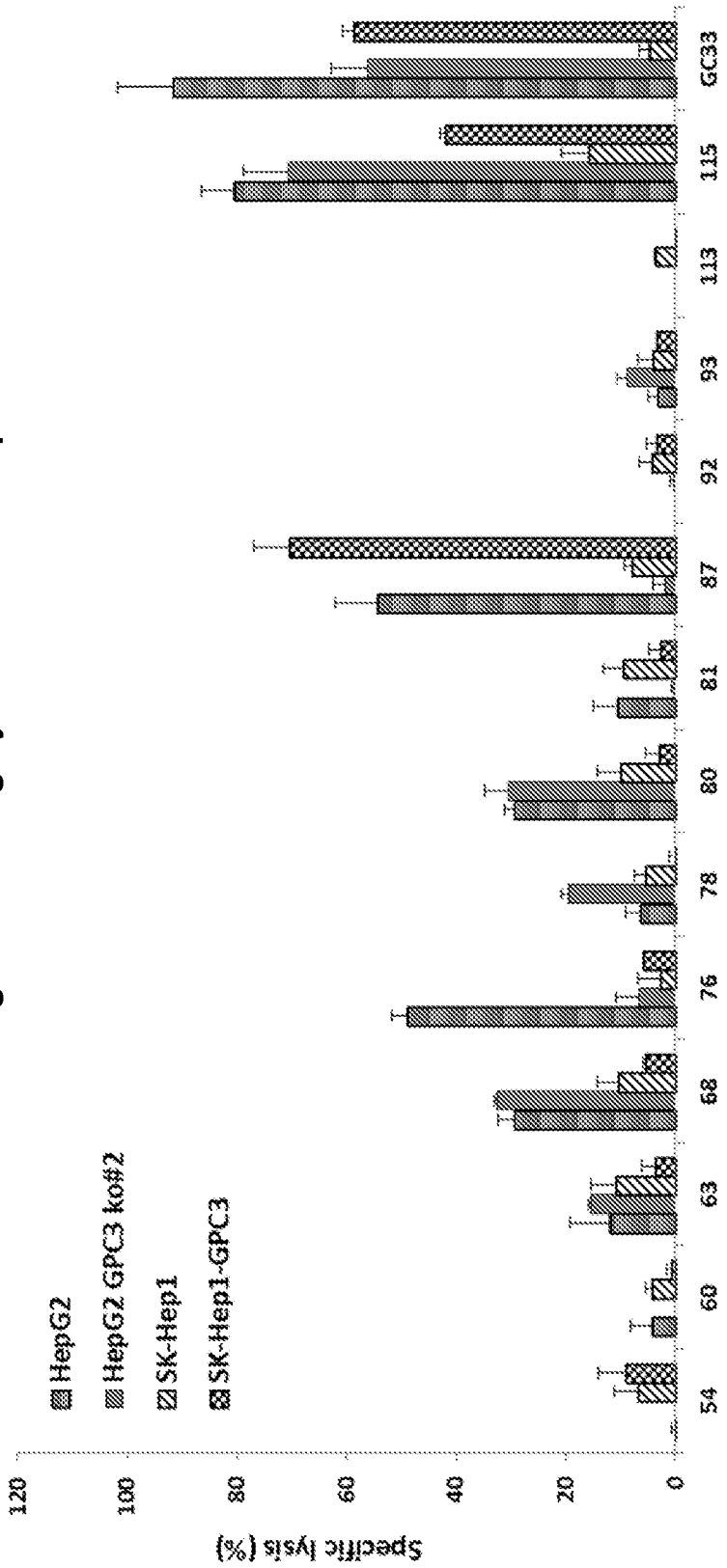
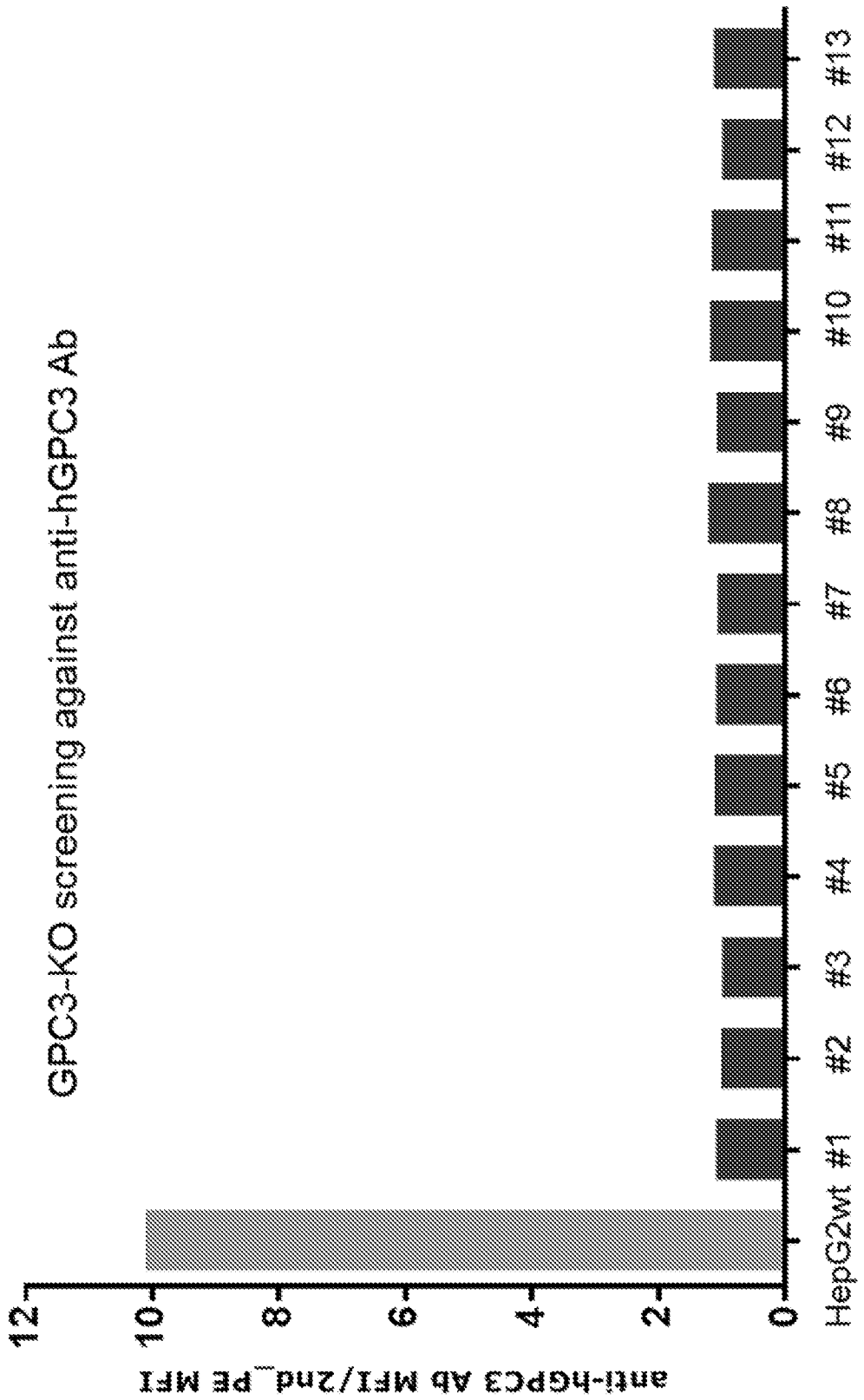

Target cell killing by GPC3B L2K bispecific antibodies
Specific lysis (%)
0
20
40
60
80
100
120
HepG2
HepG2 GPC3 ko#2
SK-Hep1
SK-Hep1-GPC3
54
60
63
68
76
78
80
81
87
92
93
113
115
GC33

FIG. 8


FACS analysis of GPC3 knockout cell lines using an anti-GPC3 antibody
GPC3-KO screening against anti-hGPC3 Ab
anti-hGPC3 Ab MFI/2nd_PE MFI
0
2
4
6
8
10
12
HepG2wt
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
#13

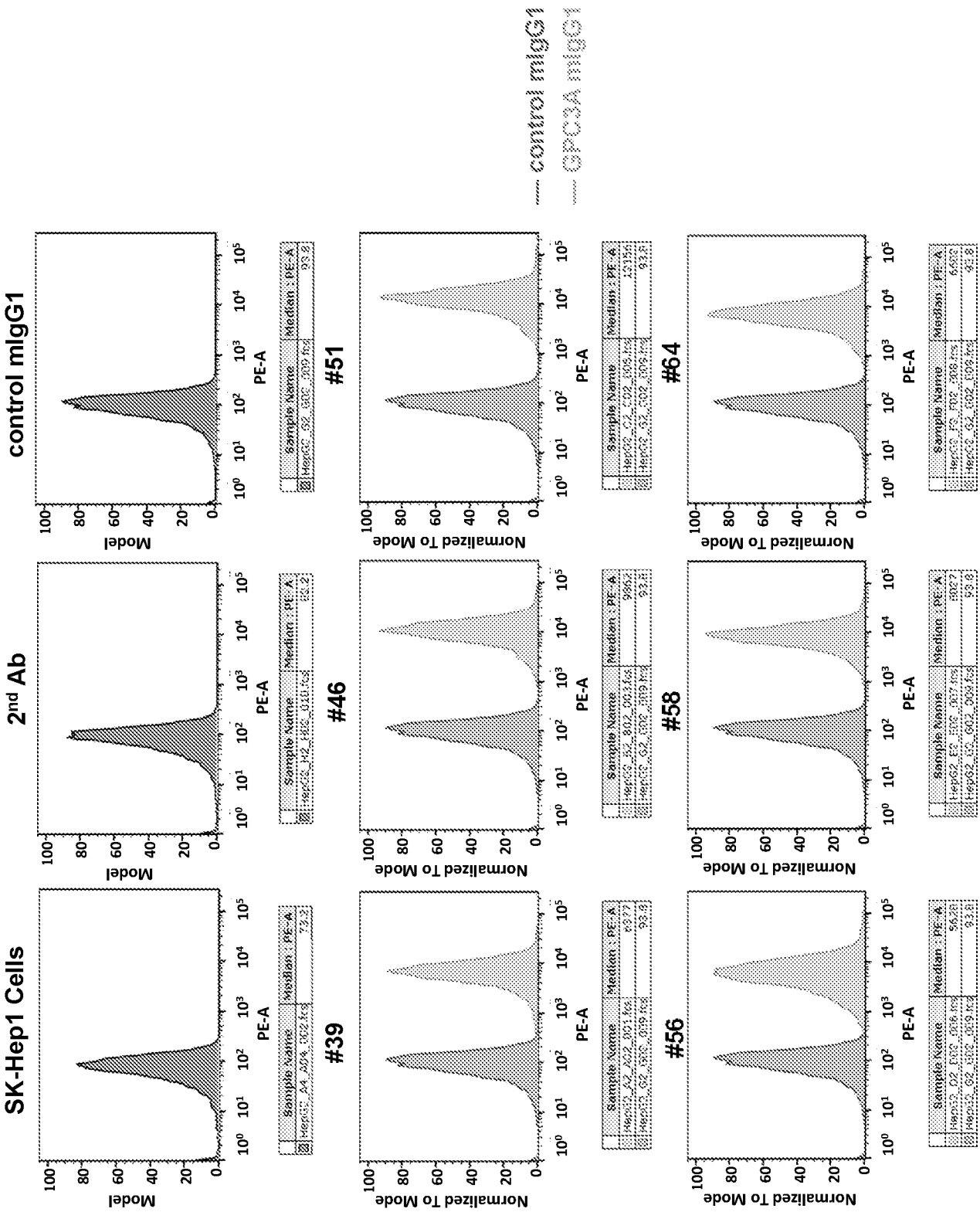

SK-Hep1 Cells
2nd Ab
control mIgG1
#39
#46
#51
#56
#58
#64
Model
Normalized To Mode
PE-A
control mIgG1
GPC3A mIgG1

FIG. 9A

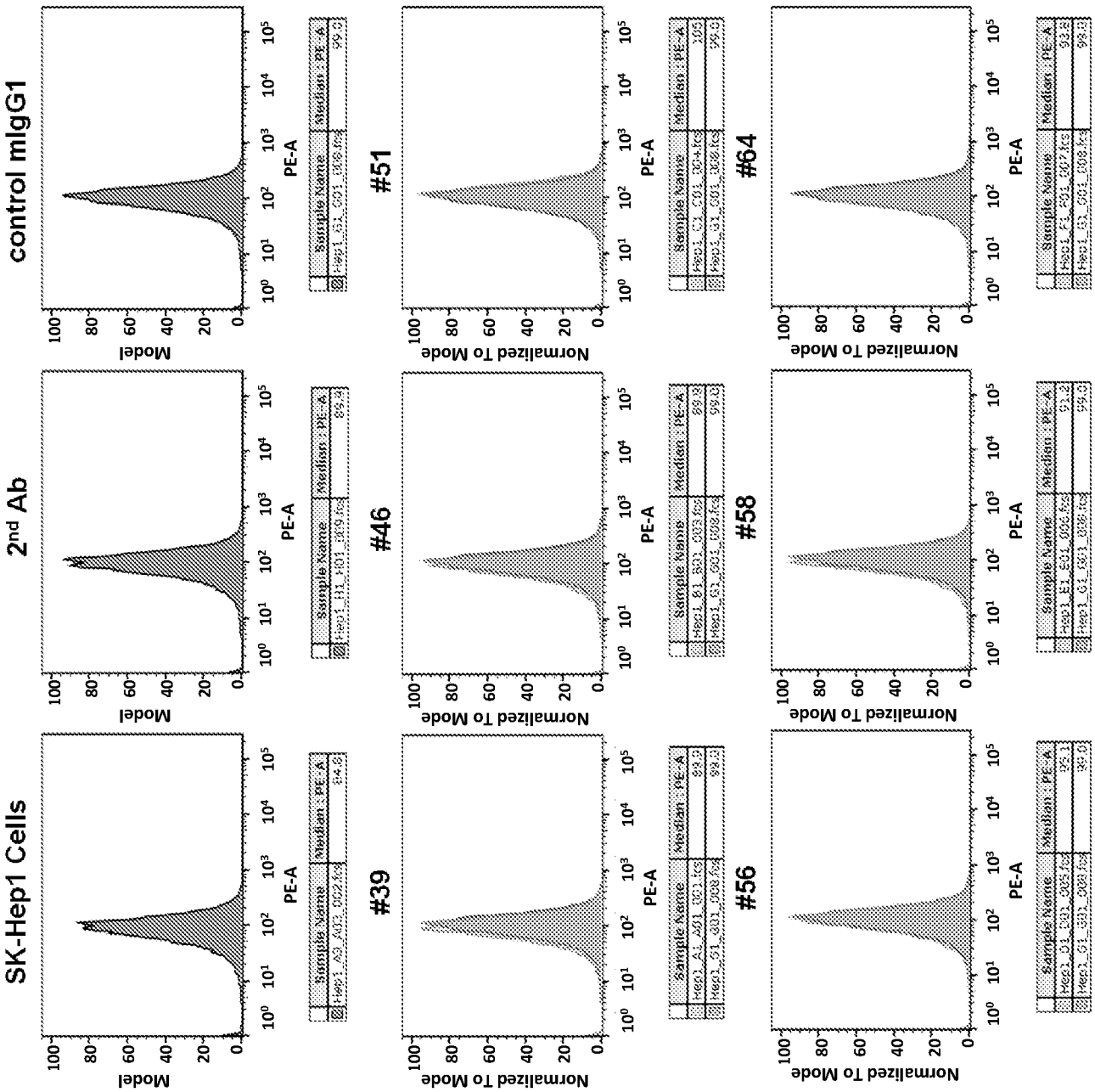

SK-Hep1 Cells
2nd Ab
control mIgG1
#39
#46
#51
#56
#58
#64
Model
Normalized To Mode
PE-A
control mIgG1
GPC3A mIgG1

FIG. 9B

FIG. 10 Competition for L2K binding to HepG2 cells by sGPC3

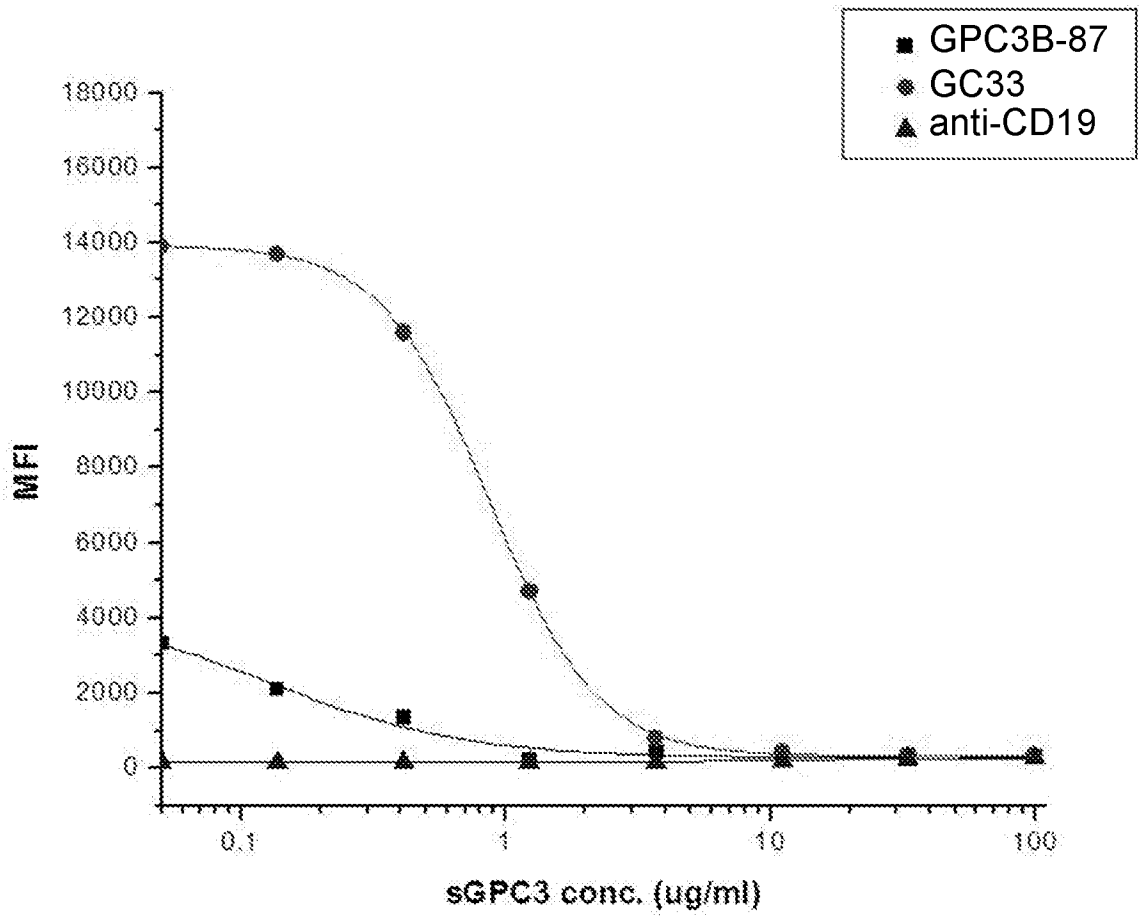


FIG. 11

Binding Affinity for HepG2 cells by L2K clones

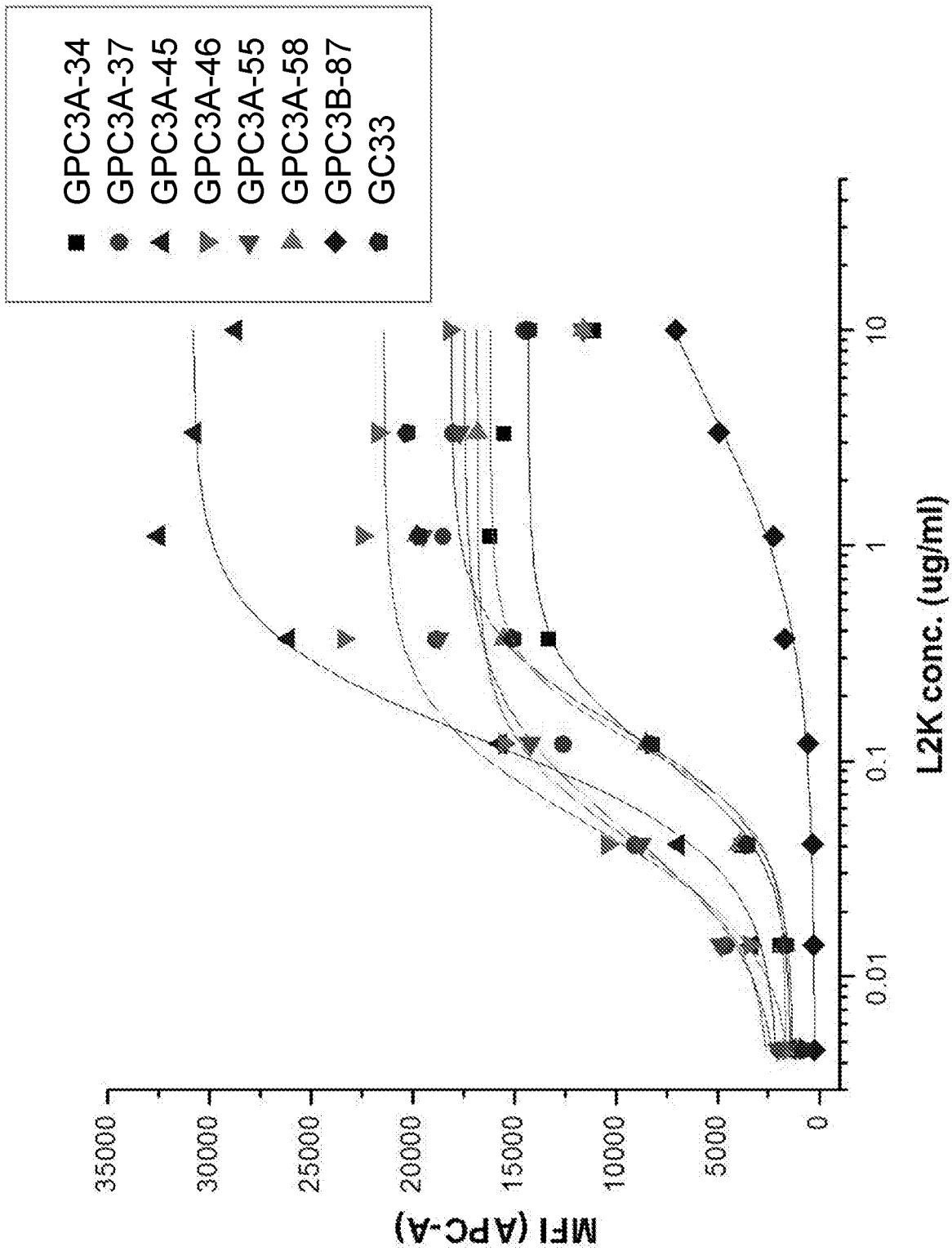


FIG. 12

Reactivity of mIgG1 clones by ELISA

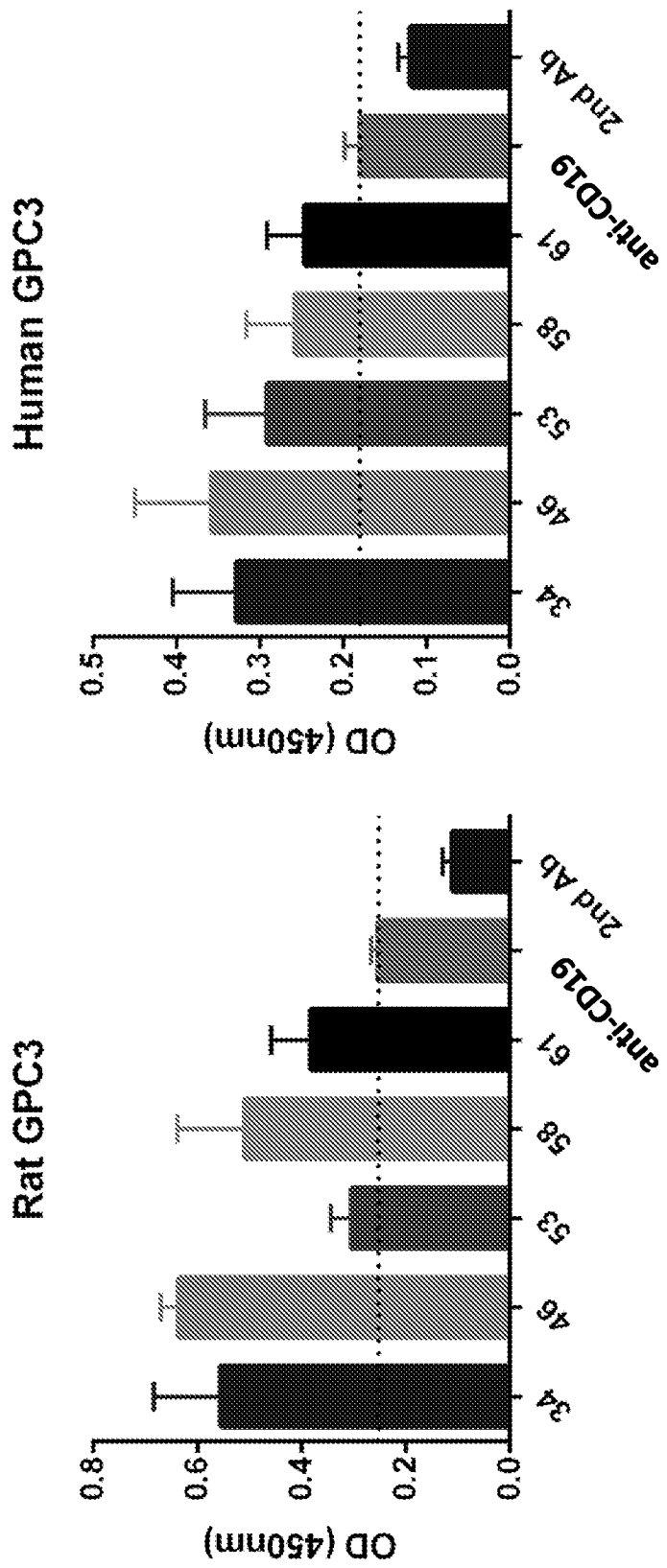


FIG. 13 **Epitope Binning Analysis**

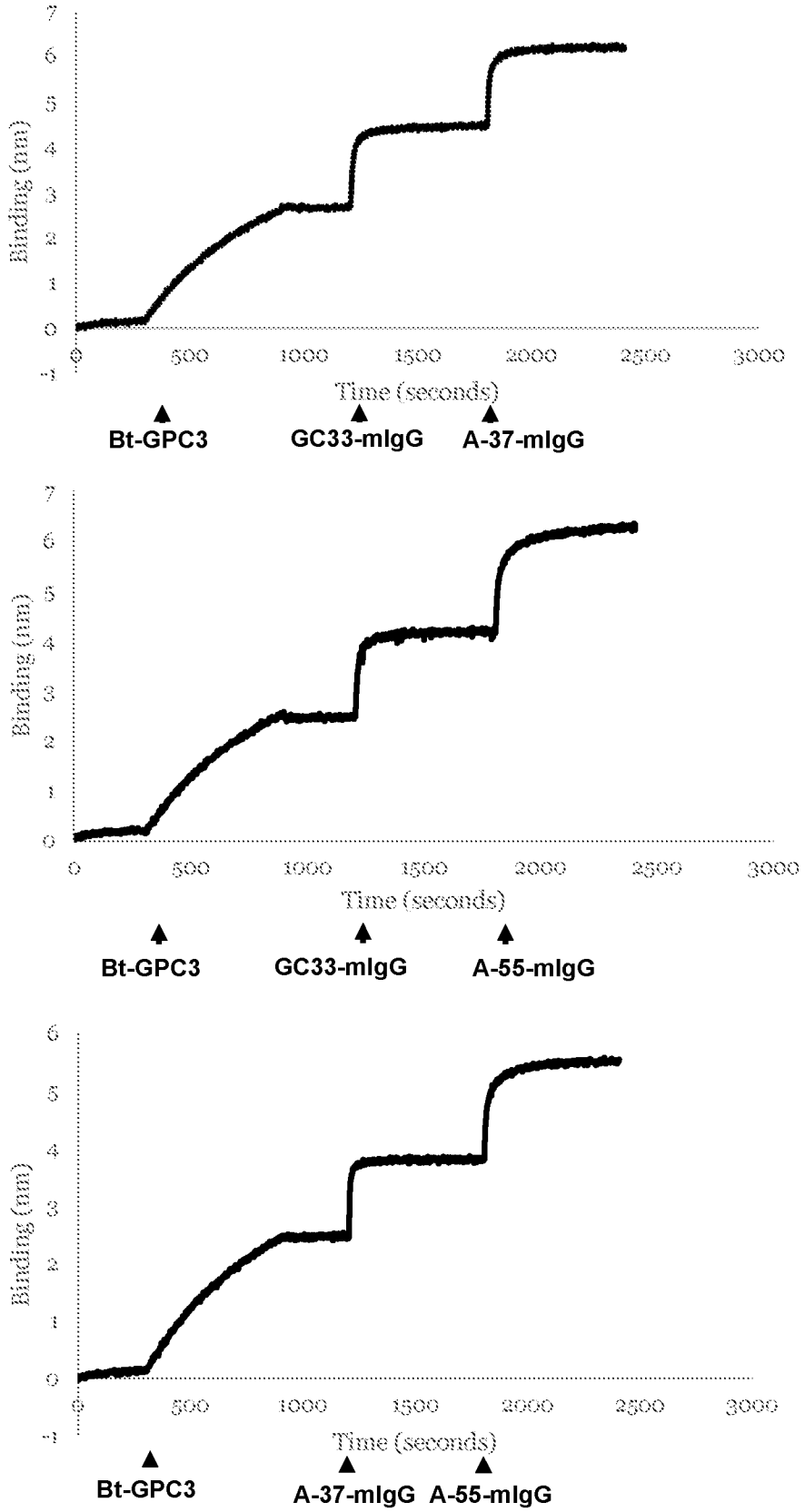
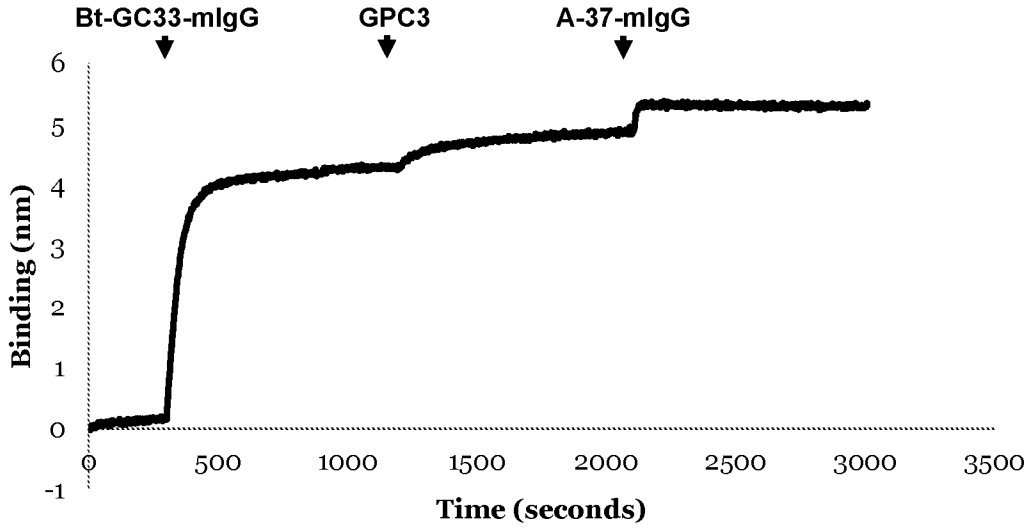
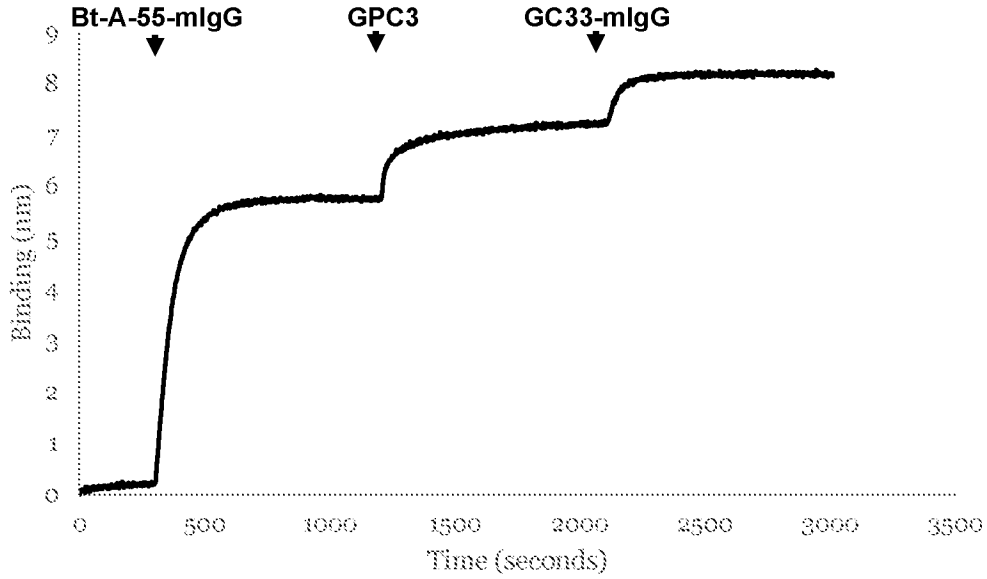


