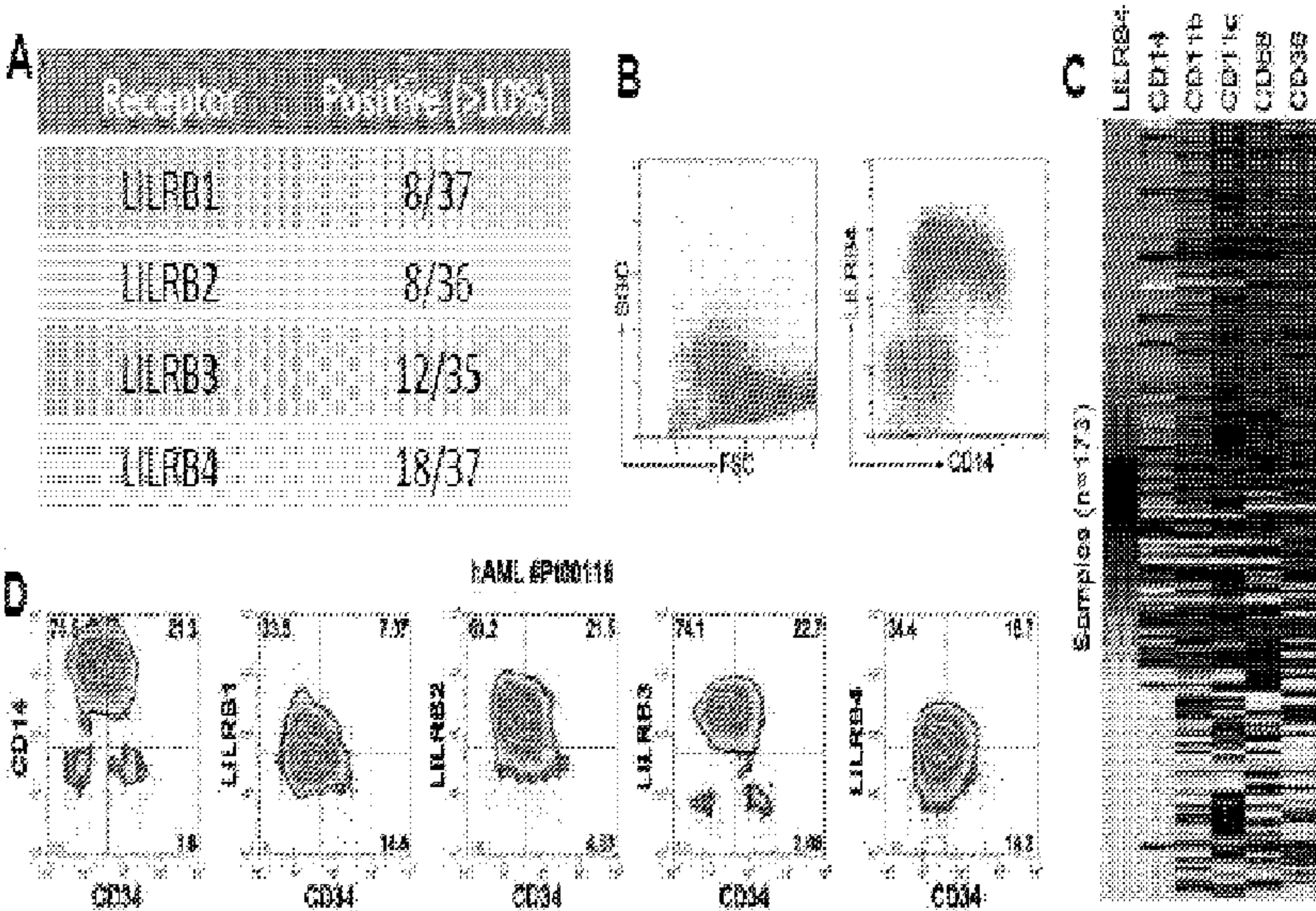




(86) Date de dépôt PCT/PCT Filing Date: 2016/03/04
(87) Date publication PCT/PCT Publication Date: 2016/09/15
(85) Entrée phase nationale/National Entry: 2017/08/22
(86) N° demande PCT/PCT Application No.: US 2016/020838
(87) N° publication PCT/PCT Publication No.: 2016/144728
(30) Priorité/Priority: 2015/03/06 (US62/129,572)

(51) Cl.Int./Int.Cl. *G01N 33/574* (2006.01),
A61K 35/15 (2015.01), *A61K 38/07* (2006.01),
A61K 39/395 (2006.01)
(71) Demandeur/Applicant:
THE BOARD OF REGENTS OF THE UNIVERSITY OF
TEXAS SYSTEM, US
(72) Inventeurs/Inventors:
ZHANG, CHENGCHENG, US;
DENG, MI, US;
AN, ZHIQIANG, US;
XIONG, WEI, US;
ZHANG, NINGYAN, US;
ZHENG, JUNKE, CN;

(54) Titre : ANTICORPS ANTI-LILRB ET LEUR UTILISATION POUR DETECTER ET TRAITER UN CANCER
(54) Title: ANTI-LILRB ANTIBODIES AND THEIR USE IN DETECTING AND TREATING CANCER



FIGS. 1A-D

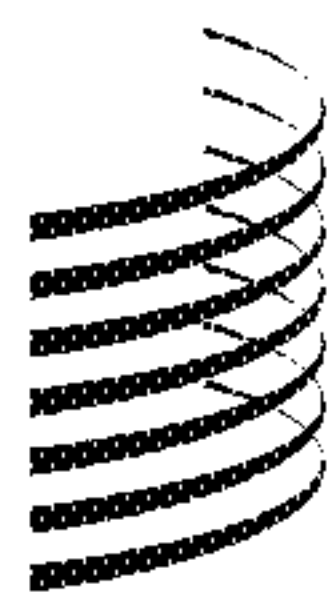
(57) Abrégé/Abstract:
The present disclosure is directed to antibodies binding to LILRBs and methods of detecting and treating cancer therewith.

(72) Inventeurs(suite)/Inventors(continued): GUI, XUN, US

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

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

(19) World Intellectual Property
Organization
International Bureau



WIPO | PCT



(10) International Publication Number

WO 2016/144728 A3

(43) International Publication Date
15 September 2016 (15.09.2016)

(51) International Patent Classification:

A61K 38/18 (2006.01) A61K 38/07 (2006.01)
A61K 35/15 (2015.01)

(21) International Application Number:

PCT/US2016/020838

(22) International Filing Date:

4 March 2016 (04.03.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/129,572 6 March 2015 (06.03.2015) US

(71) Applicant: THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West 7th Street, Austin, TX 78701 (US).

(72) Inventors: ZHANG, Chengcheng; 4501 Druid Lane, Apt. 117, Dallas, TX 75205 (US). DENG, Mi; 4712 Bull Run Drive, Plano, TX 75093 (US). AN, Zhiqiang; C/o Ut Health Science Center At Houston, 7000 Fannin Street, Suite 720, Houston, TX 77030 (US). XIONG, Wei; 3618 Abbeywood Drive, Pearland, TX 77584 (US). ZHANG, Ningyan; C/o Ut Health Science Center At Houston, 7000 Fannin Street, Suite 720, Houston, TX 77030 (US).

ZHENG, Junke; C/o Shanghai Jiao-tong University School Of Medici, Chongqing South Road 280, Shanghai, 200025 (CN). GUI, Xun; C/o Ut Health Science Center At Houston, 7000 Fannin Street, Suite 720, Houston, TX 77030 (US).

(74) Agent: HIGHLANDER, Steven, L.; Parker Highlander PLLC, 1120 S. Capital Of Texas Highway, Building One, Suite 200, Austin, TX 78746 (US).

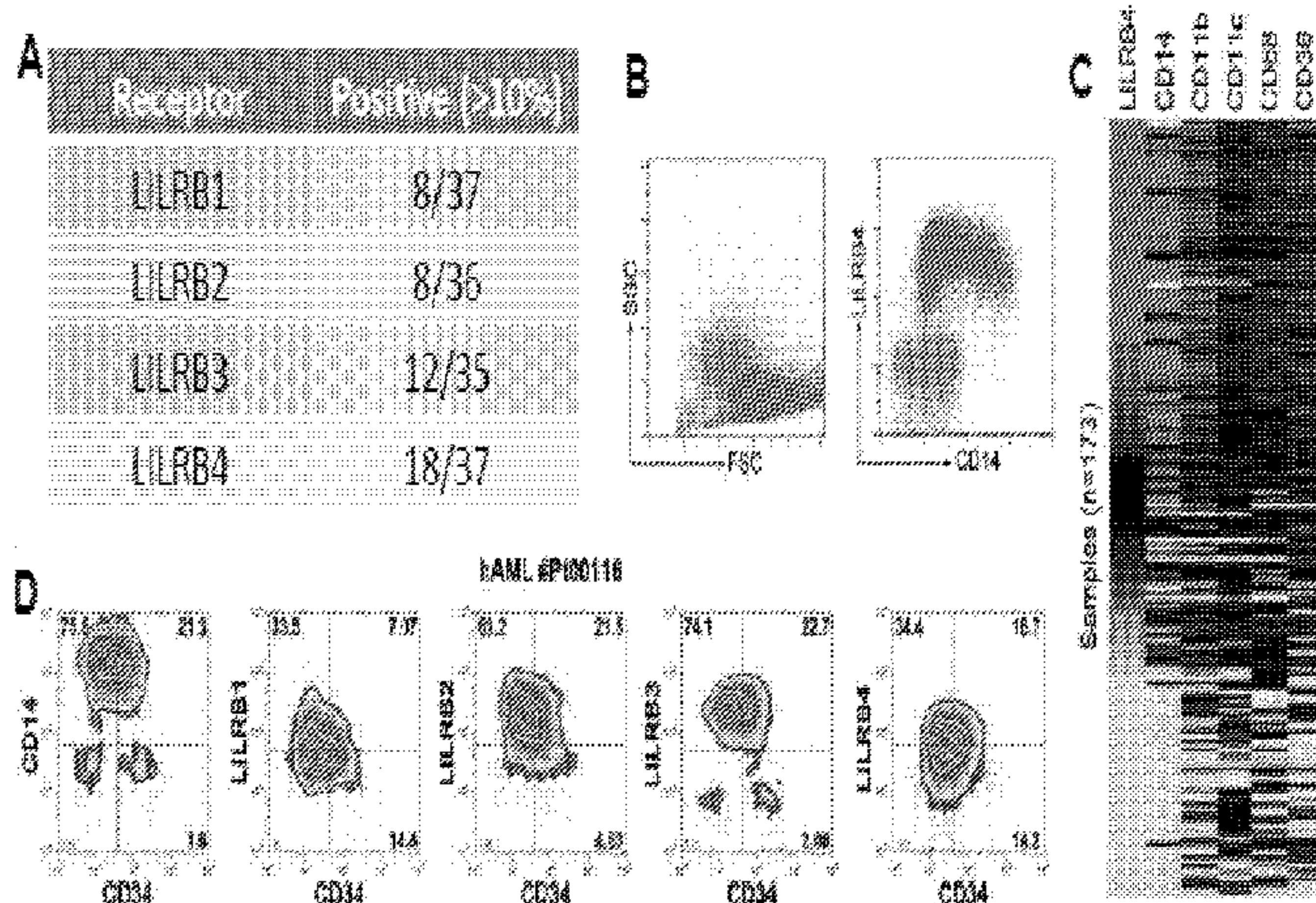
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, 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, 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,

[Continued on next page]

(54) Title: ANTI-LILRB ANTIBODIES AND THEIR USE IN DETECTING AND TREATING CANCER

(57) Abstract: The present disclosure is directed to antibodies binding to LILRBs and methods of detecting and treating cancer therewith.



FIGS. 1A-D

WO 2016/144728 A3

LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, — *before the expiration of the time limit for amending the*
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, *claims and to be republished in the event of receipt of*
GW, KM, ML, MR, NE, SN, TD, TG). *amendments (Rule 48.2(h))*

Published: (88) **Date of publication of the international search report:**
— *with international search report (Art. 21(3))* 27 October 2016

DESCRIPTION

ANTI-LILRB ANTIBODIES AND THEIR USE IN DETECTING AND TREATING CANCER

5

This application claims benefit of priority to U.S. Provisional Application Serial No. 62/129,572, filed March 6, 2015, the entire contents of which are hereby incorporated by reference.

10

BACKGROUND

1. Field of the Disclosure

The present disclosure relates generally to the fields of medicine, oncology, and immunology. More particular, the disclosure relates to antibodies that LILRBs and can treat cancers, including leukemias.

15

2. Background

Acute myeloid leukemia (AML) is the most common acute leukemia of adults. Despite continuous treatment, most patients relapse within 5 years. To effectively treat acute leukemia, new molecular targets and therapeutic approaches must be identified. Recently, the
20 inventors cloned the human leukocyte immunoglobulin-like receptor B2 (LILRB2) as a receptor for several angiopoietin-like proteins (Angptls) (Zheng *et al.*, 2012). The LILRB family receptors are type I transmembrane glycoprotein containing extracellular Ig-like domains that bind ligands and intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and are classified as inhibitory receptors because ITIM motifs can recruit
25 phosphatases SHP-1, SHP-2, or SHIP to negatively regulate immune cell activation (Takai *et al.*, 2011; Daron *et al.*, 2008; Katz *et al.*, 2006). It is known that LILRBs are expressed on myeloid cells and certain other hematopoietic cells (Mori *et al.*, 2008). Surprisingly, the inventors have shown that PirB, the mouse ortholog of LILRB2, and LAIR1, a close related ITIM-receptor, are expressed by AML stem cells (AML-SCs) and support AML development
30 (Zheng *et al.*, 2012; Kang *et al.*, in press). Although counterintuitive, this result is consistent with the generally immune-suppressive and thus tumor-promoting roles of the inhibitory receptors in the immune system (Ma *et al.*, 2011).

In recent work, the inventors found that several members of the LILRB family are highly expressed on AML cells, and their expression negatively correlates with the overall
35 survival of human AML patients. LILRBs are expressed by both AML-SCs and some

differentiated acute leukemia cells (including AML and acute lymphoblastic leukemia or ALL). There is no defect in the normal hematopoietic development in mice that do not express any individual LILRB tested. Interestingly, however, knockout of individual *LILRBs* reversed leukemia development in a number of AML and acute lymphoblastic leukemia (ALL) mouse models and abolished AML-SCs (Zheng *et al.*, 2012; Kang *et al.*, in press; also see PCT/US13/43431). In addition, inhibition of expression of several *LILRBs* individually in different human leukemia cell lines in culture and blocked leukemia development in xenografted mice (Kang *et al.*, in press; also see PCT/US13/43431).

The inventors have determined that some leukemia stem cells express high levels of ITIM-inhibitory receptors including LILRBs. The current treatment options for patients with acute leukemia, including chemotherapy, do not efficiently target cancer stem cells because these inhibitory receptors enable the leukemia stem cells to survive the conventional therapies eventually resulting in tumor relapse. The inventors theorize that LILRB signaling represents an ideal target for treating AML for several reasons: 1) several LILRBs are essential to the survival of AML cells including AML-SCs; 2) knockout of an individual LILRB does not result in overt defects in normal hematopoiesis; and 3) inhibition of LILRB activity stimulates immunity and indirectly boosts antitumor effects.

SUMMARY

Thus, in accordance with the present disclosure, there is provided a method of detecting a cancer cell or cancer stem cell in a sample or subject comprising (a) contacting a subject or a sample from said subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from FIG. 22; and (b) detecting binding of said antibody to a cancer cell or cancer stem cell in said subject or sample. The sample may be a biopsy or body fluid, such as blood, sputum, tears, saliva, mucous or serum, urine or feces. Detection may comprise immunohistochemistry, ELISA, RIA or Western blot. The method may further comprise performing steps (a) and (b) a second time and determining a change in cell levels as compared to the first assay. The antibody may be characterized by clone-paired variable sequences as set forth in FIG. 21. The antibody or antibody fragment may be encoded by light and heavy chain variable nucleic acid sequences having 70%, 80%, or 90% identity to clone-paired sequences from FIG. 21. The antibody or antibody fragment may be encoded by light and heavy chain variable amino acid sequences having 95% identity to clone-paired sequences from FIG. 21. The antibody fragment may be a recombinant ScFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The antibody may or may not bind to Ebola virus.

In another embodiment, there is provided a method of treating a subject having cancer comprising administering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from FIG. 22. The antibody may be characterized by clone-paired heavy and light chain variable nucleic acid sequences as set forth in FIG. 21. The antibody may be characterized by clone-paired heavy and light chain variable amino acid sequences as set forth in FIG. 21. The antibody or antibody fragment may be encoded by light and heavy chain variable nucleic acid sequences having 70%, 80%, or 90% identity to clone-paired sequences from FIG. 21. The antibody or antibody fragment may be encoded by light and heavy chain variable amino acid sequences having 95% identity to clone-paired sequences from FIG. 21. The antibody fragment may be a recombinant ScFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The antibody may be an IgG and/or a chimeric antibody. The cancer may be acute myeloid leukemia. Administration may be intravenous, intra-arterial or intra-tumoral.

In yet another embodiment, there is provided a monoclonal antibody or antibody fragment, wherein the antibody is characterized by clone-paired heavy and light chain CDR sequences from FIG. 22. The monoclonal antibody or antibody fragment may be encoded by

light and heavy chain variable nucleic acid sequences according to clone-paired sequences from FIG. 21. The antibody or antibody fragment may be encoded by light and heavy chain variable nucleic acid sequences having 70%, 80%, or 90% identity to clone-paired sequences from FIG. 21. The antibody or antibody fragment may be encoded by light chain and heavy variable amino acid sequences according to clone-paired sequences from FIG. 21. The antibody or antibody fragment may be encoded by light and heavy chain variable amino acid sequences having 95% identity to clone-paired sequences from FIG. 21. The antibody fragment may be a recombinant ScFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment. The antibody may be a chimeric antibody or is bispecific antibody, and/or an IgG. The antibody may be humanized and/or optimized.

Another embodiment relates to a hybridoma encoding wherein the antibody is characterized by clone-paired heavy and light chain CDR sequences from FIG. 22. The monoclonal antibody may be encoded by light and heavy chain variable nucleic acid sequences according to clone-paired sequences from FIG. 21. The antibody may be encoded by light and heavy chain variable nucleic acid sequences having 70%, 80%, or 90% identity to clone-paired sequences from FIG. 21. The antibody may be encoded by light chain and heavy variable amino acid sequences according to clone-paired sequences from FIG. 21. The antibody may be encoded by light and heavy chain variable amino acid sequences having 95% identity to clone-paired sequences from FIG. 21.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The word “about” means plus or minus 5% of the stated number.

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein. Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these drawings in combination with the detailed
5 description of specific embodiments presented herein.

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed
10 description of specific embodiments presented herein.

FIGS. 1A-D – Expression of LILRBs in primary human AML cells. (FIG. 1A) LILRB4 is expressed on ~50% tested human AML cases. (FIG. 1B) LILRB4 is a better marker than CD14 for human monocytic AML cells (M5b, peripheral blood) and LILRBs including LILRB4 can be co-expressed with the leukemia stem cell
15 marker CD34.

FIGS. 2A-D – Expression of individual LILRB1, 2, 3, 4 inversely correlates with overall survival of AML patients. Data were from the TCGA database (tcga-data.nci.nih.gov/tcga/; accessed November 5, 2012). n = 92, p <0.05.
20

FIGS. 3A-D - The expression of shRNA against LILRB3 or LILRB4 robustly inhibited cell growth. (FIG. 3A) Both THP-1 and MV4-11 human AML cells express LILRB4 on their cell surfaces. (FIG. 3B) shRNAs were designed specifically knockdown individual LILRB expression as determined by real-time RT-PCR. (FIGS. 3C-D) THP-1 (FIG. 3C) and MV4-11 (FIG. 3D) cells were respectively infected with PII3.7 lentiviruses that express shRNAs targeted to individual LILRBs or a scrambled control. The expression of shRNA against LILRB3 or LILRB4 robustly inhibited cell
25 growth.

FIG. 4 – LILRB Abs for Western blotting. Indicated mAbs bind to human LILRB2, 3, 4 respectively in Western blotting.
30

FIG. 5 – LILRB Abs' binding on LILRB-overexpressing 293T cells. Indicated 2 µg/ml mAbs bind to human LILRB2, 3, 4 overexpressing 293T cells as determined by flow cytometry.

FIG. 6 – Immobilized (coated) LILRB Abs' effects on report cells. Indicated immobilized mAbs that were developed to target human LILRB2, 3, 4 induce activation of LILRB2,3,4 respectively. Together with FIG. 7 that shows soluble anti-LILRB4 inhibits activation of the chimeric receptor reporter, the result indicate that these antibodies are blocking antibodies against LILRB2, 3, 4 respectively. Indicated mAbs were immobilized on tissue culture dishes (10 µg/ml). LILRB2, 3, 4 chimeric receptor reporter cells were cultured in these dishes. GFP induction was measured by flow cytometry at 12 hr. The chimeric receptor reporter system that can measure the activity of LILRB agonists and antagonists was described in Deng *et al.*, 2014 *Blood*, 124(6):924-35

FIG. 7 – Soluble anti-LILRB4 mAbs inhibit GFP induction of reporter cells. Antibodies including C84, C53, C92, C201 inhibit GFP induction by coated antibody in the chimeric receptor reporter system for LILRB4.

FIGS. 8-9 – THP-1 MV4-11 cells express LILRB4 on cell surface as determined by flow cytometry.

FIGS. 10-12 – C84 (anti-hLILRB4) inhibits human AML development in intraveneously xenografted NSG mice. 4×10^6 human AML cell line THP-1 that are LILRB4⁺ were intravenously implanted into NSG mice. 200 µg C84 was i.v. injected into each mouse every 3 days (for a total of 8 times) starting from the first day when THP-1 cells were implanted. PBS and mouse IgG served as controls. Tumor growth was monitored over time by examining the leukemia infiltration in recipient liver (FIGS. 10-11) and bone marrow (FIG. 12). hCD45 was used to detect human leukemia cells by flow cytometry.

FIG. 13 – C84 suppresses tumor engraftment of all tested LILRB4⁺ human leukemia cells. Only the tumors formed by the LILRB4⁺ human leukemia cells (THP-1 and MV4-11) can be effectively inhibited by C84. The tumors formed by LILRB4⁻ cells (U937) cannot be inhibited by C84. Shown is a comparison of tumor growth by

examining the leukemia infiltration in recipient liver, spleen, bone marrow, and peripheral blood implanted by THP-1, MV4-11, and U937 cells. hCD45 was used to detect human leukemia cells by flow cytometry. LILRB4 was detected on cell surface of THP-1 and MV4-11 cells but not on U937 cells by flow cytometry (lower left panel).

FIG. 14 – C84 (anti-hLILRB4) inhibits development of primary patient AML in intraveneously xenografted NSG mice (8 different patients). $4\sim 10 \times 10^6$ primary human AML cells that are LILRB4⁺ were intravenously implanted into NSG mice. 200 μ g C84 was iv injected into each mouse twice per week starting from the first day when AML cells were implanted. Mouse IgG served as controls. Tumor growth was monitored over time by examining the leukemia infiltration in recipient liver, spleen, bone marrow, and peripheral blood. hCD45 and hLILRB4 were used to detect human leukemia cells by flow cytometry.

FIG. 15 – Testing of anti-hLILRB4 in hCB-HSC-derived humanized NSG mice. 3×10^4 human cord blood CD34⁺ cells were transplanted into sublethally irradiated (250 rad) NSG mice and multi-lineage human hematopoietic reconstitution was confirmed at various time points at day 21 to day 41 post-transplantation. At day 42, 1×10^6 human THP-1-Luc-GFP (THP-1 cells that stably express Luciferase and GFP to facilitate real-time *in vivo* tracking) AML cells were intravenously implanted into NSG mice. 200 μ g C84 was i.v. injected into each mouse right after AML cells were implanted. Mouse IgG served as controls. Tumor growth was monitored over time by luminescence imaging.

FIG. 16 – The amino acid sequences of variable regions of the heavy chain and light chain of 21 rabbit anti-human LILRB4 mAbs.

FIG. 17 – C84 blocks AML development initiated by primary AML cells in xenografted mice. The amino acid sequences of CDRs of the heavy chain and light chain of 21 rabbit anti-human LILRB4 mAbs.

FIGS. 18A-U – EC₅₀ of these antibodies binding to LILRB4 as determined by ELISA and the activation abilities of the immobilized antibodies (thus the blocking abilities of the soluble antibodies) toward the chimeric LILRB4 reporter system.

FIG. 19 – Anti-leukemia potency and cross-reactivity of mAbs against LILRB1-5. The anti-leukemia potency was determined by administration of indicated antibodies into AML xenograft models.

FIG. 20 – Ig isotyping of 4 individual anti-LILRB4 antibodies.

5 **FIGS. 21-22** – Sequencing and isotyping results of variable regions of individual anti-LILRB antibodies.

FIG. 23 – Expression of chimeric anti-C84 (Chimeric ab MHC-C84) as determined by SDS-PAGE.

10 **FIG. 24** – Chimeric ab MHC-C84 binds LILRB4+ THP-1 cells as C84, as determined by flow cytometry.

FIG. 25 – Chimeric ab MHC-C84 binds LILRB4 chimeric receptor reporter cells as C84, as determined by flow cytometry.

FIG. 26 – Chimeric ab MHC-C84 is a blocking antibody for LILRB4 as C84, as determined by chimeric receptor reporter assay.

15 **FIG. 27** – Chimeric ab MHC-C84 inhibits AML development in xenograft model (same or better than original mAb C84). 3×10^6 human AML cell line THP-1-Luc-GFP (THP-1 cells that stably express Luciferase and GFP to facilitate real-time in vivo tracking) were intravenously implanted into NSG mice. 200 μ g MHC-C84 or C84 was i.v. injected into each mouse only one time 30 min-1 hr right after
20 implantation of 3×10^6 THP-1-Luc-GFP cells. PBS or mouse IgG served as controls. Tumor growth was monitored after 1 month by examining the leukemia infiltration in recipient liver, spleen, bone marrow, and peripheral blood. hCD45 was used to detect human leukemia cells by flow cytometry.

25 **FIG. 28** – Chimeric ab MHC-C84 inhibits AML development in xenograft model as determined by luminescence imaging. 3×10^6 human AML cell line THP-1 that stably express luciferase (as THP-1-Luc-GFP cells) were intravenously implanted into NSG mice. 200 μ g MHC-C84 or C84 was i.v. injected into each mouse only one time 30 min ~ 1 hr right after implantation of 3×10^6 THP-1-Luc-GFP cells. Mouse IgG served as controls. Tumor growth was monitored over time by luminescence imaging.
30 While all conditions had the same luciferase intensity at day 0 (4 hr after tumor

implantation), MHC-C84 or C84 treated mice displayed no luciferase signal over time (compare them with control at day 16, 18, and 21).

FIGS. 29A-G – Anti-LILRB4 inhibits AML cell transmigration and accelerates mobilization. (FIG. 29A) C84 doesn't inhibits MV4-11 cell plasticity. 1 x 10⁵ MV4-11 cells were cultured in the up chamber of a transwell and treated with 100 µg/ml of C84 or mIgG for 18 hrs. Cells in down chamber were counted. (FIG. 29B) C84 inhibits MV4-11 cell transmigration through endothelial cells. 3 x 10⁵ HUVEC cells were cultured on the membrane of a transwell for 3 days. 1 x 10⁵ MV4-11 cells were cultured in the up chamber of a transwell and treated with 100 µg/ml of C84 or mIgG for 18 hrs. Cells in down chamber were counted. (FIG. 29C) C84 inhibits MV4-11 cell homing. 5 x 10⁶ MV4-11 cells were i.v. injected into each NSG mouse that were scarified after 8 or 20 hrs. hCD45 was used to detect human leukemia cells by flow cytometry. The percentage of leukemia cells in recipient liver, spleen and bone marrow were normalized by that in peripheral blood. (FIG. 29D) C84 doesn't inhibits HSCs homing. 1 x 10⁷ human cord blood mononuclear cells were i.v. injected into each NSG mouse that were scarified after 20 hrs. hCD45 and hCD34 were used to detect human HSCs by flow cytometry. (FIGS. 29E-F) C84 accelerates MV4-11 mobilization to PB. 5 x 10⁶ MV4-11 cells were i.v. injected into each NSG mouse. Leukemia cells in peripheral blood were examined at Day 0 (3 days after MV4-11 cell transplantation), Day 1 and Day 4. 200 µg C84 or mIgG was i.v. injected into each mouse at Day 0 and Day 1. Mice were scarified at Day 4. hCD45 was used to detect human leukemia cells by flow cytometry. (FIG. 29G) synergic C84 treatment with chemodrug cytarabine inhibits AML development. 1 x 10⁶ human AML THP-1 cells that stably express luciferase (as THP-1-Luc-GFP cells) were intravenously implanted into NSG mice. 10 mg/kg Cytarabine was i.p. injected into each mouse every day starting from 6 days or 14 days after implantation of leukemia cells. 200 µg C84 or mIgG was i.v. injected into each mouse twice a week starting from 6 days or 14 days after implantation of leukemia cells. Mice were scarified at 21 days after implantation of leukemia cells. hCD45 was used to detect human leukemia cells by flow cytometry.

FIGS. 30A-U – Anti-LILRB4 stimulates T cell immunity for anti-cancer effects. (FIGS. 30A-C) c84 repress the inhibition of CTL by LILRB4. 5 x 10⁴ CD8⁺ T cells isolated from hPBMC of a healthy donor were stimulated with anti-

CD3/CD28/CD137-coated beads or without stimulation for 2 days. Then, 5×10^3 THP-1-Luc-GFP cells were co-cultured with these T cells and treated with 500 $\mu\text{g}/\text{ml}$ C84 or mIgG for 5 days. CD8 and CD28 were used to detect human CTL cells by flow cytometry; and GFP was used to detect human leukemia cells. (FIG. 30D) c84 increases CTL cytokine production. Cell supernatants from co-culture of stimulated CTL cells and THP-1 cells that were treated with C84 or mIgG were used to examine the cytokine production by human cytokine arrays. (FIG. 30E) THP-1 cannot engraft into PBMC-driven humanized NSG mice. 1×10^7 human PBMCs were i.v. injected into each NSG mouse. Three weeks after implantation of hPBMC, these mice had 30~50% human T cells engraftment. Then, 1×10^6 human AML THP-1 cells that stably express luciferase (as THP-1-Luc-GFP cells) were intravenously implanted into these hPBMC-humanized NSG mice or age-matched regular NSG mice. Tumor growth was monitored over time by luminescence imaging. (FIG. 30F) C84 inhibits subcutaneous implantation of THP-1 cells in PBMC-driven humanized NSG mice. 1×10^7 human PBMCs were i.v. injected into each NSG mouse. Three weeks after implantation of hPBMC, these mice had 30~50% human T cells engraftment. Then, 1×10^6 human AML THP-1 cells that stably express luciferase (as THP-1-Luc-GFP cells) were subcutaneously implanted into these hPBMC-humanized NSG mice with 200 μg C84 or mIgG treatment twice a week. Tumor growth was monitored over time by luminescence imaging. (FIGS. 30G-U) C84 inhibits leukemia development and decreases CD8⁺ T suppressor cells in human cord blood-humanized NSG mice. The hCB-humanized mice were obtained as same as we shown in FIG. 15. GFP was used to detect human leukemia cells, CD19 and CD20 were used to detect CB-derived human B cells, CD11b, CD14 and LILRB4 were used to detect CB-derived human myeloid cells, CD4 and CD8 were used to detect CB-derived human T cells, CD8, CD28 and CD40L were used to detect CB-derived human CTL and T suppressor cells,

FIG. 31 – Fc-dependent and Fc-independent effect of anti-LILRB4 on AML development. 1×10^6 human AML THP-1 cells that stably express luciferase (as THP-1-Luc-GFP cells) were intravenously implanted into NSG mice. 200 μg MHC-C84 or MHC-C84-N297A was i.v. injected into each mouse at the same day (as “Day 0”) or at 3 days (as “Day 3”) after implantation of 1×10^6 THP-1-Luc-GFP cells. Anti-CMV human IgG antibody (LX-D2-43) served as controls. Tumor growth was

monitored over time by luminescence imaging and hCD45 was used to detect human leukemia cells by flow cytometry.

FIGS. 32A-E – APOE is a potential LILRB4 ligand. (FIG. 32A) human/mouse serum, APOE and LFA-1 induce LILRB4 activation; (FIG. 32B) APOE activates human LILRB4 and mouse PIRB; (FIG. 32C) SPR shows that APOE binds to LILRB4 in a high affinity, $K_d = 2.485$ nM; (FIGS. 32D-E) APOE-Knockout delays mouse AML cells development and elongate overall survival. 1×10^6 mouse AML C1498 cells that stably express GFP were intravenously implanted into C57BL/6 mice or APOE-knockout mice. GFP was used to detect mouse leukemia cells by flow cytometry in peripheral blood at 20 days after implantation of C1498 cells.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The inventors have developed a number of anti-LILRB mAb, each of which efficiently block human AML development in various xenografted mouse models. These exciting results indicate that LILRBs support the activity of leukemia stem cells and leukemia development, but are not critical for normal hematopoiesis. In particular, for the first time, the inventors demonstrate that anti-LILRB4 inhibit development of human AML (including human patient AML developed in xenografted mice). Importantly, they obtained sequences of variable regions of ten anti-LILRB mAbs, and produced chimeric antibodies with human constant regions. The inventors showed that one such chimeric antibody (MHC-C84) not only retains the same binding properties as the original mAb C84, but has enhanced anti-leukemia activity in xenograft models. Thus, anti-LILRB antibodies can be used to treat leukemia and other cancers that express the relevant LILRB on their cell surface. They can also be used to treat immune diseases that have an LILRB component. These and other aspects of the disclosure are discussed below.

I. LILRs

The leukocyte immunoglobulin-like receptors (LILR) are a family of receptors possessing extracellular immunoglobulin domains. They are also known as CD85, ILTs and LIR, and can exert immunomodulatory effects on a wide range of immune cells. The human genes encoding these receptors are found in a gene cluster at chromosomal region 19q13.4. They include, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, LILRA6, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LILRB6 or LILRA6, and LILRB7 or LILRA5. A subset of LILRs recognize MHC class I molecules (also known as HLA class I in humans). Of these, the inhibitory receptors LILRB1 and LILRB2 show a broad specificity for classical and non-classical MHC alleles with preferential binding to b2m-associated complexes. In contrast, the activating receptors LILRA1 and LILRA3 prefer b2m-independent free heavy chains of MHC class I, and in particular HLA-C alleles.

A. LILRB1

Leukocyte immunoglobulin-like receptor subfamily B member 1 is a protein that in humans is encoded by the *LILRB1* gene. This gene is a member of the leukocyte immunoglobulin-like receptor (LIR) family, which is found in a gene cluster at chromosomal region 19q13.4. The encoded protein belongs to the subfamily B class of LIR receptors which

contain two or four extracellular immunoglobulin domains, a transmembrane domain, and two to four cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The receptor is expressed on immune cells where it binds to MHC class I molecules on antigen-presenting cells and transduces a negative signal that inhibits stimulation of an immune response. LILRB1 was also reported to be expressed in human gastric cancer cells and may enhance tumor growth (see Zhang *et al.*, 2012). It is thought to control inflammatory responses and cytotoxicity to help focus the immune response and limit autoreactivity. Multiple transcript variants encoding different isoforms have been found for this gene.

B. LILRB2

Leukocyte immunoglobulin-like receptor subfamily B member 2 is a protein that in humans is encoded by the *LILRB2* gene. This gene is a member of the leukocyte immunoglobulin-like receptor (LIR) family, which is found in a gene cluster at chromosomal region 19q13.4. The encoded protein belongs to the subfamily B class of LIR receptors which contain two or four extracellular immunoglobulin domains, a transmembrane domain, and two to four cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The receptor is expressed on immune cells where it binds to MHC class I molecules on antigen-presenting cells and transduces a negative signal that inhibits stimulation of an immune response. It is thought to control inflammatory responses and cytotoxicity to help focus the immune response and limit autoreactivity. The receptor is also expressed on human non-small cell lung cancer cells (see Sun *et al.*, 2008). Multiple transcript variants encoding different isoforms have been found for this gene. LILRB2 has been shown to interact with PTPN6.

C. LILRB3

Leukocyte immunoglobulin-like receptor subfamily B member 3 is a protein that in humans is encoded by the *LILRB3* gene. This gene is a member of the leukocyte immunoglobulin-like receptor (LIR) family, which is found in a gene cluster at chromosomal region 19q13.4. The encoded protein belongs to the subfamily B class of LIR receptors which contain two or four extracellular immunoglobulin domains, a transmembrane domain, and two to four cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The receptor is expressed on immune cells where it binds to MHC class I molecules on antigen-presenting cells and transduces a negative signal that inhibits stimulation of an immune response. It is thought to control inflammatory responses and cytotoxicity to help focus the

immune response and limit autoreactivity. Multiple transcript variants encoding different isoforms have been found for this gene.

D. LILRB4

5 Leukocyte immunoglobulin-like receptor subfamily B member 4 is a protein that in humans is encoded by the *LILRB4* gene. This gene is a member of the leukocyte immunoglobulin-like receptor (LIR) family, which is found in a gene cluster at chromosomal region 19q13.4. The encoded protein belongs to the subfamily B class of LIR receptors which contain two or four extracellular immunoglobulin domains, a transmembrane domain, and
10 two to four cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The receptor is expressed on immune cells where it binds to MHC class I molecules on antigen-presenting cells and transduces a negative signal that inhibits stimulation of an immune response. The receptor can also function in antigen capture and presentation. It is thought to control inflammatory responses and cytotoxicity to help focus the immune response and limit
15 autoreactivity. LILRB4 is also expressed in human gastric cancer cells and may enhance tumor growth (Zhang *et al.*, 2012). Multiple transcript variants encoding different isoforms have been found for this gene. LILRB4 has been shown to interact with PTPN6.

E. LAIR1

20 Leukocyte-associated immunoglobulin-like receptor 1 is a protein that in humans is encoded by the *LAIR1* gene. LAIR1 has also been designated as CD305 (cluster of differentiation 305). LAIR1 is a type I transmembrane glycoprotein that contains one extracellular Ig-like domain and two intracellular ITIMs. Like the genes that encode LILRBs, *lair1* is localized to the leukocyte receptor complex (LRC) on human chromosome 19q13.4.
25 LAIR1 binds collagens, and its ITIMs recruit SHP-1 and SHP-2. LAIR1 is expressed in T cells, B cells, natural killer (NK) cells, macrophages, and dendritic cells, as well as hematopoietic progenitors including human CD34⁺ cells. The inventors have demonstrated that LAIR1 is expressed on AML stem cells and differentiated AML and ALL cells and its inhibition blocks AML-SC activity and leukemia development (unpublished data).

30

II. Cancers

A. Cancers

While hyperproliferative diseases can be associated with any disease which causes a cell to begin to reproduce uncontrollably, the prototypical example is cancer. One of the key

elements of cancer is that the cell's normal apoptotic cycle is interrupted and thus agents that interrupt the growth of the cells are important as therapeutic agents for treating these diseases. In this disclosure, the tubulysin analogs described herein may be used to lead to decreased cell counts and as such can potentially be used to treat a variety of types of cancer lines. In some aspects, it is anticipated that the tubulysin analogs described herein may be used to treat virtually any malignancy. Here, the only requirement is the presence of LILRBs on the surface of the cancer cell, and in particular on the surface of cancer stem cells.

Cancer cells that may be treated according to the present disclosure include but are not limited to cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestinal, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, pancreas, testis, tongue, cervix, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; Paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant; sertoli cell carcinoma; Leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading

melanoma; malignant melanoma in giant pigmented nevus; epithelioid cell melanoma; blue
 nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma;
 liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar
 rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; Mullerian mixed tumor;
 5 nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; Brenner
 tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant;
 dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant;
 choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma,
 malignant; Kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma;
 10 osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant;
 mesenchymal chondrosarcoma; giant cell tumor of bone; Ewing's sarcoma; odontogenic
 tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic
 fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma;
 astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma;
 15 oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma;
 ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor;
 meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor,
 malignant; malignant lymphoma; Hodgkin's disease; paragranuloma; malignant lymphoma,
 small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular;
 20 mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis;
 multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia;
 lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia;
 myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast
 cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia. In
 25 certain aspects, the tumor may comprise an osteosarcoma, angiosarcoma, rhabdosarcoma,
 leiomyosarcoma, Ewing sarcoma, glioblastoma, neuroblastoma, or leukemia.

B. Acute Myeloid Leukemia

Acute myeloid leukemia (AML), also known as acute myelogenous leukemia or acute
 30 nonlymphocytic leukemia (ANLL), is a cancer of the myeloid line of blood cells,
 characterized by the rapid growth of abnormal white blood cells that accumulate in the bone
 marrow and interfere with the production of normal blood cells. AML is the most common
 acute leukemia affecting adults, and its incidence increases with age. Although AML is a

relatively rare disease, accounting for approximately 1.2% of cancer deaths in the United States, its incidence is expected to increase as the population ages.

The symptoms of AML are caused by replacement of normal bone marrow with leukemic cells, which causes a drop in red blood cells, platelets, and normal white blood cells. These symptoms include fatigue, shortness of breath, easy bruising and bleeding, and increased risk of infection. Several risk factors and chromosomal abnormalities have been identified, but the specific cause is not clear. As an acute leukemia, AML progresses rapidly and is typically fatal within weeks or months if left untreated.

AML has several subtypes; treatment and prognosis varies among subtypes. Five-year survival varies from 15–70%, and relapse rate varies from 33–78%, depending on subtype. AML is treated initially with chemotherapy aimed at inducing a remission; patients may go on to receive additional chemotherapy or a hematopoietic stem cell transplant. Recent research into the genetics of AML has resulted in the availability of tests that can predict which drug or drugs may work best for a particular patient, as well as how long that patient is likely to survive.

Most signs and symptoms of AML are caused by the replacement of normal blood cells with leukemic cells. A lack of normal white blood cell production makes the patient susceptible to infections; while the leukemic cells themselves are derived from white blood cell precursors, they have no infection-fighting capacity. A drop in red blood cell count (anemia) can cause fatigue, paleness, and shortness of breath. A lack of platelets can lead to easy bruising or bleeding with minor trauma.

The early signs of AML are often vague and nonspecific, and may be similar to those of influenza or other common illnesses. Some generalized symptoms include fever, fatigue, weight loss or loss of appetite, shortness of breath, anemia, easy bruising or bleeding, petechiae (flat, pin-head sized spots under the skin caused by bleeding), bone and joint pain, and persistent or frequent infections.

Enlargement of the spleen may occur in AML, but it is typically mild and asymptomatic. Lymph node swelling is rare in AML, in contrast to acute lymphoblastic leukemia. The skin is involved about 10% of the time in the form of leukemia cutis. Rarely, Sweet's syndrome, a paraneoplastic inflammation of the skin, can occur with AML.

Some patients with AML may experience swelling of the gums because of infiltration of leukemic cells into the gum tissue. Rarely, the first sign of leukemia may be the development of a solid leukemic mass or tumor outside of the bone marrow, called a

chloroma. Occasionally, a person may show no symptoms, and the leukemia may be discovered incidentally during a routine blood test.

A number of risk factors for developing AML have been identified, including: other blood disorders, chemical exposures, ionizing radiation, and genetics.

5 “Preleukemic” blood disorders, such as myelodysplastic syndrome or myeloproliferative disease, can evolve into AML; the exact risk depends on the type of MDS/MPS. Exposure to anticancer chemotherapy, in particular alkylating agents, can increase the risk of subsequently developing AML. The risk is highest about three to five years after chemotherapy. Other chemotherapy agents, specifically epipodophyllotoxins and
10 anthracyclines, have also been associated with treatment-related leukemia. These treatment-related leukemias are often associated with specific chromosomal abnormalities in the leukemic cells. Occupational chemical exposure to benzene and other aromatic organic solvents is controversial as a cause of AML. Benzene and many of its derivatives are known to be carcinogenic *in vitro*. While some studies have suggested a link between occupational
15 exposure to benzene and increased risk of AML, others have suggested the attributable risk, if any, is slight. High amounts of ionizing radiation exposure can increase the risk of AML. A hereditary risk for AML appears to exist. Multiple cases of AML developing in a family at a rate higher than predicted by chance alone have been reported. Several congenital conditions may increase the risk of leukemia; the most common is probably Down syndrome, which is
20 associated with a 10- to 18-fold increase in the risk of AML.

The first clue to a diagnosis of AML is typically an abnormal result on a complete blood count. While an excess of abnormal white blood cells (leukocytosis) is a common finding, and leukemic blasts are sometimes seen, AML can also present with isolated decreases in platelets, red blood cells, or even with a *low* white blood cell count (leukopenia).
25 While a presumptive diagnosis of AML can be made via examination of the peripheral blood smear when there are circulating leukemic blasts, a definitive diagnosis usually requires an adequate bone marrow aspiration and biopsy.

Marrow or blood is examined via light microscopy, as well as flow cytometry, to diagnose the presence of leukemia, to differentiate AML from other types of leukemia (*e.g.*,
30 acute lymphoblastic leukemia - ALL), and to classify the subtype of disease (see below). A sample of marrow or blood is typically also tested for chromosomal abnormalities by routine cytogenetics or fluorescent *in situ* hybridization. Genetic studies may also be performed to look for specific mutations in genes such as FLT3, nucleophosmin, and KIT, which may influence the outcome of the disease.

Cytochemical stains on blood and bone marrow smears are helpful in the distinction of AML from ALL, and in subclassification of AML. The combination of a myeloperoxidase or Sudan black stain and a nonspecific esterase stain will provide the desired information in most cases. The myeloperoxidase or Sudan black reactions are most useful in establishing the identity of AML and distinguishing it from ALL. The nonspecific esterase stain is used to identify a monocytic component in AMLs and to distinguish a poorly differentiated monoblastic leukemia from ALL.

The diagnosis and classification of AML can be challenging, and should be performed by a qualified hematopathologist or hematologist. In straightforward cases, the presence of certain morphologic features (such as Auer rods) or specific flow cytometry results can distinguish AML from other leukemias; however, in the absence of such features, diagnosis may be more difficult.

According to the widely used WHO criteria, the diagnosis of AML is established by demonstrating involvement of more than 20% of the blood and/or bone marrow by leukemic myeloblasts. The French–American–British (FAB) classification is a bit more stringent, requiring a blast percentage of at least 30% in bone marrow (BM) or peripheral blood (PB) for the diagnosis of AML. AML must be carefully differentiated from "preleukemic" conditions such as myelodysplastic or myeloproliferative syndromes, which are treated differently.

Because acute promyelocytic leukemia (APL) has the highest curability and requires a unique form of treatment, it is important to quickly establish or exclude the diagnosis of this subtype of leukemia. Fluorescent *in situ* hybridization performed on blood or bone marrow is often used for this purpose, as it readily identifies the chromosomal translocation [t(15;17)(q22;q12);] that characterizes APL. There is also a need to molecularly detect the presence of PML/RARA fusion protein, which is an oncogenic product of that translocation.

First-line treatment of AML consists primarily of chemotherapy, and is divided into two phases: induction and post-remission (or consolidation) therapy. The goal of induction therapy is to achieve a complete remission by reducing the number of leukemic cells to an undetectable level; the goal of consolidation therapy is to eliminate any residual undetectable disease and achieve a cure. Hematopoietic stem cell transplantation is usually considered if induction chemotherapy fails or after a patient relapses, although transplantation is also sometimes used as front-line therapy for patients with high-risk disease.

All FAB subtypes except M3 are usually given induction chemotherapy with cytarabine (ara-C) and an anthracycline (most often daunorubicin). This induction

chemotherapy regimen is known as "7+3" (or "3+7"), because the cytarabine is given as a continuous IV infusion for seven consecutive days while the anthracycline is given for three consecutive days as an IV push. Up to 70% of patients will achieve a remission with this protocol. Other alternative induction regimens, including high-dose cytarabine alone, FLAG-like regimens or investigational agents, may also be used. Because of the toxic effects of therapy, including myelosuppression and an increased risk of infection, induction chemotherapy may not be offered to the very elderly, and the options may include less intense chemotherapy or palliative care.

The M3 subtype of AML, also known as acute promyelocytic leukemia (APL), is almost universally treated with the drug *all-trans*-retinoic acid (ATRA) in addition to induction chemotherapy, usually an anthracycline. Care must be taken to prevent disseminated intravascular coagulation (DIC), complicating the treatment of APL when the promyelocytes release the contents of their granules into the peripheral circulation. APL is eminently curable, with well-documented treatment protocols.

The goal of the induction phase is to reach a complete remission. Complete remission does not mean the disease has been cured; rather, it signifies no disease can be detected with available diagnostic methods. Complete remission is obtained in about 50%–75% of newly diagnosed adults, although this may vary based on the prognostic factors described above. The length of remission depends on the prognostic features of the original leukemia. In general, all remissions will fail without additional consolidation therapy.

Even after complete remission is achieved, leukemic cells likely remain in numbers too small to be detected with current diagnostic techniques. If no further post-remission or consolidation therapy is given, almost all patients will eventually relapse. Therefore, more therapy is necessary to eliminate non-detectable disease and prevent relapse — that is, to achieve a cure.

The specific type of post-remission therapy is individualized based on a patient's prognostic factors (see above) and general health. For good-prognosis leukemias (*i.e.*, inv(16), t(8;21), and t(15;17)), patients will typically undergo an additional three to five courses of intensive chemotherapy, known as consolidation chemotherapy. For patients at high risk of relapse (*e.g.*, those with high-risk cytogenetics, underlying MDS, or therapy-related AML), allogeneic stem cell transplantation is usually recommended if the patient is able to tolerate a transplant and has a suitable donor. The best post-remission therapy for intermediate-risk AML (normal cytogenetics or cytogenetic changes not falling into good-risk or high-risk groups) is less clear and depends on the specific situation, including the age and overall

health of the patient, the patient's personal values, and whether a suitable stem cell donor is available.

For patients who are not eligible for a stem cell transplant, immunotherapy with a combination of histamine dihydrochloride (Ceplene) and interleukin 2 (Proleukin) after the
5 completion of consolidation has been shown to reduce the absolute relapse risk by 14%, translating to a 50% increase in the likelihood of maintained remission.

For patients with relapsed AML, the only proven potentially curative therapy is a hematopoietic stem cell transplant, if one has not already been performed. In 2000, the monoclonal antibody-linked cytotoxic agent gemtuzumab ozogamicin (Mylotarg) was
10 approved in the United States for patients aged more than 60 years with relapsed AML who are not candidates for high-dose chemotherapy. This drug was voluntarily withdrawn from the market by its manufacturer, Pfizer in 2010. Since treatment options for relapsed AML are so limited, palliative care may be offered.

Patients with relapsed AML who are not candidates for stem cell transplantation, or
15 who have relapsed after a stem cell transplant, may be offered treatment in a clinical trial, as conventional treatment options are limited. Agents under investigation include cytotoxic drugs such as clofarabine, as well as targeted therapies, such as farnesyl transferase inhibitors, decitabine, and inhibitors of MDR1 (multidrug-resistance protein). For relapsed acute promyelocytic leukemia (APL), arsenic trioxide has been tested in trials and approved by the
20 U.S. FDA. Like ATRA, arsenic trioxide does not work with other subtypes of AML.

While acute myeloid leukemia is a curable disease, the chance of cure for a specific patient depends on a number of prognostic factors. The single most important prognostic factor in AML is cytogenetics, or the chromosomal structure of the leukemic cell. Certain cytogenetic abnormalities are associated with very good outcomes (for example, the (15:17)
25 translocation in acute promyelocytic leukemia). About half of AML patients have "normal" cytogenetics; they fall into an intermediate risk group. A number of other cytogenetic abnormalities are known to associate with a poor prognosis and a high risk of relapse after treatment.

AML which arises from a pre-existing myelodysplastic syndrome (MDS) or
30 myeloproliferative disease (so-called secondary AML) has a worse prognosis, as does treatment-related AML arising after chemotherapy for another previous malignancy. Both of these entities are associated with a high rate of unfavorable cytogenetic abnormalities.

In some studies, age >60 years and elevated lactate dehydrogenase level were also associated with poorer outcomes. As with most forms of cancer, performance status (*i.e.*, the

general physical condition and activity level of the patient) plays a major role in prognosis as well.

FLT3 internal tandem duplications (ITDs) have been shown to confer a poorer prognosis in AML. Treating these patients with more aggressive therapy, such as stem-cell transplantation in first remission, has not been shown to enhance long-term survival. ITDs of *FLT3* may be associated with leukostasis. In 2012, the *FLT3* inhibitor quizartinib showed positive phase II trial results in AML patients with *FLT3*-ITD mutations.

Researchers are investigating the clinical significance of *c-KIT* mutations in AML. These are prevalent, and clinically relevant because of the availability of tyrosine kinase inhibitors, such as imatinib and sunitinib that can block the activity of *c-KIT* pharmacologically. Other genes being investigated as prognostic factors or therapeutic targets include *CEBPA*, *BAALC*, *ERG*, and *NPM1*.

B. Acute Lymphoblastic Leukemia (ALL)

Acute lymphoblastic leukemia (ALL) or acute lymphoid leukemia is an acute form of leukemia, or cancer of the white blood cells, characterized by the overproduction of cancerous, immature white blood cells—known as lymphoblasts. In persons with ALL, lymphoblasts are overproduced in the bone marrow and continuously multiply, causing damage and death by inhibiting the production of normal cells—such as red and white blood cells and platelets—in the bone marrow and by infiltrating to other organs. ALL is most common in childhood with a peak incidence at 2–5 years of age, and another peak in old age.

The symptoms of ALL are indicative of a reduced production of functional blood cells, because the leukemia wastes the resources of the bone marrow, which are normally used to produce new, functioning blood cells. These symptoms can include fever, increased risk of infection (especially bacterial infections like pneumonia, due to neutropenia; symptoms of such an infection include shortness of breath, chest pain, cough, vomiting, changes in bowel or bladder habits), increased tendency to bleed (due to thrombocytopenia) and signs indicative of anemia including pallor, tachycardia (high heart rate), fatigue and headache.

About 6,000 cases are reported in the U.S. every year; statistics from other countries are difficult to come by, although it is known to be more common in the United States, Italy and Costa Rica. Cure is a realistic goal and is achieved in over 80% of affected children, although only 20-40% of adults can be cured. "Acute" refers to the relatively short time

course of the disease to differentiate it from chronic lymphocytic leukemia, which has a potential time course of many years.

The symptoms are not specific to ALL, but worsen to the point that medical help is sought. They result from the lack of normal and healthy blood cells because they are crowded
5 out by malignant and immature leukocytes (white blood cells). Therefore, people with ALL experience symptoms from malfunctioning of their erythrocytes (red blood cells), leukocytes, and platelets. Laboratory tests that might show abnormalities include blood count tests, renal function tests, electrolyte tests, and liver enzyme tests.

