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

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3059820 A1 2018/11/01

(21) 3 059 820

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2018/04/24
(87) Date publication PCT/PCT Publication Date: 2018/11/01
(85) Entrée phase nationale/National Entry: 2019/10/10
(86) N° demande PCT/PCT Application No.: US 2018/029221
(87) N° publication PCT/PCT Publication No.: 2018/200586
(30) Priorité/Priority: 2017/04/26 (US62/490,586)

(51) Cl.Int./Int.Cl. *C07K 16/30* (2006.01), *A61K 39/395* (2006.01), *A61P 35/00* (2006.01), *C07K 16/46* (2006.01), *C07K 19/00* (2006.01), *G01N 33/574* (2006.01)

(71) Demandeur/Applicant:
EUREKA THERAPEUTICS, INC., US

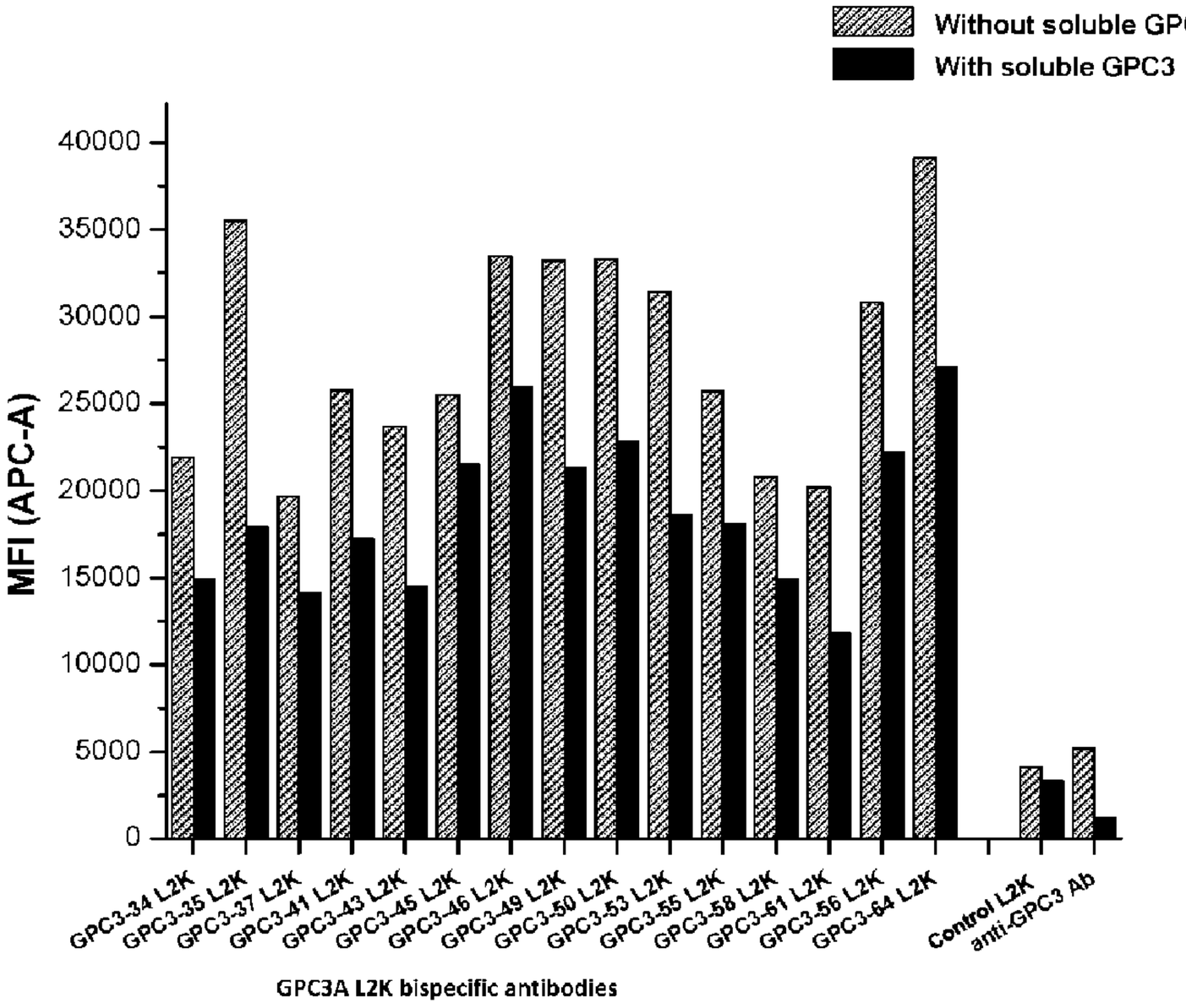
(72) Inventeurs/Inventors:
ZHANG, PENGBO, US;
XU, YIYANG, US;
MORALES, JAVIER, US;
NAKANO, YOKO, US;
LIU, HONG, US;
XIANG, JINGYI, US; ...

(54) Titre : CONSTRUCTIONS RECONNAISSANT SPECIFIQUEMENT LE GLYPICANE 3 ET UTILISATIONS DE CES DERNIERES

(54) Title: CONSTRUCTS SPECIFICALLY RECOGNIZING GLYPICAN 3 AND USES THEREOF

FIG. 6

Competition for Hep2 cell binding with and without soluble GPC3



(57) Abrégé/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.

CA 3059820 A1 2018/11/01

(21) 3 059 820

(13) A1

(72) Inventeurs(suite)/Inventors(continued): ACKER, TIMOTHY, US

(74) Agent: SMART & BIGGAR LLP

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
01 November 2018 (01.11.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/200586 A9

(51) International Patent Classification:
C07K 16/30 (2006.01) *G01N 33/574* (2006.01)
C07K 16/46 (2006.01) *A61K 39/395* (2006.01)
C07K 19/00 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/US2018/029221

(22) International Filing Date:
24 April 2018 (24.04.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/490,586 26 April 2017 (26.04.2017) US

(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).

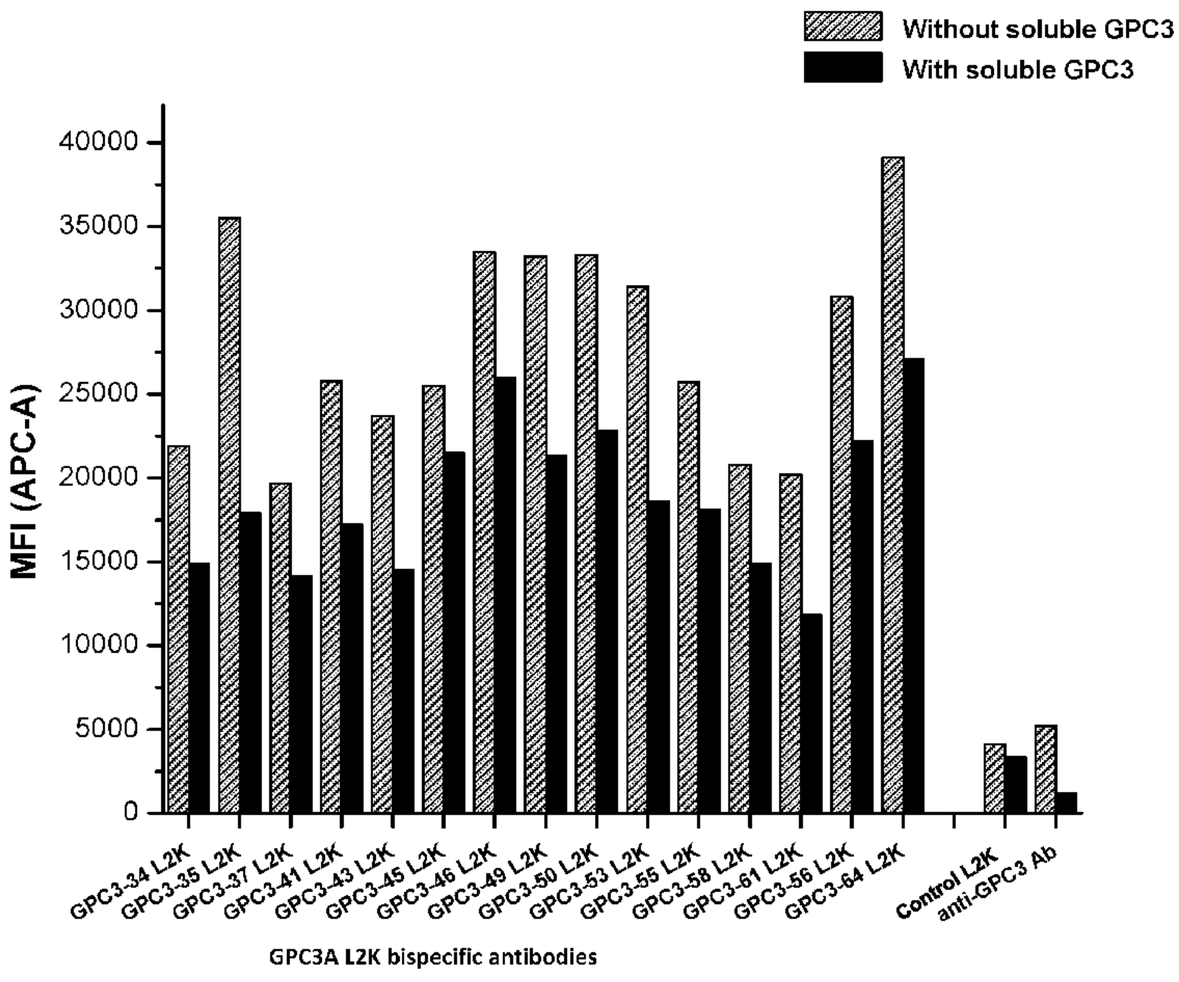
(74) Agent: **QI, Yan**; Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, California 94304-1018 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

(54) Title: CONSTRUCTS SPECIFICALLY RECOGNIZING GLYPICAN 3 AND USES THEREOF

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]

WO 2018/200586 A9

WO 2018/200586 A9

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

(48) Date of publication of this corrected version:

29 August 2019 (29.08.2019)

(15) Information about Correction:

see Notice of 29 August 2019 (29.08.2019)

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME	1	DE	2
CONTENANT LES PAGES	1	À	229

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME	1	OF	2
CONTAINING PAGES	1	TO	229

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CONSTRUCTS SPECIFICALLY RECOGNIZING GLYPICAN 3 AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 62/490,586, filed on April 26, 2017, the contents of which are 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 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] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

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.

[0009] Thus, in some embodiments, there is provided an anti-GPC3 construct (such as an isolated anti-GPC3 construct) comprising an antibody moiety specifically recognizing a cell

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 immunoconjugates 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 immunoconjugates 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[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.

[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.

<u>Anti-GPC3 antibody moiety specifically recognizing a cell surface-bound GPC3</u>

[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	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTISVDTSKNQFSLELSSVTAADTAVYYCARGYGGRFDYWGQGTLVTVSS	358	QPVLTQPPSASGTPGQRVTISCSGSSSNIGSNNVIWYQQLPGAAPKLLIYSNHRRPSGVPDRFSGSRSGTSASLAISGLQSEDEADYYCAAWDDSLDGYLFGTGTKVTVLG
GPC3A-035	308	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARSWTSGFDYWGQGTLVTVSS	359	QAVLTQPPSASQTPGQMVTISCSGTSSNIGSNYVFWYQQLPGTAPKLLIYKNFQRPSGVPGRFSGSKSGTAASLAISGLRSEDEADYFCAAWDDALSGYVFGAGTKVTVLG
GPC3A-037	309	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSVIYSGGSSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDYWGQGTLVTVSS	360	QSVLTQPPSVSAAPGQRVTISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSGIPDRFSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLG
GPC3A-038	310	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARKVTGYDSWGQGTLVTVSS	361	QSVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVLG

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	EVQLVQSGAEVKKPGASVKLSCKTSGYTFIDYYVYWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLGSEDTAVYYCARERRYSSSPSDHWGQGTLVTVSS	389	QSVLTQPPSASGTPGQRVTISCSGSNSNIGSDTVNWYQQLPGTAPKLLIYRDNQRPSGVPDRFSGSKSGTSASLAISGLQSEDEADYYCTTWDDSLNGLVFGGGTKVTVLG
GPC3B-60	339	EVQLVESGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARSLYSQPYIDGWSQGTLVTVSS	390	SYVLTQPPSVSVSPGQTATIACSGDNLENKFVYWYHQKPGQSPVLVMYEDNKRPSGIPERFSGSNSGNTAALTISGAQPMDEADYYCQTWDSPTGLFVFGTGTKVTVLG
GPC3B-63	340	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARSLHAMRWSQTMDSWGQGTLVTVSS	391	EIVLTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPTFGQGTKVEIKR
GPC3B-66	341	QVQLVQSGAEVKRPGASVKVSCKASGYTFIGQYLHWVRQAPGQGLEWMGRINPMTGVTNYAPKFQGRVTMTRDTSISTGYMEISRLRSDDTAVYYCARFSSGYSRDTWGQGTLVTVSS	392	QAVLTQPPSVSVAPGKTASITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHLYVFGTGTKVTVLG
GPC3B-68	342	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARYGYEGHDTWGQGTLVTVSS	393	QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNIKRPSGIPDRFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAGVFGGGTKLTVLG
GPC3B-71	343	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYTMNWVRQAPGKGLEWVSSISSSGSYIYYADSVKGRFTISRDNAKNSLFLQMDSLRAEDTAVYYCARQGHMWYVPVDAWGQGTLVTVSS	394	SYVLTQPPSVSVAPGKTARVTCGGNNIGSKSVHWYQQKPGQAPVLVMYYDSDRPSGIPERFSGSNSGNTATLTISSVEAGDEADYYCQVWDSSSDHYVFGTGTKVTVLG
GPC3B-76	344	QMQLVQSGAEVKEPGASVKVSCKASGYTFTDYYIHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARNYDWGQGTLVTVSS	395	QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSKSGTSATLGITGLQTGDEAAYYCGTWDSSLSAGVFGTGTKVTVLG
GPC3B-78	345	QMQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGYSSFFDSWGQGTLVTVSS	396	LPVLTQPPSVSVSPGQTASITCSGDKLGDKYAYWYQQKPGQSPVLVIYQDNKRPSGIPERFSGSNSGNTATLTISGTQAMDAADYYCQTWDRSTYVFGTGTKVTVLG
GPC3B-80	346	QVQLVQSGAEVKKPGSSVKVSCKASGGTLSRFAISWVRQAPGQGLEWMGGIIPIFRTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARMSKYYGSYSSYDEWGQGTLVTVSS	397	QAVLTQPPSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKSGTSAYLAISGLQSEDEADYYCAAWDDSLNGWGVFGGGTKLTVLG
GPC3B-81	347	EVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGRINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGLWDSWGQGTLVTVSS	398	SYELTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDLLYVFGTGTKVTVLG
GPC3B-82	348	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMEMSSLRSEDTAVYYCARYPVYMETSDFDSWGSRYSGDRLL	399	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGFDVHWYQQLPGTAPKLLIYDNNNRPSGVPDRFSGSKSDTSASLAITGLQAEDEADYYCQSFDSSLSGWVFGGGTKLTVLG
GPC3B-85	349	QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTNTGNPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARSSLYWMGSKWSRQTDMWGQGTLVTVSS	400	QAVLTQPPSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGYVFGTGTKVTVLG
GPC3B-87	350	EVQLVQSGAEVRKPGSSVKVSCQASGGTFGSYAISWVRQAPGQGLEWMGRIIPVLGRTKYAQKFQGRVTVTADTSTSTVYMELTSLTSEDTAVYYCARTNDSWGQGTLVTVSS	401	DVVMTQSPLSLPVTPGEPASVSCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKR

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-

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME	1	DE	2
CONTENANT LES PAGES	1	À	229

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME	1	OF	2
CONTAINING PAGES	1	TO	229

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CLAIMS

What is claimed is:

1. An isolated anti-Glypican 3 (GPC3) construct comprising an antibody moiety specifically recognizing a cell surface-bound GPC3.
2. The isolated anti-GPC3 construct of claim 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.
3. The isolated anti-GPC3 construct of claim 2, 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.
4. The isolated anti-GPC3 construct of any one of claims 1-3, wherein the antibody moiety specifically recognizes an epitope within human GPC3 comprising the amino acid sequence of any one of SEQ ID NOs: 460-464.
5. The isolated anti-GPC3 construct of any one of claims1-3, wherein the antibody moiety specifically recognizes an epitope within the C-terminal fragment of GPC3 lacking heparin sulfate side chain.
6. The isolated anti-GPC3 construct of any one of claims 1-5, wherein the antibody moiety does not specifically bind to the same or substantially the same GPC3 epitope competitively with GC33.
7. The isolated anti-GPC3 construct of any one of claims 1-6, wherein the GPC3 is expressed on the surface of a cancer cell.
8. An isolated anti-GPC3 construct comprising an antibody moiety specifically recognizing GPC3, wherein the antibody moiety comprises:

(a) 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; or

(b) 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.

9. The isolated anti-GPC3 construct of claim 8, wherein the antibody moiety comprises:

(a) 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; or

(b) 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.

10. 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 claims 1-9.

11. The isolated anti-GPC3 construct of any one of claims 1-10, 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).

12. The isolated anti-GPC3 construct of any one of claims 1-11, wherein the isolated anti-GPC3 construct is multispecific.
13. The isolated anti-GPC3 construct of any one of claims 1-12, wherein the antibody moiety specifically recognizing GPC3 is fused to an Fc fragment optionally via a linker.
14. The isolated anti-GPC3 construct of claim 13, wherein the isolated anti-GPC3 construct is a tandem scFv comprising two scFvs linked by a peptide linker.
15. The isolated anti-GPC3 construct of claim 13 or 14, wherein the isolated anti-GPC3 construct further comprises a second antibody moiety specifically recognizing a second antigen.
16. The isolated anti-GPC3 construct of claim 15, wherein the second antigen is an antigen on the surface of a T cell.
17. The isolated anti-GPC3 construct of claim 16, wherein the second antigen is selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM.
18. The isolated anti-GPC3 construct of claim 17, wherein the second antigen is CD3ε.
19. The isolated anti-GPC3 construct of any one of claims 1-12, 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.
20. The isolated anti-GPC3 construct of claim 19, wherein the intracellular signaling domain comprises a CD3ζ intracellular signaling sequence and a CD28 intracellular signaling sequence.
21. The isolated anti-GPC3 construct of any one of claims 1-12, 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.
22. The isolated anti-GPC3 construct of any one of claims 1-10, wherein the isolated anti-GPC3 construct is an immunoconjugate comprising the antibody moiety and an effector molecule.

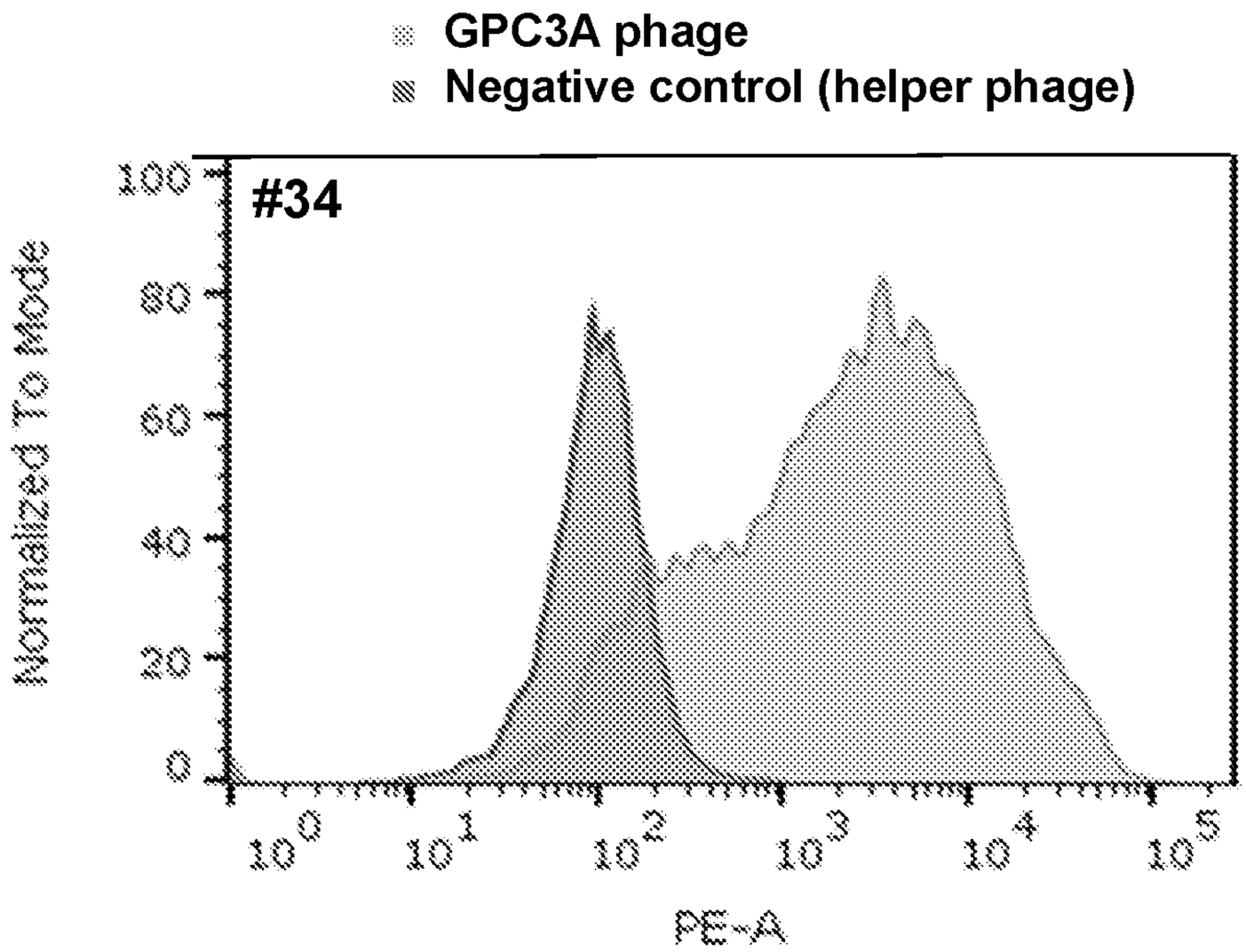
23. The isolated anti-GPC3 construct of claim 22, wherein the effector molecule is a therapeutic agent selected from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid.
24. The isolated anti-GPC3 construct of claim 22, wherein the effector molecule is a label.
25. An isolated nucleic acid encoding the polypeptide components of the isolated anti-GPC3 construct of any one of claims 1-24.
26. A vector comprising the isolated nucleic acid of claim 25.
27. An isolated host cell comprising the anti-GPC3 construct of any one of claims 1-24, isolated nucleic acid of claim 25, or the vector of 26.
28. An effector cell expressing the isolated anti-GPC3 construct of any one of claims 19-21.
29. The effector cell of claim 28, wherein the effector cell is a T cell.
30. The effector cell of claim 28 or 29, wherein the expression of the anti-GPC3 construct is induced by activation of the effector cell.
31. The effector cell of any one of claims 28-30, wherein the effector cell further comprises a chimeric antibody-T cell receptor (TCR) construct (caTCR).
32. A pharmaceutical composition comprising the isolated anti-GPC3 construct of any one of claims1-24 or the effect cell of any one of claims 28-31, and a pharmaceutically acceptable carrier.
33. A kit comprising the isolated anti-GPC3 construct of any one of claims 1-24, the isolated nucleic acid of claim 25, the vector of claim 26, the isolated host cell of claim 27, the effector cell of any one of claims 28-31, or the pharmaceutical composition of claim 32.
34. A method of detecting GPC3 in a sample, comprising contacting the sample with the isolated anti-GPC3 construct of claim 24 and detecting the presence of the label.
35. A method of treating an individual having a GPC3-positive disease, comprising administering to the individual an effective amount of the pharmaceutical composition of claim 32.
36. A method of diagnosing an individual having a GPC3-positive disease, comprising:

a) administering an effective amount of the isolated anti-GPC3 construct of claim 24 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.

37. A method of diagnosing an individual having an GPC3-positive disease, comprising:

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

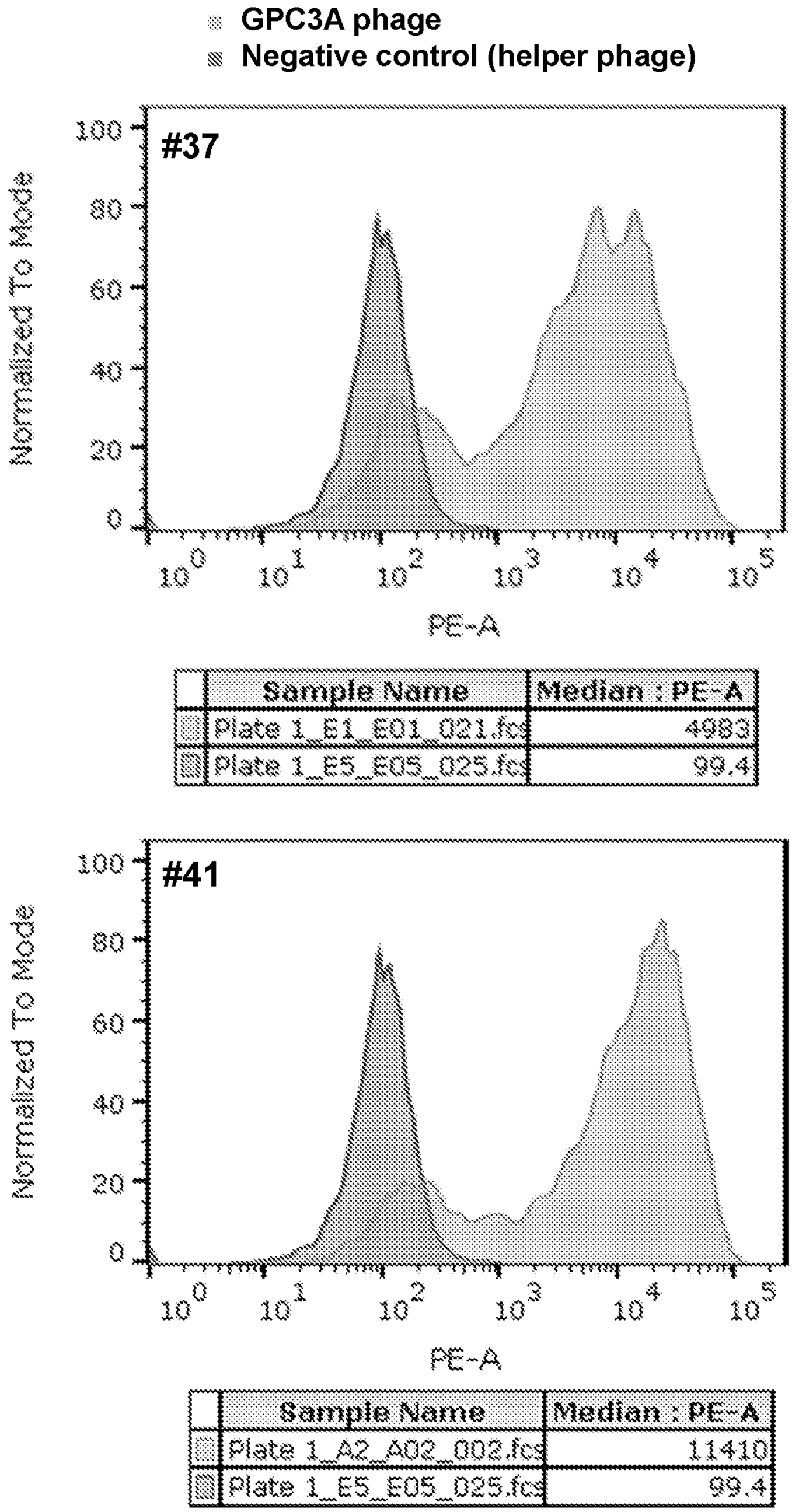

GPC3A phage
Negative control (helper phage)
#37
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
10^5
PE-A
Sample Name
Median : PE-A
Plate 1_E1_E01_021.fcs
4983
Plate 1_E5_E05_025.fcs
99.4
#41
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
10^5
PE-A
Sample Name
Median : PE-A
Plate 1_A2_A02_002.fcs
11410
Plate 1_E5_E05_025.fcs
99.4

