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Compositions and methods for the treatment of HIV are provided.



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COMPOSITIONS AND METHODS FOR THE TREATMENT OF HIV

5

This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application No. 61/513,200, filed July 29, 2011. The foregoing application is incorporated by reference herein.

FIELD OF THE INVENTION

The present invention relates to the field of HIV therapy. More specifically, the invention provides methods and compositions for the treatment, inhibition, prevention, and/or cure of an HIV infection.

BACKGROUND OF THE INVENTION

One persistent obstacle to treating HIV infection is the ability of virus to enter a latent state, inaccessible to current antiretroviral (ARV) therapy. How HIV enters latency and remains latent is only partially understood. One theory is that changes in chromatin structure, such as histone deacetylation, suppress transcription from the HIV long terminal repeat (LTR), resulting in the establishment of latency. IL-7, prostratin, and histone deacetylase inhibitors have been tested to purge HIV from the latently infected reservoir. However, no appreciable decrease of the latent reservoir is observed when these drugs are combined with ARVs. In contrast to reactivating latent provirus, a drug that blocks cellular activation might reduce the initial burst of viremia and limit establishment of the latent viral reservoir. Cyclosporin A (CSA), an immunosuppressive agent, has been shown to suppress viral replication and

restore normal CD4 T-cell levels. However, adverse effects of CSA advocate against its use in HIV disease. Development of new approaches to purge the HIV reservoir and prevent establishment of latency are critical
5 therapeutic challenges.

SUMMARY OF THE INVENTION

In accordance with one aspect of the instant invention, methods for treating, inhibiting, and/or
10 preventing a retroviral infection (e.g., HIV) in an animal are provided. In a particular embodiment, the method comprises administering to the subject at least one BET antagonist/inhibitor (e.g., in a pharmaceutically acceptable carrier). In a particular embodiment, the BET
15 antagonist is a Brd4 antagonist. In yet another embodiment, the BET antagonist is a small molecule, particularly one that specifically binds the acetylated lysine recognition pocket/binding site of the BET (see, e.g., Filippakopoulos et al. (2010) Nature 468:1067-
20 1073). The methods may further comprise the administration of at least one additional anti-retroviral (e.g., an anti-HIV) compound before, after, and/or simultaneously with the BET antagonist(s).

In accordance with another aspect of the instant
25 invention, methods for inhibiting and/or preventing a retroviral (e.g., HIV) infection are provided. In a particular embodiment, the method comprises delivering to a cell at least one BET antagonist. In a particular embodiment, the BET antagonist is a Brd4 antagonist. In
30 yet another embodiment, the BET antagonist is a small molecule, particularly one that specifically binds the acetylated lysine recognition pocket of the BET. The methods may further comprise the delivery of at least one

anti-retroviral (e.g., an anti-HIV) compound before, after, and/or simultaneously with the BET antagonist(s).

In accordance with still another aspect of the instant invention, methods for inducing retroviral (e.g.,
5 HIV) reactivation are provided. In a particular embodiment, the method comprises delivering (e.g., contacting) at least one BET antagonist to a cell which is latently infected. In a particular embodiment, the BET antagonist is a Brd4 antagonist. In yet another
10 embodiment, the BET antagonist is a small molecule, particularly one that specifically binds the acetylated lysine recognition pocket of the BET. The methods may further comprise the delivery of at least one additional anti-retroviral (e.g., an anti-HIV) compound before,
15 after, and/or simultaneously with the BET antagonist(s).

According to another aspect of the instant invention, a composition comprising at least one BET antagonist, at least one additional anti-retroviral (e.g., an anti-HIV) and/or activation agent, and at least
20 one pharmaceutically acceptable carrier are provided.

BRIEF DESCRIPTIONS OF THE DRAWING

Figure 1 provides graphs demonstrating that JQ1 inhibits HIV infection in primary CD4 T-cells. Primary
25 CD4 T-cells were activated with anti-CD3/28, infected with NL43, SG3, BAL1, or BL2 (Fig. 1A) or NL43 (Figs. 1B and 1C), and cultured in the presence of the BET antagonist JQ1(+) or the control enantiomer JQ1(-). Figure 1A provides the expression of p24Gag in cells as
30 determined by flow cytometry. Figures 1B and 1C show viral production as measured in culture supernatants by ELISA. Figure 1C shows viral production with varying amounts of JQ1(+), JQ1(-), or the carrier dimethyl sulfoxide (DMSO).

Figure 2A shows that JQ1 drives activation of the HIV LTR. CEMss LTR-GFP cells were infected with NL43 and cultured in the presence of the BET antagonists JQ1(+) (1 μ M) or the control enantiomer JQ1(-). GFP expression in
5 cells was examined by flow cytometry. A representative of three experiments is shown. Figure 2B provides a graph showing GFP expression in cells when cultured with different amounts of JQ1, as determined by flow cytometry. 0.25 μ M of JQ1(+) was sufficient to
10 dramatically increase the expression of GFP in NL43 infected CEMss LTR-GFP cells. Figures 2C and 2D show % of GFP expression (Fig. 2C) and mean fluorescence of GFP (MFI; Fig. 2D) in CEMss LTR-GFP cells cultured in the presence of JQ1(+) or JQ1(-) (1 μ M), and different amount
15 of HIV-1 Tat.

Figure 3A shows that JQ1(+) drives reactivation of the HIV LTR in a latently infected cell line. The latently infected cell line, J-Lat clone A2, was reactivated with TNF α or PHA in the presence of inactive
20 JQ1(-) or active JQ1(+) (0.5 μ M) for 16 hours. GFP expression was measured by flow cytometry. Figures 3B and 3C shown the % cells positive for GFP (Fig. 3B) and the mean MFI of GFP (Fig. 3C) in latently infected cell lines, J-Lat clones 82, A72, A7, A2, and A1, at 16 hours
25 after TNF α or PHA was added in the cultures in the presence of JQ1(+) or JQ1(-).

Figure 4 provides the chemical structures of GSK525762A (I-BET) (Figure 4A), JQ1 (tert-butyl 2-((6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-
30 f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate) (Figure 4B), and GW841819X (Figure 4C).

Figure 5A shows p24 levels per million cells of primary resting CD4 T-cells spinoculated with HIV-1 NL43 and then cultured in the presence of 1 μ M of JQ1(+) or

JQ1(-) and, optionally, IL-7 (20 ng/ml; at day 3 after infection). A representative of three experiments is shown. Figure 5B shows p24 levels per million cells of primary resting CD4 T-cells spinoculated with SG3 or
5 SG3ΔVif (SG3dVif) and then cultured in the presence of 1 μM of JQ1(+) or JQ1(-) and, optionally, IL-7 (20ng/ml; at day 3 after infection). AZT (100 μM) was added at day 1 after infection to prevent the spreading infection. The level of p24 in the media was measured by ELISA at day 3
10 (d3) and day 5 (d5) post-infection.

Figure 6A provides a graph of p24Gag levels at various time points after the infection of CEMss LTR-GFP cells with NL43, which were cultured in the presence of JQ1(+) (1 μM) or JQ1(-). A representative of three
15 experiments is shown. Figure 6B provides a graph of the ratio of % of P24⁺ cells in the GFP⁺ population. A representative of 2 experiments is shown. Figure 6C shows GFP versus p24 staining of cells 3 days after infection.

20

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods to treat, control, and/or cure retroviral infections using antagonists (e.g., cell-permeable small molecules) of
25 cellular BET (Bromodomains and Extra Terminal) protein family. HIV Vif (viral infectivity factor) interacts with two cellular proteins, Brd4 and Cdk9, to drive cells out of G1 into the S phase of the cell cycle (Wang et al. (2011) Blood 117:1260-1269). Cdk9 is a component of the
30 heterodimer p-TEFb (positive transcription elongation factor b). Brd4, a BET family member, acts by binding to acetylated lysines on histones and other nuclear factors to regulate gene expression. Brd4 recruits p-TEFb to promoters of active genes, altering gene transcription

and thus stimulating progression from G1 to S. The HIV Tat protein also recruits p-TEFb to the viral LTR, a crucial step for efficient transcriptional elongation of HIV. Overexpression of Brd4 disrupts the interaction
5 between Tat and p-TEFb and suppresses the ability of Tat to transactivate the HIV promoter (Bisgrove et al. (2007) PNAS 104:13690-13695).

The anti-HIV activity of BET antagonists is shown hereinbelow. Specifically, it is shown that BET
10 antagonism inhibits viral replication and drives activation of the HIV LTR. The BET antagonists displace BET from chromatin by competitively binding to their acetylated lysine binding sites. As such, antagonism (e.g., with small molecule antagonists) of Brd4 and other
15 BET proteins controls and cures an HIV infection. Based on work in other systems (e.g., bone marrow-derived macrophages), BET antagonists such as JQ1 may also inhibit inflammatory cytokine expression by activated T-cells in the presence or absence of infection.
20 Accordingly, without being bound by theory, both effects of BET antagonism (i.e., inhibition of cytokine expression and the disruption of Brd4 function) may lead to the inhibition of viral replication.

The present invention encompasses methods for
25 preventing, inhibiting, and/or treating a retroviral infection in an animal. In a particular embodiment, the method comprises administering at least one composition comprising at least one BET antagonist and at least one pharmaceutically acceptable carrier to an animal. The
30 animal may be a mammal, particularly a primate or human. In a particular embodiment, the retrovirus is a lentivirus. Lentiviruses include, without limitation, human immunodeficiency virus (HIV) (e.g., HIV-1, HIV-2), bovine immunodeficiency virus (BIV), feline

immunodeficiency virus (FIV), simian immunodeficiency virus (SIV), and equine infectious anemia virus (EIA). The methods may further comprise the delivery of at least one anti-retroviral (e.g., an anti-HIV) compound before, 5 after, and/or simultaneously with the BET antagonist(s). The methods may further comprise the delivery of at least one other activation (e.g., HIV activation) agent (e.g., protein kinase C activators (e.g., prostratin, bryostatin), histone deacetylase inhibitors (e.g., 10 trichostatin A, valproic acid, sodium butyrate, vorinostat, romidepsin), TNF α , PHA, Tat, and/or IL-7).

The instant invention also encompasses methods for inhibiting and/or preventing a retroviral (e.g., HIV) infection, replication, and/or viral production. In a 15 particular embodiment, the methods comprise delivering to a cell at least one BET antagonist (e.g., prior to or simultaneously with a retrovirus). The methods may further comprise the delivery of at least one anti-retroviral (e.g., an anti-HIV) compound before, after, 20 and/or simultaneously with the BET antagonist(s). The methods may further comprise the delivery of at least one other activation agent. The methods may be performed *in vitro* (e.g., cell culture) or *in vivo*.

The instant invention also encompasses methods for 25 inducing retroviral (e.g., HIV) activation. In a particular embodiment, the method comprises delivering at least one BET antagonist to a cell which is latently infected with a retrovirus. The methods may be performed *in vitro* (e.g., cell culture) or *in vivo*. The methods 30 may further comprise the delivery of at least one other activation agent. The methods may also be used to screen for HIV therapeutic agents (e.g., those with synergistic effects with the BET antagonist) by further contacting the cells with a test compound and monitoring retrovirus

levels/clearance (e.g., compared to in the absence of the test compound).

BET antagonists of the instant invention disrupt the function of BET proteins (e.g., Brd2, Brd3, Brd4). In a particular embodiment, the BET antagonist specifically
5 disrupts the function of Brd4 (bromodomain containing protein 4; see, e.g., Gene ID: 23476). In a particular embodiment, the BET antagonist specifically binds the acetylated lysine (e.g., of acetylated histones)
10 recognition pocket (e.g., within the bromodomain). In a particular embodiment, the BET antagonist is a small molecule inhibitor. Three synthetic BET antagonists that have been recently developed are JQ1, I-BET, and GW841819X (Nicodeme et al. (2010) Nature 468:1119-1123;
15 Filippakopoulos et al. (2010) Nature 468:1067-1073; Gamsjaeger et al. (2011) Mol. Cell. Biol., 31:2632-2640). In another embodiment, the BET antagonist is an inhibitory nucleic acid molecule, such as an antisense or siRNA (see, e.g., brd4 siRNA from Dharmacon (Chicago,
20 IL); brd4 siRNA from Chung et al., J. Med. Chem. (2011) 54:3827-3838 (page S20); and human analogs of the mouse brd4 siRNA provided in Nicodeme et al. (2010) Nature 468:1119-1123). Examples of small molecule BET antagonists include, without limitation, JQ1 (Figure 4),
25 I-BET (GSK525762A) (Figure 4), GW841819X (Figure 4; Chung et al., J. Med. Chem. (2011) 54:3827-3838), thienotriazolodiazepine compounds, those provided in U.S. Patent Application Publication No. 2010/0286127 (including, e.g., those of Formula (I) and compounds 1-
30 18), derivatives of the compounds of Formula (I) in U.S. Patent Application Publication No. 2010/0286127 (e.g., wherein R₃ is hydrogen, wherein the thiophene ring is replaced with a six-membered ring (e.g., an aryl or benzyl group, optionally substituted with one or more R₁

and/or R_2 groups), and/or wherein R_4 is $-(CH_2)_a-CO-O-R_9)$, and pharmaceutically acceptable salts thereof. In a particular embodiment, the BET antagonist is JQ1 or I-BET. In a particular embodiment, the BET antagonist is the JQ1(+) enantiomer. The JQ1(+) enantiomer need not be enantiomerically pure and may be in mixture with the JQ1(-) enantiomer, though it is preferable to have a composition comprising substantially pure JQ1(+) enantiomer.