The signs and symptoms of ALL are variable but follow from bone marrow
10 replacement and/or organ infiltration, and include generalized weakness and fatigue, anemia, dizziness, frequent or unexplained fever and infection, weight loss and/or loss of appetite, excessive and unexplained bruising, bone pain, joint pain (caused by the spread of "blast" cells to the surface of the bone or into the joint from the marrow cavity), breathlessness, enlarged lymph nodes, liver and/or spleen, pitting edema (swelling) in the lower limbs and/or
15 abdomen, and petechiae, which are tiny red spots or lines in the skin due to low platelet levels.

In general, cancer is caused by damage to DNA that leads to uncontrolled cellular growth and spreads throughout the body, either by increasing chemical signals that cause growth or by interrupting chemical signals that control growth. Damage can be caused through the formation of fusion genes, as well as the dysregulation of a proto-oncogene via
20 juxtaposition of it to the promoter of another gene, *e.g.*, the T-cell receptor gene. This damage may be caused by environmental factors such as chemicals, drugs or radiation, and occurs naturally during mitosis or other normal processes (although cells have numerous mechanisms of DNA repair that help to reduce this).

ALL is associated with exposure to radiation and chemicals in animals and humans.
25 High level radiation exposure is a known risk factor for developing leukemia, as found by studies of survivors of atom bomb exposure in Hiroshima and Nagasaki. In animals, exposure to benzene and other chemicals can cause leukemia. Epidemiological studies have associated leukemia with workplace exposure to chemicals, but these studies are not as conclusive. Some evidence suggests that secondary leukemia can develop in individuals treated for other
30 cancers with radiation and chemotherapy as a result of that treatment.

Diagnosing ALL begins with a medical history, physical examination, complete blood count, and blood smears. Because the symptoms are so general, many other diseases with similar symptoms must be excluded. Typically, the higher the white blood cell count the worse the prognosis. Blast cells are seen on blood smear in the majority of cases (blast cells

are precursors (stem cells) to all immune cell lines). A bone marrow biopsy is conclusive proof of ALL. A lumbar puncture (also known as a spinal tap) will indicate if the spinal column and brain have been invaded.

Pathological examination, cytogenetics (in particular the presence of Philadelphia chromosome), and immunophenotyping establish whether myeloblastic (neutrophils, eosinophils, or basophils) or lymphoblastic (B lymphocytes or T lymphocytes) cells are the problem. RNA testing can establish how aggressive the disease is; different mutations have been associated with shorter or longer survival. Immunohistochemical testing may reveal TdT or CALLA antigens on the surface of leukemic cells. TdT is a protein expressed early in the development of pre-T and pre-B cells, whereas CALLA is an antigen found in 80% of ALL cases and also in the "blast crisis" of CML. Medical imaging (such as ultrasound or CT scanning) can find invasion of other organs commonly the lung, liver, spleen, lymph nodes, brain, kidneys, and reproductive organs.

The earlier acute lymphocytic leukemia is detected, the more effective the treatment. The aim is to induce a lasting remission, defined as the absence of detectable cancer cells in the body (usually less than 5% blast cells in the bone marrow). Treatment for acute leukemia can include chemotherapy, steroids, radiation therapy, intensive combined treatments (including bone marrow or stem cell transplants), and growth factors.

Chemotherapy is the initial treatment of choice. Most ALL patients will receive a combination of different treatments. There are no surgical options, due to the body-wide distribution of the malignant cells. In general, cytotoxic chemotherapy for ALL combines multiple antileukemic drugs in various combinations. Chemotherapy for ALL consists of three phases: remission induction, intensification, and maintenance therapy.

As the chemotherapy regimens can be intensive and protracted (often about 2 years in case of the GMALL UKALL, HyperCVAD or CALGB protocols; for ALL about 3 years, 2 months for males on COG protocols; 2 years, 2 months for females - longer for males, as testicles are a potential reservoir), many patients have an intravenous catheter inserted into a large vein (termed a central venous catheter or a Hickman line), or a Portacath, a cone-shaped port with a silicone nose that is surgically planted under the skin, usually near the collar bone, and the most effective product available, due to low infection risks and the long-term viability of a portacath.

Radiation therapy (or radiotherapy) is used on painful bony areas, in high disease burdens, or as part of the preparations for a bone marrow transplant (total body irradiation). Radiation in the form of whole-brain radiation is also used for central nervous system

prophylaxis, to prevent recurrence of leukemia in the brain. Whole-brain prophylaxis radiation used to be a common method in treatment of children's ALL. Recent studies showed that CNS chemotherapy provided results as favorable but with less developmental side-effects. As a result, the use of whole-brain radiation has been more limited. Most
5 specialists in adult leukemia have abandoned the use of radiation therapy for CNS prophylaxis, instead using intrathecal chemotherapy.

For some subtypes of relapsed ALL, aiming at biological targets such as the proteasome, in combination with chemotherapy, has given promising results in clinical trials. Selection of biological targets on the basis of their combinatorial effects on the leukemic
10 lymphoblasts can lead to clinical trials for improvement in the effects of ALL treatment. In ongoing clinical trials, a CD19-CD3 bi-specific monoclonal murine antibody - Blinatumomab, is showing great promise.

Chimeric antigen receptors (CARs) have been developed as a promising therapy for ALL. This technology uses a single chain variable fragment (scFv) designed to recognize the
15 cell surface marker CD19 as a method of treating ALL. CD19 is a molecule found on all B-cells and can be used as a means of distinguishing the potentially malignant B-cell population in the patient. In this therapy, mice are immunized with the CD19 antigen and produce anti-CD19 antibodies. Hybridomas developed from the mouse spleen cells fused to a myeloma cell line can be developed as a source for the cDNA encoding the CD19 specific antibody.
20 The cDNA is sequenced and the sequence encoding the variable heavy and variable light chains of these antibodies are cloned together using a small peptide linker. This resulting sequence encodes the scFv. This can be cloned into a transgene encoding what will become the endodomain of the CAR. There are varying arrangements of subunits used as the endodomain but they generally consist of the hinge region that attaches to the scFv, a
25 transmembrane region, the intracellular region of a costimulatory molecule such as CD28, and the intracellular domain of CD3-zeta containing ITAM repeats. Other sequences frequently included are: 4-1bb and OX40. The final transgene sequence, containing the scFv and endodomain sequences is then inserted into immune effector cells that are obtained from the patient and expanded in vitro. In previous trials these have been a type of T-cell capable
30 of cytotoxicity. Inserting the DNA into the effector cell can be accomplished by several methods. Most commonly, this is done using a lentivirus which encodes the transgene. Pseudotyped, self-inactivating lentiviruses have been shown to be an effective method for the stable insertion of a desired transgene into the target cell genomic DNA. Other methods include electroporation and transfection but these are limited in their efficacy as transgene

expression will diminish over time. The gene-modified effector cells are then transplanted back into the patient. Typically this process is done in conjunction with a conditioning regiment such as cyclophosphamide which has been shown to potentiate the effects of infused T-cells. This effect has been attributed to the creation of an immunologic space niche.

5 The process as a whole results in an effector cell, typically a T-cell that can recognize a tumor cell antigen in a major histocompatibility complex independent manner and initiate a cytotoxic response

C. Chronic Lymphoblastic Leukemia (CLL)

10 B-cell chronic lymphocytic leukemia (B-CLL), also known as chronic lymphoid leukemia (CLL), is the most common type of leukemia (a type of cancer of the white blood cells) in adults. CLL affects B cell lymphocytes, which originate in the bone marrow, develop in the lymph nodes, and normally fight infection by producing antibodies. In CLL, B cells grow out of control and accumulate in the bone marrow and blood, where they crowd out
15 healthy blood cells. CLL is a stage of small lymphocytic lymphoma (SLL), a type of B-cell lymphoma, which presents primarily in the lymph nodes. CLL and SLL are considered the same underlying disease, just with different appearances. CLL is a disease of adults. Most (>75%) people newly diagnosed with CLL are over the age of 50, and the majority are men. However, in rare cases, it can occur in teenagers and occasionally in children. Some of these
20 may relate to an inherited predisposition.

Most people are diagnosed without symptoms as the result of a routine blood test that returns a high white blood cell count, but, as it advances, CLL results in swollen lymph nodes, spleen, and liver, and eventually anemia and infections. Early CLL is not treated, and late CLL is treated with chemotherapy and monoclonal antibodies.

25 DNA analysis has distinguished two major types of CLL, with different survival times. CLL that is positive for the marker ZAP-70 has an average survival of 8 years, while CLL negative for ZAP-70 has an average survival of more than 25 years. Many patients, especially older ones, with slowly progressing disease can be reassured and may not need any treatment in their lifetimes.

30 Most people are diagnosed without symptoms as the result of a routine blood test that returns a high white blood cell count. Less commonly, CLL may present with enlarged lymph nodes without a high white blood cell count or no evidence of the disease in the blood. This is referred to as *small lymphocytic lymphoma*. In some individuals the disease comes to light

only after the neoplastic cells overwhelm the bone marrow resulting in anemia producing tiredness or weakness.

CLL is usually first suspected by the presence of lymphocytosis, an increase in a type of white blood cell, on a complete blood count (CBC) test. This frequently is an incidental finding on a routine physician visit. Most often the lymphocyte count is greater than 4000 cells per microliter (μl) of blood, but can be much higher. The presence of a lymphocytosis in an elderly individual should raise strong suspicion for CLL, and a confirmatory diagnostic test, in particular flow cytometry, should be performed unless clinically unnecessary.

The diagnosis of CLL is based on the demonstration of an abnormal population of B lymphocytes in the blood, bone marrow, or tissues that display an unusual but characteristic pattern of molecules on the cell surface. This atypical molecular pattern includes the coexpression of cells surface markers cluster of differentiation 5 (CD5) and cluster of differentiation 23 (CD23). In addition, all the CLL cells within one individual are clonal, that is, genetically identical. In practice, this is inferred by the detection of only one of the mutually exclusive antibody light chains, kappa or lambda, on the entire population of the abnormal B cells. Normal B lymphocytes consist of a stew of different antibody-producing cells, resulting in a mixture of both kappa and lambda expressing cells. The lack of the normal distribution of kappa and lambda producing B cells is one basis for demonstrating clonality, the key element for establishing a diagnosis of any B cell malignancy (B cell non-Hodgkin lymphoma).

The combination of the microscopic examination of the peripheral blood and analysis of the lymphocytes by flow cytometry to confirm clonality and marker molecule expression is needed to establish the diagnosis of CLL. Both are easily accomplished on a small amount of blood. A flow cytometer is an instrument that can examine the expression of molecules on individual cells in fluids. This requires the use of specific antibodies to marker molecules with fluorescent tags recognized by the instrument. In CLL, the lymphocytes are genetically clonal, of the B cell lineage (expressing marker molecules cluster of differentiation 19 (CD19) and CD20), and characteristically express the marker molecules CD5 and CD23. These B cells resemble normal lymphocytes under the microscope, although slightly smaller, and are fragile when smeared onto a glass slide, giving rise to many broken cells, which are called "smudge" or "smear" cells.

The Matutes's CLL score allows the identification of a homogeneous subgroup of classical CLL, that differs from atypical/mixed CLL for the five markers' expression (CD5, CD23, FMC7, CD22 and immunoglobulin light chain) Matutes's CLL scoring system is very

helpful for the differential diagnosis between classical CLL and the other B cell chronic lymphoproliferative disorders, but not for the immunological distinction between mixed/atypical CLL and mantle cell lymphoma (MCL malignant B cells). Discrimination between CLL and MCL can be improved by adding non-routine markers such as CD54 and CD200. Among routine markers, the most discriminating feature is the CD20/CD23 mean fluorescence intensity ratio. In contrast, FMC7 expression can surprisingly be misleading for borderline cases.

Staging, determining the extent of the disease, is done with the Rai staging system or the Binet classification (see details) and is based primarily on the presence of a low platelet or red cell count. Early stage disease does not need to be treated.

CLL treatment focuses on controlling the disease and its symptoms rather than on an outright cure. CLL is treated by chemotherapy, radiation therapy, biological therapy, or bone marrow transplantation. Symptoms are sometimes treated surgically (splenectomy removal of enlarged spleen) or by radiation therapy ("de-bulking" swollen lymph nodes).

Initial CLL treatments vary depending on the exact diagnosis and the progression of the disease, and even with the preference and experience of the health care practitioner. Dozens of agents are used for CLL therapy. An initial treatment regimen that contains fludarabine, cyclophosphamide, and rituximab (known as FCR) has demonstrated higher overall response rates and complete response rates.

A study carried out by the researchers at the University of Pennsylvania used genetically modified T cells to attack cells that expressed the CD19 protein to fight the disease. In 2013, the researchers announced that 26 of 59 patients had achieved complete remission and that the original patient had remained tumor-free.

Leukemia is rarely associated with pregnancy, affecting only about 1 in 10,000 pregnant women. Treatment for chronic lymphocytic leukemias can often be postponed until after the end of the pregnancy. If treatment is necessary, then giving chemotherapy during the second or third trimesters is less likely to result in pregnancy loss or birth defects than treatment during the first trimester.

While generally considered incurable, CLL progresses slowly in most cases. Many people with CLL lead normal and active lives for many years—in some cases for decades. Because of its slow onset, early-stage CLL is, in general, not treated since it is believed that early CLL intervention does not improve survival time or quality of life. Instead, the condition is monitored over time to detect any change in the disease pattern.

The decision to start CLL treatment is taken when the patient's clinical symptoms or blood counts indicate that the disease has progressed to a point where it may affect the patient's quality of life. Clinical "staging systems" such as the Rai 4-stage system and the Binet classification can help to determine when and how to treat the patient. Determining when to start treatment and by what means is often difficult; studies have shown there is no survival advantage to treating the disease too early. The National Cancer Institute Working Group has issued guidelines for treatment, with specific markers that should be met before it is initiated.

Combination chemotherapy regimens are effective in both newly diagnosed and relapsed CLL. Combinations of fludarabine with alkylating agents (cyclophosphamide) produce higher response rates and a longer progression-free survival than single agents:

FC (fludarabine with cyclophosphamide)

FR (fludarabine with rituximab)

FCR (fludarabine, cyclophosphamide, and rituximab)

CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone)

Although the purine analogue fludarabine was shown to give superior response rates to chlorambucil as primary therapy, there is no evidence early use of fludarabine improves overall survival, and some clinicians prefer to reserve fludarabine for relapsed disease.

Chemoimmunotherapy with FCR has shown to improve response rates, progression-free survival and overall survival in a large randomized trial in CLL patients selected for good physical fitness. This has been the first clinical trial demonstrating that the choice of a first line therapy can improve the overall survival of patients with CLL. Alkylating agents approved for CLL include bendamustine and cyclophosphamide.

Targeted therapy attacks cancer cells at a specific target, with the aim of not harming normal cells. Monoclonal antibodies, such as alemtuzumab (directed against CD52), and rituximab and ofatumumab (directed against CD20), are used in CLL. Tyrosine kinase inhibitor therapy can also be used in CLL. In February 2014, the FDA granted ibrutinib approval to treat chronic lymphocytic leukemia. Ibrutinib is a Bruton's tyrosine kinase (BTK) inhibitor. In July 2014, the FDA and EMA granted idelalisib approval to treat different types of leukemia. Idelalisib is a PI3K inhibitor that targets the PI3K δ pathway. It is taken orally.

Autologous stem cell transplantation, using the recipient's own cells, is not curative. Younger individuals, if at high risk for dying from CLL, may consider allogeneic hematopoietic stem cell transplantation (HSCT). Myeloablative (bone marrow killing) forms of allogeneic stem cell transplantation, a high-risk treatment using blood cells from a healthy

donor, may be curative, but treatment-related toxicity is significant. An intermediate level, called reduced-intensity conditioning allogeneic stem cell transplantation, may be better tolerated by older or frail patients.

"Refractory" CLL is a disease that no longer responds favorably to treatment. In this case, more aggressive therapies, including lenalidomide, flavopiridol, and bone marrow (stem cell) transplantation, are considered. The monoclonal antibody, alemtuzumab (directed against CD52), may be used in patients with refractory, bone marrow-based disease.

Complications include Richter's syndrome, hypogammaglobulinemia leading to recurrent infection, warm autoimmune hemolytic anemia in 10–15% of patients, transformation to high grade lymphoma. Chronic lymphocytic leukemia may transform into Richter's syndrome, the development of fast-growing diffuse large B cell lymphoma, prolymphocytic leukemia, Hodgkin's lymphoma, or acute leukemia in a patient who has chronic lymphocytic leukemia. Its incidence is estimated to be around 5 percent in patients with CLL.

Gastrointestinal (GI) involvement can rarely occur with chronic lymphocytic leukemia. Some of the reported manifestations include intussusception, small intestinal bacterial contamination, colitis and others. Usually, GI complications with CLL occur after Richter transformation. There have been two case reports to date of GI involvement in chronic lymphocytic leukemia without Richter's transformation.

D. Non-Small Cell Lung Cancer

Non-small-cell lung carcinoma (NSCLC) is any type of epithelial lung cancer other than small cell lung carcinoma (SCLC). As a class, NSCLCs are relatively insensitive to chemotherapy, compared to small cell carcinoma. When possible, they are primarily treated by surgical resection with curative intent, although chemotherapy is increasingly being used both pre-operatively (neoadjuvant chemotherapy) and post-operatively (adjuvant chemotherapy).

The most common types of NSCLC are squamous cell carcinoma, large cell carcinoma, and adenocarcinoma, but there are several other types that occur less frequently, and all types can occur in unusual histologic variants and as mixed cell-type combinations. Sometimes the phrase "non-small-cell lung cancer" ("not otherwise specified", or NOS) is used generically, usually when a more specific diagnosis cannot be made. This is most often the case when a pathologist examines a small amount of malignant cells or tissue in a cytology or biopsy specimen.

Lung cancer in never-smokers is almost universally NSCLC, with a sizeable majority being adenocarcinoma. On relatively rare occasions, malignant lung tumors are found to contain components of both SCLC and NSCLC. In these cases, the tumors should be classified as combined small cell lung carcinoma (c-SCLC), and are (usually) treated like
5 "pure" SCLC.

Adenocarcinoma of the lung is currently the most common type of lung cancer in "never smokers" (lifelong non-smokers). Adenocarcinomas account for approximately 40% of lung cancers. Historically, adenocarcinoma was more often seen peripherally in the lungs than small cell lung cancer and squamous cell lung cancer, both of which tended to be more
10 often centrally located. Interestingly, however, recent studies suggest that the "ratio of centrally-to-peripherally occurring" lesions may be converging toward unity for both adenocarcinoma and squamous cell carcinoma.

Squamous cell carcinoma (SCC) of the lung is more common in men than in women. It is closely correlated with a history of tobacco smoking, more so than most other types of
15 lung cancer. According to the Nurses' Health Study, the relative risk of SCC is approximately 5.5, both among those with a previous duration of smoking of 1 to 20 years, and those with 20 to 30 years, compared to never-smokers. The relative risk increases to approximately 16 with a previous smoking duration of 30 to 40 years, and approximately 22 with more than 40 years.

20 Large cell lung carcinoma (LCLC) is a heterogeneous group of undifferentiated malignant neoplasms originating from transformed epithelial cells in the lung. LCLC's have typically comprised around 10% of all NSCLC in the past, although newer diagnostic techniques seem to be reducing the incidence of diagnosis of "classic" LCLC in favor of more poorly differentiated squamous cell carcinomas and adenocarcinomas. LCLC is, in effect, a
25 "diagnosis of exclusion", in that the tumor cells lack light microscopic characteristics that would classify the neoplasm as a small-cell carcinoma, squamous-cell carcinoma, adenocarcinoma, or other more specific histologic type of lung cancer. LCLC is differentiated from small cell lung carcinoma (SCLC) primarily by the larger size of the anaplastic cells, a higher cytoplasmic-to-nuclear size ratio, and a lack of "salt-and-pepper" chromatin.

30 More than one kind of treatment is often used, depending on the stage of the cancer, the individual's overall health, age, response to chemotherapy, and other factors such as the likely side effects of the treatment. NSCLCs are usually not very sensitive to chemotherapy and/or radiation, so surgery is the treatment of choice if diagnosed at an early stage, often

with adjuvant (ancillary) chemotherapy involving cisplatin. Other treatment choices are chemotherapy, radiation therapy (radiotherapy), and targeted therapy.

New methods of giving radiation treatment allow doctors to be more accurate in treating lung cancers. This means less radiation affects nearby healthy tissues. New methods
5 include Cyberknife and stereotactic radiosurgery (SRS). Other treatments are radiofrequency ablation and chemoembolization.

A wide variety of chemotherapies are used in advanced (metastatic) NSCLC. Some patients with particular mutations in the *EGFR* gene respond to EGFR tyrosine kinase inhibitors such as gefitinib. About 7% of NSCLC have EML4-ALK translocations; these may
10 benefit from ALK inhibitors which are in clinical trials. Crizotinib gained FDA approval in August 2011.

E. Gastric Cancer

Stomach cancer or gastric cancer is cancer developing from the lining of the stomach.
15 Early symptoms may include heartburn, upper abdominal pain, nausea and loss of appetite. Later signs and symptoms may include weight loss, yellow skin, vomiting, difficulty swallowing, and blood in the stool among others. The cancer may spread from the stomach to other parts of the body, particularly the liver, lungs, bones, lining of the abdomen and lymph nodes. The prognosis of stomach cancer is generally poor, due to the fact the tumor has often
20 metastasized by the time of discovery and the fact that most people with the condition are elderly (median age is between 70 and 75 years) at presentation. The 5-year survival rate for stomach cancer is reported to be less than 10%.

The most common cause is infection by the bacteria *Helicobacter pylori*, which accounts for more than 60% of cases. Certain types of *H. pylori* have greater risks than others.
25 Other common causes include eating pickled vegetables and smoking. About 10% of cases run in families and between 1% and 3% of cases are due to genetic syndromes inherited from a person's parents such as hereditary diffuse gastric cancer. Most cases of stomach cancers are gastric carcinomas. This type can be divided into a number of subtypes. Lymphomas and mesenchymal tumors may also develop within the stomach. Most of the time, stomach cancer
30 develops through a number of stages over a number of years. Diagnosis is usually by biopsy done during endoscopy. This is then followed by medical imaging to determine if the disease has spread to other parts of the body. Japan and South Korea, two countries that have high rates of disease, screen for stomach cancer.

A Mediterranean diet lowers the risk of cancer as does the stopping of smoking. There is tentative evidence that treating *H. pylori* decreases the future risk. If cancer is treated early, many cases can be cured. Treatments may include some combination of surgery, chemotherapy, radiation therapy, and targeted therapy. If treated late, palliative care may be
5 advised. Outcomes are often poor with a less than 10% 5-year survival rate globally. This is largely because most people with the condition present with advanced disease. In the United States 5-year survival is 28% while in South Korea it is over 65% partly due to screening efforts.

Globally stomach cancer is the fifth leading cause of cancer and the third leading
10 cause of death from cancer making up 7% of cases and 9% of deaths. In 2012 it occurred in 950,000 people and caused 723,000 deaths. Before the 1930s in much of the world, including the United States and the United Kingdom, it was the most common cause of death from cancer. Rates of death have been decreasing in many areas of the world since then. This is believed to be due to the eating of less salted and pickled foods as a result of the development
15 of refrigeration as a method of keeping food fresh. Stomach cancer occurs most commonly in East Asia and Eastern Europe and it occurs twice as often in males as in females.

Stomach cancer is often either asymptomatic (producing no noticeable symptoms) or it may cause only nonspecific symptoms (symptoms that are specific not only to stomach cancer, but also to other related or unrelated disorders) in its early stages. By the time
20 symptoms occur, the cancer has often reached an advanced stage (see below) and may have also metastasized (spread to other, perhaps distant, parts of the body), which is one of the main reasons for its relatively poor prognosis. Early cancers may be associated with indigestion or a burning sensation (heartburn). However, less than 1 in every 50 people referred for endoscopy due to indigestion has cancer. Abdominal discomfort and loss of
25 appetite, especially for meat, can occur.

Gastric cancers that have enlarged and invaded normal tissue can cause weakness, fatigue, bloating of the stomach after meals, abdominal pain in the upper abdomen, nausea and occasional vomiting, diarrhea or constipation. Further enlargement may cause weight loss or bleeding with vomiting blood or having blood in the stool, the latter apparent as black
30 discolouration (melena) and sometimes leading to anemia. Dysphagia suggests a tumour in the cardia or extension of the gastric tumor into the esophagus.

Gastric cancer is a multifactorial disease. *Helicobacter pylori* infection is an essential risk factor in 65–80% of gastric cancers, but in only 2% of such infections. The mechanism by which *H. pylori* induces stomach cancer potentially involves chronic inflammation, or the

action of *H. pylori* virulence factors such as CagA. Smoking increases the risk of developing gastric cancer significantly, from 40% increased risk for current smokers to 82% increase for heavy smokers. Gastric cancers due to smoking mostly occur in the upper part of the stomach near the esophagus. Some studies show increased risk with alcohol consumption as well.

5 Dietary factors are not proven causes, but some foods including smoked foods, salt and salt-rich foods, red meat, processed meat, pickled vegetables, and bracken are associated with a higher risk of stomach cancer. Nitrates and nitrites in cured meats can be converted by certain bacteria, including *H. pylori*, into compounds that have been found to cause stomach cancer in animals. On the other hand, fresh fruit and vegetable intake, citrus fruit intake, and
10 antioxidant intake are associated with a lower risk of stomach cancer. A Mediterranean diet is also associated with lower rates of stomach cancer as does regular aspirin use.

There is a correlation between iodine deficiency and gastric cancer. Gastric cancer shows a male predominance in its incidence as up to two males are affected for every female. Estrogen may protect women against the development of this cancer form. Approximately 10%
15 of cases show a genetic component.

People may possess certain risk factors, such as those that are physical or genetic, that can alter their susceptibility for gastric cancer. Obesity is one such physical risk factor that has been found to increase the risk of gastric adenocarcinoma by contributing to the development of gastroesophageal reflux disease (GERD). The exact mechanism by which
20 obesity causes GERD is not completely known. Studies hypothesize that increased dietary fat leading to increased pressure on the stomach and the lower esophageal sphincter, due to excess adipose tissue, could play a role, yet no statistically significant data has been collected. However, the risk of gastric cardia adenocarcinoma, with GERD present, has been found to increase more than 2 times for an obese person. A genetic risk factor for gastric cancer is a
25 genetic defect of the CDH1 gene known as hereditary diffuse gastric cancer (HDGC). The CDH1 gene, which codes for E-cadherin, lies on the 16th chromosome. When the gene experiences a particular mutation, gastric cancer develops through a mechanism that is not fully understood. This mutation is considered autosomal dominant meaning that half of a carrier's children will likely experience the same mutation. Diagnosis of hereditary diffuse
30 gastric cancer usually takes place when at least two cases involving a family member, such as a parent or grandparent, are diagnosed, with at least one diagnosed before the age of 50. The diagnosis can also be made if there are at least three cases in the family, in which case age is not considered.

The International Cancer Genome Consortium is leading efforts to identify genomic changes involved in stomach cancer. A very small percentage of diffuse-type gastric cancers (see Histopathology below) arise from an inherited abnormal *CDH1* gene. Genetic testing and treatment options are available for families at risk.

5 Other factors associated with increased risk are AIDS, diabetes, pernicious anemia, chronic atrophic gastritis, Menetrier's disease (hyperplastic, hypersecretory gastropathy), and intestinal metaplasia.

To find the cause of symptoms, the doctor asks about the patient's medical history, does a physical exam, and may order laboratory studies. Gastroscopic exam is the diagnostic
10 method of choice. This involves insertion of a fibre optic camera into the stomach to visualize it. Upper GI series (may be called barium roentgenogram). Computed tomography or CT scanning of the abdomen may reveal gastric cancer, but is more useful to determine invasion into adjacent tissues, or the presence of spread to local lymph nodes. Wall thickening of more than 1 cm that is focal, eccentric and enhancing favours malignancy.

15 Abnormal tissue seen in a gastroscope examination will be biopsied by the surgeon or gastroenterologist. This tissue is then sent to a pathologist for histological examination under a microscope to check for the presence of cancerous cells. A biopsy, with subsequent histological analysis, is the only sure way to confirm the presence of cancer cells.

Various gastroscopic modalities have been developed to increase yield of detected
20 mucosa with a dye that accentuates the cell structure and can identify areas of dysplasia. *Endocytoscopy* involves ultra-high magnification to visualise cellular structure to better determine areas of dysplasia. Other gastroscopic modalities such as optical coherence tomography are also being tested investigationally for similar applications.

A number of cutaneous conditions are associated with gastric cancer. A condition of
25 darkened hyperplasia of the skin, frequently of the axilla and groin, known as acanthosis nigricans, is associated with intra-abdominal cancers such as gastric cancer. Other cutaneous manifestations of gastric cancer include *tripe palms* (a similar darkening hyperplasia of the skin of the palms) and the Leser-Trelat sign, which is the rapid development of skin lesions known as seborrheic keratoses. Various blood tests may be performed including a complete
30 blood count (CBC) to check for anaemia, and a fecal occult blood test to check for blood in the stool.

Getting rid of *H. pylori* in those who are infected decreases the risk of stomach cancer, at least in those who are Asian. Low doses of vitamins, especially from a healthy diet,

decrease the risk of stomach cancer. A previous review of supplementation did not find supporting evidence and possibly worse outcomes.

Cancer of the stomach is difficult to cure unless it is found at an early stage (before it has begun to spread). Unfortunately, because early stomach cancer causes few symptoms, the disease is usually advanced when the diagnosis is made. Treatment for stomach cancer may include surgery, chemotherapy, and/or radiation therapy. New treatment approaches such as biological therapy and improved ways of using current methods are being studied in clinical trials.

Surgery remains the only curative therapy for stomach cancer. Of the different surgical techniques, endoscopic mucosal resection (EMR) is a treatment for early gastric cancer (tumor only involves the mucosa) that has been pioneered in Japan, but is also available in the United States at some centers. In this procedure, the tumor, together with the inner lining of stomach (mucosa), is removed from the wall of the stomach using an electrical wire loop through the endoscope. The advantage is that it is a much smaller operation than removing the stomach. Endoscopic submucosal dissection (ESD) is a similar technique pioneered in Japan, used to resect a large area of mucosa in one piece. If the pathologic examination of the resected specimen shows incomplete resection or deep invasion by tumor, the patient would need a formal stomach resection.

Those with metastatic disease at the time of presentation may receive palliative surgery and while it remains controversial, due to the possibility of complications from the surgery itself and the fact that it may delay chemotherapy the data so far is mostly positive, with improved survival rates being seen in those treated with this approach.

The use of chemotherapy to treat stomach cancer has no firmly established standard of care. Unfortunately, stomach cancer has not been particularly sensitive to these drugs, and chemotherapy, if used, has usually served to palliatively reduce the size of the tumor, relieve symptoms of the disease and increase survival time. Some drugs used in stomach cancer treatment have included: 5-FU (fluorouracil) or its analog capecitabine, BCNU (carmustine), methyl-CCNU (semustine) and doxorubicin (Adriamycin), as well as mitomycin C, and more recently cisplatin and taxotere, often using drugs in various combinations. The relative benefits of these different drugs, alone and in combination, are unclear. Clinical researchers have explored the benefits of giving chemotherapy before surgery to shrink the tumor, or as adjuvant therapy after surgery to destroy remaining cancer cells. Recently, a targeted treatment called trastuzumab has become available for use with chemotherapy for the treatment of those overexpressing the HER2 gene in their tumor cells.

Radiation therapy (also called radiotherapy) may also be used to treat stomach cancer, often as an adjuvant to chemotherapy and/or surgery.

III. Monoclonal Antibodies and Production Thereof

5 The monoclonal antibodies described herein were prepared using standard methods, followed by screening, characterization and functional assessment. Variable regions were sequenced and then subcloned into a human expression vector to produce the chimeric antibody genes, which were then expressed and purified. These chimeric antibodies were tested for antigen binding, signaling blocking, and in xenograft experiments.

A. General Methods

10 It will be understood that monoclonal antibodies binding to LILRBs will have several applications. These include the production of diagnostic kits for use in detecting and diagnosing cancer, as well as for cancer therapies. In these contexts, one may link such
15 antibodies to diagnostic or therapeutic agents, use them as capture agents or competitors in competitive assays, or use them individually without additional agents being attached thereto. The antibodies may be mutated or modified, as discussed further below. Methods for preparing and characterizing antibodies are well known in the art (see, *e.g.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; U.S. Patent 4,196,265).

20 The methods for generating monoclonal antibodies (MAbs) generally begin along the same lines as those for preparing polyclonal antibodies. The first step for both these methods is immunization of an appropriate host. As is well known in the art, a given composition for immunization may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling a peptide or polypeptide immunogen to
25 a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or rabbit serum albumin can also be used as carriers. Means for conjugating a polypeptide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimide and bis-biazotized benzidine. As also is well known
30 in the art, the immunogenicity of a particular immunogen composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Exemplary and preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

The amount of immunogen composition used in the production of polyclonal antibodies varies upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal). The production of polyclonal antibodies may be monitored by sampling blood of the immunized animal at various points following immunization. A second, booster injection, also may be given. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored, and/or the animal can be used to generate MAbs.

Following immunization, somatic cells with the potential for producing antibodies, specifically B lymphocytes (B cells), are selected for use in the MAb generating protocol. These cells may be obtained from biopsied spleens or lymph nodes, or from circulating blood. The antibody-producing B lymphocytes from the immunized animal are then fused with cells of an immortal myeloma cell, generally one of the same species as the animal that was immunized or human or human/mouse chimeric cells. Myeloma cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). Any one of a number of myeloma cells may be used, as are known to those of skill in the art (Goding, pp. 65-66, 1986; Campbell, pp. 75-83, 1984).

Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 proportion, though the proportion may vary from about 20:1 to about 1:1, respectively, in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. Fusion methods using Sendai virus have been described by Kohler and Milstein (1975; 1976), and those using polyethylene glycol (PEG), such as 37% (v/v) PEG, by Geftter *et al.* (1977). The use of electrically induced fusion methods also is appropriate (Goding, pp. 71-74, 1986). Fusion procedures usually produce viable hybrids at low frequencies, about 1×10^{-6} to 1×10^{-8} . However, this does not pose a problem, as the viable, fused hybrids are differentiated from the parental, infused cells (particularly the infused myeloma cells that would normally continue to divide indefinitely) by culturing in a selective medium. The selective medium is generally one that contains an agent that blocks the *de novo* synthesis of nucleotides in the tissue culture media. Exemplary and preferred agents are aminopterin, methotrexate, and azaserine. Aminopterin and methotrexate block *de novo* synthesis of both purines and

pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the media is supplemented with hypoxanthine and thymidine as a source of nucleotides (HAT medium). Where azaserine is used, the media is supplemented with hypoxanthine. Ouabain is added if the B cell source is an Epstein Barr virus (EBV) transformed human B cell line, in order to eliminate EBV transformed lines that have not fused to the myeloma.

The preferred selection medium is HAT or HAT with ouabain. Only cells capable of operating nucleotide salvage pathways are able to survive in HAT medium. The myeloma cells are defective in key enzymes of the salvage pathway, *e.g.*, hypoxanthine phosphoribosyl transferase (HPRT), and they cannot survive. The B cells can operate this pathway, but they have a limited life span in culture and generally die within about two weeks. Therefore, the only cells that can survive in the selective media are those hybrids formed from myeloma and B cells. When the source of B cells used for fusion is a line of EBV-transformed B cells, as here, ouabain is also used for drug selection of hybrids as EBV-transformed B cells are susceptible to drug killing, whereas the myeloma partner used is chosen to be ouabain resistant.

Culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays dot immunobinding assays, and the like. The selected hybridomas are then serially diluted or single-cell sorted by flow cytometric sorting and cloned into individual antibody-producing cell lines, which clones can then be propagated indefinitely to provide mAbs. The cell lines may be exploited for MAb production in two basic ways. A sample of the hybridoma can be injected (often into the peritoneal cavity) into an animal (*e.g.*, a mouse). Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. When human hybridomas are used in this way, it is optimal to inject immunocompromised mice, such as SCID mice, to prevent tumor rejection. The injected animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide MAbs in high concentration. The individual cell lines could also be cultured *in vitro*, where the MAbs are naturally secreted into the culture medium from which they can be readily obtained in high concentrations. Alternatively,

human hybridoma cells lines can be used *in vitro* to produce immunoglobulins in cell supernatant. The cell lines can be adapted for growth in serum-free medium to optimize the ability to recover human monoclonal immunoglobulins of high purity.

MAbs produced by either means may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as FPLC or affinity chromatography. Fragments of the monoclonal antibodies of the disclosure can be obtained from the purified monoclonal antibodies by methods which include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, monoclonal antibody fragments encompassed by the present disclosure can be synthesized using an automated peptide synthesizer.

It also is contemplated that a molecular cloning approach may be used to generate monoclonals. For this, RNA can be isolated from the hybridoma line and the antibody genes obtained by RT-PCR and cloned into an immunoglobulin expression vector. Alternatively, combinatorial immunoglobulin phagemid libraries are prepared from RNA isolated from the cell lines and phagemids expressing appropriate antibodies are selected by panning using viral antigens. The advantages of this approach over conventional hybridoma techniques are that approximately 10^4 times as many antibodies can be produced and screened in a single round, and that new specificities are generated by H and L chain combination which further increases the chance of finding appropriate antibodies.

Other U.S. patents, each incorporated herein by reference, that teach the production of antibodies useful in the present disclosure include U.S. Patent 5,565,332, which describes the production of chimeric antibodies using a combinatorial approach; U.S. Patent 4,816,567 which describes recombinant immunoglobulin preparations; and U.S. Patent 4,867,973 which describes antibody-therapeutic agent conjugates.

B. Antibodies of the Present Disclosure

Antibodies according to the present disclosure may be defined, in the first instance, by their binding specificity, which in this case is for LILRBs. Those of skill in the art, by assessing the binding specificity/affinity of a given antibody using techniques well known to those of skill in the art, can determine whether such antibodies fall within the scope of the instant claims. In one aspect, there are provided monoclonal antibodies having clone-paired CDR's from the heavy and light chains as illustrated in FIG. 22. Such antibodies may be produced by the clones discussed below in the Examples section using methods described herein.

In a second aspect, the antibodies may be defined by their variable sequence, which include additional “framework” regions. These are provided by sequences in FIG. 21 that encode or represent full variable regions. Furthermore, the antibodies sequences may vary from these sequences, particularly in regions outside the CDRs. For example, nucleic acid sequences may vary from those set out above in that (a) the variable regions may be segregated away from the constant domains of the light and heavy chains, (b) the nucleic acids may vary from those set out above while not affecting the residues encoded thereby, (c) the nucleic acids may vary from those set out above by a given percentage, *e.g.*, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology, (d) the nucleic acids may vary from those set out above by virtue of the ability to hybridize under high stringency conditions, as exemplified by low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C, (e) the amino acids may vary from those set out above by a given percentage, *e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology, or (f) the amino acids may vary from those set out above by permitting conservative substitutions (discussed below). Each of the foregoing apply to the nucleic acid sequences or amino acid sequences of FIG. 21. In another embodiment, the antibody derivatives of the present disclosure comprise V_L and V_H domains having up to 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more conservative or non-conservative amino acid substitutions, while still exhibiting the desired binding and functional properties.

While the antibodies of the present disclosure were generated as IgG's, it may be useful to modify the constant regions to alter their function. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the 30 immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system. Thus, the term “antibody” includes intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof), wherein the light chains of the immunoglobulin may be of types kappa or lambda. Within light and heavy chains, the variable and constant regions are joined by a 35 “J” region of about 12 or more amino acids, with the heavy chain also including a “D” region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)).

The present disclosure further comprises nucleic acids which hybridize to nucleic acids encoding the antibodies disclosed herein. In general, the nucleic acids hybridize under moderate or high stringency conditions to nucleic acids that encode antibodies disclosed herein and also encode antibodies that maintain the ability to specifically bind to an LILRB.

A first nucleic acid molecule is “hybridizable” to a second nucleic acid molecule when a single stranded form of the first nucleic acid molecule can anneal to the second nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (see Sambrook *et al.*, *supra*). The conditions of temperature and ionic strength determine the “stringency” of the hybridization. Typical moderate stringency hybridization conditions are 40% formamide, with 5X or 6X SSC and 0.1% SDS at 42°C. High stringency hybridization conditions are 50% formamide, 5X or 6X SSC (0.15M NaCl and 0.015M Na-citrate) at 42°C or, optionally, at a higher temperature (*e.g.*, 57°C, 59°C, 60°C, 62°C, 63°C, 65°C or 68°C). Hybridization requires that the two nucleic acids contain complementary sequences, although, depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the higher the stringency under which the nucleic acids may hybridize. For hybrids of greater than 100 nucleotides in length, equations for calculating the melting temperature have been derived (see Sambrook *et al.*, *supra*). For hybridization with shorter nucleic acids, *e.g.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook *et al.*, *supra*).

C. Engineering of Antibody Sequences

In various embodiments, one may choose to engineer sequences of the identified antibodies for a variety of reasons, such as improved expression, improved cross-reactivity or diminished off-target binding. The following is a general discussion of relevant techniques for antibody engineering.

Hybridomas may be cultured, then cells lysed, and total RNA extracted. Random hexamers may be used with RT to generate cDNA copies of RNA, and then PCR performed using a multiplex mixture of PCR primers expected to amplify all human variable gene sequences. PCR product can be cloned into pGEM-T Easy vector, then sequenced by automated DNA sequencing using standard vector primers. Assay of binding and neutralization may be performed using antibodies collected from hybridoma supernatants and purified by FPLC, using Protein G columns. Recombinant full length IgG antibodies may be generated by subcloning heavy and light chain Fv DNAs from the cloning vector into an IgG plasmid vector, transfected into 293 Freestyle cells or CHO cells, and antibodies collected and purified from the 293 or CHO cell supernatant.

The rapid availability of antibody produced in the same host cell and cell culture process as the final cGMP manufacturing process has the potential to reduce the duration of process development programs. Lonza has developed a generic method using pooled transfectants grown in CDACF medium, for the rapid production of small quantities (up to 50 g) of antibodies in CHO cells. Although slightly slower than a true transient system, the advantages include a higher product concentration and use of the same host and process as the production cell line. Example of growth and productivity of GS-CHO pools, expressing a model antibody, in a disposable bioreactor: in a disposable bag bioreactor culture (5 L working volume) operated in fed-batch mode, a harvest antibody concentration of 2 g/L was achieved within 9 weeks of transfection.

Antibody molecules will comprise fragments (such as F(ab'), F(ab')₂) that are produced, for example, by the proteolytic cleavage of the mAbs, or single-chain immunoglobulins producible, for example, via recombinant means. Such antibody derivatives are monovalent. In one embodiment, such fragments can be combined with one another, or with other antibody fragments or receptor ligands to form "chimeric" binding molecules. Significantly, such chimeric molecules may contain substituents capable of binding to different epitopes of the same molecule.

1. Antigen Binding Modifications

In related embodiments, the antibody is a derivative of the disclosed antibodies, *e.g.*, an antibody comprising the CDR sequences identical to those in the disclosed antibodies (*e.g.*, a chimeric, or CDR-grafted antibody). Alternatively, one may wish to make modifications, such as introducing conservative changes into an antibody molecule. In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

It also is understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned

to amino acid residues: basic amino acids: arginine (+3.0), lysine (+3.0), and histidine (-0.5); acidic amino acids: aspartate (+3.0 $\pm$ 1), glutamate (+3.0 $\pm$ 1), asparagine (+0.2), and glutamine (+0.2); hydrophilic, nonionic amino acids: serine (+0.3), asparagine (+0.2), glutamine (+0.2), and threonine (-0.4), sulfur containing amino acids: cysteine (-1.0) and methionine (-1.3); hydrophobic, nonaromatic amino acids: valine (-1.5), leucine (-1.8), isoleucine (-1.8), proline (-0.5 $\pm$ 1), alanine (-0.5), and glycine (0); hydrophobic, aromatic amino acids: tryptophan (-3.4), phenylalanine (-2.5), and tyrosine (-2.3).

It is understood that an amino acid can be substituted for another having a similar hydrophilicity and produce a biologically or immunologically modified protein. In such changes, the substitution of amino acids whose hydrophilicity values are within $\pm$ 2 is preferred, those that are within $\pm$ 1 are particularly preferred, and those within $\pm$ 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions generally are based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take into consideration the various foregoing characteristics are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

The present disclosure also contemplates isotype modification. By modifying the Fc region to have a different isotype, different functionalities can be achieved. For example, changing to IgG₁ can increase antibody dependent cell cytotoxicity, switching to class A can improve tissue distribution, and switching to class M can improve valency.

Modified antibodies may be made by any technique known to those of skill in the art, including expression through standard molecular biological techniques, or the chemical synthesis of polypeptides. Methods for recombinant expression are addressed elsewhere in this document.

2. Fc Region Modifications

The antibodies disclosed herein can also be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or effector function (*e.g.*, antigen-dependent cellular cytotoxicity). Furthermore, the antibodies disclosed herein can be chemically modified (*e.g.*, one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the

antibody. Each of these embodiments is described in further detail below. The numbering of residues in the Fc region is that of the EU index of Kabat. The antibodies disclosed herein also include antibodies with modified (or blocked) Fc regions to provide altered effector functions. See, *e.g.*, U.S. Patent 5,624,821; WO2003/086310; WO2005/120571; 5 WO2006/0057702. Such modification can be used to enhance or suppress various reactions of the immune system, with possible beneficial effects in diagnosis and therapy. Alterations of the Fc region include amino acid changes (substitutions, deletions and insertions), glycosylation or deglycosylation, and adding multiple Fc. Changes to the Fc can also alter the half-life of antibodies in therapeutic antibodies, enabling less frequent dosing and thus 10 increased convenience and decreased use of material. This mutation has been reported to abolish the heterogeneity of inter-heavy chain disulfide bridges in the hinge region.

In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is increased or decreased. This approach is described further in U.S. Patent 5,677,425. The number of cysteine residues in the hinge region of CH1 15 is altered, for example, to facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody. In another embodiment, the antibody is modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent 6,277,375. Alternatively, to increase the biological half-life, the antibody can be 20 altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patents 5,869,046 and 6,121,022. In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector function(s) of the antibodies. For example, one or more amino acids selected from amino acid residues 234, 25 235, 236, 237, 297, 318, 320 and 322 can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patents 5,624,821 and 5,648,260.

30 In another example, one or more amino acid residues within amino acid positions 231 and 239 are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351. In yet another example, the Fc region is modified to increase or decrease the ability of the antibodies to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase or decrease the affinity of the

antibodies for an Fcγ receptor by modifying one or more amino acids at the following positions: 238, 239, 243, 248, 249, 252, 254, 255, 256, 258, 264, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. This approach is described further in PCT Publication WO 00/42072. Moreover, the binding sites on human IgG1 for FcγR1, FcγR2, FcγR3 and FcRn have been mapped and variants with improved binding have been described. Specific mutations at positions 256, 290, 298, 333, 334 and 339 were shown to improve binding to FcγR3. Additionally, the following combination mutants were shown to improve FcγR3 binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A.

In one embodiment, the Fc region is modified to decrease the ability of the antibodies to mediate effector function and/or to increase anti-inflammatory properties by modifying residues 243 and 264. In one embodiment, the Fc region of the antibody is modified by changing the residues at positions 243 and 264 to alanine. In one embodiment, the Fc region is modified to decrease the ability of the antibody to mediate effector function and/or to increase anti-inflammatory properties by modifying residues 243, 264, 267 and 328. In still another embodiment, the antibody comprises a particular glycosylation pattern. For example, an aglycosylated antibody can be made (*i.e.*, the antibody lacks glycosylation). The glycosylation pattern of an antibody may be altered to, for example, increase the affinity or avidity of the antibody for an antigen. Such modifications can be accomplished by, for example, altering one or more of the glycosylation sites within the antibody sequence. For example, one or more amino acid substitutions can be made that result removal of one or more of the variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity or avidity of the antibody for antigen. *See, e.g.*, U.S. Patents 5,714,350 and 6,350,861.

An antibody may also be made in which the glycosylation pattern includes hypofucosylated or afucosylated glycans, such as a hypofucosylated antibodies or afucosylated antibodies have reduced amounts of fucosyl residues on the glycan. The antibodies may also include glycans having an increased amount of bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such modifications can be accomplished by, for example, expressing the antibodies in a host cell in which the glycosylation pathway was been genetically engineered to produce glycoproteins with particular glycosylation patterns. These cells have

been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (α (1,6)-fucosyltransferase), such that antibodies expressed in the Ms704, Ms705, and Ms709 cell lines lack fucose on their carbohydrates. The Ms704, Ms705, and Ms709 FUT8^{-/-} cell lines were created by the targeted disruption of the FUT8 gene in CHO/DG44 cells using two replacement vectors (*see* U.S. Patent Publication No. 20040110704. As another example, EP 1 176 195 describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation by reducing or eliminating the α -1,6 bond-related enzyme. EP 1 176 195 also describes cell lines which have a low enzyme activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody or does not have the enzyme activity, for example the rat myeloma cell line YB2/0 (ATCC CRL 1662). PCT Publication WO 03/035835 describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell. Antibodies with a modified glycosylation profile can also be produced in chicken eggs, as described in PCT Publication WO 06/089231. Alternatively, antibodies with a modified glycosylation profile can be produced in plant cells, such as *Lemna* (US Patent 7,632,983). Methods for production of antibodies in a plant system are disclosed in the U.S. Patents 6,998,267 and 7,388,081. PCT Publication WO 99/54342 describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (*e.g.*, β (1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies.

Alternatively, the fucose residues of the antibodies can be cleaved off using a fucosidase enzyme; *e.g.*, the fucosidase α -L-fucosidase removes fucosyl residues from antibodies. Antibodies disclosed herein further include those produced in lower eukaryote host cells, in particular fungal host cells such as yeast and filamentous fungi have been genetically engineered to produce glycoproteins that have mammalian- or human-like glycosylation patterns. A particular advantage of these genetically modified host cells over currently used mammalian cell lines is the ability to control the glycosylation profile of glycoproteins that are produced in the cells such that compositions of glycoproteins can be produced wherein a particular N-glycan structure predominates (*see, e.g.*, U.S. Patents

7,029,872 and 7,449,308). These genetically modified host cells have been used to produce antibodies that have predominantly particular *N*-glycan structures.

In addition, since fungi such as yeast or filamentous fungi lack the ability to produce fucosylated glycoproteins, antibodies produced in such cells will lack fucose unless the cells
 5 are further modified to include the enzymatic pathway for producing fucosylated glycoproteins (See for example, PCT Publication WO2008112092). In particular embodiments, the antibodies disclosed herein further include those produced in lower eukaryotic host cells and which comprise fucosylated and nonfucosylated hybrid and complex *N*-glycans, including bisected and multiantennary species, including but not limited
 10 to *N*-glycans such as GlcNAc(1-4)Man3GlcNAc2; Gal(1-4)GlcNAc(1-4)Man3GlcNAc2; NANA(1-4)Gal(1-4)GlcNAc(1-4)Man3GlcNAc2. In particular embodiments, the antibody compositions provided herein may comprise antibodies having at least one hybrid *N*-glycan selected from the group consisting of GlcNAcMan5GlcNAc2; GalGlcNAcMan5GlcNAc2; and NANAGalGlcNAcMan5GlcNAc2. In particular aspects, the hybrid *N*-glycan is the
 15 predominant *N*-glycan species in the composition. In further aspects, the hybrid *N*-glycan is a particular *N*-glycan species that comprises about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, or 100% of the hybrid *N*-glycans in the composition.

In particular embodiments, the antibody compositions provided herein comprise antibodies having at least one complex *N*-glycan selected from the group consisting of
 20 GlcNAcMan3GlcNAc2; GalGlcNAcMan3GlcNAc2; NANAGalGlcNAcMan3GlcNAc2; GlcNAc2Man3GlcNAc2; GalGlcNAc2Man3GlcNAc2; Gal2GlcNAc2Man3GlcNAc2; NANAGal2GlcNAc2Man3GlcNAc2; and NANA2Gal2GlcNAc2Man3GlcNAc2. In particular aspects, the complex *N*-glycan is the predominant *N*-glycan species in the composition. In further aspects, the complex *N*-glycan is a particular *N*-glycan species that
 25 comprises about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, or 100% of the complex *N*-glycans in the composition. In particular embodiments, the *N*-glycan is fucosylated. In general, the fucose is in an α 1,3-linkage with the GlcNAc at the reducing end of the *N*-glycan, an α 1,6-linkage with the GlcNAc at the reducing end of the *N*-glycan, an α 1,2-linkage with the Gal at the non-reducing end of the *N*-glycan, an α 1,3-linkage with the
 30 GlcNAc at the non-reducing end of the *N*-glycan, or an α 1,4-linkage with a GlcNAc at the non-reducing end of the *N*-glycan.

Therefore, in particular aspects of the above the glycoprotein compositions, the glycoform is in an α 1,3-linkage or α 1,6-linkage fucose to produce a glycoform selected from the

group consisting of Man5GlcNAc2(Fuc), GlcNAcMan5GlcNAc2(Fuc), Man3GlcNAc2(Fuc),
 GlcNAcMan3GlcNAc2(Fuc), GlcNAc2Man3GlcNAc2(Fuc),
 GalGlcNAc2Man3GlcNAc2(Fuc), Gal2GlcNAc2Man3GlcNAc2(Fuc),
 NANAGal2GlcNAc2Man3GlcNAc2(Fuc), and NANA2Gal2GlcNAc2Man3GlcNAc2(Fuc);
 5 in an α 1,3-linkage or α 1,4-linkage fucose to produce a glycoform selected from the group
 consisting of GlcNAc(Fuc)Man5GlcNAc2, GlcNAc(Fuc)Man3GlcNAc2, GlcNAc2(Fuc1-
 2)Man3GlcNAc2, GalGlcNAc2(Fuc1-2)Man3GlcNAc2, Gal2GlcNAc2(Fuc1-
 2)Man3GlcNAc2, NANAGal2GlcNAc2(Fuc1-2)Man3GlcNAc2, and
 NANA2Gal2GlcNAc2(Fuc1-2)Man3GlcNAc2; or in an α 1,2-linkage fucose to produce a
 10 glycoform selected from the group consisting of Gal(Fuc)GlcNAc2Man3GlcNAc2,
 Gal2(Fuc1-2)GlcNAc2Man3GlcNAc2, NANAGal2(Fuc1-2)GlcNAc2Man3GlcNAc2, and
 NANA2Gal2(Fuc1-2)GlcNAc2Man3GlcNAc2.

In further aspects, the antibodies comprise high mannose *N*-glycans, including but not
 limited to, Man8GlcNAc2, Man7GlcNAc2, Man6GlcNAc2, Man5GlcNAc2, Man4GlcNAc2,
 15 or *N*-glycans that consist of the Man3GlcNAc2 *N*-glycan structure. In further aspects of the
 above, the complex *N*-glycans further include fucosylated and non-fucosylated bisected and
 multiantennary species. As used herein, the terms "*N*-glycan" and "glycoform" are used
 interchangeably and refer to an *N*-linked oligosaccharide, for example, one that is attached by
 an asparagine-*N*-acetylglucosamine linkage to an asparagine residue of a polypeptide. *N*-
 20 linked glycoproteins contain an *N*-acetylglucosamine residue linked to the amide nitrogen of
 an asparagine residue in the protein.

