



(86) Date de dépôt PCT/PCT Filing Date: 2013/08/14
(87) Date publication PCT/PCT Publication Date: 2014/02/20
(85) Entrée phase nationale/National Entry: 2015/02/16
(86) N° demande PCT/PCT Application No.: US 2013/055015
(87) N° publication PCT/PCT Publication No.: 2014/028668
(30) Priorités/Priorities: 2012/08/14 (US61/683,155);
2012/08/17 (US61/684,654); 2012/08/27 (US61/693,712);
2012/10/17 (USPCT/US2012/060684);
2013/02/13 (USPCT/US2013/025981);
2013/06/20 (US61/837,560)

(51) Cl.Int./Int.Cl. *A61K 39/395* (2006.01),
A61P 35/00 (2006.01), *C07K 16/28* (2006.01),
C12N 5/071 (2010.01), *C12Q 1/02* (2006.01),
C40B 30/04 (2006.01), *C40B 30/06* (2006.01),
C07K 14/705 (2006.01)
(71) Demandeur/Applicant:
MINERVA BIOTECHNOLOGIES CORPORATION, US
(72) Inventeur/Inventor:
BAMDAD, CYNTHIA, US
(74) Agent: SIM & MCBURNEY

(54) Titre : COMPOSES THERAPEUTIQUES ACTIVANT LES CELLULES SOUCHES
(54) Title: STEM CELL ENHANCING THERAPEUTICS

(57) **Abrégé/Abstract:**

The present application disclose a method for treating a patient who would benefit from stimulation of the patient's stem cells, comprising administering to the patient an antibody that specifically binds to an epitope of the MUC1 protein expressed on human undifferentiated stem cells.



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

(19) World Intellectual Property Organization

International Bureau

WIPO | PCT

(10) International Publication Number

WO 2014/028668 A3

(43) International Publication Date

20 February 2014 (20.02.2014)

- (51) International Patent Classification:
A61K 39/395 (2006.01) C12N 5/02 (2006.01)
C12N 5/00 (2006.01)
- (21) International Application Number:
PCT/US2013/055015
- (22) International Filing Date:
14 August 2013 (14.08.2013)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/683,155 14 August 2012 (14.08.2012) US
61/684,654 17 August 2012 (17.08.2012) US
61/693,712 27 August 2012 (27.08.2012) US
PCT/US2012/060684
17 October 2012 (17.10.2012) US
PCT/US2013/025981
13 February 2013 (13.02.2013) US
61/837,560 20 June 2013 (20.06.2013) US

- 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, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, 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, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).
- Published:
- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))
- (88) Date of publication of the international search report:
1 May 2014

- (71) Applicant: MINERVA BIOTECHNOLOGIES CORPORATION [US/US]; 40 Bear Hill Rd., Waltham, MA 02451 (US).
- (72) Inventor: BAMDAD, Cynthia; 40 Bear Hill Rd., Waltham, MA 02451 (US).
- (74) Agent: KIM, Joseph, Hyosuk; JHK Law, P.O. Box 1078, La Canada, CA 91012-1078 (US).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

(54) Title: STEM CELL ENHANCING THERAPEUTICS

(57) Abstract: The present application disclose a method for treating a patient who would benefit from stimulation of the patient's stem cells, comprising administering to the patient an antibody that specifically binds to an epitope of the MUC1 protein expressed on human undifferentiated stem cells.

STEM CELL ENHANCING THERAPEUTICS

BACKGROUND

[0001] A problem encountered by oncologists is that they must balance the benefit of chemotherapy against the risk of killing the patient during the course of killing their cancers. One of the life-threatening side effects of chemotherapy is that cytotoxic drugs kill cancer cells but also kill healthy cells and in particular kill the patients' stem cells. Stem cells in the bone marrow are constantly regenerating to supply the body with red blood cells, which carry oxygen, white blood cells, which fight infection and platelets which cause the blood to clot. Chemo and radiation can destroy the stem cells which eventually become blood cells and thus put the patient's life at risk, which makes the physician reduce the cancer killing treatments. The problem with designing agents to counter these deleterious effects of cancer treatment is that blood cells are terminally differentiated cells – meaning that they cannot divide to replicate themselves. They developed from hematopoietic stem cells in the bone marrow. This means that it is not possible to collect some red or white blood cells from a patient and expand them *in vitro* then inject them back into the patient.

[0002] There are currently a handful of drugs on the market that are used to modulate stem cell development in a patient. The first group, known as erythropoiesis stimulating agents (ESAs), includes Epoetin, marketed under the trade names Procrit and Epogen, and Aranesp. These drugs are used to treat anemia in patients with chronic kidney disease and in cancer patients with chemotherapy-induced anemia. These drugs do not stimulate the growth of stem cells in the bone marrow but rather skew the development of the patient's stem cells such that more become blood cells of the erythroid lineage. Epogen and epogen-like drugs help cancer patients and chronic kidney disease patients by increasing the number of stem cells in the bone marrow that differentiate into red blood cells.

[0003] Another class of drugs that is used to stimulate the production of white blood cells. Colony-stimulating factors (CSFs) that include G-CSF (granulocyte-colony stimulating factors: marketed as Filgrastim) and GM-CSF (granulocyte-macrophage colony stimulating factors: Sargamostim marketed as Leukine) stimulate the production of the precursors of white blood cells, which can mature to become neutrophils, macrophages and dendritic cells and may be administered with or without cyclophosphamide. The CSFs are also stem cell mobilizers which cause blood cell progenitors to be secreted from the bone marrow. However, a drawback of G-CSF and GM-CSF is that they inhibit bone formation. Another mobilizer of hematopoietic stem cells is the CXCR4 antagonist AMD3100, which reportedly mobilizes blood cell progenitors without inhibiting bone formation.

[0004] Both erythropoiesis stimulating agents (ESAs) and CSFs can be used to accelerate the recovery of blood cells from effects of chemotherapy, used after bone marrow transplant, used before or after stem cell transplant which may be transplanted into the peripheral blood, and or to treat a patient who could benefit from increased production of hematopoietic stem cells or blood cells or their progenitors.

[0005] Both ESAs and CSFs function by skewing the maturation of stem cells toward the hematopoietic stem cell lineages and necessarily away from maturation into other types of cells. It follows that there may be unwanted and dangerous side effects stemming from the inhibition of other types of cells that are produced in the bone marrow. In fact, the FDA recently issued warnings that the use of Procrit, Epogen or Aranesp increases the risk of developing cancers and also increases the risk of heart attack and stroke.

[0006] Thus an improvement would be the development of agents that stimulate the production of or skew the development of stem cells to blood cell lineages via a pathway that is different from that of the ESAs and the CSFs. A greater improvement to the state of the art would be the development of agents that stimulate the production of stem cells in the bone marrow rather than just skewing their maturation in one direction. An even greater improvement to the state of the art would be the development of an agent(s) that would stimulate the growth of stem cells but would not stimulate the growth of cancer cells or increase the patient's risk of developing another cancer. A yet greater improvement would be the development of an anti-cancer agent that would kill the cancer cells without killing the patient's stem cells.

SUMMARY OF THE INVENTION

[0007] In one aspect, the present invention is directed to a method for treating a patient whose blood cells or bone marrow cells have been depleted, which may occur in some instances as a result of exposure to radiation, the method including administering to the patient a stem cell specific antibody. In another aspect, the present invention is directed to a method for treating a patient suffering from low blood cell count by stimulating the growth of hematopoietic stem cells or progenitor cells by administering to the patient a stem cell specific antibody.

[0008] In another aspect, the present invention is directed to a method of stimulating growth of bone marrow cells, hematopoietic stem or progenitor cells *in vitro* and then administering to a patient suffering from low blood cell count in the peripheral blood or in the bone marrow, in which the method includes administering to the patient a stem cell specific antibody.

[0009] In another aspect, the present invention is directed to a method of diagnosing cancer by testing a patient sample with an antibody that preferentially binds cancer cells compared to its binding to stem cells.

[0010] In a preferred embodiment, the cancer specific antibodies of the invention are administered to a patient whose treatment regime also includes treatment with stem cell specific antibodies.

[0011] In another aspect, the present invention is directed to a method of selecting therapeutic antibodies for the treatment of cancer involving de-selecting those antibodies that bind to stem cells. Similarly, the present invention is directed to a method of selecting therapeutic antibodies for the treatment of conditions requiring regeneration of blood cells or bone marrow cells, involving de-selecting those antibodies that bind to cancer cells.

[0012] In one aspect, the present invention is directed to a method for treating a patient who would benefit from stimulation of the patient's stem cells, comprising administering to the patient an antibody that specifically binds to an epitope of the MUC1 protein expressed on human undifferentiated stem cells. The antibody specifically may bind to at least six consecutive amino acids of the peptide of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:11. In particular, the antibody may not bind to the peptide of SEQ ID NO:3, SEQ ID NO:7 or SEQ ID NO:8. The patient may suffer from cancer. The patient may suffer from cancer and is receiving chemotherapy or radiation. Or, the patient may suffer from chronic kidney disease.

[0013] In another aspect, the invention is directed to a method for treating a patient suffering from cancer or at risk of developing cancer, comprising administering to the patient an antibody that specifically binds to an epitope of the MUC1 protein that is expressed on cancer cells but is not expressed on human undifferentiated stem cells. The antibody may specifically bind to the peptide of SEQ ID NO:3 and may not bind to the peptide of SEQ ID NO:4.

[0014] In still another aspect, the invention is directed to a method for treating a patient diagnosed with a MUC1-positive cancer comprising administering to the patient a monovalent cancer cell-specific antibody.

[0015] In another aspect, the invention is directed to a method for treating a patient diagnosed with a MUC1-positive cancer comprising administering to the patient a monovalent cancer cell-specific antibody.

[0016] In another aspect, the invention is directed to a method for treating a patient diagnosed with a MUC1-positive cancer comprising administering to the patient a monovalent cancer cell-specific antibody and bivalent stem cell specific antibody.

[0017] In another aspect, the invention is directed to a method for proliferating stem or progenitor cells, comprising contacting the cells with an antibody that specifically binds to an epitope of the MUC1 protein expressed on human stem cells, without causing proliferation of cancer cells.

[0018] In yet another aspect, the invention is directed to a method of obtaining an antibody that specifically binds to stem cells, but not to cancer cells comprising the steps of:

[0019] (i) generating a mixed set of antibodies that recognize a peptide whose sequence is that of any of the peptides having sequence of SEQ ID NOS:1-11;

[0020] (ii) selecting those antibodies that bind to a peptide of SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, but not to the peptide of SEQ ID NO:3;

[0021] (iii) selecting those antibodies that when adsorbed onto a surface facilitate the attachment of stem cells; and

[0022] (iv) selecting those antibodies that in the bivalent form stimulate the growth of stem cells and in the monovalent form inhibit the growth of stem cells, while having no effect on cancer cells.

DESCRIPTION OF THE DRAWINGS

[0023] **Figure 1** is a cartoon depicting how bivalent antibodies that recognized MUC1* receptor stimulate growth, but monovalent Fabs of those antibodies inhibit growth by blocking its interaction with its cognate ligand, dimeric NM23. Bivalent antibodies mimic activity of ligand NM23 to make cancer cells or stem cells grow; monovalent Fab blocks their growth.

[0024] **Figure 2** is a graph from an ELISA experiment showing binding of various antibody clones to either a PSMGFR peptide lacking the N-terminal 10 amino acids (N delta 10: SEQ ID NO:3) or a PSMGFR peptide lacking the C-terminal 10 amino acids (C delta 10: SEQ ID NO:4). Figure 2 shows titer of subclones on MUC1* and MUC1* on peptides SED ID NO:3, "N delta 10" and SEQ ID NO:4, "C delta 10".

[0025] **Figure 3** shows FACS showing the response of MUC1-positive T47D breast cancer cells to either cancer cell specific antibody C2 (MIN-C2) or stem cell specific antibody C3 (2D6C3) plus the data presented graphically. Figure 3 shows FACS summary of live MUC1* (+) breast cancer cells (T47D cells) – only cancer cell antibodies (C2) recognize cancer cells; stem cell antibodies (C3) do not.

[0026] **Figure 4** shows FACS showing the response of MUC1-positive ZR-75-1 breast cancer cells to either cancer specific antibody C2 (MIN-C2) or stem cell specific antibody C3 (2D6C3) plus the data presented graphically. Figure 4 shows FACS summary of live MUC1* (+) breast cancer cells (1500 (ZR-75-1 cells)); monoclonal antibody C2 (MIN-C2) binds to cancer cells. C3 (2D6C3) does not bind to breast cancer cells.

[0027] **Figure 5** shows FACS showing the response of MUC1-positive DU145 prostate cancer cells to either cancer specific antibody C2 (MIN-C2) or stem cell specific antibody C3 (2D6C3) plus the data presented graphically. Figure 5 shows FACS summary of MUC1* (+)

prostate cancer cells (DU145 cells): cancer cell antibodies C2 (MIN-C2) and E6 (MIN-E6) bind to MUC1-positive prostate cancer cells DU145; stem cell antibody C3 (2D6C3) does not.

[0028] **Figure 6** shows FACS showing the response of either MUC1-positive embryonic BGO1V/hOG human stem cells, MUC1-positive T47D breast cancer cells, or MUC1-positive DU145 prostate cancer cells to stem cell specific antibody C3 (2D6C3) plus the data presented graphically. Figure 6 shows that stem cell antibody (C3) recognizes stem cells but not cancer cells.

[0029] **Figure 7** shows photographs of BGO1V/hOG embryonic stem cells growing in plates in the presence of either the control 8nM NM23 (dimeric), 200ug/ml bivalent C3 (2D6C3) stem cell specific monoclonal antibody, or 200ug/ml bivalent C8 (2D6C8) stem cell specific monoclonal antibody. Resultant cells were measured after 72 hour growth period and measured in a Vialight assay and graphed as shown. Figure 7 shows that bivalent stem cell antibodies C3 (2D6C3) and C8 (2D6C8) stimulate stem cell growth better than the natural ligand NM23.

[0030] **Figure 8** shows photographs of BGO1V/hOG embryonic stem cells growing in plates in the presence of either the control 8nM NM23 (dimeric) or bivalent C3 (2D6C3) stem cell specific monoclonal antibody at the concentrations shown. Resultant cells were measured after 72 hour growth period and measured in a Vialight assay and graphed as shown. Cell count was measured using vialight assay.

[0031] **Figure 9** shows photographs of BGO1V/hOG embryonic stem cells growing in plates in the presence of either the control 8nM NM23 (dimeric) or bivalent C8 (2D6C8) stem cell specific monoclonal antibody at the concentrations shown. Resultant cells were measured after 72 hour growth period and measured in a Vialight assay and graphed as shown. Cell count was measured using vialight assay.

[0032] **Figure 10** shows photographs of BGO1V/hOG embryonic stem cells growing in plates in the presence of either the control 8nM NM23 (dimeric) or the Fab of either the cancer specific monoclonal antibody C2 (MIN-C2) or the Fab of stem cell specific monoclonal antibody C3 (2D6C3) at the concentrations indicated, showing that only the C3 Fab at 100ug/ml significantly inhibited the growth of stem cells.

[0033] **Figure 11** shows photographs of BGO1V/hOG embryonic stem cells growing in plates in the presence of either the control 8nM NM23 (dimeric) or the Fab of cancer specific monoclonal antibodies C2 (MIN-C2) and E6 (MIN-E6), showing that neither cancer specific antibody inhibited the growth of stem cells.

[0034] **Figure 12** shows a graph of measured Tra 1-60, a marker on undifferentiated stem cells, after stem cells were treated with either the control (Ctrl) 8nM NM23 (dimeric) or the Fab of either stem cell specific monoclonal antibodies C3 (2D6C3) and C8 (2D6C8) or with the Fab

of cancer specific monoclonal antibody E6 (MIN-E6). Results show that by another measure, the cancer specific antibody did not inhibit the growth of stem cells. Figure 12 shows that Fab's of the stem cell antibodies inhibit stem cell growth and cause loss of pluripotency marker Tra 1-60; here we show that the Fab's of stem cell monoclonal antibodies C3 and C8 decrease expression of stem cell marker Tra 1-60; the Fab of cancer cell antibody E6 has no effect on inhibition of BG01V cell growth: effect of Fab's measured at t=72 hrs.

[0035] **Figure 13** shows photographs of MUC1-positive DU145 prostate cancer cells after treatment with either the Fab of cancer specific antibody C2 (CA-C2-Fab (MIN-C2)), Fab of E6 (CA-E6-Fab (MIN-E6)) or the Fabs of stem cell specific antibodies C3 (STEM-C3-Fab (2D6C3)) or C8 (STEM-C8-Fab (2D6C8)), showing that only the Fabs of the cancer specific antibodies inhibited the growth of cancer cells. Figure 13 shows that stem cell specific mAbs (C3 and C8) do not bind to DU145 prostate cancer cells and do not inhibit cancer cell growth; Fab's of cancer specific mAbs (C2 and E6) do. The Fabs bind to and block ligand-induced dimerization of MUC1*.

[0036] **Figure 14** shows photographs and graphs of cell counts of MUC1-positive T47D breast cancer cells after treatment with either the Fab of cancer specific antibody C2 (CA-C2-Fab (MIN-C2)), Fab of E6 (CA-E6-Fab (MIN-E6)) or the Fabs of stem cell specific antibodies C3 (STEM-C3-FAB (2D6C3)) or C8 (STEM-C8-Fab (2D6C8)), showing that only the Fabs of the cancer specific antibodies inhibited the growth of cancer cells. Figure 14 shows stem cell specific mAbs (C3 and C8) do not bind to cancer cells and do not inhibit cancer cell growth; Fabs of cancer specific mAbs (C2 and E6) do.

[0037] **Figure 15** shows photographs and graphs of cell counts of MUC1-positive DU145 prostate cancer cells after treatment with either the Fab of cancer specific antibody C2 (C2-Fab (MIN-C2)), Fab of E6 (E6-Fab (MIN-E6)) or the Fabs of stem cell specific antibodies C3 (C3-Fab (2D6C3)) or C8 (C8-Fab (2D6C8)), showing that only the Fabs of the cancer specific antibodies inhibited the growth of cancer cells.

[0038] **Figure 16** is a graph of cell counts of MUC1-negative PC3 prostate cancer cells, which are MUC1 negative, after treatment with either the Fab of cancer specific antibody C2 (C2-Fab (MIN-C2)), Fab of E6 (E6-Fab (MIN-E6)) or the Fabs of stem cell specific antibodies C3 (C3-Fab (2D6C3)) or C8 (C8-Fab (2D6C8)), showing that none of the Fabs had any effect on MUC1-negative cancer cells.

[0039] **Figure 17** is the graphical representation of the results of an experiment in which nude mice were xenografted with human T47D breast tumors then treated with either vehicle (control) or 80mg/kg E6 (MIN-E6) Fab 2-times per week. Treatment commenced at Day 14 post implantation, then was suspended for 15 days, then resumed. Figure 17 shows that human breast

cancer tumors (T47D) were implanted into female Nu/Nu mice with estrogen pellets; anti-MUC1* Fab inhibited tumor growth.

[0040] **Figure 18** shows that anti-MUC1* Fab shrinks tumors and reduces expression of MUC1* growth factor receptor. Figure 18 shows photographs of two of the mice from that study.

[0041] **Figure 19** shows a graph of tumor volume for NOD/SCID mice xenografted with human prostate tumor line DU145, wherein half the mice were treated with E6 Fab at 160mg/kg every 48 hours and the other half treated with vehicle alone. Figure 19 shows anti-MUC1* Fab inhibits growth of DU145 tumors implanted into male NOD/SCID mice. miR-145 except for V8, low miR=high tumor volumes, except for Fab 5, high miR-145=low tumor volume.

[0042] **Figure 20** shows Western blot and a graph of microRNA 145 levels from tumors excised from mice of the study, showing that in general, the tumors treated with the E6 Fab changed to have less MUC1* growth factor receptor than the control group and more microRNA145 which signals cells to differentiate. Figure 20 shows that animals treated with anti-MUC1* Fab express less MUC1* growth factor receptor post treatment and become more differentiated (miR-145 increases).

[0043] **Figure 21** shows a graph of tumor volume for NOD/SCID mice xenografted with human prostate tumor line DU145, wherein half the mice began treatment when their tumors averaged over 400mm³ and the other half began treatment when their tumors were between 175mm³ and 300mm³. Half of the large tumors were treated with the E6 Fab and the other half the vehicle alone. Similarly, the second group was divided into treated with E6 Fab at 160mg/kg every 48 hours and the other half treated with vehicle alone. Results show that the Fab reduced tumor growth rate in both groups, however the group that began with the smaller tumors did much better. Figure 21 shows normalized tumor growth – DU145 human prostate cancer in NOD/SCID male mice. Groups 1 and 2 tumors were very large after 27 days growth – 350-550mm³ at start of treatment. Groups 3 and 4 tumors were 175-300mm³ at start of treatment. Despite size difference, both vehicle groups quickly merged to the same growth rate and size; smaller tumors responded better than the very large tumors as expected; 160 mg/kg every 48 hrs.

[0044] **Figure 22** shows photos of mice bearing human prostate tumors that received the mock treatment.

[0045] **Figure 23** shows photos of mice bearing human prostate tumors that received the cancer specific E6 (MIN-E6) Fab.

[0046] **Figure 24** shows anti-MUC1* IgG monoclonal antibody light chain variable region sequences.

[0047] **Figure 25** shows anti- MUC1* IgG monoclonal antibody heavy chain variable region sequences.

[0048] **Figure 26** shows the amino acid sequence of a MIN-C2 (single chain fragment variable) design (heavy chain variable – linker – light chain variable). The scFv construct was expressed in bacteria and purified using C-terminal poly-histidine (HHHHHH) tag.

[0049] **Figure 27** shows the amino acid sequence of a MIN-E6 scFv (single chain fragment variable) design heavy chain variable (MIN-E6 VH7) – linker – light chain variable). The scFv construct was expressed in bacteria and purified using C-terminal poly-histidine (HHHHHH) tag.

[0050] **Figure 28** shows amino acid sequence for the 2D6C3 Kappa Chain Variable Region. CDR1: RSSQTIVHSNGNTYLE (SEQ ID NO:70); CDR2: KVSNRFS (SEQ ID NO:71); and CDR3: FQGSHVPFT (SEQ ID NO:72).

[0051] **Figure 29** shows amino acid sequence for the 2D6C3 Heavy Chain Variable Region. CDR1: GYAMS (SEQ ID NO:73); CDR2: TISSGGTYIYYPDSVKG (SEQ ID NO:74); and CDR3: LGGDNYEY (SEQ ID NO:75).

[0052] **Figure 30** shows amino acid sequence for the 2D6C8 Kappa Chain Variable Region. CDR1: RASKSVSTSGYSYMH (SEQ ID NO:76); CDR2: LVSNLES (SEQ ID NO:77); and CDR3: QHIRELTRSE (SEQ ID NO:78).

[0053] **Figure 31** shows amino acid sequence for the 2D6C8 Heavy Chain Variable Region. CDR1: GYAMS (SEQ ID NO:79); CDR2: TISSGGTYIYYPDSVKG (SEQ ID NO:80); and CDR3: LGGDNYEY (SEQ ID NO:81).

[0054] **Figure 32** shows amino acid sequence for the 3C2B1 Kappa Chain Variable Region. CDR1: RASKSISTSDYNYIH (SEQ ID NO:82); CDR2: LASNLES (SEQ ID NO:83); and CDR3: QHSRELPLTF (SEQ ID NO:84).

[0055] **Figure 33** shows amino acid sequence for the 3C2B1 Heavy Chain Variable Region. CDR1: TYTMS (SEQ ID NO:85); CDR2: TISTGGDKTYYSDSVKG (SEQ ID NO:86); and CDR3: GTTAMYYYAM (SEQ ID NO:87).

DETAILED DESCRIPTION OF THE INVENTION

[0056] The present invention discloses antibodies and antibody variants that modulate a pathway involving MUC1* wherein one set of antibodies preferentially binds to MUC1* as it exists on stem cells but does not recognize MUC1* on cancer cells as well and another set of antibodies that preferentially binds to MUC1* as it exists on cancer cells but does not recognize MUC1* on stem cells as well. The present invention further discloses methods for identifying other antibodies that fall into these categories. The invention further discloses methods for using the first set of antibodies, hereafter referred to as “stem cell antibodies”, for stimulating stem cell growth *in vitro* and *in vivo*. The invention also discloses methods for using the second set of

antibodies, hereafter referred to as “cancer cell antibodies”, for inhibiting cancer cell growth *in vitro* and *in vivo*.

[0057] In the present application, as well as in all of the applications from which the present application claims priority, names that are given to the antibodies that are disclosed in the priority applications as well as in the present application are consistent. For instance, the cancer specific antibodies MIN-C2 (also referred to herein as well as in the applications from which the present application claims priority as “C2”) or MIN-E6 (also referred to herein as well as in the applications from which the present application claims priority as “E6”) are the same antibodies structurally and sequence-wise as referred to in the present application as in the applications from which the present application claims priority. Likewise, the stem cell specific antibodies 2D6C3 (also referred to herein as well as in the applications from which the present application claims priority as “C3”) or 2D6C8 (also referred to herein as well as in the applications from which the present application claims priority as “C8”) are the same antibodies structurally and sequence-wise as referred to in the present application as in the applications from which the present application claims priority.