FIG. 1C

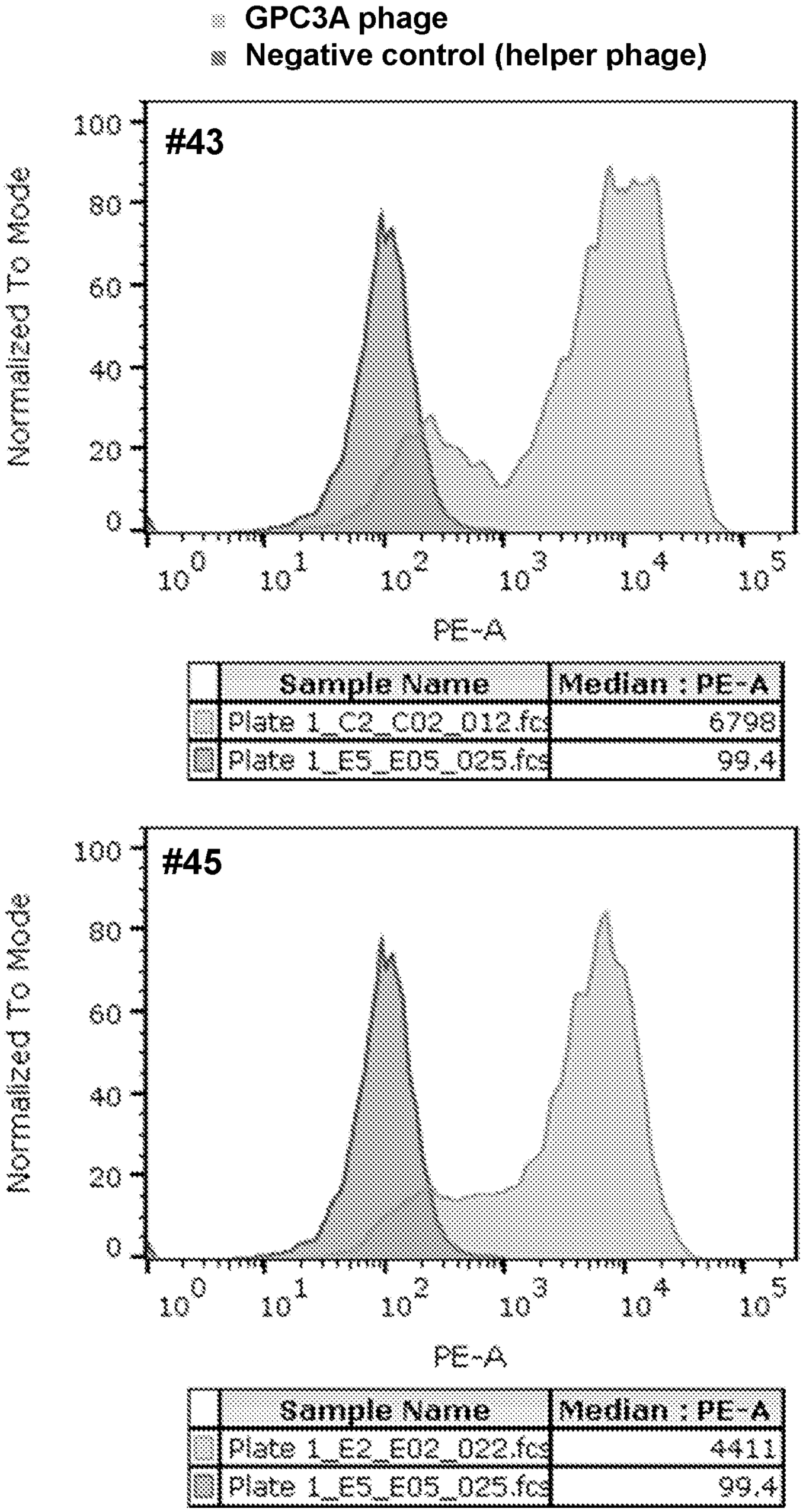

GPC3A phage
Negative control (helper phage)
#43
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_C2_C02_012.fcs
6798
Plate 1_E5_E05_025.fcs
99.4
#45
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_E2_E02_022.fcs
4411
Plate 1_E5_E05_025.fcs
99.4

FIG. 1D

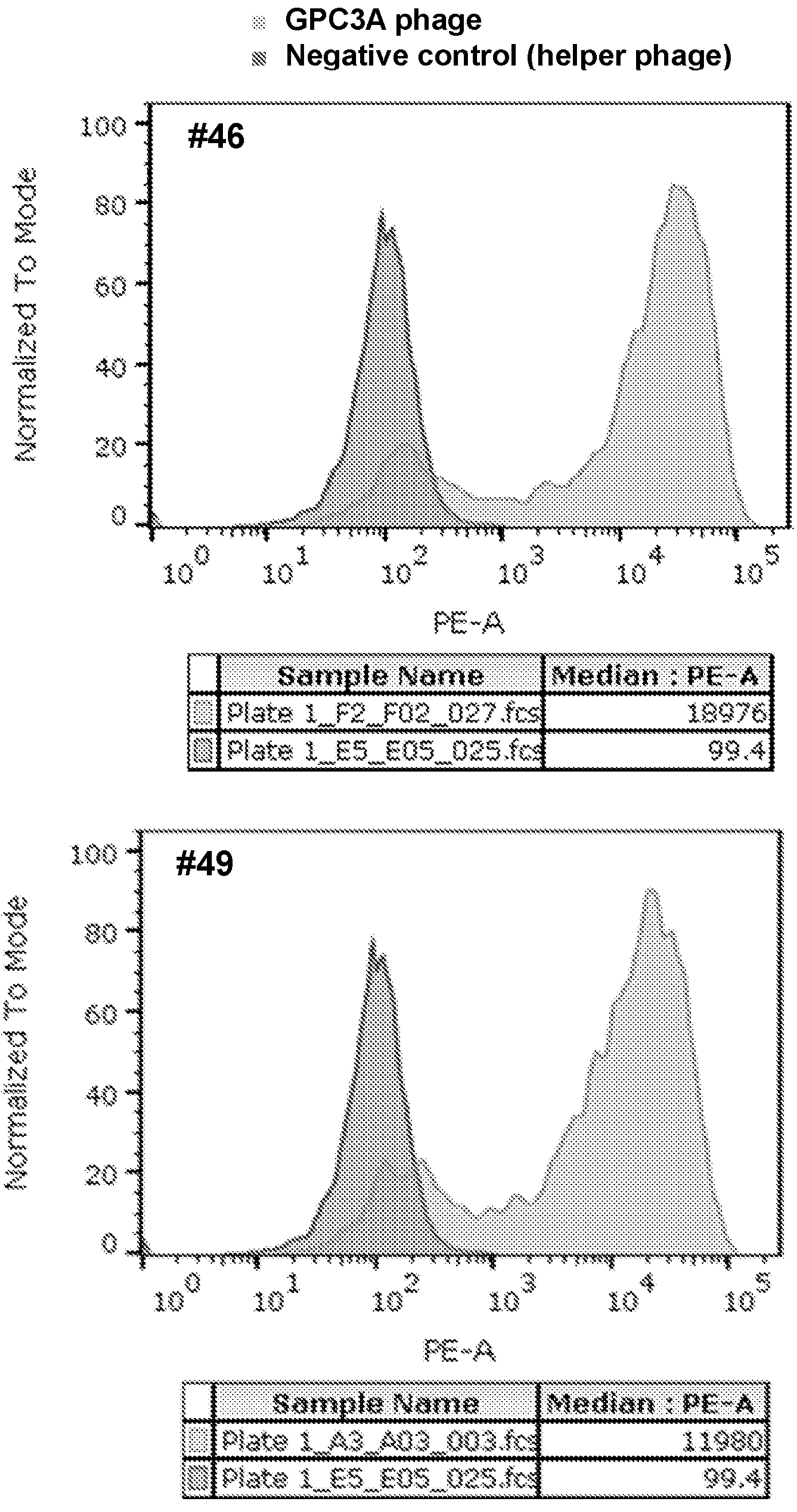

GPC3A phage
Negative control (helper phage)
#46
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
10^5
PE-A
Sample Name
Median : PE-A
Plate 1_F2_F02_027.fcs
18976
Plate 1_E5_E05_025.fcs
99.4
#49
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
10^5
PE-A
Sample Name
Median : PE-A
Plate 1_A3_A03_003.fcs
11980
Plate 1_E5_E05_025.fcs
99.4

FIG. 1E

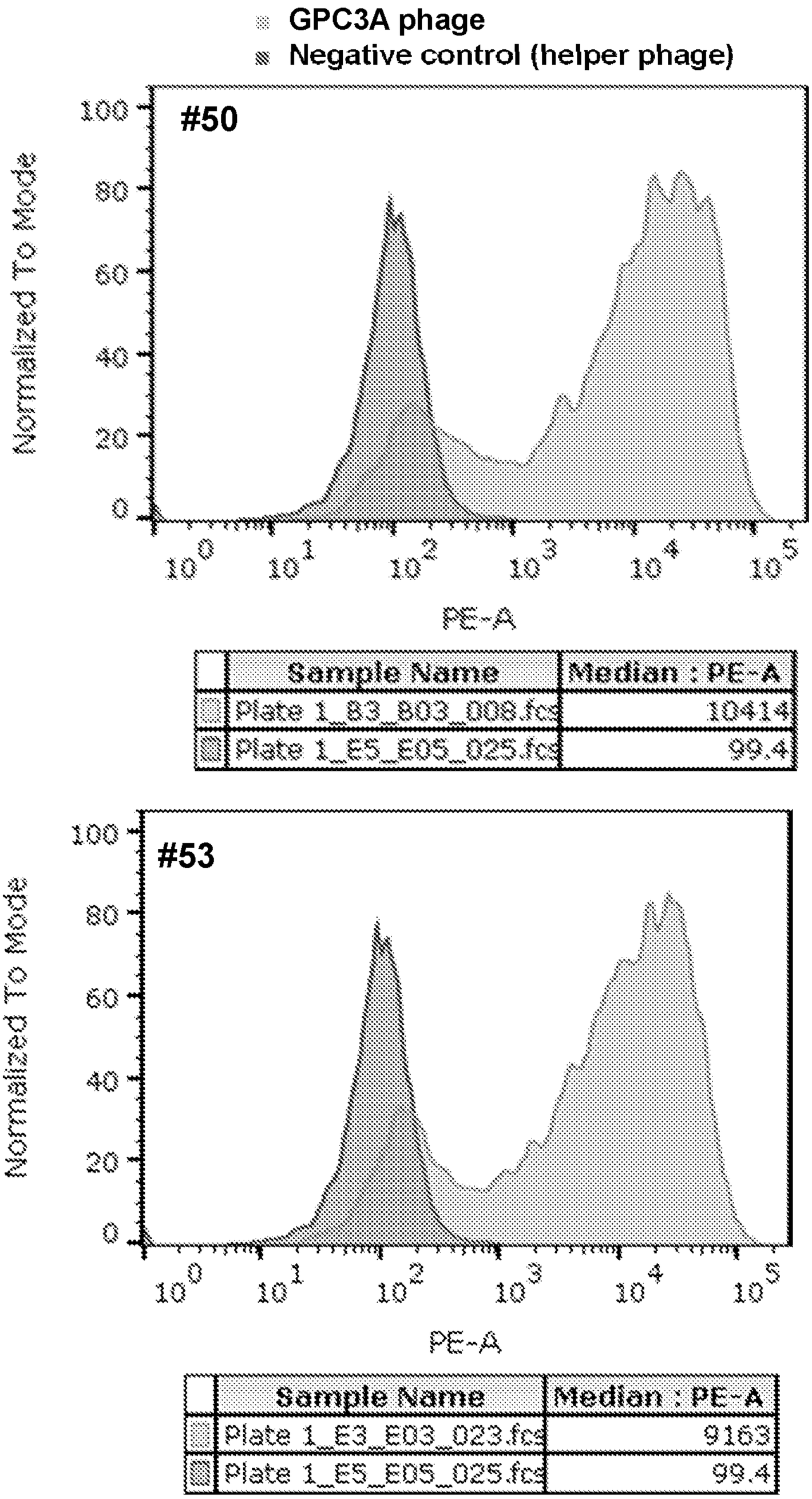

GPC3A phage
Negative control (helper phage)
#50
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_B3_B03_008.fcs
10414
Plate 1_E5_E05_025.fcs
99.4
#53
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_E3_E03_023.fcs
9163
Plate 1_E5_E05_025.fcs
99.4

FIG. 1F

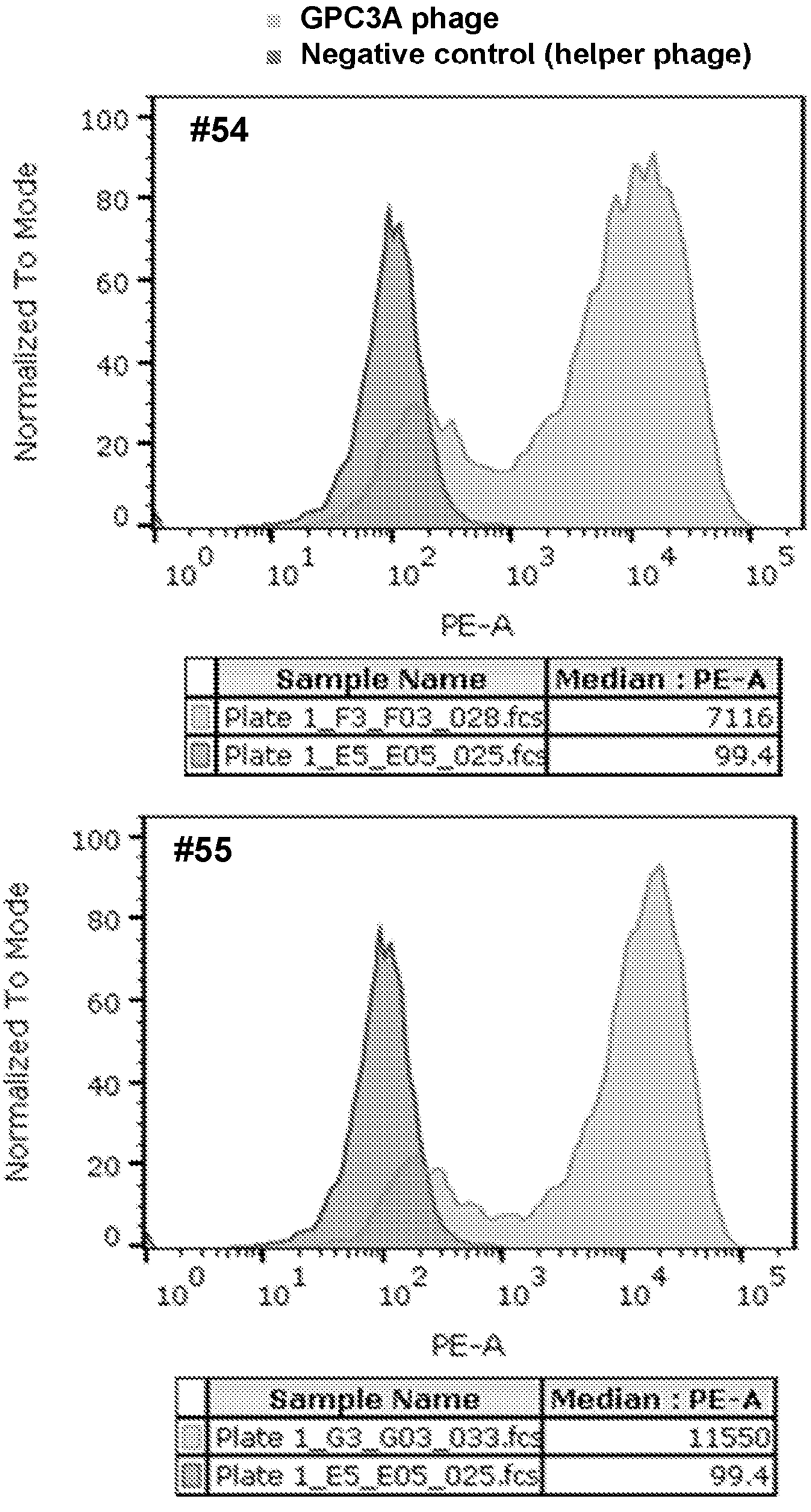
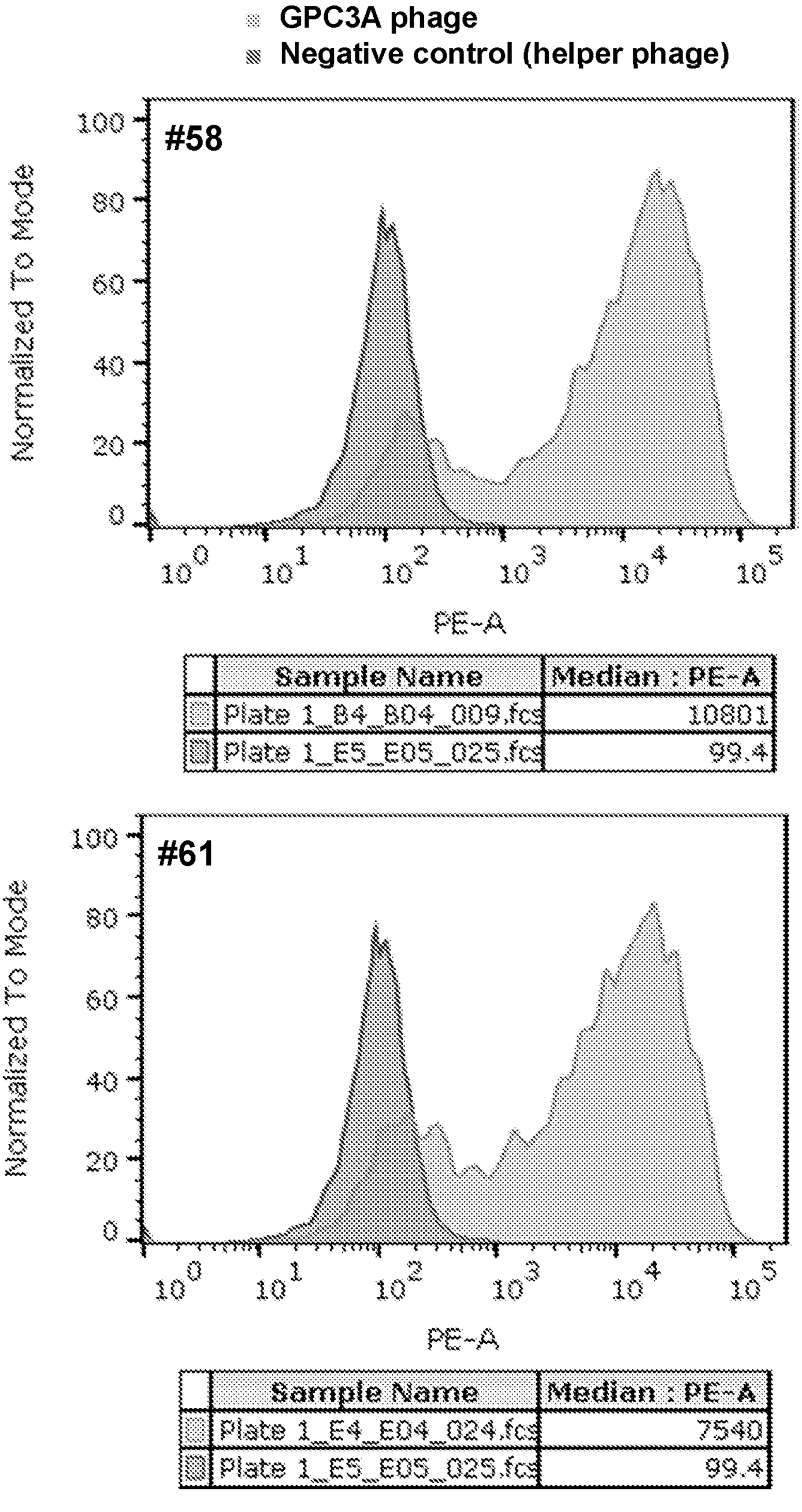

GPC3A phage
Negative control (helper phage)
#54
Normalized To Mode
0
20
40
60
80
100
10^0
10^1
10^2
10^3
10^4
10^5
PE-A
Sample Name
Median : PE-A
Plate 1_F3_F03_028.fcs
7116
Plate 1_E5_E05_025.fcs
99.4
#55
Normalized To Mode
0
20
40
60
80
100
10^0
10^1
10^2
10^3
10^4
10^5
PE-A
Sample Name
Median : PE-A
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

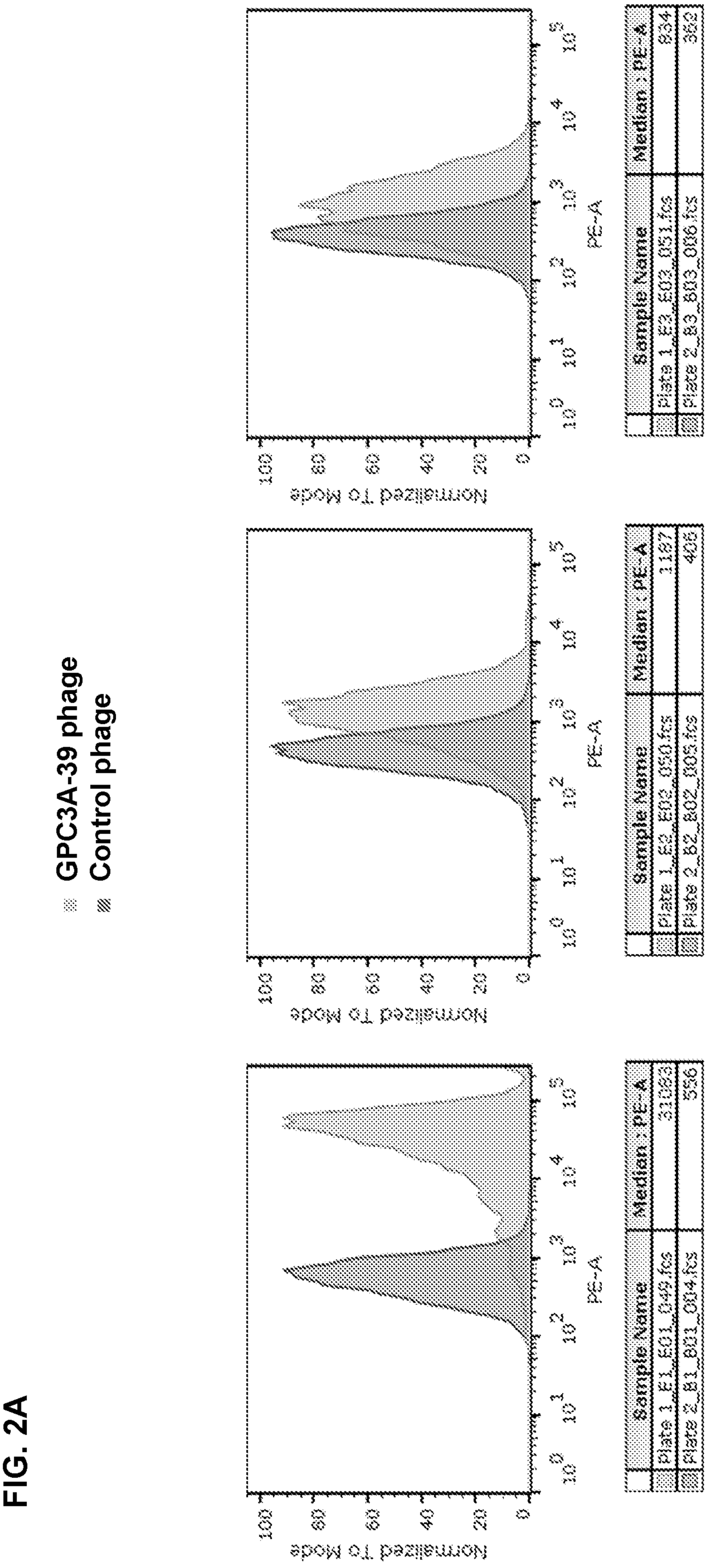

GPC3A-39 phage
Control phage
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_E3_E03_051.fcs
Plate 2_B3_B03_006.fcs
Plate 1_E2_E02_050.fcs
Plate 2_B2_B02_005.fcs
Plate 1_E1_E01_049.fcs
Plate 2_B1_B01_004.fcs
HepG2-GPC3-KO-3
HepG2-GPC3-KO-2
HepG2

FIG. 2A

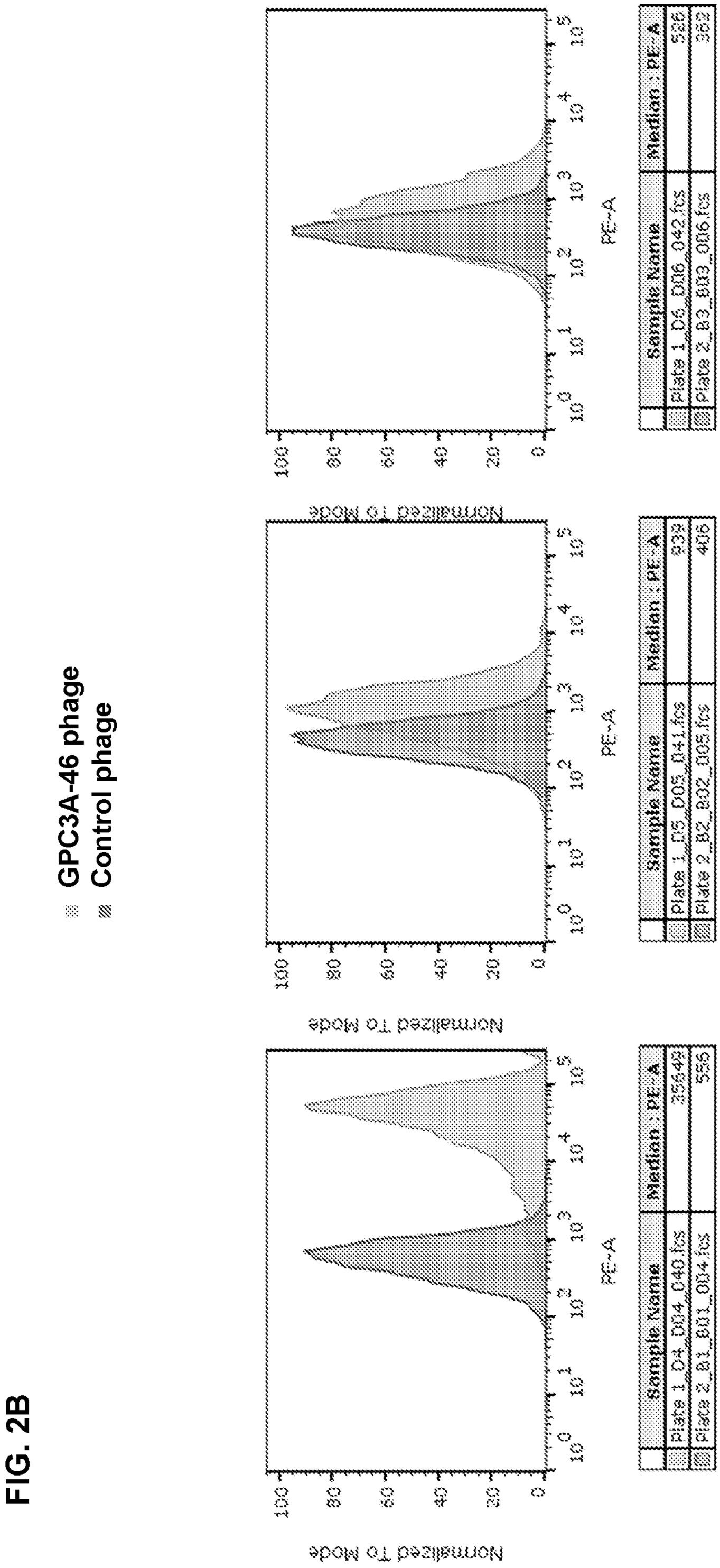

GPC3A-46 phage
Control phage
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_D5_D11_005_041.fcs
939
Plate 2_B02_B05.fcs
406
HepG2-GPC3-KO-2
Plate 1_D5_D6_006_042.fcs
586
Plate 2_B3_B6_003_006.fcs
362
HepG2-GPC3-KO-3
Plate 1_D4_D7_004_040.fcs
25649
Plate 2_B1_B01_004.fcs
555
HepG2

