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(54) Title: MICRORNA MOLECULES ASSOCIATED WITH INFLAMMATORY SKIN DISORDERS

(57) Abstract: This invention relates to the finding that inflammatory skin disorders, such as psoriasis and atopic eczema, are characterised by- changes in the expression of specific microRNA molecules (miRNAs). These miRNAs may therefore be useful as biomarkers for inflammatory skin disorders as well as therapeutic targets. Methods of diagnosis and treatment of inflammatory skin disorders are provided, as well as methods of screening for therapeutic compounds.



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microRNA Molecules Associated with Inflammatory Skin Disorders

This invention relates to microRNA molecules (miRNAs) which are associated with inflammatory skin disorders, such as psoriasis and atopic eczema.

Psoriasis and atopic eczema are the two most common chronic inflammatory skin diseases. Each disease affects 1-3% of the adult population worldwide and have a great negative impact on the patients' quality of life (Lebwohl 2003; Leung, Boguniewicz et al. 2004). A complex interplay of genetic and environmental factors together with immunoregulatory abnormalities is thought to play a critical role in the pathogenesis of both diseases. Although psoriasis and atopic eczema share several common features, the clinical characteristics and the so far identified molecular abnormalities are different. Keratinocytes and infiltrating immune cells play a cooperative role in these diseases; however the exact molecular mechanisms and the complex interactions among resident skin cells and infiltrating immunocytes are still not completely understood. Investigations analyzing the molecular background of psoriasis and atopic eczema have identified many disease-associated genes and proteins with aberrant expression (Nomura, Gao et al. 2003; Zhou, Krueger et al. 2003; Carlen, Sanchez et al. 2005), however, the underlying regulatory networks responsible for these gene expression alterations have not been fully characterized.

The present inventors have discovered that lesional skin from patients with inflammatory skin disorders such as psoriasis and atopic eczema is characterized by specific, non-random miRNA expression profiles which differ from the miRNA expression profile of healthy skin. This indicates that miRNAs represent a previously unreported, epigenetic mechanism in the pathogenesis of chronic skin inflammation and may be useful in the diagnosis and therapy of inflammatory skin disorders.

One aspect of the invention provides a method of diagnosing an inflammatory skin disorder in an individual comprising; determining the expression of one or more of the miRNAs in a sample of skin cells obtained from the individual,

5 wherein a change in expression of the one or more miRNAs in the sample relative to controls is indicative that the individual has an inflammatory skin disorder.

A method of diagnosing an inflammatory skin disorder in an
10 individual may for example, comprise;

determining the expression of one or more of the miRNAs selected from the group consisting of let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-9, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-17-5p, miR-20a, miR-21, miR-22, miR-23a, miR-23b, miR-24, miR-26a, miR-26b, miR-27a, miR-27b, miR-29a, miR-29c, miR-30a-5p, miR-30a-3p, miR-30b, miR-30c, miR-30d, miR-30e-5p, miR-30e-3p, miR-31, miR-92, miR-93, miR-95, miR-99a, miR-99b, miR-100, miR-101, miR-106a, miR-106b, miR-107, miR-122a, miR-125a, miR-125b, miR-126, miR-127, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-135b, miR-140, miR-141, miR-142-3p, miR-142-5p, miR-143, miR-145, miR-146a, miR-146b, miR-148a, miR-148b, miR-149, miR-152, miR-155, miR-181d, miR-182, miR-186, miR-187, miR-191, miR-193a, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-203, miR-204, miR-205, miR-214, miR-218, miR-222, miR-223, miR-224, miR-301, miR-320, miR-324-5p, miR-326, miR-328, miR-331, miR-335, miR-361, miR-362, miR-365, miR-375, miR-381, miR-383, miR-411, miR-422b, miR-425, miR-432, miR-451, miR-452, miR-483, miR-486, miR-487b, miR-492, miR-497, miR-501, miR-515-5p, miR-516-5p, miR-518b, miR-519d, miR-524* and miR-526b, miR-532, miR-615, miR-660, in a sample of skin cells
30 obtained from the individual,

wherein a change in expression of the one or more miRNAs in the sample relative to controls is indicative that the individual has an inflammatory skin disorder.

In some embodiments, the expression of one or more of the miRNAs selected from the group consisting of let-7c, let-7d, let-7e, let-7i, miR-1, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-17-5p, miR-20a, miR-21, miR-22, miR-24, miR-27a, miR-27b, miR-29a, miR-30c, 5 miR-30e-5p, miR-31, miR-99b, miR-100, miR-101, miR-106a, miR-106b, miR-107, miR-122a, miR-125b, miR-130a, miR-133a-133b, miR-133b, miR-141, miR-142-3p, miR-143, miR-146a, miR-146b, miR-155, miR-193a, miR-194, miR-197, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-203, miR-205, miR-222, miR-223, miR-326, miR-335, miR-361, miR-10 365, miR-381, miR-422b, miR-451, miR-483, miR-487b, miR-492, miR-515-5p, miR-516-5p, miR-518b, miR-519d, miR-524* and miR-526b may be determined.

A microRNA (miRNA) is a ribonucleic acid molecule of about 19 to 23 15 nucleotides, usually 21 to 22 nucleotides. miRNA molecules are naturally produced by higher eukaryotic cells and reduce the expression of specific protein-coding genes by targeting cognate messenger RNA for translational repression. miRNAs are transcribed from non-protein-coding genes in the form of long precursor miRNA 20 molecules. These precursors are processed by a dsRNA-specific nuclease in the cell nucleus into hairpin RNA molecules of 70-100 nucleotides. These hairpin RNA molecules are further processed in the cytosol by a second dsRNA specific nuclease to produce the mature 19 to 23 nucleotide miRNA (Ambros, 2003; Bartel and Chen, 25 2004; Czech 2006).

The sequences of preferred mature miRNAs described herein are set out in Table 2. The sequences of miRNA genes, precursors and mature miRNAs are also described in Lim LP, et al Science. 299:1540 (2003) 30 and are publicly available from the miRNA Registry (miRBase) which is maintained by the Wellcome Trust Sanger Institute, Hinxton, UK. The miRBase database is described in Griffiths-Jones S. NAR, 2004, 32, D109-D111 and Griffiths-Jones S et al NAR, 2006, 34, D140-D144) and is available online at <http://microrna.sanger.ac.uk/>. To date, 35 342 human miRNAs have been registered in mirBase 8.0.

In the art, miRNAs are generally referred to by name. An assigned miRNA name refers unambiguously to a miRNA of a specific sequence. The annotation of miRNAs is described in Ambros V et al RNA, 2003, 9(3), 277-279.

Methods of diagnosing an inflammatory skin disorder as described herein may be useful in the diagnosis or prognosis of an inflammatory skin disorder in an individual; predicting the susceptibility, onset or likely severity of an inflammatory skin disorder in an individual; or in predicting the responsiveness of an individual to therapy. For example, a change in expression of a miRNA described herein relative to controls may be indicative of the onset of the inflammatory skin disorder or may be indicative that the individual is susceptible to or has a high risk of suffering from an inflammatory skin disorder relative to control members of the population.

The expression of one or more miRNAs selected from the group consisting of miR-20a, miR-146a, miR-20a, miR-31, miR-21, miR-17-5p, miR-193a, miR-146b, miR-146a, miR-222, miR-142-3p, let-7i, miR-106a, miR-200a, miR-30e-5p, miR-203, miR-27b, miR-141, miR-130a, miR-24, miR-199b, miR-199a, miR-106b, miR-199a*, miR-15a, miR-451, miR-29a, miR-422b, miR-205, miR-361, miR-487b, miR-107, miR-451, miR-16, miR-27a and miR-155 may be determined and/or the expression of one or more miRNAs selected from the group consisting of miR-93, miR-130b, miR-132, miR-135b, miR-142-5p, miR-155, miR-187, miR-205, miR-223, miR-224, miR-301, miR-324-5p, miR-362, miR-432, miR-425 and miR-501 may be determined. An increase in expression of one or more of these miRNAs in a sample relative to controls is indicative that the individual has an inflammatory skin disorder, for example psoriasis or atopic eczema.

In some preferred embodiments, the expression of one or more miRNAs selected from the group consisting of miR-20a, miR-17-5p, miR-21,

miR-106a, let-7i, miR-106b, miR-222, miR-422b, miR-130a, miR-193a, miR-106b, miR-199b, miR-199a, and miR-27b may be determined. An increase in expression of one or more of these miRNAs in a sample relative to controls is indicative that the individual has an inflammatory skin disorder, for example psoriasis or atopic eczema. More preferably, the expression of miR-21 may be determined.

The expression of one or more miRNAs selected from the group consisting of miR-122a, miR-133a-133b, miR-197, miR-326, miR-133b, miR-524*, miR-215, miR-194, miR-1, let-7e, miR-381, miR-483, miR-10a, miR-365, miR-492, miR-99b, miR-125b, miR-100, miR-515-5p, miR-335, miR-518b and let-7c may be determined in the sample. A decrease in expression of one or more of these miRNAs relative to controls is indicative that the individual has an inflammatory skin disorder.

In some preferred embodiments, the expression of one or more miRNAs selected from the group consisting of miR-122a, miR-133a, miR-133b, miR-326, miR-125b, and miR-215 may be determined in the sample. A decrease in expression of one or more of these miRNAs relative to controls is indicative that the individual has an inflammatory skin disorder. More preferably, the expression of miR-125b may be determined.

Inflammatory skin disorders include conditions associated with chronic skin inflammation, such as atopic eczema, lupus erythematosus, lichen ruber, prurigo nodularis, psoriasiform disorders such as psoriasis, psoriasiform sarcoidosis, psoriasiform keratosis, psoriasiform-lichenoid dermatitis, pityriasis rubra pilaris, and glucagonoma syndrome.

In some embodiments, a change in the expression of one or more miRNAs is specifically indicative of psoriasis. Psoriasis is a chronic skin disorder which typically presents as well-demarcated erythematous scaling plaques most often symmetrically involving the elbows, knees, lower back, and buttocks. More than 90% of patients

who present with psoriasis have symmetrical discrete plaques, but clinical manifestations can vary greatly (RS Stern, Psoriasis, Lancet 350 (1997), pp. 349-353).

- 5 An increase in expression of one or more miRNAs set out herein may be indicative of psoriasis. A method of diagnosing psoriasis in an individual may comprise the step of determining the expression of one or more miRNAs selected from the group consisting of miR-20a, miR-146a, miR-146b, miR-31, miR-146a, miR-20a, miR-200a, miR-17-5p,
10 miR-30e-5p, miR-141, miR-203, miR-142-3p, miR-21, miR-106a, miR-487b, miR-15a, let-7i, miR-222, miR-422b, miR-130a, miR-193a, miR-106b, miR-199b, miR-199a* and miR-27b and/or one or more miRNAs selected from the group consisting of miR-135b, miR-205, miR-155, miR-223, miR-93, miR-132, miR-425, miR-362, miR-324-5p, miR-224,
15 miR-432 and miR-301 in a sample of skin cells obtained from the individual. An increase in expression of the one or more miRNAs in the sample cells relative to controls is indicative that the individual has psoriasis.
- 20 In some preferred embodiments, the expression of one or more miRNAs selected from the group consisting of miR-146a, miR-146b, miR-31, miR-20a, miR-200a, miR-30e-5p, miR-141, miR-203, miR-142-3p miR-487b, miR-15a may be determined. More preferably, the expression of miR-203 and/or miR-146a may be determined. An increase in expression
25 of the one or more miRNAs in the sample cells relative to controls is indicative that the individual has psoriasis.

- A decrease in the expression of one or more miRNAs set out herein may be indicative of psoriasis. A method of diagnosing psoriasis in
30 an individual may comprise determining the expression of one or more miRNAs selected from the group consisting of miR-125b, miR-99b, miR-122a, miR-197, miR-100, miR-381, miR-518b, miR-524*, let-7e, miR-30c, miR-365, miR-133b, miR-10a, miR-133a-133b, miR-22, miR-326, miR-215, miR-516-5p, let-7c, let-7d, miR-335, miR-492, miR-1, miR-
35 519d, miR-10b, miR-483, miR-194 and miR-526b and/or one or more

miRNAs selected from the group consisting of miR-30a-3p, miR-195, miR-30a-5p, miR-30e-5p, miR-99a, miR-193b, miR-149, miR-26a, miR-26b, miR-218, miR-30e-3p, miR-23b, let-7g, miR-411, miR-199b, miR-29c, miR-101, miR-375, miR-214, miR-375, miR-181d, miR-125a, miR-140, miR-30b, miR-152, miR-328, miR-497, miR-130a, miR-127, miR-148b, miR-186, miR-143, miR-145, miR-30d, miR-126, miR-199a, miR-196b, miR-486, miR-365, miR-29a, miR-320, miR-361, miR-95, miR-532, miR-331, miR-199a, miR-196a, miR-451, miR-193a, miR-126, miR-23a, miR-660, let-7b, miR-16, miR-182, miR-27a, miR-383, let-7a and miR-191 in a sample of skin cells obtained from the individual.

In some preferred embodiments, the expression of one or more miRNAs selected from the group consisting of miR-99b, miR-197, miR-100, miR-381, miR-518b, miR-524, let-7e, miR-30c, miR-365, miR-10a and miR-22 may be determined.

A decrease in expression of the one or more miRNAs in the sample cells relative to controls is indicative that the individual has psoriasis.

20

In other embodiments, a change in the expression of one or more miRNAs set out herein is specifically indicative of atopic eczema. Atopic eczema is a chronic inflammatory skin disease associated with cutaneous hyperreactivity to environmental triggers that are innocuous to normal nonatopic individuals. The diagnosis of atopic eczema is based on the following constellation of clinical findings: pruritus, facial and extensor eczema in infants and children, flexural eczema in adults, and chronicity of the dermatitis (Leung, Boguniewicz et al. 2004).

30

An increase in expression of one or more miRNAs set out herein may be indicative of atopic eczema. A method of diagnosing atopic eczema in an individual may comprise;

determining the expression of one or more miRNAs selected from the group consisting of miR-146a, let-7i, miR-29a, miR-222, miR-24,

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miR-193a, miR-199a, miR-27a, miR-21, miR-20a, miR-17-5p, miR-106b, miR-142-3p, miR-30e-5p, miR-107, miR-200a, miR-146b, miR-27b, miR-199b, miR-106a, miR-101, miR-451, miR-130a, miR-451, miR-141, miR-31, miR-203, miR-223, miR-199a*, miR-200c, miR-155, miR-16, miR-361,
5 miR-205, miR-143, miR-422b, miR-200b and miR-15b and/or one or more miRNAs selected from the group consisting of miR-135b, miR-187, miR-142-5p, miR-501, miR-487b, miR-362, miR-301, miR-130b, and miR-146b.

10 In some preferred embodiments, the expression of one or more miRNAs selected from the group consisting of let-7i, miR-29a, miR-222, miR-24, miR-193a, miR-199a and miR-27a may be determined.

An increase in expression of the one or more miRNAs relative to controls is indicative that the individual has atopic eczema.

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A decrease in expression of one or more miRNAs set out herein may be indicative of atopic eczema. A method of diagnosing atopic eczema in an individual may comprise:

determining the expression of one or more miRNAs selected from
20 the group consisting of miR-122a, miR-133a-133b, miR-326, miR-215, miR-483, miR-519d, miR-335, miR-133b, miR-515-5p, miR-194, miR-524* and miR-197 and/or one or more miRNAs selected from the group consisting of miR-99a, miR-383, miR-125b, miR-193b, miR-10a, miR-30a, miR-100, miR-328, miR-375, let-7c, miR-149, let-7a, miR-130a,
25 miR-196b, miR-197, miR-26b, miR-30e-3p, miR-214, let-7b, miR-26a, miR-101, miR-30c, miR-195, let-7f, miR-148a, miR-125a, miR-30e-5p, miR-29c, miR-199b, miR-365, let-7d, miR-204, miR-23a, miR-196a, miR-452, miR-143, miR-199a, miR-145, miR-23b, miR-486, miR-92, miR-615, miR-497, miR-99b, miR-9, miR-10b, miR-1 and miR-451.

30

In some preferred embodiments, the expression of one or more miRNAs selected from the group consisting of miR-483, miR-519d, miR-335 and miR-515-5p may be determined.

A decrease in expression of the one or more miRNAs relative to controls is indicative that the individual has atopic eczema.

5 The methods described above may comprise determining the expression of one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, fifteen or more or twenty or more of the listed miRNAs.

10 The skilled person is readily able to employ suitable controls for use in the methods described herein. Suitable controls include cells from healthy (i.e. non-lesional) skin which is not affected by the inflammatory skin disorder. Control cells may be obtained from a different individual to the sample cells, for example a healthy individual not suffering from or susceptible to an inflammatory skin
15 disorder.