10 The compositions of the instant invention can be administered to an animal, in particular a mammal, more particularly a human, in order to treat/inhibit/prevent a retroviral infection (e.g., the composition may be administered before, during, or after a retroviral infection). The pharmaceutical compositions of the instant invention may also comprise at least one other antiretroviral agent (e.g., an anti-HIV agent). The additional antiretroviral agent may also be administered in a separate composition from the BET antagonists of the instant invention. The compositions may be administered at the same time or at different times (e.g., sequentially (e.g., the BET antagonists may be administered prior to, after, or simultaneously with the antiretroviral agents)). In a particular embodiment of the instant invention, the BET antagonists are administered with (e.g., before, during, and/or after) highly active antiretroviral therapy (HAART).

The compositions of the instant invention may be administered, in a therapeutically effective amount, to a patient in need thereof. The pharmaceutical compositions of the present invention can be administered by any suitable route, for example, by injection (e.g., parenteral, intramuscular, intravenous, or intraperitoneal administration), by oral, pulmonary

(e.g., intratraeentially), nasal, topical, or other modes of administration such as controlled release devices. In general, pharmaceutical compositions and carriers of the present invention comprise, among other things,

5 pharmaceutically acceptable diluents, preservatives, stabilizing agents, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions can include diluents of various buffer content (e.g., saline, Tris HCl, acetate, phosphate), pH and ionic strength; and additives

10 such as detergents and solubilizing agents (e.g., Tween™ 80, Polysorbate 80), anti oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol). The compositions can be incorporated into

15 particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc., or into liposomes. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of components of a pharmaceutical

20 composition of the present invention. Exemplary pharmaceutical compositions and carriers are provided, e.g., in "Remington's Pharmaceutical Sciences" by E.W. Martin (Mack Pub. Co., Easton, PA) and "Remington: The Science and Practice of Pharmacy" by Alfonso R. Gennaro

25 (Lippincott Williams & Wilkins) which are herein incorporated by reference. The pharmaceutical composition of the present invention can be prepared, for example, in liquid form, aerosolized form, or can be in pill or dried powder form (e.g., lyophilized). The

30 concentration of the agents in the chosen medium may be varied and the medium may be chosen based on the desired route of administration of the pharmaceutical preparation. Except insofar as any conventional media or agent is incompatible with the agents to be administered,

its use in the pharmaceutical preparation is contemplated.

In yet another embodiment, the pharmaceutical compositions of the present invention can be delivered in
5 a controlled release system, such as using an intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In a particular embodiment, a pump may be used (see Medical Applications of Controlled Release, Langer and Wise
10 (eds.), CRC Press; Sefton, CRC Crit. Ref. Biomed. Eng. (1987) 14:201; Buchwald et al., Surgery (1980) 88:507; Saudek et al., N. Engl. J. Med. (1989) 321:574). In another embodiment, polymeric materials may be employed (see Medical Applications of Controlled Release, Langer
15 and Wise (eds.), CRC Press; Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley: New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. (1983) 23:61; see also Levy et al., Science (1985) 228:190;
20 During et al., Ann. Neurol. (1989) 25:351; Howard et al., J. Neurosurg. (1989) 71:105).

Pharmaceutical compositions containing an agent of the present invention as the active ingredient in intimate admixture with a pharmaceutically acceptable
25 carrier can be prepared according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous.

30 A pharmaceutical preparation of the invention may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to a physically discrete unit of the pharmaceutical preparation appropriate for the patient

undergoing treatment. Each dosage should contain a quantity of active ingredient calculated to produce the desired effect in association with the selected pharmaceutical carrier. Procedures for determining the appropriate dosage unit are well known to those skilled in the art. Dosage units may be proportionately increased or decreased based on the weight of the patient. Appropriate concentrations for alleviation of a particular pathological condition may be determined by dosage concentration curve calculations, as known in the art. The dosage ranges for the administration of the compositions of the invention are those large enough to produce the desired effect (e.g., curing, relieving, treating, and/or preventing the disease, disorder, or infection and/or the symptoms associated therewith). The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counter indications.

In accordance with the present invention, the appropriate dosage unit for the administration of compositions of the instant invention may be determined by evaluating the toxicity of the molecules or cells in animal models. Various concentrations of agents in pharmaceutical preparations may be administered to animals, and the minimal and maximal dosages may be determined based on the beneficial results and side effects observed as a result of the treatment. Appropriate dosage unit may also be determined by assessing the efficacy of the agent treatment in combination with other standard drugs. The dosage units

of the compositions may be determined individually or in combination with each treatment according to the effect detected.

The pharmaceutical preparation comprising the agents
5 of the instant invention may be administered at appropriate intervals, for example, at least once or twice a day or more. The appropriate interval in a particular case would normally depend on the condition of the patient.

10

Definitions

The term "substantially pure" refers to a preparation comprising at least 50-60% by weight of a given material (e.g., nucleic acid, oligonucleotide,
15 protein, peptide, etc.). More preferably, the preparation comprises at least 75% by weight, and most preferably 90-95% by weight of the given compound. Purity is measured by methods appropriate for the given compound (e.g., chromatographic methods, agarose or
20 polyacrylamide gel electrophoresis, HPLC analysis, and the like).

The term "isolated" is not meant to exclude artificial or synthetic mixtures with other compounds or materials, or the presence of impurities that do not
25 interfere with the fundamental activity, and that may be present, for example, due to incomplete purification, or the addition of stabilizers.

"Pharmaceutically acceptable" indicates approval by a regulatory agency of the Federal or a state government
30 or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term "treat" as used herein refers to any type of treatment that imparts a benefit to a patient

afflicted with a disease, including improvement in the condition of the patient (e.g., in one or more symptoms), delay in the progression of the condition, etc. In a particular embodiment, the treatment of a retroviral
5 infection results in at least an inhibition/reduction in the number of infected cells.

The phrase "effective amount" refers to that amount of therapeutic agent that results in an improvement in the patient's condition.

10 The term "inhibit" in the context of inhibiting an HIV infection may refer to the reduction in the incidence of or the symptoms of the HIV infection being treated or the presence or extent of the HIV infection being treated.

15 As used herein, the term "prevent" refers to the prophylactic treatment of a subject who is at risk of developing a condition resulting in a decrease (e.g., at least a significant decrease) in the probability that the subject will develop the condition.

20 A "carrier" refers to, for example, a diluent, adjuvant, excipient, auxiliary agent or vehicle with which an active agent of the present invention is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those
25 of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions.

30 Suitable pharmaceutical carriers are described in, e.g., "Remington's Pharmaceutical Sciences" (Ed. Gennaro; Mack Publishing, Easton, PA) and "Remington: The Science and Practice of Pharmacy" (Ed. Troy; Lippincott Williams & Wilkins, Baltimore, MD).

As used herein, the term "small molecule" refers to a substance or compound that has a relatively low molecular weight (e.g., less than 4,000, less than 2,000, particularly less than 1 kDa or 800 Da). Typically,
5 small molecules are organic, but are not proteins, polypeptides, or nucleic acids, though they may be amino acids or dipeptides.

As used herein, an "anti-HIV compound" is a compound which inhibits HIV. Examples of an anti-HIV compound
10 include, without limitation:

(I) nucleoside-analog reverse transcriptase inhibitors (NRTIs; e.g., AZT (zidovudine, RETROVIR®), lamivudine (3TC, EPIVIR®), emtricitabine (EMTRIVA®), dideoxycytidine (ddC, zalcitabine, HIVID®), 2',3'-
15 dideoxyinosine (ddI, VIDEX®), tenofovir DF (VIREAD®), stavudine (d4T, ZERIT®), abacavir (1592U89; ZIAGEN®), adefovir dipivoxil (bis(POM)-PMEA; PREVON®), lobucavir (BMS-180194), BCH-10652, emitricitabine, elvucitabine, and lodenosine (FddA; 2'-beta-fluoro-2',3'-
20 dideoxyadenosine)), trizivir (abacavir, zidovudine, and lamivudine),

(II) non-nucleoside reverse transcriptase inhibitors (NNRTIs; e.g., delavirdine (BHAP, U-90152; RESCRIPTOR®), efavirenz (DMP-266, SUSTIVA®), nevirapine (VIRAMUNE®),
25 PNU-142721, capravirine (S-1153, AG-1549), emivirine (+)-calanolide A (NSC-675451) and B, etravirine (TMC-125), DAPY (TMC120), BILR-355 BS, PHI-236, and PHI-443 (TMC-278)),

(III) protease inhibitors (PIs; e.g., amprenavir
30 (141W94, AGENERASE®), tipranavir (PNU-140690, APTIVUS®), indinavir (MK-639; CRIXIVAN®), saquinavir (INVIRASE®, FORTOVASE®), fosamprenavir (LEXIVA®), lopinavir (ABT-378), ritonavir (ABT-538, NORVIR®), atazanavir (REYATAZ®), nelfinavir (AG-1343, VIRACEPT®), lasinavir

(BMS-234475/CGP-61755), BMS-2322623, GW-640385X (VX-385), AG-001859, and SM-309515), Kaletra™ (lopinavir and ritonavir), and

(**IV**) fusion inhibitors (FIs; e.g., T-20 (DP-178, 5 FUZEON®) and T-1249).

As used herein, the term "nucleoside-analog reverse transcriptase inhibitors" (NRTIs) refers to nucleosides and nucleotides and analogues thereof that inhibit the activity of HIV-1 reverse transcriptase. As used herein, 10 NNRTIs are allosteric inhibitors which bind reversibly at a nonsubstrate-binding site on the HIV reverse transcriptase, thereby altering the shape of the active site or blocking polymerase activity. As used herein, the term "protease inhibitor" refers to inhibitors of the 15 HIV-1 protease. As used herein, "fusion inhibitors" are compounds, such as peptides, which act by binding to HIV envelope protein and blocking the structural changes necessary for the virus to fuse with the host cell.

Anti-HIV compounds also include HIV vaccines such 20 as, without limitation, ALVAC® HIV (vCP1521), AIDSVAX®B/E (gp120), and combinations thereof. Anti-HIV compounds also include HIV antibodies (e.g., antibodies against gp120 or gp41 (e.g., VCR01 (Zhou et al. (Science (2010) 329:811-7), PG9 and PG16 (Doores et al. (J. Virol. (2010) 25 84:10510-21), and see also Walker et al. (Science (2009) 326:285-9), particularly broadly neutralizing antibodies. Other anti-HIV agents include, without limitation, recombinant soluble CD4 (rsCD4), an anti-CD4 antibody (e.g., from Tanox, Inc.), an anti-CCR5 antibody (e.g., 30 Pro 140); a CXCR4 blocker (e.g., AMD 3100), an HIV entry inhibitor (e.g., Pro-542; Progenies), and a CCR5 blocker (e.g., SCH-C, SCH-D; Schering Plough).

As used herein, the term "highly active antiretroviral therapy" (HAART) refers to HIV therapy

with various combinations (e.g., at least three) of
therapeutics such as nucleoside reverse transcriptase
inhibitors, non-nucleoside reverse transcriptase
inhibitors, HIV protease inhibitors, and fusion
5 inhibitors. For example, HAART may include two reverse
transcriptase inhibitors and a protease inhibitor.

As used herein, the terms "activation agent" of "HIV
activation agent" refer to compounds that stimulate
proviral latent DNA to begin transcription, replication,
10 and/or production of infectious virus and/or cell surface
antigens.

The phrase "small, interfering RNA (siRNA)" refers
to a short (typically less than 30 nucleotides long,
particularly 12-30 or 20-25 nucleotides in length) double
15 stranded RNA molecule. Typically, the siRNA modulates
the expression of a gene to which the siRNA is targeted.
Methods of identifying and synthesizing siRNA molecules
are known in the art (see, e.g., Ausubel et al. (2006)
Current Protocols in Molecular Biology, John Wiley and
20 Sons, Inc). As used herein, the term siRNA may include
short hairpin RNA molecules (shRNA). Typically, shRNA
molecules consist of short complementary sequences
separated by a small loop sequence wherein one of the
sequences is complimentary to the gene target. shRNA
25 molecules are typically processed into an siRNA within
the cell by endonucleases. Exemplary modifications to
siRNA molecules are provided in U.S. Application
Publication No. 20050032733. Expression vectors for the
expression of siRNA molecules preferably employ a strong
30 promoter which may be constitutive or regulated. Such
promoters are well known in the art and include, but are
not limited to, RNA polymerase II promoters, the T7 RNA
polymerase promoter, and the RNA polymerase III promoters

U6 and H1 (see, e.g., Myslinski et al. (2001) Nucl. Acids Res., 29:2502-09).

