



(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION
(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2017/02/01
(87) Date publication PCT/PCT Publication Date: 2017/08/10
(85) Entrée phase nationale/National Entry: 2018/11/01
(86) N° demande PCT/PCT Application No.: IB 2017/000166
(87) N° publication PCT/PCT Publication No.: 2017/134525
(30) Priorité/Priority: 2016/02/02 (US62/290,298)

(51) Cl.Int./Int.Cl. *C12N 15/113* (2010.01),
A61K 31/7105 (2006.01), *A61K 45/06* (2006.01)

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(54) Titre : TRAITEMENT DE LA DERMATITE ATOPIQUE ET DE L'ASTHME EN UTILISANT DES COMPLEXES D'ARN
QUI CIBLENT L'LL4R?, TRPA1, OU F2RL1
(54) Title: TREATMENT OF ATOPIC DERMATITIS AND ASTHMA USING RNA COMPLEXES THAT TARGET LL4RA,
TRPA1, OR F2RL1

(57) **Abrégé/Abstract:**

In certain aspects, provided herein are RNA complexes (e.g., asymmetric RNA complexes, such as asiRNAs or cell penetrating asiRNAs) that inhibit IL4R α , TRPA1, and/or F2RL1 expression and are therefore useful for treating atopic dermatitis or asthma.

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

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
10 August 2017 (10.08.2017)

(10) International Publication Number
WO 2017/134525 A1

- (51) **International Patent Classification:**
C12N 15/113 (2010.01) *A61K 45/06* (2006.01)
A61K 31/7105 (2006.01)
- (21) **International Application Number:**
PCT/IB2017/000166
- (22) **International Filing Date:**
1 February 2017 (01.02.2017)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
62/290,298 2 February 2016 (02.02.2016) US
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- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).
- Published:**
— *with international search report (Art. 21(3))*

- (54) **Title:** TREATMENT OF ATOPIC DERMATITIS AND ASTHMA USING RNA COMPLEXES THAT TARGET IL4R α , TRPA1, OR F2RL1
- (57) **Abstract:** In certain aspects, provided herein are RNA complexes (e.g., asymmetric RNA complexes, such as asiRNAs or cell penetrating asiRNAs) that inhibit IL4R α , TRPA1, and/or F2RL1 expression and are therefore useful for treating atopic dermatitis or asthma.

WO 2017/134525 A1

***TREATMENT OF ATOPIC DERMATITIS AND ASTHMA USING RNA
COMPLEXES THAT TARGET IL4R α , TRPA1, OR F2RL1***

RELATED APPLICATIONS

5 This application claims the benefit of priority to U.S. Provisional Patent Application serial number 62/290,298, filed February 2, 2016, which is incorporated herein by reference in its entirety.

BACKGROUND

10 Dysregulation of the immune system can result in autoimmune diseases such as atopic dermatitis and asthma. Atopic dermatitis, also referred to as eczema, is an inflammatory disease characterized by the presence of itchy and tender skin, edema, and erythema. Atopic dermatitis is common in children and infants, although the disease can occur at any age.

15 About 70% of atopic dermatitis patients develop asthma by “atopic march,” characterized by the progression of atopic dermatitis to asthma and allergic rhinitis. Asthma is a respiratory disorder also associated with dysregulation of the immune system. More specifically, it is a chronic respiratory disease marked by respiratory spasms and obstruction due to allergic inflammation of the bronchi, causing repetitive breathing
20 shortness, wheezing and coughing. Asthma prevalence is estimated to be as high as 300 million individuals worldwide, and about 8% of the population of major developed countries are afflicted with asthma.

 IL4R α , F2RL1 and TRPA1 genes play a key role in the onset and progression of symptoms of atopic dermatitis and/or asthma. When exposed to foreign antigens, dendritic
25 cells in atopic dermatitis patients activate Th2 cells, leading to the secretion of cytokines (e.g., IL-4, IL-5, IL-10, and IL-13) by the activated Th2 cells. Among the cytokines, IL-4 and IL-13 are known to play an important role in the onset of atopic dermatitis, while IL-4 and IL-13 have been reported to worsen atopic dermatitis symptoms of through the inhibition of human beta defensin-3 and filaggrin, both of which maintain the skin barrier.
30 The receptors for IL-4 and IL-13 are heterodimers and contain IL4R α (interleukin 4

receptor, alpha, also known as IL4R α). Therefore, down-regulation of the IL4R α can block out the signals of IL-4 and IL-13.

The main cause of the itching symptom experienced by atopic dermatitis patients is the overexpression of thymic stromal lymphopoietin (TSLP) in keratinocytes, which
5 elevates the transient receptor potential (TRP) of TRP ion channels, including TRPV1 and TRPA1. Thus, the symptoms of atopic dermatitis can be treated by the inhibition of TRPA1.

Coagulation factor II (thrombin) receptor-like 1 (F2RL1, also known as protease-activated receptor 2, PAR2) is expressed by keratinocytes, activated endothelial cells, and
10 sensory nerves in the skin and is involved in various inflammation reactions, pigmentation production, and the skin barrier function. F2RL1 plays a pivotal role in the activation of proteinases, which induce inflammation reactions and the aggravated skin conditions seen in atopic dermatitis patients.

Thus, there is a need for new and improved therapeutics targeting IL4R α , TRPA1
15 and F2RL1 for the treatment of atopic dermatitis or asthma.

SUMMARY

In certain aspects, provided herein are RNA complexes that target IL4R α , TRPA1, or F2RL1 and are useful for treating and/or preventing atopic dermatitis and/or asthma. In certain aspects, provided herein are pharmaceutical compositions comprising such RNA
20 complexes and methods of using such RNA complexes and pharmaceutical compositions.

In certain aspects, provided herein is an RNA complex comprising an antisense strand having sequence complementarity to an IL4R α , TRPA1, or F2RL1 mRNA sequence and a sense strand having sequence complementarity to the antisense strand. In some embodiments, the RNA complex is capable of inhibiting IL4R α , TRPA1, or F2RL1
25 expression by a cell (*e.g.*, a keratinocyte). In some embodiments, the RNA complex is an asymmetric shorter-duplex small interfering RNA (an asiRNA). In some embodiments, the RNA complex is an RNA complex listed in Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, or Table 10. In some embodiments, the RNA complex comprises the antisense and sense strand of IL4RA#5. In some embodiments, the RNA
30 complex comprises the antisense and sense strand of TRPA1#81. In some embodiments, the RNA complex comprises the antisense and sense strand of F2RL1#22.

In some embodiments, the RNA complex provided herein comprises a chemical modification, wherein the modification facilitates the penetration of a cellular membrane in the absence of a delivery vehicle. In some embodiments, the modification is a 2'-O-methylated nucleoside, a phosphorothioate bond or a hydrophobic moiety. In some
5 embodiments, the RNA complexes provided herein comprise a hydrophobic moiety. In some embodiments, the hydrophobic moiety can be any chemical structure having hydrophobic character. For example, in some embodiments the hydrophobic moiety is a lipid, a lipophilic peptide and/or a lipophilic protein. In some embodiments, the hydrophobic moiety is a lipid, such as cholesterol, tocopherol, or a long-chain fatty acid
10 having 10 or more carbon atoms (*e.g.*, stearic acid or palmitic acid). In some embodiments, the hydrophobic moiety is cholesterol. In some embodiments, the RNA complex is a modified RNA complex listed in Table 2, Table 3, Table 5, Table 6, Table 8, Table 9, or Table 10. In certain embodiments, the RNA complex is not cytotoxic.

In certain aspects, provided herein is a pharmaceutical composition comprising an
15 RNA complex provided herein and a pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutical composition is formulated for topical delivery. In some embodiments, the pharmaceutical composition is a cream or a lotion. In some embodiments, the pharmaceutical composition is formulated for parenteral, intravenous, or oral delivery. In other embodiments, the pharmaceutical composition is formulated for
20 inhalation.

In certain aspects, provided herein is a method of inhibiting IL4R α , TRPA1, or F2RL1 expression by a cell, comprising contacting the cell with an RNA complex provided herein.

In certain aspects, provided herein is a method of inhibiting gene expression IL4R α ,
25 TRPA1, or F2RL1 in a human subject comprising administering to the subject an RNA complex or pharmaceutical composition provided herein. In certain aspects, provided herein is a method of treating a human subject for atopic dermatitis and/or asthma comprising administering to the subject an RNA complex or pharmaceutical composition provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the gene silencing efficiency of 73 exemplary asiRNAs that target IL4R α .

Figure 2 shows the gene silencing efficiency of 15 exemplary asiRNAs that target
5 IL4R α .

Figure 3 shows the gene silencing effects of 2 exemplary asiRNAs that target IL4R α .

Figure 4 shows the gene silencing efficiency of exemplary IL4R α -targeting cell-penetrating asiRNAs (IL4R α cp-asiRNAs) to which various chemical modifications have
10 been applied.

Figure 5 shows the inhibition of IL4R α protein expression by exemplary cp-asiRNAs.

Figure 6 shows the gene silencing efficiency of 4 cp-asiRNAs of different antisense strand lengths (19 or 21 nucleotides).

Figure 7 shows the inhibition of IL4R α protein expression by 4 exemplary cp-asiRNAs.
15

Figure 8 provides the human IL4R α mRNA sequence.

Figure 9 shows the gene silencing efficiency of 102 exemplary asiRNAs that target TRPA1.

Figure 10 shows the gene silencing effects of 14 exemplary asiRNAs that target
20 TRPA1.

Figure 11 shows the inhibition of TRPA1 protein expression by 14 exemplary asiRNAs that target TRPA1.

Figure 12 shows the gene silencing efficiency of exemplary TRPA1-targeting cell-penetrating asiRNAs (TRPA1 cp-asiRNAs) to which various chemical modifications have
25 been applied.

Figure 13 shows the inhibition of TRPA1 protein expression by exemplary cp-asiRNAs.

Figure 14 shows the gene silencing efficiency of 8 cp-asiRNAs of different
30 antisense strand lengths (19 or 21 nucleotides) and different sense strand chemical modifications (3 or 4 phosphorothioate bond).

Figure 15 shows the inhibition of TRPA1 protein expression by 8 exemplary cp-asiRNAs.

Figure 16 shows the inhibition of TRPA1 protein expression by 4 exemplary cp-asiRNAs. A549 cells were incubated with 1 μ M and 3 μ M cp-asiRNAs in the absence of transfection reagent.

Figure 17 provides the human TRPA1 mRNA sequence.

5 **Figure 18** shows the gene silencing efficiency of 100 exemplary asiRNAs that target F2RL1.

Figure 19 shows the gene silencing efficiency of 29 exemplary asiRNAs that target F2RL1.

10 **Figure 20** shows the gene silencing efficiency of 32 exemplary asiRNAs containing 2'-O-Methylation modifications.

Figure 21 shows the gene silencing effects of 12 exemplary asiRNAs that target F2RL1.

Figure 22 shows the inhibition of F2RL1 protein expression by 12 exemplary asiRNAs that target F2RL1.

15 **Figure 23** shows the gene silencing efficiency of exemplary F2RL1-targeting cell-penetrating asiRNAs (cp-asiRNAs or cp-asiF2RL1s) to which various chemical modifications have been applied.

Figure 24 shows the inhibition of F2RL1 mRNA expression by exemplary cp-asiRNAs.

20 **Figure 25** shows the inhibition of F2RL1 protein expression by exemplary cp-asiRNAs.

Figure 26 shows the gene silencing efficiency of 8 cp-asiRNAs of different antisense strand lengths (19 or 21 nucleotides).

25 **Figure 27** shows the inhibition of F2RL1 protein expression by 8 exemplary cp-asiRNAs.

Figure 28 shows the mRNA sequence of human F2RL1.

Figure 29 shows the experimental timeline of cp-asiRNA treatment in an induced model of atopic dermatitis.

30 **Figure 30** shows scratching time observed in *Dermatophagoides farinae* body extract (Df) cream treated samples.

Figure 31 shows intradermal injection versus cream cp-asiRNA application in rodents of atopic dermatitis.

Figure 32 shows H&E staining of skin sections and quantified epidermis area by analyzing skin section image of a rodent model of atopic dermatitis.

Figure 33 shows mast cell infiltration analysis of treated skin region.

5 DETAILED DESCRIPTION

General

In certain aspects, provided herein are asymmetric RNA complexes (*e.g.*, asiRNAs or cp-asiRNAs) that inhibit IL4R α , TRPA1, and/or F2RL1 and are therefore useful for the treatment of atopic dermatitis and/or asthma. In some embodiments, the RNA complexes
10 are chemically modified to be capable of penetrating a cell without need for a transfection vehicle. In some embodiments, the RNA complex is an RNA complex listed in Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, or Table 10. In certain aspects, provided herein are pharmaceutical compositions comprising such RNA complexes and methods of using such RNA complexes and pharmaceutical compositions.

15 In some embodiments, the RNA complexes described herein are asiRNAs or cp-asiRNAs. As used herein, the term asiRNA refers to double-stranded asymmetrical short interfering RNA molecules that have a 19-21 nt antisense strand and a 13-17 nt sense strand. Additional information on asiRNAs can be found in U.S. Pat. Pub. No. 2012/0238017 and in Chang *et al.*, *Mol. Ther.* 17:725-732 (2009), each of which is hereby
20 incorporated by reference in its entirety.

In some embodiments, the RNA complexes described herein are delivered to cells using a delivery vehicle, such as liposomes, cationic polymers, cell penetrating peptides (CPPs), protein transduction domains (PTDs), antibodies and/or aptamers. In some
25 embodiments, the RNA complex described herein is chemically modified so as to not require the use of such delivery vehicles to mediate IL4R α , TRPA1, and/or F2RL1 inhibition in a cell. Such RNA complexes are referred to herein as cell-penetrating asiRNAs (cp-asiRNAs).

Definitions

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

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

As used herein, the term “*administering*” means providing a pharmaceutical agent or composition to a subject, and includes, but is not limited to, administering by a medical professional and self-administering.

As used herein, the term “*immunomodulator*” refers to a compound or composition
5 which weakens, stimulate, or otherwise modulates the immune system. Examples include, by are not limited to leukotriene receptor agonists, immunosuppressants (*e.g.*, FK-506), or cytokines.

As used herein, the terms “*interfering nucleic acid*” and “*inhibiting nucleic acid*” are used interchangeably. Interfering nucleic acids generally include a sequence of cyclic
10 subunits, each bearing a base-pairing moiety, linked by intersubunit linkages that allow the base-pairing moieties to hybridize to a target sequence in a nucleic acid (typically RNA) by Watson-Crick base pairing, to form a nucleic acid: oligomer heteroduplex within the target sequence. Interfering RNA molecules include, but are not limited to, antisense molecules, siRNA molecules, asiRNA molecules, cp-asiRNA molecules, single-stranded siRNA
15 molecules, miRNA molecules and shRNA molecules. Such an interfering nucleic acids can be designed to block or inhibit translation of mRNA or to inhibit natural pre-mRNA splice processing, or induce degradation of targeted mRNAs, and may be said to be “directed to” or “targeted against” a target sequence with which it hybridizes. Interfering nucleic acids may include, for example, peptide nucleic acids (PNAs), locked nucleic acids (LNAs), 2'-
20 O-Methyl oligonucleotides and RNA interference agents (siRNA agents). RNAi molecules generally act by forming a heteroduplex with the target molecule, which is selectively degraded or “knocked down,” hence inactivating the target RNA. Under some conditions, an interfering RNA molecule can also inactivate a target transcript by repressing transcript translation and/or inhibiting transcription of the transcript. An interfering nucleic acid is
25 more generally said to be “targeted against” a biologically relevant target, such as a protein, when it is targeted against the nucleic acid of the target in the manner described above.

The terms “*polynucleotide*”, and “*nucleic acid*” are used interchangeably. They refer to a polymeric form of nucleotides, whether deoxyribonucleotides, ribonucleotides, or analogs thereof, in any combination and of any length. Polynucleotides may have any three-
30 dimensional structure, and may perform any function. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched

polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. A polynucleotide may be further modified, such as by conjugation with a labeling component. In all nucleic acid sequences provided herein, Unucleobases are interchangeable with T nucleobases.

The phrase “*pharmaceutically-acceptable carrier*” as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material.

An oligonucleotide “*specifically hybridizes*” to a target polynucleotide if the oligomer hybridizes to the target under physiological conditions, with a T_m substantially greater than 45 °C, or at least 50 °C, or at least 60 °C-80 °C or higher. Such hybridization corresponds to stringent hybridization conditions. At a given ionic strength and pH, the T_m is the temperature at which 50% of a target sequence hybridizes to a complementary polynucleotide. Again, such hybridization may occur with “near” or “substantial” complementarity of the antisense oligomer to the target sequence, as well as with exact complementarity.

As used herein, the term “*subject*” means a human or non-human animal selected for treatment or therapy.

The phrases “*therapeutically-effective amount*” and “*effective amount*” as used herein means the amount of an agent which is effective for producing the desired therapeutic effect in at least a sub-population of cells in a subject at a reasonable benefit/risk ratio applicable to any medical treatment.

“*Treating*” a disease in a subject or “*treating*” a subject having a disease refers to subjecting the subject to a pharmaceutical treatment, *e.g.*, the administration of a drug, such that at least one symptom of the disease is decreased or prevented from worsening.

As used herein, a therapeutic that “*prevents*” a disorder or condition refers to a compound that, when administered to a statistical sample prior to the onset of the disorder or condition, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

RNA Complexes

In certain aspects, provided herein are RNA complexes that target IL4R α , TRPA1, and/or F2RL1 mRNA and inhibit IL4R α , TRPA1, and/or F2RL1 expression by a cell, respectively. The nucleic acid sequence of human IL4R α , TRPA1, and F2RL1 mRNA is
5 provided in Figures 8, Figure 17, and Figure 28, respectively.

In certain aspects, provided herein is an RNA complex comprising an antisense strand having sequence complementarity to an IL4R α , TRPA1, or F2RL1 mRNA sequence (*e.g.*, a human IL4R α , TRPA1, or F2RL1 mRNA sequence) and a sense strand having sequence complementarity to the antisense strand. In some embodiments, the RNA
10 complex is capable of inhibiting IL4R α , TRPA1, or F2RL1 expression by a cell. In some embodiments, the RNA complex is an asymmetric shorter-duplex small interfering RNA (an asiRNA). In some embodiments, the RNA complex is an RNA complex listed in Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 8 or Table 10. The RNA complexes described herein can contain RNA bases, non-RNA bases or a mixture of RNA bases and
15 non-RNA bases. For example, certain RNA complexes provided herein can be primarily composed of RNA bases but also contain DNA bases or non-naturally occurring nucleotides.

In some embodiments, the antisense strand is at least 19 nucleotides (nt) in length. In some embodiments, the antisense strand is 19 to 21 nt in length (*i.e.*, 19, 20 or 21 nt in
20 length). In some embodiments, at least 13, 14, 15, 16, 17, 18, 19, 20 or 21 nt of the antisense strand are complementary to the IL4R α , TRPA1, or F2RL1 mRNA sequence. Perfect complementarity is not necessary. In some embodiments, the antisense strand is perfectly complementary to the IL4R α , TRPA1, or F2RL1 mRNA sequence.

In some embodiments, the antisense strand is at least 24 nt in length (*e.g.*, at least 25
25 nt in length, at least 26 nt in length, at least 27 nt in length, at least 28 nt in length, at least 29 nt in length, at least 30 nt in length or at least 31 nt in length). In some embodiments, the antisense strand is no greater than 124 nt in length (*e.g.*, no greater than 100 nt in length, no greater than 90 nt in length, no greater than 80 nt in length, no greater than 70 nt in length, no greater than 60 nt in length, no greater than 50 nt in length or no greater than 40 nt in
30 length). In some embodiments, the antisense strand is 31 nt in length. In some embodiments, at least 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 29, 30 or 31 nt of the antisense strand are complementary to the IL4R α , TRPA1, or F2RL1 mRNA sequence. Perfect

complementarity is not necessary. In some embodiments, the antisense strand is perfectly complementary to the IL4R α , TRPA1, or F2RL1 mRNA sequence.

In some embodiments, the sense strand is 15 to 17 nt in length (*i.e.*, 15 nt in length, 16 nt in length or 17 nt in length). In some embodiments, at least 15 nt, at least 16 nt or at least 17 nt of the sense strand are complementary to the sequence of the antisense strand. In some embodiments the sense strand is perfectly complementary to the sequence of the antisense strand.

In some embodiments, the antisense strand and the sense strand form a complex in which the 5' end of the antisense strand and the 3' end of the sense strand form a blunt end. In some embodiments, the antisense strand and the sense strand form a complex in which the 5' end of the antisense strand overhangs the 3' end of the sense strand (*e.g.*, by 1, 2, 3, 4 or 5 nt). In some embodiments, the antisense strand and the sense strand form a complex in which the 5' end of the sense strand overhangs the 3' end of the antisense strand (*e.g.*, by 1, 2, 3, 4 or 5 nt).

In some embodiments, the antisense strand and/or the sense strand of the RNA complex has a sense strand sequence and/or an antisense strand sequence selected from the sequences listed in Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 8 or Table 10.

In some embodiments, the RNA complex provided herein comprises a chemical modification, wherein the modification facilitates the penetration of a cellular membrane in the absence of a delivery vehicle. In some embodiments, the modification is a 2'-O-methylated nucleoside, a phosphorothioate bond or a hydrophobic moiety. In some embodiments, the chemical modification is a hydrophobic moiety. In some embodiments, the hydrophobic moiety is a cholesterol moiety. In some embodiments, the RNA complex is a modified RNA complex listed in Table 2, table 3, Table 5, Table 6, Table 8, Table 9, or Table 10. In certain embodiments, the RNA complex is not cytotoxic.

The RNA complexes described herein can employ a variety of oligonucleotide chemistries. Examples of oligonucleotide chemistries include, without limitation, peptide nucleic acid (PNA), linked nucleic acid (LNA), phosphorothioate, 2'-O-Me-modified oligonucleotides, and morpholino chemistries, including combinations of any of the foregoing. In general, PNA chemistries can utilize shorter targeting sequences because of their relatively high target binding strength relative to 2'-O-Me oligonucleotides. Phosphorothioate and 2'-O-Me-modified chemistries are often combined to generate 2'-O-

Me-modified oligonucleotides having a phosphorothioate backbone. See, *e.g.*, PCT Publication Nos. WO/2013/112053 and WO/2009/008725, each of which is hereby incorporated by reference in its entirety.

Peptide nucleic acids (PNAs) are analogs of DNA in which the backbone is structurally homomorphous with a deoxyribose backbone, consisting of N-(2-aminoethyl) glycine units to which pyrimidine or purine bases are attached. PNAs containing natural pyrimidine and purine bases hybridize to complementary oligonucleotides obeying Watson-Crick base-pairing rules, and mimic DNA in terms of base pair recognition. The backbone of PNAs is formed by peptide bonds rather than phosphodiester bonds, making them well-suited for antisense applications (see structure below). The backbone is uncharged, resulting in PNA/DNA or PNA/RNA duplexes that exhibit greater than normal thermal stability. PNAs are not recognized by nucleases or proteases.

Despite a radical structural change to the natural structure, PNAs are capable of sequence-specific binding in a helix form to DNA or RNA. Characteristics of PNAs include a high binding affinity to complementary DNA or RNA, a destabilizing effect caused by single-base mismatch, resistance to nucleases and proteases, hybridization with DNA or RNA independent of salt concentration and triplex formation with homopurine DNA. PANAGENE.TM. has developed its proprietary Bts PNA monomers (Bts; benzothiazole-2-sulfonyl group) and proprietary oligomerization process. The PNA oligomerization using Bts PNA monomers is composed of repetitive cycles of deprotection, coupling and capping. PNAs can be produced synthetically using any technique known in the art. See, *e.g.*, U.S. Pat. Nos. 6,969,766, 7,211,668, 7,022,851, 7,125,994, 7,145,006 and 7,179,896. See also U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262 for the preparation of PNAs. Further teaching of PNA compounds can be found in Nielsen *et al.*, *Science*, 254:1497-1500, 1991. Each of the foregoing is incorporated by reference in its entirety.

Interfering nucleic acids may also contain “locked nucleic acid” subunits (LNAs). “LNAs” are a member of a class of modifications called bridged nucleic acid (BNA). BNA is characterized by a covalent linkage that locks the conformation of the ribose ring in a C3'-endo (northern) sugar pucker. For LNA, the bridge is composed of a methylene between the 2'-O and the 4'-C positions. LNA enhances backbone preorganization and base stacking to increase hybridization and thermal stability.

The structures of LNAs can be found, for example, in Wengel, *et al.*, *Chemical Communications* (1998) 455; *Tetrahedron* (1998) 54:3607, and *Accounts of Chem.*

Research (1999) 32:301); Obika, *et al.*, *Tetrahedron Letters* (1997) 38:8735; (1998) 39:5401, and *Bioorganic Medicinal Chemistry* (2008) 16:9230. Compounds provided herein may incorporate one or more LNAs; in some cases, the compounds may be entirely composed of LNAs. Methods for the synthesis of individual LNA nucleoside subunits and their incorporation into oligonucleotides are described, for example, in U.S. Pat. Nos. 7,572,582, 7,569,575, 7,084,125, 7,060,809, 7,053,207, 7,034,133, 6,794,499, and 6,670,461, each of which is incorporated by reference in its entirety. Typical intersubunit linkers include phosphodiester and phosphorothioate moieties; alternatively, non-phosphorous containing linkers may be employed. One embodiment is an LNA-containing compound where each LNA subunit is separated by a DNA subunit. Certain compounds are composed of alternating LNA and DNA subunits where the intersubunit linker is phosphorothioate.

In certain embodiments, the RNA complex is linked to a cholesterol moiety. In some embodiments, the cholesterol moiety is attached to the 3' terminus of the sense strand. In some embodiments, the cholesterol moiety is attached to the 3' terminus of the antisense strand. In some embodiments, the cholesterol moiety is attached to the 5' terminus of the sense strand. In some embodiments, the cholesterol moiety is attached to the 5' terminus of the antisense strand.

In some embodiments, the RNA complex comprises a 2'-O-methylated nucleoside. 2'-O-methylated nucleosides carry a methyl group at the 2'-OH residue of the ribose molecule. 2'-O-Me-RNAs show the same (or similar) behavior as RNA, but are protected against nuclease degradation. 2'-O-Me-RNAs can also be combined with phosphothioate oligonucleotides (PTOs) for further stabilization. 2'-O-Me-RNAs (phosphodiester or phosphothioate) can be synthesized according to routine techniques in the art (see, *e.g.*, Yoo *et al.*, *Nucleic Acids Res.* 32:2008-16, 2004, which is hereby incorporated by reference).

In some embodiments, the 2'-O-methyl nucleoside is positioned at the 3' terminus of the sense strand. In some embodiments, 3' terminal region of the sense strand comprises a plurality of 2'-O-methylated nucleosides (*e.g.*, 2, 3, 4, 5 or 6 2'-O-methylated nucleosides within 6 nucleosides of the 3' terminus). In some embodiments, the 2'-O-methyl nucleoside is positioned at the 3' terminus of the antisense strand. In some embodiments, 3' terminal region of the antisense strand comprises a plurality of 2'-O-methylated nucleosides (*e.g.*, 2, 3, 4, 5 or 6 2'-O-methylated nucleosides within 6 nucleosides of the 3' terminus). In some embodiments, both the 3' terminal region of the sense strand and the 3' terminal region of

the antisense strand comprise a plurality of 2'-O-methylated nucleosides. In some
embodiments, the sense strand comprises 2'-O-methylated nucleosides that alternate with
unmodified nucleosides. In some embodiments, the sense strand comprises a contiguous
sequence of 2, 3, 4, 5, 6, 7 or 8 2'-O-methylated nucleosides that alternate with unmodified
5 nucleosides. In some embodiments, the anti-sense strand comprises 2'-O-methylated
nucleosides that alternate with unmodified nucleosides. In some embodiments, the anti-
sense strand comprises a contiguous sequence of 2, 3, 4, 5, 6, 7 or 8 2'-O-methylated
nucleosides that alternate with unmodified nucleosides.

In some embodiments, the RNA complex comprises a phosphorothioate bond.
10 “Phosphorothioates” (or S-oligos) are a variant of normal DNA in which one of the non-
bridging oxygens is replaced by a sulfur. The sulfurization of the internucleotide bond
reduces the action of endo-and exonucleases including 5' to 3' and 3' to 5' DNA POL 1
exonuclease, nucleases S1 and P1, RNases, serum nucleases and snake venom
phosphodiesterase. Phosphorothioates are made by two principal routes: by the action of a
15 solution of elemental sulfur in carbon disulfide on a hydrogen phosphonate, or by the
method of sulfurizing phosphite triesters with either tetraethylthiuram disulfide (TETD) or
3H-1,2-benzodithiol-3-one 1,1-dioxide (BDTD) (see, *e.g.*, Iyer *et al.*, *J. Org. Chem.* 55,
4693-4699, 1990). The latter methods avoid the problem of elemental sulfur's insolubility
in most organic solvents and the toxicity of carbon disulfide. The TETD and BDTD
20 methods also yield higher purity phosphorothioates.

In some embodiments, at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%,
70%, 75%, 80%, 85%, 90% or 95% of the bonds between the ribonucleotides in the sense
strand of the RNA complex are phosphorothioate bonds. In some embodiments, all of the
bonds between the ribonucleotides in the sense strand of the RNA complex are
25 phosphorothioate bonds.

In some embodiments, at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%,
70%, 75%, 80%, 85%, 90% or 95% of the bonds between the ribonucleotides in the
antisense strand of the RNA complex are phosphorothioate bonds. In some embodiments,
all of the bonds between the ribonucleotides in the antisense strand of the RNA complex are
30 phosphorothioate bonds.

The RNA complexes described herein may be contacted with a cell or administered
to an organism (*e.g.*, a human). Alternatively, constructs and/or vectors encoding the RNA

complexes may be contacted with or introduced into a cell or organism. In certain embodiments, a viral, retroviral or lentiviral vector is used.

The RNA complexes described herein can be prepared by any appropriate method known in the art. For example, in some embodiments, the RNA complexes described herein
5 are prepared by chemical synthesis or in vitro transcription.

In certain aspects, provided herein is a pharmaceutical composition comprising an RNA complex provided herein and a pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutical composition is formulated for delivery to the skin (*e.g.*, as a cream or lotion). In certain embodiments, the pharmaceutical composition is
10 formulated for delivery to the lungs (*e.g.*, as an inhaler). In some embodiments, the pharmaceutical composition is formulated for oral or parenteral delivery. In some embodiments, the pharmaceutical composition further comprises a second agent for treatment of atopic dermatitis or asthma. In some embodiments, the second agent is a steroid (*e.g.*, a corticosteroid), a long acting beta agonist (*e.g.*, salmeterol or formoterol),
15 or an immunomodulator. Examples of steroids include hydrocortisone, fluticasone, mudesonide, mometasone, beclomethasone, ciclesonide or flunisolide. Examples of immunomodulators include montelukast, zafirlukast, or zileuton. Two or more steroids, long acting beta agonists, and immunomodulators may be taken in with the pharmaceutical composition.

20 In some embodiments, the pharmaceutical composition is formulated for delivery to the skin. In some embodiments, the composition is an emulsion, cream, lotion, gel, oil, ointment, aerosol spray, or semi-solid formulation. In some embodiments, the topical formulation comprises a carrier selected from trehalose, malto-dextrin, rice flour, micro-crystalline cellulose, magnesium stearate, inositol, fructo-oligosaccharide, gluco-
25 oligosaccharide, dextrose, sucrose, talc, water, physiological salt solution, urea, methanol, ethanol, propanol, butanol, ethylene glycol, propylene glycol, white petrolatum, isopropyl myristate, lanolin, lanolin alcohol, mineral oil, lavender oil, nasturtium extract oil, sorbitan mono-oleate, cetylstearyl alcohol, hydroxypropyl cellulose, detergent, sucrose stearate, sucrose cocoate, sucrose distearate, 2-ethyl-1,3-hexanediol, polyoxypropylene-15-stearyl
30 ether, glycerol stearate, glycerin, synthetic spermaceti, cetyl alcohol, butylparaben, propylparaben, and methylparaben.

In certain embodiments, the pharmaceutical composition does not comprise a transfection vehicle. In some embodiments, the pharmaceutical composition comprises a

delivery vehicle (*e.g.*, liposomes, cationic polymers, cell penetrating peptides (CPPs), protein transduction domains (PTDs), antibodies and/or aptamers). In some embodiments, the composition includes a combination of multiple (*e.g.*, two or more) of the RNA complexes described herein.

5 Methods of preparing these formulations or compositions include the step of bringing into association an RNA complex described herein with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association an agent described herein with liquid carriers.

10 Therapeutic Methods

 In certain aspects, provided herein is a method of inhibiting IL4R α , TRPA1, or F2RL1 expression by a cell, comprising contacting the cell with an RNA complex provided herein. In some embodiments, the RNA complex is a modified RNA complex and the cell is contacted with the RNA complex in the absence of a transfection vehicle. In some
15 embodiments, the cell is contacted with the RNA complex in the presence of a delivery vehicle (*e.g.*, a liposome, cationic polymer, cell penetrating peptide (CPP), protein transduction domain (PTD), antibody and/or aptamer). In some embodiments, the cell is present in the respiratory tract of a human subject. In some embodiments, the cell is present in the skin of a human subject. In some embodiments, the subject has atopic dermatitis. In
20 some embodiments, the subject has asthma. In some embodiments, the subject is female. In some embodiments, the subject is male.

 In certain aspects, provided herein is a method of treating a human subject for atopic dermatitis and/or asthma comprising administering to the subject an RNA complex or pharmaceutical composition provided herein. In certain embodiments, the RNA complex or
25 pharmaceutical composition is administered to the respiratory tract of the subject. In certain embodiments, the RNA complex or pharmaceutical composition is administered to the skin of the subject. In some embodiments, the RNA complex or pharmaceutical composition self-administered by the subject.

 In the present methods, an RNA complex described herein can be administered to
30 the subject, for example, as nucleic acid without delivery vehicle (*e.g.*, for cp-asiRNAs), in combination with a delivery reagent, and/or as a nucleic acid comprising sequences that express the RNA complex described herein. In some embodiments, any nucleic acid delivery method known in the art can be used in the methods described herein. Suitable

delivery reagents include, but are not limited to, *e.g.*, the Mirus Transit TKO lipophilic reagent; lipofectin; lipofectamine; cellfectin; polycations (*e.g.*, polylysine), atelocollagen, nanoplexes and liposomes. The use of atelocollagen as a delivery vehicle for nucleic acid molecules is described in Minakuchi *et al. Nucleic Acids Res.*, 32(13):e109 (2004); Hanai *et al. Ann NY Acad Sci.*, 1082:9-17 (2006); and Kawata *et al. Mol Cancer Ther.*, 7(9):2904-12 (2008); each of which is incorporated herein in their entirety. Exemplary interfering nucleic acid delivery systems are provided in U.S. Patent Nos. 8,283,461, 8,313,772, 8,501,930, 8,426,554, 8,268,798 and 8,324,366, each of which is hereby incorporated by reference in its entirety.

In some embodiments of the methods described herein, liposomes are used to deliver an RNA complex described herein to a subject. Liposomes suitable for use in the methods described herein can be formed from standard vesicle-forming lipids, which generally include neutral or negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of factors such as the desired liposome size and half-life of the liposomes in the blood stream. A variety of methods are known for preparing liposomes, for example, as described in Szoka *et al.* (1980), *Ann. Rev. Biophys. Bioeng.* 9:467; and U.S. Pat. Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369, the entire disclosures of which are herein incorporated by reference.

