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(54) Title: METHODS OF TREATING CANCER

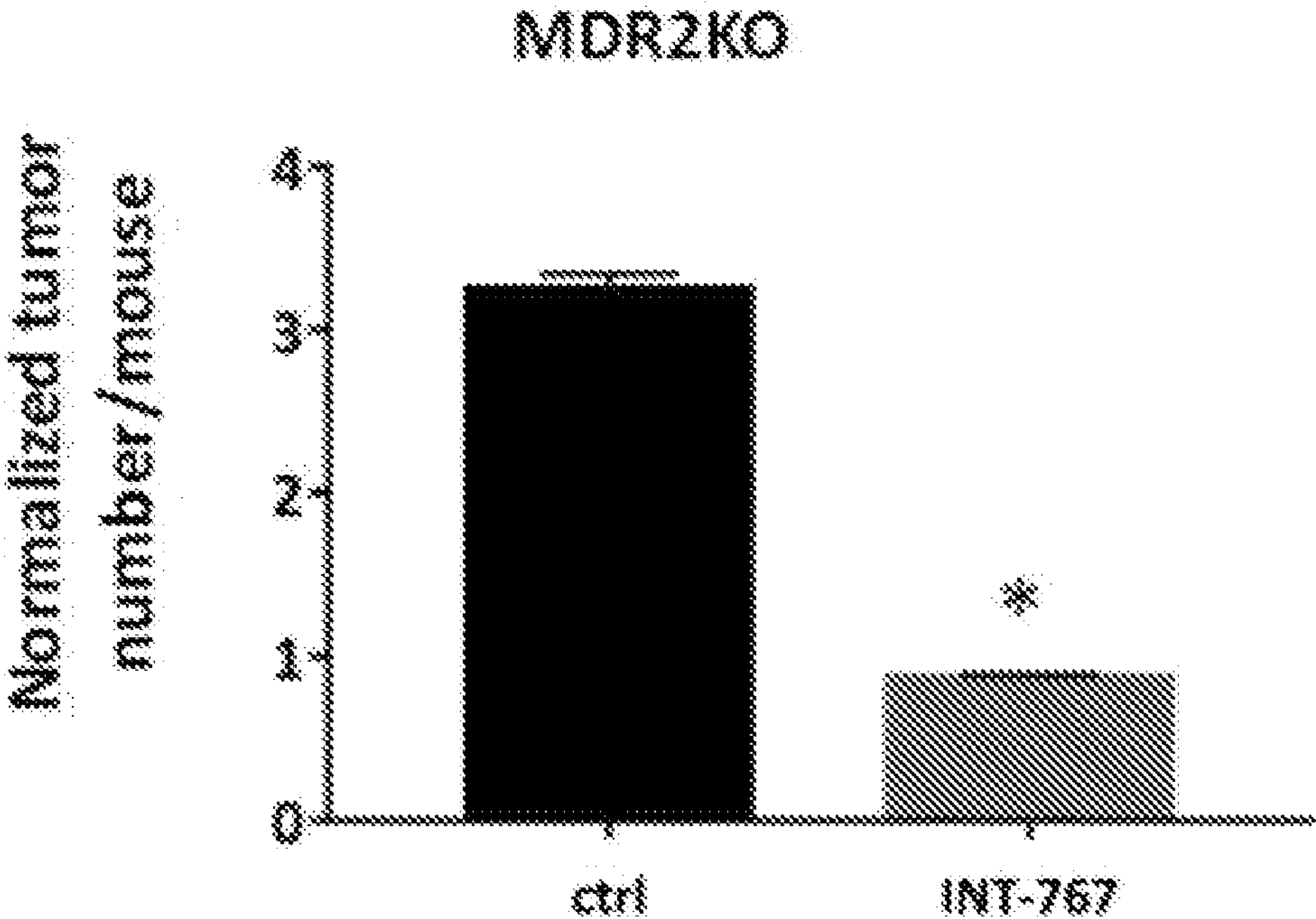


Figure 1A

(57) **Abrégé/Abstract:**
The present invention relates to methods of treating or preventing cancer in a subject in need thereof comprising administering a therapeutically effective amount of a compound of the invention.

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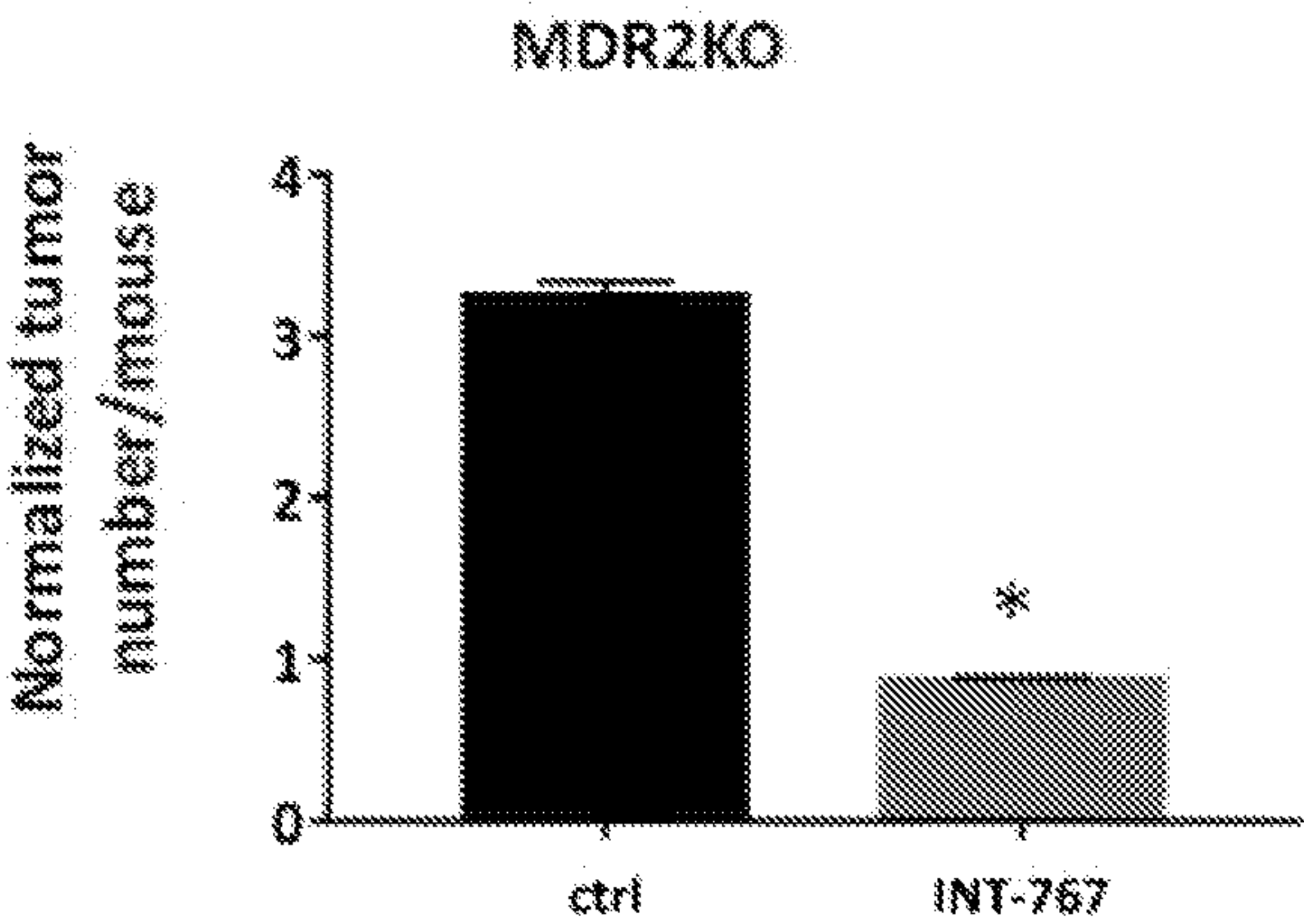


Figure 1A

(57) Abstract: The present invention relates to methods of treat-
ing or preventing cancer in a subject in need thereof comprising
administering a therapeutically effective amount of a compound
of the invention.

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METHODS OF TREATING CANCER

BACKGROUND TO THE DISCLOSURE

Cancer is characterized primarily by an increase in the number of abnormal cells
5 derived from a given normal tissue, invasion of adjacent tissues by the abnormal cells, or
lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant
sites (metastasis). Clinical data and molecular biologic studies indicate that cancer is a
multistep process that begins with minor pre-neoplastic changes, which may under certain
conditions progress to neoplasia.

10 Primary liver cancer is one of the most common forms of cancer in the world. There
are two main types of liver cancer: hepatocellular carcinoma (HCC), also known as malignant
hepatoma, and cholangiocellular carcinoma, also known as cholangiocarcinoma (CCA). HCC
is the most common form of primary liver cancer, and develops within the hepatocyte. HCC
occurs mostly in men and patients that suffer from cirrhosis. HCC is the one of the most
15 common cancers worldwide and the third most common cause of cancer-related deaths. The
disease is often diagnosed late in the course of clinical manifestation. As a result, only 10-
15% of patients are candidates for curative surgery. For the majority of HCC patients,
systemic chemotherapies or supportive therapies are the mainstay treatment options.

In contrast, cholangiocellular carcinoma or bile duct cancer which mainly develops in
20 the small bile duct epithelial cells (i.e. cholangiocytes) within the liver. This type of cancer is
more common among women. The incidence of cholangiocarcinoma (CCA) is increasing
worldwide and already represents the second most common primary liver cancer. CCA
patients show poor outcome due to late diagnosis and the refractory nature of these tumors.

Up to the present time, there are a limited number of drugs that can effectively treat
25 cancers such as HCC and/or CCA. For example, patients with metastatic hepatocellular
carcinoma or hepatocellular carcinoma, where local treatment has failed, normally survive for
only three to four months. Metastatic hepatocellular carcinoma or hepatocellular carcinoma,
where local treatment has failed, is mainly subjected to systemic therapy. The use of
doxorubicin, a high dosage of tamoxifen in combination doxorubicin or EA-PFL (etoposide,
30 adriamycin, cisplatin, fluorouracil and leucovorin), is an effective example. The remission rate
of these drugs can achieve levels between 15 and 30%. However, because the patients of

hepatocellular carcinoma usually develop complication of liver cirrhosis and other complications (such as leukopenia, thrombopenia or liver function impairment), they cannot be subject to systemic chemotherapy. Further, most chemotherapeutic agents show limited effectiveness and have not been able to significantly improve patient survival. Despite ongoing efforts, the adverse clinical course of most cancer patients underscores the needs for more efficacious chemotherapies.

Cholangiocarcinoma is a relatively rare neoplasm that is classified as an adenocarcinoma (a cancer that forms glands or secretes significant amounts of mucins). It has an annual incidence rate of 1–2 cases per 100,000 in the Western world, but rates of cholangiocarcinoma have been rising worldwide over the past few decades.

Cholangiocarcinoma is considered to be an incurable and rapidly lethal cancer unless both the primary tumor and any metastases can be fully removed by surgery. No potentially curative treatment exists except surgery, but most people have advanced stage disease at presentation and are inoperable at the time of diagnosis. People with cholangiocarcinoma are generally managed, though not cured, with chemotherapy, radiation therapy, and other palliative care measures.

The present invention addresses these needs. Therefore, it is the object of the present invention to provide improved methods of treating or preventing cancer such as hepatocellular carcinoma and cholangiocarcinoma.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a bar graph showing the effects of Compound 1-Na and control diet on the number of hepatic tumors in multidrug resistance protein 2 (Mdr2^{-/-}) knockout mice. *p<0.01 vs. control.

Figure 1B is a bar graph showing the effects of Compound 1-Na and control diet on the percent reduction of tumors (>5mm diameter) in Mdr2^{-/-} mice.

Figure 2A is a bar graph showing the effects of OCA (Compound 2) and control diet on the number of hepatic tumors Mdr2^{-/-} knockout mice.

Figure 2B is a bar graph showing the effects of OCA and control diet on the percent reduction of tumors (>5mm diameter) in Mdr2^{-/-} mice.

Figure 3A is a bar graph showing the effects of Compound 1-Na and control diet on the number of hepatic tumors in Farnesoid X Receptor (FXR^{-/-}) mice.

Figure 3B is a bar graph showing the effects of Compound 1-Na and control diet on the percent reduction of tumors (>5mm diameter) in FXR^{-/-} mice.

- 5 Figure 4 is a bar graph showing the effects of Compound 1-Na and control diet on the percent reduction on the number of hepatic tumors in FXR^{-/-} mice.

Figure 5A is a bar graph showing the effects of Compound 1-Na and control diet on the percent reduction on liver/body weight ratio in Mdr2^{-/-} mice. *p<0.01 vs. control.

- 10 Figure 5B is a bar graph showing the effects of Compound 1-Na and control diet on the percent reduction on liver/body weight ratio in FXR^{-/-} mice.

Figure 6A is a bar graph showing the effect of Compound 1-Na and control diet on alanine transaminase (ALT) levels in Mdr2^{-/-} and FXR^{-/-} mice. *p<0.01 vs. control.

Figure 6B is a bar graph showing the effect of Compound 1-Na and control diet on aspartate transaminase (AST) levels in Mdr2^{-/-} and FXR^{-/-} mice. *p<0.01 vs. control.

- 15 Figure 7A is a bar graph showing the effect of Compound 1-Na and control diet on ileal gene expression of fibroblast growth factor 15 (Fgf15) in Mdr2^{-/-} and FXR^{-/-} mice. *p<0.01 vs. control.

Figure 7B is a bar graph showing the effect of Compound 1-Na and control diet on ileal gene expression of small heterodimer partner (Shp) in Mdr2^{-/-} and FXR^{-/-} mice. *p<0.01 vs. control

- 20 Figure 8 is a bar graph showing the effect of Compound 1-Na and control diet on the downregulation of cholesterol 7 alpha-hydroxylase (cyp7a1) in Mdr2^{-/-} and FXR^{-/-} mice. *p<0.01 vs. control

- 25 Figure 9A is a bar graph showing the effect of Compound 1-Na and control diet on hepatic gene expression of small heterodimer partner (Shp) in Mdr2^{-/-} and FXR^{-/-} mice. *p<0.01 vs. control.

Figure 9B is a bar graph showing the effect of Compound 1-Na and control diet on hepatic gene expression of bile salt export pump (Bsep) in Mdr2^{-/-} and FXR^{-/-} mice. *p<0.01 vs. control

Figure 10A is a bar graph showing the effect of Compound 1-Na and control diet on the total serum bile acids in Mdr2^{-/-} mice. *p<0.01 vs. control.

Figure 10B is a bar graph showing the effect of Compound 1-Na₃ and control diet on the total serum bile acids in FXR^{-/-} mice.

5 Figure 11A shows FXR mRNA microarray expression in whole tissue of CCA tumors compared to surrounding human tissue.

Figure 11B shows FXR mRNA microarray expression in whole tissue of CCA tumors grouped upon tumor differentiation grade.

10 Figure 11C shows FXR mRNA expression (qPCR) in CCA tumors compared to normal human liver tissue and surrounding human liver tissue.

Figure 11D shows FXR mRNA expression (qPCR) in CCA tumors compared to matched-surrounding human liver tissue.

Figure 12A shows TGR mRNA microarray expression in whole tissue of CCA tumors compared to surrounding human tissue.

15 Figure 12B shows TGR5 mRNA microarray expression in whole tissue of CCA tumors upon clinico-pathological parameters: anatomical location and perineural invasion.

Figure 12C shows TGR5 mRNA expression (qPCR) in CCA tumors compared to surrounding human liver tissue.

20 Figure 12D shows TGR5 mRNA expression (qPCR) in CCA tumors compared to matched-surrounding human liver tissue.

Figure 13A shows FXR mRNA expression (qPCR) in normal human cholangiocytes and CCA cell lines.

Figure 13B shows TGR5 mRNA expression (qPCR) in normal human cholangiocytes and CCA cell lines.

25 Figure 14A shows representative MRI and liver images of untreated control mice, OCA-treated mice and Compound 4-treated mice.

Figure 14B is a bar-graph showing tumor volume fold-change quantified by MRI.

Figure 14C shows mRNA expression levels of proliferation (i.e. *Ki67* and *PCNA*), biliary (i.e. *CK19*) and epithelial (i.e. *ZO-1*) markers in liver orthotopic CCA tumors.

Figure 14D shows representative immunohistochemistry images of proliferation markers (i.e. *Ki67* and *PCNA*) in liver orthotopic CCA tumors of mice untreated or treated with OCA or
5 Compound 4.

Figure 15A shows mRNA expression levels of proliferation markers upon OCA treatment and proliferation of CCA cells (i.e. EGI1) treated with OCA at 10 or 25 μ M for 48 h compared to non-treated control cells, performed by flow cytometry using CFSE cell proliferation dye staining.

10 Figure 15B shows migration assays in CCA cells (i.e. EGI1) and representative microscope images of wound-healing assay, corresponding quantification and representative microscope images of transwell migration chambers at 24 h.

Figure 15C shows Seahorse Oxygen Consumption Rate (OCR) using mitochondrial stress test kit in CCA cells (i.e. EGI1) and a bar-graph of metabolic parameters calculated upon OCR
15 measurements.

Figure 15D shows flow cytometry-based apoptosis assays by Annexin V and Propidium Iodide staining in CCA cells (i.e. EGI1) non-treated or treated with 10 or 25 μ M of OCA and representative histograms and corresponding quantification of pooled data.

Figure 16A shows mRNA expression levels of proliferation markers upon Compound 4
20 treatment and proliferation of CCA cells (i.e. EGI1) treated with Compound 3 at 10 or 25 μ M for 48 h compared to non-treated control cells.

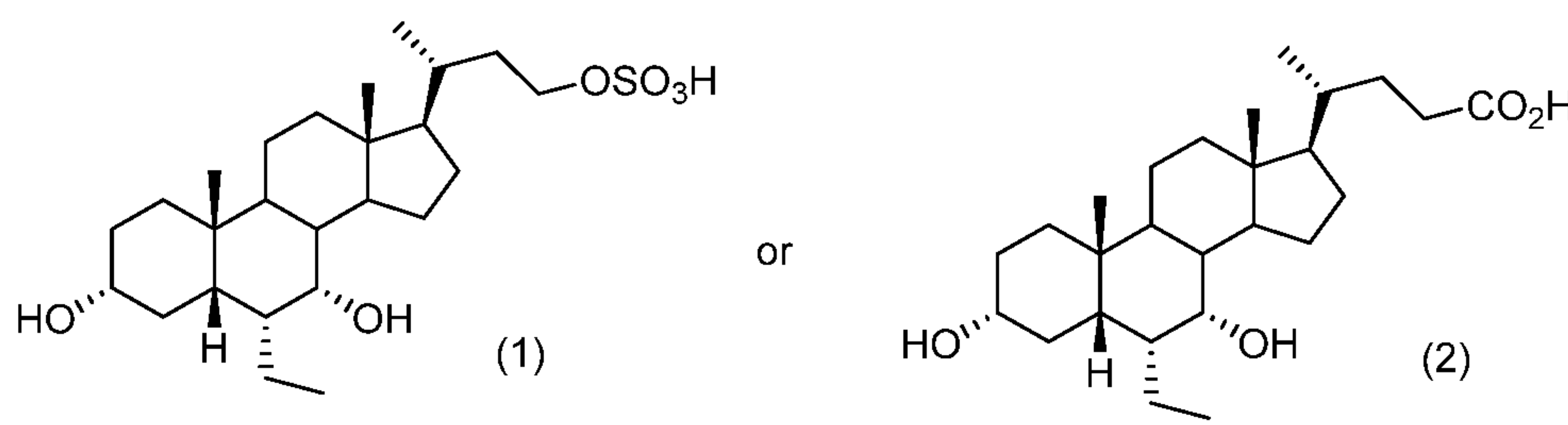
Figure 16B shows migration assays in CCA cells (i.e. EGI1), representative microscope images of wound-healing assay and corresponding quantification, and representative microscope images of transwell migration chambers at 24 h in non-treated and Compound 4-
25 treated cells and corresponding quantification.

Figure 16C shows Seahorse Oxygen Consumption Rate (OCR) using mitochondrial stress test kit in CCA cells (i.e. EGI1) non-treated or treated with Compound 4 (25 μ M) and a bar-graph of metabolic parameters calculated upon OCR measurements.

Figure 17 shows mRNA expression levels of proliferation (i.e. Cdc25a, cyclin D1 and cyclin D3) markers in liver orthotopic CCA tumors.

SUMMARY OF THE DISCLOSURE

The present application relates to methods of treating or preventing cancer in a subject in need thereof comprising administering a therapeutically effective amount of a Farnesoid X Receptor (FXR) agonist. In one embodiment, the FXR agonist is Compound 1 or Compound 2:



or a pharmaceutically acceptable salt or amino acid conjugate thereof.

In one embodiment, the cancer is selected from the group consisting of hepatocellular carcinoma, pancreatic cancer, kidney cancer, prostate cancer, esophageal cancer, breast cancer, gastric cancer, renal cancer, salivary gland cancer, ovarian cancer, uterine body cancer, bladder cancer, and lung cancer. In one embodiment, the cancer is hepatocellular carcinoma. In one embodiment, the cancer is pancreatic cancer. In one embodiment, the cancer is kidney cancer. In one embodiment, the cancer is prostate cancer. In one embodiment, the cancer is esophageal cancer. In one embodiment, the cancer is breast cancer. In one embodiment, the cancer is gastric cancer. In one embodiment, the cancer is renal cancer. In one embodiment, the cancer is salivary gland cancer. In one embodiment, the cancer is ovarian cancer. In one embodiment, the cancer is uterine body cancer. In one embodiment, the cancer is lung cancer.

In one embodiment, the FXR agonist is Compound 1 or a pharmaceutically acceptable salt thereof. In another embodiment, the FXR agonist is the sodium salt of Compound 1 (i.e., Compound 1-Na). In yet another embodiment, the FXR agonist is the N,N-diethaneamine salt of Compound 1 (i.e. Compound 1-DEA).

In another embodiment, the FXR agonist is Compound 2 or a pharmaceutically acceptable salt or amino acid conjugate thereof. In one embodiment, the FXR agonist is the glycine conjugate of Compound 2. In one embodiment, the FXR agonist is the taurine

conjugate of Compound 2. In one embodiment, the FXR agonist is the sarcosine conjugate of Compound 2.

The present invention further relates to the use of Compound 1 or a pharmaceutically acceptable salt thereof or Compound 2 or a pharmaceutically acceptable salt or amino acid conjugate thereof in the manufacture of a medicament for treating or preventing cancer in a subject in need thereof.

The present invention further relates to Compound 1 or a pharmaceutically acceptable salt thereof and Compound 2 or a pharmaceutically acceptable salt or amino acid conjugate thereof for use in treating or preventing cancer in a subject in need thereof.

The present invention also relates a pharmaceutical composition comprising Compound 1 or a pharmaceutically acceptable salt thereof and Compound 2 or a pharmaceutically acceptable salt or amino acid conjugate thereof for treating or preventing cancer in a subject in need thereof and a pharmaceutically acceptable excipient.

The present invention further relates to a kit for treating or preventing cancer in a subject in need thereof comprising Compound 1 or a pharmaceutically acceptable salt thereof and Compound 2 or a pharmaceutically acceptable salt or amino acid conjugate thereof.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present invention is based at least in part on the discovery that Compounds 1 and 2 were effective in treating cancer in animal models predictive of cancer. As described in the examples below, the inventors have discovered that a compound of the invention suppressed tumor growth in murine models of spontaneous hepatocarcinogenesis.

Definitions

For convenience, certain terms used in the specification, examples and appended claims are collected here.

The term "cancer" as used herein refers to any of the diseases characterized by the presence of cancerous tissue in a subject.

As used herein, "cancerous tissue" refers to a tissue that comprises malignant neoplastic cells, exhibits an abnormal growth of cells and/or hyperproliferative cells.

Cancerous tissue can be a primary malignant tumor, arising from a tissue or organ of origin,

or it can be a metastatic malignant tumor, growing in a body tissue which was not the source of the original tumor.

As used herein, the term "tumor" can include a solid tumor or a cancer of hematopoietic origin. In some embodiments the tumor may be characterized by its ability to
5 invade surrounding tissues, to metastasize to other parts of the body, and/or by its angiogenic activity. Exemplary tumors result from hepatocellular carcinoma, gastric cancer, renal cancer, prostate cancer, adrenal cancer, pancreatic cancer, breast cancer, bladder cancer, salivary gland cancer, ovarian cancer, uterine body cancer, and lung cancer.

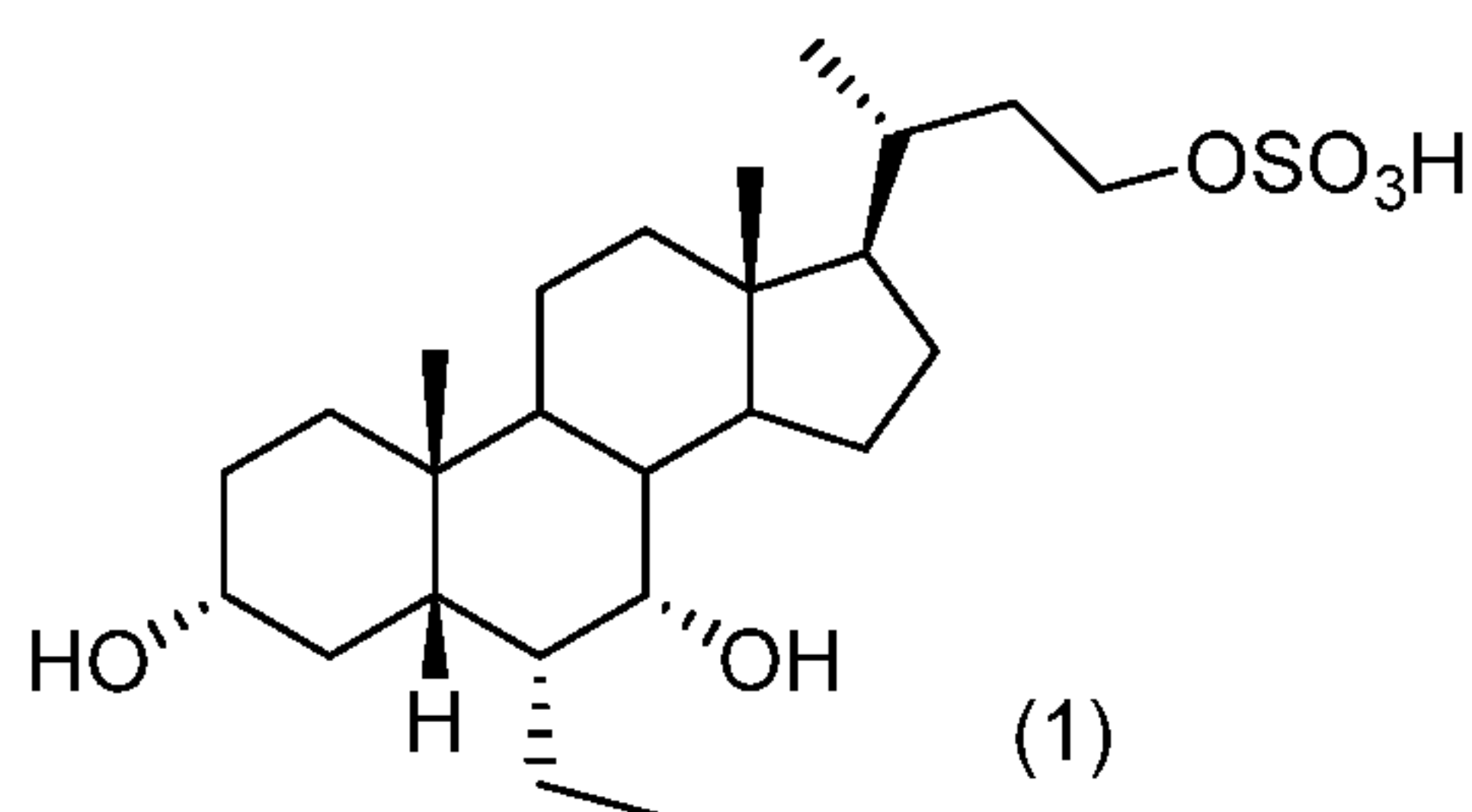
As used herein, the term "invasive" refers to the process by which a cell, a group of
10 cells, or a malignancy spreads from a site to adjacent sites.

The term "metastatic", as used herein, refers to the process by which a cell, a group of cells, or a malignancy spreads from a site to sites not adjacent to the first site.

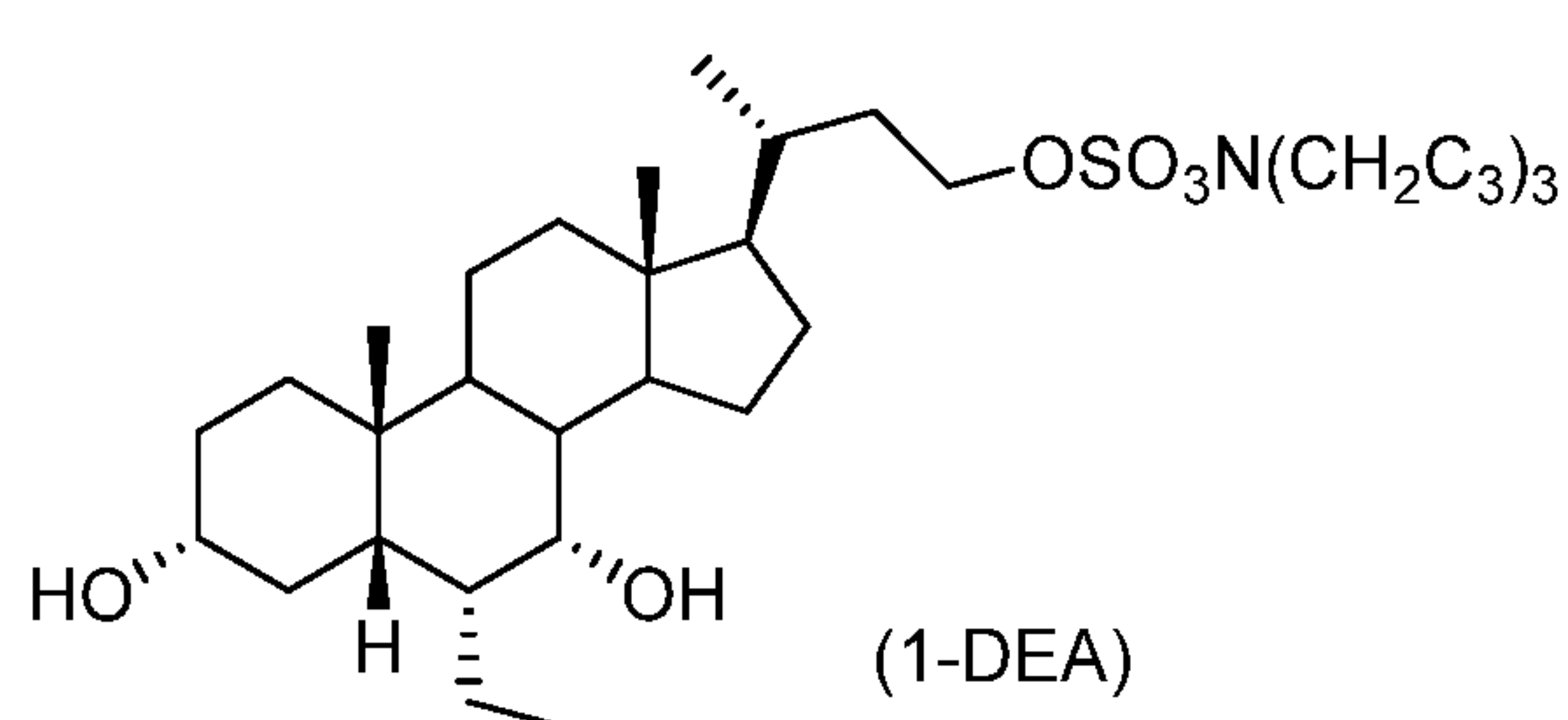
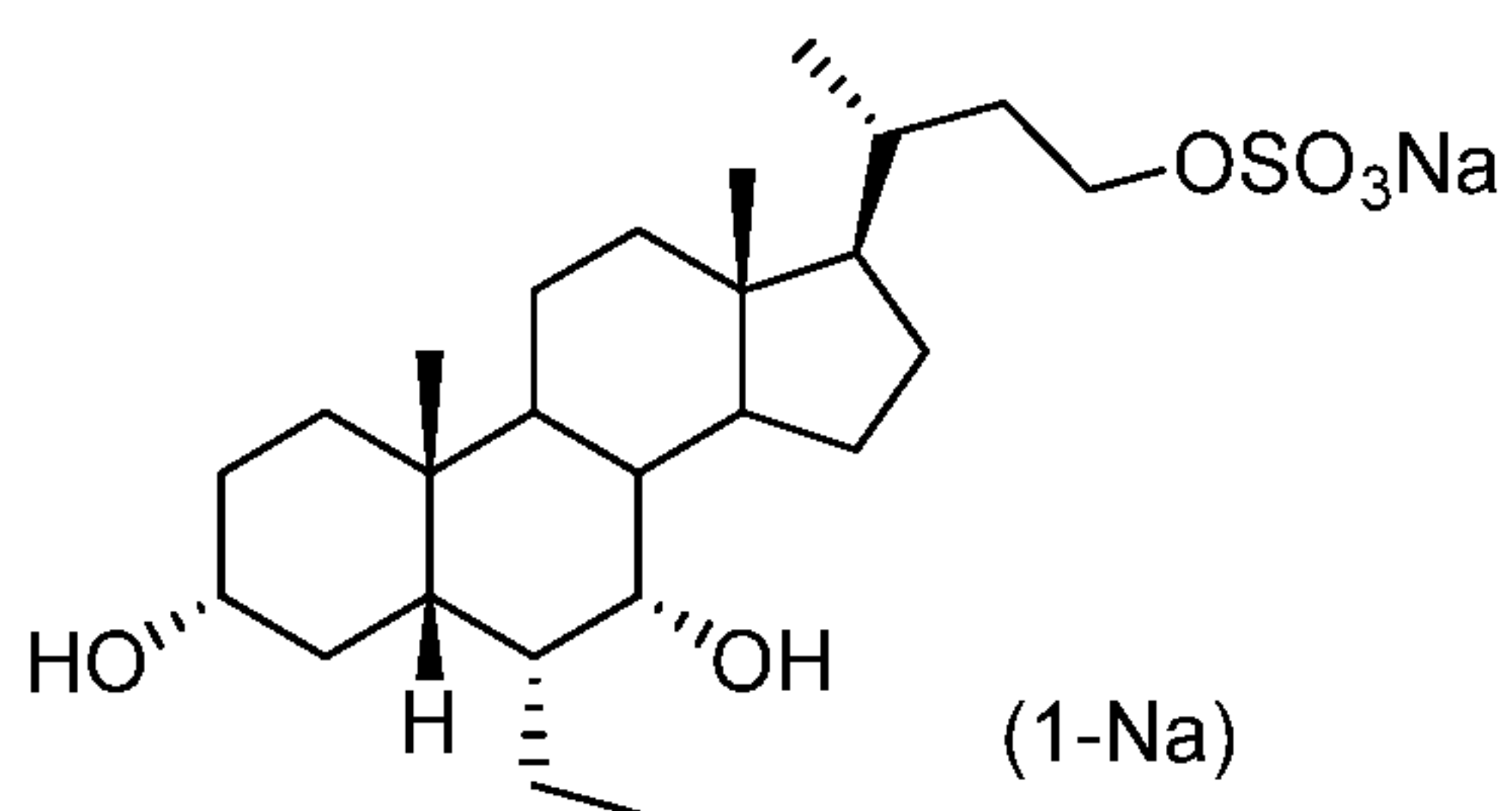
As used herein, "hepatocellular carcinoma", "HCC", and "malignant hepatoma" are used interchangeably and refer to primary and secondary (metastasized) tumors that originate
15 from the liver tissue. The term "refractory hepatocellular carcinoma" as used herein refers to hepatocellular carcinoma that fails to respond favorably to an antineoplastic treatment. Accordingly, "a hepatocellular carcinoma refractory to a treatment" as used herein refers to a hepatocellular carcinoma that fails to respond favorably to, or resistant to the treatment, or alternatively, recurs or relapses after responding favorably to the treatment.