FIG. 2B

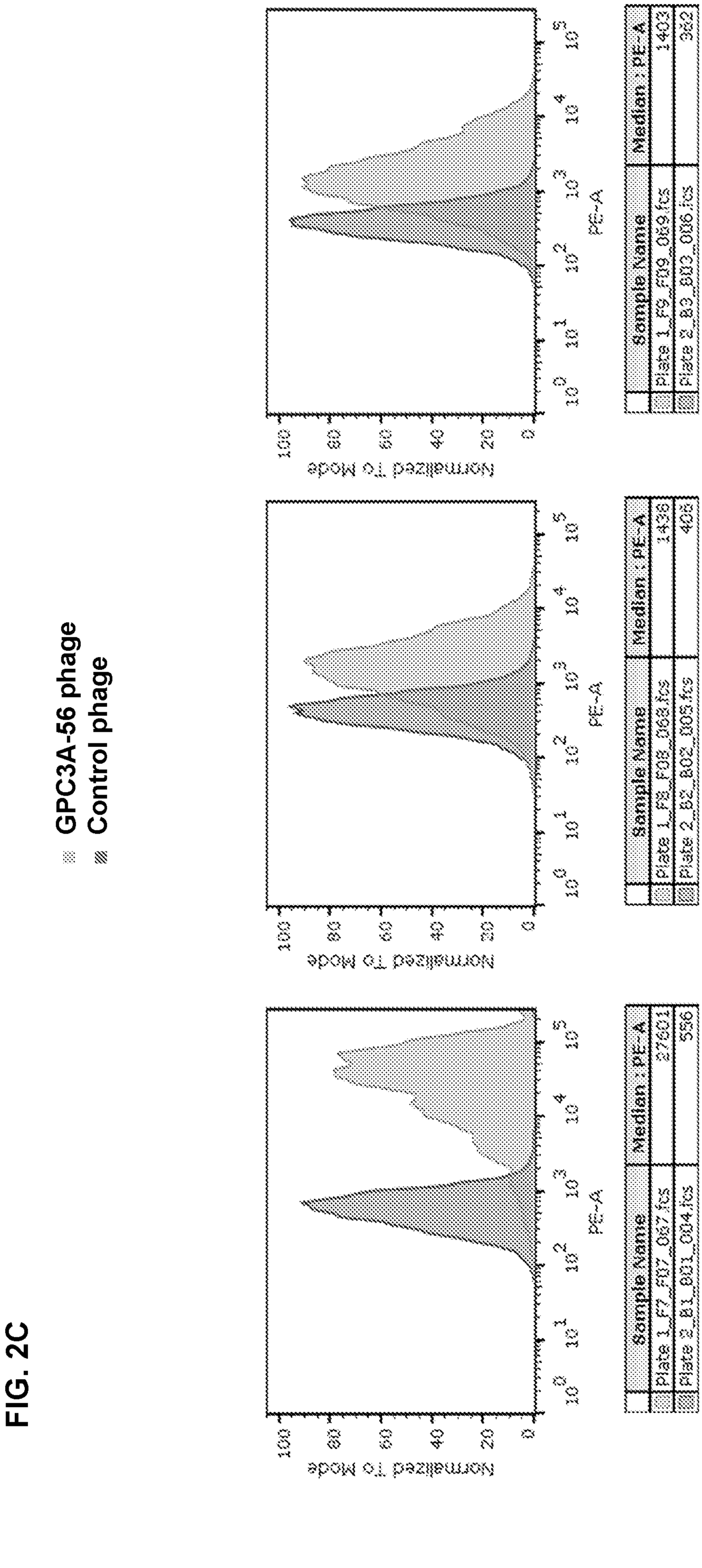

GPC3A-56 phage
Control phage
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_F9_F09_069.fcs
1403
Plate 2_B3_B03_086.fcs
322
HepG2-GPC3-KO-3
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_F8_F08_068.fcs
1436
Plate 2_B02_B05.fcs
408
HepG2-GPC3-KO-2
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_F7_F07_067.fcs
27601
Plate 2_B1_B01_004.fcs
556
HepG2

FIG. 2C

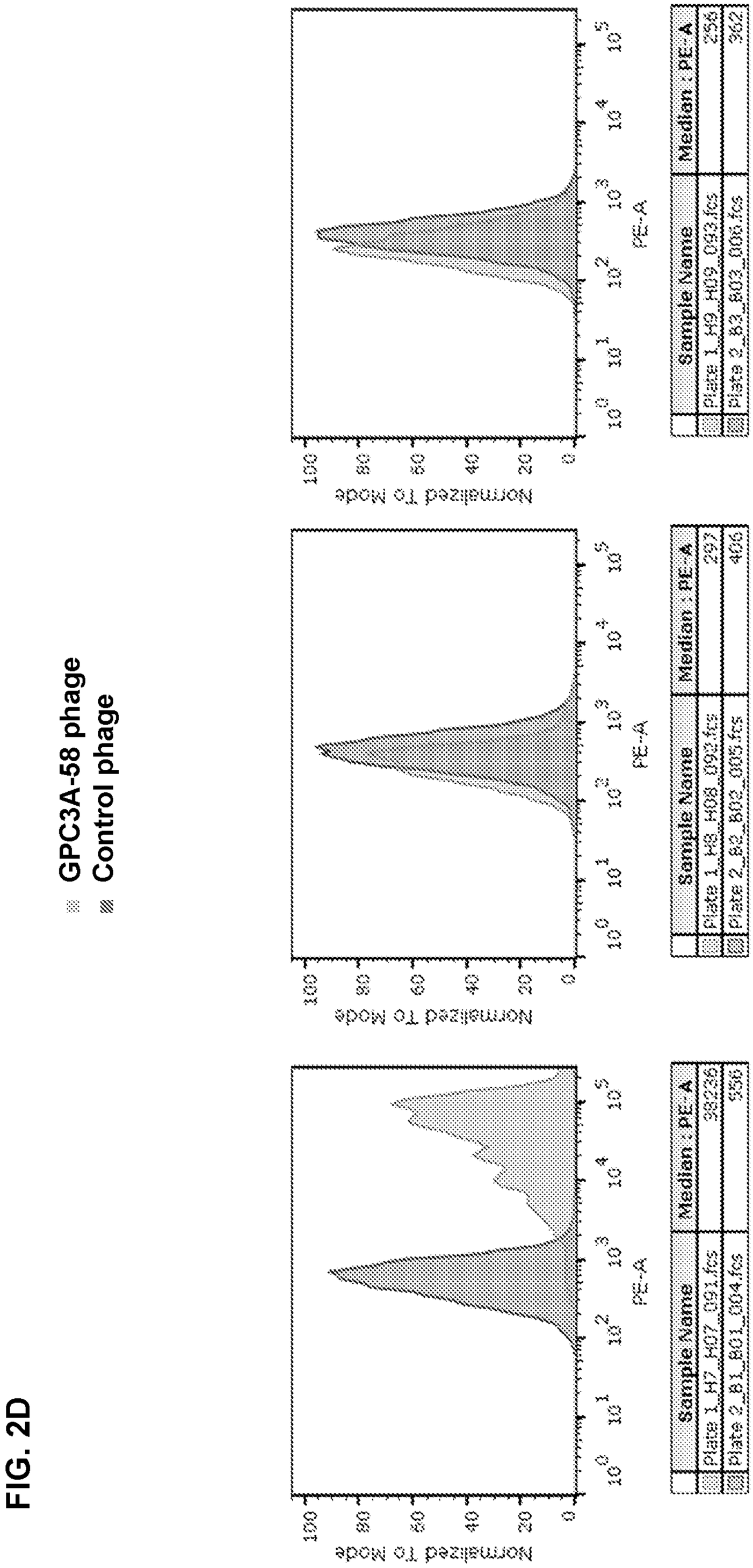

GPC3A-58 phage
Control phage
HepG2-GPC3-KO-3
HepG2-GPC3-KO-2
HepG2
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_H9_H09_093.fcs
Plate 2_B3_B03_006.fcs
Plate 1_H8_H08_092.fcs
Plate 2_B02_B05.fcs
Plate 1_H7_H07_091.fcs
Plate 2_B1_B01_B04.fcs
256
362
297
406
38236
556

FIG. 2D

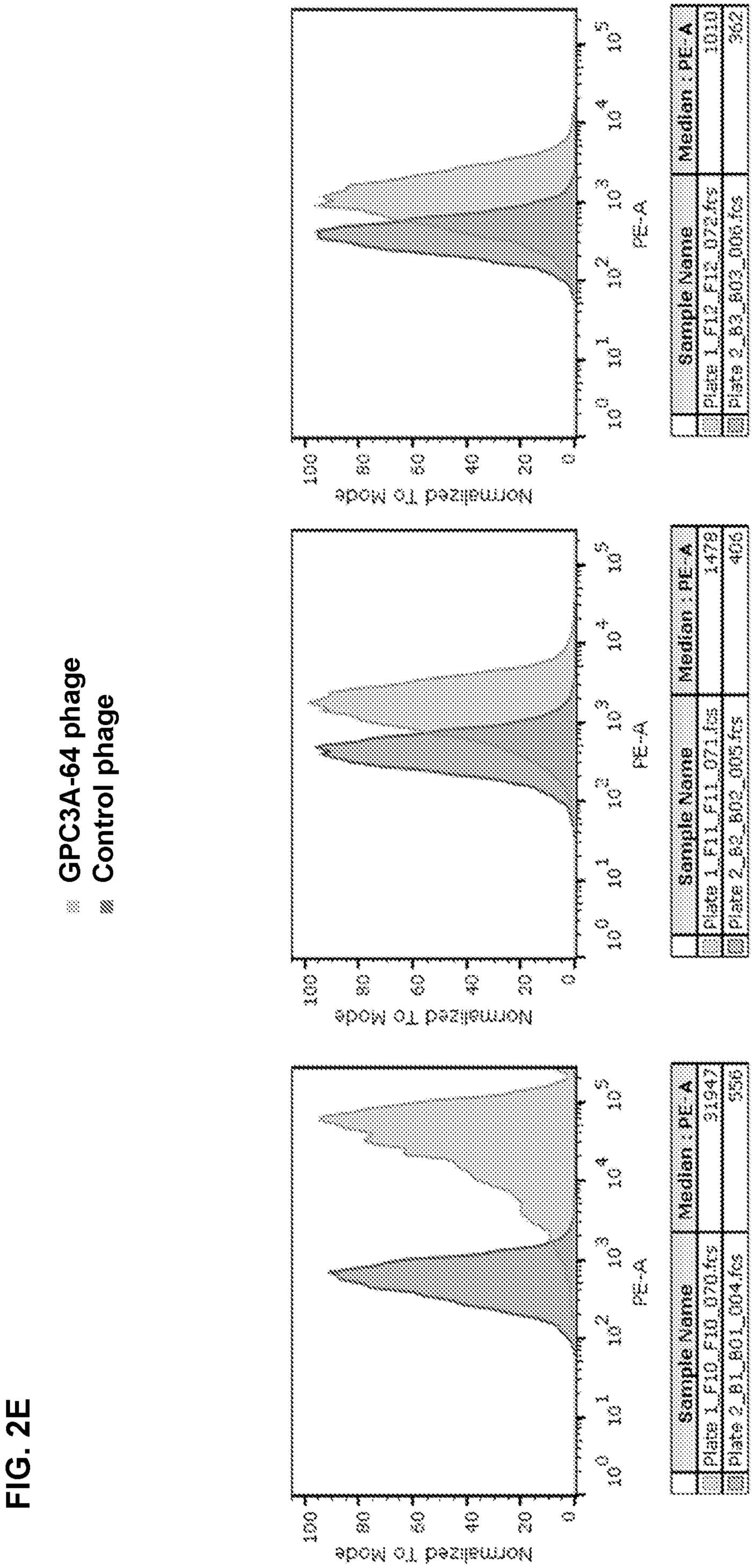
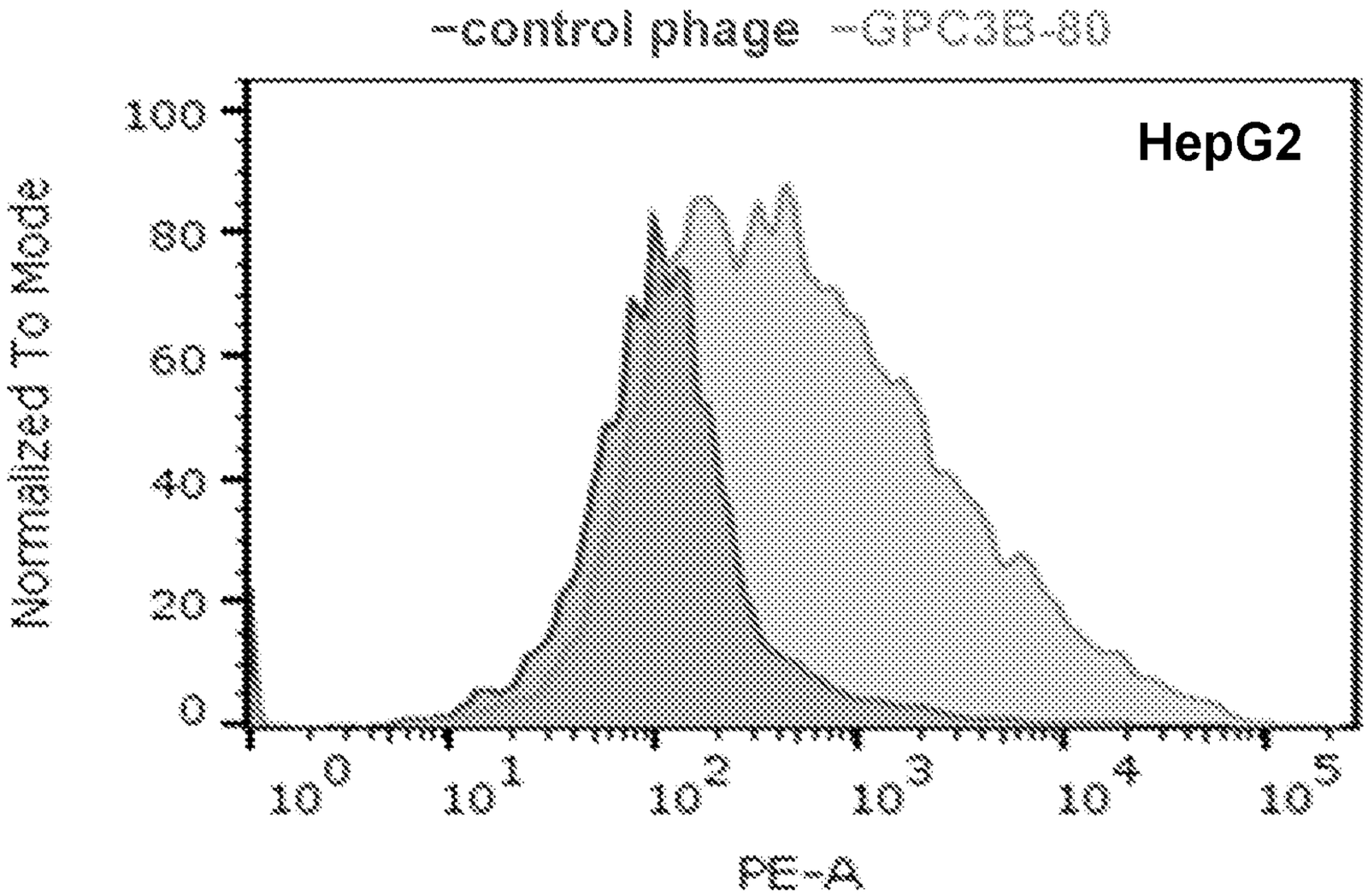
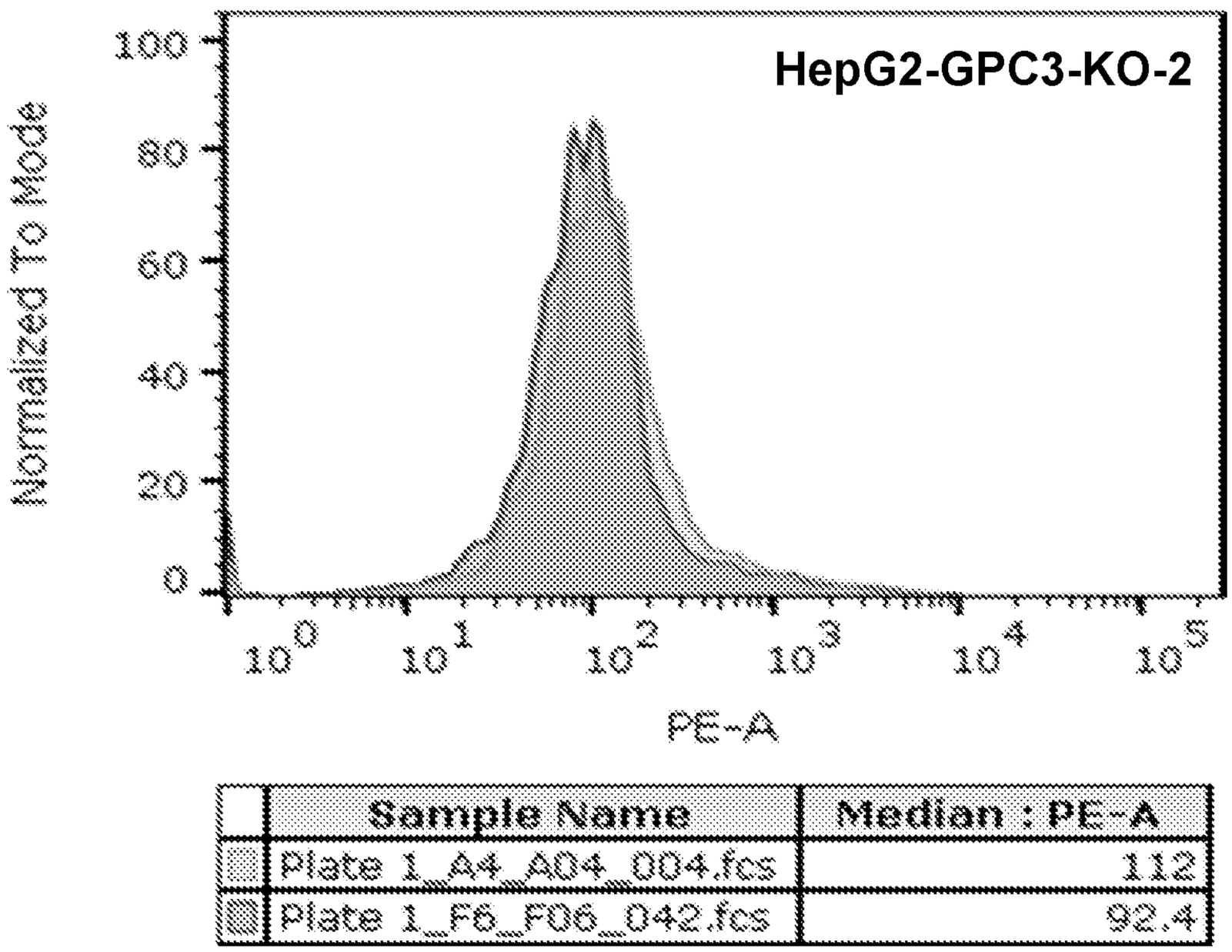

GPC3A-64 phage
Control phage
HepG2-GPC3-KO-3
HepG2-GPC3-KO-2
HepG2
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_F12_072.fcs
1010
Plate 2_B3_006.fcs
362
Plate 1_F11_071.fcs
1478
Plate 2_B2_005.fcs
406
Plate 1_F10_070.fcs
91947
Plate 2_B1_004.fcs
556

FIG. 2E

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

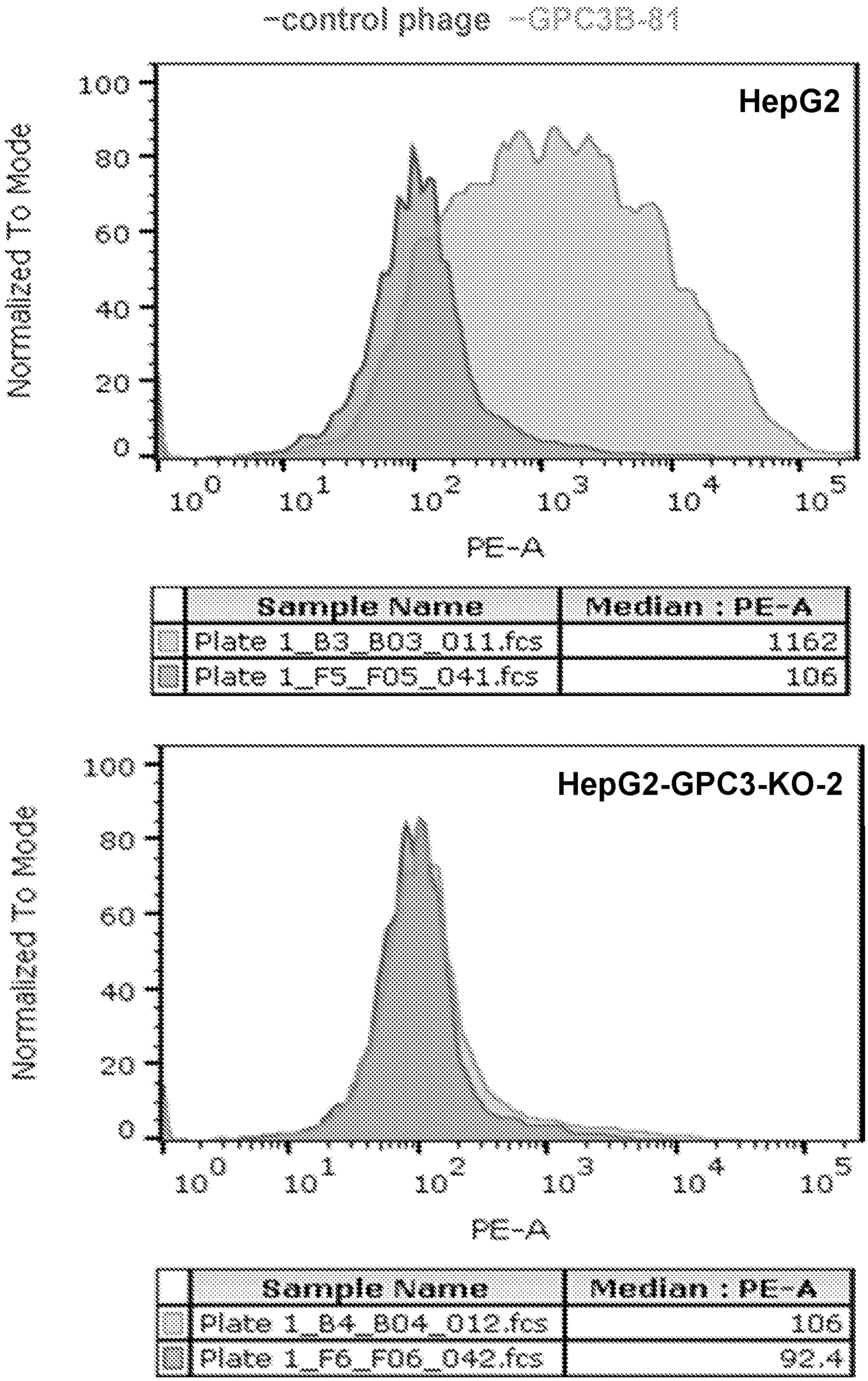

control phage
GPC3B-81
HepG2
Normalized To Mode
0
20
40
60
80
100
10^0
10^1
10^2
10^3
10^4
10^5
PE-A
Sample Name
Median : PE-A
Plate 1_B3_B03_011.fcs
1162
Plate 1_F5_F05_041.fcs
106
HepG2-GPC3-KO-2
Normalized To Mode
0
20
40
60
80
100
10^0
10^1
10^2
10^3
10^4
10^5
PE-A
Sample Name
Median : PE-A
Plate 1_B4_B04_012.fcs
106
Plate 1_F6_F06_042.fcs
92.4