The liposomes for use in the present methods can also be modified so as to avoid clearance by the mononuclear macrophage system ("MMS") and reticuloendothelial system ("RES"). Such modified liposomes have opsonization-inhibition moieties on the surface or incorporated into the liposome structure.

Opsonization-inhibiting moieties for use in preparing the liposomes described herein are typically large hydrophilic polymers that are bound to the liposome membrane. As used herein, an opsonization inhibiting moiety is "bound" to a liposome membrane when it is chemically or physically attached to the membrane, *e.g.*, by the intercalation of a lipid-soluble anchor into the membrane itself, or by binding directly to active groups of membrane lipids. These opsonization-inhibiting hydrophilic polymers form a protective surface layer that significantly decreases the uptake of the liposomes by the MMS and RES; *e.g.*, as described in U.S. Pat. No. 4,920,016, the entire disclosure of which is herein incorporated by reference.

In some embodiments, opsonization inhibiting moieties suitable for modifying liposomes are water-soluble polymers with a number-average molecular weight from about 500 to about 40,000 daltons, or from about 2,000 to about 20,000 daltons. Such polymers include polyethylene glycol (PEG) or polypropylene glycol (PPG) derivatives; *e.g.*, methoxy PEG or PPG, and PEG or PPG stearate; synthetic polymers such as polyacrylamide or poly N-vinyl pyrrolidone; linear, branched, or dendrimeric polyamidoamines; polyacrylic acids; polyalcohols, *e.g.*, polyvinylalcohol and polyxylitol to which carboxylic or amino groups are chemically linked, as well as gangliosides, such as ganglioside GM1. Copolymers of PEG, methoxy PEG, or methoxy PPG, or derivatives thereof, are also suitable. In addition, the opsonization inhibiting polymer can be a block copolymer of PEG and either a polyamino acid, polysaccharide, polyamidoamine, polyethyleneamine, or polynucleotide. The opsonization inhibiting polymers can also be natural polysaccharides containing amino acids or carboxylic acids, *e.g.*, galacturonic acid, glucuronic acid, mannuronic acid, hyaluronic acid, pectic acid, neuraminic acid, alginic acid, carrageenan; aminated polysaccharides or oligosaccharides (linear or branched); or carboxylated polysaccharides or oligosaccharides, *e.g.*, reacted with derivatives of carbonic acids with resultant linking of carboxylic groups. In some embodiments, the opsonization-inhibiting moiety is a PEG, PPG, or derivatives thereof. Liposomes modified with PEG or PEG-derivatives are sometimes called “PEGylated liposomes.”

The pharmaceutical compositions disclosed herein may be delivered by any suitable route of administration, including topically, through inhalation, orally, and parenterally. In certain embodiments, the pharmaceutical compositions are delivered systemically (*e.g.*, via oral or parenteral administration). In certain other embodiments, the pharmaceutical compositions are delivered locally through inhalation into the lungs or topically onto the skin. In some embodiments, the pharmaceutical composition is administered via intradermal injection.

Actual dosage levels of the RNA complexes in the pharmaceutical compositions may be varied so as to obtain an amount of RNA complex that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular agent employed, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being

employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

5 A physician having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could prescribe and/or administer doses of the agents employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is
10 achieved.

In general, a suitable daily dose of an RNA complex described herein will be that amount of the RNA complex which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

EXEMPLIFICATION

15 Example 1: Screening for IL4R α -specific asymmetric shorter-duplex small interfering RNAs

To identify asymmetric shorter-duplex small interfering RNAs (asiRNAs) that inhibit IL4R α with high efficiency, 73 asiRNAs were synthesized and screened. The nucleic acid sequences of the screened asiRNAs are provided in Table 1.

20 **Table 1: Nucleic acid sequences for exemplary IL4R α -targeting asiRNA.**

SEQUENCE
IL4R α #1(S) : 5' AUCACCAAGAUUAAGA 3'
IL4R α #1(AS) : 5' UCUUAAUCUUGGUGAUGCUGA 3'
IL4R α #2(S) : 5' UCACCAAGAUUAAGAA 3'
IL4R α #2(AS) : 5' UUCUUAAUCUUGGUGAUGCUG 3'
IL4R α #3(S) : 5' GCCUUCUCAAGCCUGC 3'
IL4R α #3(AS) : 5' GCAGGCUUGAGAAGGCCUUGU 3'
IL4R α #4(S) : 5' CCUUCUCAAGCCUGCU 3'
IL4R α #4(AS) : 5' AGCAGGCUUGAGAAGGCCUUG 3'
IL4R α #5(S) : 5' UGCGUCUCCGACUACA 3'
IL4R α #5(AS) : 5' UGUAGUCGGAGACGCAGGUGG 3'
IL4R α #6(S) : 5' GCGUCUCCGACUACAU 3'
IL4R α #6(AS) : 5' AUGUAGUCGGAGACGCAGGUG 3'
IL4R α #7(S) : 5' GUGGAAGGGCUCCUUC 3'
IL4R α #7(AS) : 5' GAAGGAGCCCUUCCACAGCAG 3'
IL4R α #8(S) : 5' UGGAAGGGCUCCUUCA 3'

IL4R α #8(AS) : 5' UGAAGGAGCCCUUCCACAGCA 3'
IL4R α #9(S) : 5' CAUCACCAAGAUUAAG 3'
IL4R α #9(AS) : 5' CUUAAUCUUGGUGAUGCUGAC 3'
IL4R α #10(S) : 5' CACCAAGAUUAAGAAA 3'
IL4R α #10(AS) : 5' UUUCUUAUUCUUGGUGAUGCU 3'
IL4R α #11(S) : 5' UGGGAUCAGAUUCCCA 3'
IL4R α #11(AS) : 5' UGGGAAUCUGAUCCCACCAUU 3'
IL4R α #12(S) : 5' GGGAUCAGAUUCCCAA 3'
IL4R α #12(AS) : 5' UUGGGAAUCUGAUCCCACCAU 3'
IL4R α #13(S) : 5' AAGACAGUCCUCUGGC 3'
IL4R α #13(AS) : 5' GCCAGAGGACUGUCUUGCUGA 3'
IL4R α #14(S) : 5' AGACAGUCCUCUGGCC 3'
IL4R α #14(AS) : 5' GGCCAGAGGACUGUCUUGCUG 3'
IL4R α #15(S) : 5' GACAGUCCUCUGGCCA 3'
IL4R α #15(AS) : 5' UGGCCAGAGGACUGUCUUGCUG 3'
IL4R α #16(S) : 5' ACAGUCCUCUGGCCAG 3'
IL4R α #16(AS) : 5' CUGGCCAGAGGACUGUCUUGC 3'
IL4R α #17(S) : 5' CAGUCCUCUGGCCAGA 3'
IL4R α #17(AS) : 5' UCUGGCCAGAGGACUGUCUUG 3'
IL4R α #18(S) : 5' AGUCCUCUGGCCAGAG 3'
IL4R α #18(AS) : 5' CUCUGGCCAGAGGACUGUCUU 3'
IL4R α #19(S) : 5' GUCCUCUGGCCAGAGA 3'
IL4R α #19(AS) : 5' UCUCUGGCCAGAGGACUGUCU 3'
IL4R α #20(S) : 5' CUCCAGCAUGGGGCGAG 3'
IL4R α #20(AS) : 5' CUGCCCCAUGCUGGAGGACAU 3'
IL4R α #21(S) : 5' GGCUAUCAGGAGUUUG 3'
IL4R α #21(AS) : 5' CAAACUCCUGAUAGCCACUGG 3'
IL4R α #22(S) : 5' GCUAUCAGGAGUUUGU 3'
IL4R α #22(AS) : 5' ACAAACUCCUGAUAGCCACUG 3'
IL4R α #23(S) : 5' CUUCUCAAGCCUGCUU 3'
IL4R α #23(AS) : 5' AAGCAGGCUUGAGAAGGCCUU 3'
IL4R α #24(S) : 5' AAUGGGGUGGCUUUGC 3'
IL4R α #24(AS) : 5' GCAAAGCCACCCCAUUGGGAG 3'
IL4R α #25(S) : 5' AUGGGGUGGCUUUGCUG 3'
IL4R α #25(AS) : 5' AGCAAAGCCACCCCAUUGGGA 3'
IL4R α #26(S) : 5' CGUCUCCGACUACAUG 3'
IL4R α #26(AS) : 5' CAUGUAGUCGGAGACGCAGGU 3'
IL4R α #27(S) : 5' GACAGUUCACACCAAU 3'
IL4R α #27(AS) : 5' AUUGGUGUGAACUGUCAGGUU 3'
IL4R α #28(S) : 5' ACAGUUCACACCAAUG 3'

IL4R α #28(AS) : 5' CAUUGGUGUGAACUGUCAGGU 3'
IL4R α #29(S) : 5' CAGUUCACACCAAUGU 3'
IL4R α #29(AS) : 5' ACAUUGGUGUGAACUGUCAGG 3'
IL4R α #30(S) : 5' AGUUCACACCAAUGUC 3'
IL4R α #30(AS) : 5' GACAUUGGUGUGAACUGUCAG 3'
IL4R α #31(S) : 5' CUGGAGUGAGUGGAGC 3'
IL4R α #31(AS) : 5' GCUCCACUCACUCCAGGUGGU 3'
IL4R α #32(S) : 5' CAGCAUCACCAAGAUU 3'
IL4R α #32(AS) : 5' AAUCUUGGUGAUGCUGACAUA 3'
IL4R α #33(S) : 5' AGCAUCACCAAGAUUA 3'
IL4R α #33(AS) : 5' UAAUCUUGGUGAUGCUGACAU 3'
IL4R α #34(S) : 5' GCAUCACCAAGAUUAA 3'
IL4R α #34(AS) : 5' UUAUCUUGGUGAUGCUGACA 3'
IL4R α #35(S) : 5' UAAGAAAGAAUGGUGG 3'
IL4R α #35(AS) : 5' CCACCAUUCUUUCUAAUCUU 3'
IL4R α #36(S) : 5' AAGAAAGAAUGGUGGG 3'
IL4R α #36(AS) : 5' CCCACCAUUCUUUCUAAUCU 3'
IL4R α #37(S) : 5' AGAAAGAAUGGUGGGA 3'
IL4R α #37(AS) : 5' UCCCACCAUUCUUUCUAAUC 3'
IL4R α #38(S) : 5' GAUUCCCAACCCAGCC 3'
IL4R α #38(AS) : 5' GGCUGGGUUGGGAAUCUGAUC 3'
IL4R α #39(S) : 5' AGCAAGACAGUCCUCU 3'
IL4R α #39(AS) : 5' AGAGGACUGUCUUGCUGAUCU 3'
IL4R α #40(S) : 5' GCAAGACAGUCCUCUG 3'
IL4R α #40(AS) : 5' CAGAGGACUGUCUUGCUGAUC 3'
IL4R α #41(S) : 5' CAAGACAGUCCUCUGG 3'
IL4R α #41(AS) : 5' CCAGAGGACUGUCUUGCUGAU 3'
IL4R α #42(S) : 5' GUUGUUUGAGGCCCCG 3'
IL4R α #42(AS) : 5' CGGGGCCUCAACAACUCCAC 3'
IL4R α #43(S) : 5' AACAGAGAGCCUGUUC 3'
IL4R α #43(AS) : 5' GAACAGGCUCUCUGUUAGCCG 3'
IL4R α #44(S) : 5' CUGGGAGCAGAUCUC 3'
IL4R α #44(AS) : 5' GAGGAUCUGCUCUCCAGGUUUC 3'
IL4R α #45(S) : 5' CUAUCAGGAGUUUGUA 3'
IL4R α #45(AS) : 5' UACAAACUCCUGAUAGCCACU 3'
IL4R α #46(S) : 5' GGCUGGUUACAAGGCC 3'
IL4R α #46(AS) : 5' GGCCUUGUAACCAGCCUCUCC 3'
IL4R α #47(S) : 5' GCUGGUUACAAGGCCU 3'
IL4R α #47(AS) : 5' AGGCCUUGUAACCAGCCUCUC 3'
IL4R α #48(S) : 5' CUGGUUACAAGGCCUU 3'

IL4R α #48(AS) : 5' AAGGCCUUGUAACCAGCCUCU 3'
IL4R α #49(S) : 5' UGGUUACAAGGCCUUC 3'
IL4R α #49(AS) : 5' GAAGGCCUUGUAACCAGCCUC 3'
IL4R α #50(S) : 5' GGUUACAAGGCCUUCU 3'
IL4R α #50(AS) : 5' AGAAGGCCUUGUAACCAGCCU 3'
IL4R α #51(S) : 5' GUUACAAGGCCUUCUC 3'
IL4R α #51(AS) : 5' GAGAAGGCCUUGUAACCAGCC 3'
IL4R α #52(S) : 5' UUACAAGGCCUUCUCA 3'
IL4R α #52(AS) : 5' UGAGAAGGCCUUGUAACCAGC 3'
IL4R α #53(S) : 5' GUGCGGCCACCUGAAA 3'
IL4R α #53(AS) : 5' UUUCAGGUGGCCGCACAGGUG 3'
IL4R α #54(S) : 5' GCUGUGGCUGCUGCUG 3'
IL4R α #54(AS) : 5' CAGCAGCAGCCACAGCAAGGA 3'
IL4R α #55(S) : 5' AGCCGAGCCUAGAAAC 3'
IL4R α #55(AS) : 5' GUUUCUAGGCUCGGCUUCUAG 3'
IL4R α #56(S) : 5' GGGAACAUGAAGGUCU 3'
IL4R α #56(AS) : 5' AGACCUUCAUGUUCCCAGAGC 3'
IL4R α #57(S) : 5' CUUGCAGGAGCCCACC 3'
IL4R α #57(AS) : 5' GGUGGGCUCCUGCAAGACCUU 3'
IL4R α #58(S) : 5' UUGCAGGAGCCCACCU 3'
IL4R α #58(AS) : 5' AGGUGGGCUCCUGCAAGACCU 3'
IL4R α #59(S) : 5' AGUUCACACCAAUGUC 3'
IL4R α #59(AS) : 5' GACAUUGGUGUGAACUGUCAG 3'
IL4R α #60(S) : 5' UUUCAGAAUCUAUAAC 3'
IL4R α #60(AS) : 5' GUUAUAGAUUCUGAAAUCUGC 3'
IL4R α #61(S) : 5' UAUAACGUGACCUACC 3'
IL4R α #61(AS) : 5' GGUAGGUCACGUUAUAGAUUC 3'
IL4R α #62(S) : 5' CACCUGGAGUGAGUGG 3'
IL4R α #62(AS) : 5' CCACUCACUCCAGGUGGUGUU 3'
IL4R α #63(S) : 5' ACCUGGAGUGAGUGGA 3'
IL4R α #63(AS) : 5' UCCACUCACUCCAGGUGGUGU 3'
IL4R α #64(S) : 5' UGUGCUAUGUCAGCAU 3'
IL4R α #64(AS) : 5' AUGCUGACAUAGCACAAACAGG 3'
IL4R α #65(S) : 5' GUCAGCAUCACCAAGA 3'
IL4R α #65(AS) : 5' UCUUGGUGAUGCUGACAUAGC 3'
IL4R α #66(S) : 5' UCAGCAUCACCAAGAU 3'
IL4R α #66(AS) : 5' AUCUUGGUGAUGCUGACAUAG 3'
IL4R α #67(S) : 5' UGGUGGGAUCAGAUUC 3'
IL4R α #67(AS) : 5' GAAUCUGAUCCCACCAUUCUU 3'
IL4R α #68(S) : 5' GGUGGGAUCAGAUUCC 3'

IL4R α #68(AS) : 5' GGAAUCUGAUCCCACCAUUCU 3'
IL4R α #69(S) : 5' GUGCCCACACUGGAAG 3'
IL4R α #69(AS) : 5' CUUCCAGUGUGGGCACUUGGC 3'
IL4R α #70(S) : 5' CUGGAAGAAUUGUCUU 3'
IL4R α #70(AS) : 5' AAGACAAUUCUCCAGUGUGG 3'
IL4R α #71(S) : 5' GUCCUCCAGCAUGGGG 3'
IL4R α #71(AS) : 5' CCCCAUGCUGGAGGACAUUUC 3'
IL4R α #72(S) : 5' AGUGGCUAUCAGGAGU 3'
IL4R α #72(AS) : 5' ACUCCUGAUAGCCACUGGUGG 3'
IL4R α #73(S) : 5' GUGGCUAUCAGGAGUU 3'
IL4R α #73(AS) : 5' AACUCCUGAUAGCCACUGGUG 3'

The asiRNAs listed in Table 1 were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in annealing buffer (Bioneer Inc. Korea). Proper strand annealing was confirmed via gel electrophoresis using a UV transilluminator. For the screen, A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco), 100 units/ml Penicillin and 100 µg/ml Streptomycin in a 100 mm cell culture dish. One day prior to transfection, 5 x 10³ A549 cells were seeded in 96-well plates. The A549 cells were transfected with 0.1 nM of the asiRNAs using RNAiMAX (Invitrogen Inc.) according to the manufacturer's instructions.

The IL4R α mRNA levels in the transfected cells were measured 24 hours after transfection using qRT-PCR. Specifically, total RNA was extracted and synthesized into cDNA using Super Prep Cell Lysis & RT kit for qPCR (TOYOBO) according to manufacturer's instructions. Amplification of the IL4R α gene was detected using IL4R α TaqMan® Probe (Hs00166237_m1). 18S was amplified as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

The level of IL4R α inhibition by each of the 73 asiRNAs is depicted in Figure 1. Fifteen of the asiRNA sequences, #5, #6, #20, #32, #38, #40, #41, #44, #48, #56, #58, #59, #64, #67 and #72, were selected for use in follow-up studies.

Example 2: Inhibition of IL4R α mRNA expression using IL4R α -targeting asiRNAs

The asiRNA sequences selected in Example 1 were tested for their ability to inhibit IL4R α mRNA expression.

The asiRNAs were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in annealing buffer (Bioneer Inc. Korea). Proper strand annealing was confirmed via gel electrophoresis using a UV transilluminator. For the screen, A549 cells (ATCC) that had

been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco) and 100 units/ml Penicillin and 100 µg/ml Streptomycin in a 100 mm cell culture dish. One day prior to transfection, 2.5×10^4 A549 cells were seeded in 24-well plates. The A549 cells were transfected with asiRNAs using RNAiMAX (Invitrogen Inc.) according to the manufacturer's instructions.

IL4Rα mRNA levels in A549 cells were determined using qRT-PCR 24 hours after asiRNA transfection. Specifically, total RNA was extracted using RNAiPlus® (TaKaRa) and then 500 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. Amplification of the IL4Rα was detected using IL4Rα TaqMan® Probe (Hs00166237_m1). 18S RNA was amplified as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

The level of IL4Rα inhibition of the 15 asiRNAs is provided in Figure 2. AsiRNAs #5 and #6, which exhibited 40–50% inhibition of IL4Rα mRNA, were selected for use in follow-up studies.

Example 3: Inhibition of IL4Rα protein expression using IL4Rα-targeting asiRNAs

The two asiRNAs selected in Example 2 were tested for their ability to inhibit IL4Rα protein expression.

The asiRNAs were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in annealing buffer (Bioneer Inc. Korea). Proper strand annealing was confirmed via gel electrophoresis using a UV transilluminator. For the screen, A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 units/ml Penicillin and 100 µg/ml Streptomycin in a 100 mm cell culture dish. One day prior to transfection, 2.5×10^4 A549 cells were seeded in 24-well plates. The A549 cells were transfected with 1 nM of the asiRNAs using RNAiMAX (Invitrogen Inc.) according to the manufacturer's instructions.

IL4Rα protein levels were determined via western blot 48 hours after asiRNA transfection. Briefly, the transfected A549 cells were lysed with 1% SDS lysis buffer (1% SDS, 100mM Tris pH 8.0). 15 µg of the total protein extract was loaded onto an 8% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 3% BSA (Bioworld) and then incubated overnight at 4 °C in 3% BSA containing anti-IL4Rα

antibody (Acris) and anti-GAPDH antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL substrate (Thermo scientific) for 1 minute. The IL4R α and GAPDH bands were then imaged using a Chemidoc instrument (Bio-rad). The results of the western blot are depicted in Figure 3.

Example 4: Chemical modification of asiRNAs for self-delivery

Chemical modifications were applied to the asiRNAs and the cellular delivery of the modified asiRNAs was tested in the absence of other delivery reagent. As described below, certain of the modifications improved endocytosis and stability of asiRNAs. Such cell penetrating asiRNAs (cp-asiRNAs) are able to be delivered into the cell in the absence of a delivery reagent.

Potential cp-asiRNA (Table 2) were screened for IL4R α mRNA and protein inhibition in A549 cells. Each potential cp-asiRNA was incubated with A549 cells at 1 μ M and 3 μ M without a delivery reagent and IL4R α expression levels were measured by qRT-PCR and western blot.

Table 2: Modified asiRNA sequences tested for self-delivery and IL4R α inhibition. (m= 2'-O-Methyl RNA. *= phosphorothioate bond.)

Name	SEQUENCE
IL4R α cp-asiRNA #5 (s)	5' mUGmCGmUCmUCmCGmACmUA*mC*A*Cholesterol 3'
IL4R α cp-asiRNA #5 21(2,4) (AS)	5' UGUAGUCGGAGACGmCmAG*G*U*G*G 3'
IL4R α cp-asiRNA #6 (s)	5' mGCmGUmCUmCCmGAmCUmAC*mA*U*Cholesterol 3'
IL4R α cp-asiRNA #6 21(2,4) (AS)	5' AUGUAGUCGGAGACmGmCA*G*G*U*G 3'
IL4R α cp-asiRNA #6 21(7,4) (AS)	5' AUGUAGUCGGAGACmGmCmA*mG*mG*mU*mG 3'

A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco) and 100 units/ml Penicillin and 100 μ g/ml Streptomycin in a 100 mm cell culture dish.

The potential cp-asiRNAs listed in Table 2 were incubated at 95 $^{\circ}$ C for 5 minutes and at 37 $^{\circ}$ C for 1 hour in Opti-MEM (Gibco). Proper strand annealing of the potential cp-asiRNAs was confirmed by gel electrophoresis using a UV transilluminator.

One day prior to treatment, 2.5×10^4 A549 cells were seeded in 24-well plates. Immediately before treatment, the A549 cells were washed with Dulbecco's modified Eagle's medium (DMEM, Gibco) then cultured in the presence of the potential cp-asiRNAs in OPTI-MEM buffer for 24 hours, at each point the cp-asiRNA containing OPTI-MEM media was replaced with a serum containing media.

Total RNA was extracted 48 hours after cp-asiRNA treatment, using RNAiPlus® (TaKaRa) and then 500 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. Amplification of the IL4R α was detected using IL4R α TaqMan® Probe (Hs00166237_m1). 18S was amplified as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

IL4R α protein levels were determined via western blot 72 hours after cp-asiRNA treatment. Briefly, the transfected A549 cells were lysed with 1% SDS lysis buffer (1% SDS, 100mM Tris pH 8.0). 15 μ g of the total protein extract of A549 cells were loaded onto an 8% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 3% BSA (Bioworld) and then incubated overnight at 4 °C in 3% BSA containing anti-IL4R α antibody (Acris) and anti-GAPDH antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL substrate (Thermo scientific) for 1 minute. The IL4R α and GAPDH bands were then imaged using a Chemidoc instrument (Bio-rad).

The levels of IL4R α inhibition of three potential cp-asiRNAs is provided in Figure 4 and Figure 5. As the result, cp-asiRNA #5_21(2, 4) and cp-asiRNA #6_21(2, 4) were selected for further studies.

Example 5: Additional chemical modification of cp-asiRNA structures.

Other potential IL4R α cp-asiRNA structures having different strand length was synthesized and tested for its ability to inhibit IL4R α expression (Table 3).

Table 3: Additional cp-asiRNA sequence.**(m= 2'-O-Methyl RNA. *= phosphorothioate bond.)**

Name	SEQUENCE
IL4R α cp-asiRNA #5 (s)	5' mUGmCGmUCmUCmCGmACmUA*mC*A*Cholesterol 3'
IL4R α cp-asiRNA #5 19(2,4) (AS)	5' UGUAGUCGGAGACGmC*mA*G*G*U 3'
IL4R α cp-asiRNA #5 21(2,4) (AS)	5' UGUAGUCGGAGACGmCmAG*G*U*G*G 3'
IL4R α cp-asiRNA #6 (s)	5' mGCmGUmCUmCCmGAmCUmAC*mA*U*Cholesterol 3'
IL4R α cp-asiRNA #6 19(2,4) (AS)	5' AUGUAGUCGGAGACmG*mC*A*G*G 3'
IL4R α cp-asiRNA #6 21 (2,4) (AS)	5' AUGUAGUCGGAGACmGmCA*G*G*U*G 3'

A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco) and 100 units/ml Penicillin and 100 μ g/ml Streptomycin in a 100 mm cell culture dish.

The potential cp-asiRNAs listed in Table 3 were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in Opti-MEM (Gibco). Proper strand annealing of the potential cp-asiRNAs was confirmed by gel electrophoresis using a UV transilluminator.

One day prior to treatment, 2.5×10^4 A549 cells were seeded in 24-well plates. Immediately before treatment, the A549 cells were washed with Dulbecco's modified Eagle's medium (DMEM, Gibco) then cultured in the presence of the potential cp-asiRNAs in OPTI-MEM buffer for 24 hours, at each point the cp-asiRNA containing OPTI-MEM media was replaced with a serum containing media.

Total RNA was extracted 48 hours after cp-asiRNA treatment, using RNAiPlus® (TaKaRa) and then 500 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. Amplification of the IL4R α was detected using IL4R α TaqMan® Probe (Hs00166237_m1). 18S was amplified as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

IL4R α protein levels were determined via western blot 72 hours after cp-asiRNA treatment. Briefly, the transfected A549 cells were lysed with 1% SDS lysis buffer (1% SDS, 100 mM Tris pH 8.0). 15 μ g of the total protein extract of A549 cells were loaded onto an 8% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 3% BSA (Bioworld) and then incubated overnight at 4 °C in 3% BSA containing anti-IL4R α antibody (Acris) and anti-GAPDH antibody (Santa Cruz). The

membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL substrate (Thermo scientific) for 1 minute. The IL4R α and GAPDH bands were then imaged using a Chemidoc instrument (Bio-rad).

As shown the Figure 6 and Figure 7, cp-asiRNAs with different antisense strand lengths (21 or 19 nucleotides) exhibited the similar mRNA levels of IL4R α inhibition.

Example 6: Screening for TRPA1-specific asymmetric shorter-duplex small interfering RNAs

To identify asymmetric shorter-duplex small interfering RNAs (asiRNAs) that inhibit TRPA1 with high efficiency, 102 asiRNAs were synthesized and screened. The nucleic acid sequences of the screened asiRNAs are provided in Table 4.

Table 4: Nucleic acid sequences for exemplary TRPA1-targeting asiRNA.

SEQUENCE
TRPA1#1(S) : 5' UGAAGGACGCUCUCCA 3'
TRPA1#1(AS) : 5' UGGAGAGCGUCCUUCAGAAUC 3'
TRPA1#2(S) : 5' GAAGGACGCUCUCCAC 3'
TRPA1#2(AS) : 5' GUGGAGAGCGUCCUUCAGAAU 3'
TRPA1#3(S) : 5' UGAAGGACGCUCUCCA 3'
TRPA1#3(AS) : 5' UGGAGAGCGUCCUUCAGAAUC 3'
TRPA1#4(S) : 5' AGGACGCUCUCCACUU 3'
TRPA1#4(AS) : 5' AAGUGGAGAGCGUCCUUCAGA 3'
TRPA1#5(S) : 5' GGACGCUCUCCACUUA 3'
TRPA1#5(AS) : 5' UAAGUGGAGAGCGUCCUUCAG 3'
TRPA1#6(S) : 5' GACGCUCUCCACUUAU 3'
TRPA1#6(AS) : 5' AUAAGUGGAGAGCGUCCUUCA 3'

TRPA1#7(S) : 5' UUUUGCAGCCAGUUAU 3'
TRPA1#7(AS) : 5' AUAACUGGCUGCAAAAUGCAG 3'
TRPA1#8(S) : 5' UUUGCAGCCAGUUAUG 3'
TRPA1#8(AS) : 5' CAUAACUGGCUGCAAAAUGCA 3'
TRPA1#9(S) : 5' UUGCAGCCAGUUAUGG 3'
TRPA1#9(AS) : 5' CCAUAACUGGCUGCAAAAUGC 3'
TRPA1#10(S) : 5' UGCAGCCAGUUAUGGG 3'
TRPA1#10(AS) : 5' CCCAUAACUGGCUGCAAAAUG 3'
TRPA1#11(S) : 5' GCAGCCAGUUAUGGGC 3'
TRPA1#11(AS) : 5' GCCCAUAACUGGCUGCAAAAU 3'
TRPA1#12(S) : 5' CAGCCAGUUAUGGGCG 3'
TRPA1#12(AS) : 5' CGCCCAUAACUGGCUGCAAAA 3'
TRPA1#13(S) : 5' CAUAAGUGAUACGAGG 3'
TRPA1#13(AS) : 5' CCUCGUAUCACUUAUGUCUUG 3'
TRPA1#14(S) : 5' AUAAGUGAUACGAGGC 3'
TRPA1#14(AS) : 5' AAGACAUAAGUGAUACGAGGC 3'
TRPA1#15(S) : 5' UAAGUGAUACGAGGCU 3'
TRPA1#15(AS) : 5' AGCCUCGUAUCACUUAUGUCU 3'
TRPA1#16(S) : 5' AAGUGAUACGAGGCUU 3'
TRPA1#16(AS) : 5' AAGCCUCGUAUCACUUAUGUC 3'
TRPA1#17(S) : 5' CAGUGACCACAAUGGC 3'
TRPA1#17(AS) : 5' GCCAUUGUGGUCACUGAGAAA 3'
TRPA1#18(S) : 5' AGUGACCACAAUGGCU 3'
TRPA1#18(AS) : 5' AGCCAUUGUGGUCACUGAGAA 3'
TRPA1#19(S) : 5' GUGACCACAAUGGCUG 3'

TRPA1#19(AS) : 5' CAGCCAUUGUGGUCACUGAGA 3'
TRPA1#20(S) : 5' UGACCACAAUGGCUGG 3'
TRPA1#20(AS) : 5' CCAGCCAUUGUGGUCACUGAG 3'
TRPA1#21(S) : 5' GACCACAAUGGCUGGA 3'
TRPA1#21(AS) : 5' UCCAGCCAUUGUGGUCACUGA 3'
TRPA1#22(S) : 5' ACCACAAUGGCUGGAC 3'
TRPA1#22(AS) : 5' GUCCAGCCAUUGUGGUCACUG 3'
TRPA1#23(S) : 5' CACUCAGACCAUGAAG 3'
TRPA1#23(AS) : 5' CUUCAUGGUCUGAGUGUACCC 3'
TRPA1#24(S) : 5' ACUCAGACCAUGAAGG 3'
TRPA1#24(AS) : 5' CCUUCAUGGUCUGAGUGUACC 3'
TRPA1#25(S) : 5' CUCAGACCAUGAAGGU 3'
TRPA1#25(AS) : 5' ACCUUCAUGGUCUGAGUGUAC 3'
TRPA1#26(S) : 5' UCAGACCAUGAAGGUC 3'
TRPA1#26(AS) : 5' GACCUUCAUGGUCUGAGUGUA 3'
TRPA1#27(S) : 5' CAGACCAUGAAGGUCA 3'
TRPA1#27(AS) : 5' UGACCUUCAUGGUCUGAGUGU 3'
TRPA1#28(S) : 5' AGACCAUGAAGGUCAU 3'
TRPA1#28(AS) : 5' AUGACCUUCAUGGUCUGAGUG 3'
TRPA1#29(S) : 5' GACCAUGAAGGUCAUU 3'
TRPA1#29(AS) : 5' AAUGACCUUCAUGGUCUGAGU 3'
TRPA1#30(S) : 5' ACCAUGAAGGUCAUUC 3'
TRPA1#30(AS) : 5' GAAUGACCUUCAUGGUCUGAG 3'
TRPA1#31(S) : 5' CCAUGAAGGUCAUUCU 3'
TRPA1#31(AS) : 5' AGAAUGACCUUCAUGGUCUGA 3'

TRPA1#32(S) : 5' CAUGAAGGUCAUUCUU 3'
TRPA1#32(AS) : 5' AAGAAUGACCUUCAUGGUCUG 3'
TRPA1#33(S) : 5' AUGAAGGUCAUUCUUG 3'
TRPA1#33(AS) : 5' CAAGAAUGACCUUCAUGGUCU 3'
TRPA1#34(S) : 5' UGAAGGUCAUUCUUGA 3'
TRPA1#34(AS) : 5' UCAAGAAUGACCUUCAUGGUC 3'
TRPA1#35(S) : 5' GAAGGUCAUUCUUGAU 3'
TRPA1#35(AS) : 5' AUCAAGAAUGACCUUCAUGGU 3'
TRPA1#36(S) : 5' AAGGUCAUUCUUGAUA 3'
TRPA1#36(AS) : 5' UAUCAAGAAUGACCUUCAUGG 3'
TRPA1#37(S) : 5' AGGUCAUUCUUGAUAC 3'
TRPA1#37(AS) : 5' GUAUCAAGAAUGACCUUCAUG 3'
TRPA1#38(S) : 5' GGUCAUUCUUGAUACU 3'
TRPA1#38(AS) : 5' AGUAUCAAGAAUGACCUUCAU 3'
TRPA1#39(S) : 5' GUCAUUCUUGAUACUA 3'
TRPA1#39(AS) : 5' UAGUAUCAAGAAUGACCUUCA 3'
TRPA1#40(S) : 5' UCAUUCUUGAUACUAA 3'
TRPA1#40(AS) : 5' UUAGUAUCAAGAAUGACCUUC 3'
TRPA1#41(S) : 5' CAGAAGACAAGUCCUG 3'
TRPA1#41(AS) : 5' CAGGACUUGUCUUCUGUGGAA 3'
TRPA1#42(S) : 5' UUUCCAACAGAAAAGG 3'
TRPA1#42(AS) : 5' CCUUUUCUGUUGGAAAAUUUG 3'
TRPA1#43(S) : 5' GGCAAUGUGGAGCAAU 3'
TRPA1#43(AS) : 5' AUUGCUCACACAUUGCCACUGC 3'
TRPA1#44(S) : 5' GCAGGUGGAACUUCAU 3'