As used herein, "cholangiocarcinoma" or "CCA" or "cholangiocellular carcinoma" or
20 a bile duct cancer is a form of cancer that is composed of mutated epithelial cells (or cells showing characteristics of epithelial differentiation) that originate in the bile ducts which drain bile from the liver into the small intestine. A proportion of CCAs arises under cholestatic liver conditions. Although intrahepatic accumulation of bile acids does not induce
25 carcinogenesis directly they may facilitate the cocarcinogenic effect by promoting cholangiocyte proliferation and inflammation, and by reducing FXR-dependent chemoprotection.

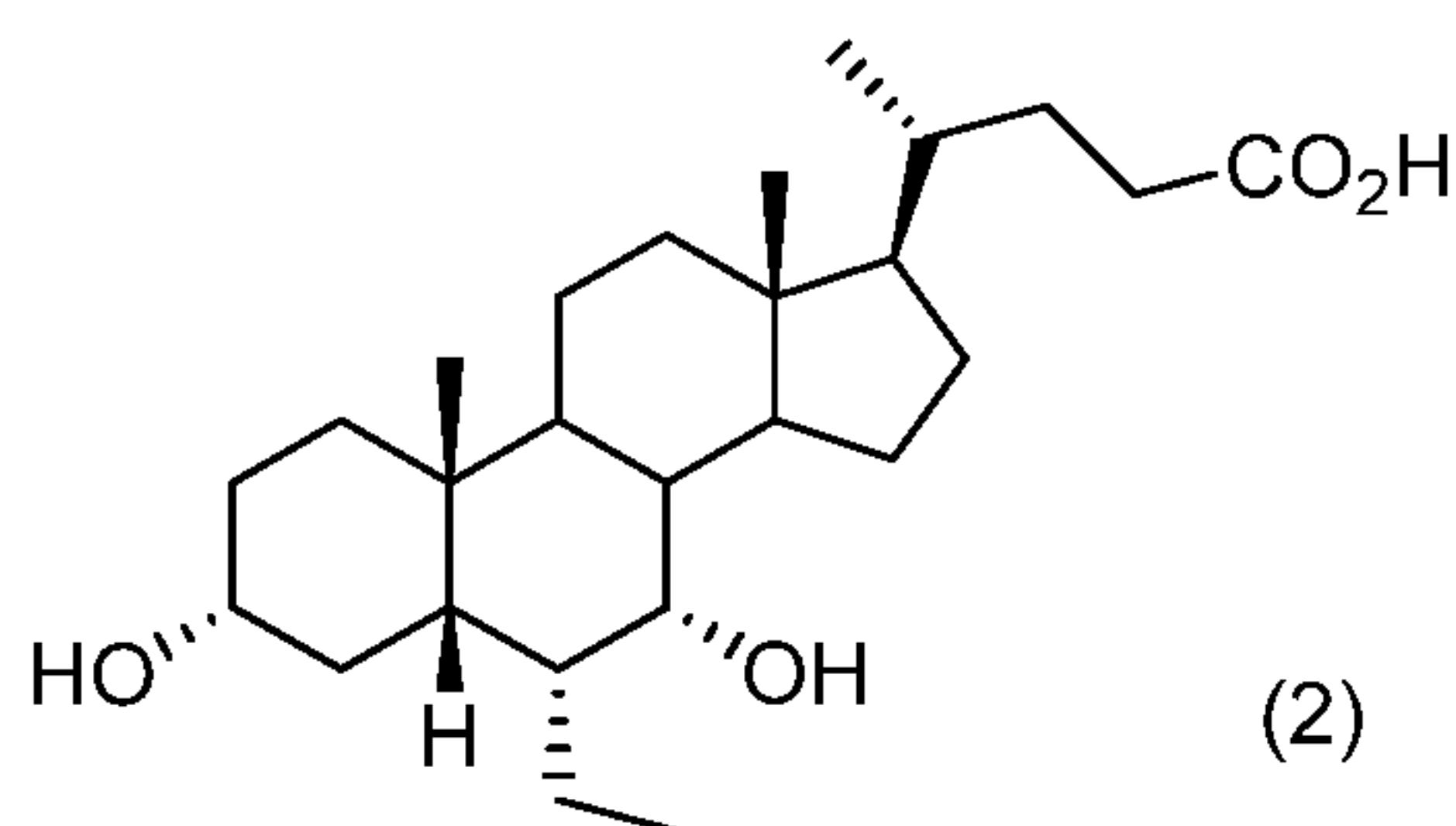
As used herein, the term "Compound 1" refers to



which is also known as 6 α -ethyl-3 α ,7 α ,23-trihydroxy-24-nor-5 β -cholan-23-hydrogen sulphate. "Compound 1-Na" or "1-Na" which is also known as 6 α -ethyl-3 α ,7 α ,23-trihydroxy-24-nor-5 β -cholan-23-sulphate sodium" are used interchangeably and refer to the sodium salt of Compound 1. As used herein, "Compound 1-DEA" or "1-DEA" which is also known as 6 α -ethyl-3 α ,7 α ,23-trihydroxy-24-nor-5 β -cholan-23-sulphate N,N-diethylethaneamine" are used interchangeably and refer to the N,N-diethylethaneamine salt of Compound 1. The structures of Compound 1-Na and Compound 1-DEA are provided below.

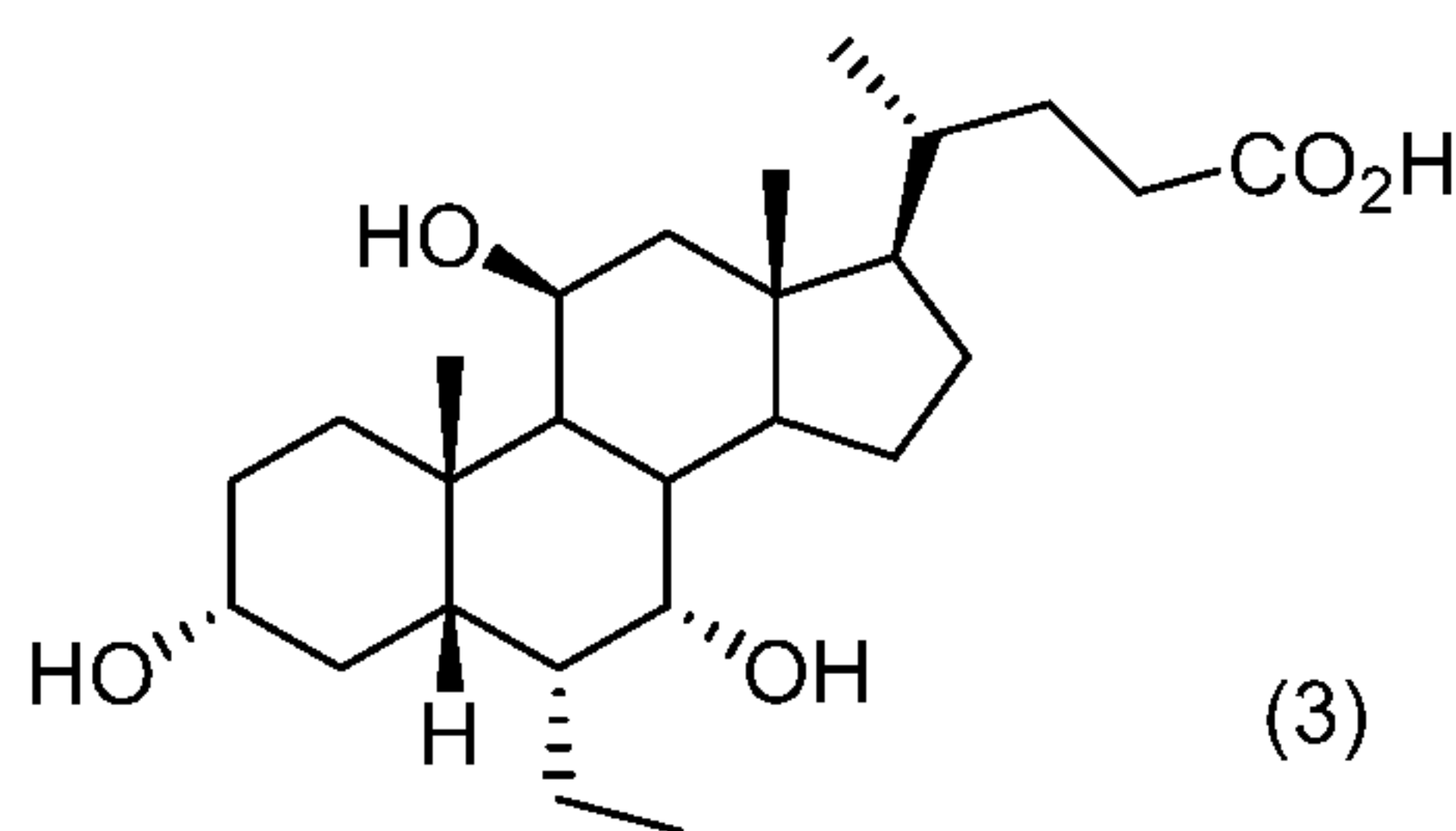


"Compound 2" as used herein refers to



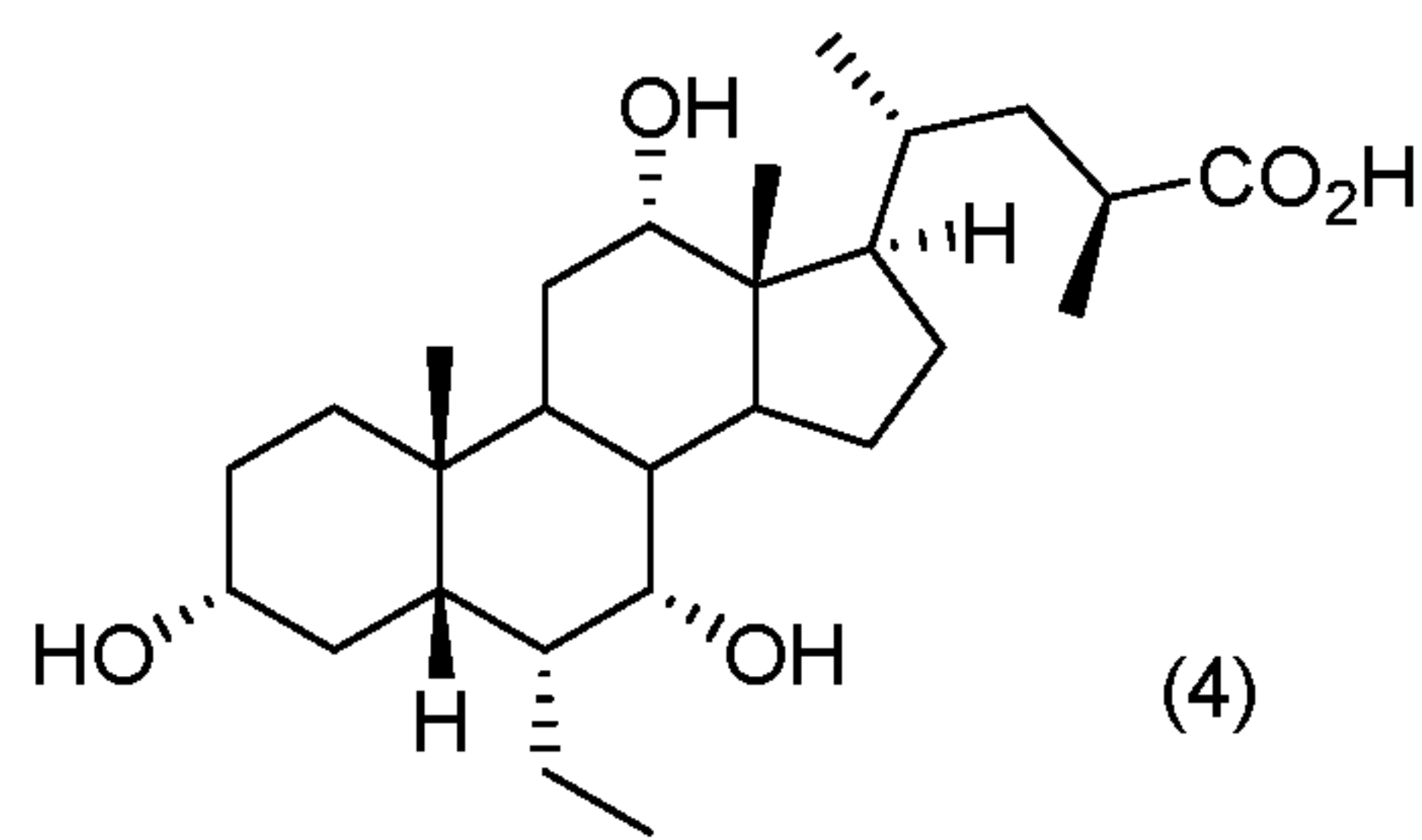
which is also known as obeticholic acid (OCA), 6-ECDC, 6- α -ethyl chenodeoxycholic acid, or 6 α -ethyl-3 α ,7 α -dihydroxy-5 β -cholan-24-oic acid.

As used herein, "Compound 3" refers to



which is also known as 3 α ,7 α ,11 β -trihydroxy-6 α -ethyl-5 β -cholan-24-oic acid.

As used herein, "Compound 4" refers to



which is also known as 6 α -ethyl-23(S)-methyl-3 α ,7 α ,12 α -trihydroxy-5 β -cholan-24-oic acid.

5 Compound 4 described in U.S. Patent No. 8, 114,862 is a TGR5 modulator. In one of the embodiments, TGR5 modulator is an agonist.

The term "TGR5 modulator" means any compound that interacts with the TGR5 receptor. The interaction is not limited to a compound acting as an antagonist, agonist, partial agonist, or inverse agonist of the TGR5 receptor.

10 Generally, the term "agonist" means a compound that enhances the activity of another molecule or receptor site. An agonist, by classical definition, whether a orthosteric, allosteric, inverse or a co-agonist has a property to bind to the receptor, alter its receptor state and result in a biological action. Consequently, agonism is defined as a property of an agonist or a ligand to produce a biological action. More specifically, a TGR5 agonist is a receptor ligand or
15 compound that binds to TGR5 and increases the concentration of cyclic adenosine monophosphate (cAMP) by at least 20% in cells expressing the receptor.

The phrase a "compound of the invention" as used herein encompasses Compounds 1, 1-Na, 1-DEA, 2, and 3, or a pharmaceutically acceptable salt or amino acid conjugate thereof.

20 The term "treating" as used herein refers to any indicia of success in the treatment or amelioration of the cancer. Treating can include, for example, reducing or alleviating the severity of one or more symptoms of the cancer, or it can include reducing the frequency with which symptoms of a disease, defect, disorder, or adverse condition, and the like, are experienced by a patient. "Treating" can also refer to reducing or eliminating a condition of a part of the body, such as a cell, tissue or bodily fluid, e.g., blood.

25 As used herein, the term "preventing" refers to the partial or complete prevention of the cancer in an individual or in a population, or in a part of the body, such as a cell, tissue or bodily fluid (e.g., blood). The term "prevention" does not establish a requirement for

complete prevention of a disease or condition in the entirety of the treated population of individuals or cells, tissues or fluids of individuals. The term "treat or prevent" is used herein to refer to a method that results in some level of treatment or amelioration of the cancer, and contemplates a range of results directed to that end, including but not restricted to prevention of the cancer entirely.

The phrase "therapeutically effective amount" as used herein refers to an effective amount comprising an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation in cancer. In some embodiments, an effective amount is an amount sufficient to delay the development of cancer. In some embodiments, an effective amount is an amount sufficient to prevent or delay recurrence. An effective amount can be administered in one or more administrations. In the case of HCC or CRC, the effective amount of the drug or composition may: (i) reduce the number of tumor cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor, and/or (vii) relieve to some extent one or more of the symptoms associated with cancer.

The term "regimen" as used herein refers to a protocol for dosing and/or timing the administration a compound of the invention for treating cancer. A regimen can include periods of active administration and periods of rest as known in the art. Active administration periods include administration of a compound of the invention in a defined course of time, including, for example, the number of and timing of dosages of the compositions. In some regimens, one or more rest periods can be included where no compound is actively administered, and in certain instances, includes time periods where the efficacy of such compounds can be minimal.

As used herein, "combination therapy" refers to a compound of the invention can be administered in conjunction with another therapeutic agent. "In conjunction with" refers to administration of one treatment modality in addition to another treatment modality, such as administration of a compound of the invention as described herein in addition to administration of another therapeutic agent to the same subject. As such, "in conjunction

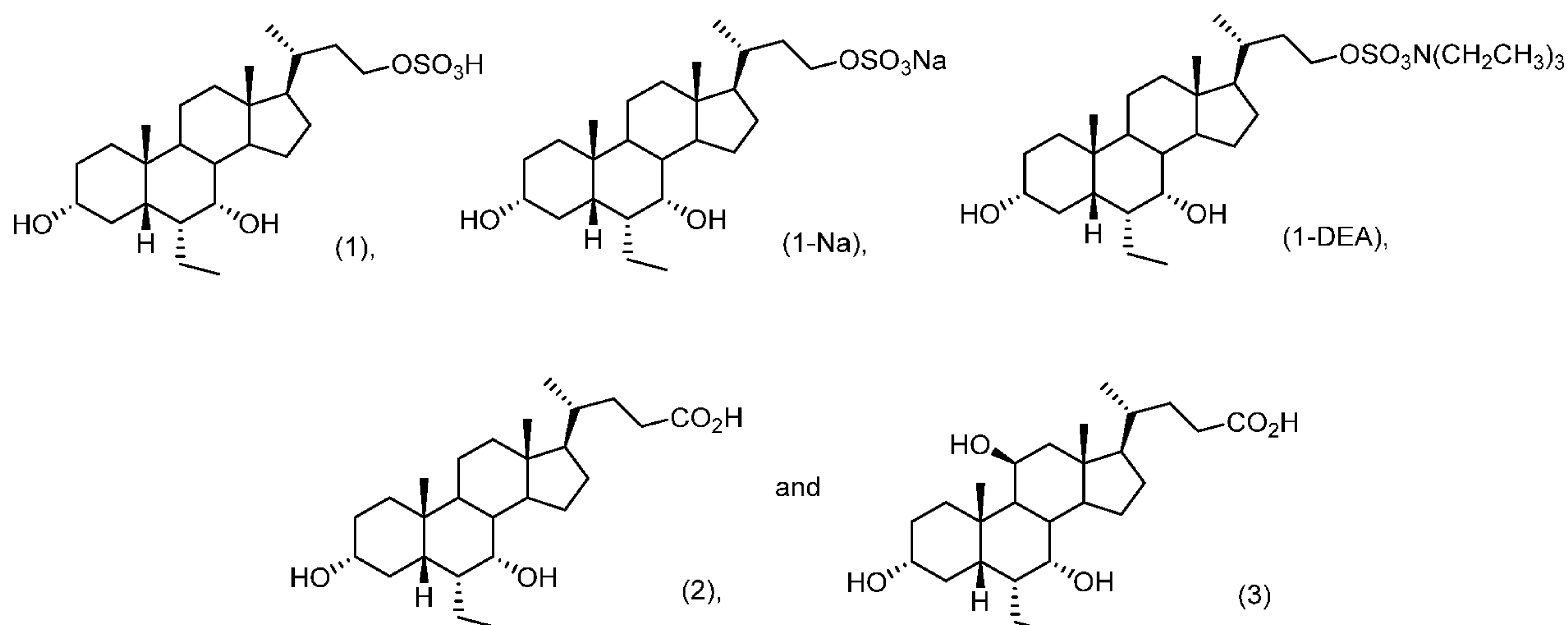
with" refers to administration of one treatment modality before, during, or after delivery of a second treatment modality to the subject.

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

The term "about" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, and in some embodiments $\pm 0.1\%$ from the specified value, as such variations are appropriate to practice the disclosed methods or to make and used the disclosed compounds and in the claimed methods.

Methods of Treating Cancer

The present invention is based at least in part based on the discovery that a compound of the invention is effective in treating cancer in murine models predictive of cancer in humans. Accordingly, the present application relates to methods of treating or preventing cancer in a subject in need thereof comprising administering therapeutically effective amount of a FXR agonist selected from the group consisting of Compounds 1, 1-Na, 1-DEA, 2, and 3:



or a pharmaceutically acceptable salt or amino acid conjugate thereof. In one embodiment, the FXR agonist is Compound 1. In one embodiment, the FXR agonist is Compound 1-Na. In another embodiment, the FXR agonist is Compound 1-DEA. In one embodiment, the FXR agonist is Compound 2. In one embodiment, the FXR agonist is Compound 3.

In the methods described herein, exemplary cancers are selected from the group consisting of hepatocellular carcinoma, cholangiocarcinoma, pancreatic cancer, prostate cancer, esophageal cancer, breast cancer, gastric cancer, renal cancer, salivary gland cancer, ovarian cancer, uterine body cancer, bladder cancer, and lung cancer. The appropriate treatment regimen for cancer depends on the type of cell from which the tumor derived, the stage and severity of the malignancy, and the genetic abnormality that contributes to the tumor.

Cancer staging systems describe the extent of cancer progression. In general, the staging systems describe how far the cancer has spread and puts patients with similar prognosis and treatment in the same staging group. In general, there are poorer prognoses for tumors that have become invasive or metastasized. In one type of staging system, cases are grouped into four stages, denoted by Roman numerals I to IV. In stage I, cancers are often localized and are usually curable. Stage II and IIIA cancers are usually more advanced and may have invaded the surrounding tissues and spread to lymph nodes. Stage IV cancers include metastatic cancers that have spread to sites outside of lymph nodes.

Another staging system is TNM staging which stands for the categories: Tumor, Nodes, and Metastases. In this system, malignancies are described according to the severity of the individual categories. For example, T classifies the extent of a primary tumor from 0 to 4

with 0 representing a malignancy that does not have invasive activity and 4 representing a malignancy that has invaded other organs by extension from the original site. N classifies the extent of lymph node involvement with 0 representing a malignancy with no lymph node involvement and 4 representing a malignancy with extensive lymph node involvement. M classifies the extent of metastasis from 0 to 1 with 0 representing a malignancy with no metastases and 1 representing a malignancy with metastases.

These staging systems or variations of these staging systems or other suitable staging systems may be used to describe a tumor. Few options are available for the treatment of cancer depending on the stage and features of the cancer. Treatments include surgery, treatment with sorafenib, and targeted therapies. In general, surgery is the first line of treatment for early stage localized cancer. Additional systemic treatments may be used to treat invasive and metastatic tumors.

In accordance with one aspect of the present invention, a method is provided for treating hepatocellular carcinoma (or malignant hepatoma). Specifically, the method comprises treating a subject in need thereof having hepatocellular carcinoma with a therapeutically effective amount of a compound of the invention. That is, the present invention is directed to the use a compound of the invention for the manufacture of medicaments for treating hepatocellular carcinoma patients identified or diagnosed as having hepatocellular carcinoma. In separate embodiments, the treatment method optionally comprises a step of diagnosing or identifying a patient as having hepatocellular carcinoma. The identified patient is then treated with or administered with a therapeutically effective amount of a compound of the invention. Hepatocellular carcinoma can be diagnosed in any conventional diagnostic methods known in the art including ultrasound, CT scan, MRI, alpha-fetoprotein testing, des-gamma carboxyprothrombin screening, and biopsy.

The present invention also provides a method of treating refractory hepatocellular carcinoma comprising treating a patient identified as having refractory hepatocellular carcinoma with a therapeutically effective amount of a compound of the invention. In specific embodiments, the patient has a hepatocellular carcinoma that is refractory to a treatment comprising one or more drugs selected from the group consisting of sorafenib, regorafenib, anthracyclines (e.g., doxorubicin, daunorubicin, epirubicin, idarubicin), platinum agents (e.g., cisplatin, carboplatin, oxaliplatin, picoplatin), 5-FU and capecitabine. The present invention

is also directed to the use of a compound of the invention for the manufacture of medicaments for treating refractory hepatocellular carcinoma, e.g., a hepatocellular carcinoma refractory to one or more drugs selected from sorafenib, regorafenib, anthracyclines (e.g., doxorubicin, daunorubicin, epirubicin, idarubicin), platinum agents (cisplatin, carboplatin, oxaliplatin, picoplatin), 5-FU and capecitabine.

To detect a refractory hepatocellular carcinoma, patients undergoing initial treatment can be carefully monitored for signs of resistance, non-responsiveness or recurring hepatocellular carcinoma. This can be accomplished by monitoring the patient's cancer's response to the initial treatment which, e.g., may include one or more drugs selected from the group consisting of sorafenib, regorafenib, doxorubicin, daunorubicin, epirubicin, idarubicin, cisplatin, carboplatin, oxaliplatin, picoplatin, 5-FU, tegafur and capecitabine. The response, lack of response or relapse of the cancer to the initial treatment can be determined by any suitable method practiced in the art. For example, this can be accomplished by the assessment of tumor size and number. An increase in tumor size or, alternatively, tumor number, indicates that the tumor is not responding to the chemotherapy or that a relapse has occurred. The determination can be done according to the "RECIST" criteria as described in detail in Therasse et al, J. Natl. Cancer Inst. 92:205-216 (2000).

In accordance with yet another aspect of the present invention, a method is provided for preventing or delaying the onset of hepatocellular carcinoma (or hepatocellular carcinoma), or preventing or delaying the recurrence of hepatocellular carcinoma, which comprises treating a patient in need of the prevention or delay with a prophylactically effective amount of a compound of the invention.

It is known that subjects with hepatitis B or hepatitis C infection, or having cirrhosis have an increased risk of developing hepatocellular carcinoma. In addition, people who have acute and chronic hepatic porphyrias (acute intermittent porphyria, porphyria cutanea tarda, hereditary coproporphyria, variegate porphyria) and tyrosinemia type I are also at an increased risk of for developing hepatocellular carcinoma. These people can all be candidates for the method of the present invention for preventing or delaying the onset of hepatocellular carcinoma using a prophylactically effective amount of a compound of the invention. In addition, patients with a family history of hepatocellular carcinoma can also be identified for the application of the present method of preventing or delaying the onset

of hepatocellular carcinoma. For purposes of preventing or delaying the recurrence of hepatocellular carcinoma, hepatocellular carcinoma patients who have been treated and are in remission or in a stable or progression free-state may be treated with a prophylactically effective amount of a compound of the invention to effectively prevent or delay the recurrence or relapse of hepatocellular carcinoma.

In accordance with one aspect of the present disclosure, a method is provided for treating cholangiocarcinoma (CCA). Specifically, the method comprises treating a subject in need thereof having cholangiocarcinoma with a therapeutically effective amount of a compound of the invention. That is, the present invention is directed to the use a compound of the invention for the manufacture of medicaments for treating cholangiocarcinoma patients identified or diagnosed as having cholangiocarcinoma. In separate embodiments, the treatment method optionally comprises a step of diagnosing or identifying a patient as having cholangiocarcinoma. The identified patient is then treated with or administered with a therapeutically effective amount of a compound of the invention. Cholangiocarcinoma can be diagnosed in any conventional diagnostic methods known in the art including ultrasound, CT scan, MRI, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) screening, and biopsy.

In accordance with yet another aspect of the present invention, a method is provided for preventing or delaying the onset of cholangiocarcinoma, or preventing or delaying the recurrence of cholangiocarcinoma, which comprises treating a patient in need of the prevention or delay with a prophylactically effective amount of a compound of the invention.

For purposes of preventing or delaying the recurrence of cholangiocarcinoma, cholangiocarcinoma patients who have been treated and are in remission or in a stable or progression free-state may be treated with a prophylactically effective amount of a compound of the invention to effectively prevent or delay the recurrence or relapse of cholangiocarcinoma.

In accordance with another aspect of the present invention, a method is provided for inhibiting proliferation and migration of CCA cells with an FXR agonist.

In accordance with another aspect of the present invention, a method is provided for inhibiting proliferation and migration of CCA cells with a therapeutically effective amount of a compound of the invention. In accordance with another aspect of the present invention, a

method is provided for inhibiting progression of CCA which comprises treating a patient in need with a therapeutically effective amount of a compound of the invention.

One embodiment is a method of treating pancreatic cancer by administering a therapeutically effective amount of a compound of the invention. Another embodiment is a method of treating prostate cancer by administering a therapeutically effective amount of a compound of the invention. Another embodiment is a method of treating kidney cancer by administering a therapeutically effective amount of a compound of the invention. Another embodiment is a method of treating prostate cancer by administering a therapeutically effective amount of a compound of the invention. In still another embodiment is a method of treating esophageal cancer by administering a therapeutically effective amount of a compound of the invention. In still another embodiment is a method of treating breast cancer by administering a therapeutically effective amount of a compound of the invention. One embodiment is a method of treating gastric cancer by administering a therapeutically effective amount of a compound of the invention. In another embodiment is a method of treating renal cancer by administering a therapeutically effective amount of a compound of the invention. In still another embodiment is a method of treating salivary gland cancer by administering a therapeutically effective amount of a compound of the invention. In still another embodiment is a method of treating ovarian cancer by administering a therapeutically effective amount of a compound of the invention. One embodiment is a method of treating uterine body cancer by administering a therapeutically effective amount of a compound of the invention. In another embodiment is a method of treating bladder cancer by administering a therapeutically effective amount of a compound of the invention. In still another embodiment is a method of treating lung cancer by administering a therapeutically effective amount of a compound of the invention.

In instances where a compound of the invention is useful for the treatment of a cancer described herein, such compounds can be co-administered with a second agent. The second agent useful in methods of treating cancers provided herein can include any known class of anti-cancer agents such as, for example, radiation therapy, operations, alkylating agents, antimetabolites, anthracyclines, camptothecins, vinca alkaloids, taxanes or platinum, as well as other antineoplastic agents known in the art. Such anti-cancer agent and antineoplastic

agent classifications are known in the art and used in accordance with their plain and ordinary meaning.

Exemplary anti-cancer agents include but are not limited to: ABRAXANE;
 abiraterone; ace-11; aclarubicin; acivicin; acodazole hydrochloride; acronine; actinomycin;
 5 acylfulvene; adecypenol; adozelesin; adriamycin; aldesleukin; all trans-retinoic acid (ATRA);
 altretamine; ambamustine; ambomycin; ametantrone acetate; amidox; amifostine;
 aminoglutethimide; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole;
 andrographolide; antarelix; anthramycin; aphidicolin glycinate; apurinic acid; ara-CDP-DL-
 PTBA; arginine deaminase; ARRY-162; ARRY-300; ARRY-142266; AS703026;
 10 asparaginase; asperlin; asulacrine; atamestane; atrimustine; AVASTIN; axinastatin 1;
 axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; azacitidine; AZD8330; azetepa;
 azotomycin; balanol; batimastat; BAY 11-7082; BAY 43-9006; BAY 869766; bendamustine;
 benzochlorins; benzodepa; benzoylstaurosporine; beta-aethine; betaclamycin B; betulinic
 acid; b-FGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bisnafide
 15 dimesylate; bistratene A; bisantrene hydrochloride; bleomycin; bleomycin sulfate; busulfan;
 bizelesin; breflate; bortezomib; brequinar sodium; broprimine; budotitane; buthionine
 sulfoximine; bryostatin; cactinomycin; calusterone; calcipotriol; calphostin C; camptothecin
 derivatives; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3;
 CARN 700; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride;
 20 carzelesin; castanospermine; cecropin B; cedefingol; celecoxib; cetorelix; chlorins;
 chloroquinoxaline sulfonamide; cicaprost; chlorambucil; Chlorofusin; cirolemycin; cisplatin;
 CI-1040; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A;
 collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816;
 crisnatol; crisnatol mesylate; cryptophycin 8; cryptophycin A derivatives; curacin A;
 25 cyclopentantraquinones; cycloplatan; cypemycin; cyclophosphamide; cytarabine; cytarabine
 ocfosfate; cytolytic factor; cytostatin; dacarbazine; dactinomycin; daunorubicin; daunorubicin
 hydrochloride; decarbazine; dacliximab; dasatinib; decitabine; dehydrodidemnin B;
 deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; dexormaplatin;
 dezaguanine; dezaguanine mesylate; diaziquone; didemnin B; didox; diethylnorspermine;
 30 dihydro 5 azacytidine; dihydrotaxol; 9-dioxamycin; diphenyl spiromustine; docosanol;
 dolasetron; docetaxel; doxorubicin; doxorubicin hydrochloride; doxifluridine; droloxifene;

droloxifene citrate; dromostanolone propionate; dronabinol; duazomycin; duocarmycin SA;
 ebselen; ecomustine; edelfosine; edrecolomab; edatrexate; eflornithine hydrochloride;
 eflornithine; elemene; emitefur; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin;
 epirubicin hydrochloride; epristeride; erbulozole; eribulin; esorubicin hydrochloride;
 5 estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate;
 etoprine; exemestane; fadrozole; fadrozole hydrochloride; fazarabine; fenretinide; filgrastim;
 finasteride; flavopiridol; flezelastine; fluasterone; floxuridine; fludarabine phosphate;
 fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fluorouracil;
 floxouridine; flurocitabine; fosquidone; fostriecin sodium; fostriecin; fotemustine; gadolinium
 10 texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine;
 geldanamycin; gossyphol; GDC-0973; GSK1120212/trametinib; herceptin; hydroxyurea;
 hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; ibrutinib;
 idarubicin; idarubicin hydrochloride; ifosfamide; canfosfamide; ilmofosine; iproplatin;
 idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imatinib (e.g., GLEEVEC);
 15 imiquimod; iniparib (BSI 201); iobenguane; iododoxorubicin; ipomeanol; irinotecan;
 irinotecan hydrochloride; irsogladine; isobengazole; isohomohalicondrin B; itasetron;
 iimofosine; interleukin IL-2 (including recombinant interleukin II; or rIL.sub.2); interferon
 alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1a; interferon
 gamma-1b; jasplakinolide; kahalalide F; lamellarin N triacetate; lanreotide; leinamycin;
 20 lenograstim; lentinan sulfate; leptolstatin; letrozole; leuprorelin; levamisole; lenalidomide;
 lenvatinib; liarozole; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine;
 losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lanreotide
 acetate; lapatinib; letrozole; leucovorin; leuprolide acetate; liarozole hydrochloride;
 lometrexol sodium; lomustine; losoxantrone hydrochloride; pomalidomide; LY294002;
 25 maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; menogaril;
 merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone;
 miltefosine; mirimostim; mitoguazone; mitolactol; mitonafide; mitoxantrone; mofarotene;
 molgramostim; mopidamol; mycaperoxide B; myriaporone; maytansine; mechlorethamine
 hydrochloride; megestrol acetate; melengestrol acetate; melphalan; mercaptopurine;
 30 methotrexate; methotrexate sodium; metoprine; meturedapa; mitindomide; mitocarcin;
 mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone

hydrochloride; mycophenolic acid; nafarelin; nagrestip; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; nocodazole; nogalamycin; oblimersen (GENASENSE); octreotide; okicenone; olaparib (LYNPARZA); oligonucleotides; onapristone; ondansetron; 5 oracin; oral cytokine inducer; ormaplatin; oxisuran; oxaloplatin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; PARP (polyADP ribose polymerase) inhibitors; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine 10 hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; porfiromycin; prednisone; prostaglandin J2; pyrazoloacridine; paclitaxel; PD035901; PD184352; PD318026; PD98059; peliomycin; pentamustine; peplomycin sulfate; PKC412; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; podophyllotoxin; polyphenol E; porfimer sodium; porfiromycin; prednimustine; procarbazine; procarbazine hydrochloride; 15 puromycin; puromycin hydrochloride; pyrazofurin; raltitrexed; ramosetron; retelliptine demethylated; rhizoxin; rituximab; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; riboprime; romidepsin; rucaparib; safingol; safingol hydrochloride; saintopin; sarcophytol A; sargramostim; semustine; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; sonermin; sorafenib; sunitinib; sparfosic 20 acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; Spongistatin 2; Spongistatin 3; Spongistatin 4; Spongistatin 5; Spongistatin 6; Spongistatin 7; Spongistatin 8; and Spongistatin 9; squalamine; stipiamide; stromelysin inhibitors; sulfinosine; suradista; suramin; swainsonine; SB239063; selumetinib/AZD6244; simtrazene; SP600125; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiroplatin; streptonigrin; streptozocin; 25 sulofenur; tallimustine; tamoxifen methiodide; talazoparib (BMN 673); tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thymalfasin; thymopoietin receptor agonist; thymotrinan; tirapazamine; titanocene bichloride; topsentin; toremifene; tretinoin; triacetyluridine; tricinibine; trimetrexate; triptorelin; 30 tropisetron; turosteride; tyrphostins; talisomycin; TAK-733; taxotere; tegafur; teloxantrone hydrochloride; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin;

tirapazamine; toremifene citrate; trastuzumab; trestolone acetate; tricitabine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; tumor necrosis factor-related apoptosis-inducing ligand (TRAIL); UBC inhibitors; ubenimex; U0126; uracil mustard; uredepa; vapreotide; variolin B; velaresol; veliparib (ABT-888); veramine; verteporfin; vinorelbine; vinxaltine; vitaxin; vinblastine; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinylicinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; wortmannin; XL518; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer; zinostatin; and zorubicin hydrochloride.