FIG. 14

Epitope Binning Analysis





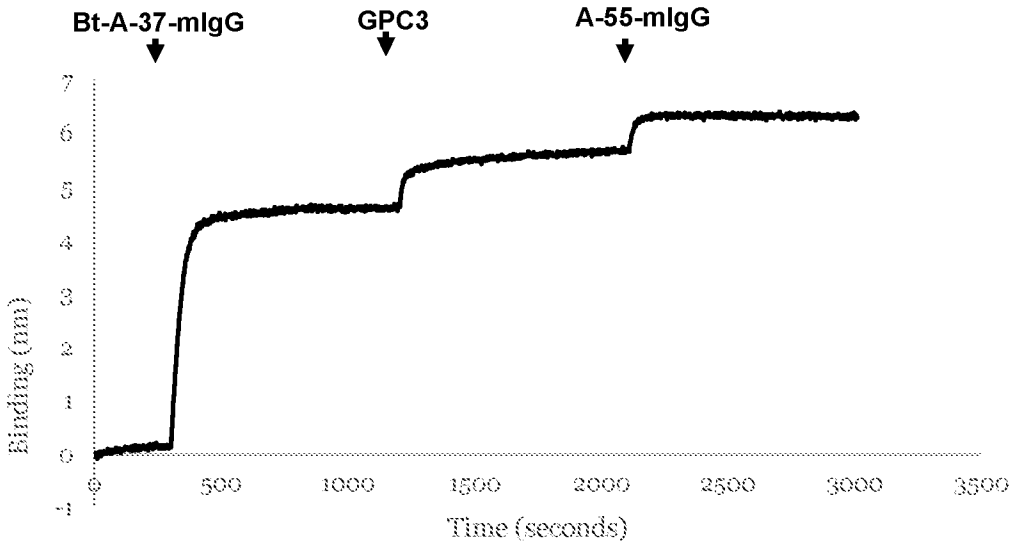


FIG. 15A **Binding kinetics between GPC3A L2K clones and GPC3 antigen**

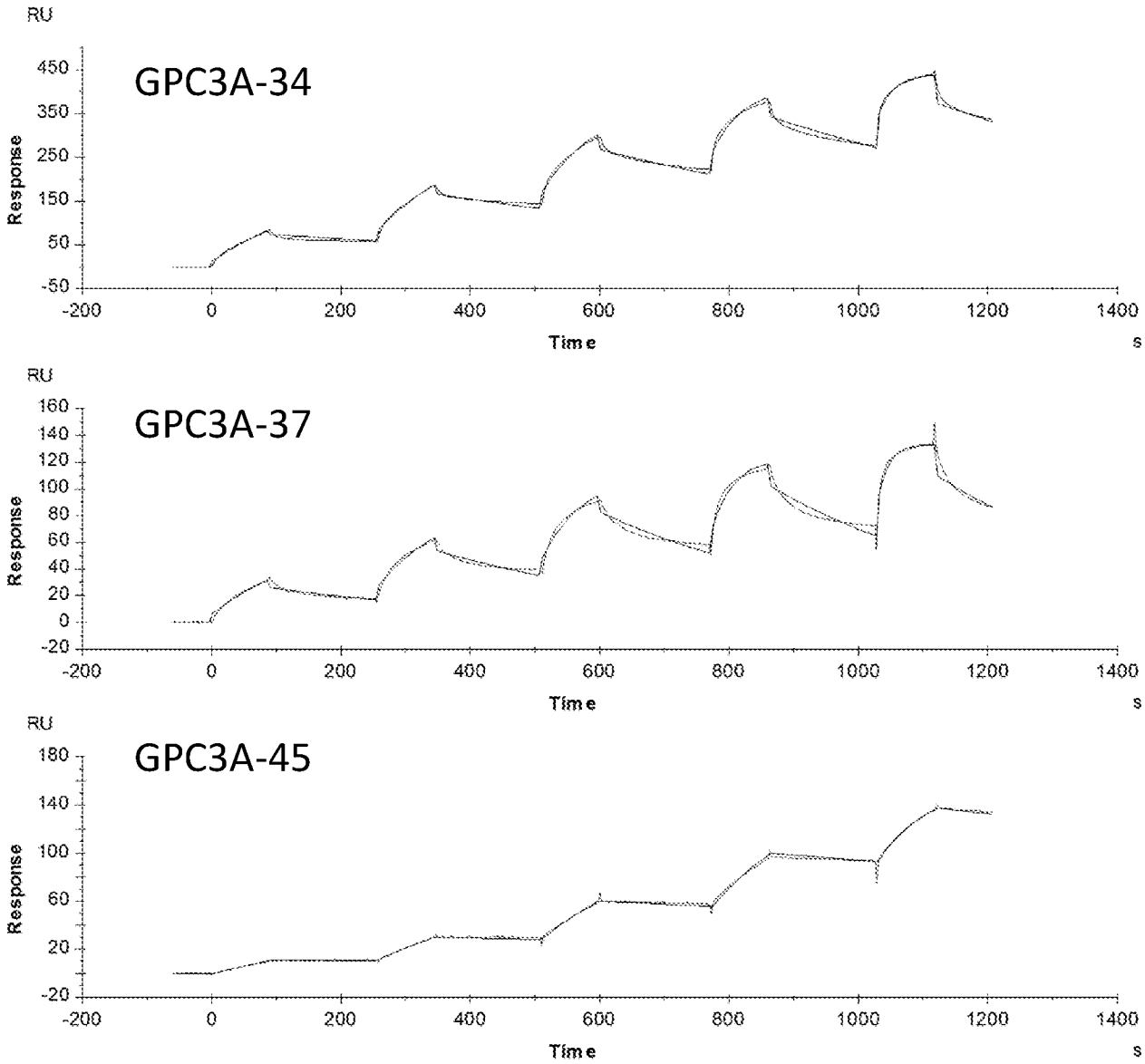
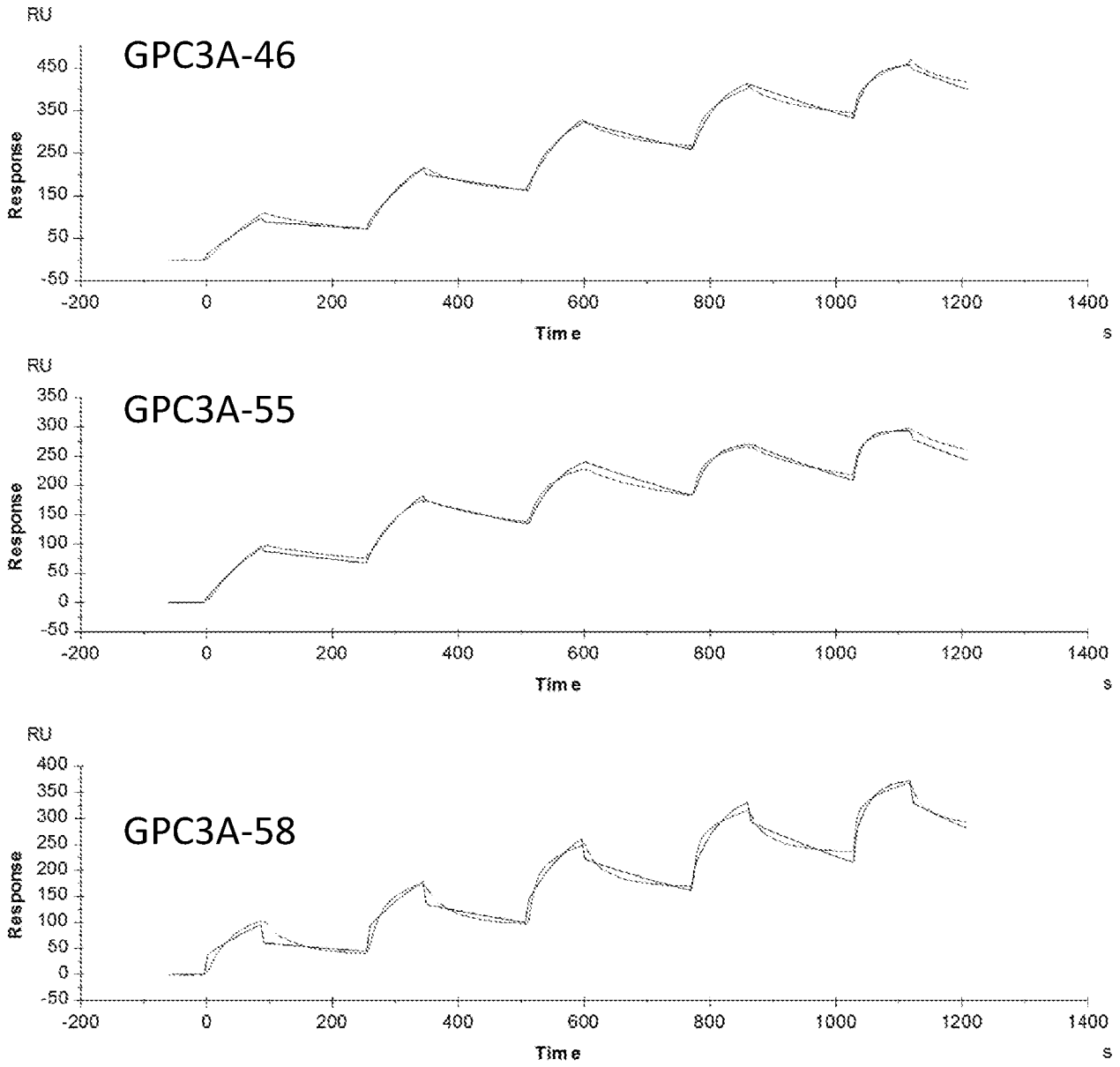


FIG. 15B **Binding kinetics between GPC3A L2K clones and GPC3 antigen**



LDH Assay of GPC3 CAR-T Cell Killing

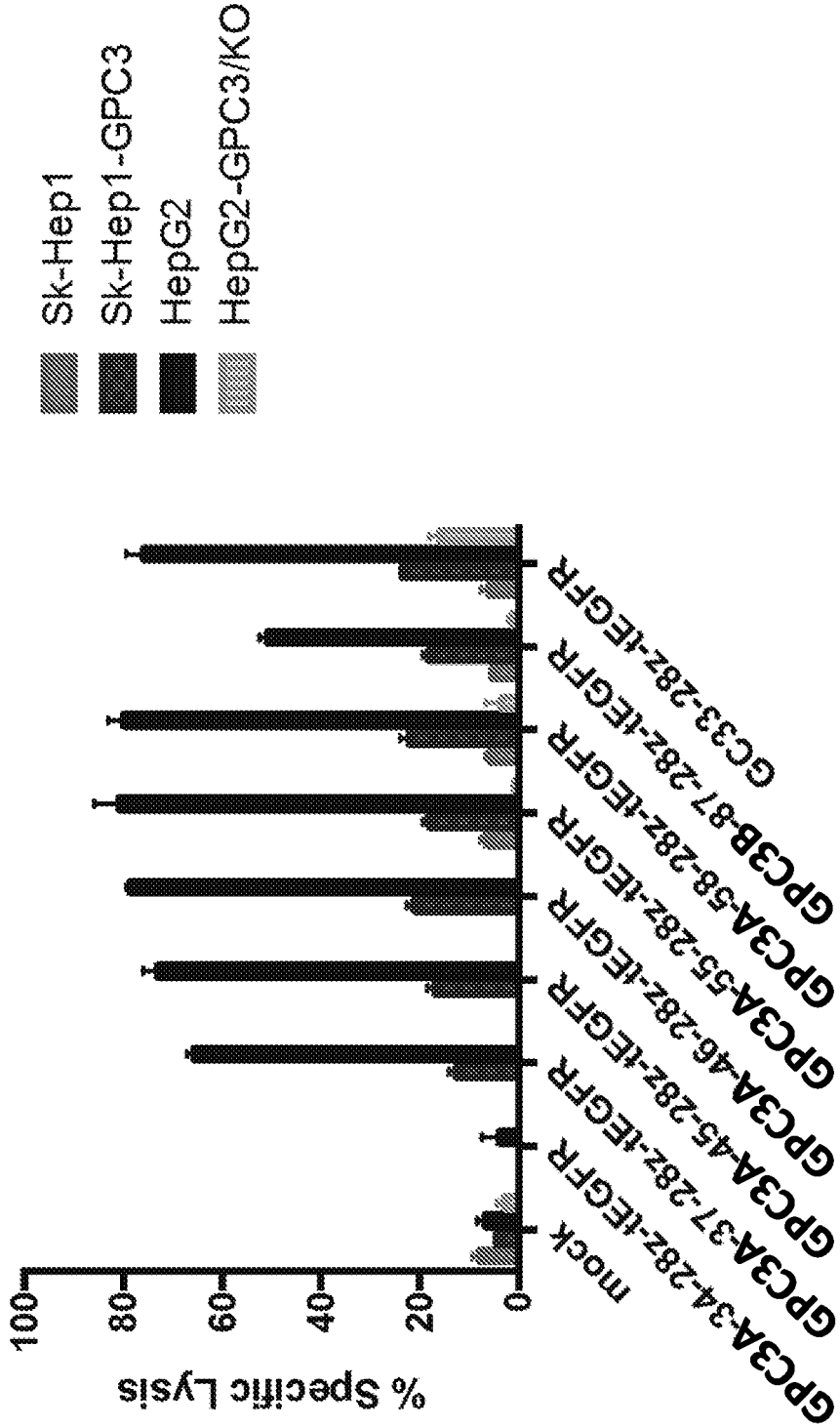


FIG. 16

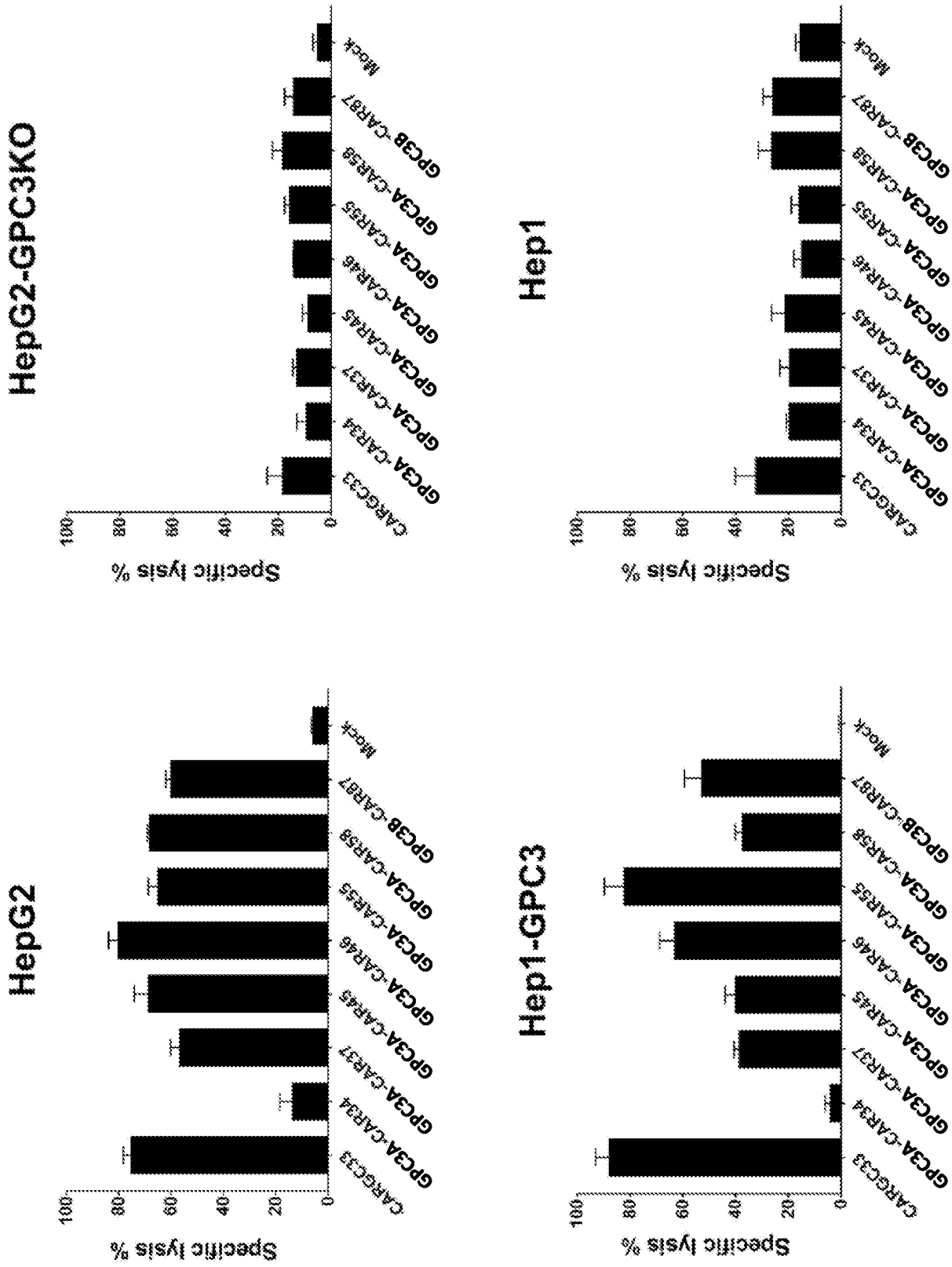

HepG2-GPC3KO
Hep1
HepG2
Hep1-GPC3
Specific lysis %
0
20
40
60
80
100
CARGC33
GPC3A-CAR34
GPC3A-CAR37
GPC3A-CAR45
GPC3A-CAR46
GPC3A-CAR55
GPC3A-CAR58
GPC3B-CAR87
Mock

FIG. 17A

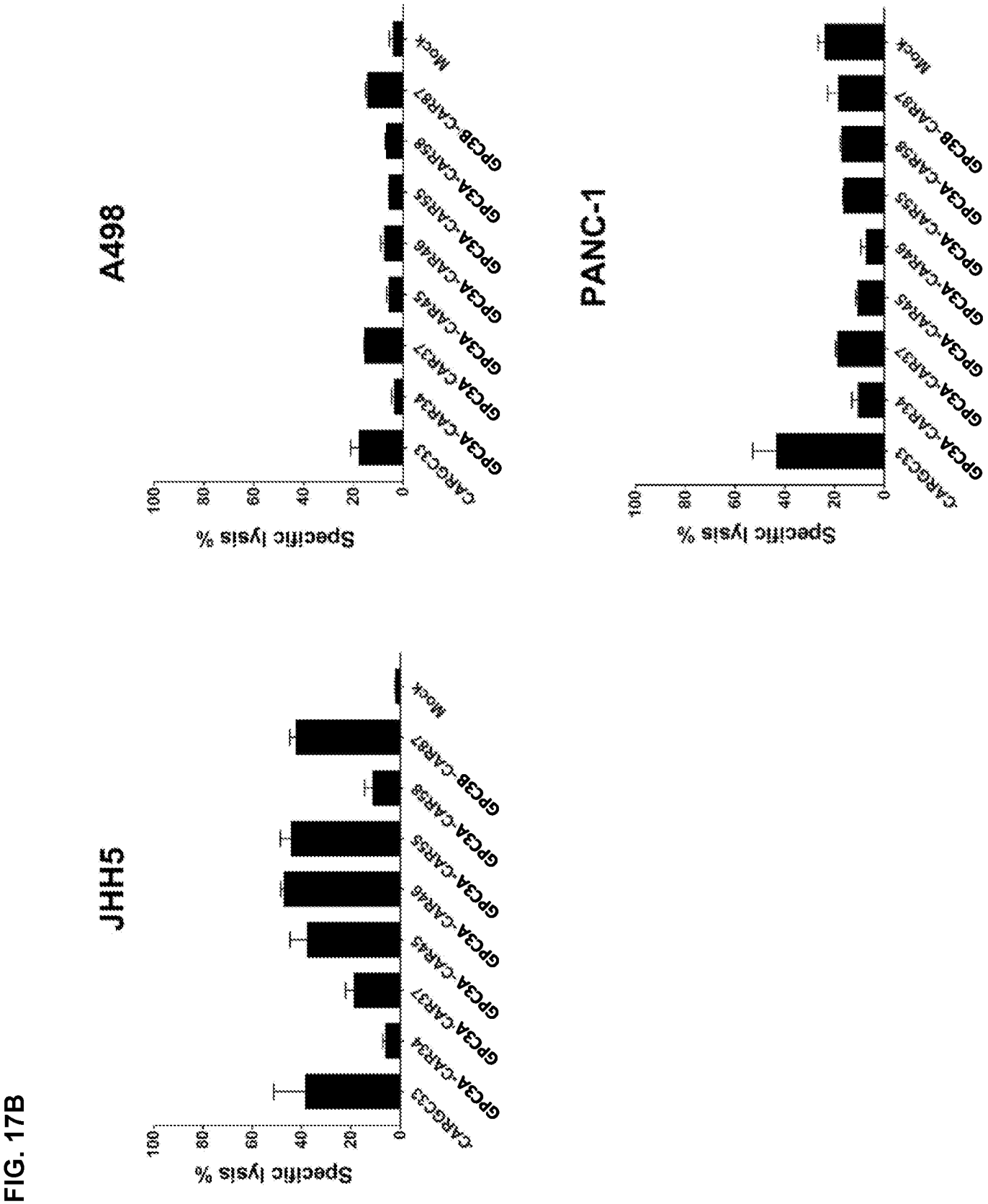

A498
PANC-1
JHH5
% Specific lysis
100
80
60
40
20
0
CARGC33
GPC3A-CAR34
GPC3A-CAR37
GPC3A-CAR45
GPC3A-CAR46
GPC3A-CAR55
GPC3A-CAR59
GPC3B-CAR87
Mock

FIG. 17B

FIG. 18

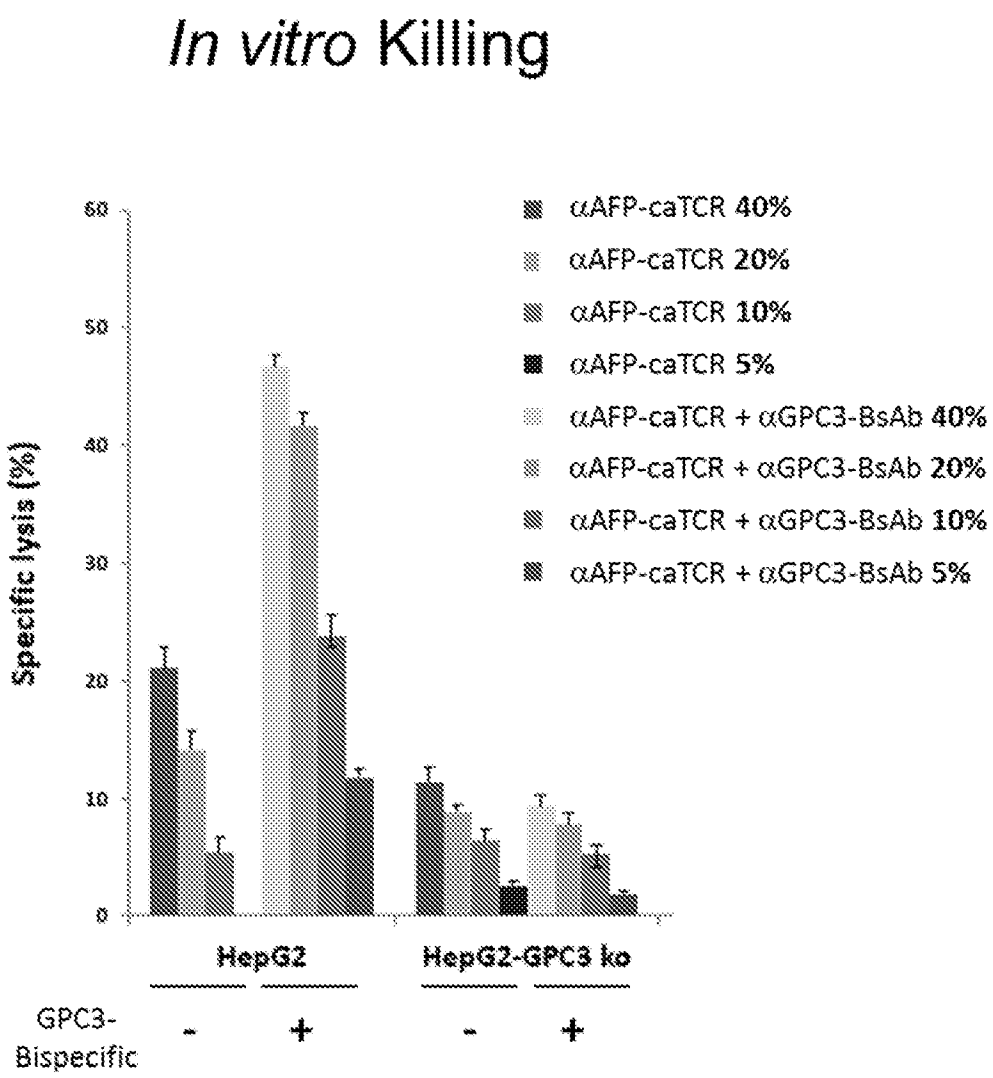

In vitro Killing
Specific lysis (%)
0
10
20
30
40
50
60
αAFP-caTCR 40%
αAFP-caTCR 20%
αAFP-caTCR 10%
αAFP-caTCR 5%
αAFP-caTCR + αGPC3-BsAb 40%
αAFP-caTCR + αGPC3-BsAb 20%
αAFP-caTCR + αGPC3-BsAb 10%
αAFP-caTCR + αGPC3-BsAb 5%
HepG2
HepG2-GPC3 ko
GPC3-Bispecific
-
+
-
+

FIG. 19

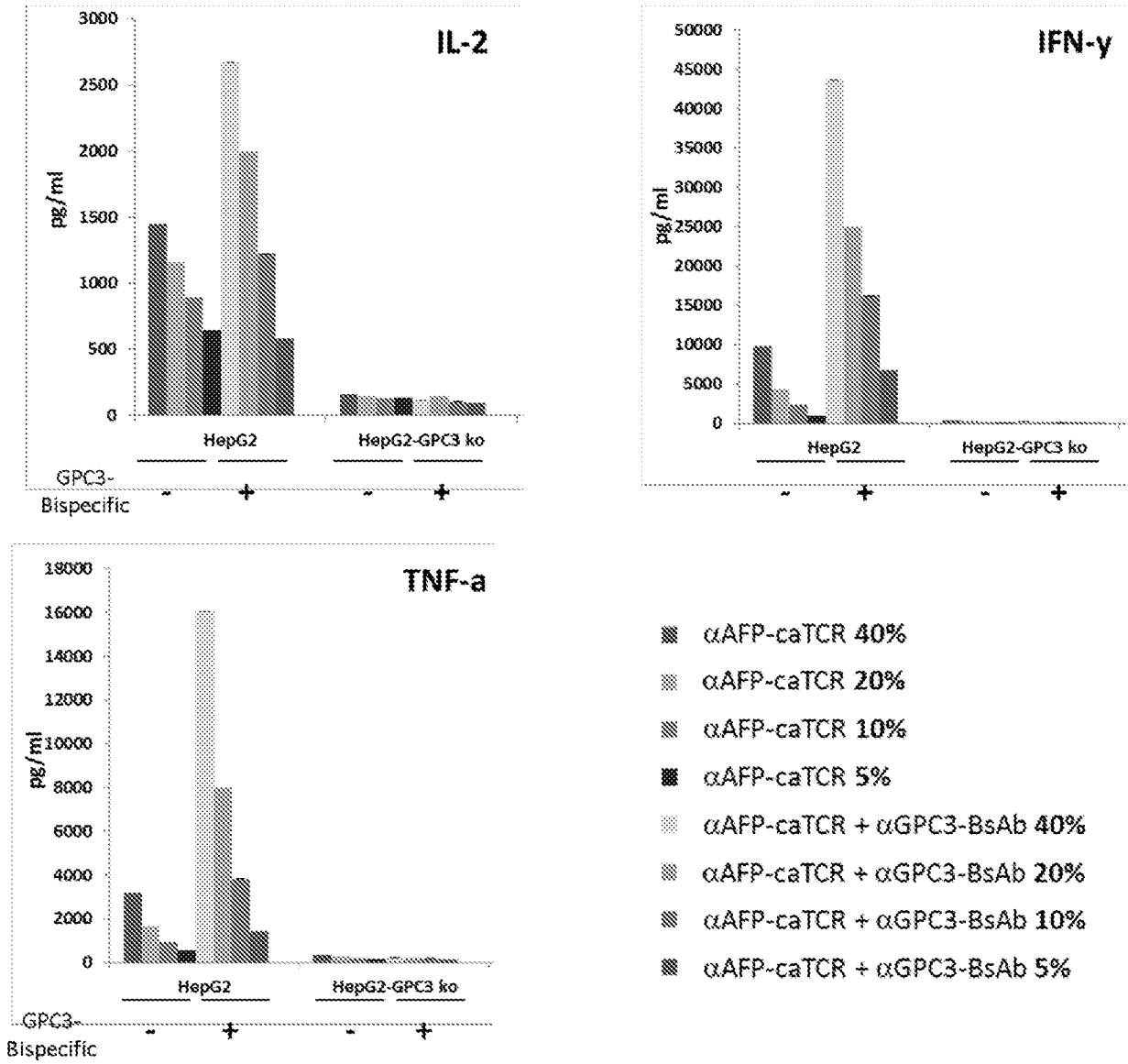

IL-2
pg/ml
3000
2500
2000
1500
1000
500
0
HepG2
HepG2-GPC3 ko
GPC3-
Bispecific
-
+
-
+
IFN-γ
pg/ml
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
HepG2
HepG2-GPC3 ko
TNF-a
pg/ml
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
HepG2
HepG2-GPC3 ko
GPC3-
Bispecific
αAFP-caTCR 40%
αAFP-caTCR 20%
αAFP-caTCR 10%
αAFP-caTCR 5%
αAFP-caTCR + αGPC3-BsAb 40%
αAFP-caTCR + αGPC3-BsAb 20%
αAFP-caTCR + αGPC3-BsAb 10%
αAFP-caTCR + αGPC3-BsAb 5%

FIG. 20

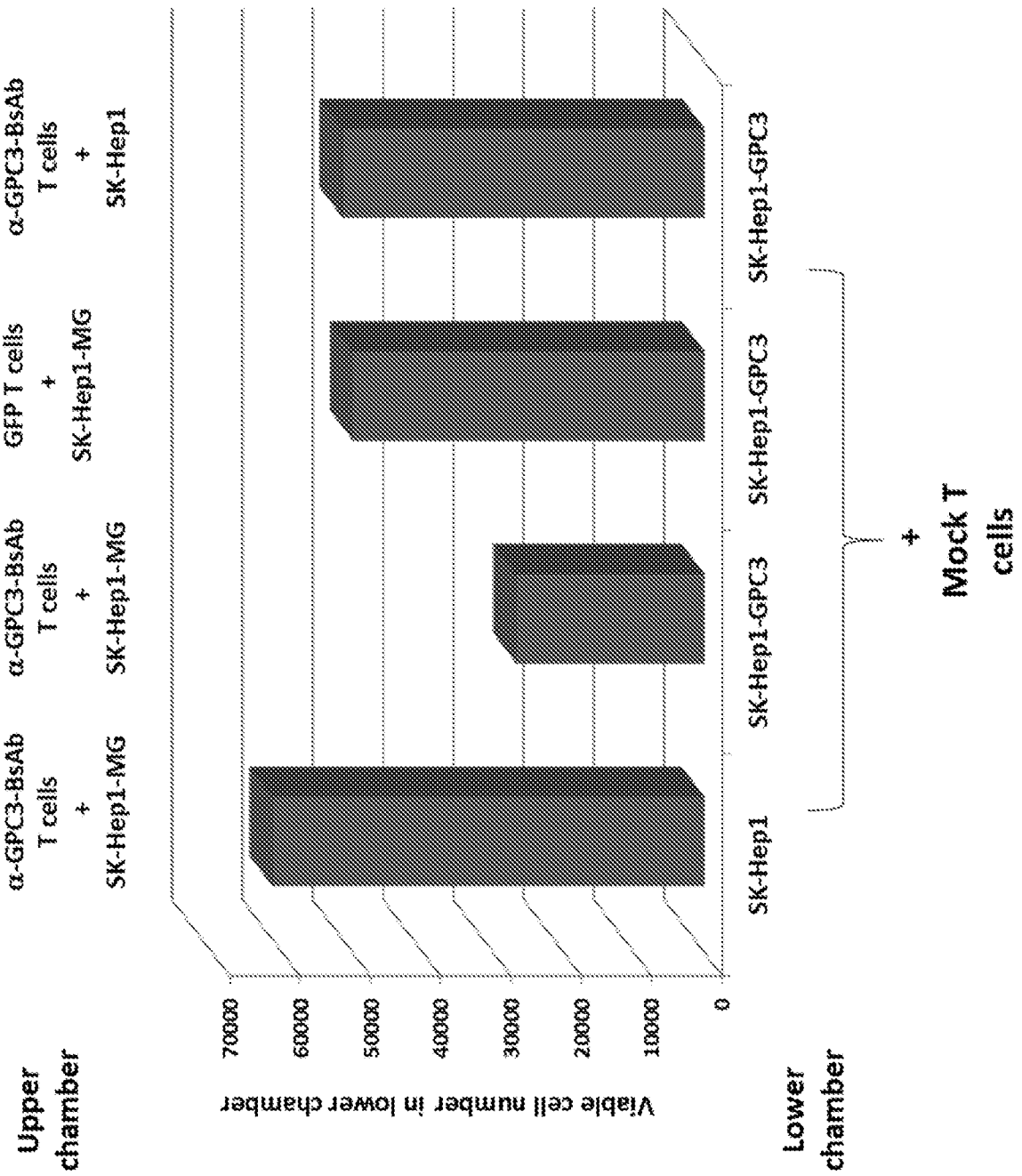

Upper chamber
αAFP-caTCR + α-GPC3-BsAb T cells + SK-Hep1-MG
αAFP-caTCR + α-GPC3-BsAb T cells + SK-Hep1-MG
αAFP-caTCR + GFP T cells + SK-Hep1-MG
αAFP-caTCR + α-GPC3-BsAb T cells + SK-Hep1
Viable cell number in lower chamber
70000
60000
50000
40000
30000
20000
10000
0
Lower chamber
SK-Hep1
SK-Hep1-GPC3
SK-Hep1-GPC3
SK-Hep1-GPC3
+
Mock T cells