TRPA1#44(AS) : 5' AUGAAGUUCCACCUGCAUAGC 3'
TRPA1#45(S) : 5' CAGGUGGAACUUCAUA 3'
TRPA1#45(AS) : 5' UAUGAAGUUCCACCUGCAUAG 3'
TRPA1#46(S) : 5' AGGUGGAACUUCAUAC 3'
TRPA1#46(AS) : 5' GUAUGAAGUUCCACCUGCAUA 3'
TRPA1#47(S) : 5' GGUGGAACUUCAUACC 3'
TRPA1#47(AS) : 5' GGUAUGAAGUUCCACCUGCAU 3'
TRPA1#48(S) : 5' GUGGAACUUCAUACCA 3'
TRPA1#48(AS) : 5' UGGUAUGAAGUUCCACCUGCA 3'
TRPA1#49(S) : 5' UGAUUAUGGAAAUACC 3'
TRPA1#49(AS) : 5' GGUAUUUCCAUAUAUCAUCCA 3'
TRPA1#50(S) : 5' AAUACCCCUCUGCAUU 3'
TRPA1#50(AS) : 5' AAUGCAGAGGGGGUAUUUCCA 3'
TRPA1#51(S) : 5' UACCCCUCUGCAUUGU 3'
TRPA1#51(AS) : 5' ACAAUGCAGAGGGGGUAUUUCC 3'
TRPA1#52(S) : 5' ACCCCUCUGCAUUGUG 3'
TRPA1#52(AS) : 5' CACAAUGCAGAGGGGGUAUUUC 3'
TRPA1#53(S) : 5' UUGUGCUGUAGAAAAA 3'
TRPA1#53(AS) : 5' UUUUUCUACAGCACAAUGCAG 3'
TRPA1#54(S) : 5' ACGCUCUCCACUUAUA 3'
TRPA1#54(AS) : 5' UAUAAGUGGAGAGCGUCCUUC 3'
TRPA1#55(S) : 5' CCACUUAUAUUAAGCAA 3'
TRPA1#55(AS) : 5' UUGCUGAAUAUAAGUGGAGAGC 3'
TRPA1#56(S) : 5' GUGCCCAAGUAGACAU 3'
TRPA1#56(AS) : 5' AUGUCUACUUGGGCACCUUUA 3'

TRPA1#57(S) : 5' UGCCCAAGUAGACAUA 3'
TRPA1#57(AS) : 5' UAUGUCUACUUGGGCACCUU 3'
TRPA1#58(S) : 5' GCCCAAGUAGACAUA 3'
TRPA1#58(AS) : 5' UUAUGUCUACUUGGGCACCU 3'
TRPA1#59(S) : 5' CCCAAGUAGACAUA 3'
TRPA1#59(AS) : 5' UUAUGUCUACUUGGGCACCU 3'
TRPA1#60(S) : 5' CAAGUAGACAUA 3'
TRPA1#60(AS) : 5' CUUUUAUGUCUACUUGGGCAC 3'
TRPA1#61(S) : 5' AAGUAGACAUA 3'
TRPA1#61(AS) : 5' UCUUUUAUGUCUACUUGGGCA 3'
TRPA1#62(S) : 5' AGUAGACAUA 3'
TRPA1#62(AS) : 5' AUCUUUUAUGUCUACUUGGGC 3'
TRPA1#63(S) : 5' AUUUAUGCAGAUGCAA 3'
TRPA1#63(AS) : 5' UUGCAUCUGCAUAAAUUCAGG 3'
TRPA1#64(S) : 5' UAUGGGCGUAUCAUA 3'
TRPA1#64(AS) : 5' UAUUGAUACGCCCAUAACUGG 3'
TRPA1#65(S) : 5' AUGGGCGUAUCAUA 3'
TRPA1#65(AS) : 5' GUAUUGAUACGCCCAUAACUG 3'
TRPA1#66(S) : 5' CGAGGCUUCUGAAUGA 3'
TRPA1#66(AS) : 5' UCAUUCAGAAGCCUCGUAUCA 3'
TRPA1#67(S) : 5' GAGGCUUCUGAAUGAA 3'
TRPA1#67(AS) : 5' UUCAUUCAGAAGCCUCGUAUC 3'
TRPA1#68(S) : 5' AGGCUUCUGAAUGAAG 3'
TRPA1#68(AS) : 5' CUUCAUUCAGAAGCCUCGUAU 3'
TRPA1#69(S) : 5' UCUCAGUGACCACAAU 3'

TRPA1#69(AS) : 5' AUUGUGGUCACUGAGAAACAA 3'
TRPA1#70(S) : 5' CUCAGUGACCACAAUG 3'
TRPA1#70(AS) : 5' CAUUGUGGUCACUGAGAAACA 3'
TRPA1#71(S) : 5' ACACUCAGACCAUGAA 3'
TRPA1#71(AS) : 5' UUCAUGGUCUGAGUGUACCCG 3'
TRPA1#72(S) : 5' ACUGUCUUGGUCUCAU 3'
TRPA1#72(AS) : 5' AUGAGACCAAGACAGUAAGAU 3'
TRPA1#73(S) : 5' CUGUCUUGGUCUCAUA 3'
TRPA1#73(AS) : 5' UAUGAGACCAAGACAGUAAGA 3'
TRPA1#74(S) : 5' UGUCUUGGUCUCAUAC 3'
TRPA1#74(AS) : 5' GUAUGAGACCAAGACAGUAAG 3'
TRPA1#75(S) : 5' AUAUUUGGGUAUUGCA 3'
TRPA1#75(AS) : 5' UGCAAUACCCAAAUAUACUUG 3'
TRPA1#76(S) : 5' GGGUAUUGCAAAGAAG 3'
TRPA1#76(AS) : 5' CUUCUUUGCAAUACCCAAAUA 3'
TRPA1#77(S) : 5' UUUUCCAACAGAAAAG 3'
TRPA1#77(AS) : 5' CUUUUCUGUUGGAAAAUUUGC 3'
TRPA1#78(S) : 5' GCAAUGUGGAGCAAUU 3'
TRPA1#78(AS) : 5' AAUUGCUCACAUUGCCACUG 3'
TRPA1#79(S) : 5' UUUUGGACUCAGCUUU 3'
TRPA1#79(AS) : 5' AAAGCUGAGUCCAAAAGCCAG 3'
TRPA1#80(S) : 5' UUUGGACUCAGCUUUU 3'
TRPA1#80(AS) : 5' AAAAGCUGAGUCCAAAAGCCA 3'
TRPA1#81(S) : 5' UUGGACUCAGCUUUUA 3'
TRPA1#81(AS) : 5' UAAAAGCUGAGUCCAAAAGCC 3'

TRPA1#82(S) : 5' CUAGGAGAUAAUCAAUU 3'
TRPA1#82(AS) : 5' AAUUGAUAAUCUCCUAGCAUCA 3'
TRPA1#83(S) : 5' UAGGAGAUAAUCAAUUA 3'
TRPA1#83(AS) : 5' UAAUUGAUAAUCUCCUAGCAUC 3'
TRPA1#84(S) : 5' GGAGAUAAUCAAUUAUC 3'
TRPA1#84(AS) : 5' GAUAAUUGAUAAUCUCCUAGCA 3'
TRPA1#85(S) : 5' GAGAUAAUCAAUUAUCG 3'
TRPA1#85(AS) : 5' CGAUAAUUGAUAAUCUCCUAGC 3'
TRPA1#86(S) : 5' AGAUAAUCAAUUAUCGA 3'
TRPA1#86(AS) : 5' UCGAUAAUUGAUAAUCUCCUAG 3'
TRPA1#87(S) : 5' AUAUUUGUCCCAAUUG 3'
TRPA1#87(AS) : 5' CAAUUGGGACAAAUAAUUGUGA 3'
TRPA1#88(S) : 5' UAUUUUGUCCCAAUUGU 3'
TRPA1#88(AS) : 5' ACAAUUGGGACAAAUAAUUGUG 3'
TRPA1#89(S) : 5' CCAAUUGUCCUCAUGA 3'
TRPA1#89(AS) : 5' UCAUGAGGACAAUUGGGACAA 3'
TRPA1#90(S) : 5' CAAUUGUCCUCAUGAA 3'
TRPA1#90(AS) : 5' UUCAUGAGGACAAUUGGGACA 3'
TRPA1#91(S) : 5' UGCUGAGGUCCAGAAA 3'
TRPA1#91(AS) : 5' UUUCUGGACCUCAGCAAUGUC 3'
TRPA1#92(S) : 5' AGAGGAUAGCUAUGCA 3'
TRPA1#92(AS) : 5' UGCAUAGCUAUCCUCUUCAAU 3'
TRPA1#93(S) : 5' GAGGAUAGCUAUGCAG 3'
TRPA1#93(AS) : 5' CUGCAUAGCUAUCCUCUUCAA 3'
TRPA1#94(S) : 5' UAUGCAGGUGGAACUU 3'

TRPA1#94(AS) : 5' AAGUUCCACCUGCAUAGCUAU 3'
TRPA1#95(S) : 5' AUGCAGGUGGAACUUC 3'
TRPA1#95(AS) : 5' GAAGUUCCACCUGCAUAGCUA 3'
TRPA1#96(S) : 5' UGCAGGUGGAACUUCA 3'
TRPA1#96(AS) : 5' UGAAGUUCCACCUGCAUAGCU 3'
TRPA1#97(S) : 5' AACAGCAUGAGCUCAU 3'
TRPA1#97(AS) : 5' AUGAGCUCAUGCUGUUUUUCC 3'
TRPA1#98(S) : 5' CAGAAGAUGGAGAUCA 3'
TRPA1#98(AS) : 5' UGAUCUCCAUCUUCUGAAUGA 3'
TRPA1#99(S) : 5' AGAAGAUGGAGAUCAU 3'
TRPA1#99(AS) : 5' AUGAUCUCCAUCUUCUGAAUG 3'
TRPA1#100(S) : 5' GAAGAUGGAGAUCAUC 3'
TRPA1#100(AS) : 5' GAUGAUCUCCAUCUUCUGAAU 3'
TRPA1#101(S) : 5' AAGAUGGAGAUCAUCU 3'
TRPA1#101(AS) : 5' AGAUGAUCUCCAUCUUCUGAA 3'
TRPA1#102(S) : 5' GAUGGAGAUCAUCUCU 3'
TRPA1#102(AS) : 5' AGAGAUGAUCUCCAUCUUCUG 3'

The asiRNAs listed in Table 4 were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in annealing buffer (Bioneer Inc. Korea). Proper strand annealing was confirmed via gel electrophoresis using a UV transilluminator. For the screen, 5X10³ A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco) and 100 units/ml Penicillin and 100 µg/ml Streptomycin in a 100 mm cell culture dish were seed 96 well plates. The A549 cells were transfected with 0.1 nM of the asiRNAs using RNAiMAX (Invitrogen Inc.) according to the manufacturer's instructions.

The TRPA1 mRNA levels in the transfected cells were measured 24 hours after transfection using qRT-PCR. Specifically, total RNA was extracted and synthesized the

cDNA using SuperPrep Cell Lysis & RT kit for qPCR (TOYOBO) according to manufacturer's instructions. qRT-PCR was performed using THUNDERBIRD® Probe qPCR Mix (TOYOBO) according to manufacturer's instructions. Amplification of the TRPA1 was detected using TRPA1 TaqMan® Probe (Hs00175798_m1). 18S was amplified
 5 as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

The level of TRPA1 inhibition by each of the 102 asiRNAs is provided in Figure 9. 14 of the asiRNA sequences, asiRNA (#32), asiRNA (#34), asiRNA (#35), asiRNA (#38), asiRNA (#40), asiRNA (#41), asiRNA (#50), asiRNA (#64), asiRNA (#66), asiRNA (#69), asiRNA (#71), asiRNA (#72), asiRNA (#78) and asiRNA (#81), were selected for use in
 10 follow-up studies.

Example 7: Inhibition of TRPA1 mRNA and protein expression using TRPA1-targeting asiRNAs

The asiRNAs selected in Example 6, asiRNA (#32), asiRNA (#34), asiRNA (#35), asiRNA (#38), asiRNA (#40), asiRNA (#41), asiRNA (#50), asiRNA (#64), asiRNA (#66),
 15 asiRNA (#69), asiRNA (#71), asiRNA (#72), asiRNA (#78) and asiRNA (#81), were tested for their ability to inhibit TRPA1 mRNA and protein expression.

The asiRNAs were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in annealing buffer (Bioneer Inc. Korea). Proper strand annealing was confirmed via gel electrophoresis using a UV transilluminator. For the screen, A549 cells (ATCC) that had
 20 been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco) and 100 units/ml Penicillin and 100 µg/ml Streptomycin in a 100 mm cell culture dish. One day prior to transfection, 2.5×10^4 A549 cells were seeded in 24-well plates. The A549 cells were transfected with 1nM of the asiRNAs using RNAiMAX (Invitrogen Inc.) according to the manufacturer's instructions.

25 24 hours after asiRNA transfection, total RNA was extracted using RNAiPlus® (TaKaRa) and then 500 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. Amplification of the TRPA1 was detected using TRPA1 TaqMan® Probe (Hs00175798_m1). 18S was amplified as an internal control using 18S
 30 TaqMan® Probe (Hs03928985_g1).

48 hours after asiRNA transfection, TRPA1 protein levels were determined via western blot. Briefly, the transfected A549 cells were lysed with 1% SDS lysis buffer (1% SDS, 100 mM Tris pH 8.0). 30 µg of the total protein extract of A549 cells were loaded

onto an 8% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 5% skim milk (Seoul Milk) and 1% BSA (Bioworld) and then incubated overnight at 4 °C in 5% skim milk and 1% BSA containing anti-TRPA1 antibody (Novus) and anti- β -actin antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL substrate (Thermo scientific) for 1 minute. The TRPA1 and β -actin bands were then imaged using a Chemidoc instrument (Bio-rad).

The level of TRPA1 inhibition of 14 asiRNAs is provided in Figure 10.

The results of western blot are depicted in Figure 11. asiRNA (#71) and asiRNA (#81) were selected for use in follow-up studies.

Example 8: Chemical modification of asiRNAs for self-delivery

Chemical modifications were applied to the asiRNAs and the cellular delivery of the modified asiRNAs was tested in the absence of other delivery reagents. As described below, certain of the modifications improved endocytosis and stability of asiRNAs. Such cell penetrating asiRNAs (cp-asiRNAs) are able to be delivered into the cell in the absence of a delivery reagent.

Potential cp-asiRNA (Table 5) screened for TRPA1 mRNA and protein inhibition in A549 cells. Each potential cp-asiRNA was incubated with A549 cells at 1 μ M and 3 μ M without a delivery reagent and TRPA1 expression levels were measured by qRT-PCR and western blot.

Table 5: Modified asiRNA sequences tested for self-delivery and TRPA1 inhibition. (m= 2'-O-Methyl RNA. *= phosphorothioate bond.)

Name	SEQUENCE
TRPA1 cp-asiRNA #71 PS4 (s)	5' mACmACmUCmAGmACmCAmU*G*mA*A*Cholesterol 3'
TRPA1 cp-asiRNA #71 21(2,4) (AS)	5' UUCAUGGUCUGAGUmGmUA*C*C*C*G 3'

TRPA1 cp-asiRNA #71 21(4,4) (AS)	5' UUCAUGGUCUGAGUmGmUmA*mC*C*C*G 3'
TRPA1 cp-asiRNA #71 21(7,4) (AS)	5' UUCAUGGUCUGAGUmGmUmA*mC*mC*mC*mG 3'
TRPA1 cp-asiRNA #81 PS4 (s)	5' mUUmGGmACmUCmAGmCUmU*U*mU*A*Cholesterol 3'
TRPA1 cp-asiRNA #81 21(2,4) (AS)	5' UAAAAGCUGAGUCCmAmAA*A*G*C*C 3'
TRPA1 cp-asiRNA #81 21(4,4) (AS)	5' UAAAAGCUGAGUCCmAmAmA*mA*G*C*C 3'
TRPA1 cp-asiRNA #81 21(7,4) (AS)	5' UAAAAGCUGAGUCCmAmAmA*mA*mG*mC*mC 3'

A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco), 100 units/ml Penicillin and 100 µg/ml Streptomycin in a 100 mm cell culture dish.

The potential cp-asiRNAs listed in Table 2 were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in Opti-MEM (Gibco). Proper strand annealing of the potential cp-asiRNAs was confirmed by gel electrophoresis using a UV transilluminator.

One day prior to treatment, 2.5×10^4 A549 cells were seeded in 24-well plates. Immediately before treatment, the A549 cells were washed with Dulbecco's modified Eagle's medium (DMEM, Gibco) then cultured in the presence of the potential cp-asiRNAs in OPTI-MEM buffer for 24 hours, at each point the cp-asiRNA containing OPTI-MEM media was replaced with a serum containing media.

Total RNA was extracted 48 hours after asiRNA transfection, using RNAiPlus® (TaKaRa) and then 500 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. Amplification of the TRPA1 was detected using TRPA1 TaqMan® Probe (Hs00175798_m1). 18S was amplified as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

TRPA1 protein levels were determined via western blot 72 hours after asiRNA transfection. Briefly, the transfected A549 cells were lysed with 1% SDS lysis buffer (1% SDS, 100 mM Tris pH8.0). 30 µg of the total protein extract of A549 cells were loaded onto an 8% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 5% skim milk (Seoul Milk) and 1% BSA (Bioworld) and then incubated overnight at 4 °C in 5% skim milk and 1% BSA containing anti-TRPA1 antibody (Novus) and anti-β-actin antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL substrate (Thermo scientific) for 1 minute. The TRPA1 and β-actin bands were then imaged using a Chemidoc instrument (Bio-rad).

The level of TRPA1 inhibition of 6 potential cp-asiRNAs is provided in Figure 12 and Figure 13. cp-asiRNA #71_21(4, 4) and cp-asiRNA #81_21(4, 4) was selected for further studies.

Example 9: Additional chemical modification of cp-asiRNA structures.

A variety of potential TRPA1 cp-asiRNA structures having different strand length and number of phosphorothioate bond and 2'-O-methylateion modifications were synthesized and tested for its ability to inhibit TRPA1 expression (Table 6).

Table 6: Additional cp-asiRNA sequence.

(m= 2'-O-Methyl RNA. *= phosphorothioate bond.)

Name	SEQUENCE
TRPA1 cp-asiRNA #71_PS3 (s)	5' mACmACmUCmAGmACmCamUG*mA*A*Cholesterol 3'
TRPA1 cp-asiRNA #71_PS4 (s)	5' mACmACmUCmAGmACmCamU*G*mA*A*Cholesterol 3'
TRPA1 cp-asiRNA #71_19(4,4) (AS)	5' UUCAUGGUCUGAGUmG*mU*mA*mC*C 3'

TRPA1 cp- asiRNA #71_21(4,4) (AS)	5' UUCAUGGUCUGAGUmGmUmA*mC*C*C*G 3'
TRPA1 cp- asiRNA #81_PS3 (s)	5' mUUmGGmACmUCmAGmCUmUU*mU*A*Cholesterol 3'
TRPA1 cp- asiRNA #81_PS4 (s)	5' mUUmGGmACmUCmAGmCUmU*U*mU*A*Cholesterol 3'
TRPA1 cp- asiRNA #81_19(4,4) (AS)	5' UAAAAGCUGAGUCCmA*mA*mA*mA*G 3'
TRPA1 cp- asiRNA #81_21(4,4) (AS)	5' UAAAAGCUGAGUCCmAmAmA*mA*G*C*C 3'

The ability of 1 μ M or 3 μ M of each of the potential cp-asiRNAs listed in Table 6 to inhibit TRPA1 mRNA and protein expression in A549 cells was tested.

5 A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco), 100 units/ml Penicillin and 100 μ g/ml Streptomycin. The potential cp-asiRNAs listed in Table 3 were incubated at 95 °C for 5 minutes and at 37 °C for 1hour in Opti-MEM (Gibco). Proper strand annealing was confirmed by gel electrophoresis using a UV transilluminator.

10 One day prior to treatment, 2.5×10^4 A549 cells were seeded in 24-well plates. Immediately before treatment, the A549 cells were washed with DMEM (Gibco) then cultured in the presence of the potential cp-asiRNAs in Opti-MEM media for 24 hours, at which point the cp-asiRNA-containing Opti-MEM media was replaced with a serum-containing media.

15 Total RNA was extracted 48 hours after asiRNA transfection using RNAiPlus® (TaKaRa) and then 500 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. Amplification of the TRPA1 was detected using TRPA1 TaqMan® Probe (Hs00175798_m1). 18S was amplified as an internal control using 18S
20 TaqMan® Probe (Hs03928985_g1).

TRPA1 protein levels were determined via western blot 72 hours after asiRNA transfection. Briefly, the transfected A549 cells were lysed with 1% SDS lysis buffer (1%

SDS, 100 mM Tris pH 8.0). 30 µg of the total protein extract of A549 cells were loaded onto an 8% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 5% skim milk (Seoul Milk) and 1% BSA (Bioworld) and then incubated overnight at 4 °C in 5% skim milk and 1% BSA containing anti-TRPA1 antibody (Novus) and anti-β-actin antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL substrate (Thermo scientific) for 1 minute. The TRPA1 and β-actin bands were then imaged using a Chemidoc instrument (Bio-rad).

As shown the Figure 14 and Figure 15, the indicated TRPA1 cp-asiRNA exhibited the similar mRNA levels of TRPA1 inhibition.

Example 10: Inhibition of TRPA1 protein expression using TRPA1-specific cp-asiRNAs

The efficacy of cp-asiRNAs for the inhibition of TRPA1 protein level was tested.

Each potential cp-asiRNA was incubated with A549 cells at 1 µM and 3 µM without a delivery reagent and TRPA1 protein levels were measured by western blot.

A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco) and 100 units/ml Penicillin and 100 µg/ml Streptomycin. The potential cp-asiRNAs were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in Opti-MEM (Gibco). Proper strand annealing was confirmed by gel electrophoresis using a UV transilluminator.

One day prior to treatment, 2.5×10^4 A549 cells were seeded in 24-well plates. Immediately before treatment, the A549 cells were washed with DMEM (Gibco) then cultured in the presence of the potential cp-asiRNAs in Opti-MEM media for 24 hours, at which point the cp-asiRNA-containing Opti-MEM media was replaced with a serum-containing media.

TRPA1 protein levels were determined via western blot 72 hours after asiRNA transfection. Briefly, the transfected A549 cells were lysed with 1% SDS lysis buffer (1% SDS, 100 mM Tris pH 8.0). 30 µg of the total protein extract of A549 cells were loaded onto an 8% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room

temperature with 5% skim milk (Seoul Milk) and 1% BSA (Bioworld) and then incubated overnight at 4 °C in 5% skim milk and 1% BSA containing anti-TRPA1 antibody (Novus) and anti-GAPDH antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 5% skim milk with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL substrate (Thermo scientific) for 1 minute. The TRPA1 and GAPDH bands were then imaged using a Chemidoc instrument (Bio-rad).

The results of the western blot assay are depicted in Figure 16. As a result, TRPA1 cp-asiRNA #81 containing 3 phosphorothioate bond on sense strand and 19 nucleotides antisense strand with 4 phosphorothioate bond and four 2'-O-Methylation (TRPA1 cp-asiRNA #81_PS3/19(4,4)) exhibited the highest levels of TRPA1 inhibition.

Example 11: Screening for F2RL1-targeting asymmetric shorter-duplex small interfering RNAs

To identify asymmetric shorter-duplex small interfering RNAs (asiRNAs) that inhibit F2RL1 with high efficiency, 100 asiRNAs were synthesized and screened. The nucleic acid sequences of the screened asiRNAs are provided in Table 7.

Table 7: Nucleic acid sequences for exemplary F2RL1-targeting asiRNA.

SEQUENCE
F2RL1#1(S) : 5'CCUCUCUGUCAUCUGG 3'
F2RL1#1(AS) : 5'CCAGAUGACAGAGAGGAGGUC 3'
F2RL1#2(S) : 5'CUCUCUGUCAUCUGGU 3'
F2RL1#2(AS) : 5'ACCAGAUGACAGAGAGGAGGU 3'
F2RL1#3(S) : 5'UCUCUGUCAUCUGGUU 3'
F2RL1#3(AS) : 5'AACCAGAUGACAGAGAGGAGG 3'
F2RL1#4(S) : 5'CUCUGUCAUCUGGUUC 3'
F2RL1#4(AS) : 5'GAACCAGAUGACAGAGAGGAG 3'
F2RL1#5(S) : 5'UCUGUCAUCUGGUUCC 3'
F2RL1#5(AS) : 5'GGAACCAGAUGACAGAGAGGA 3'
F2RL1#6(S) : 5'CUGUCAUCUGGUUCCC 3'
F2RL1#6(AS) : 5'GGGAACCAGAUGACAGAGAGG 3'
F2RL1#7(S) : 5'UGUCAUCUGGUUCCCC 3'
F2RL1#7(AS) : 5'GGGGAACCAGAUGACAGAGAG 3'
F2RL1#8(S) : 5'CACCAUCCCUUUGUAU 3'
F2RL1#8(AS) : 5'AUACAAAGGGAUGGUGACCAG 3'
F2RL1#9(S) : 5'ACCAUCCCUUUGUAUG 3'

F2RL1#9(AS) : 5'CAUACAAAGGGAUGGUGACCA 3'
F2RL1#10(S) : 5'CCAUCCCUUUGUAUGU 3'
F2RL1#10(AS) : 5'ACAUACAAAGGGAUGGUGACC 3'
F2RL1#11(S) : 5'CAUCCCUUUGUAUGUC 3'
F2RL1#11(AS) : 5'GACAUACAAAGGGAUGGUGAC 3'
F2RL1#12(S) : 5'ACAAAGGGAUGGUGAC 3'
F2RL1#12(AS) : 5'GUCACCAUCCCUUUGUAUGUC 3'
F2RL1#13(S) : 5'UUCAAUUACUUCCUCU 3'
F2RL1#13(AS) : 5'AGAGGAAGUAAUUGAACAUGU 3'
F2RL1#14(S) : 5'UCAAUUACUUCCUCUC 3'
F2RL1#14(AS) : 5'GAGAGGAAGUAAUUGAACAUG 3'
F2RL1#15(S) : 5'CUUUGUCUAUUACUUU 3'
F2RL1#15(AS) : 5'AAAGUAAUAGACAAAGGGGUC 3'
F2RL1#16(S) : 5'UUUGUCUAUUACUUUG 3'
F2RL1#16(AS) : 5'CAAAGUAAUAGACAAAGGGGU 3'
F2RL1#17(S) : 5'UUGUCUAUUACUUUGU 3'
F2RL1#17(AS) : 5'ACAAAGUAAUAGACAAAGGGG 3'
F2RL1#18(S) : 5'AUGGCCAAUCUGGCCU 3'
F2RL1#18(AS) : 5'AGGCCAGAUUGGCCAUGUAAA 3'
F2RL1#19(S) : 5'UUGGCUGACCUCCUCU 3'
F2RL1#19(AS) : 5'AGAGGAGGUCAGCCAAGGCCA 3'
F2RL1#20(S) : 5'GGCUGACCUCCUCUCU 3'
F2RL1#20(AS) : 5'AGAGAGGAGGUCAGCCAAGGC 3'
F2RL1#21(S) : 5'GCUGACCUCCUCUCUG 3'
F2RL1#21(AS) : 5'CAGAGAGGAGGUCAGCCAAGG 3'
F2RL1#22(S) : 5'CUGACCUCCUCUCUGU 3'
F2RL1#22(AS) : 5'ACAGAGAGGAGGUCAGCCAAG 3'
F2RL1#23(S) : 5'UGACCUCCUCUCUGUC 3'
F2RL1#23(AS) : 5'GACAGAGAGGAGGUCAGCCAA 3'
F2RL1#24(S) : 5'GACCUCCUCUCUGUCA 3'
F2RL1#24(AS) : 5'UGACAGAGAGGAGGUCAGCCA 3'
F2RL1#25(S) : 5'ACCUCCUCUCUGUCAU 3'
F2RL1#25(AS) : 5'AUGACAGAGAGGAGGUCAGCC 3'
F2RL1#26(S) : 5'CCUCCUCUCUGUCAUC 3'
F2RL1#26(AS) : 5'GAUGACAGAGAGGAGGUCAGC 3'
F2RL1#27(S) : 5'CUCCUCUCUGUCAUCU 3'
F2RL1#27(AS) : 5'AGAUGACAGAGAGGAGGUCAG 3'
F2RL1#28(S) : 5'UCCUCUCUGUCAUCUG 3'
F2RL1#28(AS) : 5'CAGAUGACAGAGAGGAGGUCA 3'

F2RL1#29(S) : 5'GUCAUCUGGUUCCCCU 3'
F2RL1#29(AS) : 5'AGGGGAACCAGAUGACAGAGA 3'
F2RL1#30(S) : 5'ACAUGGCAACAACUGG 3'
F2RL1#30(AS) : 5'CCAGUUGUUGCCAUGUAUGUG 3'
F2RL1#31(S) : 5'UAUUGGCUUUUUCUAU 3'
F2RL1#31(AS) : 5'AUAGAAAAAGCCAAUAAGCAC 3'
F2RL1#32(S) : 5'AUUGGCUUUUUCUAUG 3'
F2RL1#32(AS) : 5'CAUAGAAAAAGCCAAUAAGCA 3'
F2RL1#33(S) : 5'UUGGCUUUUUCUAUGG 3'
F2RL1#33(AS) : 5'CCAUAGAAAAAGCCAAUAAGC 3'
F2RL1#34(S) : 5'UUCUAUGGCAACAUGU 3'
F2RL1#34(AS) : 5'ACAUGUUGCCAUAGAAAAAGC 3'
F2RL1#35(S) : 5'UCUAUGGCAACAUGUA 3'
F2RL1#35(AS) : 5'UACAUGUUGCCAUAGAAAAAG 3'
F2RL1#36(S) : 5'CUCUUCAUGACCUGCC 3'
F2RL1#36(AS) : 5'GGCAGGUCAUGAAGAGAAUGG 3'
F2RL1#37(S) : 5'UCUUCAUGACCUGCCU 3'
F2RL1#37(AS) : 5'AGGCAGGUCAUGAAGAGAAUG 3'
F2RL1#38(S) : 5'CUUCAUGACCUGCCUC 3'
F2RL1#38(AS) : 5'GAGGCAGGUCAUGAAGAGAAU 3'
F2RL1#39(S) : 5'UUCAUGACCUGCCUCA 3'
F2RL1#39(AS) : 5'UGAGGCAGGUCAUGAAGAGAA 3'
F2RL1#40(S) : 5'UCAUGACCUGCCUCAG 3'
F2RL1#40(AS) : 5'CUGAGGCAGGUCAUGAAGAGA 3'
F2RL1#41(S) : 5'CAUGACCUGCCUCAGU 3'
F2RL1#41(AS) : 5'ACUGAGGCAGGUCAUGAAGAG 3'
F2RL1#42(S) : 5'UGCCUCAGUGUGCAGA 3'
F2RL1#42(AS) : 5'UCUGCACACUGAGGCAGGUCA 3'
F2RL1#43(S) : 5'GCCUCAGUGUGCAGAG 3'
F2RL1#43(AS) : 5'CUCUGCACACUGAGGCAGGUC 3'
F2RL1#44(S) : 5'CUCAGUGUGCAGAGGU 3'
F2RL1#44(AS) : 5'ACCUCUGCACACUGAGGCAGG 3'
F2RL1#45(S) : 5'UCAGUGUGCAGAGGUA 3'
F2RL1#45(AS) : 5'UACCUCUGCACACUGAGGCAG 3'
F2RL1#46(S) : 5'CAUCGUGAACCCCAUG 3'
F2RL1#46(AS) : 5'CAUGGGGUUCACGAUGACCCA 3'
F2RL1#47(S) : 5'AUCGUGAACCCCAUGG 3'
F2RL1#47(AS) : 5'CCAUGGGGUUCACGAUGACCC 3'
F2RL1#48(S) : 5'UCGUGAACCCCAUGGG 3'

F2RL1#48(AS) : 5'CCCAUGGGGUUCACGAUGACC 3'
F2RL1#49(S) : 5'CAGGAAGAAGGCAAAC 3'
F2RL1#49(AS) : 5'GUUUGCCUUCUUCCUGGAGUG 3'
F2RL1#50(S) : 5'AGGAAGAAGGCAAACA 3'
F2RL1#50(AS) : 5'UGUUUGCCUUCUUCCUGGAGU 3'
F2RL1#51(S) : 5'GGAAGAAGGCAAACAU 3'
F2RL1#51(AS) : 5'AUGUUUGCCUUCUUCCUGGAG 3'
F2RL1#52(S) : 5'GUCACCAUCCCUUUGU 3'
F2RL1#52(AS) : 5'ACAAAGGGAUGGUGACCAGCA 3'
F2RL1#53(S) : 5'UCACCAUCCCUUUGUA 3'
F2RL1#53(AS) : 5'UACAAAGGGAUGGUGACCAGC 3'
F2RL1#54(S) : 5'AUCCCUUUGUAUGUCG 3'
F2RL1#54(AS) : 5'CGACAUACAAAGGGAUGGUGA 3'
F2RL1#55(S) : 5'UGUAUGUCGUGAAGCA 3'
F2RL1#55(AS) : 5'UGCUUCACGACAUACAAAGGG 3'
F2RL1#56(S) : 5'GUAUGUCGUGAAGCAG 3'
F2RL1#56(AS) : 5'CUGCUUCACGACAUACAAAGG 3'
F2RL1#57(S) : 5'UAUGUCGUGAAGCAGA 3'
F2RL1#57(AS) : 5'UCUGCUUCACGACAUACAAAG 3'
F2RL1#58(S) : 5'GUCGUGAAGCAGACCA 3'
F2RL1#58(AS) : 5'UGGUCUGCUUCACGACAUACA 3'
F2RL1#59(S) : 5'UCGUGAAGCAGACCAU 3'
F2RL1#59(AS) : 5'AUGGUCUGCUUCACGACAUAC 3'
F2RL1#60(S) : 5'CGUGAAGCAGACCAUC 3'
F2RL1#60(AS) : 5'GAUGGUCUGCUUCACGACAU 3'
F2RL1#61(S) : 5'GUGAAGCAGACCAUCU 3'
F2RL1#61(AS) : 5'AGAUGGUCUGCUUCACGACAU 3'
F2RL1#62(S) : 5'GGGAGACAUGUUCAAU 3'
F2RL1#62(AS) : 5'AUUGAACAUGUCUCCCACCAA 3'
F2RL1#63(S) : 5'GGAGACAUGUUCAAUU 3'
F2RL1#63(AS) : 5'AAUUGAACAUGUCUCCCACCA 3'
F2RL1#64(S) : 5'GAGACAUGUUCAAUUA 3'
F2RL1#64(AS) : 5'UAAUUGAACAUGUCUCCCACC 3'
F2RL1#65(S) : 5'AGACAUGUUCAAUUAC 3'
F2RL1#65(AS) : 5'GUAAUUGAACAUGUCUCCCAC 3'
F2RL1#66(S) : 5'GACAUGUUCAAUUACU 3'
F2RL1#66(AS) : 5'AGUAAUUGAACAUGUCUCCCA 3'
F2RL1#67(S) : 5'ACAUGUUCAAUUACUU 3'
F2RL1#67(AS) : 5'AAGUAAUUGAACAUGUCUCCC 3'

F2RL1#68(S) : 5'CAUGUUCAAUUACUUC 3'
F2RL1#68(AS) : 5'GAAGUAAUUGAACAUGUCUCC 3'
F2RL1#69(S) : 5'AUGUUCAAUUACUUC 3'
F2RL1#69(AS) : 5'GGAAGUAAUUGAACAUGUCUC 3'
F2RL1#70(S) : 5'UGUUCAAUUACUUCCU 3'
F2RL1#70(AS) : 5'AGGAAGUAAUUGAACAUGUCU 3'
F2RL1#71(S) : 5'CAAUUACUUCCUCUCU 3'
F2RL1#71(AS) : 5'AGAGAGGAAGUAAUUGAACAU 3'
F2RL1#72(S) : 5'UUCCUCUCUCUGGCCA 3'
F2RL1#72(AS) : 5'UGGCCAGAGAGAGGAAGUAAU 3'
F2RL1#73(S) : 5'CCUCUCUCUGGCCAUU 3'
F2RL1#73(AS) : 5'AAUGGCCAGAGAGAGGAAGUA 3'
F2RL1#74(S) : 5'CUCUCUCUGGCCAUUG 3'
F2RL1#74(AS) : 5'CAAUGGCCAGAGAGAGGAAGU 3'
F2RL1#75(S) : 5'UCUCUCUGGCCAUUGG 3'
F2RL1#75(AS) : 5'CCAAUGGCCAGAGAGAGGAAG 3'
F2RL1#76(S) : 5'UGAAAACUCAGAGAAG 3'
F2RL1#76(AS) : 5'CUUCUCUGAGUUUUCAUCCAU 3'
F2RL1#77(S) : 5'GAAAACUCAGAGAAGA 3'
F2RL1#77(AS) : 5'UCUUCUCUGAGUUUUCAUCCA 3'
F2RL1#78(S) : 5'AAAACUCAGAGAAGAA 3'
F2RL1#78(AS) : 5'UUCUUCUCUGAGUUUUCAUCC 3'
F2RL1#79(S) : 5'AAACUCAGAGAAGAAA 3'
F2RL1#79(AS) : 5'UUUCUUCUCUGAGUUUUCAUC 3'
F2RL1#80(S) : 5'ACUCAGAGAAGAAAAG 3'
F2RL1#80(AS) : 5'CUUUUCUUCUCUGAGUUUUCA 3'
F2RL1#81(S) : 5'CUCAGAGAAGAAAAGG 3'
F2RL1#81(AS) : 5'CCUUUUCUUCUCUGAGUUUUC 3'
F2RL1#82(S) : 5'CUGCAUCGACCCCUUU 3'
F2RL1#82(AS) : 5'AAAGGGGUCGAUGCAGCUGUU 3'
F2RL1#83(S) : 5'UGCAUCGACCCCUUUG 3'
F2RL1#83(AS) : 5'CAAAGGGGUCGAUGCAGCUGU 3'
F2RL1#84(S) : 5'GCAUCGACCCCUUUGU 3'
F2RL1#84(AS) : 5'ACAAAGGGGUCGAUGCAGCUG 3'
F2RL1#85(S) : 5'CAUCGACCCCUUUGUC 3'
F2RL1#85(AS) : 5'GACAAAGGGGUCGAUGCAGCU 3'
F2RL1#86(S) : 5'AUCGACCCCUUUGUCU 3'
F2RL1#86(AS) : 5'AGACAAAGGGGUCGAUGCAGC 3'
F2RL1#87(S) : 5'UCGACCCCUUUGUCUA 3'

F2RL1#87(AS) : 5'UAGACAAAGGGGUCGAUGCAG 3'
F2RL1#88(S) : 5'CGACCCCUUUGUCUAU 3'
F2RL1#88(AS) : 5'AUAGACAAAGGGGUCGAUGCA 3'
F2RL1#89(S) : 5'GACCCCUUUGUCUAUU 3'
F2RL1#89(AS) : 5'AAUAGACAAAGGGGUCGAUGC 3'
F2RL1#90(S) : 5'ACCCCUUUGUCUAUUA 3'
F2RL1#90(AS) : 5'UAAUAGACAAAGGGGUCGAUG 3'
F2RL1#91(S) : 5'CCCCUUGUCUAUUAC 3'
F2RL1#91(AS) : 5'GUAAUAGACAAAGGGGUCGAU 3'
F2RL1#92(S) : 5'CCCUUUGUCUAUUACU 3'
F2RL1#92(AS) : 5'AGUAAUAGACAAAGGGGUCGA 3'
F2RL1#93(S) : 5'CCUUGUCUAUUACUU 3'
F2RL1#93(AS) : 5'AAGUAAUAGACAAAGGGGUCG 3'
F2RL1#94(S) : 5'UGUCUAUUACUUUGUU 3'
F2RL1#94(AS) : 5'AACAAAGUAAUAGACAAAGGG 3'
F2RL1#95(S) : 5'UGCCGAAGUGUCCGCA 3'
F2RL1#95(AS) : 5'UGCGGACACUUCGGCAAAGGA 3'
F2RL1#96(S) : 5'GCCGAAGUGUCCGCAC 3'
F2RL1#96(AS) : 5'GUGCGGACACUUCGGCAAAGG 3'
F2RL1#97(S) : 5'CCGAAGUGUCCGCACU 3'
F2RL1#97(AS) : 5'AGUGCGGACACUUCGGCAAAG 3'
F2RL1#98(S) : 5'CGAAGUGUCCGCACUG 3'
F2RL1#98(AS) : 5'CAGUGCGGACACUUCGGCAAA 3'
F2RL1#99(S) : 5'GAAGUGUCCGCACUGU 3'
F2RL1#99(AS) : 5'ACAGUGCGGACACUUCGGCAA 3'
F2RL1#100(S) : 5'AAGUGUCCGCACUGUA 3'
F2RL1#100(AS) : 5'UACAGUGCGGACACUUCGGCA 3'

The asiRNAs listed in Table 7 were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in 1x siRNA duplex buffer (Bioneer). Proper strand annealing was confirmed via gel electrophoresis.