Other exemplary anti-cancer agents include Erbulozole (e.g., R-55104); Dolastatin 10 (e.g., DLS-10 and NSC-376128); Mivobulin isethionate (e.g., CI-980); NSC-639829; Discodermolide (e.g., NVP-XX-A-296); ABT-751 (Abbott; e.g., E-7010); Altorhyrtin A; Altorhyrtin C; Cemadotin hydrochloride (e.g., LU-103793 and NSC-D-669356); CEP 9722; Epothilone A; Epothilone B; Epothilone C; Epothilone D; Epothilone E; Epothilone F; Epothilone B N-oxide; Epothilone A N-oxide; 16-aza-epothilone B; 21-aminoepothilone B; 21-hydroxyepothilone D; 26-fluoroepothilone; Auristatin PE (e.g., NSC-654663); Soblidotin (e.g., TZT-1027); LS-4559-P (Pharmacia; e.g., LS-4577); LS-4578 (Pharmacia; e.g., LS-477-P); LS-4477 (Pharmacia); LS-4559 (Pharmacia); RPR-112378 (Aventis); DZ-3358 (Daiichi); FR-182877 (Fujisawa; e.g., WS-9265B); GS-164 (Takeda); GS-198 (Takeda); KAR-2 (Hungarian Academy of Sciences); BSF-223651 (BASF; e.g., ILX-651 and LU-223651); SAH-49960 (Lilly/Novartis); SDZ-268970 (Lilly/Novartis); AM-97 (Armad/Kyowa Hakko); AM-132 (Armad); AM-138 (Armad/Kyowa Hakko); IDN-5005 (Indena); Cryptophycin 52 (e.g., LY-355703); AC-7739 (Ajinomoto; e.g., AVE-8063A and CS-39.HCl); AC-7700 (Ajinomoto; e.g., AVE-8062; AVE-8062A; CS-39-L-Ser.HCl; and RPR-258062A); Vitilevuamide; Tubulysin A; Canadensol; CA-170 (Curis, Inc.); Centaureidin (e.g., NSC-106969); T-138067 (Tularik; e.g., T-67; TL-138067 and TI-138067); COBRA-1 (Parker Hughes Institute; e.g., DDE-261 and WHI-261); H10 (Kansas State University); H16 (Kansas State University); Oncocidin A1 (e.g., BTO-956 and DIME); DDE-313 (Parker Hughes Institute); Fijianolide B; Laulimalide; SPA-2 (Parker Hughes Institute); SPA-1 (Parker Hughes Institute; e.g., SPIKET-P); 3-IAABU (Cytoskeleton/Mt. Sinai School of Medicine; e.g., MF-569); Narcosine (e.g., NSC-5366); Nascapine; D-24851 (Asta Medica); A-105972

(Abbott); Hemiasterlin; 3-BAABU (Cytoskeleton/Mt. Sinai School of Medicine; e.g., MF-191); TMPN (Arizona State University); Vanadocene acetylacetonate; T-138026 (Tularik); Monsatrol; Inanocine (e.g., NSC-698666); 3-IAABE (Cytoskeleton/Mt. Sinai School of Medicine); A-204197 (Abbott); T-607 (Tularik; e.g., T-900607); RPR-115781 (Aventis);

5 Eleutherobins (e.g., Desmethyleleutherobin; Desaeyleleutherobin; Isoeleutherobin A; and Z-Eleutherobin); Caribaeoside; Caribaeolin; Halichondrin B; D-64131 (Asta Medica); D-68144 (Asta Medica); Diazonamide A; A-293620 (Abbott); NPI-2350 (Nereus); Taccalonolide A; TUB-245 (Aventis); A-259754 (Abbott); Diozostatin; (-)-Phenylahistin (e.g., NSCL-96F037);

10 D-62638 (Asta Medica); D-62636 (Asta Medica); Myoseverin B; D-43411 (Zentaris; e.g., D-81862); A-289099 (Abbott); A-318315 (Abbott); HTI-286 (e.g., SPA-110; trifluoroacetate salt) (Wyeth); D-82317 (Zentaris); D-82318 (Zentaris); SC-12983 (NCI); Resverastatin phosphate sodium; BPR-OY-007 (National Health Research Institutes); and SSR-250411 (Sanofi)); goserelin; leuprolide; triptolide; homoharringtonine; topotecan; itraconazole; deoxyadenosine; sertraline; pitavastatin; clofazimine; 5-nonyloxytryptamine; vemurafenib;

15 dabrafenib; gefitinib (IRESSA); erlotinib (TARCEVA); cetuximab (ERBITUX); lapatinib (TYKERB); panitumumab (VECTIBIX); vandetanib (CAPRELSA); afatinib/BIBW2992; CI-1033/canertinib; neratinib/HKI-272; CP-724714; TAK-285; AST-1306; ARRY334543; ARRY-380; AG-1478; dacomitinib/PF299804; OSI-420/desmethyl erlotinib; AZD8931; AEE726; pelitinib/EKB-569; CUDC-101; WZ8040; WZ4002; WZ3146; AG-490; XL647;

20 PD153035; 5-azathioprine; 5-aza-2'-deoxycytidine; 17-N-Allylamino-17-Demethoxygeldanamycin (17-AAG); 20-epi-1,25 dihydroxyvitamin D3; 5 ethynyluracil; and BMS-599626.

One embodiment is a method for treating patients with hepatocellular carcinoma (optionally refractory) by administering a compound of the invention in combination with

25 capecitabine and/or PLX4032 (Plexxikon).

In another embodiment is a method for treating hepatocellular carcinoma (optionally refractory) by administering a compound of the invention in combination with capecitabine, xeloda, and/or CPT-11.

In another embodiment is a method for treating hepatocellular carcinoma (optionally

30 refractory) by administering a compound of the invention in combination with capecitabine, xeloda, and/or CPT-11.

In another embodiment is a method for treating patients with hepatocellular carcinoma (optionally refractory) or patients with unresectable or metastatic hepatocellular carcinoma by administering a compound of the invention in combination with capecitabine and irinotecan.

5 In another embodiment is a method for treating patients with unresectable or metastatic hepatocellular carcinoma by administering a compound of the invention in combination with interferon alpha or capecitabin.

In another embodiment is a method for treating patients with pancreatic cancer by administering a compound of the invention in combination with gemcitabine.

10 One of the embodiments is a method for treating patients with CCA by administering a compound of the invention in combination with another agent or agents or combination thereof.

One of the embodiments is a method for treating patients with CCA by administering a compound of the invention in combination with gemcitabine, cisplatin or combination thereof.

15 Pharmaceutical Compositions

A "pharmaceutical composition" is a formulation containing a compound of the invention in a form suitable for administration to a subject. In one embodiment, the pharmaceutical composition is in bulk or in unit dosage form. It can be advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of
20 dosage. The specification for the dosage unit forms are dictated by and directly dependent on the unique characteristics of the active reagent and the particular therapeutic effect to be achieved.

Possible formulations include those suitable for oral, sublingual, buccal, parenteral (e.g., subcutaneous, intramuscular, or intravenous), rectal, topical including transdermal,
25 intranasal and inhalation administration. Most suitable means of administration for a particular patient will depend on the nature and severity of the disease being treated or the nature of the therapy being used and on the nature of the active compound, but where possible, oral administration may be used for the prevention and treatment cancer. Formulations suitable for oral administration may be provided as discrete units, such as
30 tablets, capsules, cachets, lozenges, each containing a predetermined amount of the active

compound; as powders or granules; as solutions or suspensions in aqueous or non-aqueous liquids; or as oil-in-water or water-in-oil emulsions.

Formulations suitable for sublingual or buccal administration include lozenges comprising a compound of the invention and typically a flavored base, such as sugar and acacia or tragacanth and pastilles comprising the active compound in an inert base, such as gelatin and glycerin or sucrose acacia.

Formulations suitable for parenteral administration typically comprise sterile aqueous solutions containing a predetermined concentration of the active compound; the solution may be isotonic with the blood of the intended recipient. Additional formulations suitable for parenteral administration include formulations containing physiologically suitable co-solvents and/or complexing agents such as surfactants and cyclodextrins. Oil-in-water emulsions are also suitable formulations for parenteral formulations. Although such solutions may be administered intravenously, they may also be administered by subcutaneous or intramuscular injection.

Formulations suitable for rectal administration may be provided as unit-dose suppositories comprising a compound of the invention in one or more solid carriers forming the suppository base, for example, cocoa butter.

Formulations suitable for topical or intranasal application include ointments, creams, lotions, pastes, gels, sprays, aerosols and oils. Suitable carriers for such formulations include petroleum jelly, lanolin, polyethylene glycols, alcohols, and combinations thereof.

Formulations of the invention may be prepared by any suitable method, typically by uniformly and intimately admixing a compound of the invention with liquids or finely divided solid carriers or both, in the required proportions and then, if necessary, shaping the resulting mixture into the desired shape.

For example, a tablet may be prepared by compressing an intimate mixture comprising a powder or granules of the active ingredient and one or more optional ingredients, such as a binder, lubricant, inert diluent, or surface active dispersing agent, or by molding an intimate mixture of powdered active ingredient and inert liquid diluent. Suitable formulations for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose pressurized aerosols, nebulizers, or insufflators.

For pulmonary administration via the mouth, the particle size of the powder or droplets is typically in the range about 0.5-10 μm or 1-5 μm , to ensure delivery into the bronchial tree. For nasal administration, a particle size in the range about 10-500 μm may be used to ensure retention in the nasal cavity.

5 Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of a compound of the invention in a liquefied propellant. During use, these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from about 10 to 150 μm , to produce a fine particle spray containing the active ingredient. Suitable propellants include certain chlorofluorocarbon
10 compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The formulation may additionally contain one or more co-solvents, for example, ethanol surfactants, such as oleic acid or sorbitan trioleate, anti-oxidants and suitable flavoring agents.

 Nebulizers are commercially available devices that transform solutions or suspensions
15 of the active ingredient into a therapeutic aerosol mist either by means of acceleration of a compressed gas typically air or oxygen, through a narrow venturi orifice, or by means of ultrasonic agitation. Suitable formulations for use in nebulizers consist of the active ingredient in a liquid carrier and comprise up to about 40% w/w of the formulation, and may comprise less than about 20% w/w. The carrier is typically water or a dilute aqueous
20 alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the formulation is not prepared sterile, for example, methyl hydroxy-benzoate, anti-oxidants, flavoring agents, volatile oils, buffering agents and surfactants.

 Suitable formulations for administration by insufflation include finely comminuted
25 powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in situ and the powder delivered by air drawn through the device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active
30 ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent,

such as lactose, and an optional surfactant. The active ingredient typically comprises from about 0.1 to 100% w/w of the formulation.

In a further embodiment, the present invention provides a pharmaceutical composition comprising, as active ingredient, a compound of the invention together, and/or in admixture, with at least one pharmaceutical carrier or diluent.

The carrier is pharmaceutically acceptable and must be compatible with, *i.e.* not have a deleterious effect upon, the other ingredients in the composition. The carrier may be a solid or liquid and is preferably formulated as a unit dose formulation, for example, a tablet which may contain from about 0.05 to 95% by weight of the active ingredient. If desired, other physiologically active ingredients may also be incorporated in the pharmaceutical compositions of the invention.

In addition to the ingredients specifically mentioned above, the formulations of the present invention may include other agents known to those skilled in the art of pharmacy, having regard for the type of formulation in issue. For example, formulations suitable for oral administration may include flavoring agents and formulations suitable for intranasal administration may include perfumes.

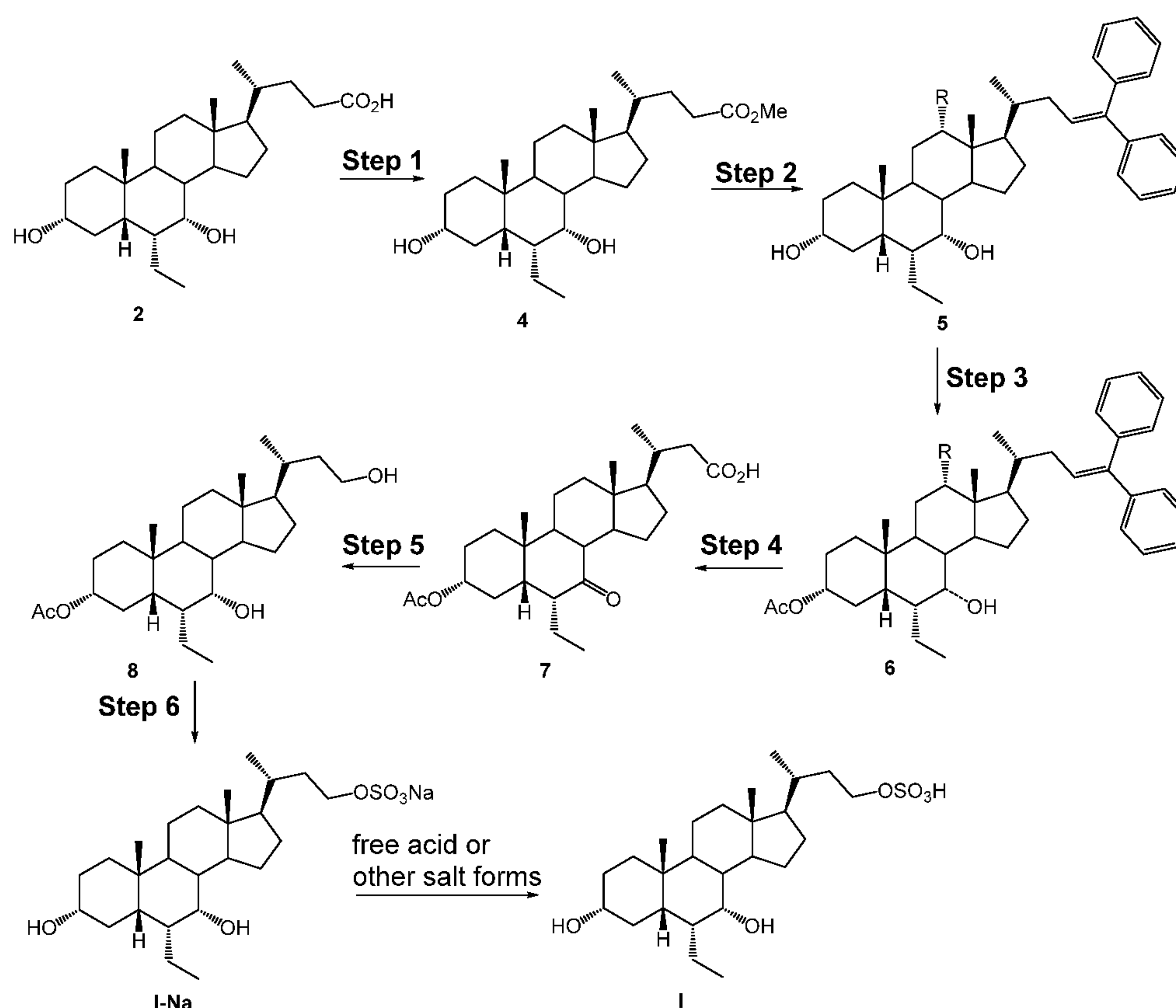
In one embodiment, a pharmaceutical composition is administered in a dosage form which comprises a compound of the invention in a daily total amount of from about 0.1-1500 mg, 0.2-1200 mg, 0.3-1000 mg, 0.4-800 mg, 0.5-600 mg, 0.6-500 mg, 0.7-400 mg, 0.8-300 mg, 1-200 mg, 1-100 mg, 1-50 mg, 1-30 mg, 4-26 mg, 5-25 mg, or 5-10mg.

A compound of the invention can be used in combination with other hepatocellular carcinoma treating drugs, such as anticancer chemotherapeutic drugs, hormones, biological response modifier(s), and other angiogenesis inhibitors; or in combination with immunotherapy or gene therapy.

Example 1. Synthesis of Compound 1

Compound 1 can be prepared by methods known in the art (*e.g.*, those described in U.S. Patent No. 7,932,244). For example, Compound 1 can be prepared by a process as shown in Scheme 1 and disclosed in WO 2014/066819.

Scheme 1

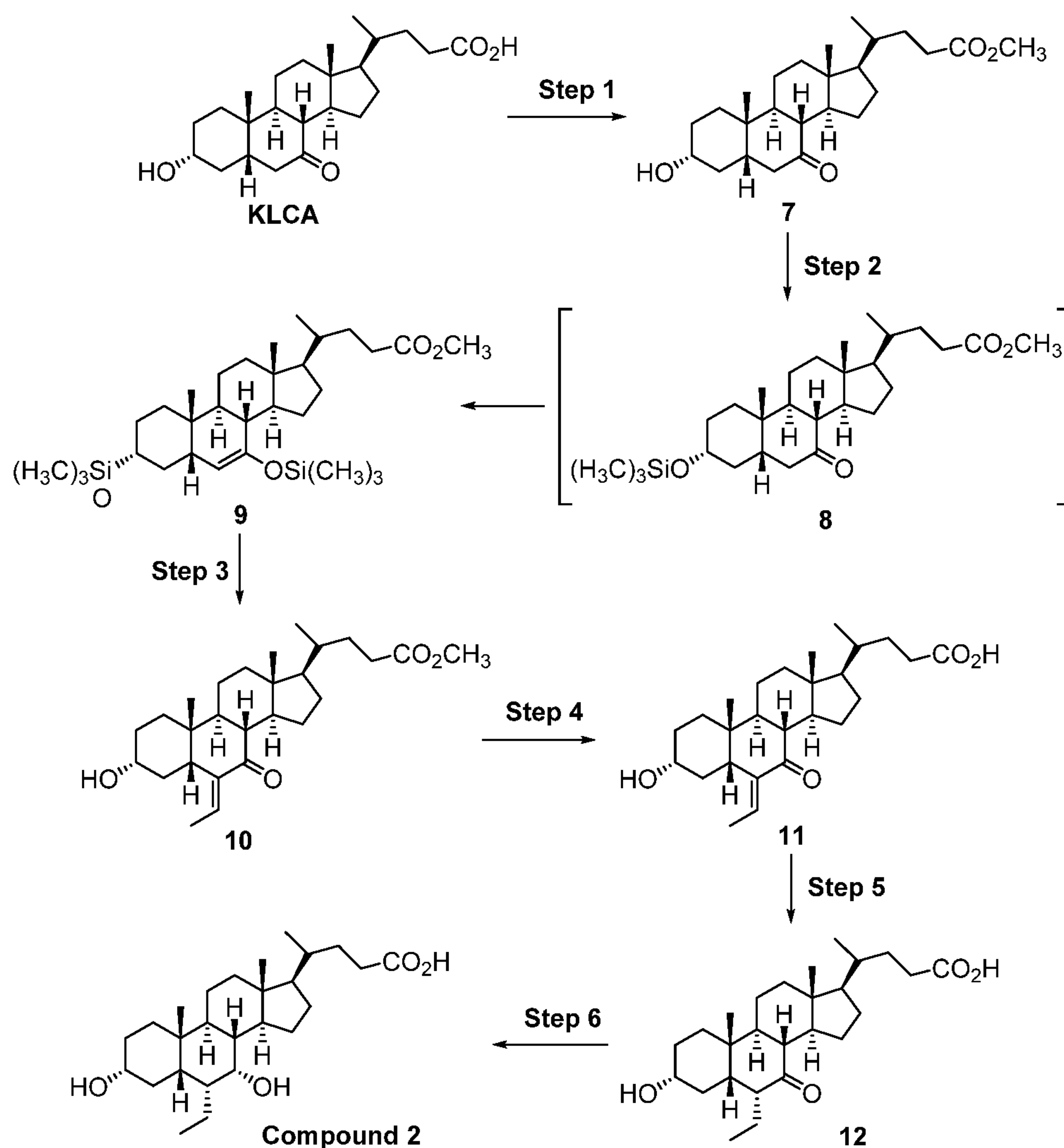


Step 1 is the esterification of Compound 2 to obtain Compound 4. Step 2 is a reaction to form Compound 5 from Compound 4. Step 3 is the protection of the hydroxy group at the C3 position of Compound 5 to afford Compound 6. Step 4 is the oxidative cleavage of Compound 6 to afford Compound 7. Step 5 is the reduction of Compound 7 to afford Compound 8. Step 6 is the sulfonation of Compound 8 to afford the sodium salt of Compound 1 (1-Na). The sodium salt of Compound 1 can be converted to its free acid form (*i.e.*, Compound 1) or other salt forms (*e.g.*, Compound 1-DEA or the N,N-diethaneamine salt of Compound 1) according to procedures known in the art.

Example 2. Synthesis of Compound 2

Compound 2 can be prepared by the conventional methods (*e.g.*, those described in U.S. Publication No. 2009/0062526, U.S. Patent No. 7,138,390, and WO 2006/122977), such as by a 6-step synthesis followed produce Compound 1 (obeticholic acid, or OCA) as shown in Scheme 2 below.

Scheme 2



The process above is a 6-step synthesis. Step 1 is the esterification of the C-24 carboxylic acid of 7-keto lithocholic acid (KLCA) using methanol in the presence of acidic catalyst and heat to produce the methyl ester Compound 7. Step 2 is silylenol ether formation from Compound a using a strong base followed by treatment with chlorosilane to produce Compound 8. Step 3 is an aldol condensation reaction of the silylenol ether of compound 8 and acetaldehyde to produce Compound 10. Step 4 is saponification of the C-24 methyl ester of Compound 10 to produce Compound 11. Step 5 is the hydrogenation of the 6-ethylidene moiety of Compound 11 to produce compound 12. Step 6 is the selective reduction of the 7-keto group of Compound 12 to a 7 α -hydroxy group to produce Compound 1.

Example 3. Hepatocarcinogenesis in Mdr2^{-/-} and FXR^{-/-} mice

The multidrug resistance protein 2 (Abcb4) is a member of the superfamily of ATP-binding cassette (ABC) transporters. The multidrug resistance protein 2 gene knockout mice (Mdr2^{-/-}) provides an in-vivo model of spontaneous hepatocarcinogenesis (Katzenellengoben, et al. Mol. Cancer Res. 2007, 5, 11, 1159-1170). Mice lacking the Abc4 protein encoded by the multidrug resistance-2 gene develop chronic periductular inflammation and cholestatic liver disease resulting in the development of hepatocellular carcinoma.

The farnesoid X receptor protein (FXR) is a nuclear receptor that functions as a bile acid sensor controlling bile acid homeostasis. This receptor is highly expressed in the liver and other organs. FXR knockout (FXR^{-/-}) mice develop hepatocellular adenoma and carcinoma after 15 months of age (Yang, et al. Cancer Res., 2007, 67, 863).

The effects of OCA, Compound 1-Na, and control diet in Mdr2^{-/-} and FXR^{-/-} mice on the development of hepatocellular carcinoma were evaluated. OCA is a FXR agonist while Compound 1-Na is a dual FXR/TGR5 agonist. The potency of Compound 1-Na for FXR is about 10 fold greater than OCA.

Study Design

Mdr2^{-/-} and FXR^{-/-} mice were randomly divided into three experimental groups. The mice were fed a specific rodent diet or a diet supplemented with OCA, Compound 1-Na, or control diet for 15 months. All mice were housed under pathogen-free conditions in a temperature-controlled room (23°C) on a 12-hour light/dark cycle and drank water ad libitum. The Ethical Committee of the University of Bari approved this experimental set-up, which also was certified by the Italian Ministry of Health in accordance with internationally accepted guidelines for animal care. After 16 months, the mice were sacrificed and serum, liver and intestine were collected. The total number of hepatic tumors was counted and the diameter of each tumor was measured.

Treatment Groups

Group 1: Control Diet

Mice (n=4) were fed control diet for 15 months.

Group 2: OCA

Mice (n=8) were fed control diet supplemented with OCA at a dose of 10 mg/kg for 15 months.

Group 3: Compound 1-Na

Mice (n=15) were fed control diet supplemented with Compound 1-Na at a dose of 10 mg/kg for 15 months.

Results

Liver inflammation and toxicity induced by bile salts in Mdr2^{-/-} mice lead to the development of hepatocyte dysplasia. By 16 months of age, nearly 100% of the Mdr2^{-/-} control mice developed liver tumors. FXR^{-/-} mice aged 16 months developed spontaneous hepatocellular carcinoma.

Tumor reduction and size

Figures 1A and 2A show the effect of Compound 1-Na, OCA, and control on the reduction of the number of tumors in Mdr2^{-/-} mice. Compound 1-Na clearly prevented hepatocellular carcinoma development in this study compared to control. Statistical significance was nearly observed (p=0.055) for the Compound 1-Na group but not achieved due to the low number of control animals (n=2) used in the study. Mdr2^{-/-} control mice and OCA treated mice displayed clearly identifiable liver tumors while minute tumors were found in Compound 1-Na group. Figures 1B and 2B show the effects of Compound 1-Na, OCA, and control on percent reduction of tumors having a diameter of >5mm. Nearly 80% of the tumors found in the OCA and control groups had a diameter larger than 5 mm in Mdr2^{-/-} mice. In contrast, FXR^{-/-} mice treated with Compound 1-Na, OCA, and control exhibited several large liver tumors indicating that the prevention of hepatocellular carcinoma development is mostly FXR dependent (Figures 3A, 3B and 4).

Liver weight/Body weight (LW/BW)

Figures 5A and 5B describe the effect of Compound 1-Na and control on the percent ratio of liver and body weight. Consistent with previously generated data, the Compound 1-Na treated Mdr2^{-/-} mice exhibited a significant reduction in LW/BW ratio compared to control and OCA treated Mdr2^{-/-} mice. No difference in LW/BW ratio was observed in the FXR^{-/-} groups.

Biochemical Parameters

In order to evaluate liver damage in Mdr2^{-/-} and FXR^{-/-} mice, the effect of Compound 1-Na and control on the levels of liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), was analyzed. As indicated in Figures 6A and 6B, treatment with

Compound 1-Na significantly reduced ALT and AST levels in Mdr2^{-/-} mice. However, no difference in ALT and AST levels were observed in FXR^{-/-} treated mice.

Ileal FXR target gene expression

To demonstrate the involvement of FXR in the prevention of hepatocellular carcinoma, the effect of Compound 1-Na and control on ileal FXR target gene expression was evaluated. As expected, both OCA and Compound 1-Na stimulated fibroblast growth factor 15 (Fgf15) and small heterodimer partner (Shp) gene expression in Mdr2^{-/-} groups only (Figures 7A and 7B).

Hepatic FXR target gene expression

Cholesterol 7 alpha-hydroxylase (cyp7a1) is the rate limiting enzyme in the classical biosynthetic pathways which convert cholesterol into bile acids. Both Compound 1-Na and OCA inhibited Cyp7a1 gene expression in Mdr2^{-/-} mice only (Figure 8). The bile salt export pump (Bsep) is a membrane protein that uses energy of ATP hydrolysis to actively transport bile acid salts. As indicated in Figures 9A and 9B, Compound 1-Na administration induced hepatic Bsep activation in Mdr2^{-/-} mice. OCA did not promote hepatic Bsep induction suggesting that in contrast to Compound 1-Na, which efficiently activates FXR in intestine and liver, OCA is less likely to have hepatic activity in Mdr2^{-/-} mice.

Serum total bile acids

Compound 1-Na significantly reduced serum bile acid levels in Mdr2^{-/-} mice (Figure 10A).

The FXR dependence of this finding was confirmed by the observation that no reduction occurred in FXR^{-/-} mice (Figure 10B).

Example 4. Differential effects of FXR or TGR5 activation in cholangiocarcinoma progression

Methods

FXR and TGR5 expression was determined in CCA human biopsies and controls by using 2 approaches (mRNA microarray and qPCR) and 2 different cohorts of patients (Copenhagen and San Sebastian), as well as in different human CCA cell lines compared to normal human cholangiocytes (NHC) (by qPCR). The growth of an orthotopic model of human CCA in immunodeficient mice was evaluated by magnetic resonance imaging (MRI) under chronic administration of the specific FXR or TGR5 agonists (OCA or Compound 4, respectively; 0,03% in chow for 2 months; Intercept Pharmaceuticals). The differential effects

of FXR or TGR5 activation was also evaluated on the proliferation, migration and mitochondrial energetic metabolism of CCA and NHC cells *in vitro*.

Results

FXR is downregulated and TGR5 upregulated in human CCA tissue samples compared to normal surrounding liver tissue and normal intrahepatic bile ducts in both Copenhagen and San Sebastian cohorts by mRNA microarray and qPCR, respectively. Activation of FXR inhibits, whereas TGR5 may promote, CCA progression through the regulation of proliferation, migration and mitochondrial energetic metabolism. Regulation of FXR and TGR5 activities represent a potential therapeutic strategy for CCA. In vitro, FXR was also found downregulated and TGR5 upregulated in different human CCA cell lines compared to NHC.

As shown in Figures 11A-11D, FXR expression is decreased in CCA tumors and correlates with tumor differentiation: Figure 11A represents FXR mRNA microarray expression in whole tissue of CCA tumors (n=104) compared to surrounding human tissue (n=60) (Copenhagen cohort) (Mann-Whitney test); Figure 11B represents FXR mRNA microarray expression in whole tissue of CCA tumors grouped upon tumor differentiation grade: well- (n=10), moderately- (n=34) or poorly- (n=9) differentiated (Copenhagen cohort) (Mann-Whitney test); Figure 11C represents FXR mRNA expression (qPCR) in CCA tumors (n=5) compared to normal human liver tissue (n=20) and surrounding human liver tissue (n=7) (San Sebastian cohort) (Mann-Whitney test); and Figure 11D represents FXR mRNA expression (qPCR) in CCA tumors compared to matched-surrounding human liver tissue (n=4) (San Sebastian cohort) (Paired T-test).

As shown in Figures 12A-12D, TGR5 expression is increased in CCA tumors, an is higher in perihilar than in intrahepatic CCAs, and correlates with perineural invasion (Figure 12A). Figure 12B represents TGR5 mRNA microarray expression in whole tissue of CCA tumors (n=104) compared to surrounding human tissue (n=60) (Copenhagen cohort) (Mann-Whitney test). Figure 12C shows TGR5 mRNA microarray expression in whole tissue of CCA tumors upon clinico-pathological parameters: anatomical location [perihilar (n=36) or intrahepatic (n=68)] and perineural invasion [negative (n=50) or positive (n=42)] (Copenhagen cohort) (Mann-Whitney test). Figure 12C represents TGR5 mRNA expression (qPCR) in CCA tumors (n=15) compared to surrounding human liver tissue (n=27) (San

Sebastian cohort) (Mann-Whitney test). TGR5 mRNA expression (qPCR) in CCA tumors compared to matched-surrounding human liver tissue (n=11) (San Sebastian cohort) (Wilcoxon matched-paires signed rank test) is shown in Figure 12D.

As shown in Figures 13A and 13B, FXR expression is decreased and TGR5 expression is increased in CCA cell lines compared to normal human cholangiocytes: Figure 13A shows FXR mRNA expression (qPCR) in normal human cholangiocytes (n=6) and CCA cell lines (n=5 and 6, respectively) (Mann-Whitney test or unpaired T-test) and Figure 13B shows TGR5 mRNA expression (qPCR) in normal human cholangiocytes (n=6) and CCA cell lines (n=5 and 4, respectively) (Unpaired T-test or Mann-Whitney test).

Chronic administration of the orthotopic CCA mouse model with the FXR agonist OCA inhibited the tumor growth compared to untreated animals, and this effect was associated with decreased PCNA and Ki67 expression within the tumors of treated animals. As shown in Figures 14A-14D, the FXR agonist obeticholic acid (OCA) inhibited tumor growth in vivo and this was associated with decreased expression of proliferation, biliary and epithelial markers. EGI1 cells were injected subcutaneously in CD1 nude male mice. Once tumor growth, tumors were orthotopically implanted in the liver of CD1 nude male mice. After two weeks, MRI analysis was performed and treatment administration was started in diet. Tumor growth was monitored at 1 and 2 months by MRI. Figure 14A shows representative MRI and liver images of untreated control mice, OCA-treated mice and Compound 4-treated mice. Bar-graph of Figure 14B shows tumor volume fold-change quantified by MRI (Control n=8, OCA n=6 and Compound 4 n=9 (Mann-Whitney test, one-tailed). Figure 14C represents mRNA expression levels of proliferation (i.e. Ki67 and PCNA), biliary (i.e. CK19) and epithelial (i.e. ZO-1) markers in liver orthotopic CCA tumors. n=5-8 (Mann-Whitney or Unpaired T-test, one-tailed). Representative immunohistochemistry images of proliferation markers (i.e. Ki67 and PCNA) in liver orthotopic CCA tumors of mice untreated or treated with OCA or Compound 4 are shown in Figure 14D. In contrast, no effects on CCA tumor growth were observed in animals treated chronically with the TGR5 agonist Compound 4.

Figure 17 shows effects of OCA and Compound 4 in liver xenograph tumoral expression of proliferation markers. mRNA expression levels of proliferation (i.e. Cdc25a, cyclin D1 and cyclin D3) markers in liver orthotopic CCA tumors. n=5-8 (Mann-Whitney or Unpaired T-test, one-tailed).

In vitro, the activation of FXR with OCA inhibited the proliferation and migration of CCA cells and these events were associated with decreased mitochondrial energetic metabolism (i.e. decreased baseline respiration, maximal respiration and ATP-linked respiration) compared to controls. On the other hand, FXR activation did not affect the survival of CCA cells. As shown in Figures 15A-15D, the FXR agonist OCA inhibits CCA cell proliferation in a dose-dependent manner and inhibits CCA cell migration, associated with decreased mitochondrial metabolism in CCA cells, without inducing apoptosis. Figure 15A shows proliferation of CCA cells (i.e. EGI1) treated with OCA (n=5) at 10 or 25 μ M for 48 h compared to non-treated control cells (n=4), performed by flow cytometry using CFSE cell proliferation dye staining (Unpaired T-test). The assay was performed at least in three independent experiments. mRNA expression of proliferation markers (i.e. Ki67, PCNA, cyclin D1 and cyclin D3) in CCA cells (i.e. EGI1) under OCA (25 μ M) treatment for 3-6-12 h compared to non-treated control cells. n=5-6, unpaired T-test to untreated control. Figure 15B shows the results of migration assays in CCA cells (i.e. EGI1). Representative microscope images of wound-healing assay at the indicated time-points and conditions (i.e. non-treated control or OCA-treated) and corresponding quantification (n=6 in each condition) (Unpaired T-test). The assay was performed in two independent experiments. Representative microscope images of transwell migration chambers at 24 h in non-treated and OCA-treated cells. The assay was performed once. Figure 15C shows Seahorse Oxygen Consumption Rate (OCR) using mitochondrial stress test kit in CCA cells (i.e. EGI1) non-treated or treated with OCA (25 μ M), with 3 h pre-treatment. Bar-graph of metabolic parameters calculated upon OCR measurements. n=11-12 for each group (unpaired T-test). The assay was performed at least in three independent experiments. Figure 15D represents flow cytometry-based apoptosis assays by Annexin V and Propidium Iodide staining in CCA cells (i.e. EGI1) non-treated or treated with 10 or 25 μ M of OCA. Representative histograms and corresponding quantification of pooled data from two independent experiments, total n=6-7 (Unpaired T-test). The assay was performed in three independent experiments.