FIG. 21

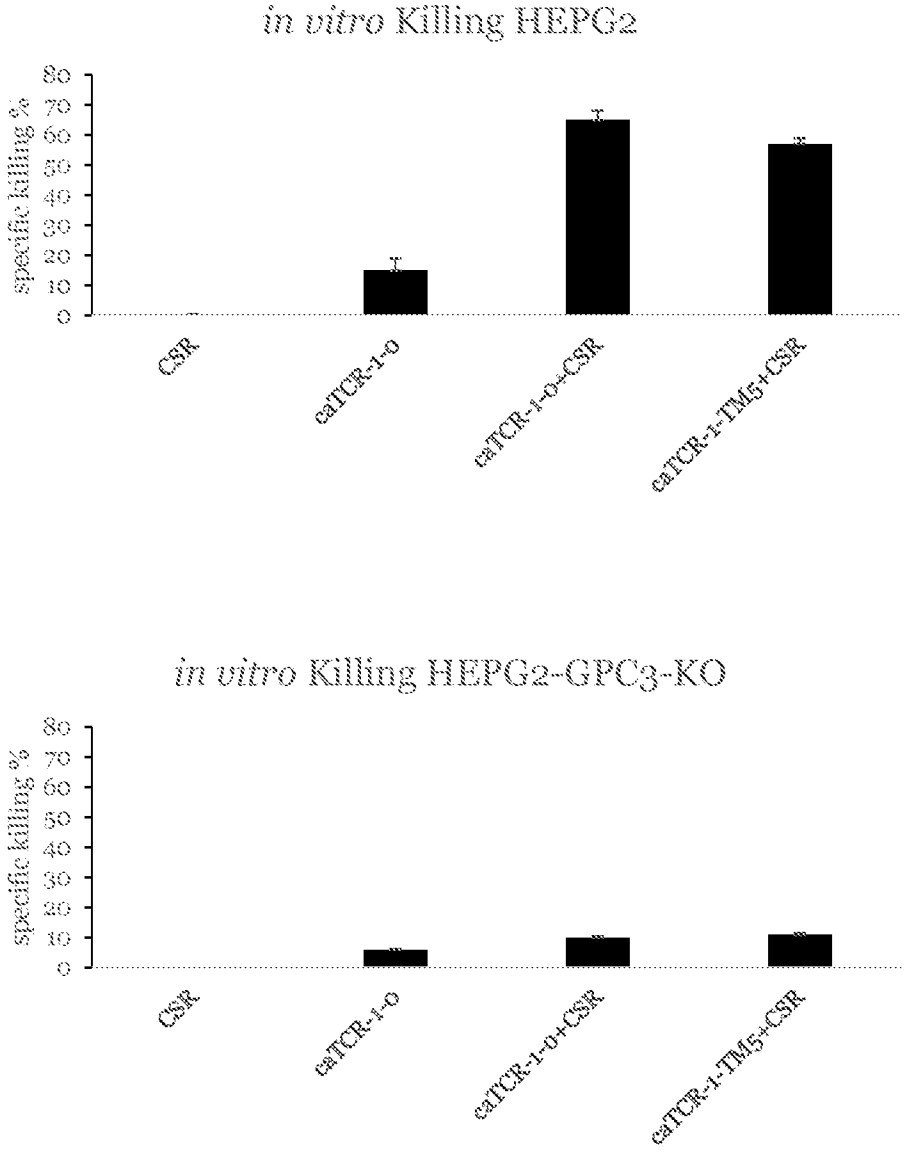
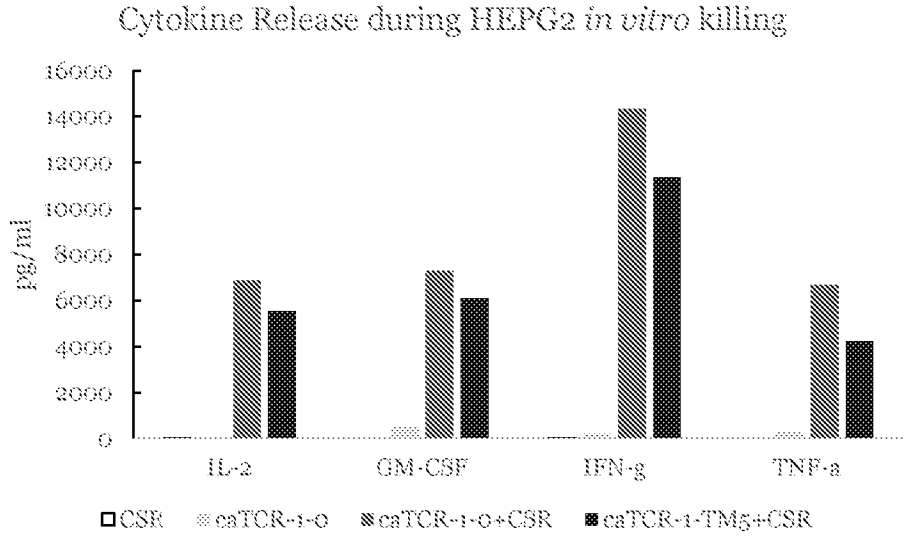

in vitro Killing HEPG2
specific killing %
0
10
20
30
40
50
60
70
80
CSR
caTCR-1-0
caTCR-1-0+CSR
caTCR-1-TM5+CSR
in vitro Killing HEPG2-GPC3-KO
specific killing %
0
10
20
30
40
50
60
70
80
CSR
caTCR-1-0
caTCR-1-0+CSR
caTCR-1-TM5+CSR

FIG. 22


Cytokine Release during HEPG2 *in vitro* killing
16000
14000
12000
10000
8000
6000
4000
2000
0
pg/ml
IL-2
GM-CSF
IFN-g
TNF-a
CSR
caTCR-1-0
caTCR-1-0+CSR
caTCR-1-TM5+CSR

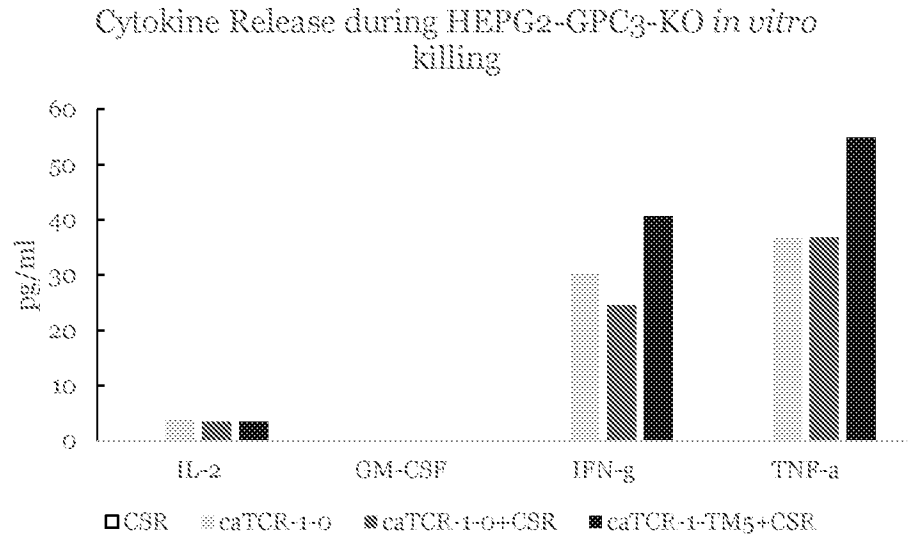
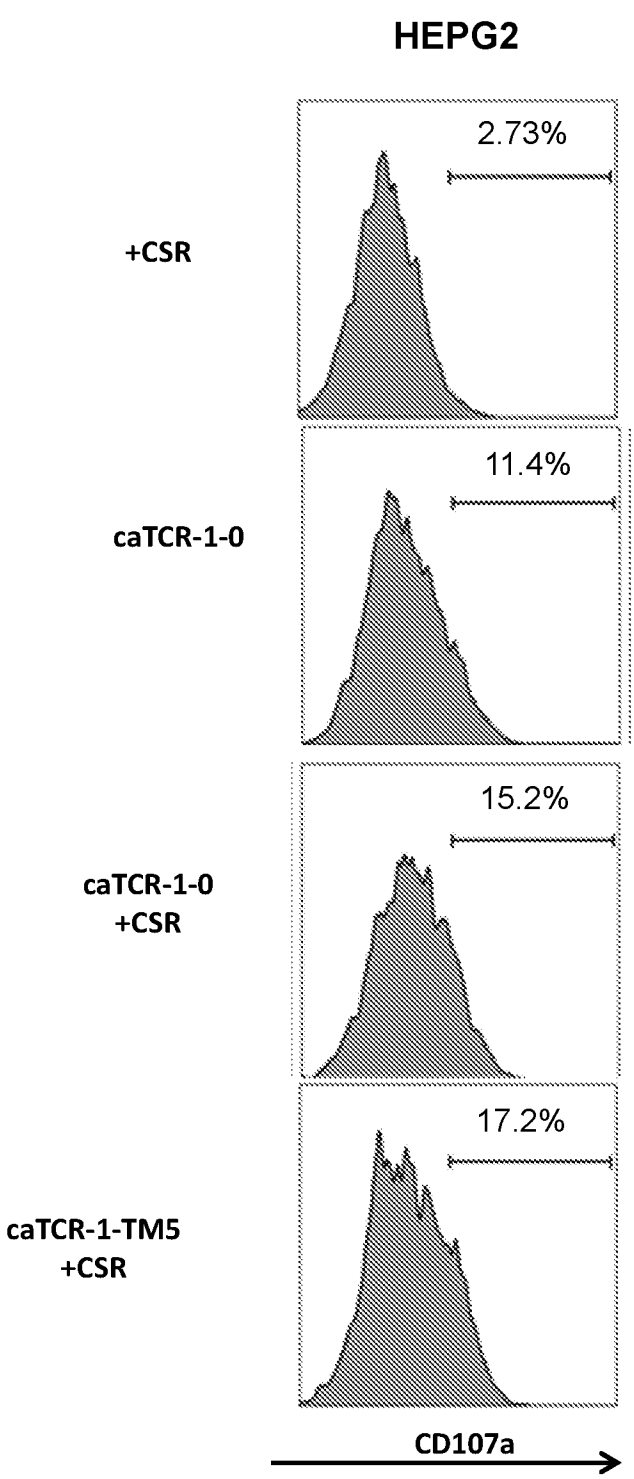

Cytokine Release during HEPG2-GPC3-KO *in vitro* killing
60
50
40
30
20
10
0
pg/ml
IL-2
GM-CSF
IFN-g
TNF-a
CSR
caTCR-1-0
caTCR-1-0+CSR
caTCR-1-TM5+CSR

FIG. 23 **CD107a Degranulation**



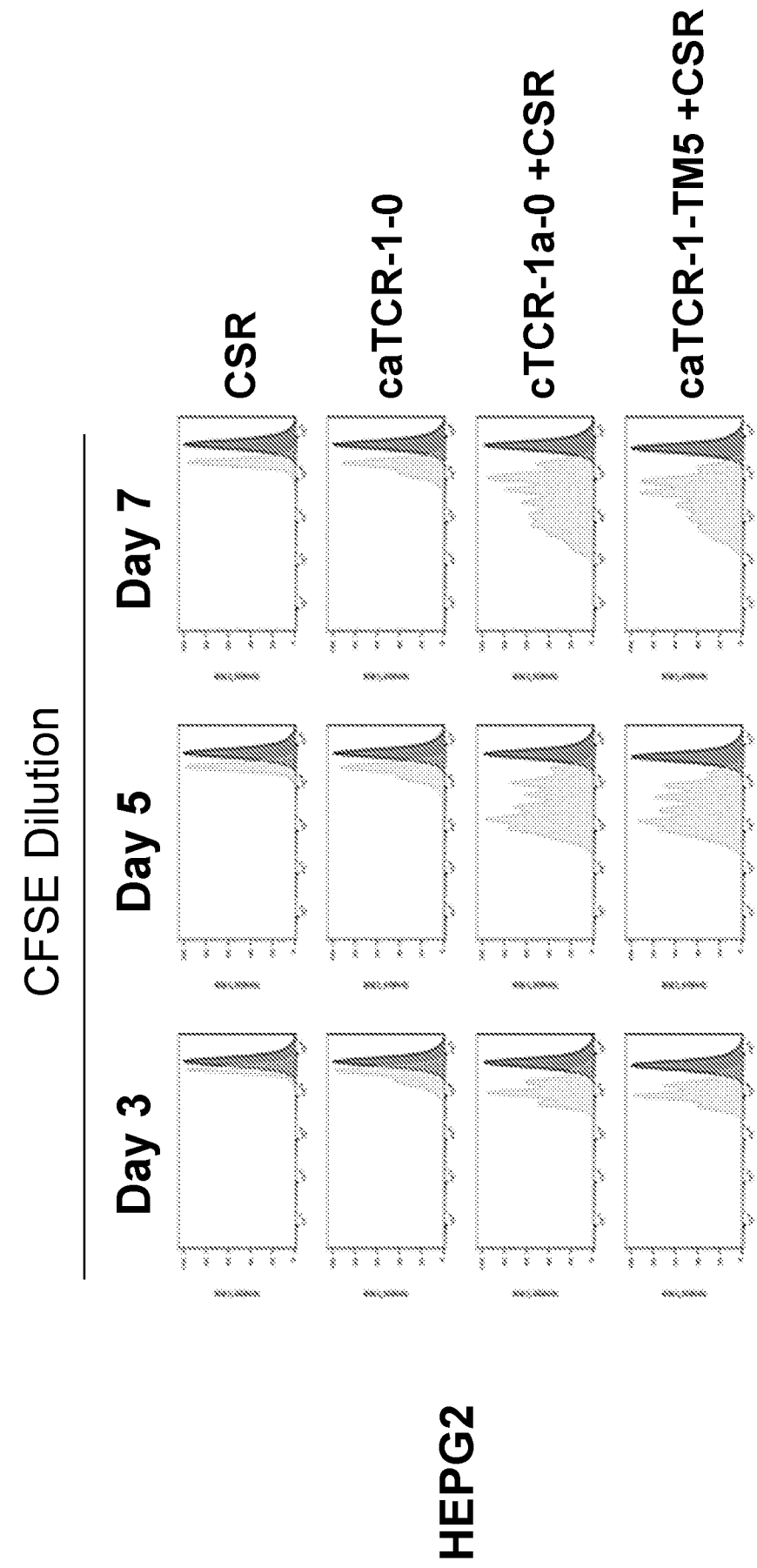
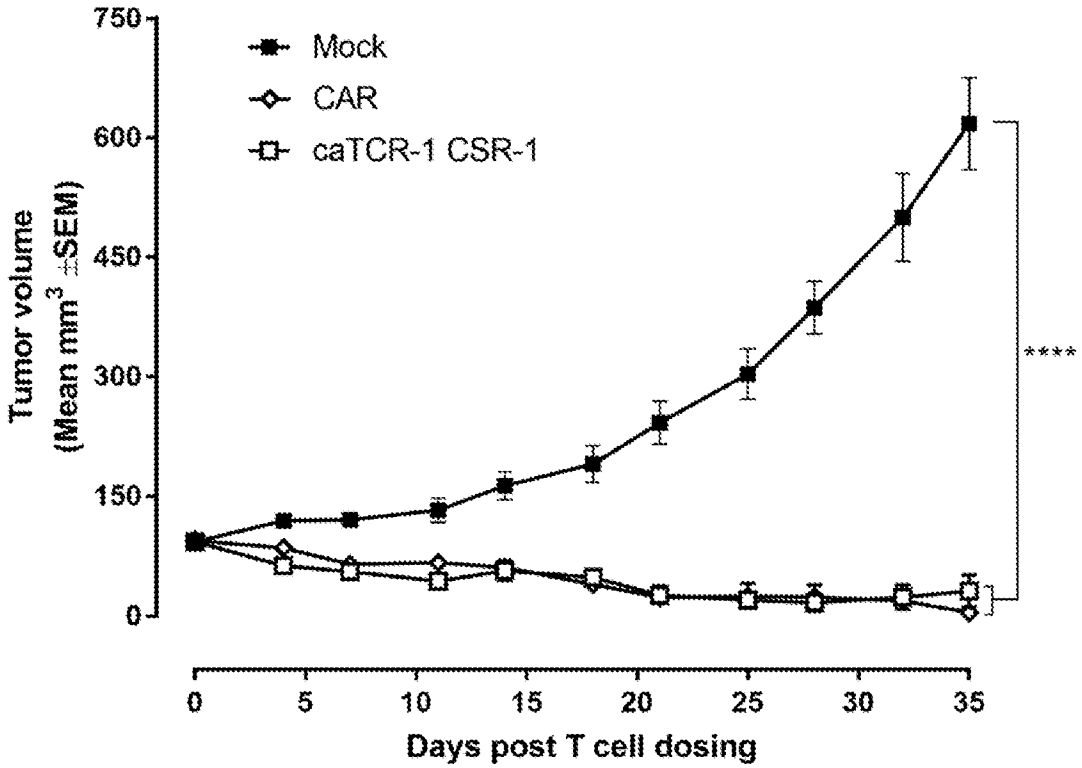

CSR
caTCR-1-0
cTCR-1a-0 +CSR
caTCR-1-TM5 +CSR
Day 7
Day 5
Day 3
CFSE Dilution
HEPG2

FIG. 24

FIG. 25


750
600
450
300
150
0
Tumor volume
(Mean mm3 ±SEM)
Mock
CAR
caTCR-1 CSR-1
****
0
5
10
15
20
25
30
35
Days post T cell dosing

a) contacting a sample derived from the individual with the isolated anti-GPC3 construct of claim 24; and

b) determining the number of cells bound with the isolated anti-GPC3 construct in the sample, wherein a value for the number of cells bound with the isolated anti-GPC3 construct above a threshold level indicates that the individual has the GPC3-positive disease.

38. The method of any one of claims 35-37, wherein the GPC3-positive disease is cancer.

39. The method of claim 38, wherein the cancer is selected from the group consisting of HCC, melanoma, lung squamous cell carcinoma, ovarian carcinoma, yolk sac tumor, choriocarcinoma, neuroblastoma, hepatoblastoma, Wilms' tumor, testicular nonseminomatous germ cell tumor, gastric carcinoma, and liposarcoma.

40. A method of producing an isolated anti-GPC3 construct, comprising:

(a) culturing a host cell comprising the isolated nucleic acid of claim 25 or the vector of claim 26, or the isolated host cell of claim 27 under conditions effective to express the anti-GPC3 construct; and

(b) obtaining the expressed anti-GPC3 construct from said host cell.

SEQUENCE LISTING

<110> Eureka Therapeutics, Inc.
Zhang, Pengbo
Xu, Yiyang
Morales, Javier
Nakano, Yoko
Liu, Hong
Xiang, Jingyi
Acker, Timothy

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 1   5

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<220>
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<400> 54
Ile Tyr Ser Gly Gly Ser Ser Thr
 1   5

<210> 55
<211> 8
<212> PRT
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<220>
<223> GPC3A-038 HCDR2

<400> 55
Ile Asn Pro Ser Gly Gly Ser Thr
 1   5

<210> 56
<211> 7
<212> PRT
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<220>
<223> GPC3A-039 HCDR2

<400> 56
Ile Gly Thr Gly Gly Gly Thr
 1   5

<210> 57
<211> 8
<212> PRT
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<220>
<223> GPC3A-040 HCDR2

<400> 57
Ile Asn Pro Asn Ser Gly Gly Thr
 1   5

<210> 58
<211> 8
<212> PRT
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<220>
<223> GPC3A-041 HCDR2

<400> 58
Ile Asn Pro Asn Ser Gly Gly Thr
 1   5

<210> 59
<211> 8
<212> PRT
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<220>
<223> GPC3A-042 HCDR2

<400> 59
Met Asn Pro Arg Ser Gly Gly Thr
 1   5

<210> 60
<211> 8
<212> PRT
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<220>
<223> GPC3A-043 HCDR2

<400> 60
Val Asp Pro Glu Asp Gly Glu Thr
 1   5

<210> 61
<211> 7
<212> PRT
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<220>
<223> GPC3A-044 HCDR2

<400> 61
Ile Asn His Ser Gly Ser Thr
 1   5

<210> 62
<211> 8
<212> PRT
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<220>
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<400> 62
Ile Ser Ser Ser Gly Ser Thr Ile
 1   5

<210> 63
<211> 8
<212> PRT
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<220>
<223> GPC3A-046 HCDR2

<400> 63
Ile Ser Gly Ser Asn Gly Asn Thr
 1   5

<210> 64
<211> 8
<212> PRT
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<220>
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<400> 64
Ile Asn Pro Asn Ser Gly Gly Thr
 1   5

<210> 65
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<220>
<223> GPC3A-048 HCDR2

<400> 65
Ile Ser Tyr Asp Gly Ser Asn Lys
 1   5

<210> 66
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<212> PRT
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<220>
<223> GPC3A-049 HCDR2

<400> 66
Ile Asn Pro Asn Ser Gly Gly Thr
 1   5

<210> 67
<211> 8
<212> PRT
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<220>
<223> GPC3A-050 HCDR2

<400> 67
Met Asn Pro Arg Ser Gly Gly Thr
 1   5

<210> 68
<211> 8
<212> PRT
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<220>
<223> GPC3A-051 HCDR2

<400> 68
Ile Ile Pro Ile Phe Gly Thr Ala
 1   5

<210> 69
<211> 8
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<220>
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<400> 69
Ile Asn Pro Asn Ser Gly Gly Thr
 1   5

<210> 70
<211> 7
<212> PRT
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<220>
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<400> 70
Ile Tyr Tyr Ser Gly Ser Thr
 1   5

<210> 71
<211> 8
<212> PRT
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<220>
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<400> 71
Ile Ser Ala Tyr Asn Gly Asn Thr
 1   5

<210> 72
<211> 8
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<220>
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<400> 72
Ile Asn Pro Ser Gly Gly Ser Thr
 1   5

<210> 73
<211> 8
<212> PRT
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<220>
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<400> 73
Ile Asn Pro Asn Asn Gly Asp Thr
 1   5

<210> 74
<211> 8
<212> PRT
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<220>
<223> GPC3A-057 HCDR2

<400> 74
Ile Ser Gly Ser Gly Gly Ser Thr
 1   5

<210> 75
<211> 8
<212> PRT
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<220>
<223> GPC3A-058 HCDR2

<400> 75
Ile Ser Pro Tyr Thr Gly Asn Thr
 1   5

<210> 76
<211> 8
<212> PRT
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<220>
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<400> 76
Ile Ser Ala Tyr Ser Asp Lys Thr
 1   5

<210> 77
<211> 8
<212> PRT
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<220>
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<400> 77
Ile Ser Ala Tyr Asn Gly Asn Thr
 1   5

<210> 78
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<212> PRT
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<220>
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<400> 78
Ile Ser Ala Tyr Asn Gly Asn Thr
 1   5

<210> 79
<211> 8
<212> PRT
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<220>
<223> GPC3A-062 HCDR2

<400> 79
Ile Ile Pro Lys Ile Gly Thr Ala
 1   5

<210> 80
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<212> PRT
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<220>
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<400> 80
Ile Asn Pro Ser Gly Gly Ser Ala
 1   5

<210> 81
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<212> PRT
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<220>
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<400> 81
Ile Ser Pro Tyr Asn Asp Asn Thr
 1   5

<210> 82
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<212> PRT
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<220>
<223> GPC3A-067 HCDR2

<400> 82
Ile Ile Pro Ile Phe Gly Ile Thr
 1   5

<210> 83
<211> 8
<212> PRT
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<220>
<223> GPC3B-54 HCDR2

<400> 83
Ile Ile Pro Ile Phe Gly Thr Ala
 1   5

<210> 84
<211> 8
<212> PRT
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<220>
<223> GPC3B-60 HCDR2

<400> 84
Ile Asn Pro Ser Gly Gly Ser Thr
 1   5

<210> 85
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-63 HCDR2

<400> 85
Ile Asn Pro Ser Gly Gly Ser Thr
 1   5

<210> 86
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-66 HCDR2

<400> 86
Ile Asn Pro Met Thr Gly Val Thr
 1   5

<210> 87
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-68 HCDR2

<400> 87
Ile Ile Pro Ile Leu Gly Ile Ala
 1   5

<210> 88
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-71 HCDR2

<400> 88
Ile Ser Ser Ser Gly Ser Tyr Ile
 1   5

<210> 89
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-76 HCDR2

<400> 89
Ile Asn Pro Asn Ser Gly Gly Thr
 1   5

<210> 90
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-78 HCDR2

<400> 90
Ile Asn Pro Ser Gly Gly Ser Thr
 1   5

<210> 91
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-80 HCDR2

<400> 91
Ile Ile Pro Ile Phe Arg Thr Ala
 1   5

<210> 92
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-81 HCDR2

<400> 92
Ile Asn Pro Asn Ser Gly Gly Thr
 1   5

<210> 93
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-82 HCDR2

<400> 93
Ile Asn Pro Ser Gly Gly Ser Thr
 1   5

<210> 94
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-85 HCDR2

<400> 94
Ile Asn Thr Asn Thr Gly Asn Pro
 1   5

<210> 95
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-87 HCDR2

<400> 95
Ile Ile Pro Val Leu Gly Arg Thr
 1   5

<210> 96
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-92 HCDR2

<400> 96
Ile Asn Pro Ser Gly Gly Thr Thr
 1   5

<210> 97
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-93 HCDR2

<400> 97
Ile Asn Pro Ser Gly Gly Ser Thr
 1   5

<210> 98
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-110 HCDR2

<400> 98
Ile Ile Pro Thr Phe Gly Thr Thr
 1   5

<210> 99
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-113 HCDR2

<400> 99
Phe Asp Pro Glu Asp Gly Glu Thr
 1   5

<210> 100
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-115 HCDR2

<400> 100
Ile Asn Ala Asn Thr Gly Gly Thr
 1   5

<210> 101
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-119 HCDR2

<400> 101
Ile Asn Thr Asn Thr Gly Asn Pro
 1   5

<210> 102
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-125 HCDR2

<400> 102
Ile Asn Thr Asn Thr Gly Asn Pro
 1   5

<210> 103
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-034 HCDR3

<400> 103
Ala Arg Gly Tyr Gly Gly Arg Phe Asp Tyr
 1   5   10

<210> 104
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-035 HCDR3

<400> 104
Ala Arg Ser Trp Thr Ser Gly Phe Asp Tyr
 1   5   10

<210> 105
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-037 HCDR3

<400> 105
Ala Arg Thr Ser Tyr Leu Asn His Gly Asp Tyr
 1   5   10

<210> 106
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-038 HCDR3

<400> 106
Ala Arg Lys Val Thr Gly Tyr Asp Ser
 1   5

<210> 107
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-039 HCDR3

<400> 107
Ala Arg Tyr Gly Arg Lys Ser Ile Asp Ala
 1   5   10

<210> 108
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-040 HCDR3

<400> 108
Ala Arg Arg Gly Tyr Tyr Gly Tyr Asp Ser
 1   5   10

<210> 109
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-041 HCDR3

<400> 109
Ala Arg Ser Gly Lys Tyr Tyr Gly Asp Lys
 1   5   10

<210> 110
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-042 HCDR3

<400> 110
Ala Arg Ser Ser Tyr Tyr Trp Ala Asp Ser
 1   5   10

<210> 111
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-043 HCDR3

<400> 111
Ala Arg Glu Leu Arg Asp Val Ala Tyr Tyr Pro Trp Gly Val Glu Asp
 1   5   10   15
Phe

<210> 112
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-044 HCDR3

<400> 112
Ala Arg Tyr Tyr Val Pro Tyr Leu Ser Asp
 1   5   10

<210> 113
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-045 HCDR3

<400> 113
Ala Arg Ala Ser Asp Leu Tyr Gly Asp
 1   5

<210> 114
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-046 HCDR3

<400> 114
Ala Arg Gly Asn Arg Arg Tyr Tyr Ser Pro Ile Ile Asp Pro

 1   5   10

<210> 115
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-047 HCDR3

<400> 115
Ala Arg Ser Asp Tyr Gly Ser Leu Tyr Asp Lys
 1   5   10

<210> 116
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-048 HCDR3

<400> 116
Ala Arg Ser Ser Phe Val Ala Thr Asp Tyr
 1   5   10

<210> 117
<211> 14
<212> PRT
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<220>
<223> GPC3A-049 HCDR3

<400> 117
Ala Arg His Gly Gly Ile Gly Ser Met Arg Ser Phe Asp Gln
 1   5   10

<210> 118
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-050 HCDR3

<400> 118
Ala Arg Ser Gly Tyr Arg Trp Leu Asp Val

1 5 10

<210> 119
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-051 HCDR3

<400> 119
Ala Arg Met Leu Tyr Leu Ser Gly Arg Tyr Tyr Trp Asp Ser
1 5 10

<210> 120
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-052 HCDR3

<400> 120
Ala Arg Ser His Ser Ser Gly Tyr Asp Lys
1 5 10

<210> 121
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-053 HCDR3

<400> 121
Ala Arg Trp Trp Ser Gly Ser Tyr Asp Thr
1 5 10

<210> 122
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-054 HCDR3

<400> 122
Ala Arg Ile Pro Met Tyr Ser Gly Ser Ser Asp Tyr

 1   5   10

<210> 123
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-055 HCDR3

<400> 123
Ala Arg Trp His Gly Gly Pro Tyr Asp Tyr
 1   5   10

<210> 124
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-056 HCDR3

<400> 124
Ala Arg Phe Ser Thr His Asn Trp Trp Trp Pro Thr Tyr Asp Tyr
 1   5   10   15

<210> 125
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-057 HCDR3

<400> 125
Ala Arg Tyr Asn Tyr Met Ser Ser Gly Phe Tyr Asp Arg
 1   5   10

<210> 126
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-058 HCDR3

<400> 126
Ala Arg Gly Lys Arg Thr Leu Ala Ser Cys Phe Asp Tyr

 1   5   10

<210> 127
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-059 HCDR3

<400> 127
Ala Arg Ser Arg Trp Ser Tyr Met Asp Val
 1   5   10

<210> 128
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-060 HCDR3

<400> 128
Ala Arg Glu Gly Tyr Gly Ser Trp Ala Met Asp Gln
 1   5   10

<210> 129
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-061 HCDR3

<400> 129
Ala Arg Lys Gly Ser Ser Gln Phe Asp Gln
 1   5   10

<210> 130
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-062 HCDR3

<400> 130
Ala Arg Met Tyr Met Asp Met Gly Trp Gly Trp Gly Tyr Trp Asp Trp

 1   5   10   15

<210> 131
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-063 HCDR3

<400> 131
Ala Arg Asp Arg Leu Ala Ser Asp Ala Phe Asp Ile
 1   5   10

<210> 132
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-064 HCDR3

<400> 132
Ala Arg Met Gly Val Gly Trp Gly Tyr Ala Gln Asp Ser
 1   5   10

<210> 133
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-067 HCDR3

<400> 133
Ala Arg Gly Ala Glu Met Ser Asp Tyr
 1   5

<210> 134
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-54 HCDR3

<400> 134
Ala Arg Glu Arg Arg Tyr Ser Ser Ser Pro Ser Asp His

 1   5   10

<210> 135
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-60 HCDR3

<400> 135
Ala Arg Ser Leu Tyr Ser Gln Pro Tyr Ile Asp Gly
 1   5   10

<210> 136
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-63 HCDR3

<400> 136
Ala Arg Ser Leu His Ala Met Arg Trp Ser Gln Thr Met Asp Ser
 1   5   10   15

<210> 137
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-66 HCDR3

<400> 137
Ala Arg Phe Ser Ser Gly Tyr Ser Arg Asp Thr
 1   5   10

<210> 138
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-68 HCDR3

<400> 138
Ala Arg Tyr Gly Tyr Glu Gly His Asp Thr

 1   5   10

<210> 139
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-71 HCDR3

<400> 139
Ala Arg Gln Gly His Met Trp Tyr Val Pro Val Asp Ala
 1   5   10

<210> 140
<211> 5
<212> PRT
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<220>
<223> GPC3B-76 HCDR3

<400> 140
Ala Arg Asn Tyr Asp
 1   5

<210> 141
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-78 HCDR3

<400> 141
Ala Arg Gly Tyr Ser Ser Phe Phe Asp Ser
 1   5   10

<210> 142
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-80 HCDR3

<400> 142
Ala Arg Met Ser Lys Tyr Tyr Gly Ser Tyr Ser Ser Tyr Asp Glu

 1   5   10   15

<210> 143
<211> 7
<212> PRT
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<220>
<223> GPC3B-81 HCDR3