“Antisense nucleic acid molecules” or “antisense oligonucleotides” include nucleic acid molecules (e.g.,
5 single stranded molecules) which are targeted (complementary) to a chosen sequence (e.g., to translation initiation sites and/or splice sites) to inhibit the expression of a protein of interest. Such antisense molecules are typically between about 15 and
10 about 50 nucleotides in length, more particularly between about 15 and about 30 nucleotides, and often span the translational start site of mRNA molecules. Antisense constructs may also be generated which contain the entire sequence of the target nucleic acid molecule in reverse
15 orientation. Antisense oligonucleotides targeted to any known nucleotide sequence can be prepared by oligonucleotide synthesis according to standard methods.

20 The following example describes illustrative methods of practicing the instant invention and is not intended to limit the scope of the invention in any way.

EXAMPLE

25 It is shown herein that HIV infections can be inhibited, controlled, and/or cured using cell-permeable small molecule antagonists of cellular BET family proteins. It is demonstrated hereinbelow that BET antagonism inhibits virus production (Figure 1) and
30 drives activation of the HIV LTR (Figures 2 & 3).

BET antagonists inhibit viral infection in primary activated CD4 T-cells. Specifically, the effect of the BET antagonist JQ1(+) on viral replication was tested in HIV-infected T-cells. Primary activated CD4 T-cells were

infected with X4-tropic virus (NL43 or SG3) or R5-tropic virus (BAL1 or BL2) in the presence or absence of JQ1(+) (1 μ M). Viral infection was measured by flow cytometry or ELISA at various days after infection. Levels of
5 infection were found to decrease in the presence of the BET antagonist, but not with DMSO or a control enantiomer JQ1(-) (Figure 1). JQ1 significantly inhibited HIV infection ($*p<0.05$) in primary CD4 T-cells at days 3 and 6 after infection (Figure 1B). The inhibition of viral
10 infection was also observed with 0.1 μ M of JQ1 (Fig. 1C). These data indicate that BET antagonists inhibit HIV infection.

BET antagonists also drive activation of the HIV LTR. Specifically, the effects of JQ1 on transcription
15 from the HIV LTR were tested. CEMss LTR-GFP cells (a reporter cell line stably transfected with GFP under the control of the HIV-1 LTR promoter) was infected with NL43, in the presence of BET antagonists or controls. GFP expression was measured at days 0, 1, 2, 3, and 6
20 post-infection. GFP expression was higher in the presence of the BET inhibitor (JQ1) compared to controls (Figure 2A). Further, by varying the amount of JQ1, it was determined that 0.25 μ M of JQ1(+) was sufficient to dramatically increase Tat-driven HIV-1 transcription
25 (Figure 2B). CEMss LTR-GFP cells were also cultured in the presence of JQ1(+) or JQ1(-) (1 μ M) and different amount of HIV-1 Tat (0 - 32 μ g/ml). Figures 2C and 2D show that 8 μ g/ml of HIV-1 Tat alone activated the express of GFP (Fig. 2C) while the mean fluorescence
30 intensities (MFI) of GFP was dramatically enhanced at 8, 16, or 32 μ g/ml of Tat in the presence of JQ1(+) (Fig. 2D).

Next, the effect of JQ1 on activation of the HIV LTR was tested using a latently infected Jurkat-derived cell

clone, J-Lat clone A2. GFP expression in J-Lat clone A2 is induced upon reactivation with tumor necrosis factor-alpha (TNF α) or phytohemagglutinin (PHA). It was determined that GFP expression (% positive cells and mean
5 fluorescence intensity) was higher in cells cultured with TNF α or PHA plus JQ1(+) than with the JQ1(-) control compound (Figure 3A), indicating that JQ1 drives reactivation of the HIV LTR in latently infected cells. Figures 3B and 3C also show that JQ1(+) drives the
10 reactivation of the HIV LTR in latently infected cell lines, J-Lat clones 82, A72, A7, A2, and A1, as determined by GFP expression (% positive cells (Fig. 3C) and mean fluorescence intensity (Fig. 3D)).

Figure 5 shows that JQ1(+) drives the reactivation
15 of the HIV LTR in latently infected primary resting CD4 T-cells. For Figure 5A, primary resting CD4 T-cells were purified and spinoculated with HIV-1 NL43 or mock control. The infected cells were then cultured in the presence of 1 μ M of JQ1(+) or JQ1(-). At day 3 after
20 infection, IL-7 (20 ng/ml) was added in some of the cultures. Cells were counted and cell supernatants were collected at day 5 after infection. The level of p24Gag in the media was measured by ELISA and p24 levels per million cells are shown in Fig. 5A. For Figure 5B,
25 primary CD4 T-cells were spinoculated with SG3 or SG3deltaVif and cultured in the presence of 1 μ M of JQ1(+) or JQ1(-). AZT (100 μ M) was added at day 1 after infection to prevent the spreading infection. IL7 (20 ng/ml) was added in some culture at day 3 after infection
30 and the level of p24 in the media was measured by ELISA at day 3 and day 5 post-infection. The data shown are summarized with triplicates. Figure 5B shows that JQ1(+) drives the reactivation of the HIV LTR in wild-type SG3

latently infected cells, but not in SG3deltaVif infected cells.

To determine if JQ1(+) affects viral integration in primary resting CD4 T-cells, the cells were purified and infected with HIV-1 NL43 or mock control. The infected cells were then cultured in the presence of 1 μ M of JQ1(+) or JQ1(-) for 48 hours. Cell pellets were collected at 0 hours and 48 hours after infection. DNA was isolated and the viral integration in resting CD4 T-cells was measured by Alu-PCR. As seen in Table 1, JQ1(+) does not significantly affect the viral integration in primary resting CD4 T-cells.

provirus/cell	T0h	T48h, JQ(-)	T48h, JQ(+)
Exp. 1	0.002 0.0002	0.527 0.064	0.559 0.005
Exp. 2	0	0.400 0.0009	0.444 0.120

Table 1

15

As seen in Figure 6, the Brd4 antagonist, JQ1(+), decreases the viral production of HIV-1 at the later time point of infection in activated primary CD4 T-cells or CEMss LTR-GFP cell lines. Without being bound by theory, this decrease in viral production may be due to 1) JQ1(+) causing G1 arrest in the cell cycle, resulting in cell death or affecting the cell fitness that contributes to the overall decreased viral production; and/or 2) JQ1(+) affecting other viral or host cellular proteins' transcriptions, thereby leading to the decreased packaging of viral particles.

Figure 6A shows that viral production was decreased in the presence of JQ1(+) at day 6 after infection. Specifically, CEMss LTR-GFP cells were infected with NL43

and cultured in the presence of BET antagonist, JQ1(+) (1 μ M) or a control JQ1 enantiomer, JQ1(-). At various time points after infection, cell supernatants were collected and the levels of p24Gag was measured by ELISA. Figures 5 6B and 6C show that the expression of p24Gag in the infected cells was decreased at day 3 after infection. The experiments were done as shown in Figure 6A and the cells were stained for the expressions of GFP and p24-PE at day 1, 2, 3, and 6 after infection. Figure 6B 10 provides a graph of the ratio of p24⁺ in the GFP+ population. A representative of two experiments is shown. Figure 6C shows a representative of GFP and p24-phycoerythrin (PE) staining at day 3 after infection.

15

A number of publications and patent documents are cited throughout the foregoing specification in order to describe the state of the art to which this invention pertains. The entire disclosure of each of these 20 citations is incorporated by reference herein.

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications 25 may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

What is claimed is:

1. A method for treating or inhibiting an HIV infection in a subject in need thereof, said method comprising administering to said subject a composition comprising at
5 least one bromodomain and extra-terminal (BET) protein antagonist and at least one pharmaceutical carrier.
2. A method for inducing HIV reactivation, said method comprising delivering at least one bromodomain and extra-
10 terminal (BET) protein antagonist to a cell which is latently infected with HIV.
3. A method for inhibiting the infection of a cell by HIV, said method comprising delivering at least one
15 bromodomain and extra-terminal (BET) protein antagonist to said cell.
4. The method of any one of claims 1 to 3, wherein said BET antagonist is a bromodomain containing protein 4
20 (Brd4) antagonist.
5. The method of any one of claims 1 to 3, wherein said BET antagonist is a small molecule.
- 25 6. The method of any one of claims 1 to 3, wherein said BET antagonist specifically binds the acetylated lysine binding domain of said BET.
7. The method of any one of claims 1 to 3, wherein said
30 BET antagonist is JQ1(+) or I-BET.
8. The method of any one of claims 1 to 3, further comprising the administration of at least one additional anti-HIV compound.

9. The method of claim 8, further comprising the administration of highly active antiretroviral therapy to said subject.

5

10. The method of any one of claims 1 to 3, further comprising the administration of at least one additional activation agent.

10 11. The method of claim 10, wherein said additional activation agent is selected from the group consisting of protein kinase C activators, histone deacetylase inhibitors, TNF α , PHA, Tat, and IL-7.

15 12. A composition comprising at least one bromodomain and extra-terminal (BET) protein antagonist and at least one additional anti-HIV compound and/or activation agent.