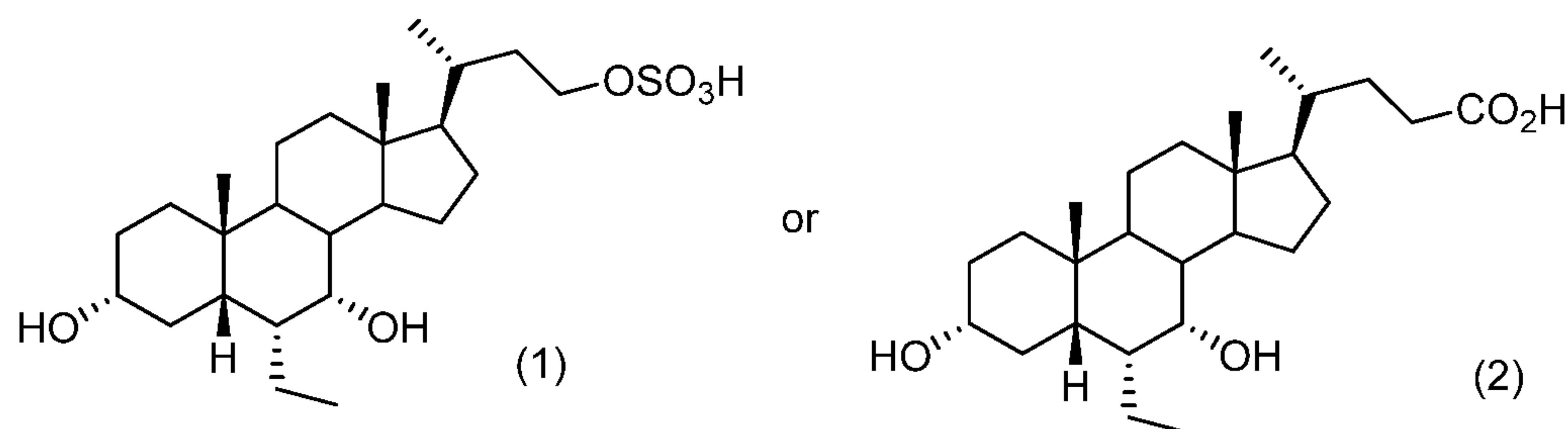
In contrast, activation of TGR5 with Compound 4 did stimulate the proliferation and migration of CCA cells and these events were associated with increased mitochondrial energetic metabolism (i.e. increased baseline respiration, proton-leak and ATP-linked respiration) compared to controls. As shown in Figures 16A-16C, the TGR5 agonist

Compound 4 slightly stimulates the proliferation, migration and mitochondrial metabolism of CCA cells. Figure 16A shows proliferation of CCA cells (i.e. EGI1) treated with Compound 4 at 10 or 25 μ M for 48 h compared to non-treated control cells, analyzed by flow cytometry using CFSE cell proliferation dye staining. n=5-6 (unpaired T-test). The assay was performed in three independent experiments. mRNA expression of proliferation markers (i.e. Ki67, PCNA, cyclin D1 and cyclin D3) in CCA cells (i.e. EGI1) under Compound 4 (25 μ M) treatment for 3-6-12 h compared to non-treated control cells. n=6 (unpaired T-test or Mann-Whitney test to untreated control). Figure 16B represents migration assays in CCA cells (i.e. EGI1). Representative microscope images of wound-healing assay at the indicated time-points and conditions (i.e. non-treated control or Compound 4-treated) and corresponding quantification. Pooled data from two independent experiments (total n=9 and 6) (Unpaired T-test). The assay was performed at least in three independent experiments. Representative microscope images of transwell migration chambers at 24 h in non-treated and Compound 4-treated cells and corresponding quantification (n=4 and 2). The assay was performed in three independent experiments. Figure 16C shows Seahorse Oxygen Consumption Rate (OCR) using mitochondrial stress test kit in CCA cells (i.e. EGI1) non-treated or treated with Compound 4 (25 μ M), with 3 h pre-treatment. Bar-graph of metabolic parameters calculated upon OCR measurements. n=11-12 for each group (unpaired T-test). The assay was performed at least in three independent experiments.

CLAIMS

Claim 1. A method of treating or preventing cancer in a subject in need thereof comprising administering a therapeutically effective amount of a FXR agonist selected from

5 Compound 1 or 2:



or a pharmaceutically acceptable salt or amino acid conjugate thereof.

Claim 2. The method of claim 1, wherein the cancer is selected from hepatocellular carcinoma, cholangiocarcinoma, pancreatic cancer, kidney cancer, prostate cancer, esophageal cancer, breast cancer, gastric cancer, renal cancer, salivary gland cancer, ovarian cancer, uterine body cancer, bladder cancer, and lung cancer.

Claim 3. The method of claim 1 or 2, wherein the cancer is hepatocellular carcinoma.

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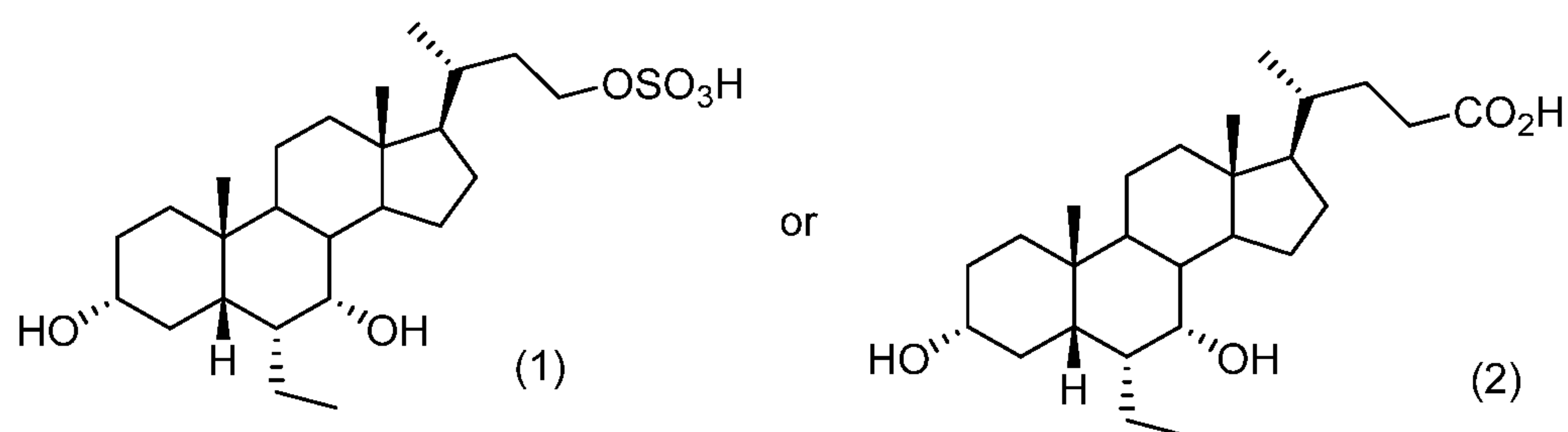
Claim 4. The method of claim 3, wherein the hepatocellular carcinoma is selected from the group consisting of early stage hepatocellular carcinoma, non-metastatic hepatocellular carcinoma, primary hepatocellular carcinoma, advanced hepatocellular carcinoma, locally advanced hepatocellular carcinoma, metastatic hepatocellular carcinoma, hepatocellular carcinoma in remission, or recurrent hepatocellular carcinoma.

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Claim 5. The method of any one of claims 1-4, wherein the FXR agonist is Compound 1 or a pharmaceutically acceptable salt thereof.

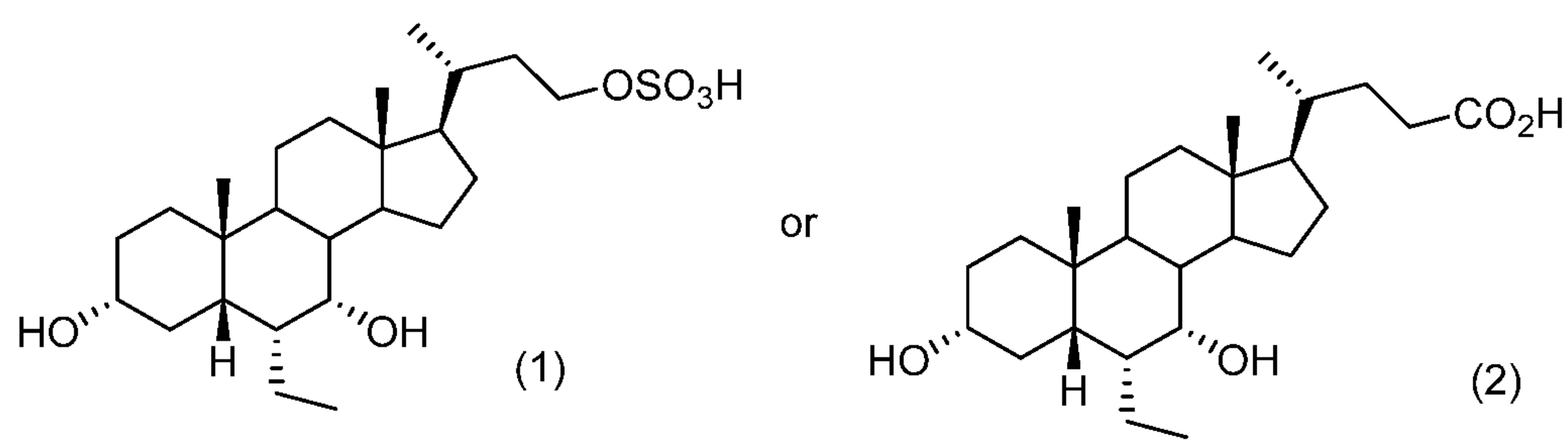
Claim 6. The method of claim 5, wherein the FXR agonist is the sodium salt of Compound 1.

25



or a pharmaceutically acceptable salt or amino acid conjugate thereof.

Claim 14. Use of a FXR agonist selected from Compound 1 or 2:



or a pharmaceutically acceptable salt or amino acid conjugate thereof in the manufacture of a medicament for treating or preventing cancer in a subject in need thereof.

Claim 15. The method of claim 1 or 2, wherein the cancer is cholangiocarcinoma.

Claim 16. The method of claim 15, wherein the FXR agonist is Compound 1 or a pharmaceutically acceptable salt thereof.

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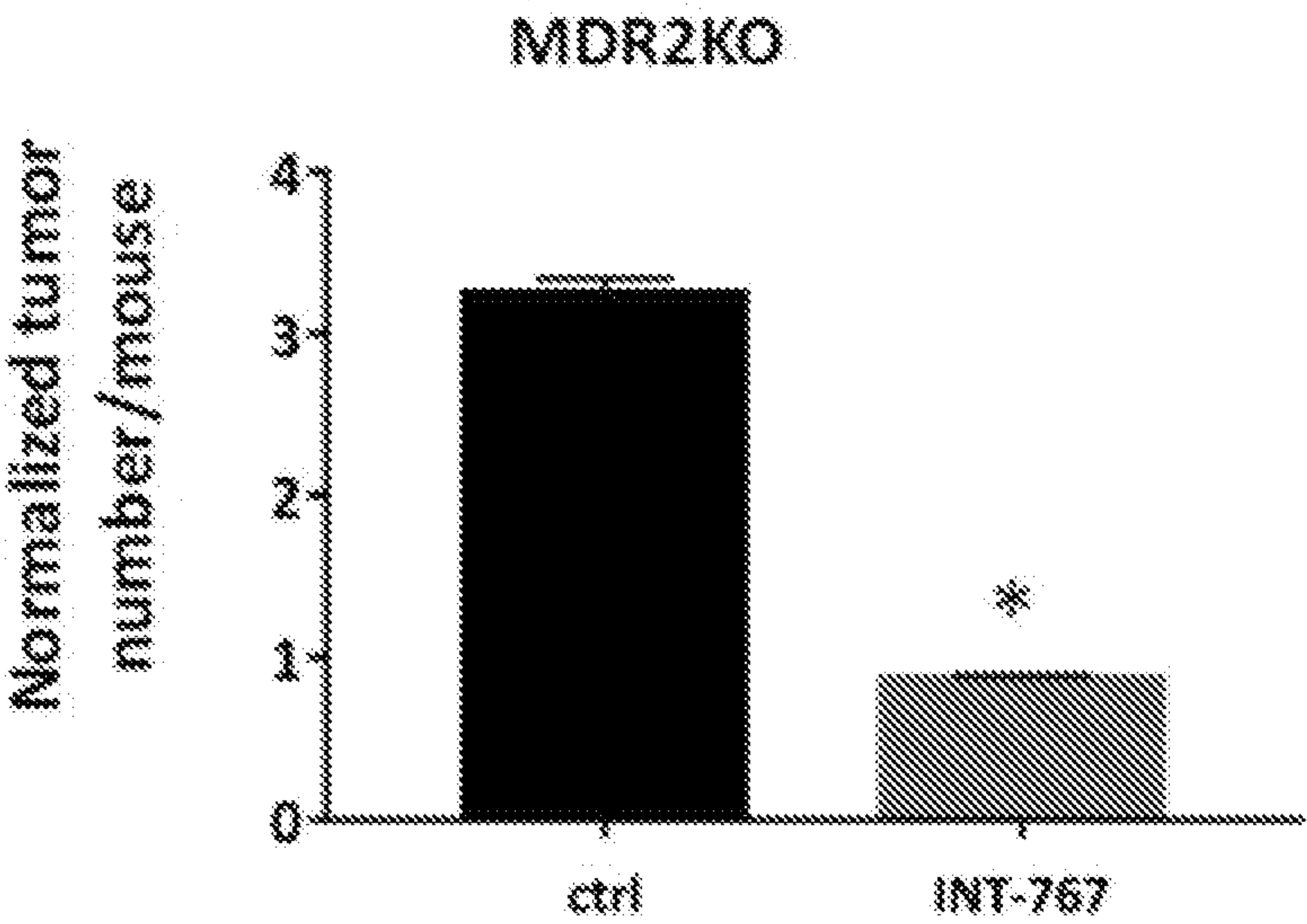


Figure 1A

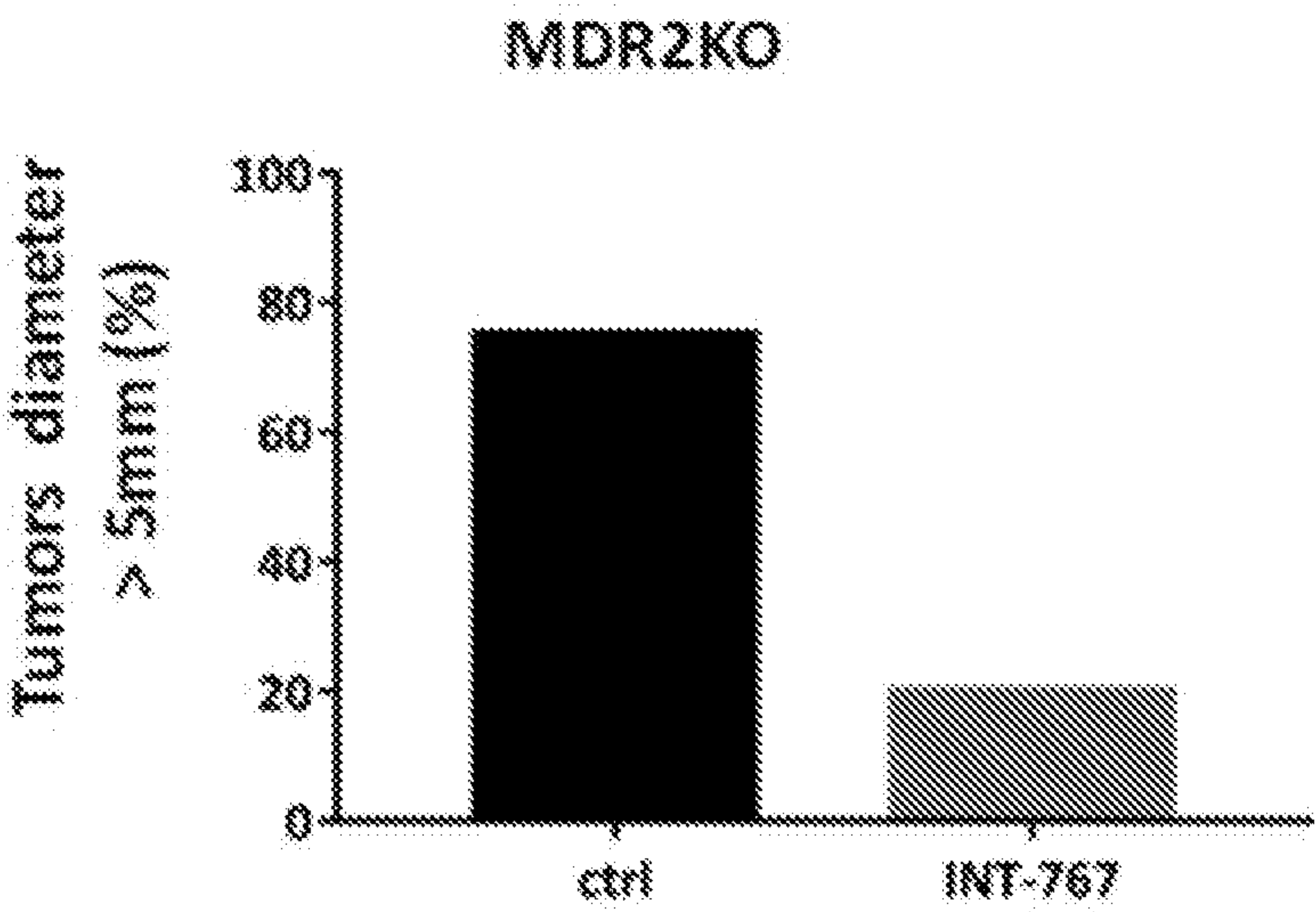


Figure 1B

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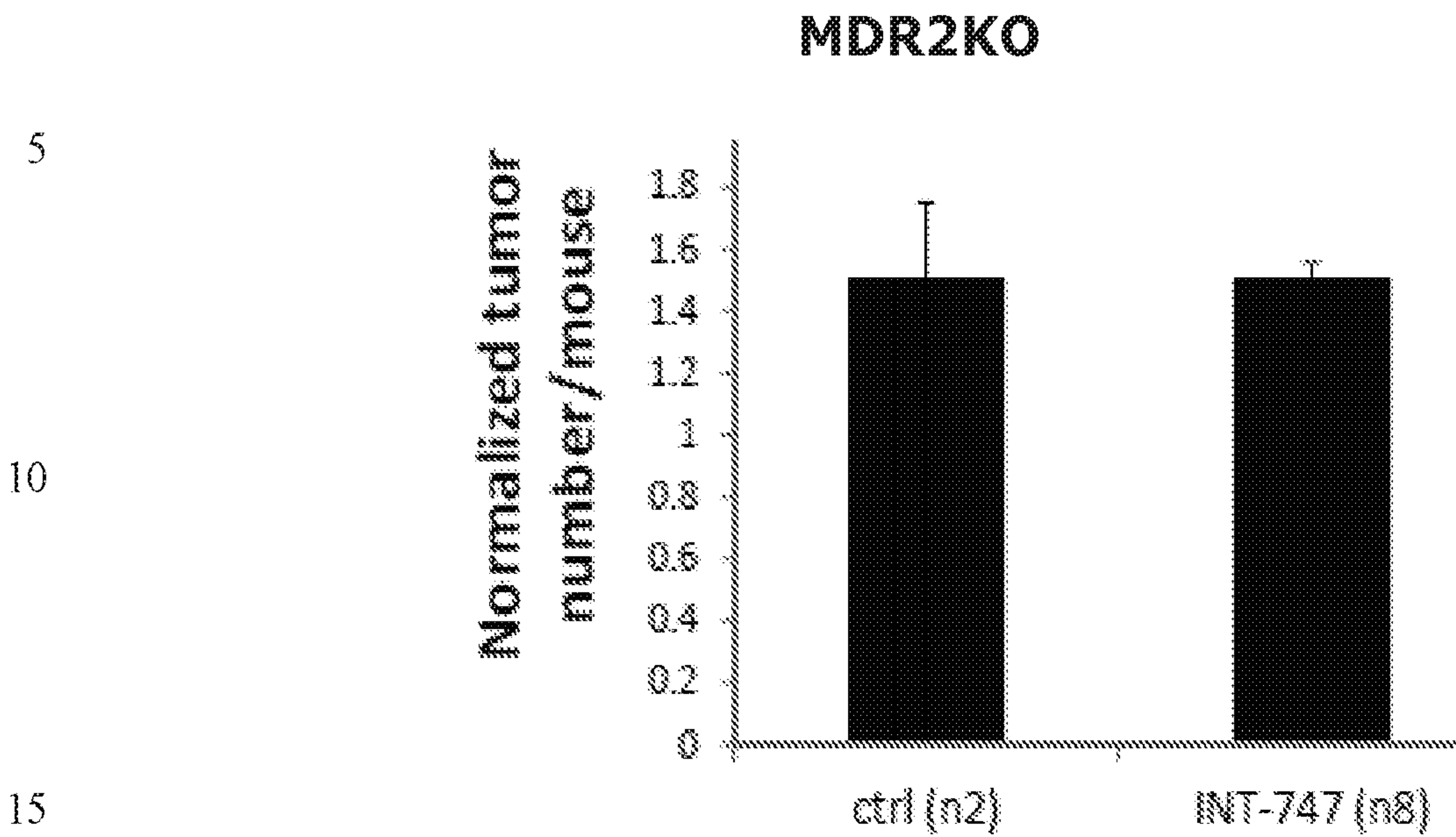


Figure 2A

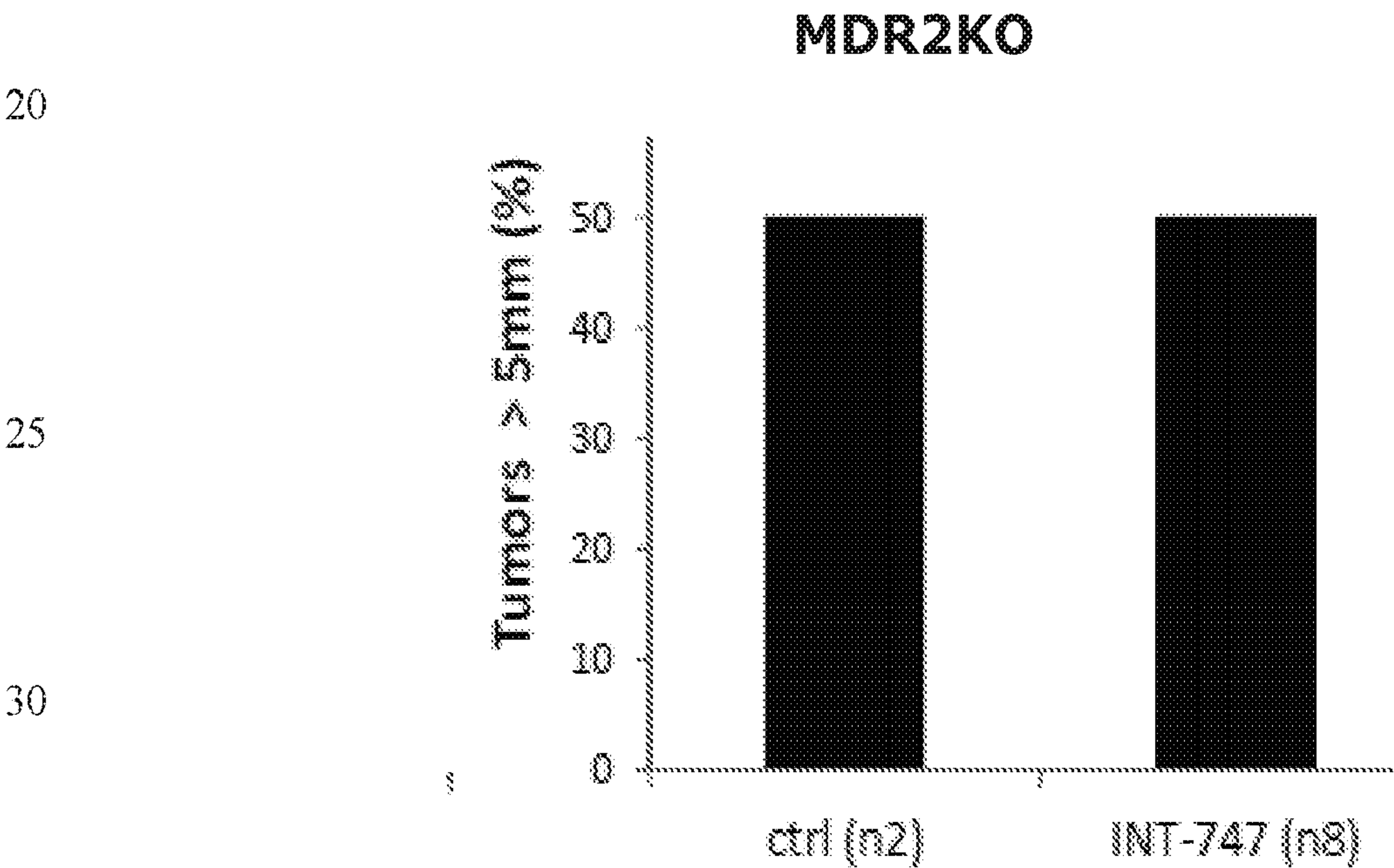


Figure 2B

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FxrKO

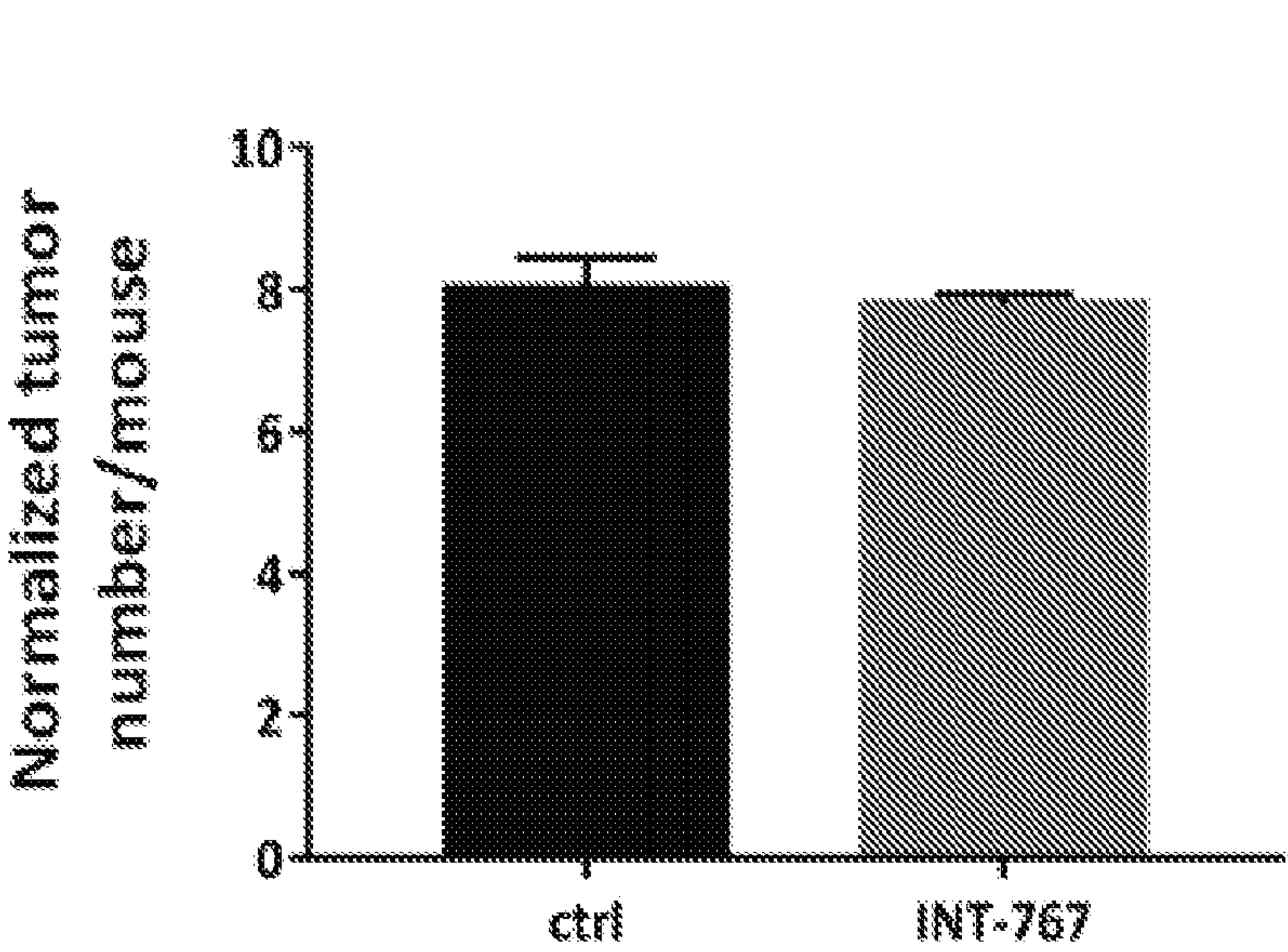


Figure 3A

FxrKO

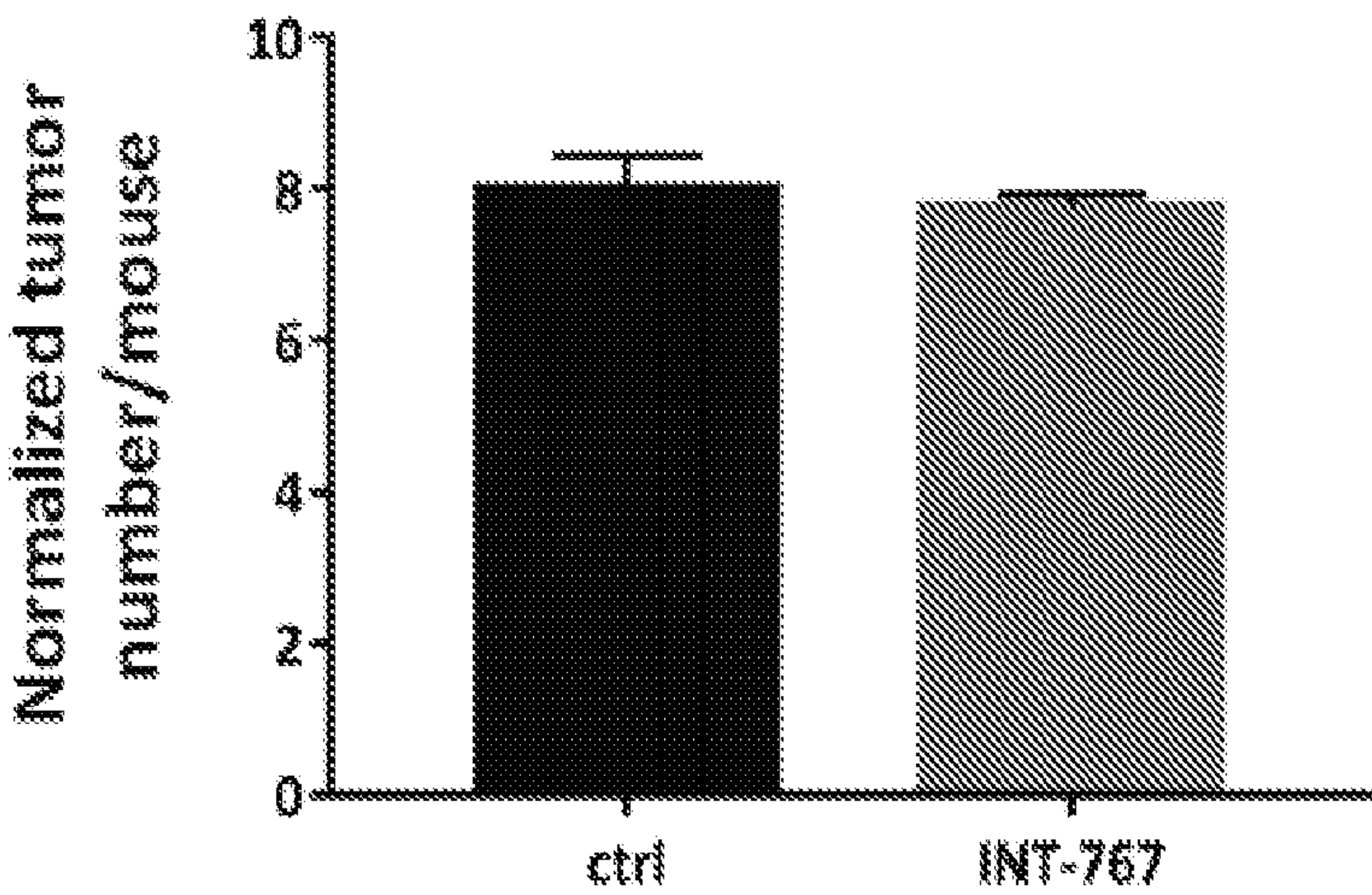


Figure 3B

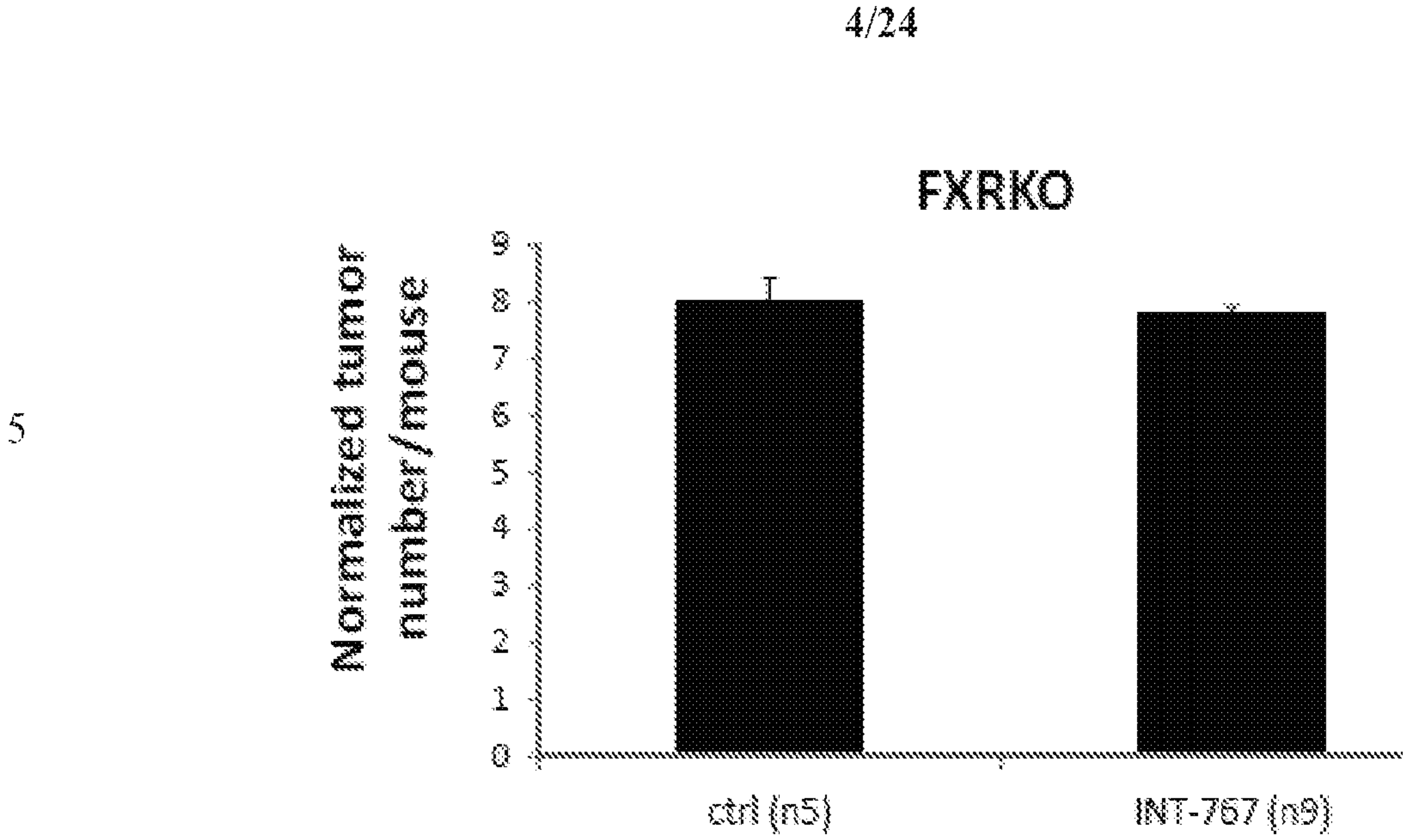


Figure 4

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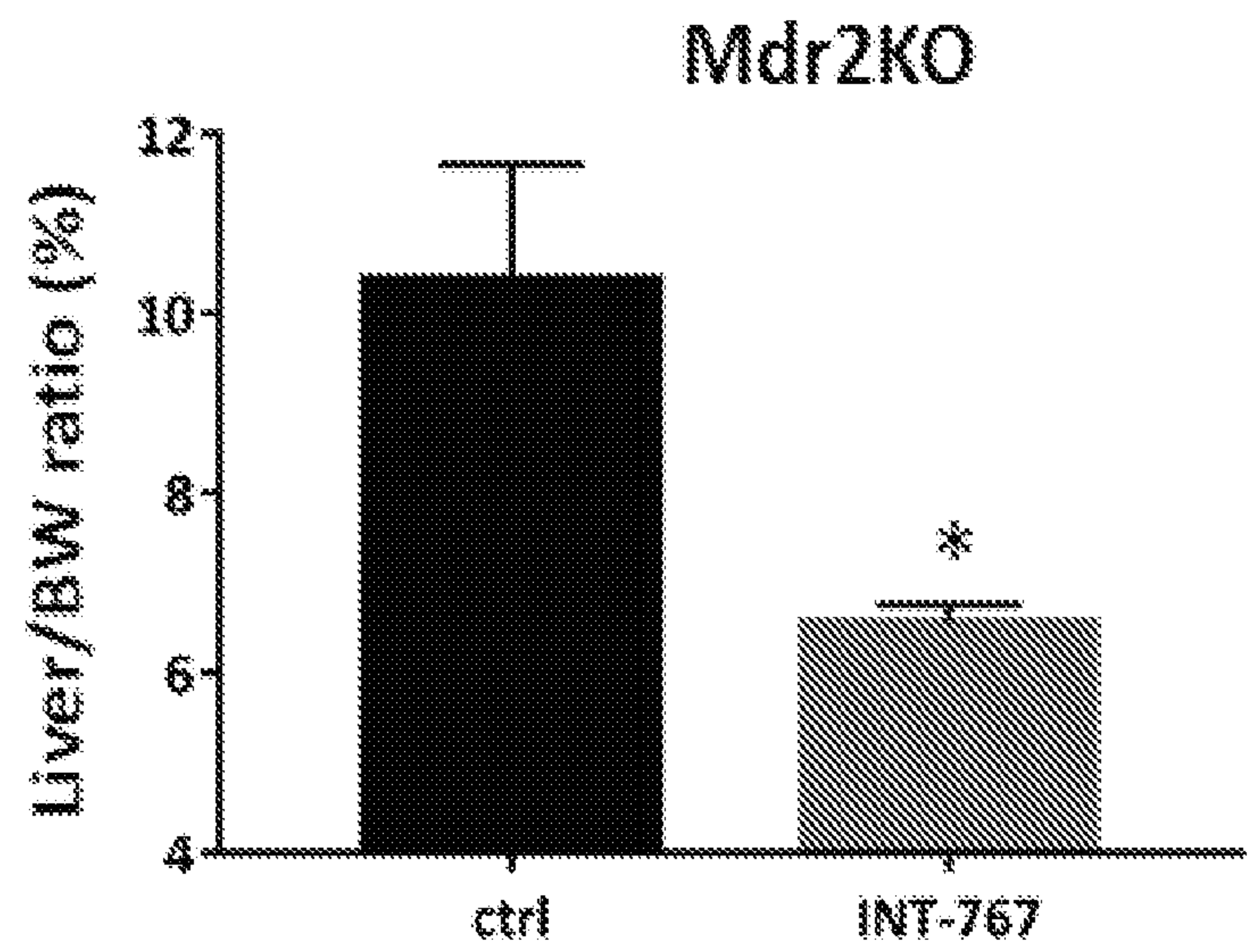