5 For the screen, A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 µg/ml penicillin / streptomycin in a 100 mm cell culture dish. One day prior to transfection, 5 x 10³ A549 cells were seeded in 96-well plates. The A549 cells were transfected with 0.1 nM of the asiRNAs using RNAiMAX (Invitrogen) according to the manufacturer's instructions.

10 The F2RL1 mRNA levels in the transfected cells were measured 24 hours after transfection using real-time PCR. Specifically, total RNA was extracted and synthesized the

cDNA using SuperPrep Cell Lysis & RT Kit for qPCR (TOYOBO), according to the manufacturer's instructions. Real-time PCR was performed using THUNDERBIRD® Probe qPCR Mix (TOYOBO) according to manufacturer's instructions. Amplification of the F2RL1 was detected using F2RL1 TaqMan® Probe (Hs00608346_m1). 18S was
 5 amplified as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

The level of F2RL1 inhibition by each of the 100 asiRNAs is provided in Figure 18. 29 of the asiRNA sequences, asiF2RL1#1, #22, #25, #26, #28, #29, #31, #34, #35, #45, #50, #51, #55, #57, #59, #64, #65, #67, #69, #73, #76, #77, #81, #84, #86, #87, #88, #92, and #100 were selected for use in follow-up studies.

10 Example 12: Inhibition of F2RL1 mRNA expression using F2RL1-targeting asiRNAs

The 29 asiRNAs selected in Example 12, asiF2RL1 #1, #22, #25, #26, #28, #29, #31, #34, #35, #45, #50, #51, #55, #57, #59, #64, #65, #67, #69, #73, #76, #77, #81, #84, #86, #87, #88, #92, and #100, were tested for their ability to inhibit F2RL1 expression.

The asiRNAs were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in 1x
 15 siRNA duplex buffer (Bioneer). Proper strand annealing was confirmed via gel electrophoresis. For the screen, A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 µg/ml penicillin / streptomycin in a 100 mm cell culture dish. One day prior to transfection, 2.5 x 10⁴ A549 cells were seeded in 24-well plates. The A549 cells were transfected with
 20 asiRNAs using RNAiMAX (Invitrogen) according to the manufacturer's instructions.

Specifically, total RNA was extracted using RNAiso Plus (TaKaRa), and then 500 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. Amplification of the F2RL1 was detected using F2RL1 TaqMan® Probe
 25 (Hs00608346_m1). 18S was amplified as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

The level of F2RL1 inhibition of 29 asiRNAs is provided in Figure 19. Twelve asiRNAs; asiF2RL1 #1, #22, #29, #50, #64, #67, #76, #77, #87, #88, #92, and #100 were selected for use in follow-up studies.

30 Example 13: Chemical modification of asiRNAs

Chemical modifications were applied to 32 asiRNAs. As described below, certain of the modifications improved endocytosis and stability of the asiRNAs.

32 asiRNAs (Table 2) were tested for F2RL1 mRNA inhibition in A549 cells.

Table 8: Modified asiRNA sequences. m = 2'-O-Methyl RNA

F2RL1#29-1 : (S) 5' mGUmCAmUCmUGmGUmUCmCCmCU 3'
F2RL1#29-1 : (AS) 5' AGGGGAACCAGAUACAGAGA 3'
F2RL1#29-2 : (S) 5' mGUmCAmUCmUGmGUmUCmCCmCU 3'
F2RL1#29-2 : (AS) 5' AGGGGAACCAGAUUmAmCAGAGA 3'
F2RL1#29-3 : (S) 5' mGUmCAmUCmUGmGUmUCmCCmCU 3'
F2RL1#29-3 : (AS) 5' AGGGGAACCAGAUUmAmCmAmGAGA 3'
F2RL1#29-4 : (S) 5' mGUmCAmUCmUGmGUmUCmCCmCU 3'
F2RL1#29-4 : (AS) 5' AGGGGAACCAGAUUmAmCmAmGmAmGmA 3'
F2RL1#50-1 : (S) 5' mAGmGAmAGmAAGmCmAmCA 3'
F2RL1#50-1 : (AS) 5' UGUUUGCCUUCUUCUGGAGU 3'
F2RL1#50-2 : (S) 5' mAGmGAmAGmAAGmCmAmCA 3'
F2RL1#50-2 : (AS) 5' UGUUUGCCUUCUUCmCmUGGAGU 3'
F2RL1#50-3 : (S) 5' mAGmGAmAGmAAGmCmAmCA 3'
F2RL1#50-3 : (AS) 5' UGUUUGCCUUCUUCmCmUmGmGAGU 3'
F2RL1#50-4 : (S) 5' mAGmGAmAGmAAGmCmAmCA 3'
F2RL1#50-4 : (AS) 5' UGUUUGCCUUCUUCmCmUmGmGmAmGmU 3'
F2RL1#57-1 : (S) 5' mUAmUGmUCmGUmGAmAGmCmGA 3'
F2RL1#57-1 : (AS) 5' UCUGCUUCACGACAUACAAAG 3'
F2RL1#57-2 : (S) 5' mUAmUGmUCmGUmGAmAGmCmGA 3'
F2RL1#57-2 : (AS) 5' UCUGCUUCACGACAmUmACAAAG 3'
F2RL1#57-3 : (S) 5' mUAmUGmUCmGUmGAmAGmCmGA 3'
F2RL1#57-3 : (AS) 5' UCUGCUUCACGACAmUmAmCmAAAG 3'
F2RL1#57-4 : (S) 5' mUAmUGmUCmGUmGAmAGmCmGA 3'
F2RL1#57-4 : (AS) 5' UCUGCUUCACGACAmUmAmCmAmAmAmG 3'
F2RL1#64-1 : (S) 5' mGAmGAmCmUGmUUmCmAmUmUA 3'
F2RL1#64-1 : (AS) 5' UAAUUGAACAUGUCUCCCACC 3'
F2RL1#64-2 : (S) 5' mGAmGAmCmUGmUUmCmAmUmUA 3'
F2RL1#64-2 : (AS) 5' UAAUUGAACAUGUCmUmCCCACC 3'
F2RL1#64-3 : (S) 5' mGAmGAmCmUGmUUmCmAmUmUA 3'
F2RL1#64-3 : (AS) 5' UAAUUGAACAUGUCmUmCmCmCACC 3'
F2RL1#64-4 : (S) 5' mGAmGAmCmUGmUUmCmAmUmUA 3'
F2RL1#64-4 : (AS) 5' UAAUUGAACAUGUCmUmCmCmCmAmCmC 3'
F2RL1#67-1 : (S) 5' mACmAmUGmUCmAAmUUmACmUU 3'
F2RL1#67-1 : (AS) 5' AAGUAAUUGAACAUGUCUCCC 3'
F2RL1#67-2 : (S) 5' mACmAmUGmUCmAAmUUmACmUU 3'
F2RL1#67-2 : (AS) 5' AAGUAAUUGAACAmGmUCUCCC 3'
F2RL1#67-3 : (S) 5' mACmAmUGmUCmAAmUUmACmUU 3'

F2RL1#67-3 : (AS) 5' AAGUAAUUGAACAUmGmUmCmUCCC 3'
F2RL1#67-4 : (S) 5' mACmAUmGUmUCmAAmUUmACmUU 3'
F2RL1#67-4 : (AS) 5' AAGUAAUUGAACAUmGmUmCmUmCmCmC 3'
F2RL1#76-1 : (S) 5' mUGmAAmAAmCUmCAmGAmGAmAG 3'
F2RL1#76-1 : (AS) 5' CUUCUCUGAGUUUUCAUCCA 3'
F2RL1#76-2 : (S) 5' mUGmAAmAAmCUmCAmGAmGAmAG 3'
F2RL1#76-2 : (AS) 5' CUUCUCUGAGUUUUmCmAUCCAU 3'
F2RL1#76-3 : (S) 5' mUGmAAmAAmCUmCAmGAmGAmAG 3'
F2RL1#76-3 : (AS) 5' CUUCUCUGAGUUUUmCmAmUmCCA 3'
F2RL1#76-4 : (S) 5' mUGmAAmAAmCUmCAmGAmGAmAG 3'
F2RL1#76-4 : (AS) 5' CUUCUCUGAGUUUUmCmAmUmCmCmAmU 3'
F2RL1#77-1 : (S) 5' mGmAAmACmUCmAGmAGmAAmGA 3'
F2RL1#77-1 : (AS) 5' UCUUCUCUGAGUUUUCAUCCA 3'
F2RL1#77-2 : (S) 5' mGmAAmACmUCmAGmAGmAAmGA 3'
F2RL1#77-2 : (AS) 5' UCUUCUCUGAGUUUUmUmCAUCCA 3'
F2RL1#77-3 : (S) 5' mGmAAmACmUCmAGmAGmAAmGA 3'
F2RL1#77-3 : (AS) 5' UCUUCUCUGAGUUUUmUmCmAmUCCA 3'
F2RL1#77-4 : (S) 5' mGmAAmACmUCmAGmAGmAAmGA 3'
F2RL1#77-4 : (AS) 5' UCUUCUCUGAGUUUUmUmCmAmUmCmCmA 3'
F2RL1#100-1 : (S) 5' mAAmGUmGUmCCmGCmACmUGmUA 3'
F2RL1#100-1 : (AS) 5' UACAGUGCGGACACUUCGGCA 3'
F2RL1#100-2 : (S) 5' mAAmGUmGUmCCmGCmACmUGmUA 3'
F2RL1#100-2 : (AS) 5' UACAGUGCGGACACmUmUCGGCA 3'
F2RL1#100-3 : (S) 5' mAAmGUmGUmCCmGCmACmUGmUA 3'
F2RL1#100-3 : (AS) 5' UACAGUGCGGACACmUmUmCmGGCA 3'
F2RL1#100-4 : (S) 5' mAAmGUmGUmCCmGCmACmUGmUA 3'
F2RL1#100-4 : (AS) 5' UACAGUGCGGACACmUmUmCmGmGmCmA 3'

The asiRNAs listed in Table 8 were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in 1x siRNA duplex buffer (Bioneer). Proper strand annealing was confirmed via gel electrophoresis. For the screen, A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 µg/ml penicillin / streptomycin in a 100 mm cell culture dish. One day prior to transfection, 2.5×10^4 A549 cells were seeded in 24-well plates. The A549 cells were transfected with 0.3 nM of the asiRNAs using RNAiMAX (Invitrogen) according to the manufacturer's instructions.

The F2RL1 mRNA levels in the transfected cells were measured 24 hours after transfection using real-time PCR. Specifically, total RNA was extracted using RNAiso

Plus(TaKaRa), and then 500 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. The synthesized cDNA was diluted and then real-time PCR was performed using THUNDERBIRD® Probe qPCR Mix (TOYOBO) according to
 5 manufacturer's instructions. Amplification of the F2RL1 was detected using F2RL1 TaqMan® Probe (Hs00608346_m1). 18S was amplified as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

The level of F2RL1 inhibition of 32 asiRNAs is provided in Figure 20.

Example 14: Inhibition of F2RL1 mRNA expression using F2RL1-targeting asiRNAs

10 The 12 asiRNAs selected in Example 12, asiF2RL1#1, #22, #29, #50, #64, #67, #76, #77, #87, #88, #92, and #100, were tested for their ability to inhibit F2RL1 expression.

The asiRNAs were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in 1x siRNA duplex buffer (Bioneer). Proper strand annealing was confirmed via gel electrophoresis. For the screen, A549 cells (ATCC) that had been cultured in Dulbecco's
 15 modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 µg/ml penicillin / streptomycin in a 100 mm cell culture dish. One day prior to transfection, 2.5×10^4 A549 cells were seeded in 24-well plates. The A549 cells were transfected with 1 nM of the asiRNAs using RNAiMAX (Invitrogen) according to the manufacturer's instructions.

Specifically, total RNA was extracted using RNAiso Plus (TaKaRa), and then 500
 20 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. Amplification of the F2RL1 was detected using F2RL1 TaqMan® Probe (Hs00608346_m1). 18S was amplified as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

25 The level of F2RL1 inhibition of 12 asiRNAs is provided in Figure 21.

Example 15: Inhibition of F2RL1 protein expression using F2RL1-targeting asiRNAs

The efficacy of asiF2RL1 for the inhibition of F2RL1 protein was tested.

The asiRNAs were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in 1x siRNA duplex buffer (Bioneer). Proper strand annealing was confirmed via gel
 30 electrophoresis.

A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 µg/ml penicillin / streptomycin in a 100 mm cell culture dish. One day prior to transfection, 2.5×10^4 A549 cells were seeded

in 24-well plates. A549 cells were transfected with 1 nM of the asiRNAs using RNAiMAX (Invitrogen) according to the manufacturer's instructions.

The levels of F2RL1 protein expression were determined via western blot 72 hours after asiRNA transfection. Briefly, the transfected A549 cells were lysed with TX-100 lysis buffer (1% TX-100, 150 mM NaCl, 100 mM Tris (pH 8.8)). 10 µg of the total protein extracts of A549 cells were loaded onto a 10% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 3% BSA (Bioworld) and then incubated overnight at 4 °C in 3% BSA containing anti-F2RL1 antibody (Abcam) and anti-GAPDH antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 1x TBST with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL for 1 minute. The F2RL1 and GAPDH bands were then imaged using a Chemidoc instrument (Bio-rad).

The results of the western blot assay are depicted in Figure 22. asiF2RL1#22, #50, #77, and #92 were selected for the chemical modification.

Example 16: Chemical modification of asiRNAs for self-delivery

Chemical modifications were applied to the 12 asiRNAs selected in Example 15 and the cellular delivery of the modified asiRNAs was tested in the absence of other delivery reagent. As described below, certain of the modifications improved endocytosis and stability of the asiRNAs. Such cell-penetrating asiRNAs (cp-asiRNAs) are able to be delivered into the cell in the absence of a delivery reagent.

12 potential cp-asiRNAs (Table 9) were screened for F2RL1 mRNA inhibition in A549 cells. Each potential cp-asiRNA was incubated with A549 cells at 1 µM and 3 µM without a delivery reagent and F2RL1 mRNA levels were measured by real-time PCR.

Table 9: Modified asiRNA sequences tested for self-delivery and F2RL1 inhibition. m = 2'-O-Methyl RNA, * = phosphorothioate bond.

F2RL1#22-PS4/21(2,4) (S) : 5' mCUmGAmCCmUCmCUmCUmC*U*mG*U*cholesterol 3'
F2RL1#22-PS4/21(2,4) (AS) : 5' ACAGAGAGGAGGUCmAmGC*C*A*A*G 3'
F2RL1#22-PS4/21(4,4) (S) : 5' mCUmGAmCCmUCmCUmCUmC*U*mG*U*cholesterol 3'
F2RL1#22-PS4/21(4,4) (AS) : 5' ACAGAGAGGAGGUCmAmGmC*mC*A*A*G 3'
F2RL1#22-PS4/21(7,4) (S) : 5' mCUmGAmCCmUCmCUmCUmC*U*mG*U*cholesterol 3'
F2RL1#22-PS4/21(7,4) (AS) : 5' ACAGAGAGGAGGUCmAmGmC*mC*mA*mA*mG 3'

F2RL1#50-PS4/21(2,4) (S) : 5' mAGmGAmAGmAAmGGmCAmA*A*mC*A*cholesterol 3'
F2RL1#50-PS4/21(2,4) (AS) : 5' UGUUUGCCUUCUUCmCmUG*G*A*G*U 3'
F2RL1#50-PS4/21(4,4) (S) : 5' mAGmGAmAGmAAmGGmCAmA*A*mC*A*cholesterol 3'
F2RL1#50-PS4/21(4,4) (AS) : 5' UGUUUGCCUUCUUCmCmUmG*mG*A*G*U 3'
F2RL1#50-PS4/21(7,4) (S) : 5' mAGmGAmAGmAAmGGmCAmA*A*mC*A*cholesterol 3'
F2RL1#50-PS4/21(7,4) (AS) : 5' UGUUUGCCUUCUUCmCmUmG*mG*mA*mG*mU 3'
F2RL1#77-PS4/21(2,4) (S) : 5' mGAmAAmACmUCmAGmAGmA*A*mG*A*cholesterol 3'
F2RL1#77-PS4/21(2,4) (AS) : 5' UCUUCUCUGAGUUUmUmCA*U*C*C*A 3'
F2RL1#77-PS4/21(4,4) (S) : 5' mGAmAAmACmUCmAGmAGmA*A*mG*A*cholesterol 3'
F2RL1#77-PS4/21(4,4) (AS) : 5' UCUUCUCUGAGUUUmUmCmA*mU*C*C*A 3'
F2RL1#77-PS4/21(7,4) (S) : 5' mGAmAAmACmUCmAGmAGmA*A*mG*A*cholesterol 3'
F2RL1#77-PS4/21(7,4) (AS) : 5' UCUUCUCUGAGUUUmUmCmA*mU*mC*mC*mA 3'
F2RL1#92-PS4/21(2,4) (S) : 5' mCCmCUmUUmGUmCUmAUmU*A*mC*U*cholesterol 3'
F2RL1#92-PS4/21(2,4) (AS) : 5' AGUAAUAGACAAAGmGmGG*U*C*G*A 3'
F2RL1#92-PS4/21(4,4) (S) : 5' mCCmCUmUUmGUmCUmAUmU*A*mC*U*cholesterol 3'
F2RL1#92-PS4/21(4,4) (AS) : 5' AGUAAUAGACAAAGmGmGmG*mU*C*G*A 3'
F2RL1#92-PS4/21(7,4) (S) : 5' mCCmCUmUUmGUmCUmAUmU*A*mC*U*cholesterol 3'
F2RL1#92-PS4/21(7,4) (AS) : 5' AGUAAUAGACAAAGmGmGmG*mU*mC*mG*mA 3'

A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 µg/ml penicillin / streptomycin in a 100 mm cell culture dish.

- 5 The potential cp-asiRNAs listed in Table 9 were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing was confirmed via gel electrophoresis.

- One day prior to treatment, 2.5×10^4 A549 cells were seeded in 24-well plates. Immediately before treatment, the A549 cells were washed with Dulbecco's modified Eagle's medium then cultured in the presence of the potential cp-asiRNAs in OPTI-MEM buffer for 8 and 24 hours, at each point the asiRNA-containing OPTI-MEM media was replaced with a serum-containing media.
- 10

- The level of F2RL1 mRNA expression was determined using real-time PCR 48 hours after asiRNAs treatment. The level of F2RL1 inhibition of cp-asiRNAs is provided in Figure 23.
- 15

Example 17: Inhibition of F2RL1 mRNA expression using F2RL1-targeting cp-asiRNAs

The efficacies of cp-asiRNAs for the inhibition of F2RL1 RNA were tested.

Each potential cp-asiRNA was incubated with A549 cells at 1 μ M and 3 μ M without a delivery reagent and F2RL1 mRNA levels were measured using real-time PCR.

A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 μ g/ml penicillin / streptomycin in
5 a 100 mm cell culture dish.

The cp-asiRNAs were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing was confirmed via gel electrophoresis.

One day prior to treatment, 2.5×10^4 A549 cells were seeded in 24-well plates. Immediately before treatment, the A549 cells were washed with Dulbecco's modified
10 Eagle's medium (Gibco) then cultured in the presence of the potential cp-asiRNAs in OPTI-MEM buffer for 24 hours, at which point the asiRNA-containing OPTI-MEM media was replaced with a serum-containing media.

The level of F2RL1 mRNA expression was determined by real-time PCR 48 hours after asiRNA treatment. Total RNA was extracted using RNAiso Plus (TaKaRa), and then
15 500 ng of the extracted RNA was used for cDNA synthesis using the High-capacity cDNA reverse transcription kit (Applied Biosystems), according to the manufacturer's instructions. Amplification of the F2RL1 was detected using F2RL1 TaqMan® Probe (Hs00608346_m1). 18S was amplified as an internal control using 18S TaqMan® Probe (Hs03928985_g1).

20 The level of F2RL1 inhibition by cp-asiRNAs is provided in Figure 24.

Example 18: Inhibition of F2RL1 protein using F2RL1-targeting cp-asiRNAs

The efficacies of cp-asiRNAs for the inhibition of F2RL1 protein were tested.

Each potential cp-asiRNA was incubated with A549 cells at 1 μ M and 3 μ M without a delivery reagent and F2RL1 protein levels were detected by western blot.

25 A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 μ g/ml penicillin / streptomycin in a 100 mm cell culture dish.

The cp-asiRNAs were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing was confirmed via gel electrophoresis.

30 One day prior to treatment, 2.5×10^4 A549 cells were seeded in 24-well plates. Immediately before treatment, the A549 cells were washed with Dulbecco's modified Eagle's medium (Gibco) then cultured in the presence of the potential cp-asiRNAs in

OPTI-MEM buffer for 24 hours, at which point the asiRNA-containing OPTI-MEM media was replaced with a serum-containing media.

The levels of F2RL1 protein expression were determined via western blot 72 hours after asiRNA transfection. Briefly, the treated A549 cells were lysed with TX-100 lysis buffer (1% TX-100, 150 mM NaCl, 100 mM Tris (pH 8.8)). 10 µg of the total protein extracts were loaded onto a 10% SDS-PAGE gel and electrophoresed at 120 V. After electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 3% BSA (Bioworld) and then incubated overnight at 4°C in 3% BSA containing anti-F2RL1 antibody (Abcam) and anti-GAPDH (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was incubated for 1 hour at the room temperature in 1x TBST with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL for 1 minute. The F2RL1 and GAPDH bands were then imaged using a Chemidoc instrument (Bio-rad).

The results of the western blot assay are depicted in Figure 25.

Example 19: Inhibition of F2RL1 mRNA expression using additional F2RL1-targeting cp-asiRNAs

A variety of potential cp-asiF2RL1#22 and #50 structures having different strand lengths and number of 2'-O-methylation modifications and phosphorothioate bond were synthesized and tested for their ability to inhibit F2RL1 expression (Table 10).

Table 10: Additional cp-asiRNA sequences. m = 2'-O-Methyl RNA, * = phosphorothioate bond.

F2RL1#50-PS3/19(5,4) (S) : 5' mAGmGAmAGmAAmGGmCAmAA*mC*A*cholesterol 3'
F2RL1#50-PS3/19(5,4) (AS) : 5' UGUUUGCCUUCUUCmC*mU*mG*mG*mA 3'
F2RL1#50-PS3/21(7,4) (S) : 5' mAGmGAmAGmAAmGGmCAmAA*mC*A*cholesterol 3'
F2RL1#50-PS3/21(7,4) (AS) : 5' UGUUUGCCUUCUUCmCmUmG*mG*mA*mG*mU 3'
F2RL1#50-PS4/19(5,4) (S) : 5' mAGmGAmAGmAAmGGmCAmA*A*mC*A*cholesterol 3'
F2RL1#50-PS4/19(5,4) (AS) : 5' UGUUUGCCUUCUUCmC*mU*mG*mG*mA 3'
F2RL1#22-PS3/19(4,4) (S) : 5' mCUmGAmCCmUCmCUmCUmCU*mG*U*cholesterol 3'
F2RL1#22-PS4/19(4,4) (AS) : 5' ACAGAGAGGAGGUCmA*mG*mC*mC*A 3'
F2RL1#22-PS3/21(4,4) (S) : 5' mCUmGAmCCmUCmCUmCUmCU*mG*U*cholesterol 3'
F2RL1#22-PS4/21(4,4) (AS) : 5' ACAGAGAGGAGGUCmAmGmC*mC*A*A*G 3'
F2RL1#22-PS4/19(4,4) (S) : 5' mCUmGAmCCmUCmCUmCUmC*U*mG*U*cholesterol 3'
F2RL1#22-PS4/19(4,4) (AS) : 5' ACAGAGAGGAGGUCmA*mG*mC*mC*A 3'

The ability of 1 μ M and 3 μ M of each of the potential cp-asiRNAs listed in Table 10 to inhibit F2RL1 mRNA in A549 cells was tested.

A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 μ g/ml penicillin / streptomycin in a 100 mm cell culture dish.

The potential cp-asiRNAs listed in Table 4 were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing was confirmed via gel electrophoresis.

One day prior to treatment, 2.5×10^4 A549 cells were seeded in 24-well plates. Immediately before treatment, the A549 cells were washed with Dulbecco's modified Eagle's medium (Gibco) then cultured in the presence of the potential cp-asiRNAs in OPTI-MEM buffer for 24 hours, at which point the asiRNA-containing OPTI-MEM media was replaced with a serum-containing media.

The level of F2RL1 mRNA expression was determined 48 hours after asiRNA treatment.

The level of F2RL1 inhibition by 8 cp-asiRNAs is provided in Figure 26.

Example 20: Inhibition of F2RL1 protein expression using additional F2RL1-targeting cp-asiRNAs

The efficacy of cp-asiRNAs for the inhibition of F2RL1 protein was tested.

Each potential cp-asiRNA was incubated with A549 cells at 1 μ M and 3 μ M without a delivery reagent and F2RL1 protein levels were measured by western blot.

A549 cells (ATCC) that had been cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 μ g/ml penicillin / streptomycin in a 100 mm cell culture dish.

The cp-asiRNAs were incubated at 95 °C for 5 minutes and at 37 °C for 1 hour in OPTI-MEM buffer (Gibco). Proper strand annealing was confirmed via gel electrophoresis.

One day prior to treatment, 2.5×10^4 A549 cells were seeded in 24-well plates. Immediately before treatment, the A549 cells were washed with Dulbecco's modified Eagle's medium (Gibco) then cultured in the presence of the potential cp-asiRNAs in OPTI-MEM buffer for 24 hours, at which point the asiRNA-containing OPTI-MEM media was replaced with a serum-containing media.

The level of F2RL1 protein expression was determined via western blot 72 hours after asiRNA treatment. Briefly, the treated A549 cells were lysed with TX-100 lysis buffer (1% TX-100, 150 mM NaCl, 100 mM Tris (pH 8.8)). 10 µg of the total protein extracts were loaded onto a 10% SDS-PAGE gel and electrophoresed at 120 V. After
 5 electrophoresis, the proteins were transferred to PVDF membrane (Bio-rad) already activated by methanol (Merck) for 1 hour at 300 mA. The membrane was blocked for 1 hour at the room temperature with 3% BSA (Bioworld) and then incubated overnight at 4 °C in 3% BSA containing anti-F2RL1 antibody (Abcam) and anti-GAPDH antibody (Santa Cruz). The membrane was then washed with 1x TBST for 10 minutes three times and was
 10 incubated for 1 hour at the room temperature in 1x TBST with HRP-conjugated secondary antibody. The membrane was washed with 1x TBST for 10 minutes and treated with 1x ECL for 1 minute. The F2RL1 and GAPDH bands were then imaged using a Chemidoc instrument (Bio-rad).

The results of the western blot assay are depicted in Figure 27.

15 Example 21: In vivo Efficacy Study

After shaving the dorsal region of NC/Nga mice, Dermatophagoides farinae body extract (Df) cream was painted in presented schedule for inducing atopic dermatitis. At day 11, 14 and 18, cp-asiRNA was administered by intradermal injection or spreading of cream emulsified cp-asiRNA before Biostir[®] AD ointment application (Figure 29). The dose of
 20 intradermal injection was 80 µg/50 µl * 4 sites/head, and the dose of the cream emulsified cp-asiRNA was 800 µg/head. Mouse behavior was recorded and scratching behavior for 480 seconds were analyzed. Increased scratching time was observed in Dermatophagoides farinae body extract (Df) cream treated samples (1XPBS +Df). In both intradermal injection (Figure 30, Part A) and cream emulsified cp-asiRNA application (Figure 30, Part B)
 25 condition, IL4RA#5-PS3/19(4,4), TRPA1#81-PS3/19(4,4), F2RL1#22-PS4/19(4,4) treated samples showed reduced scratching time compared to vehicle control (1XPBS +Df). Results are presented as bar graph (mean±S.D) in Figure 30. The results were statistically analyzed by *Student's t-test* methods (n=5).

Transepidermal water loss (TEWL) was measured using hand-held evaporimeter
 30 (VapoMeter, Delfin Technologies Ltd, Kuopio, Finland). Increased TEWL was observed in Dermatophagoides farinae body extract (Df) cream treated samples (1xPBS +Df). In both intradermal injection (Figure 31, Part A) and cream emulsified cp-asiRNA application (Figure 31, Part B) conditions, IL4RA#5-PS3/19(4,4), TRPA1#81-PS3/19(4,4), F2RL1#22-

PS4/19(4,4) treated samples showed reduced TEWL compared to vehicle control (1xPBS +Df). Data are expressed as mean±S.E.M. The results were statistically analyzed by *Student's t-test* methods (n=5).

Histological analysis of treated skin region was conducted. Upper panels show H&E staining of skin sections and lower panels show quantified epidermis area by analyzing skin section image. Increased epidermis region thickness, hyperkeratosis, and acanthosis was observed in *Dermatophagoides farinae* body extract (Df) cream treated samples (+Df). In both intradermal injection (Figure 32, Part A) and cream emulsified cp-asiRNA application (Figure 32, Part B) condition, IL4RA#5-PS3/19(4,4), TRPA1#81-PS3/19(4,4), F2RL1#22-PS4/19(4,4) treated samples showed reduced symptoms caused by Df treatment compared to vehicle control (+Df). In both intradermal injection (Figure 32, Part A) and cream emulsified cp-asiRNA application (Figure 32, Part B) conditions, IL4RA#5-PS3/19(4,4), TRPA1#81-PS3/19(4,4), F2RL1#22-PS4/19(4,4) treated samples showed reduced level of epidermis thickness compared to vehicle control (1xPBS +Df). Data are expressed as mean±S.E.M. The results were statistically analyzed by student's t-test methods (n=5).

Mast cell infiltration analysis of treated skin region was conducted. Figure 33 shows toluidine blue staining of skin sections and quantification results of the stained skin section image. Increased mast cell infiltration was observed in *Dermatophagoides farinae* body extract (Df) cream treated samples (+Df). In both intradermal injection (Figure 33, Part A) and cream emulsified cp-asiRNA application (Figure 33, Part B) conditions, IL4RA#5-PS3/19(4,4), TRPA1#81-PS3/19(4,4), F2RL1#22-PS4/19(4,4) treated samples showed reduced mast cell infiltration compared to vehicle control (+Df). In both intradermal injection and cream emulsified cp-asiRNA application conditions, IL4RA#5-PS3/19(4,4), TRPA1#81-PS3/19(4,4), F2RL1#22-PS4/19(4,4) treated samples showed reduced level of mast cell infiltration area compared to vehicle control (1xPBS +Df). Data are expressed as mean±S.E.M. The results were statistically analyzed by *Student's t-test* methods (n=5).