<400> 143
Ala Arg Gly Leu Trp Asp Ser
 1   5

<210> 144
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-82 HCDR3

<400> 144
Ala Arg Tyr Pro Val Tyr Met Glu Thr Ser Asp Phe Asp Ser
 1   5   10

<210> 145
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-85 HCDR3

<400> 145
Ala Arg Ser Ser Leu Tyr Trp Met Gly Ser Lys Trp Ser Arg Gln Thr
 1   5   10   15
Asp Met

<210> 146
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-87 HCDR3

<400> 146
Ala Arg Thr Asn Asp Ser
 1   5

<210> 147
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-92 HCDR3

<400> 147
Ala Arg Pro Ser Met Trp Thr Ser Ser Met Gly Asp Val
 1   5   10

<210> 148
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-93 HCDR3

<400> 148
Ala Arg Tyr Thr Ala Leu Lys Pro Arg Gly Ile Tyr Ser Val Asp Ser
 1   5   10   15

<210> 149
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-110 HCDR3

<400> 149
Ala Arg Tyr Tyr Trp Arg Gly Gly Ser Gly Gln Gly Ser Val Thr Ser
 1   5   10   15
Asp Tyr

<210> 150
<211> 7
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3B-113 HCDR3

<400> 150
Ala Arg Tyr Ser Gly Asp Tyr
 1   5

<210> 151
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-115 HCDR3

<400> 151
Ala Arg Ile Ser Gly Tyr His Ser Ser Gly Trp Asp Tyr
 1   5   10

<210> 152
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-119 HCDR3

<400> 152
Ala Arg Gly Tyr Tyr Gly Lys Tyr Asp Lys
 1   5   10

<210> 153
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-125 HCDR3

<400> 153
Ala Arg Gln Ser His Asp Glu
 1   5

<210> 154
<211> 8
<212> PRT
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<220>

<223> GPC3A-034 LCDR1

<400> 154
Ser Ser Asn Ile Gly Ser Asn Asn
 1   5

<210> 155
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-035 LCDR1

<400> 155
Ser Ser Asn Ile Gly Ser Asn Tyr
 1   5

<210> 156
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-037 LCDR1

<400> 156
Arg Ser Asn Ile Gly Ser Asp Tyr
 1   5

<210> 157
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-038 LCDR1

<400> 157
Asn Ile Gly Ser Lys Ser
 1   5

<210> 158
<211> 6
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3A-039 LCDR1

<400> 158
Asn Ile Gly Ser Lys Ser
 1   5

<210> 159
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-040 LCDR1

<400> 159
Ser Ser Asn Ile Gly Ser Asn Tyr
 1   5

<210> 160
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-041 LCDR1

<400> 160
Ser Ser Asn Ile Gly Ser Asn Tyr
 1   5

<210> 161
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-042 LCDR1

<400> 161
Ser Ser Asp Ile Gly Ser Asn Ser
 1   5

<210> 162
<211> 8
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3A-043 LCDR1

<400> 162
Ser Ser Asn Ile Gly Thr Asn Tyr
 1   5

<210> 163
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-044 LCDR1

<400> 163
Asn Ile Gly Tyr Lys Gly
 1   5

<210> 164
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-045 LCDR1

<400> 164
Thr Ser Asn Ile Gly Thr Asn Thr
 1   5

<210> 165
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-046 LCDR1

<400> 165
Ser Ser Asn Phe Gly Ser Asn Thr
 1   5

<210> 166
<211> 8
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3A-047 LCDR1

<400> 166
Arg Ser Asn Ile Ala Ser Asn Asp
 1   5

<210> 167
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Asn Ile Gly Ser Lys Ser
 1   5

<210> 168
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<220>
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<400> 168
Ser Ser Asn Ile Gly Ser Asn Tyr
 1   5

<210> 169
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<220>
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<400> 169
Ser Ser Asn Ile Gly Ser Asn Thr
 1   5

<210> 170
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<220>

<223> GPC3A-051 LCDR1

<400> 170
Ser Ser Asn Ile Gly Ala Gly Tyr Asp
 1   5

<210> 171
<211> 8
<212> PRT
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<220>
<223> GPC3A-052 LCDR1

<400> 171
Ser Ser Asn Ile Gly Ser Asn Tyr
 1   5

<210> 172
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<220>
<223> GPC3A-053 LCDR1

<400> 172
Ser Ser Asn Ile Gly Ser Asn Tyr
 1   5

<210> 173
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<220>
<223> GPC3A-054 LCDR1

<400> 173
Ser Ser Asn Ile Gly Ser Asn Tyr
 1   5

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<220>

<223> GPC3A-055 LCDR1

<400> 174
Asn Ile Gly Ser Lys Ser
 1   5

<210> 175
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<220>
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<400> 175
Gln Ser Ile Ser Ser Tyr
 1   5

<210> 176
<211> 6
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<220>
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<400> 176
Asn Ile Gly Ser Lys Ser
 1   5

<210> 177
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<220>
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<400> 177
Asn Ile Gly Ser Lys Ser
 1   5

<210> 178
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<220>

<223> GPC3A-059 LCDR1

<400> 178
Asn Ile Gly Ser Lys Ser
 1   5

<210> 179
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<220>
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<400> 179
Asn Ile Gly Ser Glu Ser
 1   5

<210> 180
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<220>
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<400> 180
Asn Ile Gly Ser Lys Ser
 1   5

<210> 181
<211> 9
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<220>
<223> GPC3A-062 LCDR1

<400> 181
Ser Ser Asn Ile Gly Ala Gly Tyr Asp
 1   5

<210> 182
<211> 8
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<220>

<223> GPC3A-063 LCDR1

<400> 182
Trp Ser Asn Ile Gly Ser Tyr Thr
 1   5

<210> 183
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<220>
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<400> 183
Asn Ile Gly Ser Lys Ser
 1   5

<210> 184
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<220>
<223> GPC3A-067 LCDR1

<400> 184
Asn Ile Gly Ser Lys Ser
 1   5

<210> 185
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<220>
<223> GPC3B-54 LCDR1

<400> 185
Asn Ser Asn Ile Gly Ser Asp Thr
 1   5

<210> 186
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<220>

<223> GPC3B-60 LCDR1

<400> 186
Asn Leu Glu Asn Lys Phe
 1   5

<210> 187
<211> 11
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<220>
<223> GPC3B-63 LCDR1

<400> 187
Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr
 1   5   10

<210> 188
<211> 6
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<220>
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<400> 188
Asn Ile Gly Ser Lys Ser
 1   5

<210> 189
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-68 LCDR1

<400> 189
Ser Ser Asn Ile Gly Asn Asn Tyr
 1   5

<210> 190
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<220>

<223> GPC3B-71 LCDR1

<400> 190
Asn Ile Gly Ser Lys Ser
 1   5

<210> 191
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-76 LCDR1

<400> 191
Ser Ser Asn Ile Gly Asn Asn Tyr
 1   5

<210> 192
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-78 LCDR1

<400> 192
Lys Leu Gly Asp Lys Tyr
 1   5

<210> 193
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-80 LCDR1

<400> 193
Ser Ser Asn Ile Gly Ser Asn Thr
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<210> 194
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<220>

<223> GPC3B-81 LCDR1

<400> 194
Asn Ile Gly Ser Lys Ser
 1   5

<210> 195
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<212> PRT
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<220>
<223> GPC3B-82 LCDR1

<400> 195
Ser Ser Asn Ile Gly Ala Gly Phe Asp
 1   5

<210> 196
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<212> PRT
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<220>
<223> GPC3B-85 LCDR1

<400> 196
Ser Ser Asn Ile Gly Ser Asn Thr
 1   5

<210> 197
<211> 11
<212> PRT
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<220>
<223> GPC3B-87 LCDR1

<400> 197
Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr
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<210> 198
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<220>

<223> GPC3B-92 LCDR1

<400> 198
Thr Leu Ala Lys Arg Tyr
 1   5

<210> 199
<211> 8
<212> PRT
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<220>
<223> GPC3B-93 LCDR1

<400> 199
Ser Gly Ser Ile Ala Ser Asn Tyr
 1   5

<210> 200
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<212> PRT
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<220>
<223> GPC3B-110 LCDR1

<400> 200
Ser Ser Asn Ile Gly Ser Asn Thr
 1   5

<210> 201
<211> 8
<212> PRT
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<220>
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<400> 201
Ser Ser Asn Ile Gly Ser Asn Ser
 1   5

<210> 202
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<220>

<223> GPC3B-115 LCDR1

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Asn Ile Gly Ser Lys Ser
 1   5

<210> 203
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<220>
<223> GPC3B-119 LCDR1

<400> 203
Ser Ser Asn Ile Gly Asn Asn Tyr
 1   5

<210> 204
<211> 9
<212> PRT
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<220>
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<210> 205
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<212> PRT
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<220>
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<400> 205
Ser Asn His
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<210> 206
<211> 3
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<220>

<223> GPC3A-035 LCDR2

<400> 206
Lys Asn Phe
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<210> 207
<211> 3
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<220>
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<400> 207
Gly Asp Asn
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<210> 208
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<220>
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<400> 208
Tyr Asp Ser
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<210> 209
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<220>
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<400> 209
Tyr Asp Ser
 1

<210> 210
<211> 3
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<220>

<223> GPC3A-040 LCDR2

<400> 210
Ser Asn Asn
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<210> 211
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<220>
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<400> 211
Lys Asn Phe
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<210> 212
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<213> Artificial Sequence

<220>
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<400> 212
Ser Thr Gln
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<210> 213
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-043 LCDR2

<400> 213
Arg Asn Asn
 1

<210> 214
<211> 3
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3A-044 LCDR2

<400> 214
Asp Asp Ser
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<210> 215
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<213> Artificial Sequence

<220>
<223> GPC3A-045 LCDR2

<400> 215
Ser Asn Asn
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<210> 216
<211> 3
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<213> Artificial Sequence

<220>
<223> GPC3A-046 LCDR2

<400> 216
Ser Asn Thr
 1

<210> 217
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
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<400> 217
Lys Lys Asn
 1

<210> 218
<211> 3
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<220>

<223> GPC3A-048 LCDR2

<400> 218
Tyr Asp Ser
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<210> 219
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<220>
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<400> 219
Arg Asn Asn
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<210> 220
<211> 3
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<220>
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<400> 220
Ser Asn Asn
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<210> 221
<211> 3
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<220>
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<400> 221
Gly Asn Ser
 1

<210> 222
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<223> GPC3A-052 LCDR2

<400> 222
Arg Asn Asn
 1

<210> 223
<211> 3
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<220>
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<400> 223
Gly Asn Ser
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<210> 224
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<220>
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<400> 224
Arg Asn Asn
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<210> 225
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<220>
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<400> 225
Tyr Asp Ser
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<210> 226
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<220>

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<400> 226
Ala Ala Ser
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<210> 227
<211> 3
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<220>
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<400> 227
Tyr Asp Ser
 1

<210> 228
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<220>
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<400> 228
Asp Asp Ser
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<210> 229
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<220>
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<400> 229
Tyr Asp Ser
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<210> 230
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<220>

<223> GPC3A-060 LCDR2

<400> 230
Asp Asp Asp
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<210> 231
<211> 3
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<220>
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<400> 231
Tyr Asp Ser
 1

<210> 232
<211> 3
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<220>
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<400> 232
Gly Asn Ser
 1

<210> 233
<211> 3
<212> PRT
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<220>
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<400> 233
Gly Asn Asn
 1

<210> 234
<211> 3
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<220>

<223> GPC3A-064 LCDR2

<400> 234
Asp Asp Thr
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<210> 235
<211> 3
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<220>
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<400> 235
Tyr Asp Ser
 1

<210> 236
<211> 3
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<220>
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<400> 236
Arg Asp Asn
 1

<210> 237
<211> 3
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<220>
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<400> 237
Glu Asp Asn
 1

<210> 238
<211> 3
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<220>

<223> GPC3B-63 LCDR2

<400> 238
Leu Gly Ser
 1

<210> 239
<211> 3
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<220>
<223> GPC3B-66 LCDR2

<400> 239
Tyr Asp Ser
 1

<210> 240
<211> 3
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<220>
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<400> 240
Asp Asn Ile
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<210> 241
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<220>
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<400> 241
Tyr Asp Ser
 1

<210> 242
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<220>

<223> GPC3B-76 LCDR2

<400> 242
Asp Asn Asn
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<210> 243
<211> 3
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<220>
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<400> 243
Gln Asp Asn
 1

<210> 244
<211> 3
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<220>
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<400> 244
Ser Asn Asn
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<210> 245
<211> 3
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<220>
<223> GPC3B-81 LCDR2

<400> 245
Tyr Asp Ser
 1

<210> 246
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<220>

<223> GPC3B-82 LCDR2

<400> 246
Asp Asn Asn
 1

<210> 247
<211> 3
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<220>
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<400> 247
Ser Asn Asn
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<210> 248
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<220>
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<400> 248
Leu Gly Ser
 1

<210> 249
<211> 3
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<220>
<223> GPC3B-92 LCDR2

<400> 249
Arg Asp Thr
 1

<210> 250
<211> 3
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<220>

<223> GPC3B-93 LCDR2

<400> 250
Glu Asp Asn
 1

<210> 251
<211> 3
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<220>
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<400> 251
Ser Ser Asn
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<210> 252
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<220>
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<400> 252
Ser Asn Asn
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<210> 253
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<220>
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<400> 253
Asp Asp Ser
 1

<210> 254
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<220>

<223> GPC3B-119 LCDR2

<400> 254
Asp Asn Asn
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<210> 255
<211> 3
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<220>
<223> GPC3B-125 LCDR2

<400> 255
Glu Val Ser
 1

<210> 256
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-034 LCDR3

<400> 256
Ala Ala Trp Asp Asp Ser Leu Asp Gly Tyr Leu
 1   5   10

<210> 257
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-035 LCDR3

<400> 257
Ala Ala Trp Asp Asp Ala Leu Ser Gly Tyr Val
 1   5   10

<210> 258
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3A-037 LCDR3

<400> 258
Gly Thr Trp Asp Tyr Thr Leu Asn Gly Val Val
 1   5   10

<210> 259
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-038 LCDR3

<400> 259
Gln Val Trp Asp Ser Ser Ser Asp His Val Val
 1   5   10

<210> 260
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-039 LCDR3

<400> 260
Gln Val Trp Asp Ser Ser Ser Asp His Trp Val
 1   5   10

<210> 261
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-040 LCDR3

<400> 261
Ala Ala Trp Asp Asp Ser Leu Ser Gly Tyr Val
 1   5   10

<210> 262
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3A-041 LCDR3

<400> 262
Ala Ala Trp Asp Asp Ala Leu Ser Gly Tyr Val
 1   5   10

<210> 263
<211> 11
<212> PRT
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<220>
<223> GPC3A-042 LCDR3

<400> 263
Ala Thr Trp Asp Asp Ser Leu Asn Gly Tyr Val
 1   5   10

<210> 264
<211> 11
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<220>
<223> GPC3A-043 LCDR3

<400> 264
Ala Val Trp Asp Asp Ser Leu Ser Gly Val Val
 1   5   10

<210> 265
<211> 11
<212> PRT
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<220>
<223> GPC3A-044 LCDR3

<400> 265
Gln Val Trp Asp Ser Ser Ser Asp His Val Val
 1   5   10

<210> 266
<211> 11
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<220>

<223> GPC3A-045 LCDR3

<400> 266
Ala Ala Trp Asp Asp Ser Leu Asn Gly Val Val
 1   5   10

<210> 267
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-046 LCDR3

<400> 267
Ala Ala Trp Asp Asp Ser Leu Thr Gly Val Val
 1   5   10

<210> 268
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-047 LCDR3

<400> 268
Ala Ala Trp Asp Asp Asn Leu Ser Gly Tyr Val
 1   5   10

<210> 269
<211> 11
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<220>
<223> GPC3A-048 LCDR3

<400> 269
Gln Val Trp Asp Ser Ser Ser Asp Arg Gly Val
 1   5   10

<210> 270
<211> 9
<212> PRT
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<220>

<223> GPC3A-049 LCDR3

<400> 270
Ala Ala Trp Asp Asp Ser Leu Ser Gly
 1   5

<210> 271
<211> 11
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<220>
<223> GPC3A-050 LCDR3

<400> 271
Ala Ala Trp Asp Asp Ser Leu Asn Gly Pro Val
 1   5   10

<210> 272
<211> 11
<212> PRT
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<220>
<223> GPC3A-051 LCDR3

<400> 272
Gln Ser Tyr Asp Ser Ser Leu Ser Gly Tyr Val
 1   5   10

<210> 273
<211> 11
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<220>
<223> GPC3A-052 LCDR3

<400> 273
Ala Ala Trp Asp Asp Ser Leu Ser Gly Tyr Val
 1   5   10

<210> 274
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<220>

<223> GPC3A-053 LCDR3

<400> 274
Gln Ser Tyr Asp Ser Ser Leu Ser Gly Ser Asn Val
 1   5   10

<210> 275
<211> 11
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<220>
<223> GPC3A-054 LCDR3

<400> 275
Ala Ala Trp Asp Asp Ser Leu Ser Gly Tyr Val
 1   5   10

<210> 276
<211> 11
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<220>
<223> GPC3A-055 LCDR3

<400> 276
Gln Val Trp Asp Ser Ser Ser Asp His Tyr Val
 1   5   10

<210> 277
<211> 9
<212> PRT
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<220>
<223> GPC3A-056 LCDR3

<400> 277
Gln Gln Ser Tyr Ser Thr Pro Ile Thr
 1   5

<210> 278
<211> 11
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<220>

<223> GPC3A-057 LCDR3

<400> 278
Gln Val Trp Asp Ser Ser Ser Asp His Val Val
 1   5   10

<210> 279
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-058 LCDR3

<400> 279
Gln Val Trp Asp Ser Ser Ser Asp His Val
 1   5   10

<210> 280
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-059 LCDR3

<400> 280
Gln Val Trp Asp Ser Ser Ser Asp His Val
 1   5   10

<210> 281
<211> 9
<212> PRT
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<220>
<223> GPC3A-060 LCDR3

<400> 281
Gln Thr Trp Asp Ser Ser Thr Ala Ile
 1   5

<210> 282
<211> 11
<212> PRT
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<220>

<223> GPC3A-061 LCDR3

<400> 282
Gln Val Trp Asp Ser Ser Ser Asp His Tyr Val
 1   5   10

<210> 283
<211> 12
<212> PRT
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<220>
<223> GPC3A-062 LCDR3

<400> 283
Gln Ser Tyr Asp Ser Ser Leu Ser Gly Ser Tyr Val
 1   5   10

<210> 284
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-063 LCDR3

<400> 284
Ala Ala Trp Asp Asp Asn Leu Asn Gly Val Val
 1   5   10

<210> 285
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-064 LCDR3

<400> 285
Gln Val Trp Asp Arg Ser Ser Ala His Trp Val
 1   5   10

<210> 286
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3A-067 LCDR3

<400> 286
Gln Val Trp Asp Ser Ser Ser Asp His Val Val
 1   5   10

<210> 287
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-54 LCDR3

<400> 287
Thr Thr Trp Asp Asp Ser Leu Asn Gly Leu Val
 1   5   10

<210> 288
<211> 11
<212> PRT
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<220>
<223> GPC3B-60 LCDR3

<400> 288
Gln Thr Trp Asp Ser Pro Thr Gly Leu Phe Val
 1   5   10

<210> 289
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-63 LCDR3

<400> 289
Met Gln Ala Leu Gln Thr Pro Pro Thr
 1   5

<210> 290
<211> 12
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3B-66 LCDR3

<400> 290
Gln Val Trp Asp Ser Ser Ser Asp His Leu Tyr Val
 1   5   10

<210> 291
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-68 LCDR3

<400> 291
Gly Thr Trp Asp Ser Ser Leu Ser Ala Gly Val
 1   5   10

<210> 292
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-71 LCDR3

<400> 292
Gln Val Trp Asp Ser Ser Ser Asp His Tyr Val
 1   5   10

<210> 293
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-76 LCDR3

<400> 293
Gly Thr Trp Asp Ser Ser Leu Ser Ala Gly Val
 1   5   10

<210> 294
<211> 9
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3B-78 LCDR3

<400> 294
Gln Thr Trp Asp Arg Ser Thr Tyr Val
 1   5

<210> 295
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-80 LCDR3

<400> 295
Ala Ala Trp Asp Asp Ser Leu Asn Gly
 1   5

<210> 296
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-81 LCDR3

<400> 296
Gln Val Trp Asp Ser Ser Ser Asp Leu Leu Tyr Val
 1   5   10

<210> 297
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-82 LCDR3

<400> 297
Gln Ser Phe Asp Ser Ser Leu Ser Gly Trp Val
 1   5   10

<210> 298
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3B-85 LCDR3

<400> 298
Ala Ala Trp Asp Asp Ser Leu Asn Gly Tyr Val
 1   5   10

<210> 299
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-87 LCDR3

<400> 299
Met Gln Ala Leu Gln Thr Pro Trp Thr
 1   5

<210> 300
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-92 LCDR3

<400> 300
Gln Ser Ala Asp Asn Ser Arg Thr Phe Val
 1   5   10

<210> 301
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-93 LCDR3

<400> 301
Gln Ser Tyr Asp Ser Ser Asn Trp Val
 1   5

<210> 302
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3B-110 LCDR3

<400> 302
Ala Ala Trp Asp Asp Ser Leu Asn Gly Pro Val
 1   5   10

<210> 303
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-113 LCDR3

<400> 303
Ala Ala Trp Asp Asp Ser Leu Asn Gly Val Leu
 1   5   10

<210> 304
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-115 LCDR3

<400> 304
Gln Val Trp Asp Ser Ser Ser Asp Pro Leu Tyr Val
 1   5   10

<210> 305
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-119 LCDR3

<400> 305
Ala Thr Trp Asp Asn Ser Leu Ser Ala Leu Ile
 1   5   10

<210> 306
<211> 10
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3B-125 LCDR3

<400> 306
Ser Ser Tyr Thr Ser Ser Thr Thr Val Ile
 1   5   10

<210> 307
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-034 VH

<400> 307
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
 1   5   10   15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
            20   25   30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35   40   45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50   55   60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65   70   75   80
Glu Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85   90   95
Arg Gly Tyr Gly Gly Arg Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
            100   105   110
Thr Val Ser Ser
        115

<210> 308
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-035 VH

<400> 308
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr

65 70 75 80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Ser Trp Thr Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

<210> 309
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-037 VH

<400> 309
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Val Ile Tyr Ser Gly Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Thr Ser Tyr Leu Asn His Gly Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser
115

<210> 310
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-038 VH

<400> 310
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe

    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Lys Val Thr Gly Tyr Asp Ser Trp Gly Gln Gly Thr Leu Val
            100   105   110
Thr Val Ser Ser
        115

<210> 311
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-039 VH

<400> 311
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val His Pro Gly Gly
 1   5   10   15
Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Ser Tyr
            20   25   30
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45
Ser Ala Ile Gly Thr Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val Lys
    50   55   60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65   70   75   80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                85   90   95
Arg Tyr Gly Arg Lys Ser Ile Asp Ala Trp Gly Gln Gly Thr Leu Val
            100   105   110
Thr Val Ser Ser
        115

<210> 312
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-040 VH

<400> 312
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Thr Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met

```
        35                  40                  45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Arg Gly Tyr Tyr Gly Tyr Asp Ser Trp Gly Gln Gly Thr Leu
            100                 105                 110
Val Thr Val Ser Ser
        115
```

<210> 313
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-041 VH

<400> 313

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1               5                  10                  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95
Ala Arg Ser Gly Lys Tyr Tyr Gly Asp Lys Trp Gly Gln Gly Thr Leu
            100                 105                 110
Val Thr Val Ser Ser
        115
```

<210> 314
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-042 VH

<400> 314

```
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1               5                  10                  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr
```

            20   25   30
Tyr Val Tyr Trp Met Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
        35   40   45
Gly Trp Met Asn Pro Arg Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Arg Leu Thr Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Ser Ser Tyr Tyr Trp Ala Asp Ser Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser
        115

<210> 315
<211> 124
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-043 VH

<400> 315
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Thr Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Phe Thr Asp Tyr
            20   25   30
Tyr Met His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
        35   40   45
Gly Leu Val Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Glu Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Glu Leu Arg Asp Val Ala Tyr Tyr Pro Trp Gly Val Glu Asp
            100   105   110
Phe Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 316
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-044 VH

<400> 316
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu

```
 1               5                  10                  15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
            20                  25                  30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95
Arg Tyr Tyr Val Pro Tyr Leu Ser Asp Trp Gly Gln Gly Thr Leu Val
            100                 105                 110
Thr Val Ser Ser
        115
```

<210> 317
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-045 VH

<400> 317

```
Gln Met Gln Leu Val Gln Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
            20                  25                  30
Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Ala Ser Asp Leu Tyr Gly Asp Trp Gly Gln Gly Thr Leu Val
            100                 105                 110
Thr Val Ser Ser
        115
```

<210> 318
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-046 VH

<400> 318
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Thr Val Ser Cys Lys Ala Ser Gly Tyr Arg Phe Ser Asn Tyr
            20   25   30
Gly Val Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Ser Gly Ser Asn Gly Asn Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Leu Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Thr Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Gly Asn Arg Arg Tyr Tyr Ser Pro Ile Ile Asp Pro Trp Gly
            100   105   110
Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 319
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-047 VH

<400> 319
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Pro Gly Tyr Thr Phe Thr Gly Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Ser Asp Tyr Gly Ser Leu Tyr Asp Lys Trp Gly Gln Gly Thr
            100   105   110
Leu Val Thr Val Ser Ser
        115

<210> 320
<211> 117
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3A-048 VH

<400> 320
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Glu Val Gln Pro Gly Arg
 1   5   10   15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20   25   30
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45
Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50   55   60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65   70   75   80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Ser Ser Phe Val Ala Thr Asp Tyr Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser
        115

<210> 321
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-049 VH

<400> 321
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Glu Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg His Gly Gly Ile Gly Ser Met Arg Ser Phe Asp Gln Trp Gly
            100   105   110
Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 322
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-050 VH

<400> 322
Gln Ile Thr Leu Lys Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr
            20   25   30
Tyr Val Tyr Trp Met Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
        35   40   45
Gly Trp Met Asn Pro Arg Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Arg Leu Thr Ser Asp Asp Thr Ala Thr Tyr Tyr Cys
                85   90   95
Ala Arg Ser Gly Tyr Arg Trp Leu Asp Val Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser
        115

<210> 323
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-051 VH

<400> 323
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Ala Phe Ser Ser Tyr
            20   25   30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Met Leu Tyr Leu Ser Gly Arg Tyr Tyr Trp Asp Ser Trp Gly
            100   105   110
Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 324
<211> 117

<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-052 VH

<400> 324
Glu Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Ser His Ser Ser Gly Tyr Asp Lys Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser
        115

<210> 325
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-053 VH

<400> 325
Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1   5   10   15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser
            20   25   30
Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35   40   45
Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
    50   55   60
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65   70   75   80
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85   90   95
Cys Ala Arg Trp Trp Ser Gly Ser Tyr Asp Thr Trp Gly Gln Gly Thr
            100   105   110
Leu Val Thr Val Ser Ser
        115

<210> 326
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-054 VH

<400> 326
Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20   25   30
Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
    50   55   60
Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Ile Pro Met Tyr Ser Gly Ser Ser Asp Tyr Trp Gly Gln Gly
            100   105   110
Thr Leu Val Thr Val Ser Ser
        115

<210> 327
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-055 VH

<400> 327
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Trp His Gly Gly Pro Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser
        115

<210> 328
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-056 VH

<400> 328
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys Lys Phe Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Asn Asp Tyr
            20   25   30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Asn Pro Asn Asn Gly Asp Thr Lys Tyr Glu Lys Lys Trp
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Thr Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Phe Ser Thr His Asn Trp Trp Trp Pro Thr Tyr Asp Tyr Trp
            100   105   110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 329
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-057 VH

<400> 329
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Ile Gln Pro Gly Gly
 1   5   10   15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20   25   30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
    50   55   60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65   70   75   80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Tyr Asn Tyr Met Ser Ser Gly Phe Tyr Asp Arg Trp Gly Gln
            100   105   110

Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 330
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-058 VH

<400> 330
Gln Val Gln Leu Val Gln Ser Gly Ala Asp Val Arg Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Ser His
            20   25   30
Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Leu
        35   40   45
Gly Trp Ile Ser Pro Tyr Thr Gly Asn Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Ala Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Ile Tyr Tyr Cys
                85   90   95
Ala Arg Gly Lys Arg Thr Leu Ala Ser Cys Phe Asp Tyr Trp Gly Gln
            100   105   110
Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 331
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-059 VH

<400> 331
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Thr Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20   25   30
Gly Ile Thr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Ser Ala Tyr Ser Asp Lys Thr Asn Tyr Ala Gln Lys Leu
    50   55   60
Gln Gly Arg Val Thr Met Thr Thr Asp Ile Ser Thr Asn Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Ser Arg Trp Ser Tyr Met Asp Val Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser
        115

<210> 332
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-060 VH

<400> 332
Gln Val Gln Leu Val Gln Ser Gly Gly Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Asn Ser Tyr
            20   25   30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
    50   55   60
Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Asn Thr Ala Phe
65   70   75   80
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Glu Gly Tyr Gly Ser Trp Ala Met Asp Gln Trp Gly Gln Gly
            100   105   110
Thr Leu Val Thr Val Ser Ser
        115

<210> 333
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-061 VH

<400> 333
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20   25   30
Gly Ile Ser Trp Val Arg Gln Thr Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
    50   55   60
Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65   70   75   80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Lys Gly Ser Ser Gln Phe Asp Gln Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser
        115

<210> 334
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-062 VH

<400> 334
Gln Met Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20   25   30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Gly Ile Ile Pro Lys Ile Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
                85   90   95
Ala Arg Met Tyr Met Asp Met Gly Trp Gly Trp Gly Tyr Trp Asp Trp
            100   105   110
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 335
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-063 VH

<400> 335
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Ile Ile Asn Pro Ser Gly Gly Ser Ala Ser Tyr Ala Gln Lys Phe
    50   55   60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asp Arg Leu Ala Ser Asp Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110
Thr Met Val Thr Val Ser Ser
115

<210> 336
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-064 VH

<400> 336
Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ile Tyr
20 25 30
Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Ser Pro Tyr Asn Asp Asn Thr Ile Tyr Ala Gln Lys Val
50 55 60
Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Met Gly Val Gly Trp Gly Tyr Ala Gln Asp Ser Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 337
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-067 VH

<400> 337
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Phe Gly Ile Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Gly Ala Glu Met Ser Asp Tyr Trp Gly Gln Gly Thr Leu Val
            100   105   110
Thr Val Ser Ser
        115

<210> 338
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-54 VH

<400> 338
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Thr Phe Ile Asp Tyr
            20   25   30
Tyr Val Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Gly Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Glu Arg Arg Tyr Ser Ser Ser Pro Ser Asp His Trp Gly Gln
            100   105   110
Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 339
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-60 VH

<400> 339
Glu Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20   25   30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Ser Leu Tyr Ser Gln Pro Tyr Ile Asp Gly Trp Ser Gln Gly
            100   105   110
Thr Leu Val Thr Val Ser Ser
        115

<210> 340
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-63 VH

<400> 340
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Ser Leu His Ala Met Arg Trp Ser Gln Thr Met Asp Ser Trp
            100   105   110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 341
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-66 VH

<400> 341
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Arg Pro Gly Ala
 1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ile Gly Gln
            20   25   30
Tyr Leu His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Arg Ile Asn Pro Met Thr Gly Val Thr Asn Tyr Ala Pro Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Gly Tyr
65   70   75   80
Met Glu Ile Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Phe Ser Ser Gly Tyr Ser Arg Asp Thr Trp Gly Gln Gly Thr
            100   105   110
Leu Val Thr Val Ser Ser
        115

<210> 342
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-68 VH

<400> 342
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20   25   30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Tyr Gly Tyr Glu Gly His Asp Thr Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser
        115

<210> 343
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-71 VH

<400> 343

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1   5   10   15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
            20   25   30
Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45
Ser Ser Ile Ser Ser Ser Gly Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
    50   55   60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Phe
65   70   75   80
Leu Gln Met Asp Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Gln Gly His Met Trp Tyr Val Pro Val Asp Ala Trp Gly Gln
            100   105   110
Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 344
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-76 VH

<400> 344
Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Glu Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20   25   30
Tyr Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Asn Tyr Asp Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            100   105   110

<210> 345
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-78 VH

<400> 345

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Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1               5                  10                  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
    50                  55                  60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65                  70                  75                  80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Gly Tyr Ser Ser Phe Phe Asp Ser Trp Gly Gln Gly Thr Leu
            100                 105                 110
Val Thr Val Ser Ser
        115
```

<210> 346
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-80 VH

<400> 346

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1               5                  10                  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Leu Ser Arg Phe
            20                  25                  30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
Gly Gly Ile Ile Pro Ile Phe Arg Thr Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Met Ser Lys Tyr Tyr Gly Ser Tyr Ser Ser Tyr Asp Glu Trp
            100                 105                 110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 347
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-81 VH

<400> 347
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Arg Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Gly Leu Trp Asp Ser Trp Gly Gln Gly Thr Leu Val Thr Val
            100   105   110
Ser Ser

<210> 348
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-82 VH

<400> 348
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
 1   5   10   15
Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65   70   75   80
Met Glu Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Tyr Pro Val Tyr Met Glu Thr Ser Asp Phe Asp Ser Trp Gly
            100   105   110
Ser Arg Tyr Ser Gly Asp Arg Leu Leu
        115   120

<210> 349
<211> 125
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-85 VH

<400> 349
Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20   25   30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Asn Thr Asn Thr Gly Asn Pro Thr Tyr Ala Gln Gly Phe
    50   55   60
Thr Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65   70   75   80
Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Ser Ser Leu Tyr Trp Met Gly Ser Lys Trp Ser Arg Gln Thr
            100   105   110
Asp Met Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120   125