FIG. 3C

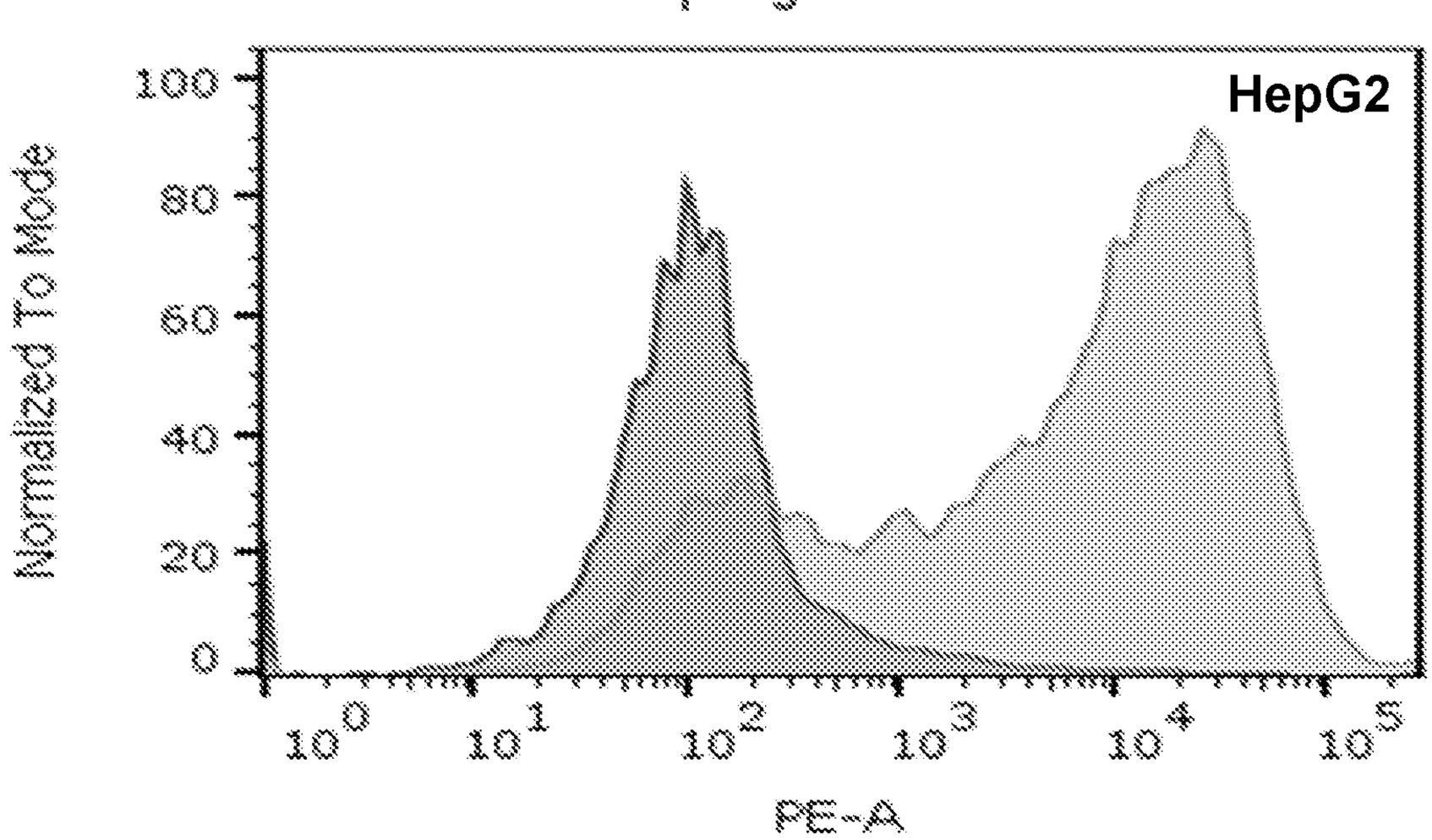
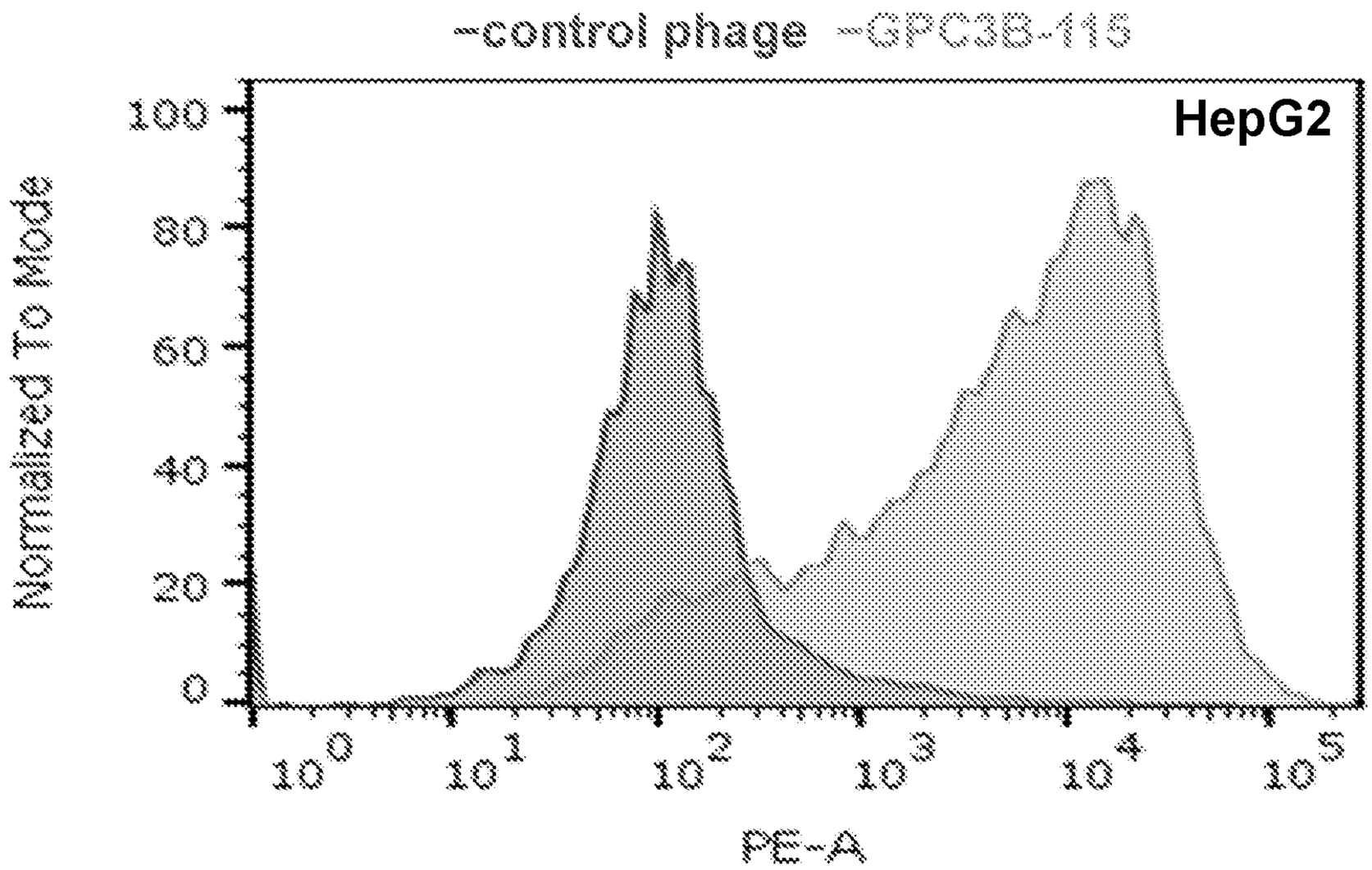
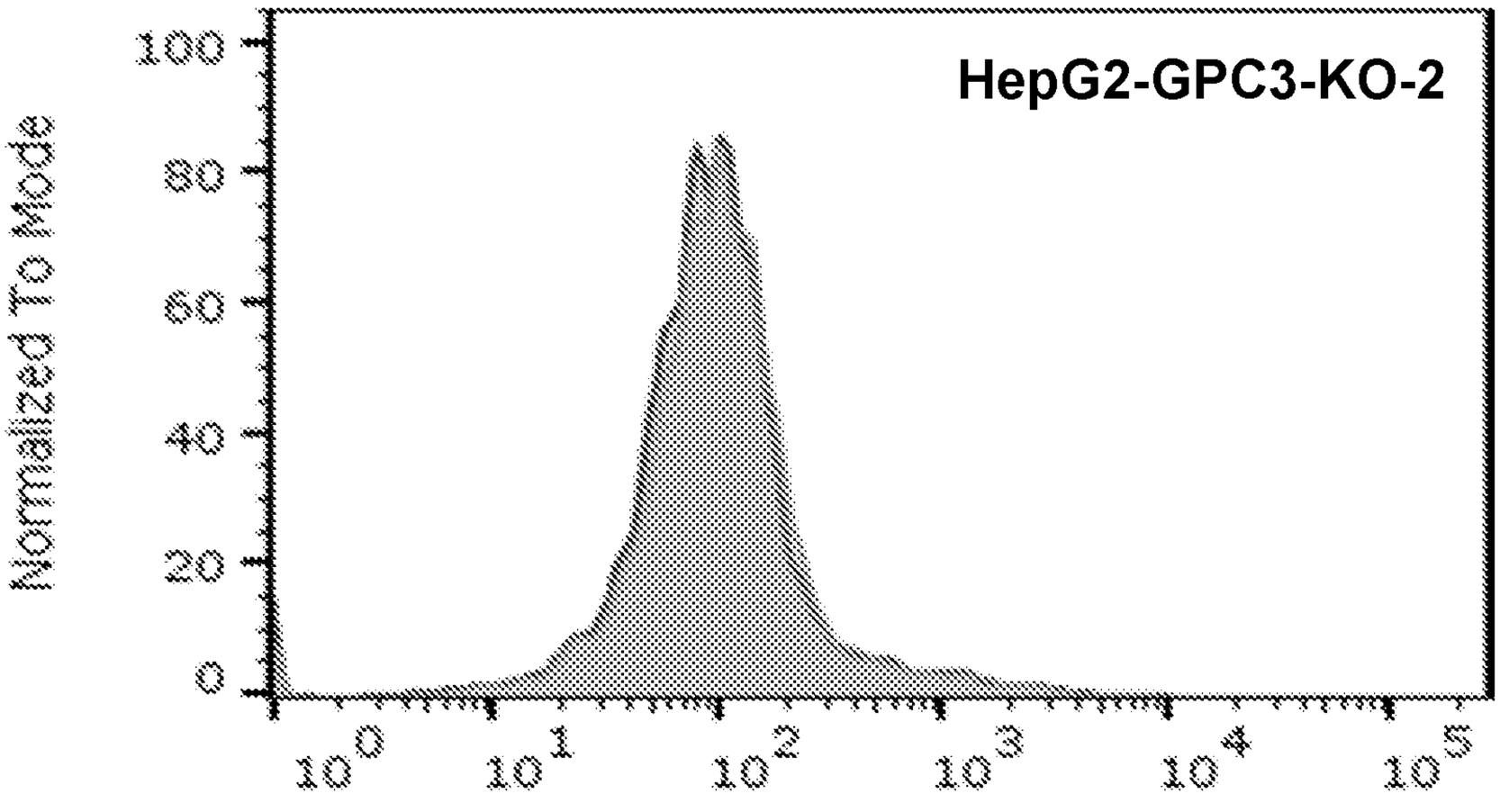

control phage
GPC3B-87
HepG2
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_E3_E03_033.fcs
8622
Plate 1_F5_F05_041.fcs
106
HepG2-GPC3-KO-2
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Plate 1_E4_E04_034.fcs
96.8
Plate 1_F6_F06_042.fcs
92.4

FIG. 3D



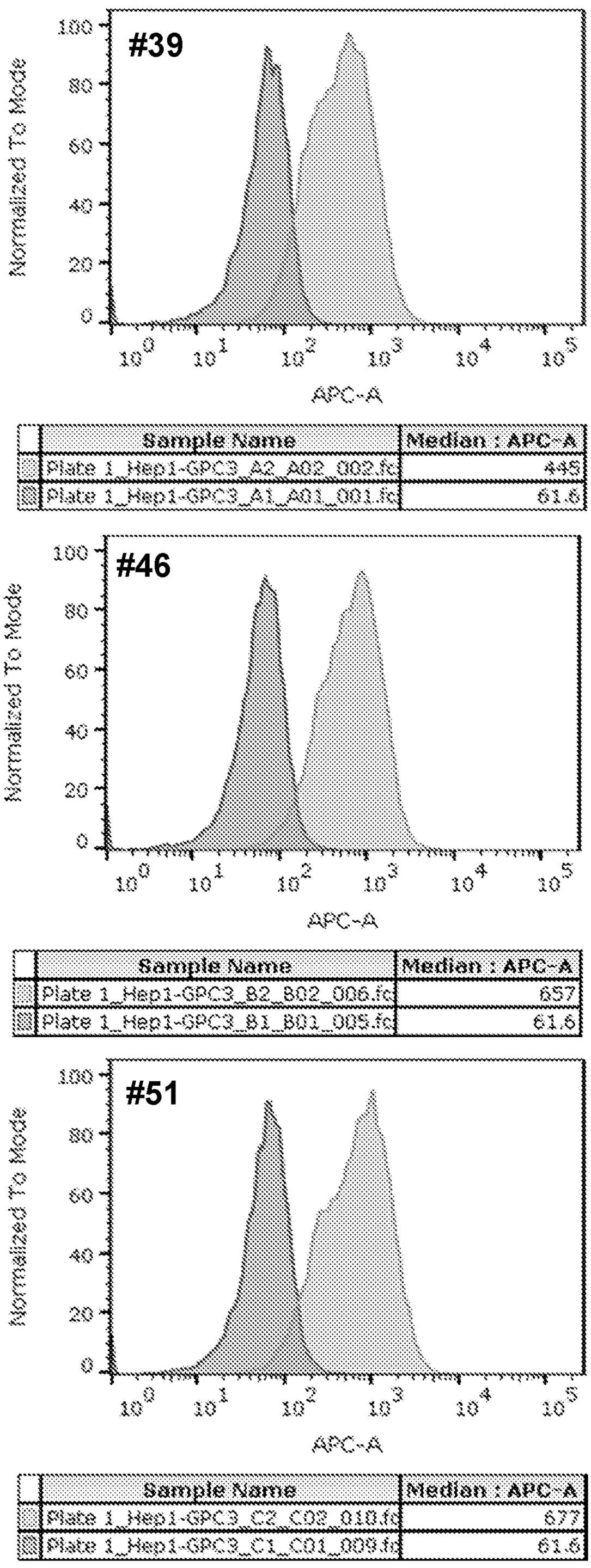
	Sample Name	Median : PE-A
	Plate 1_B5_B05_013.fcs	6319
	Plate 1_F5_F05_041.fcs	106



	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



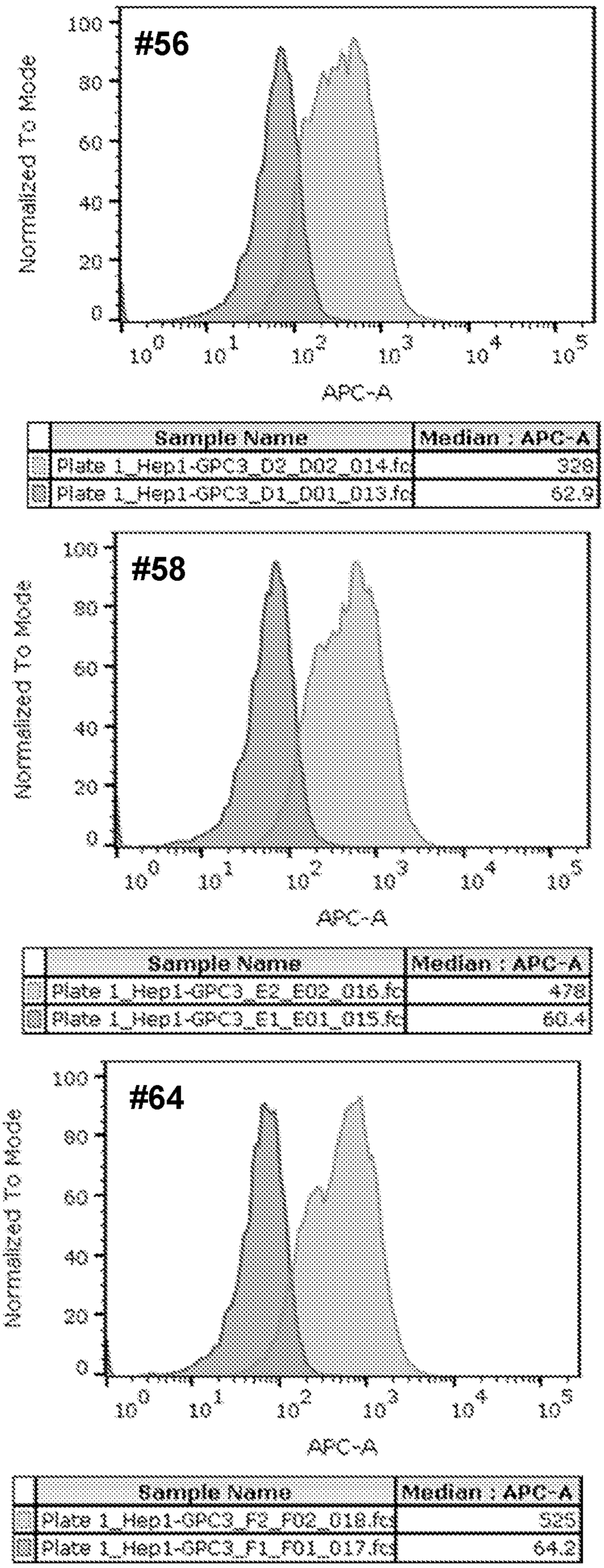
	Sample Name	Median : APC-A
	Plate 1_Hep1-GPC3_A2_A02_002.fc	445
	Plate 1_Hep1-GPC3_A1_A01_001.fc	61.6

	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	329
	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.fcs	525
	Plate 1_Hep1-GPC3_F1_F01_017.fcs	64.2

FIG. 5A

-control L2K
-GPC3A L2K

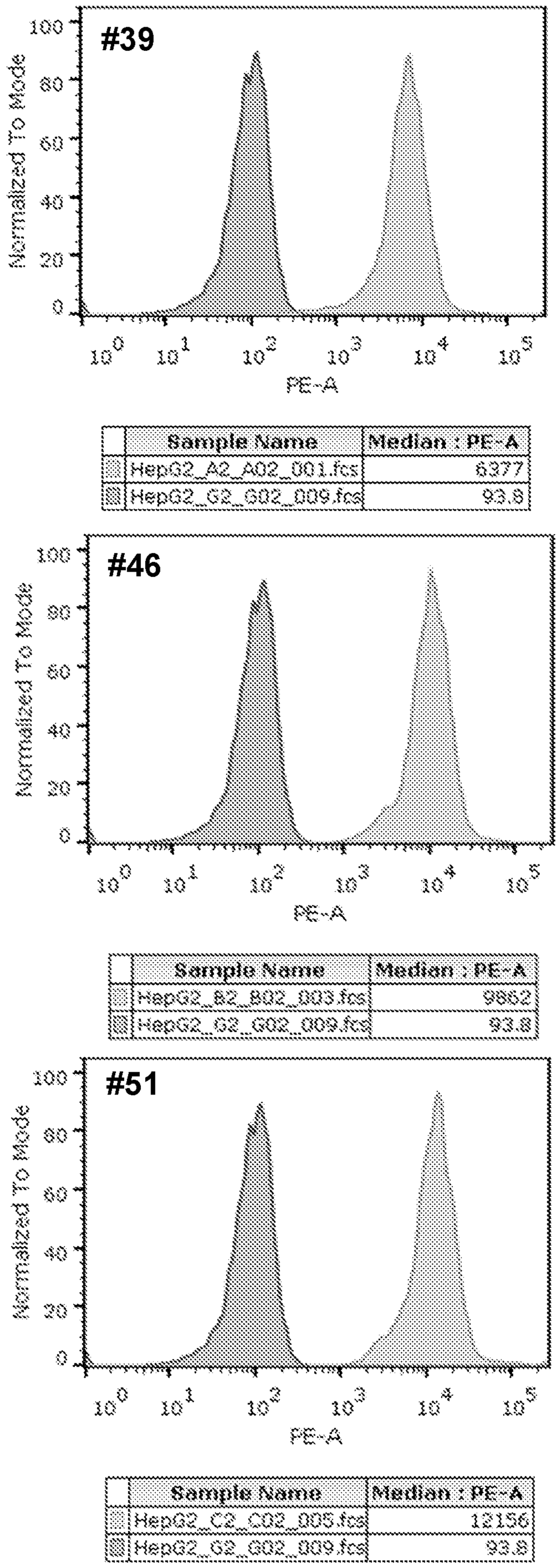


FIG. 5B

-control L2K
-GPC3A L2K

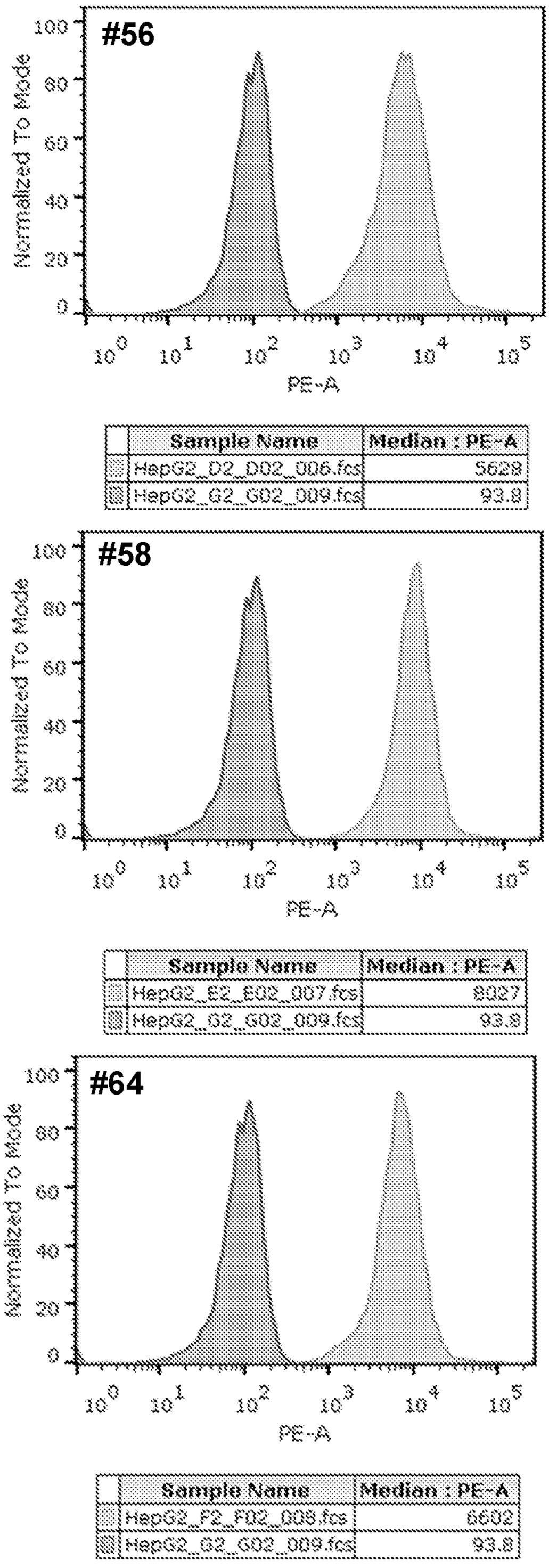
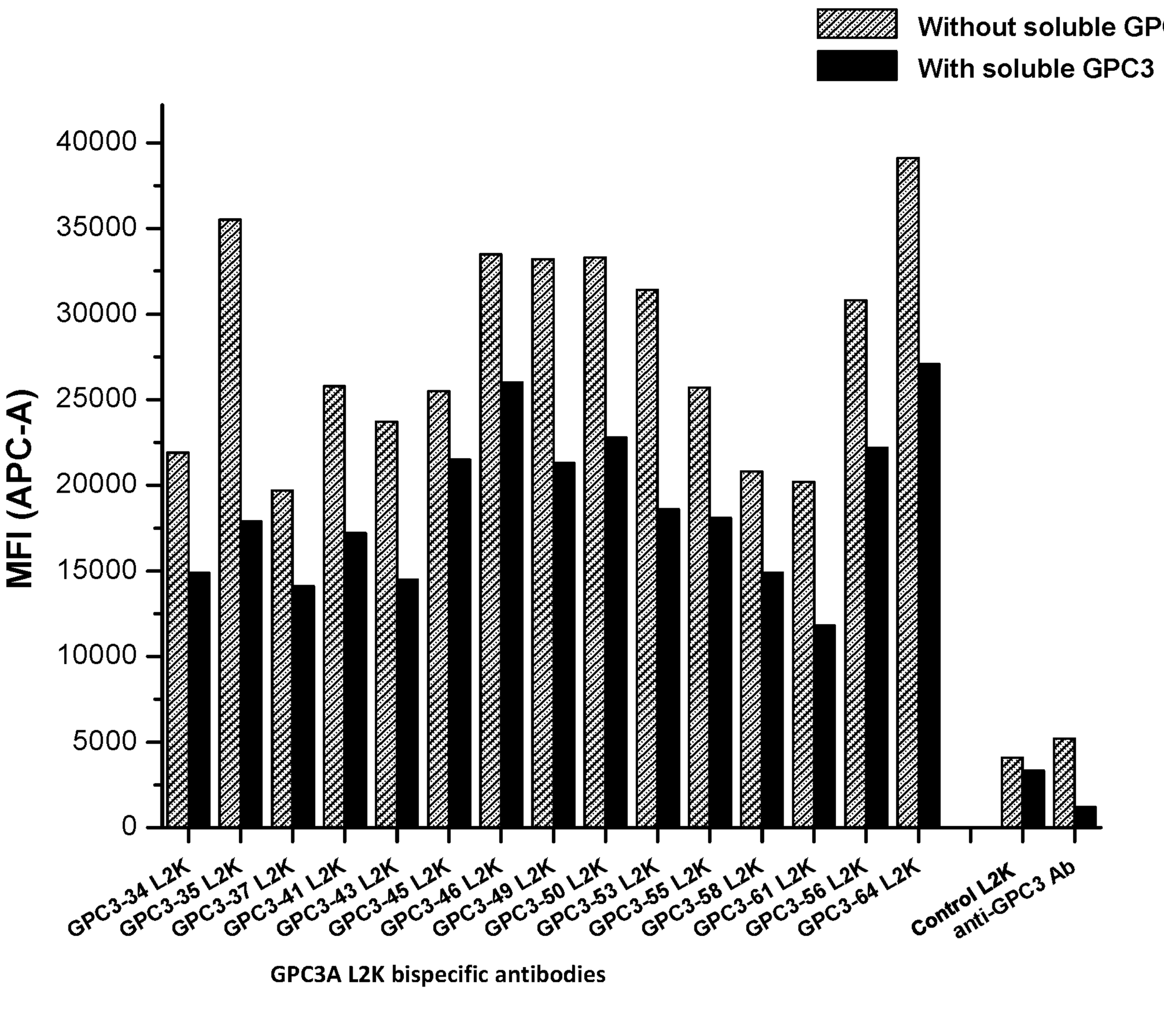


FIG. 6

Competition for Hep2 cell binding with and without soluble GPC3



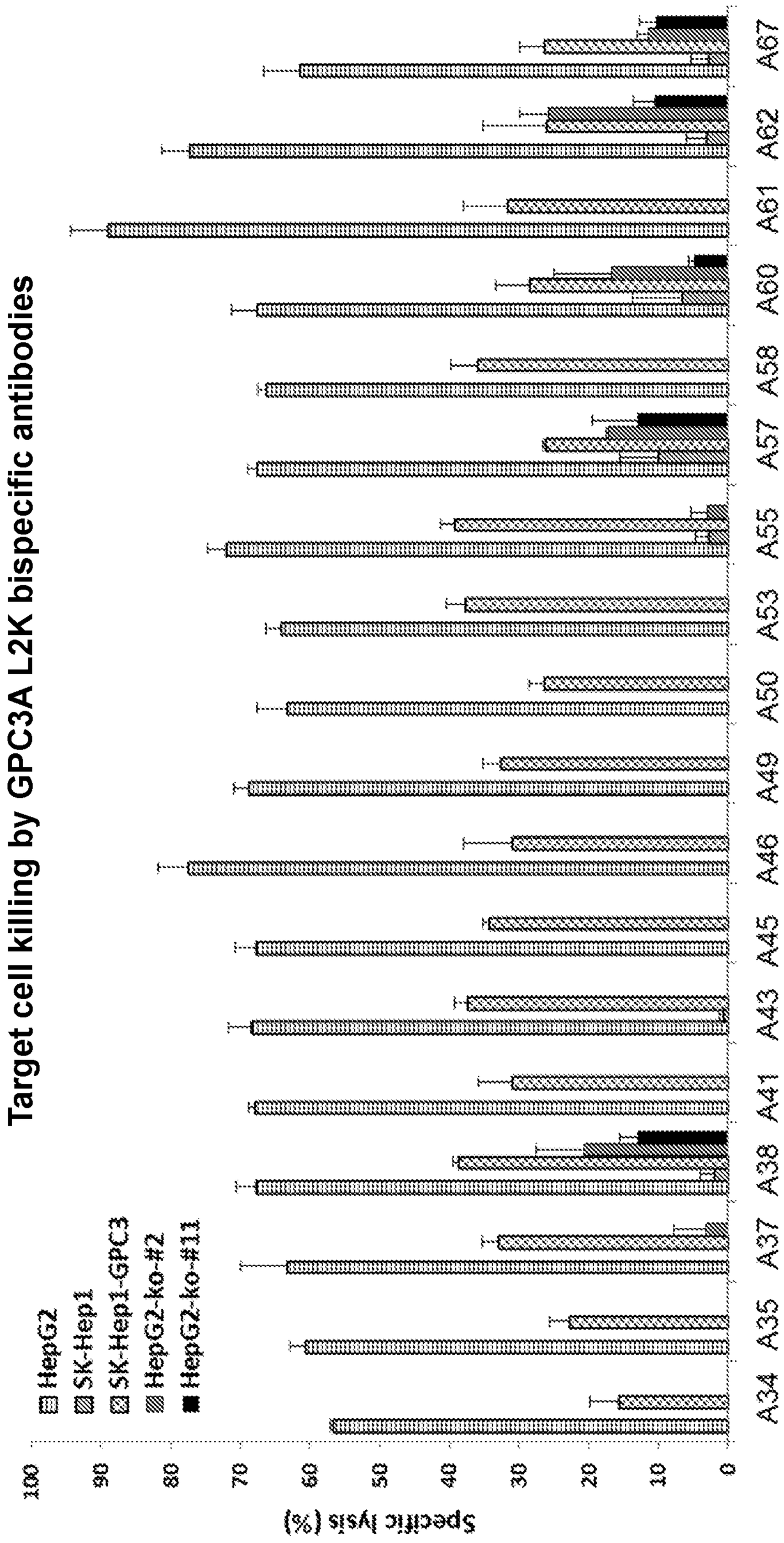

Target cell killing by GPC3A L2K bispecific antibodies
HepG2
SK-Hep1
SK-Hep1-GPC3
HepG2-ko-#2
HepG2-ko-#11
Specific lysis (%)
100
90
80
70
60
50
40
30
20
10
0
A34 A35 A37 A38 A41 A43 A45 A46 A49 A50 A53 A55 A57 A58 A60 A61 A62 A67