Incorporation by Reference

All publications, patents, and patent applications mentioned herein are hereby incorporated by reference in their entirety as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. In
5 case of conflict, the present application, including any definitions herein, will control.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention
10 described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. An RNA complex comprising an antisense strand of at least 19 nucleotides (nt) in length having sequence complementarity to an IL4R α mRNA sequence and a sense strand of 15 to 17 nt in length having sequence complementarity to the antisense strand, wherein the antisense strand and the sense strand form a complex in which the 5' end of the antisense strand and the 3' end of the sense strand form a blunt end.
2. An RNA complex comprising an antisense strand of at least 19 nucleotides (nt) in length having sequence complementarity to a TRPA1 mRNA sequence and a sense strand of 15 to 17 nt in length having sequence complementarity to the antisense strand, wherein the antisense strand and the sense strand form a complex in which the 5' end of the antisense strand and the 3' end of the sense strand form a blunt end.
3. An RNA complex comprising an antisense strand of at least 19 nucleotides (nt) in length having sequence complementarity to an F2RL1 mRNA sequence and a sense strand of 15 to 17 nt in length having sequence complementarity to the antisense strand, wherein the antisense strand and the sense strand form a complex in which the 5' end of the antisense strand and the 3' end of the sense strand form a blunt end.
4. The RNA complex of anyone of claims 1 to 3, wherein the antisense strand is 19 to 21 nt in length.
5. The RNA complex of anyone of claims 1 to 3, wherein the antisense strand is 19 nt in length.
6. The RNA complex of anyone of claims 1 to 3, wherein the antisense strand is 20 nt in length.
7. The RNA complex of anyone of claims 1 to 3, wherein the antisense strand is 21 nt in length.
8. The RNA complex of anyone of claims 1 to 3, wherein the antisense strand is at least 24 nt in length.

9. The RNA complex of any one of claims 1 to 8, wherein the sense strand is 15 nt in length.
10. The RNA complex of any one of claims 1 to 8, wherein the sense strand is 16 nt in length.
11. The RNA complex of any one of claims 1 to 8, wherein the sense strand is 17 nt in length.
12. The RNA complex of any one of claims 1 to 11, wherein the sense strand has a sequence selected from the sense strand sequences listed in Table 1, Table 2, Table 3, Table 4, table 5, Table 6, Table 7, Table 8, Table 9 and Table 10.
13. The RNA complex of any one of claims 1 to 11, wherein the antisense strand has a sequence selected from the antisense strand sequences listed in Table 1, Table 2, Table 3, Table 4, table 5, Table 6, Table 7, Table 8, Table 9 and Table 10.
14. The RNA complex of any one of claims 1 and 4 to 13, wherein the RNA complex is capable of inhibiting IL4R α expression by a cell.
15. The RNA complex of any one of claims 2 and 4 to 13, wherein the RNA complex is capable of inhibiting TRPA1 expression by a cell.
16. The RNA complex of any one of claims 3 to 13, wherein the RNA complex is capable of inhibiting F2RL1 expression by a cell.
17. The RNA complex anyone of claims 14 to 16, wherein the cell is an epithelial cell.
18. The RNA complex anyone of claims 14 to 16, wherein the cell is a keratinocyte cell.
19. The RNA complex anyone of claims 14 to 16, wherein the cell is an alveolar cell.
20. The RNA complex anyone of claims 14 to 16, wherein the cell is an A549 cell.
21. The RNA complex of claim 1, wherein the RNA complex comprises the antisense and sense strand of IL4RA#5.
22. The RNA complex of claim 3, wherein the RNA complex comprises the antisense and sense strand of TRPA1#81.

23. The RNA complex of claim 5, wherein the RNA complex comprises the antisense and sense strand of F2RL1#22.
24. The RNA complex of any one of the claims 1 to 23, wherein the RNA complex comprises a chemical modification.
25. The RNA complex of claim 24, wherein the chemical modification is a 2'-O-methylated nucleoside, a phosphorothioate bond or a hydrophobic moiety.
26. The RNA complex of claim 25, wherein the RNA complex comprises a hydrophobic moiety.
27. The RNA complex of claim 26, wherein the hydrophobic moiety is a cholesterol moiety.
28. The RNA complex of claim 27, wherein the cholesterol moiety is attached to the 3' terminus of the sense strand.
29. The RNA complex of claims 24 or 25, wherein the RNA complex comprises a 2'-O-methylated nucleoside.
30. The RNA complex of claim 29, wherein the 2'-O-methylated nucleoside is positioned at the 3' terminus of the sense strand.
31. The RNA complex of claim 30, wherein the 3' terminal region of the sense strand comprises a plurality of 2'-O-methylated nucleosides.
32. The RNA complex of claim 29, wherein the 2'-O-methylated nucleoside is positioned at the 3' terminus of the antisense strand.
33. The RNA complex of claim 32, wherein the 3' terminal region of the antisense strand comprises a plurality of 2'-O-methylated nucleosides.
34. The RNA complex of claim 29, wherein a 2'-O-methylated nucleoside is positioned at the 3' terminus of the sense strand and at the 3' terminus of the antisense strand.

35. The RNA complex of claim 34, wherein the 3' terminal region of the sense strand comprises a plurality of 2'-O-methylated nucleosides and the 3' terminal region of the antisense strand comprises a plurality of 2'-O-methylated nucleosides.
36. The RNA complex of any one of claims 24 to 35, wherein the RNA complex comprises a phosphorothioate bond.
37. The RNA complex of claim 36, wherein at least 25% of the bonds between the ribonucleotides in the sense strand of the RNA complex are phosphorothioate bonds.
38. The RNA complex of claim 36, wherein at least 50% of the bonds between the ribonucleotides in the sense strand of the RNA complex are phosphorothioate bonds.
39. The RNA complex of claim 36, wherein at least 75% of the bonds between the ribonucleotides in the sense strand of the RNA complex are phosphorothioate bonds.
40. The RNA complex of claim 36, wherein all of the bonds between the ribonucleotides in the sense strand of the RNA complex are phosphorothioate bonds.
41. The RNA complex of any one of claims 36 to 40, wherein at least 25% of the bonds between the ribonucleotides in the antisense strand of the RNA complex are phosphorothioate bonds.
42. The RNA complex of any one of claims 36 to 40, wherein at least 50% of the bonds between the ribonucleotides in the antisense strand of the RNA complex are phosphorothioate bonds.
43. The RNA complex of any one of claims 36 to 40, wherein at least 75% of the bonds between the ribonucleotides in the antisense strand of the RNA complex are phosphorothioate bonds.
44. The RNA complex of any one of claims 36 to 40, wherein all of the bonds between the ribonucleotides in the antisense strand of the RNA complex are phosphorothioate bonds.
45. The RNA complex of claim 44, wherein the RNA complex is a modified RNA complex listed in Table 2, Table 3, Table 5, Table 6, Table 8, Table 9 or Table 10.

46. The RNA complex of any one of claims 36 to 45, wherein the RNA complex is capable of penetrating the cellular membrane of a cell in the absence of a delivery vehicle.
47. The RNA complex of any one of claims 1 to 46, wherein the RNA complex is not cytotoxic.
48. A method of inhibiting IL4R α , TRPA1, or F2RL1 expression by a cell comprising contacting the cell with an RNA complex of any one of claims 1 to 47.
49. The method of claim 48, wherein the cell is an A549, an epithelial cell or a keratinocyte.
50. The method of claim 48, wherein the cell is present in the skin or respiratory tract of a human subject.
51. A method of treating atopic dermatitis or asthma in a subject comprising administering to the subject an RNA complex of any one of claims 1 to 47.
52. The method of claim 51, comprising administering the RNA complex to the skin of the subject.
53. The method of claim 51, comprising administering the RNA complex to the respiratory tract of the subject.
54. The method of claim 51, wherein the RNA complex is administered intravenously.
55. The method of claim 51, wherein the RNA complex is administered parenterally.
56. The method of claim 51 or 52, wherein the RNA complex is administered topically.
57. The method of claim 51 or 53, wherein the RNA complex is administered by inhalation.
58. A pharmaceutical composition comprising an RNA complex of any one of claims 1 to 47 and a pharmaceutically acceptable carrier.
59. The pharmaceutical composition of claim 58, wherein the pharmaceutical composition is formulated for inhalation.

60. The pharmaceutical composition of claim 58, wherein the pharmaceutical composition is formulated for an inhaler.
61. The pharmaceutical composition of claim 58, wherein the pharmaceutical composition formulated for topical administration.
62. The pharmaceutical composition of claim 58, wherein the composition is a cream or a lotion.
63. A method of treating atopic dermatitis or asthma in a subject comprising administering to the subject a pharmaceutical composition of any one of claims 58 to 62.
64. The method of claim 63, wherein the subject has atopic dermatitis.
65. The method of claim 63, wherein the subject has asthma.
66. The method of any one of claims 63 to 65, comprising administering the pharmaceutical composition to the respiratory tract of the subject.
67. The method of claim 66, wherein the pharmaceutical composition is in an inhaler.
68. The method of anyone of claims 63 to 65, comprising administering the pharmaceutical composition to the skin of the subject.
69. The method of claim 68, wherein the pharmaceutical composition is a cream or a lotion.
70. The method of any one of claims 63 to 65, comprising administering the pharmaceutical composition parenterally or intravenously.
71. The method of any one of claims 63 to 65, comprising administering the pharmaceutical composition orally.
72. The method of any one of claims 63 to 71, wherein the subject self-administers the pharmaceutical composition.
73. The method any one of claims 63 to 72, further comprising administering a second agent for the treatment of atopic dermatitis.

74. The method of anyone of claims 63 to 72, further comprising administering a second agent for the treatment of asthma.

75. The method of claim 73 or 74, wherein the second agent is a steroid or an immunomodulator.

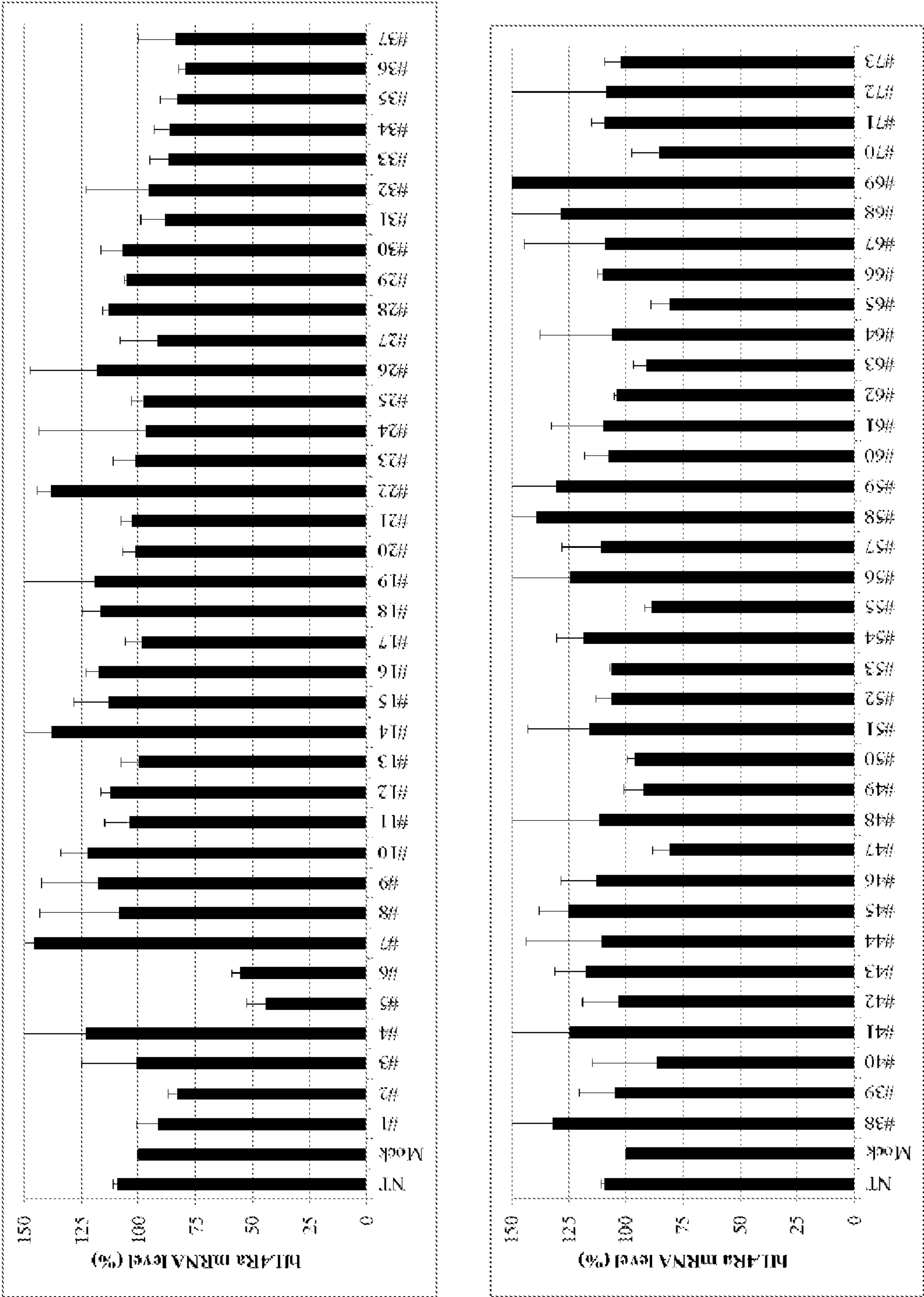


Figure 1

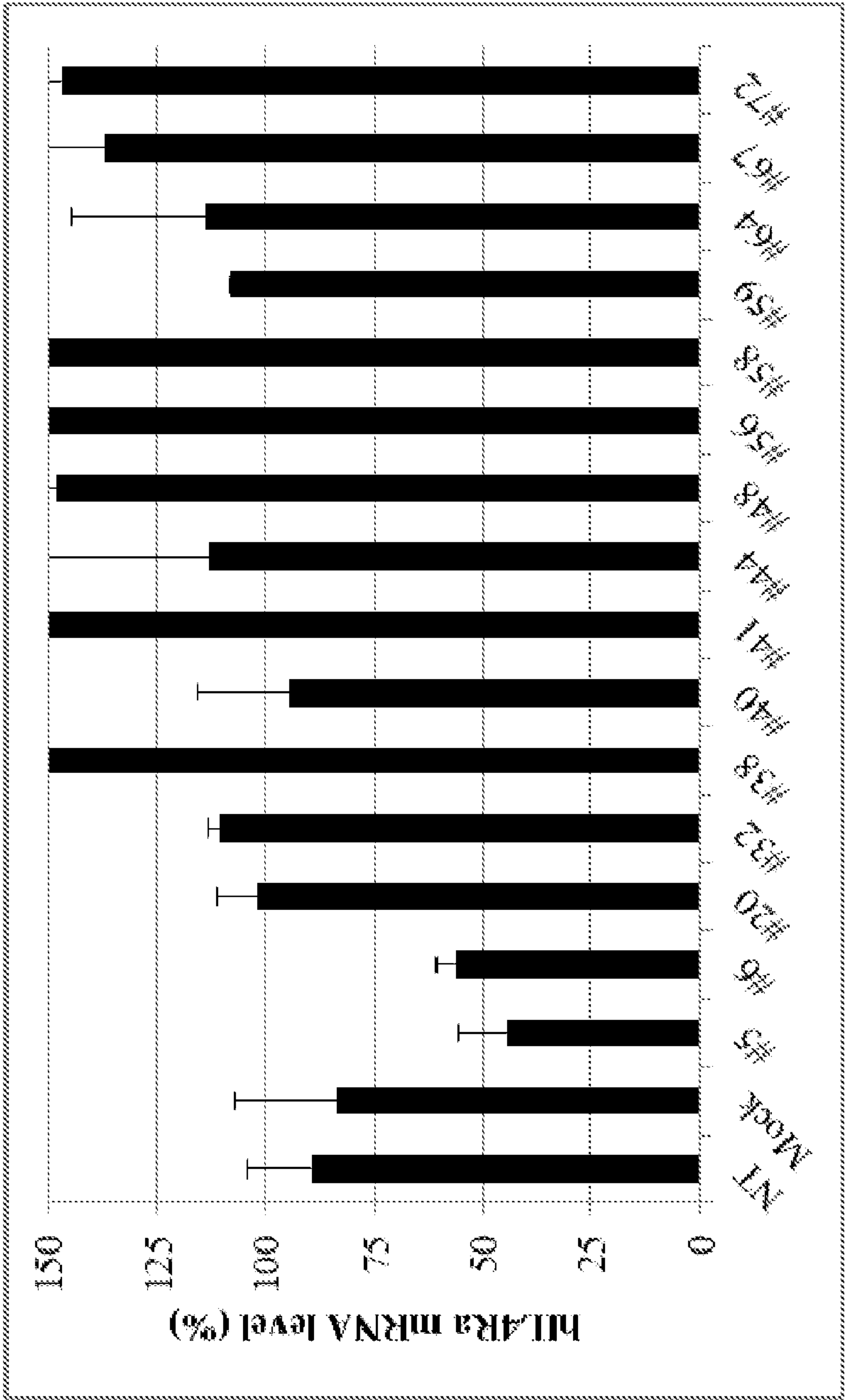


Figure 2

Figure 3

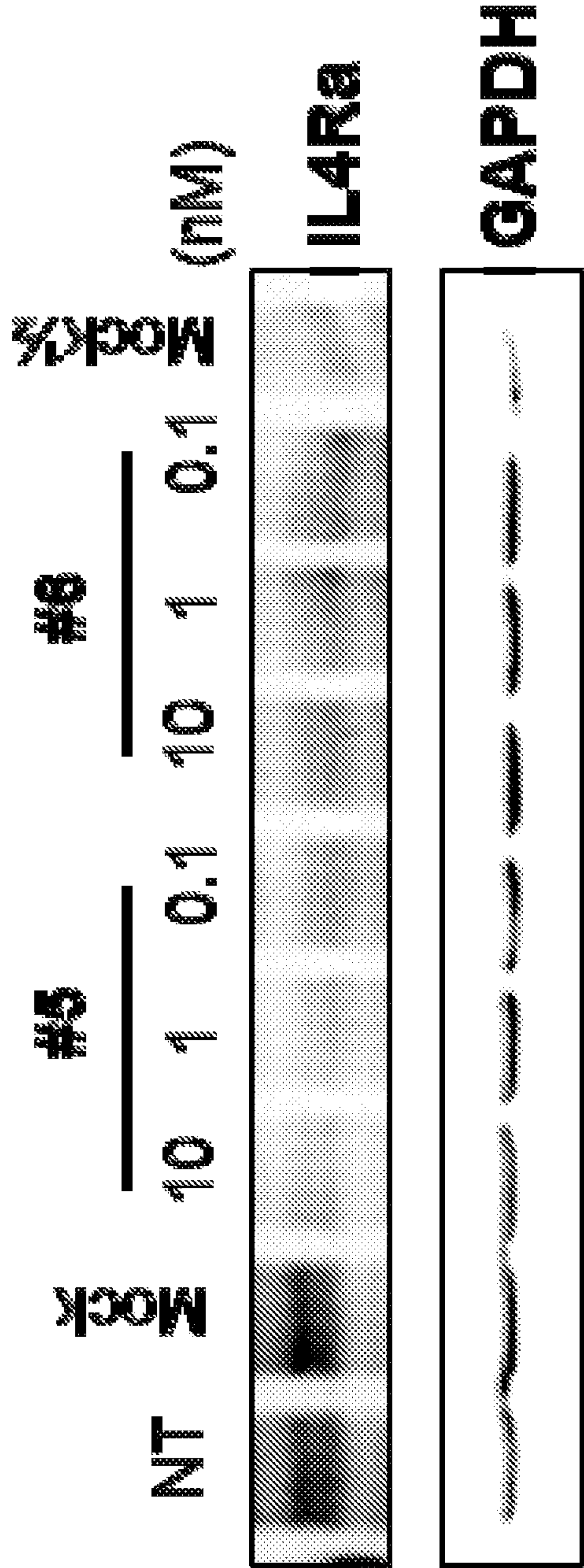


Figure 4

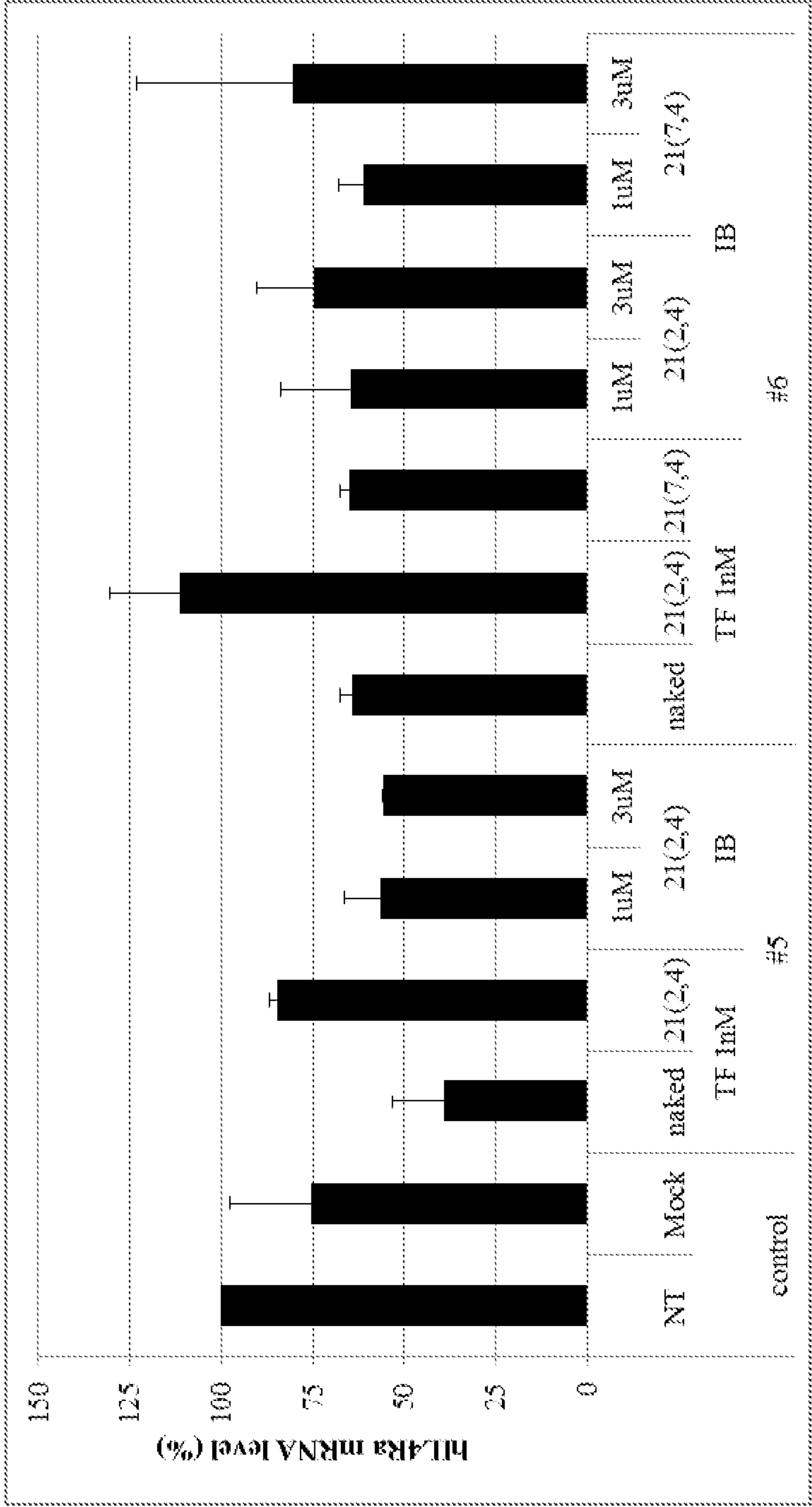


Figure 5

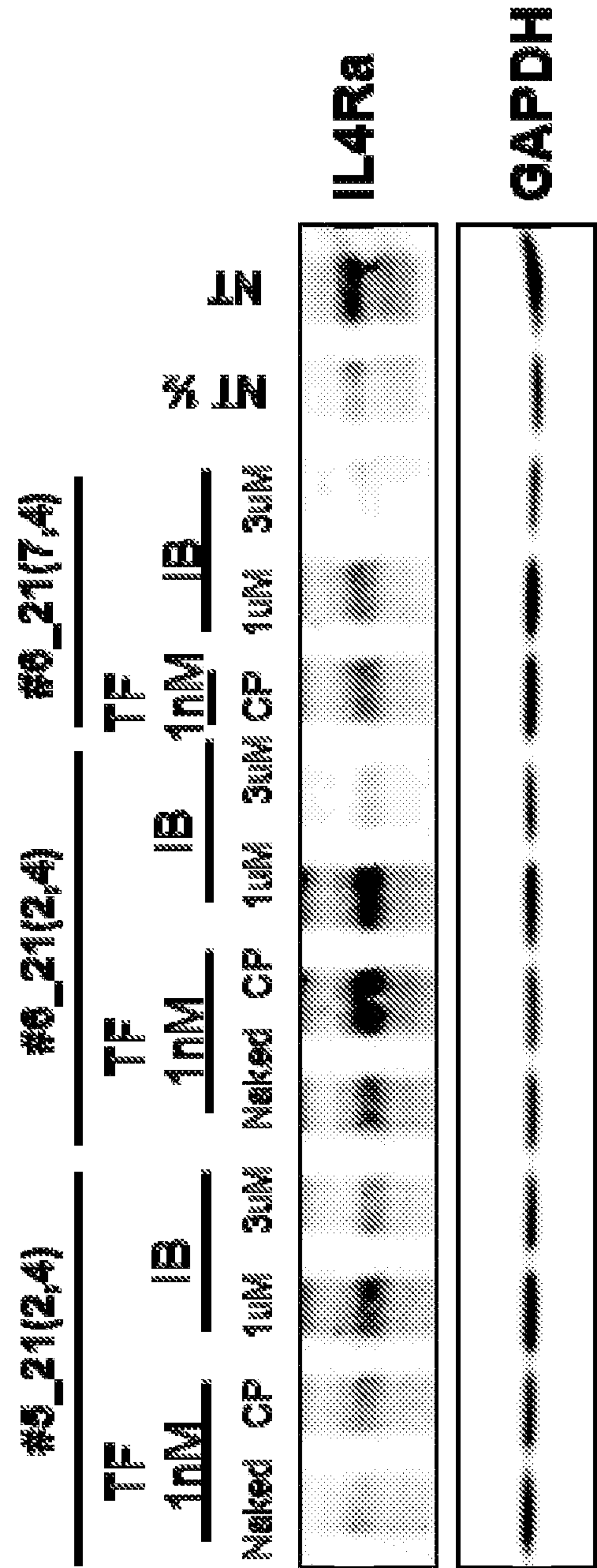


Figure 6

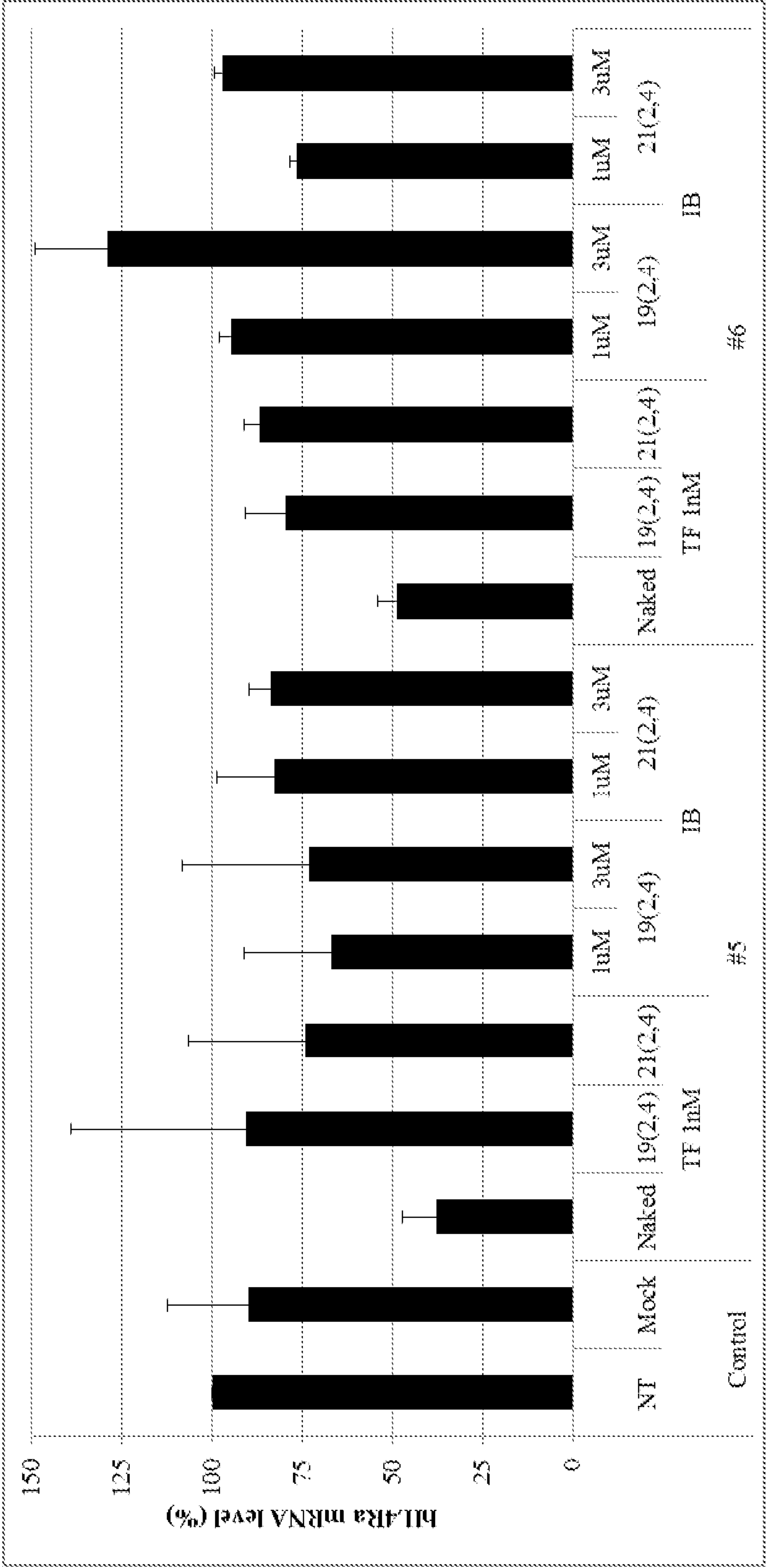


Figure 7

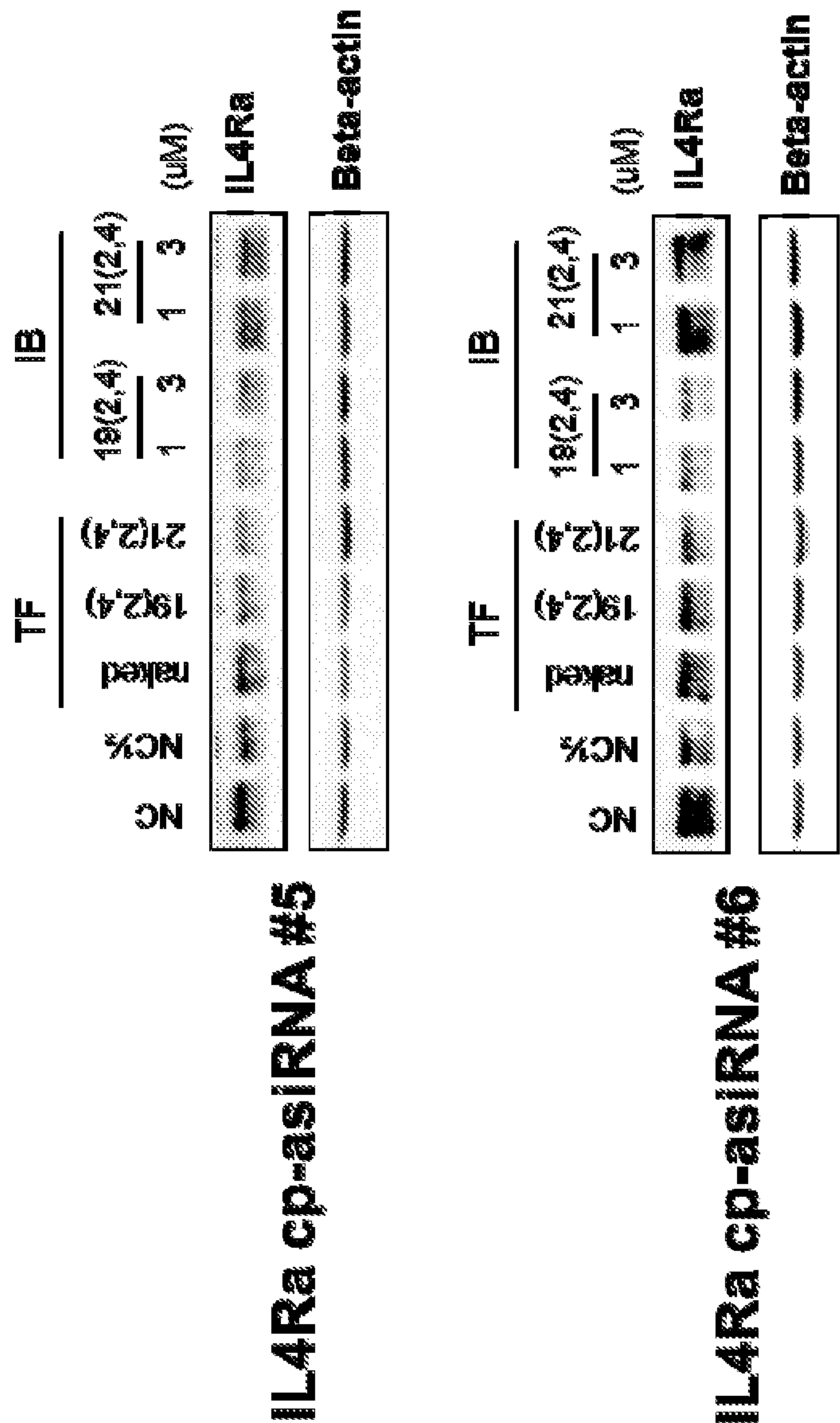


Figure 8

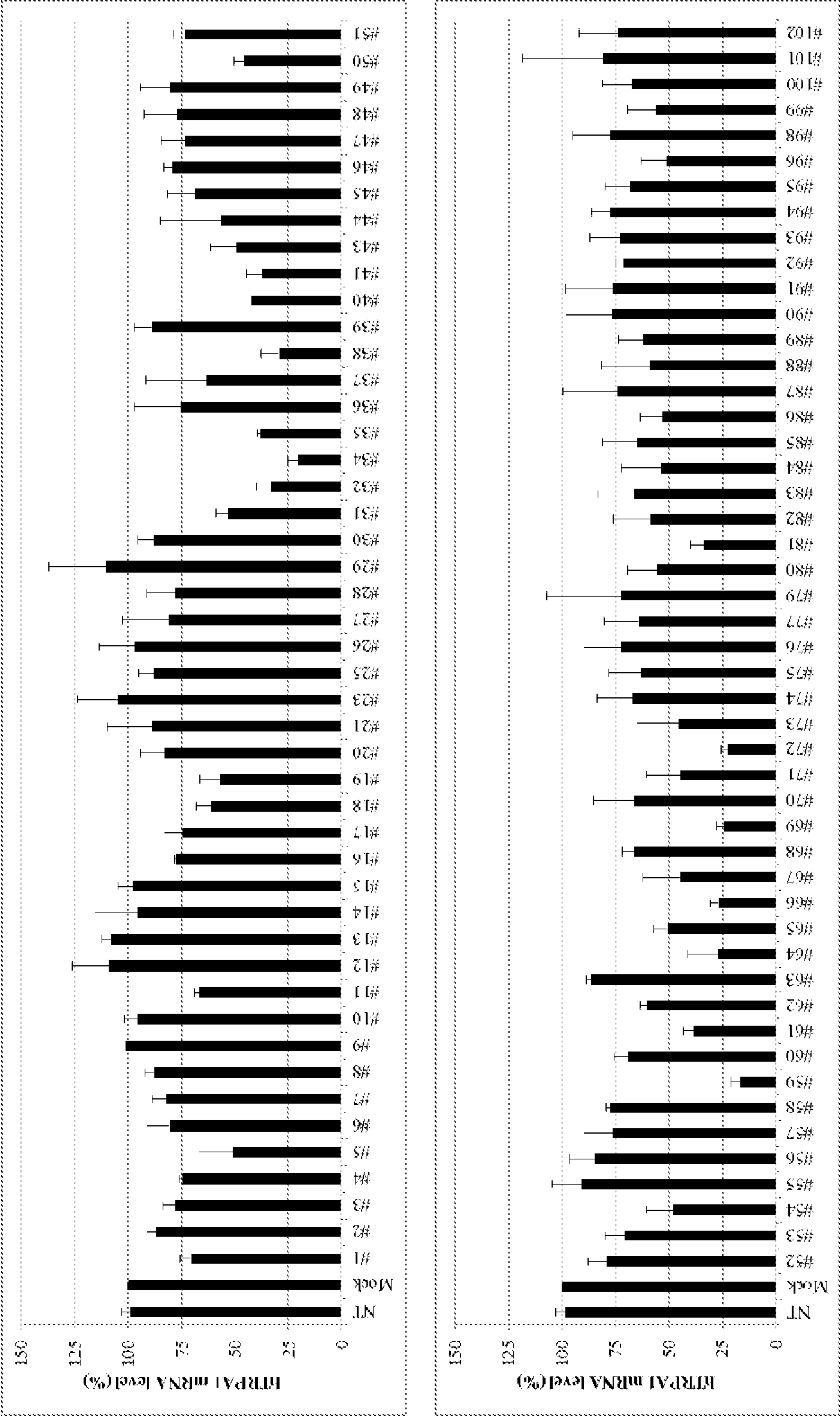
Human IL4Ra mRNA sequence (NM_000418.3).