20

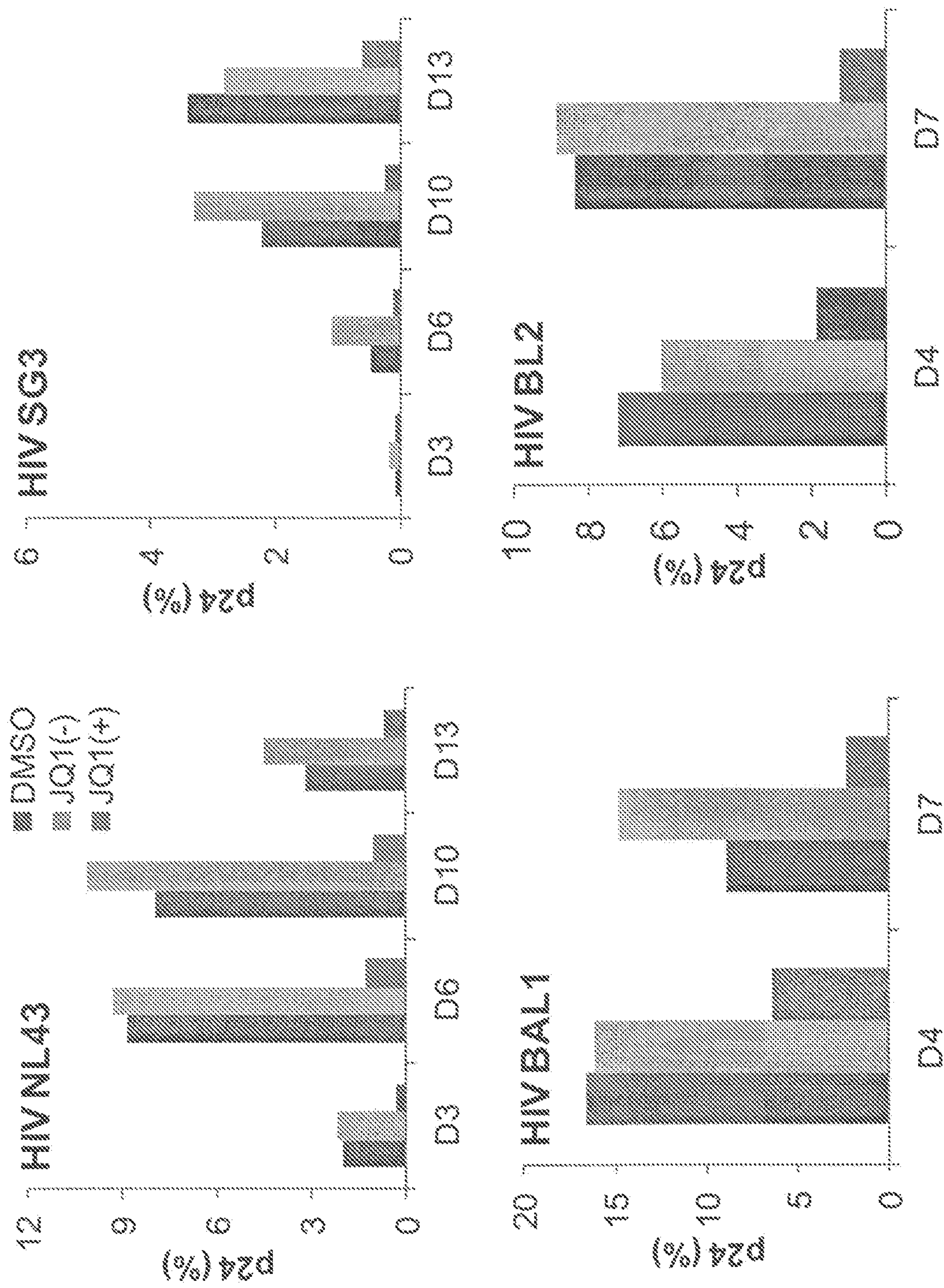


Figure 1A

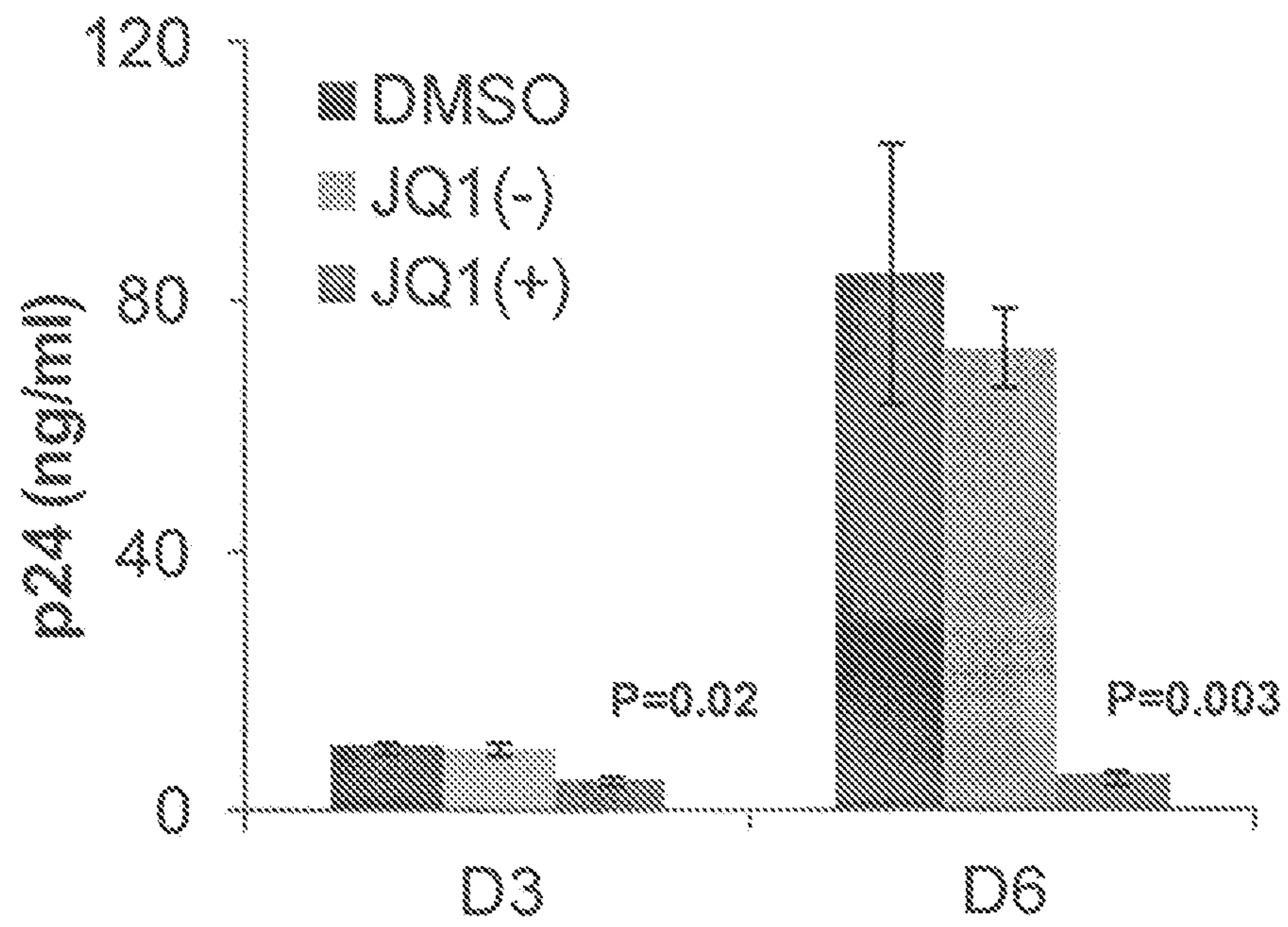


Figure 1B

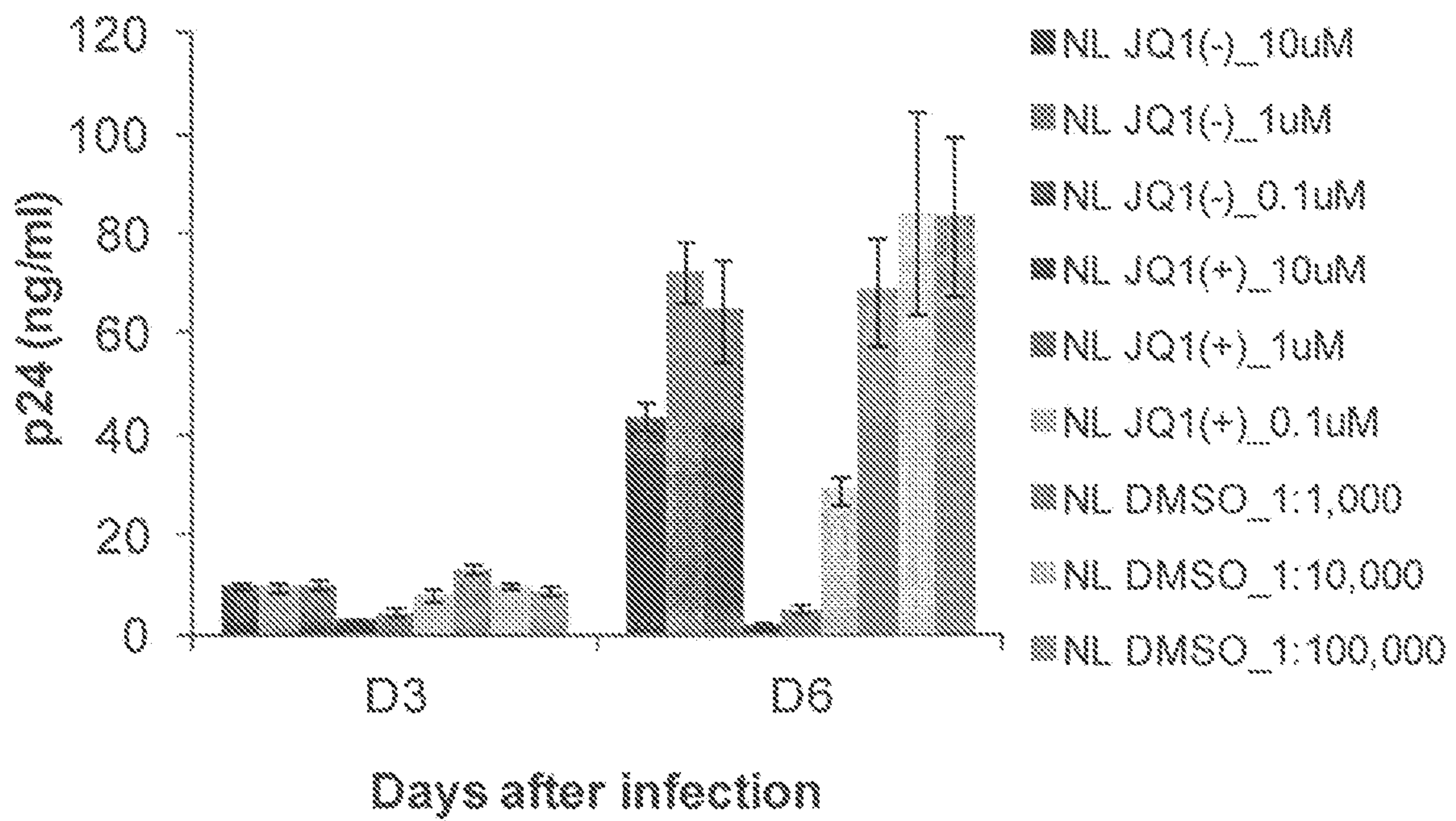


Figure 1C

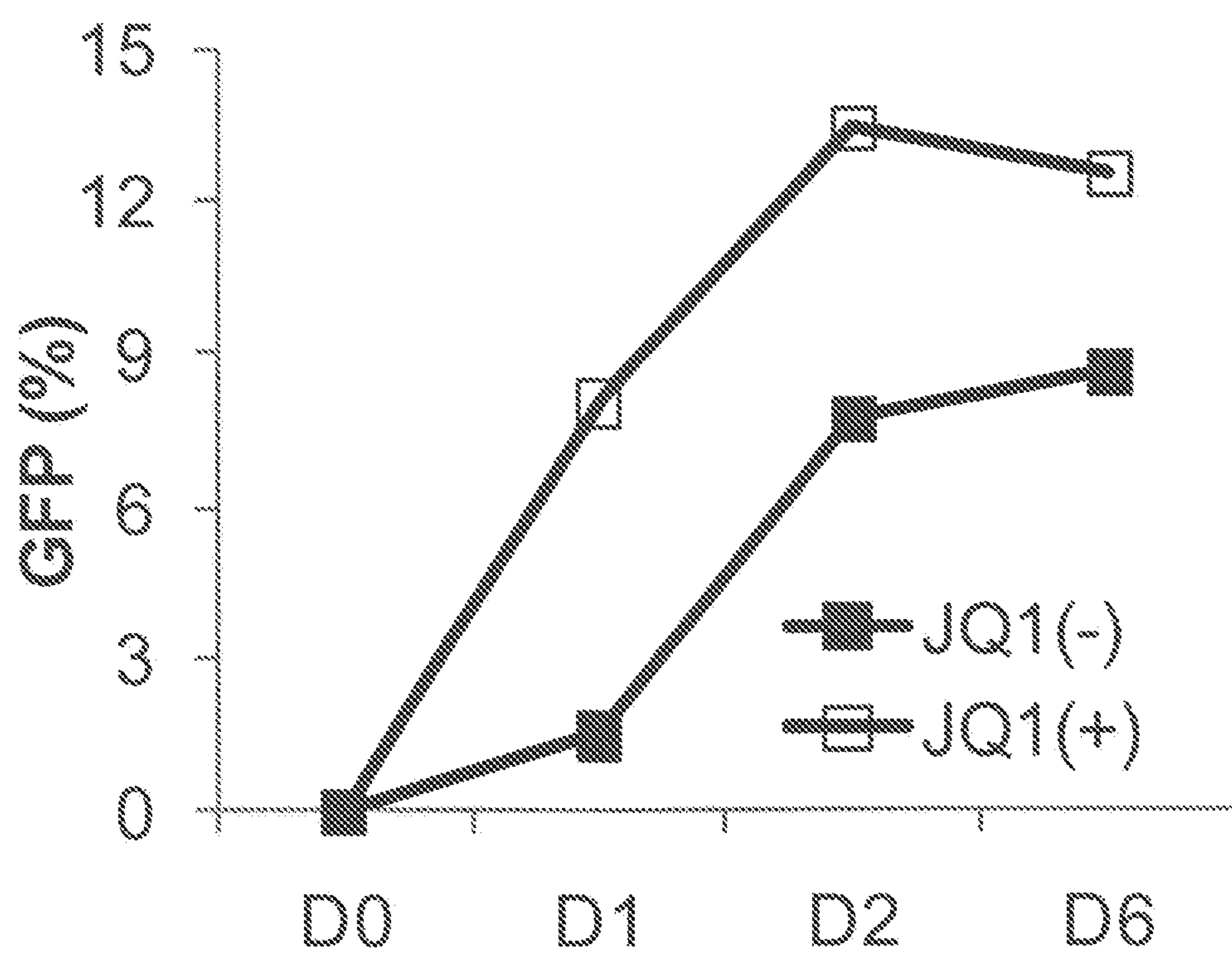