FIG. 7A

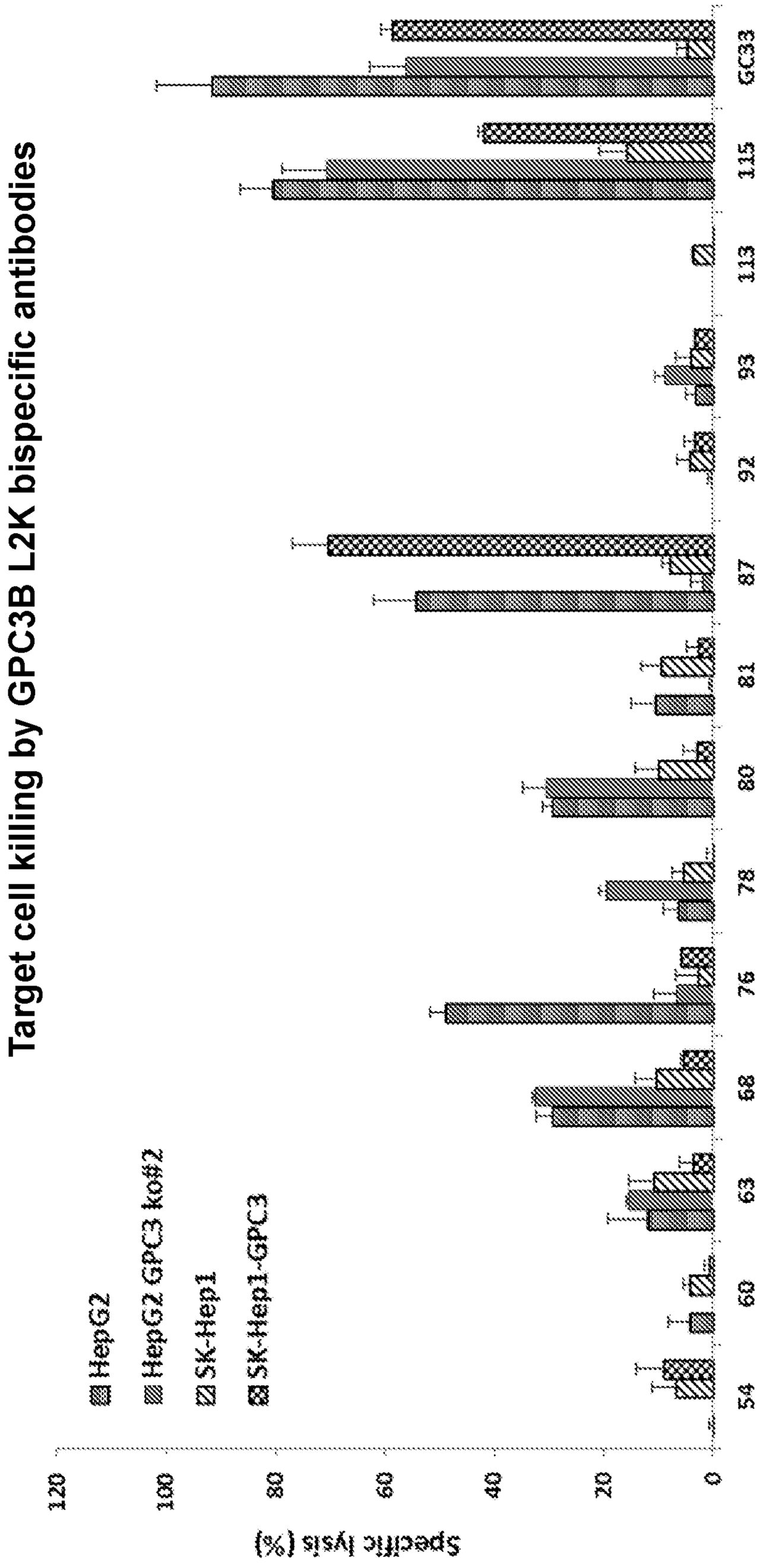

Target cell killing by GPC3B L2K bispecific antibodies
Specific lysis (%)
0
20
40
60
80
100
120
HepG2
HepG2 GPC3
SK-Hep1
SK-Hep1-GPC3 ko#2
SK-Hep1-GPC3
54
60
63
68
76
78
80
81
87
92
93
113
115
GC33

FIG. 7B

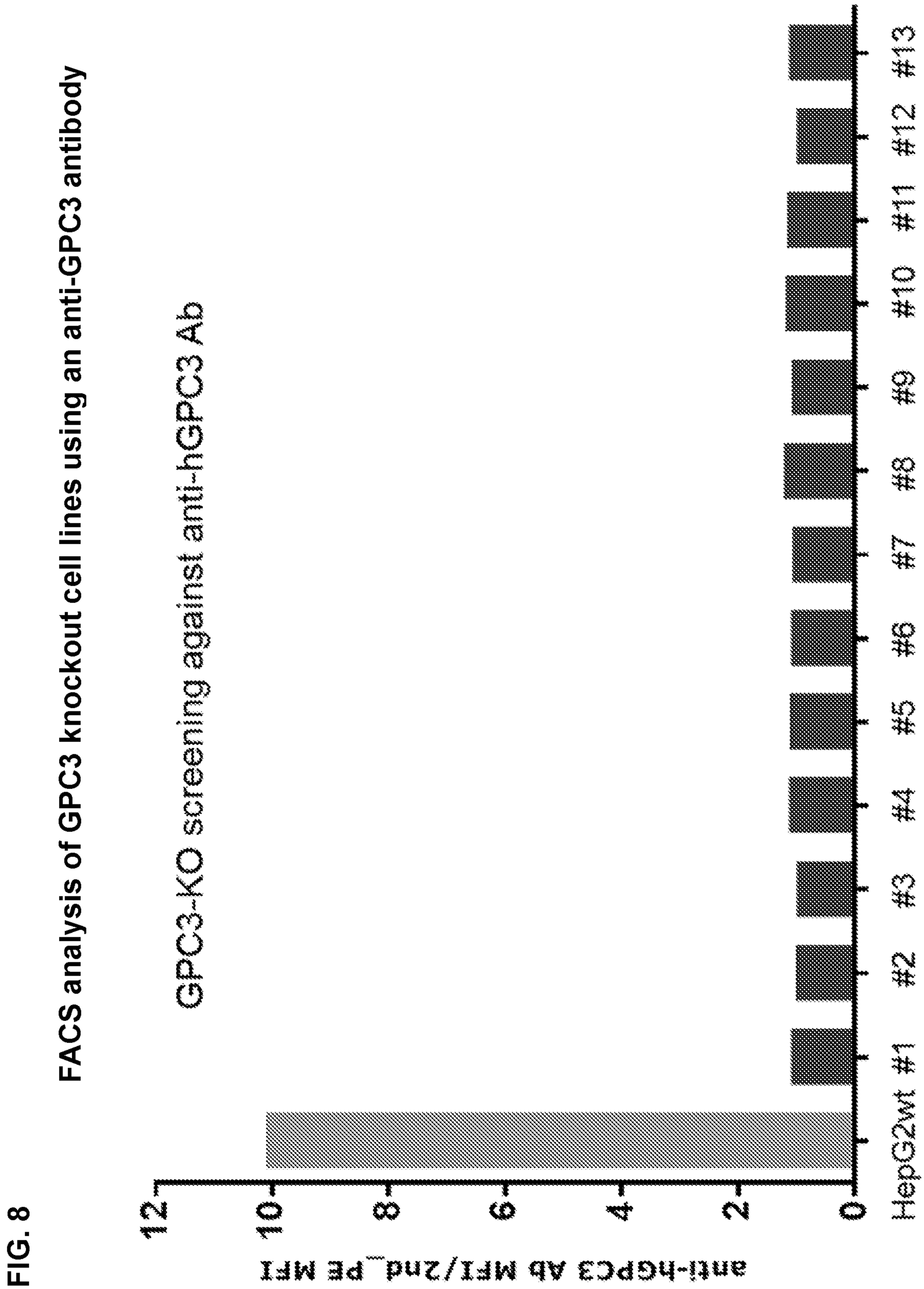

FACS analysis of GPC3 knockout cell lines using an anti-GPC3 antibody
GPC3-KO screening against anti-hGPC3 Ab
anti-hGPC3 Ab MFI/2nd_PE MFI
0
2
4
6
8
10
12
HepG2wt
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
#13

FIG. 8

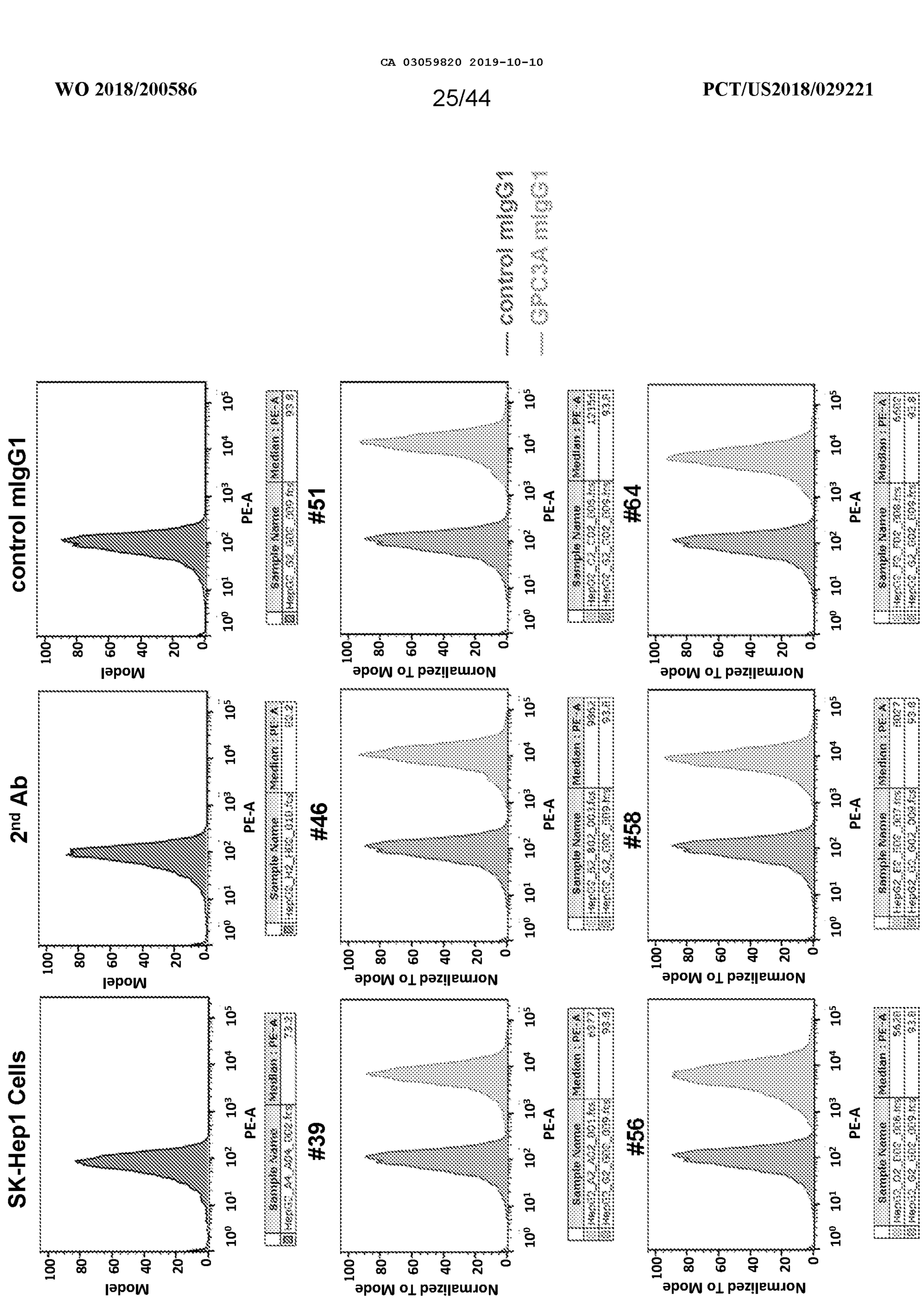

control mIgG1
2nd Ab
SK-Hep1 Cells
Model
Normalized To Mode
PE-A
Sample Name
Median : PE-A
#51
#64
#46
#58
#39
#56

FIG. 9A

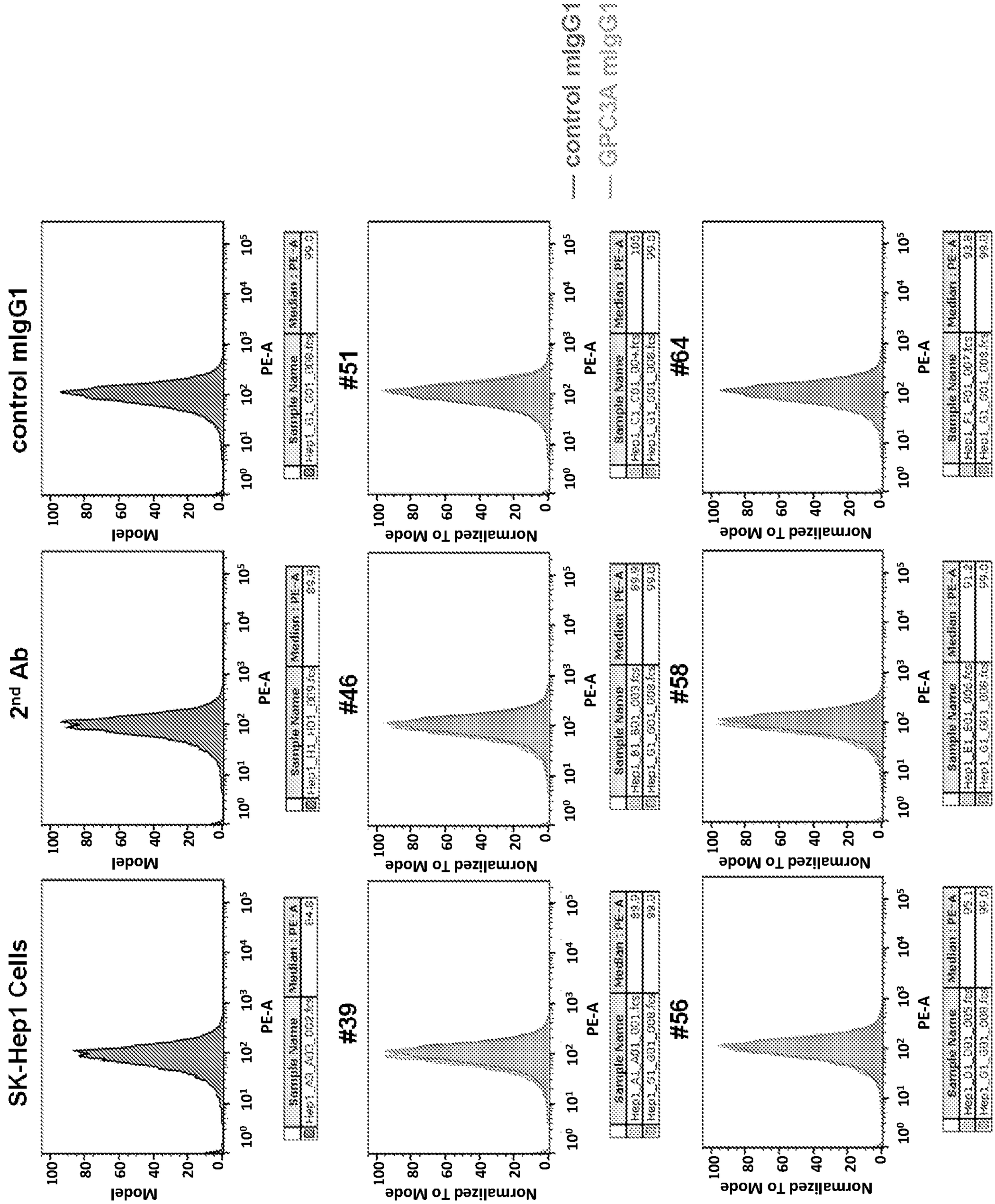
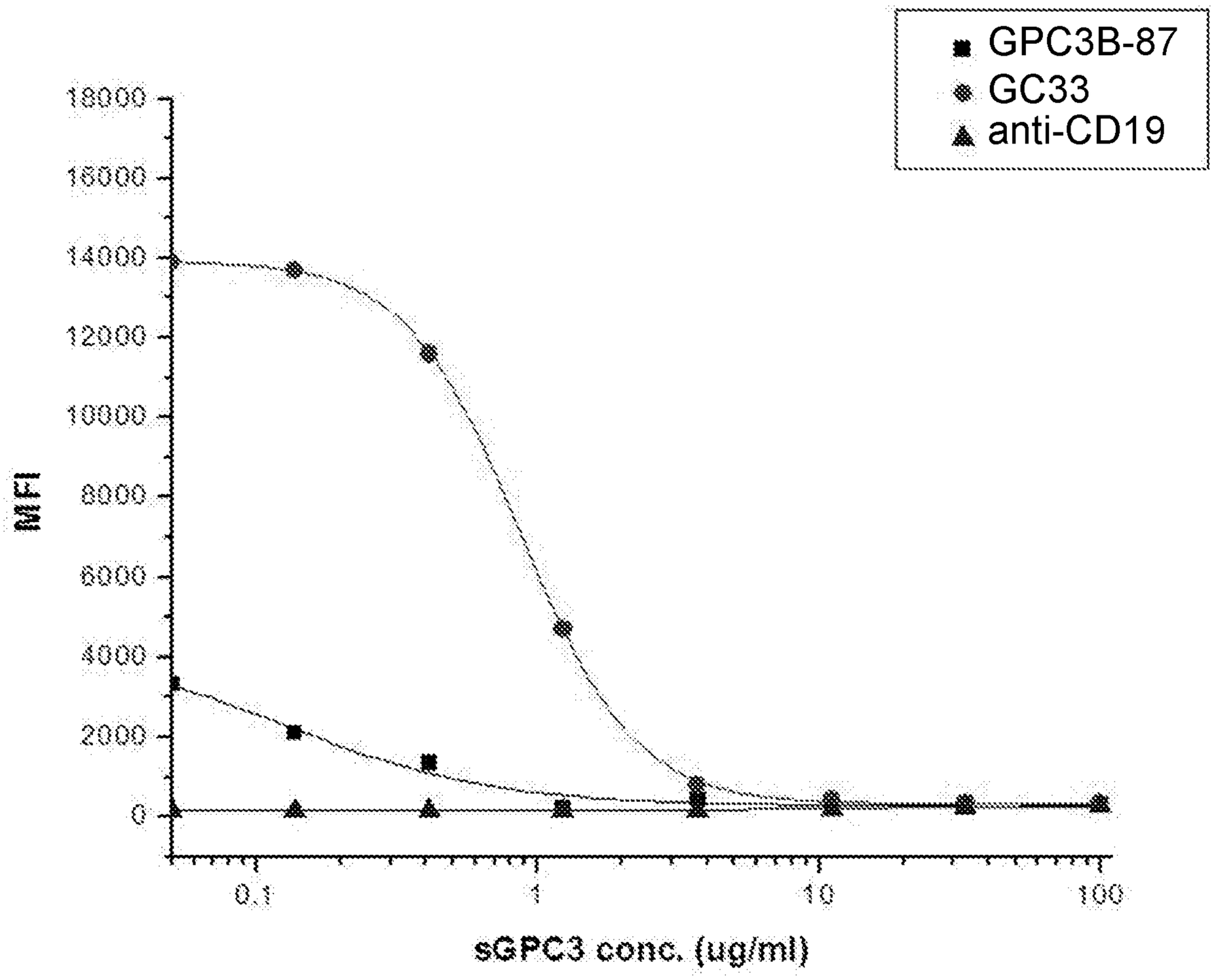

control mIgG1
anti-CD3A mAb
2nd Ab
SK-Hep1 Cells
#51
#64
#46
#58
#39
#56
Model
Normalized To Mode
PE-A
Sample Name
Median : PE-A
Hep1_C1_C01_006.fcs
Hep1_B1_B01_003.fcs
Hep1_A1_A01_001.fcs
Hep1_A2_A02_002.fcs
Hep1_H1_H01_009.fcs
Hep1_G1_G01_008.fcs

FIG. 9B

FIG. 10 Competition for L2K binding to HepG2 cells by sGPC3



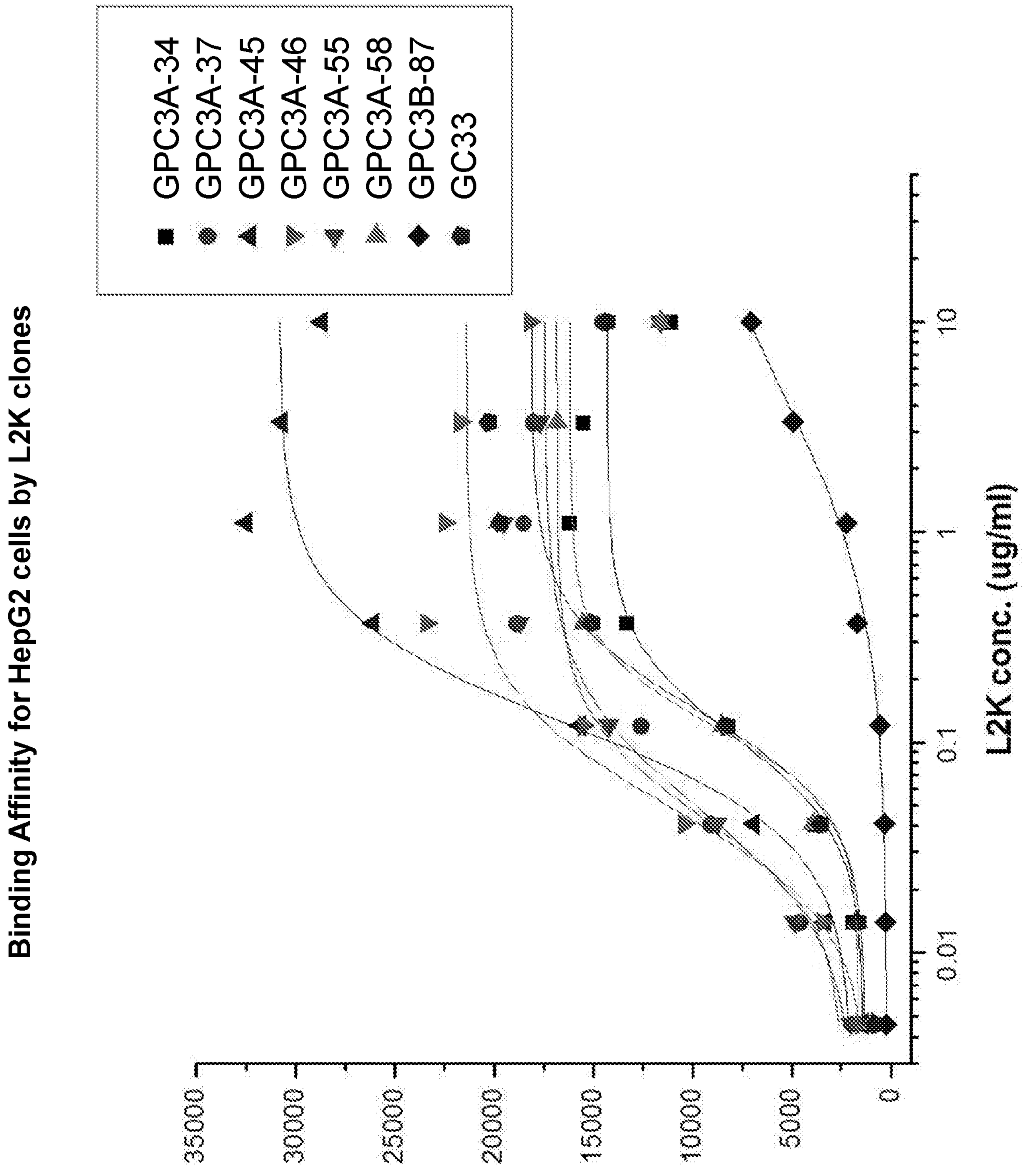

GPC3A-34
GPC3A-37
GPC3A-45
GPC3A-46
GPC3A-55
GPC3A-58
GPC3B-87
GC33
Binding Affinity for HepG2 cells by L2K clones
MFI (APC-A)
35000
30000
25000
20000
15000
10000
5000
0
0.01
0.1
1
10
L2K conc. (ug/ml)

FIG. 11

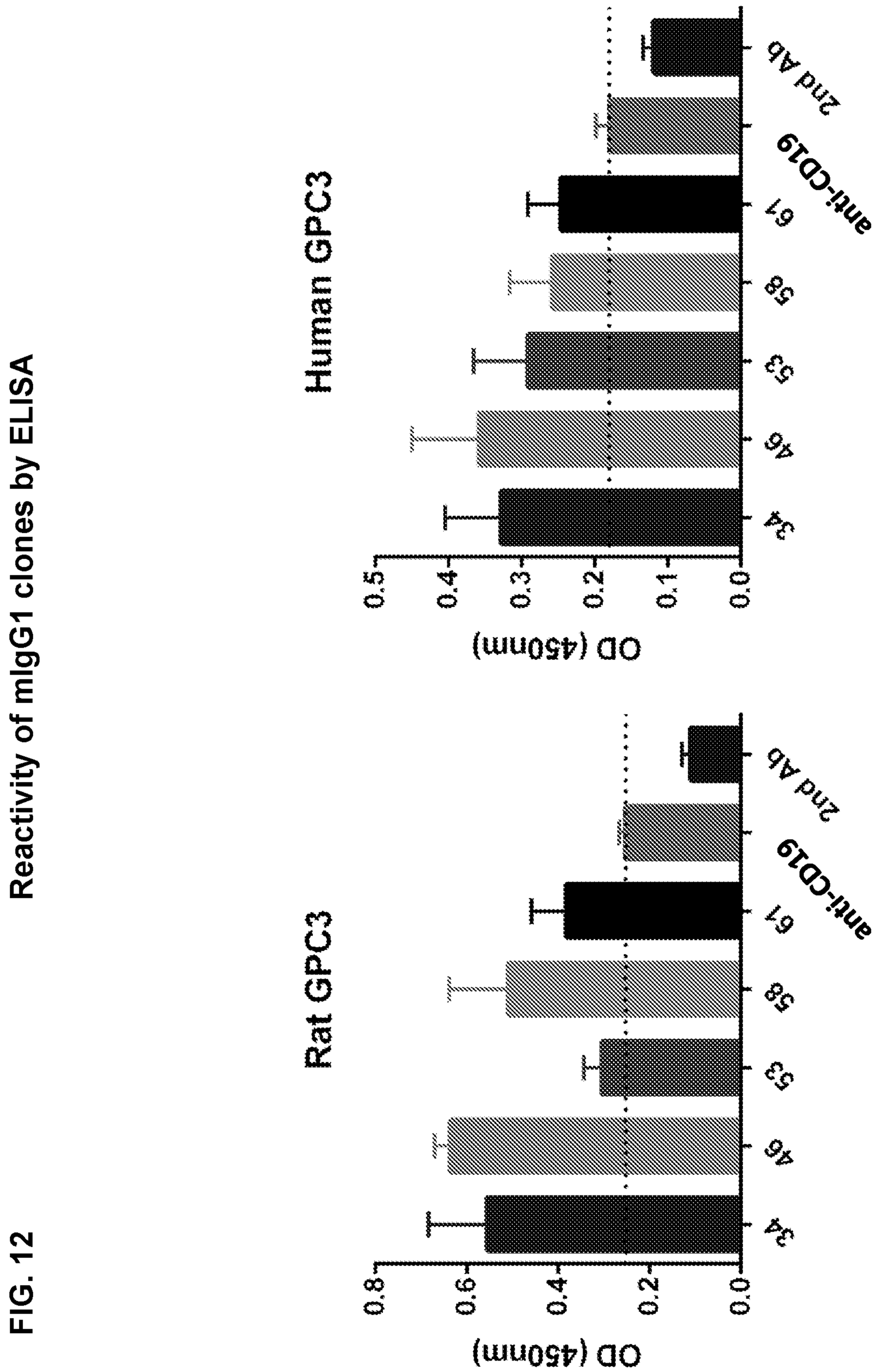

Reactivity of mIgG1 clones by ELISA
Human GPC3
Rat GPC3
OD (450nm)
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.2
0.4
0.6
0.8
34
46
53
58
61
anti-CD19
2nd Ab