[0058] In a preferred embodiment, a bivalent antibody selected from the set of stem cell antibodies is administered to a patient for stimulating the growth of the patient’s stem cells or progenitor cells. In a preferred embodiment, the progenitor cells are hematopoietic stem cells. In another embodiment, a bivalent antibody selected from the set of stem cell antibodies is used *in vivo* for stimulating the growth of a patient’s stem or progenitor cells that have been mobilized using another agent, such as a CSF. In another embodiment, a bivalent antibody selected from the set of stem cell antibodies is used *in vitro* for stimulating the growth of a person’s mobilized stem cells that have been extracted from the host for later transplantation either autologously or as donor cells transplanted into another allogeneically. In yet another embodiment, a bivalent antibody selected from the set of stem cell antibodies is used to stimulate the growth of stem cells *in vivo*. In another embodiment, a bivalent antibody selected from the set of stem cell antibodies is used for inducing pluripotency or inducing a less differentiated state in a set of cells. In another embodiment, antibodies selected from the set of stem cell antibodies is used to identify, select, isolate, or capture, including capture on a growth surface, stem or progenitor cells. In a preferred embodiment the stem and/or progenitor cells referred to above are human in origin. Stem cell antibodies can be used to accelerate the recovery of blood cells from effects of exposure to radiation, toxins or chemotherapy, used after bone marrow transplant, used before or after stem cell transplant which may be transplanted into the peripheral blood, and or to treat a patient who could benefit from increased production of hematopoietic stem cells or blood cells or their progenitors.

[0059] In one embodiment, antibodies selected from the set of cancer cell antibodies are used to treat cancer patients. In a preferred embodiment, antibodies selected from the set of cancer cell antibodies prevent the dimerization of the MUC1* portion of the MUC1 protein exemplified by most of the PSMGFR sequence N-terminus GTINVHDTVETQFNQYKTEAASRYNLTISDVSVSDVPPFSAQSGA –C terminus (SEQ ID NO:1). In a more preferred embodiment, antibodies selected from the set of cancer cell antibodies, used to treat a cancer patient, are monovalent, including Fabs, single chain constructs as well as other antibodies including engineered antibody-like agents that bind to a portion of the PSMGFR sequence and prevent its ligand-induced dimerization.

[0060] MUC1* generally refers to a MUC1 protein or alternative splice isoform that is devoid of some or all of its self-aggregation domain GFLGLSNIKFRPGSVVVQLTLAFREG (SEQ ID NO:2). In particular, MUC1* refers to MUC1 variants that lack the tandem repeat units. Most often MUC1* is a transmembrane cleavage product whose extra cellular domain consists primarily of a significant portion of the PSMGFR sequence. Because MUC1 can be cleaved at a number of positions, its exact site of cleavage may vary from one cell type to another.

[0061] In general, antibodies that belong to the group termed herein as “cancer cell antibodies” bind to the 35 amino acids that are at the C-terminal end of the PSMGFR sequence: QFNQYKTEAASRYNLTISDVSVSDVPPFSAQSGA (SEQ ID NO:3, also referred to in some figures as “N delta 10”).

[0062] In general, antibodies that belong to the group termed herein as “stem cell antibodies” bind to the 35 amino acids that are at the N-terminal end of the PSMGFR sequence: GTINVHDTVETQFNQYKTEAASRYNLTISDVSVSDV (SEQ ID NO:4, also referred to in some figures as “C delta 10”) but may also bind a peptide extended N-terminally from the peptide of SEQ ID NO:4, namely including ten (10) amino acids N-terminal to SEQ ID NO:4, which are VVQLTLAFRE (SEQ ID NO:5). Alternatively, antibodies that belong to the group of stem cell antibodies are selected based on their ability to bind to the peptide of sequence VVQLTLAFREGTINVHDTVETQFNQYKTEAASRYNLTISDVSVSDV (SEQ ID NO:6). In a preferred embodiment, the stem cell antibodies do not bind to the peptide SEQ ID NO:3.

[0063] Antibodies belonging to the set of cancer cell antibodies bind to a peptide containing 6-35 consecutive amino acids of the peptide of SEQ ID NO:3, which peptide may further contain up to 4 amino acid substitutions.

[0064] Antibodies belonging to the set of stem cell antibodies bind to a peptide containing 6-35 consecutive amino acids sequence of the peptide of SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, which peptide may further contain up to 4 amino acid substitutions.

[0065] To generate cancer cell antibodies, the immunogenic or antigenic peptide used contains 6-35 consecutive amino acids sequence of the peptide of SEQ ID NO:3, which peptide may further contain up to 4 amino acid substitutions. In a preferred embodiment, the portions of the peptide that are more C-terminal are used to generate cancer cell antibodies. For example peptides containing amino acids ASRYNLTISDVSVDVPPFSAQSGA (SEQ ID NO:7) or DVSVDVPPFSAQSGA (SEQ ID NO:8) are used to generate cancer cell antibodies. For the generation of cancer cell antibodies of the invention, the invention also includes methods for generating humanized antibodies, selecting human antibodies, or generating antibody-like proteins in which the peptide is not used to immunize an animal but rather is used, or the nucleic acids coding for it are used, to select an antibody or antibody-like protein that recognizes at least 6 consecutive amino acids from the peptide of SEQ ID NO:3, such as the peptide of SEQ ID NO:7 or SEQ ID NO:8. Further, the peptides of SEQ ID NO:3, SEQ ID NO:7 and SEQ ID NO:8 can be used to select for antibodies that specifically modulate the growth of cancer cells but not stem cells with antibodies that bind to the more C-terminal portions such as the peptide of SEQ ID NO:7, preferred wherein SEQ ID NO:8 is especially preferred. Phage display technology may be used to select antibodies or antibody-like proteins that bind to the peptides that identify it as a cancer specific antibody. Alternatively, antibodies are generated using the entire PSMGFR peptide and cancer cell antibodies are selected based on their ability to bind to peptides that contain 6-35 consecutive amino acids sequence of the peptide of SEQ ID NO:3 or SEQ ID NO:7 or SEQ ID NO:8, which peptide may further contain up to 4 amino acid substitutions.

[0066] To generate stem cell antibodies, the immunogenic or antigenic peptide used contains 6-35 consecutive amino acids sequence of the peptide of SEQ ID NO:4 or SEQ ID NO:5 or a peptide that has sequences from both, as in SEQ ID NO:6, which peptide may further contain up to 4 amino acid substitutions. In a preferred embodiment, the portions of the peptide that are more N-terminal are used to generate stem cell antibodies. For example peptides containing amino acids VVQLTLAFREGTINVHDTVETQFNQYKTEAASRYNL (SEQ ID NO:9) are preferred for the generation of stem cell antibodies. More preferred are peptides that contain consecutive amino acids from the peptide. Alternatively, antibodies are generated using the entire PSMGFR peptide and stem cell antibodies are selected based on their ability to bind to peptides that contain 6-35 consecutive amino acids sequence of the peptide of SEQ ID NO:4 or SEQ ID NO:5 or SEQ ID NO:6, which peptide may further contain up to 4 amino acid substitutions.

[0067] GTINVHDTVETQFNQYKTEAASRYNL (SEQ ID NO:10) for the generation or selection of stem cell specific antibodies. Still more preferred are peptides containing consecutive amino acids from GTINVHDTVETQFNQY (SEQ ID NO:11). For the generation of stem cell

antibodies, the invention also includes methods for generating humanized antibodies, selecting human antibodies, or generating antibody-like proteins in which the peptide is not used to immunize an animal but rather is used, or the nucleic acids coding for it are used, to select an antibody or antibody-like protein that recognizes at least 6 consecutive amino acids from the peptide of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, wherein SEQ ID NO:10 is especially preferred. Phage display technology may be used to select antibodies or antibody-like proteins that bind to the peptides that identify it as a stem cell specific antibody.

[0068] In a preferred embodiment, antibodies belonging to the set of cancer cell antibodies will bind to MUC1-positive cancer cells as determined by ICC, FACS or other similar analysis including ELISAs and phage display in which the antibody binds to a peptide containing some or all of the amino acids of SEQ ID NO:3. In a preferred embodiment, the antibody binds to a peptide containing the last 20 amino acids at the C-terminus of SEQ ID NO:3. In a more preferred embodiment the antibody binds to a peptide containing only the last 10 amino acids at the C-terminus of the peptide of SEQ ID NO:3. In a still more preferred embodiment, antibodies belonging to the set of cancer cell antibodies bind to MUC1-positive cancer cells but not to stem or progenitor cells. In addition, antibodies belonging to the cancer cell antibody set are selected by virtue of their ability to stimulate cancer cell growth when they are bivalent and inhibit cancer cell growth when monovalent, e.g. Fab. In a yet more preferred embodiment neither the bivalent nor the monovalent form of the antibody will affect the growth of stem cells.

[0069] In a preferred embodiment, antibodies belonging to the set of stem cell antibodies bind to stem and/or progenitor cells as determined by ICC, FACS or other similar analysis including ELISAs and phage display in which the antibody binds to a peptide containing some or all of the amino acids of SEQ ID NO:4. In a preferred embodiment, the stem and/or progenitor cells are human in origin. In another preferred embodiment, the antibody binds to a peptide containing only the 20 amino acids at the N-terminus of SEQ ID NO:4. In a more preferred embodiment the antibody will bind to a peptide containing only the 10 amino acids at the N-terminus of the peptide of SEQ ID NO:4. Alternatively, the antibody will bind to the peptide of SEQ ID NO:5, SEQ ID NO:6, or a peptide containing consecutive amino acids from the combined SEQ ID NOS:4 and 5. In a still more preferred embodiment, antibodies belonging to the set of stem cell antibodies will bind to human stem and/or progenitor cells but not to MUC1-positive cancer cells. In addition, antibodies belonging to the stem cell antibody set will be selected by virtue of their ability to stimulate stem and/or progenitor cell growth when they are bivalent and inhibit stem and/or progenitor cell growth when monovalent, e.g. Fab. In a yet more

preferred embodiment neither the bivalent nor the monovalent form of the antibody will affect the growth of cancer cells.

[0070] Monoclonal antibodies were generated by standard methods and antibody-producing hybridomas were selected based upon the selection criteria set out herein. Monoclonal antibodies 2D6C3 and 2D6C8 (also referred to herein as C3 and C8 respectively) were identified as stem cell antibodies. Their sequences are given in Figures 28 to 33. Monoclonal antibodies C2 and E6 were identified as cancer cell specific antibodies. The sequences of monoclonal antibodies C2 (MIN-C2) and E6 (MIN-E6) are given in Figures 24 to 27.

[0071] gaggtccagctggaggagtcagggggaggcttagtgaagcctggagggtccctgaaactctcctgtgcagcctctggatt cactttcagtggtatgccatgtcttgggttcgccagactccggagaagaggctggagtgggtcgcaaccattagtagtggtggtacttatc tactatccagacagtgtgaaggggcgattcaccatctccagagacaatgccaagaacaccctgtacctgcaaatgagcagtctgaggtctga ggacacggccatgtattactgtgcaagacttgggggggataattactacgaatacttcgatgtctggggcgcagggaccacggtcaccgtct cctccgcaaaacgacacccccatctgtctat (SEQ ID NO:12) describes MIN-C2 Heavy chain variable region.

[0072] EVQLEESGGGLVKPGGSLKLSCAASGFTFSGYAMSWVRQTPEKRLEWVATIS SGGTYIYYPD SVKGRFTISRDN AKNTLYLQMSSLRSED TAMY YCARLGGDNY YEYFDV WGAGTTVT VSSAKTTPPSVY (SEQ ID NO:13) describes MIN-C2 Heavy chain variable region.

[0073] gacattgtgatcacacagtctacagcttccttaggtgtatctctggggcagagggccaccatctcatgcagggccagcaaaa gtgtcagtacatctggctatagttatatgcactggtaccaacagagaccaggacagccacccaaactcctcatctatcttgcaccaacctaga atctgggggtccctgccaggttcagtggcagtgggtctgggacagacttcaccctcaacatccatcctgtggaggaggaggatgctgcaacc tattactgtcagcacagtagggagcttccgttcacgttcggaggggggaccaagctggagataaaacgggctgatgctgcaccaactgtatc c (SEQ ID NO:14) describes MIN-C2 Kappa chain variable region.

[0074] DIVITQSTASLG VSLGQRATISCRASKSVSTSGYSYMHWYQQRPGQPPKLLIY LASNLESGV PARFSGSGSGTDFTLNIHPVEEEDAATYYCQHSRELPFTFGGGTKLEIKRA DAAPT VS (SEQ ID NO:15) describes MIN-C2 Kappa chain variable region.

[0075] gaggttaagctggaggagtctgggggagacttagtgaagcctggagggtccctgaaactctcctgtgcagcctctggattc actttcagtagatatggcatgtcttgggttcgccagactccagacaagaggctggagtgggtcgcaaccattagtagtggtggtacttacatc actatccagacagtgtgaaggggcgattcaccatctccagagacaatgccaagaacaccctgtacctgcaaatgagcagtctgaagtctga ggacacagccatgtattactgtgcaagggataactacggtagtagctacgactatgctatggactactggggtaaggaacctcagtcaccg tctcctcagccaaaacaacagccccatcggtctat (SEQ ID NO:16) describes MIN-E6 Heavy chain-7 variable region.

[0076] EVKLEESGGDLVKPGGSLKLSCAASGFTFSRYGMSWVRQTDPDKRLEWVATIS SGGTYIYYPD SVKGRFTISRDN AKNTLYLQMSSLKSED TAMY YCARDNYGSSYDYAMD

YWGQGTSVTVSSAKTTAPSVY (SEQ ID NO:17) describes MIN-E6 Heavy chain-7 variable region.

[0077] gaggtaaagctggaggagtctgggggagacttagtgaagcctggagggtccctgaaactctcctgtgtagtctctggattc
actttcagtagatatggcatgtcttgggttcgccagactccaggcaagaggctggagtgggtcgcaaccattagtgggtggcggtacttacatc
tactatccagacagtgtgaaggggcgattcaccatctccagagacaatgccaagaacaccctgtacctgcaaatgagcagtctgaagtctga
ggacacagccatgtatcactgtacaagggataactacggtaggaactacgactacggatggactactgggggtcaaggaacctcagtcacc
gtctcctcagccaaaacaacagcccccacgggtctatccactggccccctgtgtgtggagatacaactggctcctcggtgactctaggatgcctg
gtcaag (SEQ ID NO:18) describes MIN-E6 Heavy chain-8 variable region.

[0078] EVKLEESGGDLVKPGGSLKLSCVVSFGFTFSRYGMSWVRQTPGKRLEWVATIS
GGGTYYIYPDSVKGRFTISRDNKNTLYLQMSSLKSEDTAMYHCTRDNYGRNYDYGM
DYWGQGTSVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVK (SEQ ID NO:19)
describes MIN-E6 Heavy chain-8 variable region.

[0079] gatattgtgatcaccagactacagcaatcatgtctgcattctccaggggaggaggtcaccctaacctgcagtgccacctcaa
gtgtaagttacatacactgggtccagcagaggccaggcacttctccaaaactctggatttatagcacatccaacctggcttctggagtcctgtt
cgcttcagtggcagtggatatgggacctcttactctctcacaatcagccgaatggagggtgaagatgctgccacttattactgccagcaaagg
agtagttccccattcacgttcggctcggggacaaagttggaataaaaacgggctgatgctgcaccaactgtatcc (SEQ ID NO:20)
describes MIN-E6 Kappa chain variable region.

[0080] DIVITQTTAIMSASPGEEVTLTCSATSSVSYIHWFQQRPGTSPKLWIYSTSNLAS
GVPVRFSGSGYGTSYSLTISRMEAEDAATYYCQQRSSSPFTFGSGTKLEIKRADAAPTVS
(SEQ ID NO:21) describes MIN-E6 Kappa chain variable region.

[0081] gaggtccagctggaggagtctgggggaggcttagtgaagcctggagggtccctgaaactctcctgtgcagcctctggatt
cactttcagtggctatgccatgtcttgggttcgccagactccggagaagaggctggagtgggtcgcaaccattagttagtggtggtacttatc
tactatccagacagtgtgaaggggcgattcaccatctccagagacaatgccaagaacaccctgtacctgcaaatgagcagtctgaggtctga
ggacacggccatgtattactgtgcaagacttgggggggataattactacgaatacttcgatgtctggggcgagggaccacgggtcaccgtct
cctccgcaaaaacgacacccccatctgtctatccactggccccctggatctgctgccc aaactaactccatgggtgacctgggatgcctggtc
aagggtatttcctgagccagtgcagtgacctggaactctggatccctgtccagcggtgtgcacacctcccagctgtcctgcagtctgac
ctctacactctgagcagctcagtgccttccagcactggccccagcgagaccgtcacctgcaacgttggccaccagccagcagg
accgcg (SEQ ID NO:22) describes MIN-C2 Fab Heavy chain.

[0082] gacattgtgatcacacagtctacagcttccttaggtgtatctctggggcagagggccaccatctcatgcagggccagcaaaa
gtgtcagtacatctggctatagttatatgcactggtaccaacagagaccaggacagccacccaaactcctcatctatcttgcaccaacctaga
atctgggggtccctgccaggttcagtggcagtgggtctgggacagacttcacctcaacatccatcctgtggaggaggaggatgctgcaacc
tattactgtcagcacagtagggagcttccgttcacgttcggaggggggaccaagctggagataaaaacgggctgatgctgcaccaactgtatc
catcttcccaccatccagtgcagcttaacatctggagggtgcctcagtcgtgtgcttctgaacaacttctaccccaaagacatcaatgtcaagt
ggaagattgatggcagtgaacgacaaaatggcgtcctgaacagttggactgatcaggacagcaaaagacagcacctacagcatgagcagc

accctcacgttgaccaaggacgagtatgaacgacataacagctatacctgtgaggccactcacaagacatcaacttcacccattgtcaagag
cttcaacaggaatgagtgt (SEQ ID NO:23) describes MIN-C2 Fab Kappa chain.

[0083] EVQLEESGGGLVKPGGSLKLSCAASGFTFSGYAMSWVRQTPEKRLEWVATIS
SGGTYYIYPDSVKGRFTISRDNKNTLYLQMSSLRSEDAMYYCARLGGDNYEYFDV
WGAGTTVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSS
GVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASRTA (SEQ ID NO:24)
describes MIN-C2 Fab Heavy chain.

[0084] DIVITQSTASLGVS LGQRATISCRASKSVSTSGYSYMHWYQQRPGQPPKLLIY
LASNLESGVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHSRELPFTFGGGTKLEIKRA
DAAPTVSIFPPSSEQLTSGGASVVCFLN NFYPKDINV KWKIDG SERQNGVLNSWTDQDS
KDSTYSMSSTLT LTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO:25)
describes MIN-C2 Fab Kappa chain.

[0085] RADAAPTVSIFPPSSEQLTSGGASVVCFLN NFYPKDINV KWKIDG SERQNGVL
NSWTDQDSKDSTYSMSSTLT LTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID
NO:26) describes MIN-C2 light CL region amino acid sequence.

[0086] FDVWGAGTTVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVT
VTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASRTA (SEQ ID
NO:27) describes MIN-C2 heavy chain CH1 region amino acid sequence.

[0087] DIVITQSTASLGVS LGQRATISC (SEQ ID NO:28) describes MIN-C2 light chain
variable framework region 1 (FWR1) amino acid sequence.

[0088] DIVITQTTAIMSASPGEVTLTC (SEQ ID NO:29) describes MIN-E6 light chain
variable framework region 1 (FWR1) amino acid sequence.

[0089] RASKSVSTSGYSYMH (SEQ ID NO:30) describes MIN-C2 light chain variable
complementarity determining region 1 (CDR1) amino acid sequence.

[0090] SATSSVSYIH (SEQ ID NO:31) describes MIN-E6 light chain variable
complementarity determining region 1 (CDR1) amino acid sequence.

[0091] WYQQRPGQPPKLLIY (SEQ ID NO:32) describes MIN-C2 light chain variable
framework region 2 (FWR2) amino acid sequence.

[0092] WFQQRPGTSPKLWIY (SEQ ID NO:33) describes MIN-E6 light chain variable
framework region 2 (FWR2) amino acid sequence.

[0093] LASNLES (SEQ ID NO:34) describes MIN-C2 light chain variable complementarity
determining region 2 (CDR2) amino acid sequence.

[0094] STSNLAS (SEQ ID NO:35) describes MIN-E6 light chain variable complementarity
determining region 2 (CDR2) amino acid sequence.

- [0095] GVPARFSGSGSGTDFTLNIHPVEEEDAATYYC (SEQ ID NO:36) describes MIN-C2 light chain variable framework region 3 (FWR3) amino acid sequence.
- [0096] GVPVRFSGSGYGTSYSLTISRMEAEDAATYYC (SEQ ID NO:37) describes MIN-E6 light chain variable framework region 3 (FWR3) amino acid sequence.
- [0097] QHSRELPFT (SEQ ID NO:38) describes MIN-C2 light chain variable complementarity determining region 3 (CDR3) amino acid sequence.
- [0098] QQRSSSPFT (SEQ ID NO:39) describes MIN-E6 light chain variable complementarity determining region 3 (CDR3) amino acid sequence.
- [0099] EVQLEESGGGLVKPGGSLKLSCAASGFTFS (SEQ ID NO:40) describes MIN-C2 heavy chain variable framework region 1 (FWR1) amino acid sequence.
- [00100] EVKLEESGGDLVKPGGSLKLSCAASGFTFS (SEQ ID NO:41) describes MIN-E6-7 heavy chain variable framework region 1 (FWR1) amino acid sequence.
- [00101] EVKLEESGGDLVKPGGSLKLSCVVS GFTFS (SEQ ID NO:42) describes MIN-E6-8 heavy chain variable framework region 1 (FWR1) amino acid sequence.
- [00102] GYAMS (SEQ ID NO:43) describes MIN-C2 heavy chain variable complementarity determining region 1 (CDR1) amino acid sequence.
- [00103] RYGMS (SEQ ID NO:44) describes MIN-E6-7 heavy chain variable complementarity determining region 1 (CDR1) amino acid sequence.
- [00104] RYGMS (SEQ ID NO:45) describes MIN-E6-8 heavy chain variable complementarity determining region 1 (CDR1) amino acid sequence.
- [00105] WVRQTPEKRLEWVA (SEQ ID NO:46) describes MIN-C2 heavy chain variable framework region 2 (FWR2) amino acid sequence.
- [00106] WVRQTPDKRLEWVA (SEQ ID NO:47) describes MIN-E6-7 heavy chain variable framework region 2 (FWR2) amino acid sequence.
- [00107] WVRQTPGKRLEWVA (SEQ ID NO:48) describes MIN-E6-8 heavy chain variable framework region 2 (FWR2) amino acid sequence.
- [00108] TISSGGTYIYYPDSVKG (SEQ ID NO:49) describes MIN-C2 heavy chain variable complementarity determining region 2 (CDR2) amino acid sequence.
- [00109] TISSGGTYIYYPDSVKG (SEQ ID NO:50) describes MIN-E6-7 heavy chain variable complementarity determining region 2 (CDR2) amino acid sequence.
- [00110] TISGGGTIYIYPDSVKG (SEQ ID NO:51) describes MIN-E6-8 heavy chain variable complementarity determining region 2 (CDR2) amino acid sequence.
- [00111] RFTISRDN AKNTLYLQMSSLRSED TAMYYCAR (SEQ ID NO:52) describes MIN-C2 heavy chain variable framework region 3 (FWR3) amino acid sequence.

- [00112]** RFTISRDN AKNTLYLQMSSLKSEDTAMYYCAR (SEQ ID NO:53) describes MIN-E6-7 heavy chain variable framework region 3 (FWR3) amino acid sequence.
- [00113]** RFTISRDN AKNTLYLQMSSLKSEDTAMYHCTR (SEQ ID NO:54) describes MIN-E6-8 heavy chain variable framework region 3 (FWR3) amino acid sequence.
- [00114]** LGGDNYEY (SEQ ID NO:55) describes MIN-C2 heavy chain variable complementarity determining region 3 (CDR3) amino acid sequence.
- [00115]** DNYGSSYDYA (SEQ ID NO:56) describes MIN-E6-7 heavy chain variable complementarity determining region 3 (CDR3) amino acid sequence.
- [00116]** DNYGRNYDYG (SEQ ID NO:57) describes MIN-E6-8 heavy chain variable complementarity determining region 3 (CDR3) amino acid sequence.
- [00117]** EVQLVESGGGLVKPGGSLRLSCA ASGFTFS (SEQ ID NO:58) describes IGHV3 (name from Igblast): FWR1: Human IgG antibody framework region sequence with 84.7% homology (249/294) to variable heavy chain region of MIN-C2.
- [00118]** WVRQAPGKGLEWVS (SEQ ID NO:59) describes IGHV3 (name from Igblast): FWR2: Human IgG antibody framework region sequence with 84.7% homology (249/294) to variable heavy chain region of MIN-C2.
- [00119]** RFTISRDN AKNSLYLQMNSLRAEDTAV (SEQ ID NO:60) describes IGHV3 (name from Igblast): FWR3: Human IgG antibody framework region sequence with 84.7% homology (249/294) to variable heavy chain region of MIN-C2.
- [00120]** DIVLTQSPASLAVSPGQRATITC (SEQ ID NO:61) describes IGkV7 (name from Igblast): FWR1: Human IgG antibody framework region sequence with 76.4% homology (226/296) to variable light chain region of MIN-C2.
- [00121]** WYQQKPGQPPLLIY (SEQ ID NO:62) describes IGkV7 (name from Igblast): FWR2: Human IgG antibody framework region sequence with 76.4% homology (226/296) to variable light chain region of MIN-C2.
- [00122]** GVPARFSGSGSGTDFTLTNPVEANDTANY (SEQ ID NO:63) describes IGkV7 (name from Igblast): FWR 3: Human IgG antibody framework region sequence with 76.4% homology (226/296) to variable light chain region of MIN-C2.
- [00123]** EVQLVESGGGLVKPGGSLRLSCAASGFTFS (SEQ ID NO:64) describes IGHV3 (name from Igblast): FWR1: Human IgG antibody framework region sequence with 84.1% homology (249/296) to variable heavy chain region of MIN-E6.
- [00124]** WVRQAPGKGLEWVS (SEQ ID NO:65) describes IGHV3 (name from Igblast): FWR2: Human IgG antibody framework region sequence with 84.1% homology (249/296) to variable heavy chain region of MIN-E6.

[00125] RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:66) describes IGHV3 (name from Igblast): FWR3: Human IgG antibody framework region sequence with 84.1% homology (249/296) to variable heavy chain region of MIN-E6.