Figure 5A

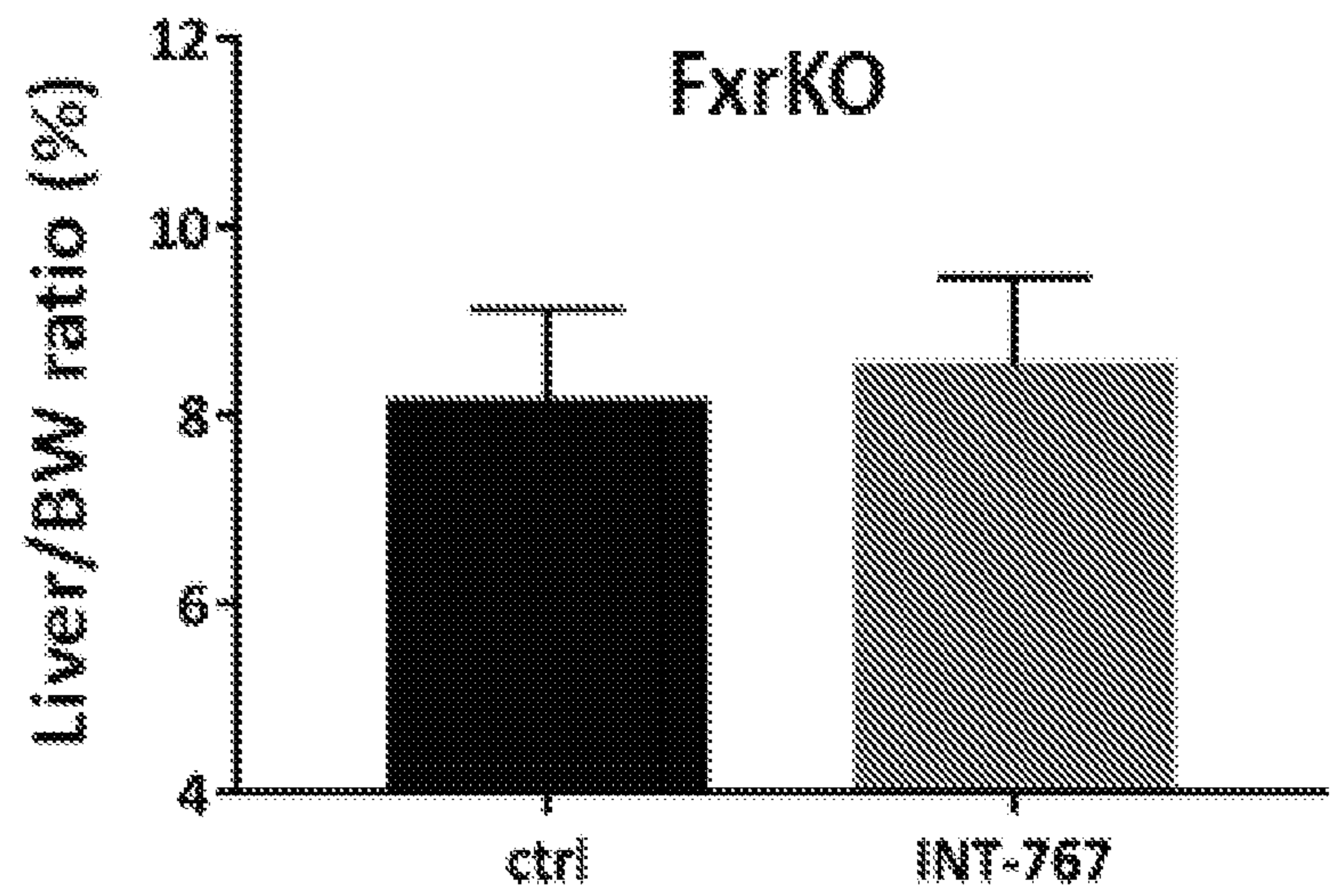


Figure 5B

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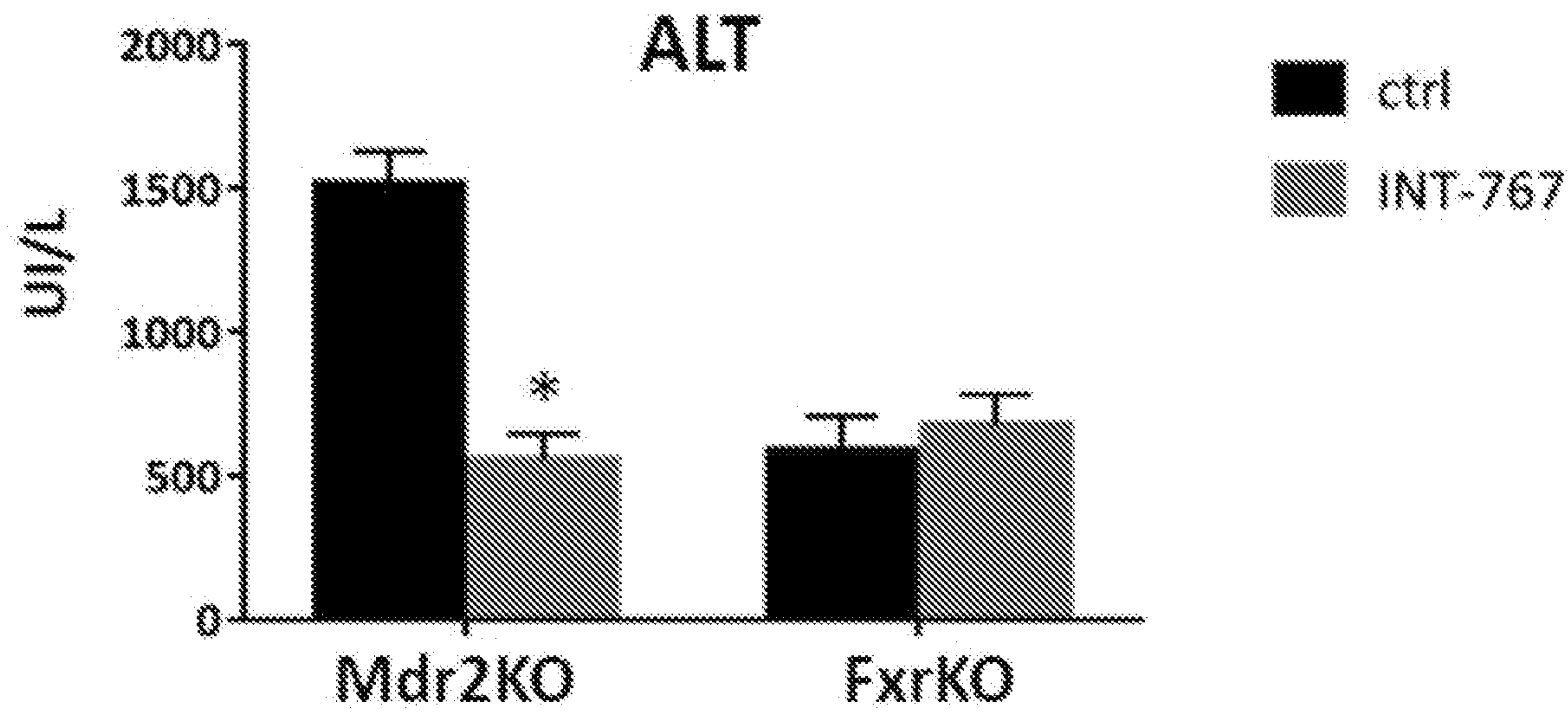


Figure 6A

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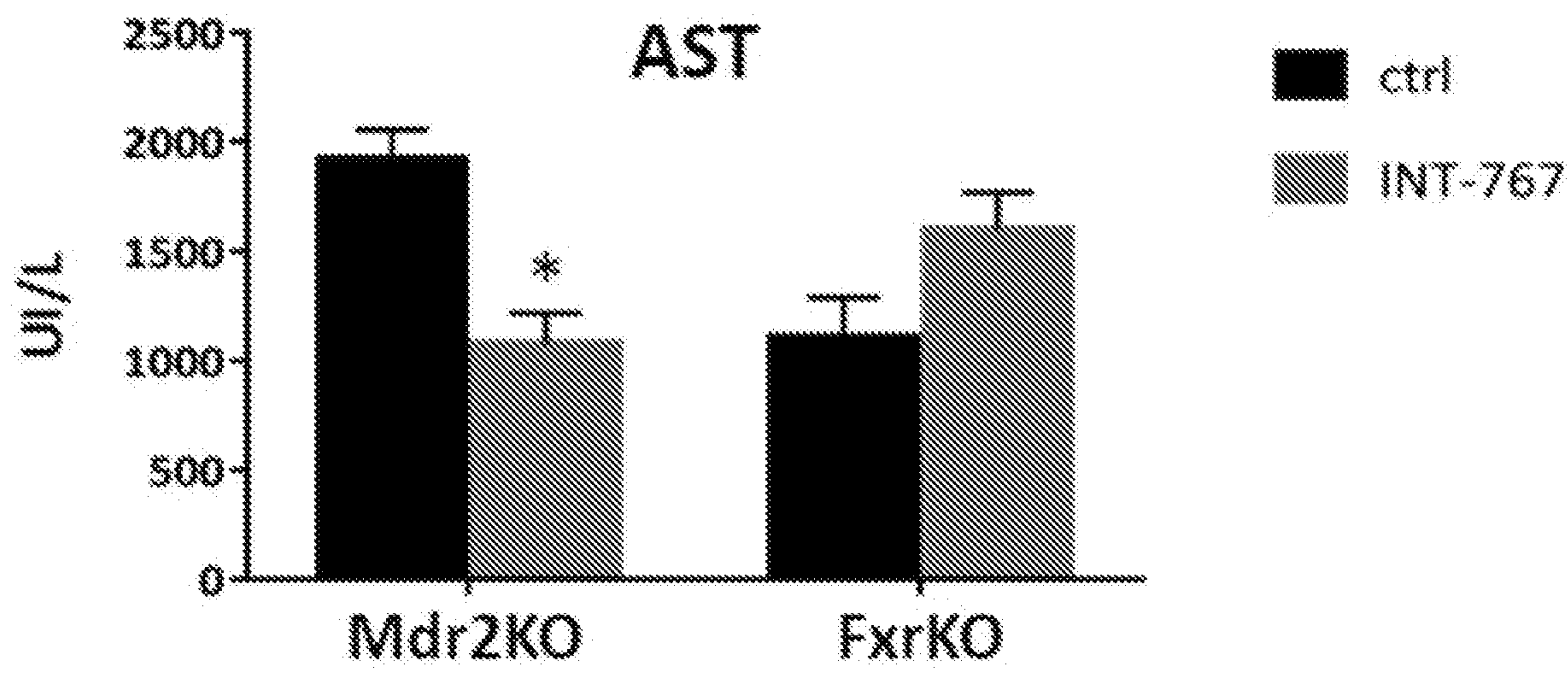


Figure 6B

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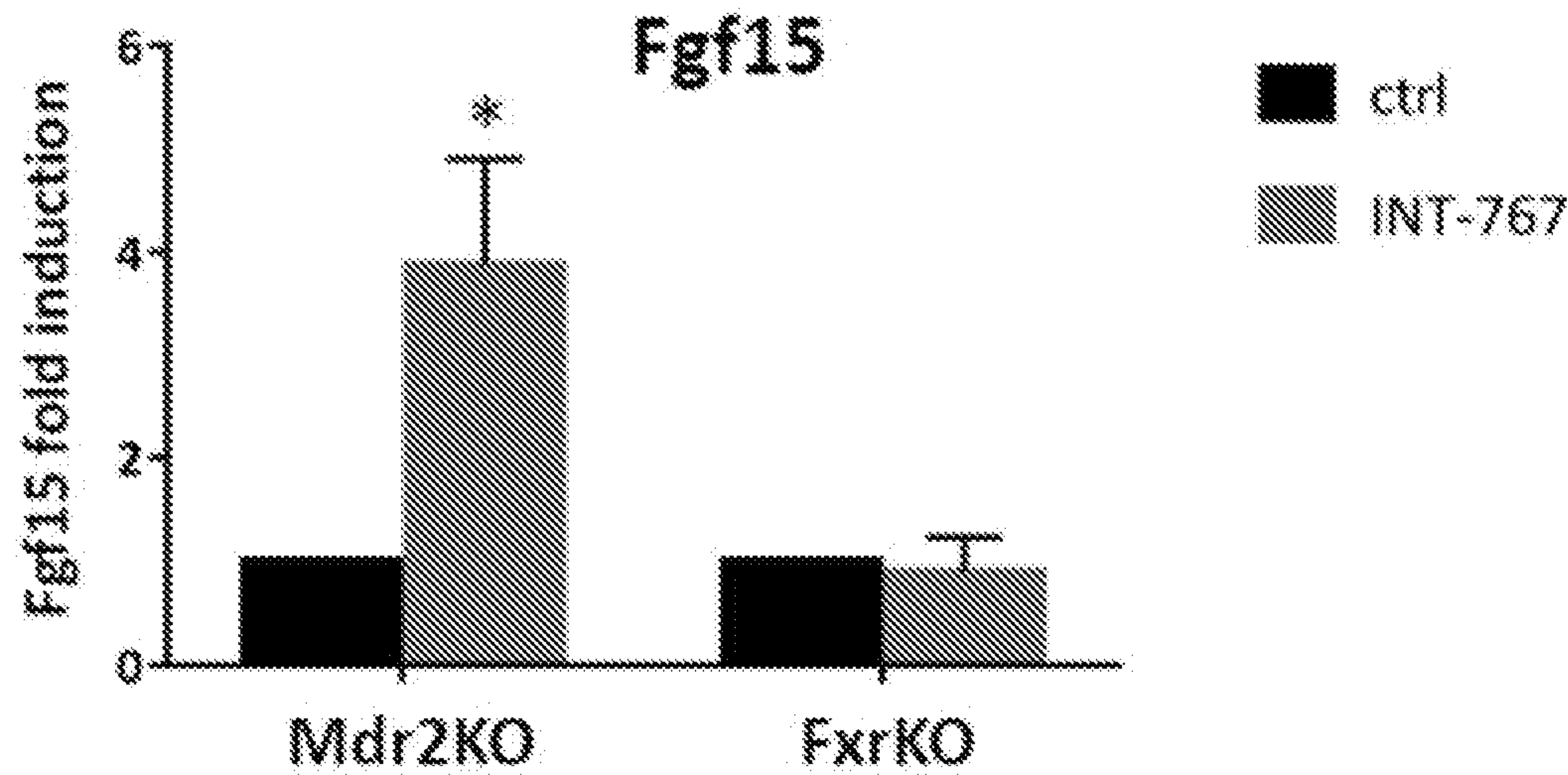


Figure 7A

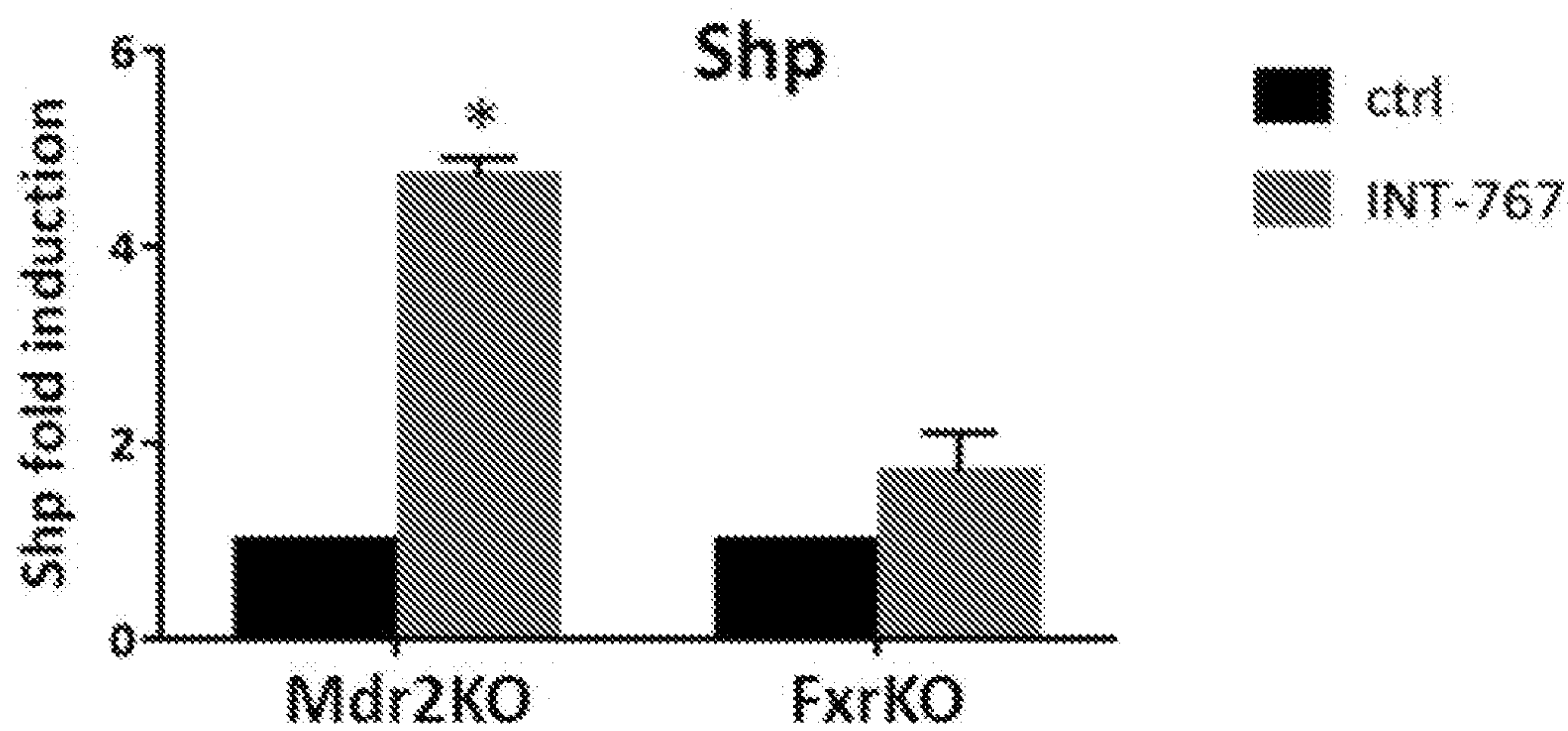


Figure 7B

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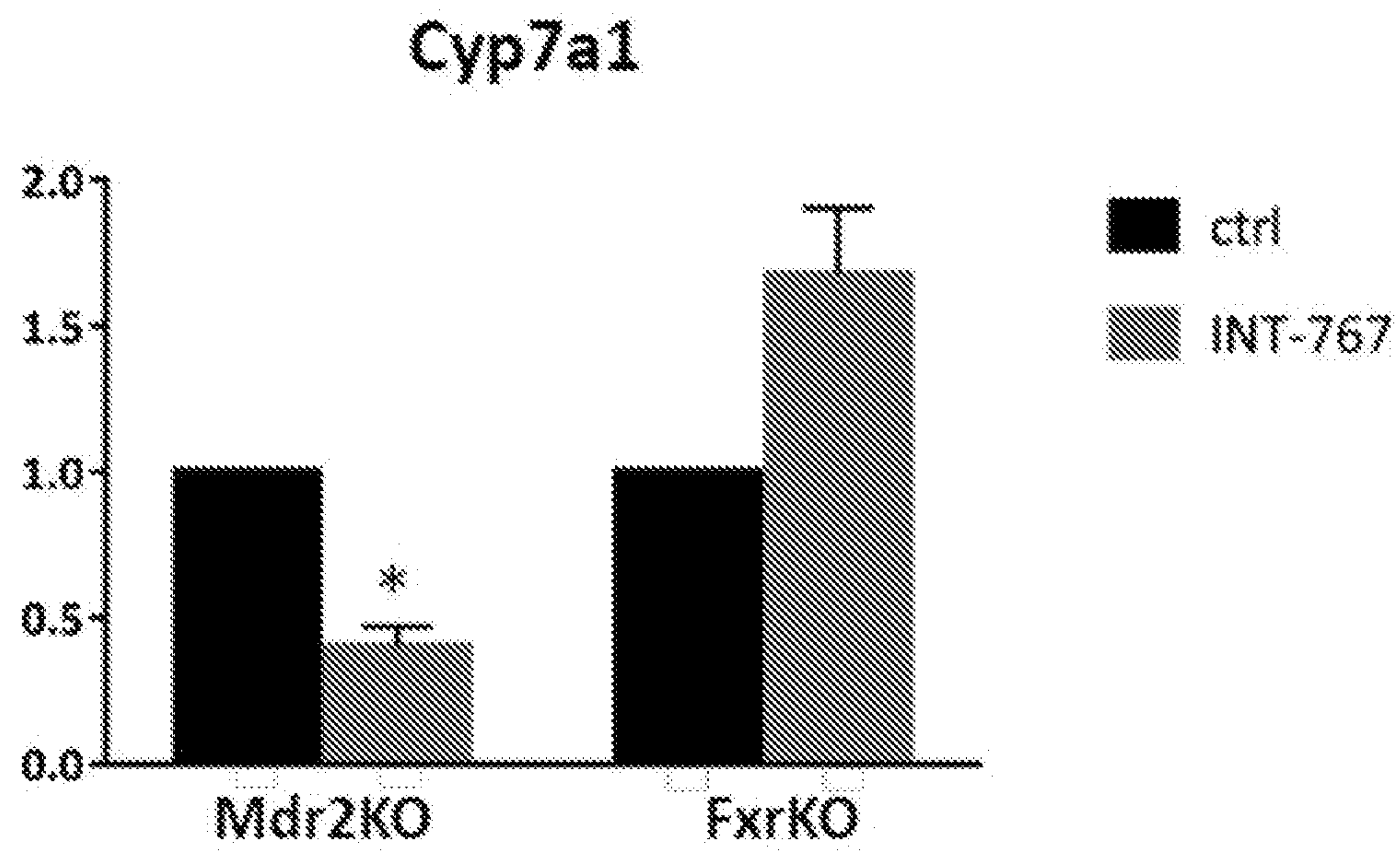


Figure 8

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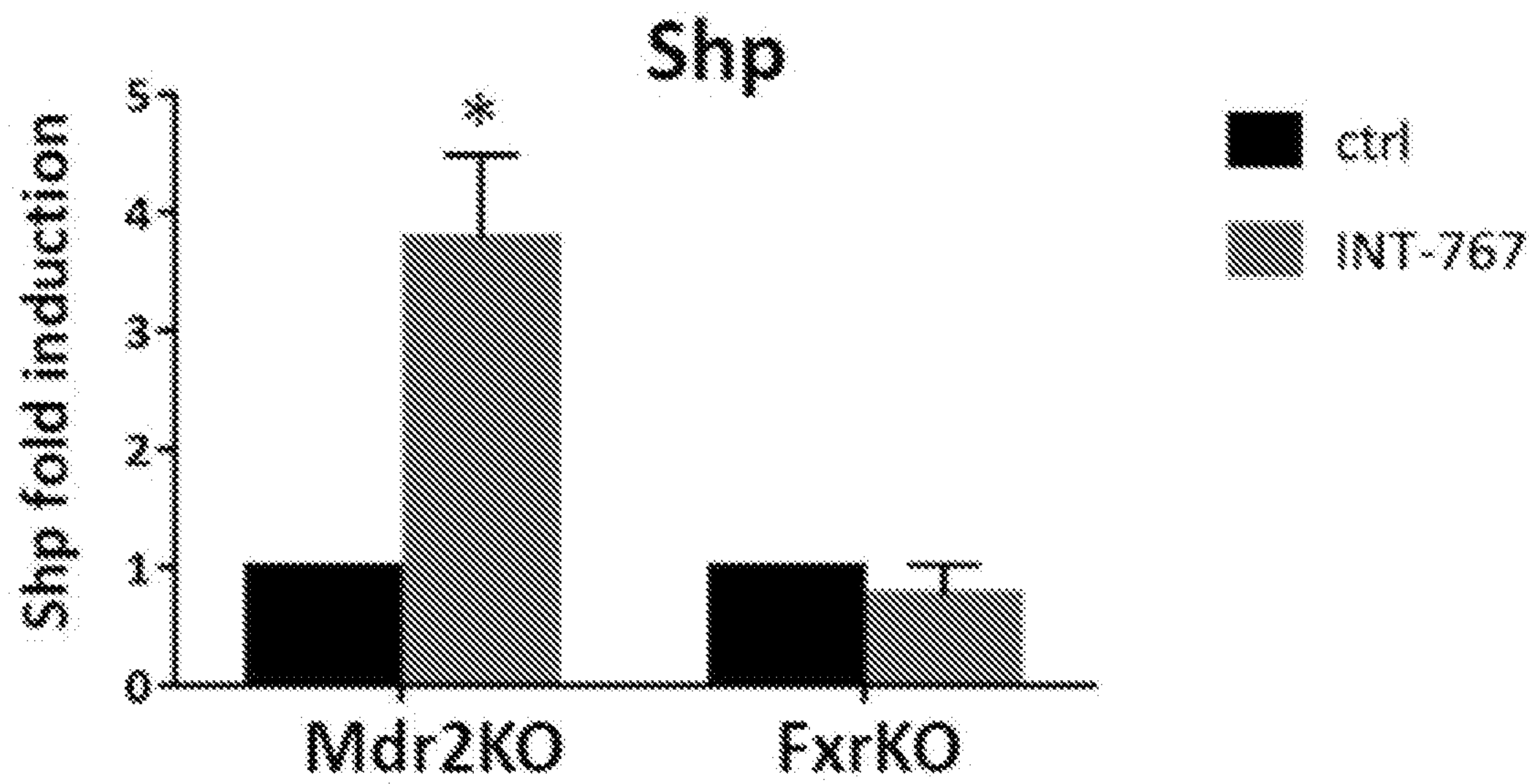


Figure 9A

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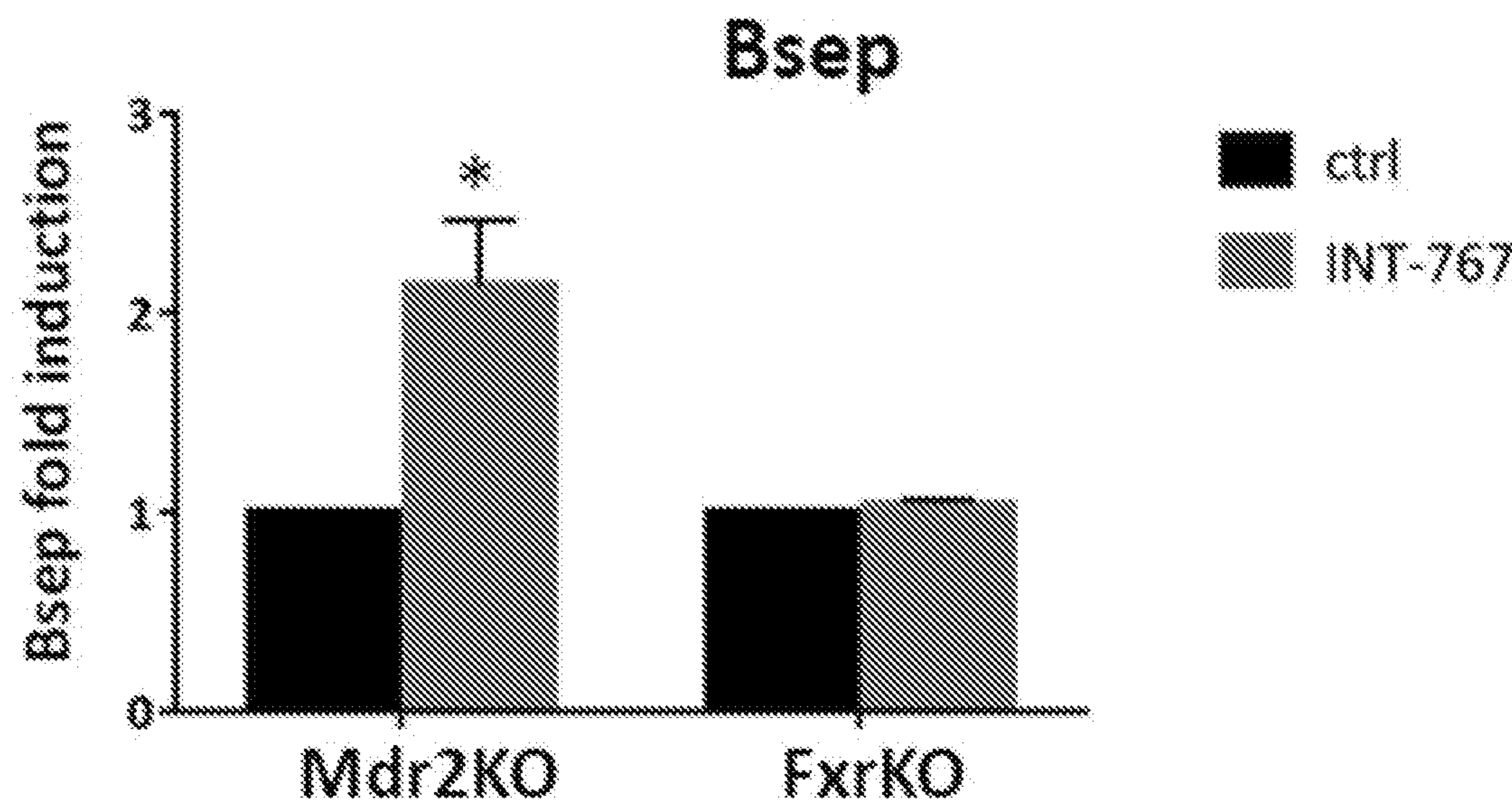


Figure 9B

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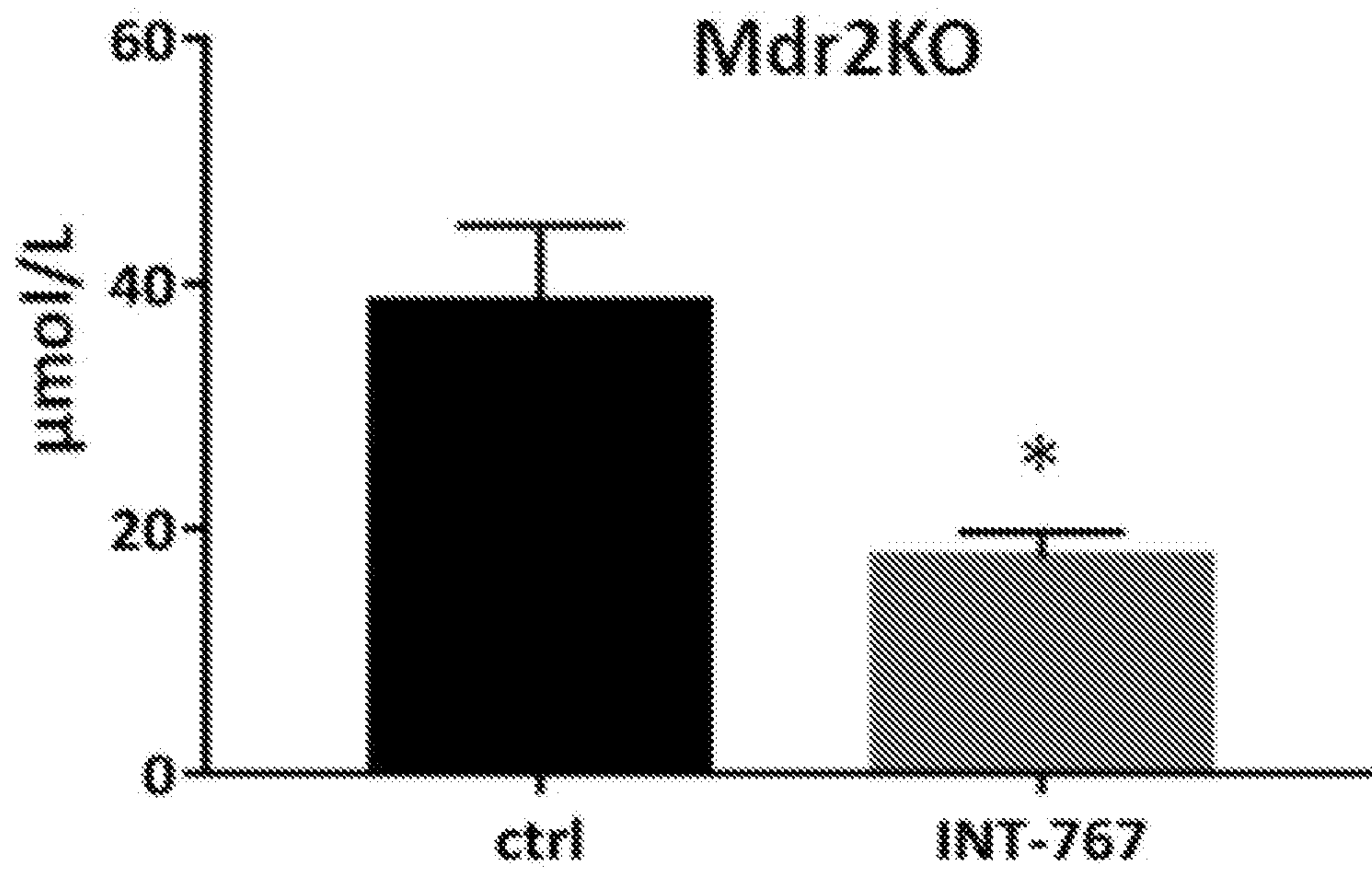