A suitable sample of skin cells may be taken from a lesion or other site on the skin of the individual which displays one or more symptoms of an inflammatory disorder, such as inflammation, scaling
20 and/or pruritus (i.e. a lesional sample). RNA may be isolated from the skin cells using methods well known in the art (see, e.g., Lagos-Quintana et al, Science 294:853-858(2001); Grad et al, Mol Cell 11: 1253-1263 (2003); Mourelatos et al, Genes Dev 16:720-728(2002); Lagos-Quintana et al, Curr Biol 12:735-739(2002); Lagos-
25 Quintana et al, RNA 9:175-179(2003)).

The expression of a miRNA in a cell may be determined by measuring the amount of miRNA precursor or, more preferably the amount of mature miRNA, which is present in the cells.

30

The amount of miRNA in a cell may be conveniently measured by any convenient technique, including for example quantitative PCR, bead-based flow cytometry, microarrays, northern blotting, dot blotting, RNase protection assays, primer extension analysis, miRNA *in situ*
35 hybridization, and InvaderTM assays. Suitable techniques are

described in Liu et al. (2004); Thomson et al. (2004); Babak et al. (2004), Chen, Ridzon et al. (2005); Castoldi, Schmidt et al. (2006) and Kim et al (2006); Kloosterman et al, Nature Methods, 3 (1), 27 - 29 (2006). Suitable reagents for miRNA *in situ* hybridization are
5 commercially available (e.g. Exiqon A/S, Denmark).

In some embodiments, the expression of one or more miRNAs in a sample may be determined by microarray techniques. Microarrays generally comprise nucleic acid probes of different sequences
10 immobilised in a predetermined arrangement on a solid support. Because different nucleic acid probes are immobilised at different locations on the support, the binding of a label which is observed at a particular location is indicative of specific binding to the nucleic acid probe immobilised at that location.

15 Microarrays may be synthesised using conventional techniques by synthesising nucleic acid probes and then attaching the probes to the support in a site-specific fashion, or by synthesising the nucleic acid probes *in situ* at predetermined locations on the
20 support. Microarrays for use in the detection of human miRNAs are also commercially available (e.g. TaqMan® Human microRNA Array v1.0; Applied Biosystems, CA USA).

In use, a microarray is contacted with a sample under conditions
25 that promote specific binding of miRNAs in the sample to one or more of the immobilised nucleic acid molecules on the microarray. The miRNAs in the sample bind to one or more different locations on the microarray, via the nucleic acid molecules immobilised at those locations to produce a particular binding pattern. This binding
30 pattern can then be detected by any convenient technique. For example all nucleic acid molecules, including miRNA molecules, in the sample may be labelled with a suitable label, typically a fluorescent label, and the locations at which label is present on the microarray following exposure to the sample can be observed.
35 Since the immobilised nucleic acid sequences have different

predetermined locations on the microarray, the observed binding pattern is indicative of the presence and/or concentration of a particular miRNA in the sample. Techniques for detecting binding to microarrays are well known in the art (see for example, US5763870, 5 US5945679 and US5721435).

A method of determining the expression of one or more miRNAs may, for example, comprise: a) contacting a sample with a microarray comprising immobilised probes for said one or more miRNAs under 10 conditions sufficient for specific binding to occur between the miRNA and its corresponding immobilised probe; and b) interrogating the microarray to determine the presence or amount of binding of one or more miRNAs in the sample.

15 A suitable sample for contacting a microarray may be a sample of RNA isolated from skin cells obtained from the individual. Techniques for the preparation of RNA isolates from cells are well known in the art.

20 Conveniently, a Locked Nucleic Acid (LNA)-based miRNA microarray may be employed. Typically, total RNA is isolated from the sample skin cells, labelled and hybridized onto a microarray containing LNA (Locked Nucleic Acid)-modified probes for each known miRNA. The high affinity LNA technology provides the LNA Array with high 25 sensitivity, high specificity and T_m-normalized probes. LNA microarrays are available commercially (e.g. miRCURY™, Exiqon).

In some embodiments, the expression of one or more miRNAs in a sample may be determined by bead-based flow cytometry methods such 30 as FlexmiR™ (Exiqon A/S, Copenhagen) (Lu et al Nature 2005 435 834-838). This involves marking individual beads with fluorescence tags, each representing a single miRNA, and coupling the beads to probes that are complementary to miRNAs of interest. miRNAs are ligated to 5' and 3' adaptors, reverse-transcribed, amplified by PCR using a 35 common biotinylated primer, hybridized to the capture beads, and

stained with a suitable reagent such as streptavidin-phycoerythrin. The beads are then analyzed using a flow cytometer capable of measuring bead color (denoting miRNA identity) and phycoerythrin intensity (denoting miRNA abundance). Because hybridization takes
5 place in solution, bead-based flow cytometry methods may allow more specific detection of closely related miRNAs than microarray techniques.

In some embodiments, the expression of one or more miRNAs in a
10 sample may be determined by miRNA-specific quantitative real-time PCR. For this, total RNA is isolated from the skin biopsy, reverse transcribed using miRNA-specific stem-loop primers, and then amplified by real-time PCR, for example using TaqMan® probes. The assays target only mature microRNAs, not their precursors, ensuring
15 biologically relevant results. Techniques for real-time PCR are well known in the art (Livak et al PCR Methods Appl (1995) 4 357-362) and reagents for use in such techniques are commercially available (e.g. Applied Biosystems, CA USA). Conveniently, real time PCR assays may be analysed on microarrays, for example on a micro-fluidic card.
20 Suitable microarrays are commercially available (e.g. TaqMan® Human microRNA Array v1.0; Applied Biosystems, CA USA).

Methods of diagnosing an inflammatory skin disorder as described herein may also be useful in determining the responsiveness of an
25 individual to a therapy for an inflammatory skin disorder, such as psoriasis or atopic eczema. A method of assessing the efficacy of a therapy for an inflammatory skin disorder in an individual or the responsiveness of an individual to an inflammatory skin disorder therapy may comprise:

30 determining the expression of one or more of the miRNAs set out above in one or more cells obtained from an individual subjected to a regimen of treatment with the inflammatory skin disorder therapy.

A control tissue sample may be obtained before the regimen of
35 inflammatory skin disorder therapy is initiated. A change, for

example, an increase or decrease in expression of one or more of the miRNAs set out above after initiation of the inflammatory skin disorder therapy regimen is indicative that the regimen normalises miRNA levels in a cell and is therefore efficacious for the treatment of the individual.

The absence of any change in the expression of the one or more of the miRNAs set out above after initiation of the the regimen of inflammatory skin disorder therapy may be indicative that the regimen is not efficacious for the treatment of the individual.

The expression of the one or more of the miRNAs may be measured in samples obtained at one or more, two or more, or three or more time points during or after the treatment. The amount of change in the expression of the one or more of the miRNAs may be indicative of the level of responsiveness of the individual to the regimen.

Inflammatory skin disorder therapies are well-known in the art and may include topical or systemic treatments. Suitable treatments include anthralin, calcipotriene, betamethasone, accutane, hydreia, mycophenolate, mofetil, sulfasalazine, 6-thioguanine, dipropionate, tazarotene corticosteroids, cyclosporine, methotrexate, soriatone, IFNgamma, pimecrolimus, tacrolimus, biological agents such as alefacept, etanercept, adalimumab, efalizumab and infliximab and phototherapy, such as UV light therapy and chemophototherapy, for example using psoralen.

Other aspects of the invention relate to methods of treatment of an inflammatory skin disorder. A method of treatment of an inflammatory skin disorder, such as psoriasis or atopic eczema, in an individual may comprise;

increasing or reducing the expression or activity of one or more miRNAs selected from the group consisting of let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-9, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-17-5p, miR-20a, miR-21,

miR-22, miR-23a, miR-23b, miR-24, miR-26a, miR-26b, miR-27a, miR-27b, miR-29a, miR-29c, miR-30a-5p, miR-30a-3p, miR-30b, miR-30c, miR-30d, miR-30e-5p, miR-30e-3p, miR-31, miR-92, miR-93, miR-95, miR-99a, miR-99b, miR-100, miR-101, miR-106a, miR-106b, miR-107, 5 miR-122a, miR-125a, miR-125b, miR-126, miR-127, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-135b, miR-140, miR-141, miR-142-3p, miR-142-5p, miR-143, miR-145, miR-146a, miR-146b, miR-148a, miR-148b, miR-149, miR-152, miR-155, miR-181d, miR-182, miR-186, miR-187, miR-191, miR-193a, miR-193b, miR-194, miR-195, miR-196a, miR-10 196b, miR-197, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-203, miR-204, miR-205, miR-214, miR-218, miR-222, miR-223, miR-224, miR-301, miR-320, miR-324-5p, miR-326, miR-328, miR-331, miR-335, miR-361, miR-362, miR-365, miR-375, miR-381, miR-383, miR-411, miR-422b, miR-425, miR-432, miR-451, miR-452, miR-483, miR-486, miR-15 487b, miR-492, miR-497, miR-501, miR-515-5p, miR-516-5p, miR-518b, miR-519d, miR-524* and miR-526b, miR-532, miR-615 and miR-660 in cells of the individual.

The expression or activity of one or more miRNAs selected from the 20 group consisting of let-7c, let-7d, let-7e, let-7i, miR-1, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-17-5p, miR-20a, miR-21, miR-22, miR-24, miR-27a, miR-27b, miR-29a, miR-30c, miR-30e-5p, miR-31, miR-99b, miR-100, miR-101, miR-106a, miR-106b, miR-107, miR-122a, miR-125b, miR-130a, miR-133a-133b, miR-133b, miR-141, miR-142-3p, 25 miR-143, miR-146a, miR-146b, miR-155, miR-193a, miR-194, miR-197, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-203, miR-205, miR-222, miR-223, miR-326, miR-335, miR-361, miR-365, miR-381, miR-422b, miR-451, miR-483, miR-487b, miR-492, miR-515-5p, miR-516-5p, miR-518b, miR-519d, miR-524* and miR-526b may be increased or 30 reduced in the cells.

For example, the expression or activity of miR-203 and/or miR-146a may be increased or reduced.

The expression or activity of one or more miRNAs selected from the group consisting of miR-20a, miR-146a, miR-20a, miR-31, miR-21, miR-17-5p, miR-193a, miR-146b, miR-146a, miR-222, miR-142-3p, let-7i, miR-106a, miR-200a, miR-30e-5p, miR-203, miR-27b, miR-141, miR-130a, 5 miR-24, miR-199b, miR-199a, miR-106b, miR-199a*, miR-15a, miR-451, miR-29a, miR-422b, miR-205, miR-361, miR-487b, miR-107, miR-451, miR-16, miR-27a and miR-155; more preferably one or more miRNAs selected from the group consisting of miR-20a, miR-17-5p, miR-21, miR-106a, and miR-106b; in cells, preferably lesional skin cells, of 10 the individual may be reduced. For example, the expression or activity of miR-21 may be reduced.

A method of treatment of psoriasis in an individual may comprise; increasing or reducing the expression or activity of one or more miRNAs selected from the group consisting of miR-20a, miR-146a, 15 miR-146b, miR-31, miR-146a, miR-20a, miR-200a, miR-17-5p, miR-30e-5p, miR-141, miR-203, miR-142-3p, miR-21, miR-106a, miR-487b, miR-15a, let-7i, miR-222, miR-422b, miR-130a, miR-193a, miR-106b, miR-199b, miR-199a*, miR-27b, miR-135b, miR-205, miR-155, miR-223, miR-93, miR-132, miR-425, miR-362, miR-324-5p, miR-224, miR-432, miR-301 20 miR-125b, miR-99b, miR-122a, miR-197, miR-100, miR-381, miR-518b, miR-524*, let-7e, miR-30c, miR-365, miR-133b, miR-10a, miR-133a-133b, miR-22, miR-326, miR-215, miR-516-5p, let-7c, let-7d, miR-335, miR-492, miR-1, miR-519d, miR-10b, miR-483, miR-194, miR-526b, miR-30a-3p, miR-195, miR-30a-5p, miR-30e-5p, miR-99a, miR-193b, miR-149, 25 miR-26a, miR-26b, miR-218, miR-30e-3p, miR-23b, let-7g, miR-411, miR-199b, miR-29c, miR-101, miR-375, miR-214, miR-375, miR-181d, miR-125a, miR-140, miR-30b, miR-152, miR-328, miR-497, miR-130a, miR-127, miR-148b, miR-186, miR-143, miR-145, miR-30d, miR-126, miR-199a, miR-196b, miR-486, miR-365, miR-29a, miR-320, miR-361, miR-95, 30 miR-532, miR-331, miR-199a, miR-196a, miR-451, miR-193a, miR-126, miR-23a, miR-660, let-7b, miR-16, miR-182, miR-27a, miR-383, let-7a and miR-191 in cells of the individual.

In some embodiments, a method of treatment of psoriasis may 35 comprise;

reducing the expression or activity of one or more miRNAs selected from the group consisting of miR-20a, miR-146a, miR-146b, miR-31, miR-146a, miR-20a, miR-200a, miR-17-5p, miR-30e-5p, miR-141, miR-203, miR-142-3p, miR-21, miR-106a, miR-487b, miR-15a, let-7i, 5 miR-222, miR-422b, miR-130a, miR-193a, miR-106b, miR-199b, miR-199a* and miR-27b and/or one or more miRNAs selected from the group consisting of miR-135b, miR-205, miR-155, miR-223, miR-93, miR-132, miR-425, miR-362, miR-324-5p, miR-224, miR-432 and miR-301; more preferably one or more miRNAs selected from the group consisting of 10 miR-146a, miR-146b, miR-31, miR-20a, miR-200a, miR-30e-5p, miR-141, miR-203 and miR-142-3p in cells, of the individual.

For example, the expression or activity of miR-203 and/or miR-146a may be reduced.

15

A method of treatment of atopic eczema in an individual may comprise;

increasing or reducing the expression or activity of one or more miRNAs selected from the group consisting of miR-146a, let-7i, 20 miR-29a, miR-222, miR-24, miR-193a, miR-199a, miR-27a, miR-21, miR-20a, miR-17-5p, miR-106b, miR-142-3p, miR-30e-5p, miR-107, miR-200a, miR-146b, miR-27b, miR-199b, miR-106a, miR-101, miR-451, miR-130a, miR-451, miR-141, miR-31, miR-203, miR-223, miR-199a*, miR-200c, miR-155, miR-16, miR-361, miR-205, miR-143, miR-422b, miR-200b, miR- 25 15b, miR-135b, miR-187, miR-142-5p, miR-501, miR-487b, miR-362, miR-301, miR-130b, and miR-146b miR-122a, miR-133a-133b, miR-326, miR-215, miR-483, miR-519d, miR-335, miR-133b, miR-515-5p, miR-194, miR-524*, miR-197, miR-99a, miR-383, miR-125b, miR-193b, miR-10a, miR-30a, miR-100, miR-328, miR-375, let-7c, miR-149, let-7a, miR-130a, 30 miR-196b, miR-197, miR-26b, miR-30e-3p, miR-214, let-7b, miR-26a, miR-101, miR-30c, miR-195, let-7f, miR-148a, miR-125a, miR-30e-5p, miR-29c, miR-199b, miR-365, let-7d, miR-204, miR-23a, miR-196a, miR-452, miR-143, miR-199a, miR-145, miR-23b, miR-486, miR-92, miR-615, miR-497, miR-99b, miR-9, miR-10b, miR-1 and miR-451 in cells of the 35 individual.

In some embodiments, a method of treatment of atopic eczema may comprise;

reducing the expression or activity of one or more miRNAs
5 selected from the group consisting of miR-146a, let-7i, miR-29a,
miR-222, miR-24, miR-193a, miR-199a, miR-27a, miR-21, miR-20a, miR-
17-5p, miR-106b, miR-142-3p, miR-30e-5p, miR-107, miR-200a, miR-
146b, miR-27b, miR-199b, miR-106a, miR-101, miR-451, miR-130a, miR-
451, miR-141, miR-31, miR-203, miR-223, miR-199a*, miR-200c, miR-
10 155, miR-16, miR-361, miR-205, miR-143, miR-422b, miR-200b and miR-
15b and/or one or more miRNAs selected from the group consisting of
miR-135b, miR-187, miR-142-5p, miR-501, miR-487b, miR-362, miR-301,
miR-130b, and miR-146b; more preferably one or more miRNAs selected
from the group consisting of let-7i, miR-29a, miR-222, miR-24, miR-
15 193a, miR-199a and miR-27a in cells, of the individual.

The expression or activity of the one or more miRNAs may be
increased or reduced in skin cells from the individual, preferably
lesional skin cells.

20

Lesional skin cells are skin cells located within lesions at which
the symptoms of the inflammatory disorder are displayed. Cells
located at skin lesions may be of any skin cell type, including
keratinocytes, melanocytes and dermal fibroblasts, or infiltrating
25 immune cells, such as CD4+, CD8+ and CD4CD25high T cell subsets, NK
cells, granulocytes, B cells, dendritic cells and mast cells. miRNA
activity or expression in skin cells may be modulated by topical
administration of therapeutic agents as described below.