[00126] EIVMTQSPATLSVSPGERATLSC (SEQ ID NO:67) describes IGKV3 (name from Igblast): FWR1: Human IgG antibody framework region sequence with 69.5% homology (187/269) to variable light chain region of MIN-E6.

[00127] WFQQRPGTSPK LLIY (SEQ ID NO:68) describes IGKV3 (name from Igblast): FWR2: Human IgG antibody framework region sequence with 69.5% homology (187/269) to variable light chain region of MIN-E6.

[00128] GIPARFSGSGSGTEFTLTISLQSEDFAVYYC (SEQ ID NO:69) describes IGKV3 (name from Igblast): FWR3: Human IgG antibody framework region sequence with 69.5% homology (187/269) to variable light chain region of MIN-E6.

[00129] Antibodies produced by the various clones were tested first by ELISA. Ideally, cancer cell specific antibodies would bind to the peptide SEQ ID NO:3 lacking the last 10 N-terminal amino acids of the PSMGFR peptide (SEQ ID NO:1), but not to the peptide of SEQ ID NO:4, lacking the last 10 amino acids at the C-terminus of the PSMGFR peptide. Ideally, the stem cell specific antibodies would have the opposite binding pattern; they should bind to peptide of SEQ ID NO:4 but not to peptide of SEQ ID NO:3. By ELISA, monoclonal antibodies 2D6C3 and 2D6C8 (also referred to herein as C3 and C8 respectively) bound to the peptide of SEQ ID NO:4 but not to the peptide of SEQ ID NO:3. Conversely, monoclonal antibodies C2 and E6 bound to the peptide of SEQ ID NO:3 but not to the peptide of SEQ ID NO:4, see Figure 2.

[00130] In another method to identify monoclonal antibodies that were specific for stem cells, antibodies were coated onto the surface of a cell culture plate. Because human stem cells are not adherent, they should not attach to the plate unless the plate is coated with an antibody that recognizes a receptor on the surface of the stem cells. In this way, clones 2D6C3 and 2D6C8 (also referred to herein as C3 and C8 respectively) were identified as being stem cell specific antibodies. In a preferred embodiment, the antibodies bound to a peptide whose sequence corresponds to the MUC1* extra cellular domain but not to the membrane proximal portion (SEQ ID NO:3). In a more preferred embodiment, the antibodies bound to the distal portion peptide (SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6) of the PSMGFR peptide and not to the membrane proximal portion (SEQ ID NO:3),

[00131] The monoclonal antibodies can additionally be tested for binding specificity by FACS to determine if they bound to cancer cells or stem cells. Figures 3-6 show FACS data that collectively show that the cancer specific antibodies C2 (MIN-C2) and E6 (MIN-E6) only bind to

MUC1-positive cancer cells but not to human stem cells. Conversely, Figures 3-6 show that stem cell antibodies 2D6C3 and 2D6C8 only bind to stem cells and not to cancer cells.

[00132] As yet another test to determine the specificity of monoclonal antibodies, their effect on the growth of either stem cells or cancer cells can be tested. As can be seen in the cartoon of Figure 1, bivalent anti-MUC1* antibodies dimerize the extra cellular domain of MUC1* and stimulate growth, while the monovalent Fabs inhibit growth. The bivalent forms of the antibodies were tested for their ability to stimulate the growth of human stem cells and the monovalent Fabs were tested for their ability to inhibit the growth of the stem cells. Figures 7-9 show photographs and plotted data showing that bivalent stem cell antibodies 2D6C3 and 2D6C8 (C3 and C8) stimulate human stem cell growth. Figures 10 - 12 show that the Fab of the stem cell antibody C3 at 100uM inhibits growth of stem cells but the Fabs of the cancer specific antibodies C2 (MIN-C2) and E6 (MIN-E6) do not. Figures 13 - 15 show that the Fabs of the cancer specific antibodies C2 (MIN-C2) and E6 (MIN-E6) inhibit the growth of MUC1-positive cancer cells but the Fabs of the stem cell specific antibodies 2D6C3 and 2D6C8 (C3 and C8) do not. Figure 16 shows that none of the Fabs of the cancer specific or stem specific antibodies have any effect on MUC1-negative cells, such as PC3 prostate cancer cells.

[00133] As yet a further test of binding specificity, antibodies selected to be stem specific can be tested *in vivo* and assayed for their ability to stimulate stem or progenitor cell growth. Cancer specific antibodies can be tested *in vivo* for their ability to inhibit cancer cell growth. In one example, to test the *in vivo* effects of a cancer cell antibody identified using methods of the invention, female nude mice, into which estrogen pellets had been implanted, were xenografted with human breast tumor cells (T47D). Tumors were allowed to engraft for 14 days before the mice were treated with 80mg/kg of the E6 Fab 2-times per week. An equal number of control mice were injected with buffer alone. Treatment was suspended during the 14 day period between Day 31 and Day 45 to determine whether the treated mice would develop a resistance to the treatment. The graph of Figure 17 shows that the E6 Fab effectively reduced tumor growth rate and volume compared to the control mice. The fact that after a 14 day lapse of treatment, the mice continued to respond to the E6 Fab shows that they did not develop a resistance to the drug. Figure 18 shows photographs of two of the mice from the study.

[00134] In another *in vivo* study, male NOD/SCID mice were xenografted with human prostate tumors (DU-145). Tumors were allowed to develop for 14 days before treatment or mock treatment was begun. 60 days after tumor implantation the groups were switched; the mice receiving the cancer cell antibody E6 Fab were then given the buffer alone and the control group began receiving the E6 Fab. Treatment was with 160mg/kg of the E6 Fab given every 24 hours. The graph of Figure 19 shows that the treated group had significantly smaller tumors than the

control group. Before the treatment groups were swapped, the tumors in the control group were more than 3-times larger than the treated group. In addition, only one of the treated mice had an increase in tumor growth when treatment was halted. Figure 20 is a Western blot for MUC1* that shows that even after 2 weeks without treatment, the treated group on average had less MUC1* than the control group. Additionally, the graph of Figure 20 shows that the treated group had an increase in the amount of microRNA-145 (miR-145) that they produced, which indicates that the E6 Fab treatment caused the cancer cells to differentiate which limited their ability to self-replicate.

[00135] In another animal study to test the efficacy of the cancer cell antibodies identified using methods of the invention, male NOD/SCID mice were xenografted with human prostate tumors (DU-145). Tumors were allowed to develop for 27 days before treatment or mock treatment was begun. The animals were divided into four groups wherein groups 1 and 2 had tumor volumes 350-500mm³ and groups 3 and 4 had tumor volumes 175-300mm³. Treatment was 160mg/kg of the E6 Fab given every 24 hours (groups 1 and 3) and the mock treatment was buffer alone (groups 2 and 4). The graph of Figure 21 shows that the tumors in the control mice grew at the same rate regardless of their starting volume. Although both treated groups had tumors that were much smaller than the control groups, mice whose tumors were in the 175-300mm³ responded better than the groups bearing tumors 350-500mm³ before treatment began. Figure 22 shows representative photographs of the mice from the control group and Figure 23 shows representative photographs of the mice from the treated group.

[00136] Thus, stem cell specific antibodies 2D6C3 and 2D6C8 (C3 and C8), as well as other monoclonal, polyclonals and antibody variants identified or selected using methods of the invention can be used much in the same way that ESAs and CSFs like Epogen and Leukine are used, including to accelerate the recovery of blood cells from effects of chemotherapy, used after bone marrow transplant, used before or after stem cell transplant which may be transplanted into the peripheral blood, and or to treat a patient who could benefit from increased production of hematopoietic stem cells or blood cells or their progenitors. There are several methods for humanizing stem cell specific antibodies 2D6C3 and 2D6C8 (C3 and C8), as well as other monoclonal, polyclonals and antibody variants identified or selected using methods of the invention, wherein constant regions of the mouse monoclonal antibodies are replaced with homologous human constant regions and wherein the variable region may be left as the mouse sequences or replaced by homologous human sequences. Phage display techniques can be used along with peptides of the invention to select the antibodies that bind best to the preferred MUC1* peptide. Alternatively, fully human antibody libraries can be screened *de novo* for their ability to bind to either the peptides used for selection of stem specific antibodies or the peptides

used for selection of the cancer specific antibodies. If a stem specific antibody is desired, candidate antibodies can be screened for their ability to bind to cancer specific peptides in a de-selection process.

[00137] Cancer specific antibodies such as C2 (MIN-C2) and E6 (MIN-E6) as well as other monoclonal, polyclonals and antibody variants identified or selected using methods of the invention can be used to treat cancer patients with reduced risk of killing the patient's stem cells or blood cell precursors and progenitors. There are several methods for humanizing cancer specific antibodies C2 (MIN-C2) and E6 (MIN-E6), as well as other monoclonal, polyclonals and antibody variants identified or selected using methods of the invention, wherein constant regions of the mouse monoclonal antibodies are replaced with homologous human constant regions and wherein the variable region may be left as the mouse sequences or replaced by homologous human sequences. Phage display techniques can be used along with peptides of the invention to select the antibodies that bind best to the preferred MUC1* peptide. Alternatively, fully human antibody libraries can be screened *de novo* for their ability to bind to either the peptides used for selection of stem specific antibodies or the peptides used for selection of the cancer specific antibodies. If a cancer specific antibody is desired, candidate antibodies can be screened for their ability to bind to stem specific peptides in a de-selection process.

[00138] The invention includes antibodies as well as antibody-like proteins, including but not limited to polyclonal, monoclonal, chimeras, humanized, single chain, antibody fragments and the like. In addition, the invention includes the use of protein scaffolds for generating antibody mimics to obtain proteins that can be characterized by binding assays described herein and according to methods of the invention as being stem cell antibodies or cancer cell antibodies and thus being able to bind specifically to either MUC1* as it exists on cancer cells, or bind to MUC1* as it exists on stem cells, progenitor cells or engineered cells that express a form of MUC1*. The invention further includes using methods set forth here to identify antibodies that recognize specific epitopes, within the MUC1* extra cellular domain, that are expressed on different types of progenitor cells or non-tumor cancer cells.

[00139] In general, antibodies for the treatment of cancer should inhibit dimerization of MUC1* receptor. Thus in a preferred embodiment, antibodies for the treatment of MUC1-positive cancers are monovalent, such as Fabs, single chain antibodies, or bispecific antibodies. The invention does contemplate the use of antibodies such as pentavalent IgMs for the treatment of cancers because the multivalency acts to re-cluster the MUC1* extra cellular domain. However, bivalent antibodies at high enough concentration bind one antibody to each receptor rather than one antibody per each one receptor and in so doing are also inhibitory to cancer growth and development.

[00140] In general, antibodies of the invention that are suitable for stimulating stem and progenitor cell growth are bivalent such that they activate growth, survival and pluripotency pathways by dimerizing the MUC1* extra cellular domain. Stem and progenitor cell specific antibodies can be used to treat patient suffering from anemia, low white blood cell count, low platelets or any blood cell or blood progenitor cell deficiency. Additionally, the invention contemplates the use of stem and progenitor specific antibodies for use as anti-aging therapeutics, agents to promote overall health, agents to enhance stem or progenitor cell growth or to enhance engraftment of cells. Stem and progenitor specific antibodies described here may be administered to the patient systemically or locally, as an injection or as a topical treatment, as in a medicament for the eye or other areas of the body that are mechanically accessible.

[00141] Bivalent stem cell specific antibodies 2D6C3 and 2D6C8 (C3 and C8), as well as other monoclonal, polyclonals and antibody variants identified or selected using methods of the invention, can be used to stimulate stem cell growth *in vitro* or *in vivo* or can alternatively be used to enhance engraftment of stem cell transplants, irrespective of the source. In another embodiment, stem cell specific bivalent antibodies of the invention are used to stimulate the growth of hematopoietic stem cells *in vitro* or *in vivo*. In some cases stem cell antibodies are used to treat patients suffering from or at risk of developing anemia or low white blood count. These patients may be suffering from cancer and may be simultaneously treated with a cancer cell antibody of the invention for inhibiting their cancer cell growth.

[00142] In another aspect, antibodies and peptides of the invention are used for diagnosis. Contacting a patient's cells with a cancer specific antibody and getting significant binding would indicate that the patient has cancer. Similarly, contacting a patient's cells with a stem specific antibody and getting significant binding would indicate that the patient does not have cancer and would further identify those cells as stem or progenitor cells. A patient's cells can be contacted by both types of antibodies in order to distinguish stem cells from cancer cells. Such diagnosis can be carried out *in vitro* or *in vivo*. *In vitro*, a tissue specimen, blood sample, or bodily fluid sample can be analyzed using cancer or stem specific antibodies. *In vivo*, imaging agents can be attached to the antibodies of the invention to enable identification of cancer or stem cells in a patient.

EXAMPLES

[00143] Example 1 – Development of monoclonal antibodies, 2D6C8 and 2D6C3 (also referred to here as C3 and C8) that facilitate human stem cell attachment to surfaces

[00144] MUC1* monoclonal antibodies were identified that preferentially bound to the portion of the MUC1* extra cellular domain that is more distal from the cell surface and these

monoclonals were shown to better facilitate the attachment of human ES and iPS cells to surfaces. Mice were immunized with a peptide that is defined by the PSMGFR sequence. Supernatants of hybridoma clones were tested by ELISA for their ability to bind to the PSMGFR peptide and by FACS to determine which bound to live, MUC1* positive cells. Hybridomas were further selected if they preferentially bound to the PSMGFR peptide lacking 10 C-terminal amino acids, but did not bind if the peptide lacked the 10 N-terminal peptides. In addition, hybridomas were screened for their ability to facilitate stem cell attachment to a surface such as a plastic cell culture plate. Of these clones two, 2D6C8 and 2D6C3 were selected that when coated onto a surface captured stem cells and facilitated their growth.

What is claimed:

1. A method for treating a patient who would benefit from stimulation of the patient's stem cells, comprising administering to the patient an antibody that specifically binds to an epitope of the MUC1 protein expressed on human undifferentiated stem cells.
2. The method according to Claim 1, wherein the antibody specifically binds to at least six consecutive amino acids of the peptide of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:11.
3. The method according to Claim 1, wherein the antibody does not bind or binds significantly less to the peptide of SEQ ID NO:3, SEQ ID NO:7 or SEQ ID NO:8.
4. The method according to Claim 1, wherein the patient suffers from cancer.
5. The method according to Claim 4, wherein the patient suffers from cancer and is receiving chemotherapy or radiation.
6. The method according to Claim 1, wherein the patient suffers from chronic kidney disease.
7. A method for treating a patient suffering from cancer or at risk of developing cancer, comprising administering to the patient an antibody that specifically binds to an epitope of the MUC1 protein that is expressed on cancer cells but is not expressed on human undifferentiated stem cells.
8. The method according to Claim 7, wherein the antibody specifically binds to the peptide of SEQ ID NO:3 and does not bind to or binds significantly less to the peptide of SEQ ID NO:4.
9. A method for treating a patient diagnosed with a MUC1-positive cancer comprising administering to the patient a monovalent cancer cell-specific antibody.
10. The method according to claim 9, wherein the cancer cell-specific antibody specifically binds to the peptide of SEQ ID NO:3 and does not bind to or binds significantly less to the peptide of SEQ ID NO:4.

11. A method for treating a patient diagnosed with a MUC1-positive cancer comprising administering to the patient a monovalent cancer cell-specific antibody and bivalent stem cell specific antibody.
12. The method according to claim 11, wherein the cancer cell-specific antibody specifically binds to the peptide of SEQ ID NO:3 and does not bind to or binds significantly less to the peptide of SEQ ID NO:4.
13. The method according to claim 11, wherein the stem cell specific antibody specifically binds to at least six consecutive amino acids of the peptide of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:11, and does not bind to or binds significantly less to the peptide of SEQ ID NO:3, SEQ ID NO:7 or SEQ ID NO:8.
14. A method for proliferating stem or progenitor cells, comprising contacting the cells with an antibody that specifically binds to an epitope of the MUC1 protein expressed on human stem cells, without causing proliferation of cancer cells.
15. The method according to claim 14, wherein the antibody specifically binds to at least six consecutive amino acids of the peptide of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10 or SEQ ID NO:11.
16. The method according to Claim 15, wherein the antibody does not bind to or binds significantly less to the peptide of SEQ ID NO:3, SEQ ID NO:7 or SEQ ID NO:8.
17. A method of obtaining an antibody that specifically binds to stem cells, but not to cancer cells comprising the steps of:
- (i) generating a mixed set of antibodies that recognize a peptide whose sequence is that of any of the peptides having sequence of SEQ ID NOS:1-11;
 - (ii) selecting those antibodies that bind to a peptide of SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, but not to the peptide of SEQ ID NO:3;
 - (iii) selecting those antibodies that when adsorbed onto a surface facilitate the attachment of stem cells; and
 - (iv) selecting those antibodies that in the bivalent form stimulate the growth of stem cells and in the monovalent form inhibit the growth of stem cells, while having no effect on cancer cells.

18. A method of obtaining an antibody that specifically binds to cancer cells, but not to stem cells comprising the steps of:

(i) generating a mixed set of antibodies that recognize a peptide whose sequence is that of any of the peptides having sequence of SEQ ID NOS:1-11;

(ii) selecting those antibodies that bind to a peptide of SEQ ID NO:3, but not to the peptide of SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6;

(iii) selecting those antibodies that when adsorbed onto a surface facilitate the attachment of cancer cells; and

(iv) selecting those antibodies that in the bivalent form stimulate the proliferation of cancer cells and in the monovalent form inhibit the growth of cancer cells, while having no effect on the proliferation of stem cells.