Figure 10A

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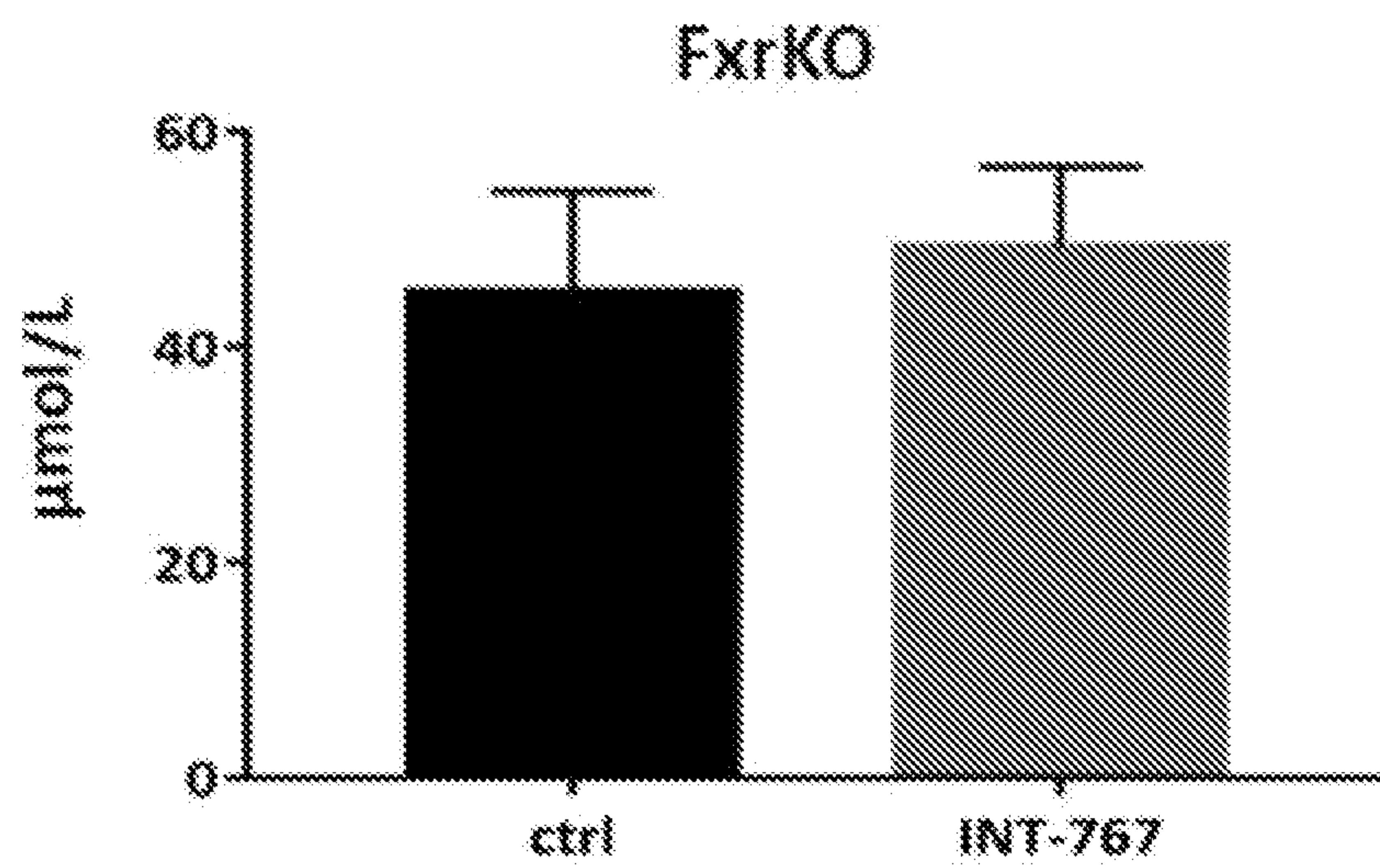


Figure 10B

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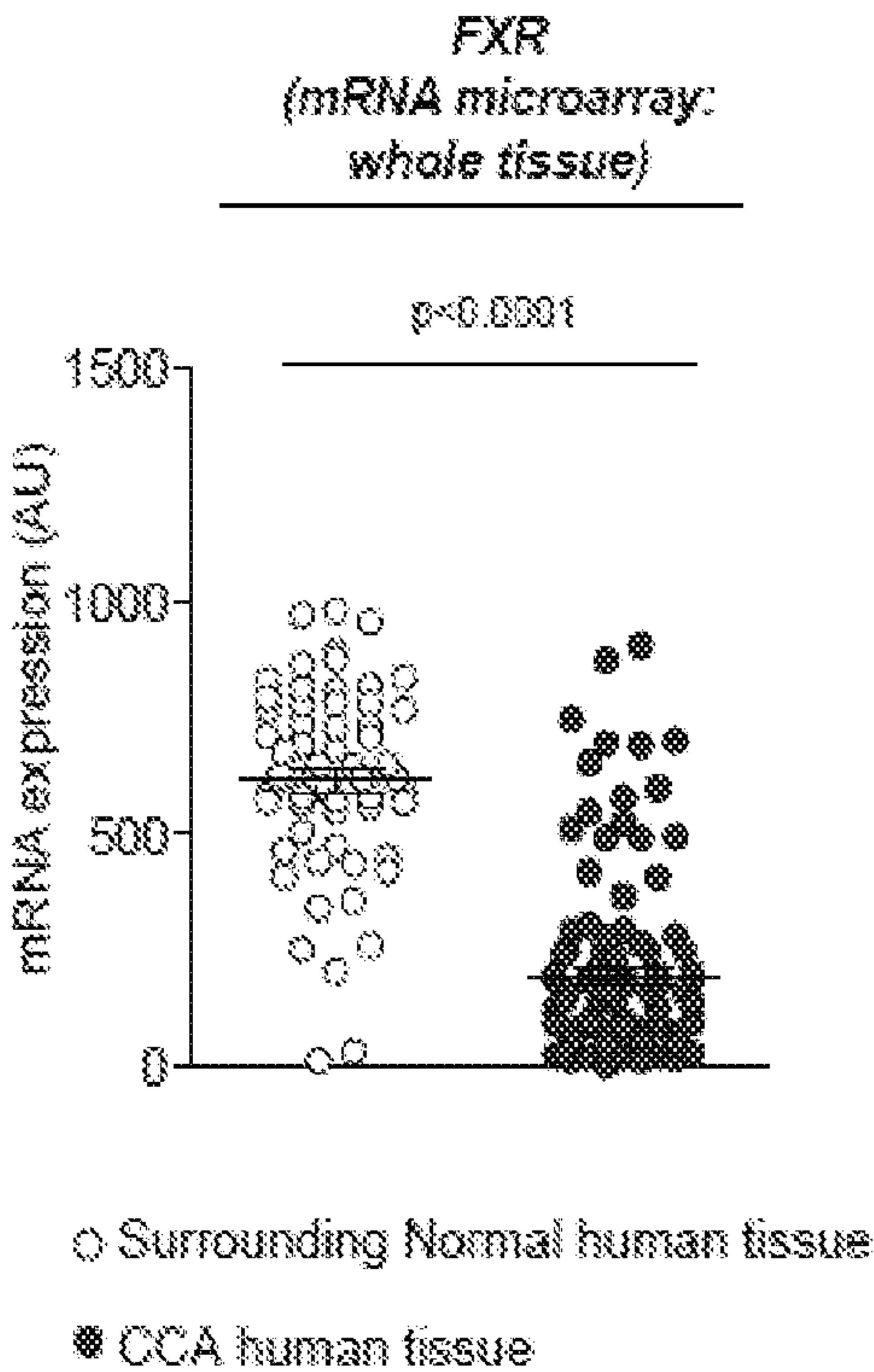


Figure 11A

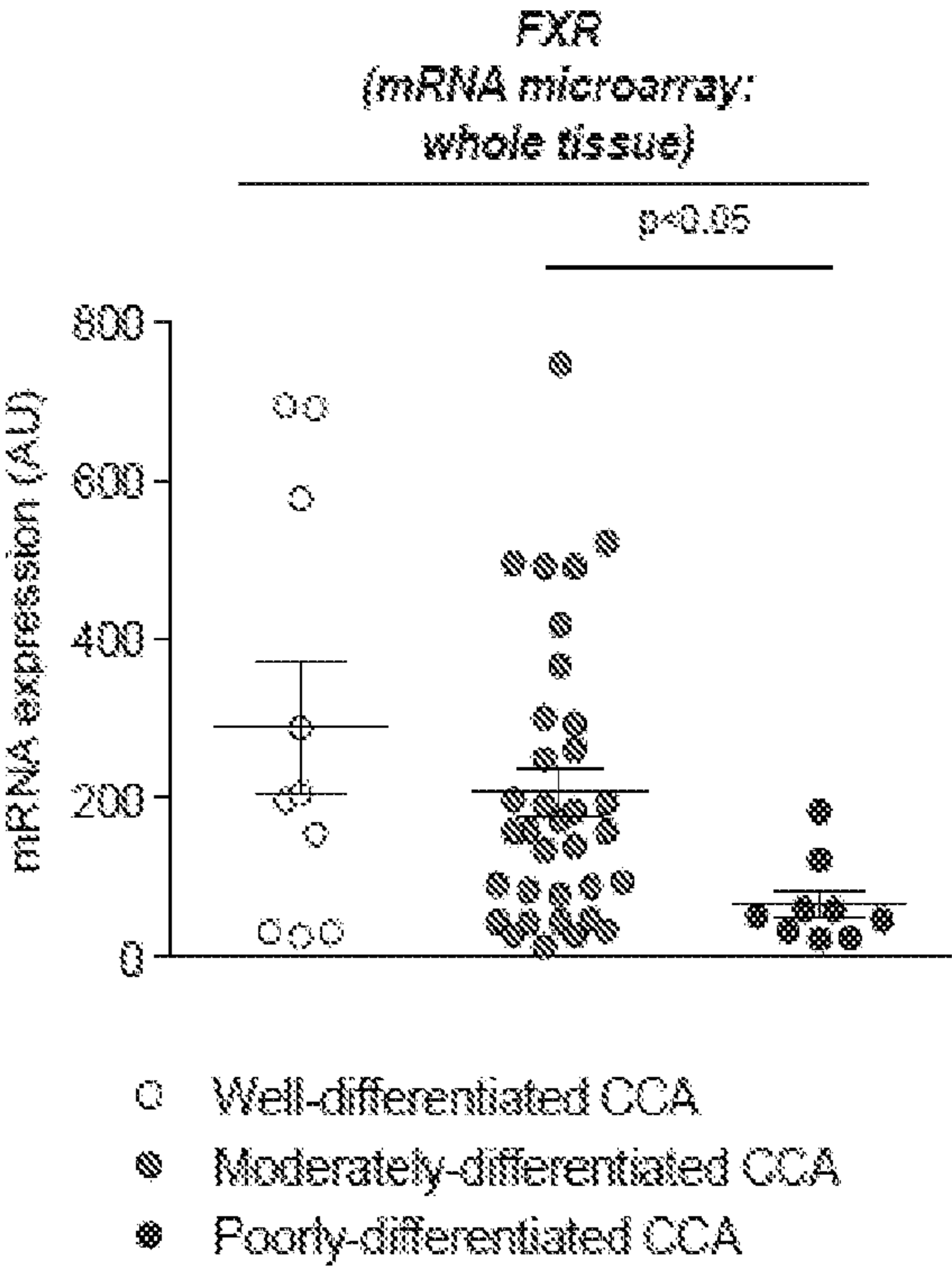


Figure 11B

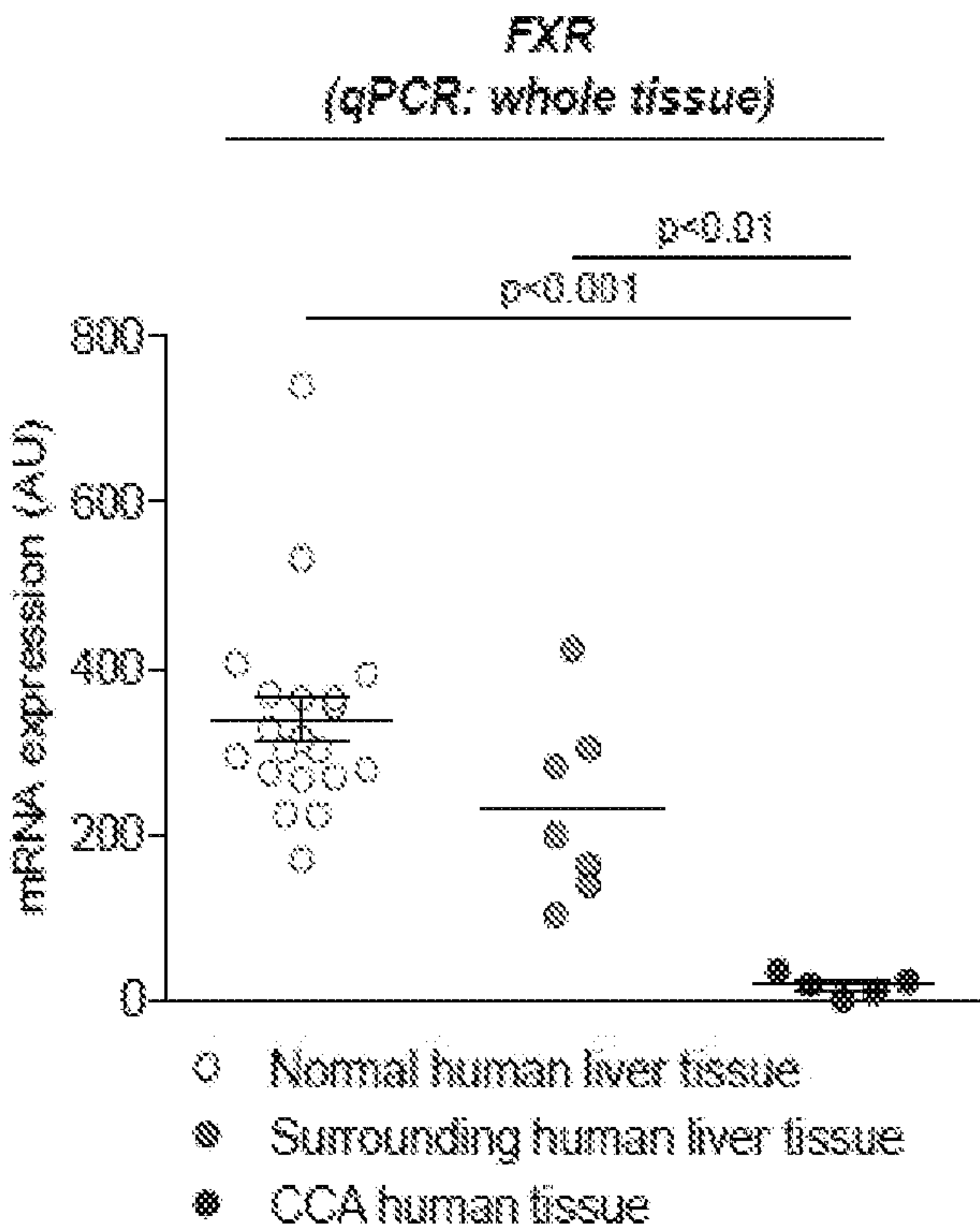


Figure 11C

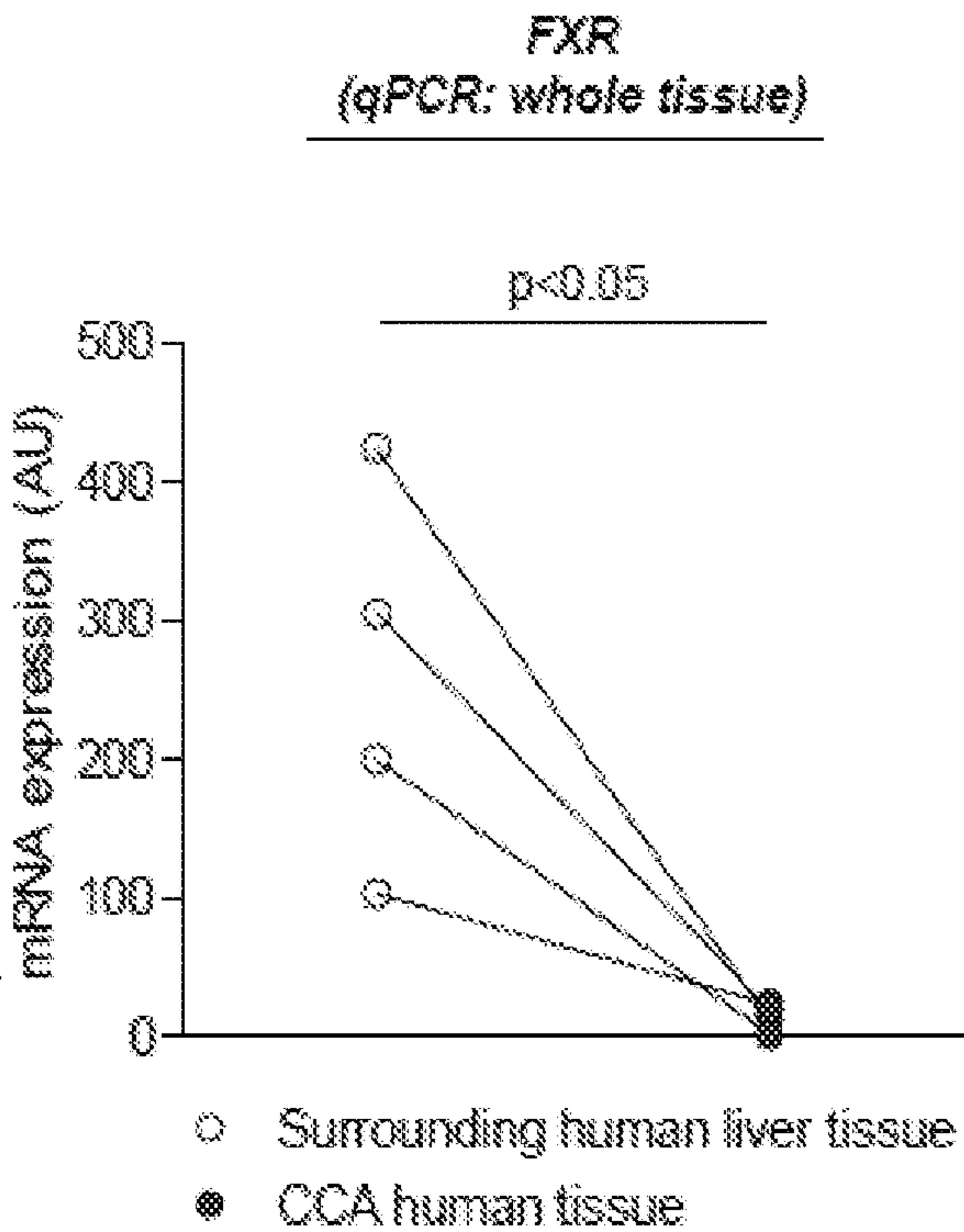


Figure 11D

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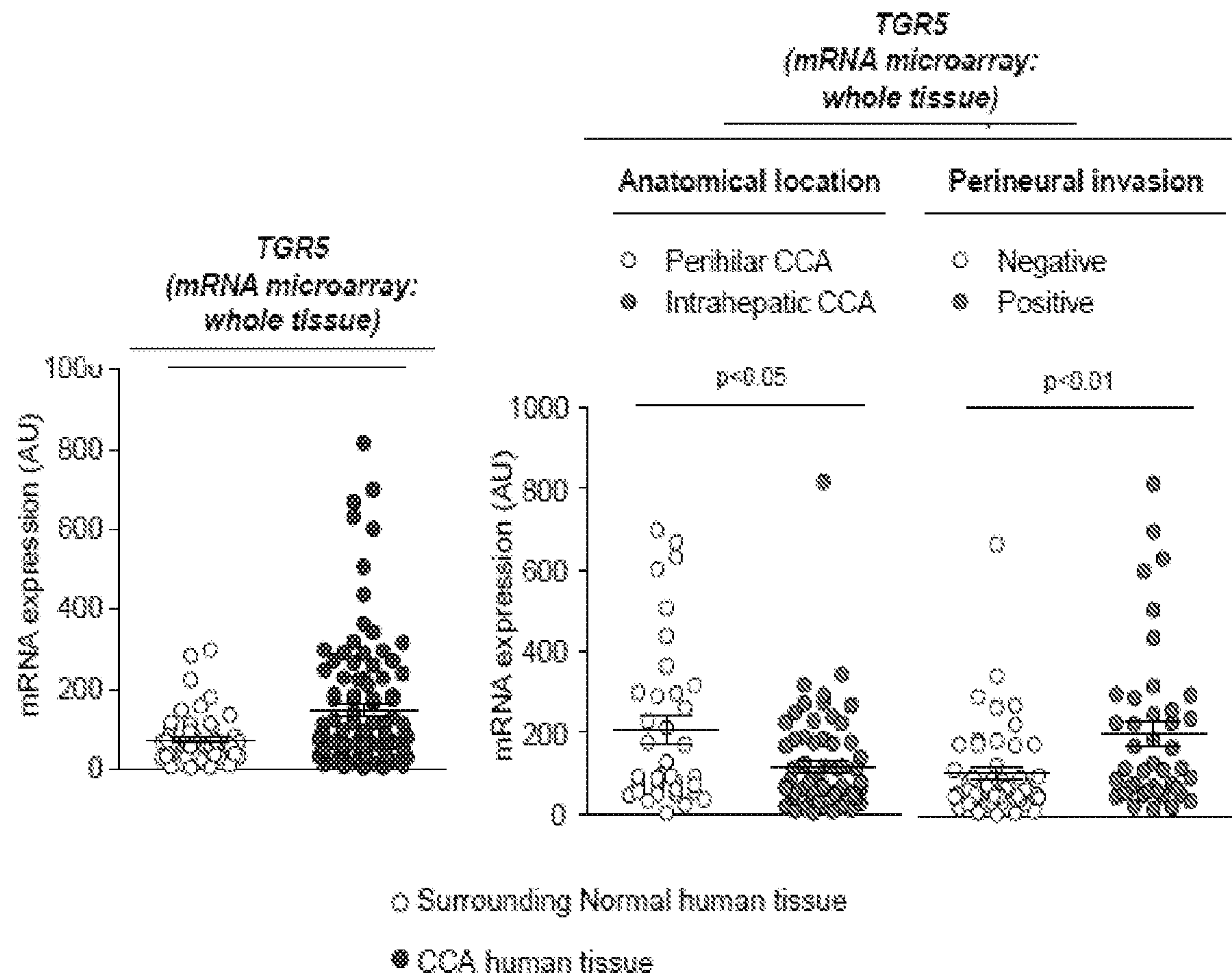


Figure 12A

Figure 12B

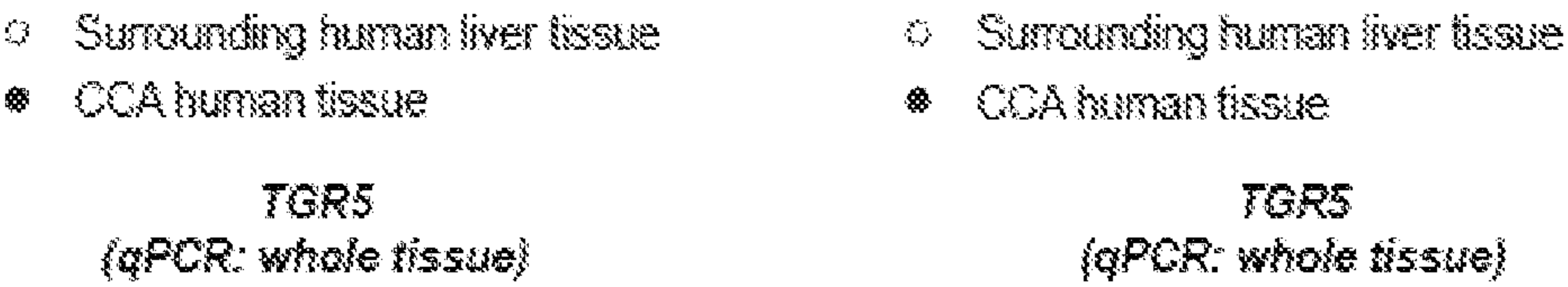


Figure 12C

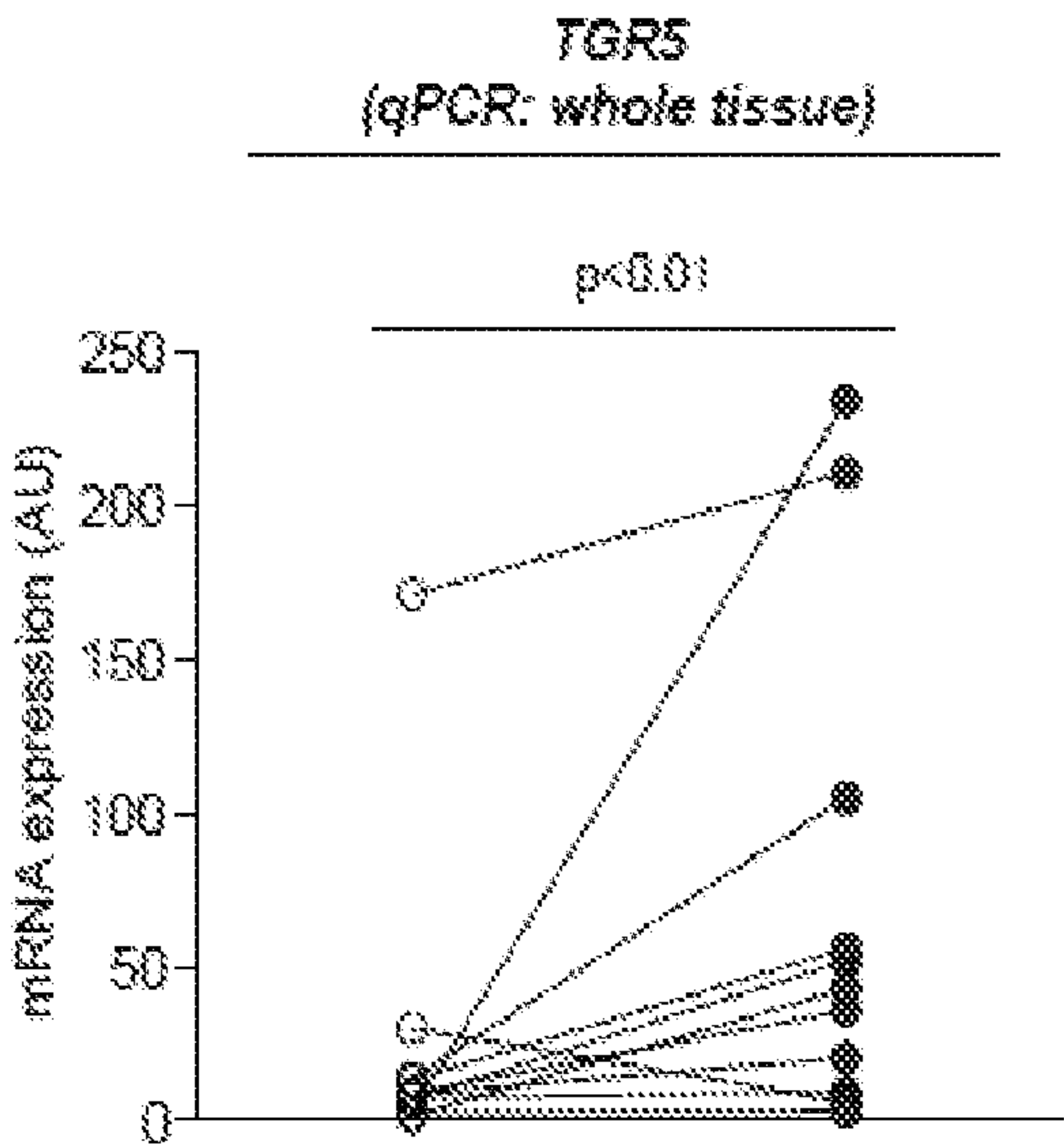


Figure 12D

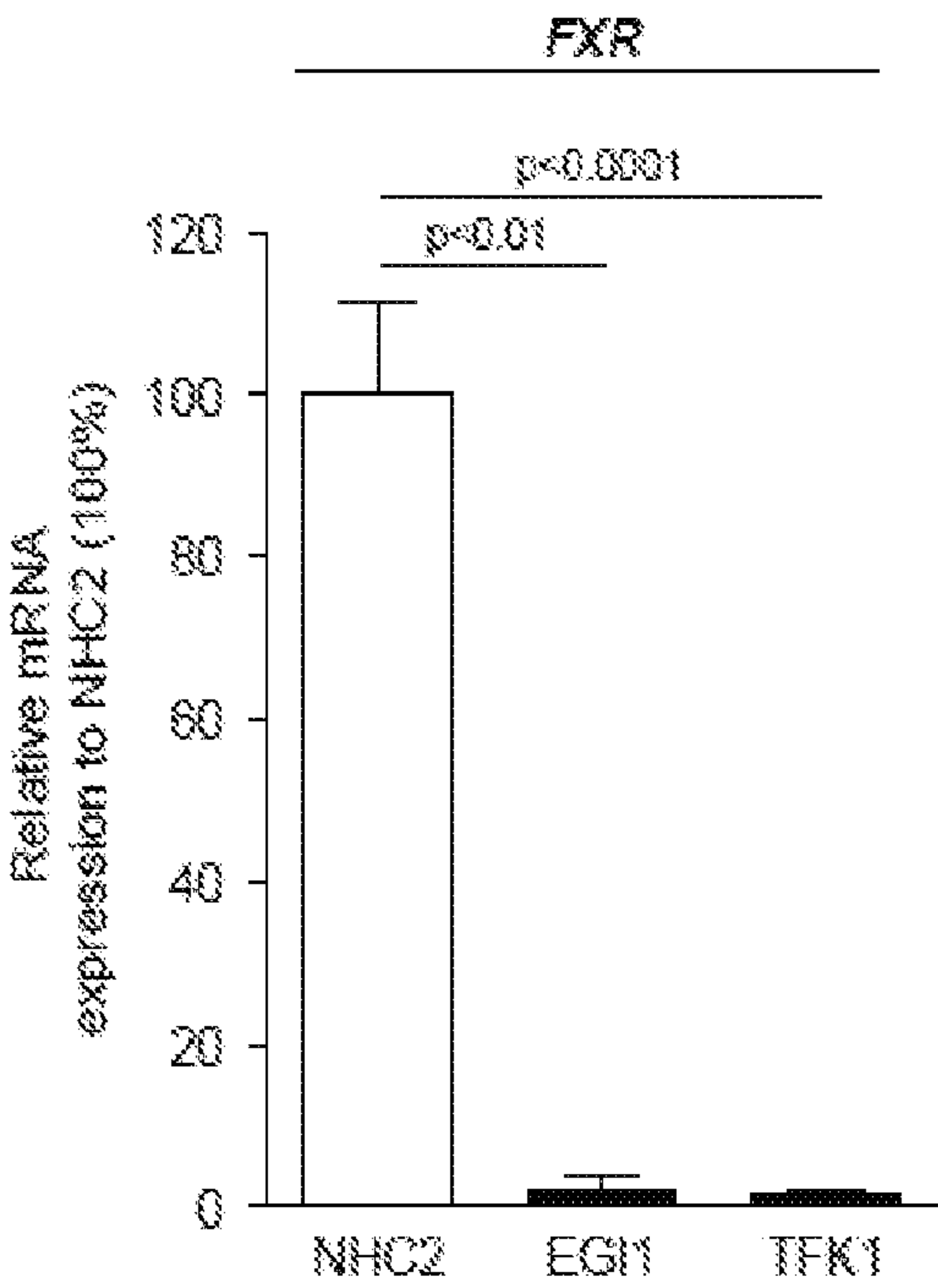


Figure 13A

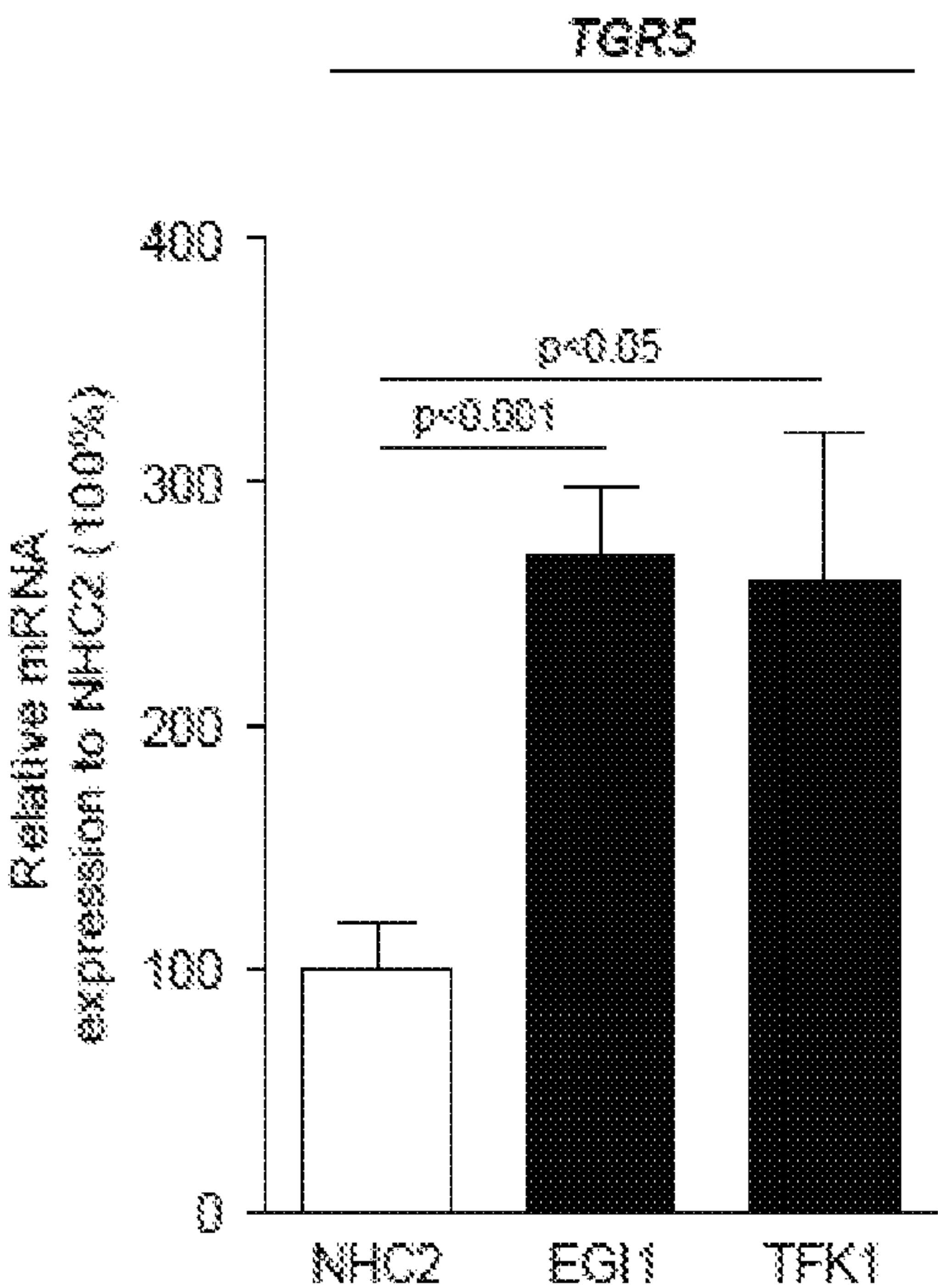


Figure 13B

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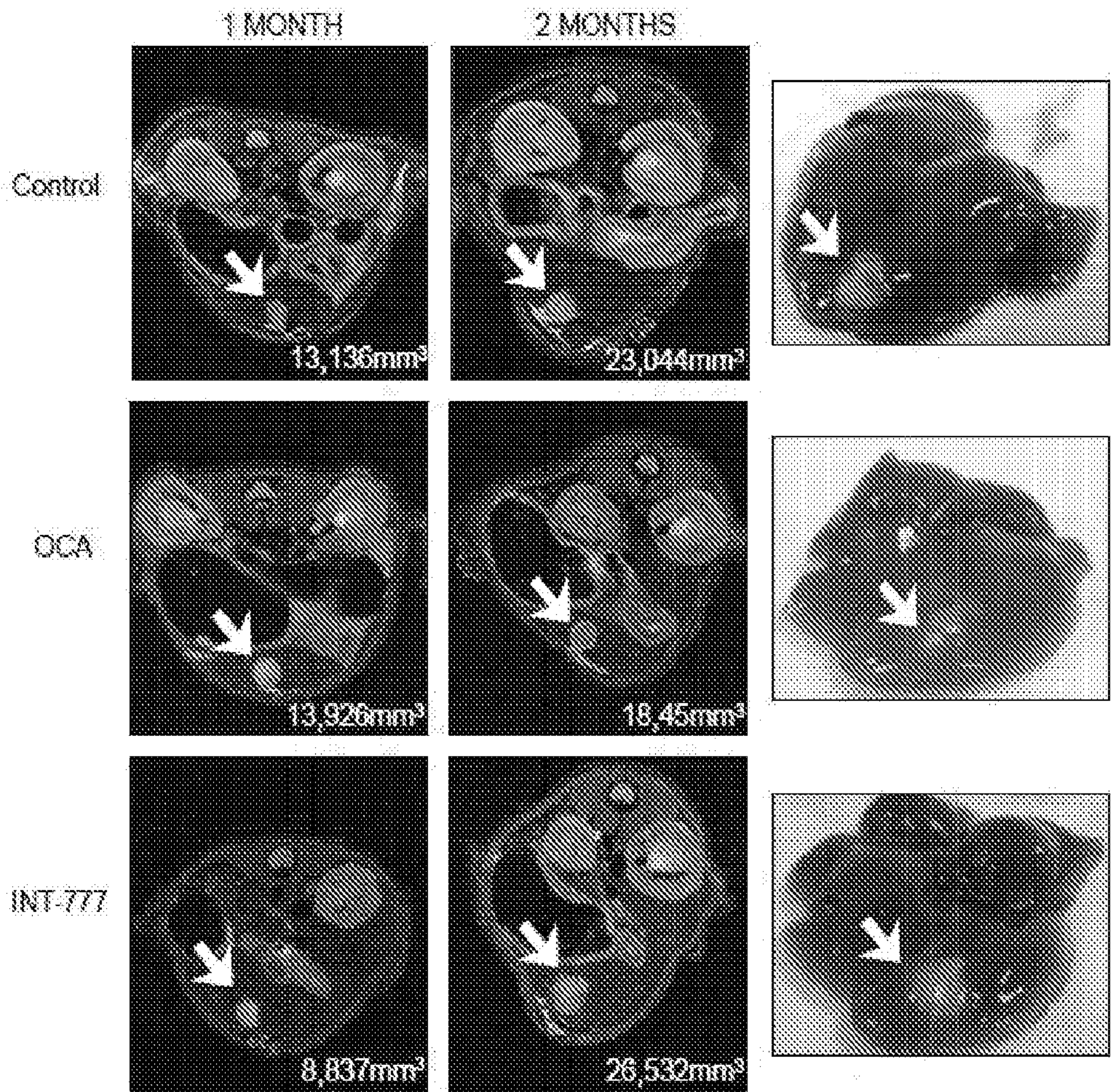