30 The expression or activity of the one or more miRNAs may be
increased or reduced in blood cells from the individual, preferably
white blood cells, such as T cells. miRNA activity or expression in
blood cells may be modulated by parental administration, for example
intravenous injection, of therapeutic agents as described below.

35

The expression or activity of a target miRNA may be reduced by decreasing in total amount of the target miRNA in the cell or by decreasing the amount of the target miRNA which is present in the cell in an active form.

5

In some embodiments, the expression or activity of the target miRNA may be reduced by administering a therapeutically effective amount of a miRNA inhibitor to an individual in need thereof.

- 10 An inhibitor of a target miRNA is a compound which reduces or represses the activity or expression of the target miRNA. Preferably, the inhibitor has no effect or substantially no effect on non-target miRNAs. Suitable inhibitors may be readily designed by the skilled person from the sequence of the target miRNA.
- 15 Sequences of target miRNAs are available from the miRNA Registry and are set out in Table 2.

- Suitable inhibitors may include single or double stranded oligonucleotides which are able to bind to mature miRNA or its precursor forms and inhibit the activity of mature miRNA, prevent or inhibit its production or increase its rate of depletion. Suitable oligonucleotides may be oligodeoxyribonucleotides, oligoribonucleotides or modified oligonucleotides as described below
- 20
- 25 In some embodiments, the activity of a mature miRNA may be inhibited by the binding of a single stranded oligonucleotide which has a sequence which is sufficiently complementary to the sequence of the miRNA to hybridise to the target miRNA by Watson-Crick base-pairing. The use of such 'antisense' oligonucleotides is well-established in
- 30 the art.

- Oligonucleotides may be generated *in vitro* or *ex vivo* for administration or anti-sense RNA may be generated *in vivo* within cells in which inhibition is desired. Thus, double-stranded DNA may
- 35 be placed under the control of a promoter in a "reverse orientation"

such that transcription of the anti-sense strand of the DNA yields RNA which is complementary to the precursor miRNA. The complementary anti-sense RNA sequence may then bind with the target miRNA, inhibiting its cellular activity (see for example, Applied Antisense Oligonucleotide Technology C A. Stein (1998) Wiley & Sons).

A suitable oligonucleotide for inhibition of an miRNA may have about 10 to 30 nucleotides, preferably about 20 nucleotides e.g. 14-23 nucleotides, for example about 15, 16 or 17.

The construction of anti-sense sequences and their use is well known in the art and is described for example in Peyman and Ulman, Chemical Reviews, 90:543-584, (1990) and Crooke, Ann. Rev. Pharmacol. Toxicol. 32:329-376, (1992).

Nucleotides comprise a base portion, generally a heterocyclic base such as a purine or pyrimidine, which is covalently linked to a sugar group, typically a pentofuranosyl sugar, which further comprises a phosphate group. The phosphate group is generally linked to the 2', 3' or 5' hydroxyl moiety of the sugar. The phosphate groups covalently link adjacent nucleotides to one another to form an oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleotide backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage

Single-stranded oligonucleotides for the inhibition of miRNA activity may be chemically modified. Modified oligonucleotides are described in more detail below.

Examples of modified oligonucleotides which may be used to inhibit target miRNA molecules include LNA Knockdown probes, (Orom, Kauppinen et al. 2006), 2'-O-methyl modified RNA oligonucleotides (Cheng, Byrom et al. 2005), and "antagomirs" (Krutzfeldt, Rajewsky et al. 2005 Mattes et al 2007).

Antagomirs are chemically modified, single-stranded RNA analogues conjugated to cholesterol. An antagomir typically comprises at least 19 nucleotides which are complementary to the sequence of a target miRNA which allow hybridisation between the antagomir and the target miRNA, thereby inhibiting the activity of the miRNA target.

Antagomirs can discriminate between single nucleotide mismatches of the targeted miRNA and have been shown to silence specific miRNAs *in vivo* (Krutzfeldt, Rajewsky et al. 2005). Antagomirs have also been shown to efficiently target miRNAs when injected locally into the mouse cortex (Krutzfeldt, Kuwajima et al. 2007).

Other useful inhibitors include oligonucleotides which cause inactivation or cleavage of mature miRNA or its precursor forms.

Suitable oligonucleotides may be chemically modified, or have enzyme activity, which causes cleavage of a nucleic acid at a specific site - thus influencing activity of miRNAs. Examples include ribozymes, EDTA-tethered oligonucleotides, or covalently bound oligonucleotides, such as a psoralen or other cross-linking reagent-bound oligonucleotides. Background references for ribozymes include Kashani-Sabet and Scanlon, 1995, *Cancer Gene Therapy*, 2(3): 213-223, and Mercola and Cohen, 1995, *Cancer Gene Therapy*, 2(1), 47-59.

In some embodiments, the activity of a mature miRNA may be inhibited using a double-stranded oligonucleotide which comprises a sequence which is complementary to a target miRNA. A suitable double-stranded oligonucleotide may comprise about 10 to 30 nucleotides, preferably about 20 nucleotides e.g. 18-23 nucleotides. Techniques for inhibiting target miRNAs using double-stranded inhibitory oligonucleotides are known in the art (Soutschek, J. et al *Nature* 432, 173-178 (2004), Vermeulen, Robertson et al. 2007 and US20050182005).

Other useful inhibitors include double- or single-stranded DNA or double- or single-stranded RNA "aptamers" that bind to specific

targets via interactions other than Watson-Crick base pairing. Suitable oligonucleotides (e.g., RNA oligonucleotides) that bind a specific miRNA can be generated using the techniques of SELEX (Tuerk, 1997, Methods Mol Biol 67, 2190). In this technique, a very large pool (10^6 - 10^9) of random sequence nucleic acids is bound to the target using conditions that cause a large amount of discrimination between molecules with high affinity and low affinity for binding the target. The bound molecules are separated from unbound, and the bound molecules are amplified by virtue of a specific nucleic acid sequence included at their termini and suitable amplification reagents. This process is reiterated several times until a relatively small number of molecules remain that possess high binding affinity for the target. These molecules can then be tested for their ability to modulate miRNA activity as described herein.

A modified oligonucleotide may contain one or more modified backbone linkages. Backbone linkages in a modified oligonucleotide may include, for example, non-phosphodiester linkages, such as phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogues of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Modified oligonucleotides may comprise linkages which lack phosphate groups and may comprise short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages, for example morpholino; siloxane; sulfide, sulfoxide, sulfone; formacetyl; thioformacetyl; methylene formacetyl; thioformacetyl; alkene containing; sulfamate; methyleneimino;

methylenehydrazino; sulfonate; sulfonamide; amide; or other linkages comprising N, O, S and/or CH₂ groups.

Suitable modified oligonucleotides may comprise phosphorothioate

5 backbones or heteroatom backbones, and in particular --CH₂--NH--O--CH₂--, --CH₂--N(CH₃)--O--CH₂--, -CH₂--O--N(CH₃)--CH₂--, -CH₂--N(CH₃)--N(CH₃)--CH₂-- and --O--N(CH₃)--CH₂--CH₂-- [wherein the native phosphodiester backbone is represented as --O--P--O--CH₂--].

10 Modified oligonucleotides may also contain one or more substituted sugar moieties. Suitable sugar moieties may comprise one of the following at the 2' position: OH; F; O--, S--, or N-alkyl; O--, S--, or N-alkenyl; O--, S-- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C₁ to
15 C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Particularly suitable are O[(CH₂)_nO]_mCH₃, O[(CH₂)_nO]_mOCH₃, O[(CH₂)_nO]_mNH₂, O[(CH₂)_n]_mCH₃, O[(CH₂)_nO]_mONH₂ and O[(CH₂)_nO]_mON(CH₂)_nCH₃]₂, where n and m are from 1 to about 10.

20 Modified sugar moieties may comprise one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF, OCF, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted
25 silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Suitable modifications include 2'-methoxyethoxy
30 (2'-O--CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chim. Acta, 1995, 78, 486 504) i.e. an alkoxyalkoxy group, 2'-dimethylaminoethoxy, i.e., a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, 2'-methoxy (2'-O--CH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F).

35 Similar modifications may also be made at other positions on the

oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide.

5 Modified oligonucleotides may also contain one or more sugar mimetics instead of a pentofuranosyl sugar. Suitable sugar mimetics include cyclobutyl moieties, azido-ribose, carbocyclic sugar analogues α -anomeric sugars; epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, and
10 sedoheptulose.

Modified oligonucleotides may also include base modifications or substitutions. Modified nucleotide bases can be used instead of or in addition to the naturally occurring bases i.e. the purine bases
15 adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). For example, modified bases may increase the stability of the molecule. Modified bases known in the art include alkylated purines and pyrimidines, acylated purines and pyrimidines, and other heterocycles. These classes of pyrimidines
20 and purines are known in the art and include pseudoisocytosine, N⁴,N⁴-ethanocytosine, 8-hydroxy-N⁶-methyladenine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5 fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyl uracil, dihydrouracil, inosine, N⁶-
25 isopentyl-adenine, 1- methyladenine, 1-methylpseudouracil, 1-methylguanine, 2,2-dimethylguanine, 2methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N⁶-methyladenine, 7-methylguanine, 5-methylaminomethyl uracil, 5-methoxy amino methyl-2-thiouracil, -D-mannosylqueosine, 5-methoxycarbonylmethyluracil,
30 5methoxyuracil, 2 methylthio-N⁶-isopentenyladenine, uracil-5-oxyacetic acid methyl ester, psueouracil, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5methyluracil, N-uracil-5-oxyacetic acid methylester, uracil 5-oxyacetic acid, queosine, 2-thiocytosine, 5-propyluracil, 5-propylcytosine, 5-ethyluracil,
35 5ethylcytosine, 5-butyluracil, 5-pentyluracil, 5-pentylcytosine, and

2,6-diaminopurine, methylpsuedouracil, 1-methylguanine and 1-methylcytosine.

In some embodiments, both the sugar and the backbone linkage of one
5 or more, preferably all of the nucleotides in a modified
oligonucleotide may be replaced with non-natural groups. The bases
are maintained for hybridization with the target miRNA. Suitable
modified oligonucleotides may include peptide nucleic acids (PNA).
In PNA, the oligonucleotide sugar-backbone is replaced with an amide
10 containing backbone, in particular an aminoethylglycine backbone.
The bases are retained and are bound directly or indirectly to azanitrogen atoms of the amide portion of the backbone.

Modified oligonucleotides may be chemically linked to one or more
15 moieties or groups which enhance the activity, cellular distribution
or cellular uptake of the oligonucleotide. Suitable moieties include
lipid moieties such as cholesterol, cholic acid, a thioether, e.g.,
hexyl-S-tritylthiol, a thiocholesterol, an aliphatic chain, e.g.,
dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-
20 rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-
H-phosphonate, a polyamine or a polyethylene glycol chain, or
adamantane acetic acid, a palmityl moiety, or an octadecylamine or
hexylamino-carbonyl-oxycholesterol moiety.

25 miRNA inhibitors may be transferred into the cell using a variety of
techniques well known in the art. For example, oligonucleotide
inhibitors can be delivered into the cytoplasm without specific
modification. Alternatively, they may be delivered by the use of
liposomes which fuse with the cellular membrane or are endocytosed,
30 i.e. by employing ligands such as antibodies which are attached to
the liposome or directly to the oligonucleotide and which bind to
surface membrane protein receptors of the cell, resulting in
endocytosis. Alternatively, the cells may be permeabilized to
enhance transport of the oligonucleotides into the cell, without

injuring the host cells or a DNA binding protein, e.g., HBGF-1, which transports oligonucleotides into a cell may be employed.

In other embodiments, a method of treatment of an inflammatory skin disorder in an individual may comprise;

increasing the amount or activity of one or more miRNAs selected from the group consisting of miR-122a, miR-133a-133b, miR-197, miR-326, miR-133b, miR-524*, miR-215, miR-194, miR-1, let-7e, miR-381, miR-483, miR-10a, miR-365, miR-492, miR-99b, miR-125b, miR-100, miR-515-5p, miR-335, miR-518b and let-7c; more preferably one or more miRNAs selected from the group consisting of miR-122a, miR-133b, miR-133a-133b, miR-326, miR-125b, and miR-215 in cells of the individual.

In some preferred embodiments, the level or activity of miR-125b may be increased.

In some embodiments, a method of treatment of psoriasis in an individual may comprise;

increasing the amount or activity of one or more miRNAs selected from the group consisting of miR-125b, miR-99b, miR-122a, miR-197, miR-100, miR-381, miR-518b, miR-524*, let-7e, miR-30c, miR-365, miR-133b, miR-10a, miR-133a-133b, miR-22, miR-326, miR-215, miR-516-5p, let-7c, let-7d, miR-335, miR-492, miR-1, miR-519d, miR-10b, miR-483, miR-194 and miR-526b and/or one or more miRNAs selected from the group consisting of miR-30a-3p, miR-195, miR-30a-5p, miR-30e-5p, miR-99a, miR-193b, miR-149, miR-26a, miR-26b, miR-218, miR-30e-3p, miR-23b, let-7g, miR-411, miR-199b, miR-29c, miR-101, miR-375, miR-214, miR-375, miR-181d, miR-125a, miR-140, miR-30b, miR-152, miR-328, miR-497, miR-130a, miR-127, miR-148b, miR-186, miR-143, miR-145, miR-30d, miR-126, miR-199a, miR-196b, miR-486, miR-365, miR-29a, miR-320, miR-361, miR-95, miR-532, miR-331, miR-199a, miR-196a, miR-451, miR-193a, miR-126, miR-23a, miR-660, let-7b, miR-16, miR-182, miR-27a, miR-383, let-7a and miR-191; more preferably one or more miRNAs selected from the group consisting of

miR-99b, miR-197, miR-100, miR-381, miR-518b, miR-524, let-7e, miR-30c, miR-365, miR-10a and miR-22 in cells of the individual.

In some embodiments, a method of treatment of atopic eczema
5 individual may comprise;

increasing the amount or activity of one or more miRNAs
selected from the group consisting of miR-122a, miR-133a-133b, miR-326, miR-215, miR-483, miR-519d, miR-335, miR-133b, miR-515-5p, miR-194, miR-524* and miR-197 and/or one or more miRNAs selected from
10 the group consisting of miR-99a, miR-383, miR-125b, miR-193b, miR-10a, miR-30a, miR-100, miR-328, miR-375, let-7c, miR-149, let-7a, miR-130a, miR-196b, miR-197, miR-26b, miR-30e-3p, miR-214, let-7b, miR-26a, miR-101, miR-30c, miR-195, let-7f, miR-148a, miR-125a, miR-30e-5p, miR-29c, miR-199b, miR-365, let-7d, miR-204, miR-23a, miR-15
196a, miR-452, miR-143, miR-199a, miR-145, miR-23b, miR-486, miR-92, miR-615, miR-497, miR-99b, miR-9, miR-10b, miR-1 and miR-451; more preferably one or more miRNAs selected from the group consisting of miR-483, miR-519d, miR-335 and miR-515-5p in cells of the individual.

20

The expression or activity of a target miRNA may be increased by administering to an individual in need thereof a therapeutically effective amount of;

- (i) the target miRNA or a precursor thereof,
- 25 (ii) a nucleic acid encoding the target miRNA or a precursor thereof,
- (iii) an analogue, derivative or modified form of the target miRNA which retains activity.

30 Nucleic acid sequences encoding a target miRNA or a target miRNA precursor may be comprised within a vector. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences which will drive transcription in the target cell, including promoter sequences, terminator fragments, polyadenylation sequences,
35 enhancer sequences, marker genes and other sequences as appropriate.

A vector may comprise a selectable marker to facilitate selection of the transgenes under an appropriate promoter. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 3rd edition, Sambrook & Russell, 2001, Cold Spring Harbor Laboratory Press.

Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds. John Wiley & Sons, 1992.

A nucleic acid vector may be introduced into a host cell, for example a lesional skin cell. Suitable techniques for transporting the constructor vector into the cell are well known in the art and include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or lentivirus.

The particular choice of a transformation technology will be determined by its efficiency to transform the particular host cells employed as well as the experience and preference of the operator with a particular methodology of choice.

An analogue, derivative or modified form of a miRNA retains the biological activity of the mature miRNA (i.e. a miRNA agonist) and may be a oligoribonucleotide or oligodeoxyribonucleotide with one or more modifications which improve the stability, transport or other pharmacological properties. Suitable modifications include modifications to the backbone linkages, bases or sugar moieties of one or more of the constituent nucleotides and are described in more detail above.

The term "treatment" in the context of treating a inflammatory skin disorder, pertains generally to treatment and therapy, whether of a human or an animal (e.g. in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition
5 of the progress of the disorder, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the disorder, and cure of the disorder. Treatment as a prophylactic measure (i.e. prophylaxis) is also included.