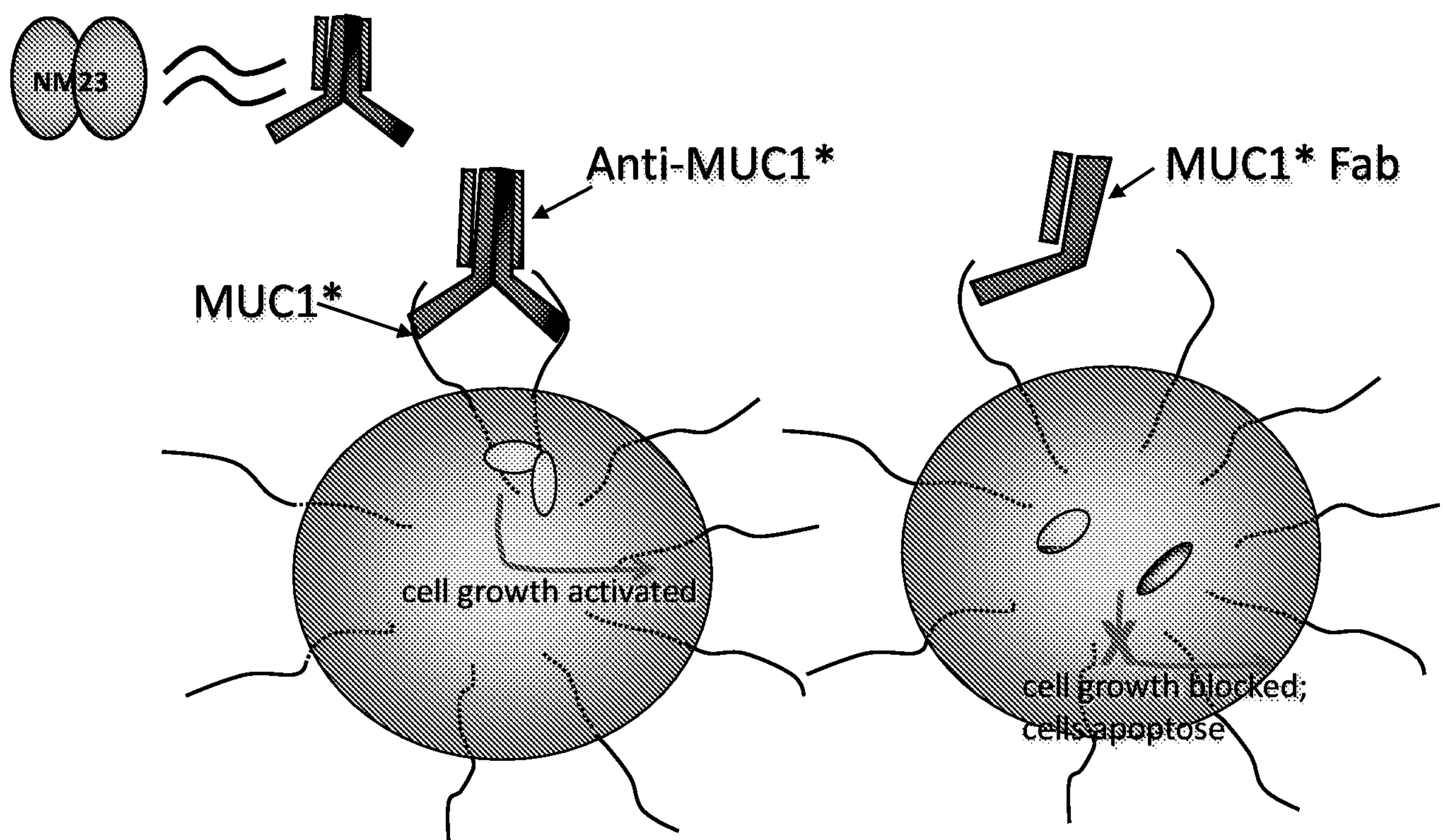


Fig. 1

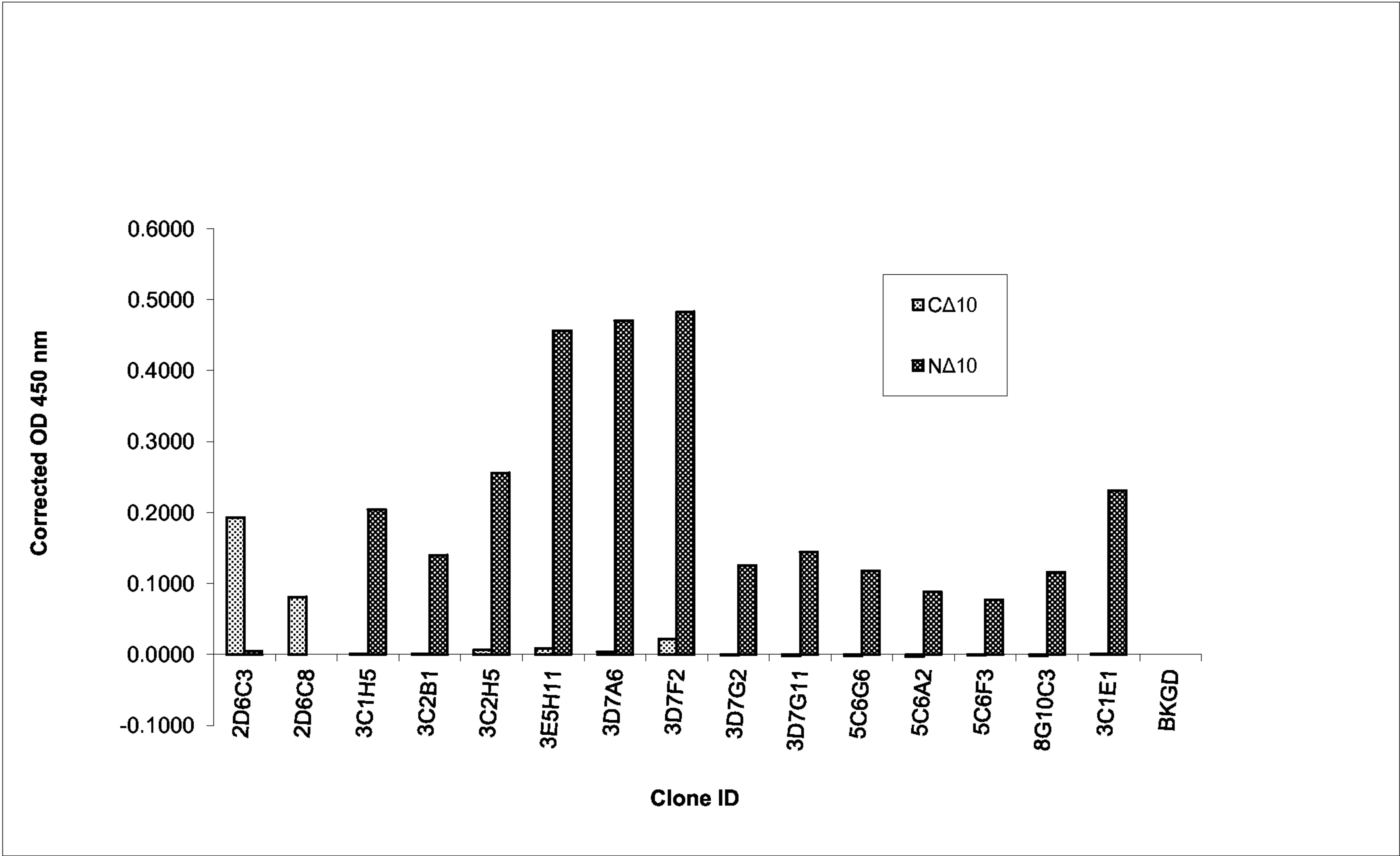


Fig. 2

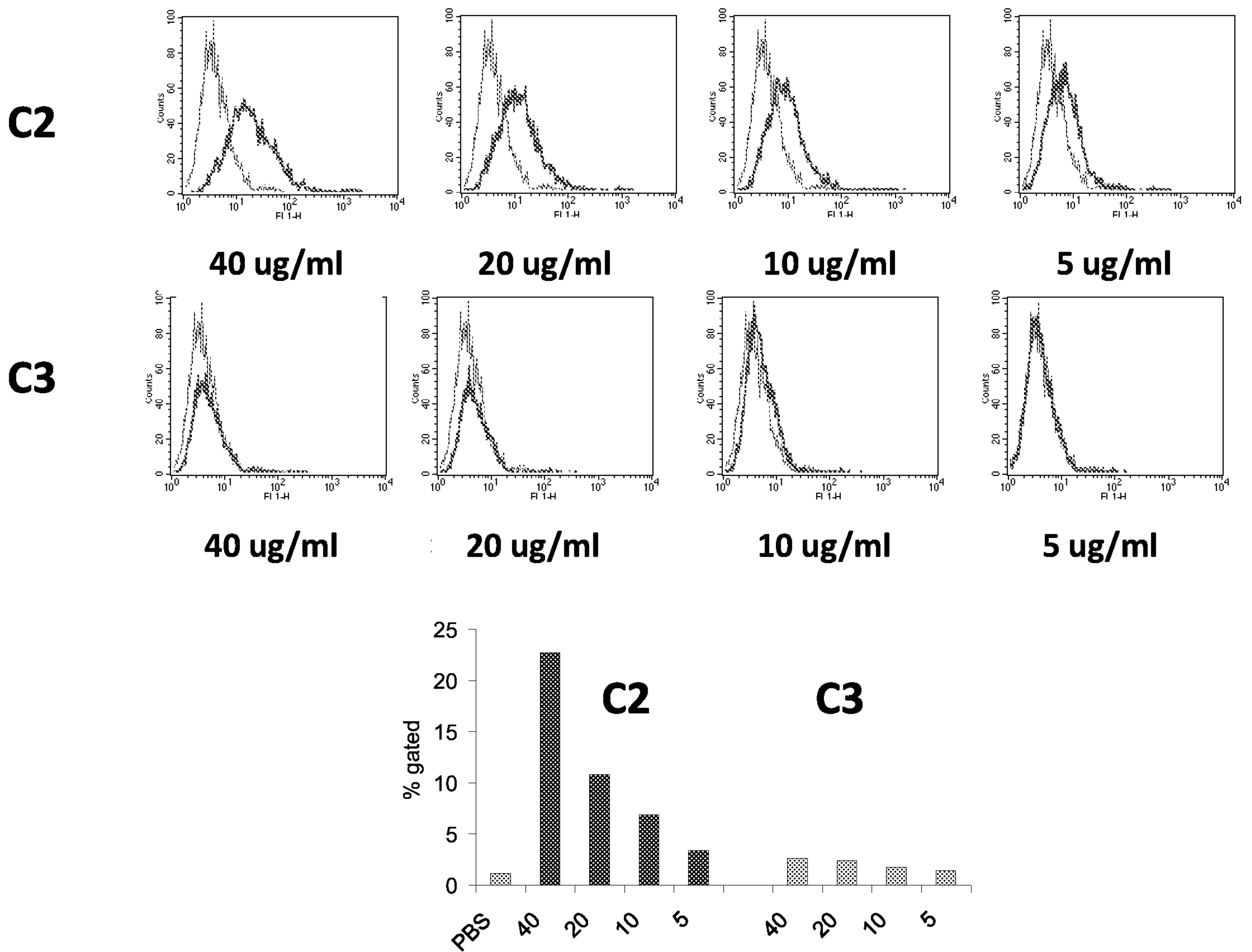


Fig. 3

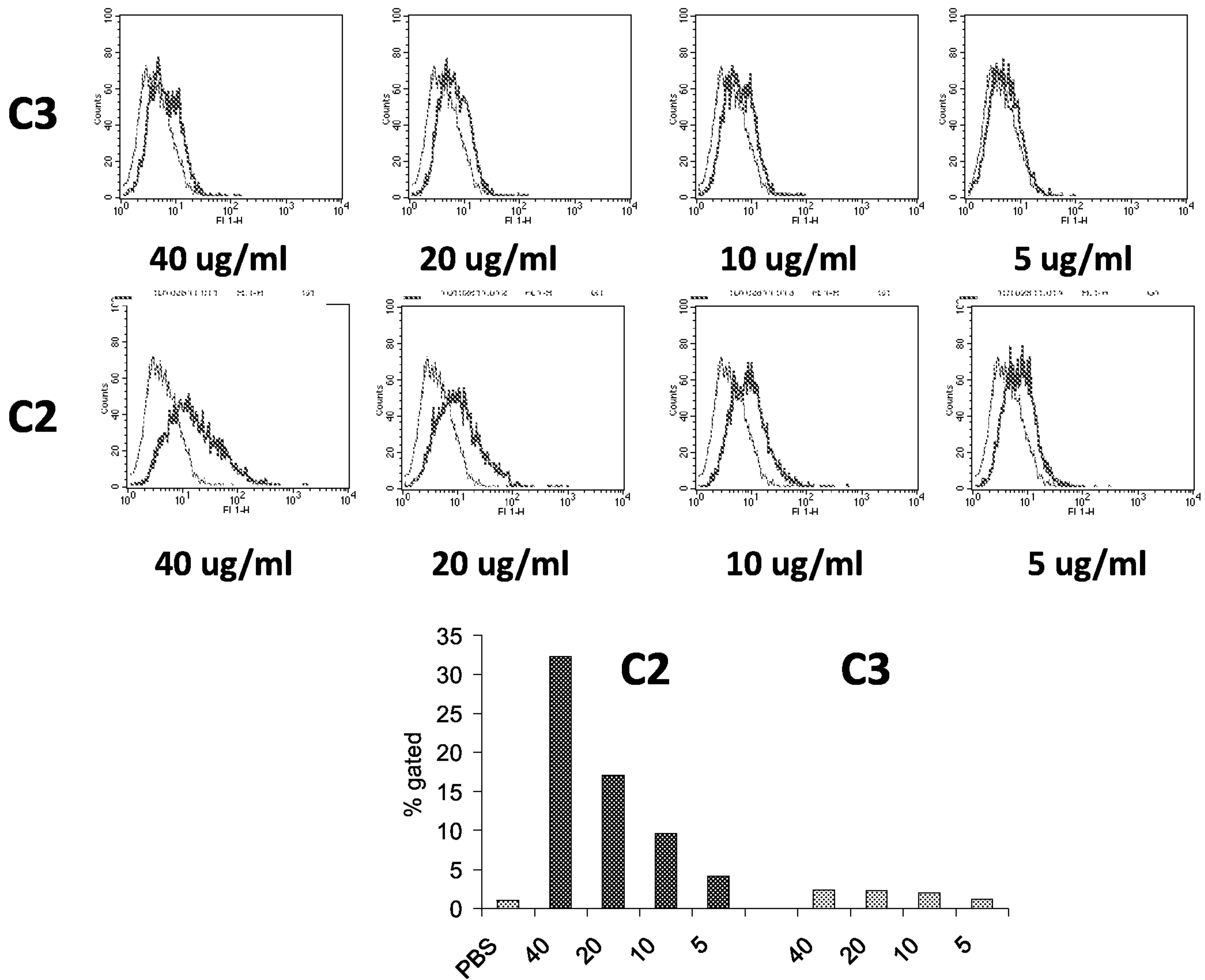


Fig. 4

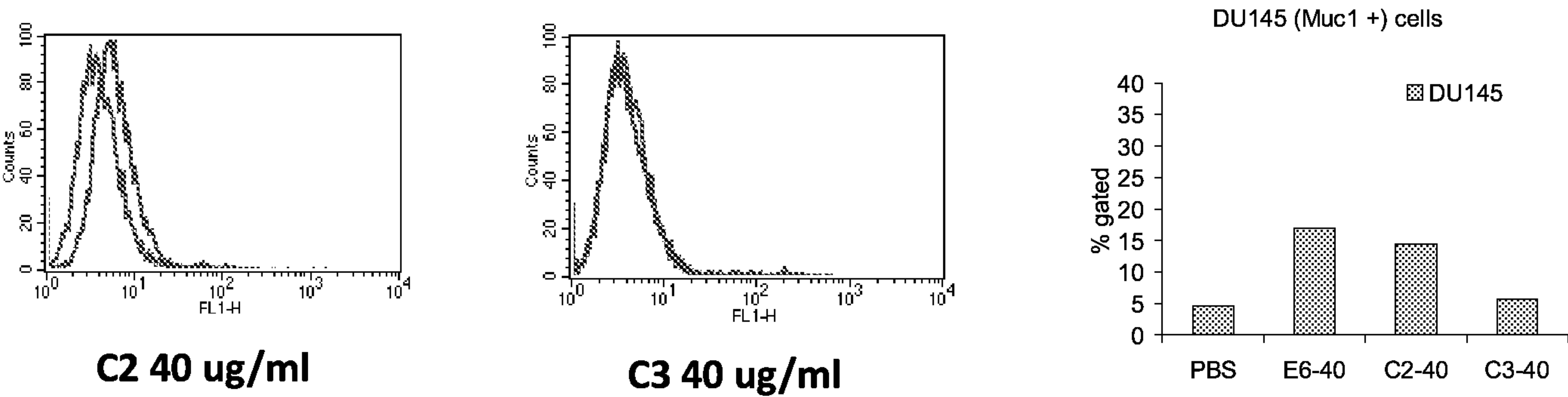


Fig. 5

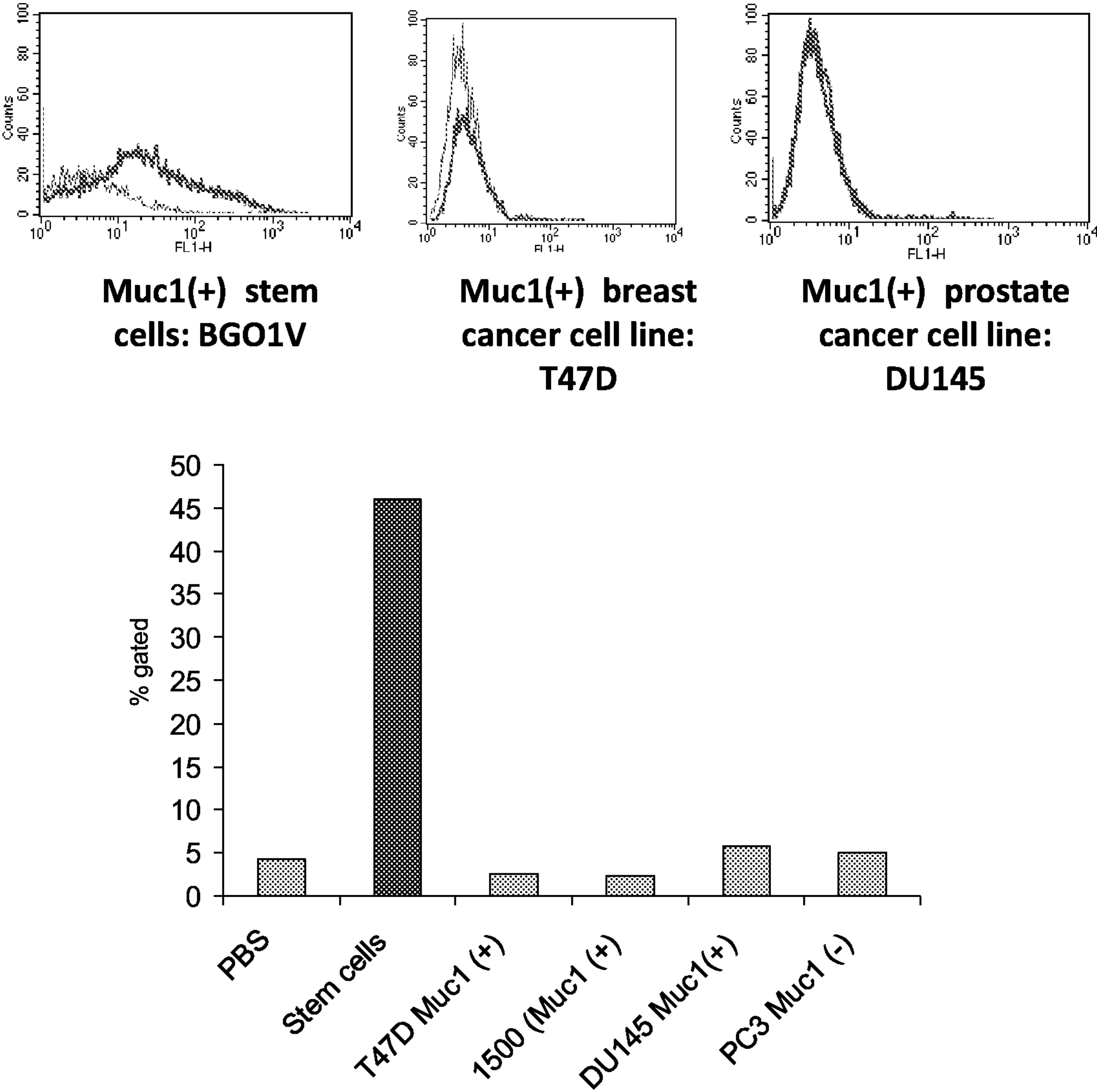


Fig. 6

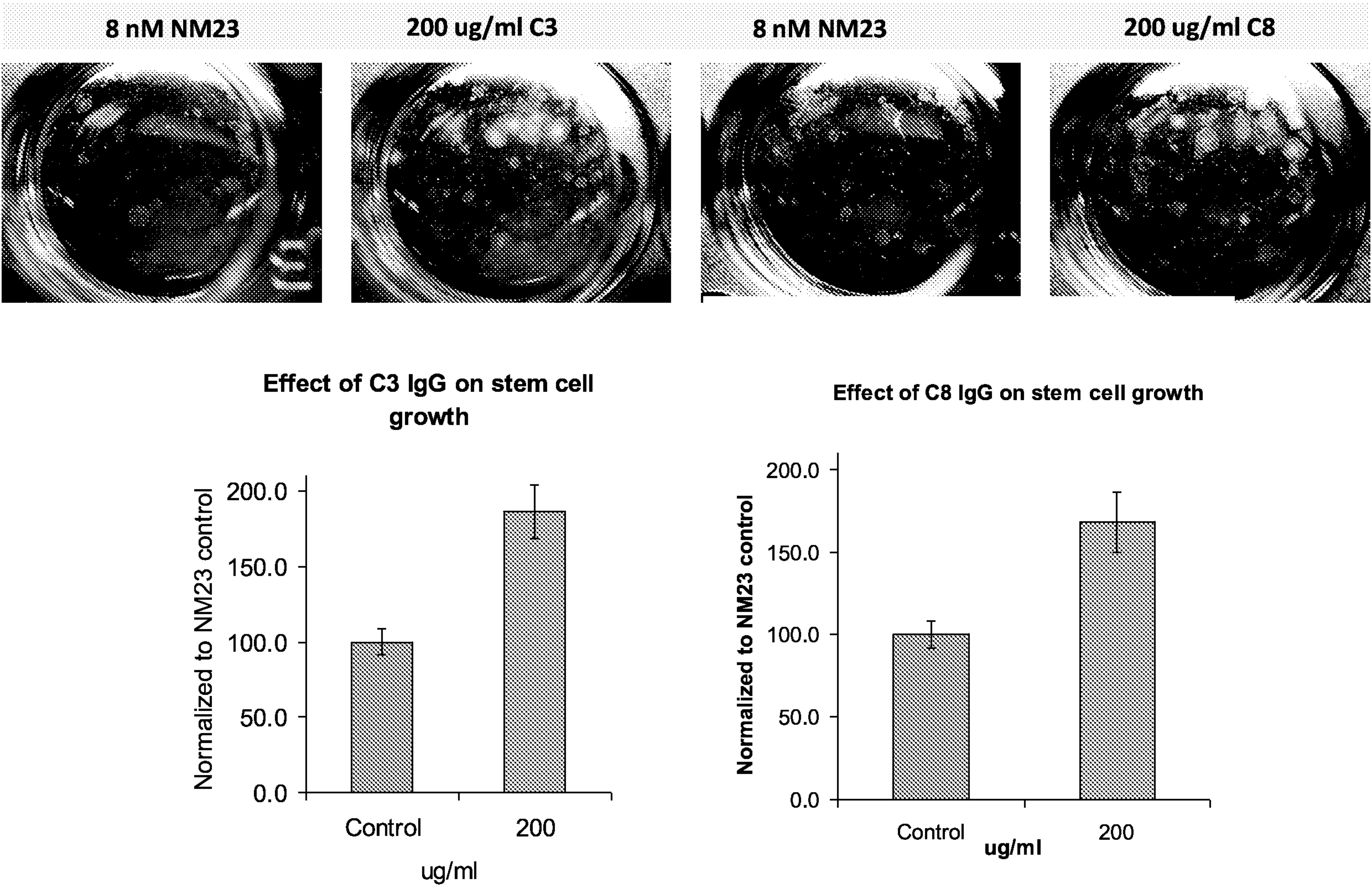
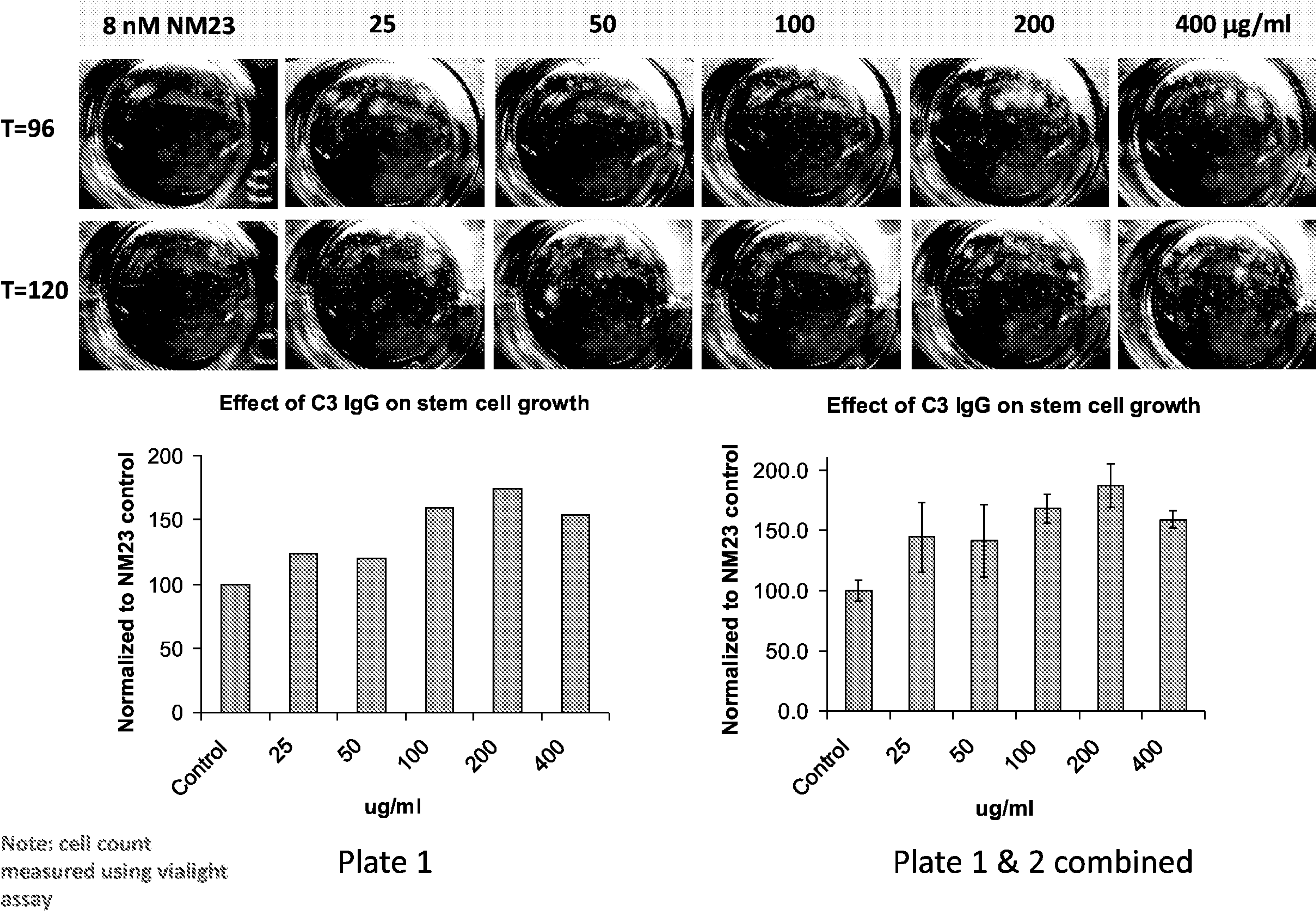
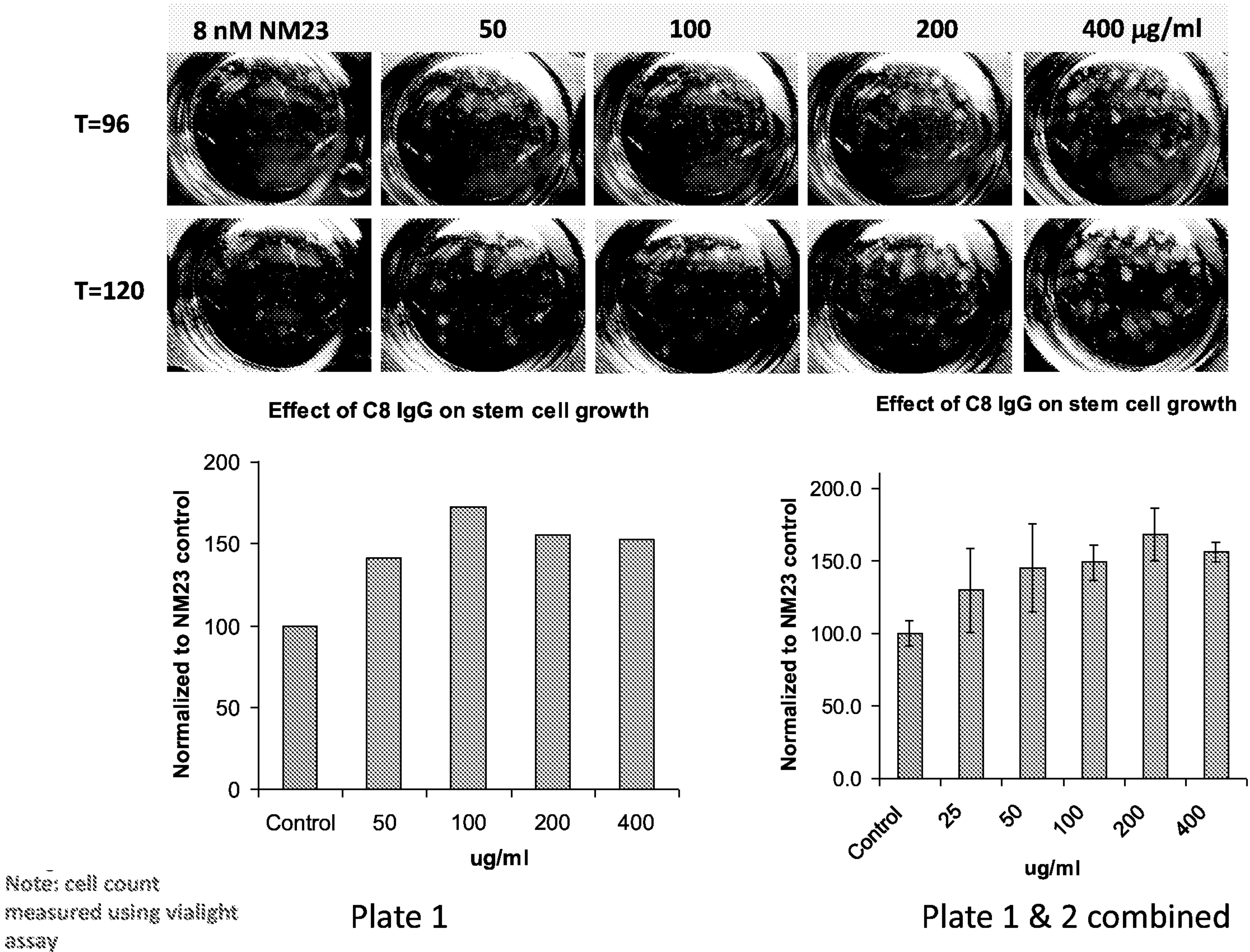
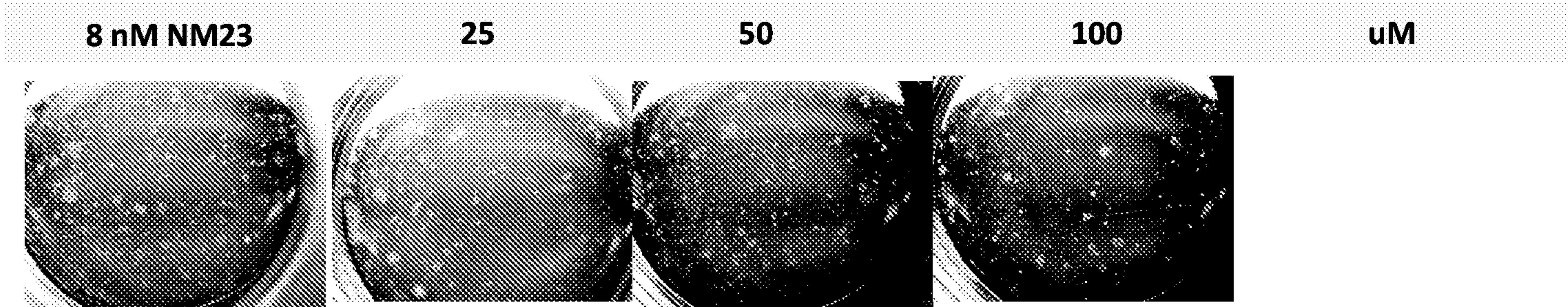


Fig. 7





The Fab of cancer cell antibody C2-FAB has no effect on growth of BGO1V stem cell growth



The Fab of stem cell antibody C3-FAB inhibits stem cell growth as a function of Fab concentration