D. Single Chain Antibodies

A Single Chain Variable Fragment (scFv) is a fusion of the variable regions of the
 25 heavy and light chains of immunoglobulins, linked together with a short (usually serine,
 glycine) linker. This chimeric molecule retains the specificity of the original immunoglobulin,
 despite removal of the constant regions and the introduction of a linker peptide. This
 modification usually leaves the specificity unaltered. These molecules were created
 historically to facilitate phage display where it is highly convenient to express the antigen
 30 binding domain as a single peptide. Alternatively, scFv can be created directly from
 subcloned heavy and light chains derived from a hybridoma. Single chain variable fragments
 lack the constant Fc region found in complete antibody molecules, and thus, the common
 binding sites (*e.g.*, protein A/G) used to purify antibodies. These fragments can often be

purified/immobilized using Protein L since Protein L interacts with the variable region of kappa light chains.

Flexible linkers generally are comprised of helix- and turn-promoting amino acid residues such as alanine, serine and glycine. However, other residues can function as well. Tang *et al.* (1996) used phage display as a means of rapidly selecting tailored linkers for single-chain antibodies (scFvs) from protein linker libraries. A random linker library was constructed in which the genes for the heavy and light chain variable domains were linked by a segment encoding an 18-amino acid polypeptide of variable composition. The scFv repertoire (approx. 5×10^6 different members) was displayed on filamentous phage and subjected to affinity selection with hapten. The population of selected variants exhibited significant increases in binding activity but retained considerable sequence diversity. Screening 1054 individual variants subsequently yielded a catalytically active scFv that was produced efficiently in soluble form. Sequence analysis revealed a conserved proline in the linker two residues after the V_H C terminus and an abundance of arginines and prolines at other positions as the only common features of the selected tethers.

The recombinant antibodies of the present disclosure may also involve sequences or moieties that permit dimerization or multimerization of the receptors. Such sequences include those derived from IgA, which permit formation of multimers in conjunction with the J-chain. Another multimerization domain is the Gal4 dimerization domain. In other embodiments, the chains may be modified with agents such as biotin/avidin, which permit the combination of two antibodies.

In a separate embodiment, a single-chain antibody can be created by joining receptor light and heavy chains using a non-peptide linker or chemical unit. Generally, the light and heavy chains will be produced in distinct cells, purified, and subsequently linked together in an appropriate fashion (*i.e.*, the N-terminus of the heavy chain being attached to the C-terminus of the light chain via an appropriate chemical bridge).

Cross-linking reagents are used to form molecular bridges that tie functional groups of two different molecules, *e.g.*, a stabilizing and coagulating agent. However, it is contemplated that dimers or multimers of the same analog or heteromeric complexes comprised of different analogs can be created. To link two different compounds in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation.

An exemplary hetero-bifunctional cross-linker contains two reactive groups: one reacting with primary amine group (*e.g.*, N-hydroxy succinimide) and the other reacting with a thiol group (*e.g.*, pyridyl disulfide, maleimides, halogens, *etc.*). Through the primary amine

reactive group, the cross-linker may react with the lysine residue(s) of one protein (*e.g.*, the selected antibody or fragment) and through the thiol reactive group, the cross-linker, already tied up to the first protein, reacts with the cysteine residue (free sulfhydryl group) of the other protein (*e.g.*, the selective agent).

5 It is preferred that a cross-linker having reasonable stability in blood will be employed. Numerous types of disulfide-bond containing linkers are known that can be successfully employed to conjugate targeting and therapeutic/preventative agents. Linkers that contain a disulfide bond that is sterically hindered may prove to give greater stability *in vivo*, preventing release of the targeting peptide prior to reaching the site of action. These linkers
10 are thus one group of linking agents.

Another cross-linking reagent is SMPT, which is a bifunctional cross-linker containing a disulfide bond that is “sterically hindered” by an adjacent benzene ring and methyl groups. It is believed that steric hindrance of the disulfide bond serves a function of protecting the bond from attack by thiolate anions such as glutathione which can be present in
15 tissues and blood, and thereby help in preventing decoupling of the conjugate prior to the delivery of the attached agent to the target site.

The SMPT cross-linking reagent, as with many other known cross-linking reagents, lends the ability to cross-link functional groups such as the SH of cysteine or primary amines (*e.g.*, the epsilon amino group of lysine). Another possible type of cross-linker includes the
20 hetero-bifunctional photoreactive phenylazides containing a cleavable disulfide bond such as sulfosuccinimidyl-2-(p-azido salicylamido) ethyl-1,3'-dithiopropionate. The N-hydroxy-succinimidyl group reacts with primary amino groups and the phenylazide (upon photolysis) reacts non-selectively with any amino acid residue.

In addition to hindered cross-linkers, non-hindered linkers also can be employed in
25 accordance herewith. Other useful cross-linkers, not considered to contain or generate a protected disulfide, include SATA, SPDP and 2-iminothiolane (Wawrzynczak & Thorpe, 1987). The use of such cross-linkers is well understood in the art. Another embodiment involves the use of flexible linkers.

U.S. Patent 4,680,338, describes bifunctional linkers useful for producing conjugates
30 of ligands with amine-containing polymers and/or proteins, especially for forming antibody conjugates with chelators, drugs, enzymes, detectable labels and the like. U.S. Patents 5,141,648 and 5,563,250 disclose cleavable conjugates containing a labile bond that is cleavable under a variety of mild conditions. This linker is particularly useful in that the agent of interest may be bonded directly to the linker, with cleavage resulting in release of the

active agent. Particular uses include adding a free amino or free sulfhydryl group to a protein, such as an antibody, or a drug.

U.S. Patent 5,856,456 provides peptide linkers for use in connecting polypeptide constituents to make fusion proteins, *e.g.*, single chain antibodies. The linker is up to about 50 amino acids in length, contains at least one occurrence of a charged amino acid (preferably arginine or lysine) followed by a proline, and is characterized by greater stability and reduced aggregation. U.S. Patent 5,880,270 discloses aminooxy-containing linkers useful in a variety of immunodiagnostic and separative techniques.

E. Purification

In certain embodiments, the antibodies of the present disclosure may be purified. The term “purified,” as used herein, is intended to refer to a composition, isolatable from other components, wherein the protein is purified to any degree relative to its naturally-obtainable state. A purified protein therefore also refers to a protein, free from the environment in which it may naturally occur. Where the term “substantially purified” is used, this designation will refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the proteins in the composition.

Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the polypeptide from other proteins, the polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; isoelectric focusing. Other methods for protein purification include, precipitation with ammonium sulfate, PEG, antibodies and the like or by heat denaturation, followed by centrifugation; gel filtration, reverse phase, hydroxylapatite and affinity chromatography; and combinations of such and other techniques.

In purifying an antibody of the present disclosure, it may be desirable to express the polypeptide in a prokaryotic or eukaryotic expression system and extract the protein using denaturing conditions. The polypeptide may be purified from other cellular components using an affinity column, which binds to a tagged portion of the polypeptide. As is generally known in the art, it is believed that the order of conducting the various purification steps may be

changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

Commonly, complete antibodies are fractionated utilizing agents (*i.e.*, protein A) that bind the Fc portion of the antibody. Alternatively, antigens may be used to simultaneously
5 purify and select appropriate antibodies. Such methods often utilize the selection agent bound to a support, such as a column, filter or bead. The antibodies is bound to a support, contaminants removed (*e.g.*, washed away), and the antibodies released by applying conditions (salt, heat, *etc.*).

Various methods for quantifying the degree of purification of the protein or peptide
10 will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by SDS/PAGE analysis. Another method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity. The actual
15 units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed protein or peptide exhibits a detectable activity.

It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi *et al.*, 1977). It will therefore be appreciated that
20 under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified expression products may vary.

IV. Treatment of Cancer

A. Formulation and Administration

25 The present disclosure provides pharmaceutical compositions comprising anti-LILRB antibodies and antigens for generating the same. Such compositions comprise a prophylactically or therapeutically effective amount of an antibody or a fragment thereof, and a pharmaceutically acceptable carrier. In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or
30 listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is

a particular carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Other suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, *etc.* Examples of suitable pharmaceutical agents are described in "Remington's Pharmaceutical Sciences." Such compositions will contain a prophylactically or therapeutically effective amount of the antibody or fragment thereof, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration, which can be oral, intravenous, intraarterial, intrabuccal, intranasal, nebulized, bronchial inhalation, or delivered by mechanical ventilation.

Antibodies of the present disclosure, as described herein, can be formulated for parenteral administration, *e.g.*, formulated for injection *via* the intradermal, intravenous, intramuscular, subcutaneous, intra-tumoral or even intraperitoneal routes. The antibodies could alternatively be administered by a topical route directly to the mucosa, for example by nasal drops, inhalation, or by nebulizer. Pharmaceutically acceptable salts, include the acid salts and those which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Passive transfer of antibodies, known as artificially acquired passive immunity, generally will involve the use of intravenous injections. The forms of antibody can be human or animal blood plasma or serum, as pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use, as high-titer human IVIG or IG from immunized or from donors recovering from disease, and as monoclonal antibodies (MAb). Such immunity generally

lasts for only a short period of time, and there is also a potential risk for hypersensitivity reactions, and serum sickness, especially from gamma globulin of non-human origin. However, passive immunity provides immediate protection. The antibodies will be formulated in a carrier suitable for injection, *i.e.*, sterile and syringeable.

5 Generally, the ingredients of compositions of the disclosure are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade
10 water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

 The compositions of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived
15 from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

B. Combination Therapies

20 It may also be desirable to provide combination treatments using antibodies of the present disclosure in conjunction with additional anti-cancer therapies. These therapies would be provided in a combined amount effective to achieve a reduction in one or more disease parameter. This process may involve contacting the cells/subjects with the both agents/therapies at the same time, *e.g.*, using a single composition or pharmacological
25 formulation that includes both agents, or by contacting the cell/subject with two distinct compositions or formulations, at the same time, wherein one composition includes the antibody and the other includes the other agent.

 Alternatively, the antibody may precede or follow the other treatment by intervals ranging from minutes to weeks. One would generally ensure that a significant period of time
30 did not expire between the time of each delivery, such that the therapies would still be able to exert an advantageously combined effect on the cell/subject. In such instances, it is contemplated that one would contact the cell with both modalities within about 12-24 hours of each other, within about 6-12 hours of each other, or with a delay time of only about 12 hours. In some situations, it may be desirable to extend the time period for treatment

significantly; however, where several 10 days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

It also is conceivable that more than one administration of either the anti-DC-HIL antibody or the other therapy will be desired. Various combinations may be employed, where
 5 the antibody is “A,” and the other therapy is “B,” as exemplified below:

A/B/A B/A/B B/B/A A/A/B B/A/A A/B/B B/B/B/A B/B/A/B
 A/A/B/B A/B/A/B A/B/B/A B/B/A/A B/A/B/A B/A/A/B B/B/B/A
 A/A/A/B B/A/A/A A/B/A/A A/A/B/A A/B/B/B B/A/B/B B/B/A/B

10

Other combinations are contemplated. To kill cells, inhibit cell growth, inhibit metastasis, inhibit angiogenesis or otherwise reverse or reduce the malignant phenotype of tumor cells, using the methods and compositions of the present invention, one may contact a target cell or site with an antibody and at least one other therapy. These therapies would be provided in a
 15 combined amount effective to kill or inhibit proliferation of cancer cells. This process may involve contacting the cells/site/subject with the agents/therapies at the same time.

Particular agents contemplated for combination therapy with antibodies of the present disclosure include chemotherapy and hematopoietic stem cell transplantation. Chemotherapy may include cytarabine (ara-C) and an anthracycline (most often daunorubicin), high-dose
 20 cytarabine alone, all-*trans*-retinoic acid (ATRA) in addition to induction chemotherapy, usually an anthracycline, histamine dihydrochloride (Ceplene) and interleukin 2 (Proleukin) after the completion of consolidation therapy, gemtuzumab ozogamicin (Mylotarg) for patients aged more than 60 years with relapsed AML who are not candidates for high-dose chemotherapy, clofarabine, as well as targeted therapies, such as kinase inhibitors, farnesyl
 25 transferase inhibitors, decitabine, and inhibitors of MDR1 (multidrug-resistance protein), or arsenic trioxide or relapsed acute promyelocytic leukemia (APL).

V. Antibody Conjugates

Antibodies of the present disclosure may be linked to at least one agent to form an
 30 antibody conjugate. In order to increase the efficacy of antibody molecules as diagnostic or therapeutic agents, it is conventional to link or covalently bind or complex at least one desired molecule or moiety. Such a molecule or moiety may be, but is not limited to, at least one effector or reporter molecule. Effector molecules comprise molecules having a desired activity, *e.g.*, cytotoxic activity. Non-limiting examples of effector molecules which have

been attached to antibodies include toxins, anti-tumor agents, therapeutic enzymes, radionuclides, antiviral agents, chelating agents, cytokines, growth factors, and oligo- or polynucleotides. By contrast, a reporter molecule is defined as any moiety which may be detected using an assay. Non-limiting examples of reporter molecules which have been
5 conjugated to antibodies include enzymes, radiolabels, haptens, fluorescent labels, phosphorescent molecules, chemiluminescent molecules, chromophores, photoaffinity molecules, colored particles or ligands, such as biotin.

Antibody conjugates are generally preferred for use as diagnostic agents. Antibody diagnostics generally fall within two classes, those for use in *in vitro* diagnostics, such as in a
10 variety of immunoassays, and those for use *in vivo* diagnostic protocols, generally known as "antibody-directed imaging." Many appropriate imaging agents are known in the art, as are methods for their attachment to antibodies (see, for *e.g.*, U.S. Patents 5,021,236, 4,938,948, and 4,472,509). The imaging moieties used can be paramagnetic ions, radioactive isotopes, fluorochromes, NMR-detectable substances, and X-ray imaging agents.

15 In the case of paramagnetic ions, one might mention by way of example ions such as chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and/or erbium (III), with gadolinium being particularly preferred. Ions useful in other contexts, such as X-ray imaging, include but are not limited to
20 lanthanum (III), gold (III), lead (II), and especially bismuth (III).

In the case of radioactive isotopes for therapeutic and/or diagnostic application, one might mention astatine²¹¹, ¹⁴carbon, ⁵¹chromium, ³⁶chlorine, ⁵⁷cobalt, ⁵⁸cobalt, copper⁶⁷, ¹⁵²Eu, gallium⁶⁷, ³hydrogen, iodine¹²³, iodine¹²⁵, iodine¹³¹, indium¹¹¹, ⁵⁹iron, ³²phosphorus, rhenium¹⁸⁶, rhenium¹⁸⁸, ⁷⁵selenium, ³⁵sulphur, technetium^{99m} and/or yttrium⁹⁰. ¹²⁵I is often
25 being preferred for use in certain embodiments, and technetium^{99m} and/or indium¹¹¹ are also often preferred due to their low energy and suitability for long range detection. Radioactively labeled monoclonal antibodies of the present disclosure may be produced according to well-known methods in the art. For instance, monoclonal antibodies can be iodinated by contact with sodium and/or potassium iodide and a chemical oxidizing agent such as sodium
30 hypochlorite, or an enzymatic oxidizing agent, such as lactoperoxidase. Monoclonal antibodies according to the disclosure may be labeled with technetium^{99m} by ligand exchange process, for example, by reducing pertechnetate with stannous solution, chelating the reduced technetium onto a Sephadex column and applying the antibody to this column. Alternatively, direct labeling techniques may be used, *e.g.*, by incubating pertechnetate, a reducing agent such

as SNCl_2 , a buffer solution such as sodium-potassium phthalate solution, and the antibody. Intermediary functional groups which are often used to bind radioisotopes which exist as metallic ions to antibody are diethylenetriaminepentaacetic acid (DTPA) or ethylene diaminetetracetic acid (EDTA).

5 Among the fluorescent labels contemplated for use as conjugates include Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy3, Cy5,6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, Renographin, ROX, TAMRA, TET,
10 Tetramethylrhodamine, and/or Texas Red.

Another type of antibody conjugates contemplated in the present disclosure are those intended primarily for use *in vitro*, where the antibody is linked to a secondary binding ligand and/or to an enzyme (an enzyme tag) that will generate a colored product upon contact with a chromogenic substrate. Examples of suitable enzymes include urease, alkaline phosphatase,
15 (horseradish) hydrogen peroxidase or glucose oxidase. Preferred secondary binding ligands are biotin and avidin and streptavidin compounds. The use of such labels is well known to those of skill in the art and are described, for example, in U.S. Patents 3,817,837, 3,850,752, 3,939,350, 3,996,345, 4,277,437, 4,275,149 and 4,366,241.

Yet another known method of site-specific attachment of molecules to antibodies
20 comprises the reaction of antibodies with hapten-based affinity labels. Essentially, hapten-based affinity labels react with amino acids in the antigen binding site, thereby destroying this site and blocking specific antigen reaction. However, this may not be advantageous since it results in loss of antigen binding by the antibody conjugate.

Molecules containing azido groups may also be used to form covalent bonds to
25 proteins through reactive nitrene intermediates that are generated by low intensity ultraviolet light (Potter and Haley, 1983). In particular, 2- and 8-azido analogues of purine nucleotides have been used as site-directed photoprobes to identify nucleotide binding proteins in crude cell extracts (Owens & Haley, 1987; Atherton *et al.*, 1985). The 2- and 8-azido nucleotides have also been used to map nucleotide binding domains of purified proteins (Khatoon *et al.*,
30 1989; King *et al.*, 1989; Dholakia *et al.*, 1989) and may be used as antibody binding agents.

Several methods are known in the art for the attachment or conjugation of an antibody to its conjugate moiety. Some attachment methods involve the use of a metal chelate complex employing, for example, an organic chelating agent such a diethylenetriaminepentaacetic acid anhydride (DTPA); ethylenetriaminetetraacetic acid; N-chloro-p-toluenesulfonamide; and/or

tetrachloro-3 α -6 α -diphenylglycouril-3 attached to the antibody (U.S. Patents 4,472,509 and 4,938,948). Monoclonal antibodies may also be reacted with an enzyme in the presence of a coupling agent such as glutaraldehyde or periodate. Conjugates with fluorescein markers are prepared in the presence of these coupling agents or by reaction with an isothiocyanate. In
5 U.S. Patent 4,938,948, imaging of breast tumors is achieved using monoclonal antibodies and the detectable imaging moieties are bound to the antibody using linkers such as methyl-p-hydroxybenzimidate or N-succinimidyl-3-(4-hydroxyphenyl)propionate.

In other embodiments, derivatization of immunoglobulins by selectively introducing sulfhydryl groups in the Fc region of an immunoglobulin, using reaction conditions that do
10 not alter the antibody combining site are contemplated. Antibody conjugates produced according to this methodology are disclosed to exhibit improved longevity, specificity and sensitivity (U.S. Patent 5,196,066, incorporated herein by reference). Site-specific attachment of effector or reporter molecules, wherein the reporter or effector molecule is conjugated to a carbohydrate residue in the Fc region have also been disclosed in the literature (O'Shannessy
15 *et al.*, 1987). This approach has been reported to produce diagnostically and therapeutically promising antibodies which are currently in clinical evaluation.

VI. Immunodetection Methods

In still further embodiments, the present disclosure concerns immunodetection
20 methods for binding, purifying, removing, quantifying and otherwise generally detecting LILRB-related cancers. While such methods can be applied in a traditional sense, another use will be in quality control and monitoring of vaccine and other virus stocks, where antibodies according to the present disclosure can be used to assess the amount or integrity (*i.e.*, long term stability) of H1 antigens in viruses. Alternatively, the methods may be used to screen
25 various antibodies for appropriate/desired reactivity profiles.

Some immunodetection methods include enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoradiometric assay, fluoroimmunoassay, chemiluminescent assay, bioluminescent assay, and Western blot to mention a few. In particular, a competitive assay for the detection and quantitation of LILRBs also is provided.
30 The steps of various useful immunodetection methods have been described in the scientific literature, such as, *e.g.*, Doolittle and Ben-Zeev (1999), Gulbis and Galand (1993), De Jager *et al.* (1993), and Nakamura *et al.* (1987). In general, the immunobinding methods include obtaining a sample suspected of containing LILRB-related cancers, and contacting the sample

with a first antibody in accordance with the present disclosure, as the case may be, under conditions effective to allow the formation of immunocomplexes.

These methods include methods for detecting or purifying LILRBs or LILRB-related cancer cells from a sample. The antibody will preferably be linked to a solid support, such as in the form of a column matrix, and the sample suspected of containing the LILRB-related cancer cells will be applied to the immobilized antibody. The unwanted components will be washed from the column, leaving the LILRB-expressing cells immunocomplexed to the immobilized antibody, which is then collected by removing the organism or antigen from the column.

The immunobinding methods also include methods for detecting and quantifying the amount of LILRB-related cancer cells or related components in a sample and the detection and quantification of any immune complexes formed during the binding process. Here, one would obtain a sample suspected of containing LILRB-related cancer cells, and contact the sample with an antibody that binds LILRBs or components thereof, followed by detecting and quantifying the amount of immune complexes formed under the specific conditions. In terms of antigen detection, the biological sample analyzed may be any sample that is suspected of containing LILRB-related cancers, such as a tissue section or specimen, a homogenized tissue extract, a biological fluid, including blood and serum, or a secretion, such as feces or urine.

Contacting the chosen biological sample with the antibody under effective conditions and for a period of time sufficient to allow the formation of immune complexes (primary immune complexes) is generally a matter of simply adding the antibody composition to the sample and incubating the mixture for a period of time long enough for the antibodies to form immune complexes with, *i.e.*, to bind to LILRBs. After this time, the sample-antibody composition, such as a tissue section, ELISA plate, dot blot or Western blot, will generally be washed to remove any non-specifically bound antibody species, allowing only those antibodies specifically bound within the primary immune complexes to be detected.

In general, the detection of immunocomplex formation is well known in the art and may be achieved through the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any of those radioactive, fluorescent, biological and enzymatic tags. Patents concerning the use of such labels include U.S. Patents 3,817,837, 3,850,752, 3,939,350, 3,996,345, 4,277,437, 4,275,149 and 4,366,241. Of course, one may find additional advantages through the use of a secondary binding ligand

such as a second antibody and/or a biotin/avidin ligand binding arrangement, as is known in the art.

The antibody employed in the detection may itself be linked to a detectable label, wherein one would then simply detect this label, thereby allowing the amount of the primary immune complexes in the composition to be determined. Alternatively, the first antibody that becomes bound within the primary immune complexes may be detected by means of a second binding ligand that has binding affinity for the antibody. In these cases, the second binding ligand may be linked to a detectable label. The second binding ligand is itself often an antibody, which may thus be termed a “secondary” antibody. The primary immune complexes are contacted with the labeled, secondary binding ligand, or antibody, under effective conditions and for a period of time sufficient to allow the formation of secondary immune complexes. The secondary immune complexes are then generally washed to remove any non-specifically bound labeled secondary antibodies or ligands, and the remaining label in the secondary immune complexes is then detected.

Further methods include the detection of primary immune complexes by a two-step approach. A second binding ligand, such as an antibody that has binding affinity for the antibody, is used to form secondary immune complexes, as described above. After washing, the secondary immune complexes are contacted with a third binding ligand or antibody that has binding affinity for the second antibody, again under effective conditions and for a period of time sufficient to allow the formation of immune complexes (tertiary immune complexes). The third ligand or antibody is linked to a detectable label, allowing detection of the tertiary immune complexes thus formed. This system may provide for signal amplification if this is desired.

One method of immunodetection uses two different antibodies. A first biotinylated antibody is used to detect the target antigen, and a second antibody is then used to detect the biotin attached to the complexed biotin. In that method, the sample to be tested is first incubated in a solution containing the first step antibody. If the target antigen is present, some of the antibody binds to the antigen to form a biotinylated antibody/antigen complex. The antibody/antigen complex is then amplified by incubation in successive solutions of streptavidin (or avidin), biotinylated DNA, and/or complementary biotinylated DNA, with each step adding additional biotin sites to the antibody/antigen complex. The amplification steps are repeated until a suitable level of amplification is achieved, at which point the sample is incubated in a solution containing the second step antibody against biotin. This second step antibody is labeled, as for example with an enzyme that can be used to detect the presence of

the antibody/antigen complex by histoenzymology using a chromogen substrate. With suitable amplification, a conjugate can be produced which is macroscopically visible.

Another known method of immunodetection takes advantage of the immuno-PCR (Polymerase Chain Reaction) methodology. The PCR method is similar to the Cantor method up to the incubation with biotinylated DNA, however, instead of using multiple rounds of streptavidin and biotinylated DNA incubation, the DNA/biotin/streptavidin/antibody complex is washed out with a low pH or high salt buffer that releases the antibody. The resulting wash solution is then used to carry out a PCR reaction with suitable primers with appropriate controls. At least in theory, the enormous amplification capability and specificity of PCR can be utilized to detect a single antigen molecule.

1. ELISAs

Immunoassays, in their most simple and direct sense, are binding assays. Certain preferred immunoassays are the various types of enzyme linked immunosorbent assays (ELISAs) and radioimmunoassays (RIA) known in the art. Immunohistochemical detection using tissue sections is also particularly useful. However, it will be readily appreciated that detection is not limited to such techniques, and western blotting, dot blotting, FACS analyses, and the like may also be used.

In one exemplary ELISA, the antibodies of the disclosure are immobilized onto a selected surface exhibiting protein affinity, such as a well in a polystyrene microtiter plate. Then, a test composition suspected of containing the LILRB-related cancer cells is added to the wells. After binding and washing to remove non-specifically bound immune complexes, the bound antigen may be detected. Detection may be achieved by the addition of another anti-LILRB antibody that is linked to a detectable label. This type of ELISA is a simple “sandwich ELISA.” Detection may also be achieved by the addition of a second anti-LILRB antibody, followed by the addition of a third antibody that has binding affinity for the second antibody, with the third antibody being linked to a detectable label.

In another exemplary ELISA, the samples suspected of containing the LILRB-related cancer cells are immobilized onto the well surface and then contacted with the anti-LILRB antibodies of the disclosure. After binding and washing to remove non-specifically bound immune complexes, the bound anti-LILRB antibodies are detected. Where the initial anti-LILRB antibodies are linked to a detectable label, the immune complexes may be detected directly. Again, the immune complexes may be detected using a second antibody that has

binding affinity for the first anti- LILRB antibody, with the second antibody being linked to a detectable label.

Irrespective of the format employed, ELISAs have certain features in common, such as coating, incubating and binding, washing to remove non-specifically bound species, and
5 detecting the bound immune complexes. These are described below.

In coating a plate with either antigen or antibody, one will generally incubate the wells of the plate with a solution of the antigen or antibody, either overnight or for a specified period of hours. The wells of the plate will then be washed to remove incompletely adsorbed material. Any remaining available surfaces of the wells are then “coated” with a nonspecific
10 protein that is antigenically neutral with regard to the test antisera. These include bovine serum albumin (BSA), casein or solutions of milk powder. The coating allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific binding of antisera onto the surface.

In ELISAs, it is probably more customary to use a secondary or tertiary detection
15 means rather than a direct procedure. Thus, after binding of a protein or antibody to the well, coating with a non-reactive material to reduce background, and washing to remove unbound material, the immobilizing surface is contacted with the biological sample to be tested under conditions effective to allow immune complex (antigen/antibody) formation. Detection of the immune complex then requires a labeled secondary binding ligand or antibody, and a
20 secondary binding ligand or antibody in conjunction with a labeled tertiary antibody or a third binding ligand.

“Under conditions effective to allow immune complex (antigen/antibody) formation” means that the conditions preferably include diluting the antigens and/or antibodies with solutions such as BSA, bovine gamma globulin (BGG) or phosphate buffered saline
25 (PBS)/Tween. These added agents also tend to assist in the reduction of nonspecific background.

The “suitable” conditions also mean that the incubation is at a temperature or for a period of time sufficient to allow effective binding. Incubation steps are typically from about 1 to 2 to 4 hours or so, at temperatures preferably on the order of 25°C to 27°C, or may be
30 overnight at about 4°C or so.

Following all incubation steps in an ELISA, the contacted surface is washed so as to remove non-complexed material. A preferred washing procedure includes washing with a solution such as PBS/Tween, or borate buffer. Following the formation of specific immune

complexes between the test sample and the originally bound material, and subsequent washing, the occurrence of even minute amounts of immune complexes may be determined.

To provide a detecting means, the second or third antibody will have an associated label to allow detection. Preferably, this will be an enzyme that will generate color development upon incubating with an appropriate chromogenic substrate. Thus, for example, one will desire to contact or incubate the first and second immune complex with a urease, glucose oxidase, alkaline phosphatase or hydrogen peroxidase-conjugated antibody for a period of time and under conditions that favor the development of further immune complex formation (*e.g.*, incubation for 2 hours at room temperature in a PBS-containing solution such as PBS-Tween).

After incubation with the labeled antibody, and subsequent to washing to remove unbound material, the amount of label is quantified, *e.g.*, by incubation with a chromogenic substrate such as urea, or bromocresol purple, or 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid (ABTS), or H₂O₂, in the case of peroxidase as the enzyme label. Quantification is then achieved by measuring the degree of color generated, *e.g.*, using a visible spectra spectrophotometer.

2. Western Blot

The Western blot (alternatively, protein immunoblot) is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) or by the 3-D structure of the protein (native/ non-denaturing conditions). The proteins are then transferred to a membrane (typically nitrocellulose or PVDF), where they are probed (detected) using antibodies specific to the target protein.

Samples may be taken from whole tissue or from cell culture. In most cases, solid tissues are first broken down mechanically using a blender (for larger sample volumes), using a homogenizer (smaller volumes), or by sonication. Cells may also be broken open by one of the above mechanical methods. However, it should be noted that bacteria, virus or environmental samples can be the source of protein and thus Western blotting is not restricted to cellular studies only. Assorted detergents, salts, and buffers may be employed to encourage lysis of cells and to solubilize proteins. Protease and phosphatase inhibitors are often added to prevent the digestion of the sample by its own enzymes. Tissue preparation is often done at cold temperatures to avoid protein denaturing.

The proteins of the sample are separated using gel electrophoresis. Separation of proteins may be by isoelectric point (pI), molecular weight, electric charge, or a combination of these factors. The nature of the separation depends on the treatment of the sample and the nature of the gel. This is a very useful way to determine a protein. It is also possible to use a two-dimensional (2-D) gel which spreads the proteins from a single sample out in two dimensions. Proteins are separated according to isoelectric point (pH at which they have neutral net charge) in the first dimension, and according to their molecular weight in the second dimension.

In order to make the proteins accessible to antibody detection, they are moved from within the gel onto a membrane made of nitrocellulose or polyvinylidene difluoride (PVDF). The membrane is placed on top of the gel, and a stack of filter papers placed on top of that. The entire stack is placed in a buffer solution which moves up the paper by capillary action, bringing the proteins with it. Another method for transferring the proteins is called electroblotting and uses an electric current to pull proteins from the gel into the PVDF or nitrocellulose membrane. The proteins move from within the gel onto the membrane while maintaining the organization they had within the gel. As a result of this blotting process, the proteins are exposed on a thin surface layer for detection (see below). Both varieties of membrane are chosen for their non-specific protein binding properties (*i.e.*, binds all proteins equally well). Protein binding is based upon hydrophobic interactions, as well as charged interactions between the membrane and protein. Nitrocellulose membranes are cheaper than PVDF, but are far more fragile and do not stand up well to repeated probings. The uniformity and overall effectiveness of transfer of protein from the gel to the membrane can be checked by staining the membrane with Coomassie Brilliant Blue or Ponceau S dyes. Once transferred, proteins are detected using labeled primary antibodies, or unlabeled primary antibodies followed by indirect detection using labeled protein A or secondary labeled antibodies binding to the Fc region of the primary antibodies.

3. Immunohistochemistry

The antibodies of the present disclosure may also be used in conjunction with both fresh-frozen and/or formalin-fixed, paraffin-embedded tissue blocks prepared for study by immunohistochemistry (IHC). The method of preparing tissue blocks from these particulate specimens has been successfully used in previous IHC studies of various prognostic factors, and is well known to those of skill in the art (Brown *et al.*, 1990; Abbondanzo *et al.*, 1990; Allred *et al.*, 1990).

Briefly, frozen-sections may be prepared by rehydrating 50 ng of frozen “pulverized” tissue at room temperature in phosphate buffered saline (PBS) in small plastic capsules; pelleting the particles by centrifugation; resuspending them in a viscous embedding medium (OCT); inverting the capsule and/or pelleting again by centrifugation; snap-freezing in -70°C isopentane; cutting the plastic capsule and/or removing the frozen cylinder of tissue; securing the tissue cylinder on a cryostat microtome chuck; and/or cutting 25-50 serial sections from the capsule. Alternatively, whole frozen tissue samples may be used for serial section cuttings.

Permanent-sections may be prepared by a similar method involving rehydration of the 50 mg sample in a plastic microfuge tube; pelleting; resuspending in 10% formalin for 4 hours fixation; washing/pelleting; resuspending in warm 2.5% agar; pelleting; cooling in ice water to harden the agar; removing the tissue/agar block from the tube; infiltrating and/or embedding the block in paraffin; and/or cutting up to 50 serial permanent sections. Again, whole tissue samples may be substituted.

4. Immunodetection Kits

In still further embodiments, the present disclosure concerns immunodetection kits for use with the immunodetection methods described above. As the antibodies may be used to detect LILRB-related cancer cells, the antibodies may be included in the kit. The immunodetection kits will thus comprise, in suitable container means, a first antibody that binds to an LILRB, and optionally an immunodetection reagent.

In certain embodiments, the antibody may be pre-bound to a solid support, such as a column matrix and/or well of a microtitre plate. The immunodetection reagents of the kit may take any one of a variety of forms, including those detectable labels that are associated with or linked to the given antibody. Detectable labels that are associated with or attached to a secondary binding ligand are also contemplated. Exemplary secondary ligands are those secondary antibodies that have binding affinity for the first antibody.

Further suitable immunodetection reagents for use in the present kits include the two-component reagent that comprises a secondary antibody that has binding affinity for the first antibody, along with a third antibody that has binding affinity for the second antibody, the third antibody being linked to a detectable label. As noted above, a number of exemplary labels are known in the art and all such labels may be employed in connection with the present disclosure.

The kits may further comprise a suitably aliquoted composition of LILRBs, whether labeled or unlabeled, as may be used to prepare a standard curve for a detection assay. The

kits may contain antibody-label conjugates either in fully conjugated form, in the form of intermediates, or as separate moieties to be conjugated by the user of the kit. The components of the kits may be packaged either in aqueous media or in lyophilized form.

The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the antibody may be placed, or preferably, suitably aliquoted. The kits of the present disclosure will also typically include a means for containing the antibody, antigen, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

VII. Examples

The following examples are included to demonstrate preferred embodiments. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to function well in the practice of embodiments, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

EXAMPLE 1

LILRBs and LAIR1 are highly expressed on AML cells including AML-SCs and their expression inversely correlates with survival of AML patients. The inventors analyzed the surface expression of LILRB1-4 on human AML patients and found that they are expressed by significant percentages of these patients (FIG. 1, top). LILRB1,2,3,4 are expressed on 8, 8, 12, and 18 out of a total of 37 tested human AML cases respectively. LILRBs including LILRB4 can be co-expressed with the leukemia stem cell marker CD34 (FIG. 1, lower panels). The inventors' *in silico* analysis indicated that the expression of several closely related LILRB family members, LILRBs 1, 2, 3, and 4, and LAIR1 inversely correlates with the overall survival of AML patients (FIG. 2). These receptors are more highly expressed on human AML cells than on normal counterparts. Importantly, the LILRB⁺/LAIR1⁺ cells are enriched for the activity of AML-SCs (Kang *et al.*, in press).

LILRBs and LAIR1 are essential for growth and xenograft of human leukemia cells. To study the potential function of LILRBs and LAIR1 in human leukemia, the inventors

knockdown expression of LILRBs and LAIR1 individually using shRNAs in human leukemia lines that have high levels of surface expression of these receptors including MV4-11 (AML), THP-1 (AML), U937 (AML), 697 (B-ALL), Kasumi2 (B-ALL), and RCH-ACV (B-ALL). The silencing of expression of individual LILRB2, 3, 4 or LAIR1 dramatically inhibited cell growth. Representative data from THP-1 and MV4-11, two AML lines with a rearranged MLL gene, are shown in FIG. 3. Importantly, inhibition of expression of LILRBs or LAIR1 in any of seven primary human AML samples almost completely abolished leukemia development in xenografted NSG mice (Kang *et al.*, in press). These results indicate that several LILRBs are essential for the growth of human AML cells.

To gain a deeper understanding of the mechanism by which LILRBs support AML development, the inventors studied acute leukemia development in *LILRB3*-null, *LILRB4*-null, or *LAIR1*-null mice (Tang *et al.*, 2012; Rojo *et al.*, 2000, Zheng *et al.*, 2012). These mice have normal hematopoiesis and HSC activity (Tang *et al.*, 2012; Rojo *et al.*, 2000, Zheng *et al.*, 2012). The inventors used MLL-AF9, AML1-ETO, or N-Myc transplantation AML or acute lymphoblastic leukemia (ALL) mouse models (Sugihara *et al.*, 2011; Kristov *et al.*, 2006; Somervaille and Clearly, 2006; Yan *et al.*, 2006) for this study. The mice transplanted with *LILRB*- or *LAIR1*-null leukemia cells developed the disease more slowly upon serial transplantation than did controls and eventually were free of leukemia. The survival and self-renewal of *LILRB*- or *LAIR1*-null phenotypic AML-SC enriched cells decreased over time whereas their ability to differentiate increased. The inventors further discovered that SHP-1 and CAMKI are key components in the LILRB/LAIR1 signaling pathway in AML-SCs (Kang *et al.* in press). These results indicate that LILRBs and LAIR1 support the activity of AML-SCs.

Identifying anti-LILRB monoclonal antibodies (mAbs). The inventors developed antibodies against individual LILRBs as determined by their binding to LILRBs in Western blotting (FIG. 4) and flow cytometry (FIG. 5).

Because there was no reporter for LILRB/LAIR1-mediated signaling, the inventors generated a stable chimeric receptor reporter cell system to test the ability of an antibody to bind to the extracellular domain (ECD) of LILRB or LAIR1 and trigger the activation or inhibition of the chimerically fused intracellular domain of paired immunoglobulin-like receptor β , which signals through the adaptor DAP-12 to activate NFAT promoter-driven

GFP expression (FIG. 6 right and FIG. 7, left). This reporter system serves as a sensitive surrogate that enables us to screen for blocking antibodies and stimulating antibodies.

Using this system, the inventors have identified a group of novel monoclonal antibodies (mAbs) that inhibit the LILRB2-4 signaling activation (FIGS. 6-7). For example, soluble anti-LILRB4 mAbs including C84, C53, C92, C201 inhibit GFP induction in the chimeric receptor reporter system for LILRB4 (FIG. 7).

Anti-LILRB mAbs block leukemia development. The inventors identified several anti-LILRB mAbs effectively block AML development in xenograft models. For example, C84, an anti-LILRB4 mAb, blocked AML development in xenografted mouse models. In these models, human leukemia cells THP-1 or MV4-11 (positive for LILRB4 surface expression as shown in FIGS. 8-9) or primary human AML cells from three individual patients the inventors subcutaneously (*sc*) or intravenously (*iv*) transplanted into the immune-deficient NSG mice (FIGS. 10-13 for AML cell lines implanted xenograft models; FIGS. 14-18 for patient AML xenograft models). By contrast, C84 administration did not inhibit cancer development if LILRB4 is not expressed by the cancer cells (FIG. 13). Therefore, the anti-LILRB4 mAb C84 had a striking specific inhibitory effect on development of human AML that express LILRB4. Importantly, six independent experiments using different xenografted models gave similar results.

In addition to C84, the inventors identified additional nine mAbs C201, C53, C92, C39, C3, C193, C290, C102, and C287 that are against LILRBs have anti-leukemia effects in xenograft models. The anti-leukemia potency and the cross-reactivity of these mAbs are summarized in FIG. 19.

Isotyping and sequencing of variable regions of mAbs that are effective to inhibit leukemia development. The inventors obtained the isotypes and sequencing of variable regions of mAbs C84, C201, C53, C92, C39, C3, C193, C290, C102, and C287 (FIGS. 20-22).

Production of chimeric antibodies and demonstration of anti-leukemia potency. To express the chimeric antibodies, the inventors subcloned the variable regions of mouse mAbs into an expression vector encoding human constant regions, and transfected into 293T cells. The inventors collected the conditioned medium from the transfected 293T cells and

purified the chimeric antibodies. FIG. 23 shows the expression and purification of chimeric anti-C84 (Chimeric ab MHC-C84) as determined by SDS-PAGE.

The inventors compared the LILRB binding, signaling blocking, and leukemia inhibition activities of chimeric antibodies and the original mAbs. Chimeric ab MHC-C84
5 binds LILRB4 on THP-1 cells (FIG. 24) and on LILRB4 chimeric receptor reporter cells (FIG. 25) respectively as C84, as determined by flow cytometry. The inventors also found that the chimeric ab MHC-C84 is a blocking antibody for LILRB4 as C84, as determined by chimeric receptor reporter assay (FIG. 26). Most importantly, the chimeric ab MHC-C84 inhibits AML development in xenograft models (with the same or better effect than the
10 original mAb C84) as determined by flow cytometry analysis of liver, bone marrow, spleen, and peripheral blood of AML xenografted mice (FIG. 27) or by luminescence imaging of tumor development in AML xenografted mice over time (FIG. 28). Strikingly, in these experiments, the inventors only administrated MHC-C84 or C84 one time (a total of 200 µg antibody/mouse) for the whole experiment period. These results indicate that chimeric anti-
15 LILRBs have the same or greater capacities to block leukemia development than the original mAbs.

In summary, these data demonstrated that: 1) LILRB/LAIR1 are highly expressed by human AML cells and LILRB⁺/LAIR1⁺ cells are enriched in AML-SC activity; 2) LILRB/LAIR1 expression inversely correlates with the overall survival of AML patients; 3)
20 LILRB/LAIR1 are essential for growth of primary and immortalized human leukemia cells in vitro and in vivo; and 4) the inventors have identified several novel anti-human LILRB4 mAb and chimeric antibodies that essentially block human AML development in various xenograft models including in patient AML derived xenograft models. It is noteworthy that there is no apparent effect of knockout of individual LILRB genes or *lair1* on normal hematopoiesis
25 (Tang *et al.*, 2012; Rojo *et al.*, 2000, Zheng *et al.*, 2012). In addition, inhibition of LILRB stimulated immunity and indirectly boosted antitumor effects. Therefore, LILRB/LAIR1 represent ideal targets for treating AML.

As the inventors identified multiple potential ligand binding sites for LILRB2 (Deng *et al.*, 2014), they envision that a large molecule such as a blocking antibody rather than a
30 small molecule chemical will be a more appropriate LILRB blocker. Indeed, as they demonstrated, even a single time administration of an anti-LILRB mAb or chimeric antibody was capable of very efficiently blocking human AML development in xenograft models.

These results provide proof-of-principle that anti-LILRB mAbs or chimeric antibodies are promising drug candidates for treating AML.

* * * * *

5 All of the compositions and methods disclosed and claimed herein can be made and
executed without undue experimentation in light of the present disclosure. While the
compositions and methods of this disclosure have been described in terms of preferred
embodiments, it will be apparent to those of skill in the art that variations may be applied to
the compositions and methods and in the steps or in the sequence of steps of the method
10 described herein without departing from the concept, spirit and scope of the disclosure. More
specifically, it will be apparent that certain agents which are both chemically and
physiologically related may be substituted for the agents described herein while the same or
similar results would be achieved. All such similar substitutes and modifications apparent to
those skilled in the art are deemed to be within the spirit, scope and concept of the disclosure
15 as defined by the appended claims.

VIII. REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

U.S. Patent 3,817,837

U.S. Patent 3,850,752

U.S. Patent 3,939,350

U.S. Patent 3,996,345

U.S. Patent 4,196,265

U.S. Patent 4,275,149

U.S. Patent 4,277,437

U.S. Patent 4,366,241

U.S. Patent 4,472,509

U.S. Patent 4,554,101

U.S. Patent 4,680,338

U.S. Patent 4,816,567

U.S. Patent 4,867,973

U.S. Patent 4,938,948

U.S. Patent 5,021,236

U.S. Patent 5,141,648

U.S. Patent 5,196,066

U.S. Patent 5,563,250

U.S. Patent 5,565,332

U.S. Patent 5,856,456

U.S. Patent 5,880,270

“Antibodies: A Laboratory Manual,” Cold Spring Harbor Press, Cold Spring Harbor, NY, 1988.

Abbondanzo *et al.*, *Am. J. Pediatr. Hematol. Oncol.*, 12(4), 480-489, 1990.

Abe and Kufe, *Cancer Res.*, 49(11):2834-2839, 1989.

Ahmad *et al.*, *Nat. Cell Biol.*, 9:1419-1427, 2007.

Allred *et al.*, *Arch. Surg.*, 125(1), 107-113, 1990.

Atherton *et al.*, *Biol. of Reproduction*, 32, 155-171, 1985.

Baldus *et al.*, *Clin. Cancer Res.*, 10(8):2790-2796, 2004.

- Beidler *et al.*, *J. Immunol.*, 141(11):4053-4060, 1988.
- Brown *et al.*, *J. Immunol. Meth.*, 12;130(1), :111-121, 1990.
- Campbell, *In: Monoclonal Antibody Technology, Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 13, Burden and Von Knippenberg, Eds. pp. 75-83, Amsterdam, Elsevier, 1984.
- Capaldi *et al.*, *Biochem. Biophys. Res. Comm.*, 74(2):425-433, 1977.
- Daeron *et al.*, *Immunol Rev* 224, 11-43, 2008.
- De Jager *et al.*, *Semin. Nucl. Med.* 23(2), 165-179, 1993.
- Deng *et al.* *Blood*, 124(6):924-35, 2014.
- Dholakia *et al.*, *J. Biol. Chem.*, 264, 20638-20642, 1989.
- Doolittle and Ben-Zeev, *Methods Mol. Biol.*, 109, :215-237, 1999.
- Duraisamy *et al.*, *Gene*, 373:28-34, 2006.
- EP Application 125,023
- EP Application 171,496
- EP Application 173,494
- EP Application 184,187
- Gefter *et al.*, *Somatic Cell Genet.*, 3:231-236, 1977.
- Gefter *et al.*, *Somatic Cell Genet.*, 3:231-236, 1977.
- Goding, *In: Monoclonal Antibodies: Principles and Practice*, 2d ed., Orlando, Fla., Academic Press, 60-61, 65-66, 71-74, 1986.
- Gulbis and Galand, *Hum. Pathol.* 24(12), 1271-1285, 1993.
- Guo *et al.*, *Sci. Transl. Med.* 3:99 ra85, 2001.
- Hodel *et al.*, *Mol. Cell*, 10(2):347-58, 2002.
- Huang *et al.*, *Cancer Biol Ther.*, 2:702-706, 2003.
- Huang *et al.*, *Cancer Res.*, 65:10413-10422, 2005.
- Jones *et al.*, *Nature*, 321:522-525, 1986.
- Kau *et al.*, *Nat. Rev. Cancer*, 4(2):106-17, 2004.
- Khatoon *et al.*, *Ann. of Neurology*, 26, 210-219, 1989.
- King *et al.*, *J. Biol. Chem.*, 269, 10210-10218, 1989.
- Kinlough *et al.*, *J. Biol. Chem.*, 279(51):53071-53077, 2004.
- Kinoshita *et al.*, *Biochem. Biophys. Res. Commun.*, 394:205-210, 2010.
- Kohler and Milstein, *Eur. J. Immunol.*, 6, 511-519, 1976.
- Kang *et al.*, *Nature Cell Biology* (in press) 2015.

- Katz, H.R., *Adv Immunol* 91, 251-272, 2006.
- Kohler and Milstein, *Nature*, 256, 495-497, 1975.
- Kufe *et al.*, *Hybridoma*, 3:223-232, 1984.
- Kyte and Doolittle, *J. Mol. Biol.*, 157(1):105-132, 1982.
- Leng *et al.*, *J. Biol. Chem.*, 282:19321-19330, 2007.
- Levitan *et al.*, *J. Biol. Chem.*, 280:33374-33386, 2005.
- Li *et al.*, *Cancer Biol. Ther.*, 2:187-193, 2003b.
- Li *et al.*, *J. Biol. Chem.*, 276:35239-35242, 2001.
- Li *et al.*, *J. Biol. Chem.*, 276:6061-6064, 2001.
- Li *et al.*, *Mol. Cancer Res.*, 1:765-775, 2003c.
- Li *et al.*, *Mol. Cell Biol.*, 18:7216-7224, 1998.
- Li *et al.*, *Oncogene*, 22:6107-6110, 2003a.
- Ligtenberg *et al.*, *J. Biol. Chem.*, 267, 6171-6177, 1992.
- Krivtsov *et al.*, *Nature*, 442(7104): p. 818-22, 2006.
- Ma *et al.*, *Immunity* 34, 385-395, 2011.
- Macao, *Nat. Struct. Mol. Biol.*, 13, 71-76, 2006.
- Merlo *et al.*, *Cancer Res.*, 49, 6966-6971, 1989.
- Mori *et al.*, *J Immunol* 181, 4742-4751, 2008.
- Morrison, *Science*, 229(4719):1202-1207, 1985.
- Nakamura *et al.*, In: *Enzyme Immunoassays: Heterogeneous and Homogeneous Systems*, Chapter 27, 1987.
- O'Shannessy *et al.*, *J. Immun. Meth.*, 99, 153-161, 1987.
- Owens and Haley, *J. Biol. Chem.*, 259, 14843-14848, 1987.
- Owens and Haley, *J. Biol. Chem.*, 259:14843-14848, 1987.
- PCT Application PCT/US86/02269
- PCT Application WO 86/01533
- Percipalle *et al.*, *J. Mol. Biol.*, (4):722-32, 1997.
- Perey *et al.*, *Cancer Res.*, 52(22):6365-6370, 1992.
- Persic *et al.*, *Gene* 187:1, 1997
- Posner *et al.*, *Hybridoma* 6, 611-625, 1987.
- Potter and Haley, *Meth. Enzymol.*, 91, 613-633, 1983.
- Raina *et al.*, *EMBO J.*, 25:3774-3783, 2006.
- Raina *et al.*, *J. Biol. Chem.*, 279:20607-20612, 2004.
- Remington's Pharmaceutical Sciences, 15th Ed., 3:624-652, 1990.

- Ren *et al.*, *Cancer Cell*, 5:163-175, 2004.
- Ren *et al.*, *J. Biol. Chem.*, 277:17616-17622, 2002.
- Rojo *et al.*, *Mol Cell Biol*, 20(19): p. 7178-82, 2000.
- Ryan and Wente, *Curr. Opin. Cell Biol.*, 12(3):361-71, 2000.
- Schroeder *et al.*, *J. Biol. Chem.*, 276(16):13057-13064, 2001.
- Schroeder *et al.*, *Oncogene*, 23:5739–5747, 2004.
- Shaw *et al.*, *J. Natl. Cancer Inst.*, 80(19):1553-1559, 1988.
- Siddiqui *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:2320–2323, 1988.
- Somerville and Cleary, *Cancer Cell*, 10(4): p. 257-68, 2006.
- Sugihara *et al.*, *Oncogene*, 31:2849-61, 2011.
- Suh and Gumbiner, *Exp. Cell Res.*, 290(2):447-56, 2003.
- Sun *et al.*, *J. Steroid Biochem.*, 26(1):83-92, 1987.
- Sun *et al.*, *Chest* 134: 783-788, 2008.
- Takai *et al.*, T, *J Biomed Biotechnol*, 2011:275302, 2011.
- Tang *et al.*, *J Immunol*, 188(2): p. 548-58, 2012.
- Tang *et al.*, *J. Biol. Chem.*, 271:28324-28330, 1996.
- Truscott *et al.*, *J Cell Biol.*, 163(4):707-713, 2003.
- Verhoeyen *et al.*, *Science*, 239(4847):1534-1536, 1988.
- Vermeer *et al.*, *Nature*, 422(6929):322-6, 2003.
- Wawrzynczak & Thorpe, In: *Immunoconjugates, Antibody Conuugates In Radioimaging And Therapy Of Cancer*, Vogel (Ed.), NY, Oxford University Press, 28, 1987.
- Wei *et al.*, *Cancer Cell*, 7:167-178, 2005.
- Weis, *Cell*, 112(4):441-51, 2003.
- Wen *et al.*, *J. Biol. Chem.*, 278:38029–38039, 2003.
- Wood *et al.*, *J. Clin. Lab. Immunol.*, 17(4):167-171, 1985.
- Yamamoto *et al.*, *J. Biol. Chem.*, 272:12492-12494, 1997.
- Yan *et al.*, *Nat Med*, 12(8): p. 945-9, 2006.
- Yin *et al.*, *J. Biol. Chem.*, 278:35458-35464, 2003.
- Yin *et al.*, *J. Biol. Chem.*, 279:45721-45727, 2004.
- Yin *et al.*, *J. Biol. Chem.*, 282:257-266, 2007.
- Young *et al.*, *Cell*. 112(1):41-50, 2003.
- Zheng *et al.*, *Nature*, 485(7400): p. 656-60, 2012.