10 While it is possible for an active compound such as an miRNA agonist or antagonist as described above, to be administered alone, it is preferable to present it as a pharmaceutical composition (e.g., formulation) comprising at least one active compound, as defined above, together with one or more pharmaceutically acceptable
15 carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, preservatives, lubricants, or other materials well known to those skilled in the art and optionally other therapeutic or prophylactic agents.

20 Pharmaceutical compositions comprising a miRNA agonist or antagonist as defined above, for example, admixed or formulated together with one or more pharmaceutically acceptable carriers, excipients, buffers, adjuvants, stabilisers, or other materials, as described herein, may be used in the methods described herein.

25 The term "pharmaceutically acceptable" as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g., human) without
30 excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

Suitable carriers, excipients, etc. can be found in standard pharmaceutical texts, for example, Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, Easton, Pa., 1990.

- 5 The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well-known in the art of pharmacy. Such methods include the step of bringing the active compound into association with a carrier which may constitute one or more accessory ingredients. In general, the formulations are
10 prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations may be in the form of liquids, solutions, suspensions,
15 emulsions, elixirs, syrups, tablets, lozenges, granules, powders, capsules, cachets, pills, ampoules, suppositories, pessaries, ointments, gels, pastes, creams, sprays, mists, foams, lotions, oils, boluses, electuaries, or aerosols.

- 20 The miRNA agonist or antagonist (s) or pharmaceutical composition comprising the miRNA agonist or antagonist (s) may be administered to a subject by any convenient route of administration, whether systemically/ peripherally or at the site of desired action, including but not limited to, oral (e.g. by ingestion); topical
25 (including e.g. transdermal, intranasal, ocular, buccal, and sublingual); pulmonary (e.g. by inhalation or insufflation therapy using, e.g. an aerosol, e.g. through mouth or nose); parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac,
30 intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot, for example, subcutaneously or intramuscularly.

In preferred embodiments, an active compound is administered directly at the site of action by topical administration to lesional skin cells.

5 Formulations suitable for topical administration (e.g. transdermal, intranasal, ocular, buccal, and sublingual) may be formulated as an ointment, cream, suspension, lotion, powder, solution, past, gel, spray, aerosol, or oil. Alternatively, a formulation may comprise a patch or a dressing such as a bandage or adhesive plaster
10 impregnated with active compounds and optionally one or more excipients or diluents.

Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic, pyrogen-free, sterile injection solutions
15 which may contain anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate
20 systems which are designed to target the compound to blood components or one or more organs. Examples of suitable isotonic vehicles for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the active compound in the solution
25 is from about 1 ng/ml to about 10 µg/ml, for example, from about 10 ng/ml to about 1 µg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for
30 example water for injections, immediately prior to use.

It will be appreciated that appropriate dosages of the miRNA agonist or antagonist(s), and compositions comprising the miRNA agonist or antagonist(s), can vary from patient to patient. Determining the
35 optimal dosage will generally involve the balancing of the level of

diagnostic benefit against any risk or deleterious side effects of the administration. The selected dosage level will depend on a variety of factors including, but not limited to, the route of administration, the time of administration, the rate of excretion of the miRNA agonist or antagonist(s), the amount of contrast required, other drugs, compounds, and/or materials used in combination, and the age, sex, weight, condition, general health, and prior medical history of the patient. The amount of miRNA agonist or antagonist(s) and route of administration will ultimately be at the discretion of the physician, although generally the dosage will be to achieve concentrations of the miRNA agonist or antagonist (s) at a lesion site without causing substantial harmful or deleterious side-effects.

Administration in vivo can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals). Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the physician. Other aspects of the invention relate to screening for compounds useful in the treatment of inflammatory skin disorders.

A method of screening for a compound useful in the treatment of an inflammatory skin disorder, such as psoriasis or atopic eczema, may comprise;

contacting a cell with a test compound and;

determining the activity or expression of one or more miRNAs selected from the group consisting of let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-9, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-17-5p, miR-20a, miR-21, miR-22, miR-23a, miR-23b, miR-24, miR-26a, miR-26b, miR-27a, miR-27b, miR-29a, miR-29c, miR-30a-5p, miR-30a-3p, miR-30b, miR-30c, miR-30d, miR-30e-

5p, miR-30e-3p, miR-31, miR-92, miR-93, miR-95, miR-99a, miR-99b, miR-100, miR-101, miR-106a, miR-106b, miR-107, miR-122a, miR-125a, miR-125b, miR-126, miR-127, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-135b, miR-140, miR-141, miR-142-3p, miR-142-5p, miR-143, miR-145, miR-146a, miR-146b, miR-148a, miR-148b, miR-149, miR-152, miR-155, miR-181d, miR-182, miR-186, miR-187, miR-191, miR-193a, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-203, miR-204, miR-205, miR-214, miR-218, miR-222, miR-223, miR-224, miR-301, miR-320, miR-324-5p, miR-326, miR-328, miR-331, miR-335, miR-361, miR-362, miR-365, miR-375, miR-381, miR-383, miR-411, miR-422b, miR-425, miR-432, miR-451, miR-452, miR-483, miR-486, miR-487b, miR-492, miR-497, miR-501, miR-515-5p, miR-516-5p, miR-518b, miR-519d, miR-524*, miR-526b, miR-532, miR-615, and miR-660 relative to controls, wherein an increase or decrease in activity or expression in the presence of the test compound is indicative that the compound is useful in the treatment of an inflammatory skin disorder.

The in activity or expression of one or more miRNAs selected from the group consisting of let-7c, let-7d, let-7e, let-7i, miR-1, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-17-5p, miR-20a, miR-21, miR-22, miR-24, miR-27a, miR-27b, miR-29a, miR-30c, miR-30e-5p, miR-31, miR-99b, miR-100, miR-101, miR-106a, miR-106b, miR-107, miR-122a, miR-125b, miR-130a, miR-133a-133b, miR-133b, miR-141, miR-142-3p, miR-143, miR-146a, miR-146b, miR-155, miR-193a, miR-194, miR-197, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-203, miR-205, miR-222, miR-223, miR-326, miR-335, miR-361, miR-365, miR-381, miR-422b, miR-451, miR-483, miR-487b, miR-492, miR-515-5p, miR-516-5p, miR-518b, miR-519d, miR-524* and miR-526b may be determined relative to controls.

In some embodiments, in activity or expression of one or more microRNAs selected from the group consisting of one or more of the miRNAs selected from the group consisting of miR-20a, miR-146a, miR-20a, miR-31, miR-21, miR-17-5p, miR-193a, miR-146b, miR-146a, miR-

222, miR-142-3p, let-7i, miR-106a, miR-200a, miR-30e-5p, miR-203, miR-27b, miR-141, miR-130a, miR-24, miR-199b, miR-199a, miR-106b, miR-199a*, miR-15a, miR-451, miR-29a, miR-422b, miR-205, miR-361, miR-487b, miR-107, miR-451, miR-16, miR-27a and miR-155 may be
5 determined in the cell,

wherein a decrease in in activity or expression in the presence of the test compound relative to its absence is indicative that the compound is useful in the treatment of an inflammatory skin disorder.

10

For example, expression of miR-21 may be determined.

In some embodiments, in activity or expression of one or more microRNAs selected from the group consisting of miR-122a, miR-133a-
15 133b, miR-197, miR-326, miR-133b, miR-524*, miR-215, miR-194, miR-1, let-7e, miR-381, miR-483, miR-10a, miR-365, miR-492, miR-99b, miR-125b, miR-100, miR-515-5p, miR-335, miR-518b and let-7c may be determined in the cell,

wherein an increase in in activity or expression of the one or
20 more microRNAs in the presence of the test compound relative to its absence is indicative that the compound is useful in the treatment of an inflammatory skin disorder.

For example, expression or activity of miR-125b may be determined.

25

A method of screening for a compound useful in the treatment of psoriasis may comprise;

contacting a cell with a test compound and;

determining the in activity or expression of one or more
30 miRNAs selected from the group consisting of miR-20a, miR-146a, miR-146b, miR-31, miR-146a, miR-20a, miR-200a, miR-17-5p, miR-30e-5p, miR-141, miR-203, miR-142-3p, miR-21, miR-106a, miR-487b, miR-15a, let-7i, miR-222, miR-422b, miR-130a, miR-193a, miR-106b, miR-199b, miR-199a* and miR-27b and/or one or more miRNAs selected from the
35 group consisting of miR-135b, miR-205, miR-155, miR-223, miR-93,

miR-132, miR-425, miR-362, miR-324-5p, miR-224, miR-432 and miR-301;
in the cell,

wherein a decrease in activity or expression in the presence
of the test compound relative to its absence is indicative that the
5 compound is useful in the treatment of psoriasis.□□

For example, the activity or expression of miR-203 and/or miR-146a
may be determined in the cell. □□

10 A method of screening for a compound useful in the treatment of
psoriasis may comprise

contacting a cell with a test compound and;

determining the activity or expression of one or more miRNAs
selected from the group consisting of miR-125b, miR-99b, miR-122a,
15 miR-197, miR-100, miR-381, miR-518b, miR-524*, let-7e, miR-30c, miR-
365, miR-133b, miR-10a, miR-133a-133b, miR-22, miR-326, miR-215,
miR-516-5p, let-7c, let-7d, miR-335, miR-492, miR-1, miR-519d, miR-
10b, miR-483, miR-194 and miR-526b and/or one or more miRNAs
selected from the group consisting of miR-30a-3p, miR-195, miR-30a-
20 5p, miR-30e-5p, miR-99a, miR-193b, miR-149, miR-26a, miR-26b, miR-
218, miR-30e-3p, miR-23b, let-7g, miR-411, miR-199b, miR-29c, miR-
101, miR-375, miR-214, miR-375, miR-181d, miR-125a, miR-140, miR-
30b, miR-152, miR-328, miR-497, miR-130a, miR-127, miR-148b, miR-
186, miR-143, miR-145, miR-30d, miR-126, miR-199a, miR-196b, miR-
25 486, miR-365, miR-29a, miR-320, miR-361, miR-95, miR-532, miR-331,
miR-199a, miR-196a, miR-451, miR-193a, miR-126, miR-23a, miR-660,
let-7b, miR-16, miR-182, miR-27a, miR-383, let-7a and miR-191; in
the cell,

wherein an increase in activity or expression in the presence
30 of the test compound relative to its absence is indicative that
the compound is useful in the treatment of psoriasis.□□

A method of screening for a compound useful in the treatment of
atopic eczema may comprise;

35 contacting a cell with a test compound and;

determining the activity or expression of one or more microRNAs selected from the group consisting of miR-146a, let-7i, miR-29a, miR-222, miR-24, miR-193a, miR-199a, miR-27a, miR-21, miR-20a, miR-17-5p, miR-106b, miR-142-3p, miR-30e-5p, miR-107, miR-200a, 5 miR-146b, miR-27b, miR-199b, miR-106a, miR-101, miR-451, miR-130a, miR-451, miR-141, miR-31, miR-203, miR-223, miR-199a*, miR-200c, miR-155, miR-16, miR-361, miR-205, miR-143, miR-422b, miR-200b and miR-15b and/or one or more miRNAs selected from the group consisting of miR-135b, miR-187, miR-142-5p, miR-501, miR-487b, miR-362, miR-10 301, miR-130b, and miR-146b,

wherein a decrease in activity or expression in the presence of the test compound relative to its absence is indicative that the compound is useful in the treatment of atopic eczema.

15 For example, the activity or expression of one or more miRNAs selected from the group consisting of miR-483, miR-519d, miR-335 and miR-515-5p.

A method of screening for a compound useful in the treatment of 20 atopic eczema may comprise;

determining the activity or expression of one or more miRNAs selected from the group consisting of miR-122a, miR-133a-133b, miR-326, miR-215, miR-483, miR-519d, miR-335, miR-133b, miR-515-5p, miR-194, miR-524* and miR-197 and/or one or more miRNAs selected from 25 the group consisting of miR-99a, miR-383, miR-125b, miR-193b, miR-10a, miR-30a, miR-100, miR-328, miR-375, let-7c, miR-149, let-7a, miR-130a, miR-196b, miR-197, miR-26b, miR-30e-3p, miR-214, let-7b, miR-26a, miR-101, miR-30c, miR-195, let-7f, miR-148a, miR-125a, miR-30e-5p, miR-29c, miR-199b, miR-365, let-7d, miR-204, miR-23a, miR-30 196a, miR-452, miR-143, miR-199a, miR-145, miR-23b, miR-486, miR-92, miR-615, miR-497, miR-99b, miR-9, miR-10b, miR-1 and miR-451; in the cell,

wherein an increase in activity or expression in the presence of the test compound relative to its absence is indicative that the 35 compound is useful in the treatment of treatment of atopic eczema.

Techniques for determining the amount of expression of a target miRNA in a cell are described in more detail above.

- 5 The cell is contacted with the test compound in vitro and may be an isolated cell, for example a cell from a cultured cell line or may be comprised in or obtained from a tissue sample which is obtained from an individual.
- 10 Suitable cells for use in the present methods may be higher eukaryotic cells, preferably mammalian cells, such as human cells. In preferred embodiments, the cell may be an a T cell or a human skin cell, for example a keratinocyte, melanocyte or dermal fibroblast, or an infiltrating immune cell, such as CD4+, CD8+ and
- 15 CD4CD25high T cell subset, NK cell, granulocyte, B cell, dendritic cell or mast cell.

The precise format for performing the methods described herein may be varied by those of skill in the art using routine skill and

20 knowledge.

Compounds which may be screened using the methods described herein may be natural or synthetic chemical compounds used in drug screening programmes. Extracts of plants, microbes or other

25 organisms which contain several characterised or uncharacterised components may also be used.

Combinatorial library technology provides an efficient way of testing a potentially vast number of different compounds for ability

30 to modulate an interaction. Such libraries and their use are known in the art, for all manner of natural products, small molecules and peptides, among others. The use of peptide libraries may be preferred in certain circumstances.

In some embodiments, the test compound may be an analogue, variant or derivative of a target miRNA as described above.

5 The amount of test compound or compound which may be added to a method of the invention will normally be determined by serial dilution experiments. Typically, from about 0.001 nM to 1 mM or more of putative inhibitor compound may be used, for example from 0.01 nM to 100µM, e.g. 0.1 to 50 µM, such as about 10 µM.

10 In some embodiments, a method may comprise identifying the test compound as a miRNA inhibitor or antagonist as described above. Such a compound may, for example, be useful in reducing the expression and/or activity of the target miRNA, for example in the treatment of an inflammatory skin disorder, as described herein.

15 In other embodiments, a method may comprise identifying the test compound as an agonist (i.e. a promoter or enhancer) of a miRNA described above. Such a compound may, for example, be useful in increasing the expression and/or activity of the target miRNA, for
20 example in the treatment of an inflammatory skin disorder, as described herein.

A test compound identified using one or more initial screens as having ability to modulate the expression and/or activity of one or
25 more target miRNAs, may be assessed further using one or more secondary screens. A secondary screen may, for example, involve testing for a biological function such as an effect on skin lesions in an animal model of an inflammatory skin disorder.

30 The test compound may be isolated and/or purified or alternatively, it may be synthesised using conventional techniques of recombinant expression or chemical synthesis. Furthermore, it may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition such as a medicament, pharmaceutical
35 composition or drug. These may be administered to individuals for

the treatment of a inflammatory skin disorder. Methods of the invention may thus comprise formulating the test compound in a pharmaceutical composition with a pharmaceutically acceptable excipient, vehicle or carrier for therapeutic application, as
5 discussed further below.

Following identification of a compound which inhibits the expression or activity of a target miRNA described herein and which may therefore be useful in treating an inflammatory skin disorder, a
10 method may further comprise modifying the compound to optimise the pharmaceutical properties thereof.

The modification of a 'lead' compound identified as biologically active is a known approach to the development of pharmaceuticals and
15 may be desirable where the active compound is difficult or expensive to synthesise or where it is unsuitable for a particular method of administration, e.g. peptides are not well suited as active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Modification of a known active
20 compound (for example, to produce a mimetic) may be used to avoid randomly screening large number of molecules for a target property.

Modification of a 'lead' compound to optimise its pharmaceutical properties commonly comprises several steps. Firstly, the
25 particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, e.g. by substituting each residue in turn. These parts or residues constituting the active region of the
30 compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modelled according its physical properties, e.g. stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g.
35 spectroscopic techniques, X-ray diffraction data and NMR.

Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modelling process.

5

In a variant of this approach, the three-dimensional structure of the compound which modulates the expression and/or activity of a target miRNA described herein is modelled. This can be especially useful where the compound changes conformation, allowing the model to take account of this in the optimisation of the lead compound.

10

A template molecule is then selected, onto which chemical groups that mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the modified compound is easy to synthesise, is likely to be pharmacologically acceptable, and does not degrade in vivo, while retaining the biological activity of the lead compound. The modified compounds found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Modified compounds include mimetics of the lead compound.

15

20

Further optimisation or modification can then be carried out to arrive at one or more final compounds for in vivo or clinical testing.

25

As described above, a compound identified and/or obtained using the present methods may be formulated into a pharmaceutical composition.