FIG. 12

FIG. 13

Epitope Binning Analysis

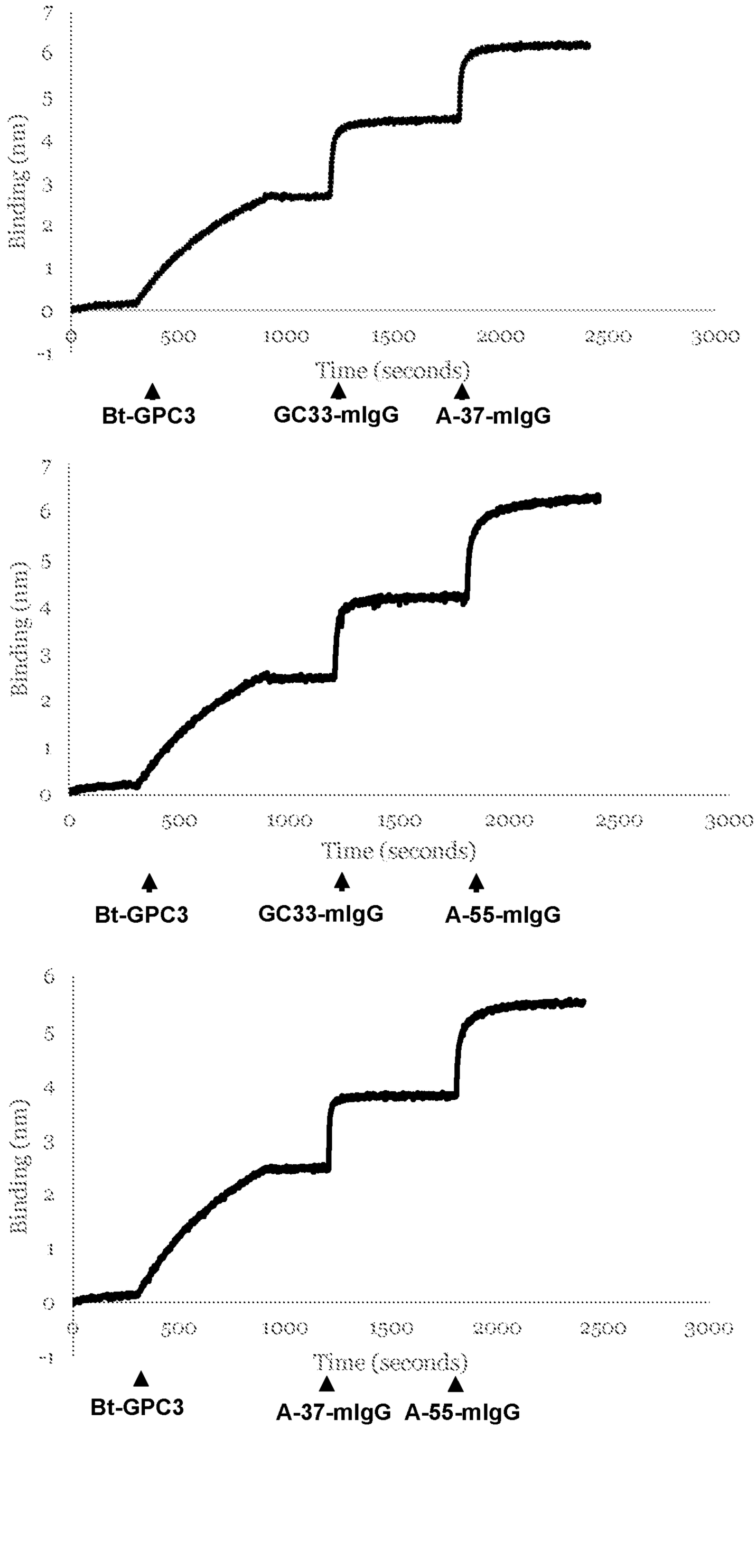


FIG. 14

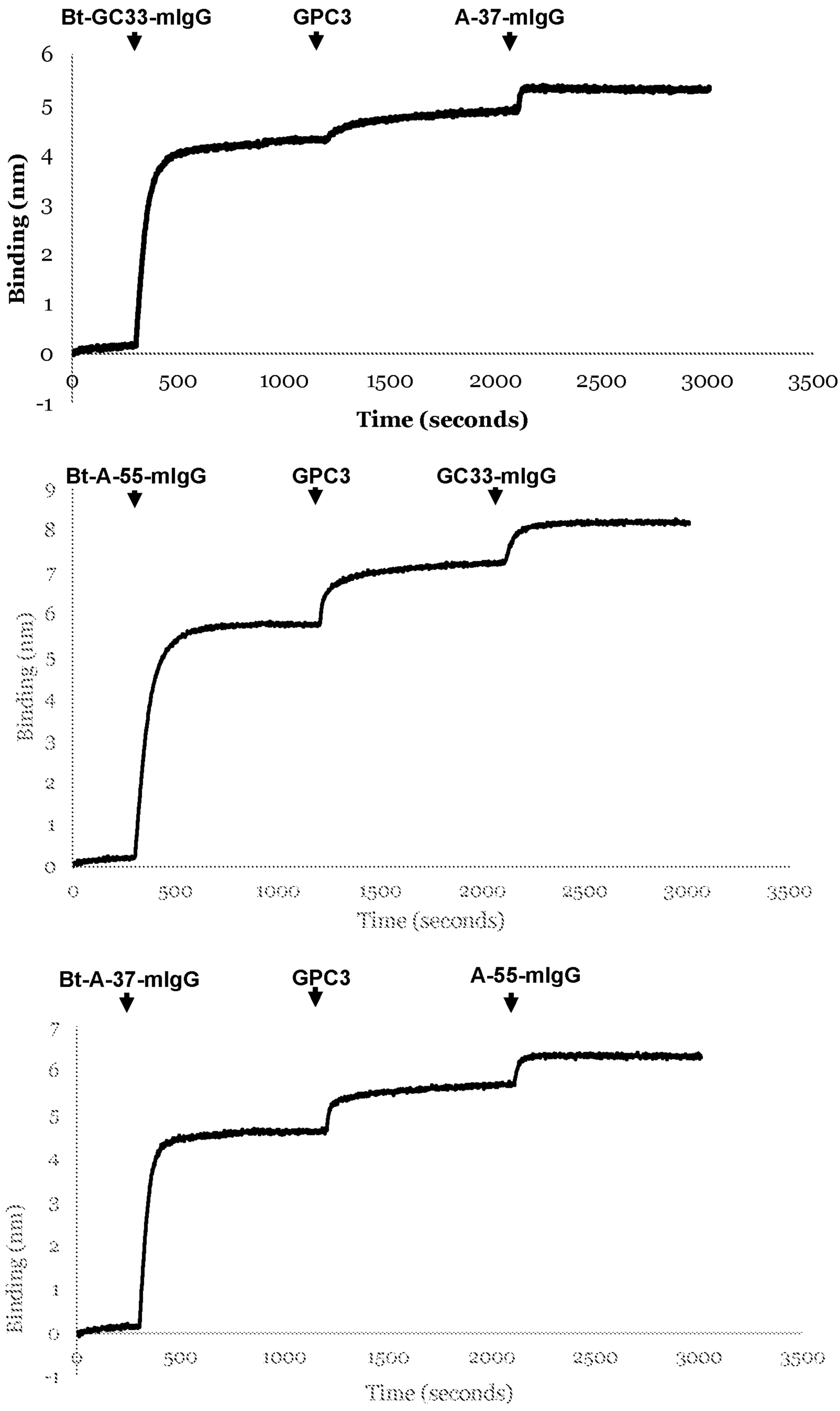

Epitope Binning Analysis
Bt-GC33-mIgG
GPC3
A-37-mIgG
Binding (nm)
Time (seconds)
Bt-A-55-mIgG
GPC3
GC33-mIgG
Binding (nm)
Time (seconds)
Bt-A-37-mIgG
GPC3
A-55-mIgG
Binding (nm)
Time (seconds)

FIG. 15A

Binding kinetics between GPC3A L2K clones and GPC3 antigen

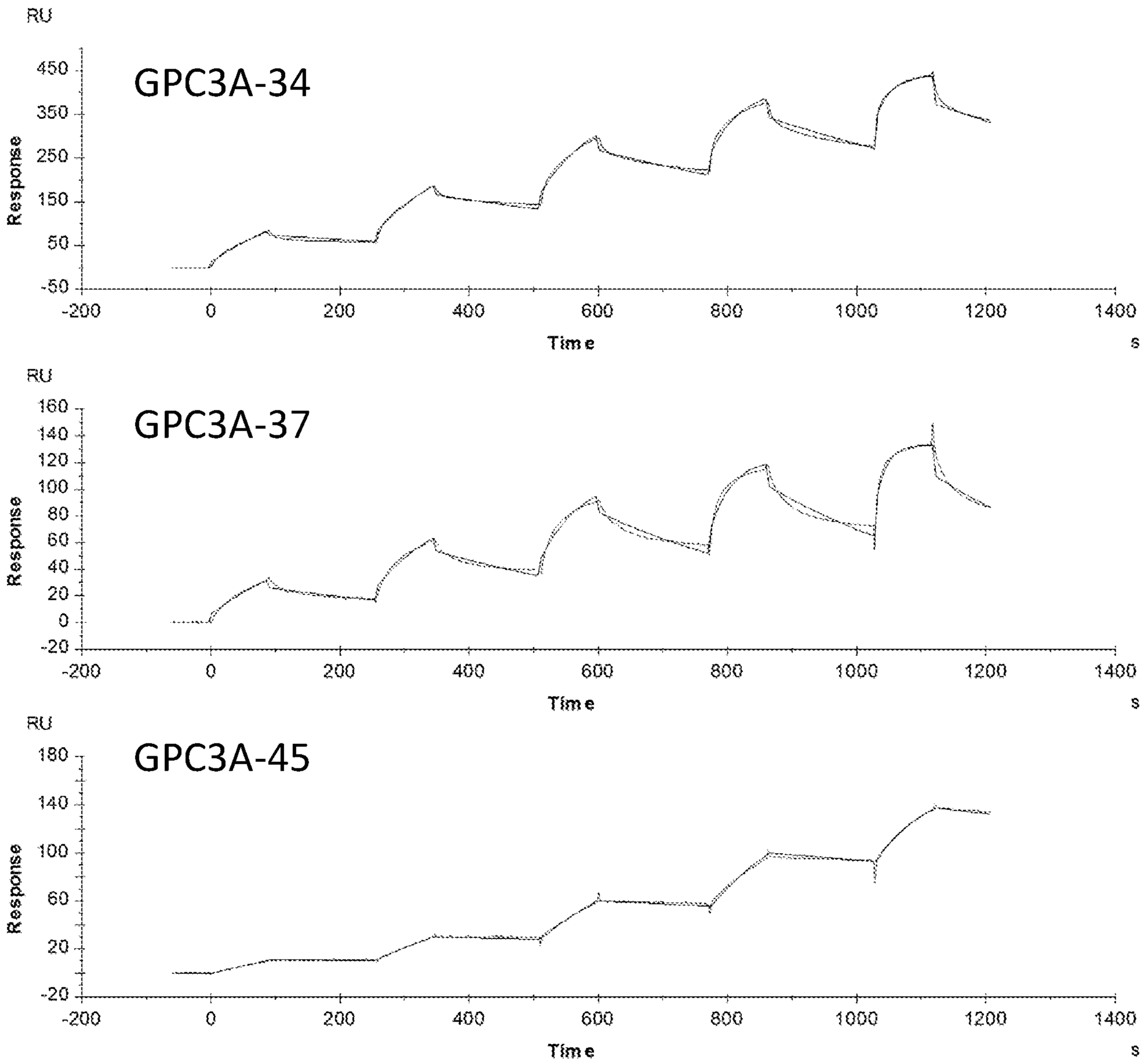
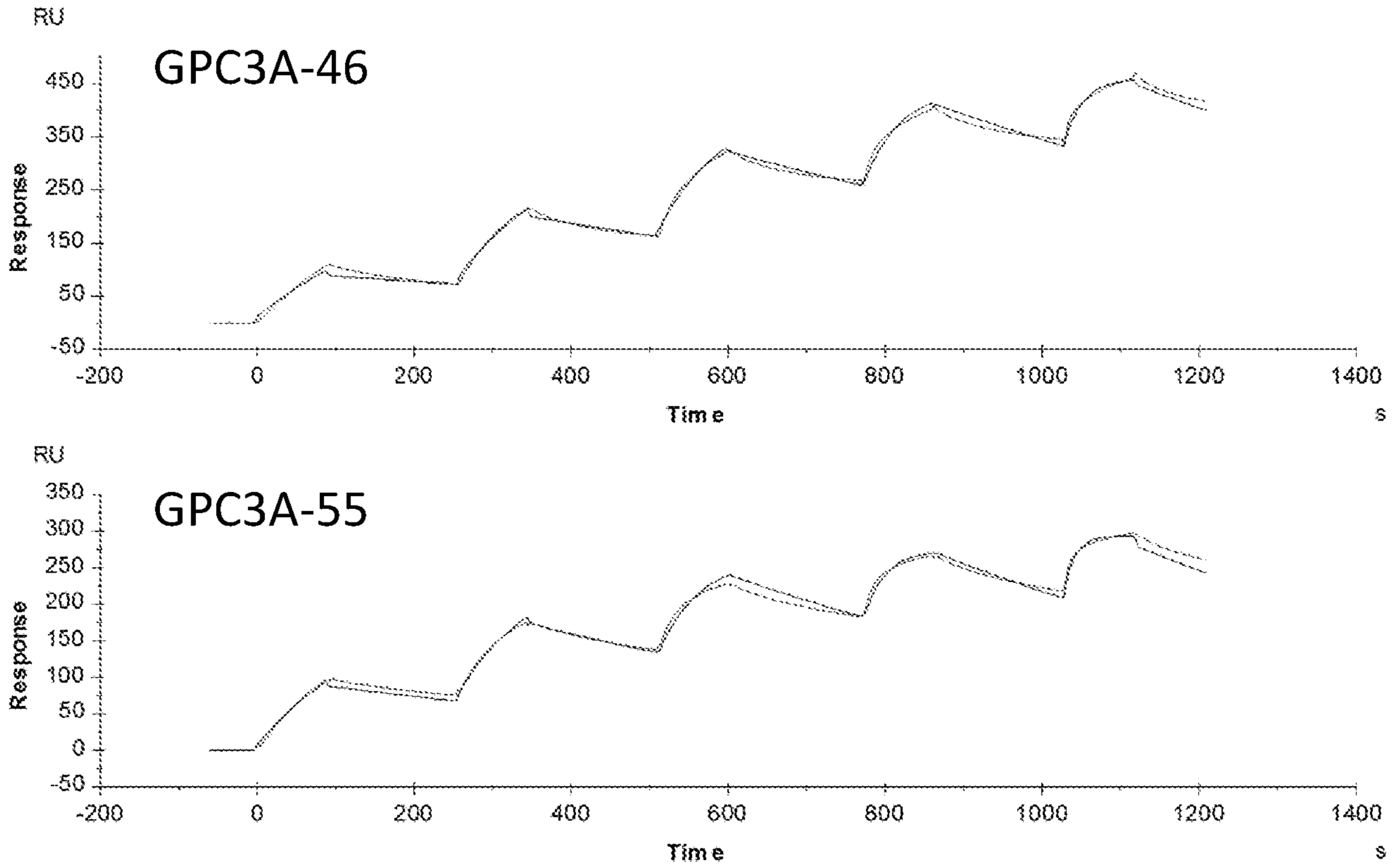


FIG. 15B

Binding kinetics between GPC3A L2K clones and GPC3 antigen



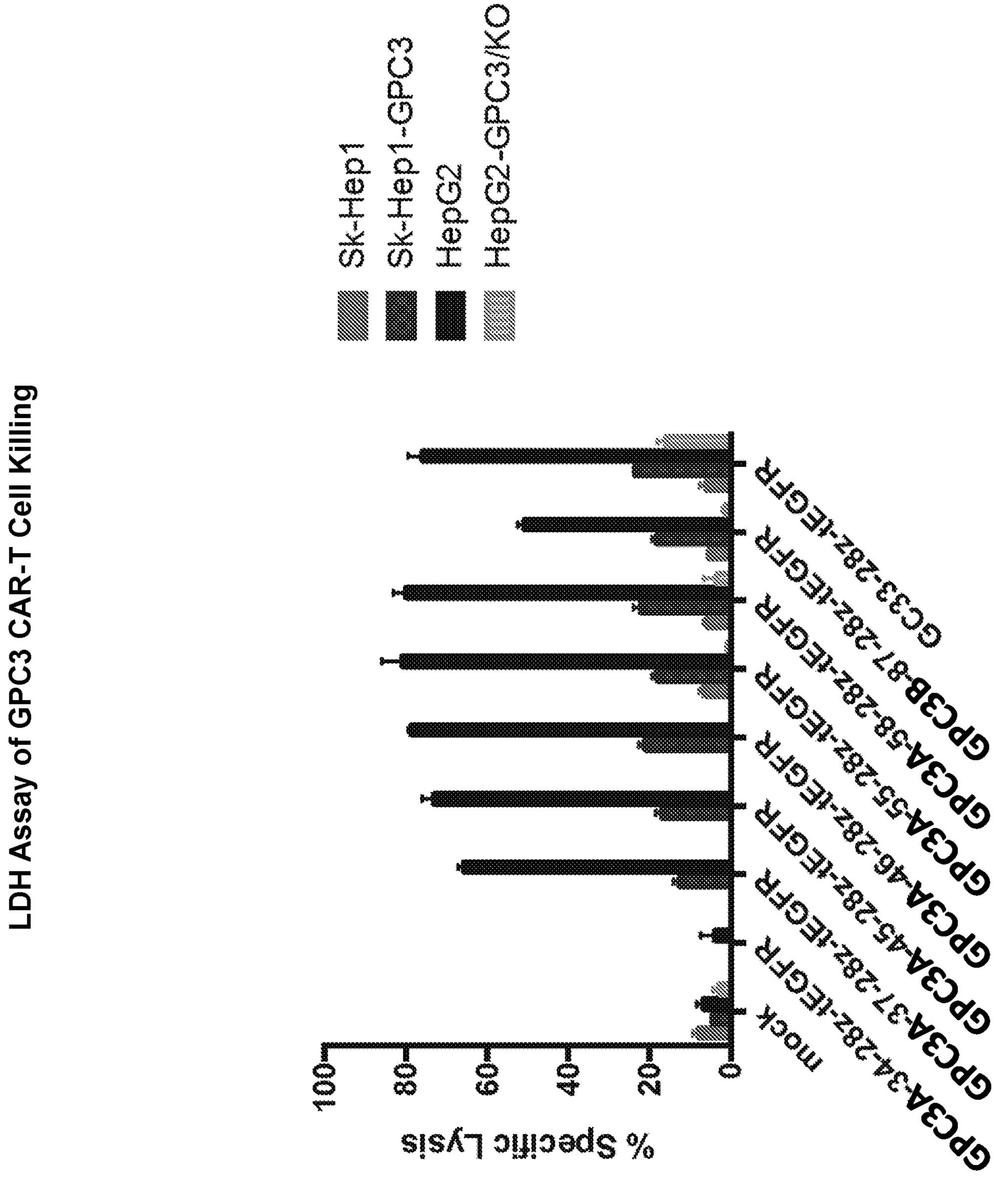
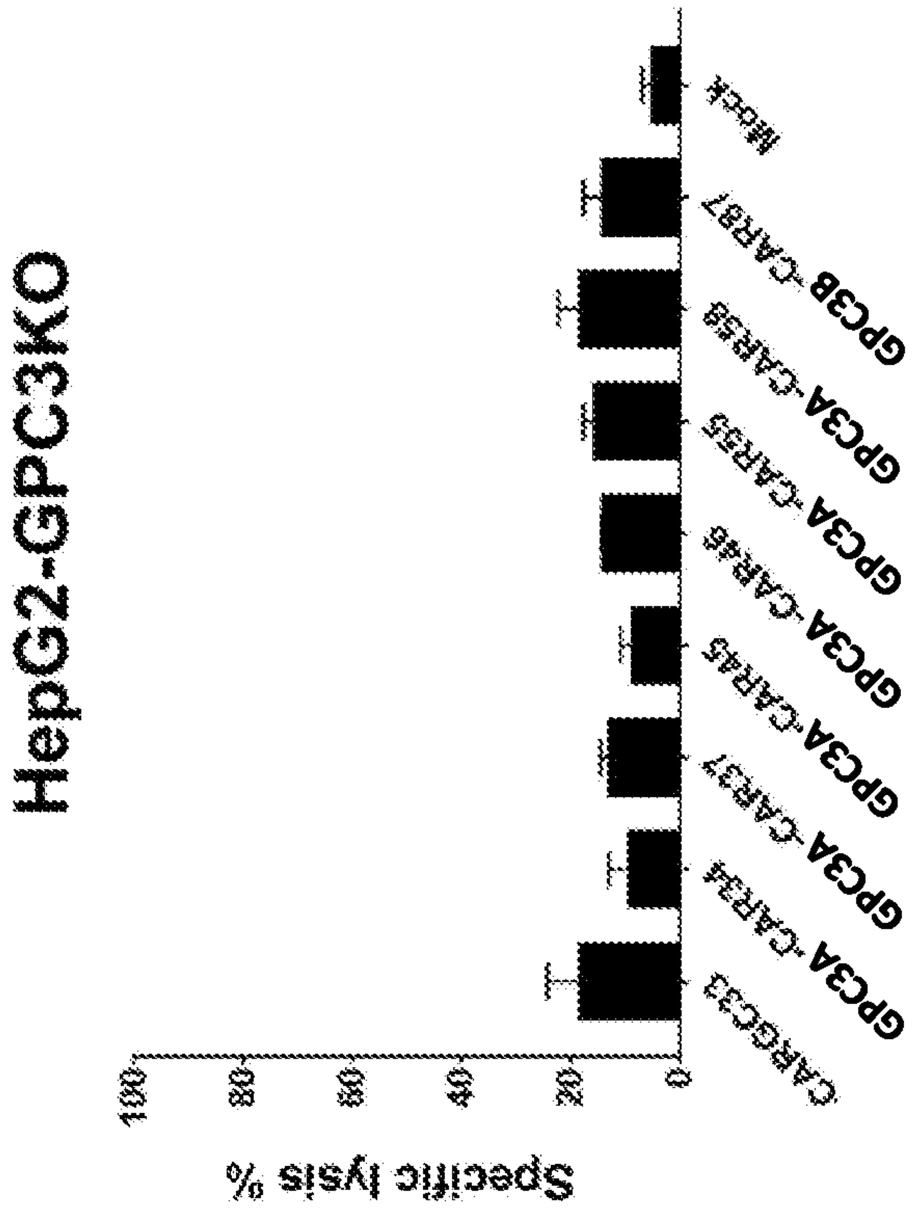
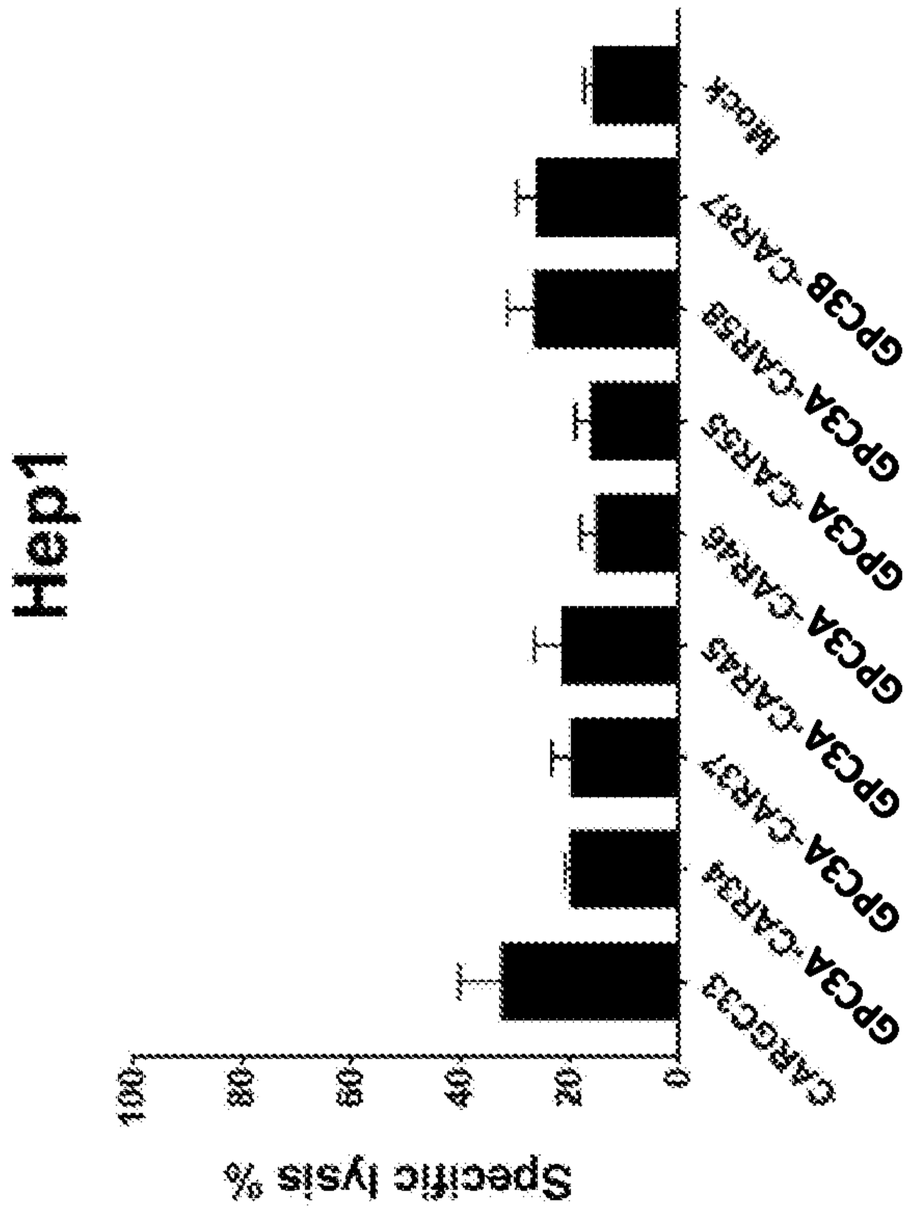
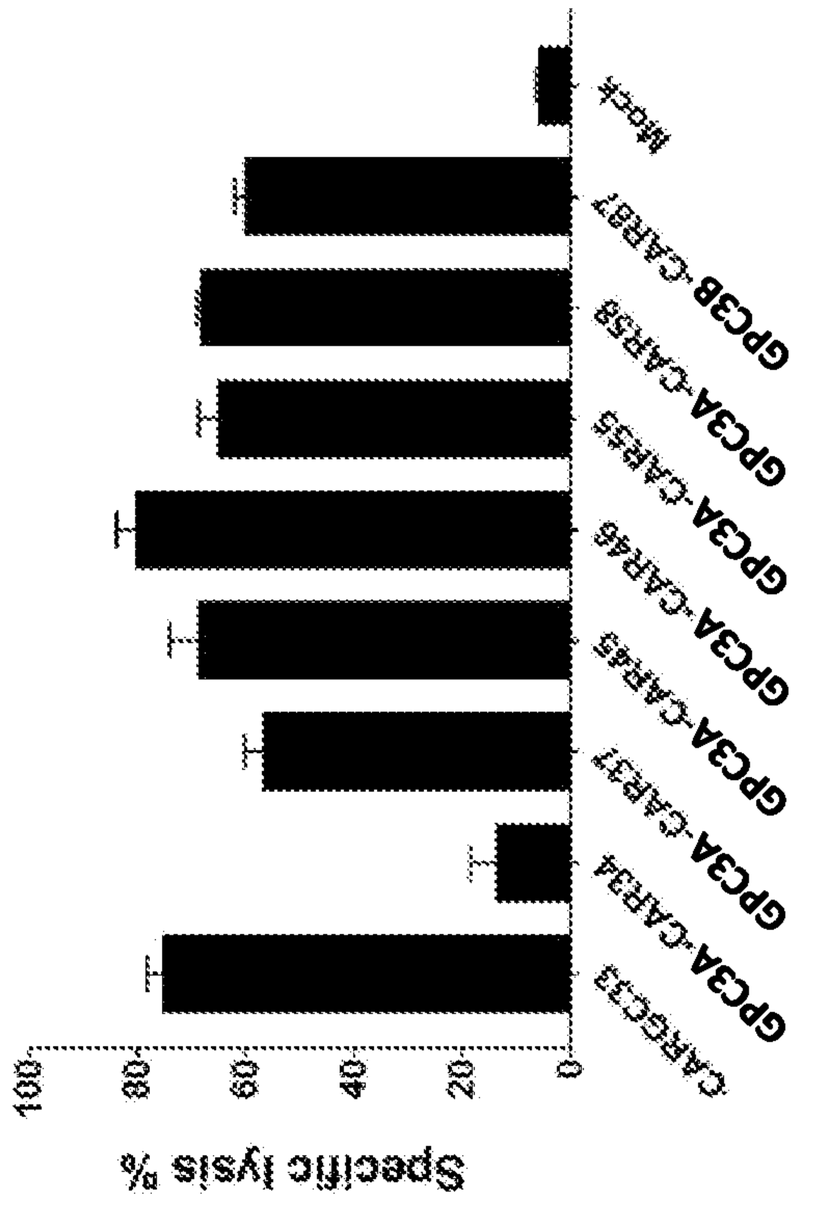

LDH Assay of GPC3 CAR-T Cell Killing
% Specific Lysis
100
80
60
40
20
0
mock
GPC3A-34-28z-tEGFR
GPC3A-37-28z-tEGFR
GPC3A-45-28z-tEGFR
GPC3A-46-28z-tEGFR
GPC3A-55-28z-tEGFR
GPC3A-58-28z-tEGFR
GPC3B-87-28z-tEGFR
GC33-28z-tEGFR
HepG2
HepG2-GPC3/KO
Hep3B
Hep3B-GPC3