Figure 2A

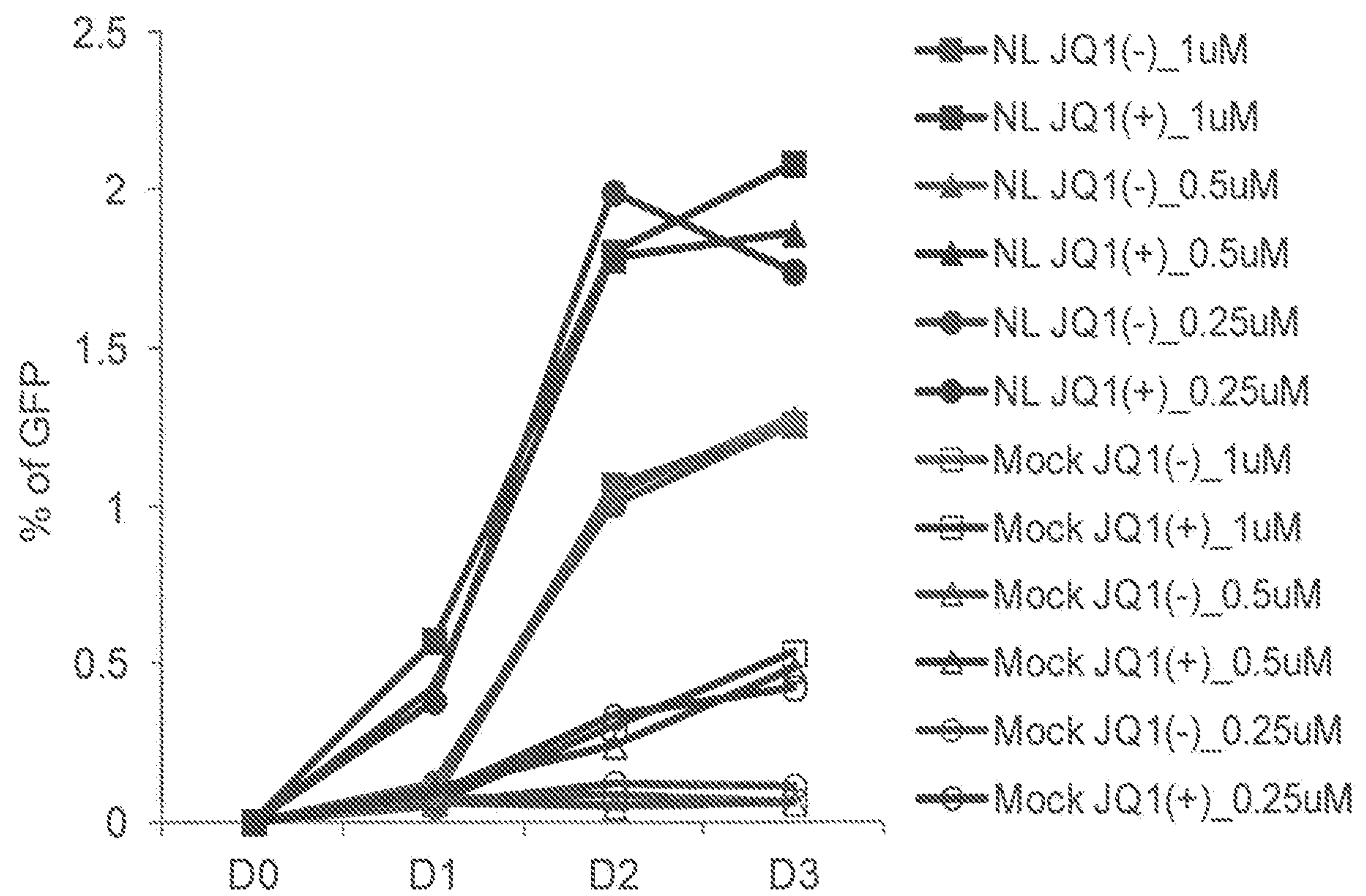


Figure 2B

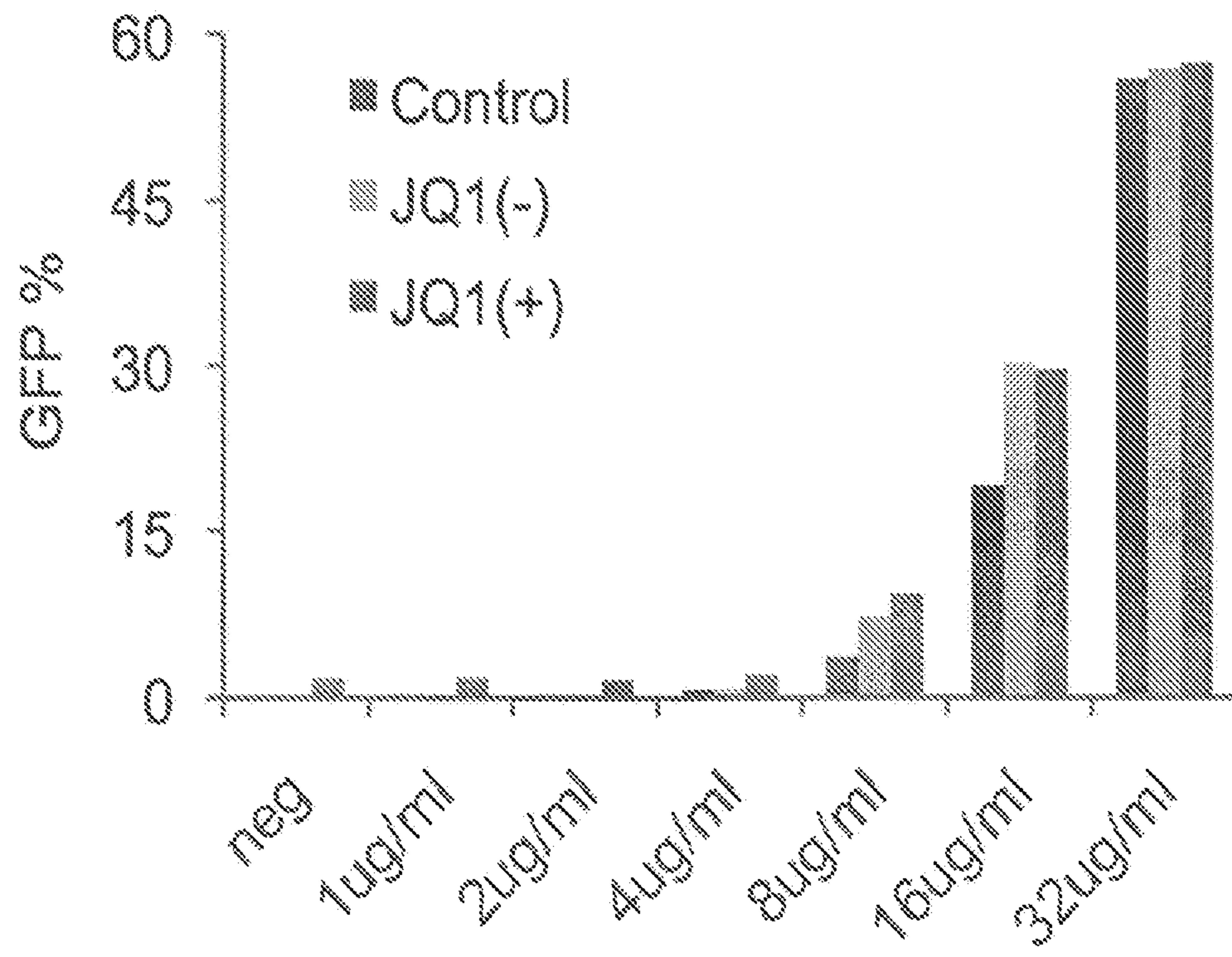


Figure 2C

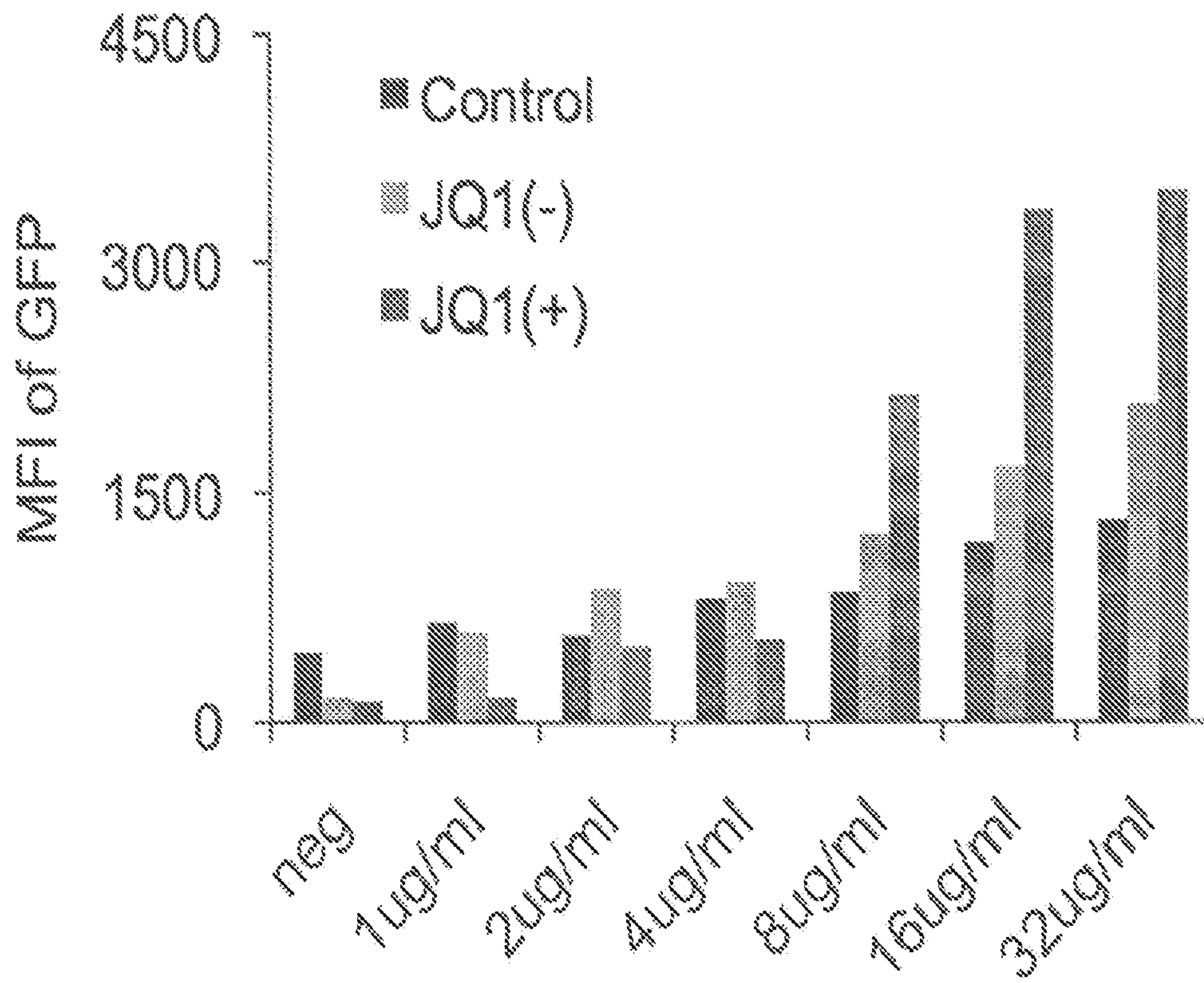


Figure 2D

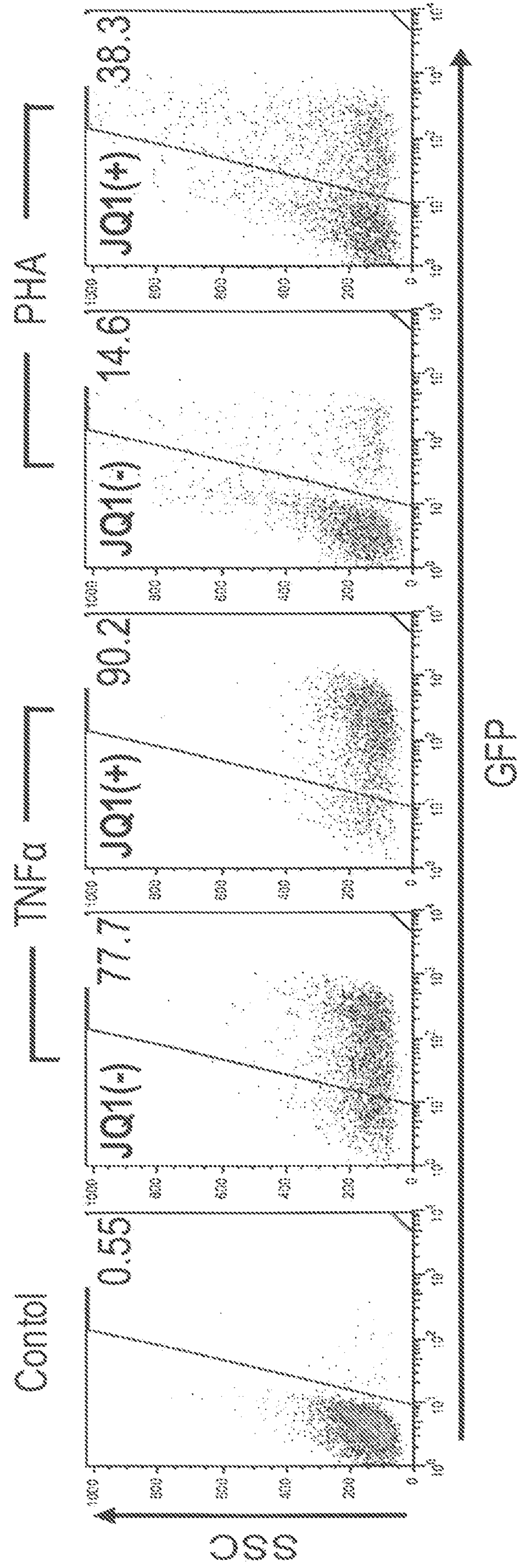