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121 agatttacac acagctggaa gaaatcatag agaagccggg cgtggtggct catgcctata
181 atccagcac ttttgaggc tgaggcgggc agatcactg agatcaggag ttcgagacca
241 gcctggtgcc ttggcatctc ccaatggggg ggctttgctc tgggctcctg ttcctgtga
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481 cgtgtatccc tgagaacaac ggaggcgcgg ggtgcgtgtg ccacctgctc atgtagacg
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Figure 8 (Continued)

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 2641 catcctcca tctgcccct ggcaatgctc agagctcaag ccagaccccc aaaatcgtga
 2701 actttgtctc cgtgggacc acatacatga ggtctctta ggtgcatgtc ctctgttgc
 2761 tgagtctgca gatgaggact agggcttctc catgcctggg aatgccacc tctggaagg
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 3241 acttcaggga ggggtgtcat tgcctagagg tgctcattca ttaacagag cttccttagg
 3301 ttgatgctgg aggcagaatc ccggctgtca aggggtgttc agttaagggg agcaacagag
 3361 gacatgaaaa attgctatga ctaaagcagg gacaattgc tgccaaacac ccatgccag
 3421 ctgtatggct gggggctcct cgtatgcatg gaacccccag aataaatatg ctcagccacc
 3481 ctgtgggccc ggcaatccag acagcaggca taaggcacca gttaccctgc atgttgccc
 3541 agacctcagg tgctagggaa ggcgggaacc ttgggtgag taatgctcgt ctgtgtgtt
 3601 tagtttcatc acctgttctc tgtgttctg gaggagagt gaacagaagg ggtggagtt
 3661 tgtataaata aagtttctt gtctctttaa aaaaaaaaaa aaaaaaaaaa

Figure 9



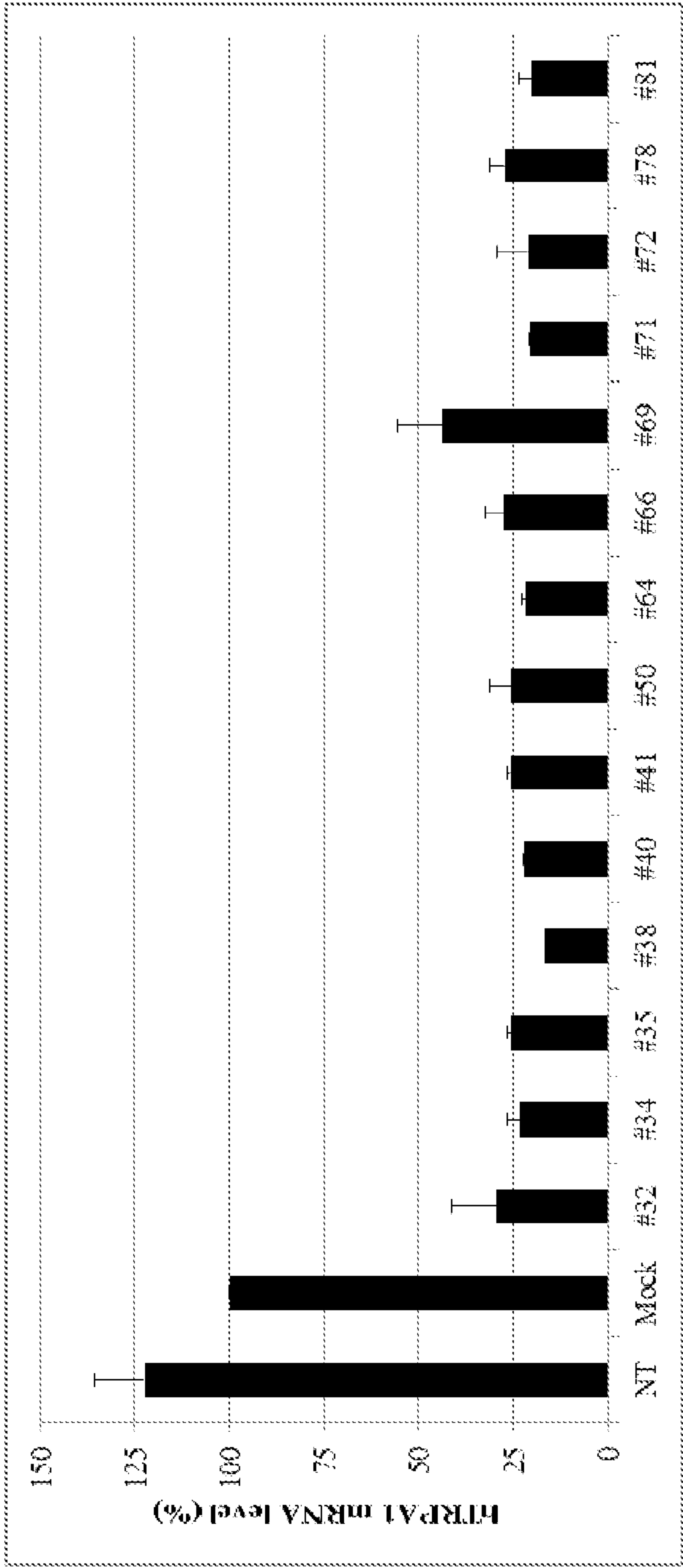


Figure 10

Figure 11

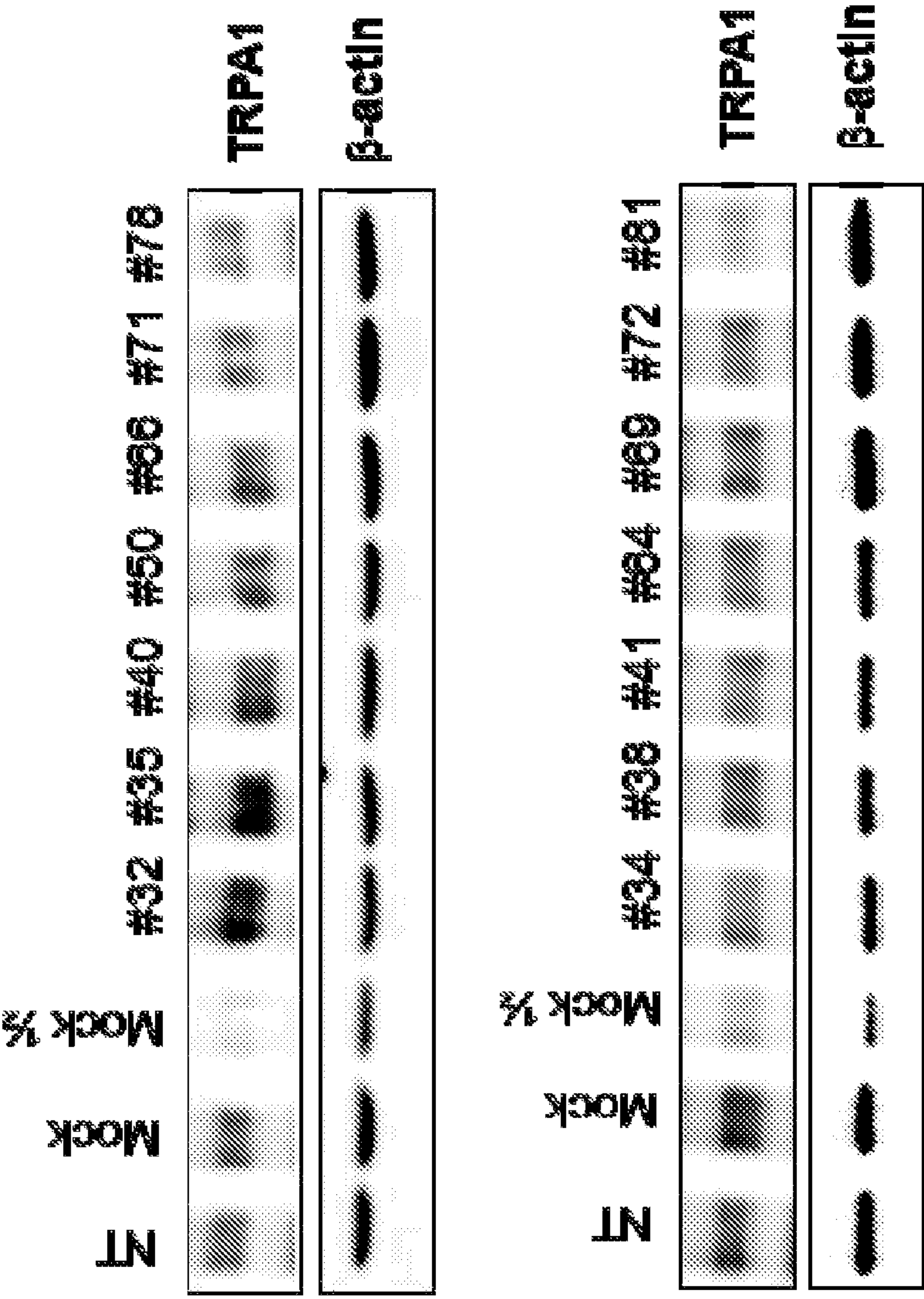


Figure 12

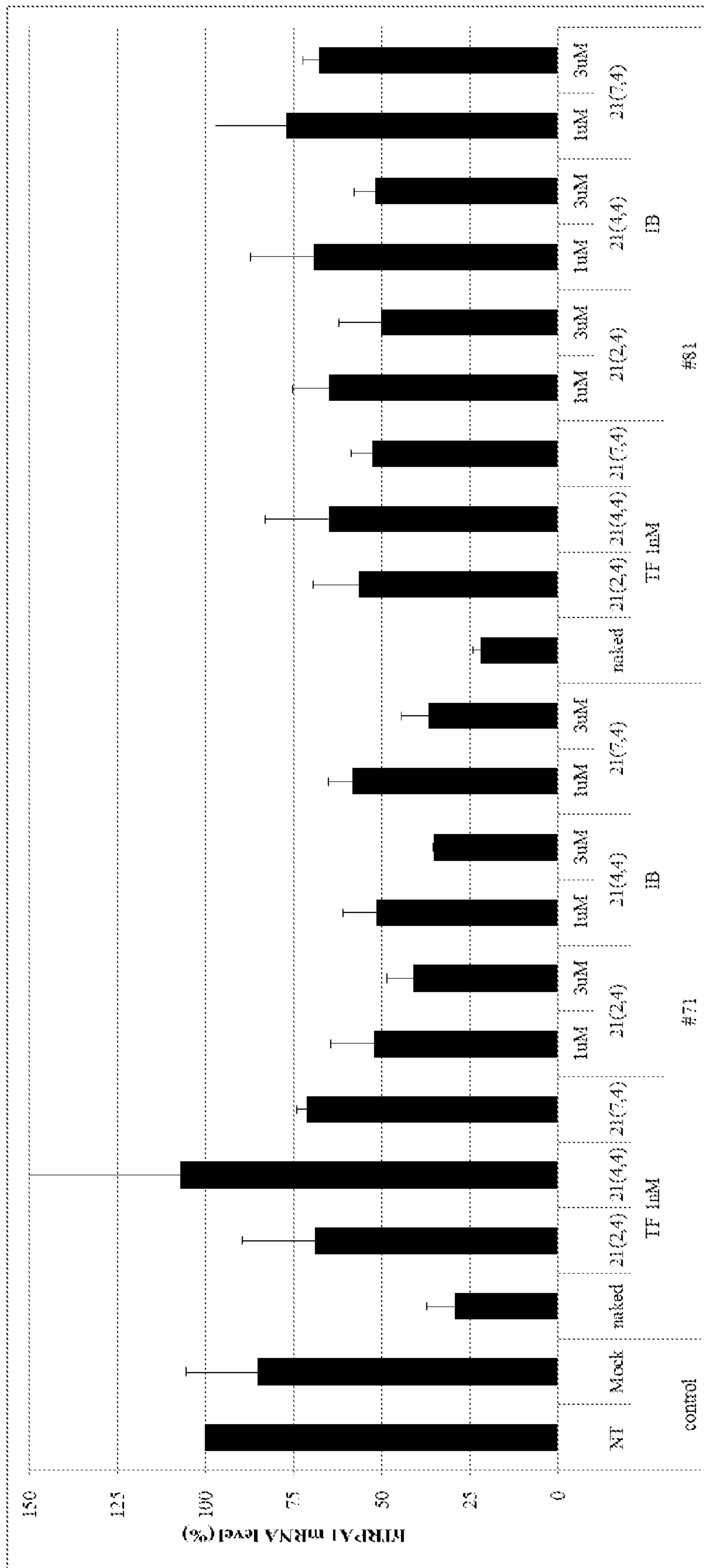


Figure 13

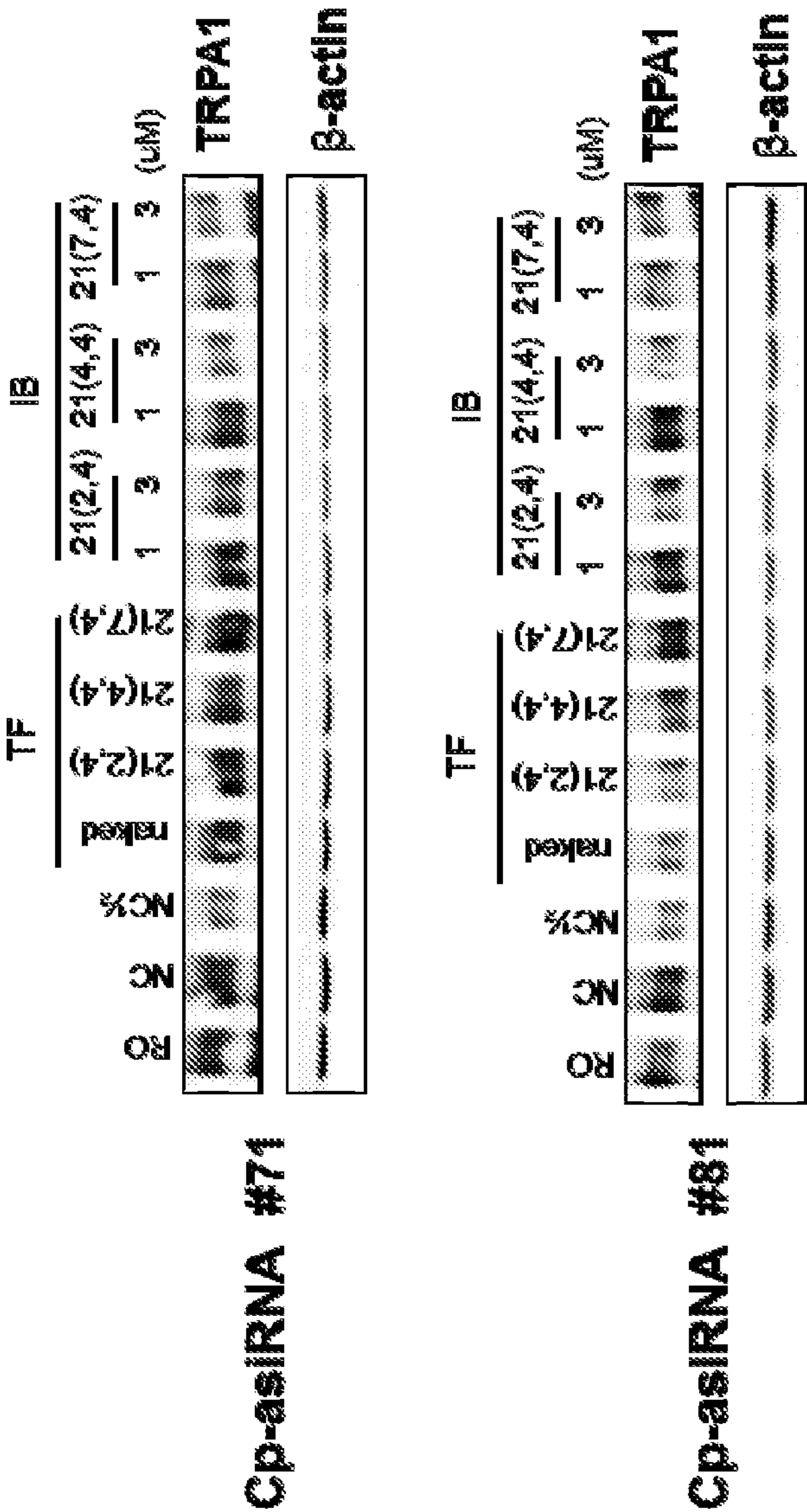


Figure 14

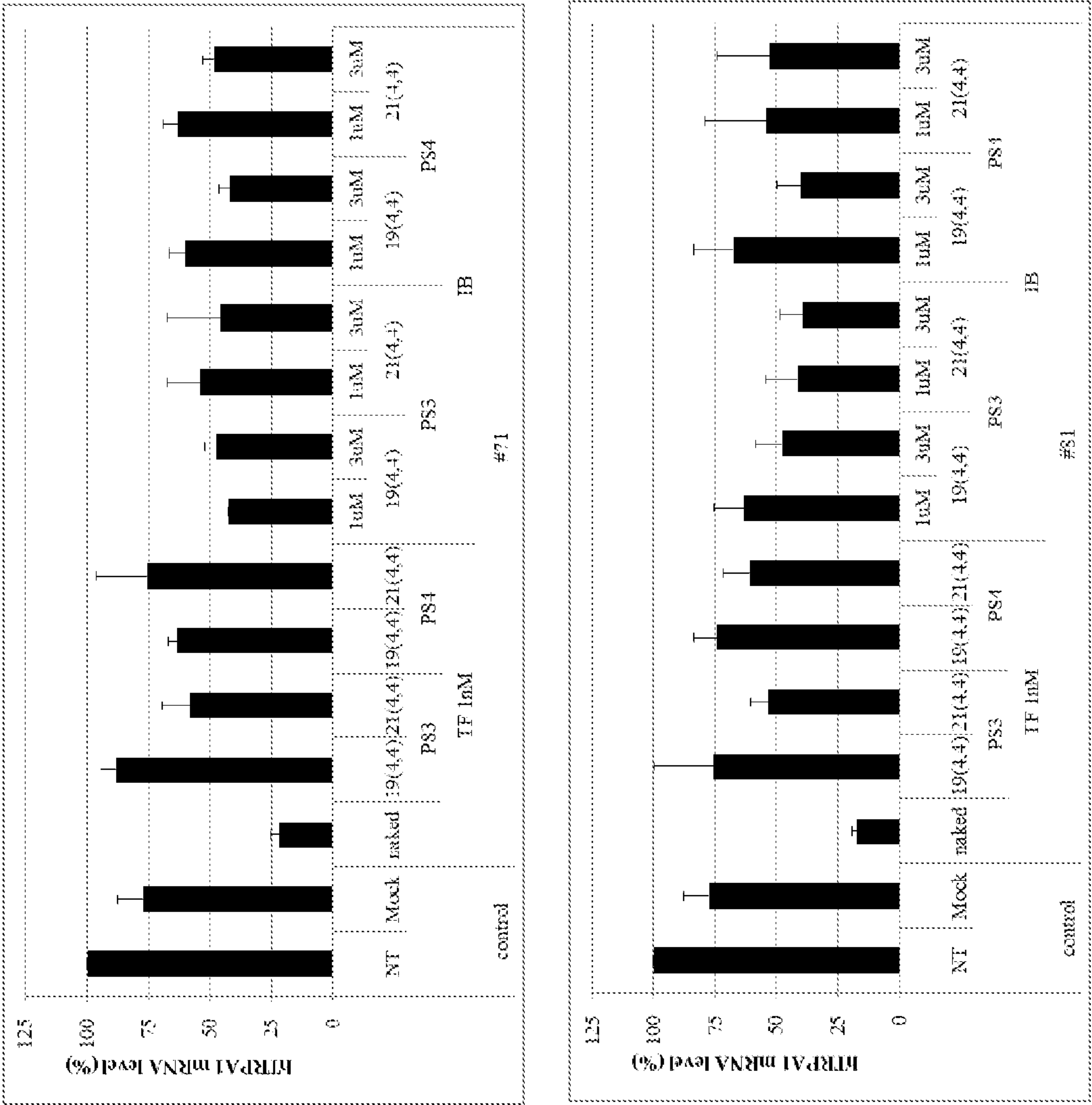
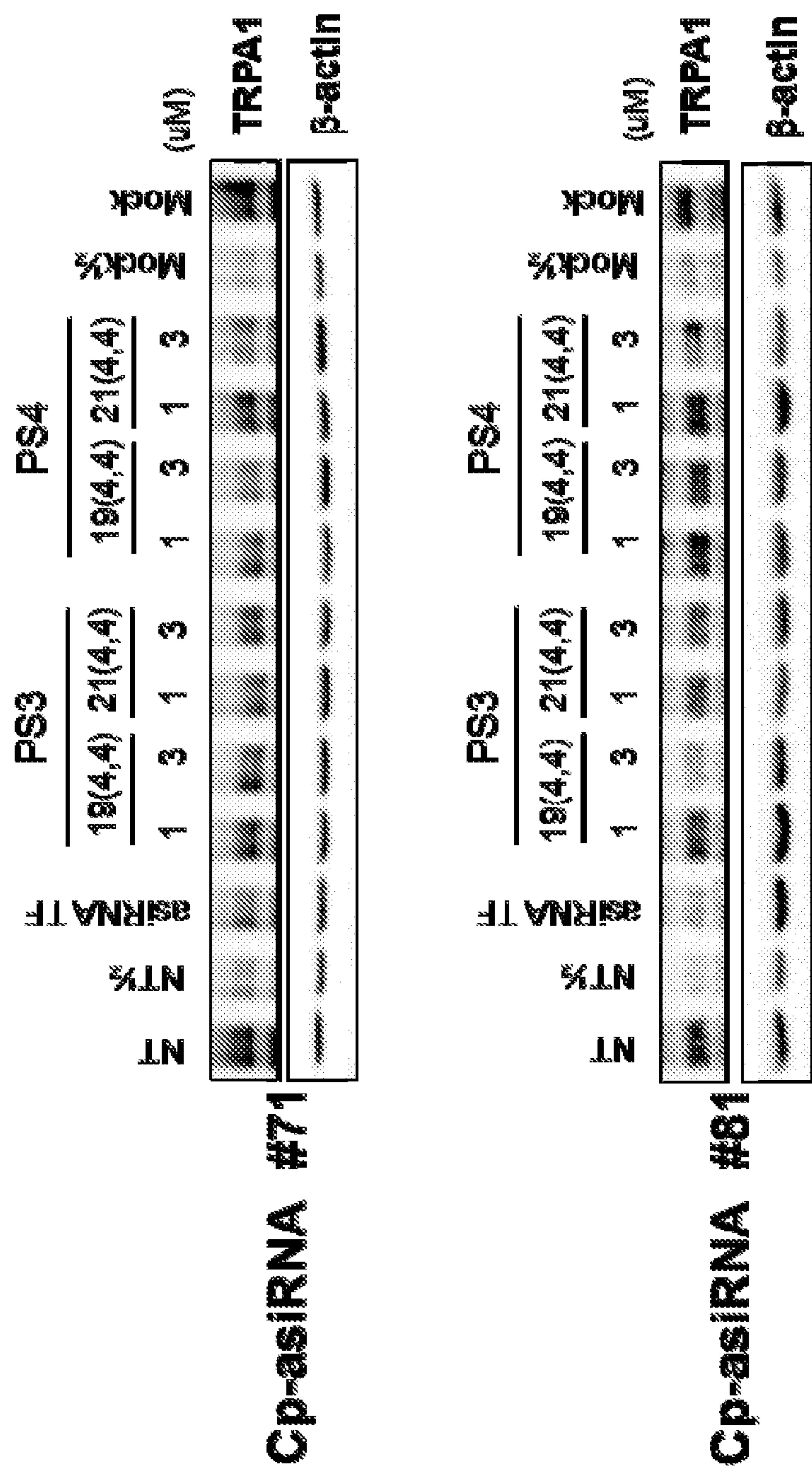


Figure 15



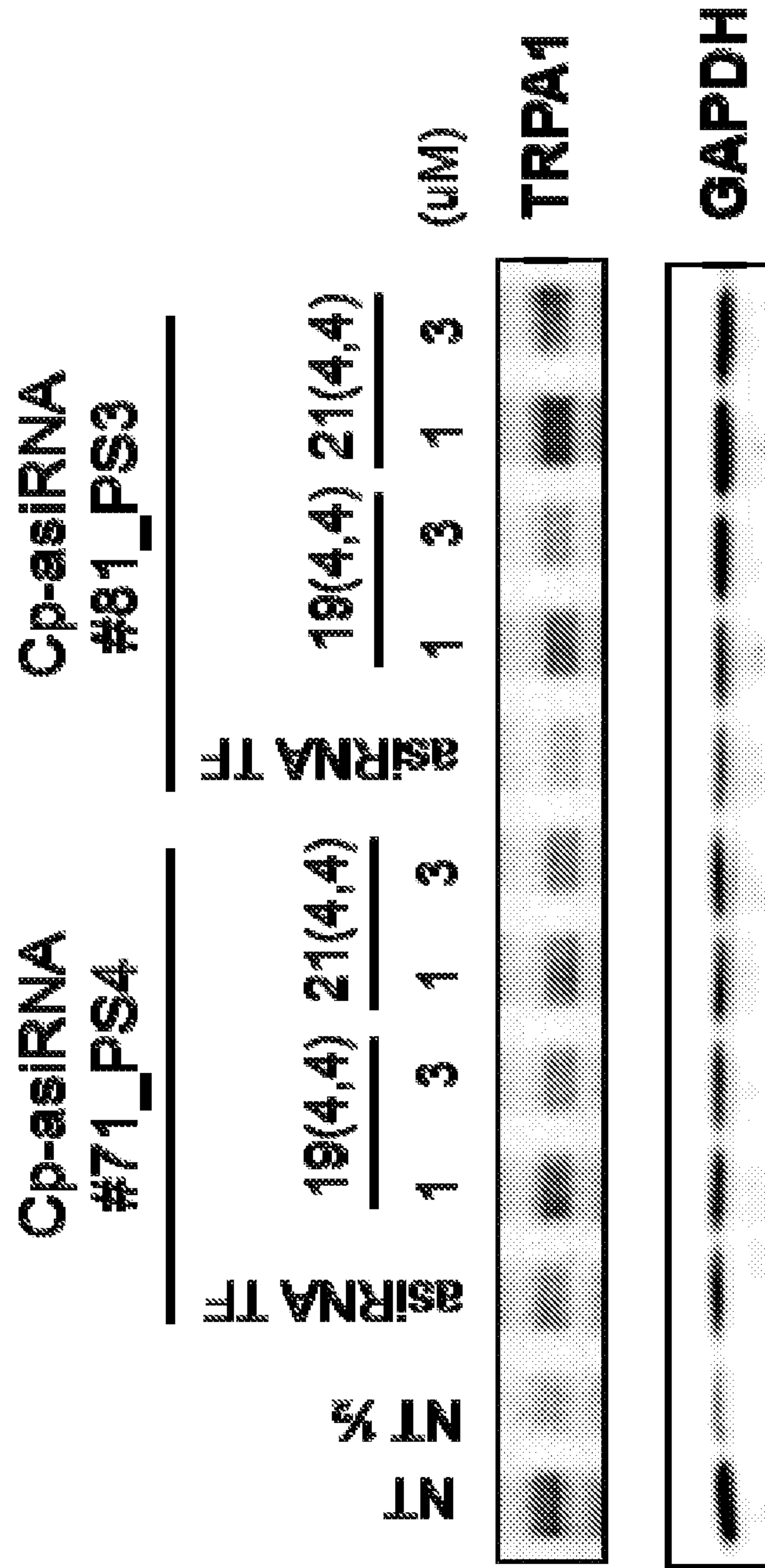


Figure 17

Human TRPA1 mRNA sequence (NM_007332)

1 ccagaagttc tccagggctt ccgcagagcg actttttcgc tgctgtgag ctgcagcgcg
61 ggagagctcg ggctcgcgcg gaccccagcg cctggcaggc tgacagcgct ctctcgcccc
121 aggtgcccgc gcgcgtggtg agcagctgca ccaggtggcg tccgggggtgg ggtcaatgaa
181 gcgcagcctg aggaagatgt ggcgccctgg agaaaagaag gagccccagg gcgttgtcta
241 tgaggatgtg ccggacgaca cggaggattt caaggaatcg ctttaagggtg ttttgaagg
301 aagtgcataat ggattacaaa actttaataa gcaaaagaaa ttaaaaagat gtgacgatat
361 ggacaccttc ttcttgcat atgctgcagc agaaggccaa attgagctaa tggagaagat
421 caccagagat tcctctttgg aagtgtgca tgaaatggat gattatggaa atacccctct
481 gcattgtgct gtagaaaaaa accaaattga aagcgtaag tttcttctca gcagaggagc
541 aaacccaaat ctccgaaact tcaacatgat ggctcctctc cacatagctg tgcagggcat
601 gaataatgag gtgatgaagg tcttgcttga gcatagaact attgatgtta atttgaagg
661 agaaaatgga aacacagctg tgatcattgc gtgcaccaca aataatagcg aagcattgca
721 gattttgctt aaaaaaggag ctaagccatg taaatcaaataaatggggat gttccctat
781 tcaccaagct gcattttcag gtccaaaga atgcatggaa ataatactaa ggtttggtga
841 agagcatggg tacagtagac agttgcacat taactttatg aataatggga aagccacccc
901 tctccacctg gctgtgcaaa atggtgactt ggaaatgatc aaaatgtgcc tggacaatgg
961 tgcacaaata gaccagtgga agaagggaag gtgcacagcc attcattttg ctgccacca
1021 gggagccact gagattgtta aactgatgat atcgtcctat tctggtagcg tggatattgt
1081 taacacaacc gatggatgtc atgagaccat gcttcacaga gcttcattgt ttgatcacca
1141 tgagctagca gactatttaa tttcagtggg agcagatatt aataagatcg attctgaagg
1201 acgctctcca ctatattag caactgcttc tgcattcttg aatattgtta atttgctact
1261 ctctaaaggt gcccaagtag acataaaaga taattttgga cgtaatttc tgcatttaac
1321 tgtacagcaa ccttatggat taaaaaatct gcgacctgaa tttatgcaga tgcaacagat
1381 caaagagctg gtaatggatg aagacaacga tgggtgtact cctctacatt atgcatgtag
1441 acaggggggc cctggttctg taaataacct acttggtttt aatgtgtcca ttcattccaa
1501 aagcaaagat aagaaatcac ctctgcattt tgcagccagt tatgggcgta tcaataacctg

Figure 17 (Continued)

1561 tcagggtc ctacaagaca taagtatac gaggttctg aatgaagtg acctcatgg
 1621 aatgactcct ctccatctgg cagcaaagaa tggacatgat aaagtagtic agcttctct
 1681 gaaaaaagg gcatgtttc tcagtacca caatggctgg acagctttgc atcatgcgc
 1741 catgggcggg tacactcaga ccatgaagg cattcttgat actaattga agtgcacaga
 1801 tcgcctggat gaagacggga aactgcact tcactttgct gcaagggaag gccacgcaa
 1861 agccgttgcg ctctctctga gccacaatgc tgacatagtc ctgaacaagc agcaggcctc
 1921 cttttgcac ctgcacttc acaataagag gaaggagggt gttcttacga tcatcaggag
 1981 caaagatgg gatgaatgc ttaagattt cagtcataat tctccaggca ataatgtcc
 2041 aattacagaa atgataaat acctccctga atgcatgaag gtacttttag attctgcat
 2101 gttgcattcc acagaagaca agtctgccc agactattat atcgagtata atttcaaata
 2161 tcttcaatgt ccattagaat tcacaaaaa aacacctaca caggatgta tatatgaacc
 2221 gcttacagcc ctcaacgcaa tggtaaaaa taaccgcata gagcttctca atcatcctgt
 2281 gtgtaaagaa tattactca tgaaatggt ggcttatgga ttagagctc atatgatgaa
 2341 ttaggatct tactgtctg gtctcatacc tatgaccatt ctggtgtca atataaacc
 2401 aggaatggct ttaactcaa ctggcatcat caatgaaact agtgatcatt cagaaatact
 2461 agataccagc aattcatatc taataaaaac ttgtatgatt ttagtgttt tatcaagtat
 2521 atttgggtat tgcaaagaag cggggcaaatt ttccaacag aaaaggaatt attttatgga
 2581 tataagcaat gttctgaat ggattatcta cagcagggc atcattttg tgctgccctt
 2641 gttgttgaa ataccagctc atctgcagt gcaatgtgga gcaattgctg ttacttcta
 2701 ttgatgaat ttctattgt atctcaaag attgaaaat tgtggaatt ttattgtat
 2761 gttggaggta atttgaaaa cttgttgag gtctacagt gtatttatct tcttctct
 2821 ggcttttga ctacgtttt acatcctcct gaattacag gatccctca gctctcatt
 2881 gctttctata atccagacct tcagcatgat gctaggagat atcaattatc gagagtcctt
 2941 cctagaacca tatctgagaa atgaattggc acatccagt ctgtccttg cacaactgt
 3001 ttcttcaca atattgtcc caattgcct catgaattta ctattggtt tggcagttgg
 3061 cgacattgct gaggtccaga aacatgcac attgaagagg atagctatgc aggtggaact
 3121 tcataccagc ttagagaaga agctgccact ttggttcta cgcaaagtgg atcagaaatc