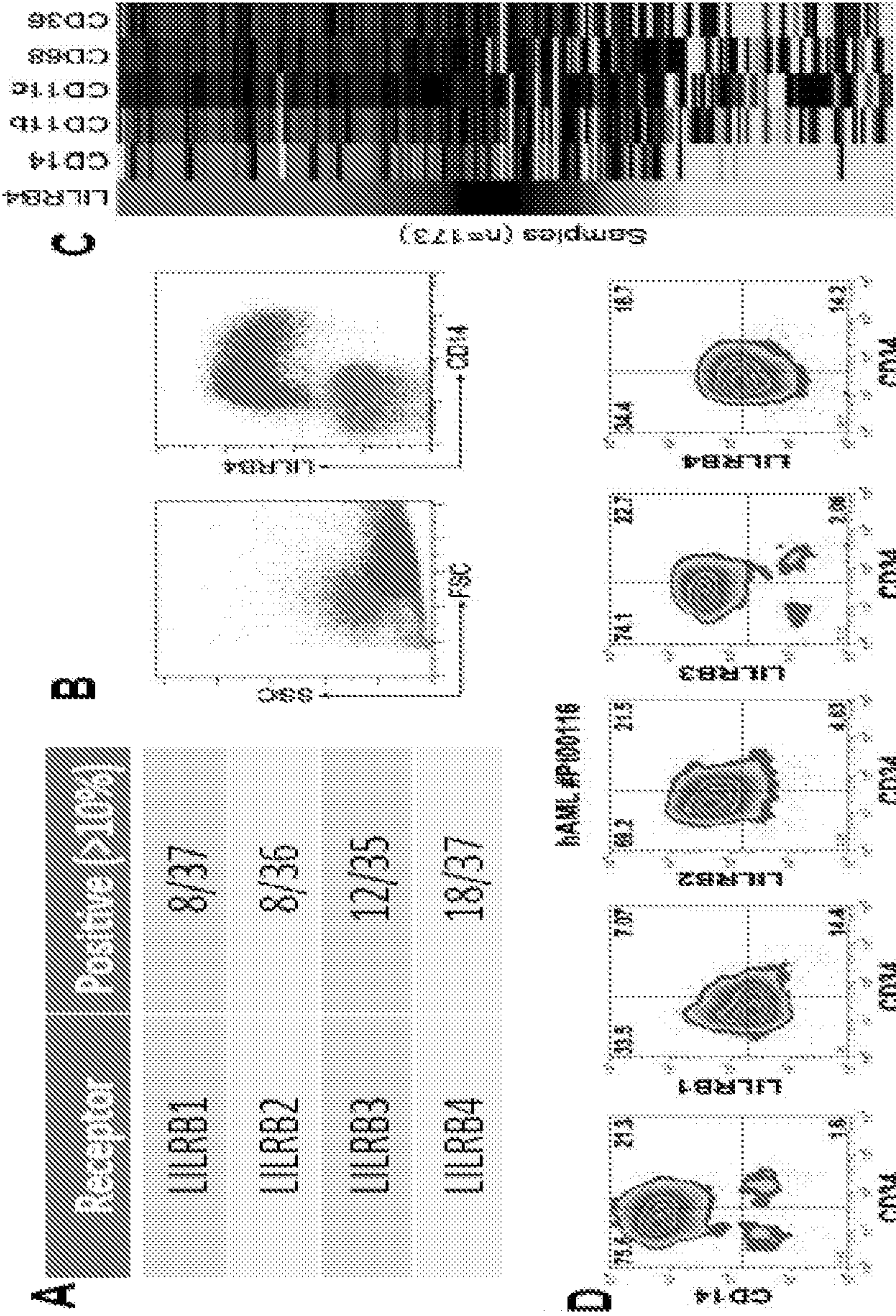
WHAT IS CLAIMED IS:

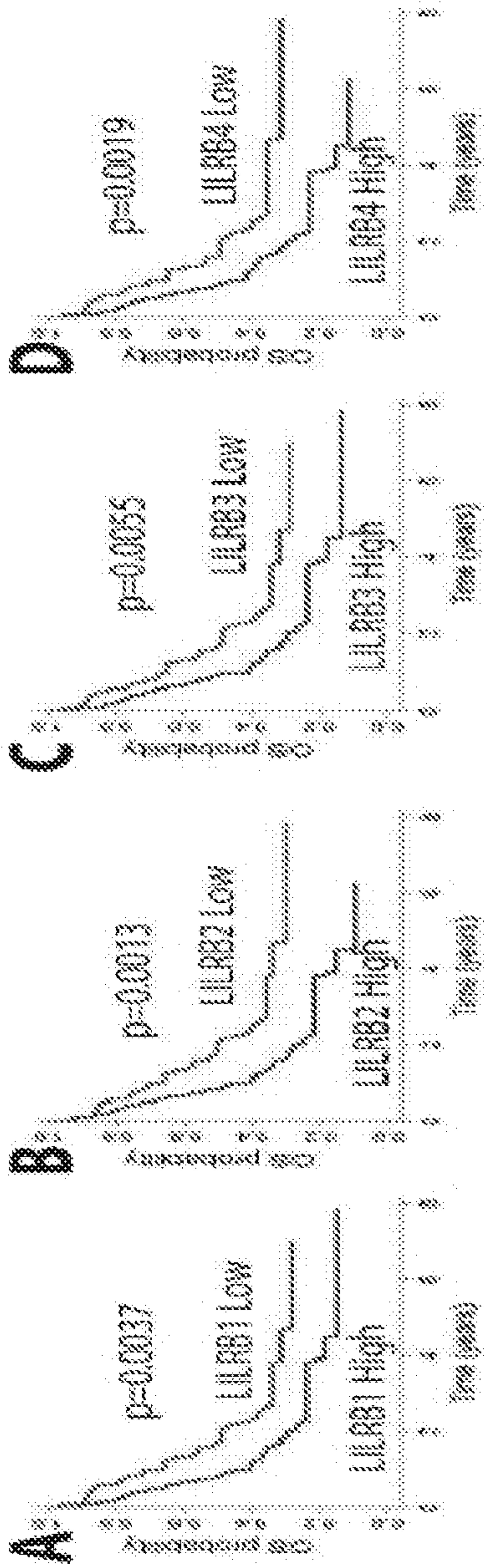
1. A method of detecting a cancer cell or cancer stem cell in a sample or subject comprising:
 - (a) contacting a subject or a sample from said subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from FIG. 22; and
 - (b) detecting binding of said antibody to a cancer cell or cancer stem cell in said subject or sample.
2. The method of claim 1, wherein said sample is a body fluid or biopsy.
3. The method of claim 1, wherein said sample is blood, sputum, tears, saliva, mucous or serum, urine or feces.
4. The method of claim 1, wherein detection comprises immunohistochemistry, ELISA, RIA or Western blot.
5. The method of claim 1, further comprising performing steps (a) and (b) a second time and determining a change in cell levels as compared to the first assay.
6. The method of claim 1, wherein the antibody is characterized by clone-paired variable sequences as set forth in FIG. 21.
7. The method of claim 1, wherein said antibody or antibody fragment is encoded by light and heavy chain variable nucleic acid sequences having 70%, 80%, or 90% identity to clone-paired sequences from FIG. 21.
8. The method of claim 1, wherein said antibody or antibody fragment is encoded by light and heavy chain variable amino acid sequences having 95% identity to clone-paired sequences from FIG. 21.

9. The method of claim 1, wherein the antibody fragment is a recombinant ScFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
10. The method of claim 1, wherein said antibody does not bind to Ebola virus.
11. A method of treating a subject having cancer comprising administering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from FIG. 22.
12. The method of claim 11, the antibody is characterized by clone-paired heavy and light chain variable nucleic acid sequences as set forth in FIG. 21.
13. The method of claim 11, the antibody is characterized by clone-paired heavy and light chain variable amino acid sequences as set forth in FIG. 21.
14. The method of claim 12, wherein said antibody or antibody fragment is encoded by light and heavy chain variable nucleic acid sequences having 70%, 80%, or 90% identity to clone-paired sequences from FIG. 21.
15. The method of claim 13, wherein said antibody or antibody fragment is encoded by light and heavy chain variable amino acid sequences having 95% identity to clone-paired sequences from FIG. 21.
16. The method of claim 11, wherein the antibody fragment is a recombinant ScFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
17. The method of claim 11, wherein said antibody is an IgG.
18. The method of claim 11, wherein said antibody is a chimeric antibody.
19. The method of claim 11, wherein said cancer is acute myeloid leukemia.
20. The method of claim 11, wherein administration is intravenous, intra-arterial or intra-tumoral.

21. A monoclonal antibody or antibody fragment, wherein the antibody is characterized by clone-paired heavy and light chain CDR sequences from FIG. 22.
22. The monoclonal antibody or antibody fragment of claim 21, wherein said antibody or antibody fragment is encoded by light and heavy chain variable nucleic acid sequences according to clone-paired sequences from FIG. 21.
23. The monoclonal antibody or antibody fragment of claim 22, wherein said antibody or antibody fragment is encoded by light and heavy chain variable nucleic acid sequences having 70%, 80%, or 90% identity to clone-paired sequences from FIG. 21.
24. The monoclonal antibody or antibody fragment of claim 21, wherein said antibody or antibody fragment is encoded by light chain and heavy variable amino acid sequences according to clone-paired sequences from FIG. 21.
25. The monoclonal antibody or antibody fragment of claim 24, wherein said antibody or antibody fragment is encoded by light and heavy chain variable amino acid sequences having 95% identity to clone-paired sequences from FIG. 21.
26. The monoclonal antibody or antibody fragment of claim 21, wherein the antibody fragment is a recombinant ScFv (single chain fragment variable) antibody, Fab fragment, F(ab')₂ fragment, or Fv fragment.
27. The monoclonal antibody or antibody fragment of claim 21, wherein said antibody is a chimeric antibody or is bispecific antibody.
28. The monoclonal antibody or antibody fragment of claim 21, wherein said antibody is an IgG.
29. The monoclonal antibody or antibody fragment of claim 21, wherein said antibody is humanized.

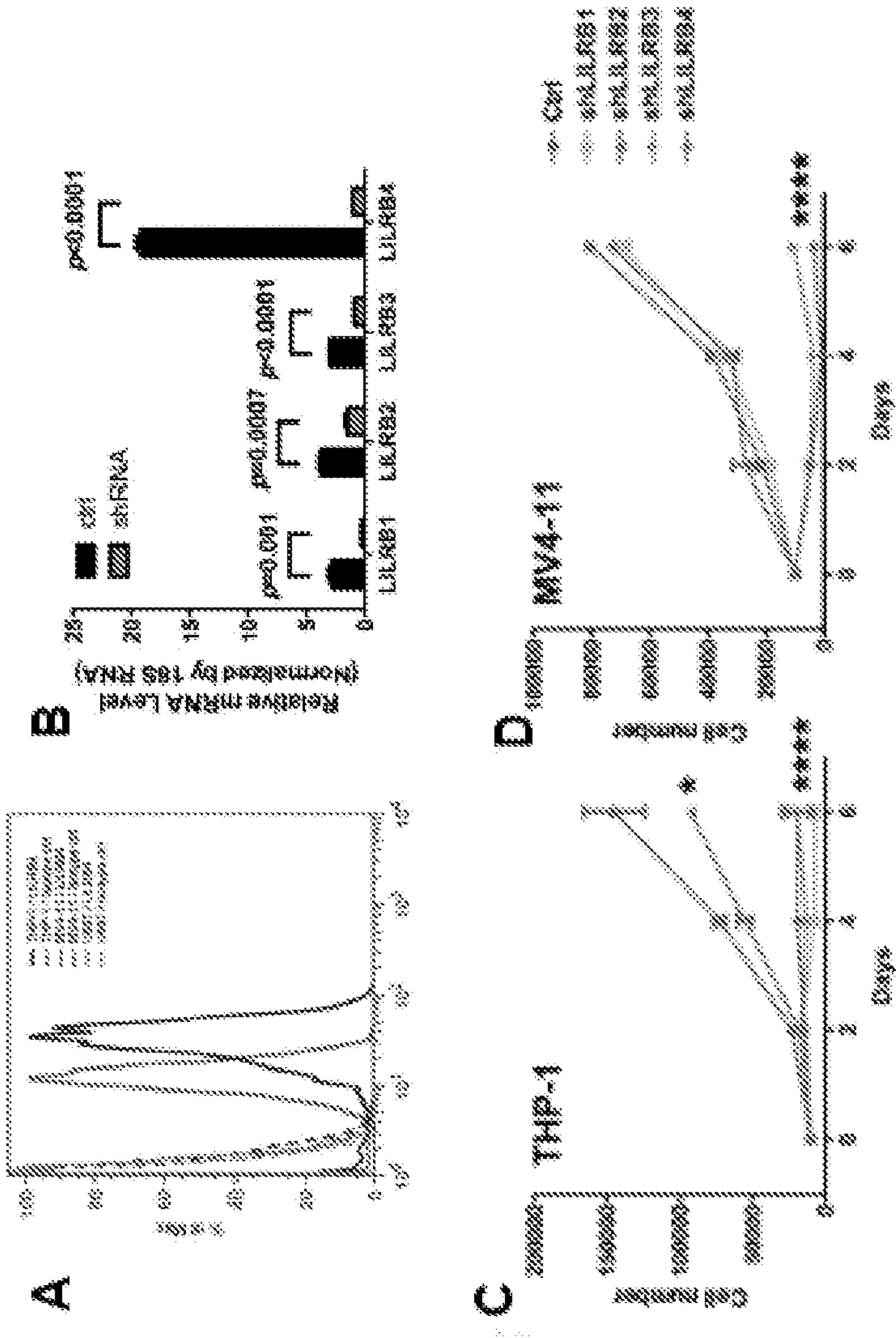
30. The monoclonal antibody or antibody fragment of claim 21, wherein said antibody optimized.
31. A hybridoma encoding wherein the antibody is characterized by clone-paired heavy and light chain CDR sequences from FIG. 22.
32. The hybridoma of claim 31, wherein said antibody is encoded by light and heavy chain variable nucleic acid sequences according to clone-paired sequences from FIG. 21.
33. The hybridoma of claim 32, wherein said antibody is encoded by light and heavy chain variable nucleic acid sequences having 70%, 80%, or 90% identity to clone-paired sequences from FIG. 21.
34. The hybridoma of claim 31, wherein said antibody is encoded by light chain and heavy variable amino acid sequences according to clone-paired sequences from FIG. 21.
35. The hybridoma of claim 34, wherein said antibody is encoded by light and heavy chain variable amino acid sequences having 95% identity to clone-paired sequences from FIG. 21.





Data from TCGA database ; AML; n=92

FIGS. 2A-D



FIGS. 3A-D

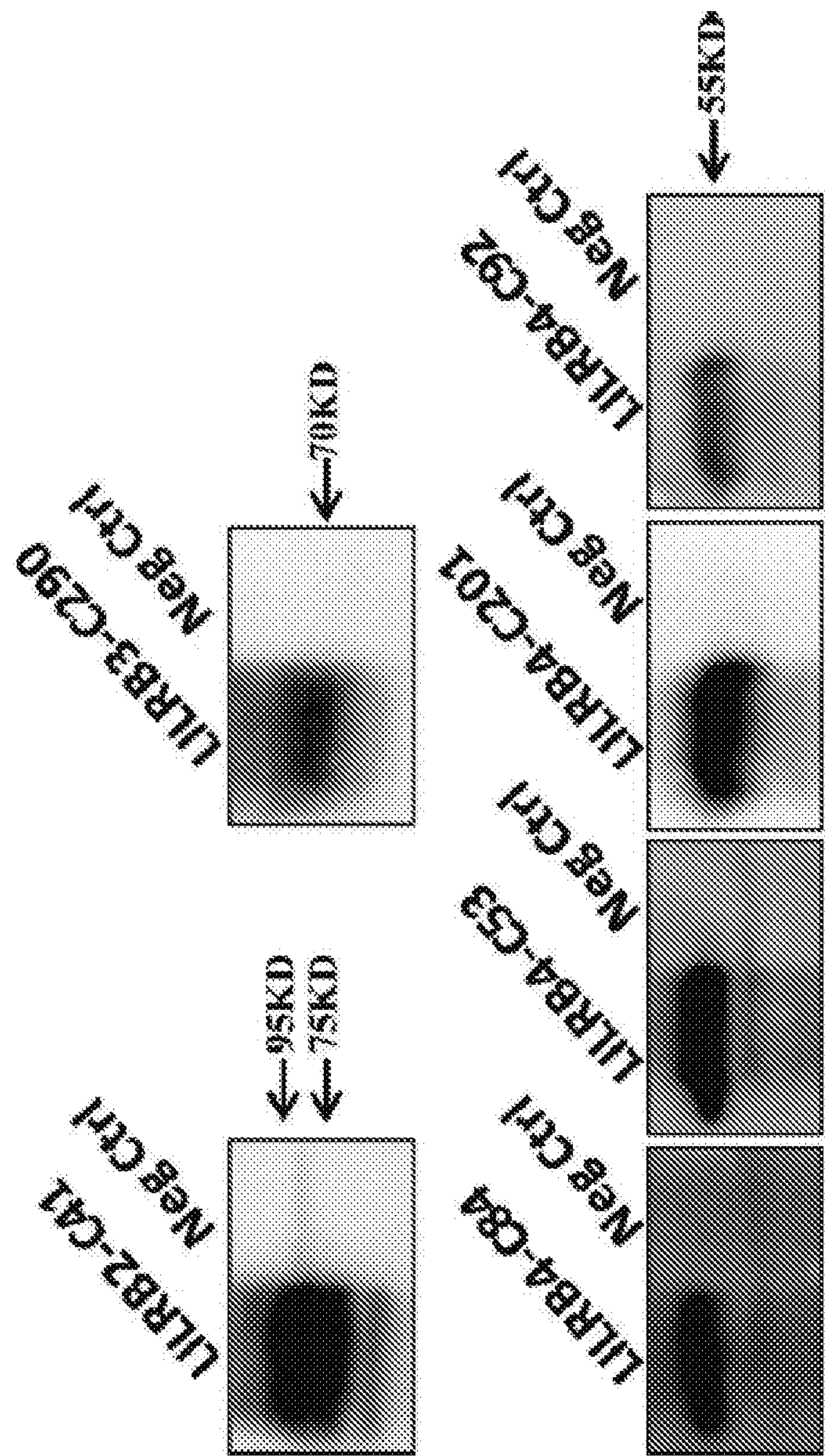


FIG. 4

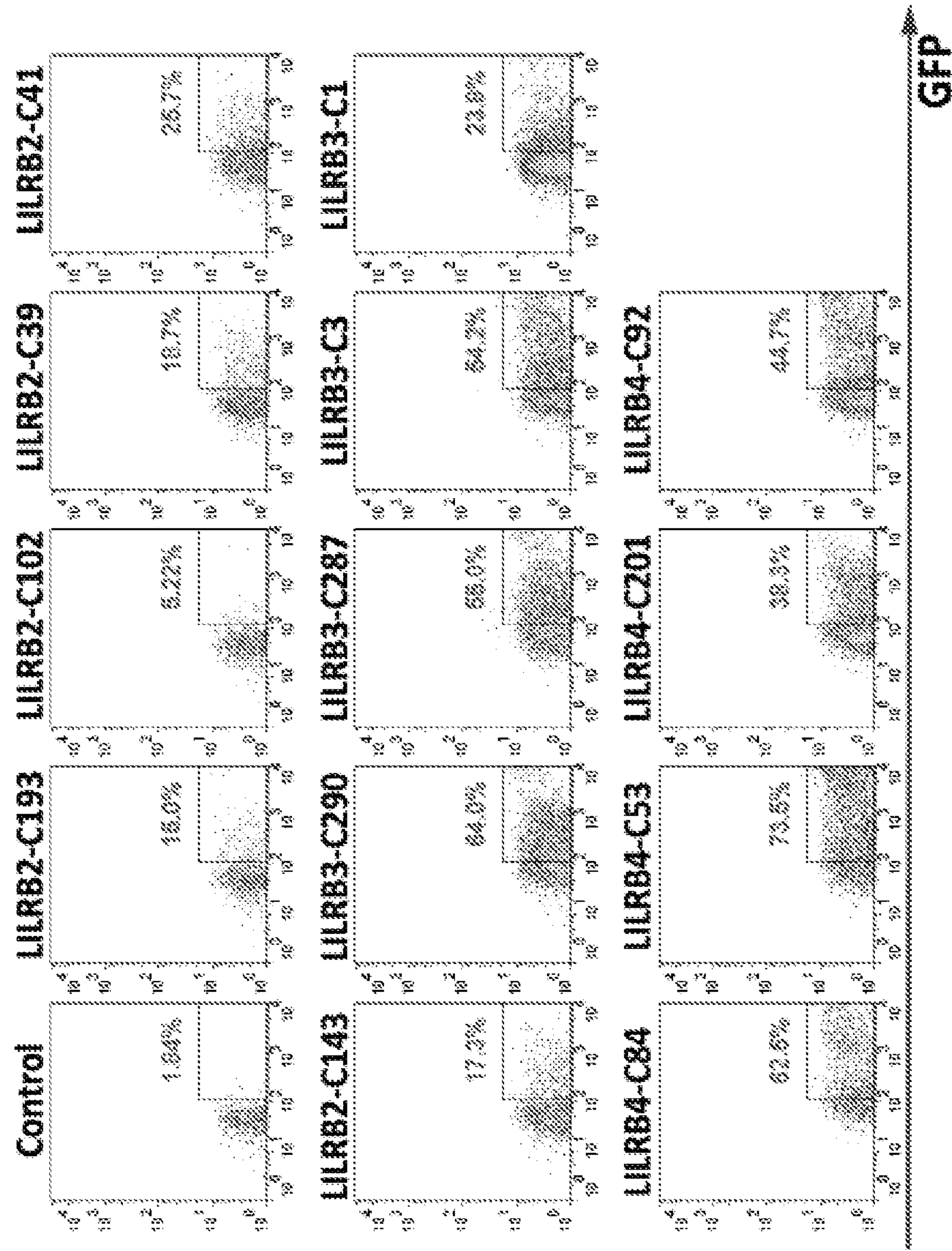
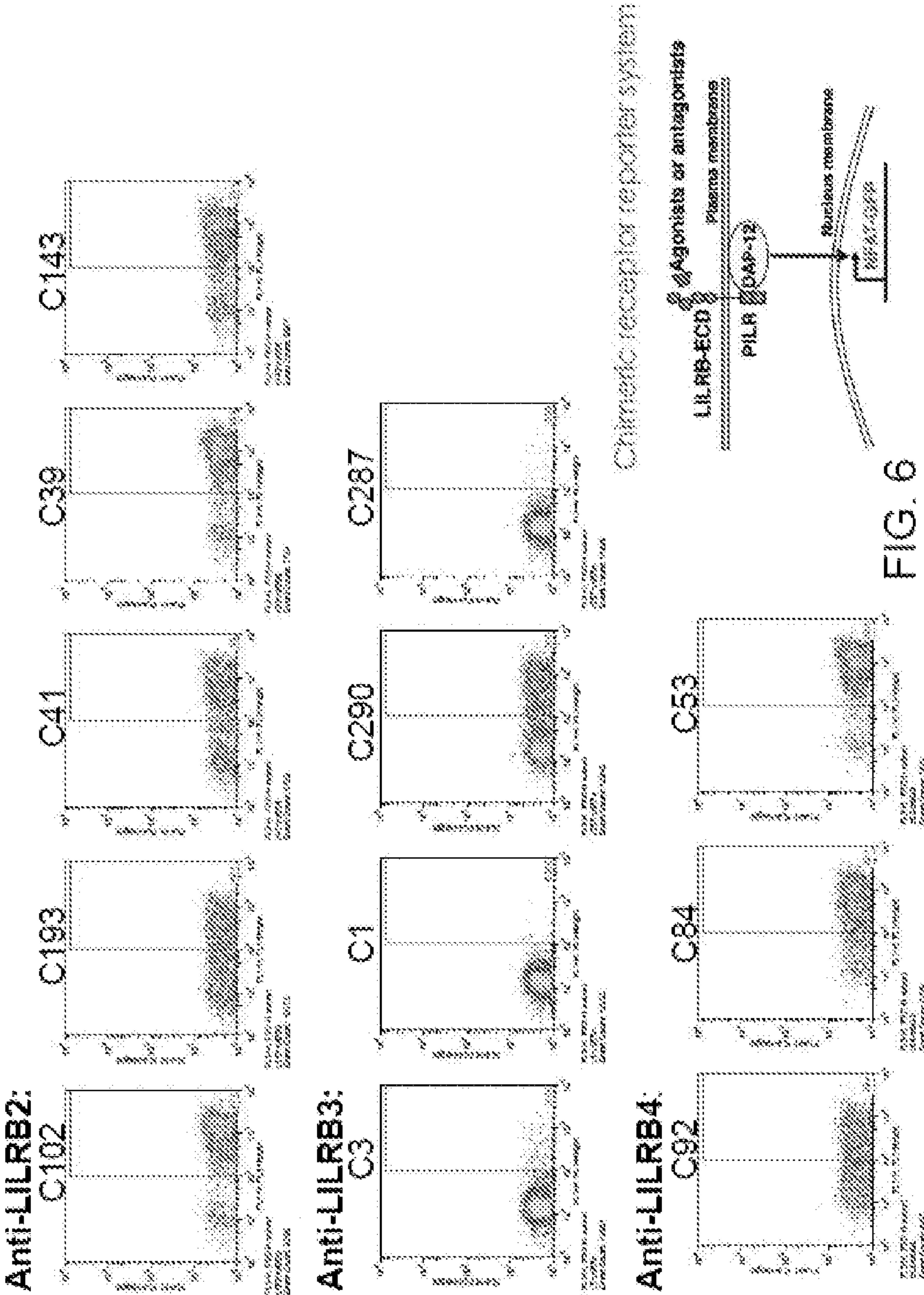


FIG. 5



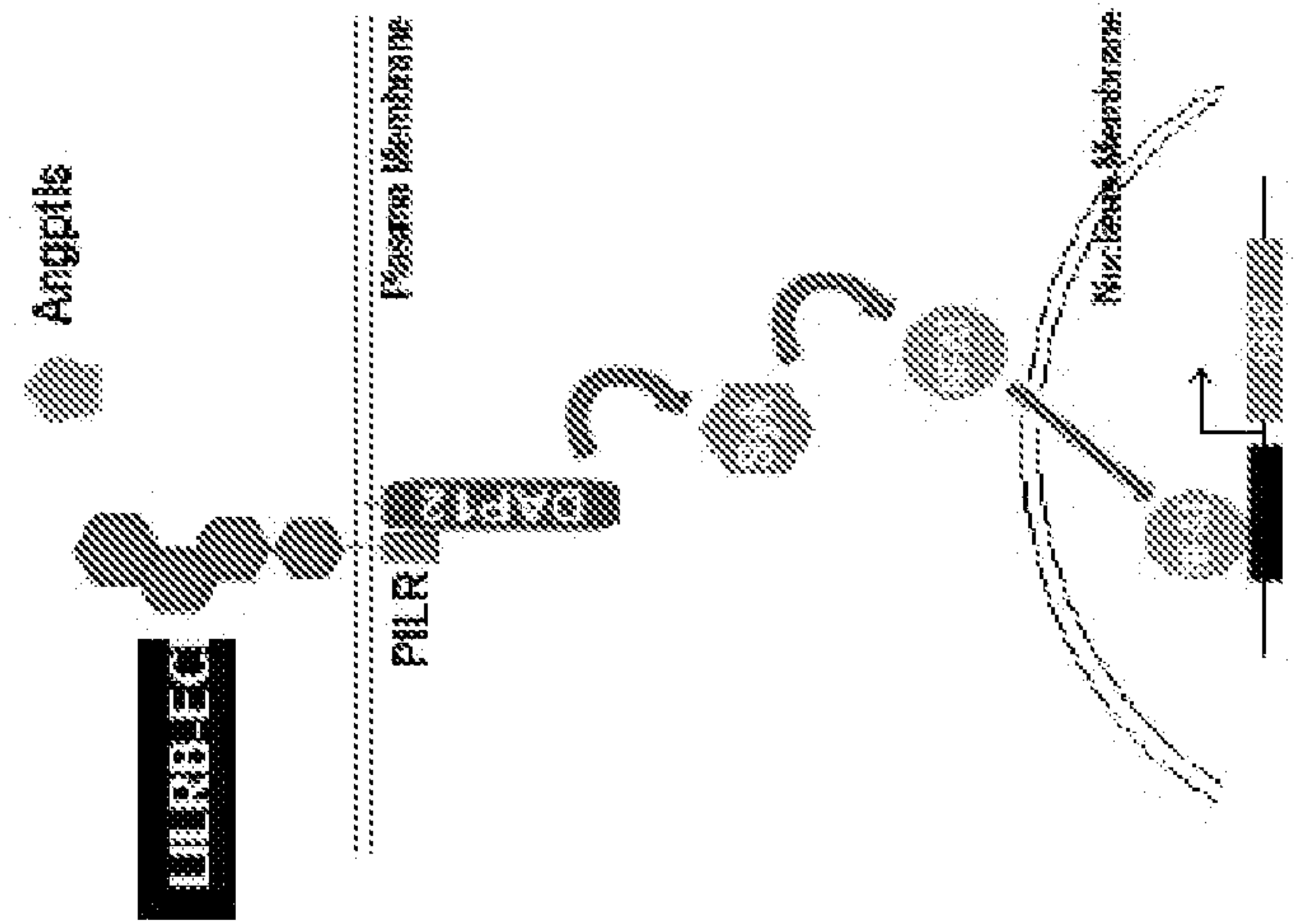
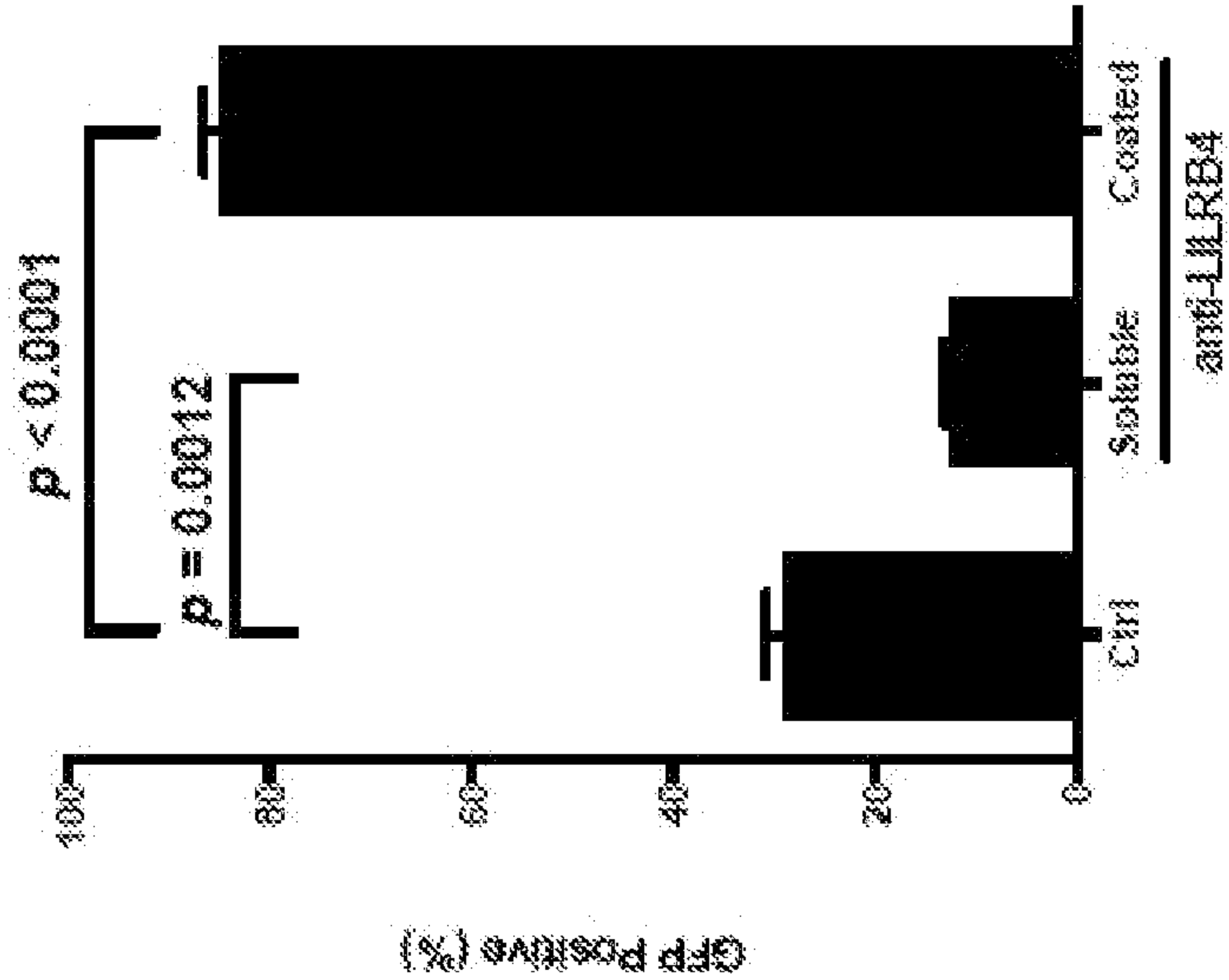


FIG. 7





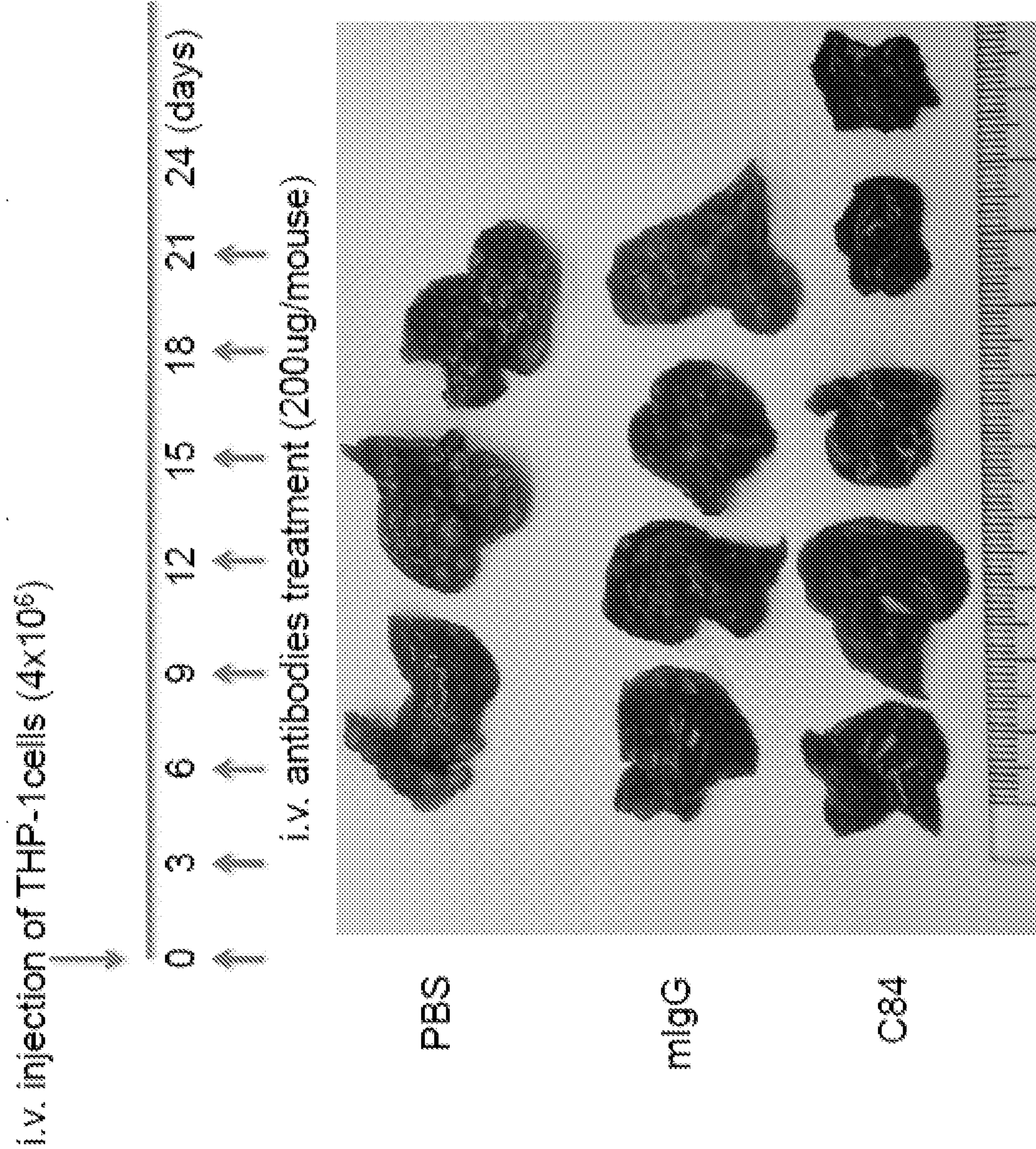


FIG. 10

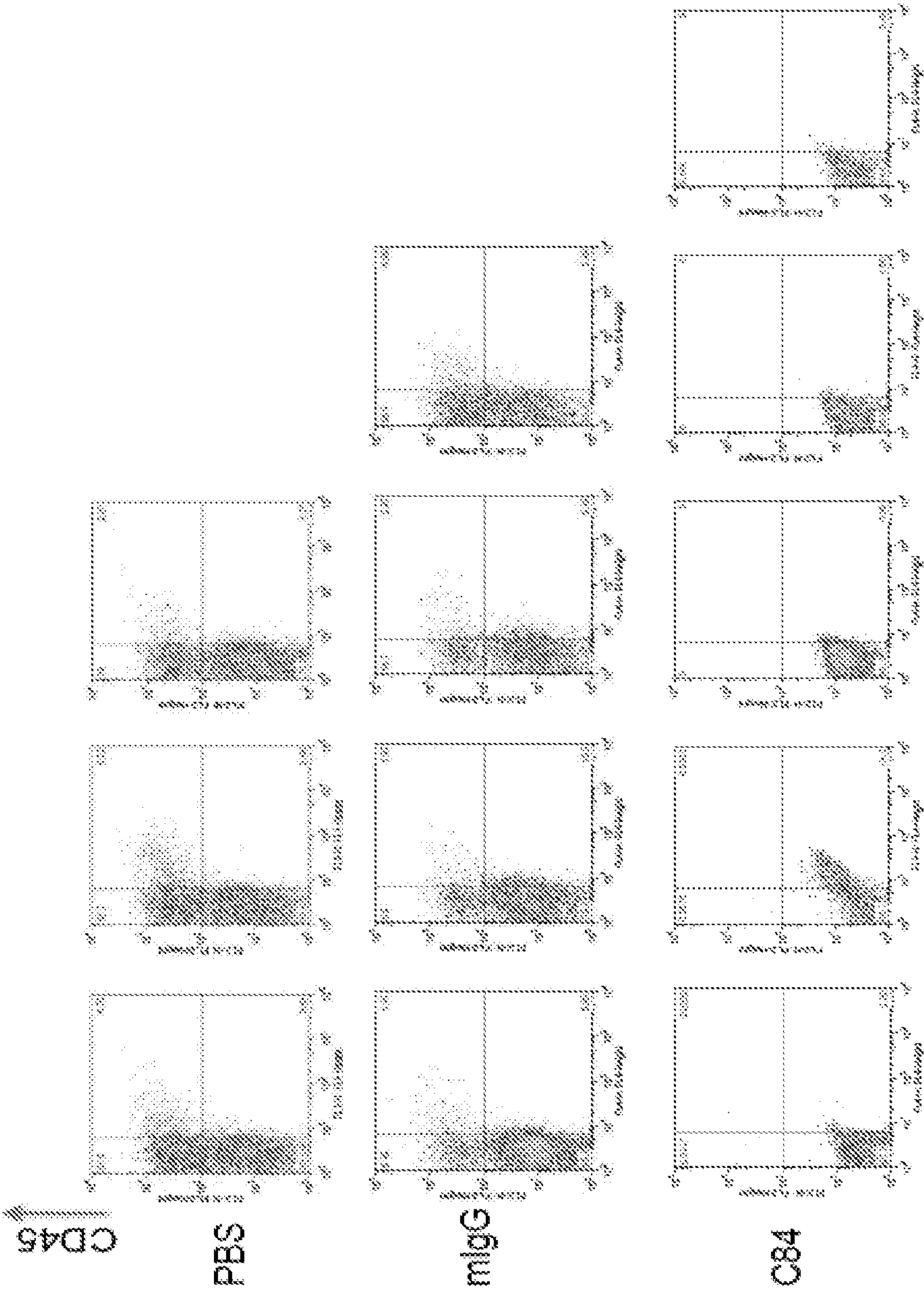


FIG. 11

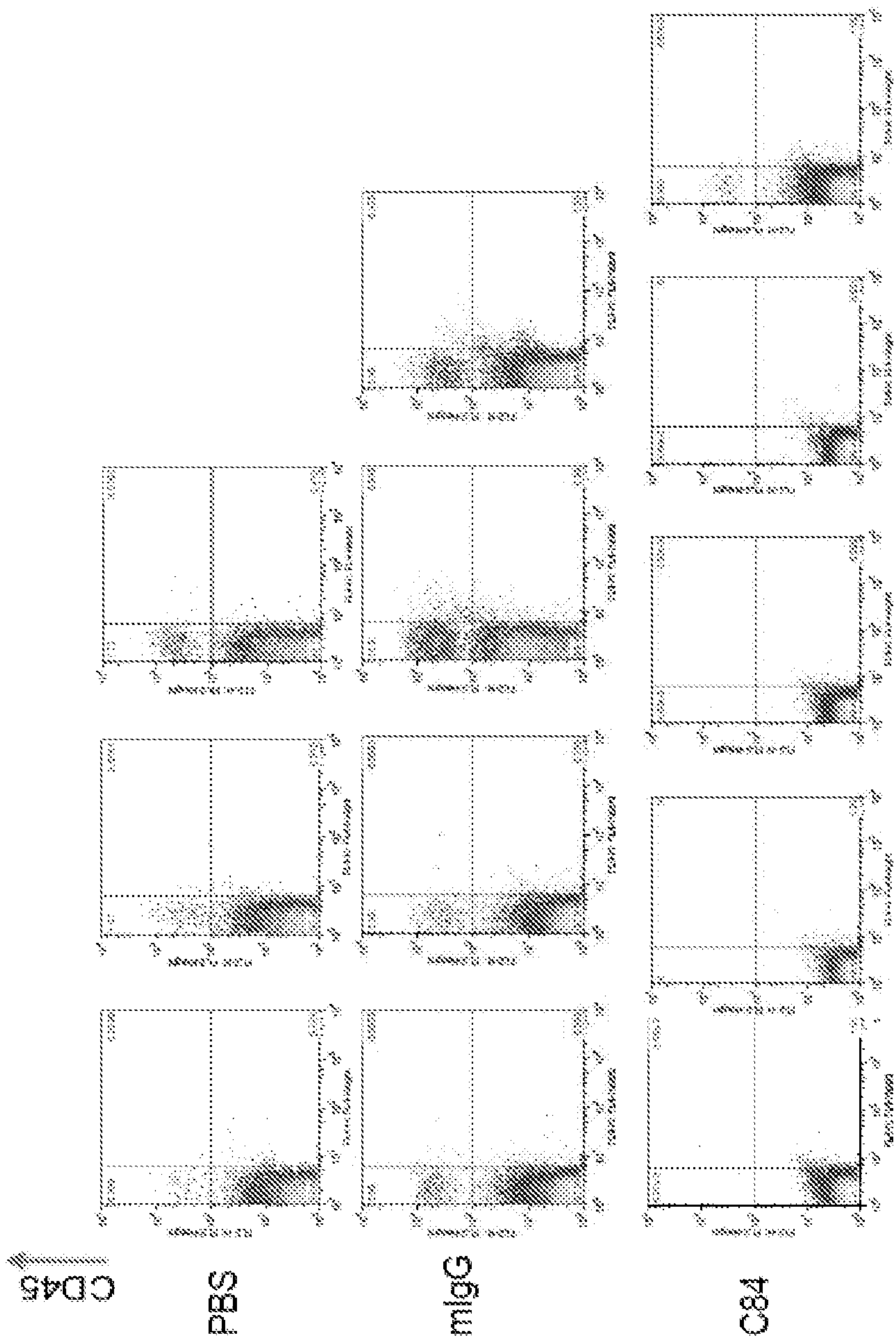


FIG. 12

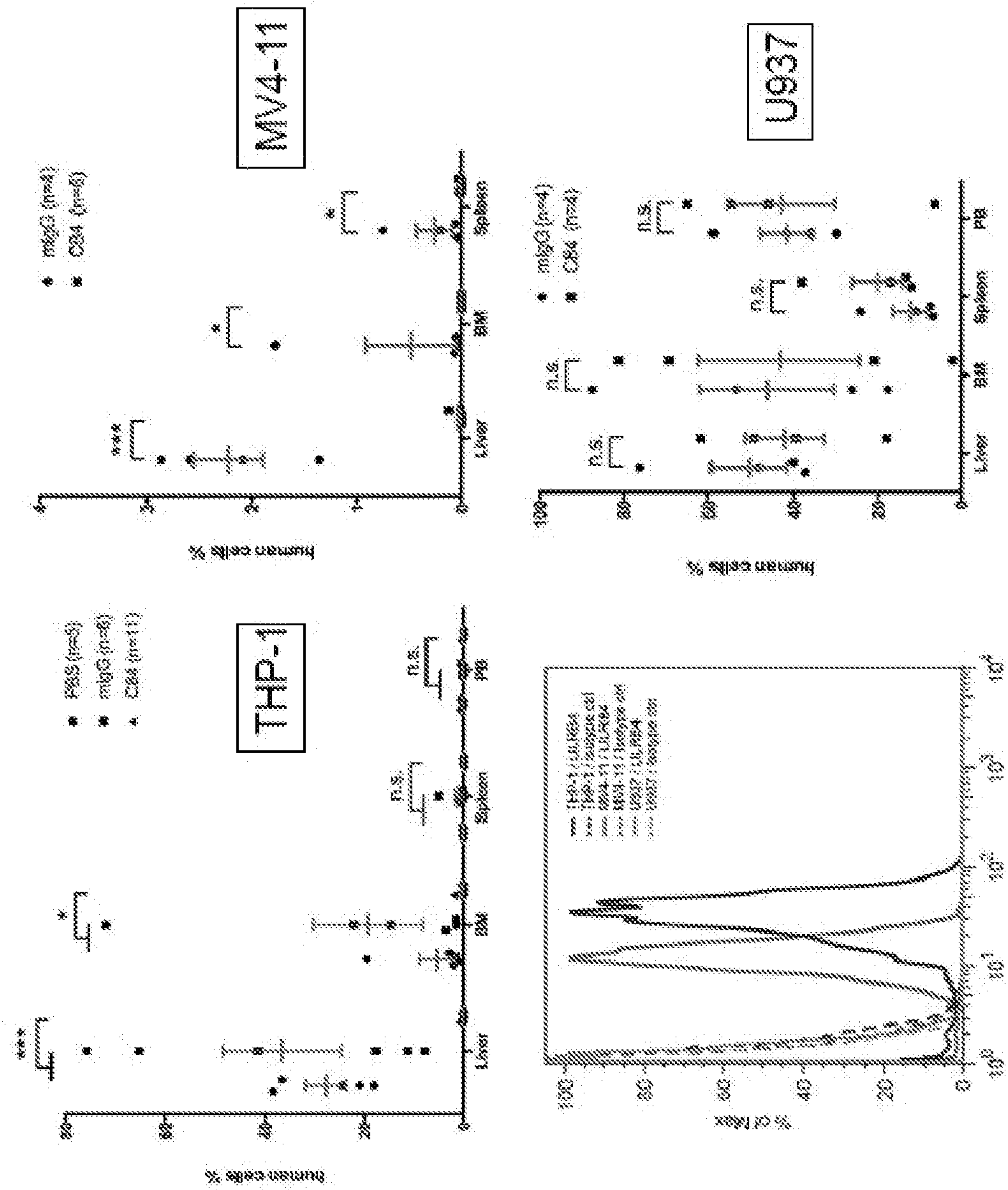


FIG. 13

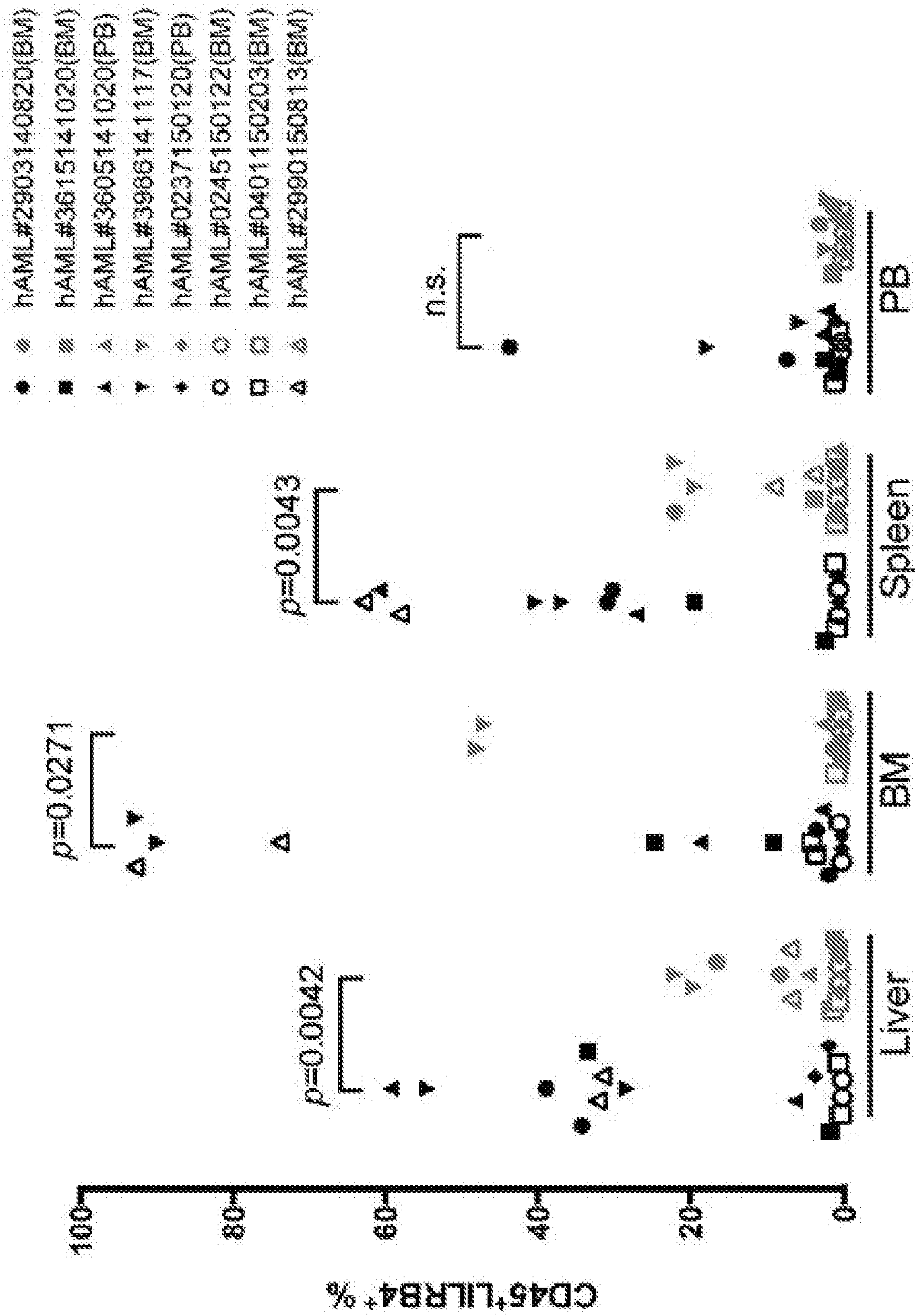


FIG. 14

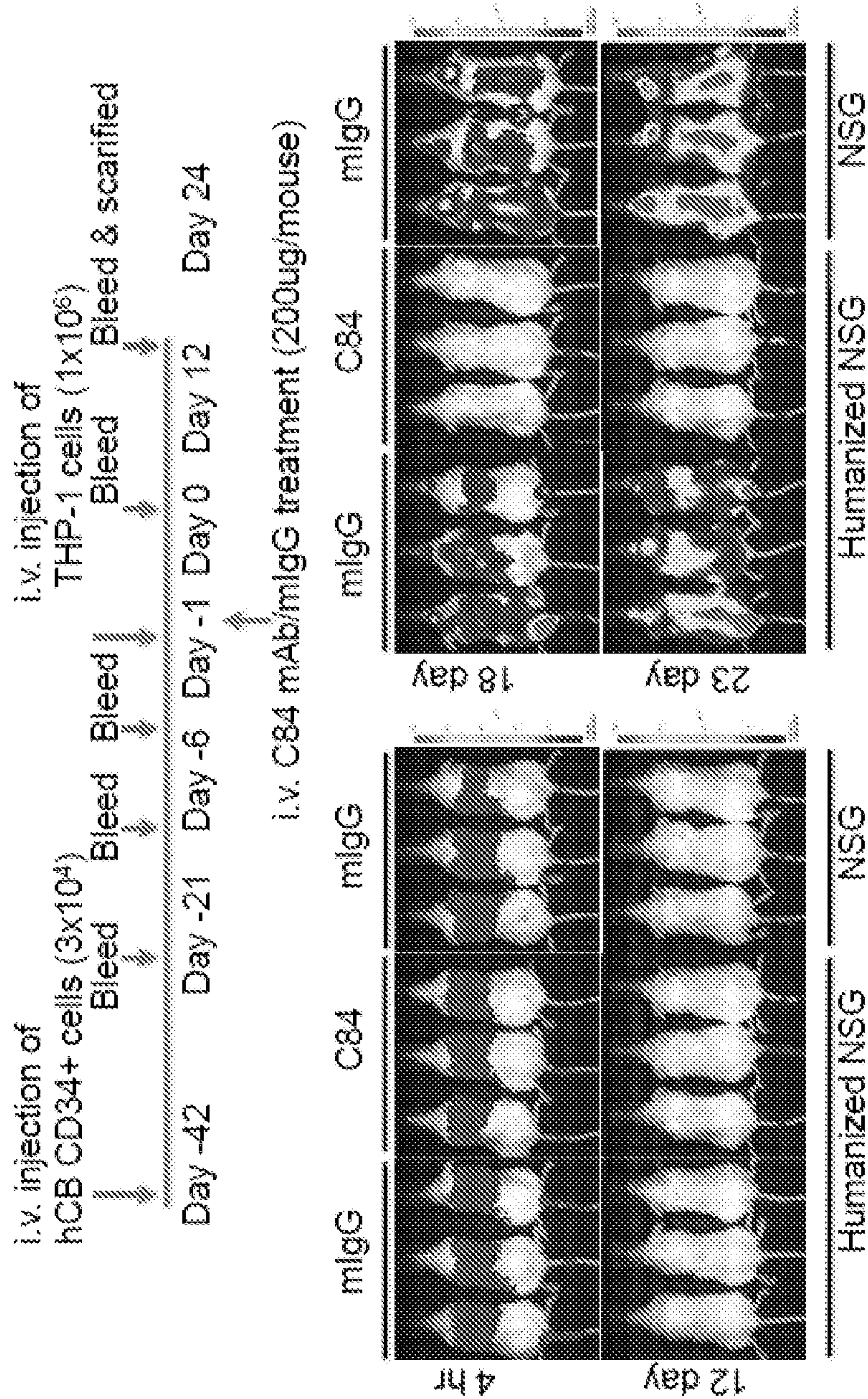


FIG. 15

>#192 Heavy

EQSLEESGGGLVTPGGTLTLTCTVSGFSLNNAMSWVRQAPGKGLEWIGVLLAGPTYYASWAKGRFTISKSTTTVDLKITSPTTEDTATYFCAIAGSRPFALWGQGTLV

TISS

>#192 Light

ELVLTQTPSS TSAAVGGTVTINCQSSQSVYSNWLSWFQQKPGQPPKPLIYLASTLASGVPSRFSGSGGTQFTLTISDVQCDDAATYYCQGGYNGNIYTFGGGTEVVVK

>#161-1 Heavy

QSVKESGGRLVTPGTPLTLTCTVSGIDLSVYLMSWVRHSPGKGLEYIGFINSAGITAYATWAKGRFIISKSTTTVDLKVTSPTTETATYFCARNWIRLDLWGQGTLVTISS

>#161-1 Light

ELDLTQTPASVSAAVGGTVTISCQSSETIYKNYLSWFQQKPGQPPKLLIYESSKLASGVPSRFSGSGGTQFTLTISDVQCDDAATYYCLGGYTDGRDTIFGGGTELEIK

>#161-2 Heavy

QSVKESGGRLVTPGTPLTLTCTVSGIDLSVYLMSWVRHSPGKGLEYIGFINSAGITAYATWAKGRFIISKSTTTVDLKVTSPTTETATYFCARNWIRLDLWGQGTLVTISS

>#161-2 Light

IQTPASVSAAVGGTVTISCQSSETIYKNYLSWFQQKPGQPPKLLIYESSKLASGVPSRFSGSGGTQFTLTISDVQCDDAATYYCLGGYTDGRDTIFGGGTEVEIK

>#208 Heavy

EQSVEESGGGLVTPGGTLTLTCTVSGFSLNNAMSWVRQAPGKGLEWIGVLLSGTTYASWAKGRFTISKSTTTVDLKITSPTTEDTATYFCAIAGSRPFALWGQGTLV

TISS

FIG. 16

>#208 Light

ELVMTQTPSS TSAAVGGTVTINCSSQSVYSNWLSWFQQKPGQPKPLIYLASTLASGVP SRFSGSGTGQFTLTISDVQCDDAATYYCQGGYNGNIYTFGGGTEVVVK

>#120 Heavy

QSLEESGGR LVTGTP LTLTCTVSGIDL NKYAMTWVRQAPGKGLEYIGFINIVGIAGYATWAKGRFTISK TSTTVDLKVTSP TTEDATYFCARNWIRLDLWGQGLTVTS

S

>#120 Light

ELDLTQTPASVSAAVGGTVTISCQSS ESLYKKNYLSWFQQKPGQPKLLIFEASKLASGVP SRFSGSGTGQFTLTISDVQCDDAASY YCLG DYTNGRD TTFFGGGTEVVVK

>#78 Heavy

QSLQESGGR LVTGTP LTLTCTVSGIDLSVYLM SWVRQSPGK GLEYIGFINSAGITAYATWAKGRFIISK TSTTVDLKVTSP TTEDATYFCARNWIRLDLWGQGLVTISS

>#78 Light

ELVLTQTPASVSAAVGGTVTISCQSS ESIYKNYLSWFQQKPGQPKLLIYETSKLASGVP SRFSGSGTGQFTLTISDVQCDDAATYYC LGGYTNGRD TIFGGGTELEIK

>#8 Heavy

QSVKESGGGLVTPGGTLT LTCTASGFSLISYDMY WVRQAPGKGLEYIGIYSDGYTFYATGAKGRITISRTSTTVDLKITSP TTEDATYFCATNAFALWGQGLVTISS

>#8 Light

ELDLTQTPSSVSAAVGGTVTISCQSSQNVYNNNWLVWLQQKPGQPKRLIYTASSLASGVP SRFAGSGSATQFTLTISDLECGDAATYYCAGGYS GPIYTFGGGTEVEIK

FIG. 16 (cont.)