Figure 3A

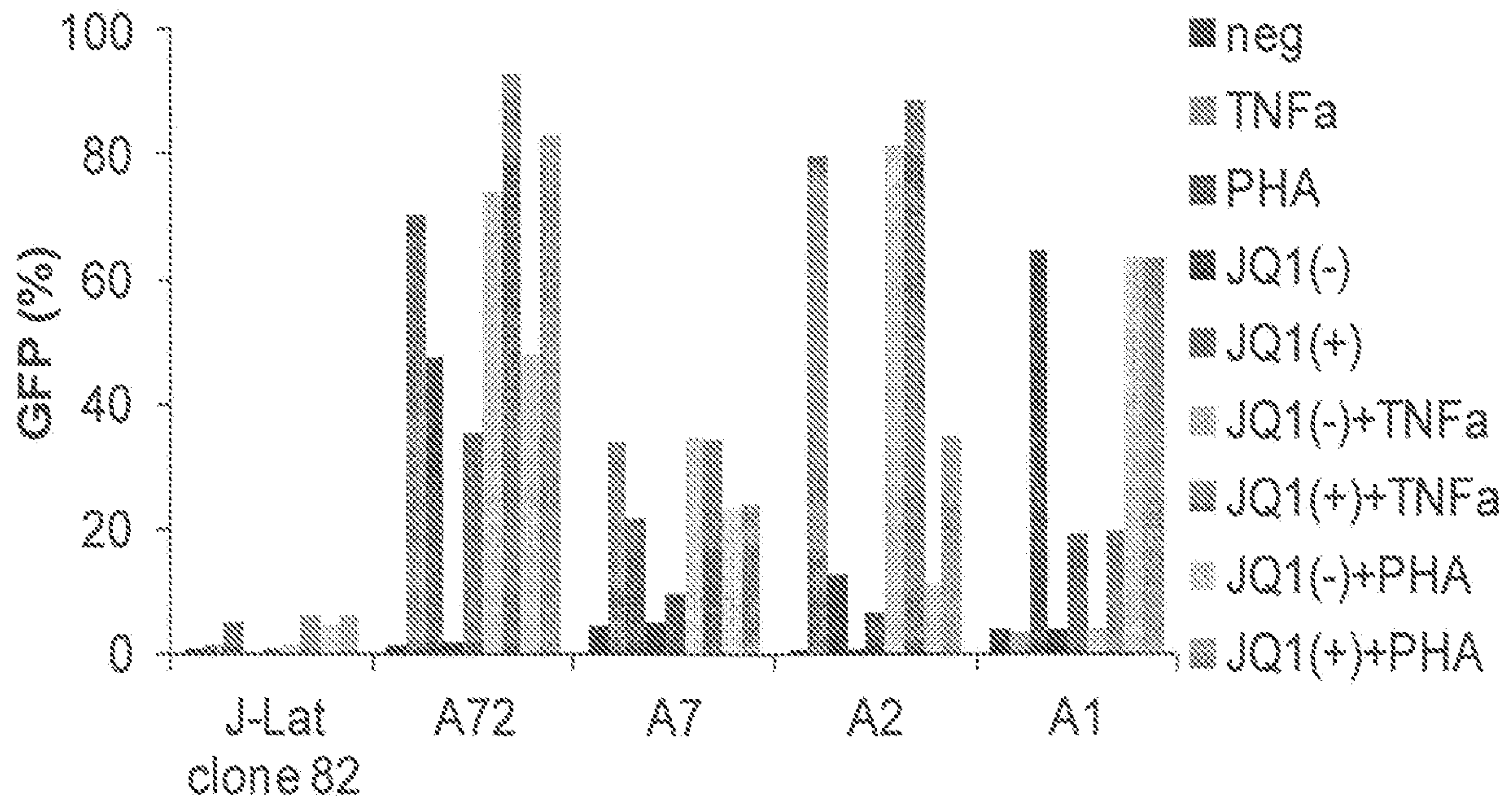


Figure 3B

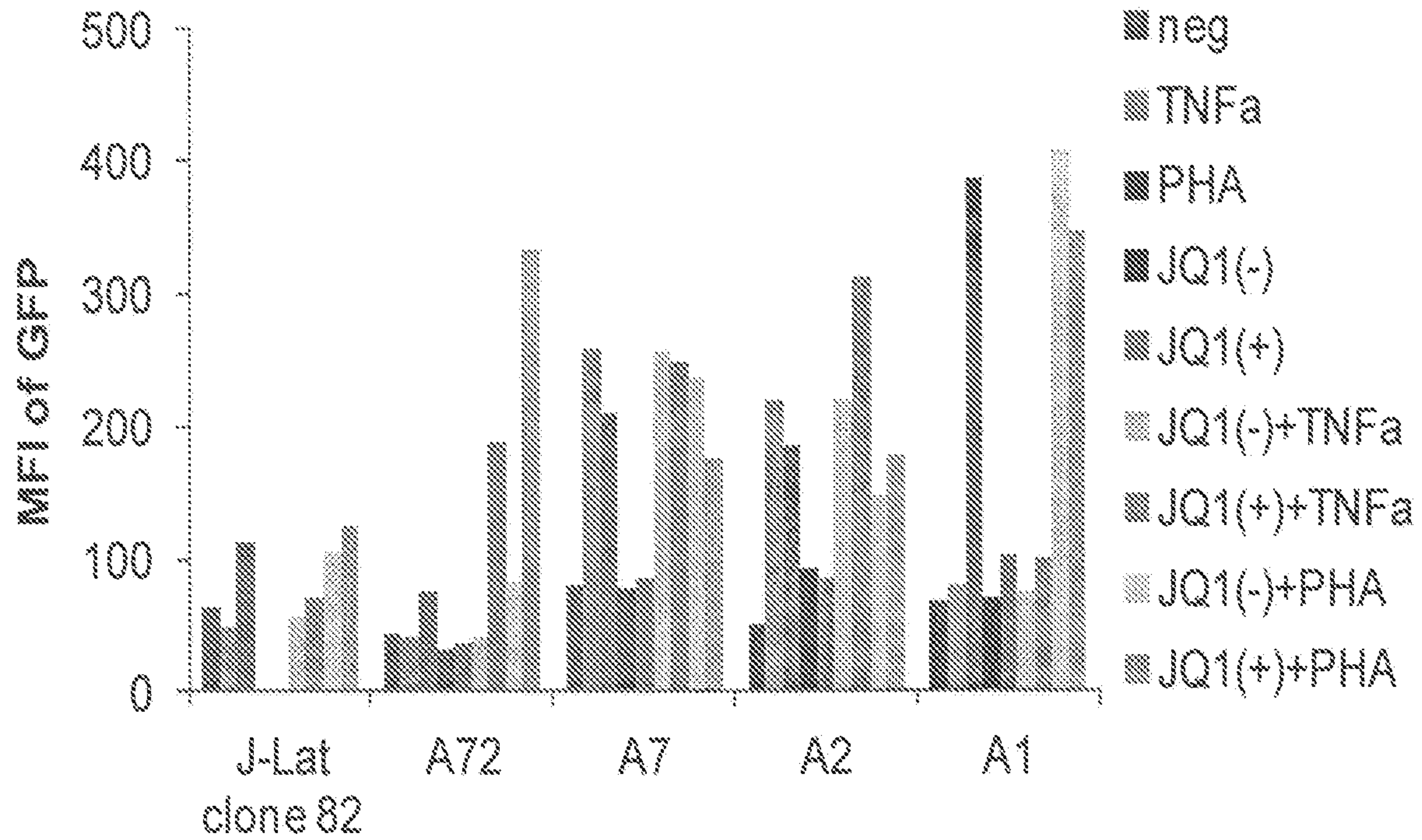
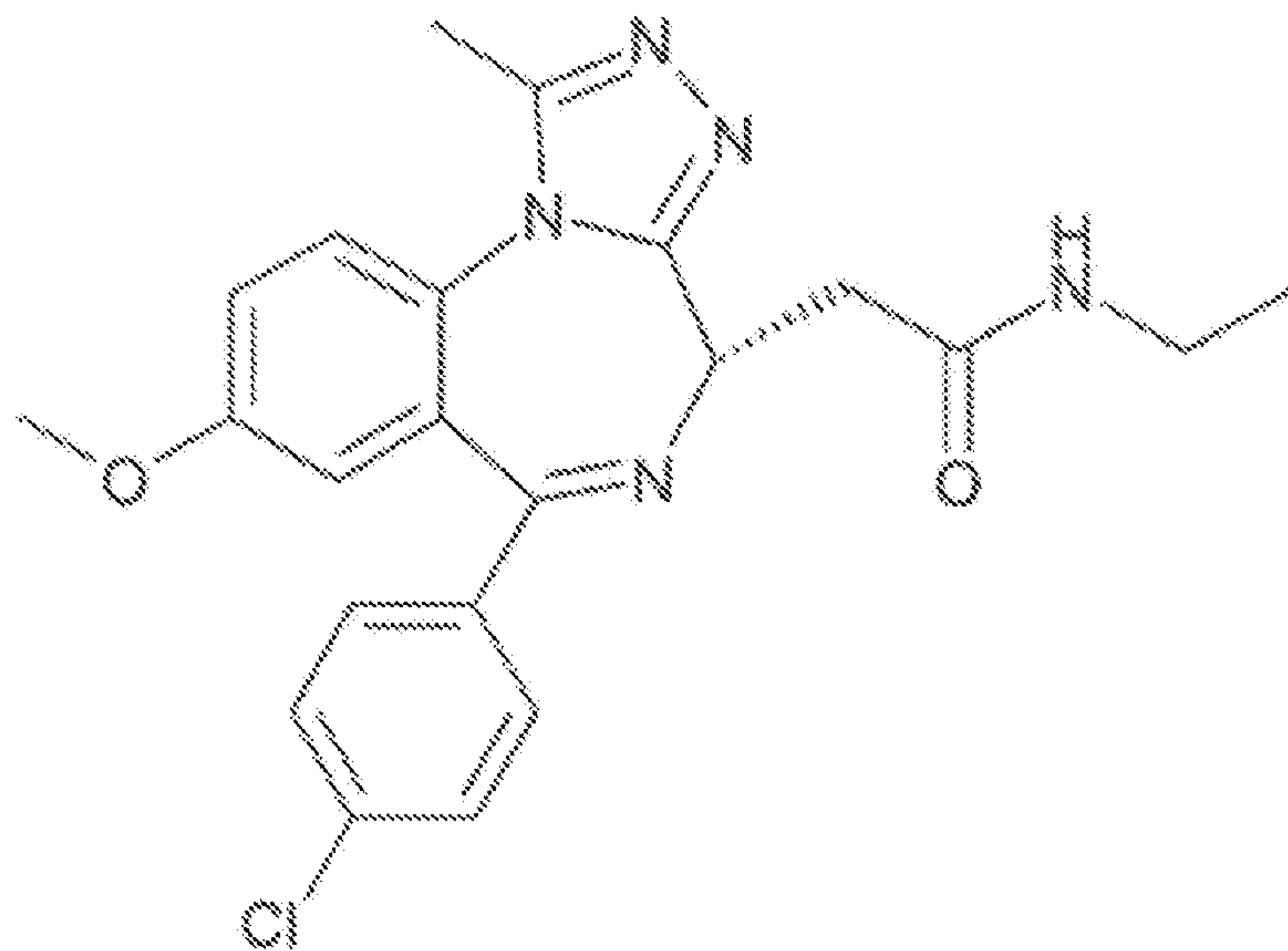
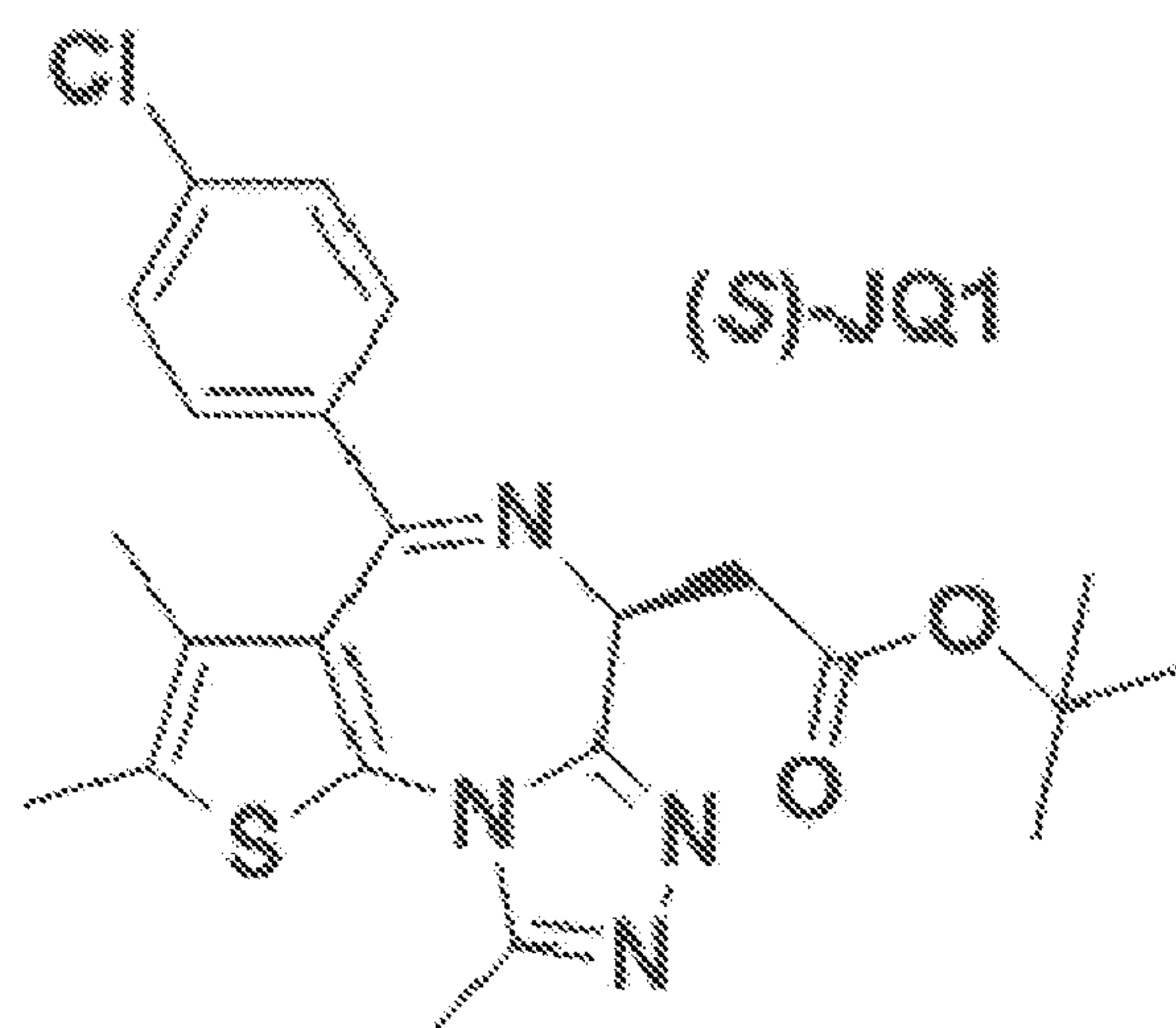