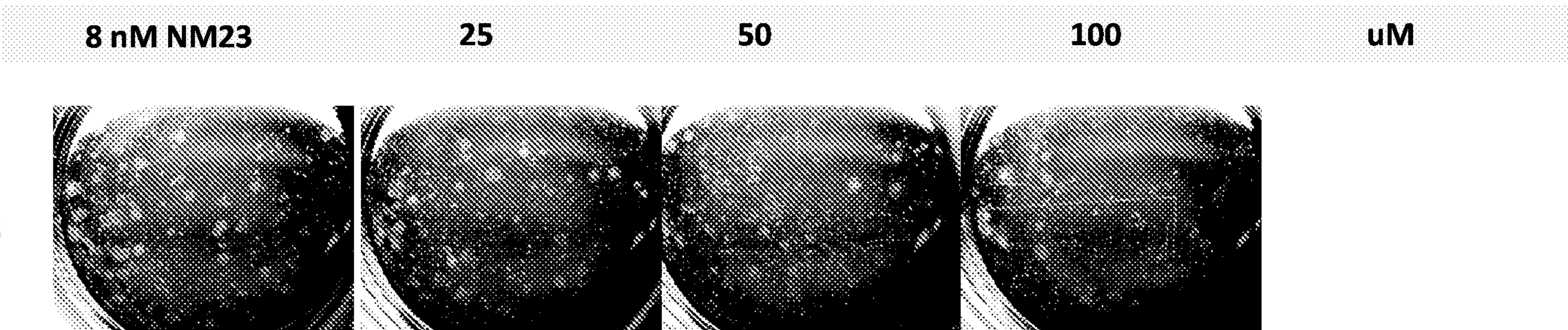
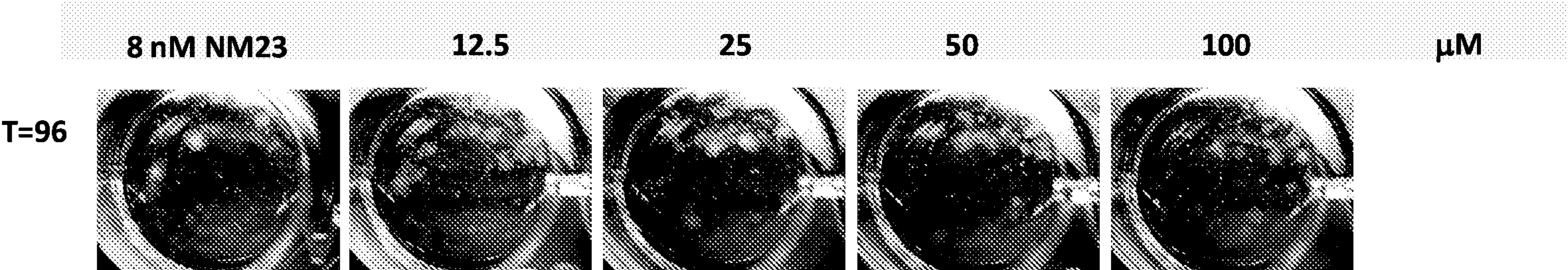


Fig. 10

The Fab of cancer cell antibody C2-FAB has no effect on growth ofBGO1V stem cell growth



The Fab of cancer cell antibody E6-FAB has no effect on growth ofBGO1V stem cell growth

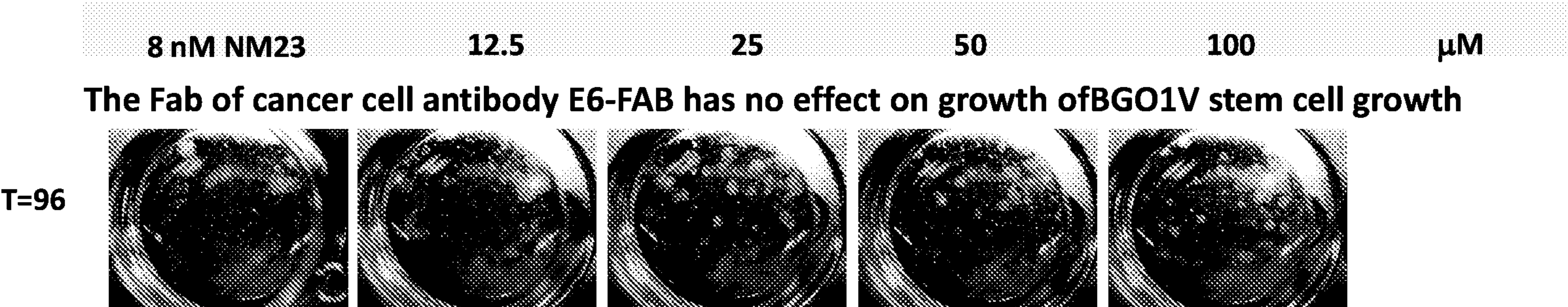


Fig. 11

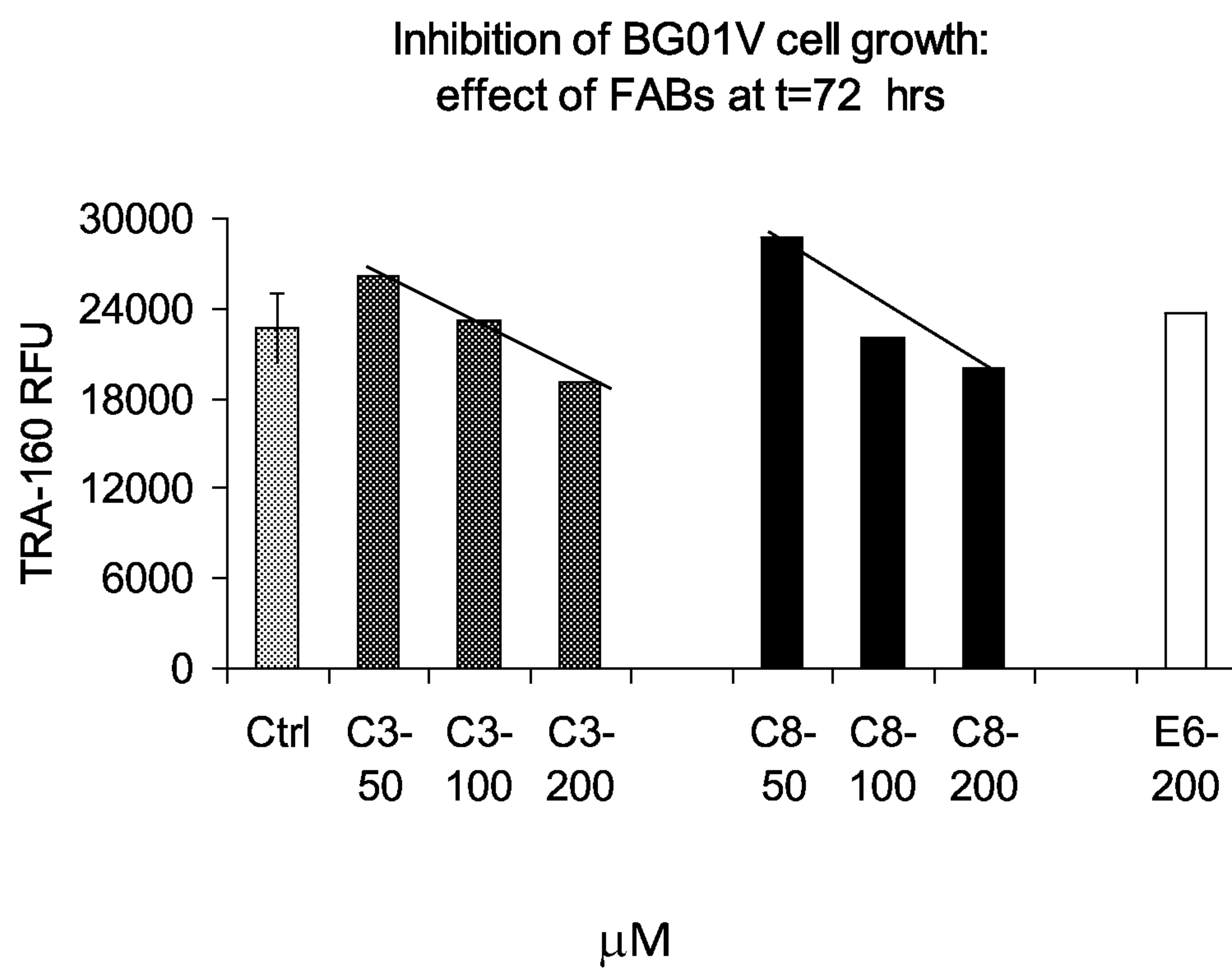
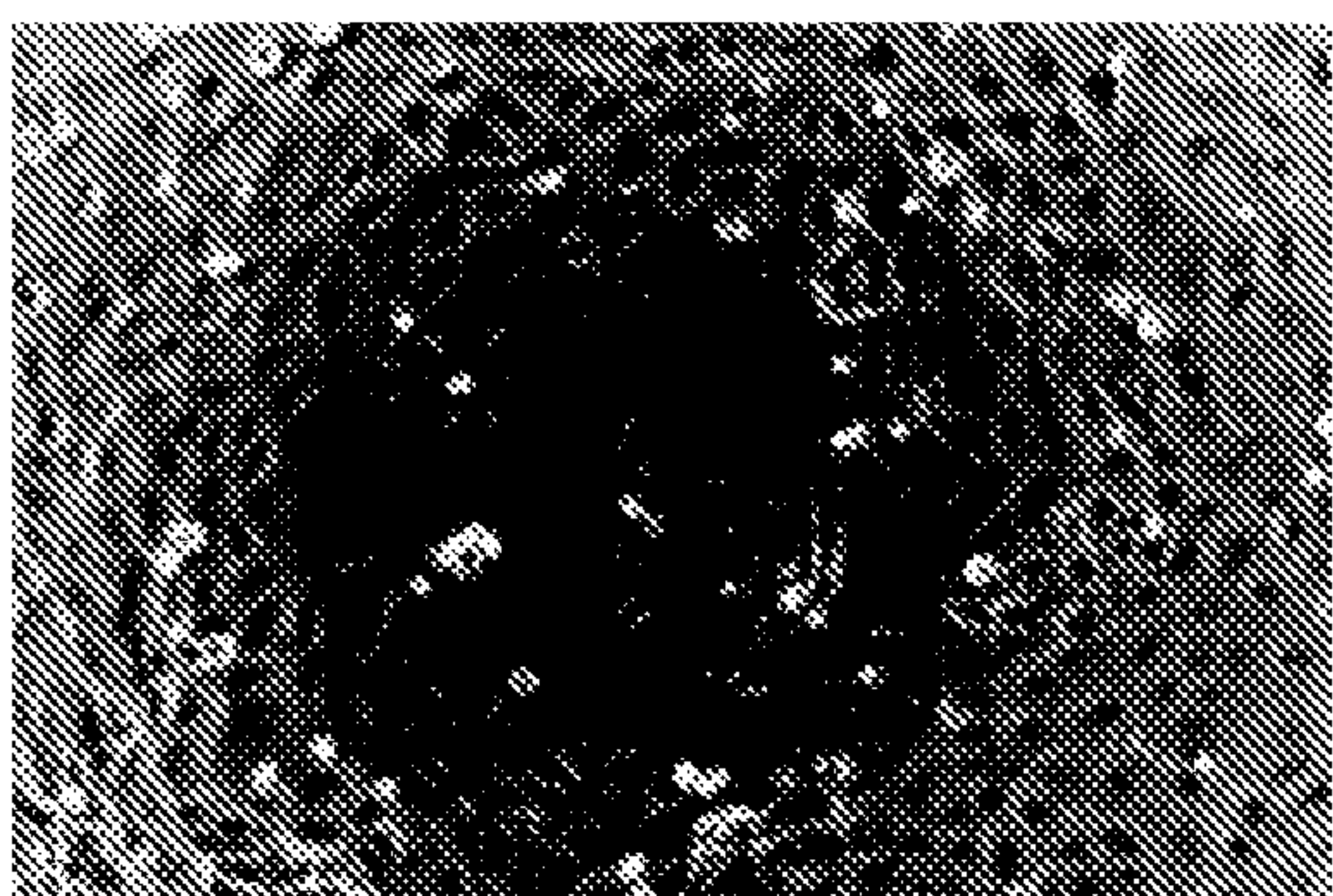
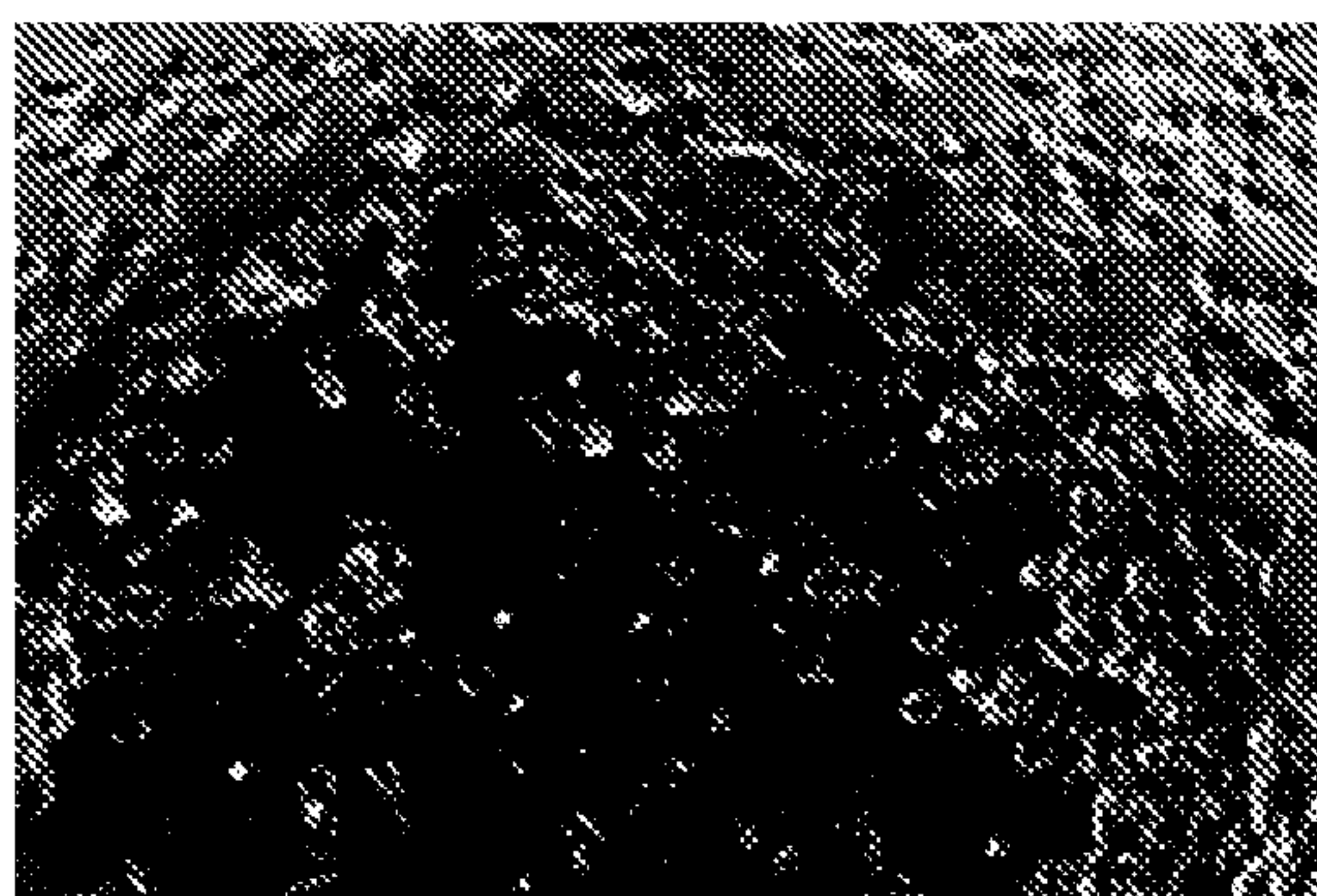
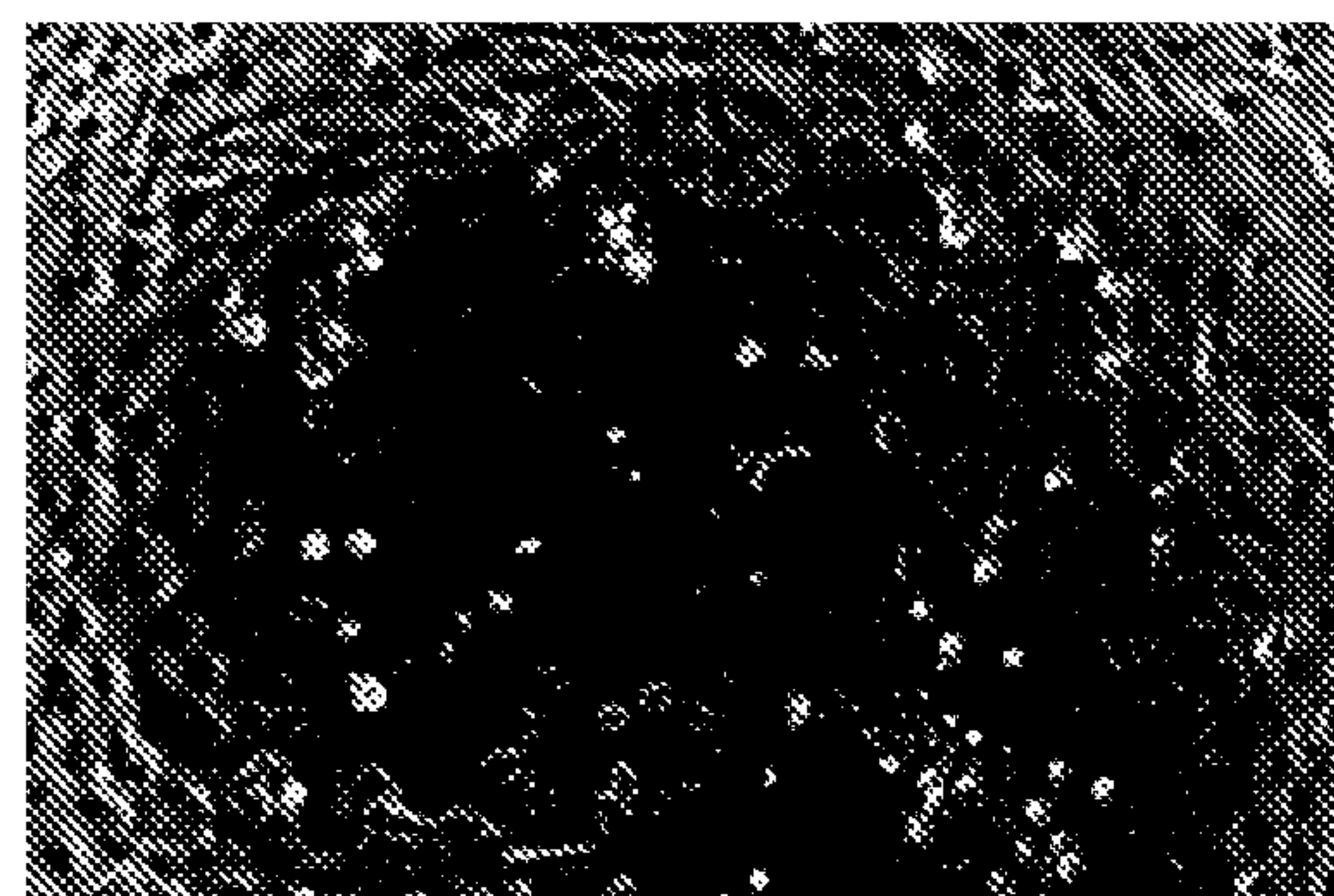
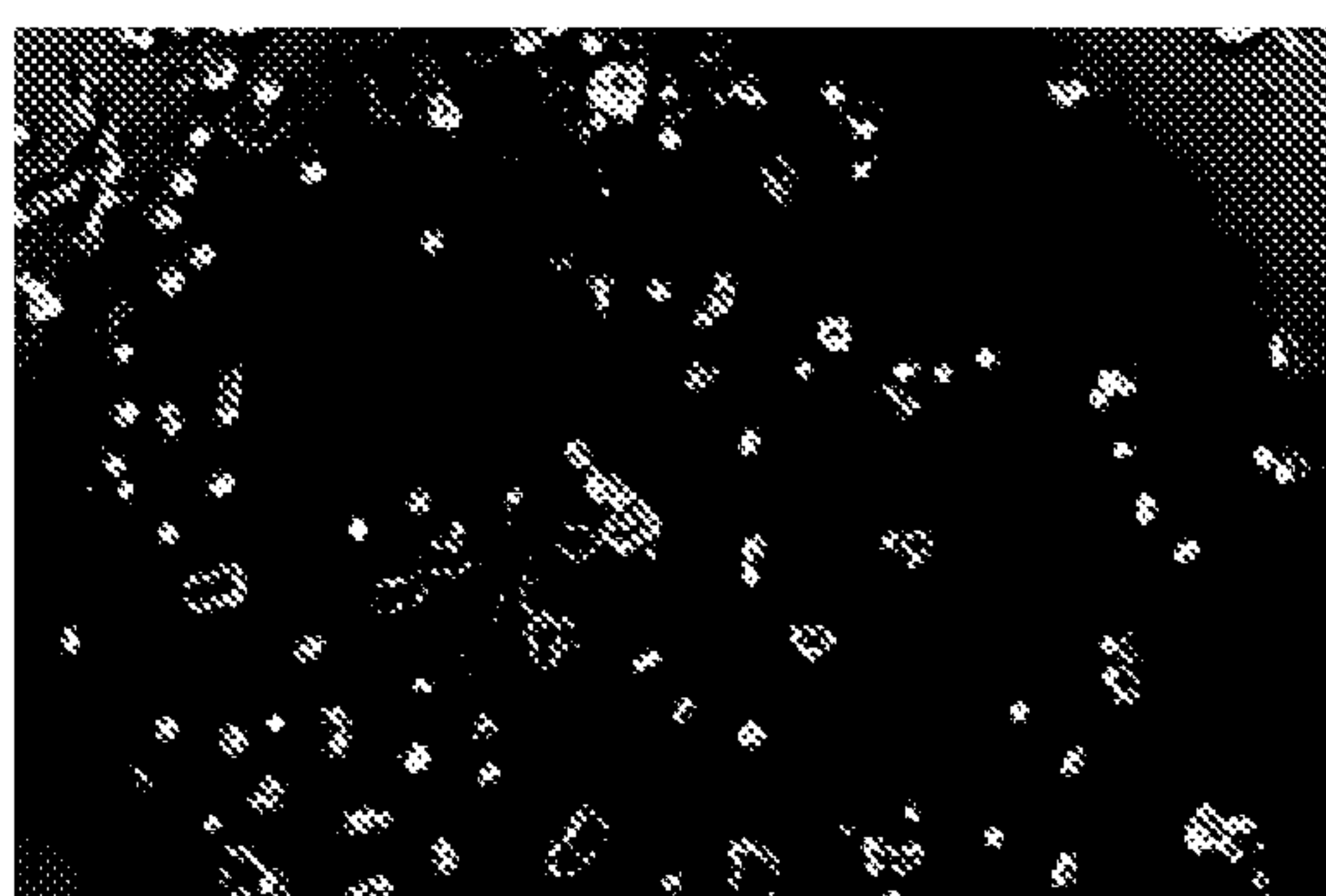
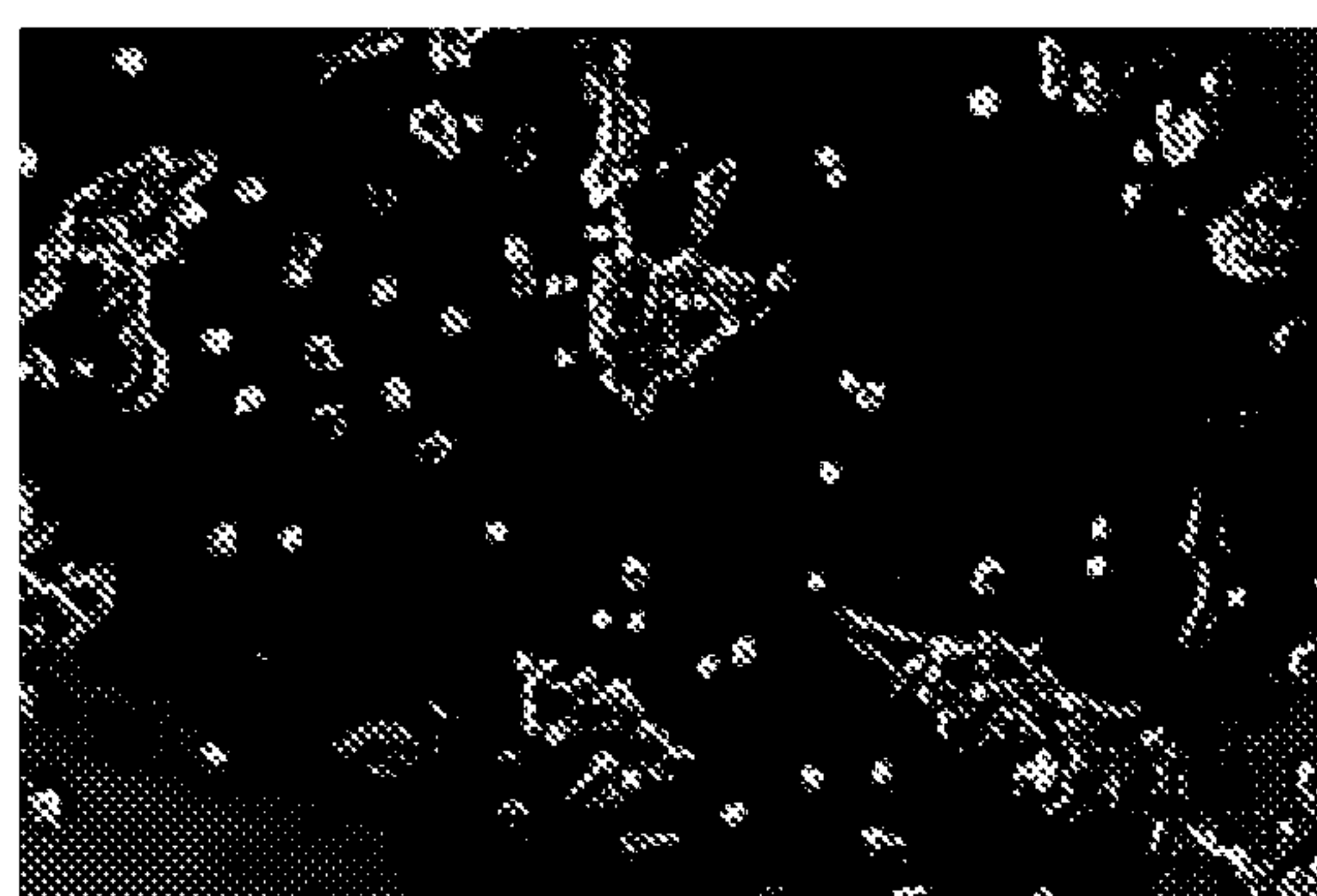