FIG. 16


HepG2-GPC3
Specific lysis %
100
80
60
40
20
0
CARGC33
GPC3A-CAR34
GPC3A-CAR37
GPC3A-CAR45
GPC3A-CAR46
GPC3A-CAR55
GPC3A-CAR58
GPC3B-CAR87
Mock


Hep1
Specific lysis %
100
80
60
40
20
0
CARGC33
GPC3A-CAR34
GPC3A-CAR37
GPC3A-CAR45
GPC3A-CAR46
GPC3A-CAR55
GPC3A-CAR58
GPC3B-CAR87
Mock


HepG2
Specific lysis %
100
80
60
40
20
0
CARGC33
GPC3A-CAR34
GPC3A-CAR37
GPC3A-CAR45
GPC3A-CAR46
GPC3A-CAR55
GPC3A-CAR58
GPC3B-CAR87
Mock

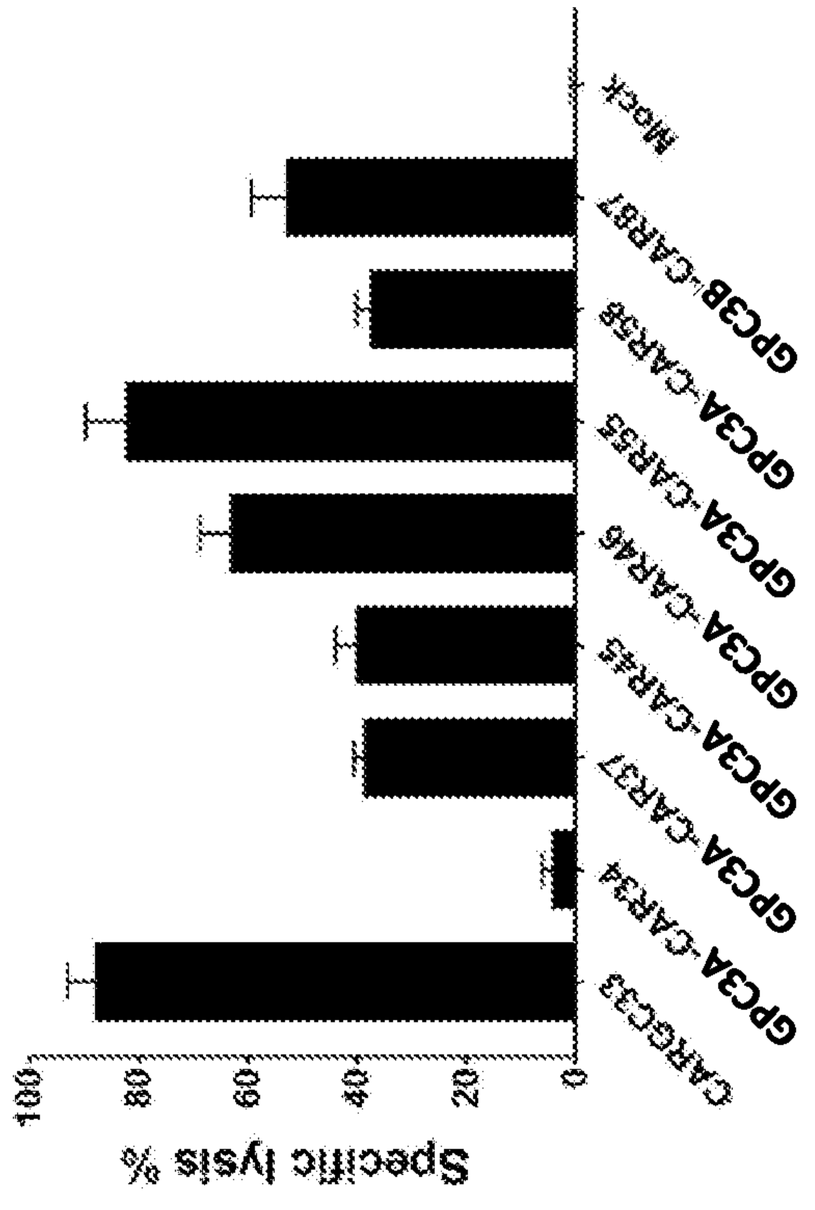
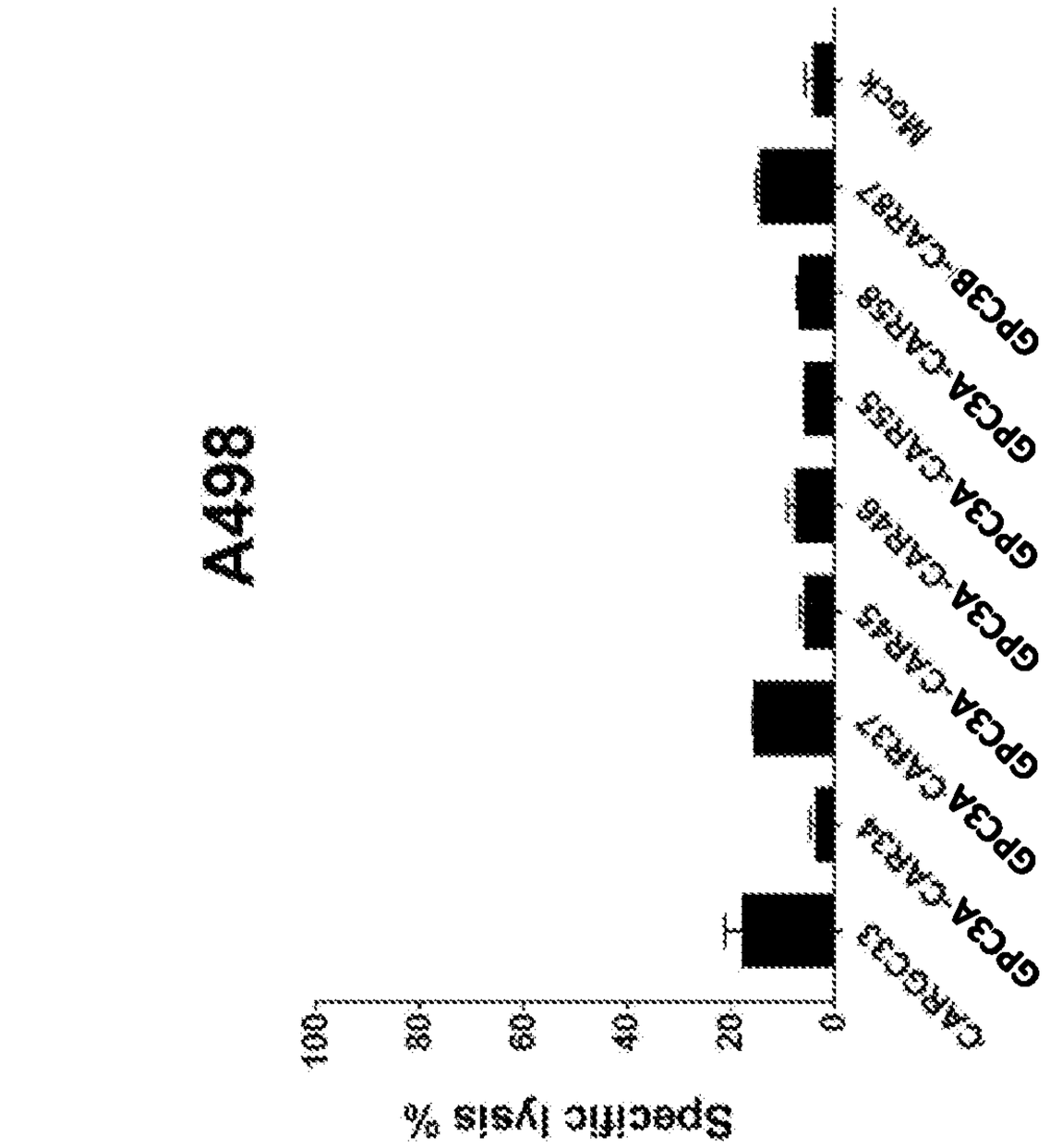
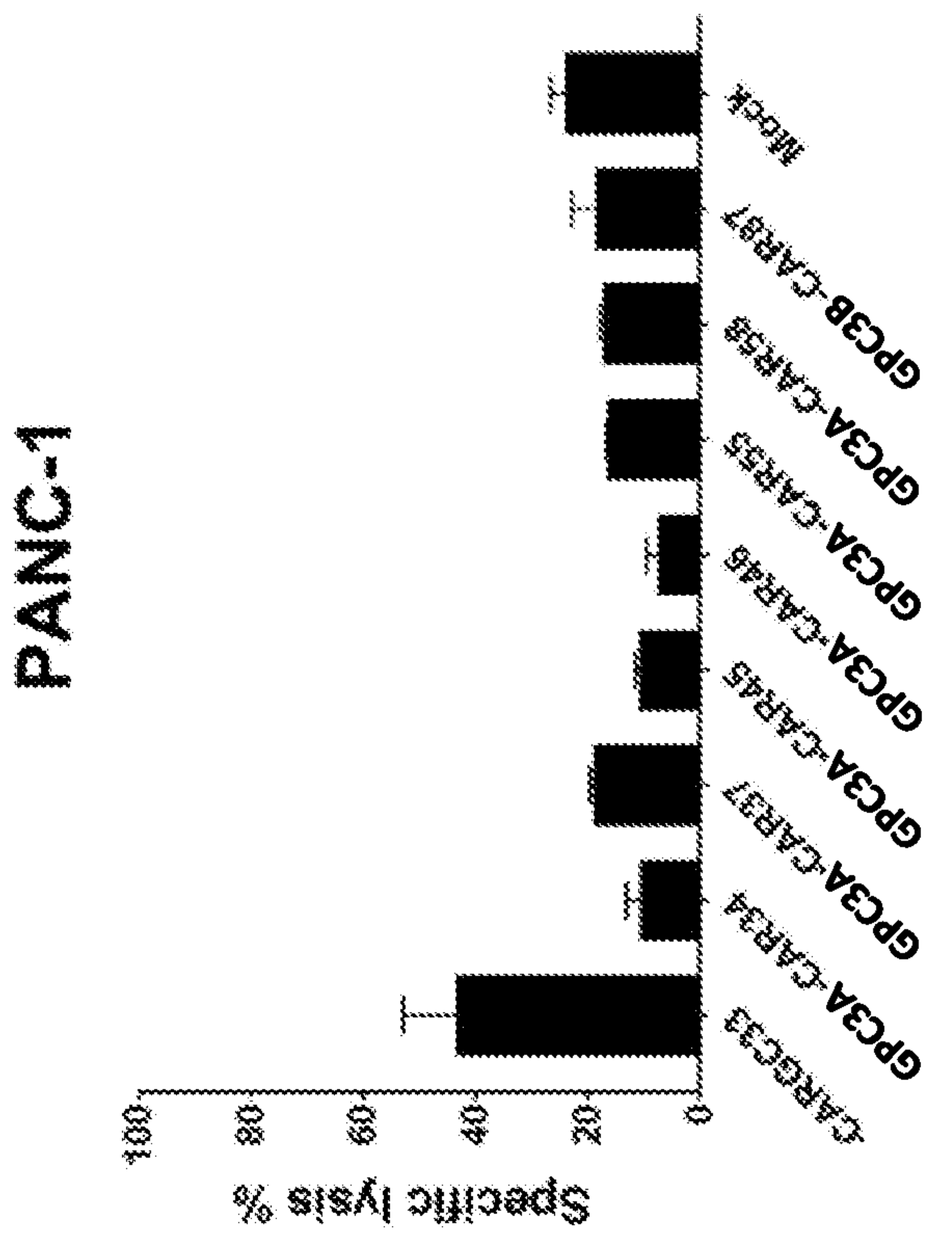

Hep1-GPC3
Specific lysis %
100
80
60
40
20
0
CARGC33
GPC3A-CAR34
GPC3A-CAR37
GPC3A-CAR45
GPC3A-CAR46
GPC3A-CAR55
GPC3A-CAR58
GPC3B-CAR87
Mock

FIG. 17A


A498
Specific lysis %
100
80
60
40
20
0
CARGC33
GPC3A-CAR34
GPC3A-CAR37
GPC3A-CAR45
GPC3A-CAR46
GPC3A-CAR55
GPC3B-CAR87
Mock


PANC-1
Specific lysis %
100
80
60
40
20
0
CARGC33
GPC3A-CAR34
GPC3A-CAR37
GPC3A-CAR45
GPC3A-CAR46
GPC3A-CAR55
GPC3B-CAR87
Mock

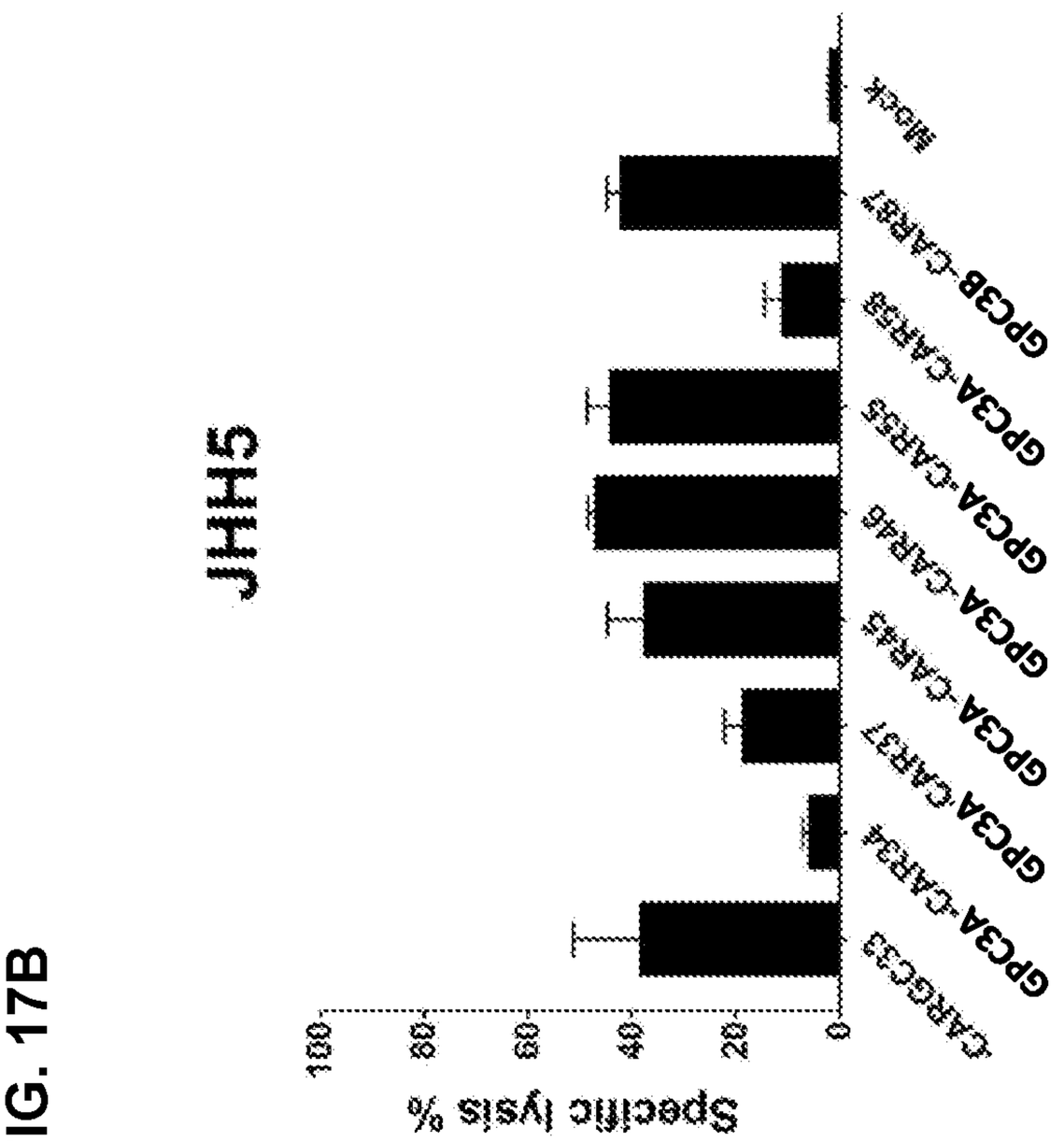

JHH5
Specific lysis %
100
80
60
40
20
0
CARGC33
GPC3A-CAR34
GPC3A-CAR37
GPC3A-CAR45
GPC3A-CAR46
GPC3A-CAR55
GPC3B-CAR87
Mock

FIG. 17B

FIG. 18

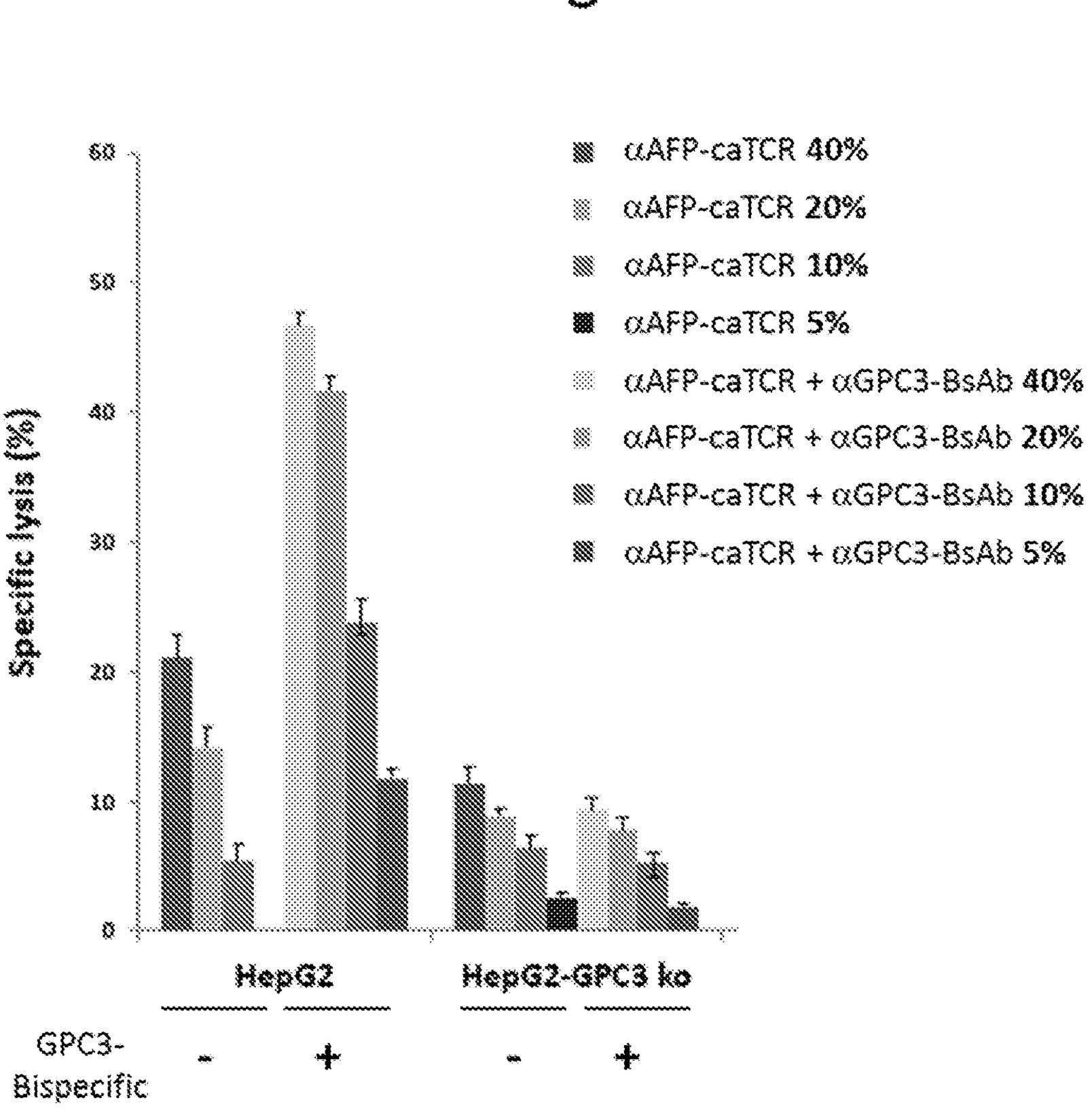
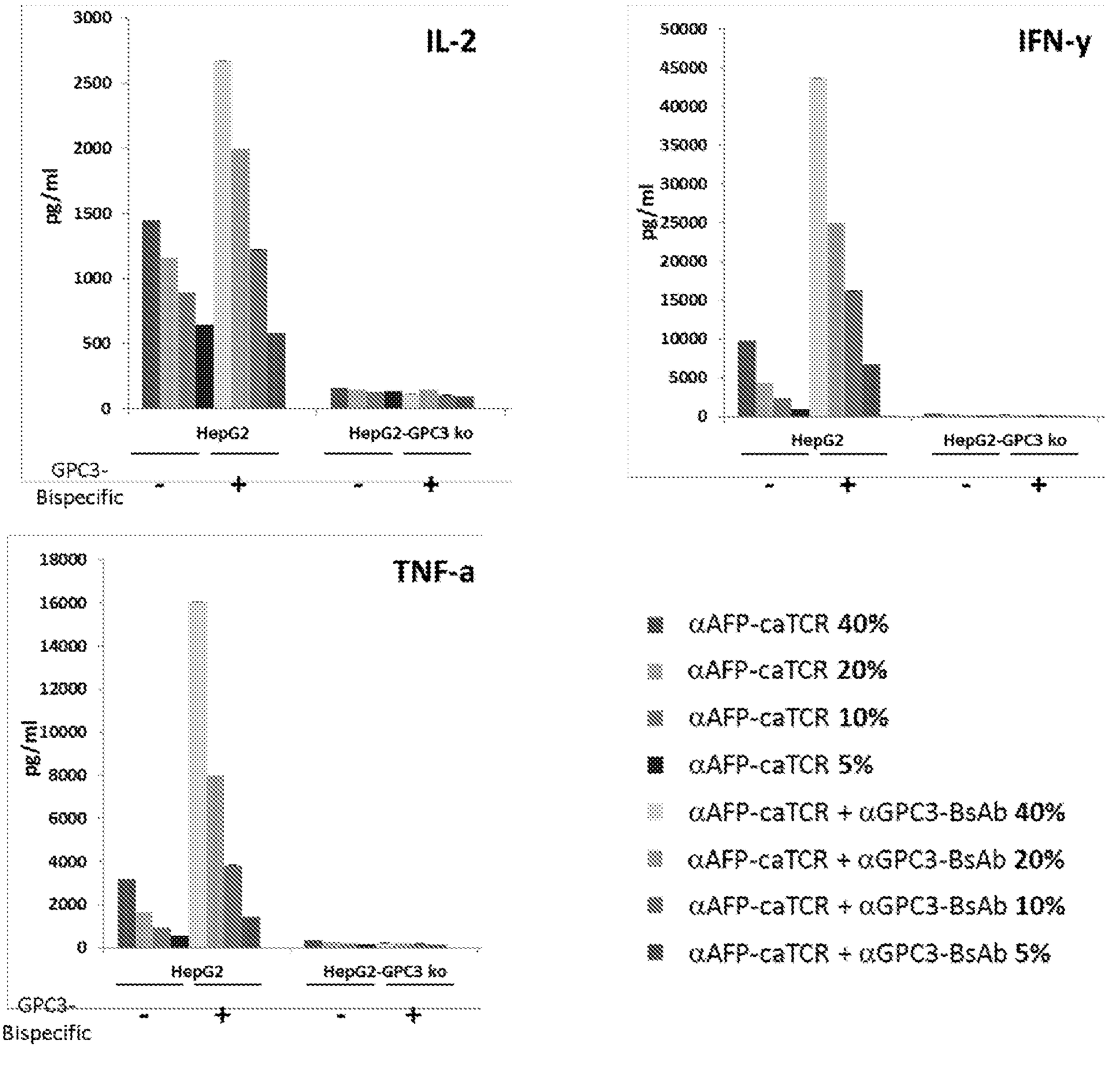

In vitro Killing
Specific lysis (%)
60
50
40
30
20
10
0
αAFP-caTCR 40%
αAFP-caTCR 20%
αAFP-caTCR 10%
αAFP-caTCR 5%
αAFP-caTCR + αGPC3-BsAb 40%
αAFP-caTCR + αGPC3-BsAb 20%
αAFP-caTCR + αGPC3-BsAb 10%
αAFP-caTCR + αGPC3-BsAb 5%
HepG2
HepG2-GPC3 ko
GPC3-Bispecific
-
+
-
+