Figure 3C

A



B



C

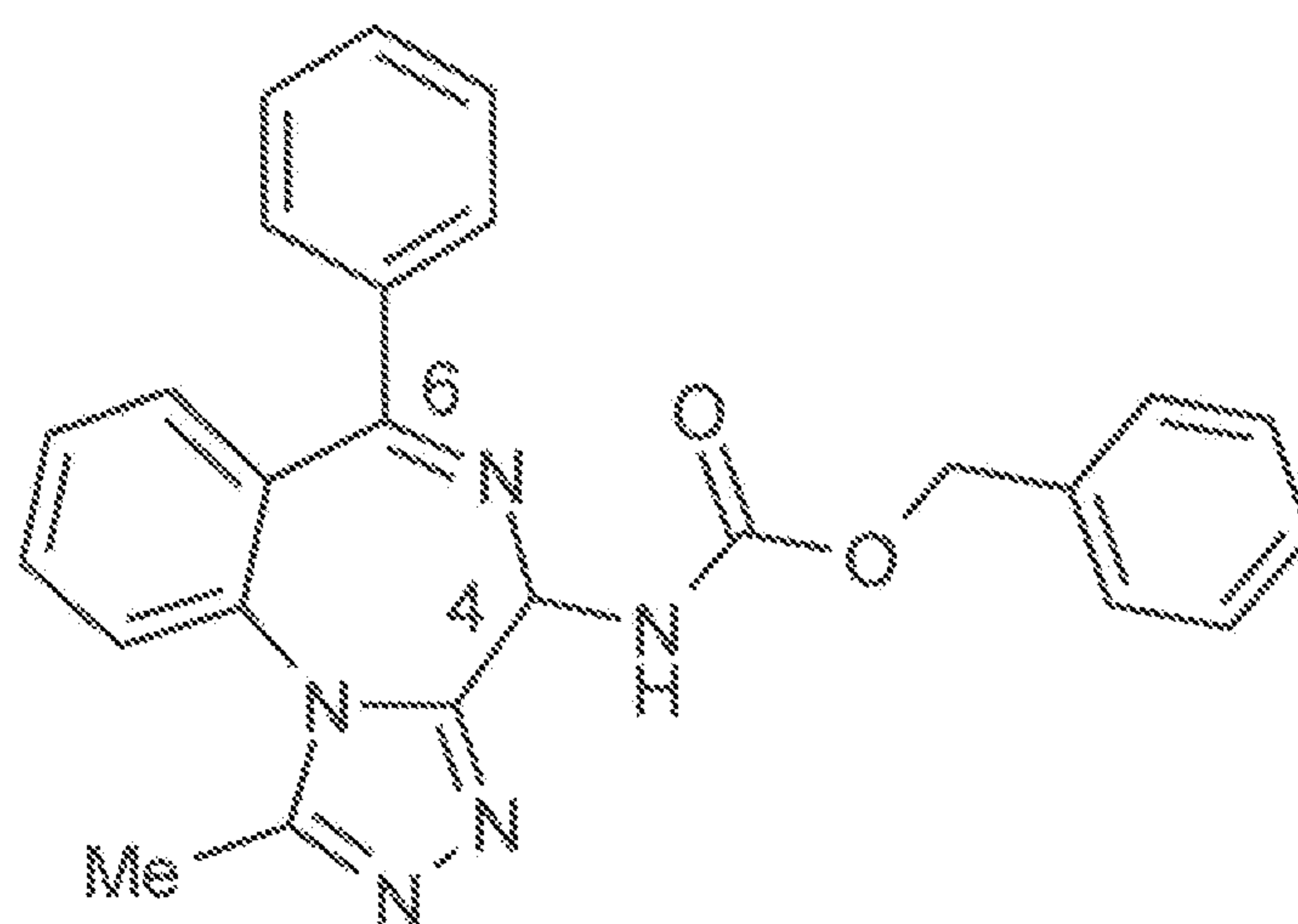


Figure 4

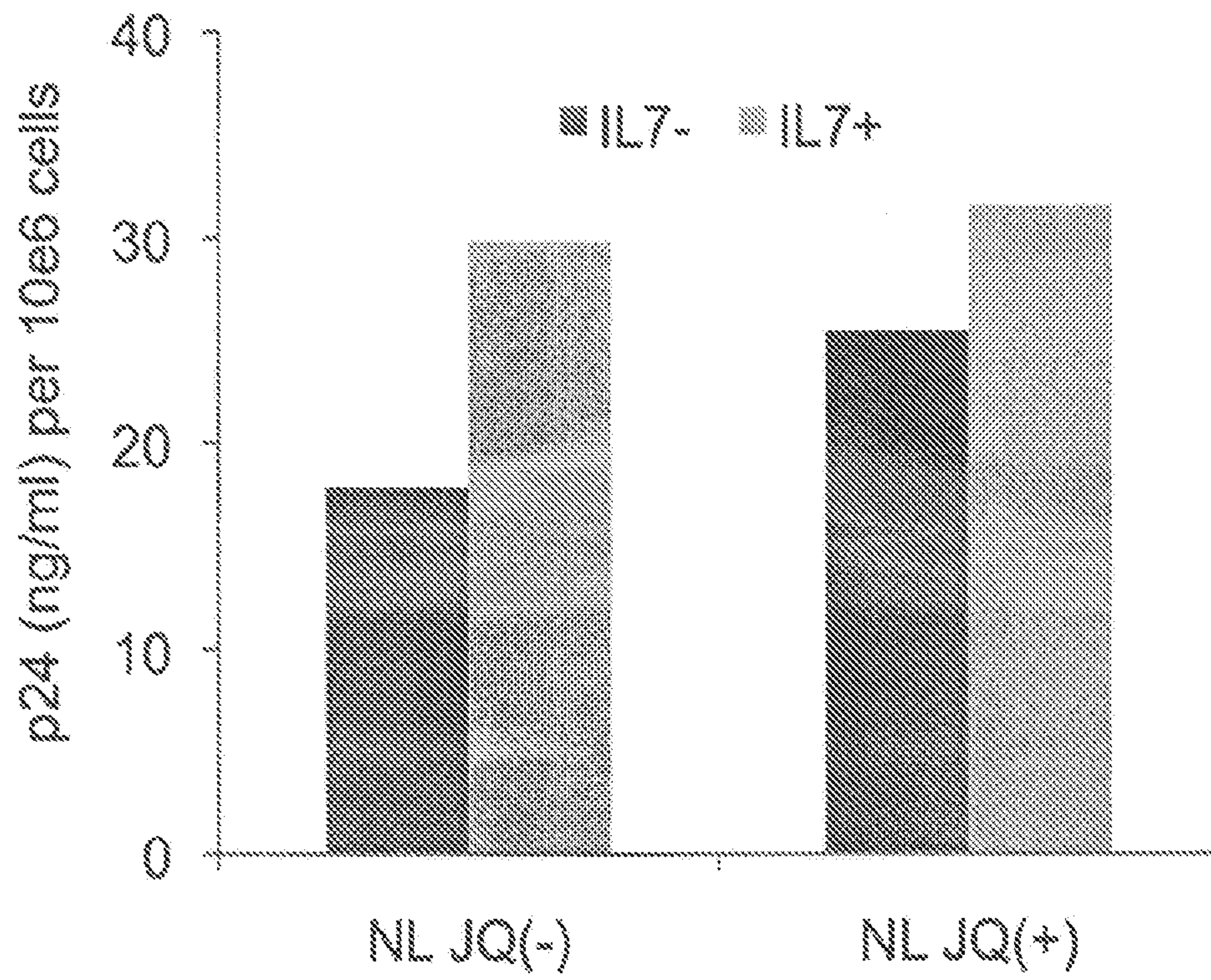


Figure 5A

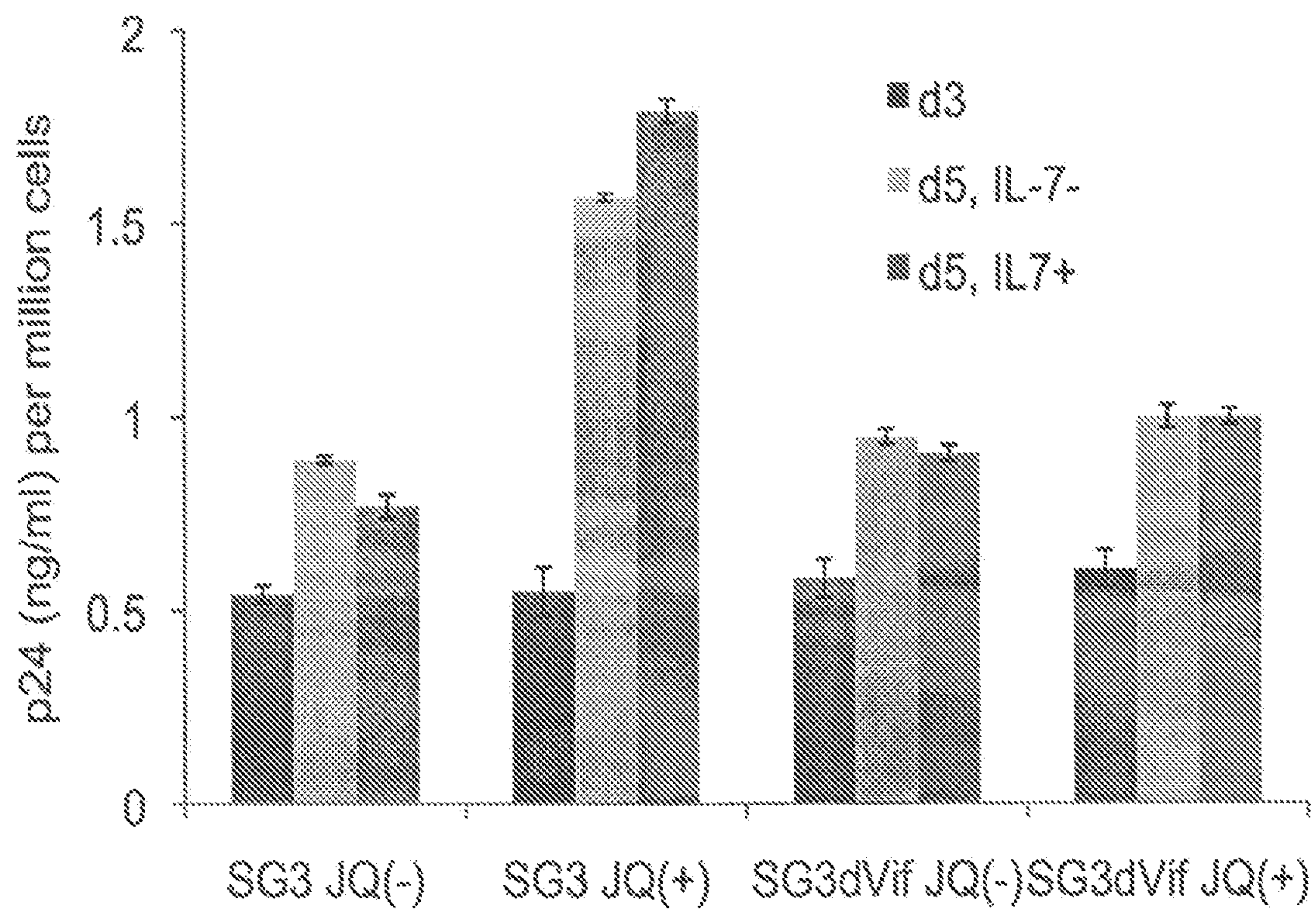