Fig. 12

CONTROL**STEM-C3-FAB****STEM-C8-FAB****CA-C2-FAB****CA-E6-FAB**

DU-145
Prostate
Cancer

Fig. 13

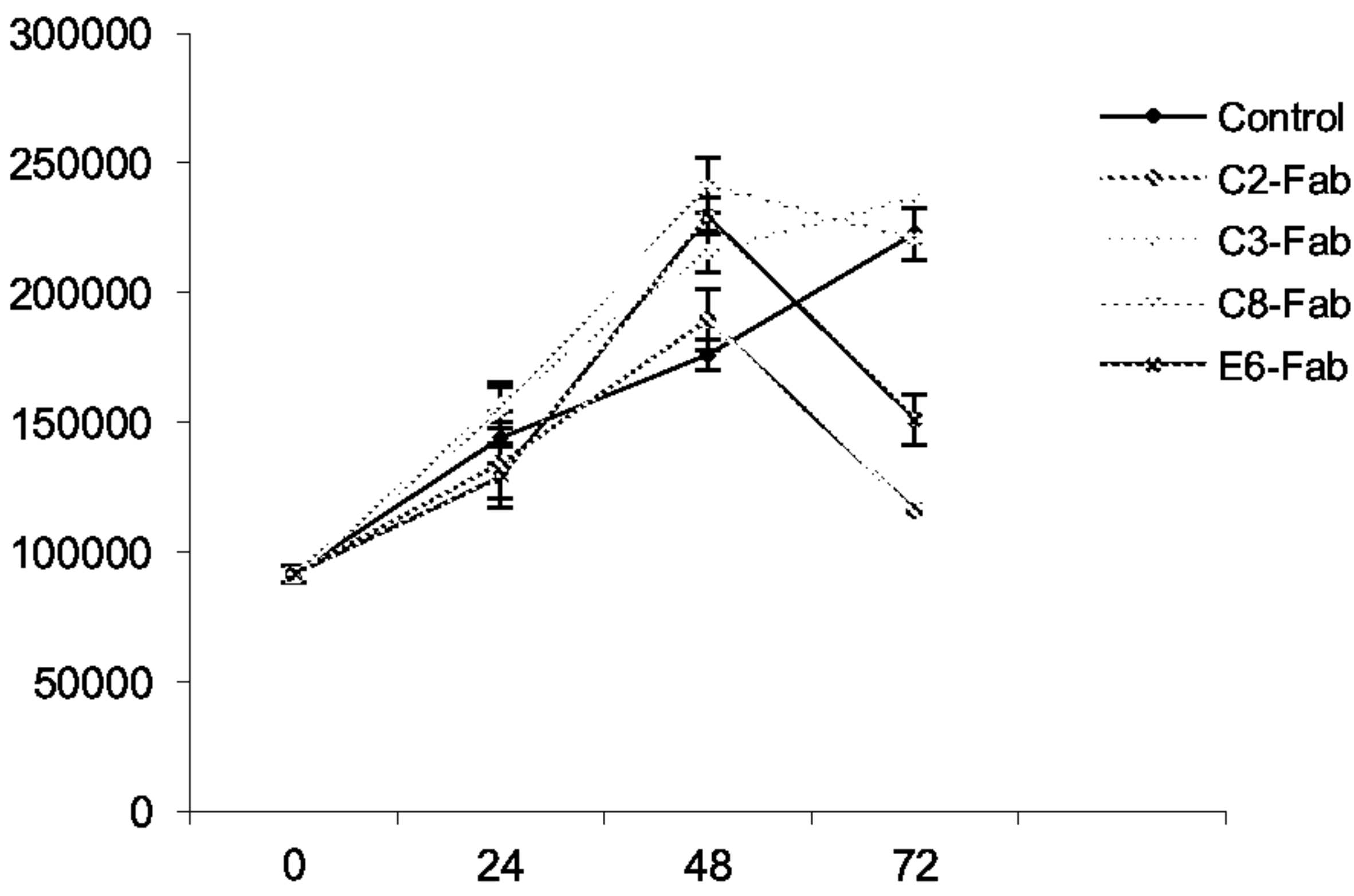
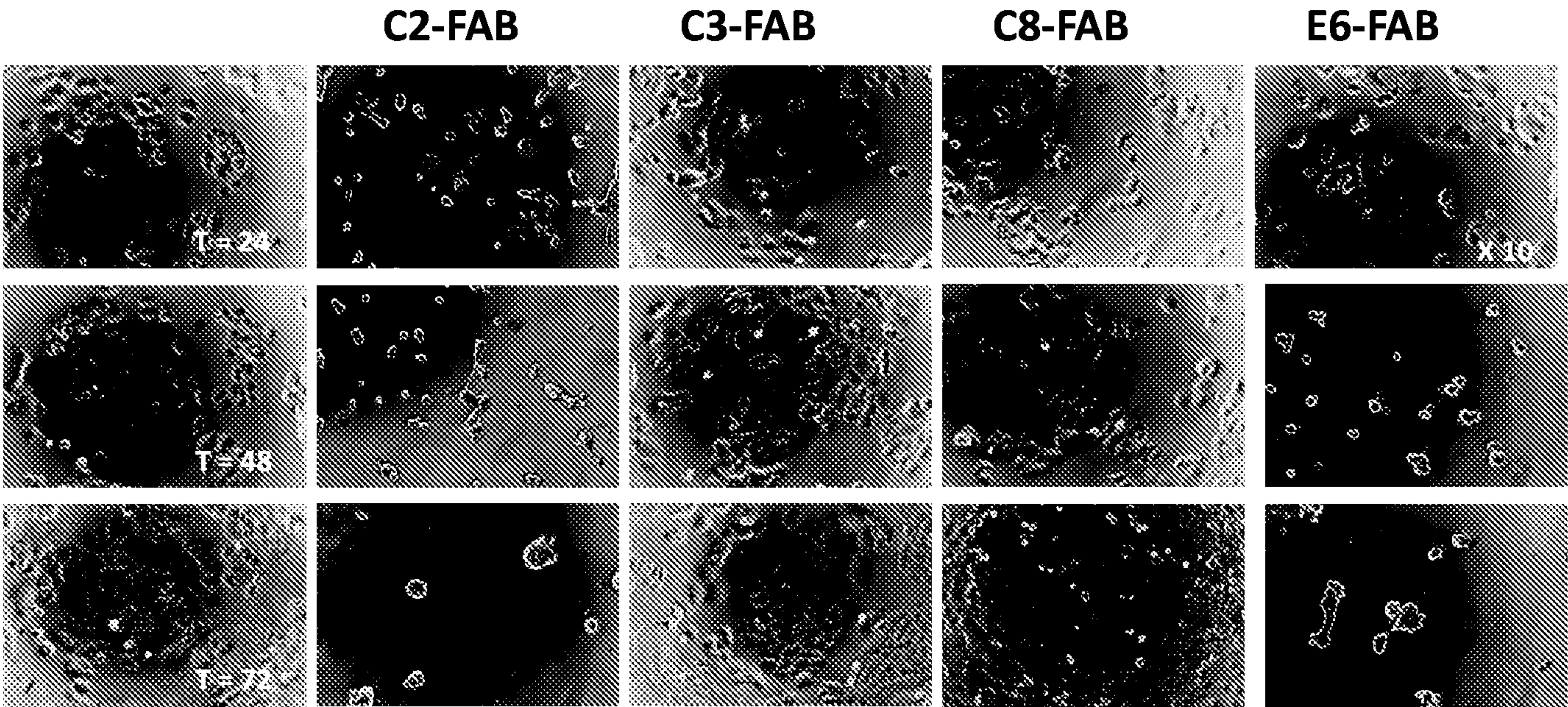


Fig. 14

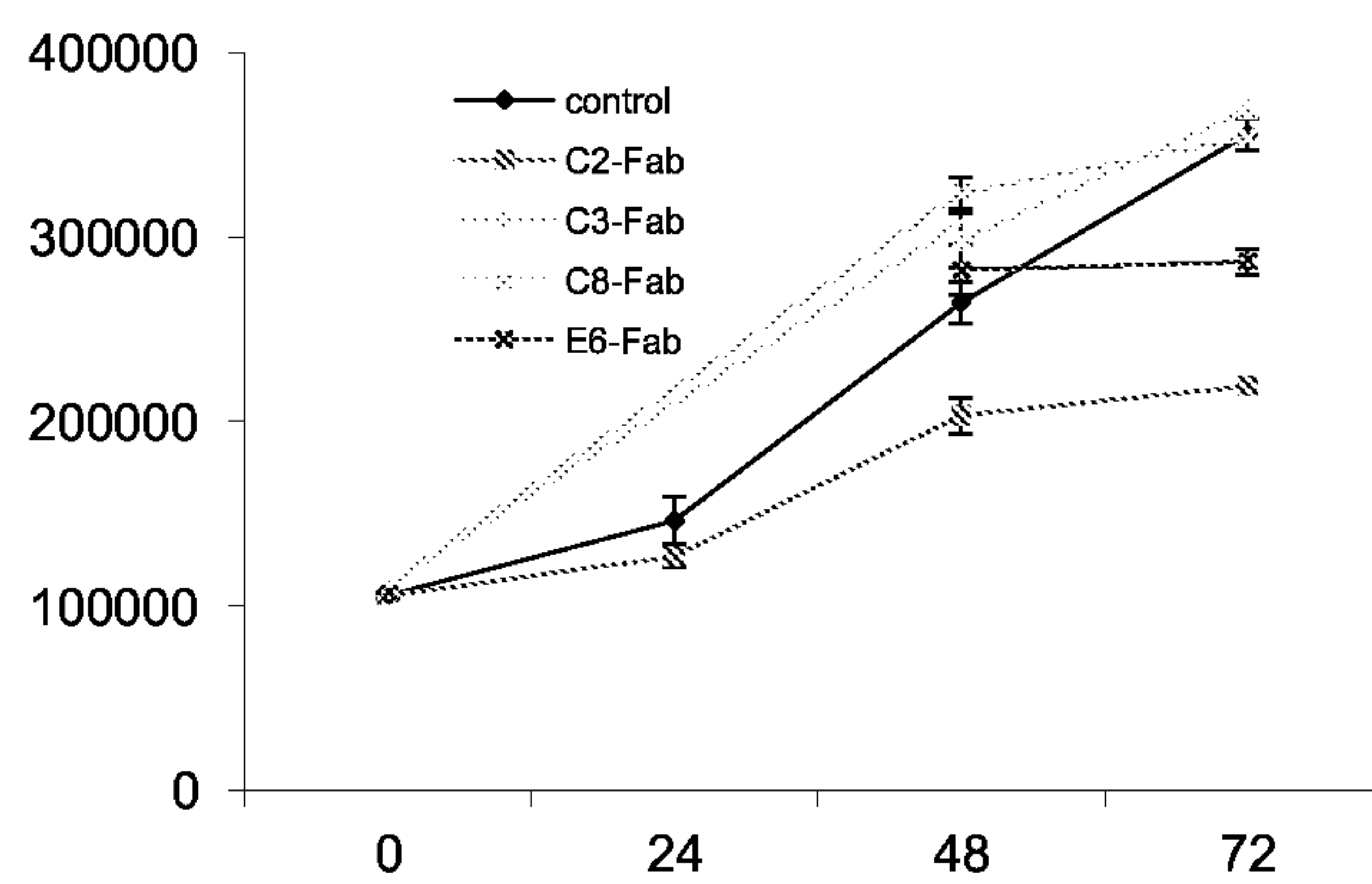
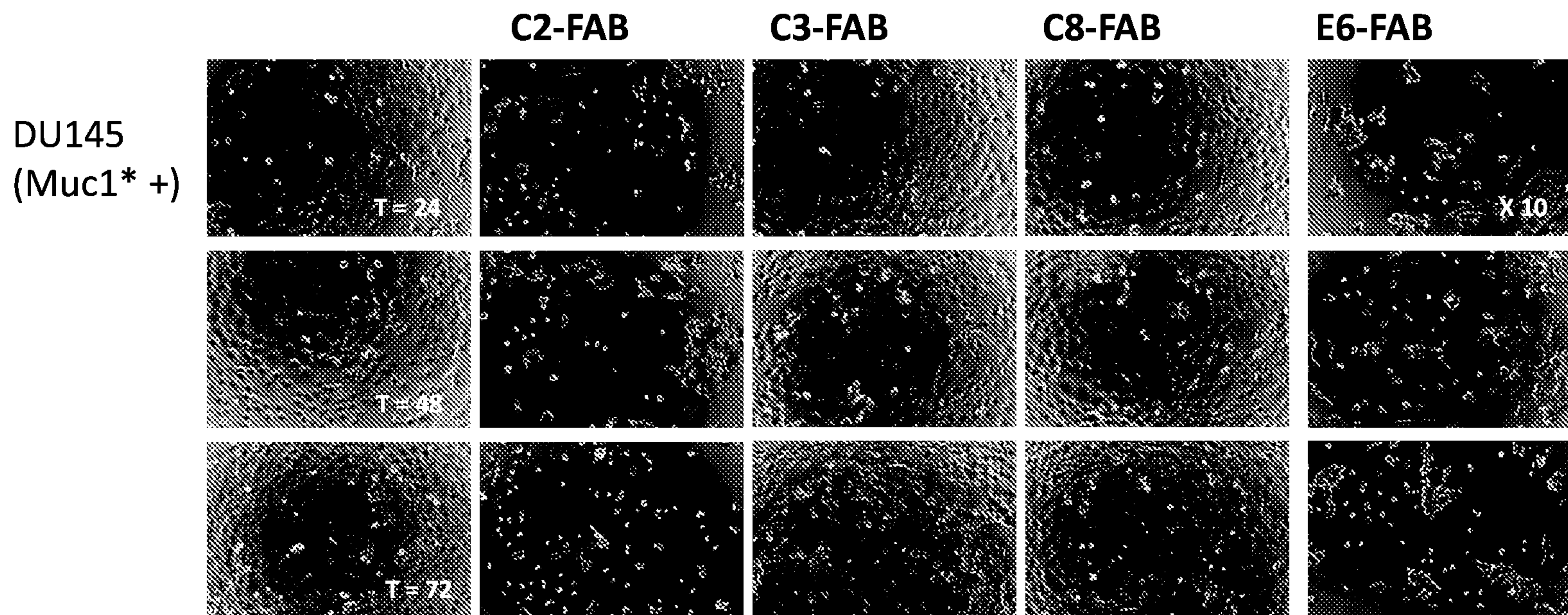


Fig. 15

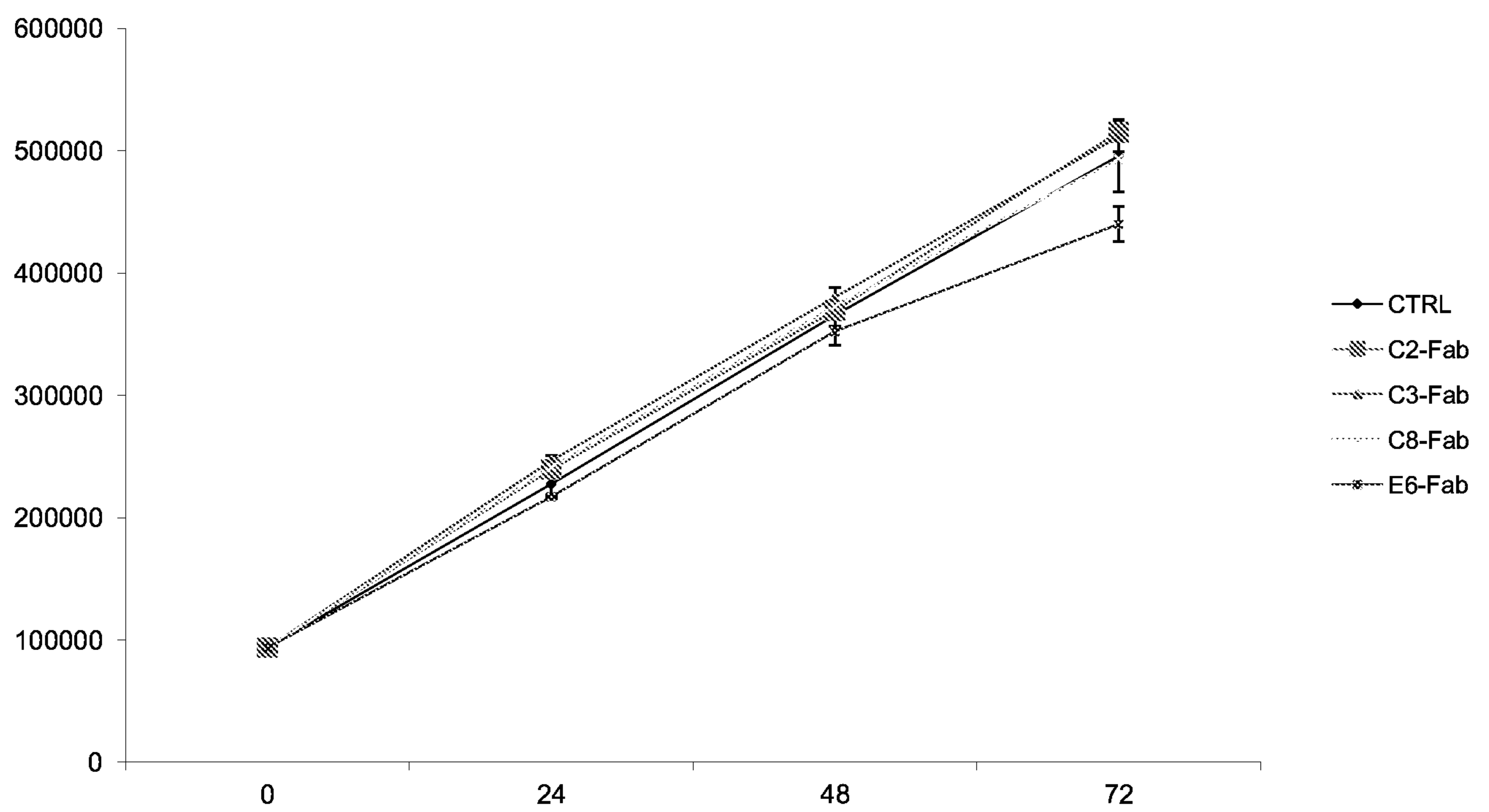


Fig. 16

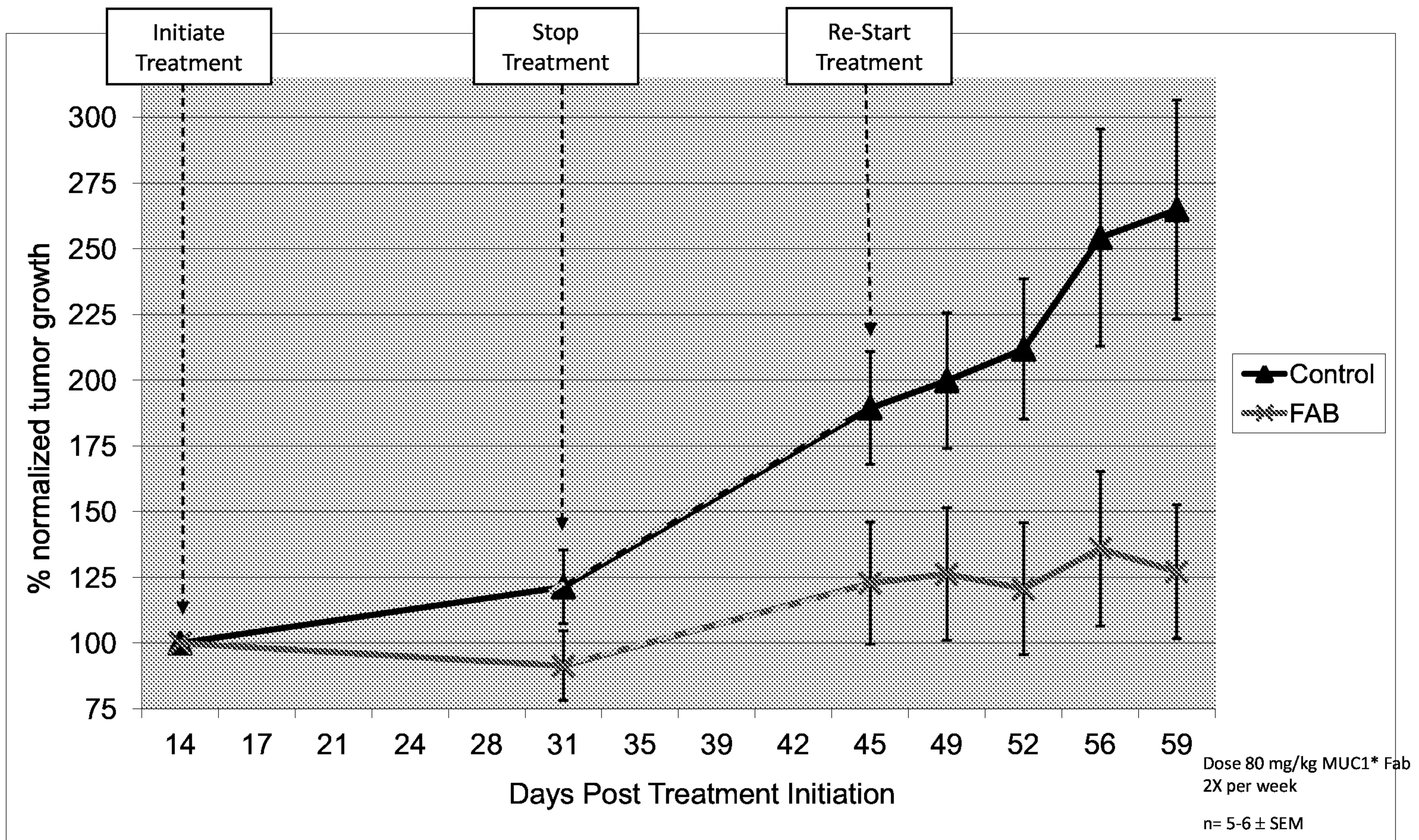


Fig. 17

Treated

Un-Treated

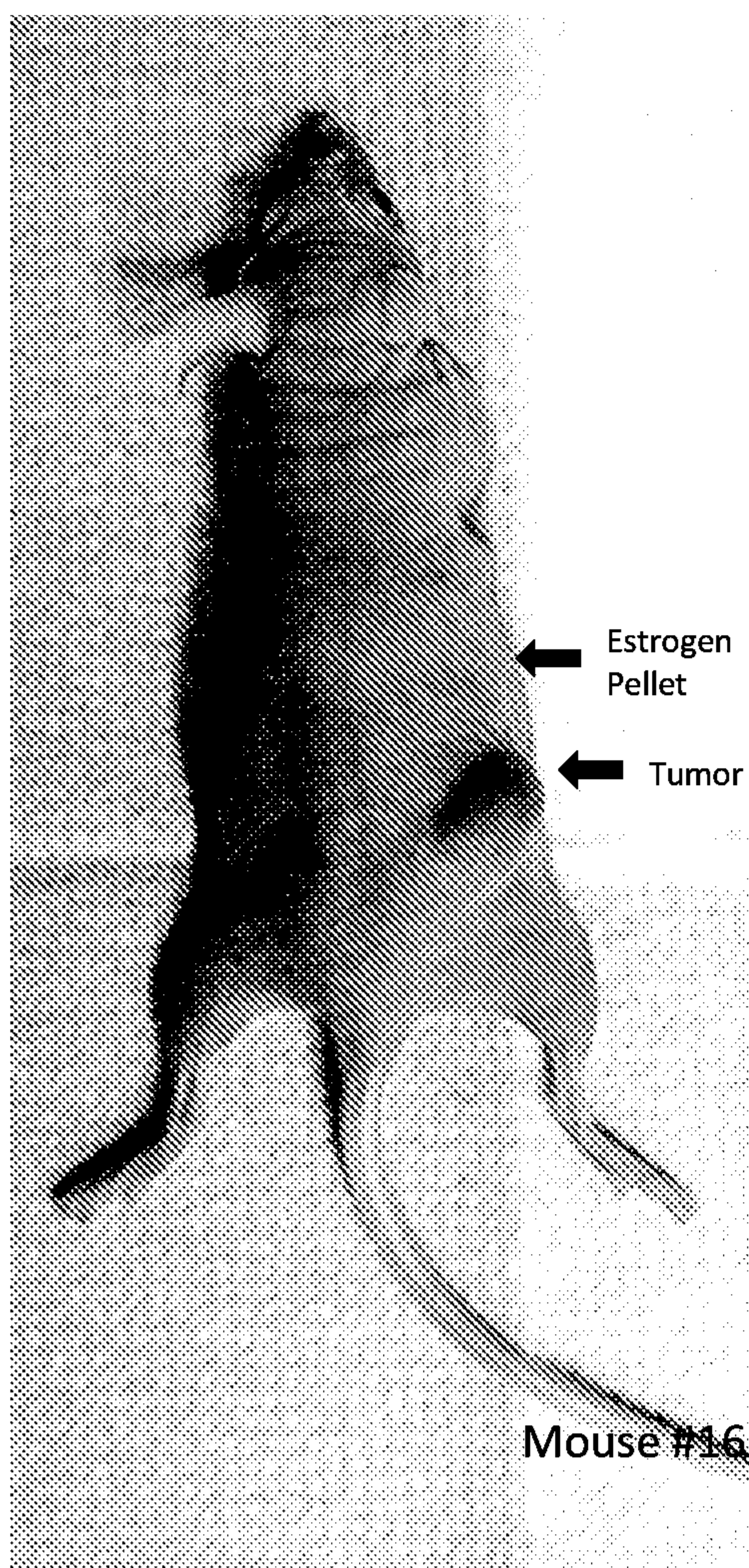
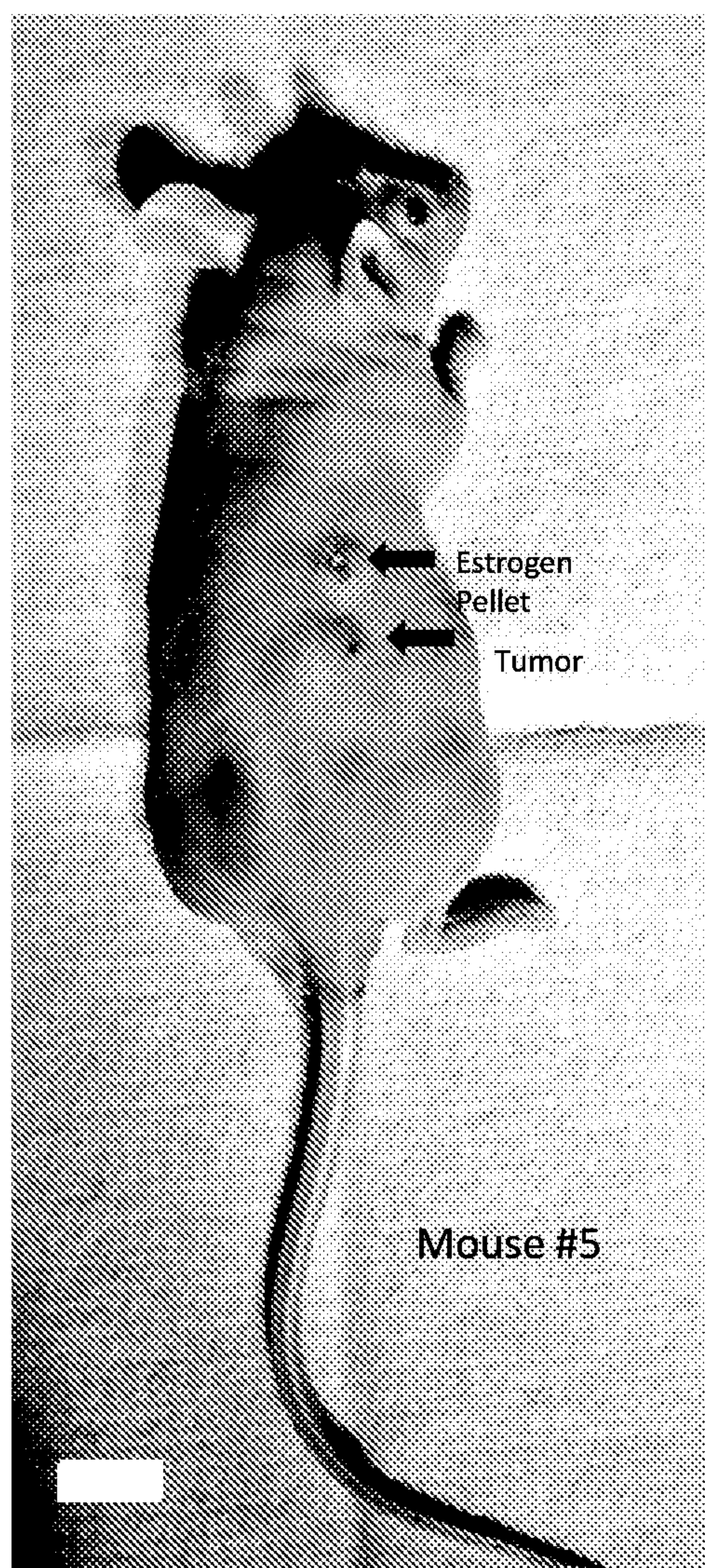