>#128-1 Heavy

EQSVEESEGRLVTPGGSLTLTCTVSGFGLSSWAMAWRQAPGKGLEWIGIVSGKIYYPTWAKRFTISRTSTTVDLKIASPTTEDTATYFCAREPYGDSLWGQGTLVTI

SS

>#128-1 Light

ELVMTQTPSPSAAVGGTVISCQSSQNISTYLSWYQQKPGQPPKFLIYQASKLASGVSSRFKGSGTQFTLISDVQCDDAASYCAGWKSYSNDDNDFGGGTEVV

K

>#128-3 Heavy

EQSLEESGDLVTPGASLTLTCKASGIDFSNHYIYWVRQAPGKGLEWIGCIFSGDSASTYYASWAKGRFTISKSSPTVTLQMTSLTAADTATYFCARGMSTNDWASDL

WGPGTMVTVSS

>#128-3 Light

ELVMTQTPASVSAAVGGTVINCASESINSILAWYQQKPGQRPKLIYRASTLASGVSSRFKGSASGTGFTLISDLECADAATYCQQSYDWGDVENTFGGGTEVEIK

>#140 Heavy

EQSLKESGGRLVTPGTPLTLTCTASGFSLTYDMSWVRQAPGKGLEWIGISTSIYTYASWAKGRFTISRTSSTTVDLKMTSLTTEDTATYFCVGGGKLNSVYIRG

LRFWGQGTLVTISS

>#140 Light

ELVMTQTPSTSAAVGGTVINCASQSVYDDNWCSWYQQKPGQPKLIYDASTLPSGVPSRFKGSGTQFTLISDLECDDAATYCAGAYSDNIYVFGGGTEVEIK

FIG. 16 (cont.)

>#216-1 Heavy

EQSVEESGGGLVKPGASLTLTCIASGFSFSSSYVMCWVRQAPGKGLELIACIHAGGSGSTYYASWAKGRFTISKTSSTTVTLQMTSLTAADTATYFCARYNYHFYYIGDYS

DLWGPGLVTVSS

>#216-1 Light

ELVMTQTPSSVAAVGGTVTIKCQASEDIDSYLAWYQQKPGQRPKVLIRASTLASGVPSRFKSGSGTEFTLTISGLECDDAATYYCQQGWSNDVGSNAFGGGTKVEI

K

>#216-2 Heavy

EQSLKESGGRLLVTPGTPLTLTCTVSGIDLSSYAMTWVRQVPGKGLEYIGFINIVGIAGYATWAKGRFTISKTSSTTVDLKVTSPPTEDTATYFCARNWIRLDLWGQGLLTV

SS

>#216-2 Light

ELVMTQTPASVSAAVGGTVTISCQSSELYKKNYLSWFQQKPGQPPKLLIYEASKLASGVPSRFSGSGTGQFTLTISDVQCDDAASYCYCLGDTNGRDITTFGGGTEVEIK

>#101 Heavy

HSVKESGGGLVTPGESLKLICKASGMDFSKYWICWVRQAPGKGLEWIACIDTGRSAITVYAKWAKGRFTISKTSSTTVTLQMTTLTAADTATYFCETSVDLWGPGLVTI

SS

>#101 Light

ELDLTQTPASVEAAVGGTVTIKCQASESIYSGLAWYKQKPGQPPKFLILSASTQASGVPSRFKSGSGTEFTLTISDLECADAATYYCQSRHYGSSRSYGFAFGGTEVEIK

FIG. 16 (cont.)

>#223 Heavy

EQSVKESGGRLVTPGTPLTLTICIVSGFSLNNYAISWVRQAPEKGLEWIGMIRSDGHHVDYATWAKGRFTISKTSITTVDLKM TSLTTEDTATYFCARGGHFFNPWGPGLV

TISS

>#223 Light

ELVMTQTTPSPVSAAVGGTVTISCQSSQTVYNNYNELAWYQQKPGQPKLLIDAASTLASGVPSRFSGSGGTQFTLKISEVQCDDAATYYCQGTYYISGWYYTFGGGTEV

VVK

>#139 Heavy

EQSLEESGGRLVTPGTPLTLTCTVSGFSLNSYAMAWVRQAPGKGLEWIGIIGLSTMTYYASWVNGRFTISKTSITTVDLKM TSLTTEDTATYFCVRNDVYWAFNLWGQG

TLVTVSS

>#139 Light

ELDLTQTTPSSVAGVGGTVSISQSSSVNNHALSWYQQKPGQPKLLIYKASTLASGVPSRFSGSGGAQFTLTISGVQCDDAATYYCQGGFYSGISDYPFGGGTEVEI

K

>#156-1 Heavy

QSVKESEGLVKPGASLTLTCRASGFSFSSDYNMCWVRQAAGKGLEWIAICIGVGTSGKTAYATWAKGRFTISRSSSTTVALQMTSLTVADTATYFCARPSYSGSGGEGG

GSGLWGPGLVTISS

>#156-1 Light

ELDLTQTASPSEPVG GTVTIKCQASQSIHSDLA WYQQKPGQRPKLLIYVASYLASGVPSRFSGSGGTEYTLTISDLECADAAATYYCQSTYYGSDYVGGAFGGGTEVVVK

FIG. 16 (cont.)

>#129 Heavy

EQSVKESGGGLVKPGASLTLTCTASGFSSSYHMCWVRQAPGKGLEWIGCIATGYGSTYYASWAKGRFTISKTSSTTVTLQVPSLTAADTATCFCARGYYRYTSDSYGYF

DLWGPGLVTISS

>#129 Light

ELVLTQTASPVSAAVGGTVTVNCQASQSISGYSWYQQKPGQPPKLLIYYASRLASGVPSRFSGSGSGTEYTLTISGVQCDDAATYYCLVVSYDGTITTDNAFGGGTEVEI

K

>#214 Heavy

QSVKESGGRLVTPGTPLTLTCTVSGFSLSSYWMHWWVRQAPGKGLEWVGVSVSGNFYYATWAKGRFTISKSTTVDLKITSPTIEDTAFYCTMSFALWGQGTLVTISS

>#214 Light

ELVMTQTASPVSAAVGGTVTINCQSSQSVYSNNLGWLKQKPGQPPKELIYYASTLASGVPSRFKGS GSGTQFTLTISDLECDDAATYYCVGGYGCSSADCSVFGGGTE

VEIK

>#210 Heavy

EQSVEESGGRLVTPGTPLTLTCTVSGFSLSSYIMGWVRQARGKGLEYIGAINTDGATYYATWAKGRFTISRTSTTVHLKVTSPTTEDTATYFCARSLAPGDSNINLWGQGT

LVTISS

>#210 Light

ELVLTQTSPVSAAVGGTVTISCQSSSVYRNRLAWYQQKPGQPPKRLIYLASTLASGVPSRFKGS GSGTQFTLTISDVQCDDAAAAYCAGDSGVGIIAFGGGTEVEIK

FIG. 16 (cont.)

>#6 Heavy

QSVKESGGRLVTPGTPLTLICTASGFSLSYYIDWVRQAPGRGLEIYIGVINPGGSVYATWAKGRFTISRTSTTVDLKMTSLTTEDTATYFCARGWSRGDLWGQGTLVTI

SS

>#6 Light

ELDMTQTPSPVSAAVGGTVTIKCQSSQNVYDDDTLSWYQQKPGQPPKLLIYDASKLASGVPSRFKGS GSGTQFTLTISGVQCDDAATYYCLGVFHDAADNAFGGGTEL

EIK

>#71-1 Heavy

QSVEEGGGLVKPGASLTLICTASGFSFSSYHMCWVRQAPGKGLEWIGCIATGYGSTYYASWAKGRFTISKTSSTTVTLQVPSLTAADTATCFCARGYYRYTSDSYGYFD

LWGPGLVTISS

>#71-1 Light

ELDLTQTASPVSAAVGGTVTVNCQASQSISSGYLSWYQQKPGQPPKLLIYASRLASGVPSRFSGSGSGTEYTLTISGVQCDDAATYYCLVVSYSGTTTIDNAFGGGTDVEI

K

FIG. 16 (cont.)

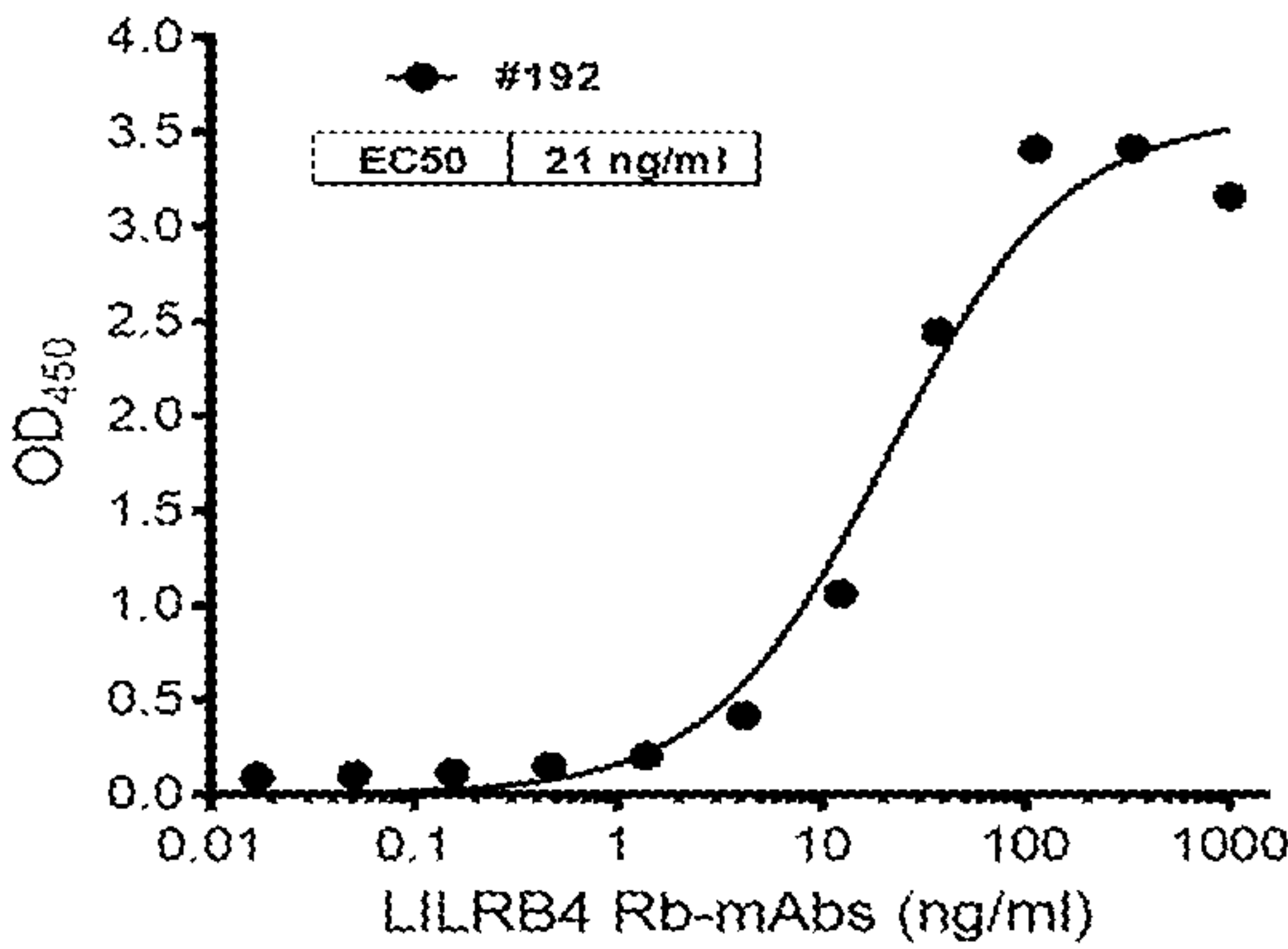
	Heavy Chain				Light Chain		
mAbs	CDR1	CDR2	CDR3		CDR1	CDR2	CDR3
#192	GFSL...SNNA	ILLA...GPT	AIAIGSRPFAL		QSVY....SNW	LA.....S	QGGYNGNIYT
#161-1	GIDL...SVYL	INSA...GIT	ARNWIRLDL		ETIY....KNY	ES.....S	LGGYTDGRDTI
#161-2	GIDL...SVYL	INSA...GIT	ARNWIRLDL		ETIY....KNY	ES.....S	LGGYTDGRDTI
#208	GFSL...SNNA	ILLS...GTT	AIAIGSRPFAL		QSVY....SNW	LA.....S	QGGYNGNIYT
#120	GIDL...NKYA	INIV...GIA	ARNWIRLDL		ESLY....KKNY	EA.....S	LGDYTNGRDTT
#78	GIDL...SVYL	INSA...GIT	ARNWIRLDL		ESIY....KNY	ET.....S	LGGYTNGRDTI
#8	GFSL...ISYD	IYSD...GYT	ATNAFAL		QNVY...NNNW	TA.....S	AGGYSGPIYT
#128-1	GFGL...SSWA	IGVS...GKI	AREPYGDSL		QNI.....STY	QA.....S	AGWKSYSNDDND
#128-3	GIDFS...NHYY	IFSGD.SAST	ARGMSTNDWASDL		ESIN....SIY	RA.....S	QQSYDWGDVENT
#140	GFSL...TYDM	STSYS.IYIT	VGGGKLNsvvYIRGLRF		QSVY....DDNW	DA.....S	AGAYSDNIYV
#216-1	GFDFS...SSYV	IHAGG.SGST	ARYNYHFYIGDYSDL		EDI.....DSY	RA.....S	QQGWSNDVGSNA
#216-2	GIDL...SSYA	INIV...GIA	ARNWIRLDL		ESLY....KKNY	EA.....S	LGDYTNGRDTT
#101	GMDF...SKYW	IDTG..RSAI	ETSVDL		ESI.....YSG	SA.....S	QSRHYGSSRSYGF A
#223	GFSL...NNYA	IRSD...GHV	ARGGHFFNP		QTVY...NYNE	AA.....S	QGTYYISGWYYT
#139	GFSL...NSYA	IGLS...TMT	VRNDVYWAFNL		ESV...NNHA	KA.....S	QGGFYSGISDYP
#156-1	GFDFS...SDYN	IGVGT.SGKT	ARPSYGSGGEGGSGL		QSI.....HSD	VA.....S	QSTYYGSDYVVGGA
#129	GFDFS...SSYH	IATG...YGS	ARGYYRYTSDSYGYFDL		QSI.....SGY	YA.....S	LYVSYDGTTT DNA
#214	GFSL...SSYW	VSVS...GNF	TMSFAL		QSVY...SNNN	YA.....S	VGGYGCSSADCSV
#210	GFSL...SSYI	INTD...GAT	ARSLAPGDSNINL		ESVY...RNNR	LA.....S	AGDSGVGIIA
#6	GFSL....STYY	INPG...GSA	ARGWSRGDL		QNVY....DDDT	DA.....S	LGVFHDAADNA
#71-1	GFDFS...SSYH	IATG...YGS	ARGYYRYTSDSYGYFDL		QSI.....SGY	YA.....S	LYVSYSGTTT DNA

FIG. 17

FIG. 18A

#192

(1)



(2)

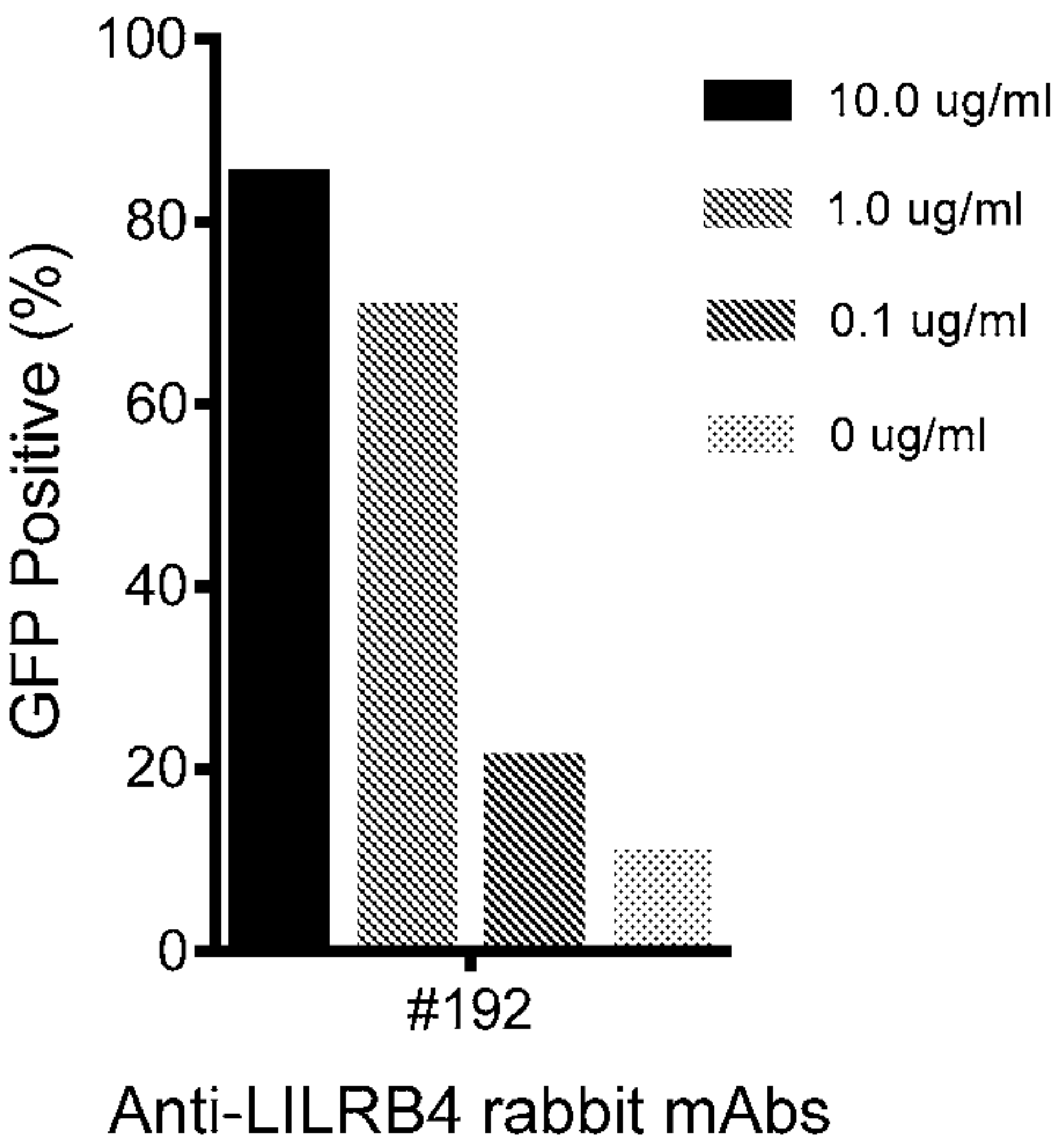
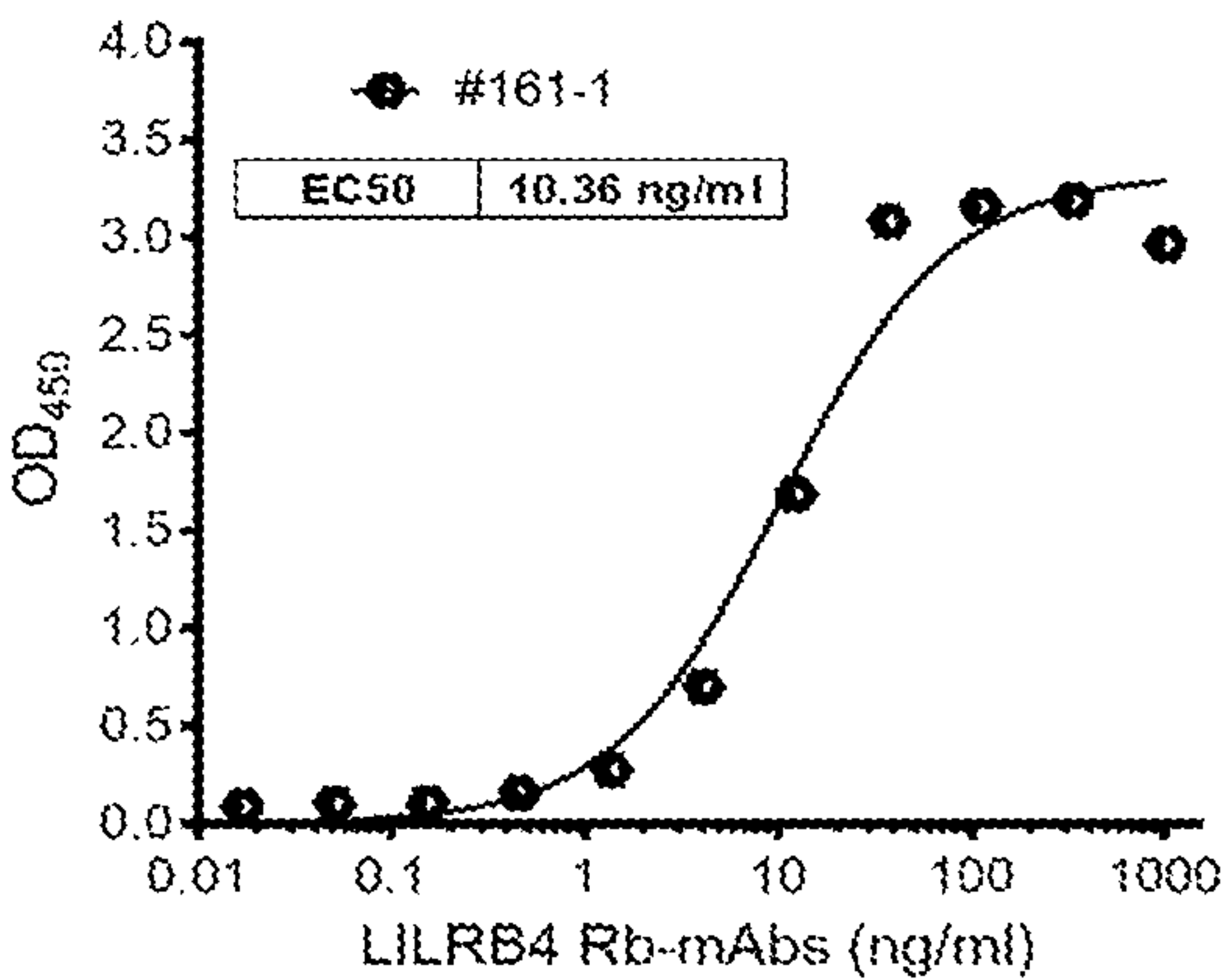


FIG. 18B

#161-1

(1)



(2)

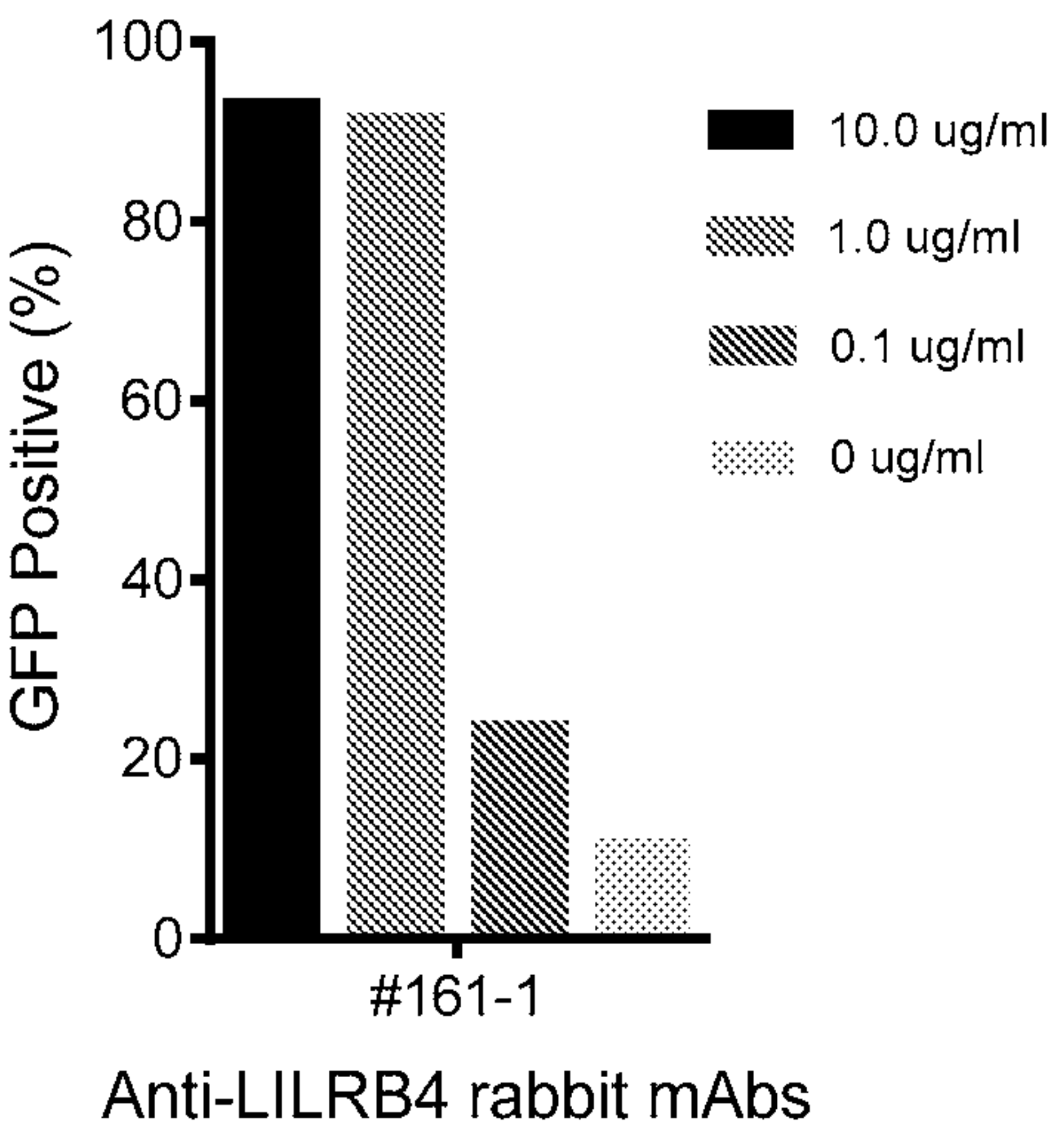


FIG. 18C

#161-2

(2)

(1)

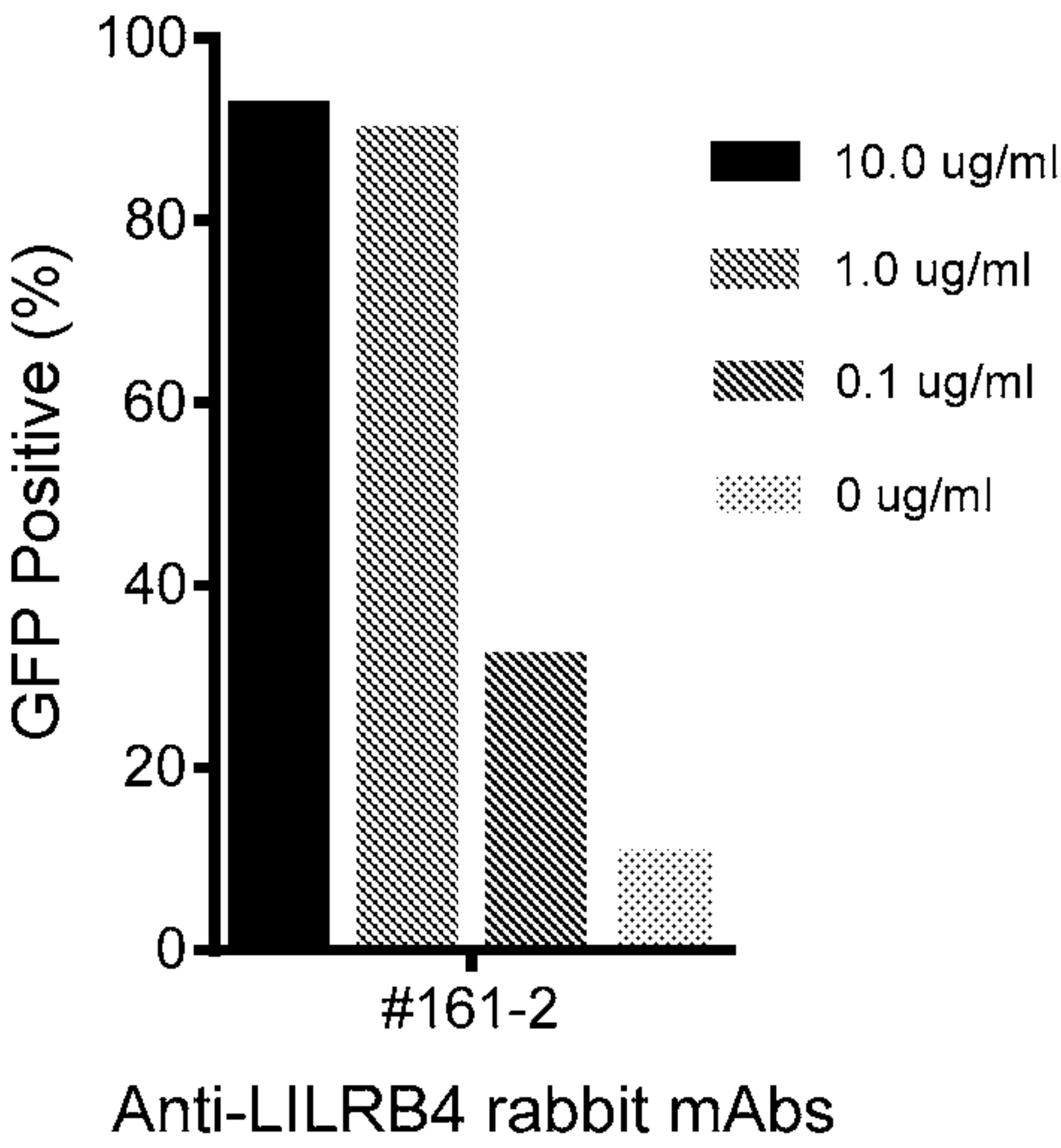
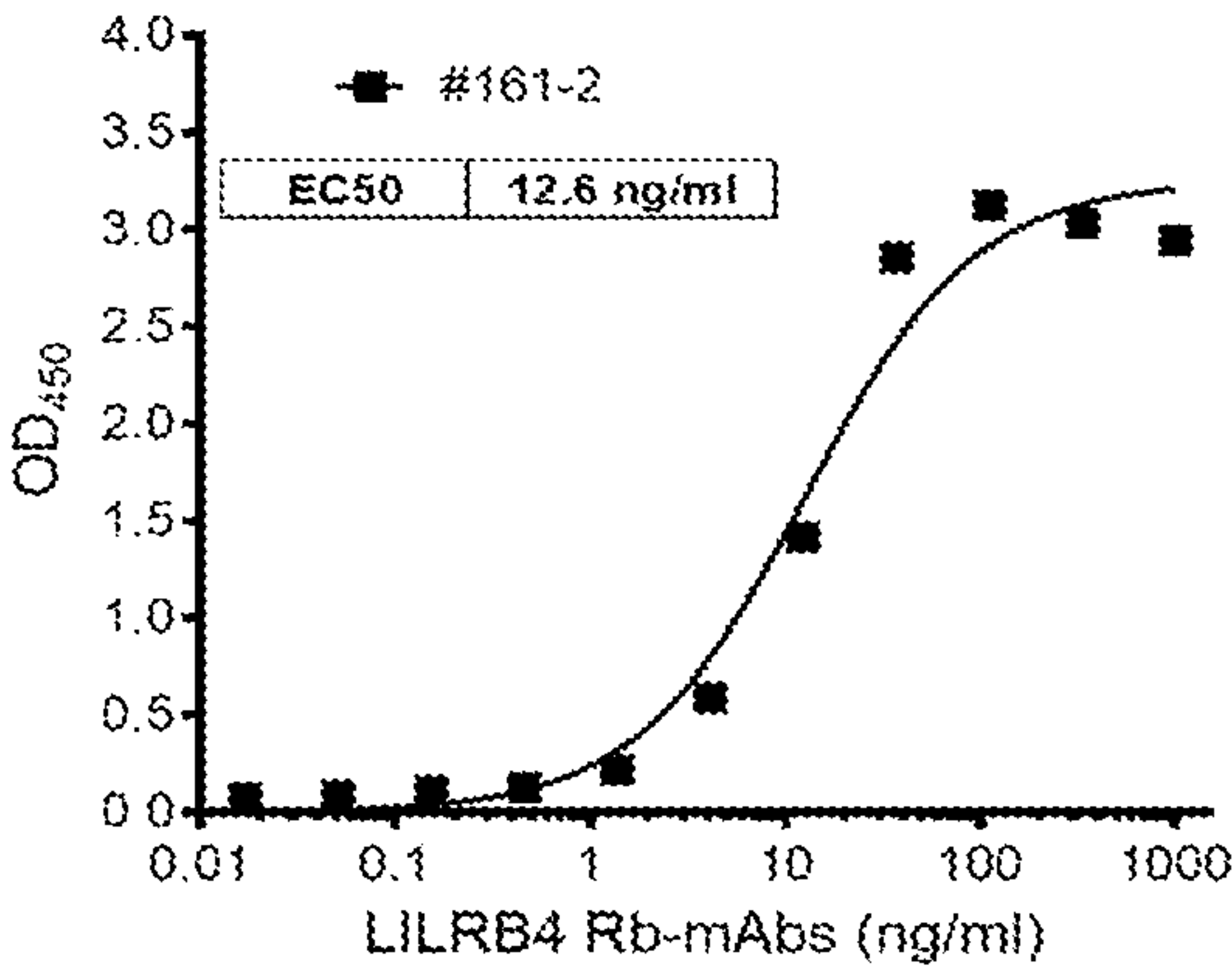


FIG. 18D

#208

(2)

(1)

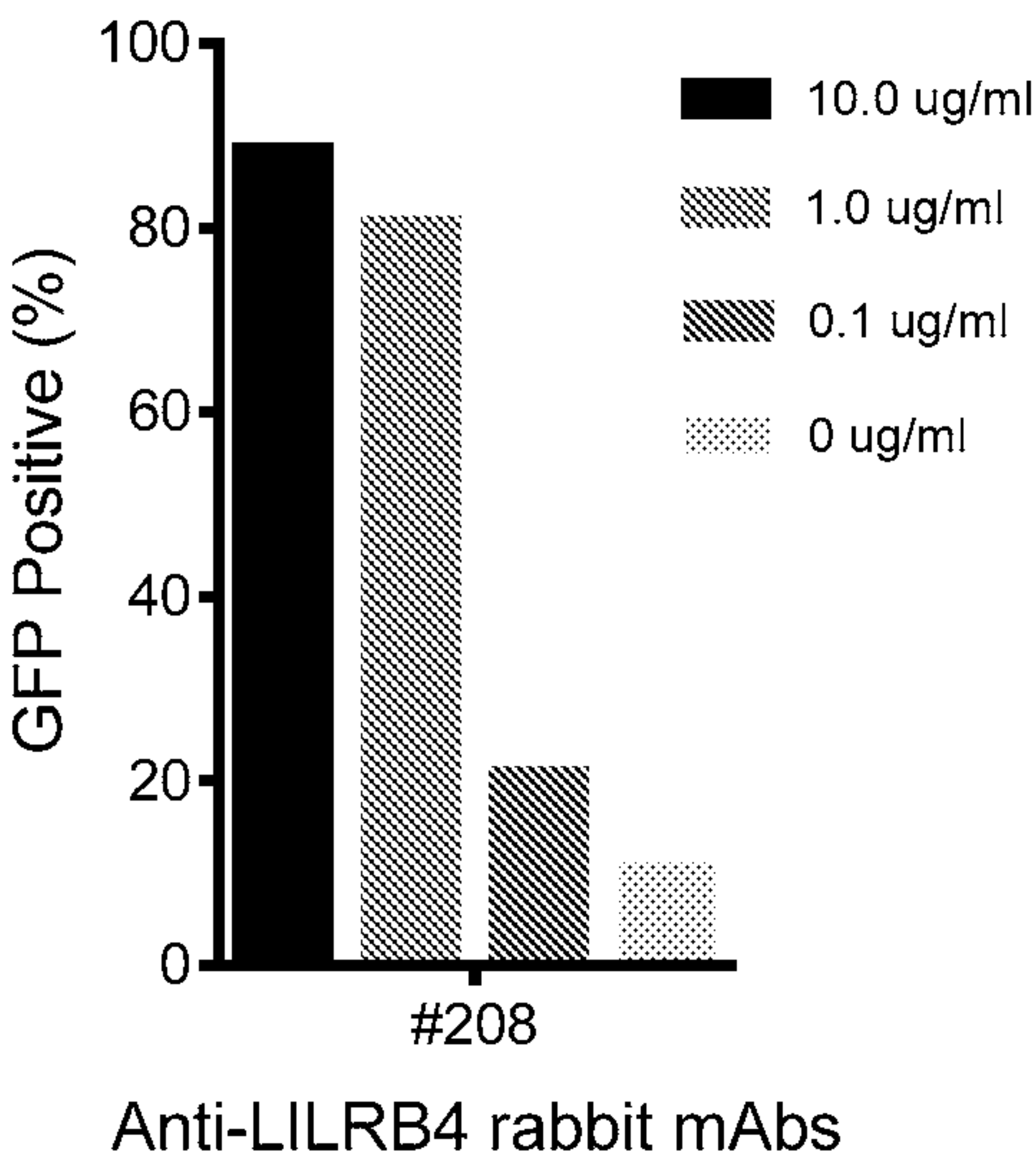
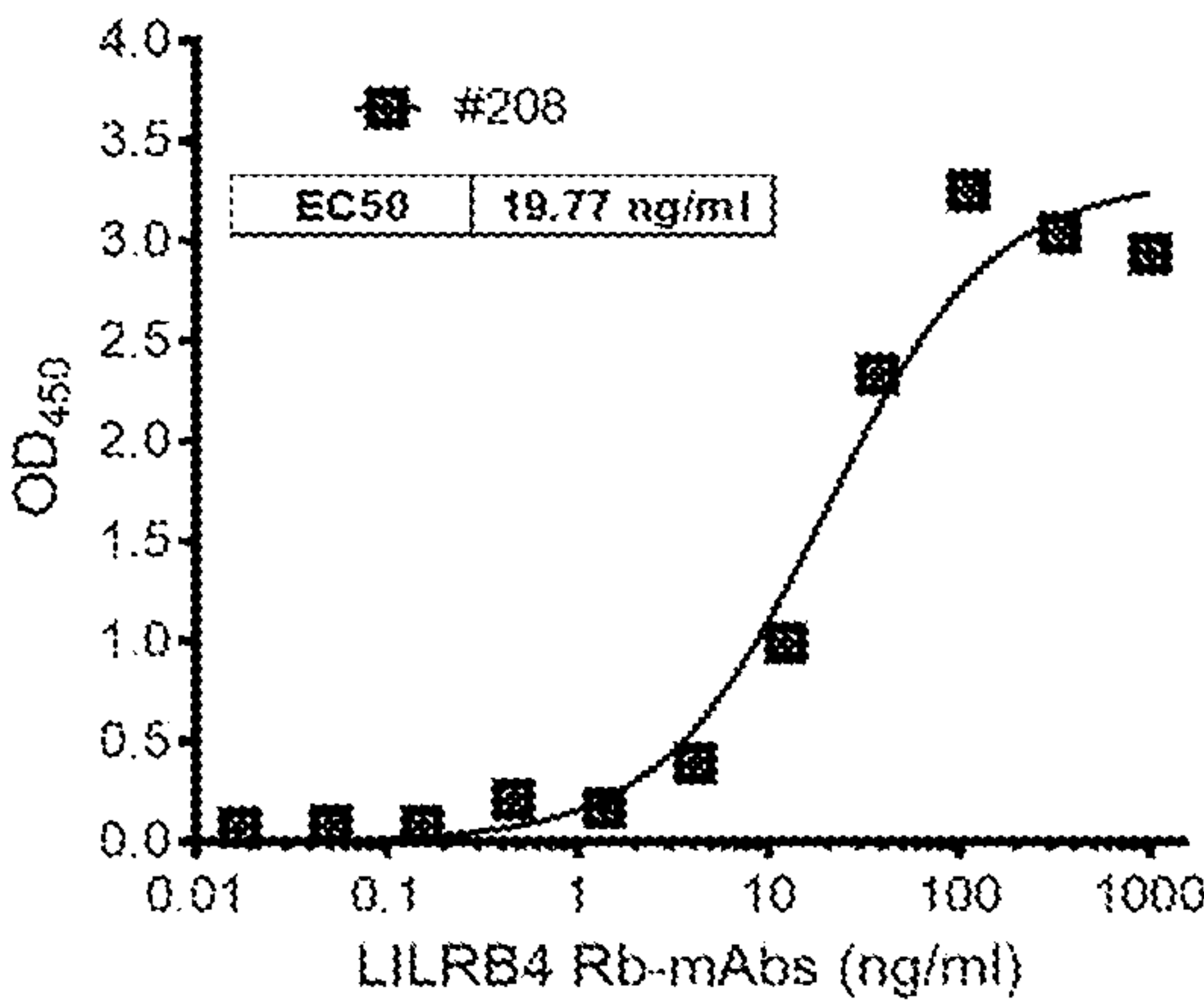


FIG. 18E

#120

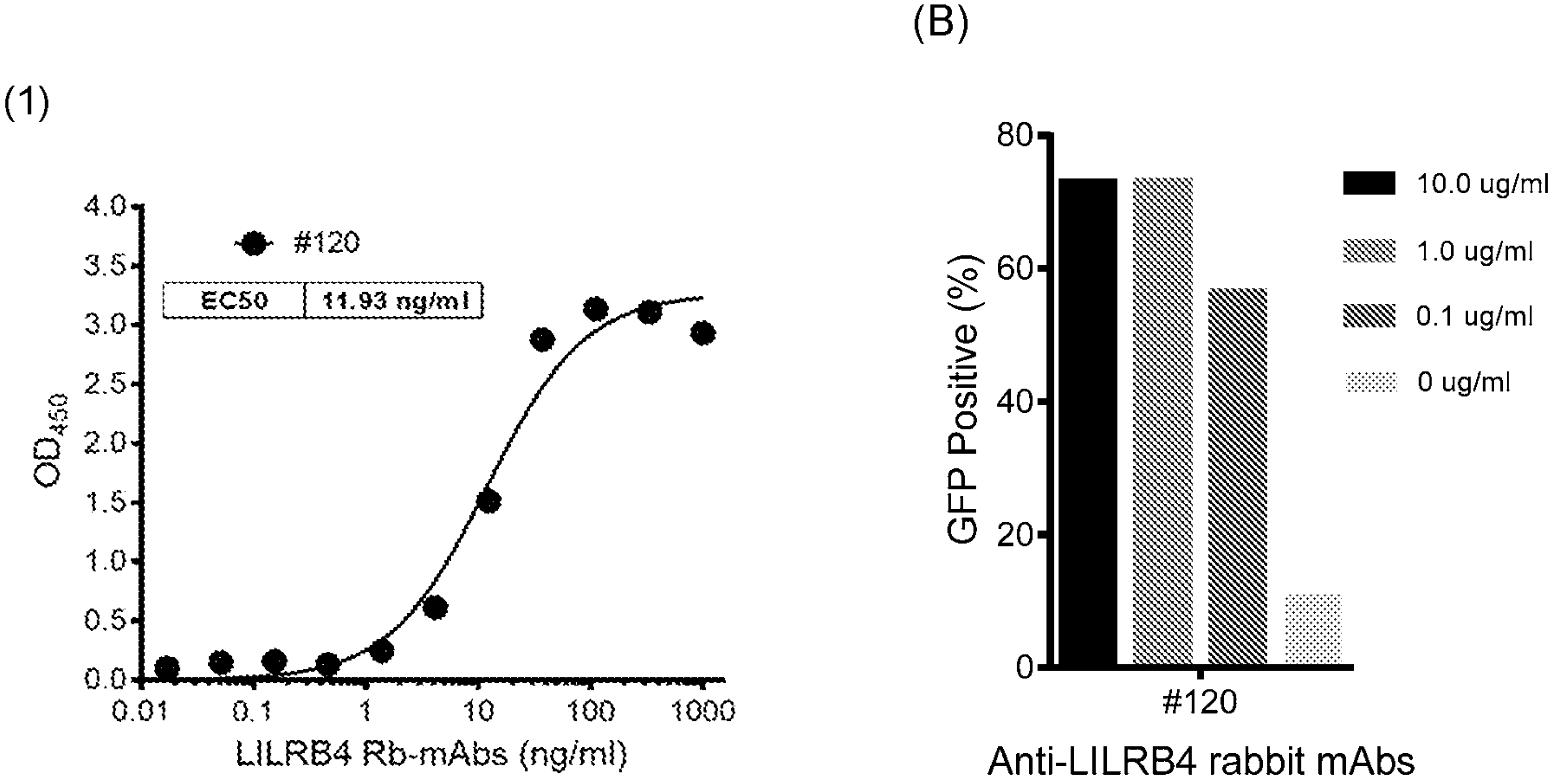


FIG. 18F

#78

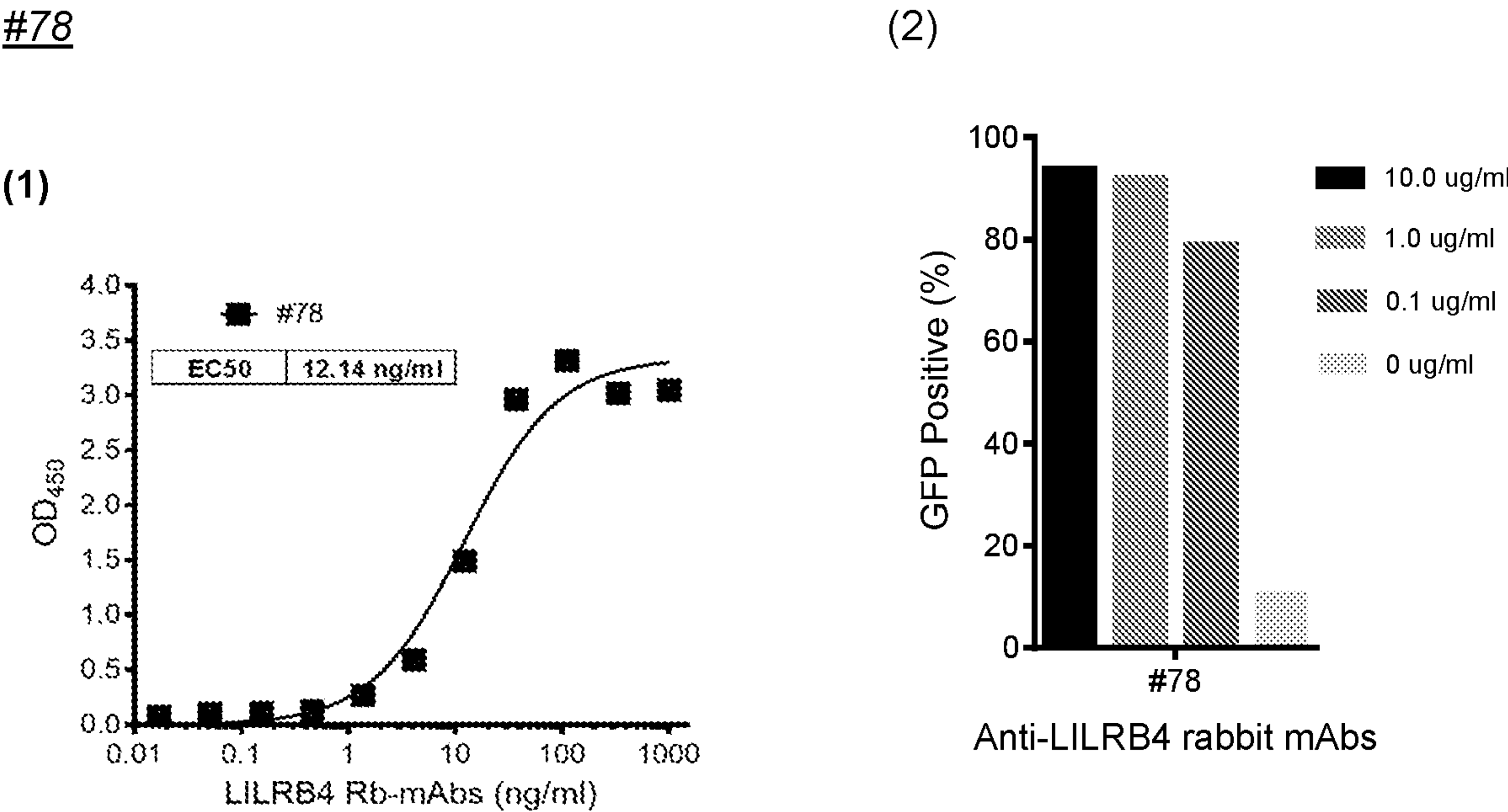
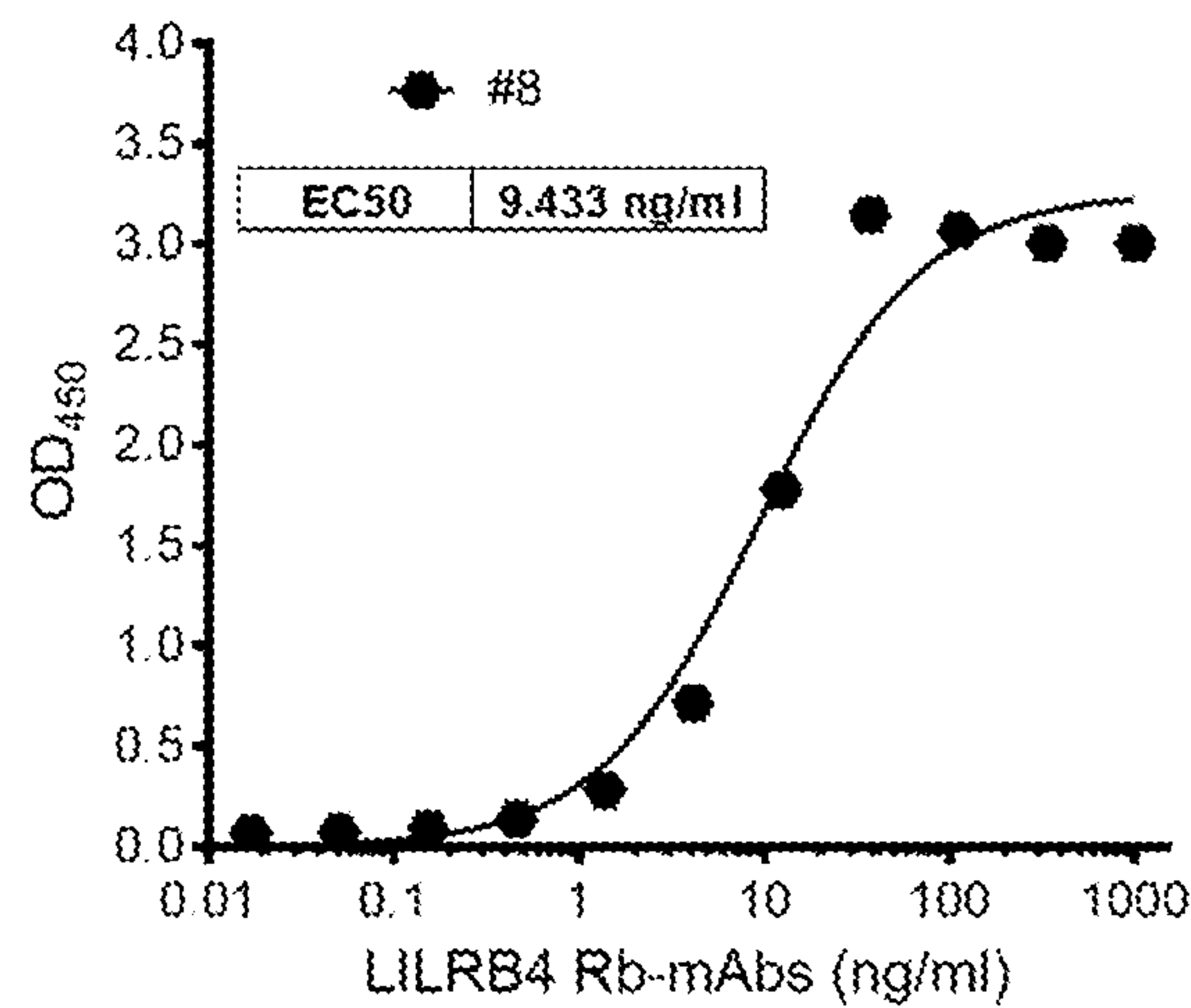