Pharmaceutical compositions are described in more detail above. Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

30

All documents mentioned in this specification and the miRNA registry entries for all the miRNAs mentioned are incorporated herein by reference in their entirety.

5 "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

10

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

15

Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the figures and tables described below.

20 Figure 1 shows that miRNAs are differentially expressed between chronic inflammatory skin diseases and healthy skin. The expressions of the functionally active, mature forms of four miRNAs were analyzed using quantitative real-time PCR in the skin of 26 healthy individuals, and lesional skin samples of 20 patients with atopic
25 eczema and 25 patients with psoriasis. The results for individual patients and mean are shown. Data are expressed in relative units compared to U48 RNA. *** $p < 0.001$.

Figure 2 shows the expression of miR-203, miR-146a, miR-21 and miR-
30 125b in human organs. The expressions of the functionally active, mature forms of miR-203, miR-21, miR-146a and miR-125b were analyzed using quantitative real-time PCR in 20 healthy organs and tissues (each a pool of three donors) as well as in healthy skin (n=26), lesional atopic eczema (n=20) and psoriasis (n=25) skin samples.

Error bars represent the standard error of the mean. Data are expressed in relative units compared to U48 RNA. *** $p < 0.001$.

Figure 3 shows the expression of miR-203, miR-146a, miR-21 and miR-125b in the cellular constituents of the skin. The expressions of the functionally active, mature forms of miR-203, miR-146a, miR-21 and miR-125b were analyzed in the cellular constituents of the skin including primary adult keratinocytes, dermal fibroblasts, melanocytes, monocyte-derived dendritic cells (MDDCs), polymorphonuclear leukocytes (PMN), granulocytes, eosinophils, CD69⁺ cells, CD19⁺ cells, CD56⁺ cells, CD4⁺CD25^{high} cells, CD8⁺ cells, CD4⁺ cells and mast cells using quantitative real-time PCR. Data are expressed in relative units compared to U48 RNA.

Figure 4 shows that SOCS3 may be a molecular target of mir-203 post-transcriptional repression. (A) SOCS3 is an evolutionarily conserved target of miR-203. The putative targets site of miR-203 is highly conserved among species. The 8mer seed sequence in the 3'UTR of SOCS3 gene corresponding to miR-203 binding site is underlined. (B) In situ hybridization with LNA-oligonucleotide probes specific to miR-203 in skin sections from healthy skin and psoriatic lesional skin. Data are representative of 6 healthy and 6 psoriatic individuals. Note strong staining in the suprabasal layers of the epidermis in psoriatic skin. The expression of SOCS3 in healthy and psoriasis skin samples was detected using immunohistochemistry. Original magnification 200x. (C) The expression of SOCS-3 in skin from healthy individuals or lesional skin of psoriatic patients was analyzed using Western blot analysis. SOCS-3 protein levels are expressed as relative units. Bars represent means of SOCS-3 protein levels in healthy skin (n=6) and psoriatic lesional skin (n=11) $\pm$ SEM. ** $p < 0.01$.

Figure 5 shows quantitative real-time PCR results which indicate that miR-155 is overexpressed both in atopic eczema (AE) and in

psoriasis (PSO) in comparison with healthy skin (H). Data are expressed in relative units compared to U48 RNA. ***p<0.001.

Figure 6 shows the expression of miR-155 in 20 human organs and
5 tissues.

Figure 7 shows the expression of miR-155 in different cellular constituents of the skin.

10 Figure 8 shows the induction of miR-155 during the differentiation of naïve T cells into mature Th1, Th2 or Treg-like cells.

Figure 9 shows the expression of miR-155 in dendritic cells after treatment with LPS, IL-6 or IFN-gamma.

15

Figure 10 shows the expression of miR-155 during the development of monocytes into dendritic cells.

Figure 11 shows the expression of miR-155 in the PBMCs of atopic
20 eczema patients and healthy individuals following stimulation with SEB or LPS. LPS induces higher MiR-155 expression in the PBMCs of atopic eczema patients relative to healthy individuals.

Figure 12 shows the shows the induction of miR-155 during the
25 following treatment with TGFbeta and anti-CD3/anti-CD28. TGFbeta treated naïve T cells are a model for regulatory T cells (Treg cells) and anti-CD3/anti-CD28 treated naïve T cells represent activated T cells.

30 Figure 13 shows the overexpression of miR-155 in bone marrow-derived mast cells after IgE-crosslinking and CpG exposure.

Figure 14 shows the effect of the cotransfection of specific precursors of miR-155 with CTLA4 3'UTR-luciferase reporter

constructs on luciferase activity (top panel) and the effect of miR-155 precursors on CTLA4 expression (bottom panel).

Figure 15 shows allergen exposure protocol for the murine atopic dermatitis/eczema model. Exposure to ovalbumin (OVA) represents a model for cutaneous allergen exposure.

Figure 16 shows the expression of miR-155 in a mouse model of atopic dermatitis/eczema. Exposure to ovalbumin (OVA) represents a model for cutaneous allergen exposure, while staphylococcal enterotoxin B (SEB) represents a model for cutaneous exposure to superantigen-producing strains of *Staphylococcus aureus*.

Figure 17 shows the kinetics of miR-155 expression after allergen exposure.

Figure 18 shows quantitative real-time PCR results which indicate that miR-21 is overexpressed both in atopic eczema (AE) and in psoriasis (PSO) in comparison with healthy skin (H). Data are expressed in relative units compared to U48 RNA. *** $p < 0.001$.

Figure 19 shows the expression of miR-21 in 20 human organs and tissues.

Figure 20 shows the expression of miR-21 in different cellular constituents of the skin.

Figure 21 shows the induction of miR-21 during the differentiation of naïve T cells into mature Th1, Th2 or Treg-like cells.

Figure 22 shows the expression of miR-21 in monocytes-derived dendritic cells (MDDCs) after treatment with LPS, IL-6 or IFN- γ .

Figure 23 shows the expression of miR-21 during the development of monocytes into dendritic cells.

Figure 24 shows the expression of miR-21 in a mouse model of atopic dermatitis/eczema. Exposure to ovalbumin (OVA) represents a model for cutaneous allergen exposure, while staphylococcal enterotoxin B (SEB) represents a model for cutaneous exposure to superantigen-producing strains of *Staphylococcus aureus*.

Figure 25 shows the effect of miR-203 on the expression of a luciferase reporter coupled to the SOCS-3 3'UTR or SOCS-3 3'UTR mutated to disrupt the predicted miR-203 binding site.

Figures 26 to 40 show the results of quantitative real-time PCR using the Taqman[™] microRNA assay on 27 healthy, 20 atopic eczema and 25 psoriarsis samples showing expression of miR-125b, miR-133a, miR-142-3p, miR-146a, miR-155, miR-17-5p, miR-193, miR-200a, miR-203, miR-205, miR-21, miR-30c, miR-31, miR-326 and miR-99b, respectively. Data are expressed in relative units compared to U48 RNA. ***p<0.001.

Figures 41 to 55 show the results of quantitative real-time PCR using the Taqman[™] microRNA assay on 20 different healthy tissue/organs (each from a pool of 3 individuals), 27 healthy, 20 atopic eczema and 25 psoriarsis samples. Figures 41 to 55 show expression of miR-125b, miR-133a, miR-142-3p, miR-146a, miR-155, miR-17-5p, miR-193, miR-200a, miR-203, miR-205, miR-21, miR-30c, miR-31, miR-326 and miR-99b, respectively, in each tissue/organ sample. Data are expressed in relative units compared to U48 RNA. ***p<0.001.

Table 1 shows miRNAs differentially expressed between healthy skin and psoriasis (left) and healthy skin and atopic eczema (right) according to the SAM algorithm with the cut-off filter set at greater than 1.7-fold down or up-regulation. miRNAs that are over-expressed in both psoriasis and in atopic eczema are highlighted in

light grey, miRNAs that are down-regulated in both diseases are highlighted in dark grey.

Table 2 shows the miRBase accession numbers and sequences of the
5 differentially expressed miRNAs.

Table 3 shows miRNAs which were differentially expressed in atopic eczema relative to healthy tissue as identified by significance analysis of microarray results.

10

Table 4 shows results from further patients setting out miRNAs showing significant increases in expression in atopic eczema lesions relative to healthy tissue as identified by significance analysis of microarray results.

15

Table 5 shows results from further patients setting out miRNAs showing significant decreases in expression in atopic eczema lesions relative to healthy tissue as identified by significance analysis of microarray results.

20

Table 6 shows miRNAs which were differentially expressed in psoriasis relative to healthy tissue as identified by significance analysis of microarray results.

25

Table 7 shows results from further patients setting out miRNAs showing significant increases in expression in psoriasis lesions relative to healthy tissue as identified by significance analysis of microarray results.

30

Table 8 shows results from further patients setting out miRNAs showing significant decreases in expression in psoriasis lesions relative to healthy tissue as identified by significance analysis of microarray results.

Table 9 shows miRNAs which were differentially expressed in either psoriasis or atopic eczema relative to healthy tissue as identified by significance analysis of microarray results.

- 5 Table 10 shows putative targets of miR-155 identified using the targetScan 3.0 algorithm.

Experiments

1. Methods

10 1.1 Cells

Primary human keratinocytes, dermal fibroblasts and melanocytes were isolated from healthy skin using standard protocols and cultured as described (21, 22). Monocytes were isolated from PBMCs from healthy blood donors (Karolinska University Hospital Blood Bank, Stockholm, Sweden) using MACS separation. Immature MDDCs were generated by culturing separated monocytes in the presence of GM-CSF (550 IU/ml), and IL-4 (800 IU/ml) (Biosource International, Camarillo, CA, USA) for 6 days. CD4+, CD8+, CD4+CD25high, CD56+ NK, CD19+, and CD69+ positive cells were isolated from PBMCs from healthy blood donors by FACS sorting using a Becton Dickinson (BD) FACSaria cell sorting system and BD FACSDiva software v 4.1.2. Granulocytes and eosinophils were FACS sorted from whole blood following RBC lysis with ACK lysis buffer.

25 1.2 Patients

Both patients and healthy controls were of Caucasian origin, between 18-65 years old. Patients had not received systemic immunosuppressive treatment or PUVA/solarium/UV, for at least 1 month, and topical therapy for at least 2 weeks before skin biopsy. Four-millimeter punch biopsies were taken, after informed consent, from lesional skin of patients with moderate or severe chronic plaque psoriasis (n=25), lesional skin of patients with moderate to severe chronic atopic eczema (n=20), and from uninflamed, unirritated skin of healthy individuals (n=26). The study was

approved by the local ethics committee, and conducted according to the Declaration of Helsinki's principles.

1.3 Microarray experiments

5 Total RNA from lesional skin of psoriasis patients (n=3) and atopic eczema patients (n=3) and skin of healthy individuals (n=4) was isolated by Trizol (Invitrogen). Two µg of total RNA from each sample were labeled using the miRCURY™ Hy3™/Hy5™ labelling kit and hybridized on the miRCURY™ LNA Array (v.8.0) (Exiqon, Vedbaek,
10 Denmark).

1.4 Statistical and Bioinformatics Analysis of Microarray Data

Signal intensities were normalized using the global Lowess regression algorithm. Flagged spots corresponding to absent or low-
15 quality signals were removed from the analysis before global median normalization. For subsequent analysis, we used the log₂ of the background subtracted, normalized median spot intensities of ratios from the two channels (Hy3/Hy5). To find consistently differentially expressed genes, the data were subjected to SAM analysis as
20 described (Tusher, Tibshirani et al. 2001). For visualization of differentially expressed miRNAs, a heat map was generated using TreeView (currently available on-line at <http://jtreeview.sourceforge.net>).

25 To identify significance, One-way Analysis of Variance (ANOVA), the Kruskal-Wallis test (Non-parametric ANOVA), Dunn's Multiple Comparisons Test and Bonferroni Multiple Comparisons Test were also employed.

30 1.5 Quantitative real-time PCR

Total RNA of skin biopsies and cells was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA). RNA from 20 different normal human organs was obtained from Ambion (FirstChoice® Human Total RNA Survey Panel). Quantification of miRNAs by TaqMan® Real-Time PCR was
35 carried out as described by the manufacturer (Applied Biosystems,

Foster City, CA). Briefly, 10 ng of template RNA was reverse transcribed using the TaqMan® MicroRNA Reverse Transcription Kit and miRNA-specific stem-loop primers (Applied Biosystems). 1.5 µl RT product was introduced into the 20 µl PCR reactions which were
5 incubated in 384-well plates on the ABI 7900HT thermocycler (Applied Biosystems) at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Target gene expression was normalized between different samples based on the values of U48 RNA expression.

10 1.6 *In situ hybridization*

In situ transcriptional levels of miR-203 were determined on frozen sections (10 µm) of skin biopsy specimens from six psoriasis patients and six healthy individuals according to the manufacturer's instructions (Exiqon). Sections were hybridized o/n with
15 digoxigenin-labeled miRCURY LNA probes (Exiqon) and incubated with anti-digoxigenin antibody conjugated with alkaline phosphatase for 1h. Sections were visualized by using BM purple substrate together with 2mM Levamisole. The color reaction was performed o/n. We followed the protocol recommended by the manufacturer (Exiqon). The
20 stained sections were reviewed with a Zeiss microscope.

1.7 *Murine model of atopic eczema*

The murine model of atopic eczema was generated as previously described (Savinko, Lauerma et al. 2005). Briefly, mice were
25 topically exposed to SEB, OVA, a combination of OVA and SEB (OVA/SEB), or PBS for different time intervals. Skin specimens were obtained from the patched areas, RNA was isolated and the expression of microRNAs was determined by microRNA-specific real-time PCR:

30 2 Results

2.1 *A characteristic miRNA signature identified in psoriasis skin*

At present, the expression and function of miRNAs in human skin is largely unknown. To determine whether miRNAs are involved in the pathogenesis of psoriasis, we performed a comprehensive analysis of
35 all human miRNAs registered in mirBase 8.0 (342 known human miRNAs)

in skin lesions of patients with psoriasis (n=3) and compared it to healthy human skin (n=4) or to lesional skin from patients with a nonpsoriatic chronic inflammatory skin disease, atopic eczema (n=3). Analysis of the microarray data showed that miRNAs are expressed in a non-random manner in psoriasis, healthy, or atopic eczema skin (Fig. 1). Using the Significance Analysis of Microarrays (SAM) algorithm (Tusher, Tibshirani et al. 2001), we identified 29 genes that were consistently differentially expressed between psoriasis and healthy skin (Table 1). Among the genes identified in the SAM analysis there were (I) miRNAs specifically up-regulated in psoriasis (e.g. miR-203), (II) miRNAs with increased expression in both psoriasis and atopic eczema (e.g. miR-21), (III) miRNAs specifically down-regulated in psoriasis (e.g. miR-99b), and (IV) miRNAs uniformly down-regulated in both skin diseases (e.g. miR-122a), as compared to healthy skin.

To confirm the results obtained by microarray profiling, we performed quantitative real-time PCR analysis of miR-203, miR-146a, miR-21 and miR-125b expression on RNA samples obtained from lesional skin of patients with psoriasis (n=25), healthy skin (n=26) or atopic eczema lesions (n=20). For this, we used primers designed to amplify specifically the mature, biologically active form of these miRNAs (Fig. 1). In accordance with the microarray data, quantitative real-time PCR results showed significantly ($p < 0.001$) increased miR-203 levels in psoriasis skin when compared with healthy skin. No significant up-regulation of miR-203 was observed in atopic eczema skin lesions compared with healthy skin (Fig. 1). Similarly, miR-146a was significantly over-expressed in psoriatic lesional skin ($p < 0.001$) but not in atopic eczema lesions when compared with healthy skin (Fig. 1). The psoriasis-specific overexpression of miR-203 and miR-146 provides indication that they play specific roles in the pathogenesis of psoriasis and not only a general role in skin inflammation. In contrast to miR-203 and miR-146a, miR-21 was significantly up-regulated both in psoriasis ($p < 0.001$) and atopic eczema ($p < 0.001$) as compared with healthy skin.

miR-125b showed the opposite expression pattern to miR-21: the level of this miRNA significantly ($p < 0.001$ for both) decreased both in psoriasis and atopic eczema. Taken together, psoriasis is characterized by a distinct miRNA expression profile in comparison with healthy skin or with atopic eczema. Of note, one of the identified psoriasis-specific miRNAs, miR-146a was recently shown to regulate the expression of proteins involved in TNF- α -signaling pathway (Taganov, Boldin et al. 2006), which is known to play a central role in psoriatic skin inflammation (Lowe, Bowcock et al. 2007). However, virtually nothing is known about the function of miR-203.

Further quantitative real-time PCR analysis was performed on larger samples (27 healthy, 20 atopic eczema and 25 psoriasis). The results are shown in figures 26 to 40.