Figure 17 (Continued)

3181 caccatcgtg tatcccaaca aaccagatc tgggtgggatg ttattccata tattctgtt
 3241 tttatttgc actggggaaa taagacaaga aataccaaat gctgataaat cttagaaat
 3301 ggaaatatta aagcagaaat accggctgaa ggatcttact tttctcctgg aaaaacagca
 3361 tgagctcatt aaactgatca ttcagaagat ggagatcatc tctgagacag aggatgatga
 3421 tagccattgt tctttcaag acaggttaa gaaagagcag atggaacaaa ggaatagcag
 3481 atggaatact gtgttgagag cagtcaaggc aaaaacacac catcttgagc cttagctcct
 3541 cagaccttca gtgaggcttc taatgggggg tgcatgactt gctggttcta actttcaatt
 3601 taaaagagt gaggaagaag cagaatgatt catttgctg cgtgtgaaat catggttct
 3661 gcatgctgta taaaagtaaa ccatcttta tctctattc atatttcta ccaatcacta
 3721 tgtattgggg atatcttgc agatatgtc aaattggact ggactttgat gagatataat
 3781 ctcatattt gaatgggtag aaaatgaatt tgctagaaca cacatttta atgaaaagaa
 3841 gtaataaatg taactattaa gctaaaatgc aatgtcagt actgaattcc tgcttgtaa
 3901 ttacataata tgtgatgctc tagaaaatag tcacaagtat taataatgcc ttagatgata
 3961 gtcttaaata ttaggttgag gtctaccta cctaagctgc ttctggaaa gcttcatgtt
 4021 gaaagaacct atgggtggca ccatgtggac tttctgtcc ctactgtgat gaatagcccc
 4081 acccttctg ctgtcccaa cacacctgat gtcacttga gccatatagt tgaagtacaa
 4141 attaataggc ctatgatat gcacgaattt tactatagat aatatatgtt gttctggtt
 4201 ttgttgcca atgagcataa taaatgtaaa acctatatag tatccctgtg attattgtat
 4261 gagccttgt ttgagattg aaaacaacat ggctccatca catattcct ttttcttt
 4321 gatgtctact caaatcatga attaataca tacctcatca ttaatcttt caagtcctt
 4381 ctattgttt gtctgattt ctccatcatc ctgattagca tgttattcc ctactacc
 4441 ccaggagata ttcactgtaa tgaatatgtc ttggctatg tatgtgtcct tgtgttatgt
 4501 tgtacagtgt tgtttgagt ctgttattat ttacacagat gttattatgc tatagcttct
 4561 atttctgtt ttgctctta tttctttat aattctact tatttctat ttttctact
 4621 catttctatt tttactcct tttactgga catgatgtt acaagataca actgtgttac
 4681 tgtattccat ctagtacggg gccttggtg tggttacta ttcattgtg tgcaccacc
 4741 caccaccac actggactt ttagagatg gacagcttg ttacctccac cttcctgcac

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Figure 17 (Continued)

4801 tcattctcaa acatactgat gttcatacaa accagcagag tgctgaggga cgatatgtac
4861 tattacaaaa ccagacactt ttacattcat ggtccaacag atcacatggc ctagaggcaa
4921 tgttgcatat accttaatct ttgatatgaa taatatcttt gttctttata tttcttaaaa
4981 cagaaagggt ggaaaatcac tatacagaag caatatccaa agatctcctg atcataaaga
5041 caaggggtct tttcagtctt ccctctcctc aaaccttgtag tagcattgca caatatagat
5101 ctcaagtcaac attcactgag tgccaagaat gtgagaaaca ctgtaccatg cctgtcatgc
5161 gaaatattta aataaacaga ttgtcttaca

Figure 18

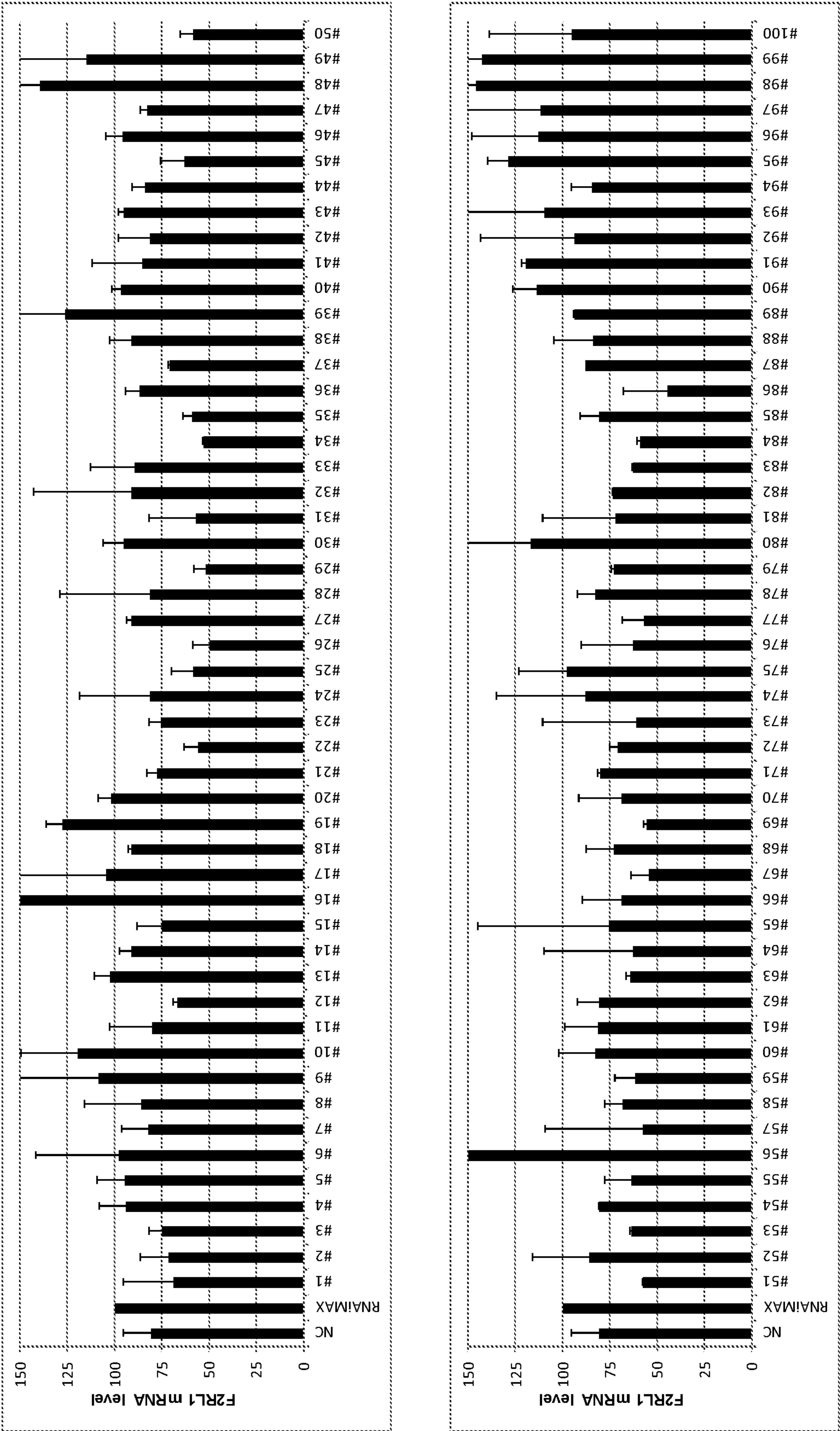
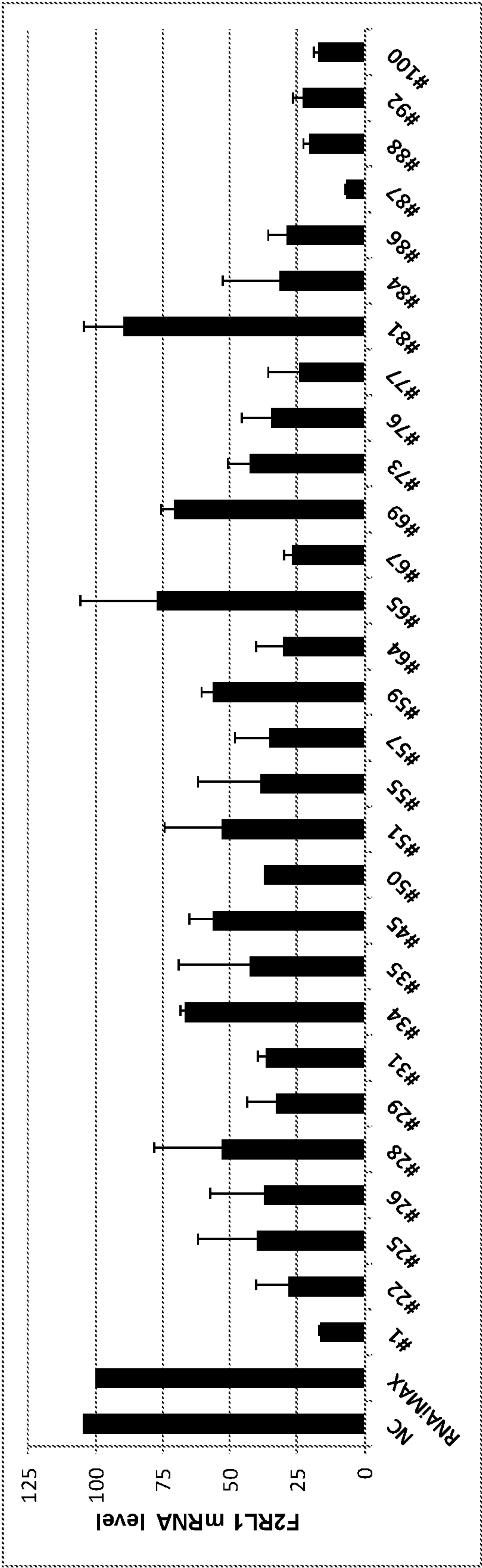


Figure 19



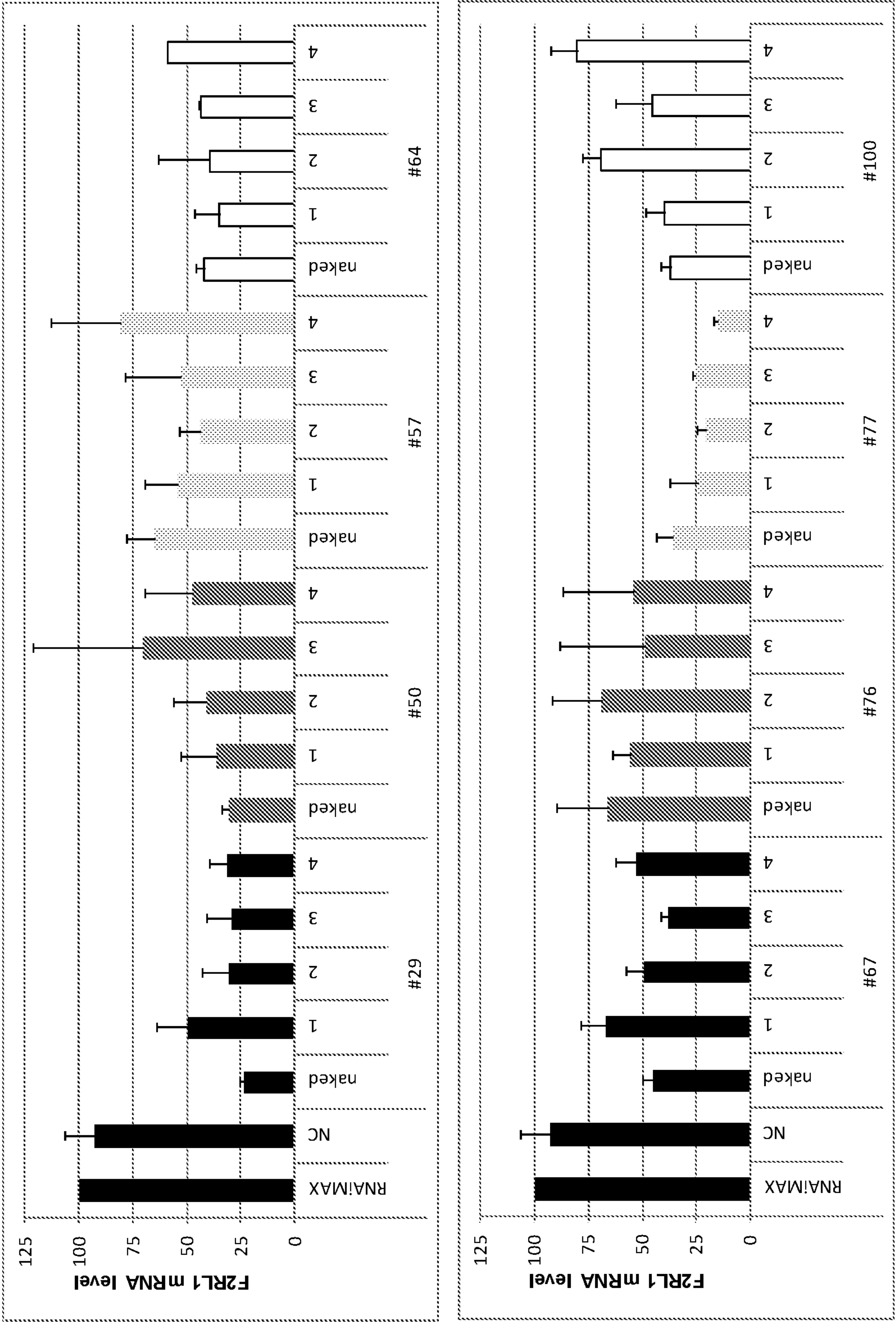


Figure 20

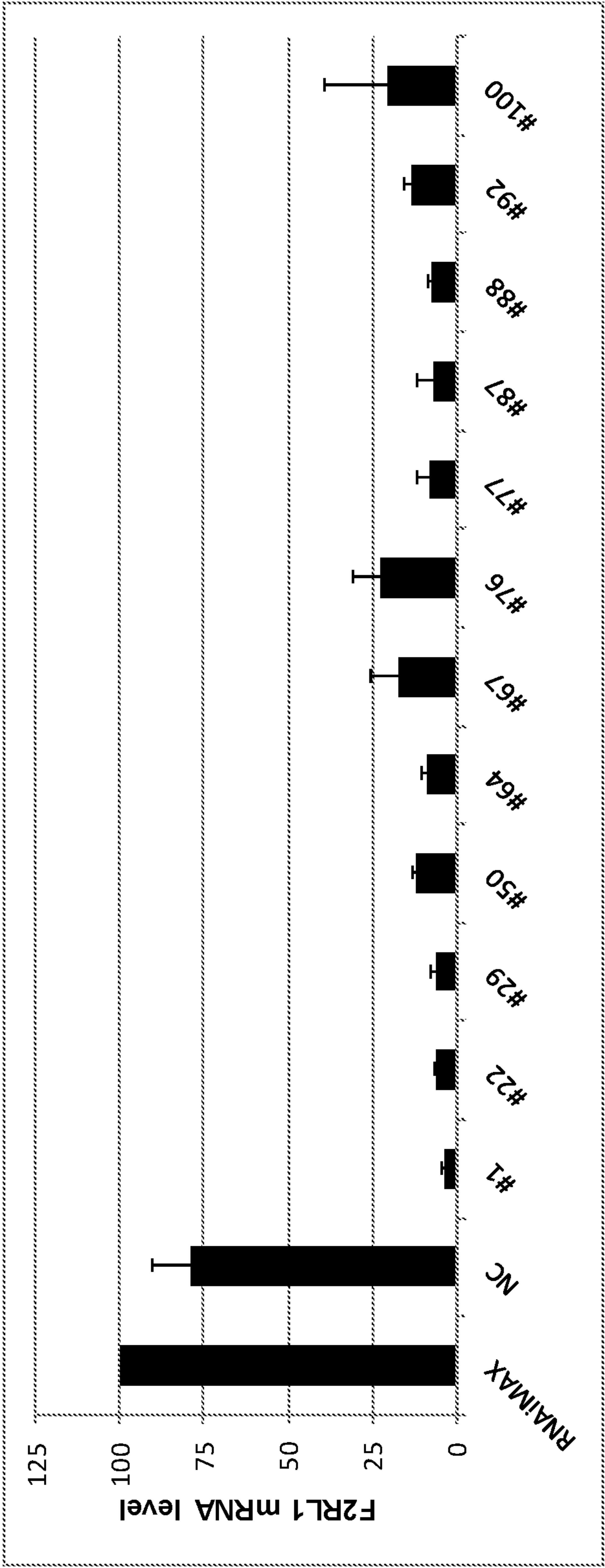


Figure 21

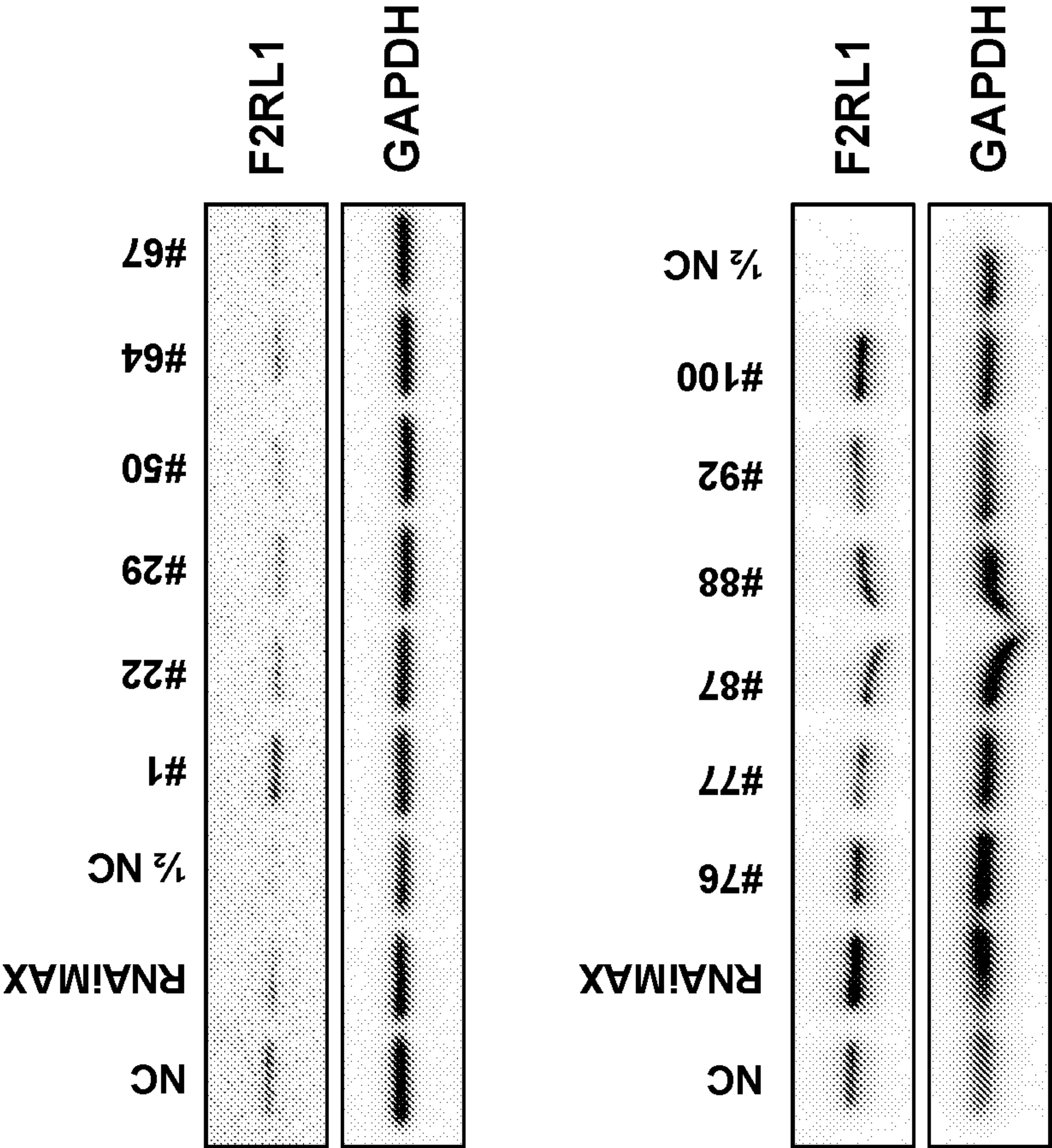


Figure 22

Figure 23

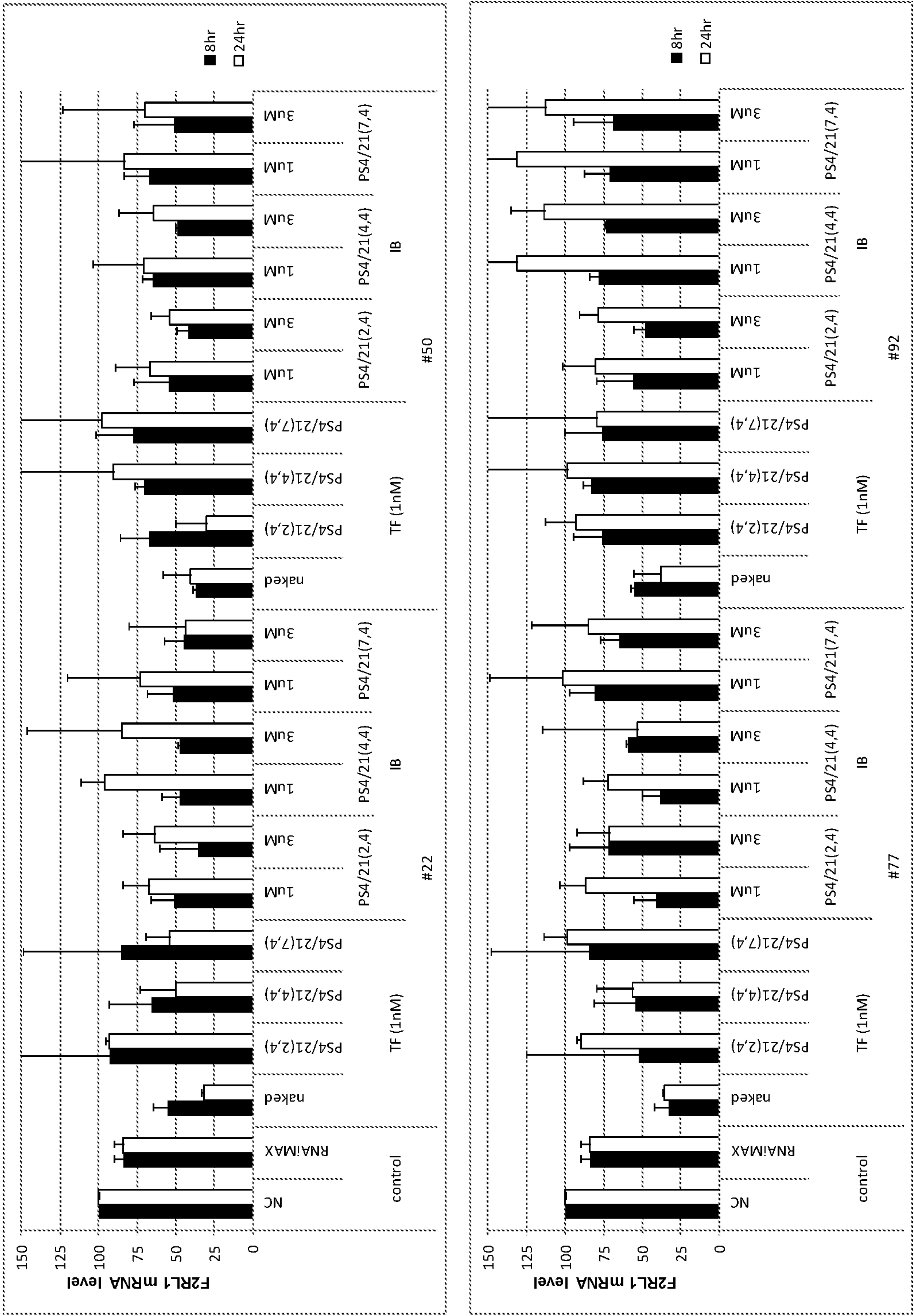


Figure 24

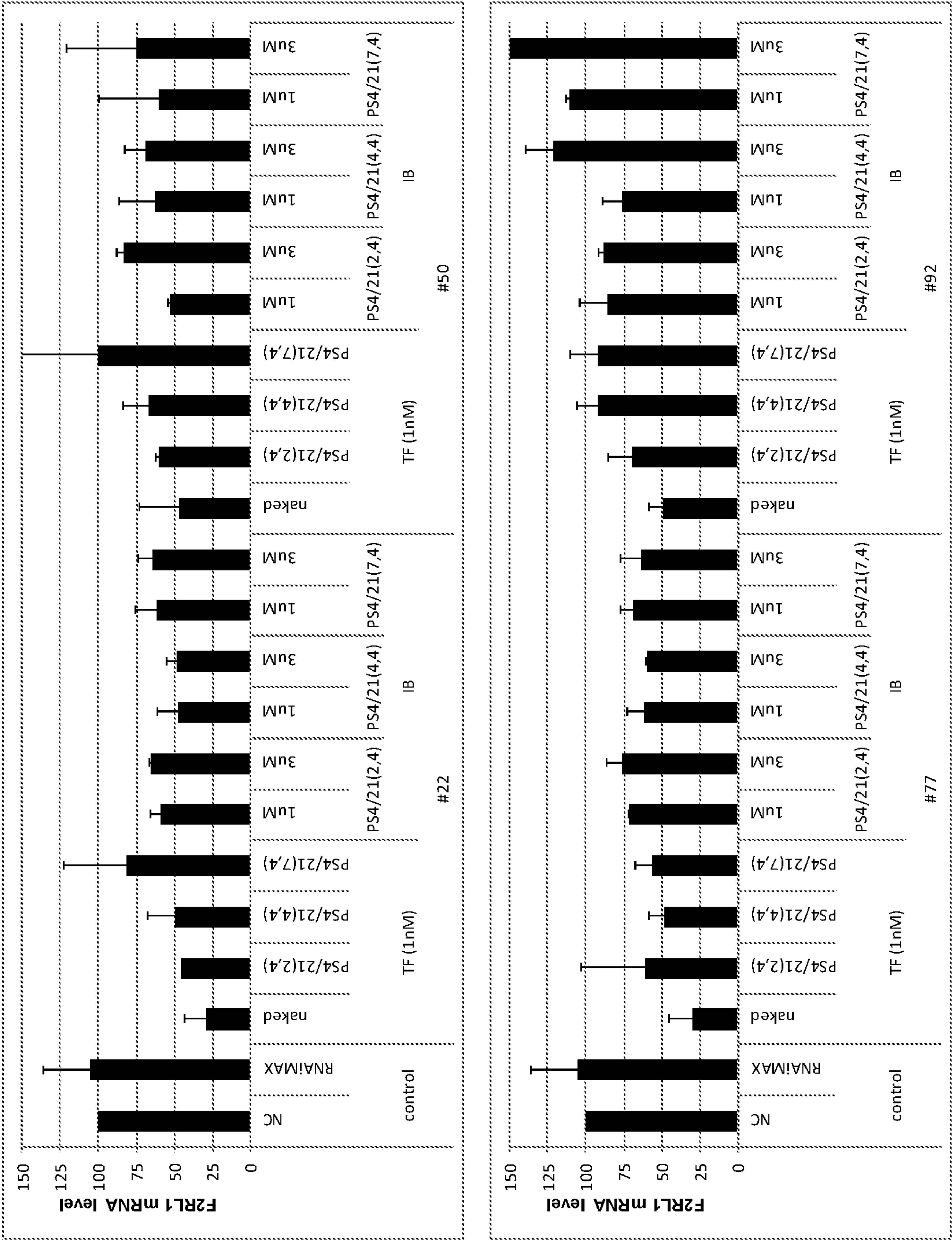


Figure 25

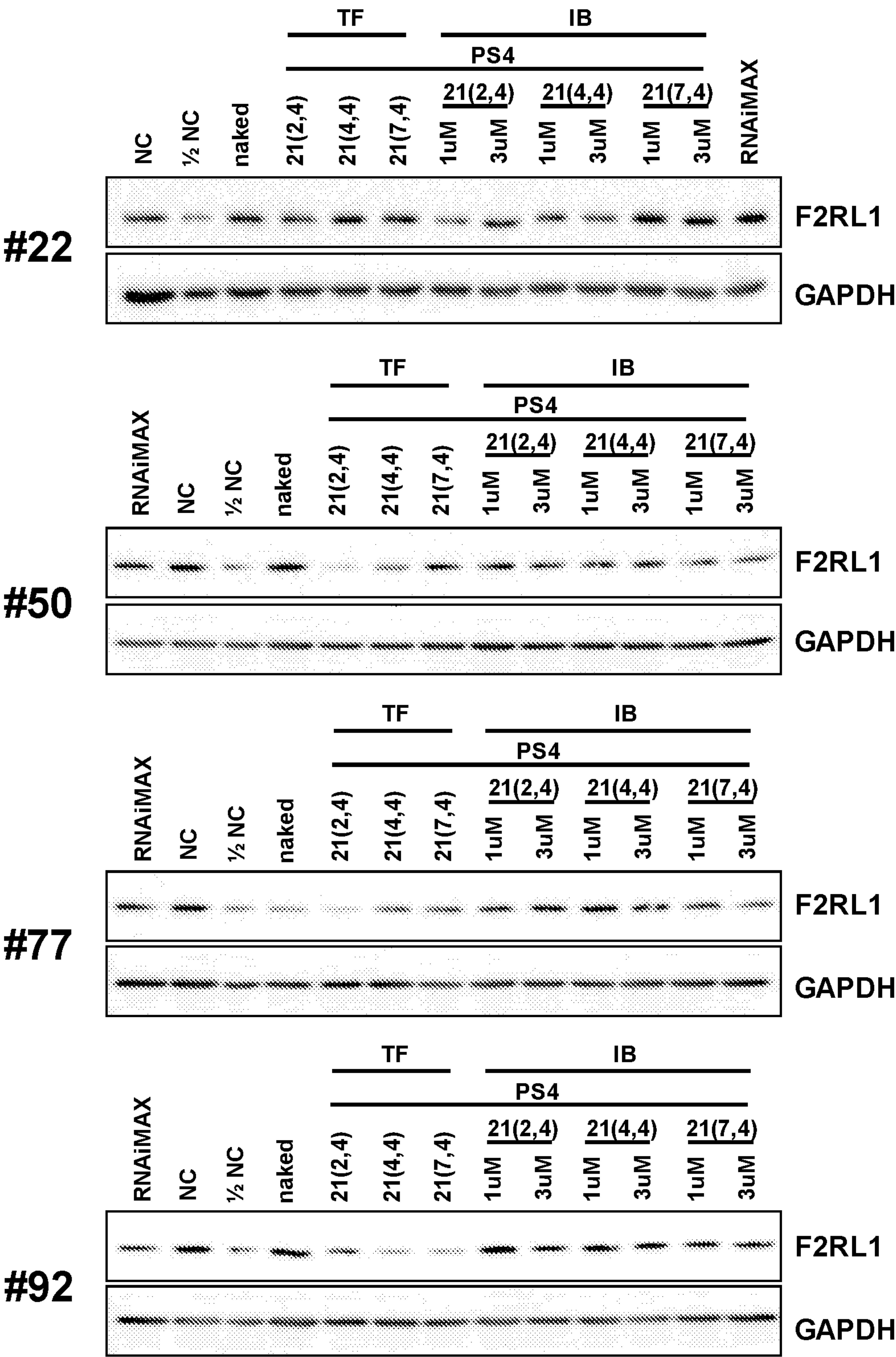


Figure 26

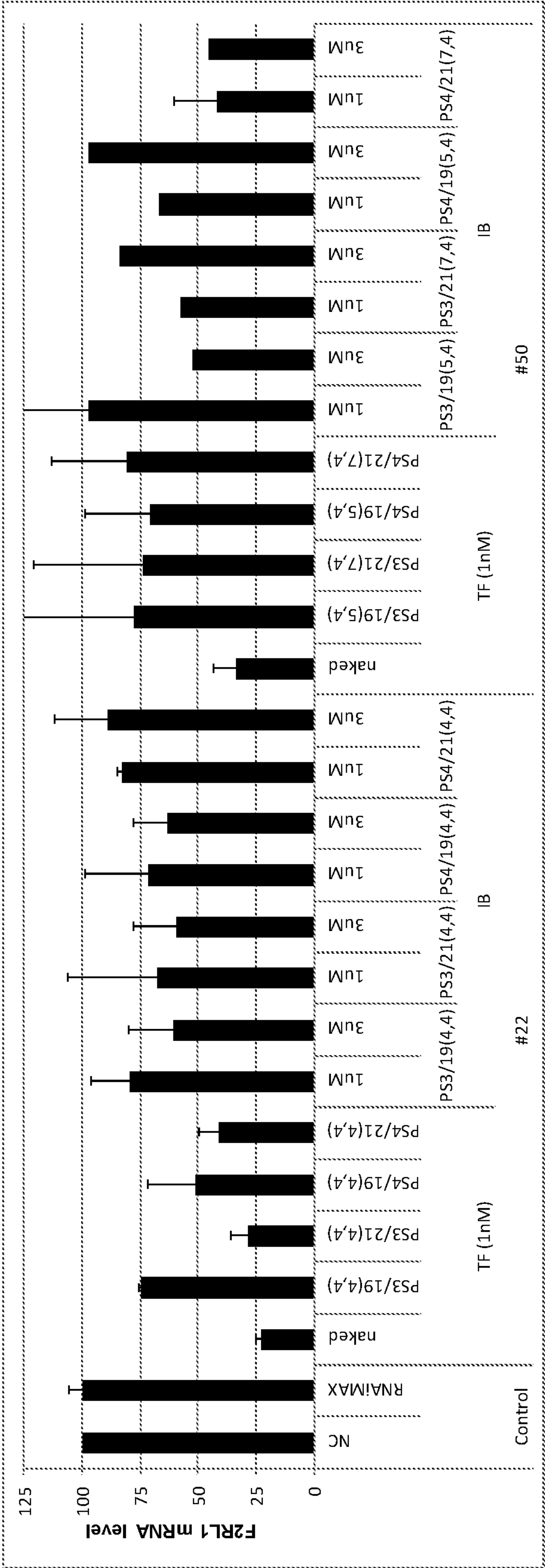


Figure 27

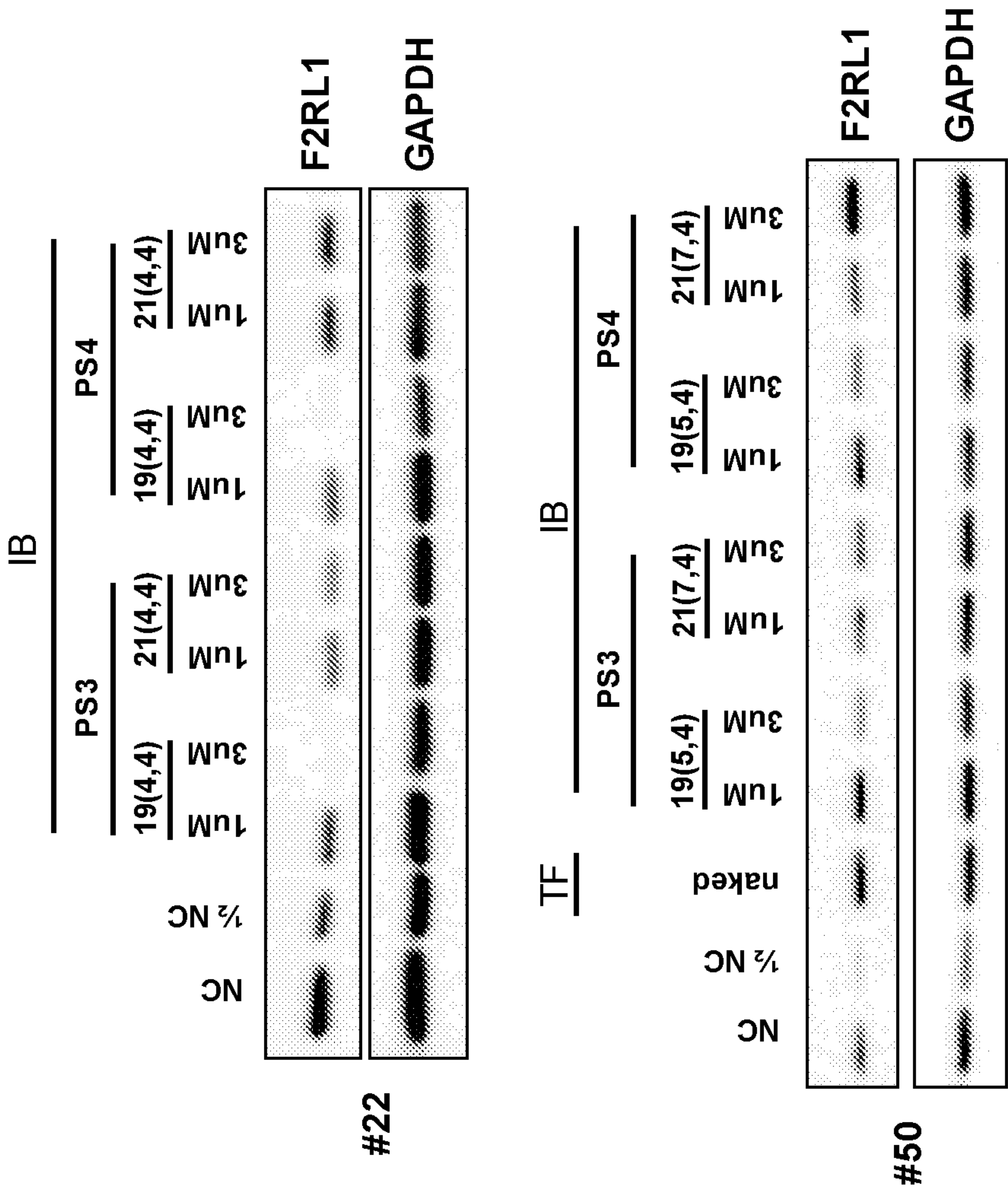


Figure 28

Human F2RL1 mRNA sequence. (NM_005242)