Figure 5B

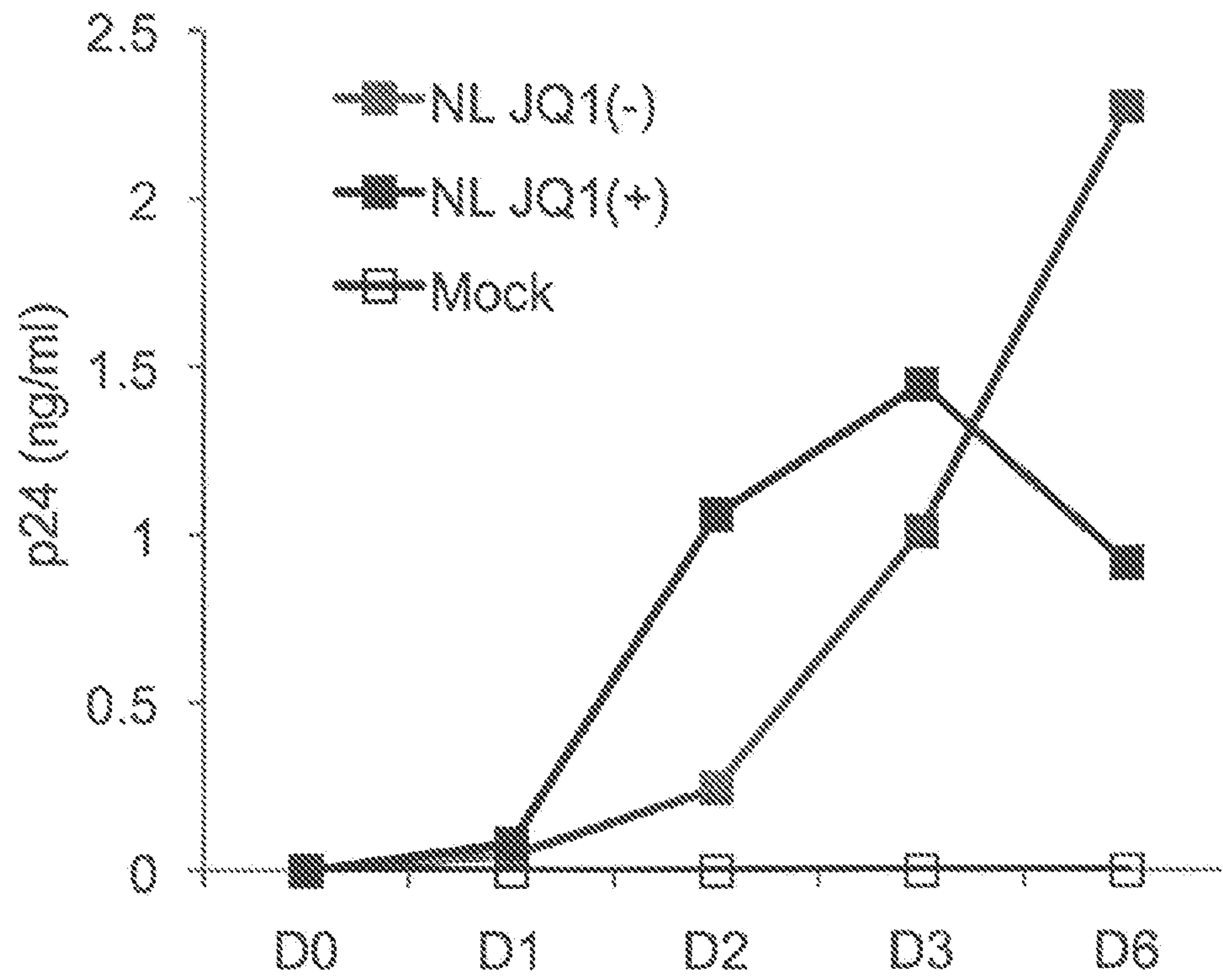


Figure 6A

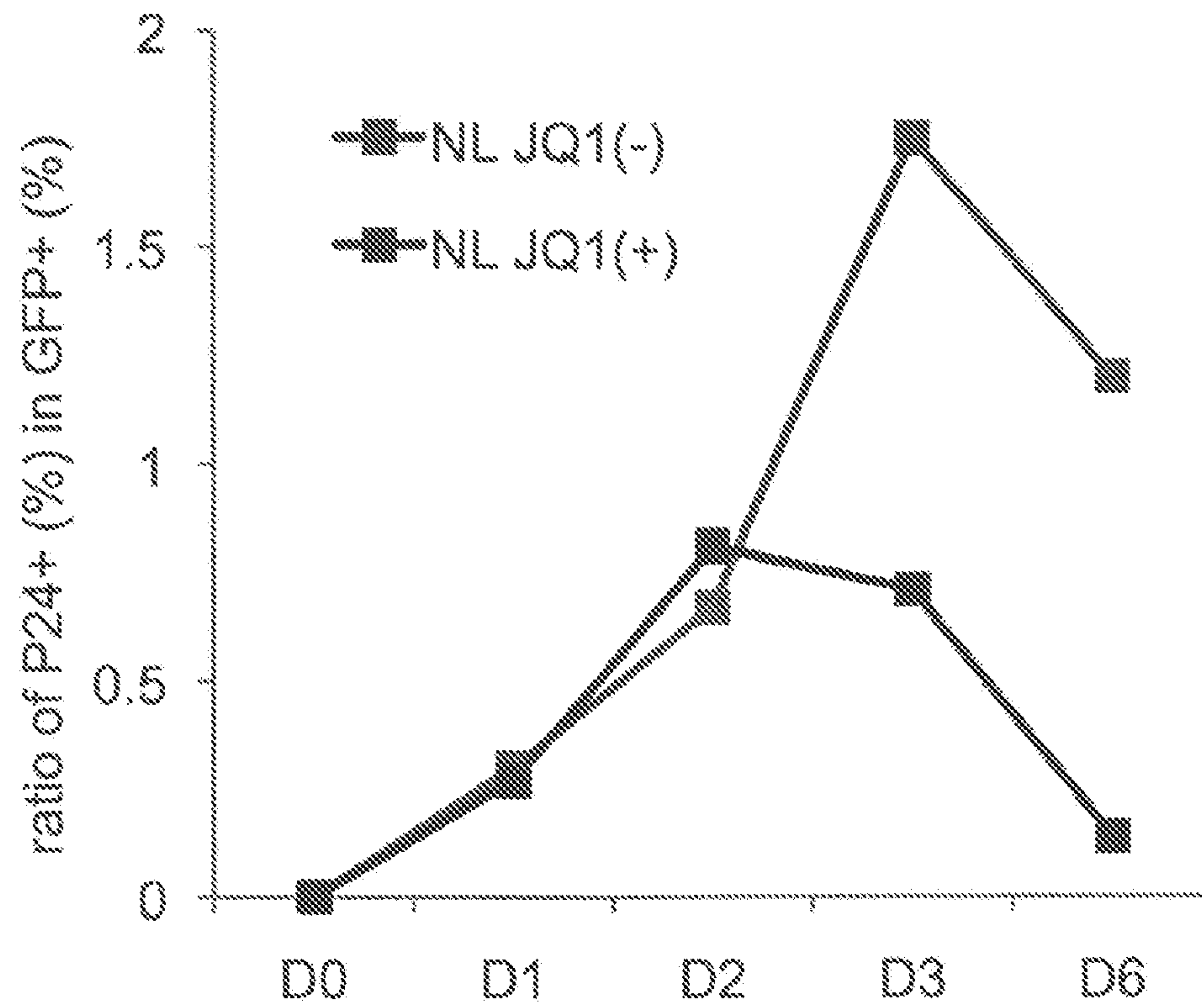


Figure 6B

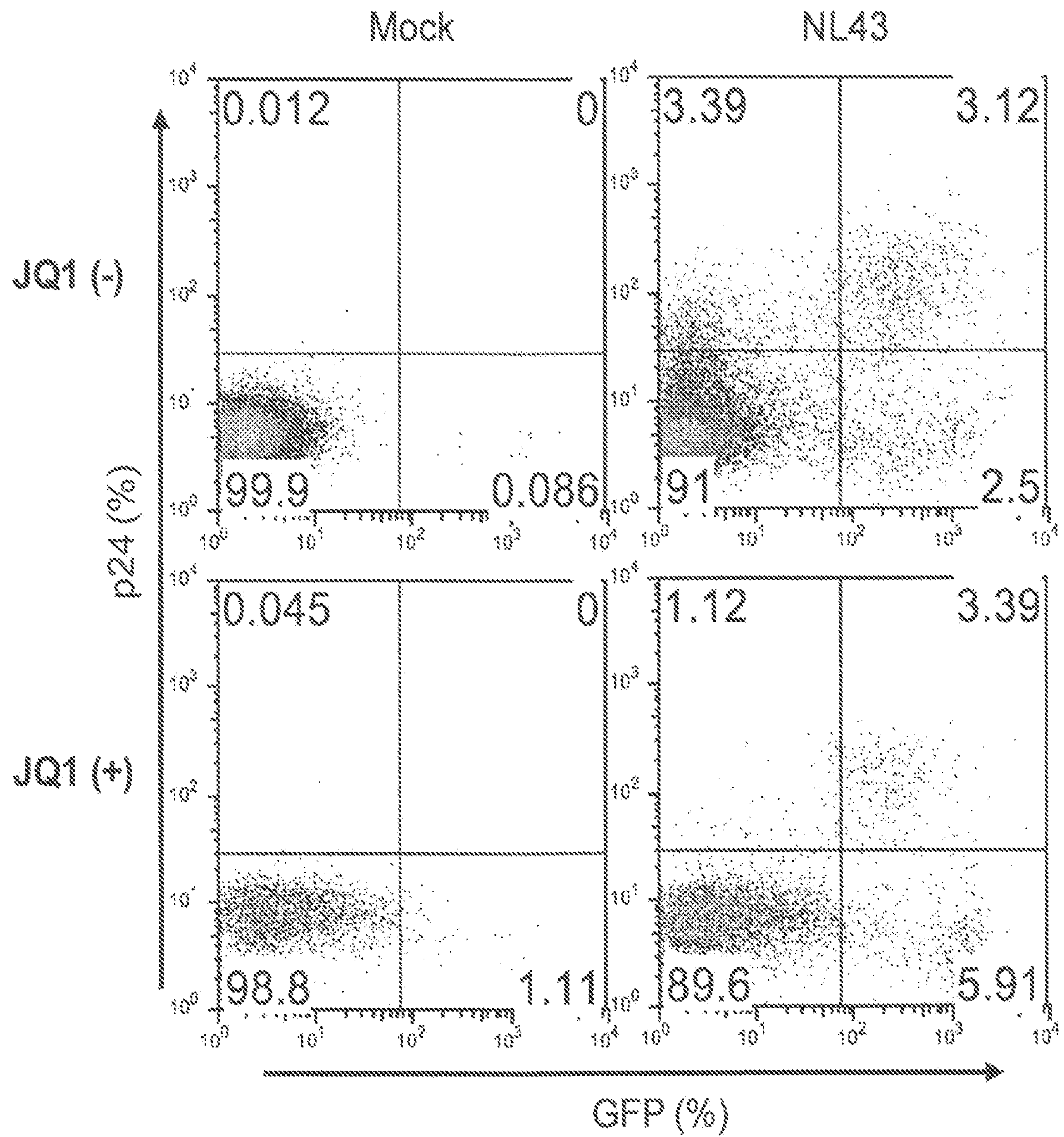


Figure 6C