The expression of miR-125b, miR-133a, miR-142-3p, miR-155, miR-17-5p, miR-193, miR-21, miR-30c, miR-31 and miR-326 were shown to be significantly associated with both psoriasis and atopic eczema, relative to healthy patients. miR-99b was found to be significantly associated with both psoriasis and atopic eczema relative to healthy patients and with psoriasis relative to atopic eczema patients. miR-146a, miR-200a and miR-203 were found to be significantly associated with psoriasis relative to both healthy and atopic eczema patients. miR-17-5p was found to be significantly associated with psoriasis relative to healthy individuals. miR-205 was found to be significantly associated with psoriasis relative to atopic eczema patients.

2.2 miR-203, miR-21, miR-146a and miR-125b show distinct expression patterns in human organs and cells types

At present, the expression pattern of the miRNAs we identified in skin is largely unknown in different organs and tissues. To obtain further insights into the function of the psoriasis-associated

miRNAs, we systematically analyzed the expression of miR-203, miR-146a, miR-21 and miR-125b in skin and a panel of 20 additional human organs obtained from healthy individuals (Fig. 2). Quantitative real-time PCR analysis showed that miR-203, a miRNA specifically up-regulated in psoriasis, was expressed more than 100-fold higher in skin compared with most other organs. In addition to skin, miR-203 was only expressed at lower levels in organs that also contain squamous epithelium, esophagus and cervix. These findings provide indication for a specific function for this miRNA in the formation or function of squamous epithelia. In contrast to miR-203, the mature forms of miR-146a, miR-21 and miR-125b were detected in all studied organs, however, their expression showed distinct patterns. miR-146a was highly expressed in organs containing significant number of leukocytes such as the thymus and the spleen, and showed low expression in healthy skin, providing indication that infiltrating cells express miR-146a in the skin (Fig. 2). MiR-21 showed highest expression in the lung, trachea, colon, prostate and bladder (Fig. 2), while miR-125b, a miRNA downregulated in both psoriasis and atopic eczema, was expressed mostly in organs that contain cells of ectodermal origin, including cervix, brain and bladder.

In psoriasis, there is evidence for the pathogenic relevance of several different cell types that normally occur in skin: keratinocytes (Sano, Chan et al. 2005), fibroblasts (Dimon-Gadal, Gerbaud et al. 2000), monocyte-derived immunocytes (Nestle, Conrad et al. 2005; Lowes, Bowcock et al. 2007), T cells (Nickoloff and Wrone-Smith 1999), and mast cells (Fischer, Harvima et al. 2006). Therefore it is likely that this disease is the outcome of aberrantly activated mechanisms that do not necessarily depend on one single cell type but involve a variety of different cell populations (Stratis, Pasparakis et al. 2006; Lowes, Bowcock et al. 2007). To identify the cell types expressing the identified psoriasis-associated miRNAs in the skin, we systematically analyzed the expression of miR-203, miR-146a, miR-21 and miR-125b in a panel of

cells present in healthy and/or inflamed skin including both resident cells (keratinocytes, dermal fibroblasts and melanocytes) and leukocyte/immune cell subsets (CD4+, CD8+ and CD4CD25^{high} T cell subsets, NK cells, granulocytes, B cells, dendritic cells and mast cells). In accordance with their expression profiles in different organs, the identified miRNAs showed a distinctive expression pattern in the studied cell types. miR-203, which was specifically expressed in skin among 21 different human organs, showed a keratinocyte-specific expression being virtually absent in all other cell types analyzed (Fig. 3). This observation provides indication for a role for this miRNA in keratinocyte functions in healthy skin as well as in psoriasis. By contrast, miR-146a was absent from keratinocytes and dermal fibroblasts, and it was preferentially expressed by immune cells (Fig. 3), in accordance with its high expression in immune organs (Fig. 2) and in the psoriatic inflamed skin. In particular, CD4CD25^{high} regulatory T cells, monocyte-derived dendritic cells (MDDCs) and mast cells expressed miR-146a at a high level. The abundant expression of miR-146a in regulatory T cells provides indication that this miRNA might influence the function of regulatory T cells in psoriatic skin. miR-21, a microRNA upregulated in both psoriasis and atopic eczema, was expressed both by structural and inflammatory cells (Fig. 2). The expression pattern of miR-125b was complementary to that of miR-146a: it was expressed at a very low level in inflammatory cells in comparison to structural cells: fibroblasts, keratinocytes and melanocytes (Fig. 2).

The expression patterns of miR-125b, miR-133a, miR-142-3p, miR-146a, miR-155, miR-17-5p, miR-193, miR-200a, miR-203, miR-205, miR-21, miR-30c, miR-31, miR-326 and miR-99b were analysed in 20 different healthy tissue/organs in a sample of 27 healthy, 20 atopic eczema and 25 psoriasis patients. The results are shown in figures 41 to 55.

It is widely accepted that psoriasis is not a disease caused by one cell type but a consequence of impaired cross talk between the immune system and the structural cells of the skin (Lowes, Bowcock et al. 2007). Investigating the cellular distribution of miRNAs deregulated in psoriasis we found that these master switches of gene expression are expressed in cells with key roles in the pathogenesis of psoriasis. Deregulation of both keratinocyte- and leukocyte-specific miRNAs in psoriasis indicates that altered miRNA-mediated gene regulation may contribute to the disturbed cross talk between keratinocytes and immune cells. One of the most important mediators in leukocyte-keratinocyte interactions in psoriasis is tumor necrosis factor alpha (TNF- α) as evidenced by the effectiveness of TNF- α inhibitors in the treatment of psoriasis (Lowes, Bowcock et al. 2007). Interestingly, a recent study showed that miR-146a, one of the psoriasis-specific miRNAs, inhibits the expression of IRAK-1 and TRAF-6 proteins both of which are regulators of the TNF- α signaling pathway (Taganov, Boldin et al. 2006). Hence, it is conceivable that miR-146a is involved in the pathogenesis of psoriasis via the modulation of TNF- α signaling in the skin. In contrast to miR-146, nothing is known about the function of the keratinocyte-specific miRNA, miR-203.

2.3 miR-203 and keratinocyte dysfunction in psoriasis through the regulation of suppressor of cytokine signalling 3 (SOCS-3)

Epidermal keratinocytes are active participants in the formation of psoriasis plaques (Lowes, Bowcock et al. 2007). Psoriatic keratinocytes show abnormal differentiation and proliferation, have aberrant cell signaling and produce mediators that contribute to the recruitment and activation of immune cells (Lowes, Bowcock et al. 2007). The specific expression of miR-203 in skin and in keratinocytes (Fig. 2 and 3) as well as its specific up-regulation in psoriasis (Fig. 1) indicates that this miRNA plays a role in the regulation of keratinocyte functions. Thus, we next focused on characterizing miR-203 in more detail.

Since miRNAs exert their effect by regulating the expression of protein-coding genes, their function can be interpreted as the sum of the function of the genes they regulate (Bartel 2004). To understand the functions of miR-203 we used a two-step sequential approach: (I) using algorithms based on a systematic analysis of the structural requirements for target site function in vivo, we predicted genes that can be regulated by this miRNA; (II) we investigated the biological functions of the predicted target genes. To explore whether the presence of miR-203 binding sites in the 3' untranslated region (UTR) of mRNAs correlates with gene function, we determined if putative miR-203 targets contain significantly more or fewer genes from any given biological process than expected given the gene ontology (GO) category's frequency in the 3'UTR database. Out of the several thousand GO categories, the top significant (p<0.01) target categories were dominated by processes related to signal transduction, cell cycle, morphogenesis and cell growth indicating a role for miR-203 in the regulation of these biological processes in the skin. Of note, these GO categories show significant overlap with the biological processes that are strongly perturbed in the psoriatic skin lesions (Zhou, Krueger et al. 2003).

Among the target genes of miR-203, we focused on the suppressor of cytokine signaling-3 (SOCS-3), an evolutionarily conserved high-score target of miR-203 with a 10-nucleotide complementarity to the mature, biologically active form of miR-203 in human, mouse, rat and dog (Figure 4A). SOCS-3 is part of a negative feedback loop in cytokine signaling inhibiting the activation of STAT3, a transcription factor whose activation in keratinocytes is essential for the development of psoriatic plaques (Sano, Chan et al. 2005; Lowes, Bowcock et al. 2007). However, SOCS-3 has not been associated with psoriasis.

Next, we analyzed the expression pattern of miR-203 and SOCS-3 in lesional skin of psoriasis patients and in healthy skin (Fig. 4B). In situ hybridization using LNA-modified nucleotide probes revealed

that miR-203 is expressed in the suprabasal layers of the epidermis in normal skin, confirming its keratinocyte-specific expression observed in vitro (Fig. 4B). In comparison to healthy skin, the expression of miR-203 was increased in psoriatic lesional skin in all epidermal layers (Fig. 4B), consistent with the real-time PCR results. Analysis of SOCS-3 protein expression by immunohistochemistry showed a complementary pattern with the miR-203 expression (Fig. 4B). SOCS-3 was strongly expressed by the basal layer of keratinocytes in healthy skin, while it was suppressed in the epidermis of psoriasis lesions. Down-regulation of SOCS-3 expression in psoriatic lesional skin was further confirmed by Western blot analysis, demonstrating a significant ($p < 0.01$) decrease in SOCS-3 protein levels in psoriatic plaques as compared to healthy skin (Fig. 4C). Since the decrease of SOCS3 protein in psoriatic skin could be due to decreased transcriptional activity of the gene, we analyzed SOCS3 mRNA levels in psoriatic and healthy skin. However, quantitative real time PCR analysis showed no significant difference in SOCS-3 mRNA expression between psoriatic and healthy skin, providing indication that the down-regulation of SOCS-3 in psoriasis occurs at the posttranscriptional level.

SOCS-3 deficiency leads to sustained activation of STAT-3 in response to IL-6 (Croker, Krebs et al. 2003), a cytokine present in the psoriasis lesions (Lowe, Bowcock et al. 2007). This provides indication that the suppression of SOCS-3 by miR-203 in psoriatic lesions would in turn lead to constant activation of STAT3. Indeed, the psoriatic hyperplastic epidermis shows increased STAT3 activation and constitutively active STAT3 in keratinocytes leads to the spontaneous development of psoriasis in transgenic mice (Sano, Chan et al. 2005). Thus, the up-regulation of miR-203 may have important implications for psoriasis pathogenesis by preventing the up-regulation of SOCS-3 in response to cytokines.

Suppression of SOCS-3 in psoriatic keratinocytes may lead to sustained activation of the STAT3 pathway, leading to the infiltration of leukocytes and the development of psoriatic plaques.

5 In addition to the modulation of inflammatory responses, SOCS-3 has also been implicated in the regulation of keratinocyte proliferation and differentiation. It has been shown that overexpression of SOCS-3 in keratinocytes leads to final differentiation and inhibits serum-stimulated proliferation (Goren, Linke et al. 2006). miRNA-mediated
10 suppression of SOCS-3 expression in keratinocytes may therefore not only modulate cytokine signaling but also contribute to keratinocyte hyperproliferation and alteration in keratinocyte differentiation in psoriatic plaques.

15 Significant repression of the reporter was observed when the SOCS-3 3'UTR was coupled to a luciferase reporter and cotransfected with the miR-203 precursor into HeLa cells. This repression was alleviated when the predicted binding site was mutated (figure 25).

20 *2.4 miR-155 is expressed in T-cells and dendritic cells and induced in a mouse model of atopic eczema*

The expression of the functionally active, mature form of miR-155 was analyzed using quantitative real-time PCR in the skin of 26 healthy individuals, and lesional skin samples of 20 patients with
25 atopic eczema and 25 patients with psoriasis. The results for individual patients and mean are shown in Figure 5. Data are expressed in relative units compared to U48 RNA (**p<0.001). miR-155 was found to be overexpressed both in atopic eczema and in psoriasis in comparison with healthy skin.

30

In order to compare the expression of miR-155 in skin to that of other organs, we analyzed its expression in 21 different tissue and organ samples. miR-155 showed the highest expression in lymphoid organs such as thymus, spleen, lung and colon, while its expression
35 in health skin was relatively low (Figure 6), providing indication

that miR-155 is mainly expressed in immune cells. Among the cellular constituents of the skin, miR-155 was found to be expressed by regulatory T cells, dendritic cells (MDDCs), fibroblasts, monocytes and mast cells (Figure 7). These results show that miR-155 is
5 expressed in multiple immune cell types.

A significant, ~10-fold induction during the differentiation of naïve T cells into mature Th1, Th2 or Treg-like cells (Figure 8) and tightly regulated expression of miR-155 was observed during T- cell
10 differentiation (Figure 12). A dramatic increase in miR-155 in dendritic cells was observed after LPS treatment, which induces the maturation of dendritic cells. However, IL-6 or IFN-gamma had no effect (Figure 9). We also observed a significant ~10-fold down-regulation in miR-155 during the development of monocytes into
15 dendritic cells (Figure 10).

Expression of MiR-155 in the PBMCs of atopic eczema patients was increased relative to healthy individuals following stimulation with SEB or LPS (Figure 11). The difference became detectable 24 hours
20 after stimulation with SEB or LPS. LPS, IL-4 had no effect on miR-155 expression in monocytes but LPS, and IgE cross-linking induced miR-155 in human mast cells (CBMCs) (Figure 13).

Patients with atopic eczema have repeated cutaneous exposure to both
25 environmental allergens and superantigen-producing strains of *Staphylococcus aureus*. Topical exposure of mouse skin to SEB, OVA, or a combination of OVA and SEB of mice results in skin inflammation resembling atopic eczema and thus represents an established animal model for investigating this disease. A suitable protocol to
30 generate the model is shown in Figure 15.

We have analyzed miR-155 expression in the skin of animals sensitized to SEB or OVA using real-time PCR after exposure to SEB or OVA/SEB. Both SEB and OVA/SEB induced miR-155 expression (Figure
35 16), indicating that (1) allergens and superantigens may contribute

to the overexpression of miR-155 in human atopic eczema lesions (2) this mouse model is suitable for further *in vivo* studies of miR-155 functions in atopic eczema. The kinetics of miR-155 expression following allergen exposure are shown in Figure 17.

5

Putative targets of miR-155 were identified using targetScan 3.0 algorithm (table 10). The occurrence of GO terms associated with miR-155 targets were analyzed using the Gene ontology Tree Machine and the Gene Set Analysis Toolkit

10 (<http://bioinfo.vanderbilt.edu/webgestalt>; Vanderbilt University). Based on the predicted targets of miR-155, this miRNA was found to play a role in inter alia in the positive regulation of interleukin-1 biosynthesis [Gene Ontology annotation GO:0045362] and lymphocyte differentiation [GO:0030098]. Gene Ontology Consortium annotations
15 are described in Nature Genet. (2000) 25: 25-29.

Preliminary analysis using TargetScan and PicTar software (Sonkoly, E., et al., PLoS ONE, 2007. 2(7): p. e610) has identified Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4), an inhibitory molecule present
20 mainly on CD4⁺ T cells, as one of the evolutionary conserved putative targets of miR-155. Cotransfection of specific precursors of miR-155 with CTLA4 3'UTR-luciferase reporter constructs was found to result in decreased luciferase activity (figure 14). Furthermore, transfection of T cells with miR-155 precursors result in decreased
25 CTLA4 expression (figure 14). These results show that CTLA4 is a target of miR-155.

The highly regulated expression of miR-155 during T cell and dendritic cell differentiation together with the significant
30 enrichment of target genes associated with differentiation provides indication that miR-155 regulates T cell and dendritic cell differentiation. The interactions between T cells and dendritic cells are crucial in the development of skin inflammation in both psoriasis and atopic eczema. Therefore, miR-155 represents a

potential therapeutic target in the treatment of these chronic inflammatory skin diseases.

2.5 miR-21 is expressed by both structural and immune cells and induced in a mouse model of atopic eczema

miR-21 was found to be overexpressed both in atopic eczema and in psoriasis in comparison with healthy skin. The expression of the functionally active, mature forms of miR-21 was analyzed using quantitative real-time PCR in the skin of 26 healthy individuals, and lesional skin samples of 20 patients with atopic eczema and 25 patients with psoriasis. The results for individual patients and mean are shown in Figure 18. Data are expressed in relative units compared to U48 RNA (**p<0.001).

Among 20 human organs and tissues, miR-21 was found to be expressed ubiquitously, with highest levels in bladder, lung, prostate and trachea (Figure 19). Among the cellular constituents of the skin, miR-21 was expressed by structural cells (keratinocytes, fibroblasts, melanocytes), monocytes and dendritic cells, CD4+CD25high regulatory T cells and mast cells (Figure 20).

A significant, induction during the differentiation of naïve T cells into mature Th1, Th2 or Treg-like cells was observed (Figure 21) but no significant change was observed in miR-21 levels in dendritic cells after LPS treatment, which induces the maturation of dendritic cells. IL-6 or IFN-gamma also did not affect miR-21 expression (Figure 22). A slight up-regulation of miRNA was observed during the development of monocytes into dendritic cells (Figure 23) and miR-21 was significantly upregulated in a mouse model of atopic eczema (figure 24).