Fig. 18

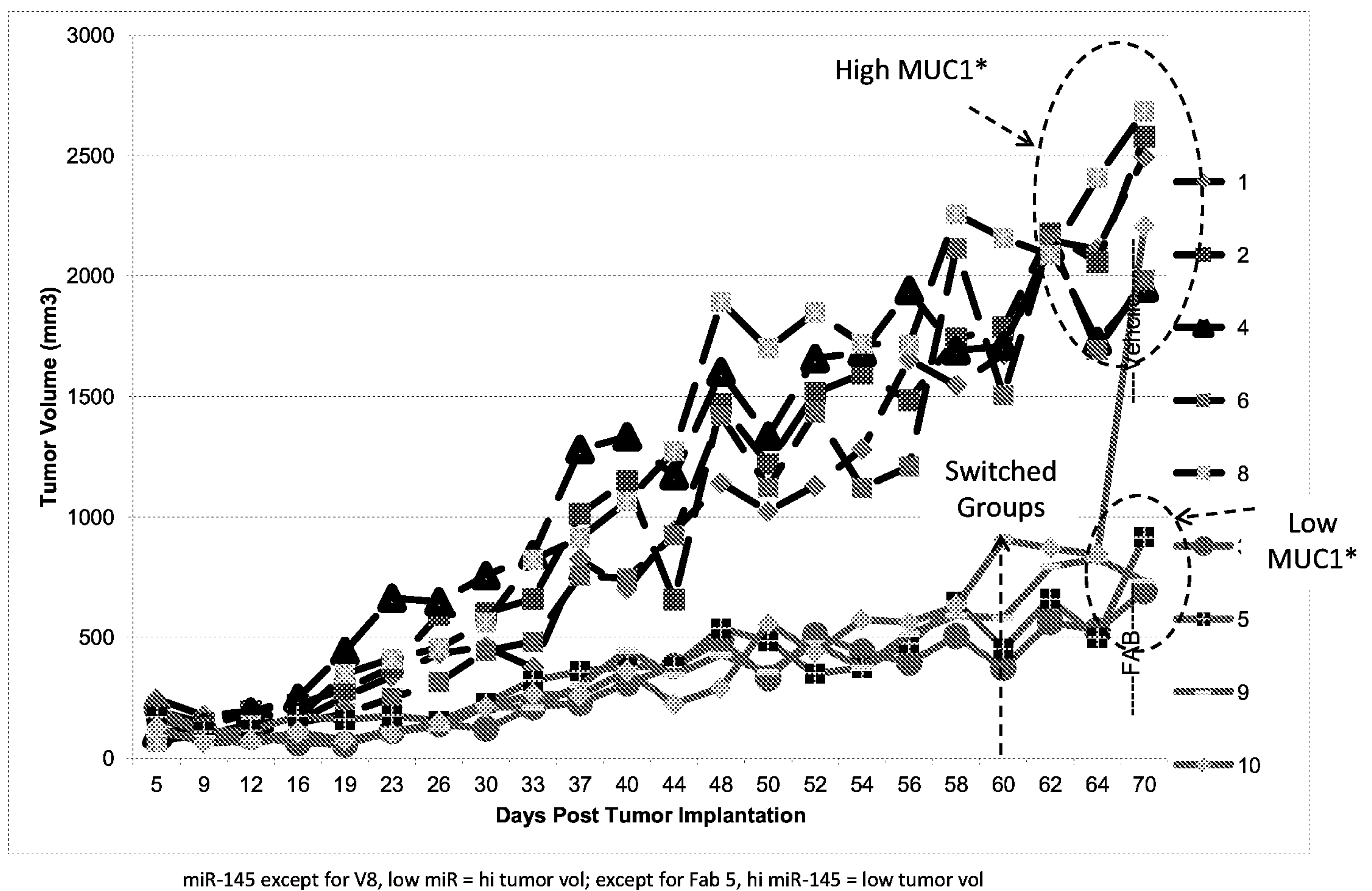
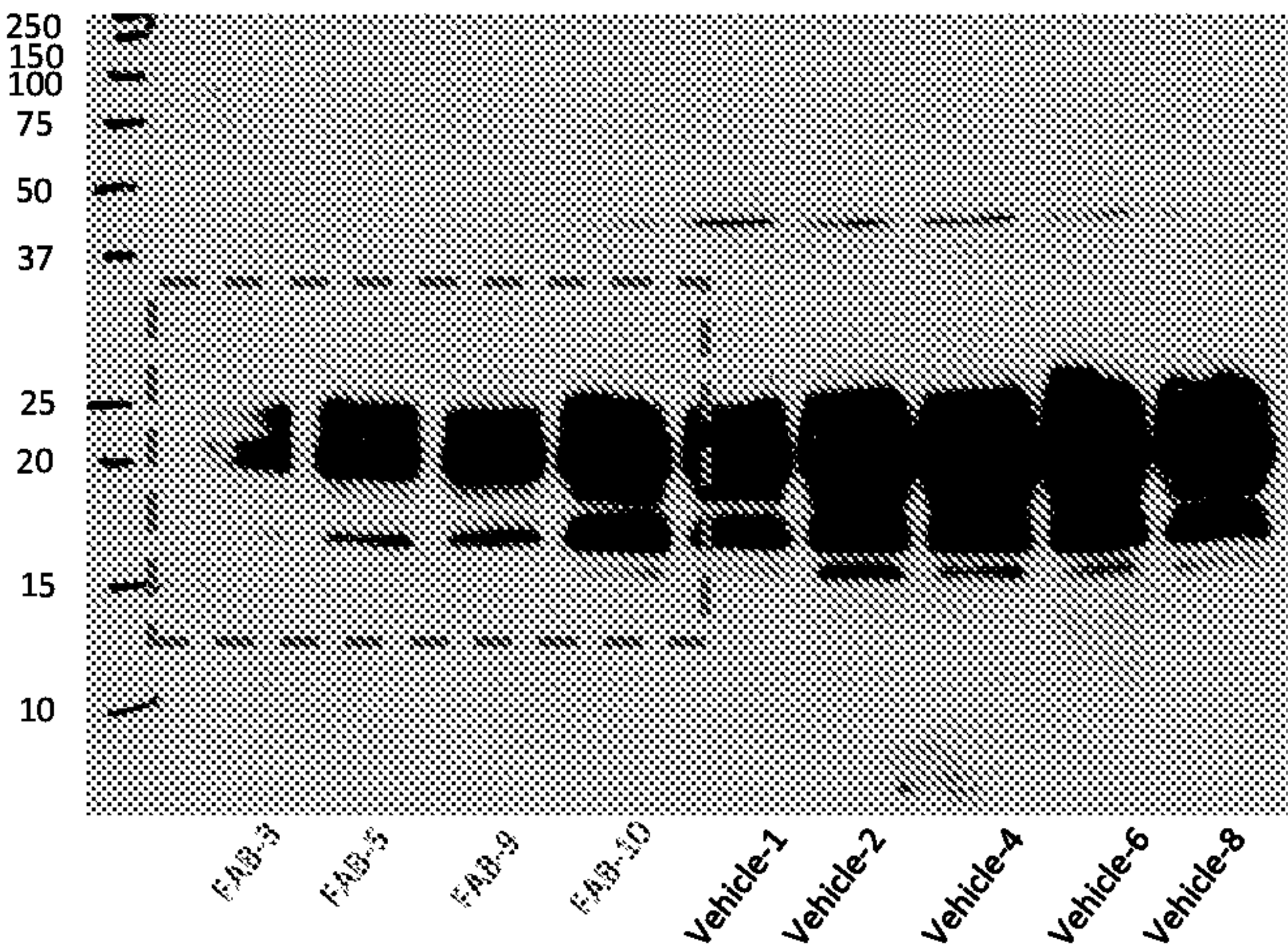


Fig. 19



Cleaved MUC1*
(growth factor receptor form)

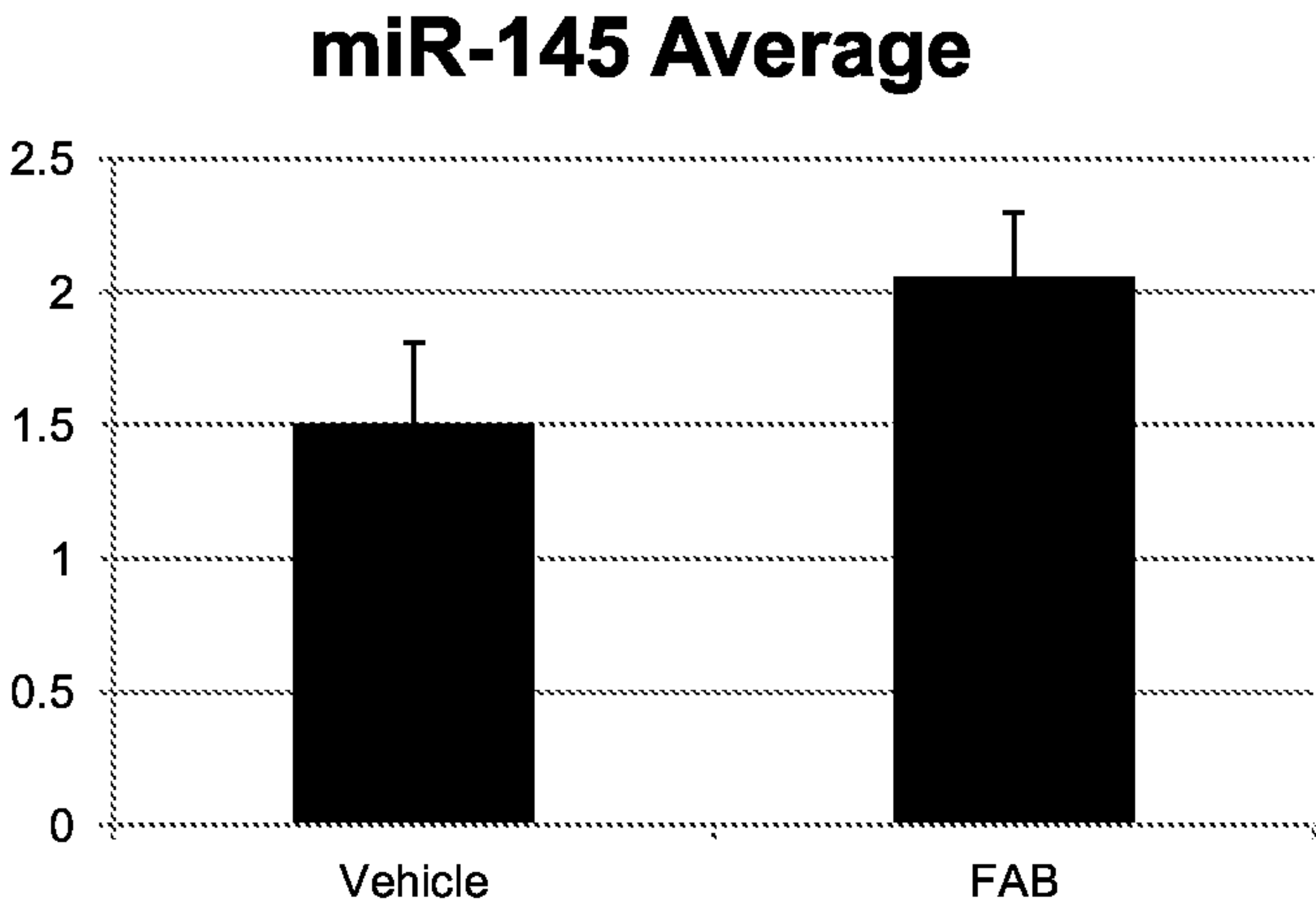


Fig. 20

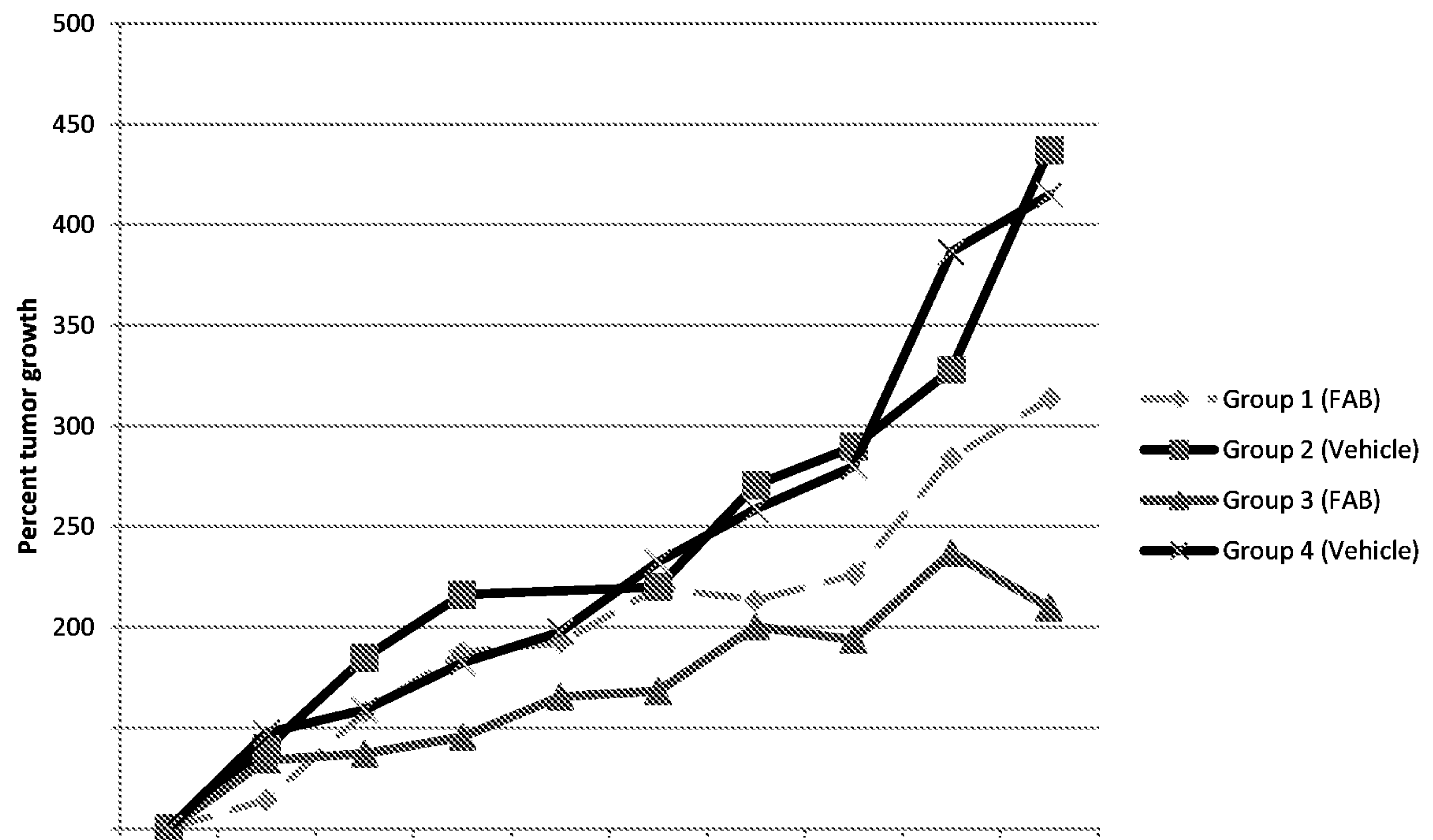
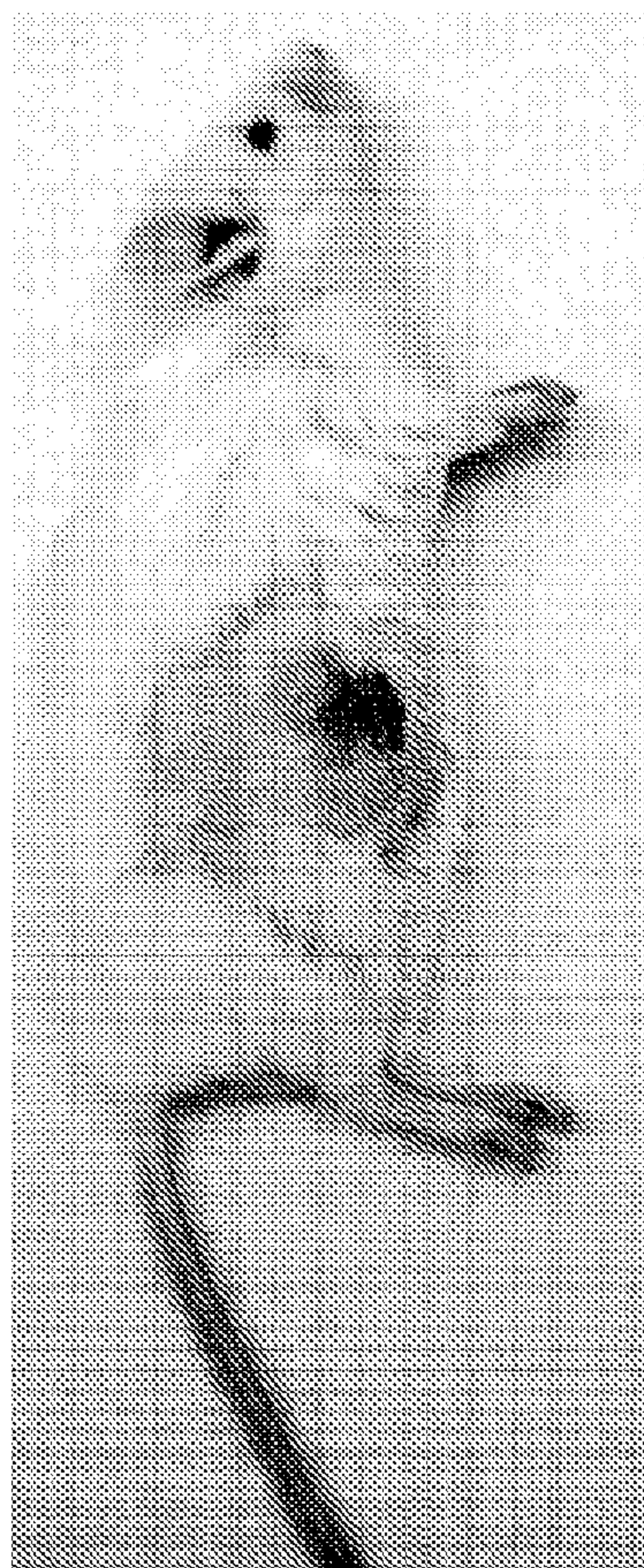
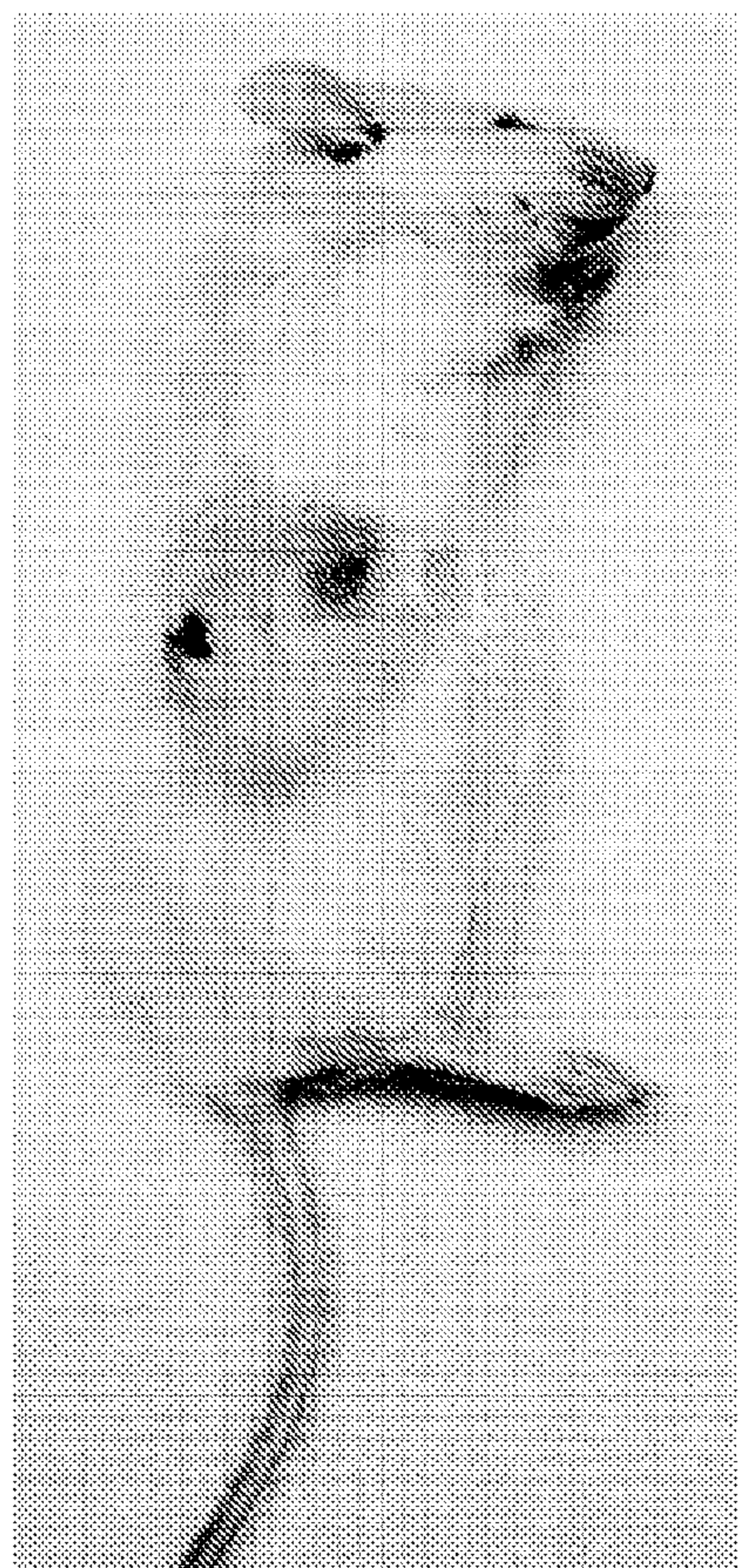