FIG. 18G

#8

(1)



(2)

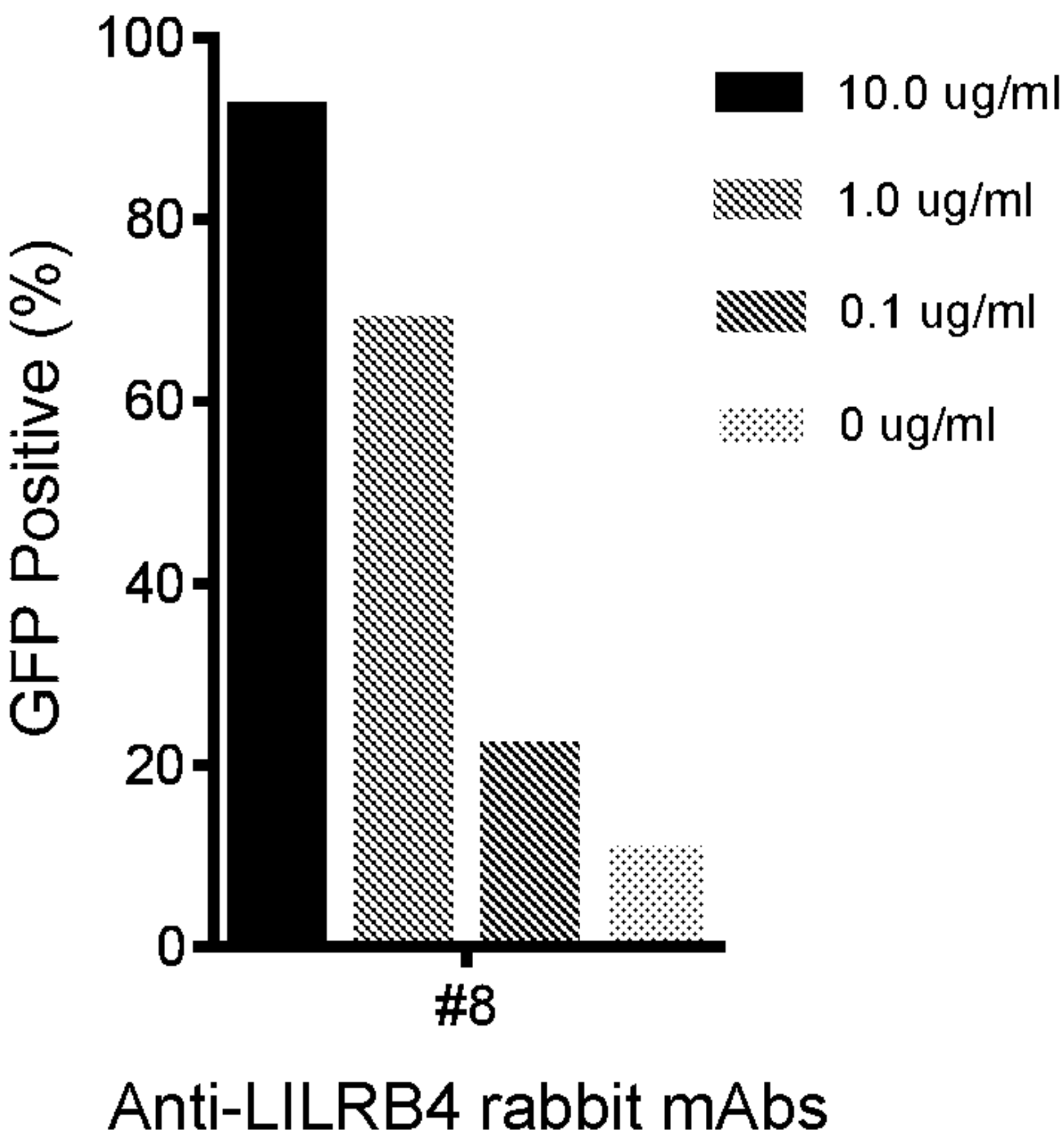
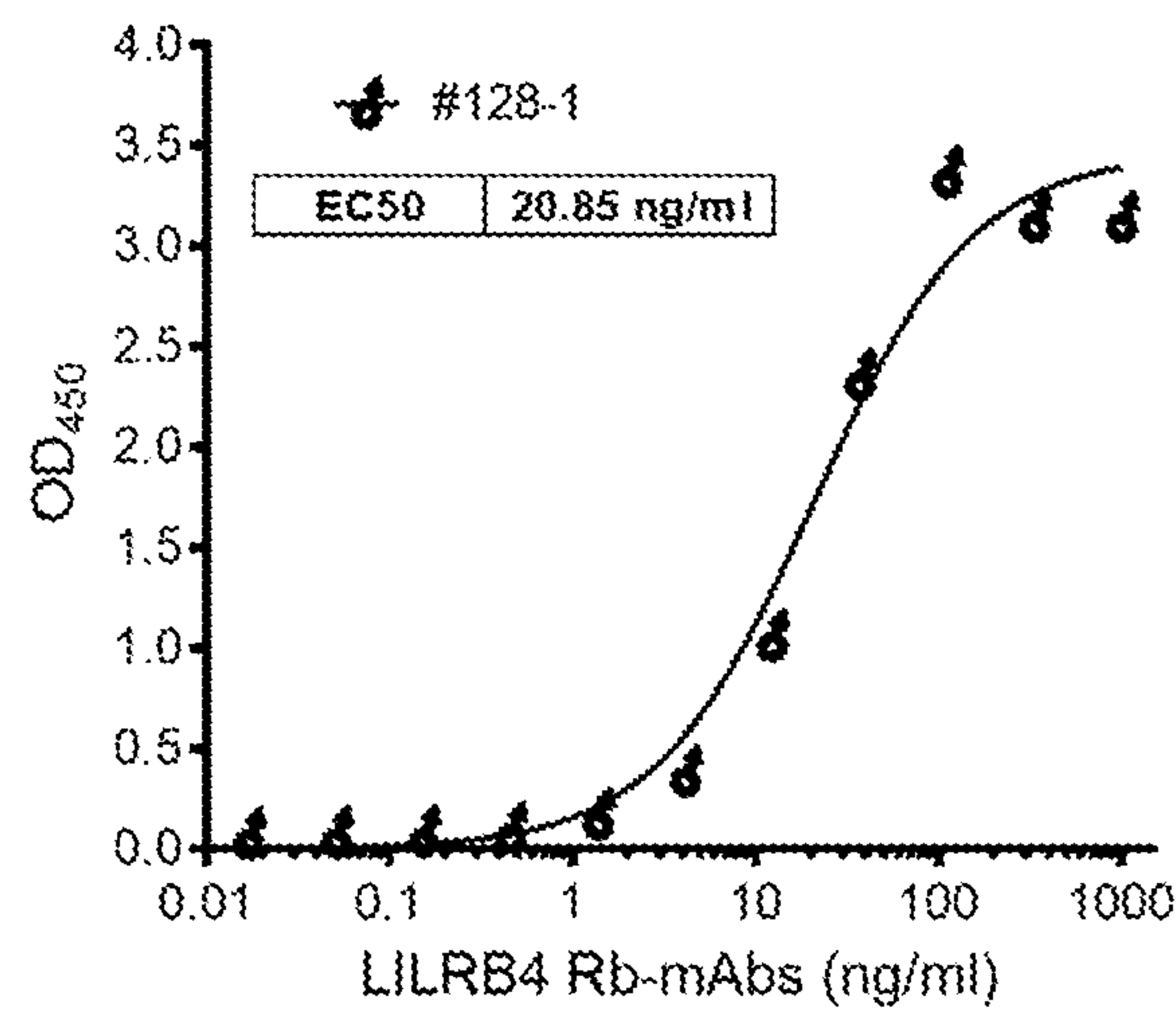


FIG. 18H

#128-1

(1)



(2)

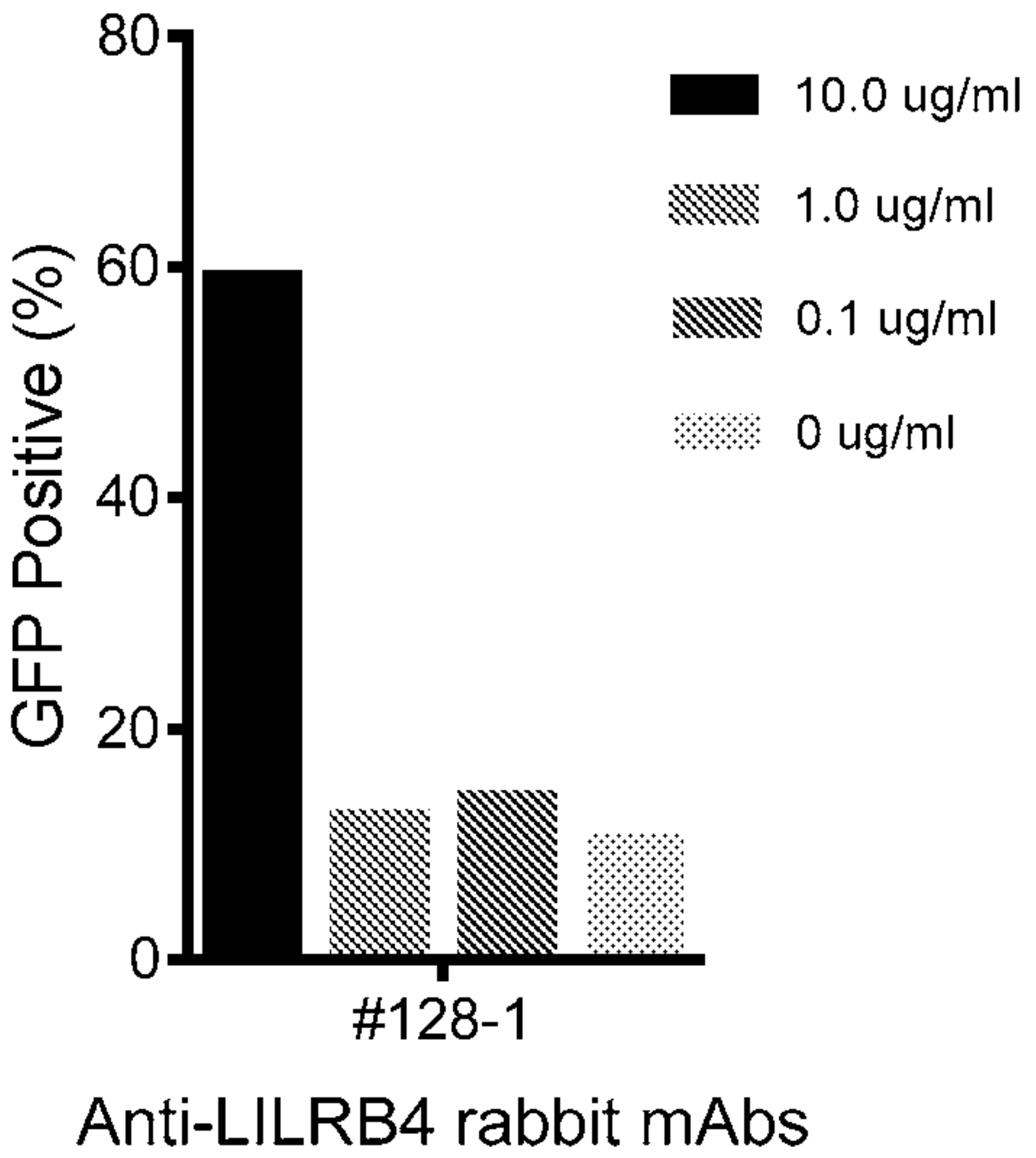


FIG. 18I

#128-3

(2)

(1)

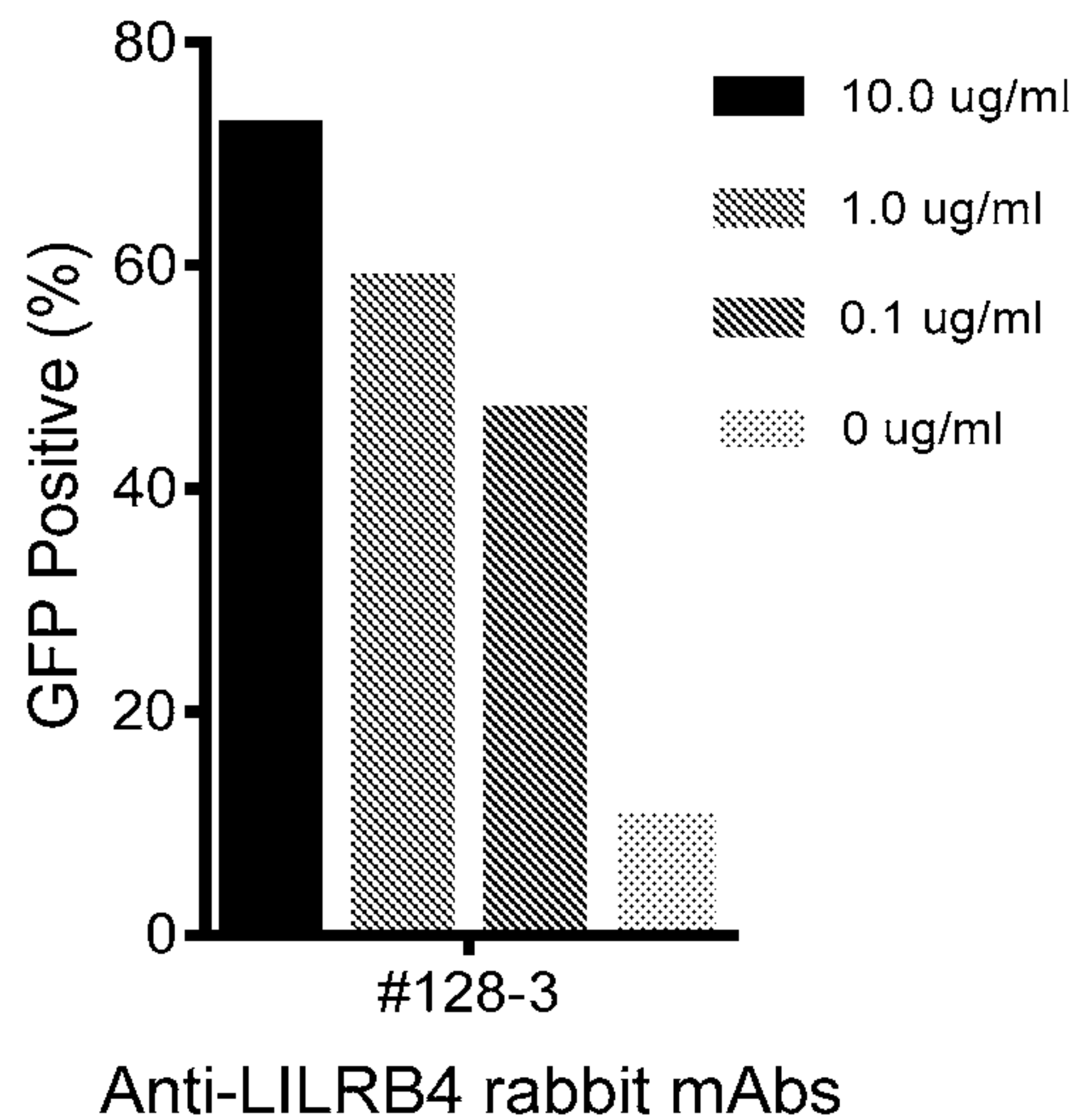
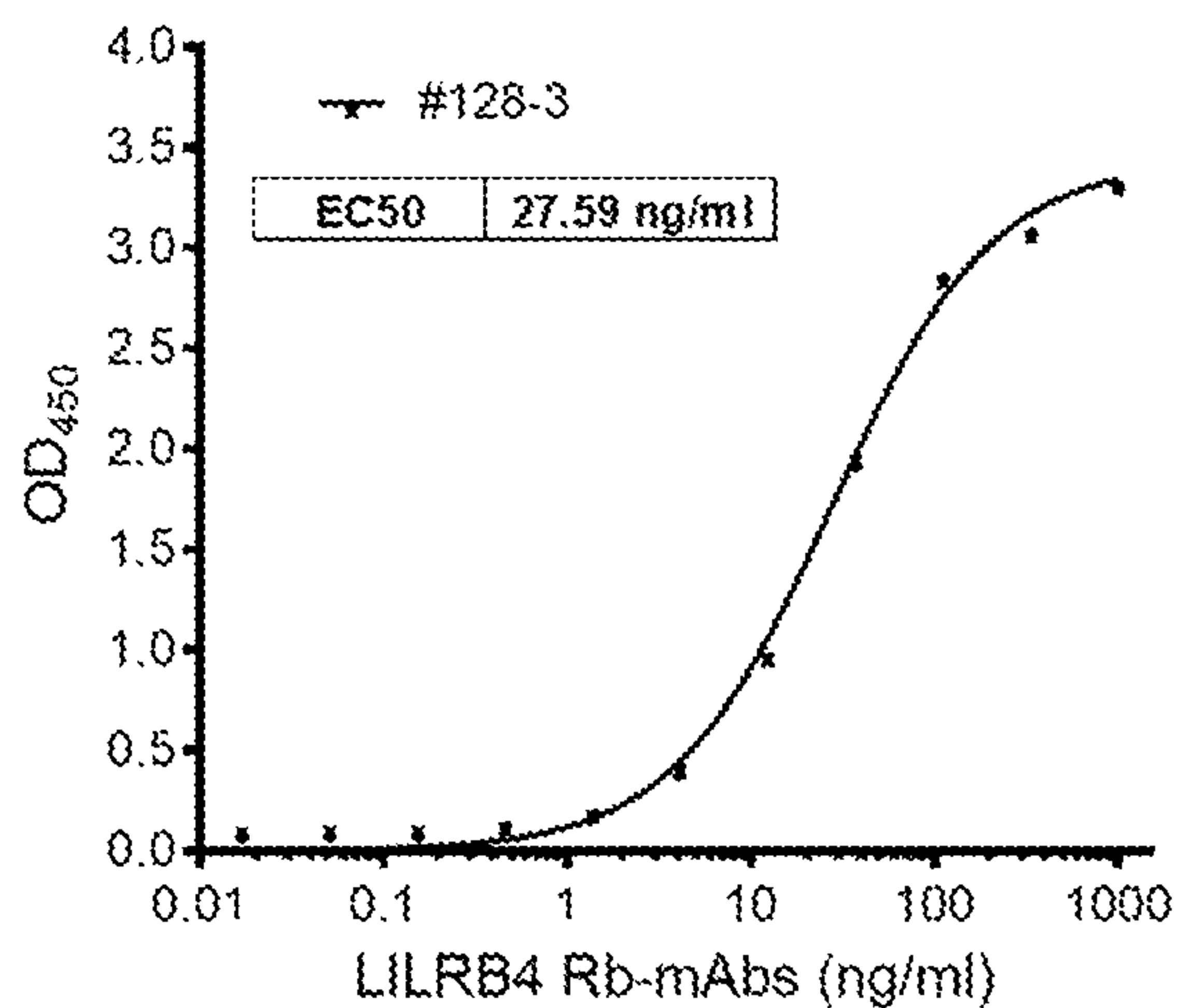


FIG. 18J

#140

(2)

(1)

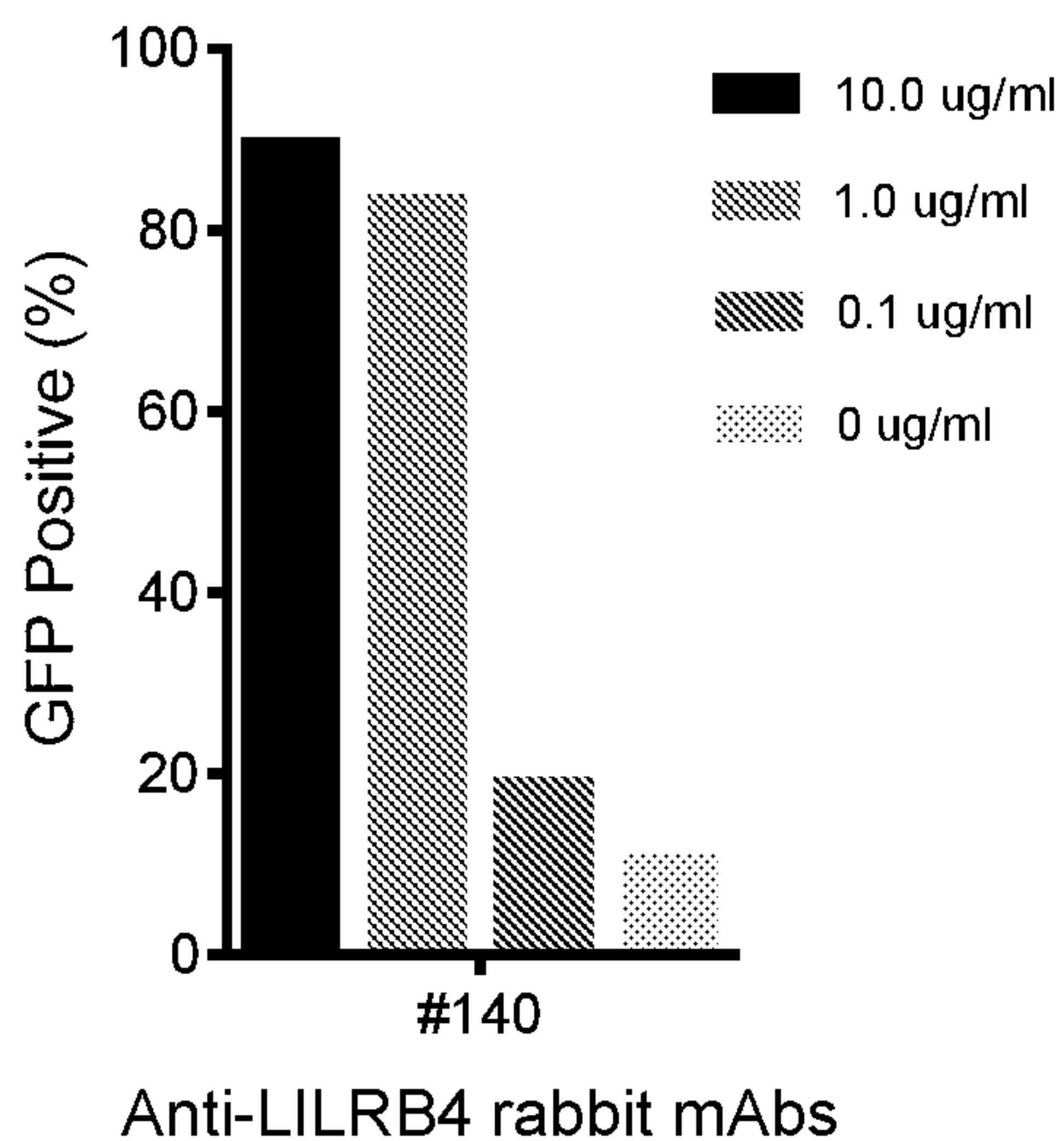
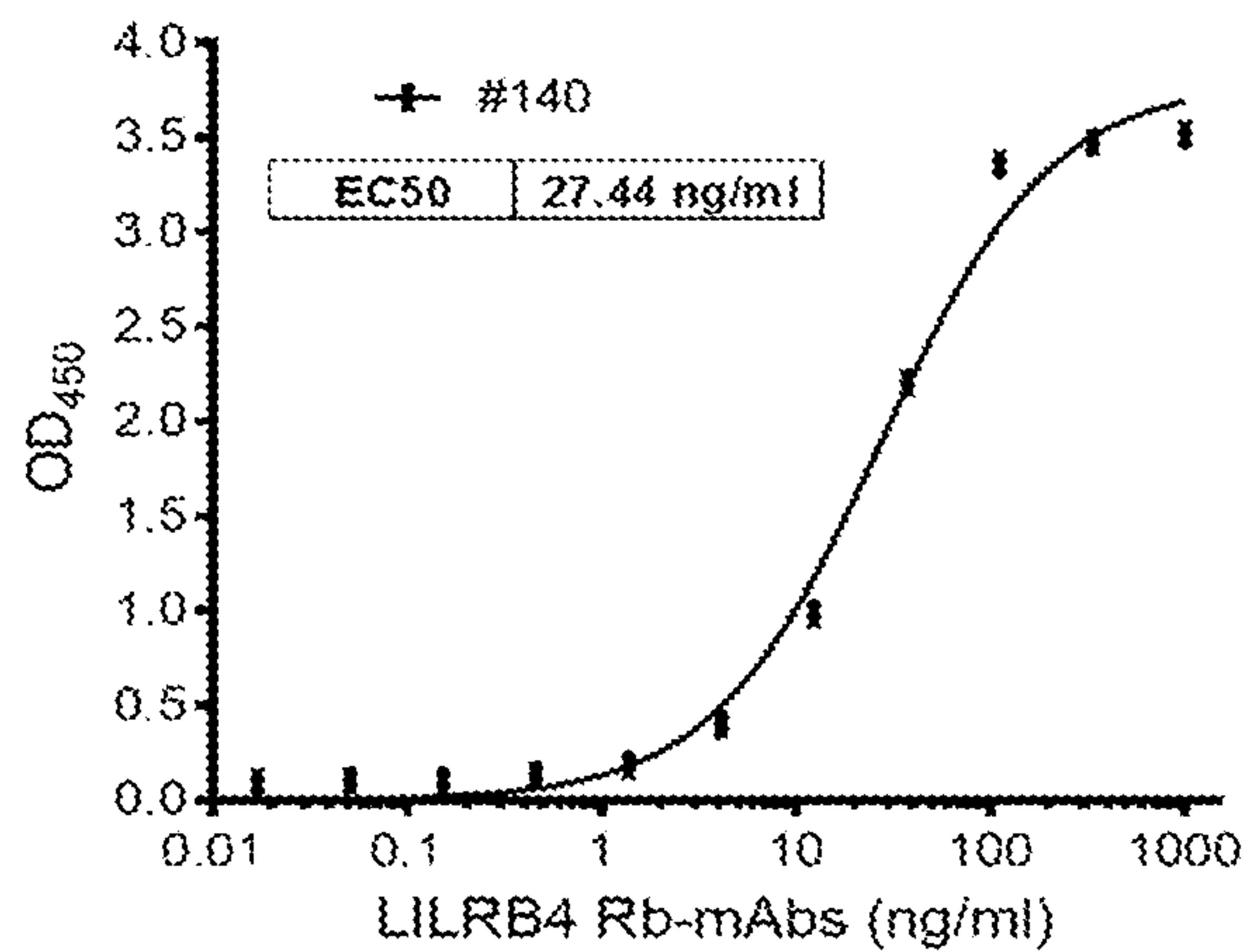


FIG. 18K

#216-1

(2)

(1)

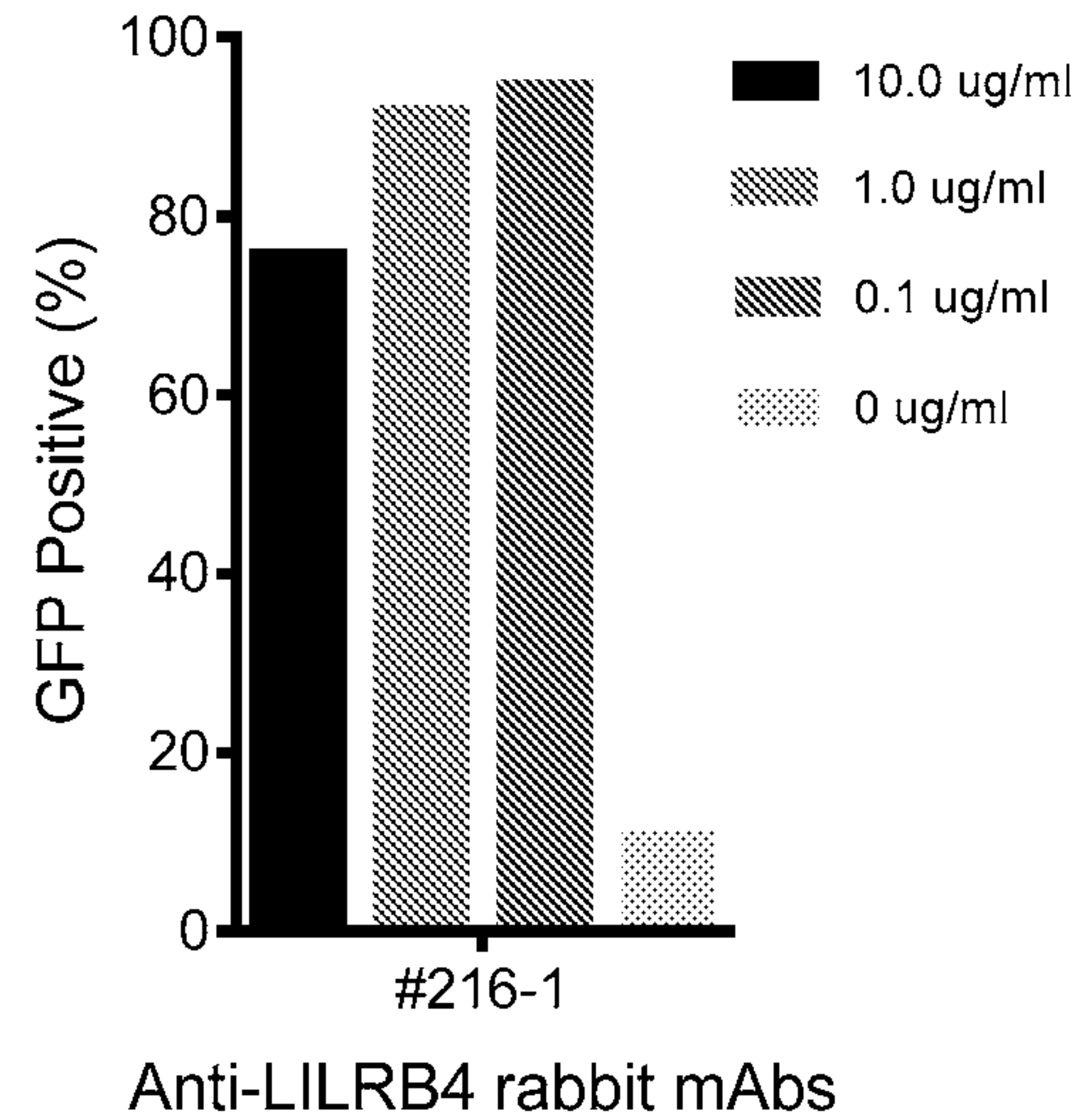
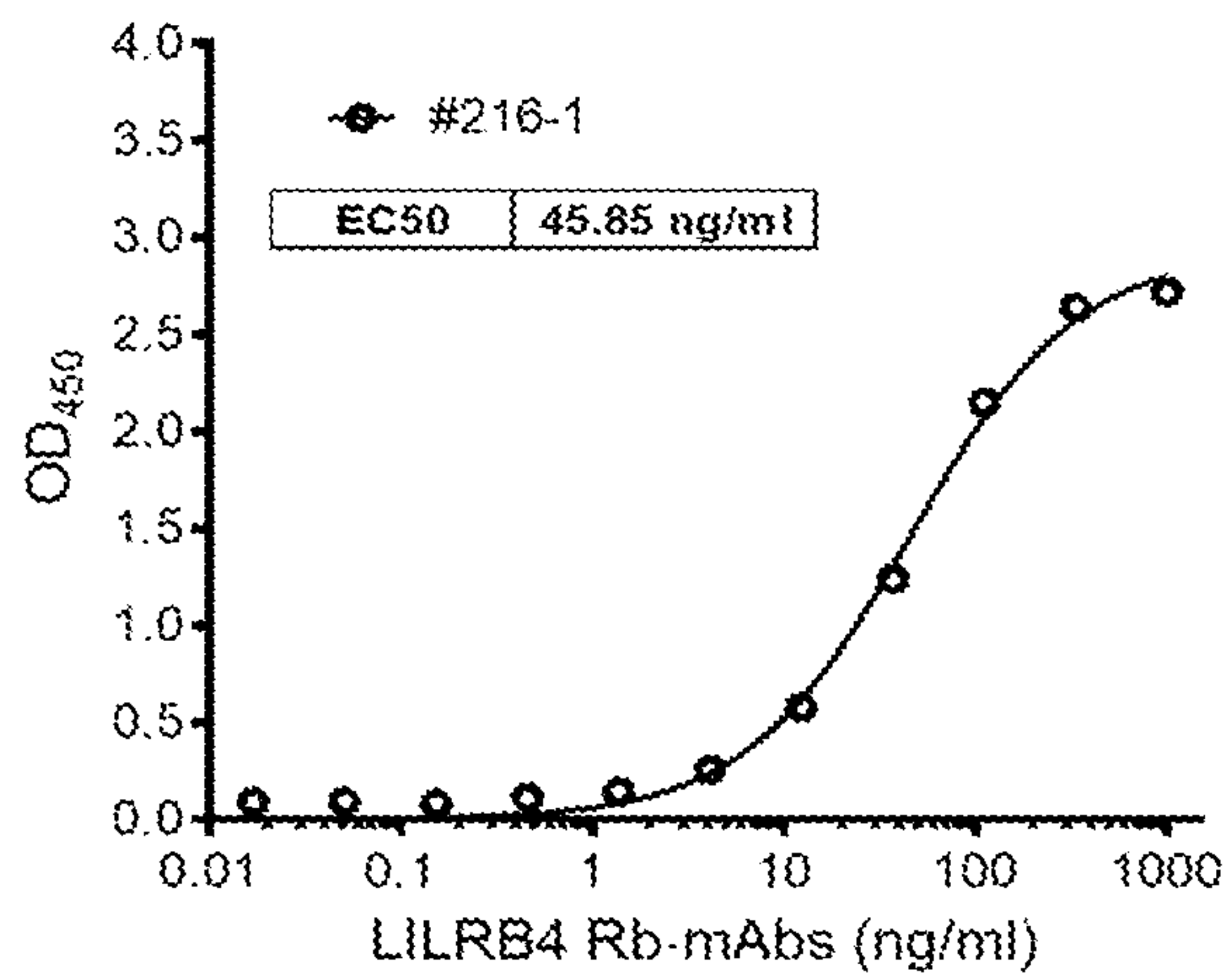


FIG. 18L

#216-2

(2)

(1)

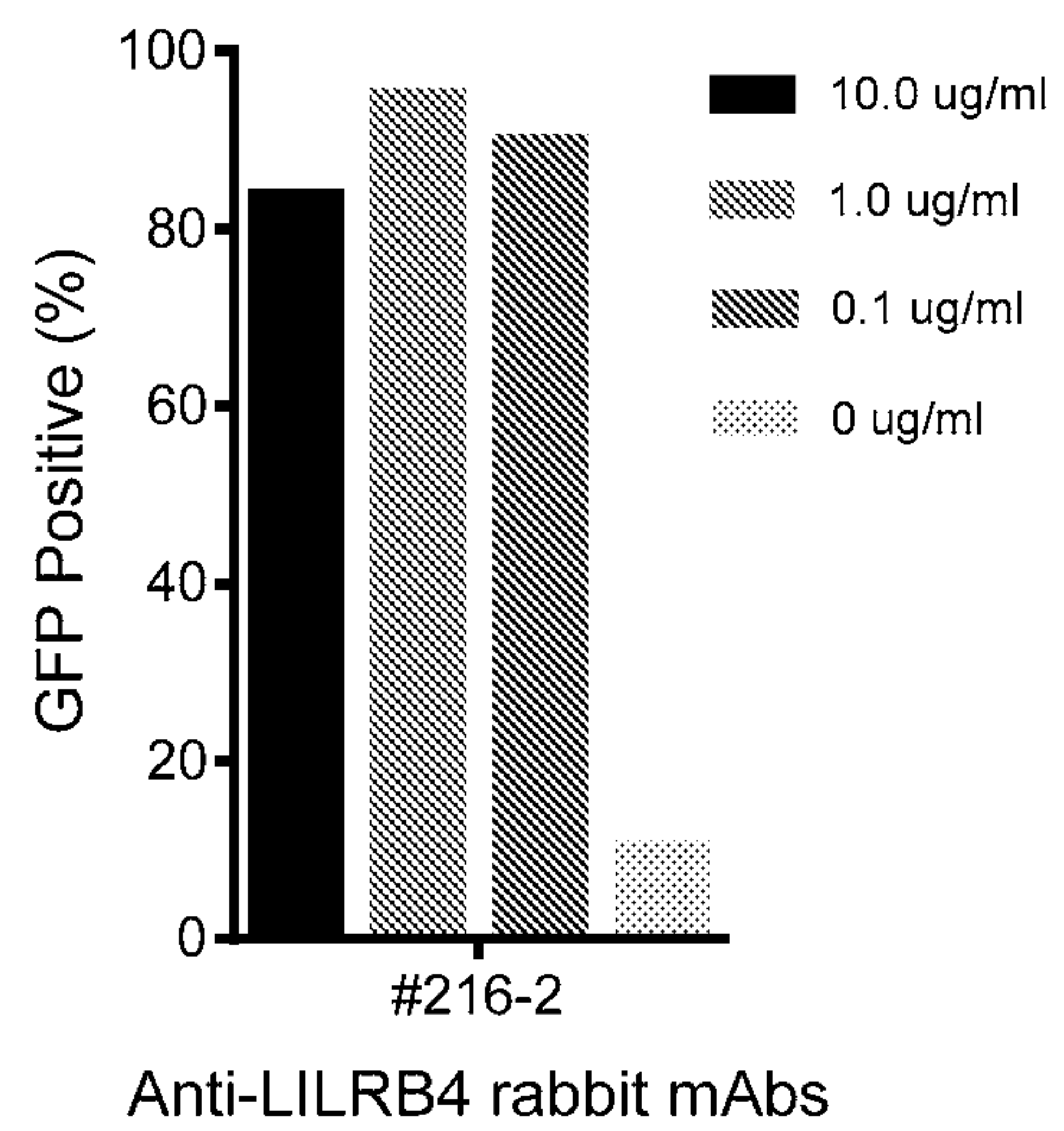
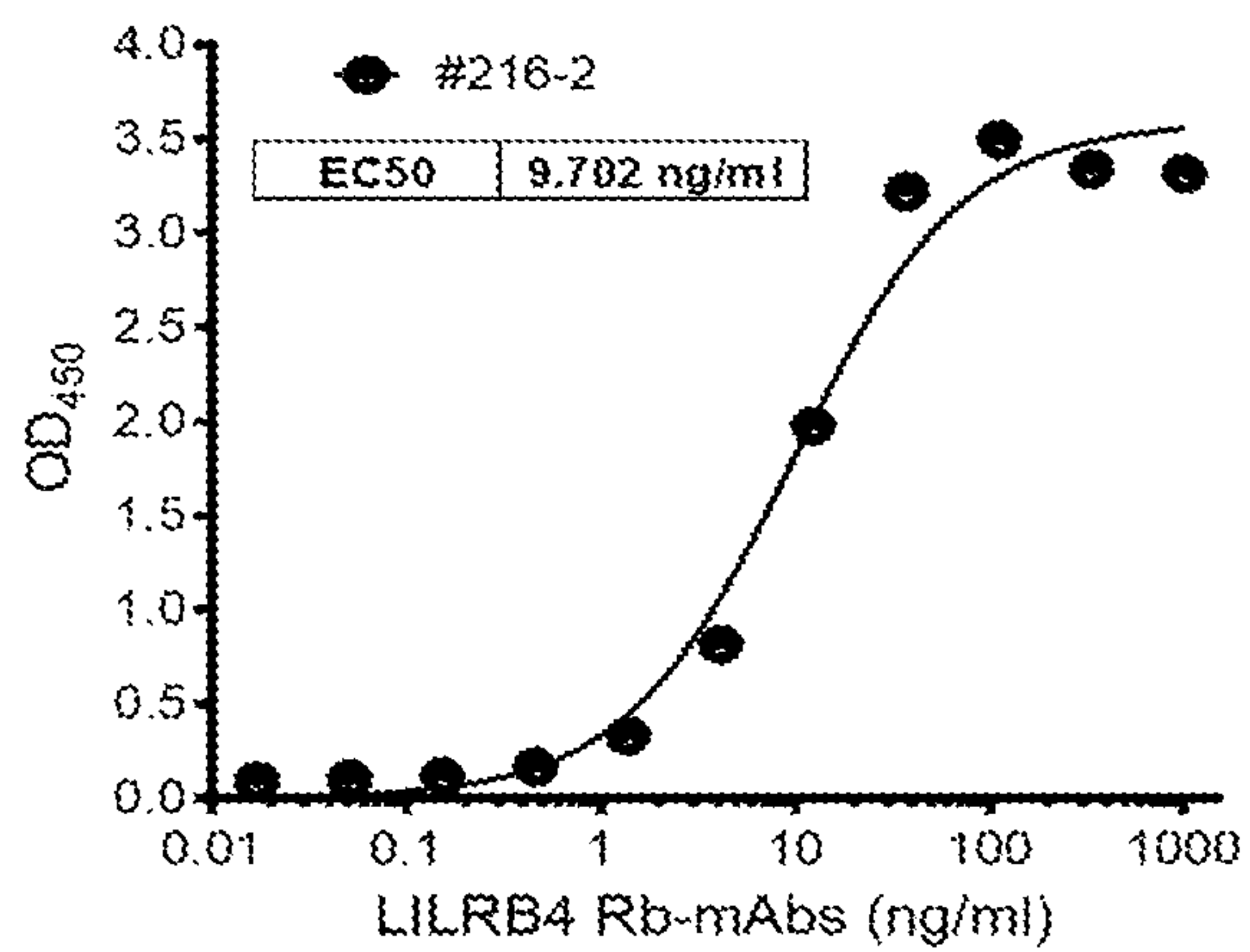


FIG. 18M

#101

(2)

(1)

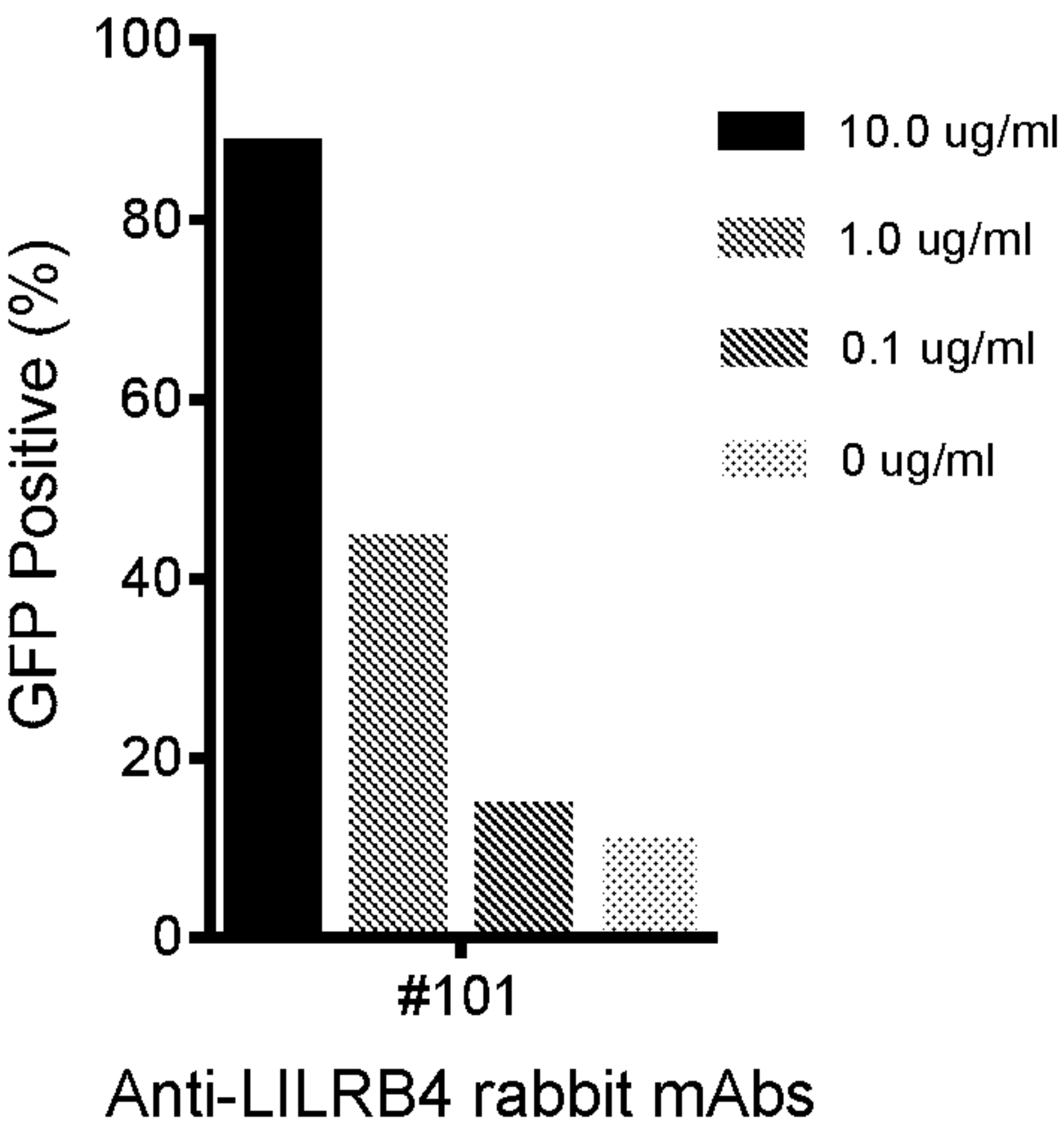
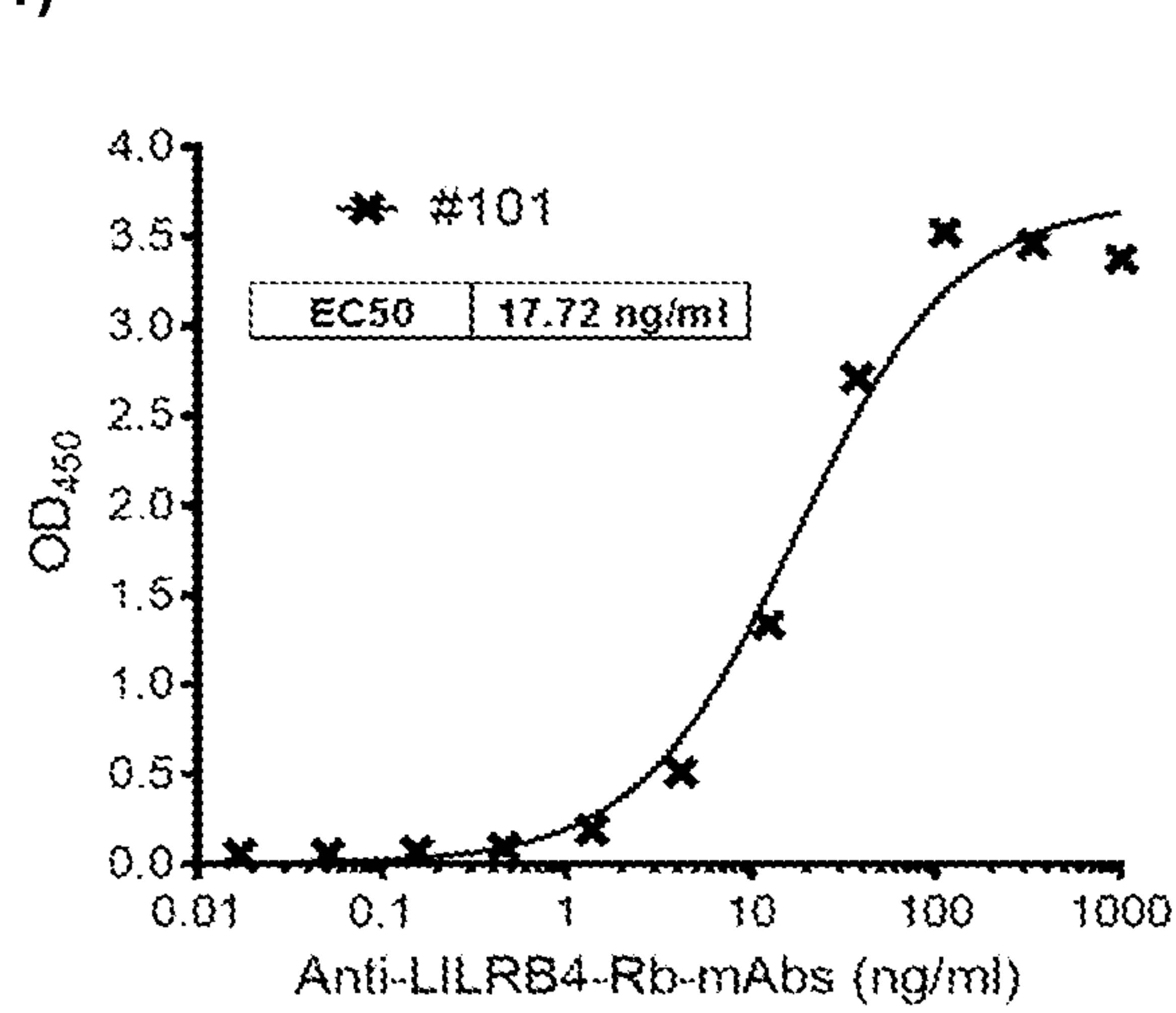


FIG. 18N

#223

(2)

(1)

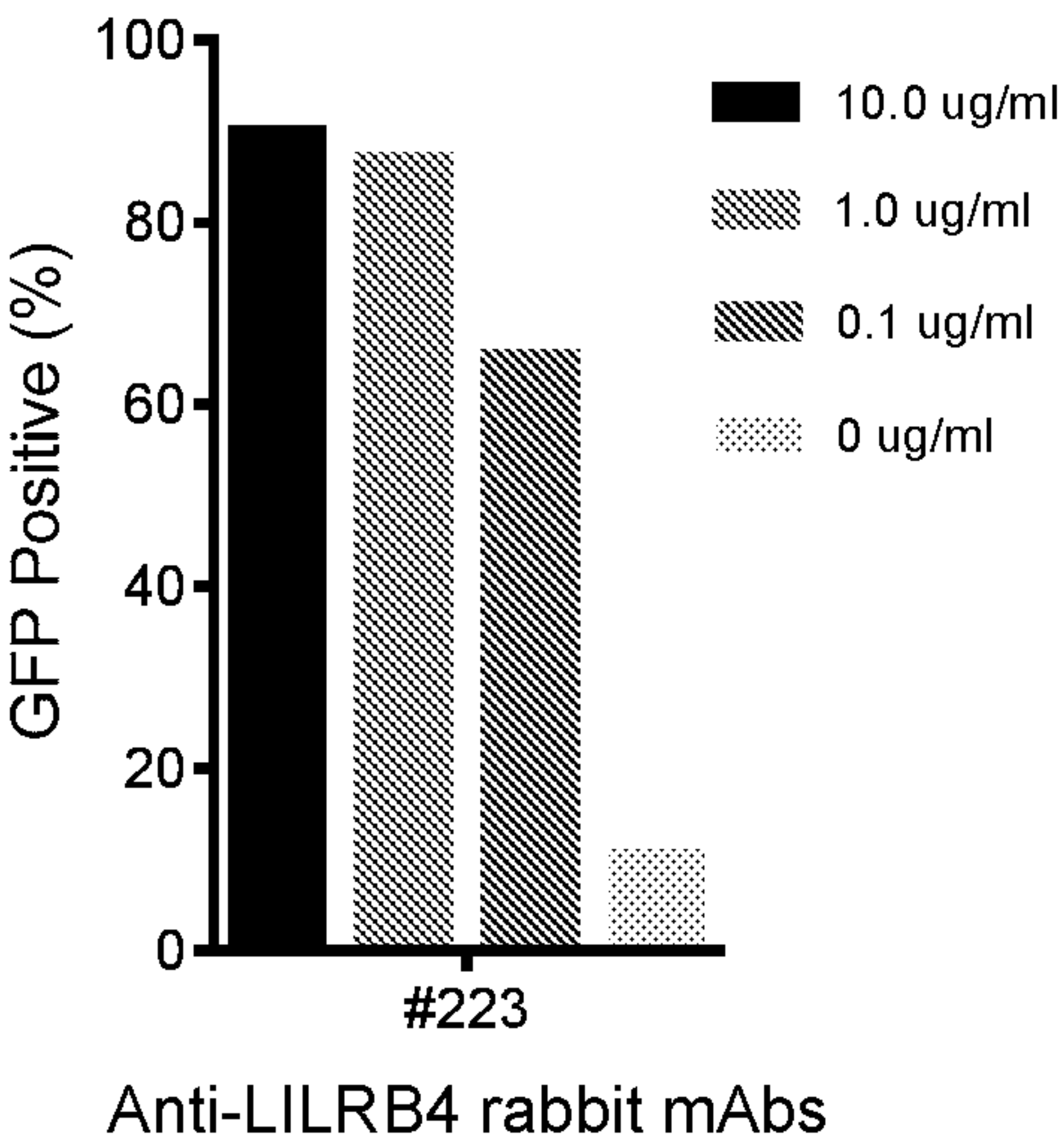
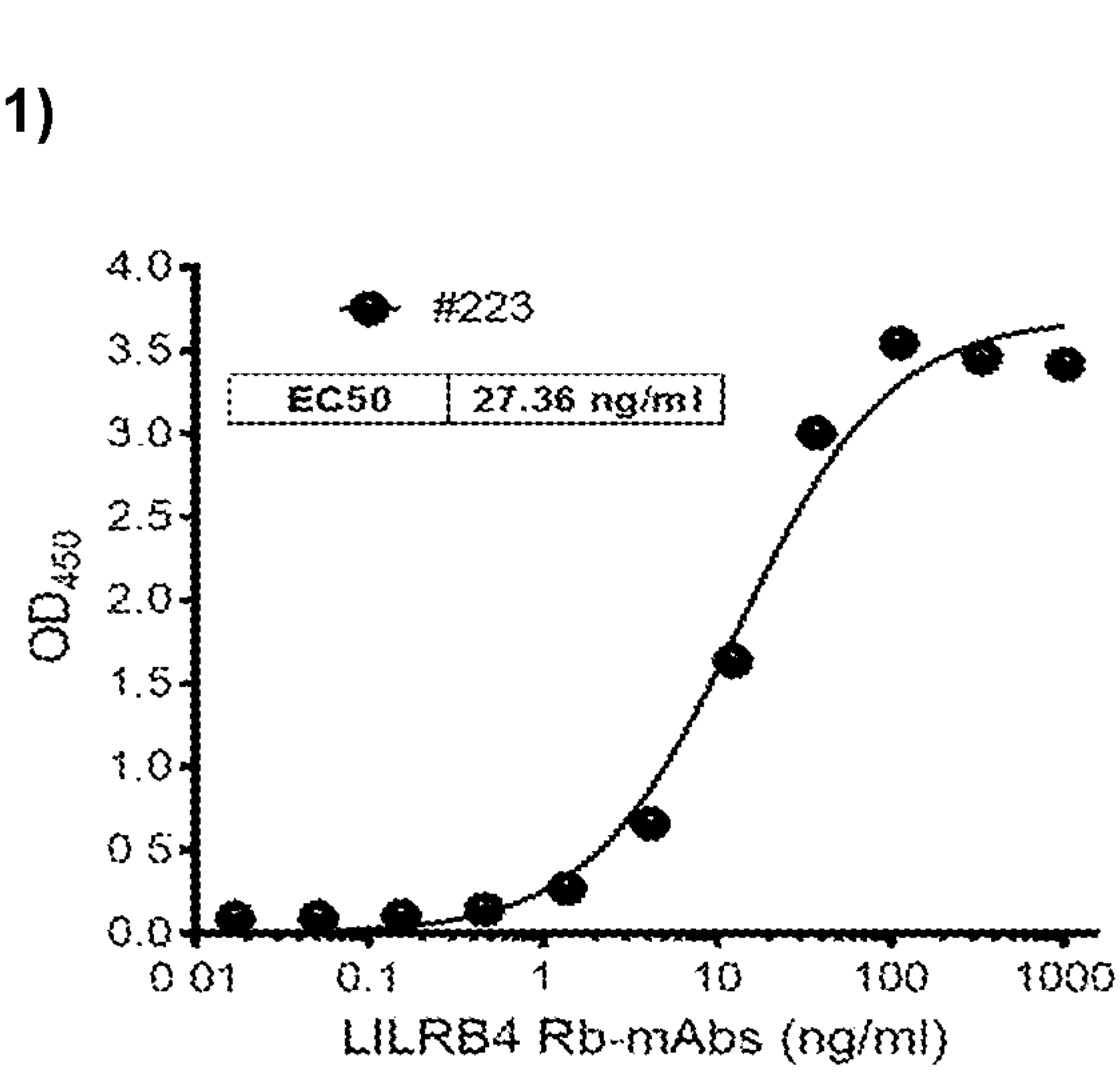
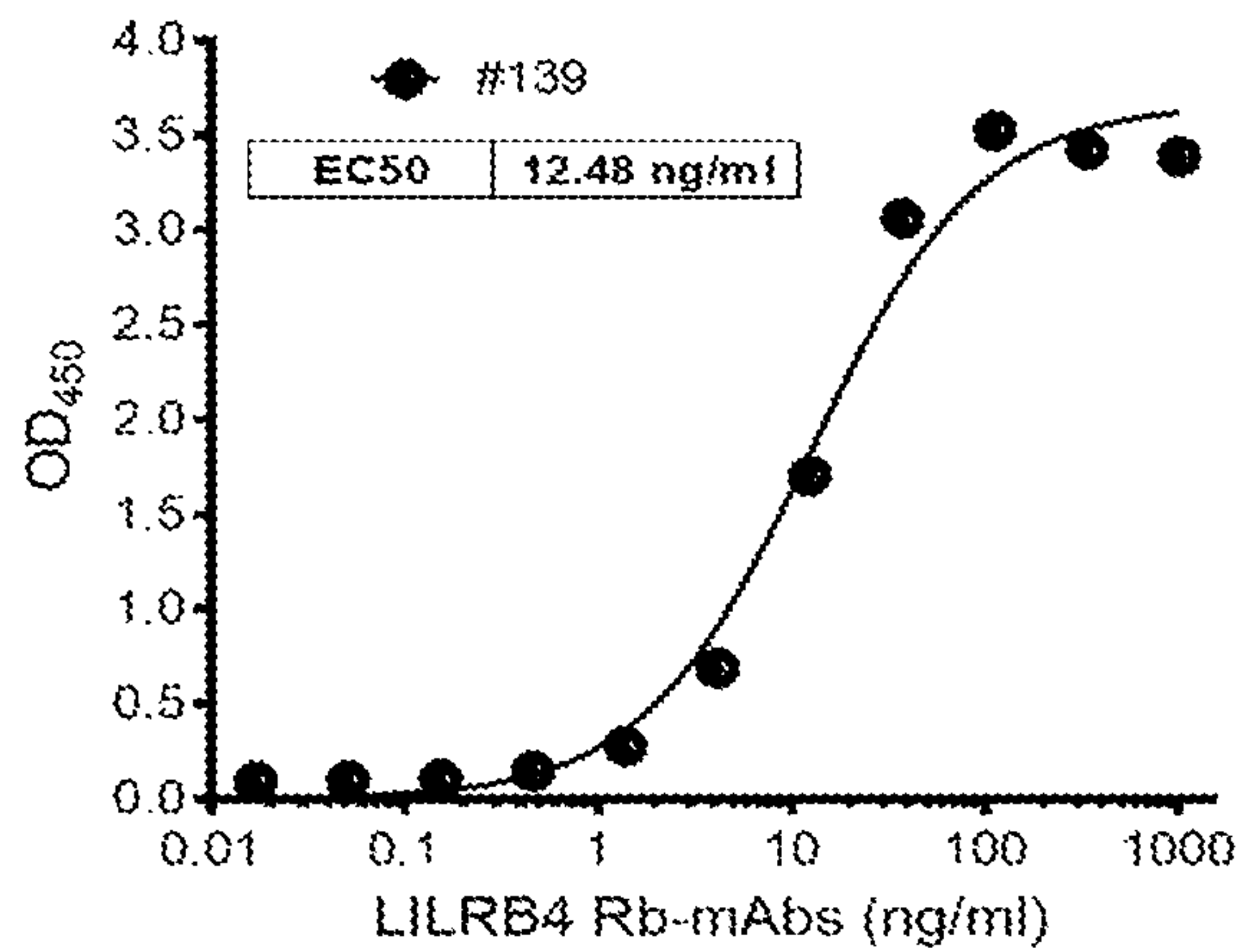