Putative targets of miR-21 were identified using targetScan 3.0 algorithm. The occurrence of GO terms associated with miR-21 targets were analyzed using the Gene ontology Tree Machine and the Gene Set Analysis Toolkit (<http://bioinfo.vanderbilt.edu/webgestalt>;

Vanderbilt University). Based on the predicted targets of miR-21, this miRNA plays a role *inter alia* in the regulation of transcription factor activity [GO:0051090], nervous system development [GO:0007399], negative regulation of signal transduction [GO:0009968], negative regulation of G-protein coupled receptor protein signalling pathway [GO:0045744], T cell receptor signaling pathway [GO:0050852], regulation of cell migration [GO:0030334], apoptosis [GO:0006915], regulation of growth [GO:0040008], cell cycle [GO:0007049], cell proliferation [GO:0008283], positive regulation of alpha-beta T cell proliferation [GO:0046641], JAK-STAT cascade [GO:0007259] and signal transduction [GO:0007165]. Predicted target genes of miR-21 are involved in the regulation of growth (fibroblast growth factor), differentiation (Jagged-1, Kerato-epithelin), apoptosis (SAMD7) and cell migration (IL-1a, Ephrin B2)

These results provide indication that miR-21 is involved in the regulation of growth, differentiation, apoptosis and cell migration. These cellular processes are altered in chronic inflammatory skin diseases. Therefore, miR-21 represents a potential therapeutic target in the treatment of these chronic inflammatory skin diseases.

Taken together, miRNA expression patterns distinguish psoriasis from healthy skin and from another chronic inflammatory skin disease, atopic eczema. Results reported here reveal a new layer of regulatory mechanisms in the pathogenesis of chronic inflammatory skin diseases. Our data provide indication that miR-203 plays a specific role in the pathogenesis of psoriasis by regulating inflammation-, proliferation- and morphogenesis-associated processes in the skin. Interestingly, miRNAs have been recently implicated in the morphogenesis of murine skin (Yi, O'Carroll et al. 2006). Since miRNAs are master switches that ultimately affect complex cellular processes and functions through the regulation of several proteins, miRNA-based therapies may be more effective than drugs targeting single proteins. The disease-specific miRNAs identified in our study

represent potential therapeutic targets in the treatment of chronic skin inflammation.

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Psoriasis			Atopic eczema			INDUCED
miRNA	Score	fold change	miRNA	Score	fold change	
miR-20a	5.17	3.15	miR-146a	2.85	1.68	
miR-146a	5.06	2.93	let-7i	2.50	2.04	
miR-146b	4.68	3.31	miR-29a	2.39	1.83	
miR-31	3.47	4.69	miR-222	2.22	1.67	
miR-146a	3.04	3.67	miR-24	2.20	1.85	
miR-20a	2.99	2.64	miR-193a	2.14	2.27	
miR-200a	2.85	2.75	miR-199a	2.13	1.72	
miR-17-5p	2.67	3.77	miR-27a	2.04	1.72	
miR-30e-5p	2.61	3.61	miR-21	2.04	3.26	
miR-141	2.26	3.45	miR-20a	2.03	2.43	
miR-203	2.23	5.86	miR-20a	2.01	2.28	
miR-142-3p	2.22	2.55	miR-146a	1.84	2.75	
miR-21	2.21	2.51	miR-17-5p	1.82	2.58	
miR-106a	2.16	2.37	miR-106b	1.78	1.72	
						SUPPRESSED
miR-125b	-5.31	0.55	miR-122a	-2.75	0.19	
miR-99b	-3.32	0.58	miR-133a-133b	-2.73	0.28	
miR-122a	-3.09	0.18	miR-326	-2.50	0.39	
miR-197	-2.69	0.64	miR-215	-2.48	0.42	
miR-100	-2.62	0.59	miR-483	-1.89	0.57	
miR-381	-2.60	0.71	miR-519d	-1.86	0.48	
miR-518b	-2.55	0.56	miR-335	-1.78	0.63	
miR-524*	-2.40	0.50	miR-133b	-1.68	0.23	
let-7e	-2.25	0.60	miR-515-5p	-1.57	0.43	
miR-30c	-1.98	0.63				
miR-365	-1.87	0.62				
miR-133b	-1.78	0.22				
miR-10a	-1.78	0.67				
miR-133a-133b	-1.76	0.40				
miR-22	-1.68	0.61				
miR-326	-1.61	0.56				
miR-215	-1.59	0.56				

Table 1

miRNA (hsa-miR)	Accession No	Sequence	SEQ ID NO:
miR-1	MIMAT0000416	UGGAAUGUAAAGAAGUAUGUA	1
miR-9	MIMAT0000441	UCUUUGGUUAUCUAGCUGUAUGA	2
miR-10a	MIMAT0000253	UACCCUGUAGAACCAGAAUUUGUG	3
miR-10b	MIMAT0000254	UACCCUGUAGAACCAGAAUUUGU	4
miR-15a	MIMAT0000068	UAGCAGCACAUAAUGGUUUUGUG	5
miR-15b	MIMAT0000417	UAGCAGCACAUCAUGGUUUACA	6
miR-16	MIMAT0000069	UAGCAGCACGUAAAUAUUGGCG	7
miR-17-5p	MIMAT0000070	CAAAGUGCUUACAGUGCAGGUAG	8
miR-20a	MIMAT0000075	UAAAGUGCUUUAUAGUGCAGGUAG	9
miR-21	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA	10
miR-22	MIMAT0000077	AAGCUGCCAGUUGAAGAACUGU	11
miR-23a	MIMAT0000078	AUCACAUUGCCAGGGAUUUCC	12
miR-23b	MIMAT0000418	AUCACAUUGCCAGGGAUUACC	13
miR-24	MIMAT0000079	GUGCCUACUGAGCUGAUUACAGU	14
miR-26a	MIMAT0000082	UUCAAGUAAUCCAGGAUAGGCU	15
miR-26b	MIMAT0000083	UUCAAGUAAUUCAGGAUAGGU	16
miR-27a	MIMAT0000084	UUCACAGUGGCUAAGUUCGCG	17
miR-27b	MIMAT0000419	UUCACAGUGGCUAAGUUCUGC	18
miR-29a	MIMAT0000086	UAGCACCAUCUGAAAUCGGUU	19
miR-29c	MIMAT0000681	UAGCACCAUUGAAAUCGGUUA	20
miR-30a-5p	MIMAT0000087	UGUAAACAUCUCGACUGGAAG	21
miR-30a-3p	MIMAT0000088	CUUUCAGUCGGAUGUUUGCAGC	22
miR-30b	MIMAT0000420	UGUAAACAUCUACACUCAGCU	23
miR-30c	MIMAT0000244	UGUAAACAUCUACACUCAGC	24
miR-30d	MIMAT0000245	UGUAAACAUCUCCGACUGGAAG	25
miR-30e-3p	MIMAT0000693	CUUUCAGUCGGAUGUUUACAGC	26
miR-30e-5p	MIMAT0000692	UGUAAACAUCUUGACUGGA	27
miR-31	MIMAT0000089	GGCAAGAUGCUGGCAUAGCUG	28
miR-92	MIMAT0000092	UAUUGCACUUGUCCCGGCCUGU	29
miR-93	MIMAT0000093	CAAAGUGCUGUUCGUGCAGGUAG	30
miR-95	MIMAT0000094	UUCAACGGGUAAUUUAUUGAGCA	31
miR-99a	MIMAT0000097	AACCCGUAGAUCGGAUCUUGUG	32
miR-99b	MIMAT0000689	CACCCGUAGAACCAGCCUUGCG	33
miR-100	MIMAT0000098	AACCCGUAGAUCGGAACUUGUG	34
miR-101	MIMAT0000099	UACAGUACUGUGAUAAACUGAAG	35
miR-106a	MIMAT0000103	AAAAGUGCUUACAGUGCAGGUAGC	36
miR-106b	MIMAT0000680	UAAAGUGCUGACAGUGCAGAU	37
miR-107	MIMAT0000104	AGCAGCAUUGUACAGGGCUAUC	38
miR-122a	MIMAT0000421	UGGAGUGUGACAAUGGUGUUUGU	39
miR-125a-5p	MIMAT0000443	UCCCUGAGACCCUUUAACCUGUGA	40
miR-125a-3p	MIMAT0004602	ACAGGUGAGGUUCUUGGGAGCC	41
miR-125b	MIMAT0000423	UCCCUGAGACCCUAACUUGUGA	42
miR-126	MIMAT0000445	UCGUACCGUGAGUAAUAUGCG	43
miR-127-3p	MIMAT0000446	UCGGAUCCGUCUGAGCUUGGCU	44
miR-127-5p	MIMAT0004604	CUGAAGCUCAGAGGGCUCUGAU	45
miR-130a	MIMAT0000425	CAGUGCAAUGUAAAAGGGCAU	46
miR-130b	MIMAT0000691	CAGUGCAAUGAUGAAAGGGCAU	47
miR-132	MIMAT0000426	UAACAGUCUACAGCCAUGGUCG	48
miR-133a	MIMAT0000427	UUGGUCCCCUUAACCAGCUGU	49
miR-133b	MIMAT0000770	UUGGUCCCCUUAACCAGCUA	50
miR-135b	MIMAT0000758	UAUGGCUUUUCAUCCUAUGUGA	51

miR-140-5p	MIMAT0000431	CAGUGGUUUUACCCUAUGGUAG	52
miR-40-3p	MIMAT0004597	UACCACAGGGUAGAACCACGG	53
miR-141	MIMAT0000432	UAACACUGUCUGGUAAAGAUGG	54
miR-142-3p	MIMAT0000434	UGUAGUGUUUCCUACUUUAUGGA	55
miR-142-5p	MIMAT0000433	CAUAAAGUAGAAAGCACUACU	56
miR-143	MIMAT0000435	UGAGAUGAAGCACUGUAGCUCA	57
miR-145	MIMAT0000437	GUCCAGUUUCCCAGGAAUCCCU	58
miR-146a	MIMAT0000449	UGAGAACUGAAUUCUAGGGUU	59
miR-146b	MIMAT0002809	UGAGAACUGAAUUCUAGGGCU	60
miR-148a	MIMAT0000243	UCAGUGCACUACAGAACUUUGU	61
miR-148b	MIMAT0000759	UCAGUGCAUCACAGAACUUUGU	62
miR-149	MIMAT0000450	UCUGGCUCGUGUCUUCACUCCC	63
miR-152	MIMAT0000438	UCAGUGCAUGACAGAACUUGG	64
miR-155	MIMAT0000646	UUA AUGCUAAUCGUGAUAGGGG	65
miR-181d	MIMAT0002821	AACAUUCAUUGUUGUCGGUGGGU	66
miR-182	MIMAT0000259	UUUGGCAAUGGUAGAACUCACACU	67
miR-186	MIMAT0000456	CAAAGAAUUCUCCUUUUGGGCU	68
miR-187	MIMAT0000262	UCGUGUCUUGUGUUGCAGCCGG	69
miR-191	MIMAT0000440	CAACGGAAUCCCAAAGCAGCUG	70
miR-193a	MIMAT0000459	AACUGGCCUACAAAGUCCCAG	71
miR-193b	MIMAT0002819	AACUGGCCUCAAAGUCCCGCU	72
miR-194	MIMAT0000460	UGUAAACAGCAACUCCAUGUGGA	73
miR-195	MIMAT0000461	UAGCAGCAGAGAAUAUUGGC	74
miR-196a	MIMAT0000226	UAGGUAGUUUCAUGUUGUUGGG	75
miR-196b	MIMAT0001080	UAGGUAGUUUCCUGUUGUUGGG	76
miR-197	MIMAT0000227	UUCACCACCUUCCACCCAGC	77
miR-199a	MIMAT0000231	CCCAGUGUUCAGACUACCUGUUC	78
miR-199b	MIMAT0000263	CCCAGUGUUUAGACUAUCUGUUC	79
miR-200a	MIMAT0000682	UAACACUGUCUGGUAAACGAUGU	80
miR-200b	MIMAT0000318	UAAUACUGCCUGGUAAUGAUGAC	81
miR-200c	MIMAT0000617	UAAUACUGCCGGGUAAUGAUGG	82
miR-203	MIMAT0000264	GUGAAAUGUUUAGGACCACUAG	83
miR-204	MIMAT0000265	UUCUUUUGUCAUCCUAUGCCU	84
miR-205	MIMAT0000266	UCCUUCAUUCCACCGGAGUCUG	85
miR-214	MIMAT0000271	ACAGCAGGCACAGACAGGCAGU	86
miR-215	MIMAT0000272	AUGACCUAUGAAUUGACAGAC	87
miR-218-1	MIMAT0000275	UUGUGCUUGAUCUAACCAUGU	88
miR-218-2			
miR-222	MIMAT0000279	AGCUACAUCUGGCUACUGGGUCUC	89
miR-223	MIMAT0000280	UGUCAGUUUGUCAAAUACCCC	90
miR-224	MIMAT0000281	CAAGUCACUAGUGGUUCCGUU	91
miR-301a	MIMAT0000688	CAGUGCAAUAGUAUUGUCAAGC	92
miR-301b	MIMAT0004958	CAGUGCAAUAGUAUUGUCAAGC	93
miR-320a	MIMAT0000510	AAAAGCUGGGUUGAGAGGGCGA	94
miR-320b	MIMAT0005792	AAAAGCUGGGUUGAGAGGGCAA	95
miR-320c	MIMAT0005793	AAAAGCUGGGUUGAGAGGGU	96
miR-320d	MIMAT0006764	AAAAGCUGGGUUGAGAGGA	97
miR-324-5p	MIMAT0000761	CGCAUCCCCUAGGGCAUUGGUGU	98
miR-324-3p	MIMAT0000762	ACUGCCCCAGGUGCUGCUGG	99
miR-326	MIMAT0000756	CCUCUGGGCCCUUCCUCCAG	100
miR-328	MIMAT0000752	CUGGCCUCUCUGCCCUUCCGU	101
miR-331-3p	MIMAT0000760	GCCCCUGGGCCUAUCCUAGAA	102
miR-331-5p	MIMAT0004700	CUAGGU AUGGUCCAGGGAUCC	103
miR-335	MIMAT0000765	UCAAGAGCAAUAACGAAAAUGU	104
miR-361	MIMAT0000703	UUAUCAGAAUCCAGGGGUAC	105

miR-362-5p	MIMAT0000705	AAUCCUUGGAACCUAGGUGUGAGU	106
miR-362-3p	MIMAT0004683	AACACACCUAUUCAAGGAUUA	107
miR-365	MIMAT0000710	UAAUGCCCCUAAAAAUCCUUAU	108
miR-375	MIMAT0000728	UUUGUUCGUUCGGCUCGCGUGA	109
miR-381	MIMAT0000736	UAUACAAGGGCAAGCUCUCUGU	110
miR-383	MIMAT0000738	AGAUCAGAAGGUGAUUGUGGCU	111
miR-411	MIMAT0003329	UAGUAGACCGUAUAGCGUACG	112
miR-422b	MIMAT0000732	CUGGACUUGGAGUCAGAAGGCC	113
miR-425	MIMAT0003393	AAUGACACGAUCACUCCCGUUGA	114
miR-432	MIMAT0002814	UCUUGGAGUAGGUCAUUGGGUGG	115
miR-451	MIMAT0001631	AAACCGUUACCAUUAUCUGAGUUU	116
miR-452	MIMAT0001635	AACUGUUUGCAGAGGAAACUGA	117
miR-483	MIMAT0002173	UCACUCCUCUCCUCCCGUCUUCU	118
miR-486-5p	MIMAT0002177	UCCUGUACUGAGCUGCCCCGAG	119
miR-486-3p	MIMAT0004762	CGGGGCAGCUCAGUACAGGAU	120
miR-487b	MIMAT0003180	AAUCGUACAGGGUCAUCCACUU	121
miR-492	MIMAT0002812	AGGACCUGCGGGACAAGAUUCUU	122
miR-497	MIMAT0002820	CAGCAGCACACUGUGGUUUUGU	123
miR-501-5p	MIMAT0002872	AAUCCUUUGUCCUUGGGUGAGA	124
miR-501-3p	MIMAT0004774	AAUGCACCCGGGCAAGGAUUCU	125
miR-515-5p	MIMAT0002826	UUCUCCAAAAGAAAGCACUUUCUG	126
miR-516-5p	MIMAT0002859	CAUCUGGAGGUAAAGAGCACUUU	127
miR-518b	MIMAT0002844	CAAAGCGCUCUUUUUAGAGGU	128
miR-519d	MIMAT0002853	CAAAGUGCCUCCUUUUAGAGUGU	129
miR-524	MIMAT0002850	GAAGGCGCUUCCUUUGGAGU	130
miR-526b	MIMAT0002835	CUCUUGAGGGAAGCACUUUCUGUU	131
miR-532-5p	MIMAT0002888	CAUGCCUUGAGUGUAGGACCGU	132
miR-532-3p	MIMAT0004780	CCUCCACACCCAAGGCUUGCA	133
miR-615-5p	MIMAT0004804	GGGGGUCCCCGGUGCUCGGAUC	134
miR-615-3p	MIMAT0003283	UCCGAGCCUGGGUCUCCCUUUU	135
miR-660	MIMAT0003338	UACCAUUGCAUAUCGGAGUUG	136
Let-7a	MIMAT0000062	UGAGGUAGUAGGUUGUAUAGUU	137
Let-7b	MIMAT0000063	UGAGGUAGUAGGUUGUGUGGUU	138
Let-7c	MIMAT0000064	UGAGGUAGUAGGUUGUAUGGUU	139
Let-7d	MIMAT0000065	AGAGGUAGUAGGUUGCAUAGU	140
Let-7e	MIMAT0000066	UGAGGUAGGAGGUUGUAUAGU	141
Let-7f	MIMAT0000067	UGAGGUAGUAGAUUGUAUAGUU	142
Let-7g	MIMAT0000414	UGAGGUAGUAGUUUGUACAGUU	143
Let-7i	MIMAT0000415	UGAGGUAGUAGUUUGUCUGU	144