<210> 350
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-87 VH

<400> 350
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Arg Lys Pro Gly Ser
 1   5   10   15
Ser Val Lys Val Ser Cys Gln Ala Ser Gly Gly Thr Phe Gly Ser Tyr
            20   25   30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Arg Ile Ile Pro Val Leu Gly Arg Thr Lys Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Val Thr Ala Asp Thr Ser Thr Ser Thr Val Tyr
65   70   75   80
Met Glu Leu Thr Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Thr Asn Asp Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100   105   110
Ser

<210> 351
<211> 120
<212> PRT

<213> Artificial Sequence

<220>
<223> GPC3B-92 VH

<400> 351
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Asn Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Ile Ile Asn Pro Ser Gly Gly Thr Thr Thr Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Pro Ser Met Trp Thr Ser Ser Met Gly Asp Val Trp Gly Gln
            100   105   110
Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 352
<211> 123
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-93 VH

<400> 352
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20   25   30
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Arg Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Tyr Thr Ala Leu Lys Pro Arg Gly Ile Tyr Ser Val Asp Ser
            100   105   110
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 353

<211> 125
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-110 VH

<400> 353
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1   5   10   15
Ser Val Lys Val Ser Cys Gln Ala Ser Gly Gly Thr Phe Thr Thr Tyr
            20   25   30
Ser Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Gly Ile Ile Pro Thr Phe Gly Thr Thr Asn Tyr Ala Gln Asn Phe
    50   55   60
Gln Asp Arg Val Thr Ile Ser Ala Asp Glu Ser Thr Asn Thr Ala Tyr
65   70   75   80
Met Glu Leu Thr Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Tyr Tyr Trp Arg Gly Gly Ser Gly Gln Gly Ser Val Thr Ser
            100   105   110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120   125

<210> 354
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-113 VH

<400> 354
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
            20   25   30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
        35   40   45
Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
    50   55   60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Tyr Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
            100   105   110
Ser Ser

<210> 355
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-115 VH

<400> 355
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Arg Pro Gly Ala
 1   5   10   15
Thr Ile Lys Val Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Ala Tyr
            20   25   30
Tyr Thr His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Val
        35   40   45
Gly Arg Ile Asn Ala Asn Thr Gly Gly Thr Asp Tyr Ala Pro Lys Phe
    50   55   60
Arg Asp Arg Val Ile Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Gly Arg Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Ile Ser Gly Tyr His Ser Ser Gly Trp Asp Tyr Trp Gly Gln
            100   105   110
Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 356
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-119 VH

<400> 356
Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20   25   30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Asn Thr Asn Thr Gly Asn Pro Thr Tyr Ala Gln Gly Phe
    50   55   60
Thr Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65   70   75   80
Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Gly Tyr Tyr Gly Lys Tyr Asp Lys Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser

115

<210> 357
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-125 VH

<400> 357
Gln Met Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20   25   30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45
Gly Trp Ile Asn Thr Asn Thr Gly Asn Pro Thr Tyr Ala Gln Gly Phe
    50   55   60
Thr Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65   70   75   80
Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95
Ala Arg Gln Ser His Asp Glu Trp Gly Gln Gly Thr Leu Val Thr Val
            100   105   110
Ser Ser

<210> 358
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-034 VL

<400> 358
Gln Pro Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Asn Val Ile Trp Tyr Gln Gln Leu Pro Gly Ala Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn His Arg Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Arg Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Asp Gly Tyr Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly

100 105 110

<210> 359
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-035 VL

<400> 359
Gln Ala Val Leu Thr Gln Pro Pro Ser Ala Ser Gln Thr Pro Gly Gln
1 5 10 15
Met Val Thr Ile Ser Cys Ser Gly Thr Ser Ser Asn Ile Gly Ser Asn
20 25 30
Tyr Val Phe Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Lys Asn Phe Gln Arg Pro Ser Gly Val Pro Gly Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ala Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80
Ser Glu Asp Glu Ala Asp Tyr Phe Cys Ala Ala Trp Asp Asp Ala Leu
85 90 95
Ser Gly Tyr Val Phe Gly Ala Gly Thr Lys Val Thr Val Leu Gly
100 105 110

<210> 360
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-037 VL

<400> 360
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Thr Arg Ser Asn Ile Gly Ser Asp
20 25 30
Tyr Val Ser Trp Tyr Gln His Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Val Tyr Gly Asp Asn Leu Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
50 55 60
Ala Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65 70 75 80
Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Tyr Thr Leu
85 90 95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

<210> 361
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-038 VL

<400> 361
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105

<210> 362
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-039 VL

<400> 362
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105

<210> 363

<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-040 VL

<400> 363
Leu Pro Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Ser Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 364
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-041 VL

<400> 364
Gln Ala Val Leu Thr Gln Pro Pro Ser Ala Ser Gln Thr Pro Gly Gln
 1   5   10   15
Met Val Thr Ile Ser Cys Ser Gly Thr Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Tyr Val Phe Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Lys Asn Phe Gln Arg Pro Ser Gly Val Pro Gly Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ala Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Phe Cys Ala Ala Trp Asp Asp Ala Leu
                85   90   95
Ser Gly Tyr Val Phe Gly Ala Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 365
<211> 111
<212> PRT

<213> Artificial Sequence

<220>
<223> GPC3A-042 VL

<400> 365
Ser Tyr Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Phe Gly Ser Ser Ser Asp Ile Gly Ser Asn
            20   25   30
Ser Val Phe Trp Tyr Gln Gln Leu Pro Gly Ala Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Thr Gln Tyr Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Glu Tyr His Cys Ala Thr Trp Asp Asp Ser Leu
                85   90   95
Asn Gly Tyr Val Phe Gly Ser Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 366
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-043 VL

<400> 366
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Thr Asn
            20   25   30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Ile
        35   40   45
Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Glu Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Val Trp Asp Asp Ser Leu
                85   90   95
Ser Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105   110

<210> 367
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-044 VL

<400> 367
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asp Asn Ile Gly Tyr Lys Gly Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35   40   45
Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105

<210> 368
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-045 VL

<400> 368
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Ile Ile Ser Cys Pro Gly Ser Thr Ser Asn Ile Gly Thr Asn
            20   25   30
Thr Val Asn Trp Tyr Gln Gln Phe Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105   110

<210> 369
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-046 VL

<400> 369
Gln Ala Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Phe Gly Ser Asn
            20   25   30
Thr Val His Trp Tyr Gln Gln Val Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Phe Ser Asn Thr Gln Arg Pro Ser Glu Ile Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Thr Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105   110

<210> 370
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-047 VL

<400> 370
Leu Pro Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Ala Ser Asn
            20   25   30
Asp Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Arg Leu
        35   40   45
Ile Tyr Lys Lys Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Ala Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Asn Leu
                85   90   95
Ser Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 371
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-048 VL

<400> 371

Gln Pro Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Phe Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp Arg
                85   90   95
Gly Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105

<210> 372
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-049 VL

<400> 372
Gln Ala Val Leu Thr Gln Pro Ser Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Gly Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Ser Gly Trp Val Phe Gly Gly Arg Thr Lys Leu Thr Val Leu Gly
            100   105   110

<210> 373
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-050 VL

<400> 373
Gln Ala Val Leu Thr Gln Pro Ser Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Asn Gly Pro Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 374
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-051 VL

<400> 374
Gln Ala Val Leu Thr Gln Pro Ser Ser Ala Ser Gly Ala Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Thr Gly Gly Ser Ser Asn Ile Gly Ala Gly
            20   25   30
Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
        35   40   45
Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
    50   55   60
Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65   70   75   80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
                85   90   95
Leu Ser Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 375
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-052 VL

<400> 375
Gln Ala Val Leu Thr Gln Pro Ser Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30

Tyr Val Tyr Trp Tyr Gln Gln Leu Ser Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Ser Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 376
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-053 VL

<400> 376
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Ala Ala Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Thr Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Tyr Val Trp Trp Tyr Gln Gln Leu Pro Gly Ala Ala Pro Arg Leu Leu
        35   40   45
Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65   70   75   80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
                85   90   95
Ser Gly Ser Asn Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 377
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-054 VL

<400> 377
Ser Tyr Glu Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Ser Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 378
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-055 VL

<400> 378
Gln Pro Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105

<210> 379
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-056 VL

<400> 379
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1   5   10   15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
            20   25   30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Ile
85 90 95
Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg
100 105

<210> 380
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-057 VL

<400> 380
Glu Ile Val Leu Thr Gln Ser Pro Ser Val Ser Val Ala Pro Gly Lys
1 5 10 15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65 70 75 80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
85 90 95
Val Val Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 381
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-058 VL

<400> 381
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
1 5 10 15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
35 40 45
Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105

<210> 382
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-059 VL

<400> 382
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105

<210> 383
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-060 VL

<400> 383
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Leu Thr Cys Gly Gly Asn Asn Ile Gly Ser Glu Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Leu Leu Val Val Tyr
        35   40   45
Asp Asp Asp Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Glu Asp Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Leu
65   70   75   80
Asp Glu Ala Glu Tyr Tyr Cys Gln Thr Trp Asp Ser Ser Thr Ala Ile
                85   90   95

Phe Gly Thr Gly Thr Lys Leu Thr Val Leu Gly
100 105

<210> 384
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-061 VL

<400> 384
Gln Pro Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
1 5 10 15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65 70 75 80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
85 90 95
Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105

<210> 385
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-062 VL

<400> 385
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30
Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45
Leu Ile Tyr Gly Asn Ser Asn Trp Pro Ser Gly Val Pro Asp Arg Phe
50 55 60
Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65 70 75 80
Arg Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
85 90 95
Leu Ser Gly Ser Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu
100 105 110

Gly

<210> 386
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-063 VL

<400> 386
Gln Thr Val Val Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Trp Ser Asn Ile Gly Ser Tyr
            20   25   30
Thr Val Asn Trp Tyr Gln His Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Ser Gly Asn Asn Gln Arg Pro Ser Gly Val Pro Gly Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr His Cys Ala Ala Trp Asp Asp Asn Leu
                85   90   95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105   110

<210> 387
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-064 VL

<400> 387
Leu Pro Val Leu Thr Gln Pro Pro Ser Leu Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Leu Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr His Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35   40   45
Asp Asp Thr Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Ala Leu Thr Ile Ser Arg Val Glu Val Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Arg Ser Ser Ala His
                85   90   95
Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105

<210> 388
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-067 VL

<400> 388
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105

<210> 389
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-54 VL

<400> 389
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Asn Ser Asn Ile Gly Ser Asp
            20   25   30
Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Arg Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Thr Thr Trp Asp Asp Ser Leu
                85   90   95
Asn Gly Leu Val Phe Gly Gly Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 390
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-60 VL

<400> 390
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
 1   5   10   15
Thr Ala Thr Ile Ala Cys Ser Gly Asp Asn Leu Glu Asn Lys Phe Val
            20   25   30
Tyr Trp Tyr His Gln Lys Pro Gly Gln Ser Pro Val Leu Val Met Tyr
        35   40   45
Glu Asp Asn Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Ala Leu Thr Ile Ser Gly Ala Gln Pro Met
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Thr Trp Asp Ser Pro Thr Gly Leu
                85   90   95
Phe Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105

<210> 391
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-63 VL

<400> 391
Glu Ile Val Leu Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1   5   10   15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20   25   30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35   40   45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50   55   60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65   70   75   80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
                85   90   95
Leu Gln Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105   110
Arg

<210> 392
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-66 VL

<400> 392
Gln Ala Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Ser Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Leu Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 393
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-68 VL

<400> 393
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1   5   10   15
Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20   25   30
Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Asp Asn Ile Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65   70   75   80
Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
                85   90   95
Ser Ala Gly Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105   110

<210> 394
<211> 109

<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-71 VL

<400> 394
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Val Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Met Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Ser Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105

<210> 395
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-76 VL

<400> 395
Gln Ser Val Val Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1   5   10   15
Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20   25   30
Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65   70   75   80
Thr Gly Asp Glu Ala Ala Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
                85   90   95
Ser Ala Gly Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 396
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-78 VL

<400> 396
Leu Pro Val Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
 1   5   10   15
Thr Ala Ser Ile Thr Cys Ser Gly Asp Lys Leu Gly Asp Lys Tyr Ala
            20   25   30
Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Ile Tyr
        35   40   45
Gln Asp Asn Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Met
65   70   75   80
Asp Ala Ala Asp Tyr Tyr Cys Gln Thr Trp Asp Arg Ser Thr Tyr Val
                85   90   95
Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105

<210> 397
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-80 VL

<400> 397
Gln Ala Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Tyr Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Asn Gly Trp Gly Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105   110

<210> 398
<211> 110
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3B-81 VL

<400> 398
Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp Leu
                85   90   95
Leu Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 399
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-82 VL

<400> 399
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
            20   25   30
Phe Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
        35   40   45
Leu Ile Tyr Asp Asn Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
    50   55   60
Ser Gly Ser Lys Ser Asp Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65   70   75   80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Phe Asp Ser Ser
                85   90   95
Leu Ser Gly Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105   110

<210> 400
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-85 VL

<400> 400
Gln Ala Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Asn Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100   105   110

<210> 401
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-87 VL

<400> 401
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1   5   10   15
Glu Pro Ala Ser Val Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20   25   30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35   40   45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50   55   60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65   70   75   80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
                85   90   95
Leu Gln Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105   110
Arg

<210> 402
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-92 VL

<400> 402
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
 1   5   10   15
Thr Ala Arg Ile Thr Cys Ser Gly Glu Thr Leu Ala Lys Arg Tyr Ala
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Leu Ile Tyr
        35   40   45
Arg Asp Thr Glu Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Ser Ser Gly Thr Thr Ile Thr Leu Thr Ile Thr Gly Val Gln Ala Glu
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Ala Asp Asn Ser Arg Thr Phe
                85   90   95
Val Phe Gly Pro Gly Thr Lys Val Thr Val Leu Gly
            100   105

<210> 403
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-93 VL

<400> 403
Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
 1   5   10   15
Thr Val Thr Ile Ser Cys Thr Gly Ser Ser Gly Ser Ile Ala Ser Asn
            20   25   30
Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Ser Ala Pro Thr Thr Val
        35   40   45
Ile Tyr Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65   70   75   80
Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser
                85   90   95
Ser Asn Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100   105   110

<210> 404
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-110 VL

<400> 404
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln

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 1               5                  10                  15
Arg Val Thr Val Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20                  25                  30
Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45
Leu Tyr Ser Ser Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Arg Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65                  70                  75                  80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85                  90                  95
Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100                 105                 110


<210> 405
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-113 VL

<400> 405
Ser Tyr Glu Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20                  25                  30
Ser Val Ser Trp Tyr Gln His Leu Pro Gly Val Ala Pro Lys Leu Leu
        35                  40                  45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Lys Thr Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65                  70                  75                  80
Ser Glu Asp Glu Gly Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85                  90                  95
Asn Gly Val Leu Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100                 105                 110


<210> 406
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-115 VL

<400> 406
Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1               5                  10                  15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
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            20                  25                  30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45
Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp Pro
                85                  90                  95
Leu Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100                 105                 110


<210> 407
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-119 VL

<400> 407
Gln Ser Val Val Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1               5                  10                  15
Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20                  25                  30
Tyr Val Ser Trp Tyr Gln Gln Ile Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45
Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Arg Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65                  70                  75                  80
Thr Gly Asp Glu Ala His Tyr Tyr Cys Ala Thr Trp Asp Asn Ser Leu
                85                  90                  95
Ser Ala Leu Ile Phe Gly Gly Gly Thr Lys Val Thr Val Leu Gly
            100                 105                 110


<210> 408
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-125 VL

<400> 408
Gln Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
 1               5                  10                  15
Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
            20                  25                  30
Asn Tyr Val Ser Trp Phe Gln Gln His Pro Gly Lys Ala Pro Lys Leu
```

```
        35                  40                  45
Ile Ile Tyr Glu Val Ser Asn Arg Pro Ser Gly Val Ser Asp Arg Phe
    50                  55                  60
Ser Gly Ser Lys Ser Gly Ser Thr Ala Ser Leu Thr Ile Ser Gly Leu
65                  70                  75                  80
Gln Ala Glu Asp Glu Ala Asn Tyr Tyr Cys Ser Ser Tyr Thr Ser Ser
                85                  90                  95
Thr Thr Val Ile Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100                 105                 110


<210> 409
<211> 248
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-034 scFv

<400> 409
Gln Pro Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20                  25                  30
Asn Val Ile Trp Tyr Gln Gln Leu Pro Gly Ala Ala Pro Lys Leu Leu
        35                  40                  45
Ile Tyr Ser Asn His Arg Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Arg Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65                  70                  75                  80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85                  90                  95
Asp Gly Tyr Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser
            100                 105                 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125
Leu Glu Met Ala Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu
    130                 135                 140
Lys Pro Ser Glu Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser
145                 150                 155                 160
Phe Ser Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly
                165                 170                 175
Leu Glu Trp Ile Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn
            180                 185                 190
Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn
        195                 200                 205
Gln Phe Ser Leu Glu Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
    210                 215                 220
Tyr Tyr Cys Ala Arg Gly Tyr Gly Gly Arg Phe Asp Tyr Trp Gly Gln
225                 230                 235                 240
Gly Thr Leu Val Thr Val Ser Ser
                245
```

<210> 410
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-035 scFv

<400> 410
Gln Ala Val Leu Thr Gln Pro Pro Ser Ala Ser Gln Thr Pro Gly Gln
 1   5   10   15
Met Val Thr Ile Ser Cys Ser Gly Thr Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Tyr Val Phe Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Lys Asn Phe Gln Arg Pro Ser Gly Val Pro Gly Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ala Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Phe Cys Ala Ala Trp Asp Asp Ala Leu
                85   90   95
Ser Gly Tyr Val Phe Gly Ala Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130   135   140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145   150   155   160
Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr
            180   185   190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile
        195   200   205
Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Ser Trp Thr Ser Gly Phe Asp Tyr Trp Gly
225   230   235   240
Gln Gly Thr Leu Val Thr Val Ser Ser
                245

<210> 411
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-037 scFv

<400> 411
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Thr Arg Ser Asn Ile Gly Ser Asp
            20   25   30
Tyr Val Ser Trp Tyr Gln His Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Val Tyr Gly Asp Asn Leu Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50   55   60
Ala Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65   70   75   80
Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Tyr Thr Leu
                85   90   95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
    130   135   140
Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145   150   155   160
Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
                165   170   175
Leu Glu Trp Val Ser Val Ile Tyr Ser Gly Gly Ser Ser Thr Tyr Tyr
            180   185   190
Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
        195   200   205
Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Thr Ser Tyr Leu Asn His Gly Asp Tyr Trp
225   230   235   240
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250

<210> 412
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-038 scFv

<400> 412
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser

    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser Arg Gly
            100   105   110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
        115   120   125
Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
    130   135   140
Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
145   150   155   160
Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
                165   170   175
Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln
            180   185   190
Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr
        195   200   205
Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr
    210   215   220
Tyr Cys Ala Arg Lys Val Thr Gly Tyr Asp Ser Trp Gly Gln Gly Thr
225   230   235   240
Leu Val Thr Val Ser Ser
                245

<210> 413
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-039 scFv

<400> 413
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser Arg Gly
            100   105   110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
        115   120   125

Met Ala Gln Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val His Pro
    130   135   140
Gly Gly Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser
145   150   155   160
Ser Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
                165   170   175
Trp Val Ser Ala Ile Gly Thr Gly Gly Gly Thr Tyr Tyr Ala Asp Ser
            180   185   190
Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu
        195   200   205
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
    210   215   220
Cys Ala Arg Tyr Gly Arg Lys Ser Ile Asp Ala Trp Gly Gln Gly Thr
225   230   235   240
Leu Val Thr Val Ser Ser
                245

<210> 414
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-040 scFv

<400> 414
Leu Pro Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Ser Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130   135   140
Lys Pro Gly Ala Ser Val Thr Val Ser Cys Lys Ala Ser Gly Tyr Thr
145   150   155   160
Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr
            180   185   190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile

195 200 205

Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala
210 215 220

Val Tyr Tyr Cys Ala Arg Arg Gly Tyr Tyr Gly Tyr Asp Ser Trp Gly
225 230 235 240

Gln Gly Thr Leu Val Thr Val Ser Ser
245

<210> 415
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-041 scFv

<400> 415

Gln Ala Val Leu Thr Gln Pro Pro Ser Ala Ser Gln Thr Pro Gly Gln
1 5 10 15

Met Val Thr Ile Ser Cys Ser Gly Thr Ser Ser Asn Ile Gly Ser Asn
20 25 30

Tyr Val Phe Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Lys Asn Phe Gln Arg Pro Ser Gly Val Pro Gly Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ala Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Phe Cys Ala Ala Trp Asp Asp Ala Leu
85 90 95

Ser Gly Tyr Val Phe Gly Ala Gly Thr Lys Val Thr Val Leu Gly Ser
100 105 110

Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125

Leu Glu Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
130 135 140

Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145 150 155 160

Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly
165 170 175

Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr
180 185 190

Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile
195 200 205

Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala
210 215 220

Met Tyr Tyr Cys Ala Arg Ser Gly Lys Tyr Tyr Gly Asp Lys Trp Gly
225 230 235 240

Gln Gly Thr Leu Val Thr Val Ser Ser
245

<210> 416
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-042 scFv

<400> 416
Ser Tyr Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Phe Gly Ser Ser Ser Asp Ile Gly Ser Asn
            20   25   30
Ser Val Phe Trp Tyr Gln Gln Leu Pro Gly Ala Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Thr Gln Tyr Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Glu Tyr His Cys Ala Thr Trp Asp Asp Ser Leu
                85   90   95
Asn Gly Tyr Val Phe Gly Ser Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys
    130   135   140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser
145   150   155   160
Phe Thr Gly Tyr Tyr Val Tyr Trp Met Arg Gln Ala Pro Gly Lys Gly
                165   170   175
Leu Glu Trp Met Gly Trp Met Asn Pro Arg Ser Gly Gly Thr Asn Tyr
            180   185   190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile
        195   200   205
Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Thr Ser Asp Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Ser Ser Tyr Tyr Trp Ala Asp Ser Trp Gly
225   230   235   240
Gln Gly Thr Leu Val Thr Val Ser Ser
                245

<210> 417
<211> 256
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-043 scFv

<400> 417

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Thr Asn
            20                  25                  30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Ile
        35                  40                  45
Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Glu Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65                  70                  75                  80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Val Trp Asp Asp Ser Leu
                85                  90                  95
Ser Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100                 105                 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125
Leu Glu Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130                 135                 140
Lys Pro Gly Ala Thr Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr
145                 150                 155                 160
Phe Thr Asp Tyr Tyr Met His Trp Val Gln Gln Ala Pro Gly Lys Gly
                165                 170                 175
Leu Glu Trp Met Gly Leu Val Asp Pro Glu Asp Gly Glu Thr Ile Tyr
            180                 185                 190
Ala Glu Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr
        195                 200                 205
Asp Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
    210                 215                 220
Val Tyr Tyr Cys Ala Arg Glu Leu Arg Asp Val Ala Tyr Tyr Pro Trp
225                 230                 235                 240
Gly Val Glu Asp Phe Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245                 250                 255


<210> 418
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-044 scFv

<400> 418
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1               5                  10                  15
Thr Ala Arg Ile Thr Cys Gly Gly Asp Asn Ile Gly Tyr Lys Gly Val
            20                  25                  30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35                  40                  45
Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
```

65 70 75 80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
85 90 95
Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser Arg Gly
100 105 110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
115 120 125
Met Ala Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro
130 135 140
Ser Glu Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser
145 150 155 160
Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
165 170 175
Trp Ile Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser
180 185 190
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
195 200 205
Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
210 215 220
Cys Ala Arg Tyr Tyr Val Pro Tyr Leu Ser Asp Trp Gly Gln Gly Thr
225 230 235 240
Leu Val Thr Val Ser Ser
245

<210> 419
<211> 248
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-045 scFv

<400> 419
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Ile Ile Ser Cys Pro Gly Ser Thr Ser Asn Ile Gly Thr Asn
20 25 30
Thr Val Asn Trp Tyr Gln Gln Phe Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65 70 75 80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
85 90 95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
100 105 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125
Leu Glu Met Ala Gln Met Gln Leu Val Gln Ser Gly Gly Gly Leu Val
130 135 140

Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145 150 155 160
Phe Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly
165 170 175
Leu Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr
180 185 190
Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
195 200 205
Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
210 215 220
Val Tyr Tyr Cys Ala Arg Ala Ser Asp Leu Tyr Gly Asp Trp Gly Gln
225 230 235 240
Gly Thr Leu Val Thr Val Ser Ser
245

<210> 420
<211> 253
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-046 scFv

<400> 420
Gln Ala Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Phe Gly Ser Asn
20 25 30
Thr Val His Trp Tyr Gln Gln Val Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Phe Ser Asn Thr Gln Arg Pro Ser Glu Ile Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65 70 75 80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
85 90 95
Thr Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
100 105 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
130 135 140
Lys Pro Gly Ala Ser Val Thr Val Ser Cys Lys Ala Ser Gly Tyr Arg
145 150 155 160
Phe Ser Asn Tyr Gly Val Ser Trp Val Arg Gln Ala Pro Gly Gln Gly
165 170 175
Leu Glu Trp Met Gly Trp Ile Ser Gly Ser Asn Gly Asn Thr Asn Tyr
180 185 190
Ala Gln Lys Phe Leu Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr
195 200 205
Thr Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala

    210   215   220
Val Tyr Tyr Cys Ala Arg Gly Asn Arg Arg Tyr Tyr Ser Pro Ile Ile
225   230   235   240
Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250

<210> 421
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-047 scFv

<400> 421
Leu Pro Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Ala Ser Asn
            20   25   30
Asp Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Arg Leu
        35   40   45
Ile Tyr Lys Lys Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Ala Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Asn Leu
                85   90   95
Ser Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130   135   140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Pro Gly Tyr Thr
145   150   155   160
Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr
            180   185   190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile
        195   200   205
Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Ser Asp Tyr Gly Ser Leu Tyr Asp Lys Trp
225   230   235   240
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250

<210> 422
<211> 247

<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-048 scFv

<400> 422
Gln Pro Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Phe Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp Arg
                85   90   95
Gly Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser Arg Gly
            100   105   110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
        115   120   125
Met Ala Glu Val Gln Leu Val Glu Ser Gly Gly Gly Glu Val Gln Pro
    130   135   140
Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
145   150   155   160
Ser Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
                165   170   175
Trp Val Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp
            180   185   190
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
        195   200   205
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
    210   215   220
Tyr Cys Ala Arg Ser Ser Phe Val Ala Thr Asp Tyr Trp Gly Gln Gly
225   230   235   240
Thr Leu Val Thr Val Ser Ser
                245

<210> 423
<211> 253
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-049 scFv

<400> 423
Gln Ala Val Leu Thr Gln Pro Ser Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15

Arg Val Thr Ile Ser Cys Ser Gly Gly Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Ser Gly Trp Val Phe Gly Gly Arg Thr Lys Leu Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130   135   140
Glu Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145   150   155   160
Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr
            180   185   190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile
        195   200   205
Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg His Gly Gly Ile Gly Ser Met Arg Ser Phe
225   230   235   240
Asp Gln Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250

<210> 424
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-050 scFv

<400> 424
Gln Ala Val Leu Thr Gln Pro Ser Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu

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                85                  90                  95
Asn Gly Pro Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser
            100                 105                 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125
Leu Glu Met Ala Gln Ile Thr Leu Lys Glu Ser Gly Ala Glu Val Lys
    130                 135                 140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser
145                 150                 155                 160
Phe Thr Gly Tyr Tyr Val Tyr Trp Met Arg Gln Ala Pro Gly Lys Gly
                165                 170                 175
Leu Glu Trp Met Gly Trp Met Asn Pro Arg Ser Gly Gly Thr Asn Tyr
            180                 185                 190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile
        195                 200                 205
Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Thr Ser Asp Asp Thr Ala
    210                 215                 220
Thr Tyr Tyr Cys Ala Arg Ser Gly Tyr Arg Trp Leu Asp Val Trp Gly
225                 230                 235                 240
Gln Gly Thr Leu Val Thr Val Ser Ser
                245


<210> 425
<211> 254
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-051 scFv

<400> 425
Gln Ala Val Leu Thr Gln Pro Ser Ser Ala Ser Gly Ala Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Ile Ser Cys Thr Gly Gly Ser Ser Asn Ile Gly Ala Gly
            20                  25                  30
Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
        35                  40                  45
Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
    50                  55                  60
Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65                  70                  75                  80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
                85                  90                  95
Leu Ser Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
            100                 105                 110
Ser Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
        115                 120                 125
Ser Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val
    130                 135                 140
Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly
145                 150                 155                 160
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Ala Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln
                165   170   175
Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn
            180   185   190
Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser
        195   200   205
Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr
    210   215   220
Ala Val Tyr Tyr Cys Ala Arg Met Leu Tyr Leu Ser Gly Arg Tyr Tyr
225   230   235   240
Trp Asp Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250

<210> 426
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-052 scFv

<400> 426
Gln Ala Val Leu Thr Gln Pro Ser Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Tyr Val Tyr Trp Tyr Gln Gln Leu Ser Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Ser Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Glu Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys
    130   135   140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145   150   155   160
Phe Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr
            180   185   190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile
        195   200   205
Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Ser His Ser Ser Gly Tyr Asp Lys Trp Gly

225 230 235 240
Gln Gly Thr Leu Val Thr Val Ser Ser
245

<210> 427
<211> 251
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-053 scFv

<400> 427
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Ala Ala Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Thr Ser Ser Asn Ile Gly Ser Asn
20 25 30
Tyr Val Trp Trp Tyr Gln Gln Leu Pro Gly Ala Ala Pro Arg Leu Leu
35 40 45
Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
85 90 95
Ser Gly Ser Asn Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110
Ser Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125
Ser Leu Glu Met Ala Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu
130 135 140
Val Lys Pro Ser Glu Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly
145 150 155 160
Ser Ile Ser Ser Ser Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro
165 170 175
Gly Lys Gly Leu Glu Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr
180 185 190
Tyr Tyr Asn Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr
195 200 205
Ser Lys Asn Gln Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp
210 215 220
Thr Ala Val Tyr Tyr Cys Ala Arg Trp Trp Ser Gly Ser Tyr Asp Thr
225 230 235 240
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
245 250

<210> 428
<211> 251
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-054 scFv

<400> 428
Ser Tyr Glu Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Ser Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130   135   140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145   150   155   160
Phe Thr Ser Tyr Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr
            180   185   190
Ala Gln Lys Leu Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr
        195   200   205
Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Ile Pro Met Tyr Ser Gly Ser Ser Asp Tyr
225   230   235   240
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250

<210> 429
<211> 247
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-055 scFv

<400> 429
Gln Pro Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30

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His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35                  40                  45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95
Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly
            100                 105                 110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
        115                 120                 125
Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
    130                 135                 140
Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
145                 150                 155                 160
Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
                165                 170                 175
Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln
            180                 185                 190
Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr
        195                 200                 205
Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr
    210                 215                 220
Tyr Cys Ala Arg Trp His Gly Gly Pro Tyr Asp Tyr Trp Gly Gln Gly
225                 230                 235                 240
Thr Leu Val Thr Val Ser Ser
                245
```

<210> 430
<211> 251
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-056 scFv

<400> 430
```
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
            20                  25                  30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Ile
                85                  90                  95
Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Ser Arg Gly Gly
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            100                 105                 110
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu Met
        115                 120                 125
Ala Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys Lys Phe Gly
    130                 135                 140
Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Asn Asp
145                 150                 155                 160
Tyr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
                165                 170                 175
Met Gly Trp Ile Asn Pro Asn Asn Gly Asp Thr Lys Tyr Glu Lys Lys
            180                 185                 190
Trp Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Thr Thr Ala
        195                 200                 205
Tyr Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr
    210                 215                 220
Cys Ala Arg Phe Ser Thr His Asn Trp Trp Trp Pro Thr Tyr Asp Tyr
225                 230                 235                 240
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245                 250