FIG. 18O

#139

(A)



(B)

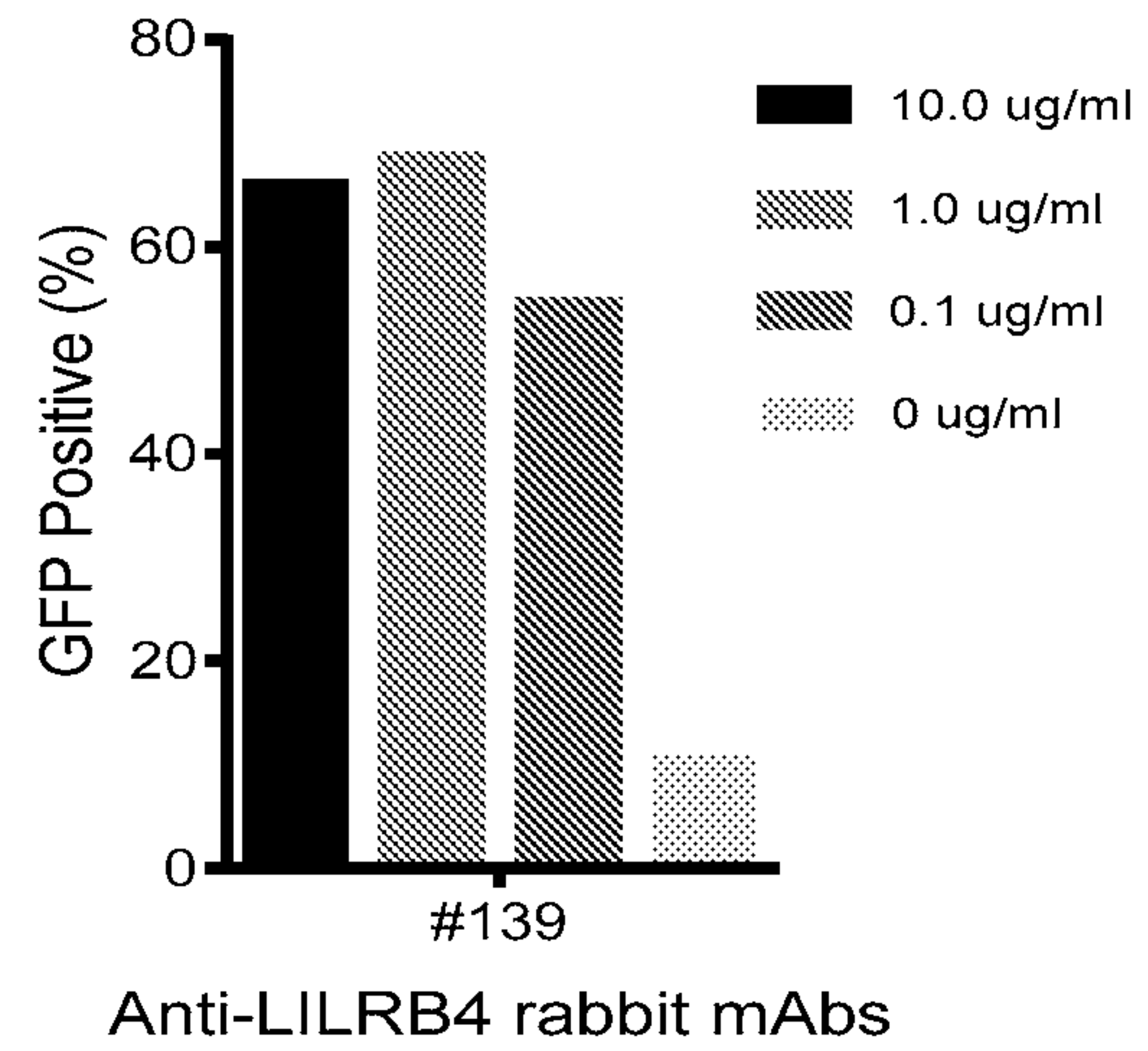
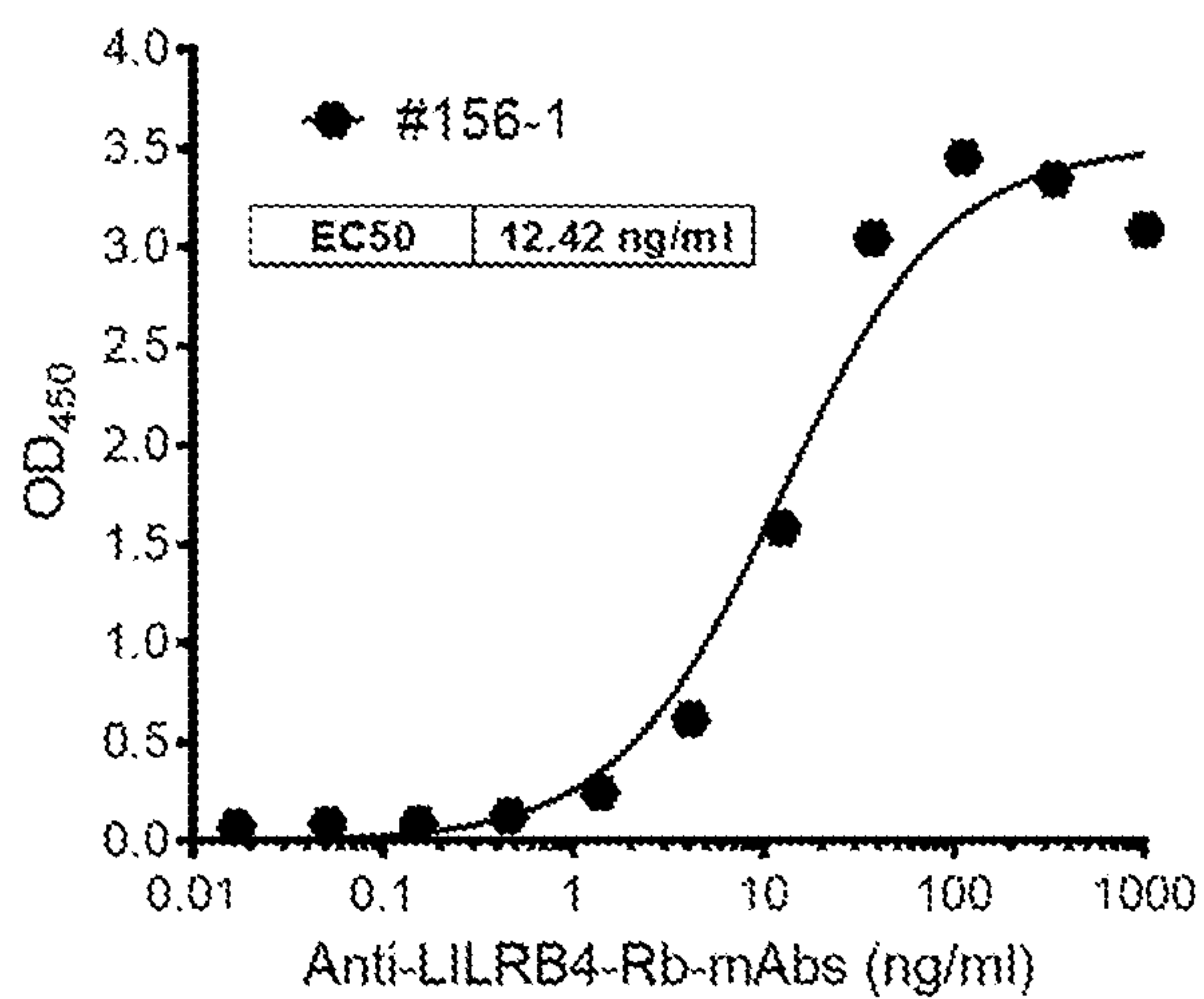


FIG. 18P

#156-1

(1)



(2)

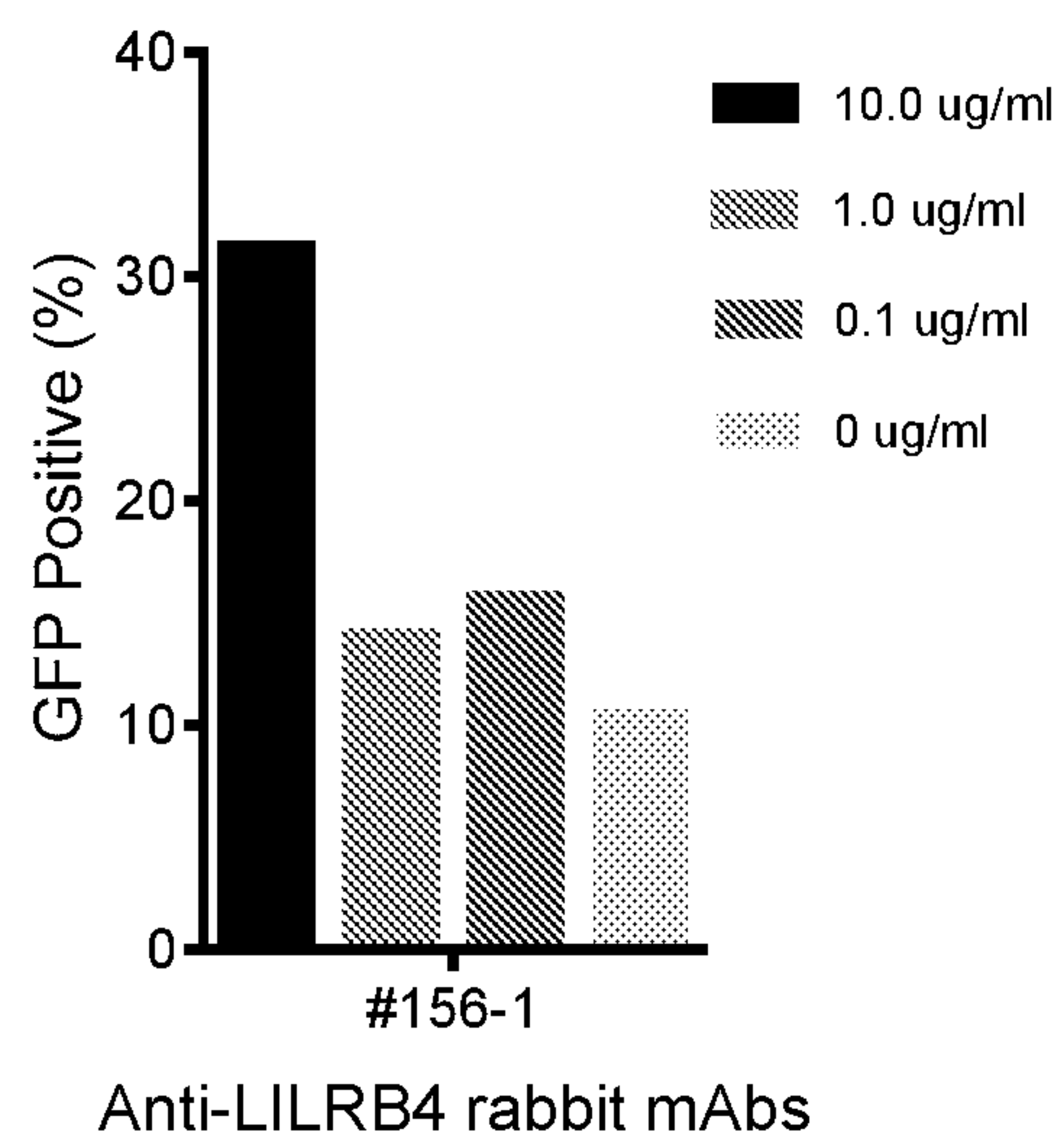
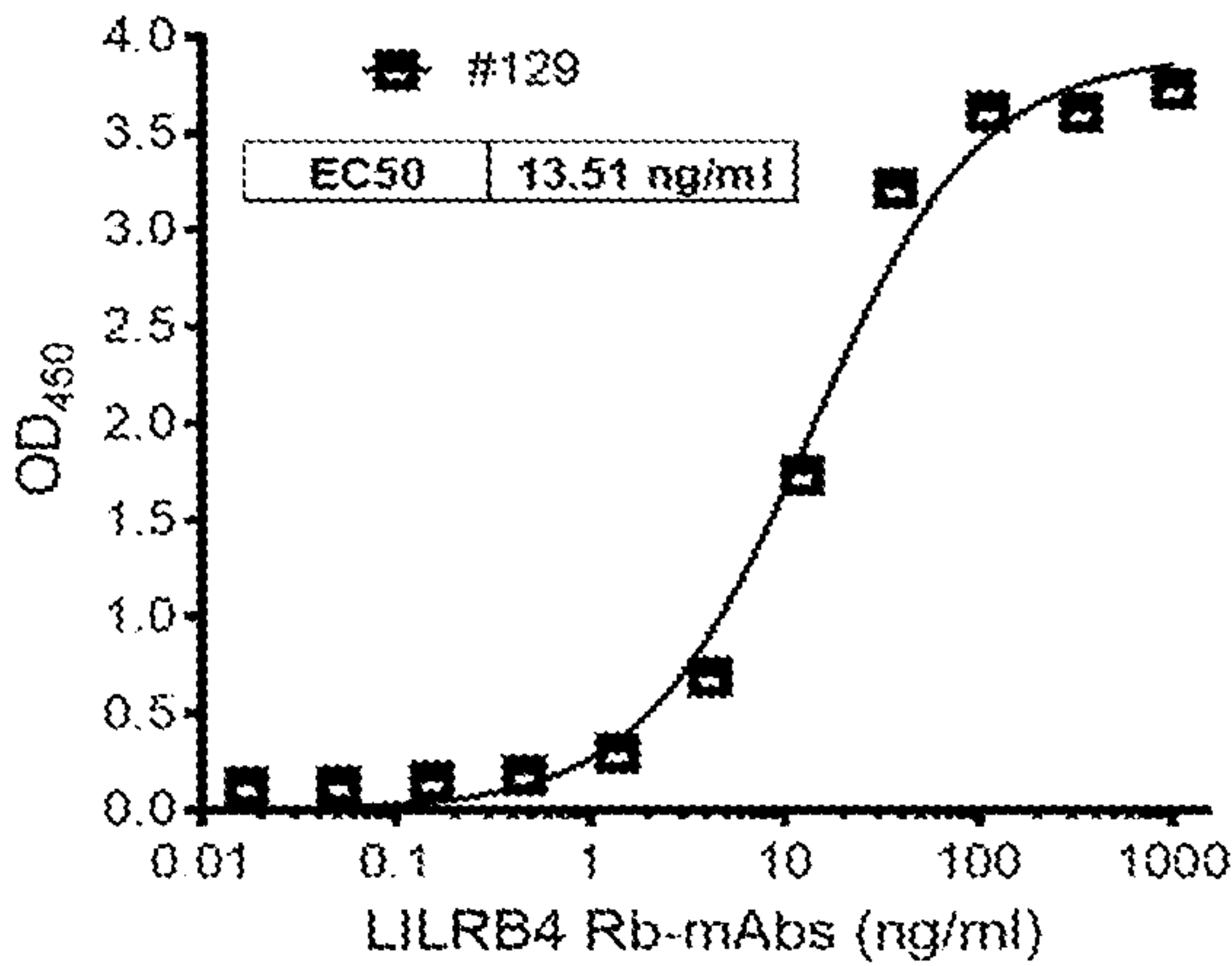


FIG. 18Q

#129

(A)



(B)

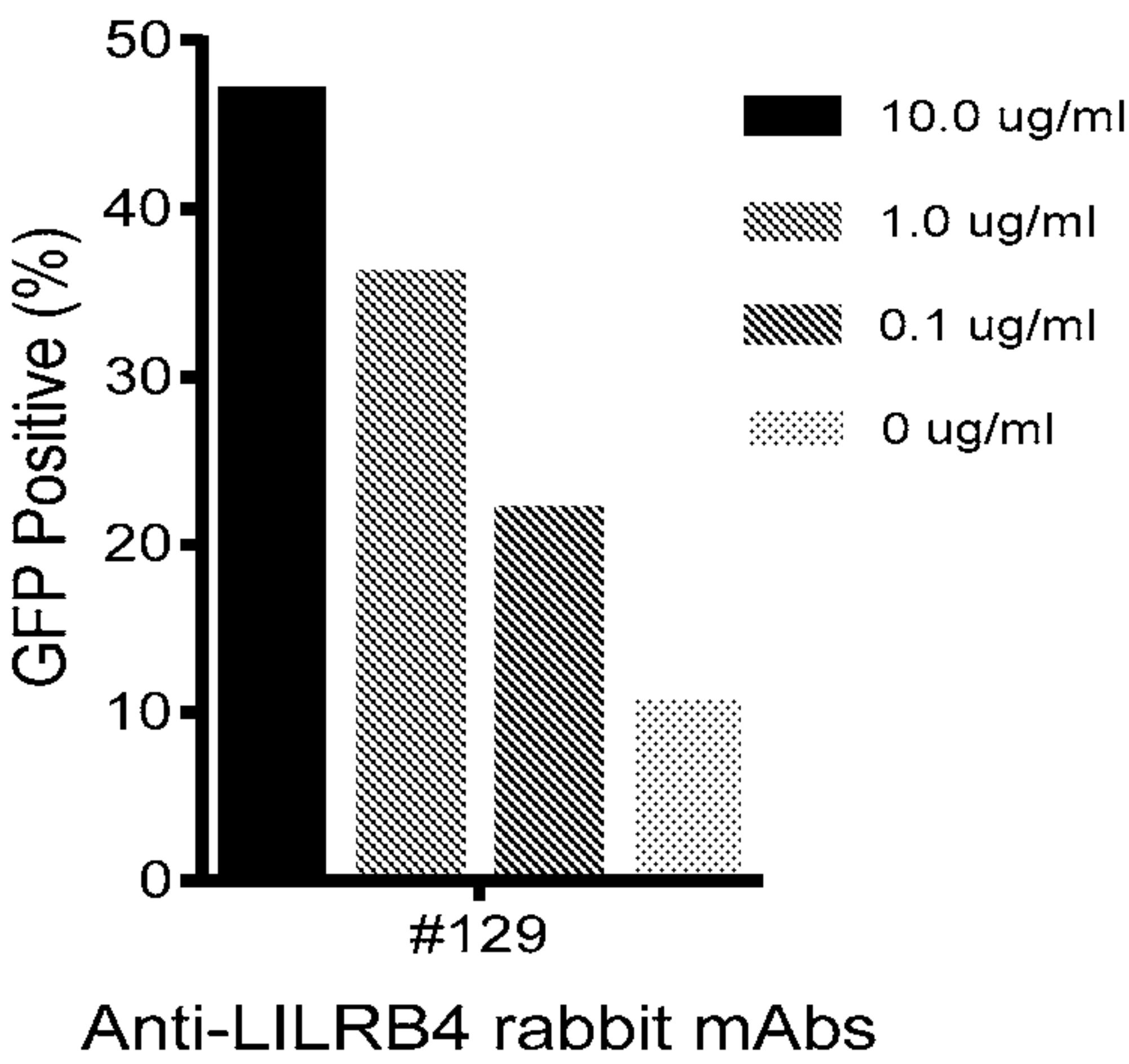
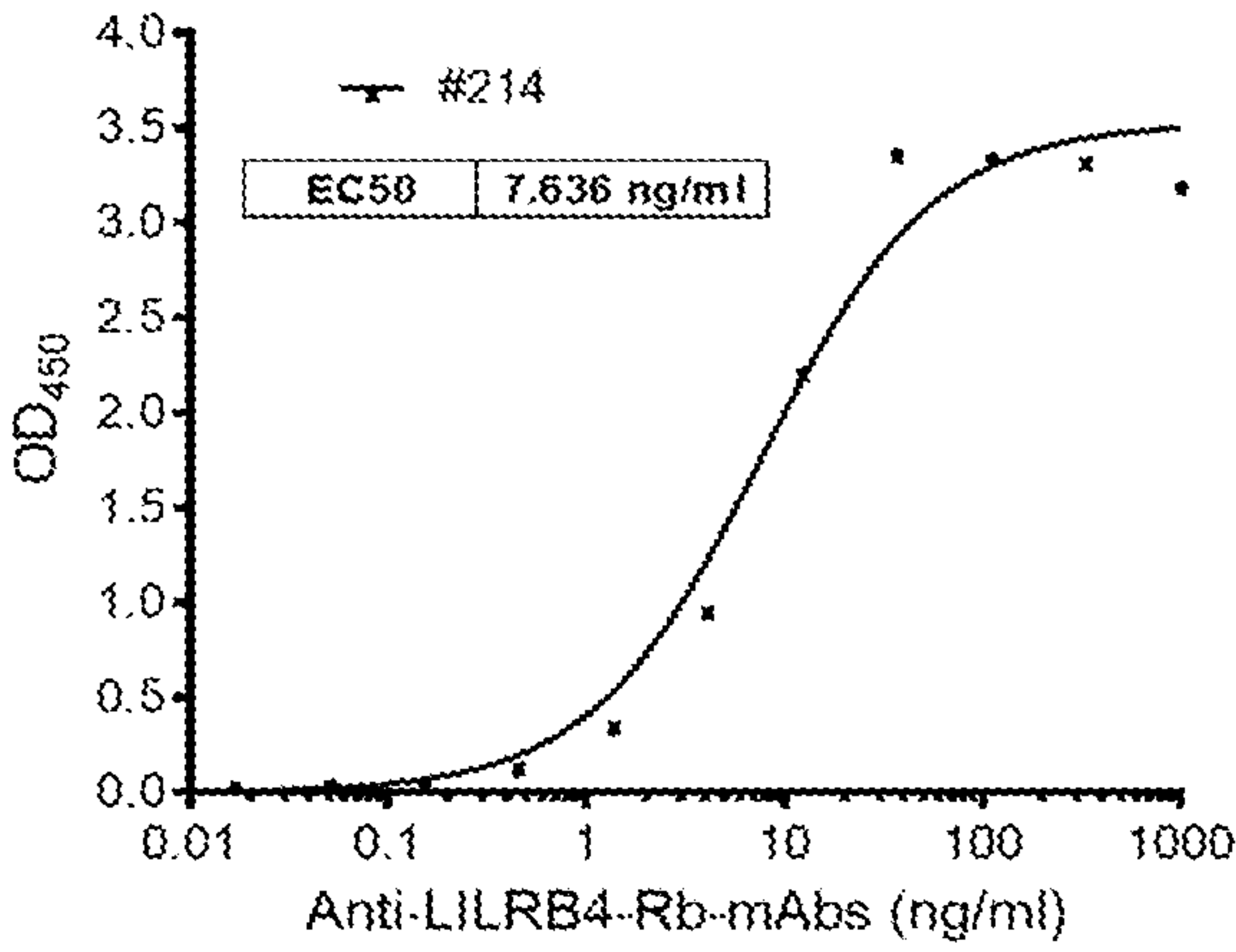


FIG. 18R

#214

(1)



(2)

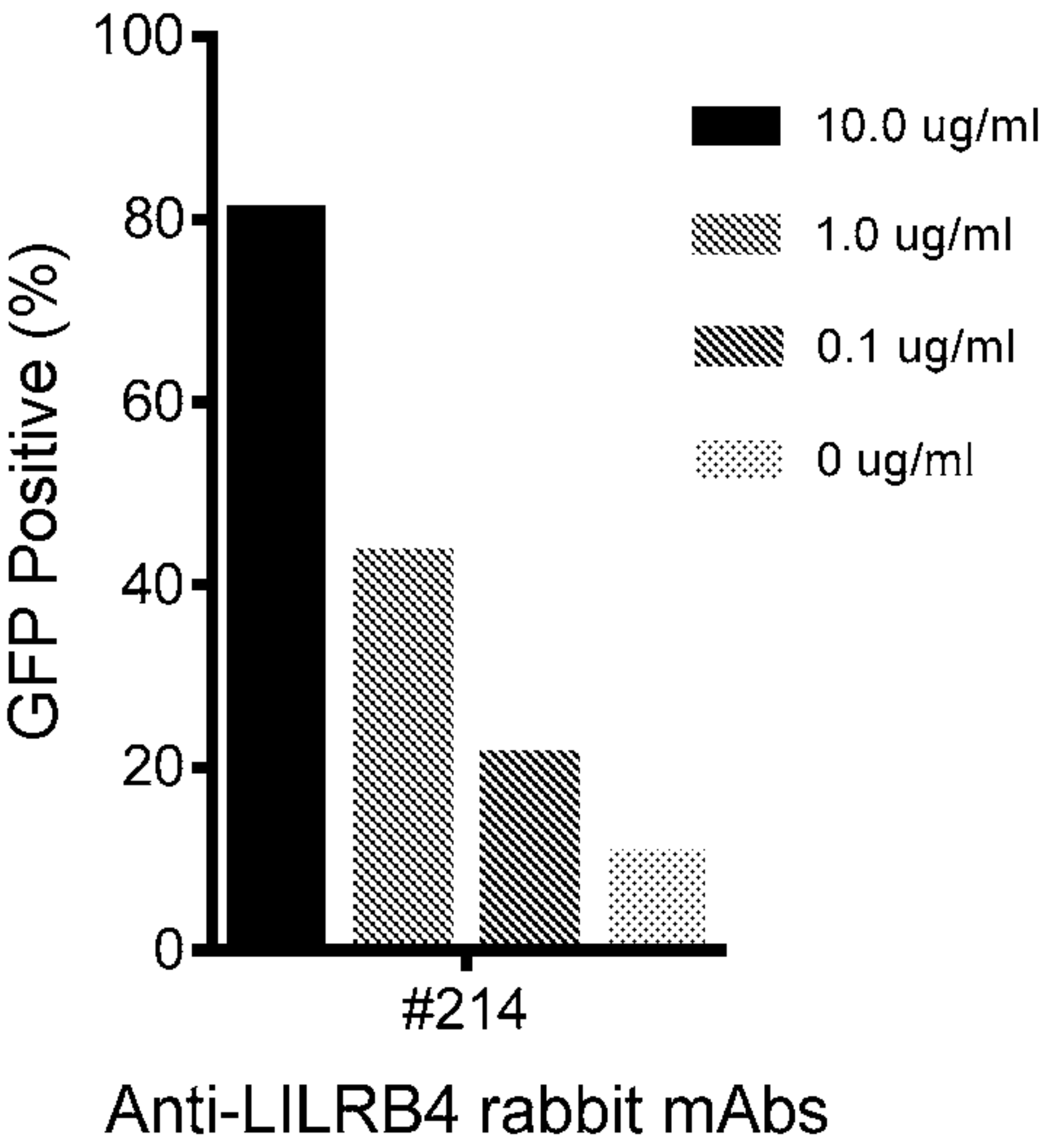


FIG. 18S

#210

(2)

(1)

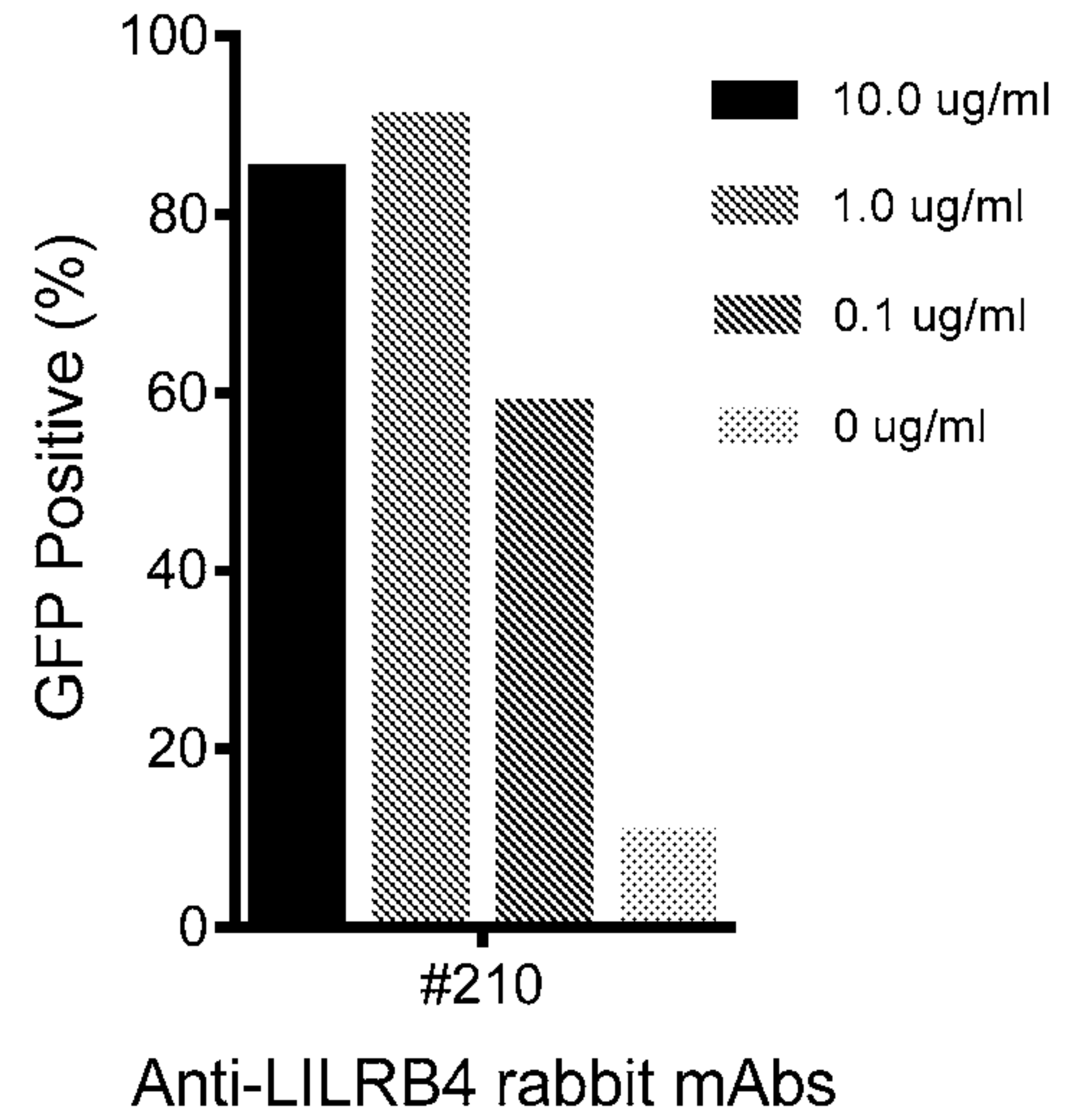
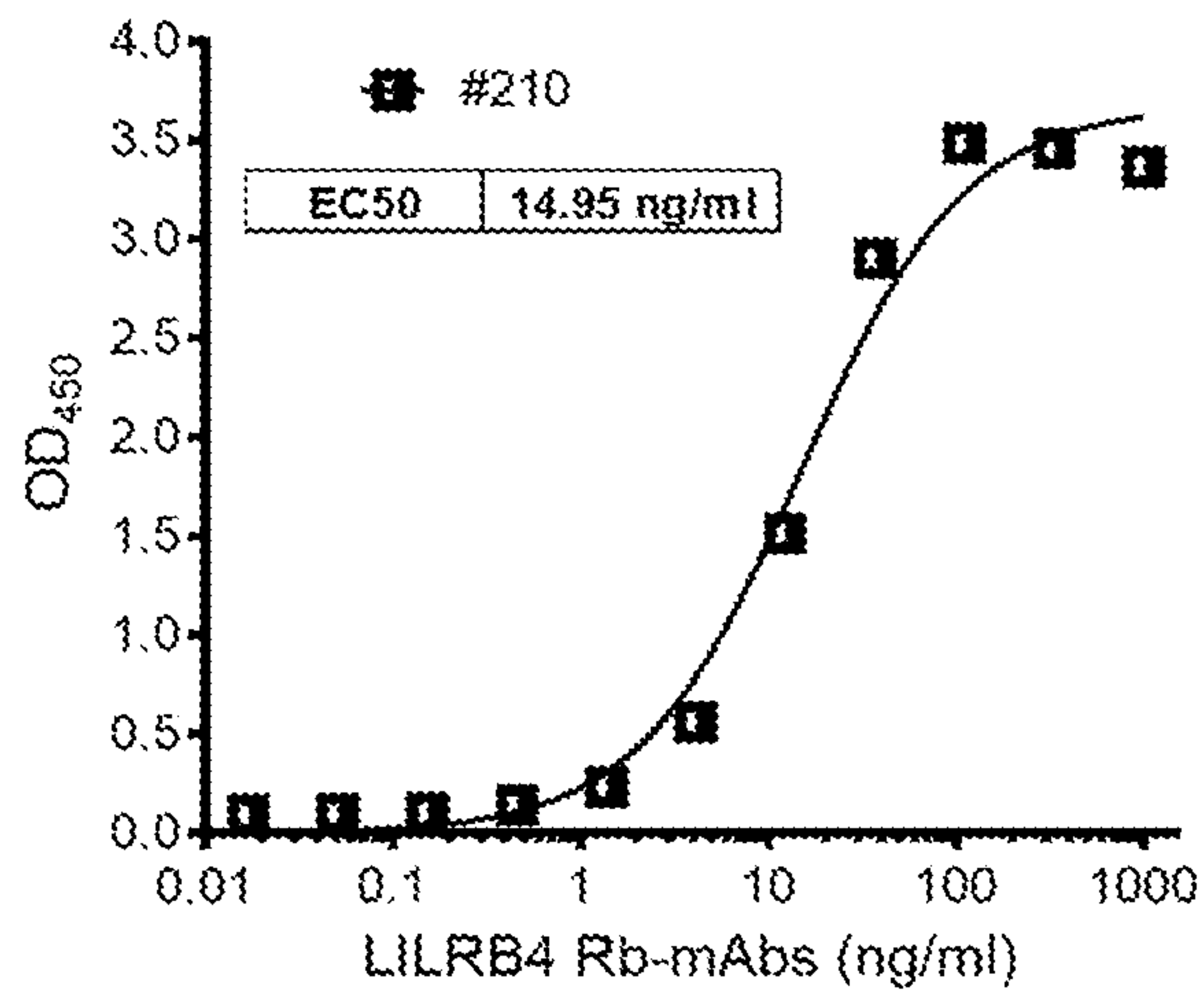


FIG. 18T

#6

(1)

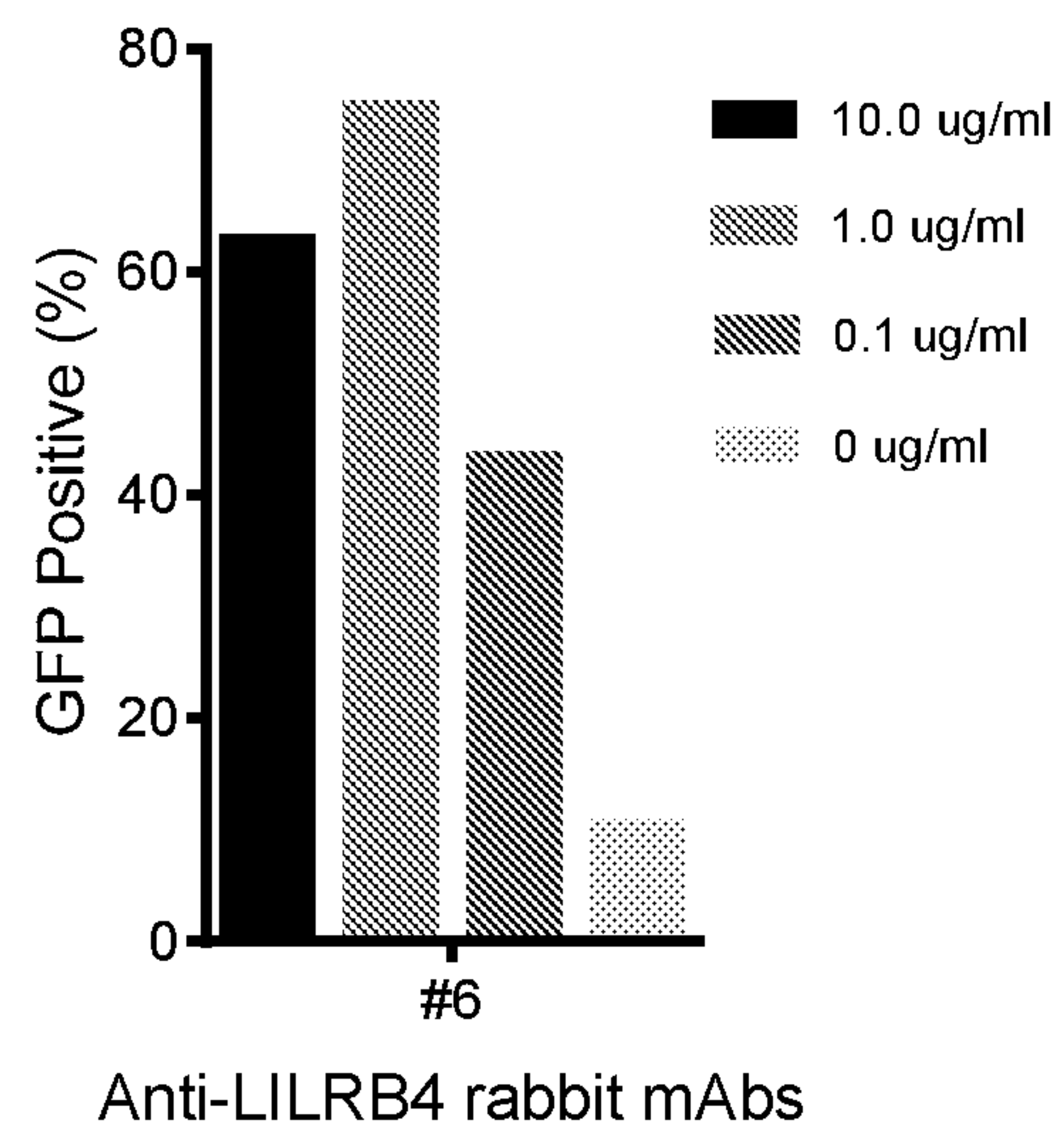
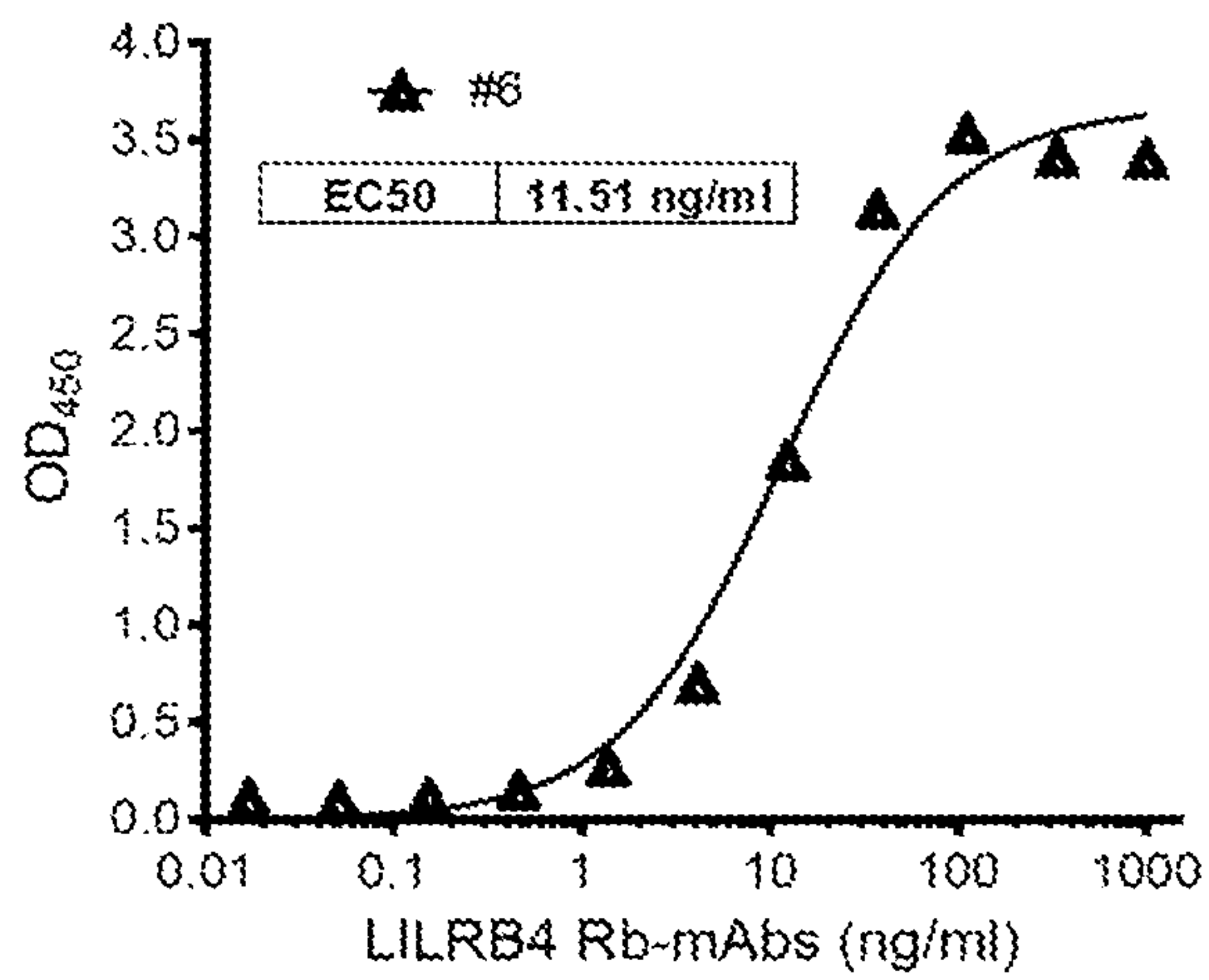
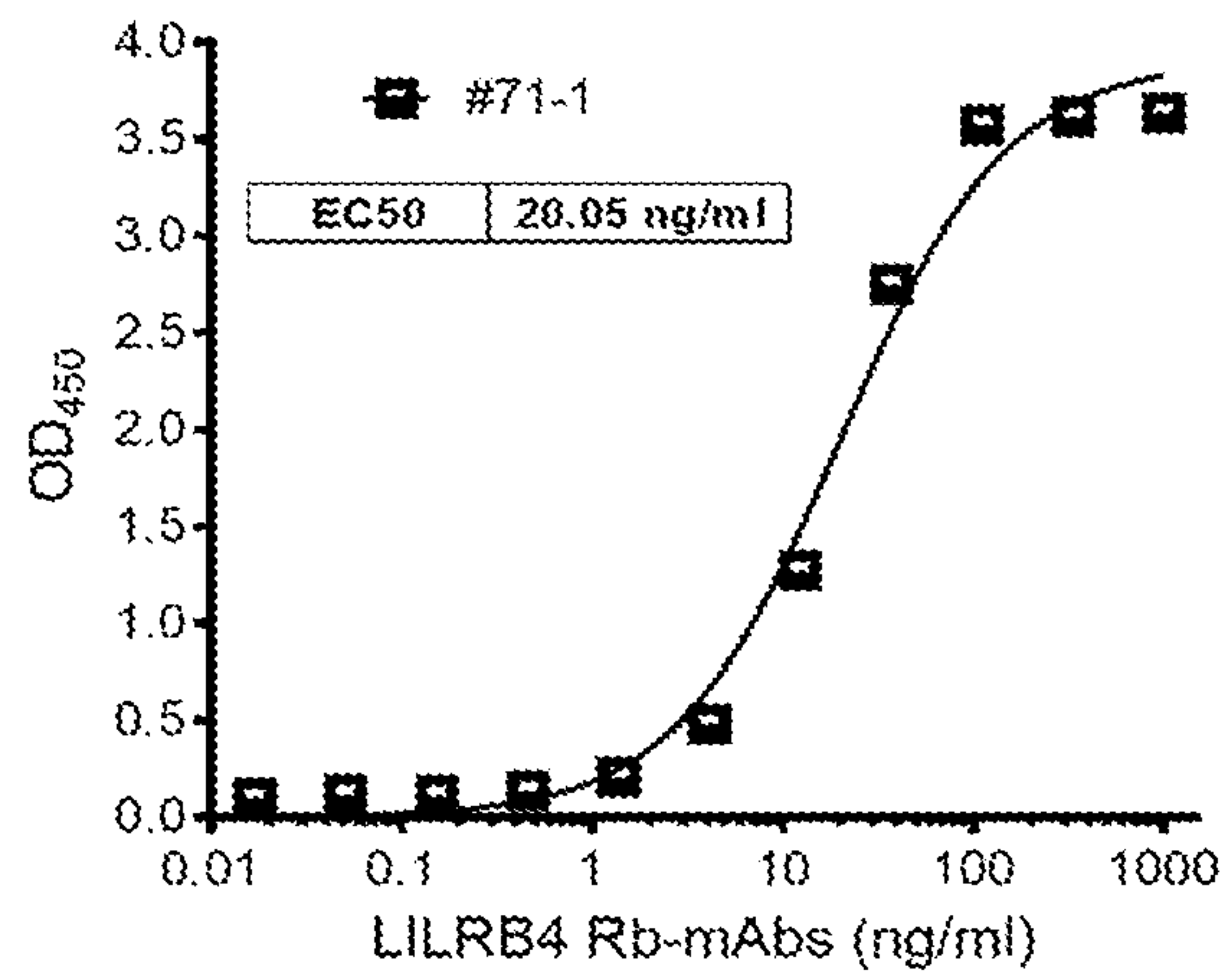


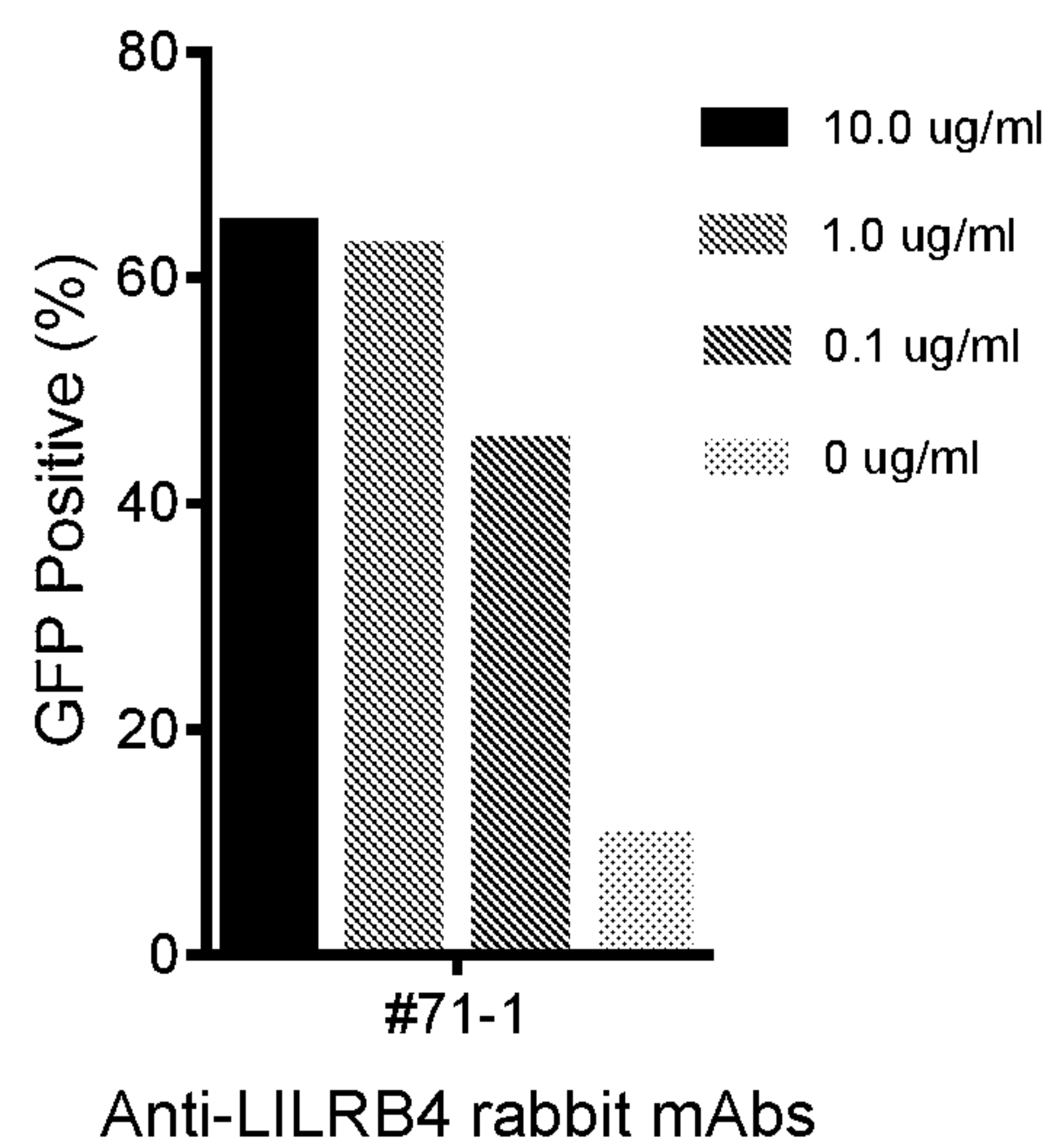
FIG. 18U

#71-1

(1)



(2)



Antibody	Reactivity to individual LILRBs					AML inhibition
	LILRB1	LILRB2	LILRB3	LILRB4	LILRB5	
C193		+				++
C102		+				+
C41						
C39		++	++	+	+	+++
C143						
C290		+	++	+	+	++
C287		++				+
C3				+		+++
C1						
C84		+		++		++++
C53				++		++++
C201		++		++	+	++++
C92				++		++++