Fig. 21

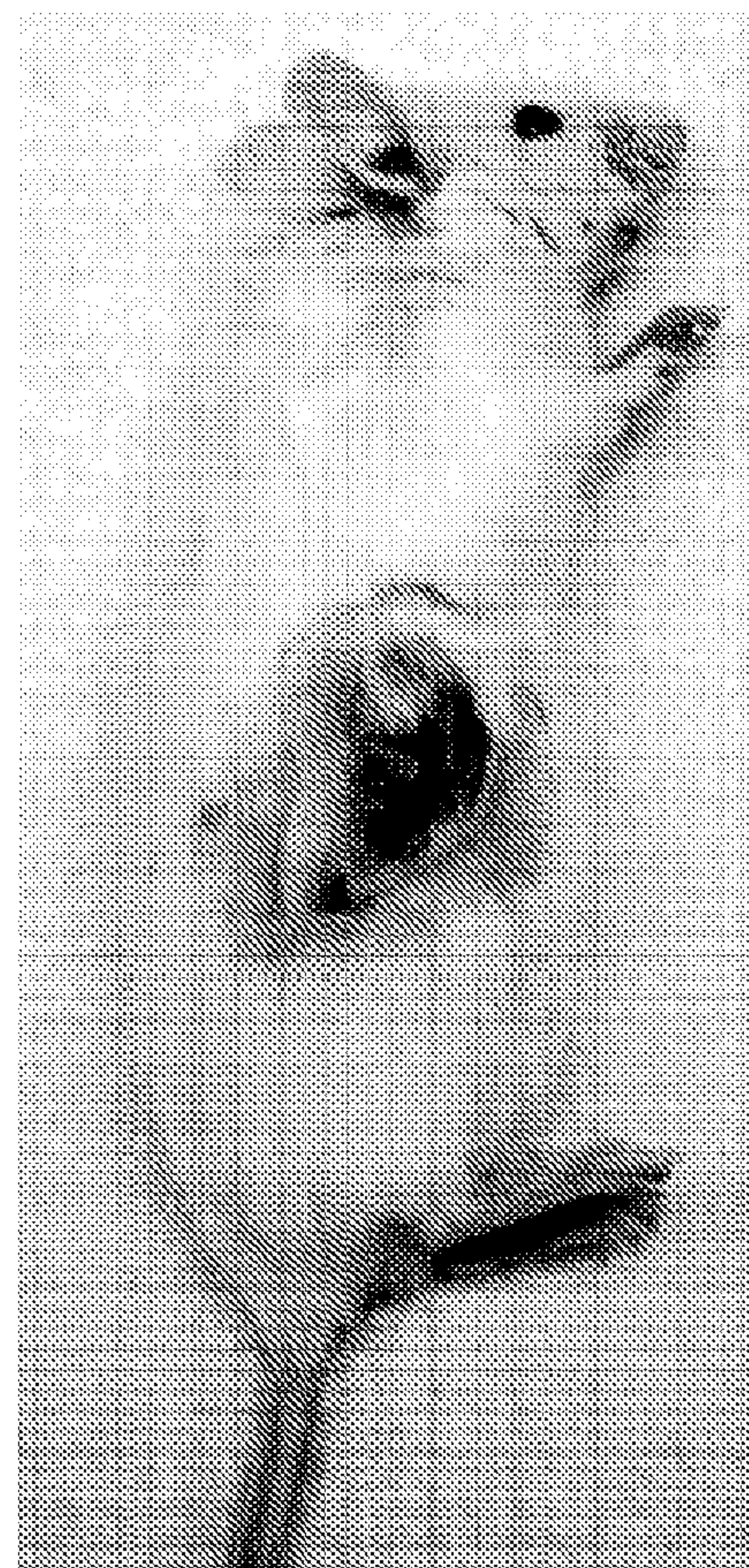
22/33



Mouse 2



Mouse 5



Mouse 12

Group 2 Mice
Untreated (Vehicle)

Fig. 22

23/33



Mouse 4



Mouse 22

Group 3 Mice
FAB Treated

Fig. 23

Anti MUC1* IgG monoclonal antibody light chain variable region sequences

	FWR1	CDR1	FWR2	CDR2
MIN-C2	DIVITQSTASLGVSLGQRATISC	RASKSVSTSGYSYMH	WYQQRPQGPPKLLIY	LASNLES
MIN-E6	DIVITQTTAIMSAPGEEVTLTC	SATSSV-----SYIH	WFQQRPGTSPKLWIY	STSNLAS
MIN-A2-1	DIVLTQSTEIMSAPGEKVTITC	SASSSI-----SYIH	WFQQKPGTSPKLWIF	GTSNLAS
MIN-A2-2	DIVMTQSPAIMSAPGEKVTMTC	SASSSV-----SYMH	WFQQKPGTSPKLWIY	STSNLAS
MIN-C9-1	DIVLTQTTAIMSAPGEKVTITC	SASSSV-----SYMY	WFQQKPGTSPKLWIY	STSNLAS
MIN-C9-2	DIVITQSTAIMSAPGEKVTITC	SASSSV-----SYTY	WFQQKPGTSPKLWIY	STSNLAS
MIN-D7-1	DIVITQTPAIMSAPGEKVTMTC	SASSSV-----SYMH	WFQQKPGTSPKLWIY	STSNLAS
MIN-D7-2	DIVLTQSTAIMSAPGEKVTMTC	SASSSV-----SYMH	WFQQKPGTSPKLWIY	STSNLAS
MIN-F2-1	DIVMTQSPAIMSAPGEKVTITC	SASSSI-----SYIH	WFQQKPGTSPKLWIF	GTSNLAS
MIN-F2-2	DIVITQSTEIMSAPGEKVTITC	SASSSI-----SYIH	WFQQKPGTSPKLWIF	GTSNLAS

	FWR3	CDR3
MIN-C2	GVPARFSGSGSGTDFTLNIHPVEEEDAATYYC	QHSRELPT
MIN-E6	GVPVRFSGSGYGTSYSLTISRMEAEEDAATYYC	QQRSSSPFT
MIN-A2-1	GVPARFSGSGSGTSYSLTVSRMEAEEDTATYYC	QQRSNYPFT
MIN-A2-2	GAPARFSGSGSGTSYSLTVSRMESEDAATYYC	QQRSSYPST
MIN-C9-1	GVPARFSGSGSGTSYSLTISRMEAEEDAATYYC	QQRSSYPST
MIN-C9-2	GVPARFSGSGSGTSYSLTISRMEAEEDAATYYC	QQRSSYPST
MIN-D7-1	GVPARFSGSGSGTSYSLTVSRMESEDAATYYC	QQRSSYPST
MIN-D7-2	GVPARFSGSGSGTSYSLTVSRMESEDAATYYC	QQRSSYPST
MIN-F2-1	GVPARFSGSGSGTSYSLTVSRMEAEEDTATYYC	QQRSNYPFT
MIN-F2-2	GVPARFSGSGSGTSYSLTVSRMEAEEDTATYYC	QQRSNYPFT

Fig. 24

Anti MUC1* IgG monoclonal antibody heavy chain variable region sequences

	FWR1				CDR1	FWR2	CDR2
MIN-C2	EVQLEESGGGLVKPGGSLKLSCAASGFTFS				GYAMS	WVRQTPEKRLWVA	TISSGGTYIYY
MIN-E6-7	EVKLEESGGDLVKPGGSLKLSCAASGFTFS				RYGMS	WVRQTPDKRLEWVA	TISSGGTYIYY
MIN-E6-8	EVKLEESGGDLVKPGGSLKLSCVVSGFTFS				RYGMS	WVRQTPGKRLEWVA	TISGGGYIYY
MIN-A2-1	EVKLQESGPPELKKPGETVEISCKASGYTFT				NYGMN	WVKQAPGKGLKWMG	WINTYTGEPTY
MIN-A2-2	EVQLQQSGPELKKPGETVKISCKASGYTFT				NYGMN	WVKQAPGKGLKWMG	WINTYTGEPTY
MIN-C9-1	QVQLQESGPPELKQPGETVKISCKASGYTFT				NNGMN	WVKQAPGKGLKWMG	WINTYTGEPTY
MIN-C9-2	QVQLQQSGPELKQPGETVKISCKASGYTFT				NNGMN	WVKQAPGKGLKWMG	WINTYTGEPTY
MIN-D7-1	EVQLQQSGPELKKPGETVKISCKASGYTFI				NYGMN	WVKQAPGKGLKWMG	WINTYTGEPTY
MIN-D7-2	EVQLQQSGPELKKPGETVKISCKASGYTFT				NYGMN	WVKQAPGKGLKWMG	WINTYTGEPTY
MIN-F2-1	EVKLEESGPPELKKPGETVKISCKASGYTFI				NYGMN	WVKQAPGKGLKWMG	WINTYTGEPTY
MIN-F2-2	EVQLQQSGAELVRPGASVKLSCKALGYTFT				DYEMH	WVKQTPVHGLEWIG	AIHPGSGGTAY
MIN-F2-3	RCRLQQSGPELKKPGETVKISCKASGYTFI				NYGMN	WVKQAPGKGLKWMG	WINTYTGEPTY
MIN-F2-4	EVQLQQSGPELKKPGETVKISCKASGYTFI				NYGMN	WVKQAPGKGLKWMG	WINTYTGEPTY

	FWR3			CDR3
MIN-C2	PDSVKG	RFTISRDNAKNTLYLQMSSLRSEDTAMYYCAR		LGGDNYY-EY
MIN-E6-7	PDSVKG	RFTISRDNAKNTLYLQMSSLKSEDTAMYYCAR		DNYGSSYDYA
MIN-E6-8	PDSVKG	RFTISRDNAKNTLYLQMSSLKSEDTAMYHCTR		DNYGRNYDYG
MIN-A2-1	AGDFKG	RFAFSLETSASTAYLQINTLKNEDTATYFCAR		SGDGYWY-YA
MIN-A2-2	AGDFKG	RFAFSLETSASTAYLQINTLKNEDTATYFCAR		SGDGYWY-YA
MIN-C9-1	ADDFKG	RFAFSLDTSASTAYLQINNLLKNEDMATYFCAR		TGTARAF-YA
MIN-C9-2	ADDFKG	RFAFSLGTSASTAYLQINNLLKNEDMATYFCAR		TGTARAF-YA
MIN-D7-1	VDDFKG	RFAFSLETSASTAYLQINNLLKNEDMATYFCAR		TGTTAIL-NG
MIN-D7-2	AGDFKG	RFAFSLETSASTAYLQINTLKNEDTATYFCAR		SGDGYWY-YA
MIN-F2-1	VDDFKG	RFAFSLETSASTAYLQINNLLKNEDMATYFCAR		TGTTAIL-NG
MIN-F2-2	NQKFKG	KATLTADKSSSTAYMELSSLTSEDSAVYYCTN		YGSFA----
MIN-F2-3	VDDFKG	RFAFSLETSASTAYLQINNLLKNEDMATYFCAR		TGTTAIL-NG
MIN-F2-4	VDDFKG	RFAFSLETSASTAYLQINNLLKNEDMATYFCAR		TGTTAIL-NG

Fig. 25

FWR1		CDR1		FWR2		CDR2	
EVQLEESGGGLVKPGGSLKLSAASGFTFS		GYAMS		WVRQTPEKRLWVA		TSSGGTYIYPPDSVKG	
FWR3				CDR3			
RFTISRDNAKNTLYLQMSSLRSED		TAMYYCAR		LGGDNYEY		FDVWGAGTTVTSSAKTTPPSVYKL	
linker		FWR1		CDR1		FWR2	
GGGSGGGSGGGGSSS		DIVITQSTASLGVSLGQRATISCR		ASKSVSTSGYSYMH		WYQQRPQPP	
CDR2		FWR3		CDR3			
KLIIY	LASNLES	GVPARFSGSGGTDFTLN		IHPVEE		DAATYYCQHSRELPFT	
						FGGGTKLE	

IKRADAPT_{TVSVD}

Fig. 26

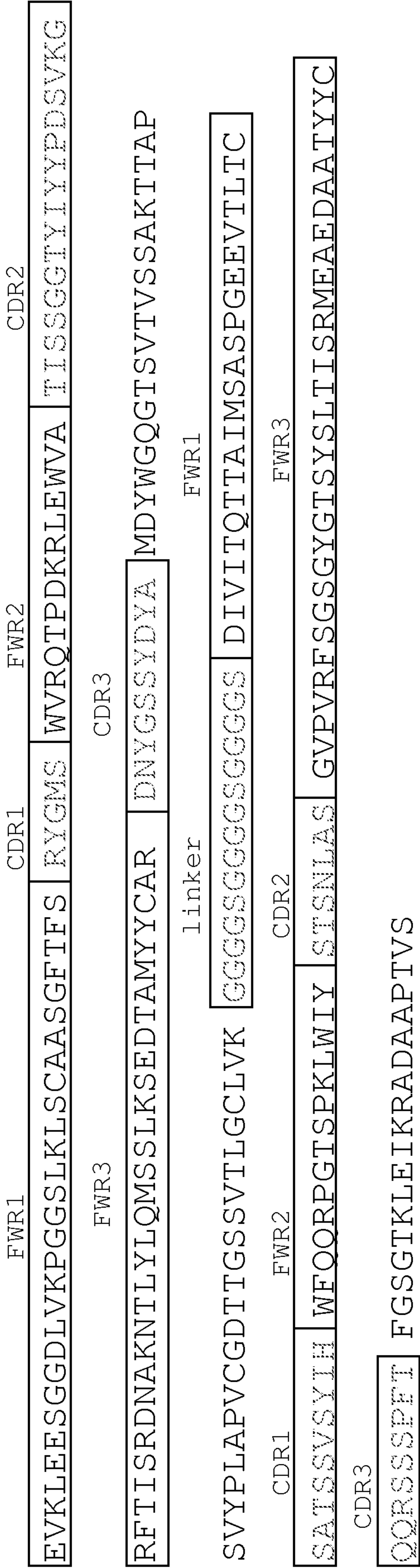


Fig. 27

2D6C3 Kappa Chain Variable Region

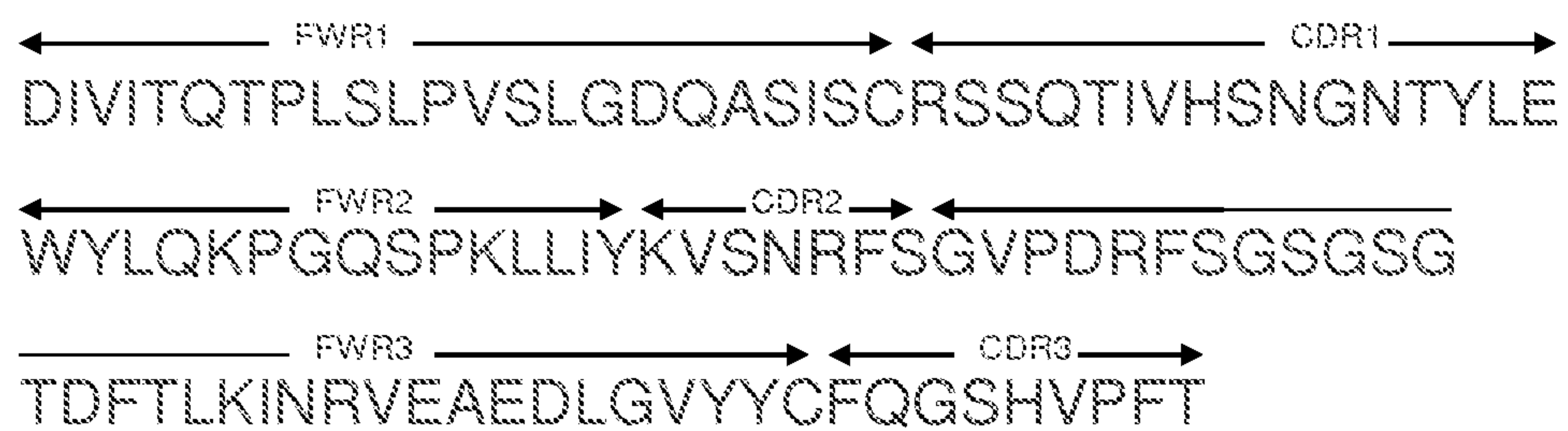


Fig. 28

2D6C3 Heavy Chain Variable Region

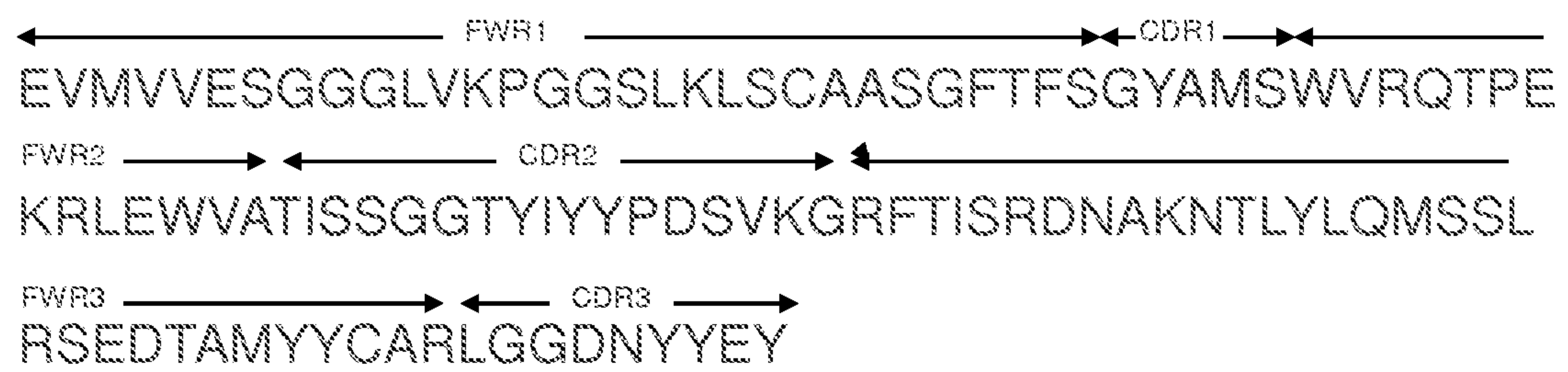


Fig. 29

2D6C8 Kappa Chain Variable Region

→

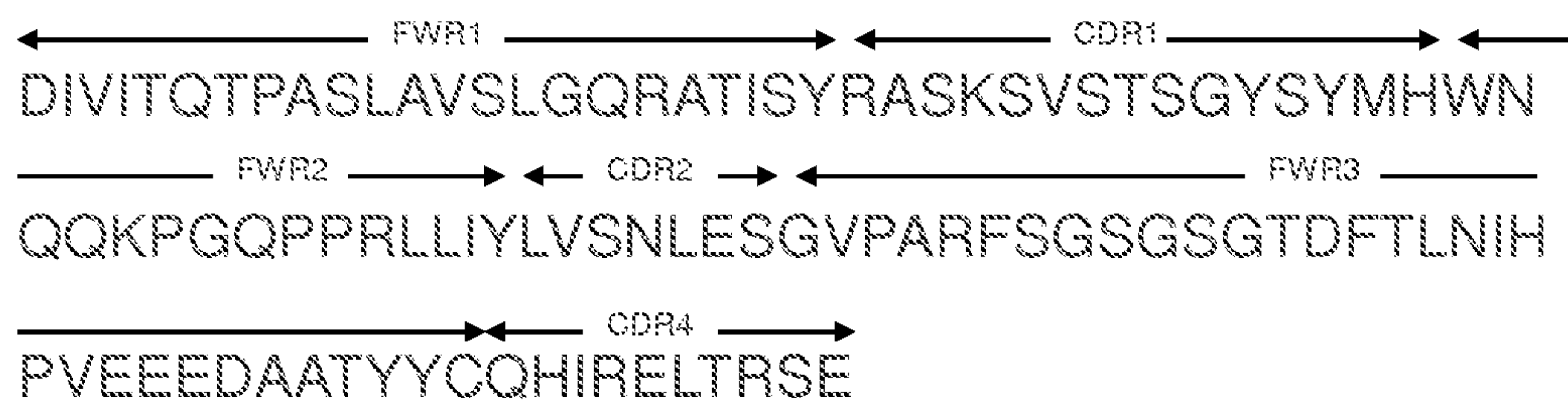


Fig. 30

2D6C8 Heavy Chain Variable Region

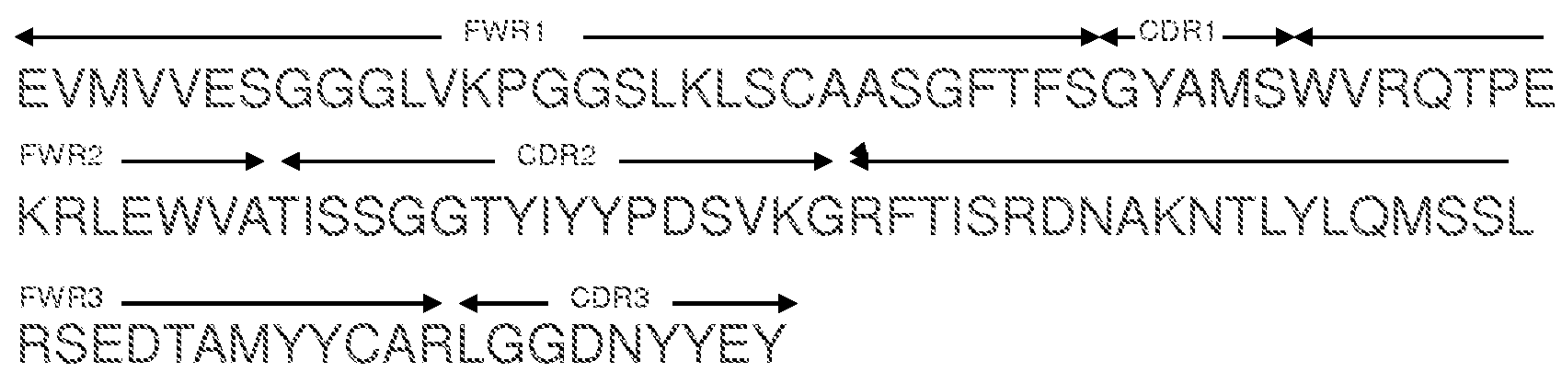


Fig. 31

3C2B1 Kappa Chain Variable Region

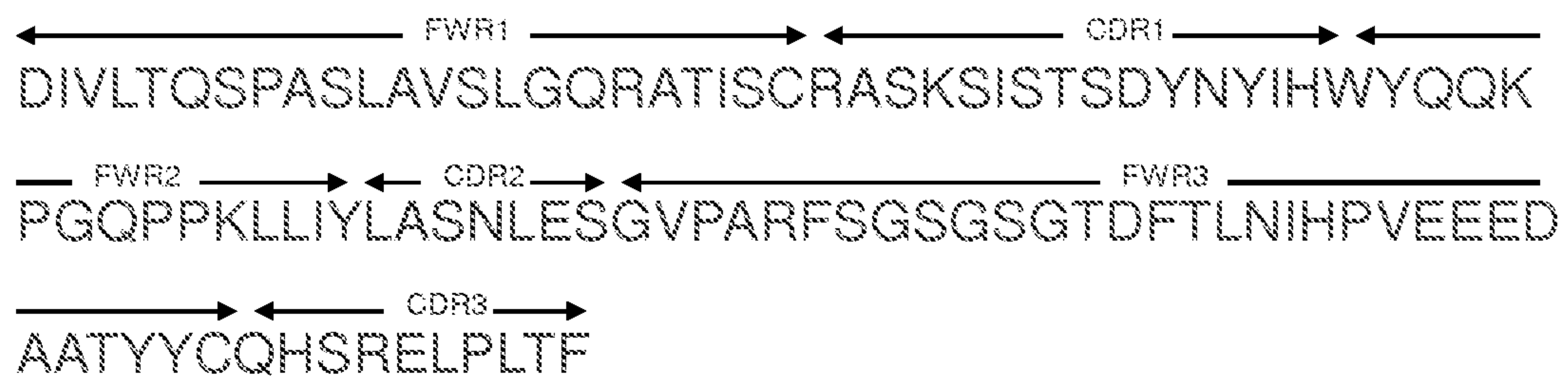


Fig. 32

3C2B1 Heavy Chain Variable Region

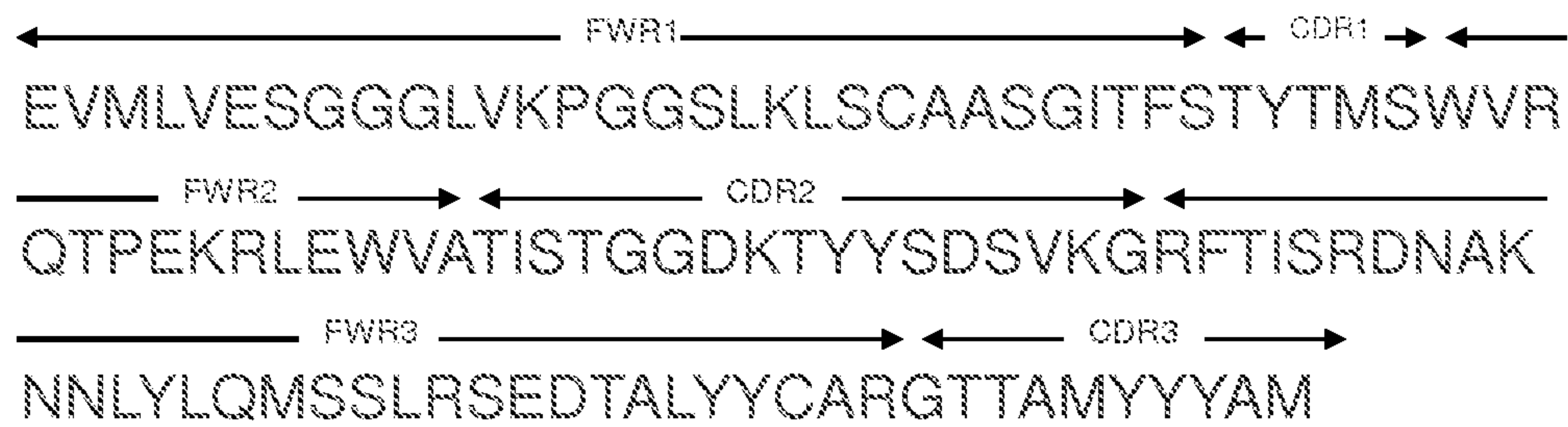


Fig. 33