<210> 431
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-057 scFv

<400> 431
Glu Ile Val Leu Thr Gln Ser Pro Ser Val Ser Val Ala Pro Gly Lys
 1               5                  10                  15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35                  40                  45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95
Val Val Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Ser Arg Gly
            100                 105                 110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
        115                 120                 125
Met Ala Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Ile Gln Pro
    130                 135                 140
Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
145                 150                 155                 160
Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
                165                 170                 175
```

Trp Val Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp
            180   185   190
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
        195   200   205
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
    210   215   220
Tyr Cys Ala Arg Tyr Asn Tyr Met Ser Ser Gly Phe Tyr Asp Arg Trp
225   230   235   240
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250

<210> 432
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-058 scFv

<400> 432
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35   40   45
Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly Gly
            100   105   110
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu Met
        115   120   125
Ala Gln Val Gln Leu Val Gln Ser Gly Ala Asp Val Arg Lys Pro Gly
    130   135   140
Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Ser
145   150   155   160
His Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
                165   170   175
Leu Gly Trp Ile Ser Pro Tyr Thr Gly Asn Thr Asn Tyr Ala Gln Lys
            180   185   190
Phe Gln Gly Arg Val Thr Met Ala Thr Asp Thr Ser Thr Ser Thr Ala
        195   200   205
Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Ile Tyr Tyr
    210   215   220
Cys Ala Arg Gly Lys Arg Thr Leu Ala Ser Cys Phe Asp Tyr Trp Gly
225   230   235   240
Gln Gly Thr Leu Val Thr Val Ser Ser

245

<210> 433
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-059 scFv

<400> 433
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly Gly
            100   105   110
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu Met
        115   120   125
Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
    130   135   140
Ala Ser Val Thr Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg
145   150   155   160
Tyr Gly Ile Thr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
                165   170   175
Met Gly Trp Ile Ser Ala Tyr Ser Asp Lys Thr Asn Tyr Ala Gln Lys
            180   185   190
Leu Gln Gly Arg Val Thr Met Thr Thr Asp Ile Ser Thr Asn Thr Ala
        195   200   205
Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr
    210   215   220
Cys Ala Arg Ser Arg Trp Ser Tyr Met Asp Val Trp Gly Gln Gly Thr
225   230   235   240
Leu Val Thr Val Ser Ser
                245

<210> 434
<211> 247
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3A-060 scFv

<400> 434
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Leu Thr Cys Gly Gly Asn Asn Ile Gly Ser Glu Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Leu Leu Val Val Tyr
        35   40   45
Asp Asp Asp Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Glu Asp Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Leu
65   70   75   80
Asp Glu Ala Glu Tyr Tyr Cys Gln Thr Trp Asp Ser Ser Thr Ala Ile
                85   90   95
Phe Gly Thr Gly Thr Lys Leu Thr Val Leu Gly Ser Arg Gly Gly Gly
            100   105   110
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu Met Ala
        115   120   125
Gln Val Gln Leu Val Gln Ser Gly Gly Glu Val Lys Lys Pro Gly Ala
    130   135   140
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Asn Ser Tyr
145   150   155   160
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
                165   170   175
Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
            180   185   190
Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Asn Thr Ala Phe
        195   200   205
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
    210   215   220
Ala Arg Glu Gly Tyr Gly Ser Trp Ala Met Asp Gln Trp Gly Gln Gly
225   230   235   240
Thr Leu Val Thr Val Ser Ser
                245

<210> 435
<211> 247
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-061 scFv

<400> 435
Gln Pro Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45

Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly
            100   105   110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
        115   120   125
Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
    130   135   140
Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
145   150   155   160
Ser Tyr Gly Ile Ser Trp Val Arg Gln Thr Pro Gly Gln Gly Leu Glu
                165   170   175
Trp Met Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln
            180   185   190
Lys Leu Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr
        195   200   205
Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr
    210   215   220
Tyr Cys Ala Arg Lys Gly Ser Ser Gln Phe Asp Gln Trp Gly Gln Gly
225   230   235   240
Thr Leu Val Thr Val Ser Ser
                245

<210> 436
<211> 257
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-062 scFv

<400> 436
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
            20   25   30
Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
        35   40   45
Leu Ile Tyr Gly Asn Ser Asn Trp Pro Ser Gly Val Pro Asp Arg Phe
    50   55   60
Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65   70   75   80
Arg Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
                85   90   95
Leu Ser Gly Ser Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu
            100   105   110
Gly Ser Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly

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        115                 120                 125
Gly Ser Leu Glu Met Ala Gln Met Gln Leu Val Gln Ser Gly Ser Glu
    130                 135                 140
Leu Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
145                 150                 155                 160
Gly Thr Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly
                165                 170                 175
Gln Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Lys Ile Gly Thr Ala
            180                 185                 190
Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Glu
        195                 200                 205
Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp
    210                 215                 220
Thr Ala Met Tyr Tyr Cys Ala Arg Met Tyr Met Asp Met Gly Trp Gly
225                 230                 235                 240
Trp Gly Tyr Trp Asp Trp Trp Gly Gln Gly Thr Leu Val Thr Val Ser
                245                 250                 255
Ser
```

<210> 437
<211> 251
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-063 scFv

<400> 437

```
Gln Thr Val Val Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Ile Ser Cys Ser Gly Ser Trp Ser Asn Ile Gly Ser Tyr
            20                  25                  30
Thr Val Asn Trp Tyr Gln His Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45
Ile Ser Gly Asn Asn Gln Arg Pro Ser Gly Val Pro Gly Arg Phe Ser
    50                  55                  60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65                  70                  75                  80
Ser Glu Asp Glu Ala Asp Tyr His Cys Ala Ala Trp Asp Asp Asn Leu
                85                  90                  95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100                 105                 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130                 135                 140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145                 150                 155                 160
Phe Thr Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly
                165                 170                 175
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Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Ala Ser Tyr
            180   185   190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr
        195   200   205
Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Asp Arg Leu Ala Ser Asp Ala Phe Asp Ile
225   230   235   240
Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
                245   250

<210> 438
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-064 scFv

<400> 438
Leu Pro Val Leu Thr Gln Pro Pro Ser Leu Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Leu Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr His Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35   40   45
Asp Asp Thr Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Ala Leu Thr Ile Ser Arg Val Glu Val Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Arg Ser Ser Ala His
                85   90   95
Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser Arg Gly
            100   105   110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
        115   120   125
Met Ala Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
    130   135   140
Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
145   150   155   160
Ile Tyr Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
                165   170   175
Trp Met Gly Trp Ile Ser Pro Tyr Asn Asp Asn Thr Ile Tyr Ala Gln
            180   185   190
Lys Val Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr
        195   200   205
Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr
    210   215   220
Tyr Cys Ala Arg Met Gly Val Gly Trp Gly Tyr Ala Gln Asp Ser Trp
225   230   235   240
Gly Gln Gly Thr Leu Val Thr Val Ser Ser

245 250

<210> 439
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-067 scFv

<400> 439
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
1 5 10 15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65 70 75 80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
85 90 95
Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser Arg Gly
100 105 110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
115 120 125
Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
130 135 140
Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser
145 150 155 160
Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
165 170 175
Trp Met Gly Arg Ile Ile Pro Ile Phe Gly Ile Thr Asn Tyr Ala Gln
180 185 190
Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr
195 200 205
Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr
210 215 220
Tyr Cys Ala Arg Gly Ala Glu Met Ser Asp Tyr Trp Gly Gln Gly Thr
225 230 235 240
Leu Val Thr Val Ser Ser
245

<210> 440
<211> 252
<212> PRT
<213> Artificial Sequence

<220>

<223> GPC3B-54 scFv

<400> 440
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Asn Ser Asn Ile Gly Ser Asp
            20   25   30
Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Arg Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Thr Thr Trp Asp Asp Ser Leu
                85   90   95
Asn Gly Leu Val Phe Gly Gly Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130   135   140
Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Thr
145   150   155   160
Phe Ile Asp Tyr Tyr Val Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr
            180   185   190
Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr
        195   200   205
Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Gly Ser Glu Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Glu Arg Arg Tyr Ser Ser Ser Pro Ser Asp
225   230   235   240
His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250

<210> 441
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-60 scFv

<400> 441
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
 1   5   10   15
Thr Ala Thr Ile Ala Cys Ser Gly Asp Asn Leu Glu Asn Lys Phe Val
            20   25   30
Tyr Trp Tyr His Gln Lys Pro Gly Gln Ser Pro Val Leu Val Met Tyr
        35   40   45

Glu Asp Asn Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Ala Leu Thr Ile Ser Gly Ala Gln Pro Met
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Thr Trp Asp Ser Pro Thr Gly Leu
                85   90   95
Phe Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly
            100   105   110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
        115   120   125
Met Ala Glu Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro
    130   135   140
Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
145   150   155   160
Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
                165   170   175
Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln
            180   185   190
Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr
        195   200   205
Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr
    210   215   220
Tyr Cys Ala Arg Ser Leu Tyr Ser Gln Pro Tyr Ile Asp Gly Trp Ser
225   230   235   240
Gln Gly Thr Leu Val Thr Val Ser Ser
                245

<210> 442
<211> 256
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-63 scFv

<400> 442
Glu Ile Val Leu Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1   5   10   15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20   25   30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35   40   45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50   55   60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65   70   75   80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
                85   90   95
Leu Gln Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105   110
Arg Ser Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly

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        115                 120                 125
Gly Ser Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu
    130                 135                 140
Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
145                 150                 155                 160
Tyr Thr Phe Thr Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly
                165                 170                 175
Gln Gly Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr
            180                 185                 190
Ser Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
        195                 200                 205
Ser Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp
    210                 215                 220
Thr Ala Val Tyr Tyr Cys Ala Arg Ser Leu His Ala Met Arg Trp Ser
225                 230                 235                 240
Gln Thr Met Asp Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245                 250                 255


<210> 443
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-66 scFv

<400> 443
Gln Ala Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1               5                  10                  15
Thr Ala Ser Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35                  40                  45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95
Leu Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg
            100                 105                 110
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu
        115                 120                 125
Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Arg
    130                 135                 140
Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
145                 150                 155                 160
Ile Gly Gln Tyr Leu His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
                165                 170                 175
Glu Trp Met Gly Arg Ile Asn Pro Met Thr Gly Val Thr Asn Tyr Ala
            180                 185                 190
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Pro Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser
        195   200   205
Thr Gly Tyr Met Glu Ile Ser Arg Leu Arg Ser Asp Asp Thr Ala Val
    210   215   220
Tyr Tyr Cys Ala Arg Phe Ser Ser Gly Tyr Ser Arg Asp Thr Trp Gly
225   230   235   240
Gln Gly Thr Leu Val Thr Val Ser Ser
                245

<210> 444
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-68 scFv

<400> 444
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1   5   10   15
Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20   25   30
Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Asp Asn Ile Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65   70   75   80
Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
                85   90   95
Ser Ala Gly Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130   135   140
Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr
145   150   155   160
Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr
            180   185   190
Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr
        195   200   205
Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Tyr Gly Tyr Glu Gly His Asp Thr Trp Gly
225   230   235   240
Gln Gly Thr Leu Val Thr Val Ser Ser
                245

<210> 445
<211> 250
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-71 scFv

<400> 445
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Val Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Met Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Ser Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly
            100   105   110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
        115   120   125
Met Ala Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro
    130   135   140
Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
145   150   155   160
Arg Tyr Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
                165   170   175
Trp Val Ser Ser Ile Ser Ser Ser Gly Ser Tyr Ile Tyr Tyr Ala Asp
            180   185   190
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser
        195   200   205
Leu Phe Leu Gln Met Asp Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
    210   215   220
Tyr Cys Ala Arg Gln Gly His Met Trp Tyr Val Pro Val Asp Ala Trp
225   230   235   240
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250

<210> 446
<211> 244
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-76 scFv

<400> 446
Gln Ser Val Val Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1   5   10   15
Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20   25   30
Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65   70   75   80
Thr Gly Asp Glu Ala Ala Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
                85   90   95
Ser Ala Gly Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130   135   140
Glu Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145   150   155   160
Phe Thr Asp Tyr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr
            180   185   190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile
        195   200   205
Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Asn Tyr Asp Trp Gly Gln Gly Thr Leu Val
225   230   235   240
Thr Val Ser Ser

<210> 447
<211> 245
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-78 scFv

<400> 447
Leu Pro Val Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
 1   5   10   15
Thr Ala Ser Ile Thr Cys Ser Gly Asp Lys Leu Gly Asp Lys Tyr Ala
            20   25   30
Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Ile Tyr
        35   40   45
Gln Asp Asn Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60

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Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Met
65                  70                  75                  80
Asp Ala Ala Asp Tyr Tyr Cys Gln Thr Trp Asp Arg Ser Thr Tyr Val
                85                  90                  95
Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly Gly Gly
            100                 105                 110
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu Met Ala
        115                 120                 125
Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
    130                 135                 140
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
145                 150                 155                 160
Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
                165                 170                 175
Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
            180                 185                 190
Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
        195                 200                 205
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
    210                 215                 220
Ala Arg Gly Tyr Ser Ser Phe Phe Asp Ser Trp Gly Gln Gly Thr Leu
225                 230                 235                 240
Val Thr Val Ser Ser
                245


<210> 448
<211> 255
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-80 scFv

<400> 448
Gln Ala Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20                  25                  30
Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Lys Ser Gly Thr Ser Ala Tyr Leu Ala Ile Ser Gly Leu Gln
65                  70                  75                  80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85                  90                  95
Asn Gly Trp Gly Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100                 105                 110
Ser Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
        115                 120                 125
Ser Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val
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    130   135   140
Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly
145   150   155   160
Thr Leu Ser Arg Phe Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln
                165   170   175
Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Arg Thr Ala Asn
            180   185   190
Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser
        195   200   205
Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr
    210   215   220
Ala Val Tyr Tyr Cys Ala Arg Met Ser Lys Tyr Tyr Gly Ser Tyr Ser
225   230   235   240
Ser Tyr Asp Glu Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250   255

<210> 449
<211> 245
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-81 scFv

<400> 449
Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp Leu
                85   90   95
Leu Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg
            100   105   110
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu
        115   120   125
Glu Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
    130   135   140
Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
145   150   155   160
Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
                165   170   175
Glu Trp Met Gly Arg Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala
            180   185   190
Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser
        195   200   205

Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val
210 215 220
Tyr Tyr Cys Ala Arg Gly Leu Trp Asp Ser Trp Gly Gln Gly Thr Leu
225 230 235 240
Val Thr Val Ser Ser
245

<210> 450
<211> 254
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-82 scFv

<400> 450
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30
Phe Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45
Leu Ile Tyr Asp Asn Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60
Ser Gly Ser Lys Ser Asp Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65 70 75 80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Phe Asp Ser Ser
85 90 95
Leu Ser Gly Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110
Ser Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125
Ser Leu Glu Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val
130 135 140
Lys Lys Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr
145 150 155 160
Ser Phe Thr Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln
165 170 175
Gly Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser
180 185 190
Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
195 200 205
Thr Ser Thr Val Tyr Met Glu Met Ser Ser Leu Arg Ser Glu Asp Thr
210 215 220
Ala Val Tyr Tyr Cys Ala Arg Tyr Pro Val Tyr Met Glu Thr Ser Asp
225 230 235 240
Phe Asp Ser Trp Gly Ser Arg Tyr Ser Gly Asp Arg Leu Leu
245 250

<210> 451

<211> 257
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-85 scFv

<400> 451
Gln Ala Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Asn Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys
    130   135   140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145   150   155   160
Phe Thr Ser Tyr Ala Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Asn Pro Thr Tyr
            180   185   190
Ala Gln Gly Phe Thr Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val
        195   200   205
Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Ser Ser Leu Tyr Trp Met Gly Ser Lys Trp
225   230   235   240
Ser Arg Gln Thr Asp Met Trp Gly Gln Gly Thr Leu Val Thr Val Ser
                245   250   255
Ser

<210> 452
<211> 247
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-87 scFv

<400> 452
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1   5   10   15
Glu Pro Ala Ser Val Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20   25   30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35   40   45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50   55   60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65   70   75   80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
                85   90   95
Leu Gln Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105   110
Arg Ser Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        115   120   125
Gly Ser Leu Glu Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu
    130   135   140
Val Arg Lys Pro Gly Ser Ser Val Lys Val Ser Cys Gln Ala Ser Gly
145   150   155   160
Gly Thr Phe Gly Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly
                165   170   175
Gln Gly Leu Glu Trp Met Gly Arg Ile Ile Pro Val Leu Gly Arg Thr
            180   185   190
Lys Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Val Thr Ala Asp Thr
        195   200   205
Ser Thr Ser Thr Val Tyr Met Glu Leu Thr Ser Leu Thr Ser Glu Asp
    210   215   220
Thr Ala Val Tyr Tyr Cys Ala Arg Thr Asn Asp Ser Trp Gly Gln Gly
225   230   235   240
Thr Leu Val Thr Val Ser Ser
                245

<210> 453
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-92 scFv

<400> 453
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
 1   5   10   15
Thr Ala Arg Ile Thr Cys Ser Gly Glu Thr Leu Ala Lys Arg Tyr Ala
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Leu Ile Tyr
        35   40   45
Arg Asp Thr Glu Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60

Ser Ser Gly Thr Thr Ile Thr Leu Thr Ile Thr Gly Val Gln Ala Glu
65 70 75 80
Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Ala Asp Asn Ser Arg Thr Phe
85 90 95
Val Phe Gly Pro Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly Gly
100 105 110
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu Met
115 120 125
Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
130 135 140
Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Asn
145 150 155 160
Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
165 170 175
Met Gly Ile Ile Asn Pro Ser Gly Gly Thr Thr Thr Tyr Ala Gln Lys
180 185 190
Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val
195 200 205
Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr
210 215 220
Cys Ala Arg Pro Ser Met Trp Thr Ser Ser Met Gly Asp Val Trp Gly
225 230 235 240
Gln Gly Thr Leu Val Thr Val Ser Ser
245

<210> 454
<211> 255
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-93 scFv

<400> 454
Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15
Thr Val Thr Ile Ser Cys Thr Gly Ser Ser Gly Ser Ile Ala Ser Asn
20 25 30
Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Ser Ala Pro Thr Thr Val
35 40 45
Ile Tyr Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80
Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser
85 90 95
Ser Asn Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
100 105 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125
Leu Glu Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys

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    130                 135                 140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145                 150                 155                 160
Phe Thr Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly
                165                 170                 175
Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Arg Tyr
            180                 185                 190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr
        195                 200                 205
Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
    210                 215                 220
Val Tyr Tyr Cys Ala Arg Tyr Thr Ala Leu Lys Pro Arg Gly Ile Tyr
225                 230                 235                 240
Ser Val Asp Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245                 250                 255


<210> 455
<211> 257
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-110 scFv

<400> 455
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Val Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20                  25                  30
Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45
Leu Tyr Ser Ser Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Arg Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65                  70                  75                  80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85                  90                  95
Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100                 105                 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125
Leu Glu Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130                 135                 140
Lys Pro Gly Ser Ser Val Lys Val Ser Cys Gln Ala Ser Gly Gly Thr
145                 150                 155                 160
Phe Thr Thr Tyr Ser Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly
                165                 170                 175
Leu Glu Trp Met Gly Gly Ile Ile Pro Thr Phe Gly Thr Thr Asn Tyr
            180                 185                 190
Ala Gln Asn Phe Gln Asp Arg Val Thr Ile Ser Ala Asp Glu Ser Thr
        195                 200                 205
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Asn Thr Ala Tyr Met Glu Leu Thr Ser Leu Arg Ser Glu Asp Thr Ala
210 215 220
Val Tyr Tyr Cys Ala Arg Tyr Tyr Trp Arg Gly Gly Ser Gly Gln Gly
225 230 235 240
Ser Val Thr Ser Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
245 250 255
Ser

<210> 456
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-113 scFv

<400> 456
Ser Tyr Glu Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30
Ser Val Ser Trp Tyr Gln His Leu Pro Gly Val Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Thr Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65 70 75 80
Ser Glu Asp Glu Gly Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
85 90 95
Asn Gly Val Leu Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
100 105 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
130 135 140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr
145 150 155 160
Leu Thr Glu Leu Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
165 170 175
Leu Glu Trp Met Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr
180 185 190
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr
195 200 205
Asp Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
210 215 220
Val Tyr Tyr Cys Ala Arg Tyr Ser Gly Asp Tyr Trp Gly Gln Gly Thr
225 230 235 240
Leu Val Thr Val Ser Ser
245

<210> 457
<211> 251
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-115 scFv

<400> 457
Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35   40   45
Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp Pro
                85   90   95
Leu Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg
            100   105   110
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu
        115   120   125
Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Arg
    130   135   140
Pro Gly Ala Thr Ile Lys Val Ser Cys Lys Thr Ser Gly Tyr Thr Phe
145   150   155   160
Thr Ala Tyr Tyr Thr His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
                165   170   175
Glu Trp Val Gly Arg Ile Asn Ala Asn Thr Gly Gly Thr Asp Tyr Ala
            180   185   190
Pro Lys Phe Arg Asp Arg Val Ile Met Thr Arg Asp Thr Ser Ile Ser
        195   200   205
Thr Ala Tyr Met Glu Leu Gly Arg Leu Thr Ser Glu Asp Thr Ala Val
    210   215   220
Tyr Tyr Cys Ala Arg Ile Ser Gly Tyr His Ser Ser Gly Trp Asp Tyr
225   230   235   240
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245   250

<210> 458
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-119 scFv

<400> 458
Gln Ser Val Val Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1   5   10   15
Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20   25   30
Tyr Val Ser Trp Tyr Gln Gln Ile Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Arg Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65   70   75   80
Thr Gly Asp Glu Ala His Tyr Tyr Cys Ala Thr Trp Asp Asn Ser Leu
                85   90   95
Ser Ala Leu Ile Phe Gly Gly Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys
    130   135   140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145   150   155   160
Phe Thr Ser Tyr Ala Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Asn Pro Thr Tyr
            180   185   190
Ala Gln Gly Phe Thr Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val
        195   200   205
Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Gly Tyr Tyr Gly Lys Tyr Asp Lys Trp Gly
225   230   235   240
Gln Gly Thr Leu Val Thr Val Ser Ser
                245

<210> 459
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3B-125 scFv

<400> 459
Gln Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
 1   5   10   15
Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
            20   25   30
Asn Tyr Val Ser Trp Phe Gln Gln His Pro Gly Lys Ala Pro Lys Leu
        35   40   45
Ile Ile Tyr Glu Val Ser Asn Arg Pro Ser Gly Val Ser Asp Arg Phe
    50   55   60

Ser Gly Ser Lys Ser Gly Ser Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80
Gln Ala Glu Asp Glu Ala Asn Tyr Tyr Cys Ser Ser Tyr Thr Ser Ser
85 90 95
Thr Thr Val Ile Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
100 105 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125
Leu Glu Met Ala Gln Met Gln Leu Val Gln Ser Gly Ser Glu Leu Lys
130 135 140
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
145 150 155 160
Phe Thr Ser Tyr Ala Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly
165 170 175
Leu Glu Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Asn Pro Thr Tyr
180 185 190
Ala Gln Gly Phe Thr Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val
195 200 205
Ser Thr Ala Tyr Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala
210 215 220
Val Tyr Tyr Cys Ala Arg Gln Ser His Asp Glu Trp Gly Gln Gly Thr
225 230 235 240
Leu Val Thr Val Ser Ser
245

<210> 460
<211> 580
<212> PRT
<213> Artificial Sequence

<220>
<223> P51654 human GPC3 full-length (UNIPROT)

<400> 460
Met Ala Gly Thr Val Arg Thr Ala Cys Leu Val Val Ala Met Leu Leu
1 5 10 15
Ser Leu Asp Phe Pro Gly Gln Ala Gln Pro Pro Pro Pro Pro Pro Asp
20 25 30
Ala Thr Cys His Gln Val Arg Ser Phe Phe Gln Arg Leu Gln Pro Gly
35 40 45
Leu Lys Trp Val Pro Glu Thr Pro Val Pro Gly Ser Asp Leu Gln Val
50 55 60
Cys Leu Pro Lys Gly Pro Thr Cys Cys Ser Arg Lys Met Glu Glu Lys
65 70 75 80
Tyr Gln Leu Thr Ala Arg Leu Asn Met Glu Gln Leu Leu Gln Ser Ala
85 90 95
Ser Met Glu Leu Lys Phe Leu Ile Ile Gln Asn Ala Ala Val Phe Gln
100 105 110
Glu Ala Phe Glu Ile Val Val Arg His Ala Lys Asn Tyr Thr Asn Ala
115 120 125
Met Phe Lys Asn Asn Tyr Pro Ser Leu Thr Pro Gln Ala Phe Glu Phe

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    130                 135                 140
Val Gly Glu Phe Phe Thr Asp Val Ser Leu Tyr Ile Leu Gly Ser Asp
145                 150                 155                 160
Ile Asn Val Asp Asp Met Val Asn Glu Leu Phe Asp Ser Leu Phe Pro
                165                 170                 175
Val Ile Tyr Thr Gln Leu Met Asn Pro Gly Leu Pro Asp Ser Ala Leu
            180                 185                 190
Asp Ile Asn Glu Cys Leu Arg Gly Ala Arg Arg Asp Leu Lys Val Phe
        195                 200                 205
Gly Asn Phe Pro Lys Leu Ile Met Thr Gln Val Ser Lys Ser Leu Gln
    210                 215                 220
Val Thr Arg Ile Phe Leu Gln Ala Leu Asn Leu Gly Ile Glu Val Ile
225                 230                 235                 240
Asn Thr Thr Asp His Leu Lys Phe Ser Lys Asp Cys Gly Arg Met Leu
                245                 250                 255
Thr Arg Met Trp Tyr Cys Ser Tyr Cys Gln Gly Leu Met Met Val Lys
            260                 265                 270
Pro Cys Gly Gly Tyr Cys Asn Val Val Met Gln Gly Cys Met Ala Gly
        275                 280                 285
Val Val Glu Ile Asp Lys Tyr Trp Arg Glu Tyr Ile Leu Ser Leu Glu
    290                 295                 300
Glu Leu Val Asn Gly Met Tyr Arg Ile Tyr Asp Met Glu Asn Val Leu
305                 310                 315                 320
Leu Gly Leu Phe Ser Thr Ile His Asp Ser Ile Gln Tyr Val Gln Lys
                325                 330                 335
Asn Ala Gly Lys Leu Thr Thr Thr Ile Gly Lys Leu Cys Ala His Ser
            340                 345                 350
Gln Gln Arg Gln Tyr Arg Ser Ala Tyr Tyr Pro Glu Asp Leu Phe Ile
        355                 360                 365
Asp Lys Lys Val Leu Lys Val Ala His Val Glu His Glu Glu Thr Leu
    370                 375                 380
Ser Ser Arg Arg Arg Glu Leu Ile Gln Lys Leu Lys Ser Phe Ile Ser
385                 390                 395                 400
Phe Tyr Ser Ala Leu Pro Gly Tyr Ile Cys Ser His Ser Pro Val Ala
                405                 410                 415
Glu Asn Asp Thr Leu Cys Trp Asn Gly Gln Glu Leu Val Glu Arg Tyr
            420                 425                 430
Ser Gln Lys Ala Ala Arg Asn Gly Met Lys Asn Gln Phe Asn Leu His
        435                 440                 445
Glu Leu Lys Met Lys Gly Pro Glu Pro Val Val Ser Gln Ile Ile Asp
    450                 455                 460
Lys Leu Lys His Ile Asn Gln Leu Leu Arg Thr Met Ser Met Pro Lys
465                 470                 475                 480
Gly Arg Val Leu Asp Lys Asn Leu Asp Glu Glu Gly Phe Glu Ser Gly
                485                 490                 495
Asp Cys Gly Asp Asp Glu Asp Glu Cys Ile Gly Gly Ser Gly Asp Gly
            500                 505                 510
Met Ile Lys Val Lys Asn Gln Leu Arg Phe Leu Ala Glu Leu Ala Tyr
        515                 520                 525
Asp Leu Asp Val Asp Asp Ala Pro Gly Asn Ser Gln Gln Ala Thr Pro
    530                 535                 540
Lys Asp Asn Glu Ile Ser Thr Phe His Asn Leu Gly Asn Val His Ser
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545 550 555 560
Pro Leu Lys Leu Leu Thr Ser Met Ala Ile Ser Val Val Cys Phe Phe
565 570 575
Phe Leu Val His
580

<210> 461
<211> 560
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acids 1-560 of SEQ ID NO: 460
human GPC3 full-length (UNIPROT)

<400> 461
Met Ala Gly Thr Val Arg Thr Ala Cys Leu Val Val Ala Met Leu Leu
1 5 10 15
Ser Leu Asp Phe Pro Gly Gln Ala Gln Pro Pro Pro Pro Pro Pro Asp
20 25 30
Ala Thr Cys His Gln Val Arg Ser Phe Phe Gln Arg Leu Gln Pro Gly
35 40 45
Leu Lys Trp Val Pro Glu Thr Pro Val Pro Gly Ser Asp Leu Gln Val
50 55 60
Cys Leu Pro Lys Gly Pro Thr Cys Cys Ser Arg Lys Met Glu Glu Lys
65 70 75 80
Tyr Gln Leu Thr Ala Arg Leu Asn Met Glu Gln Leu Leu Gln Ser Ala
85 90 95
Ser Met Glu Leu Lys Phe Leu Ile Ile Gln Asn Ala Ala Val Phe Gln
100 105 110
Glu Ala Phe Glu Ile Val Val Arg His Ala Lys Asn Tyr Thr Asn Ala
115 120 125
Met Phe Lys Asn Asn Tyr Pro Ser Leu Thr Pro Gln Ala Phe Glu Phe
130 135 140
Val Gly Glu Phe Phe Thr Asp Val Ser Leu Tyr Ile Leu Gly Ser Asp
145 150 155 160
Ile Asn Val Asp Asp Met Val Asn Glu Leu Phe Asp Ser Leu Phe Pro
165 170 175
Val Ile Tyr Thr Gln Leu Met Asn Pro Gly Leu Pro Asp Ser Ala Leu
180 185 190
Asp Ile Asn Glu Cys Leu Arg Gly Ala Arg Arg Asp Leu Lys Val Phe
195 200 205
Gly Asn Phe Pro Lys Leu Ile Met Thr Gln Val Ser Lys Ser Leu Gln
210 215 220
Val Thr Arg Ile Phe Leu Gln Ala Leu Asn Leu Gly Ile Glu Val Ile
225 230 235 240
Asn Thr Thr Asp His Leu Lys Phe Ser Lys Asp Cys Gly Arg Met Leu
245 250 255
Thr Arg Met Trp Tyr Cys Ser Tyr Cys Gln Gly Leu Met Met Val Lys
260 265 270
Pro Cys Gly Gly Tyr Cys Asn Val Val Met Gln Gly Cys Met Ala Gly

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        275                 280                 285
Val Val Glu Ile Asp Lys Tyr Trp Arg Glu Tyr Ile Leu Ser Leu Glu
    290                 295                 300
Glu Leu Val Asn Gly Met Tyr Arg Ile Tyr Asp Met Glu Asn Val Leu
305                 310                 315                 320
Leu Gly Leu Phe Ser Thr Ile His Asp Ser Ile Gln Tyr Val Gln Lys
                325                 330                 335
Asn Ala Gly Lys Leu Thr Thr Thr Ile Gly Lys Leu Cys Ala His Ser
            340                 345                 350
Gln Gln Arg Gln Tyr Arg Ser Ala Tyr Tyr Pro Glu Asp Leu Phe Ile
        355                 360                 365
Asp Lys Lys Val Leu Lys Val Ala His Val Glu His Glu Glu Thr Leu
    370                 375                 380
Ser Ser Arg Arg Arg Glu Leu Ile Gln Lys Leu Lys Ser Phe Ile Ser
385                 390                 395                 400
Phe Tyr Ser Ala Leu Pro Gly Tyr Ile Cys Ser His Ser Pro Val Ala
                405                 410                 415
Glu Asn Asp Thr Leu Cys Trp Asn Gly Gln Glu Leu Val Glu Arg Tyr
            420                 425                 430
Ser Gln Lys Ala Ala Arg Asn Gly Met Lys Asn Gln Phe Asn Leu His
        435                 440                 445
Glu Leu Lys Met Lys Gly Pro Glu Pro Val Val Ser Gln Ile Ile Asp
    450                 455                 460
Lys Leu Lys His Ile Asn Gln Leu Leu Arg Thr Met Ser Met Pro Lys
465                 470                 475                 480
Gly Arg Val Leu Asp Lys Asn Leu Asp Glu Glu Gly Phe Glu Ser Gly
                485                 490                 495
Asp Cys Gly Asp Asp Glu Asp Glu Cys Ile Gly Gly Ser Gly Asp Gly
            500                 505                 510
Met Ile Lys Val Lys Asn Gln Leu Arg Phe Leu Ala Glu Leu Ala Tyr
        515                 520                 525
Asp Leu Asp Val Asp Asp Ala Pro Gly Asn Ser Gln Gln Ala Thr Pro
    530                 535                 540
Lys Asp Asn Glu Ile Ser Thr Phe His Asn Leu Gly Asn Val His Ser
545                 550                 555                 560


<210> 462
<211> 556
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acids 25-580 of SEQ ID NO: 460
human GPC3 full-length (UNIPROT)

<400> 462
Gln Pro Pro Pro Pro Pro Pro Asp Ala Thr Cys His Gln Val Arg Ser
 1               5                  10                  15
Phe Phe Gln Arg Leu Gln Pro Gly Leu Lys Trp Val Pro Glu Thr Pro
            20                  25                  30
Val Pro Gly Ser Asp Leu Gln Val Cys Leu Pro Lys Gly Pro Thr Cys
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        35                  40                  45
Cys Ser Arg Lys Met Glu Glu Lys Tyr Gln Leu Thr Ala Arg Leu Asn
    50                  55                  60
Met Glu Gln Leu Leu Gln Ser Ala Ser Met Glu Leu Lys Phe Leu Ile
65                  70                  75                  80
Ile Gln Asn Ala Ala Val Phe Gln Glu Ala Phe Glu Ile Val Val Arg
                85                  90                  95
His Ala Lys Asn Tyr Thr Asn Ala Met Phe Lys Asn Asn Tyr Pro Ser
            100                 105                 110
Leu Thr Pro Gln Ala Phe Glu Phe Val Gly Glu Phe Phe Thr Asp Val
        115                 120                 125
Ser Leu Tyr Ile Leu Gly Ser Asp Ile Asn Val Asp Asp Met Val Asn
    130                 135                 140
Glu Leu Phe Asp Ser Leu Phe Pro Val Ile Tyr Thr Gln Leu Met Asn
145                 150                 155                 160
Pro Gly Leu Pro Asp Ser Ala Leu Asp Ile Asn Glu Cys Leu Arg Gly
                165                 170                 175
Ala Arg Arg Asp Leu Lys Val Phe Gly Asn Phe Pro Lys Leu Ile Met
            180                 185                 190
Thr Gln Val Ser Lys Ser Leu Gln Val Thr Arg Ile Phe Leu Gln Ala
        195                 200                 205
Leu Asn Leu Gly Ile Glu Val Ile Asn Thr Thr Asp His Leu Lys Phe
    210                 215                 220
Ser Lys Asp Cys Gly Arg Met Leu Thr Arg Met Trp Tyr Cys Ser Tyr
225                 230                 235                 240
Cys Gln Gly Leu Met Met Val Lys Pro Cys Gly Gly Tyr Cys Asn Val
                245                 250                 255
Val Met Gln Gly Cys Met Ala Gly Val Val Glu Ile Asp Lys Tyr Trp
            260                 265                 270
Arg Glu Tyr Ile Leu Ser Leu Glu Glu Leu Val Asn Gly Met Tyr Arg
        275                 280                 285
Ile Tyr Asp Met Glu Asn Val Leu Leu Gly Leu Phe Ser Thr Ile His
    290                 295                 300
Asp Ser Ile Gln Tyr Val Gln Lys Asn Ala Gly Lys Leu Thr Thr Thr
305                 310                 315                 320
Ile Gly Lys Leu Cys Ala His Ser Gln Gln Arg Gln Tyr Arg Ser Ala
                325                 330                 335
Tyr Tyr Pro Glu Asp Leu Phe Ile Asp Lys Lys Val Leu Lys Val Ala
            340                 345                 350
His Val Glu His Glu Glu Thr Leu Ser Ser Arg Arg Arg Glu Leu Ile
        355                 360                 365
Gln Lys Leu Lys Ser Phe Ile Ser Phe Tyr Ser Ala Leu Pro Gly Tyr
    370                 375                 380
Ile Cys Ser His Ser Pro Val Ala Glu Asn Asp Thr Leu Cys Trp Asn
385                 390                 395                 400
Gly Gln Glu Leu Val Glu Arg Tyr Ser Gln Lys Ala Ala Arg Asn Gly
                405                 410                 415
Met Lys Asn Gln Phe Asn Leu His Glu Leu Lys Met Lys Gly Pro Glu
            420                 425                 430
Pro Val Val Ser Gln Ile Ile Asp Lys Leu Lys His Ile Asn Gln Leu
        435                 440                 445
Leu Arg Thr Met Ser Met Pro Lys Gly Arg Val Leu Asp Lys Asn Leu
```

```
    450                 455                 460
Asp Glu Glu Gly Phe Glu Ser Gly Asp Cys Gly Asp Asp Glu Asp Glu
465                 470                 475                 480
Cys Ile Gly Gly Ser Gly Asp Gly Met Ile Lys Val Lys Asn Gln Leu
                485                 490                 495
Arg Phe Leu Ala Glu Leu Ala Tyr Asp Leu Asp Val Asp Asp Ala Pro
            500                 505                 510
Gly Asn Ser Gln Gln Ala Thr Pro Lys Asp Asn Glu Ile Ser Thr Phe
        515                 520                 525
His Asn Leu Gly Asn Val His Ser Pro Leu Lys Leu Leu Thr Ser Met
    530                 535                 540
Ala Ile Ser Val Val Cys Phe Phe Phe Leu Val His
545                 550                 555


<210> 463
<211> 536
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acids 25-560 of SEQ ID NO: 460
human GPC3 full-length (UNIPROT)

<400> 463
Gln Pro Pro Pro Pro Pro Pro Asp Ala Thr Cys His Gln Val Arg Ser
 1               5                  10                  15
Phe Phe Gln Arg Leu Gln Pro Gly Leu Lys Trp Val Pro Glu Thr Pro
            20                  25                  30
Val Pro Gly Ser Asp Leu Gln Val Cys Leu Pro Lys Gly Pro Thr Cys
        35                  40                  45
Cys Ser Arg Lys Met Glu Glu Lys Tyr Gln Leu Thr Ala Arg Leu Asn
    50                  55                  60
Met Glu Gln Leu Leu Gln Ser Ala Ser Met Glu Leu Lys Phe Leu Ile
65                  70                  75                  80
Ile Gln Asn Ala Ala Val Phe Gln Glu Ala Phe Glu Ile Val Val Arg
                85                  90                  95
His Ala Lys Asn Tyr Thr Asn Ala Met Phe Lys Asn Asn Tyr Pro Ser
            100                 105                 110
Leu Thr Pro Gln Ala Phe Glu Phe Val Gly Glu Phe Phe Thr Asp Val
        115                 120                 125
Ser Leu Tyr Ile Leu Gly Ser Asp Ile Asn Val Asp Asp Met Val Asn
    130                 135                 140
Glu Leu Phe Asp Ser Leu Phe Pro Val Ile Tyr Thr Gln Leu Met Asn
145                 150                 155                 160
Pro Gly Leu Pro Asp Ser Ala Leu Asp Ile Asn Glu Cys Leu Arg Gly
                165                 170                 175
Ala Arg Arg Asp Leu Lys Val Phe Gly Asn Phe Pro Lys Leu Ile Met
            180                 185                 190
Thr Gln Val Ser Lys Ser Leu Gln Val Thr Arg Ile Phe Leu Gln Ala
        195                 200                 205
Leu Asn Leu Gly Ile Glu Val Ile Asn Thr Thr Asp His Leu Lys Phe
```

```
    210                 215                 220
Ser Lys Asp Cys Gly Arg Met Leu Thr Arg Met Trp Tyr Cys Ser Tyr
225                 230                 235                 240
Cys Gln Gly Leu Met Met Val Lys Pro Cys Gly Gly Tyr Cys Asn Val
                245                 250                 255
Val Met Gln Gly Cys Met Ala Gly Val Val Glu Ile Asp Lys Tyr Trp
            260                 265                 270
Arg Glu Tyr Ile Leu Ser Leu Glu Glu Leu Val Asn Gly Met Tyr Arg
        275                 280                 285
Ile Tyr Asp Met Glu Asn Val Leu Leu Gly Leu Phe Ser Thr Ile His
    290                 295                 300
Asp Ser Ile Gln Tyr Val Gln Lys Asn Ala Gly Lys Leu Thr Thr Thr
305                 310                 315                 320
Ile Gly Lys Leu Cys Ala His Ser Gln Gln Arg Gln Tyr Arg Ser Ala
                325                 330                 335
Tyr Tyr Pro Glu Asp Leu Phe Ile Asp Lys Lys Val Leu Lys Val Ala
            340                 345                 350
His Val Glu His Glu Glu Thr Leu Ser Ser Arg Arg Arg Glu Leu Ile
        355                 360                 365
Gln Lys Leu Lys Ser Phe Ile Ser Phe Tyr Ser Ala Leu Pro Gly Tyr
    370                 375                 380
Ile Cys Ser His Ser Pro Val Ala Glu Asn Asp Thr Leu Cys Trp Asn
385                 390                 395                 400
Gly Gln Glu Leu Val Glu Arg Tyr Ser Gln Lys Ala Ala Arg Asn Gly
                405                 410                 415
Met Lys Asn Gln Phe Asn Leu His Glu Leu Lys Met Lys Gly Pro Glu
            420                 425                 430
Pro Val Val Ser Gln Ile Ile Asp Lys Leu Lys His Ile Asn Gln Leu
        435                 440                 445
Leu Arg Thr Met Ser Met Pro Lys Gly Arg Val Leu Asp Lys Asn Leu
    450                 455                 460
Asp Glu Glu Gly Phe Glu Ser Gly Asp Cys Gly Asp Asp Glu Asp Glu
465                 470                 475                 480
Cys Ile Gly Gly Ser Gly Asp Gly Met Ile Lys Val Lys Asn Gln Leu
                485                 490                 495
Arg Phe Leu Ala Glu Leu Ala Tyr Asp Leu Asp Val Asp Asp Ala Pro
            500                 505                 510
Gly Asn Ser Gln Gln Ala Thr Pro Lys Asp Asn Glu Ile Ser Thr Phe
        515                 520                 525
His Asn Leu Gly Asn Val His Ser
    530                 535
```