Figure 14A

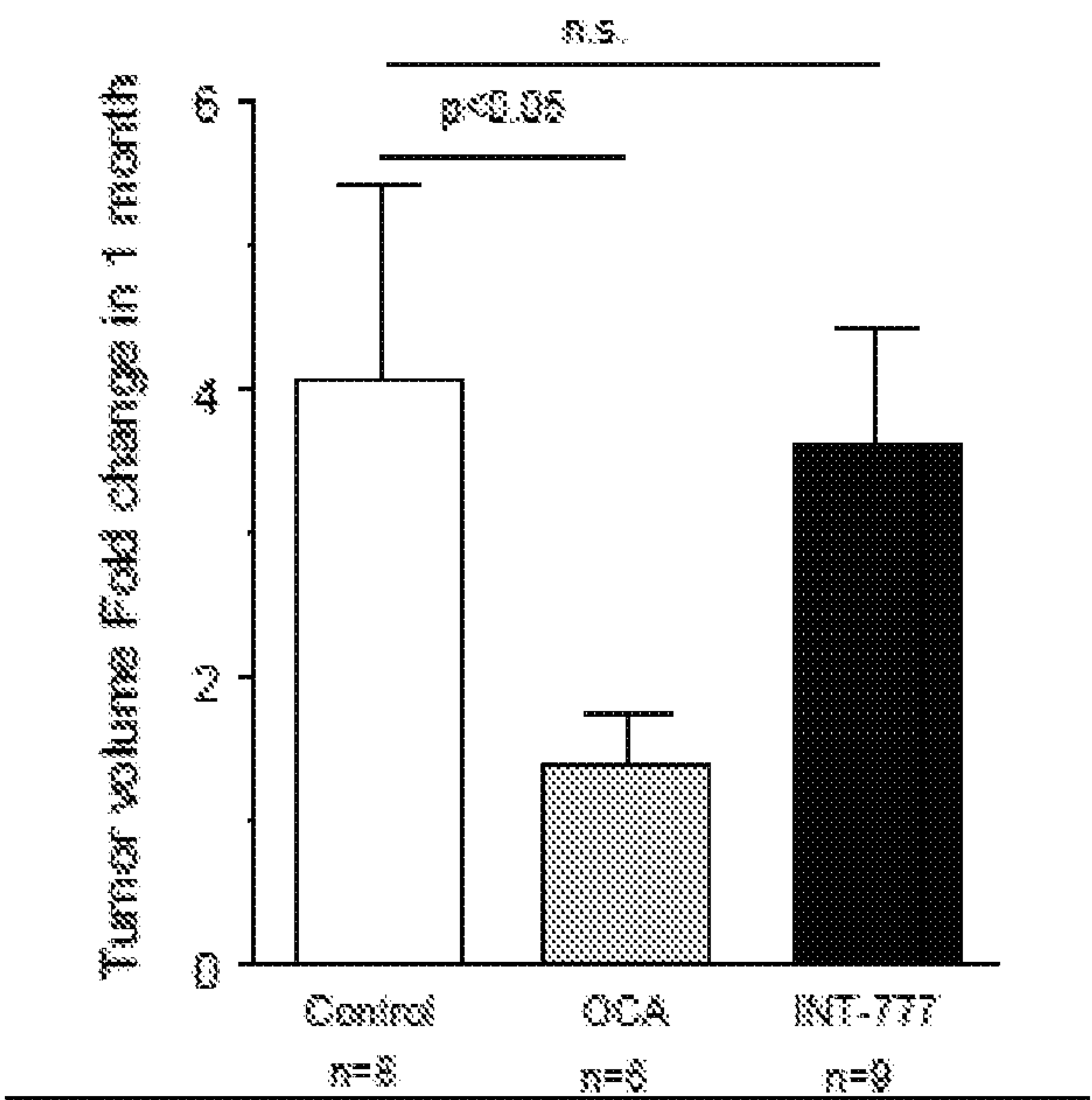


Figure 14B

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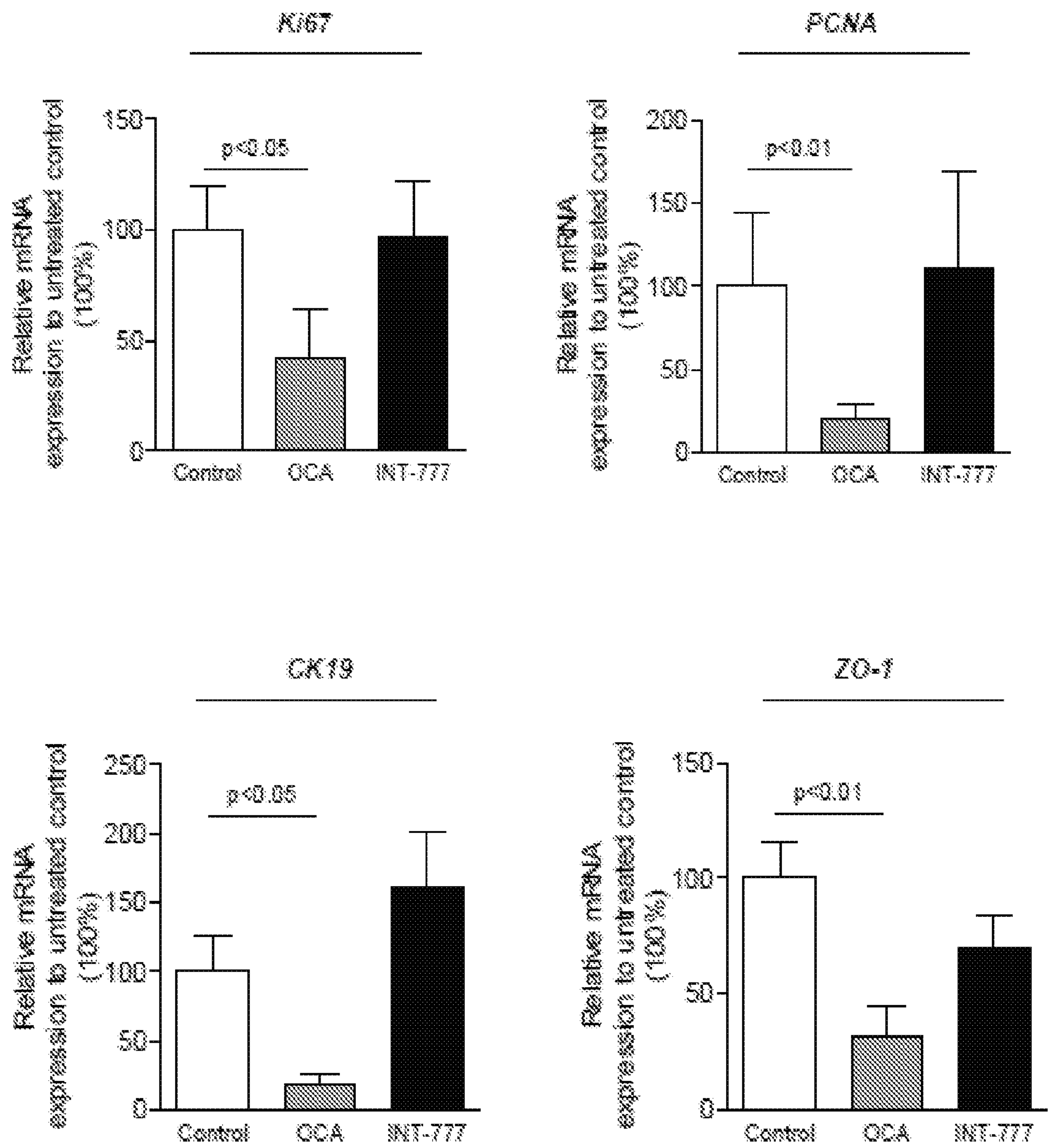


Figure 14C

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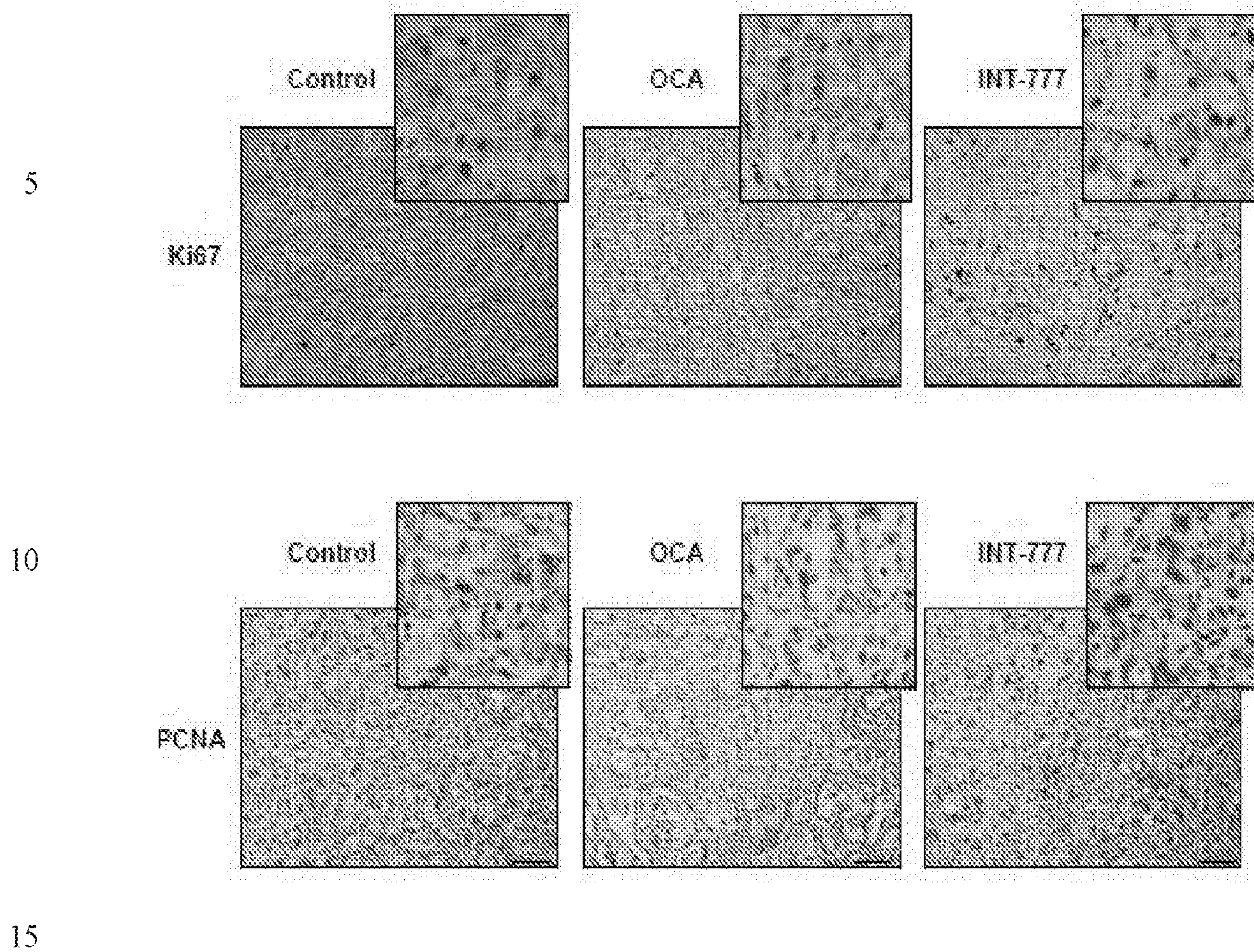


Figure 14D

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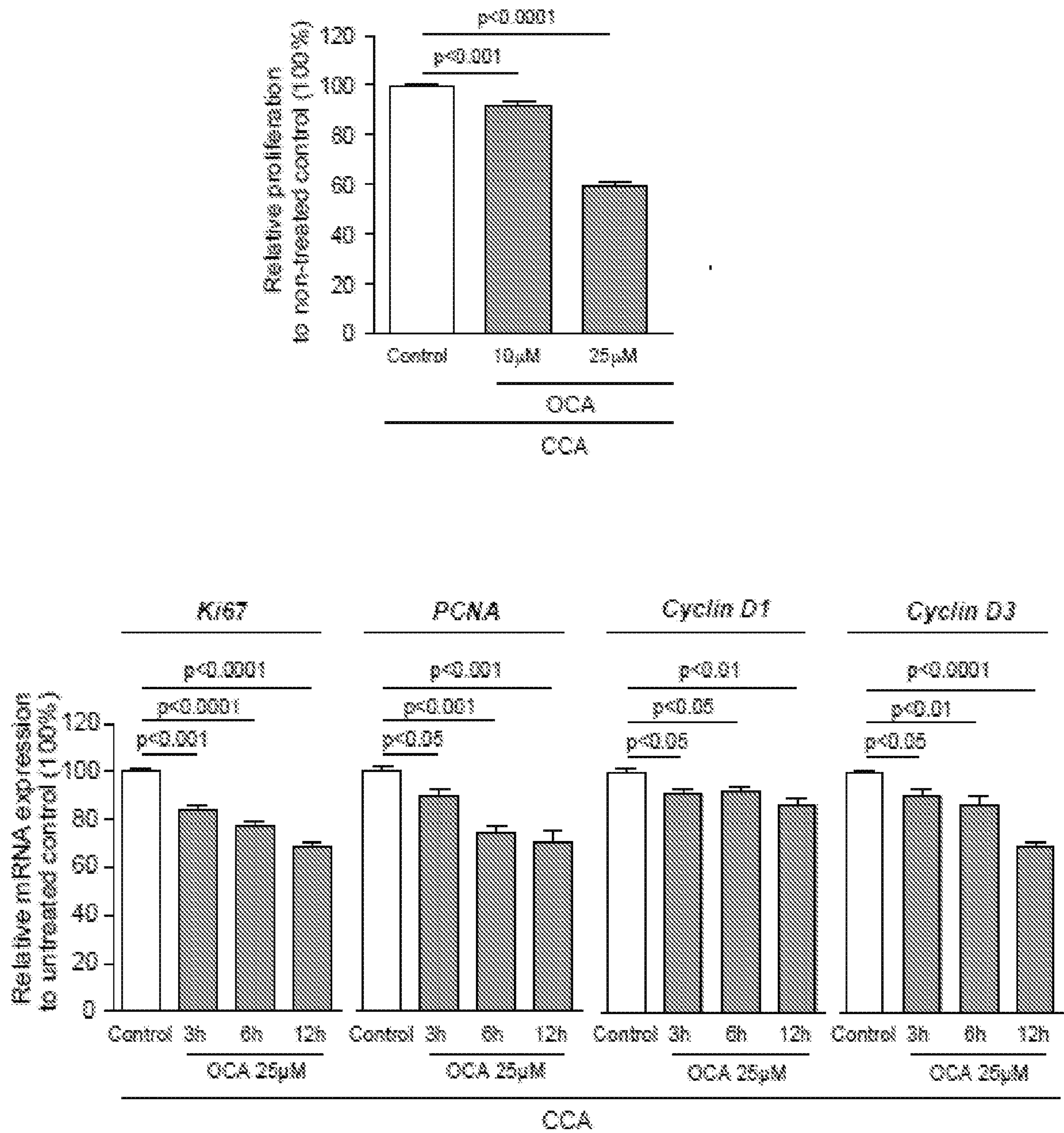


Figure 15A

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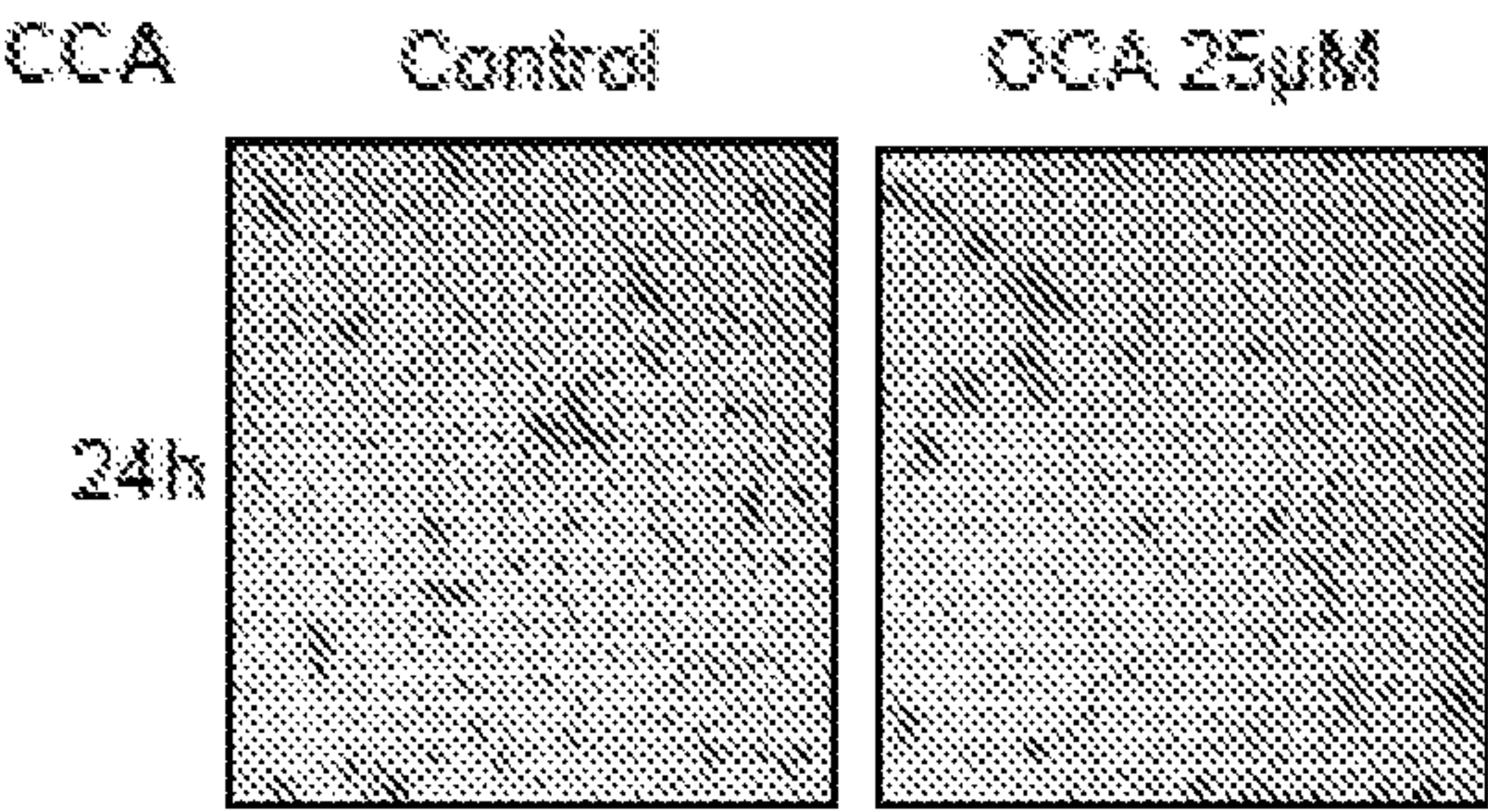
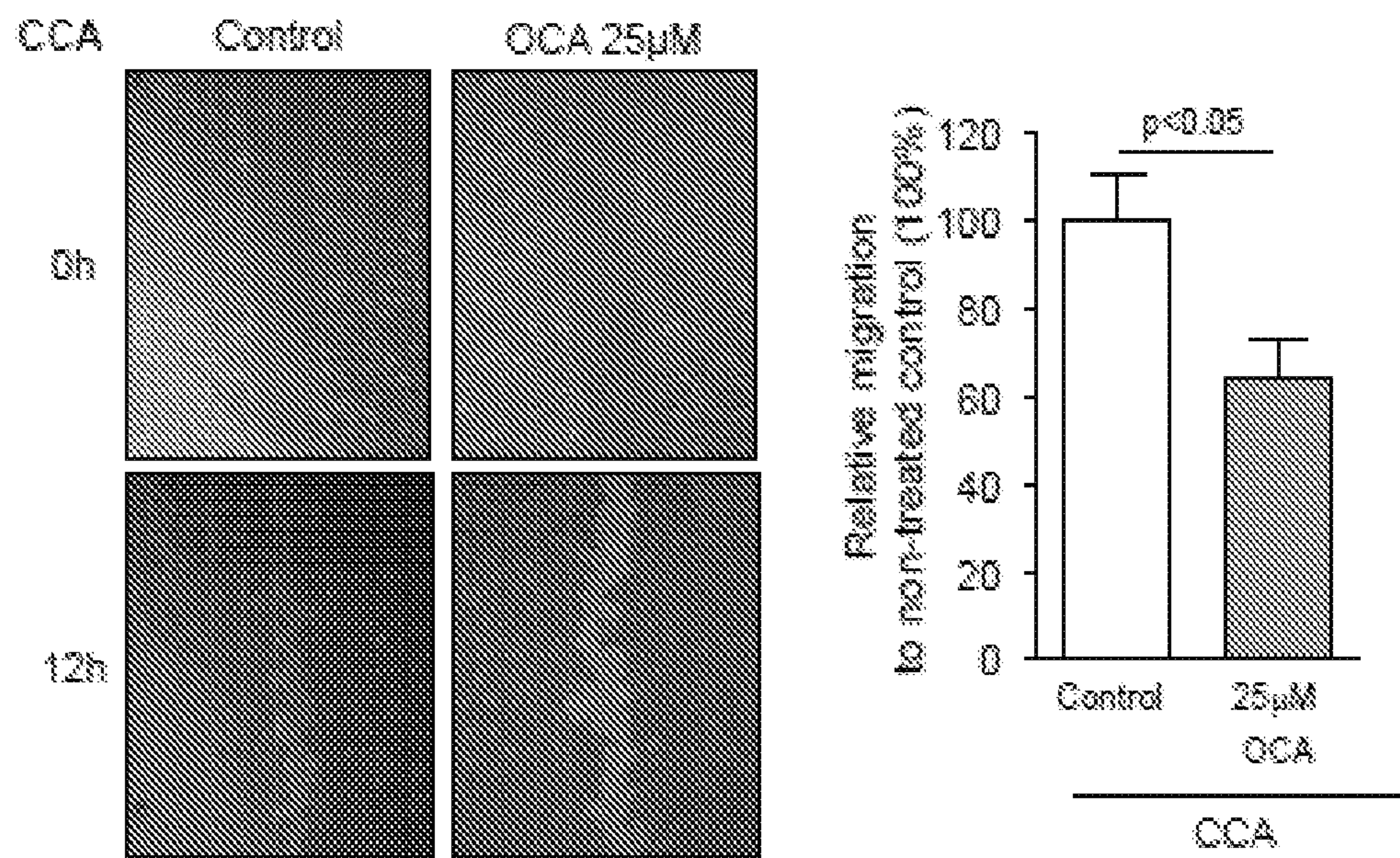


Figure 15B

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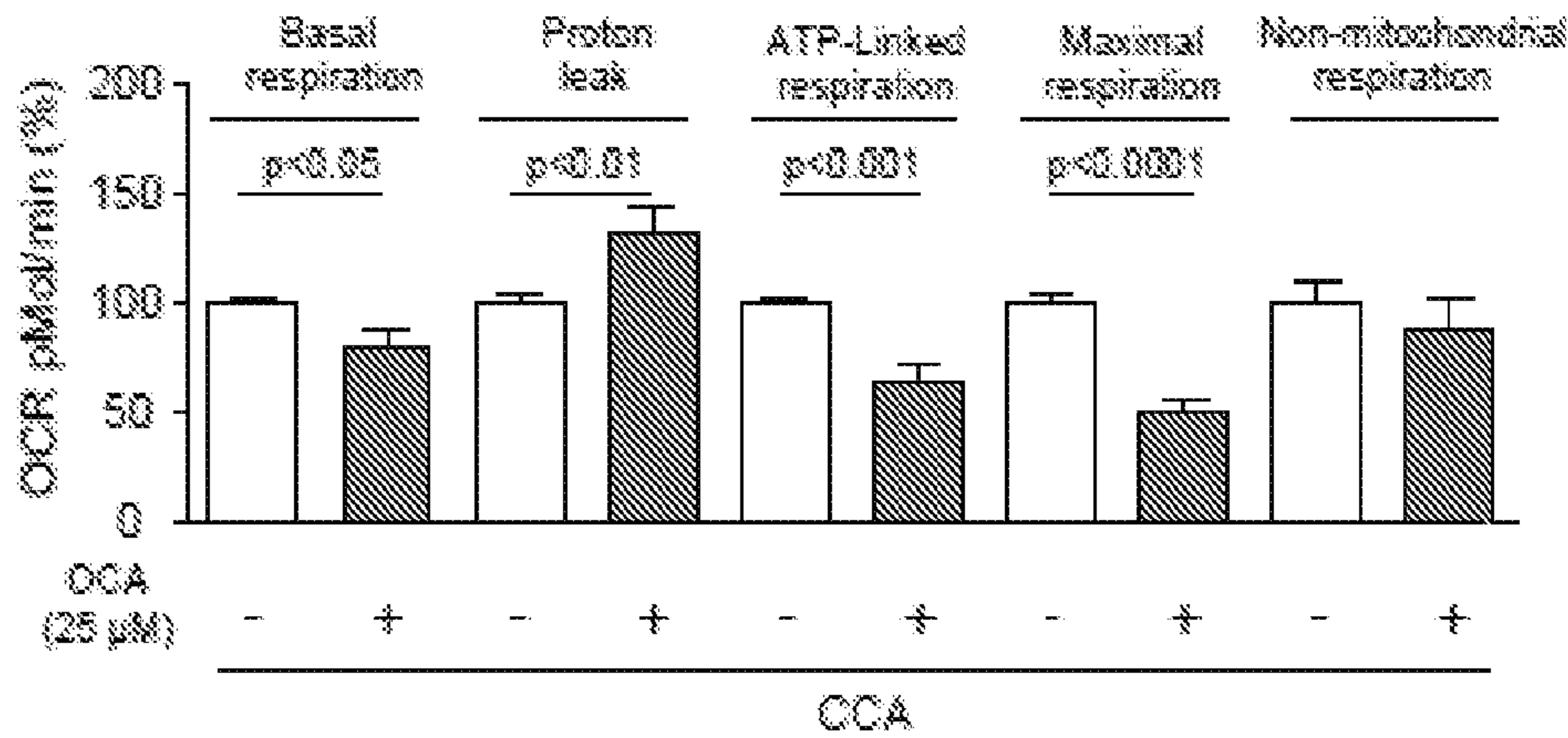
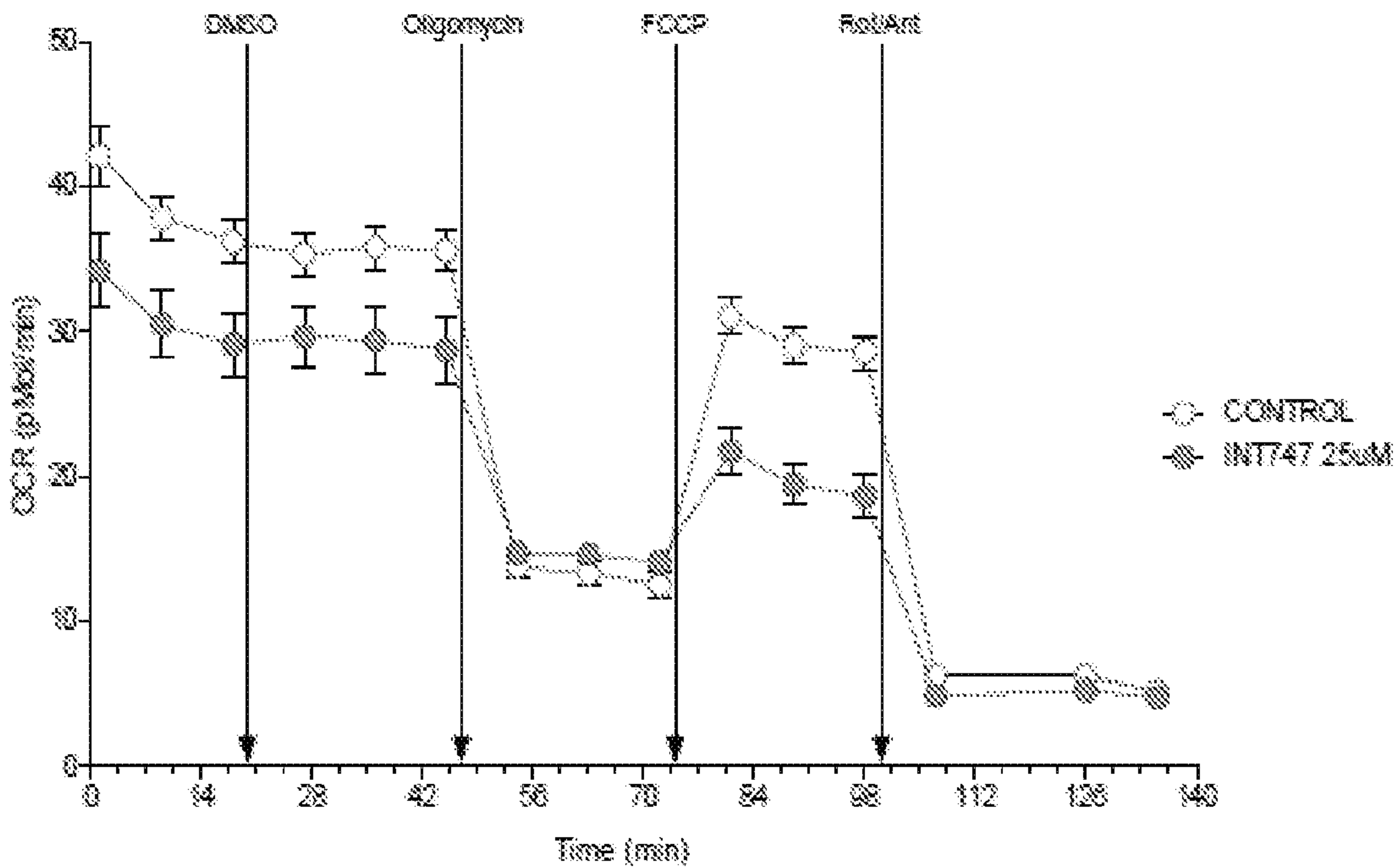
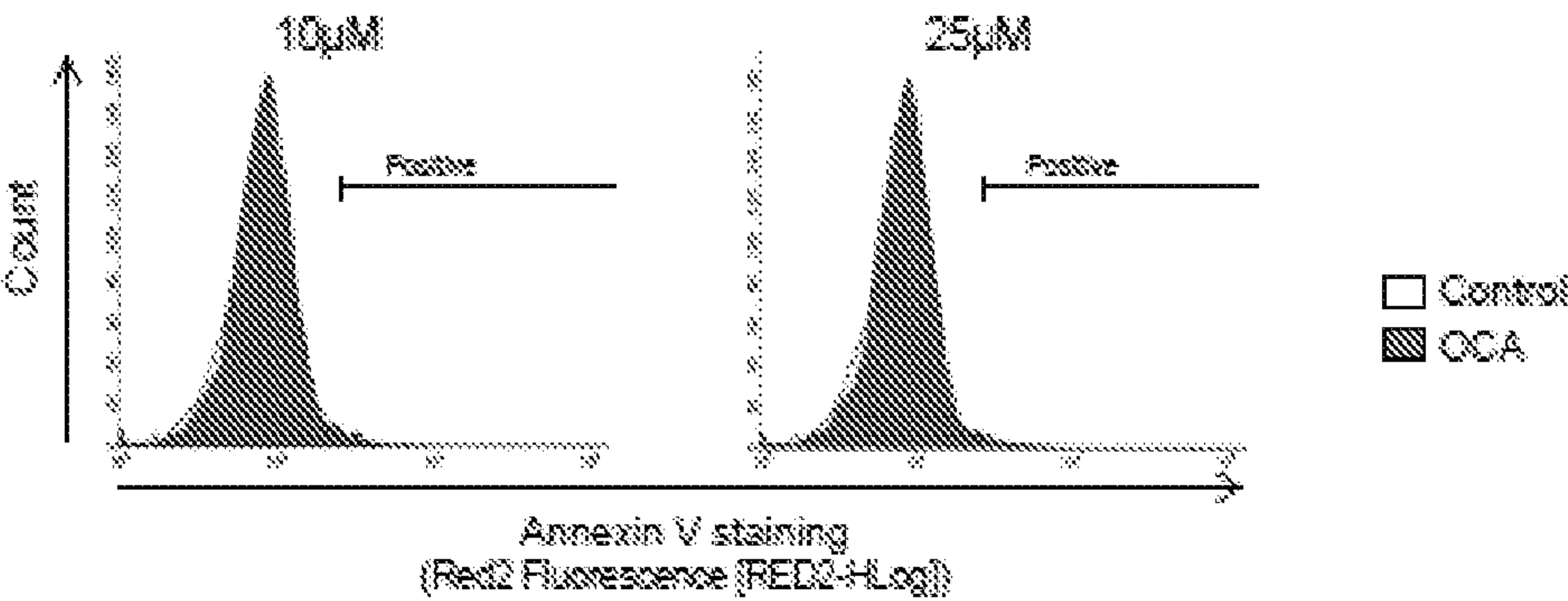