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121 ttcgaatcgg cggcggcgga ttccccgcgc gcccggcgtc ggggcttcca ggaggatgcg
181 gagccccagc gcggcgtggc tgctgggggc cgccatctg ctagcagcct ctctctctg
241 cagtggcacc atccaaggaa ccaatagatc ctctaaagga agaagcctta ttgtaaggt
301 tgatggcaca tcccacgtca ctggaaaagg agttacagtt gaaacagtct tttctgtgga
361 tgagtttct gcactgtcc tcttggaag actgaccact gtcttcctc caattgtcta
421 cacaattgtg tttgtggtgg gtttgccaag taacggcatg gccctgtggg tctttcttt
481 ccgaactaag aagaagcacc ctgctgtgat ttacatggcc aatctggcct tggtgacct
541 cctctctgtc atctggttcc cttgaagat tgcctatcac atacatggca acaactggat
601 ttatggggaa gctctttgta atgtgcttat tggcttttc tatggcaaca tgtactgtc
661 cattctctc atgacctgcc tcagtgtgca gaggtattgg gtcacgtga acccatggg
721 gcactccagg aagaaggcaa acattgcat tggcatctcc ctggcaatat ggctgctgat
781 tctgctggc accatccctt tgtatgtcgt gaagcagacc atcttcattc ctgccctgaa
841 catcacgacc tgtcatgatg tttgcctga gcagctctg gtgggagaca tgttcaatta
901 ctctctctc ctggccattg gggctttct gtcccagcc ttctcacag cctctgccta
961 tgtgctgatg atcagaatgc tgcgatctc tgccatggat gaaaactcag agaagaaaag
1021 gaagagggcc atcaaactca ttgtcactgt cctggccatg tacctgatct gcttcaactc
1081 tagtaacctt ctgcttggg tgcattattt tctgattaag agccagggcc agagccatgt
1141 ctatgccctg tacattgtag ccctctgcct ctctaccctt aacagctgca tcgaccctt
1201 tgtctattac tttgtttcac atgatttcag ggatcatgca aagaacgctc tccttgccg
1261 aagtgtccgc actgtaaagc agatgcaagt atccctcacc tcaaagaaac actccaggaa
1321 atccagctct tactcttcaa gttaaccac tgtaagacc tctattgag tttccaggt
1381 cctcagatgg gaattgcaca gtaggatgtg gaacctgtt aatgttatga ggacgtgtc
1441 gttatttctt aatcaaaaag gtctcaccac ataccatgtg gatgcagcac ctctcaggat
1501 tgctaggagc tcccctgtt gcatagaaa agtagtccc caaattaaca tcagtgtctg
1561 ttcagaatc tctctactca gatgaccca gaaactgaac caacagaagc agactttca
1621 gaagatggtg aagacagaaa cccagtaact tgcaaaaagt agacttggtg tgaagactca
1681 ctctcagct gaaattatat atatacat atatatatt tacatctggg atcatgatg
1741 acttgtagg gctcaaggc cctcagat gatcagcca actgaacgac cttaaaatg
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Figure 28 (Continued)

1801 aggaaaccaa gataaatgag ctgccagaat caggtttcca atcaacagca gtgagttggg
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1921 atggatcttg gctgggtgcg gtgcctcatg cctgtaatcc tagcactttg ggaggctgag
1981 gcaggcaatc acttgaggtc aggagttcga gaccagcctg gccatcatgg cgaaacctca
2041 tctctactaa aaatacaaaa gttaaccagg tgtgtggtgc acgtttgtaa tcccagttac
2101 tcaggaggct gaggcacaag aattgagtat cactttaact caggaggcag aggttgacgt
2161 gagccgagat tgcaccactg cactccagct tgggtgataa aataaaataa aatagtcgtg
2221 aatcttggtc aaaatgcaga ttctcagat tcaataatga gagctcagac tgggaacagg
2281 gcccaggaat ctgtgtggtg caaacctgca tgggtgttat gcacacagag atttgagaac
2341 cattgttctg aatgctgctt ccatttgaca aagtgccgtg ataattttg aaaagagaag
2401 caaacaatgg tgtctctttt atgttcagct tataatgaaa tctgtttgtt gacttattag
2461 gactttgaat tatttcttta ttaacctctt gagttttgt atgtattatt attaaagaaa
2521 aatgcaatca ggattttaa catgtaaata caaattttgt ataactttg atgacttcag
2581 tgaaattttc aggtagtctg agtaatagat tgttttgcca cttagaatag catttgccac
2641 ttagtatttt aaaaaataat tgttgagta ttattgtca gttttgtca cttgttatct
2701 aatacaaaat tataaagcct tcagagggtt tggaccacat ctctttggaa aatagtttgc
2761 aacatattta agagatactt gatgccaaaa tgactttata caacgattgt attgtgact
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2881 tta

Figure 29

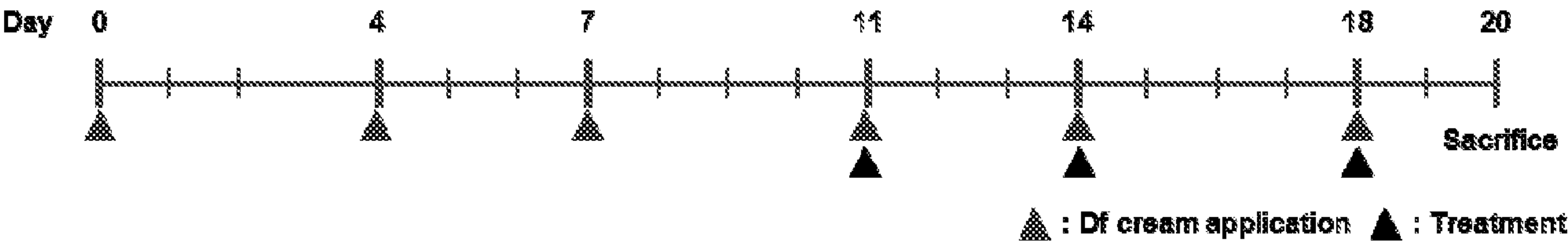
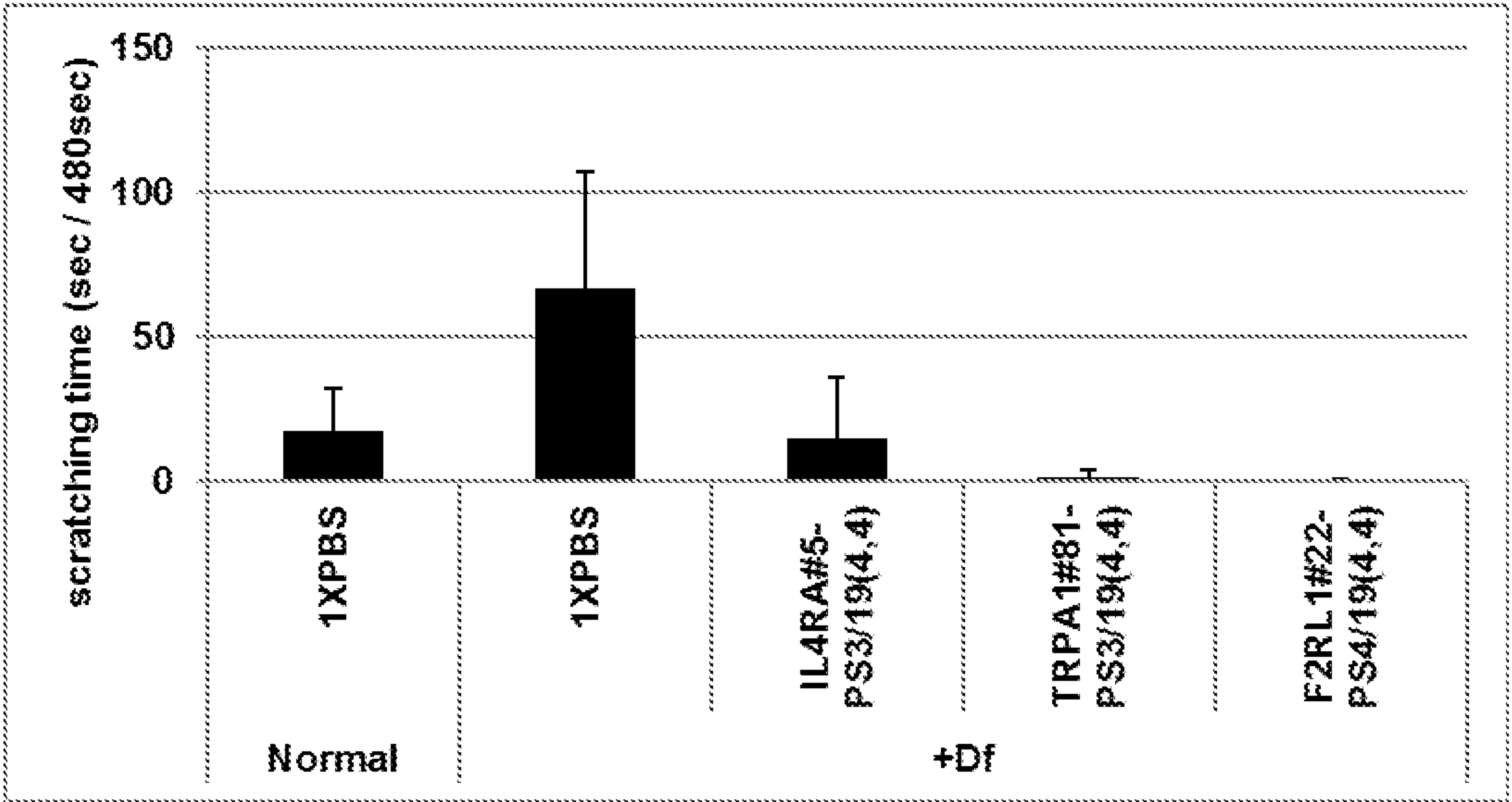


Figure 30

A. Intradermal injection



B. Cream emulsified cp-asiRNA application

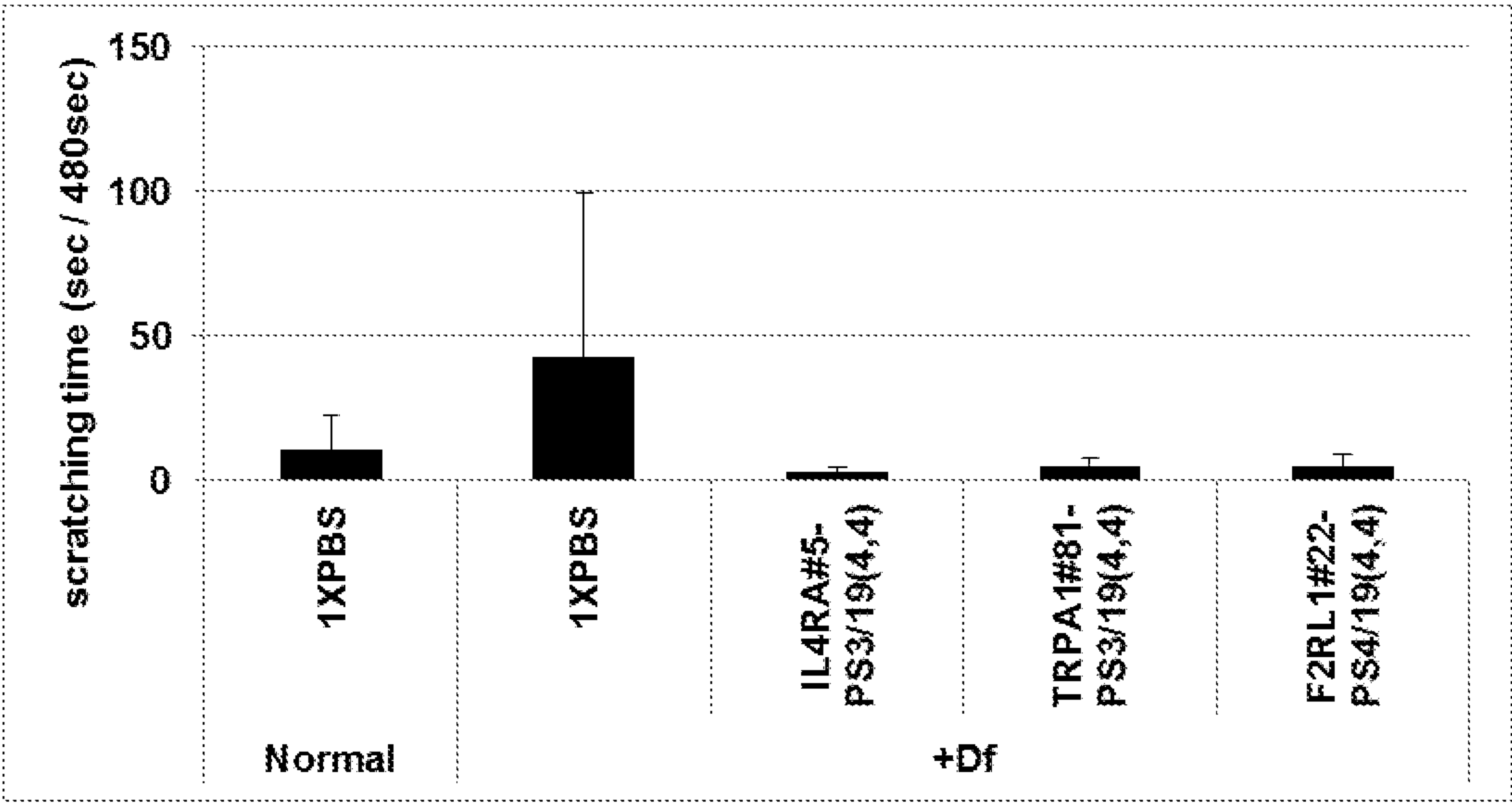
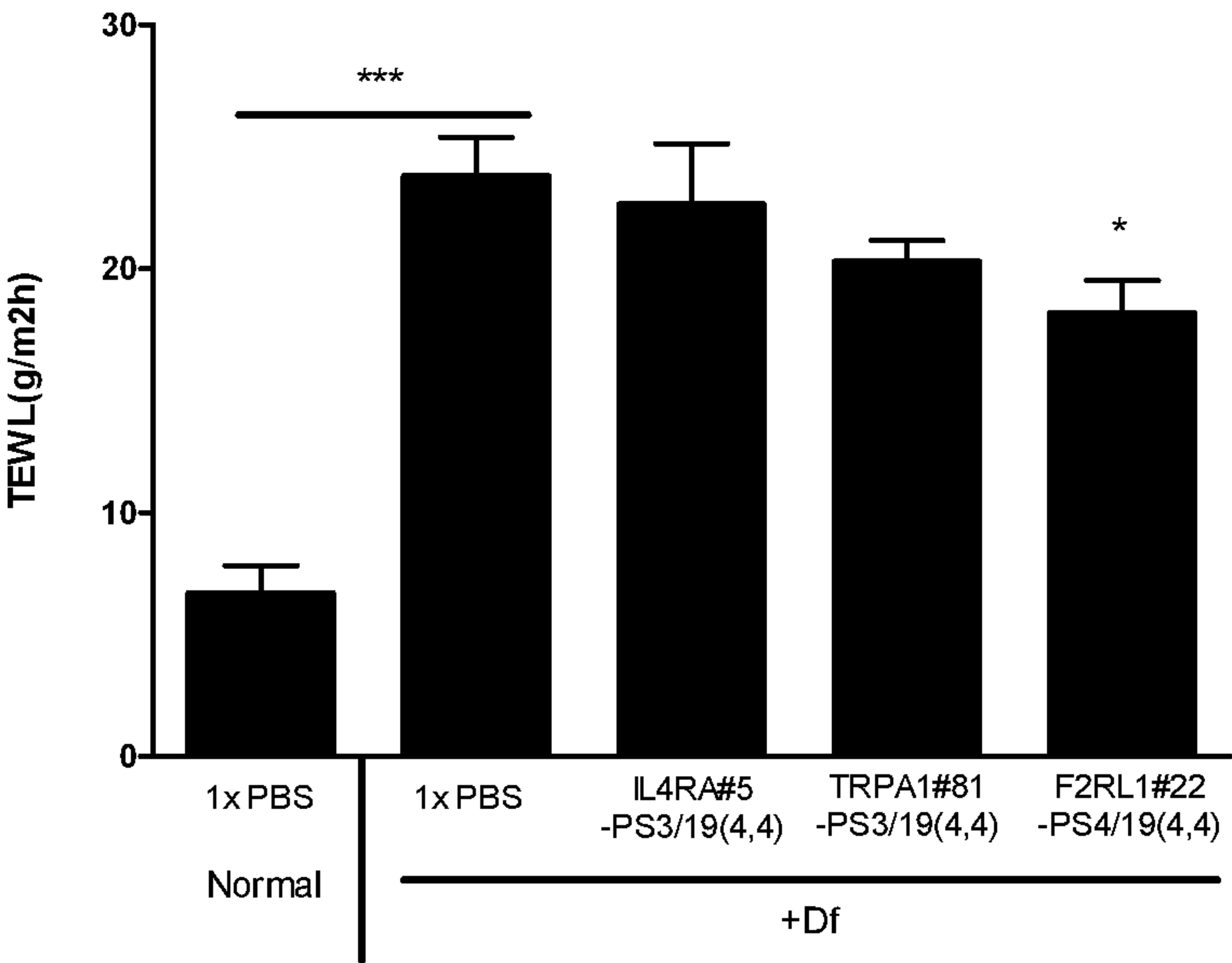
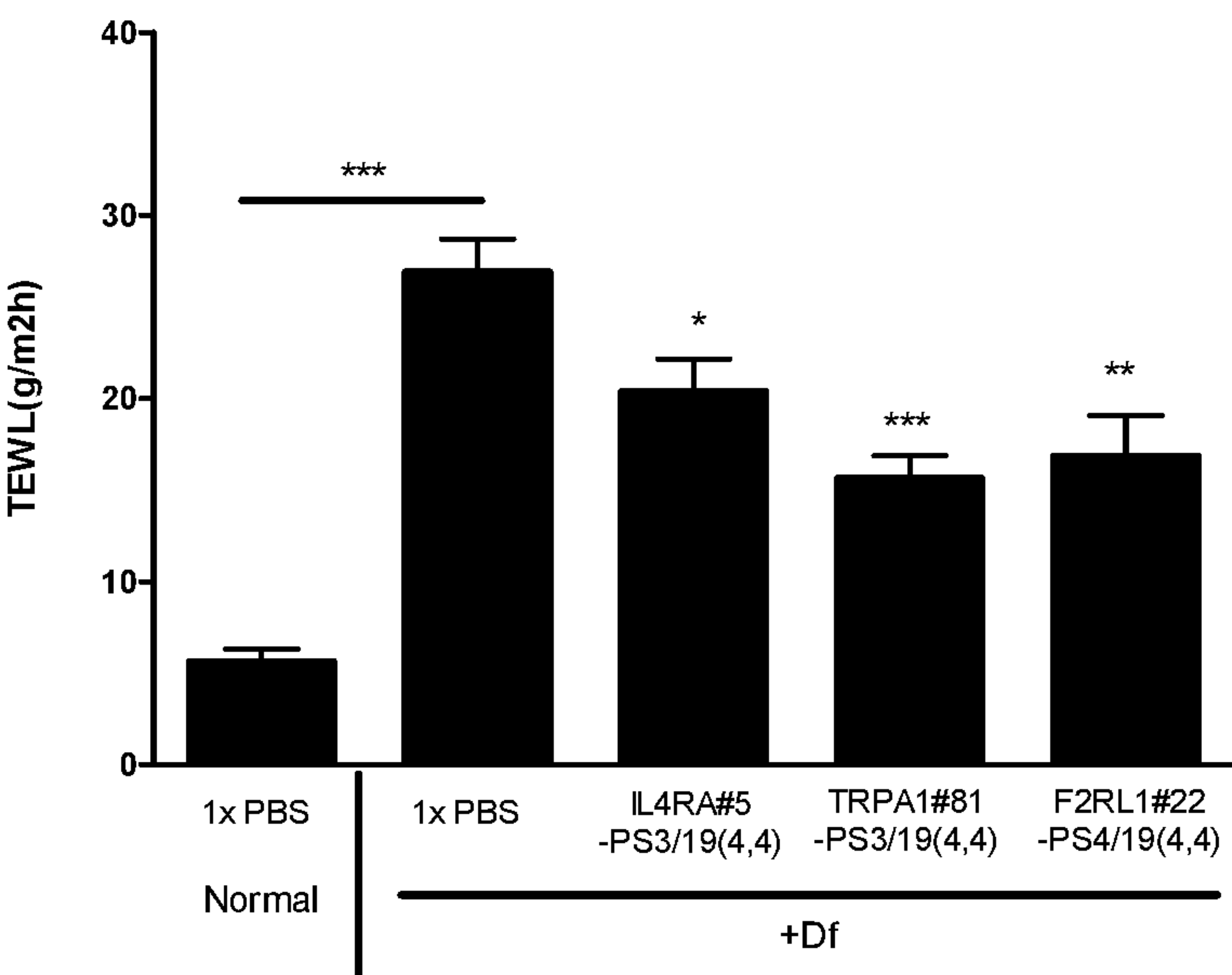


Figure 31

A. Intradermal injection



B. Cream emulsified cp-siRNA application



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Figure 32

A. Intradermal injection

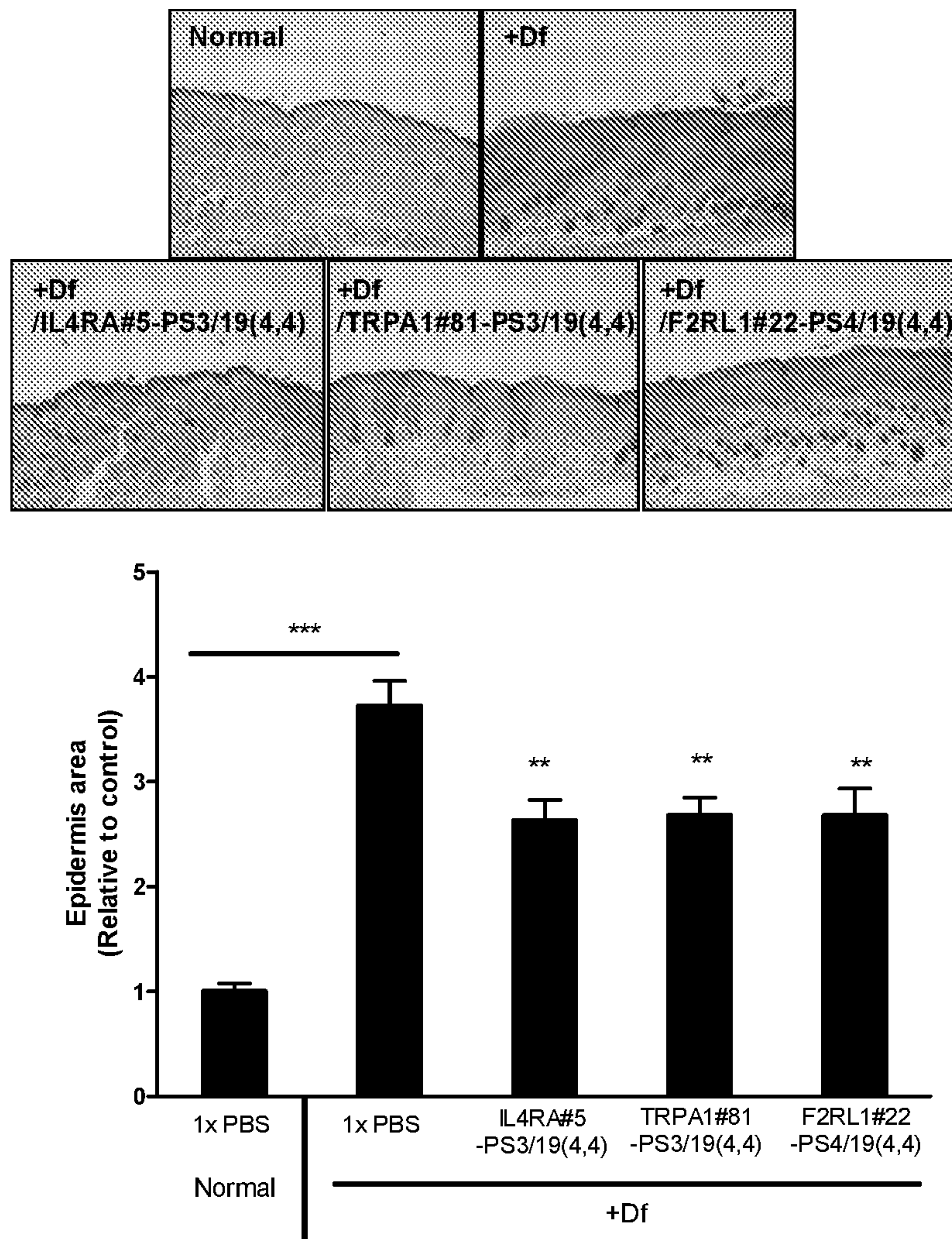


Figure 32 (Cont.)

**B. Cream emulsified cp-asRNA a
pplication**

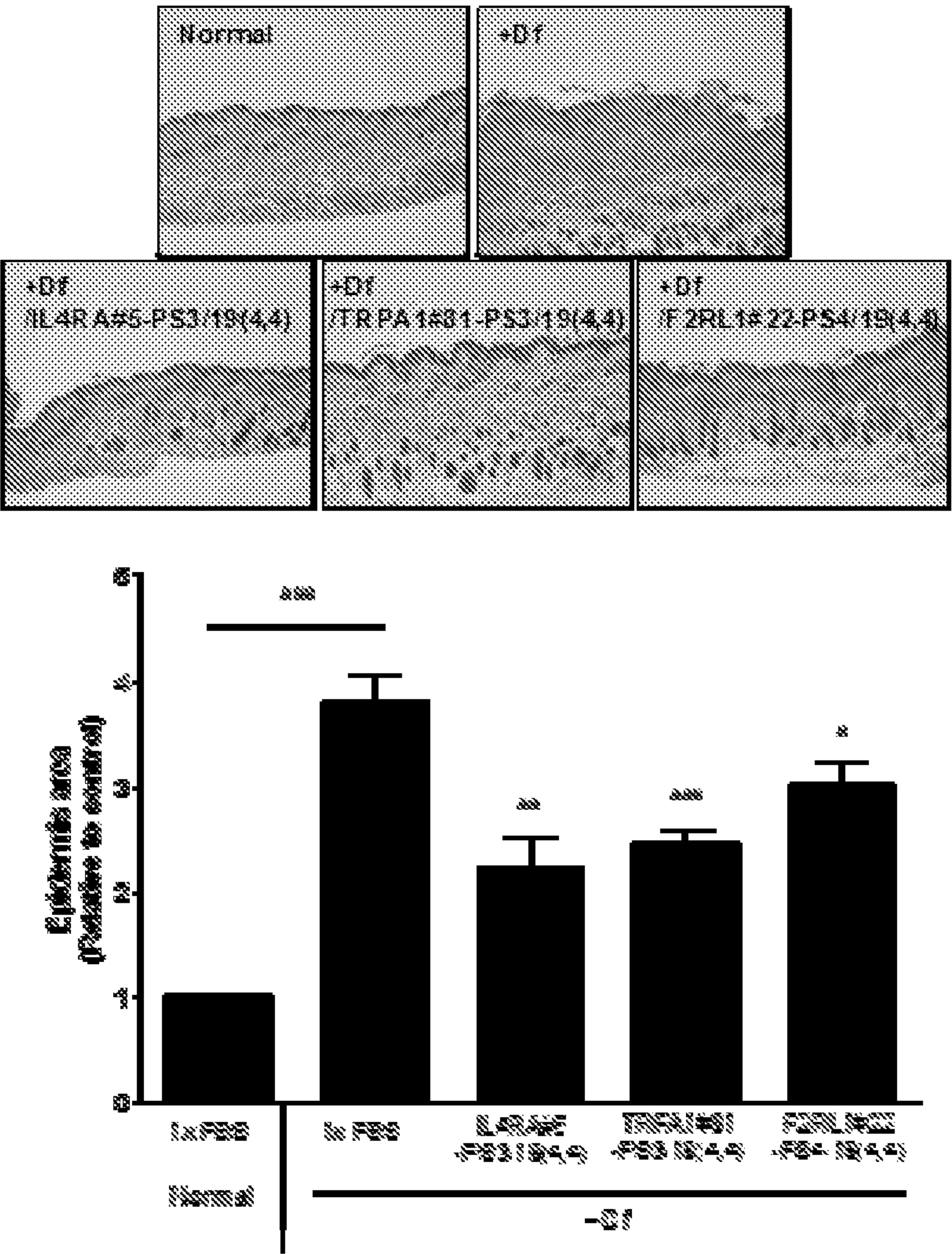


Figure 33

A. Intradermal injection

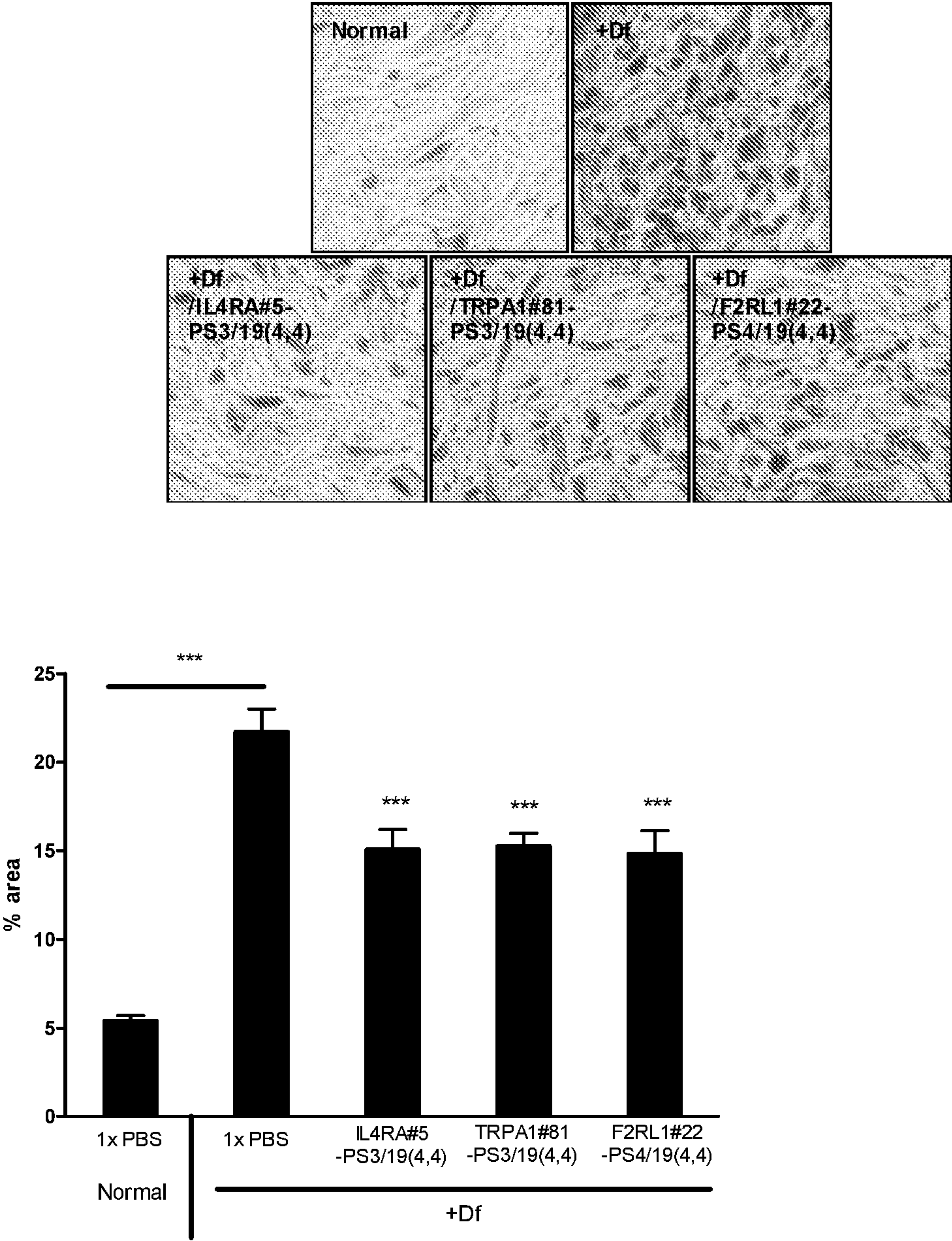


Figure 33 (Cont.)

B. Cream emulsified cp-asiRNA application