<210> 464
<211> 334
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acids 25-358 of SEQ ID NO: 460
human GPC3 full-length (UNIPROT)

<400> 464

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Gln Pro Pro Pro Pro Pro Pro Asp Ala Thr Cys His Gln Val Arg Ser
 1               5                  10                  15
Phe Phe Gln Arg Leu Gln Pro Gly Leu Lys Trp Val Pro Glu Thr Pro
            20                  25                  30
Val Pro Gly Ser Asp Leu Gln Val Cys Leu Pro Lys Gly Pro Thr Cys
        35                  40                  45
Cys Ser Arg Lys Met Glu Glu Lys Tyr Gln Leu Thr Ala Arg Leu Asn
    50                  55                  60
Met Glu Gln Leu Leu Gln Ser Ala Ser Met Glu Leu Lys Phe Leu Ile
65                  70                  75                  80
Ile Gln Asn Ala Ala Val Phe Gln Glu Ala Phe Glu Ile Val Val Arg
                85                  90                  95
His Ala Lys Asn Tyr Thr Asn Ala Met Phe Lys Asn Asn Tyr Pro Ser
            100                 105                 110
Leu Thr Pro Gln Ala Phe Glu Phe Val Gly Glu Phe Phe Thr Asp Val
        115                 120                 125
Ser Leu Tyr Ile Leu Gly Ser Asp Ile Asn Val Asp Asp Met Val Asn
    130                 135                 140
Glu Leu Phe Asp Ser Leu Phe Pro Val Ile Tyr Thr Gln Leu Met Asn
145                 150                 155                 160
Pro Gly Leu Pro Asp Ser Ala Leu Asp Ile Asn Glu Cys Leu Arg Gly
                165                 170                 175
Ala Arg Arg Asp Leu Lys Val Phe Gly Asn Phe Pro Lys Leu Ile Met
            180                 185                 190
Thr Gln Val Ser Lys Ser Leu Gln Val Thr Arg Ile Phe Leu Gln Ala
        195                 200                 205
Leu Asn Leu Gly Ile Glu Val Ile Asn Thr Thr Asp His Leu Lys Phe
    210                 215                 220
Ser Lys Asp Cys Gly Arg Met Leu Thr Arg Met Trp Tyr Cys Ser Tyr
225                 230                 235                 240
Cys Gln Gly Leu Met Met Val Lys Pro Cys Gly Gly Tyr Cys Asn Val
                245                 250                 255
Val Met Gln Gly Cys Met Ala Gly Val Val Glu Ile Asp Lys Tyr Trp
            260                 265                 270
Arg Glu Tyr Ile Leu Ser Leu Glu Glu Leu Val Asn Gly Met Tyr Arg
        275                 280                 285
Ile Tyr Asp Met Glu Asn Val Leu Leu Gly Leu Phe Ser Thr Ile His
    290                 295                 300
Asp Ser Ile Gln Tyr Val Gln Lys Asn Ala Gly Lys Leu Thr Thr Thr
305                 310                 315                 320
Ile Gly Lys Leu Cys Ala His Ser Gln Gln Arg Gln Tyr Arg
                325                 330
```

<210> 465
<211> 202
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acids 359-560 of SEQ ID NO: 460

human GPC3 full-length (UNIPROT)

<400> 465
Ser Ala Tyr Tyr Pro Glu Asp Leu Phe Ile Asp Lys Lys Val Leu Lys
 1   5   10   15
Val Ala His Val Glu His Glu Glu Thr Leu Ser Ser Arg Arg Arg Glu
            20   25   30
Leu Ile Gln Lys Leu Lys Ser Phe Ile Ser Phe Tyr Ser Ala Leu Pro
        35   40   45
Gly Tyr Ile Cys Ser His Ser Pro Val Ala Glu Asn Asp Thr Leu Cys
    50   55   60
Trp Asn Gly Gln Glu Leu Val Glu Arg Tyr Ser Gln Lys Ala Ala Arg
65   70   75   80
Asn Gly Met Lys Asn Gln Phe Asn Leu His Glu Leu Lys Met Lys Gly
                85   90   95
Pro Glu Pro Val Val Ser Gln Ile Ile Asp Lys Leu Lys His Ile Asn
            100   105   110
Gln Leu Leu Arg Thr Met Ser Met Pro Lys Gly Arg Val Leu Asp Lys
        115   120   125
Asn Leu Asp Glu Glu Gly Phe Glu Ser Gly Asp Cys Gly Asp Asp Glu
    130   135   140
Asp Glu Cys Ile Gly Gly Ser Gly Asp Gly Met Ile Lys Val Lys Asn
145   150   155   160
Gln Leu Arg Phe Leu Ala Glu Leu Ala Tyr Asp Leu Asp Val Asp Asp
                165   170   175
Ala Pro Gly Asn Ser Gln Gln Ala Thr Pro Lys Asp Asn Glu Ile Ser
            180   185   190
Thr Phe His Asn Leu Gly Asn Val His Ser
        195   200

<210> 466
<211> 222
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acids 359-580 of SEQ ID NO: 460
human GPC3 full-length (UNIPROT)

<400> 466
Ser Ala Tyr Tyr Pro Glu Asp Leu Phe Ile Asp Lys Lys Val Leu Lys
 1   5   10   15
Val Ala His Val Glu His Glu Glu Thr Leu Ser Ser Arg Arg Arg Glu
            20   25   30
Leu Ile Gln Lys Leu Lys Ser Phe Ile Ser Phe Tyr Ser Ala Leu Pro
        35   40   45
Gly Tyr Ile Cys Ser His Ser Pro Val Ala Glu Asn Asp Thr Leu Cys
    50   55   60
Trp Asn Gly Gln Glu Leu Val Glu Arg Tyr Ser Gln Lys Ala Ala Arg
65   70   75   80
Asn Gly Met Lys Asn Gln Phe Asn Leu His Glu Leu Lys Met Lys Gly

```
                85                  90                  95
Pro Glu Pro Val Val Ser Gln Ile Ile Asp Lys Leu Lys His Ile Asn
            100                 105                 110
Gln Leu Leu Arg Thr Met Ser Met Pro Lys Gly Arg Val Leu Asp Lys
        115                 120                 125
Asn Leu Asp Glu Glu Gly Phe Glu Ser Gly Asp Cys Gly Asp Asp Glu
    130                 135                 140
Asp Glu Cys Ile Gly Gly Ser Gly Asp Gly Met Ile Lys Val Lys Asn
145                 150                 155                 160
Gln Leu Arg Phe Leu Ala Glu Leu Ala Tyr Asp Leu Asp Val Asp Asp
                165                 170                 175
Ala Pro Gly Asn Ser Gln Gln Ala Thr Pro Lys Asp Asn Glu Ile Ser
            180                 185                 190
Thr Phe His Asn Leu Gly Asn Val His Ser Pro Leu Lys Leu Leu Thr
        195                 200                 205
Ser Met Ala Ile Ser Val Val Cys Phe Phe Phe Leu Val His
    210                 215                 220
```

<210> 467
<211> 51
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acids 510-560 of SEQ ID NO: 460
human GPC3 full-length (UNIPROT)

<400> 467

```
Gly Asp Gly Met Ile Lys Val Lys Asn Gln Leu Arg Phe Leu Ala Glu
 1               5                  10                  15
Leu Ala Tyr Asp Leu Asp Val Asp Asp Ala Pro Gly Asn Ser Gln Gln
            20                  25                  30
Ala Thr Pro Lys Asp Asn Glu Ile Ser Thr Phe His Asn Leu Gly Asn
        35                  40                  45
Val His Ser
    50
```

<210> 468
<211> 358
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acids 1-358 of SEQ ID NO: 460
human GPC3 full-length (UNIPROT)

<400> 468

```
Met Ala Gly Thr Val Arg Thr Ala Cys Leu Val Val Ala Met Leu Leu
 1               5                  10                  15
Ser Leu Asp Phe Pro Gly Gln Ala Gln Pro Pro Pro Pro Pro Pro Asp
```

```
            20                  25                  30
Ala Thr Cys His Gln Val Arg Ser Phe Phe Gln Arg Leu Gln Pro Gly
        35                  40                  45
Leu Lys Trp Val Pro Glu Thr Pro Val Pro Gly Ser Asp Leu Gln Val
    50                  55                  60
Cys Leu Pro Lys Gly Pro Thr Cys Cys Ser Arg Lys Met Glu Glu Lys
65                  70                  75                  80
Tyr Gln Leu Thr Ala Arg Leu Asn Met Glu Gln Leu Leu Gln Ser Ala
                85                  90                  95
Ser Met Glu Leu Lys Phe Leu Ile Ile Gln Asn Ala Ala Val Phe Gln
            100                 105                 110
Glu Ala Phe Glu Ile Val Val Arg His Ala Lys Asn Tyr Thr Asn Ala
        115                 120                 125
Met Phe Lys Asn Asn Tyr Pro Ser Leu Thr Pro Gln Ala Phe Glu Phe
    130                 135                 140
Val Gly Glu Phe Phe Thr Asp Val Ser Leu Tyr Ile Leu Gly Ser Asp
145                 150                 155                 160
Ile Asn Val Asp Asp Met Val Asn Glu Leu Phe Asp Ser Leu Phe Pro
                165                 170                 175
Val Ile Tyr Thr Gln Leu Met Asn Pro Gly Leu Pro Asp Ser Ala Leu
            180                 185                 190
Asp Ile Asn Glu Cys Leu Arg Gly Ala Arg Arg Asp Leu Lys Val Phe
        195                 200                 205
Gly Asn Phe Pro Lys Leu Ile Met Thr Gln Val Ser Lys Ser Leu Gln
    210                 215                 220
Val Thr Arg Ile Phe Leu Gln Ala Leu Asn Leu Gly Ile Glu Val Ile
225                 230                 235                 240
Asn Thr Thr Asp His Leu Lys Phe Ser Lys Asp Cys Gly Arg Met Leu
                245                 250                 255
Thr Arg Met Trp Tyr Cys Ser Tyr Cys Gln Gly Leu Met Met Val Lys
            260                 265                 270
Pro Cys Gly Gly Tyr Cys Asn Val Val Met Gln Gly Cys Met Ala Gly
        275                 280                 285
Val Val Glu Ile Asp Lys Tyr Trp Arg Glu Tyr Ile Leu Ser Leu Glu
    290                 295                 300
Glu Leu Val Asn Gly Met Tyr Arg Ile Tyr Asp Met Glu Asn Val Leu
305                 310                 315                 320
Leu Gly Leu Phe Ser Thr Ile His Asp Ser Ile Gln Tyr Val Gln Lys
                325                 330                 335
Asn Ala Gly Lys Leu Thr Thr Thr Ile Gly Lys Leu Cys Ala His Ser
            340                 345                 350
Gln Gln Arg Gln Tyr Arg
        355


<210> 469
<211> 2
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker amino acid sequence
```

<220>
<221> Variant
<222> (1)..(2)
<223> Can be in repeat of at least one and up to ten or more

<400> 469
Gly Ser
 1

<210> 470
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker amino acid sequence

<220>
<221> Variant
<222> (1)..(5)
<223> Can be in repeat of at least one and up to ten or more

<400> 470
Gly Ser Gly Gly Ser
 1   5

<210> 471
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker amino acid sequence

<220>
<221> Variant
<222> (1)..(5)
<223> Can be in repeat of at least one and up to ten or more

<400> 471
Gly Gly Gly Gly Ser
 1   5

<210> 472
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker amino acid sequence

<220>
<221> Variant
<222> (1)..(4)
<223> Can be in repeat of at least one and up to ten or more

<400> 472
Gly Gly Gly Ser
 1

<210> 473
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker amino acid sequence

<400> 473
Ser Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 1   5   10   15
Ser Leu Glu Met Ala
            20

<210> 474
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker amino acid sequence

<400> 474
Thr Ser Gly Gly Gly Gly Ser
 1   5

<210> 475
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker amino acid sequence

<400> 475
Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys
 1   5   10   15

Glu Ala Ala Ala Lys Ala
            20

<210> 476
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> His-tag amino acid sequence

<400> 476
His His His His His His
 1   5

<210> 477
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> His-tag and HA-tag amino acid sequence

<400> 477
Thr Ser Gly Gln Ala Gly Gln His His His His His His Gly Ala Tyr
 1   5   10   15
Pro Tyr Asp Val Pro Asp Tyr Ala Ser
            20   25

<210> 478
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Fc hinge amino acid sequence

<400> 478
Cys Pro Pro Cys Pro
 1   5

<210> 479
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-CD3 antibody HC-CDR1 amino acid sequence

<400> 479
Gly Tyr Thr Phe Thr Arg Tyr Thr
 1   5

<210> 480
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-CD3 antibody HC-CDR2 amino acid sequence

<400> 480
Ile Asn Pro Ser Arg Gly Tyr Thr
 1   5

<210> 481
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-CD3 antibody HC-CDR3 amino acid sequence

<400> 481
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr
 1   5   10

<210> 482
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-CD3 antibody LC-CDR1 amino acid sequence

<400> 482
Gln Ser Val Ser Tyr
 1   5

<210> 483
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-CD3 antibody LC-CDR2 amino acid sequence

<400> 483
Asp Thr Ser
 1

<210> 484
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-CD3 antibody LC-CDR3 amino acid sequence

<400> 484
Gln Gln Trp Ser Ser Asn Pro Leu Thr
 1   5

<210> 485
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-CD3 antibody VH amino acid sequence

<400> 485
Asp Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20   25   30
Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
        35   40   45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Ala Asp Ser Val
    50   55   60
Lys Gly Arg Phe Thr Ile Thr Thr Asp Lys Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
                85   90   95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100   105   110
Thr Thr Val Thr Val Ser Ser
        115

<210> 486
<211> 106
<212> PRT
<213> Artificial Sequence

<220>

<223> Anti-CD3 antibody VL amino acid sequence

<400> 486
Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1   5   10   15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Tyr Met
            20   25   30
Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35   40   45
Asp Thr Ser Lys Val Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50   55   60
Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Asn Ser Leu Glu Ala Glu
65   70   75   80
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr
                85   90   95
Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100   105

<210> 487
<211> 243
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-CD3 scFv amino acid sequence

<400> 487
Asp Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20   25   30
Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
        35   40   45
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Ala Asp Ser Val
    50   55   60
Lys Gly Arg Phe Thr Ile Thr Thr Asp Lys Ser Thr Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
                85   90   95
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100   105   110
Thr Thr Val Thr Val Ser Ser Gly Glu Gly Thr Ser Thr Gly Ser Gly
        115   120   125
Gly Ser Gly Gly Ser Gly Gly Ala Asp Asp Ile Val Leu Thr Gln Ser
    130   135   140
Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys
145   150   155   160
Arg Ala Ser Gln Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro
                165   170   175
Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
            180   185   190

Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser
        195   200   205
Leu Thr Ile Asn Ser Leu Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
    210   215   220
Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys Val
225   230   235   240
Glu Ile Lys

<210> 488
<211> 498
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-nGPC3 (GPC3A-034) scFv-anti-CD3 scFv
amino acid sequence

<400> 488
Gln Pro Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20   25   30
Asn Val Ile Trp Tyr Gln Gln Leu Pro Gly Ala Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn His Arg Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Arg Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Asp Gly Tyr Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu
    130   135   140
Lys Pro Ser Glu Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser
145   150   155   160
Phe Ser Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly
                165   170   175
Leu Glu Trp Ile Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn
            180   185   190
Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn
        195   200   205
Gln Phe Ser Leu Glu Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
    210   215   220
Tyr Tyr Cys Ala Arg Gly Tyr Gly Gly Arg Phe Asp Tyr Trp Gly Gln
225   230   235   240
Gly Thr Leu Val Thr Val Ser Ser Thr Ser Gly Gly Gly Gly Ser Asp
                245   250   255

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Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser
            260                 265                 270
Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr
        275                 280                 285
Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly
    290                 295                 300
Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Ala Asp Ser Val Lys
305                 310                 315                 320
Gly Arg Phe Thr Ile Thr Thr Asp Lys Ser Thr Ser Thr Ala Tyr Met
                325                 330                 335
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr Cys Ala
            340                 345                 350
Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr
        355                 360                 365
Thr Val Thr Val Ser Ser Gly Glu Gly Thr Ser Thr Gly Ser Gly Gly
    370                 375                 380
Ser Gly Gly Ser Gly Gly Ala Asp Asp Ile Val Leu Thr Gln Ser Pro
385                 390                 395                 400
Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg
                405                 410                 415
Ala Ser Gln Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly
            420                 425                 430
Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly
        435                 440                 445
Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu
    450                 455                 460
Thr Ile Asn Ser Leu Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
465                 470                 475                 480
Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu
                485                 490                 495
Ile Lys
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<210> 489
<211> 497
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-GPC3 (GPC3B-87) scFv-anti-CD3 scFv
amino acid sequence

<400> 489

```
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1               5                  10                  15
Glu Pro Ala Ser Val Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20                  25                  30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50                  55                  60
```

```
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
                85                  90                  95
Leu Gln Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110
Arg Ser Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        115                 120                 125
Gly Ser Leu Glu Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu
    130                 135                 140
Val Arg Lys Pro Gly Ser Ser Val Lys Val Ser Cys Gln Ala Ser Gly
145                 150                 155                 160
Gly Thr Phe Gly Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly
                165                 170                 175
Gln Gly Leu Glu Trp Met Gly Arg Ile Ile Pro Val Leu Gly Arg Thr
            180                 185                 190
Lys Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Val Thr Ala Asp Thr
        195                 200                 205
Ser Thr Ser Thr Val Tyr Met Glu Leu Thr Ser Leu Thr Ser Glu Asp
    210                 215                 220
Thr Ala Val Tyr Tyr Cys Ala Arg Thr Asn Asp Ser Trp Gly Gln Gly
225                 230                 235                 240
Thr Leu Val Thr Val Ser Ser Thr Ser Gly Gly Gly Gly Ser Asp Val
                245                 250                 255
Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val
            260                 265                 270
Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met
        275                 280                 285
His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr
    290                 295                 300
Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Ala Asp Ser Val Lys Gly
305                 310                 315                 320
Arg Phe Thr Ile Thr Thr Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu
                325                 330                 335
Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr Cys Ala Arg
            340                 345                 350
Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr
        355                 360                 365
Val Thr Val Ser Ser Gly Glu Gly Thr Ser Thr Gly Ser Gly Gly Ser
    370                 375                 380
Gly Gly Ser Gly Gly Ala Asp Asp Ile Val Leu Thr Gln Ser Pro Ala
385                 390                 395                 400
Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala
                405                 410                 415
Ser Gln Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys
            420                 425                 430
Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val
        435                 440                 445
Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr
    450                 455                 460
Ile Asn Ser Leu Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln
465                 470                 475                 480
```

Trp Ser Ser Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
485 490 495
Lys

<210> 490
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker amino acid sequence

<400> 490
Gly Glu Gly Thr Ser Thr Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly
1 5 10 15
Ala Asp

<210> 491
<211> 470
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-034 scFv-CAR amino acid sequence

<400> 491
Gln Pro Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30
Asn Val Ile Trp Tyr Gln Gln Leu Pro Gly Ala Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Ser Asn His Arg Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Arg Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65 70 75 80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
85 90 95
Asp Gly Tyr Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser
100 105 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125
Leu Glu Met Ala Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu
130 135 140
Lys Pro Ser Glu Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser
145 150 155 160
Phe Ser Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly
165 170 175

```
Leu Glu Trp Ile Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn
            180                 185                 190
Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn
        195                 200                 205
Gln Phe Ser Leu Glu Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
    210                 215                 220
Tyr Tyr Cys Ala Arg Gly Tyr Gly Gly Arg Phe Asp Tyr Trp Gly Gln
225                 230                 235                 240
Gly Thr Leu Val Thr Val Ser Ser Ala Ala Ala Ile Glu Val Met Tyr
                245                 250                 255
Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser Asn Gly Thr Ile Ile His
            260                 265                 270
Val Lys Gly Lys His Leu Cys Pro Ser Pro Leu Phe Pro Gly Pro Ser
        275                 280                 285
Lys Pro Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr
    290                 295                 300
Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys
305                 310                 315                 320
Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg
                325                 330                 335
Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp
            340                 345                 350
Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
        355                 360                 365
Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
    370                 375                 380
Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
385                 390                 395                 400
Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
                405                 410                 415
Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
            420                 425                 430
Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
        435                 440                 445
Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
    450                 455                 460
Gln Ala Leu Pro Pro Arg
465                 470


<210> 492
<211> 287
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-034 (VHCH)-TCR delta amino acid sequence

<400> 492
Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
 1               5                  10                  15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
```

```
            20                  25                  30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80
Glu Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95
Arg Gly Tyr Gly Gly Arg Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110
Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
        115                 120                 125
Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
    130                 135                 140
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145                 150                 155                 160
Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175
Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            180                 185                 190
Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
        195                 200                 205
Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Glu Val Lys Thr Asp
    210                 215                 220
Ser Thr Asp His Val Lys Pro Lys Glu Thr Glu Asn Thr Lys Gln Pro
225                 230                 235                 240
Ser Lys Ser Cys His Lys Pro Lys Ala Ile Val His Thr Glu Lys Val
                245                 250                 255
Asn Met Met Ser Leu Thr Val Leu Gly Leu Arg Met Leu Phe Ala Lys
            260                 265                 270
Thr Val Ala Val Asn Phe Leu Leu Thr Ala Lys Leu Phe Phe Leu
        275                 280                 285


<210> 493
<211> 283
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-034 (VLCL)-TCR gamma amino acid sequence

<400> 493
Gln Pro Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20                  25                  30
Asn Val Ile Trp Tyr Gln Gln Leu Pro Gly Ala Ala Pro Lys Leu Leu
        35                  40                  45
Ile Tyr Ser Asn His Arg Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60
```

Gly Ser Arg Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65 70 75 80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
85 90 95
Asp Gly Tyr Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Gln
100 105 110
Pro Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
115 120 125
Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
130 135 140
Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro Val Lys
145 150 155 160
Ala Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn Lys Tyr
165 170 175
Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
180 185 190
Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
195 200 205
Thr Val Ala Pro Thr Glu Cys Ser Pro Ile Lys Thr Asp Val Ile Thr
210 215 220
Met Asp Pro Lys Asp Asn Cys Ser Lys Asp Ala Asn Asp Thr Leu Leu
225 230 235 240
Leu Gln Leu Thr Asn Thr Ser Ala Tyr Tyr Met Tyr Leu Leu Leu Leu
245 250 255
Leu Lys Ser Val Val Tyr Phe Ala Ile Ile Thr Cys Cys Leu Leu Arg
260 265 270
Arg Thr Ala Phe Cys Cys Asn Gly Glu Lys Ser
275 280

<210> 494
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<223> CD28-CD3 zeta CAR domain fusion amino acid sequence

<400> 494
Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser Asn
1 5 10 15
Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro Leu
20 25 30
Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly Gly
35 40 45
Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe
50 55 60
Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn
65 70 75 80
Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr
85 90 95
Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser

```
            100                 105                 110
Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr
        115                 120                 125
Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys
    130                 135                 140
Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn
145                 150                 155                 160
Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu
                165                 170                 175
Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly
            180                 185                 190
His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr
        195                 200                 205
Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
    210                 215


<210> 495
<211> 68
<212> PRT
<213> Artificial Sequence

<220>
<223> TCR delta chain amino acid sequence

<400> 495
Glu Val Lys Thr Asp Ser Thr Asp His Val Lys Pro Lys Glu Thr Glu
 1               5                  10                  15
Asn Thr Lys Gln Pro Ser Lys Ser Cys His Lys Pro Lys Ala Ile Val
            20                  25                  30
His Thr Glu Lys Val Asn Met Met Ser Leu Thr Val Leu Gly Leu Arg
        35                  40                  45
Met Leu Phe Ala Lys Thr Val Ala Val Asn Phe Leu Leu Thr Ala Lys
    50                  55                  60
Leu Phe Phe Leu
65


<210> 496
<211> 67
<212> PRT
<213> Artificial Sequence

<220>
<223> TCR gamma chain amino acid sequence

<400> 496
Pro Ile Lys Thr Asp Val Ile Thr Met Asp Pro Lys Asp Asn Cys Ser
 1               5                  10                  15
Lys Asp Ala Asn Asp Thr Leu Leu Leu Gln Leu Thr Asn Thr Ser Ala
            20                  25                  30
Tyr Tyr Met Tyr Leu Leu Leu Leu Leu Lys Ser Val Val Tyr Phe Ala
```

35 40 45
Ile Ile Thr Cys Cys Leu Leu Arg Arg Thr Ala Phe Cys Cys Asn Gly
50 55 60
Glu Lys Ser
65

<210> 497
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> GC33 antibody HC-CDR1 amino acid sequence

<400> 497
Asp Tyr Glu Met His
1 5

<210> 498
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> GC33 antibody HC-CDR2 amino acid sequence

<400> 498
Ala Leu Asp Pro Lys Thr Gly Asp Thr Ala Tyr Ser Gln Lys Phe Lys
1 5 10 15
Gly

<210> 499
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> GC33 antibody HC-CDR3 amino acid sequence

<400> 499
Phe Tyr Ser Tyr Thr Tyr
1 5

<210> 500
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> GC33 antibody LC-CDR1 amino acid sequence

<400> 500
Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His
 1   5   10   15

<210> 501
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> GC33 antibody LC-CDR2 amino acid sequence

<400> 501
Lys Val Ser Asn Arg Phe Ser
 1   5

<210> 502
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> GC33 antibody LC-CDR3 amino acid sequence

<400> 502
Ser Gln Asn Thr His Val Pro Pro Thr
 1   5

<210> 503
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> GC33 antibody VH amino acid sequence

<400> 503
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20   25   30
Glu Met His Trp Val Lys Gln Thr Pro Val His Gly Leu Lys Trp Met
        35   40   45
Ile Gly Ala Leu Asp Pro Lys Thr Gly Asp Thr Ala Tyr Ser Gln Lys
    50   55   60

Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala
65 70 75 80
Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
85 90 95
Cys Thr Arg Phe Tyr Ser Tyr Thr Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110
Thr Val Ser Ala
115

<210> 504
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> GC33 antibody VL amino acid sequence

<400> 504
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Asn
85 90 95
Thr His Val Pro Pro Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 505
<211> 440
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-039 mouse heavy chain amino acid sequence

<400> 505
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val His Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Gly Thr Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val Lys
50 55 60