FIG. 19


IL-2
IFN-γ
TNF-α
pg/ml
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%

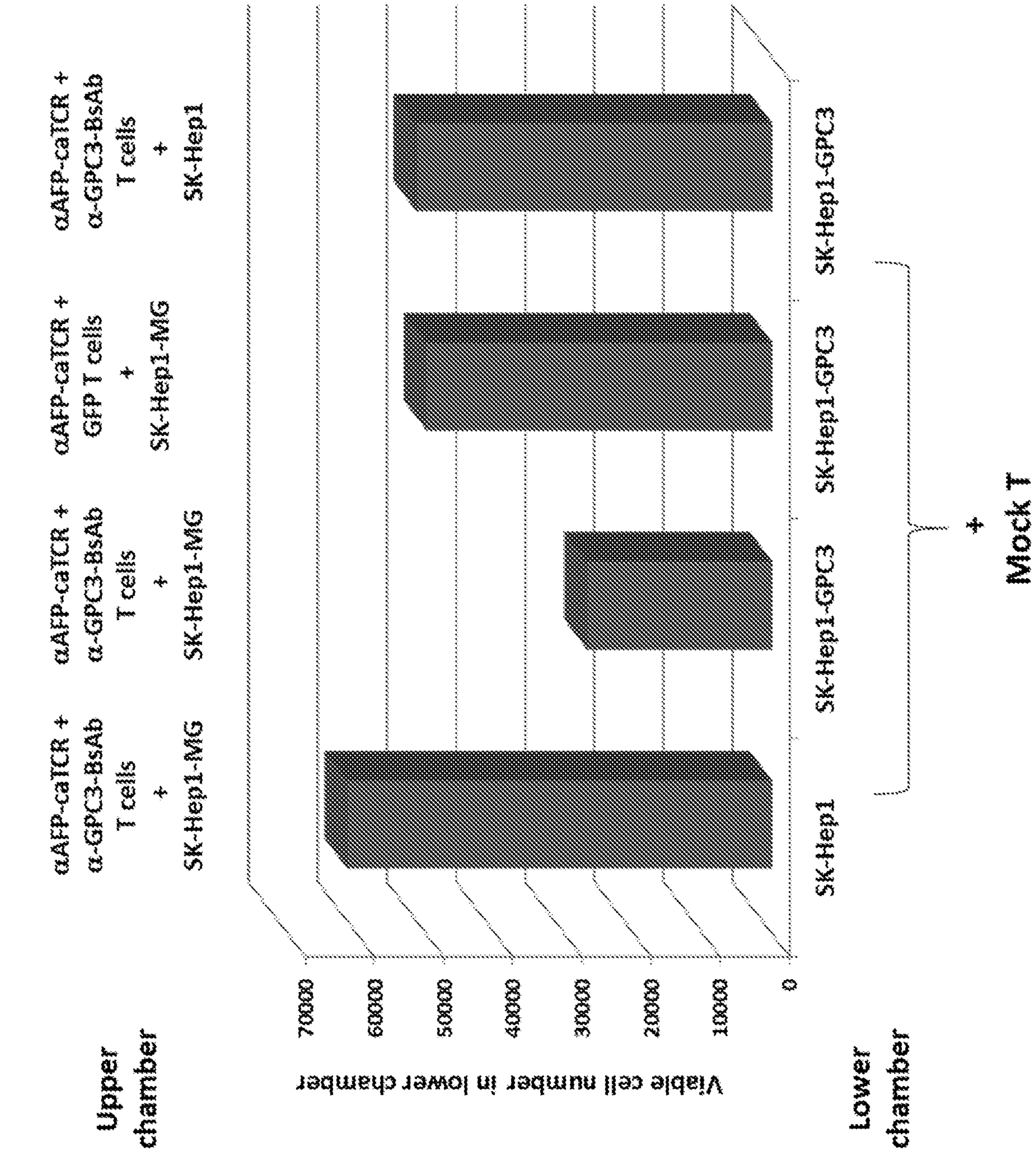
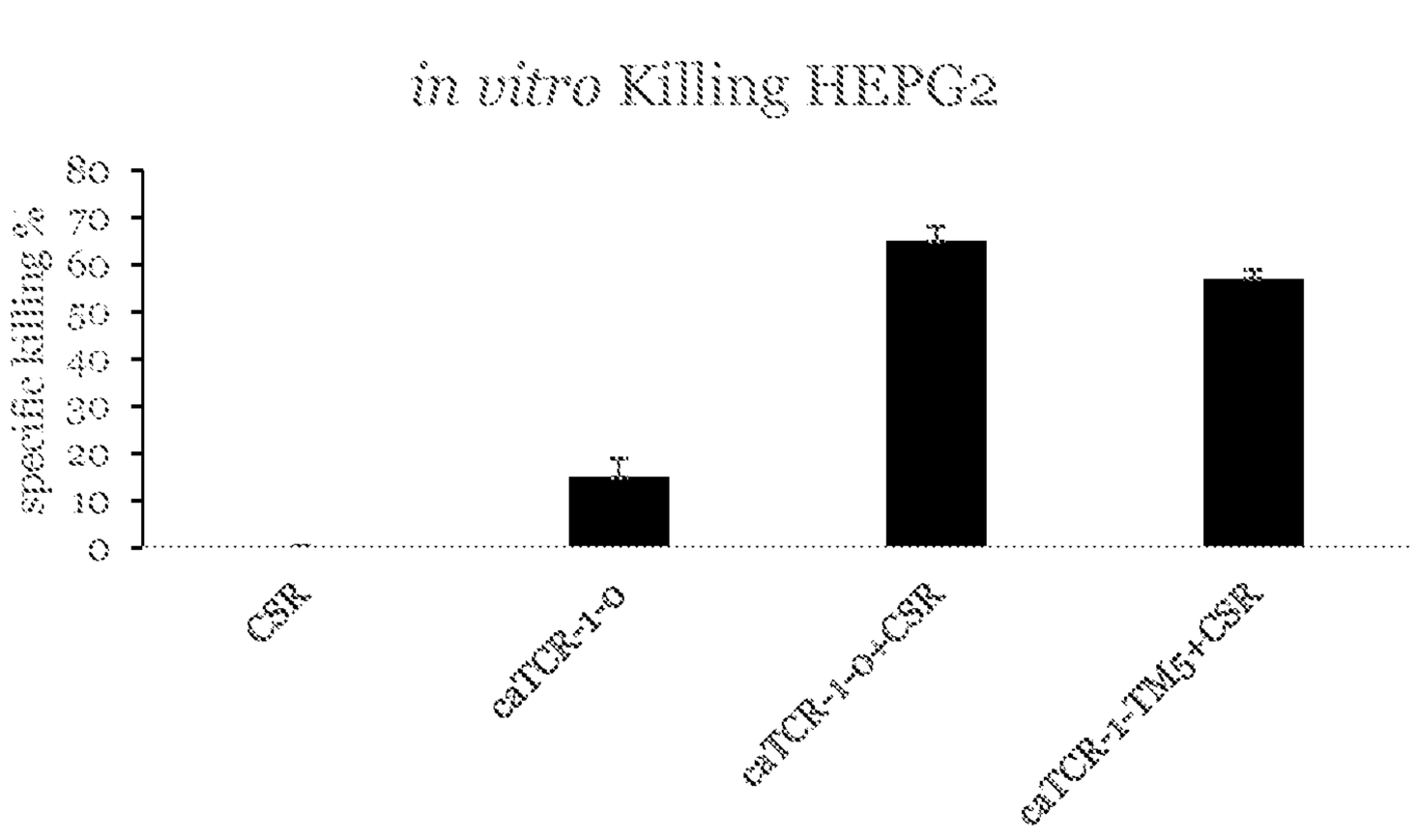

Upper chamber
Viable cell number in lower chamber
70000
60000
50000
40000
30000
20000
10000
0
αAFP-caTCR + α-GPC3-BsAb T cells + SK-Hep1-MG
αAFP-caTCR + GFP T cells + SK-Hep1-MG
αAFP-caTCR + α-GPC3-BsAb T cells + SK-Hep1
SK-Hep1-GPC3
SK-Hep1-GPC3
SK-Hep1
Lower chamber
Mock T cells + SK-Hep1-GPC3

FIG. 20

FIG. 21


in vitro Killing HEPG2
specific killing %
0
10
20
30
40
50
60
70
80
CSR
caTCR-1-O
caTCR-1-O-CSR
caTCR-1-TM5+CSR

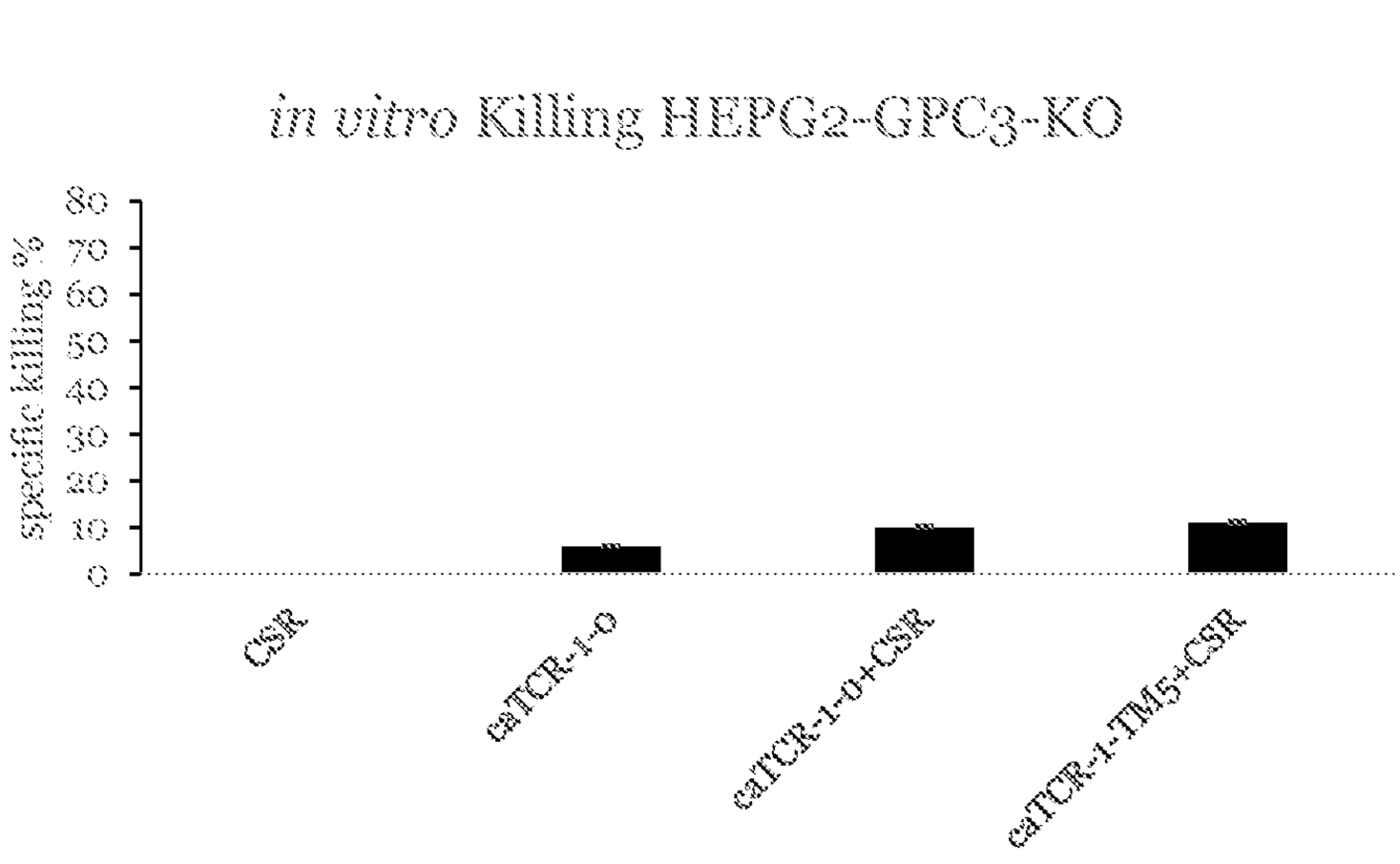
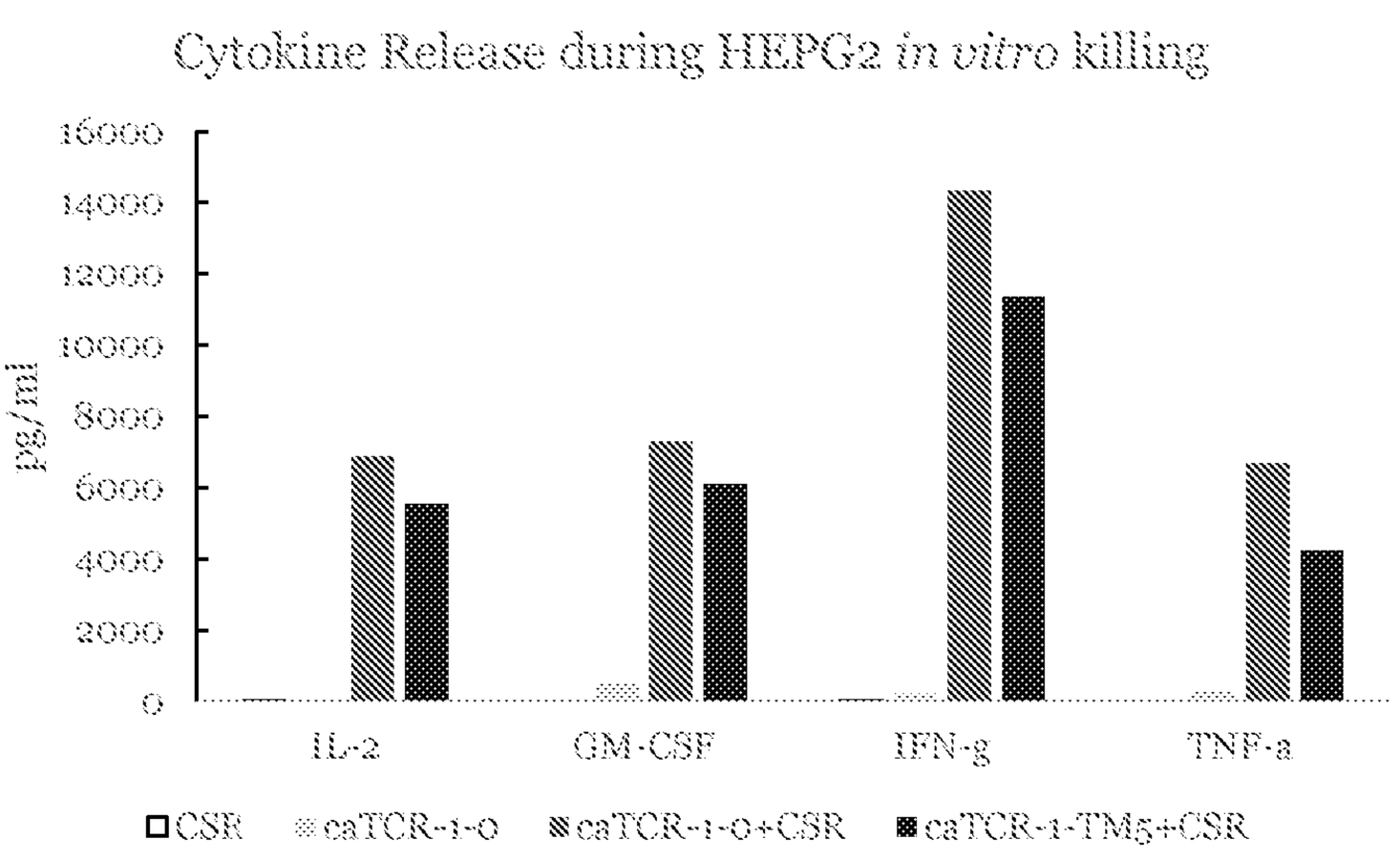

in vitro Killing HEPG2-GPC3-KO
specific killing %
0
10
20
30
40
50
60
70
80
CSR
caTCR-1-O
caTCR-1-O+CSR
caTCR-1-TM5+CSR

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

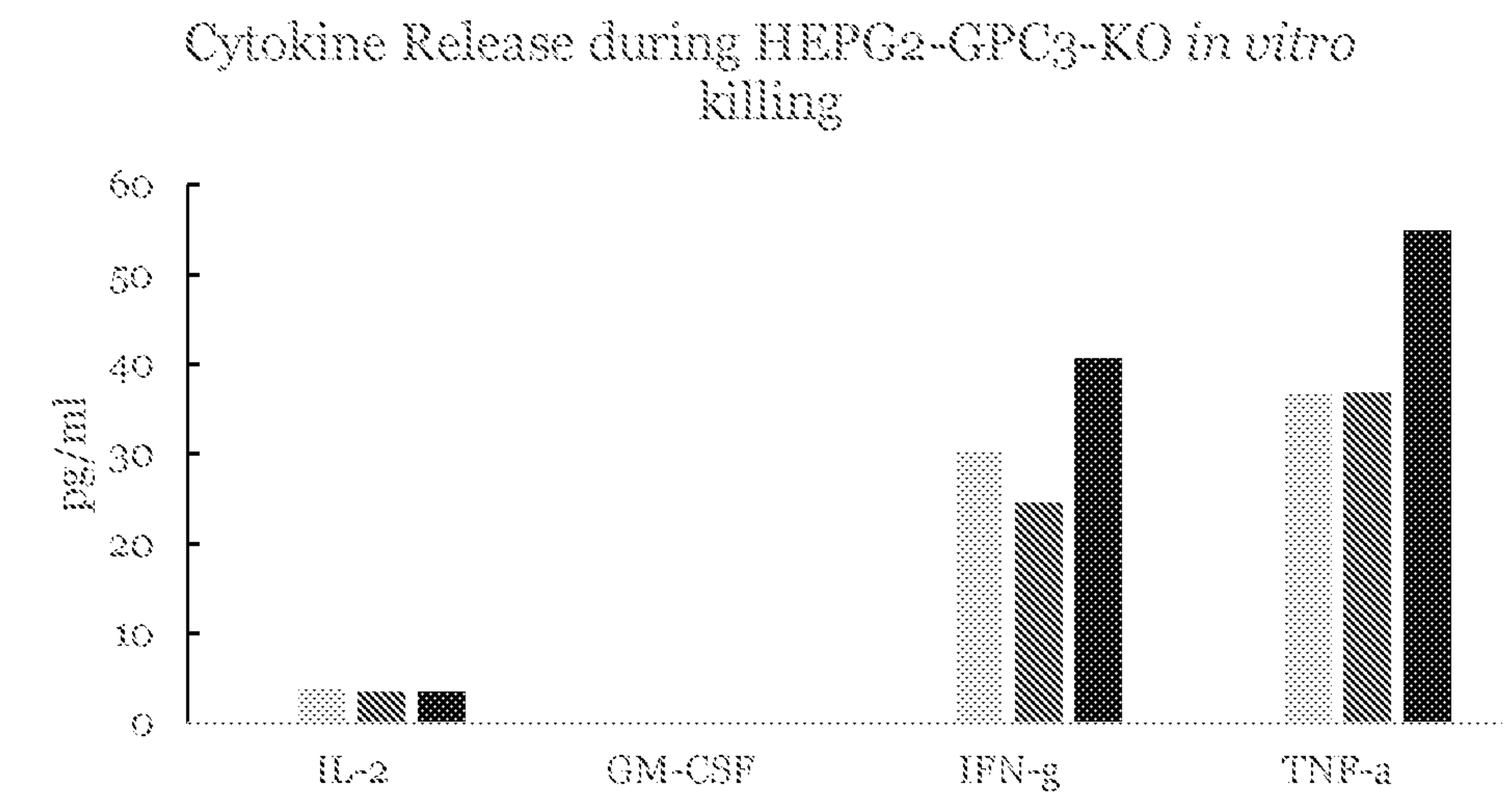
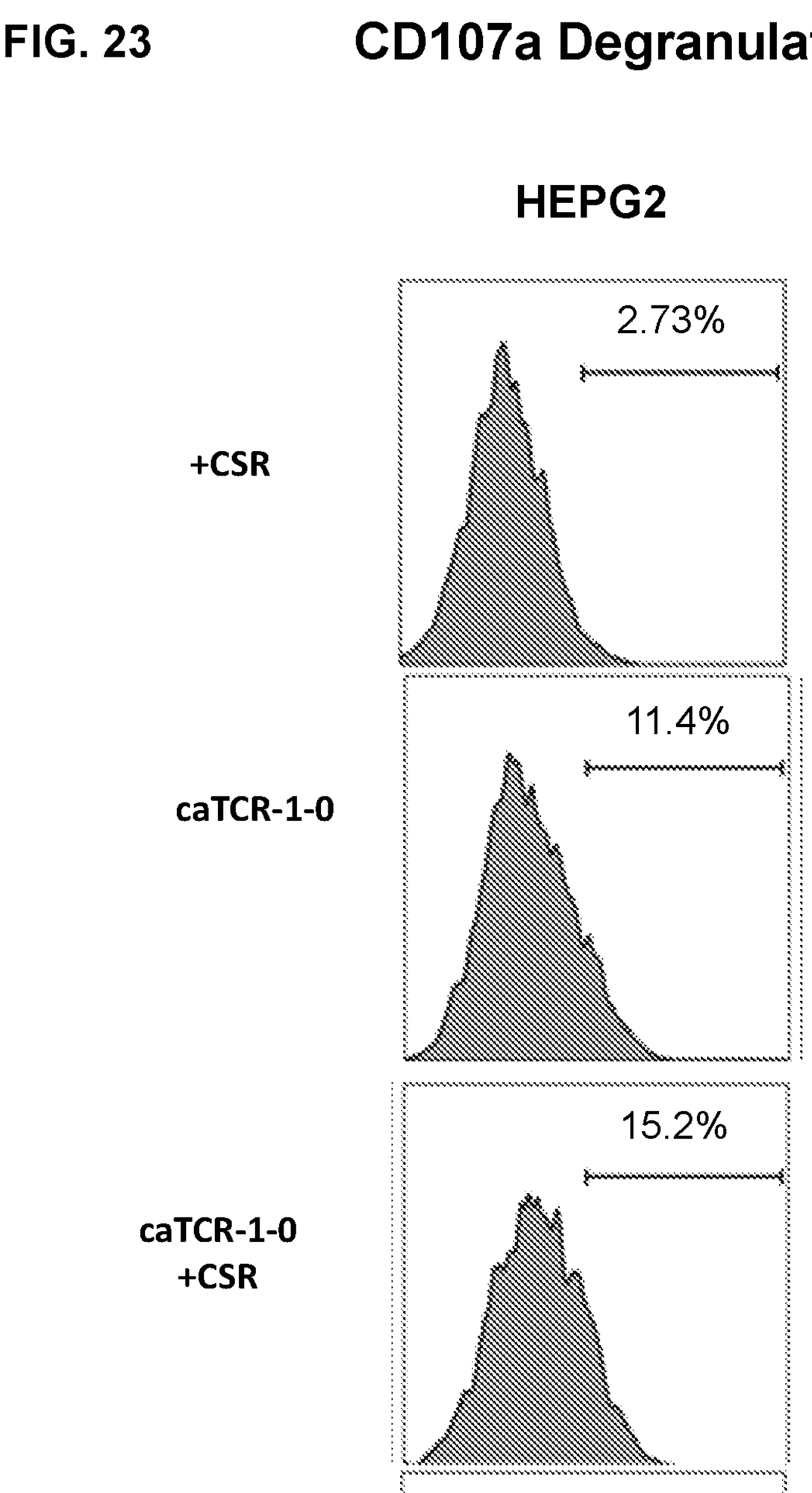

Cytokine Release during HEPG2-GPC3-KO *in vitro* killing
60
50
40
30
20
10
0
pg/ml
IL-2
GM-CSF
IFN-g
TNF-a
CSR
caTCR-1-0
caTCR-1-0+CSR
caTCR-1-TM5+CSR

FIG. 23


CD107a Degranulation
HEPG2
+CSR
2.73%
caTCR-1-0
11.4%
caTCR-1-0
+CSR
15.2%
caTCR-1-TM5
+CSR
17.2%
CD107a

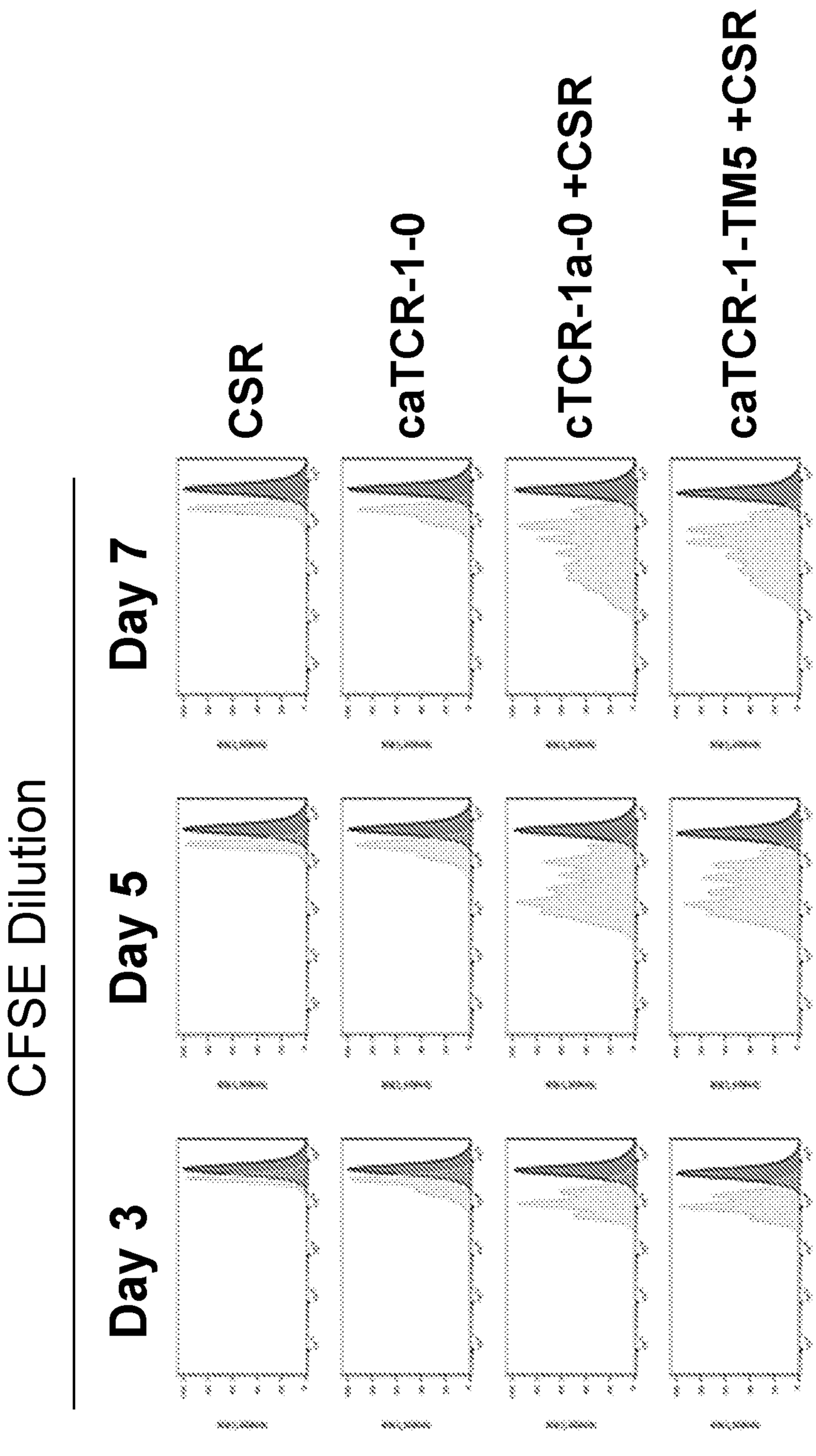
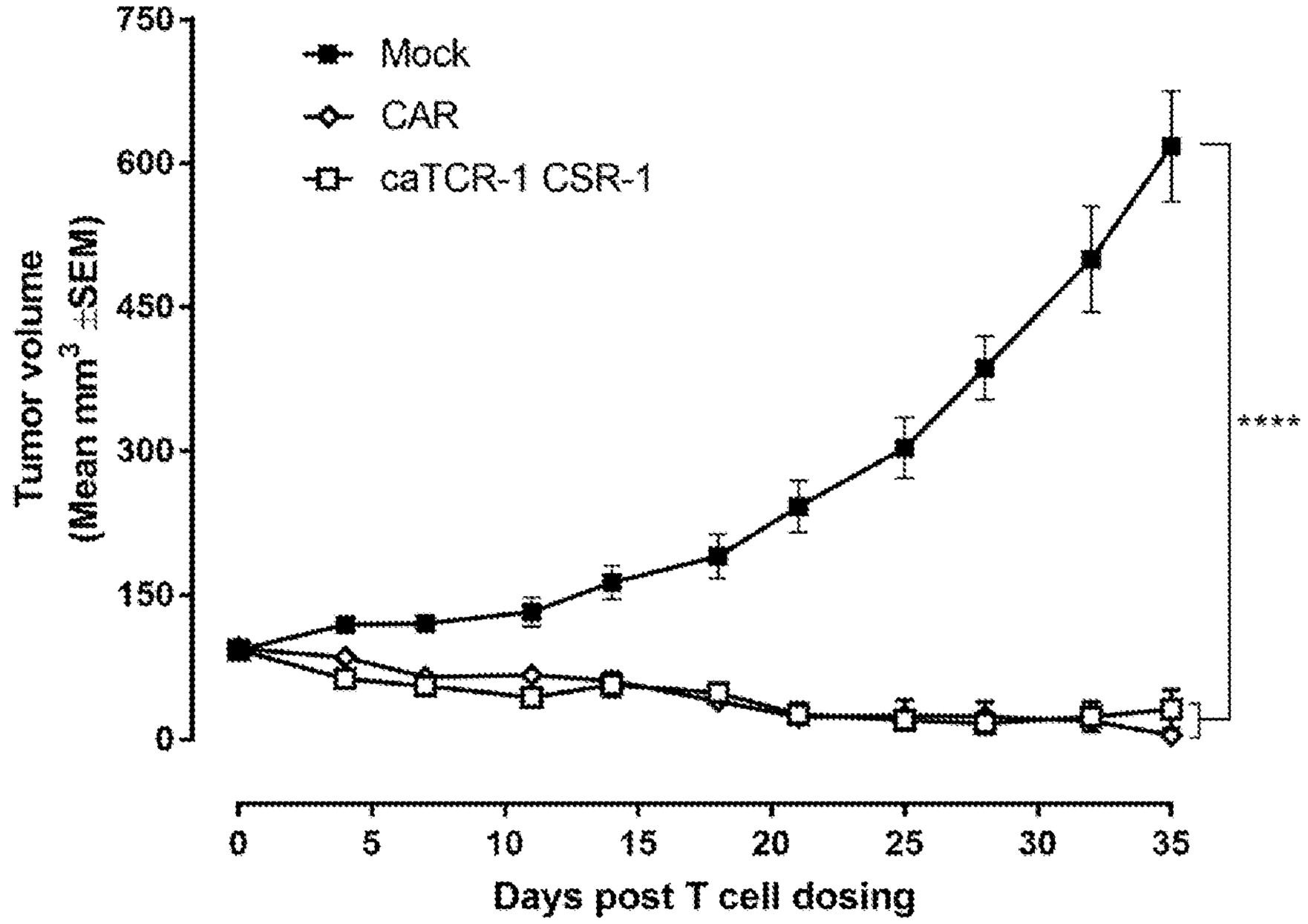

HEPG2
CFSE Dilution
Day 3
Day 5
Day 7
CSR
caTCR-1-0
caTCR-1a-0 +CSR
caTCR-1-TM5 +CSR

FIG. 24

FIG. 25


Mock
CAR
caTCR-1 CSR-1
Tumor volume
(Mean mm3 ±SEM)
750
600
450
300
150
0
****
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.

FIG. 6

Competition for Hep2 cell binding with and without soluble GPC3