FIG. 19

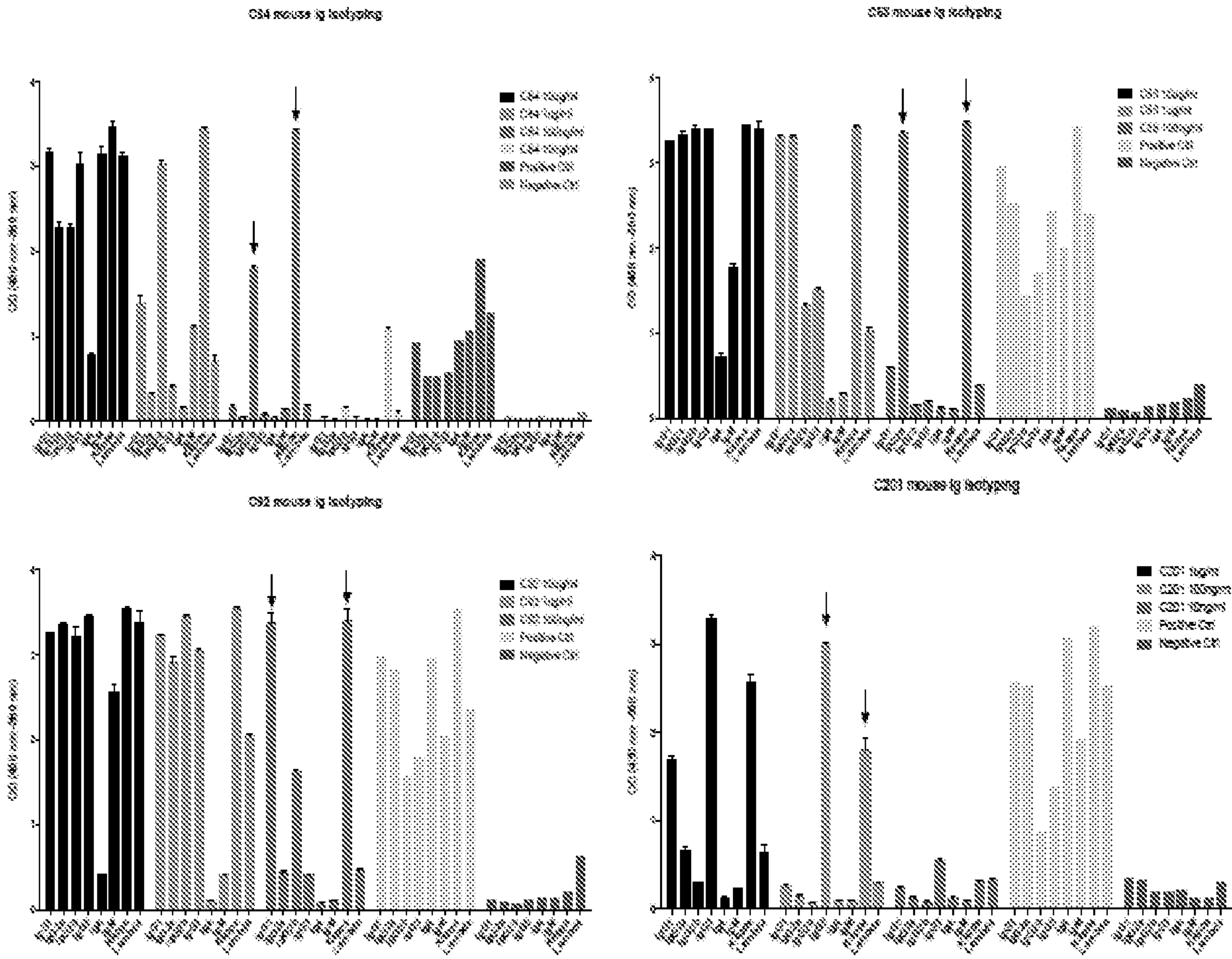


FIG. 20

		HEAVY CHAIN			LIGHT CHAIN		
		CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
C53	IgG2a, k	GFTFSNY WMN	EIRLKYNMYATH YAESVKG	TRYGSSLDY	KSSQNLFY5 TNQKNYLA	WASTRES	QQYNYPL T
C84	IgG2b, k	GYTFTNY GMN	WINTYTGEPTY ADDFKG	IYYHTSLWY FDV	RSSQNIWH SNGNTYLE	RVSNRFS	FGSHVPL T
C92	IgG1, k	GYTFTNY GMN	WINTYTGEPTY ADDFKG	IYYHTSLWY FDV	RSSQSLVH SNGNTYLH	KVSNRFS	SQSTHVPY T
C201	IgG3, k	GYSITSQY AWN	YISYSGGTSYNP SLKS	LHYGYDY	RSSQSIVHS NGNTYLE	KVSNRFS	FGGLHVPP T
C3	IgG2b, k	GFTFNMY AMN	RIRSKRNMYAT HYDDSVKD	DGPYAMD Y	KSSQSLLYS SNQKNYLA	WASTRES	QQYYSYPY T
C193	IgG2a, k	GFIFNTYA MN	RIRSKSNKYATY YVDSVKD	DGI	KSSQSLLNS GNQKNYLT	WASTRES	QNDYSYPL T
C287	IgG1, k	GYTFTNY GIN	WVKQAPGKGL KWMG	GSAKGGFF Y	KSSQSLLYS NGRTYLN	LVSKLDS	VQGTDFPY T
C39	IgG3, k	GFTFSSYG MS	TID5NGGGTYT PDSVKG	DGGG5YGY YYAMDY	KSSQSLLNS RTRKSYLA	WASTRES	KQSYNLPW T
C102	IgG3, k	GYTFTEYT MH	GINPNNGGTSY NQKFKG	YWDYFDY	RSSQSIVHS NGNTYLE	KVSNRFS	FGGSQIPP T
C290	IgG3, k	GYTFTEYT MH	GINTNNGGTSY NQKFKG	DKRSRGEL DSTMDY	RSSKSLLHS NGNTYLY	RMSNLAS	MQHLEYPF T

FIG. 22

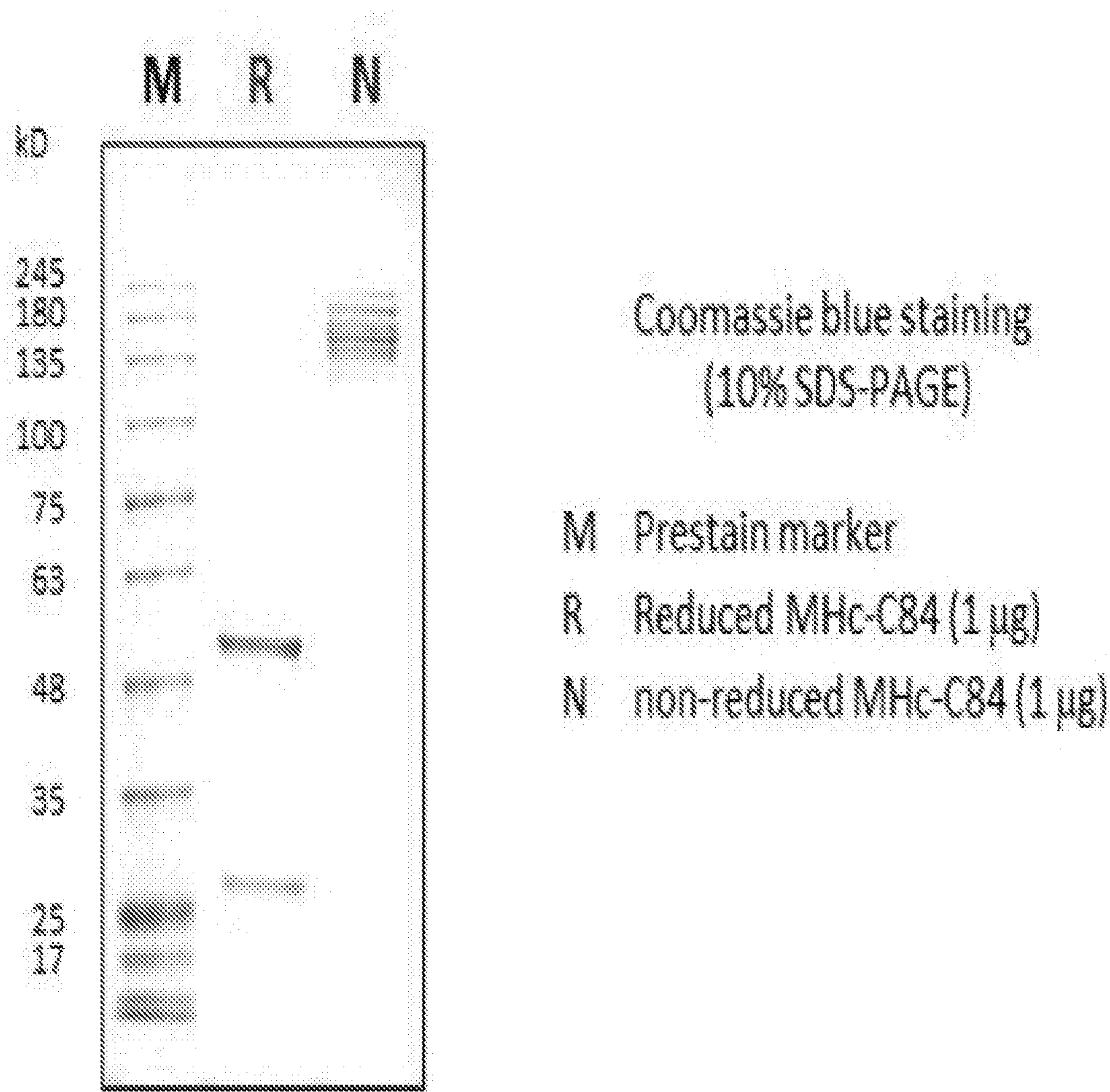
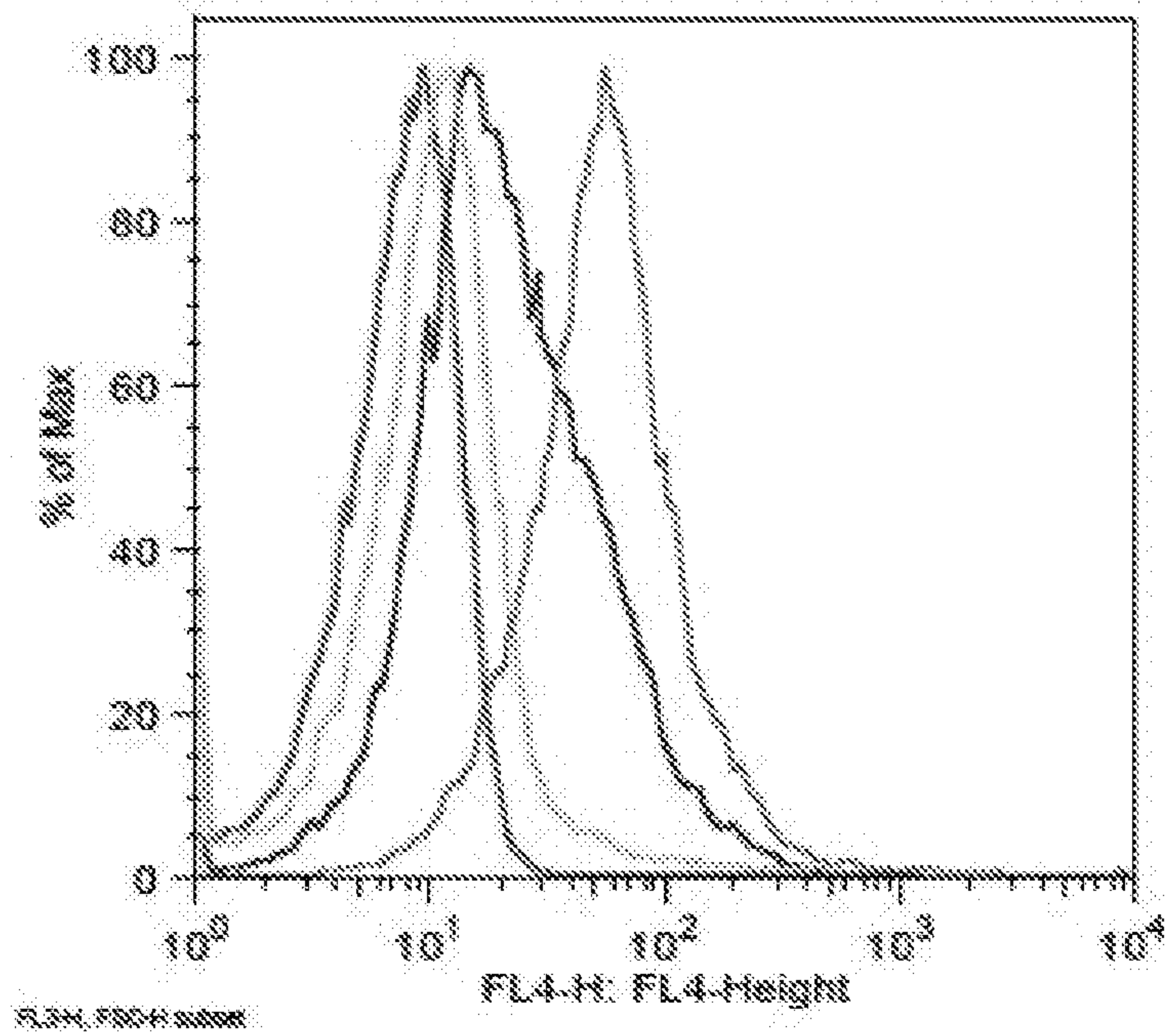
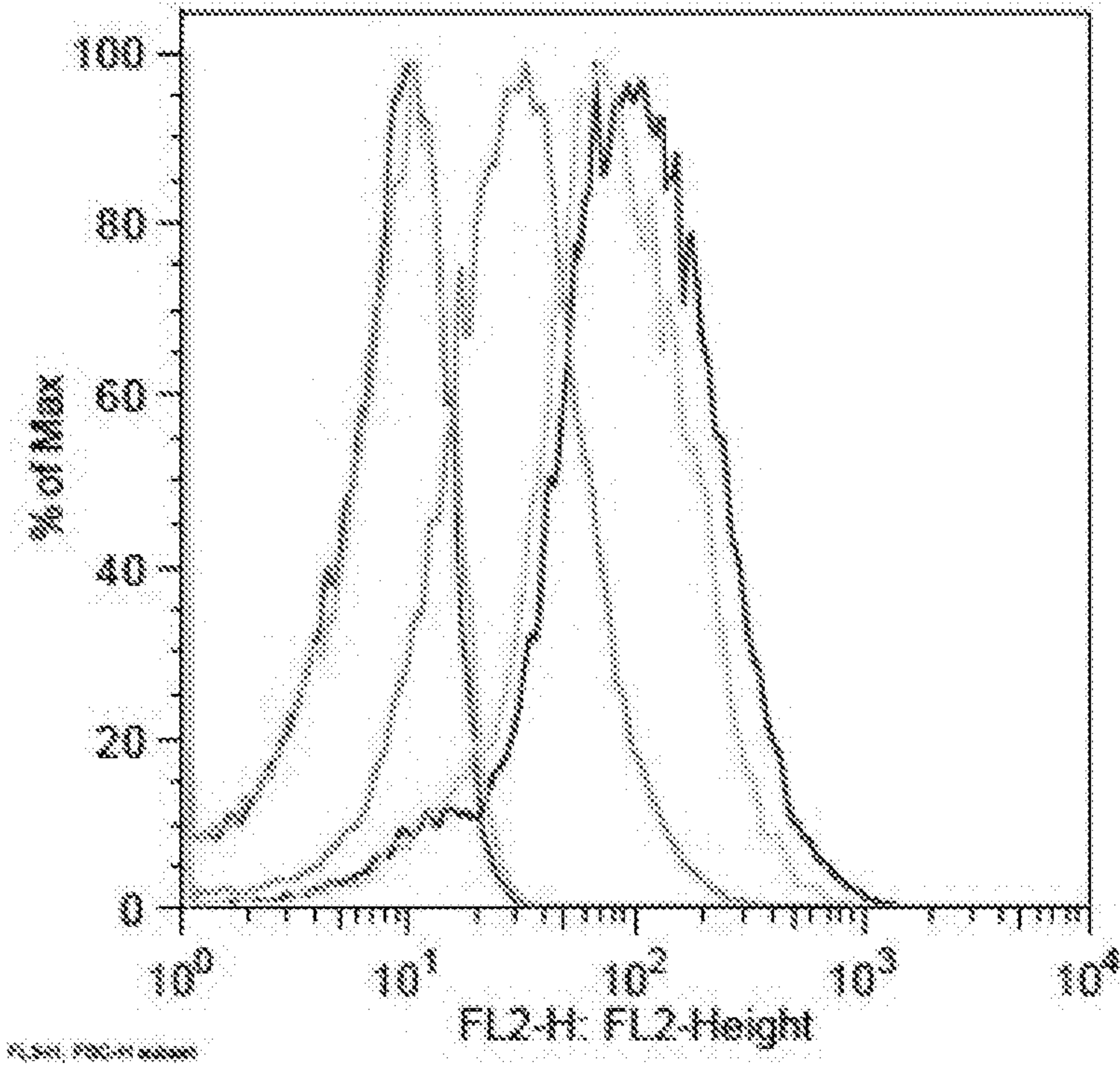


FIG. 23



Sample	%	Median:FL4-H
thp1 84721 mhc-c84	83.2	55.7
thp1 84721 hu	81.4	10.7
thp1 84721 c84	83.9	20
thp1 84721 ms	83.8	7.7

FIG. 24



Sample	Median:FL2-H
rep-k hu-pe mhc-c84-10ug	77
rep-k hu-pe mhc-c84-2ug	28.9
rep-k hu-pe	8.58
rep-k ms-pe c84-10ug	99.1
rep-k ms-pe	8.35

FIG. 25

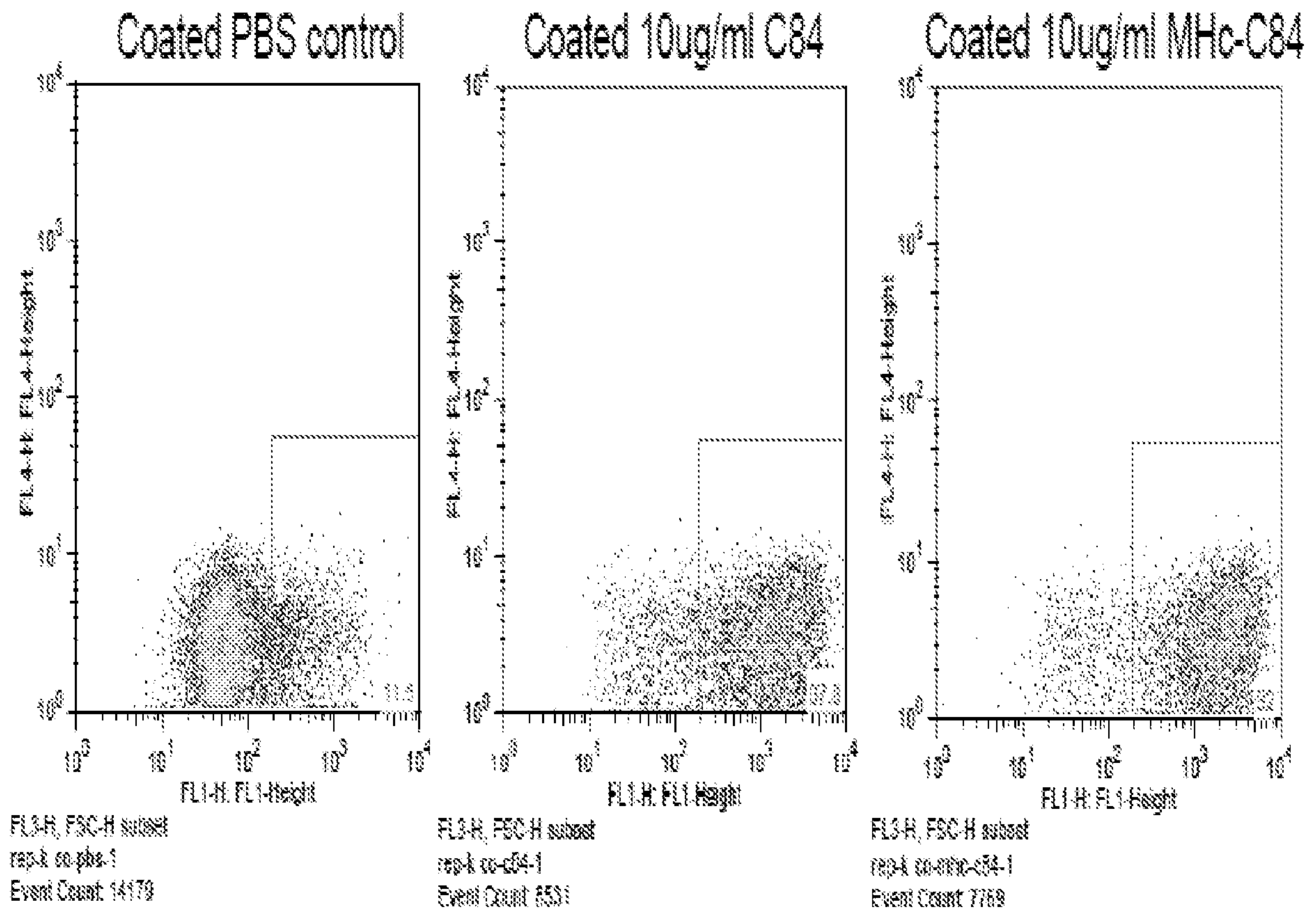


FIG. 26

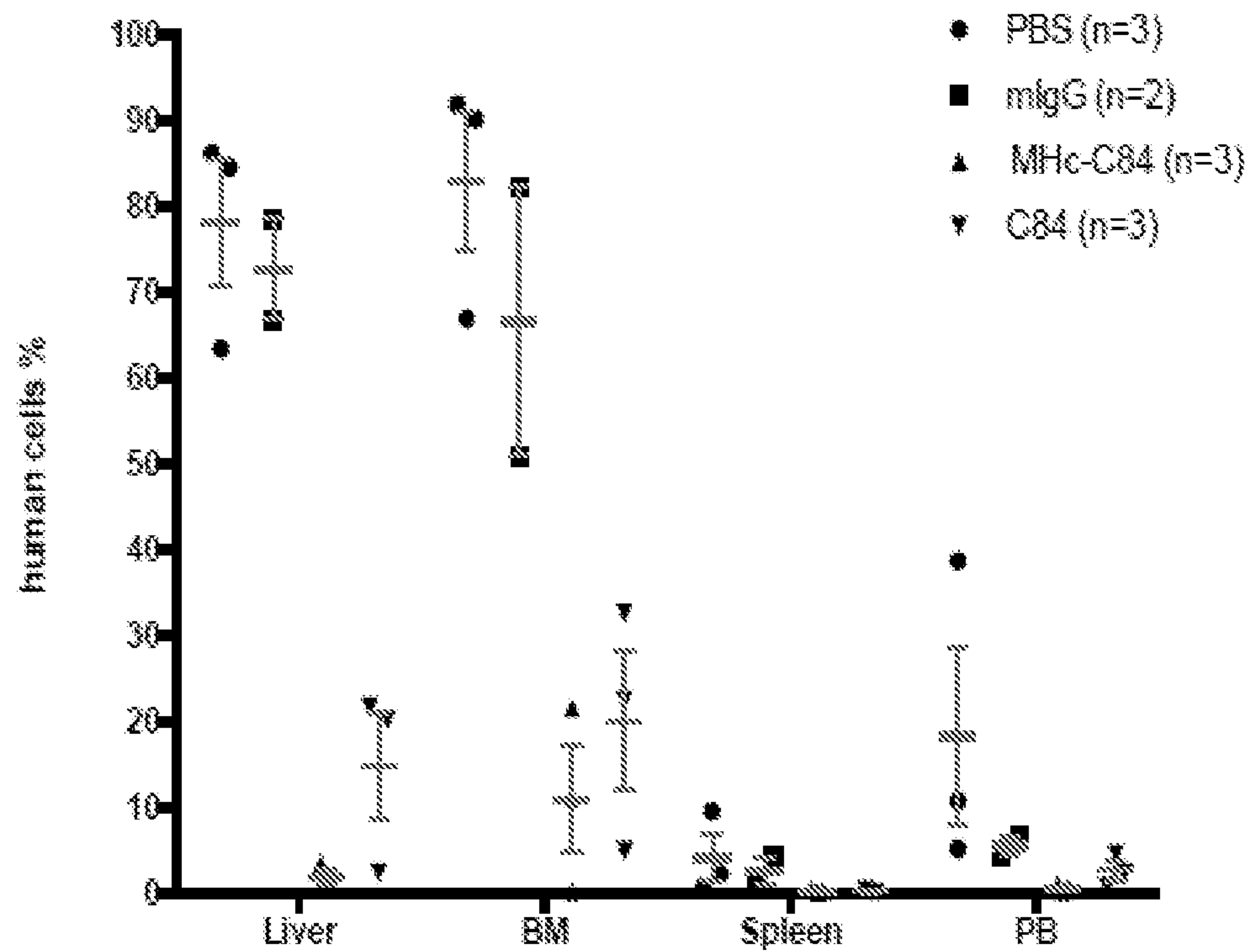


FIG. 27

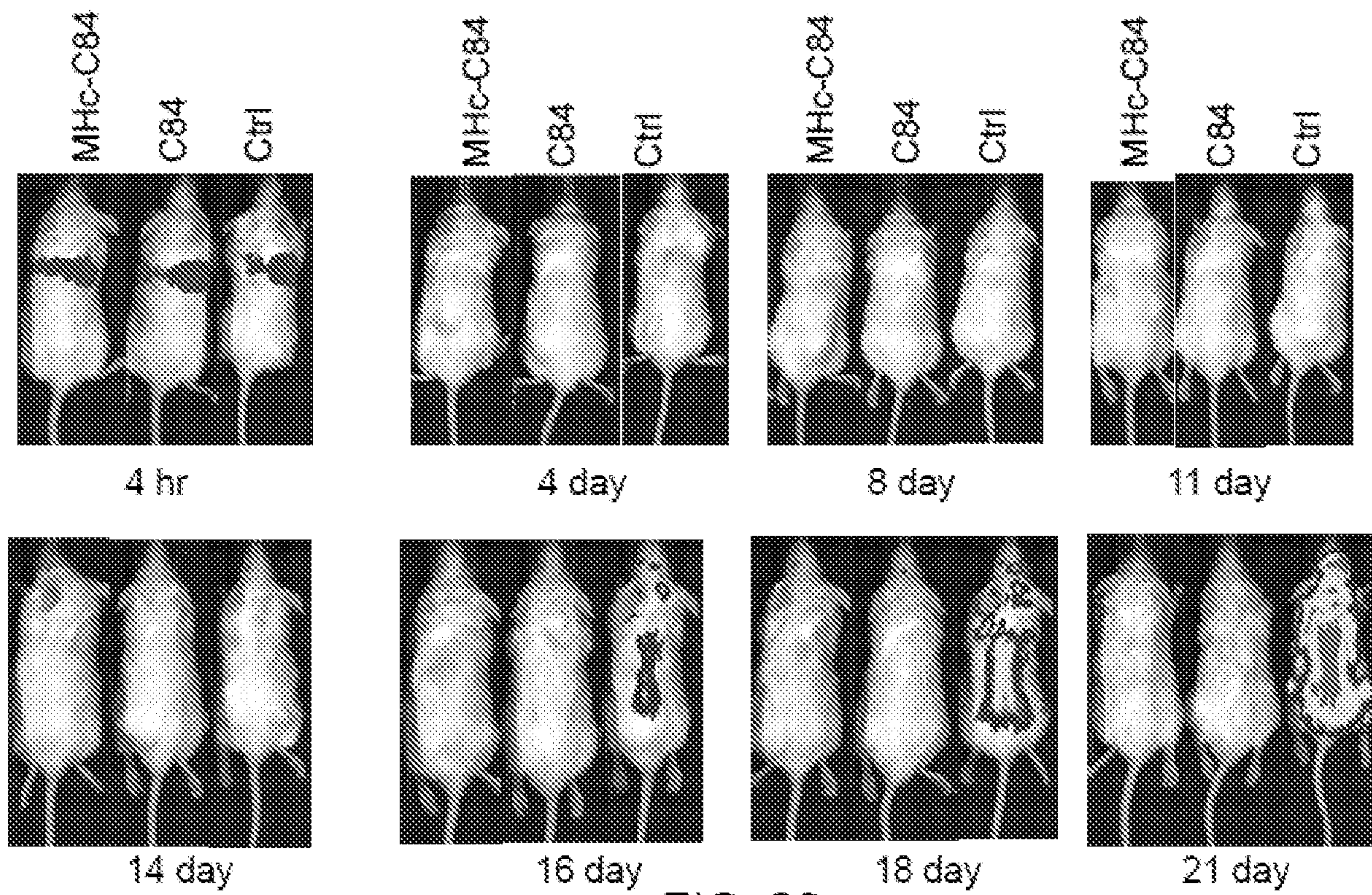


FIG. 28

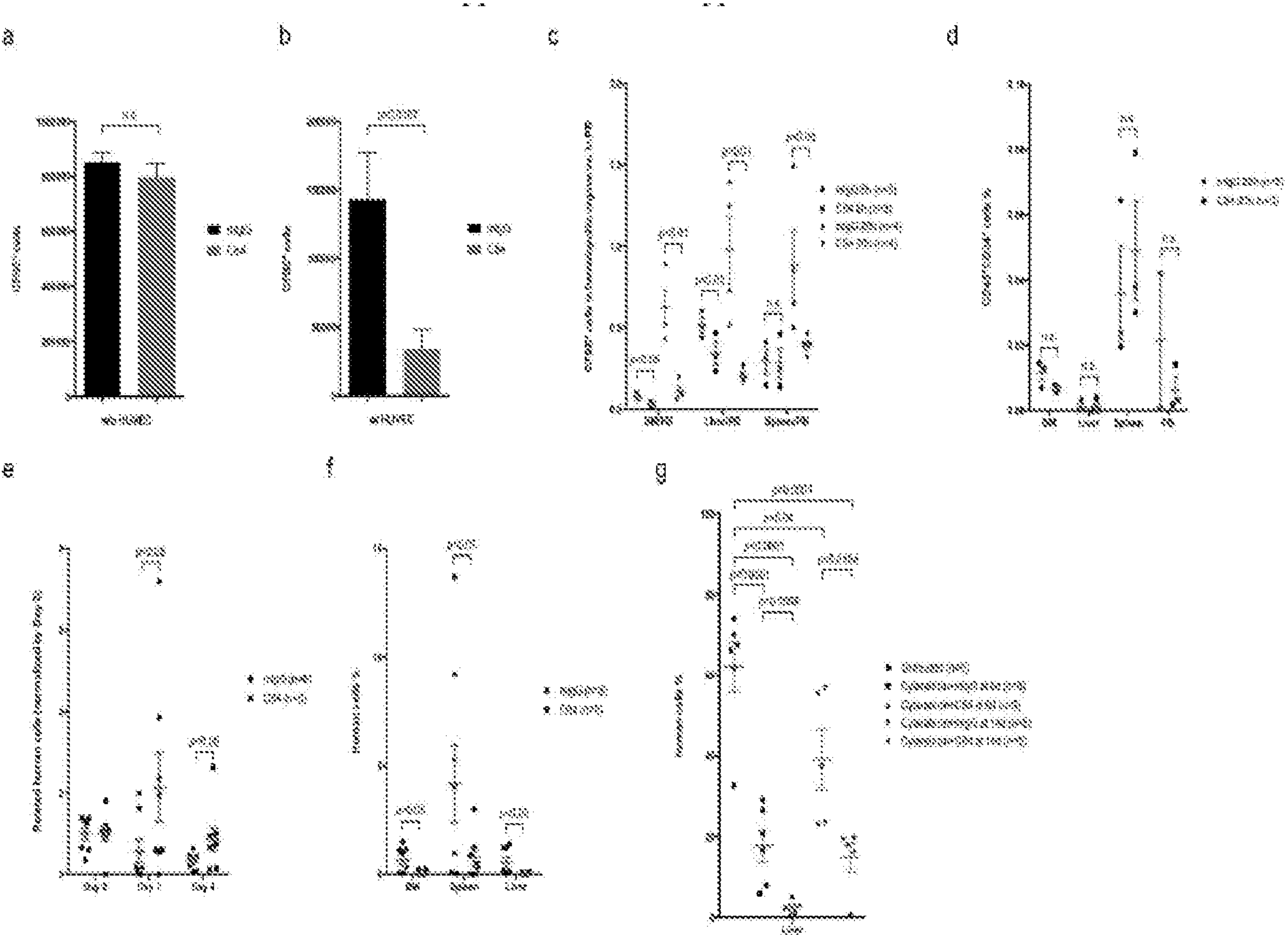
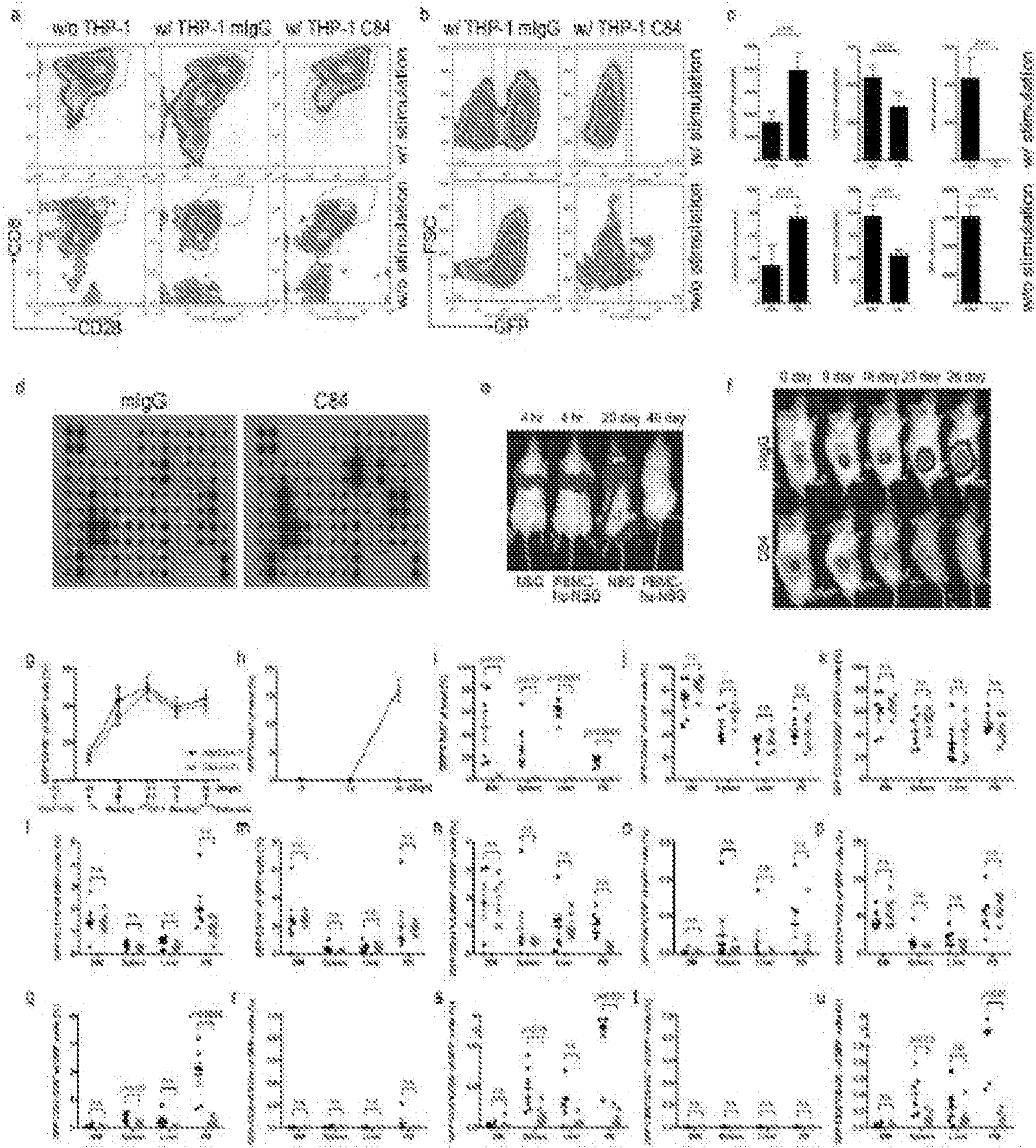


FIG. 29A-G



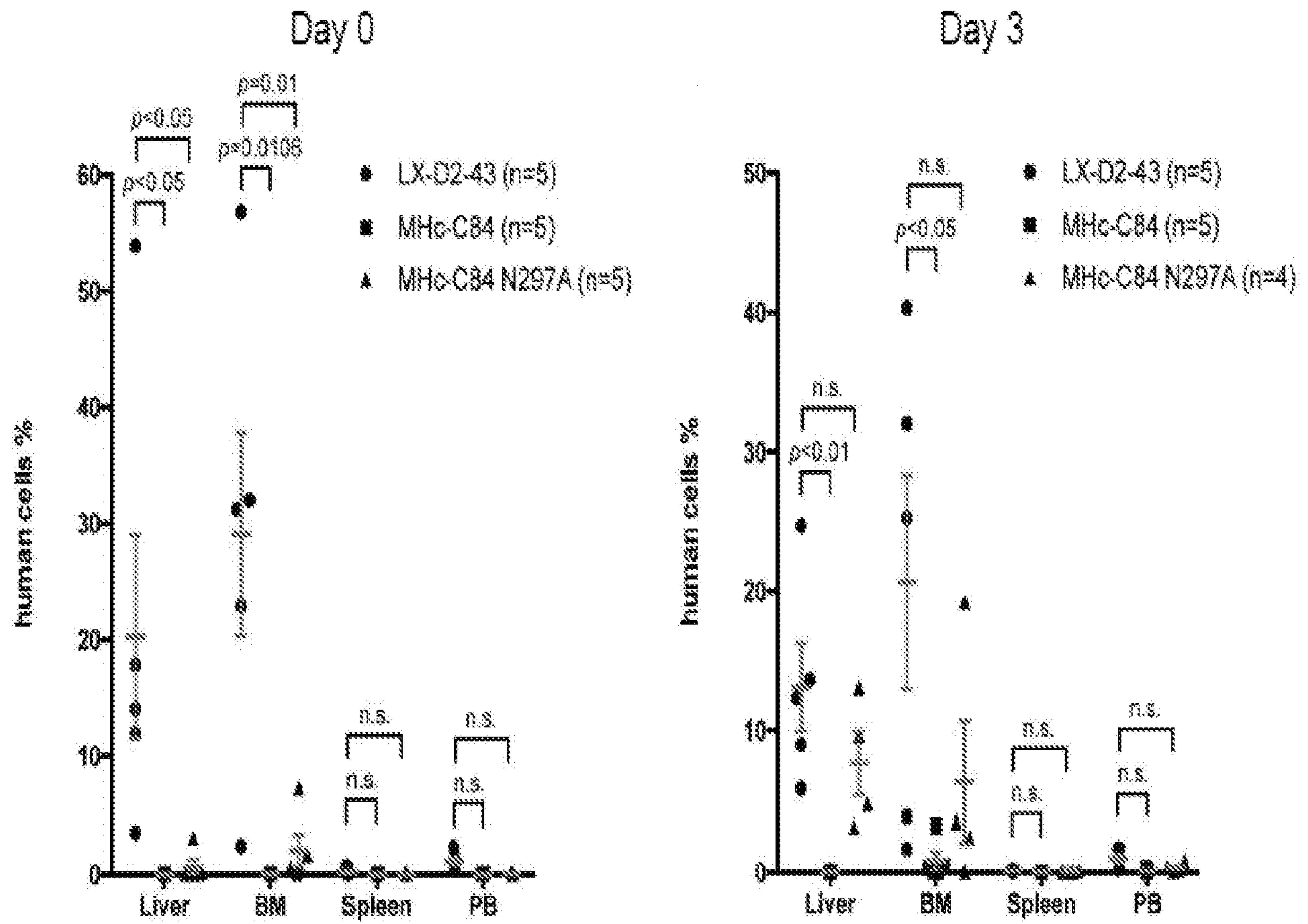
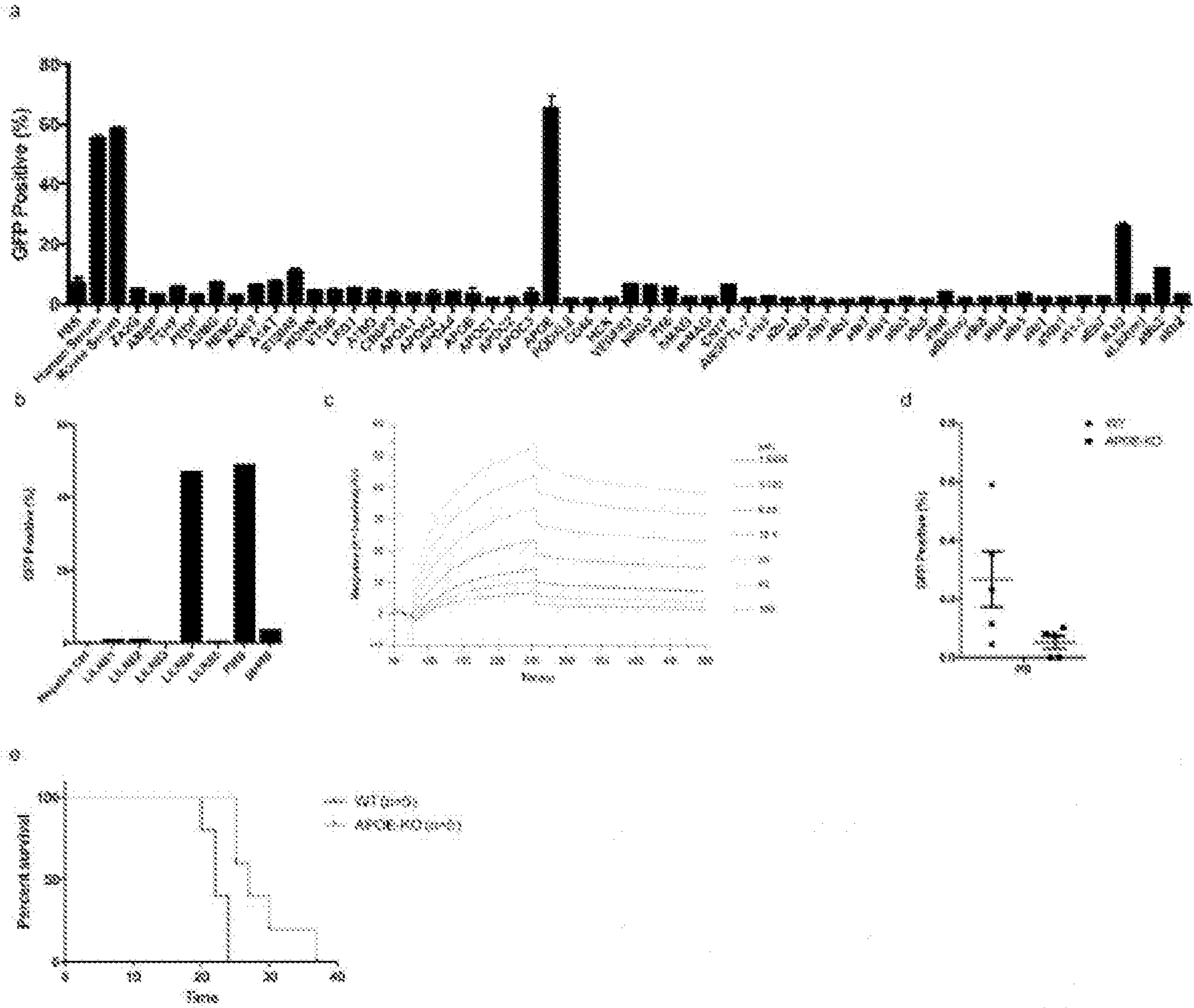
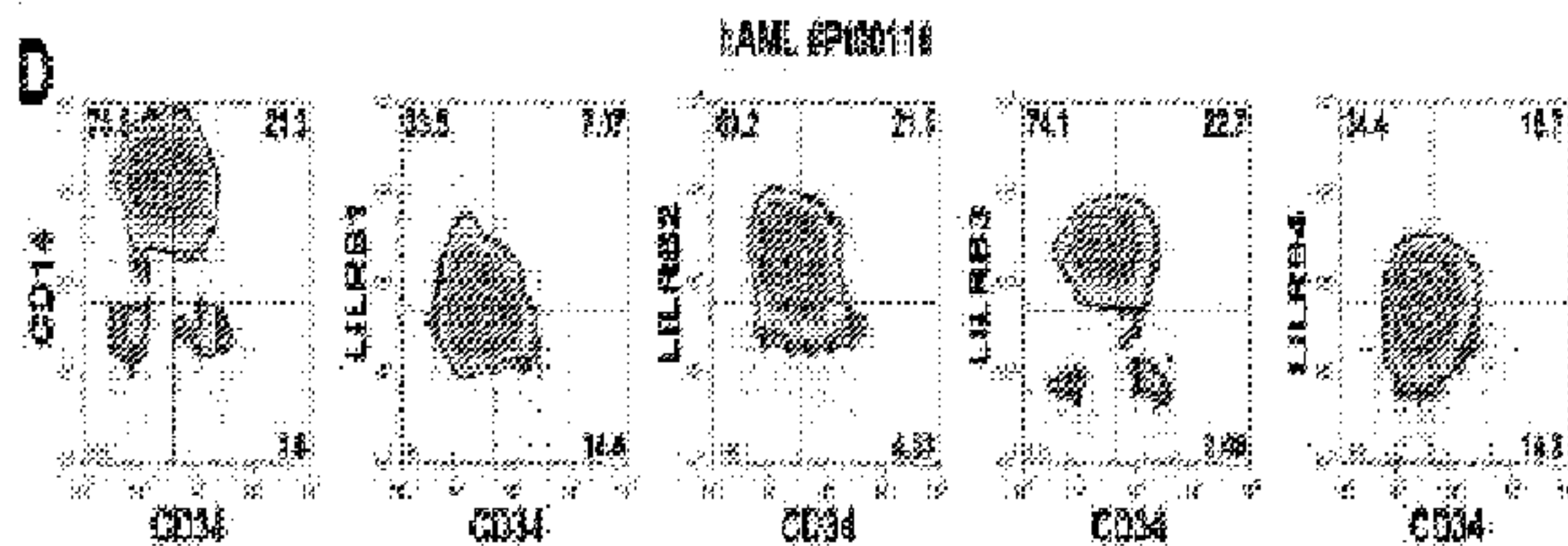
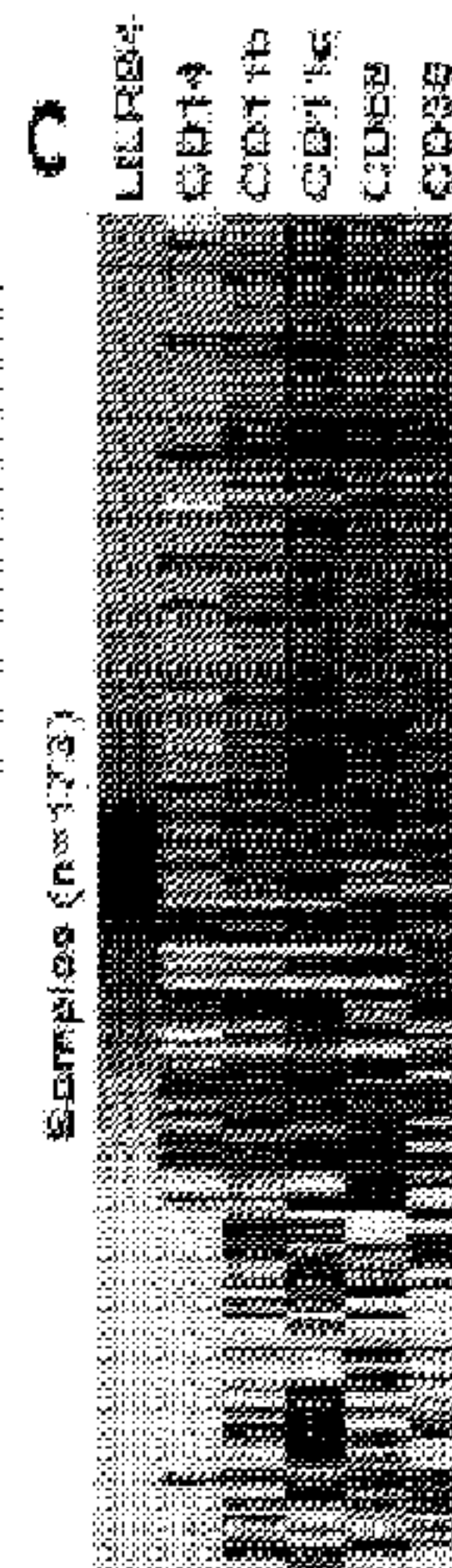
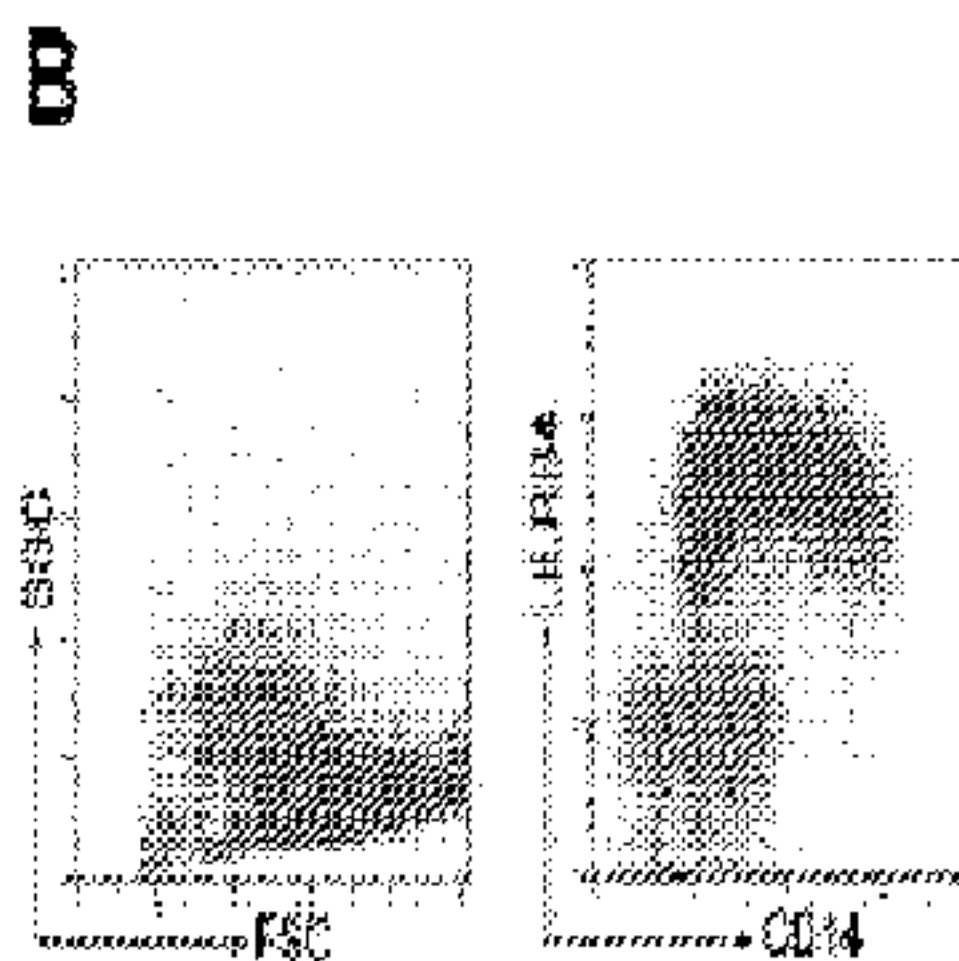
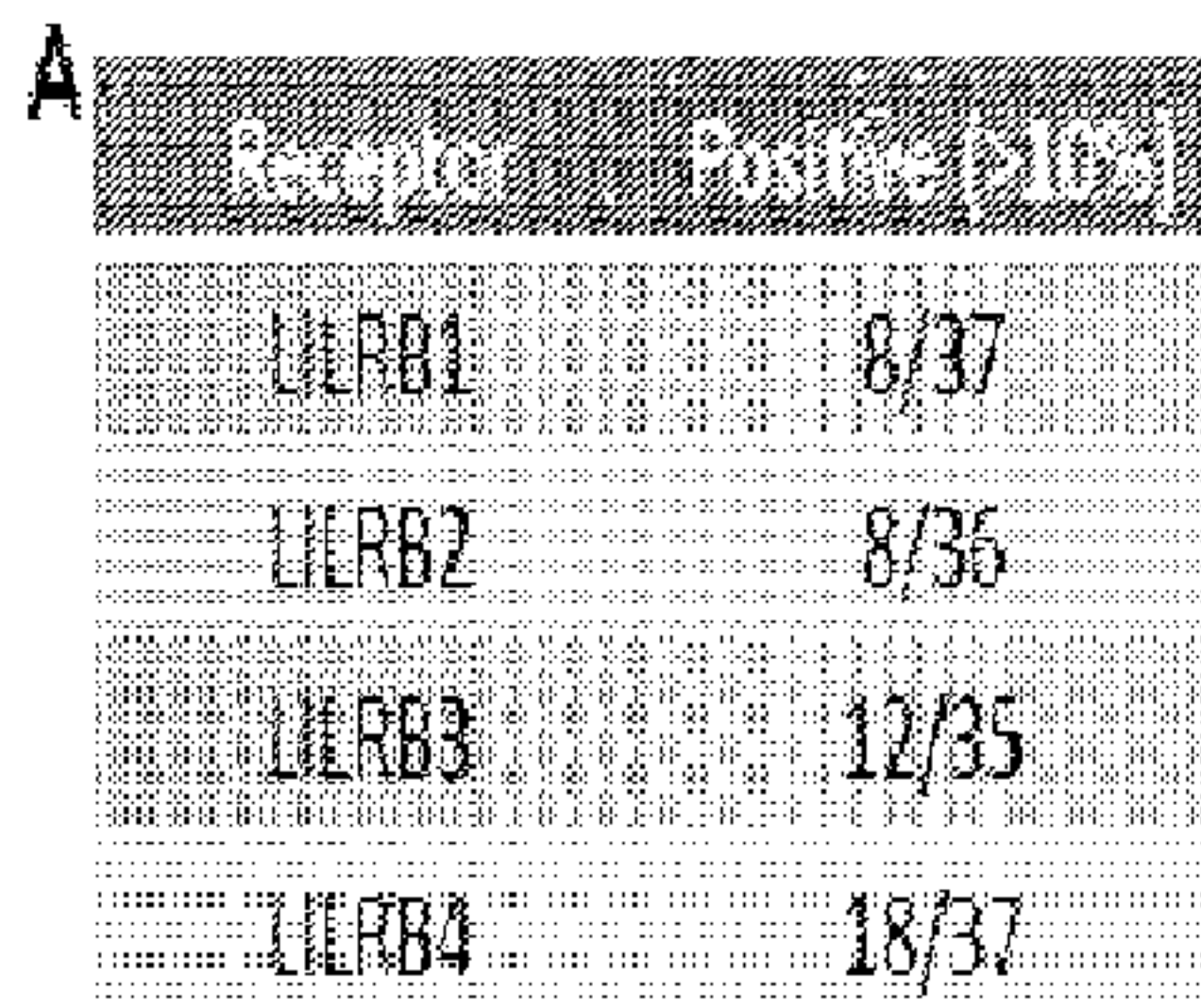


FIG. 31





FIGS. 1A-D