```
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65                  70                  75                  80
Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95
Arg Tyr Gly Arg Lys Ser Ile Asp Ala Trp Gly Gln Gly Thr Leu Val
            100                 105                 110
Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala
        115                 120                 125
Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu
    130                 135                 140
Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly
145                 150                 155                 160
Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp
                165                 170                 175
Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro
            180                 185                 190
Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys
        195                 200                 205
Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile
    210                 215                 220
Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro
225                 230                 235                 240
Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val
                245                 250                 255
Val Ala Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val
            260                 265                 270
Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln
        275                 280                 285
Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln
    290                 295                 300
Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala
305                 310                 315                 320
Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro
                325                 330                 335
Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala
            340                 345                 350
Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu
        355                 360                 365
Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr
    370                 375                 380
Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr
385                 390                 395                 400
Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe
                405                 410                 415
Thr Cys Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys
            420                 425                 430
Ser Leu Ser His Ser Pro Gly Lys
        435                 440
```

<210> 506
<211> 214

<212> PRT
<213> Artificial Sequence

<220>
<223> GPC3A-039 mouse light chain amino acid sequence

<400> 506
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys
            100   105   110
Ser Ser Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Glu
        115   120   125
Thr Asn Lys Ala Thr Leu Val Cys Thr Ile Thr Asp Phe Tyr Pro Gly
    130   135   140
Val Val Thr Val Asp Trp Lys Val Asp Gly Thr Pro Val Thr Gln Gly
145   150   155   160
Met Glu Thr Thr Gln Pro Ser Lys Gln Ser Asn Asn Lys Tyr Met Ala
                165   170   175
Ser Ser Tyr Leu Thr Leu Thr Ala Arg Ala Trp Glu Arg His Ser Ser
            180   185   190
Tyr Ser Cys Gln Val Thr His Glu Gly His Thr Val Glu Lys Ser Leu
        195   200   205
Ser Arg Ala Asp Cys Ser
    210

<210> 507
<211> 324
<212> PRT
<213> Artificial Sequence

<220>
<223> Mouse IgG1 heavy chain constant domain
amino acid sequence

<400> 507
Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala
 1   5   10   15
Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
            20   25   30
Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser

```
        35                  40                  45
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
    50                  55                  60
Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val
65                  70                  75                  80
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
                85                  90                  95
Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro
            100                 105                 110
Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu
        115                 120                 125
Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Ala Ile Ser
    130                 135                 140
Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu
145                 150                 155                 160
Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr
                165                 170                 175
Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn
            180                 185                 190
Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro
        195                 200                 205
Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln
    210                 215                 220
Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val
225                 230                 235                 240
Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val
                245                 250                 255
Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln
            260                 265                 270
Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn
        275                 280                 285
Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val
    290                 295                 300
Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
305                 310                 315                 320
Ser Pro Gly Lys
```

<210> 508
<211> 105
<212> PRT
<213> Artificial Sequence

<220>
<223> Mouse lambda light chain constant domain
amino acid sequence

<400> 508

```
Gln Pro Lys Ser Ser Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
 1               5                  10                  15
Glu Leu Glu Thr Asn Lys Ala Thr Leu Val Cys Thr Ile Thr Asp Phe
```

            20   25   30
Tyr Pro Gly Val Val Thr Val Asp Trp Lys Val Asp Gly Thr Pro Val
        35   40   45
Thr Gln Gly Met Glu Thr Thr Gln Pro Ser Lys Gln Ser Asn Asn Lys
    50   55   60
Tyr Met Ala Ser Ser Tyr Leu Thr Leu Thr Ala Arg Ala Trp Glu Arg
65   70   75   80
His Ser Ser Tyr Ser Cys Gln Val Thr His Glu Gly His Thr Val Glu
                85   90   95
Lys Ser Leu Ser Arg Ala Asp Cys Ser
            100   105

<210> 509
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Signal peptide amino acid sequence

<400> 509
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1   5   10   15
Gly Ser Thr Gly
            20

<210> 510
<211> 228
<212> PRT
<213> Artificial Sequence

<220>
<223> GC33 antibody amino acid sequence

<400> 510
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
 1   5   10   15
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20   25   30
Glu Met His Trp Val Lys Gln Thr Pro Val His Gly Leu Lys Trp Met
        35   40   45
Ile Gly Ala Leu Asp Pro Lys Thr Gly Asp Thr Ala Tyr Ser Gln Lys
    50   55   60
Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala
65   70   75   80
Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
                85   90   95
Cys Thr Arg Phe Tyr Ser Tyr Thr Tyr Trp Gly Gln Gly Thr Leu Val
            100   105   110
Thr Val Ser Ala Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro

```
        115                 120                 125
Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
    130                 135                 140
Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys
145                 150                 155                 160
Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
                165                 170                 175
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
            180                 185                 190
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe
        195                 200                 205
Cys Ser Gln Asn Thr His Val Pro Pro Thr Phe Gly Ser Gly Thr Lys
    210                 215                 220
Leu Glu Ile Lys
225


<210> 511
<211> 500
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-nGPC3 (GPC3A-037) scFv-anti-CD3 scFv
amino acid sequence

<400> 511
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Ile Ser Cys Ser Gly Thr Arg Ser Asn Ile Gly Ser Asp
            20                  25                  30
Tyr Val Ser Trp Tyr Gln His Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45
Val Tyr Gly Asp Asn Leu Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60
Ala Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65                  70                  75                  80
Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Tyr Thr Leu
                85                  90                  95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100                 105                 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125
Leu Glu Met Ala Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
    130                 135                 140
Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145                 150                 155                 160
Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
                165                 170                 175
Leu Glu Trp Val Ser Val Ile Tyr Ser Gly Gly Ser Ser Thr Tyr Tyr
            180                 185                 190
Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
```

```
        195                 200                 205
Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
    210                 215                 220
Val Tyr Tyr Cys Ala Arg Thr Ser Tyr Leu Asn His Gly Asp Tyr Trp
225                 230                 235                 240
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Thr Ser Gly Gly Gly Gly
                245                 250                 255
Ser Asp Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
            260                 265                 270
Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg
        275                 280                 285
Tyr Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
    290                 295                 300
Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Ala Asp Ser
305                 310                 315                 320
Val Lys Gly Arg Phe Thr Ile Thr Thr Asp Lys Ser Thr Ser Thr Ala
                325                 330                 335
Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr
            340                 345                 350
Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln
        355                 360                 365
Gly Thr Thr Val Thr Val Ser Ser Gly Glu Gly Thr Ser Thr Gly Ser
    370                 375                 380
Gly Gly Ser Gly Gly Ser Gly Gly Ala Asp Asp Ile Val Leu Thr Gln
385                 390                 395                 400
Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser
                405                 410                 415
Cys Arg Ala Ser Gln Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys
            420                 425                 430
Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala
        435                 440                 445
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
    450                 455                 460
Ser Leu Thr Ile Asn Ser Leu Glu Ala Glu Asp Ala Ala Thr Tyr Tyr
465                 470                 475                 480
Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys
                485                 490                 495
Val Glu Ile Lys
            500


<210> 512
<211> 498
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-nGPC3 (GPC3A-045) scFv-anti-CD3 scFv
amino acid sequence

<400> 512
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
```

```
 1               5                  10                  15
Arg Val Ile Ile Ser Cys Pro Gly Ser Thr Ser Asn Ile Gly Thr Asn
            20                  25                  30
Thr Val Asn Trp Tyr Gln Gln Phe Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65                  70                  75                  80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85                  90                  95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100                 105                 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125
Leu Glu Met Ala Gln Met Gln Leu Val Gln Ser Gly Gly Gly Leu Val
    130                 135                 140
Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145                 150                 155                 160
Phe Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly
                165                 170                 175
Leu Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr
            180                 185                 190
Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
        195                 200                 205
Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
    210                 215                 220
Val Tyr Tyr Cys Ala Arg Ala Ser Asp Leu Tyr Gly Asp Trp Gly Gln
225                 230                 235                 240
Gly Thr Leu Val Thr Val Ser Ser Thr Ser Gly Gly Gly Gly Ser Asp
                245                 250                 255
Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser
            260                 265                 270
Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr
        275                 280                 285
Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly
    290                 295                 300
Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Ala Asp Ser Val Lys
305                 310                 315                 320
Gly Arg Phe Thr Ile Thr Thr Asp Lys Ser Thr Ser Thr Ala Tyr Met
                325                 330                 335
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr Cys Ala
            340                 345                 350
Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr
        355                 360                 365
Thr Val Thr Val Ser Ser Gly Glu Gly Thr Ser Thr Gly Ser Gly Gly
    370                 375                 380
Ser Gly Gly Ser Gly Gly Ala Asp Asp Ile Val Leu Thr Gln Ser Pro
385                 390                 395                 400
Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg
                405                 410                 415
Ala Ser Gln Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly
```

            420   425   430
Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly
        435   440   445
Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu
    450   455   460
Thr Ile Asn Ser Leu Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
465   470   475   480
Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu
                485   490   495
Ile Lys

<210> 513
<211> 503
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-nGPC3 (GPC3A-046) scFv-anti-CD3 scFv
amino acid sequence

<400> 513
Gln Ala Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Phe Gly Ser Asn
            20   25   30
Thr Val His Trp Tyr Gln Gln Val Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Phe Ser Asn Thr Gln Arg Pro Ser Glu Ile Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Thr Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130   135   140
Lys Pro Gly Ala Ser Val Thr Val Ser Cys Lys Ala Ser Gly Tyr Arg
145   150   155   160
Phe Ser Asn Tyr Gly Val Ser Trp Val Arg Gln Ala Pro Gly Gln Gly
                165   170   175
Leu Glu Trp Met Gly Trp Ile Ser Gly Ser Asn Gly Asn Thr Asn Tyr
            180   185   190
Ala Gln Lys Phe Leu Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr
        195   200   205
Thr Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Gly Asn Arg Arg Tyr Tyr Ser Pro Ile Ile

225 230 235 240
Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Thr Ser Gly
245 250 255
Gly Gly Gly Ser Asp Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
260 265 270
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr
275 280 285
Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly
290 295 300
Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr
305 310 315 320
Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Thr Thr Asp Lys Ser Thr
325 330 335
Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala
340 345 350
Thr Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr
355 360 365
Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Gly Thr Ser
370 375 380
Thr Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ala Asp Asp Ile Val
385 390 395 400
Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala
405 410 415
Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Tyr Met Asn Trp Tyr
420 425 430
Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser
435 440 445
Lys Val Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
450 455 460
Thr Asp Tyr Ser Leu Thr Ile Asn Ser Leu Glu Ala Glu Asp Ala Ala
465 470 475 480
Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Gly
485 490 495
Gly Thr Lys Val Glu Ile Lys
500

<210> 514
<211> 497
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-nGPC3 (GPC3A-055) scFv-anti-CD3 scFv
amino acid sequence

<400> 514
Gln Pro Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
1 5 10 15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr

```
        35                  40                  45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65                  70                  75                  80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85                  90                  95
Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly
            100                 105                 110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
        115                 120                 125
Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
    130                 135                 140
Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
145                 150                 155                 160
Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
                165                 170                 175
Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln
            180                 185                 190
Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr
        195                 200                 205
Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr
    210                 215                 220
Tyr Cys Ala Arg Trp His Gly Gly Pro Tyr Asp Tyr Trp Gly Gln Gly
225                 230                 235                 240
Thr Leu Val Thr Val Ser Ser Thr Ser Gly Gly Gly Gly Ser Asp Val
                245                 250                 255
Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val
            260                 265                 270
Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met
        275                 280                 285
His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr
    290                 295                 300
Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Ala Asp Ser Val Lys Gly
305                 310                 315                 320
Arg Phe Thr Ile Thr Thr Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu
                325                 330                 335
Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr Cys Ala Arg
            340                 345                 350
Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr
        355                 360                 365
Val Thr Val Ser Ser Gly Glu Gly Thr Ser Thr Gly Ser Gly Gly Ser
    370                 375                 380
Gly Gly Ser Gly Gly Ala Asp Asp Ile Val Leu Thr Gln Ser Pro Ala
385                 390                 395                 400
Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala
                405                 410                 415
Ser Gln Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys
            420                 425                 430
Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val
        435                 440                 445
Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr
```

    450   455   460
Ile Asn Ser Leu Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln
465   470   475   480
Trp Ser Ser Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
                485   490   495
Lys

<210> 515
<211> 499
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-nGPC3 (GPC3A-058) scFv-anti-CD3 scFv
amino acid sequence

<400> 515
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1   5   10   15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20   25   30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
        35   40   45
Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50   55   60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65   70   75   80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
                85   90   95
Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly Gly
            100   105   110
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu Met
        115   120   125
Ala Gln Val Gln Leu Val Gln Ser Gly Ala Asp Val Arg Lys Pro Gly
    130   135   140
Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Ser
145   150   155   160
His Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
                165   170   175
Leu Gly Trp Ile Ser Pro Tyr Thr Gly Asn Thr Asn Tyr Ala Gln Lys
            180   185   190
Phe Gln Gly Arg Val Thr Met Ala Thr Asp Thr Ser Thr Ser Thr Ala
        195   200   205
Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Ile Tyr Tyr
    210   215   220
Cys Ala Arg Gly Lys Arg Thr Leu Ala Ser Cys Phe Asp Tyr Trp Gly
225   230   235   240
Gln Gly Thr Leu Val Thr Val Ser Ser Thr Ser Gly Gly Gly Gly Ser
                245   250   255
Asp Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala

```
            260                 265                 270
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
        275                 280                 285
Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
    290                 295                 300
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Ala Asp Ser Val
305                 310                 315                 320
Lys Gly Arg Phe Thr Ile Thr Thr Asp Lys Ser Thr Ser Thr Ala Tyr
                325                 330                 335
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
            340                 345                 350
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
        355                 360                 365
Thr Thr Val Thr Val Ser Ser Gly Glu Gly Thr Ser Thr Gly Ser Gly
    370                 375                 380
Gly Ser Gly Gly Ser Gly Gly Ala Asp Asp Ile Val Leu Thr Gln Ser
385                 390                 395                 400
Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys
                405                 410                 415
Arg Ala Ser Gln Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro
            420                 425                 430
Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser
        435                 440                 445
Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser
    450                 455                 460
Leu Thr Ile Asn Ser Leu Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
465                 470                 475                 480
Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Gly Gly Thr Lys Val
                485                 490                 495
Glu Ile Lys
```

<210> 516
<211> 472
<212> PRT
<213> Artificial Sequence

<220>
<223> ET230-37-28Z CAR amino acid sequence

<400> 516

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Ile Ser Cys Ser Gly Thr Arg Ser Asn Ile Gly Ser Asp
            20                  25                  30
Tyr Val Ser Trp Tyr Gln His Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45
Val Tyr Gly Asp Asn Leu Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60
Ala Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65                  70                  75                  80
```

```
Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Tyr Thr Leu
                85                  90                  95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100                 105                 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125
Leu Glu Met Ala Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
    130                 135                 140
Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145                 150                 155                 160
Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
                165                 170                 175
Leu Glu Trp Val Ser Val Ile Tyr Ser Gly Gly Ser Ser Thr Tyr Tyr
            180                 185                 190
Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
        195                 200                 205
Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
    210                 215                 220
Val Tyr Tyr Cys Ala Arg Thr Ser Tyr Leu Asn His Gly Asp Tyr Trp
225                 230                 235                 240
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ala Ala Ile Glu Val
                245                 250                 255
Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser Asn Gly Thr Ile
            260                 265                 270
Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro Leu Phe Pro Gly
        275                 280                 285
Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala
    290                 295                 300
Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg
305                 310                 315                 320
Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro
                325                 330                 335
Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro
            340                 345                 350
Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala
        355                 360                 365
Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu
    370                 375                 380
Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly
385                 390                 395                 400
Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu
                405                 410                 415
Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser
            420                 425                 430
Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly
        435                 440                 445
Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu
    450                 455                 460
His Met Gln Ala Leu Pro Pro Arg
465                 470
```

<210> 517
<211> 248
<212> PRT
<213> Artificial Sequence

<220>
<223> ET230-45-28Z CAR amino acid sequence

<400> 517
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1   5   10   15
Arg Val Ile Ile Ser Cys Pro Gly Ser Thr Ser Asn Ile Gly Thr Asn
            20   25   30
Thr Val Asn Trp Tyr Gln Gln Phe Pro Gly Thr Ala Pro Lys Leu Leu
        35   40   45
Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50   55   60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65   70   75   80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85   90   95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100   105   110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115   120   125
Leu Glu Met Ala Gln Met Gln Leu Val Gln Ser Gly Gly Gly Leu Val
    130   135   140
Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145   150   155   160
Phe Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly
                165   170   175
Leu Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr
            180   185   190
Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
        195   200   205
Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
    210   215   220
Val Tyr Tyr Cys Ala Arg Ala Ser Asp Leu Tyr Gly Asp Trp Gly Gln
225   230   235   240
Gly Thr Leu Val Thr Val Ser Ser
                245

<210> 518
<211> 253
<212> PRT
<213> Artificial Sequence

<220>
<223> ET230-46-28Z CAR amino acid sequence

<400> 518

```
Gln Ala Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1               5                  10                  15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Phe Gly Ser Asn
            20                  25                  30
Thr Val His Trp Tyr Gln Gln Val Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45
Ile Phe Ser Asn Thr Gln Arg Pro Ser Glu Ile Pro Asp Arg Phe Ser
    50                  55                  60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
65                  70                  75                  80
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
                85                  90                  95
Thr Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
            100                 105                 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125
Leu Glu Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
    130                 135                 140
Lys Pro Gly Ala Ser Val Thr Val Ser Cys Lys Ala Ser Gly Tyr Arg
145                 150                 155                 160
Phe Ser Asn Tyr Gly Val Ser Trp Val Arg Gln Ala Pro Gly Gln Gly
                165                 170                 175
Leu Glu Trp Met Gly Trp Ile Ser Gly Ser Asn Gly Asn Thr Asn Tyr
            180                 185                 190
Ala Gln Lys Phe Leu Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr
        195                 200                 205
Thr Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala
    210                 215                 220
Val Tyr Tyr Cys Ala Arg Gly Asn Arg Arg Tyr Tyr Ser Pro Ile Ile
225                 230                 235                 240
Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245                 250
```

<210> 519
<211> 247
<212> PRT
<213> Artificial Sequence

<220>
<223> ET230-55-28Z CAR amino acid sequence

<400> 519

```
Gln Pro Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
 1               5                  10                  15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
            20                  25                  30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35                  40                  45
Tyr Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
```

65 70 75 80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
85 90 95
Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly
100 105 110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu
115 120 125
Met Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro
130 135 140
Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
145 150 155 160
Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
165 170 175
Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln
180 185 190
Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr
195 200 205
Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr
210 215 220
Tyr Cys Ala Arg Trp His Gly Gly Pro Tyr Asp Tyr Trp Gly Gln Gly
225 230 235 240
Thr Leu Val Thr Val Ser Ser
245

<210> 520
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> ET230-58-28Z CAR amino acid sequence

<400> 520
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Lys
1 5 10 15
Thr Ala Arg Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val
20 25 30
His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr
35 40 45
Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65 70 75 80
Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser Asp His
85 90 95
Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Ser Arg Gly Gly
100 105 110
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu Met
115 120 125
Ala Gln Val Gln Leu Val Gln Ser Gly Ala Asp Val Arg Lys Pro Gly
130 135 140

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Ser
145 150 155 160
His Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
165 170 175
Leu Gly Trp Ile Ser Pro Tyr Thr Gly Asn Thr Asn Tyr Ala Gln Lys
180 185 190
Phe Gln Gly Arg Val Thr Met Ala Thr Asp Thr Ser Thr Ser Thr Ala
195 200 205
Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Ile Tyr Tyr
210 215 220
Cys Ala Arg Gly Lys Arg Thr Leu Ala Ser Cys Phe Asp Tyr Trp Gly
225 230 235 240
Gln Gly Thr Leu Val Thr Val Ser Ser
245

<210> 521
<211> 247
<212> PRT
<213> Artificial Sequence

<220>
<223> ET230-87-28Z CAR amino acid sequence

<400> 521
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15
Glu Pro Ala Ser Val Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95
Leu Gln Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Ser Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125
Gly Ser Leu Glu Met Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu
130 135 140
Val Arg Lys Pro Gly Ser Ser Val Lys Val Ser Cys Gln Ala Ser Gly
145 150 155 160
Gly Thr Phe Gly Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly
165 170 175
Gln Gly Leu Glu Trp Met Gly Arg Ile Ile Pro Val Leu Gly Arg Thr
180 185 190
Lys Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Val Thr Ala Asp Thr
195 200 205
Ser Thr Ser Thr Val Tyr Met Glu Leu Thr Ser Leu Thr Ser Glu Asp

210 215 220
Thr Ala Val Tyr Tyr Cys Ala Arg Thr Asn Asp Ser Trp Gly Gln Gly
225 230 235 240
Thr Leu Val Thr Val Ser Ser
245

<210> 522
<211> 288
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-AFP158/HLA-A*02:01-caTCR-6MD delta

<400> 522
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15
Ser Leu Thr Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Pro Asn Tyr
20 25 30
Trp Ile Thr Trp Val Arg Gln Met Ser Gly Gly Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Asp Pro Gly Asp Ser Tyr Thr Thr Tyr Asn Pro Ser Phe
50 55 60
Gln Gly His Val Thr Ile Ser Ile Asp Lys Ser Thr Asn Thr Ala Tyr
65 70 75 80
Leu His Trp Asn Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95
Ala Arg Tyr Tyr Val Ser Leu Val Asp Ile Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195 200 205
Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Glu Val Lys Thr
210 215 220
Asp Ser Thr Asp His Val Lys Pro Lys Glu Thr Glu Asn Thr Lys Gln
225 230 235 240
Pro Ser Lys Ser Cys His Lys Pro Lys Ala Ile Val His Thr Glu Lys
245 250 255
Val Asn Met Met Ser Leu Thr Val Leu Gly Leu Arg Met Leu Phe Ala
260 265 270
Lys Thr Val Ala Val Asn Phe Leu Leu Thr Ala Lys Leu Phe Phe Leu
275 280 285

<210> 523
<211> 283
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-AFP158/HLA-A*02:01-caTCR-6MD gamma

<400> 523
Gln Ser Val Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
 1   5   10   15
Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
            20   25   30
Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
        35   40   45
Met Ile Tyr Asp Val Asn Asn Arg Pro Ser Glu Val Ser Asn Arg Phe
    50   55   60
Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65   70   75   80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Thr Gly
                85   90   95
Ser Arg Ala Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
            100   105   110
Pro Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
        115   120   125
Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
    130   135   140
Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro Val Lys
145   150   155   160
Ala Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn Lys Tyr
                165   170   175
Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
            180   185   190
Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
        195   200   205
Thr Val Ala Pro Thr Glu Cys Ser Pro Ile Lys Thr Asp Val Ile Thr
    210   215   220
Met Asp Pro Lys Asp Asn Cys Ser Lys Asp Ala Asn Asp Thr Leu Leu
225   230   235   240
Leu Gln Leu Thr Asn Thr Ser Ala Tyr Tyr Met Tyr Leu Leu Leu Leu
                245   250   255
Leu Lys Ser Val Val Tyr Phe Ala Ile Ile Thr Cys Cys Leu Leu Arg
            260   265   270
Arg Thr Ala Phe Cys Cys Asn Gly Glu Lys Ser
        275   280

<210> 524
<211> 180
<212> DNA

<213> Artificial Sequence

<220>
<223> 6NFAT response element

<400> 524
ggaggaaaaa ctgtttcata cagaaggcgt ggaggaaaaa ctgtttcata cagaaggcgt 60
ggaggaaaaa ctgtttcata cagaaggcgt ggaggaaaaa ctgtttcata cagaaggcgt 120
ggaggaaaaa ctgtttcata cagaaggcgt ggaggaaaaa ctgtttcata cagaaggcgt 180

<210> 525
<211> 149
<212> DNA
<213> Artificial Sequence

<220>
<223> TA promoter

<400> 525
gccgccccga ctgcatctgc gtgttccaat tcgccaatga caagacgctg ggcggggttt 60
gtgtcatcat agaactaaag acatgcaaat atatttcttc cggggacacc gccagcaaac 120
gcgagcaacg ggccacgggg atgaagcag 149

<210> 526
<211> 335
<212> DNA
<213> Artificial Sequence

<220>
<223> NFAT-derived promoter

<400> 526
ggaggaaaaa ctgtttcata cagaaggcgt ggaggaaaaa ctgtttcata cagaaggcgt 60
ggaggaaaaa ctgtttcata cagaaggcgt ggaggaaaaa ctgtttcata cagaaggcgt 120
ggaggaaaaa ctgtttcata cagaaggcgt ggaggaaaaa ctgtttcata cagaaggcgt 180
ctcgaggccg ccccgactgc atctgcgtgt tccaattcgc caatgacaag acgctgggcg 240
gggtttgtgt catcatagaa ctaaagacat gcaaatatat ttcttccggg gacaccgcca 300
gcaaacgcga gcaacgggcc acggggatga agcag 335

<210> 527
<211> 544
<212> DNA
<213> Artificial Sequence

<220>
<223> EF1-alpha promoter

<400> 527
ggatctgcga tcgctccggt gcccgtcagt gggcagagcg cacatcgccc acagtccccg 60
agaagttggg gggaggggtc ggcaattgaa cgggtgccta gagaaggtgg cgcggggtaa 120
actgggaaag tgatgtcgtg tactggctcc gccttttttcc cgagggtggg ggagaaccgt 180

atataagtgc agtagtcgcc gtgaacgttc tttttcgcaa cgggtttgcc gccagaacac 240
agctgaagct tcgaggggct cgcatctctc cttcacgcgc ccgccgccct acctgaggcc 300
gccatccacg ccggttgagt cgcgttctgc cgcctcccgc ctgtggtgcc tcctgaactg 360
cgtccgccgt ctaggtaagt ttaaagctca ggtcgagacc gggcctttgt ccggcgctcc 420
cttggagcct acctagactc agccggctct ccacgctttg cctgaccctg cttgctcaac 480
tctacgtctt tgtttcgttt tctgttctgc gccgttacag atccaagctg tgaccggcgc 540
ctac 544

<210> 528
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> EF1-alpha promoter

<400> 528
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
 1   5   10   15
Ser Leu Thr Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Pro Asn Tyr
            20   25   30
Trp Ile Thr Trp Val Arg Gln Met Ser Gly Gly Gly Leu Glu Trp Met
        35   40   45
Gly Arg Ile Asp Pro Gly Asp Ser Tyr Thr Thr Tyr Asn Pro Ser Phe
    50   55   60
Gln Gly His Val Thr Ile Ser Ile Asp Lys Ser Thr Asn Thr Ala Tyr
65   70   75   80
Leu His Trp Asn Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85   90   95
Ala Arg Tyr Tyr Val Ser Leu Val Asp Ile Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser
        115

<210> 529
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> IgVL domain of anti-AFP antibody

<400> 529
Gln Ser Val Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
 1   5   10   15
Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
            20   25   30
Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
        35   40   45
Met Ile Tyr Asp Val Asn Asn Arg Pro Ser Glu Val Ser Asn Arg Phe
    50   55   60

Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Thr Gly
85 90 95
Ser Arg Ala Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 530
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> CSR1

<400> 530
Ala Ala Ala Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu
1 5 10 15
Lys Ser Asn Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro
20 25 30
Ser Pro Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val
35 40 45
Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe
50 55 60
Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp
65 70 75 80
Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr
85 90 95
Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser
100 105 110

<210> 531
<211> 360
<212> PRT
<213> Artificial Sequence

<220>
<223> Anti-GPC3 CSR

<400> 531
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Thr Arg Ser Asn Ile Gly Ser Asp
20 25 30
Tyr Val Ser Trp Tyr Gln His Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Val Tyr Gly Asp Asn Leu Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
50 55 60
Ala Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65 70 75 80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Tyr Thr Leu
85 90 95
Asn Gly Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser
100 105 110
Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125
Leu Glu Met Ala Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
130 135 140
Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145 150 155 160
Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
165 170 175
Leu Glu Trp Val Ser Val Ile Tyr Ser Gly Gly Ser Ser Thr Tyr Tyr
180 185 190
Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
195 200 205
Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
210 215 220
Val Tyr Tyr Cys Ala Arg Thr Ser Tyr Leu Asn His Gly Asp Tyr Trp
225 230 235 240
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ala Ala Ile Glu Val
245 250 255
Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser Asn Gly Thr Ile
260 265 270
Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro Leu Phe Pro Gly
275 280 285
Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala
290 295 300
Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg
305 310 315 320
Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro
325 330 335
Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro
340 345 350
Arg Asp Phe Ala Ala Tyr Arg Ser
355 360

<210> 532
<211> 288
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-AFP158/HLA-A*02:01-caTCR-1-0 delta

<400> 532
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15
Ser Leu Thr Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Pro Asn Tyr
20 25 30
Trp Ile Thr Trp Val Arg Gln Met Ser Gly Gly Gly Leu Glu Trp Met

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        35                  40                  45
Gly Arg Ile Asp Pro Gly Asp Ser Tyr Thr Thr Tyr Asn Pro Ser Phe
    50                  55                  60
Gln Gly His Val Thr Ile Ser Ile Asp Lys Ser Thr Asn Thr Ala Tyr
65                  70                  75                  80
Leu His Trp Asn Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95
Ala Arg Tyr Tyr Val Ser Leu Val Asp Ile Trp Gly Gln Gly Thr Leu
            100                 105                 110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205
Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Glu Val Lys Thr
    210                 215                 220
Asp Ser Thr Asp His Val Lys Pro Lys Glu Thr Glu Asn Thr Lys Gln
225                 230                 235                 240
Pro Ser Lys Ser Cys His Lys Pro Lys Ala Ile Val His Thr Glu Lys
                245                 250                 255
Val Asn Met Met Ser Leu Thr Val Leu Gly Leu Arg Met Leu Phe Ala
            260                 265                 270
Lys Thr Val Ala Val Asn Phe Leu Leu Thr Ala Lys Leu Phe Phe Leu
        275                 280                 285


<210> 533
<211> 283
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-AFP158/HLA-A*02:01-caTCR-1-0 gamma

<400> 533
Gln Ser Val Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
 1               5                  10                  15
Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
            20                  25                  30
Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
        35                  40                  45
Met Ile Tyr Asp Val Asn Asn Arg Pro Ser Glu Val Ser Asn Arg Phe
    50                  55                  60
Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65                  70                  75                  80
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Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Thr Gly
                85   90   95
Ser Arg Ala Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
            100   105   110
Pro Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
        115   120   125
Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
    130   135   140
Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro Val Lys
145   150   155   160
Ala Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn Lys Tyr
                165   170   175
Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
            180   185   190
Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
        195   200   205
Thr Val Ala Pro Thr Glu Cys Ser Pro Ile Lys Thr Asp Val Ile Thr
    210   215   220
Met Asp Pro Lys Asp Asn Cys Ser Lys Asp Ala Asn Asp Thr Leu Leu
225   230   235   240
Leu Gln Leu Thr Asn Thr Ser Ala Tyr Tyr Met Tyr Leu Leu Leu Leu
                245   250   255
Leu Lys Ser Val Val Tyr Phe Ala Ile Ile Thr Cys Cys Leu Leu Arg
            260   265   270
Arg Thr Ala Phe Cys Cys Asn Gly Glu Lys Ser
        275   280

<210> 534
<211> 288
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-AFP158/HLA-A*02:01-caTCR-1-TM5 delta

<400> 534
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
 1   5   10   15
Ser Leu Thr Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Pro Asn Tyr
            20   25   30
Trp Ile Thr Trp Val Arg Gln Met Ser Gly Gly Gly Leu Glu Trp Met
        35   40   45
Gly Arg Ile Asp Pro Gly Asp Ser Tyr Thr Thr Tyr Asn Pro Ser Phe
    50   55   60
Gln Gly His Val Thr Ile Ser Ile Asp Lys Ser Thr Asn Thr Ala Tyr
65   70   75   80
Leu His Trp Asn Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85   90   95
Ala Arg Tyr Tyr Val Ser Leu Val Asp Ile Trp Gly Gln Gly Thr Leu
            100   105   110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu

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        115                 120                 125
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205
Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Glu Val Lys Thr
    210                 215                 220
Asp Ser Thr Asp His Val Lys Pro Lys Glu Thr Glu Asn Thr Lys Gln
225                 230                 235                 240
Pro Ser Lys Ser Cys His Lys Pro Lys Ala Ile Val His Thr Glu Lys
                245                 250                 255
Val Asn Met Met Ser Leu Thr Val Leu Gly Leu Arg Val Leu Phe Ala
            260                 265                 270
Lys Thr Val Ala Val Asn Phe Leu Leu Thr Ala Lys Leu Phe Phe Leu
        275                 280                 285


<210> 535
<211> 283
<212> PRT
<213> Artificial Sequence

<220>
<223> anti-AFP158/HLA-A*02:01-caTCR-1-TM5 gamma

<400> 535
Gln Ser Val Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
 1               5                  10                  15
Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
            20                  25                  30
Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
        35                  40                  45
Met Ile Tyr Asp Val Asn Asn Arg Pro Ser Glu Val Ser Asn Arg Phe
    50                  55                  60
Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65                  70                  75                  80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Thr Gly
                85                  90                  95
Ser Arg Ala Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln
            100                 105                 110
Pro Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
        115                 120                 125
Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
    130                 135                 140
Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro Val Lys
145                 150                 155                 160
```

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Ala Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn Lys Tyr
                165                 170                 175
Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
            180                 185                 190
Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
        195                 200                 205
Thr Val Ala Pro Thr Glu Cys Ser Pro Ile Lys Thr Asp Val Ile Thr
    210                 215                 220
Met Asp Pro Lys Asp Asn Cys Ser Lys Asp Ala Asn Asp Thr Leu Leu
225                 230                 235                 240
Leu Gln Leu Thr Asn Thr Ser Ala Tyr Leu Val Tyr Leu Leu Leu Leu
                245                 250                 255
Leu Lys Ser Val Val Tyr Phe Val Ile Val Thr Cys Cys Leu Leu Arg
            260                 265                 270
Arg Thr Ala Phe Cys Cys Asn Gly Glu Lys Ser
        275                 280
```

<210> 536
<211> 43
<212> PRT
<213> Artificial Sequence

<220>
<223> GPC33 fragment

<400> 536

```
Leu Ala Glu Leu Ala Tyr Asp Leu Asp Val Asp Asp Ala Pro Gly Asn
 1               5                  10                  15
Ser Gln Gln Ala Thr Pro Lys Asp Asn Glu Ile Ser Thr Phe His Asn
            20                  25                  30
Leu Gly Asn Val His Ser Pro Leu Lys Leu Leu
        35                  40
```