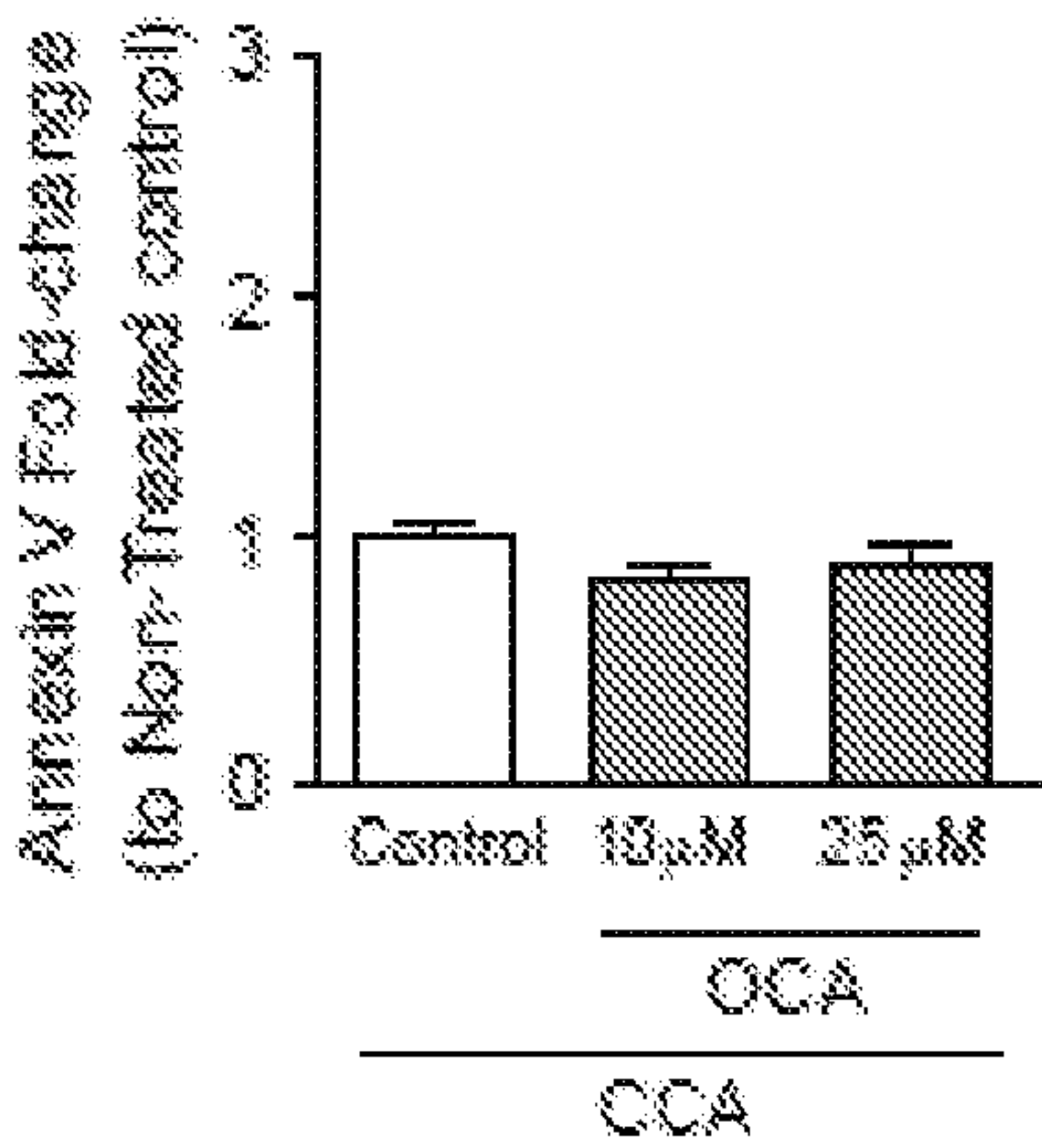
Figure 15C

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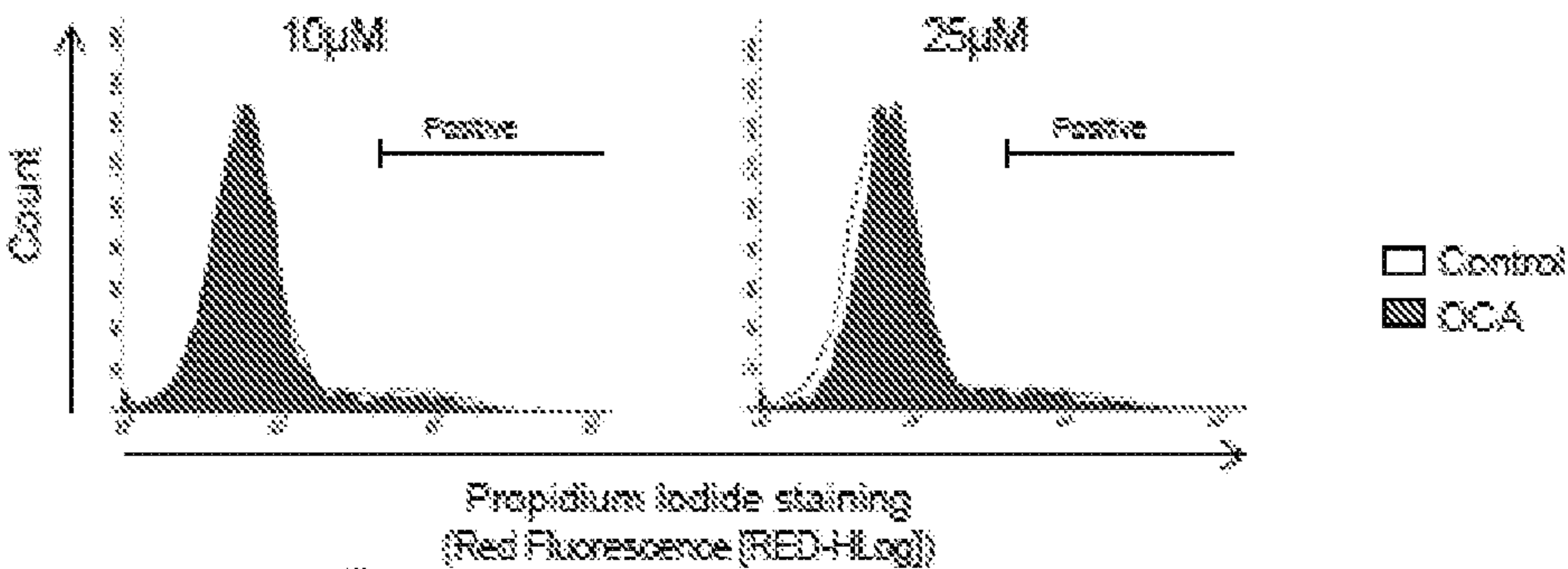
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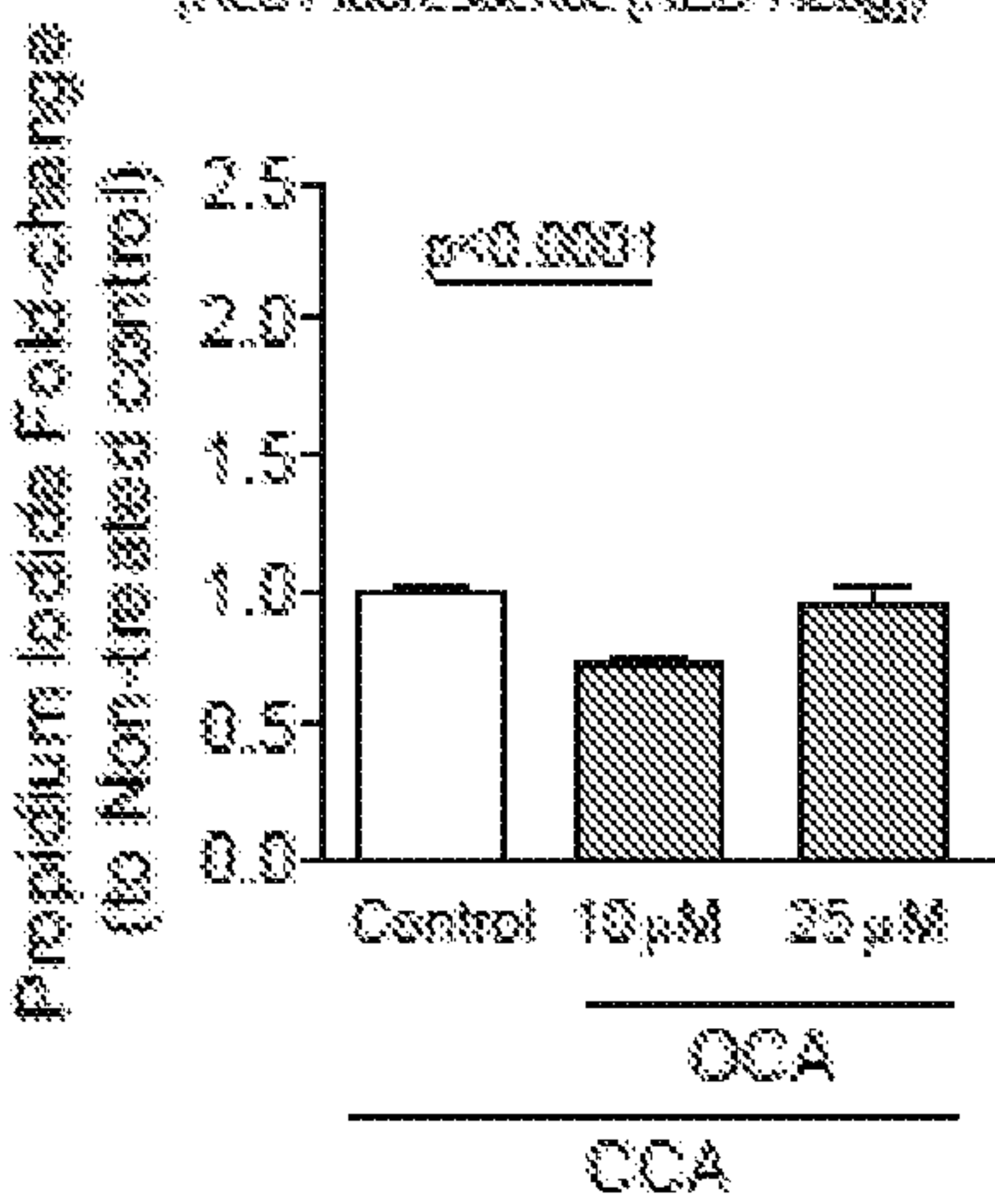
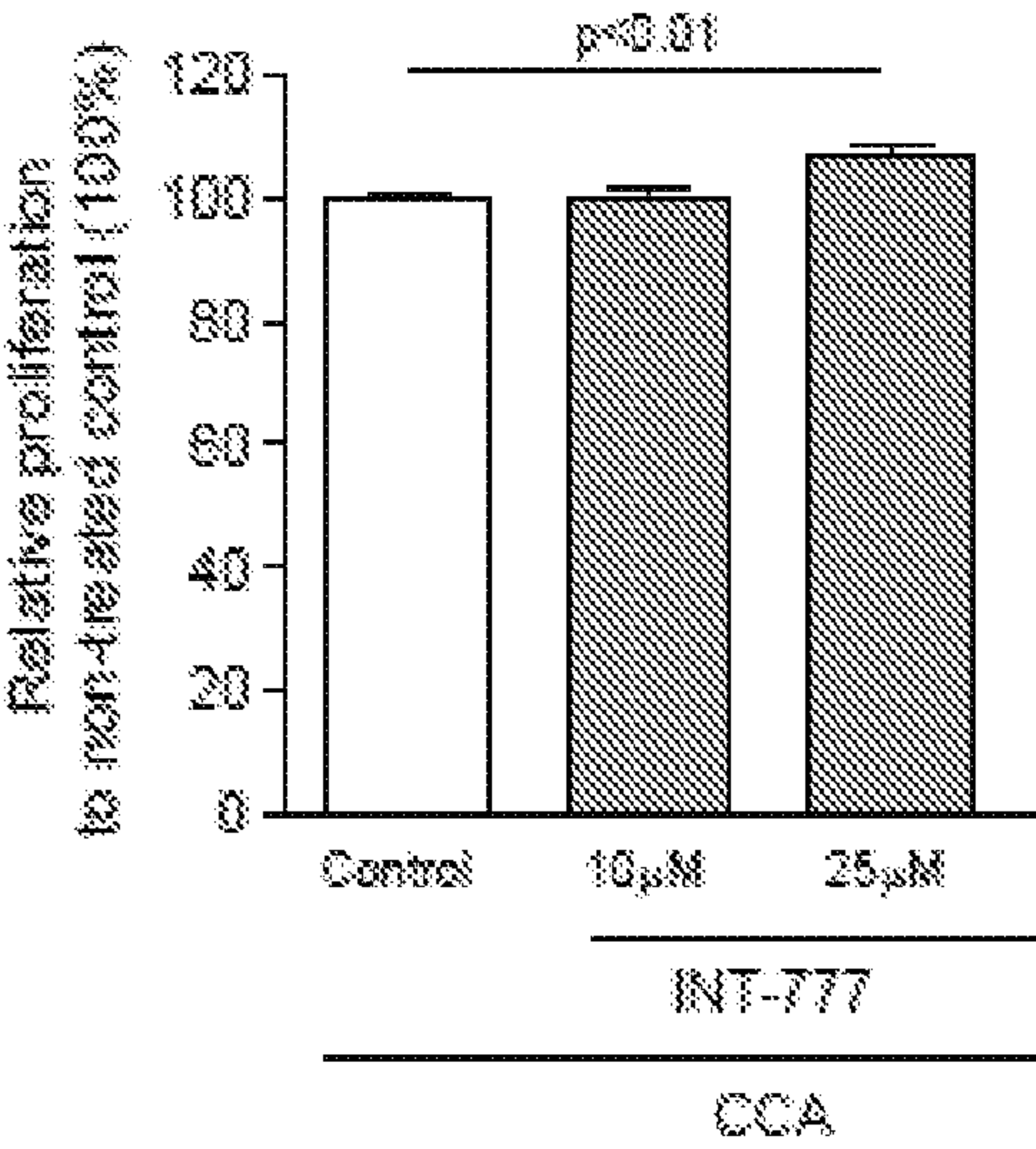


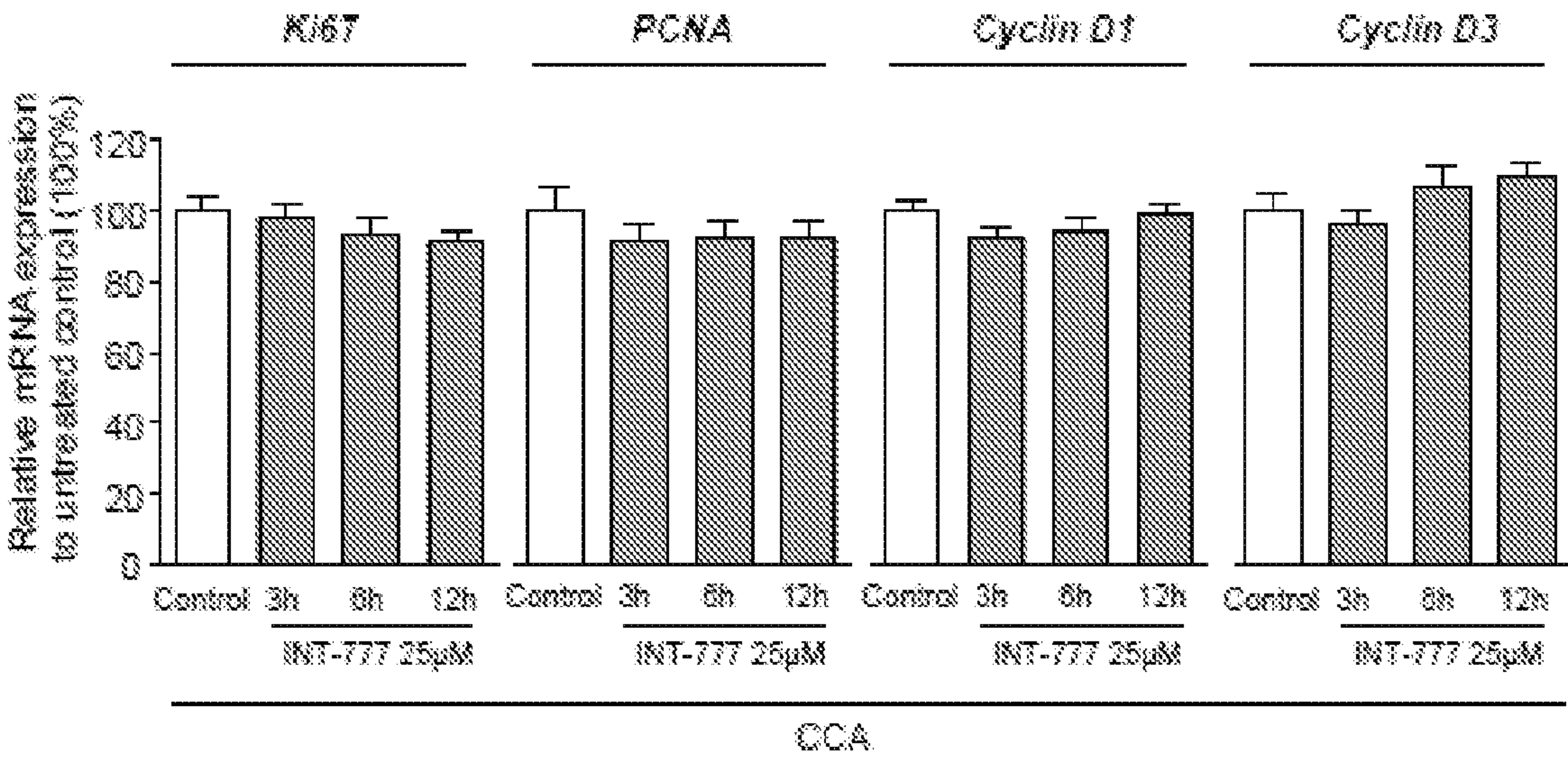
Figure 15D

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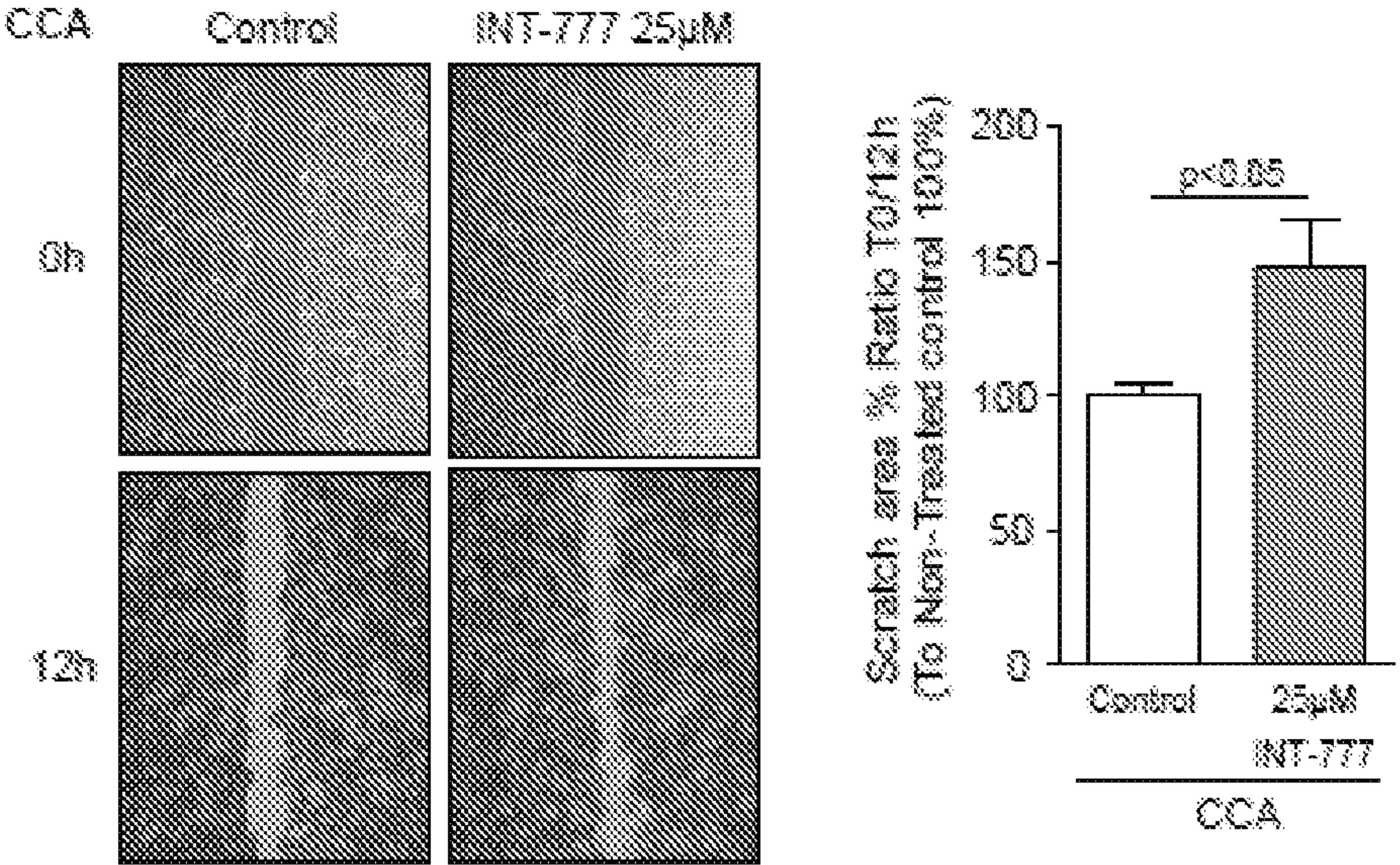
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Figure 16A

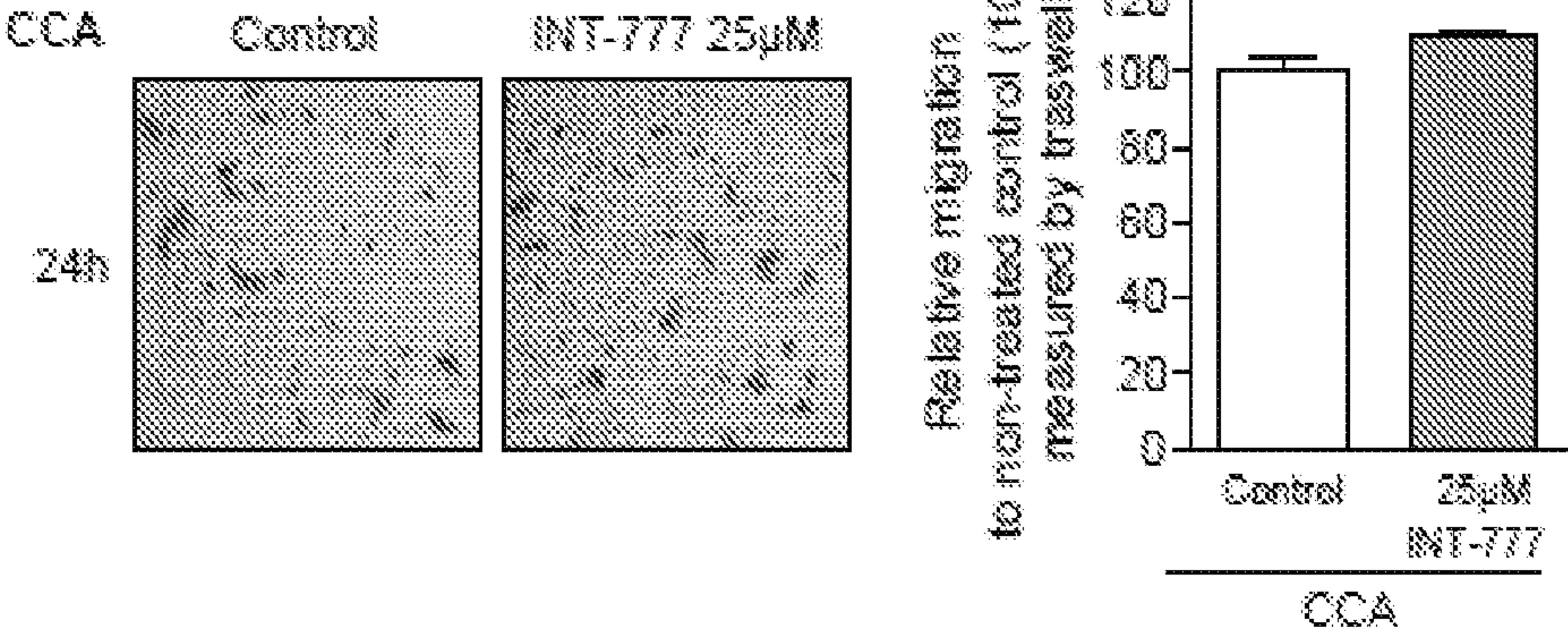
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Figure 16B

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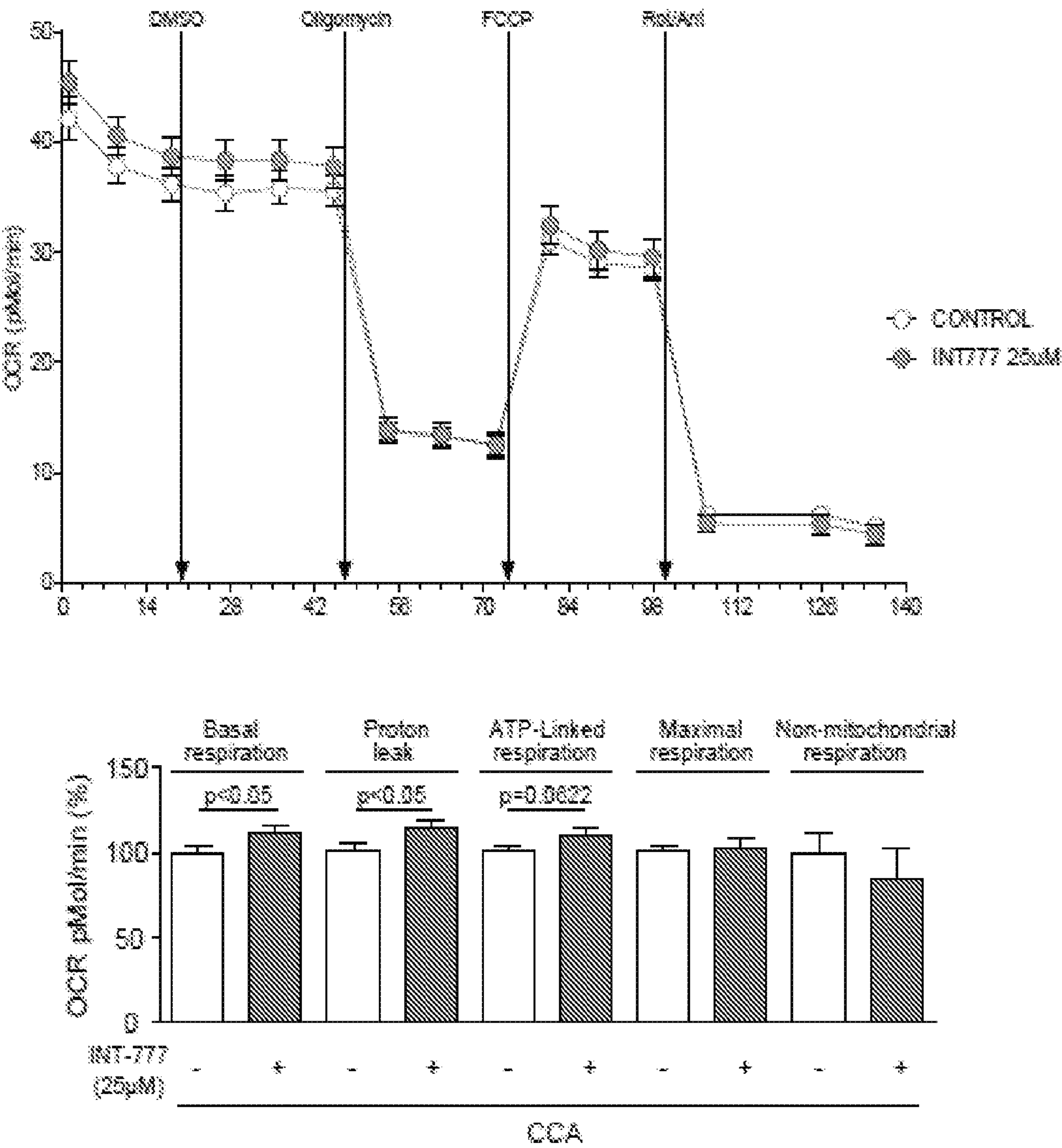


Figure 16C

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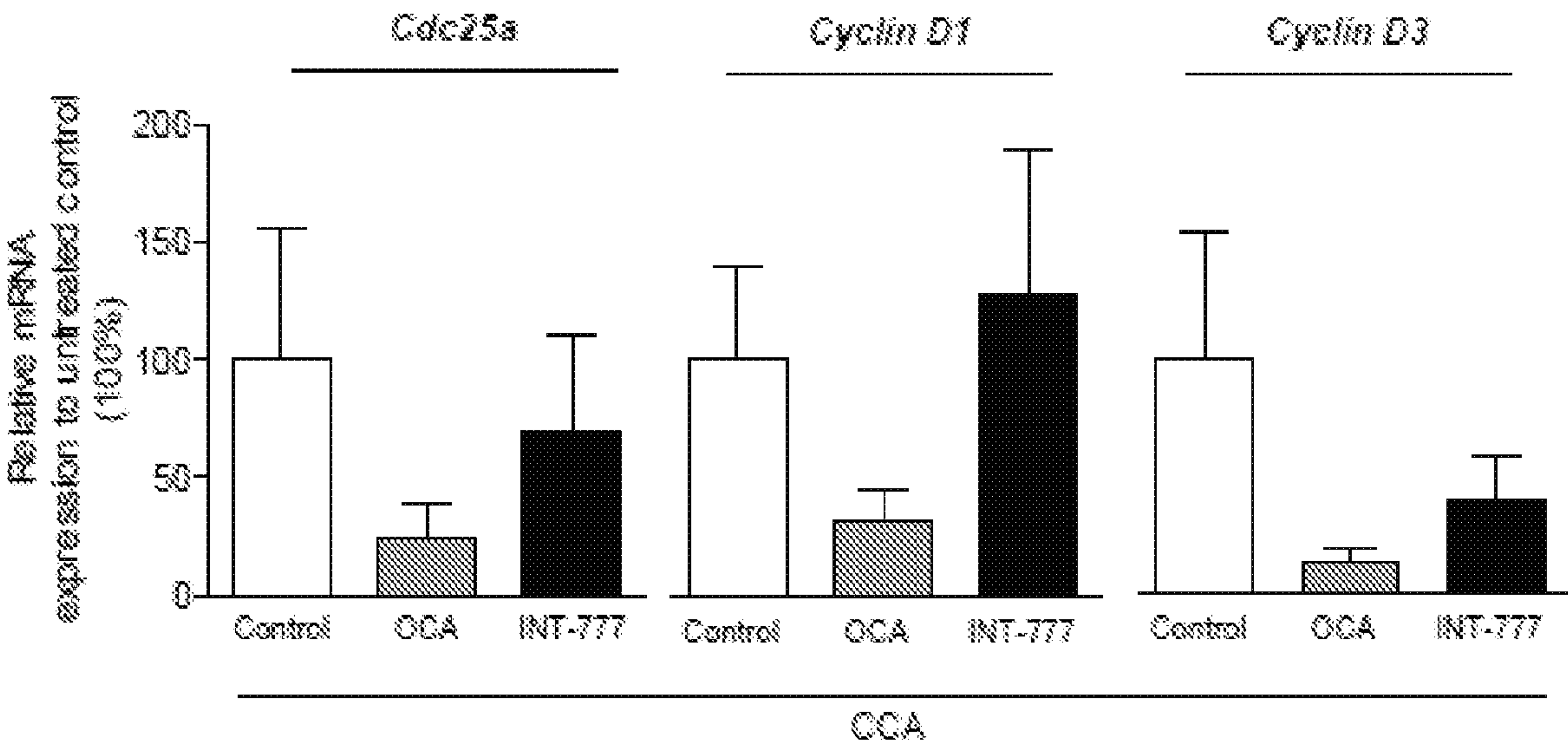


Figure 17

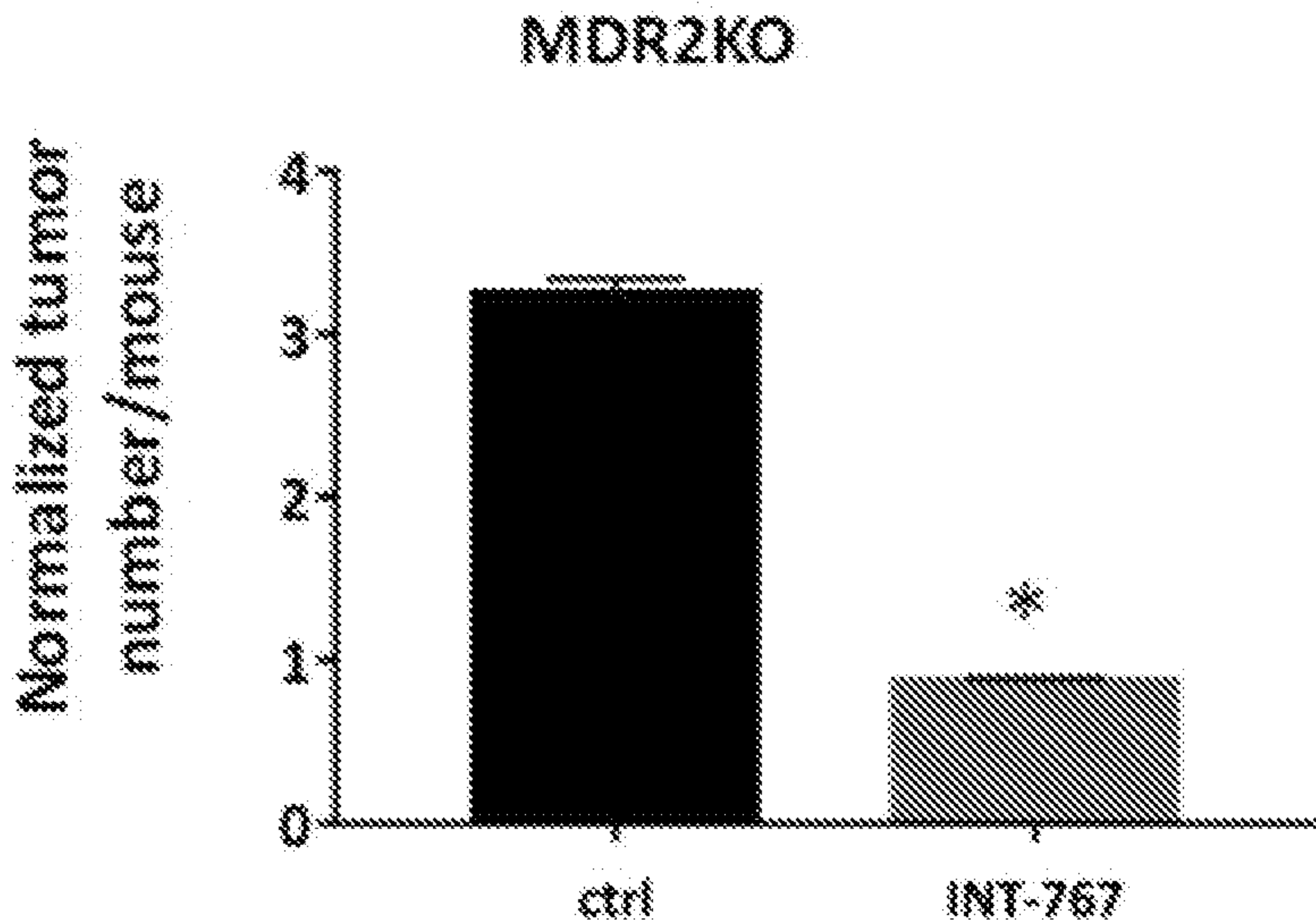


Figure 1A