Table 2

List of Significant Genes for Delta = 0.313

Significance Analysis of Microarray results:

The microRNA expression profile of healthy skin was compared with that of atopic eczema samples

Positive genes (40)

Row	Gene ID	Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(%)
36	hsa-miR-146a	hsa-miR-146a	2,847800754	0,75427571	0,26486253	1,68126002	0
14	hsa-let-7i	hsa-let-7i	2,604257309	1,01799114	0,406504211	2,03625559	0
77	hsa-miR-29a	hsa-miR-29a	2,391953097	0,87786575	0,367007929	1,82824873	0
68	hsa-miR-222	hsa-miR-222	2,219985456	0,7549702	0,340235218	1,86873008	0
72	hsa-miR-24	hsa-miR-24	2,195206472	0,91805404	0,418213073	1,85248263	0
46	hsa-miR-193a	hsa-miR-193a	2,137919416	1,3278388	0,621089263	2,2672356	0
52	hsa-miR-199a	hsa-miR-199a	2,129125085	0,79753185	0,374767975	1,72424482	0
75	hsa-miR-27a	hsa-miR-27a	2,040294894	0,79882624	0,391409789	1,71657941	0
6	hsa-miR-21	hsa-miR-21	2,036000493	1,81383848	0,890005853	3,26259701	0
63	hsa-miR-20a	hsa-miR-20a	2,030996142	1,14949948	0,565978171	2,42876149	0
55	hsa-miR-20a	hsa-miR-20a	2,013823139	1,05659988	0,524673522	2,27959077	0
37	hsa-miR-146a	hsa-miR-146a	1,84420326	1,26278817	0,684733725	2,74916966	3,5971223
128	hsa-miR-17-5p	hsa-miR-17-5p	1,824869564	1,34047332	0,734558431	2,58146493	3,5971223
19	hsa-miR-106b	hsa-miR-106b	1,783402935	0,74734878	0,419057718	1,72170737	3,5971223
34	hsa-miR-142-3p	hsa-miR-142-3p	1,749243431	1,63454499	0,934429685	3,43157103	3,5971223
129	hsa-miR-30e-5p	hsa-miR-30e-5p	1,699767753	1,18117764	0,695314493	2,47096251	3,5971223
20	hsa-miR-107	hsa-miR-107	1,665502598	0,57416894	0,344740945	1,4713302	5,1798561
58	hsa-miR-200a	hsa-miR-200a	1,624346538	0,84227346	0,518530645	1,72555934	5,1798561
8	hsa-miR-146b	hsa-miR-146b	1,611926434	0,65266365	0,404866571	1,56077801	5,1798561
130	hsa-miR-27b	hsa-miR-27b	1,61081792	0,842562	0,523083361	1,70499957	5,1798561
54	hsa-miR-199b	hsa-miR-199b	1,488496805	0,77152341	0,519024729	1,70722886	5,1798561
18	hsa-miR-108a	hsa-miR-108a	1,431371335	0,7234806	0,505445778	1,67899243	7,4700463
17	hsa-miR-101	hsa-miR-101	1,33381555	0,54101869	0,405678147	1,43945942	8,9307884
121	hsa-miR-451	hsa-miR-451	1,277468897	0,96044135	0,751631546	1,94272973	10,116906
28	hsa-miR-130a	hsa-miR-130a	1,25887724	0,48144493	0,382429938	1,37623735	10,116906
100	hsa-miR-451	hsa-miR-451	1,227893705	0,65341448	0,532142529	1,60701289	11,7724
33	hsa-miR-141	hsa-miR-141	1,191641058	0,82780351	0,694675468	1,91357827	13,390512
84	hsa-miR-31	hsa-miR-31	1,126639035	0,8099328	0,719892698	1,59359516	14,389489
60	hsa-miR-203	hsa-miR-203	1,072778216	1,66264156	1,54994923	1,87078808	17,489514
69	hsa-miR-223	hsa-miR-223	1,026156621	0,53151074	0,517962594	1,47626816	22,07325
53	hsa-miR-199a*	hsa-miR-199a*	1,008190984	0,76241748	0,757728405	1,50424222	22,07325
59	hsa-miR-200c	hsa-miR-200c	1,001088271	0,72725498	0,728457129	1,48498483	22,07325
40	hsa-miR-155	hsa-miR-155	0,98894048	0,67675886	0,699463605	1,42764502	23,021583
43	hsa-miR-16	hsa-miR-16	0,888957791	0,66309207	0,768419013	1,39490375	23,021583
138	hsa-miR-351	hsa-miR-351	0,873353389	0,45010074	0,515370693	1,37926987	27,39347
61	hsa-miR-205	hsa-miR-205	0,830984822	0,99314164	1,195138122	1,46811545	27,39347
131	hsa-miR-143	hsa-miR-143	0,829695815	0,48428817	0,58439799	1,28262814	27,39347
97	hsa-miR-422b	hsa-miR-422b	0,808410934	0,31844853	0,394896092	1,21690006	27,39347
57	hsa-miR-200b	hsa-miR-200b	0,804845158	0,53430154	0,864021326	1,32222337	27,39347
42	hsa-miR-15b	hsa-miR-15b	0,794999094	0,43930235	0,552582212	1,26155122	27,39347

Negative genes (12)

Row	Gene ID	Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(%)
23	hsa-miR-122a	hsa-miR-122a	-2,749245741	-2,24837574	0,817815487	0,1878036	0
30	hsa-miR-133a-133b	hsa-miR-133a-133b	-2,732418887	-1,6955356	0,620525501	0,279006	0
87	hsa-miR-326	hsa-miR-326	-2,50117378	-1,27859774	0,510369466	0,39304256	0
65	hsa-miR-215	hsa-miR-215	-2,481879021	-1,21822866	0,490849736	0,42030862	0
132	hsa-miR-483	hsa-miR-483	-1,885807135	-0,86536877	0,458884028	0,58944872	8,9307884
115	hsa-miR-519d	hsa-miR-519d	-1,861389454	-1,03229843	0,554585403	0,47559914	8,9307884
89	hsa-miR-335	hsa-miR-335	-1,778102342	-0,64853741	0,365146418	0,6312991	10,116906
31	hsa-miR-133b	hsa-miR-133b	-1,683955284	-1,65823882	0,984728535	0,2270142	12,94964
112	hsa-miR-515-5p	hsa-miR-515-5p	-1,586990919	-1,05110262	0,679777734	0,43240828	17,371469
48	hsa-miR-194	hsa-miR-194	-1,508287708	-1,04653859	0,694780012	0,41926544	17,371469
10	hsa-miR-524*	hsa-miR-524*	-1,487588872	-0,596361	0,400891009	0,66356818	17,371469
50	hsa-miR-197	hsa-miR-197	-1,461762755	-0,81234898	0,418911333	0,66050391	17,371469

Table 3

Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(%)
<u>hsa-miR-142-3p-4373136</u>	4.378755	1.607882	0.367201	3.084592	0
<u>hsa-miR-155-4373124</u>	4.045666	2.201913	0.544265	4.622148	0
<u>hsa-miR-31-4373190</u>	4.045172	2.949023	0.729023	8.503418	0
<u>hsa-miR-135b-4373139</u>	3.97071	2.075938	0.522813	4.161518	0
<u>hsa-miR-223-4373075</u>	3.024304	2.567422	0.84893	7.796735	0.95264
<u>hsa-miR-187-4373111</u>	2.864714	2.313972	0.80775	5.282102	0.95264
<u>hsa-miR-142-5p-4373135</u>	2.496498	1.454419	0.582584	2.870536	3.104901
<u>hsa-miR-501-4373226</u>	2.421184	2.028121	0.837657	3.142973	3.104901
<u>hsa-miR-487b-4378102</u>	2.411741	0.890143	0.369087	1.823577	3.104901
<u>hsa-miR-362-4378092</u>	2.40528	0.898964	0.373746	1.81384	3.104901
<u>hsa-miR-301-4373064</u>	2.063725	0.84708	0.410462	1.837104	5.408538
<u>hsa-miR-130b-4373144</u>	2.039959	1.009641	0.494932	1.976644	5.408538
<u>hsa-miR-146b-4373178</u>	2.02866	1.288674	0.635234	2.852587	5.408538

Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(%)
<u>hsa-miR-99a-4373008</u>	-6.04738	-2.0183	0.333748	0.248652	0
<u>hsa-miR-383-4373018</u>	-4.77089	-3.18162	0.666883	0.095693	0
<u>hsa-miR-125b-4373148</u>	-3.87755	-1.56497	0.403597	0.344311	0
<u>hsa-miR-193b-4373185</u>	-3.84871	-2.16266	0.561918	0.244698	0
<u>hsa-miR-10a-4373153</u>	-3.81751	-2.06867	0.541889	0.225324	0
<u>hsa-miR-30a-3p-4373062</u>	-3.81637	-1.22445	0.320841	0.433119	0
<u>hsa-miR-100-4373160</u>	-3.80818	-1.5392	0.404182	0.359408	0
<u>hsa-miR-328-4373049</u>	-3.77431	-1.87846	0.497698	0.288528	0
<u>hsa-miR-375-4373027</u>	-3.65756	-2.75268	0.7526	0.164098	0
<u>hsa-let-7c-4373167</u>	-3.63714	-1.29772	0.356798	0.412749	0
<u>hsa-miR-149-4373128</u>	-3.5306	-1.75469	0.496996	0.313165	0
<u>hsa-let-7a-4373169</u>	-3.49817	-1.26355	0.361203	0.415284	0
<u>hsa-miR-130a-4373145</u>	-3.3135	-0.75955	0.229227	0.593078	0
<u>hsa-miR-196b-4373103</u>	-3.27877	-1.12147	0.342039	0.472103	0
<u>hsa-miR-197-4373102</u>	-3.14883	-0.97294	0.308985	0.505505	0
<u>hsa-miR-26b-4373069</u>	-3.13817	-0.88531	0.282111	0.541842	0
<u>hsa-miR-30e-3p-4373057</u>	-3.03191	-1.06231	0.350376	0.485263	0
<u>hsa-miR-214-4373085</u>	-2.85054	-0.99412	0.348746	0.506723	0
<u>hsa-let-7b-4373168</u>	-2.75513	-0.91065	0.330529	0.530145	0
<u>hsa-miR-26a-4373070</u>	-2.74665	-0.94222	0.343042	0.527914	0
<u>hsa-miR-101-4373159</u>	-2.73899	-1.29598	0.47316	0.43303	0
<u>hsa-miR-30c-4373060</u>	-2.69194	-0.71693	0.266323	0.606057	0
<u>hsa-miR-195-4373105</u>	-2.65536	-1.13527	0.427539	0.477336	0
<u>hsa-let-7f-4373164</u>	-2.62864	-0.99246	0.377557	0.492843	0
<u>hsa-miR-148a-4373130</u>	-2.5669	-0.86253	0.336019	0.560532	1.397206
<u>hsa-miR-125a-4373149</u>	-2.55992	-0.8137	0.317859	0.565006	1.397206
<u>hsa-miR-30e-5p-4373058</u>	-2.40463	-0.76863	0.319647	0.596565	1.397206
<u>hsa-miR-29c-4373289</u>	-2.31281	-0.68918	0.297985	0.624465	1.397206
<u>hsa-miR-335-4373045</u>	-2.30675	-1.03751	0.449769	0.466339	1.397206
<u>hsa-miR-199b-4373100</u>	-2.24244	-1.01818	0.454052	0.523841	1.397206
<u>hsa-miR-365-4373194</u>	-2.17202	-1.24449	0.572964	0.464332	1.397206
<u>hsa-let-7d-4373166</u>	-2.02807	-1.00011	0.493137	0.510683	1.397206
<u>hsa-miR-204-4373094</u>	-1.99469	-1.45447	0.729173	0.350249	1.397206
<u>hsa-miR-23a-4373074</u>	-1.98014	-1.08338	0.547122	0.510837	1.397206
<u>hsa-miR-196a-4373104</u>	-1.95493	-1.35207	0.691622	0.452161	1.397206
<u>hsa-miR-452-4378077</u>	-1.94633	-1.07419	0.551906	0.520588	1.397206
<u>hsa-miR-143-4373134</u>	-1.91854	-1.01914	0.531203	0.479496	1.397206
<u>hsa-miR-199a-4378068</u>	-1.91765	-0.75679	0.394645	0.590972	1.397206
<u>hsa-miR-145-4373133</u>	-1.87997	-0.88982	0.473314	0.507434	1.397206
<u>hsa-miR-23b-4373073</u>	-1.87852	-0.92947	0.494788	0.563197	1.397206
<u>hsa-miR-486-4378096</u>	-1.64369	-1.68641	1.025988	0.265357	3.104901
<u>hsa-miR-92-4373013</u>	-1.63478	-0.79137	0.484082	0.598864	3.104901
<u>hsa-miR-615-4380991</u>	-1.60544	-1.57382	0.980301	0.445292	3.104901
<u>hsa-miR-497-4373222</u>	-1.59269	-0.72665	0.456242	0.613462	3.104901
<u>hsa-miR-99b-4373007</u>	-1.55486	-0.71492	0.459797	0.624682	3.810561
<u>hsa-miR-9-4373285</u>	-1.40531	-1.01605	0.723009	0.46856	5.058848
<u>hsa-miR-10b-4373152</u>	-1.35584	-0.77149	0.569011	0.620912	5.058848
<u>hsa-miR-1-4373161</u>	-1.28859	-0.999	0.775269	0.403283	5.058848
<u>hsa-miR-451-4373209</u>	-1.11603	-1.08736	0.974303	0.392322	5.408538

Table 5

List of Significant Genes for Delta = 0.497

Significance Analysis of Microarray results:

The microRNA expression profile of healthy skin was compared with that of psoriasis samples

Positive genes (25)						
Row	Gene ID	Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change q-value(%)
55	hsa-miR-2	<u>hsa-miR-20a</u>	5,170411954	1,62447184	0,314186152	3,14945009 0
36	hsa-miR-1	<u>hsa-miR-146a</u>	5,058578965	1,57115956	0,310593069	2,92702779 0
8	hsa-miR-1	<u>hsa-miR-146b</u>	4,675413034	1,74589906	0,373421353	3,31096122 0
84	hsa-miR-3	<u>hsa-miR-31</u>	3,473376049	2,36599204	0,681179351	4,68651364 0
37	hsa-miR-1	<u>hsa-miR-146a</u>	3,040941498	1,87952335	0,618072841	3,66798762 0
63	hsa-miR-2	<u>hsa-miR-20a</u>	2,990800951	1,39302887	0,465771174	2,64322314 0
56	hsa-miR-2	<u>hsa-miR-200a</u>	2,854728326	1,5347068	0,537601699	2,7493251 0
128	hsa-miR-1	<u>hsa-miR-17-5p</u>	2,67025785	1,89194925	0,708526799	3,77246059 0
129	hsa-miR-3	<u>hsa-miR-30a-5p</u>	2,611400855	1,79358072	0,686827042	3,61090198 0
33	hsa-miR-1	<u>hsa-miR-141</u>	2,264261118	1,61882182	0,714944847	3,44625503 3,4009156
60	hsa-miR-2	<u>hsa-miR-203</u>	2,227372204	3,31926781	1,490216948	5,86052607 3,4009156
34	hsa-miR-1	<u>hsa-miR-142-3p</u>	2,216758209	1,51428402	0,683107437	2,55218154 3,4009156
6	hsa-miR-2	<u>hsa-miR-21</u>	2,209169714	1,60495806	0,726498308	2,50634692 3,4009156
18	hsa-miR-1	<u>hsa-miR-106a</u>	2,163687633	1,22347959	0,565460363	2,36519222 3,4009156
135	hsa-miR-4	<u>hsa-miR-487b</u>	2,055234968	0,8970352	0,436463573	1,87341258 5,1600099
41	hsa-miR-1	<u>hsa-miR-15a</u>	1,900393906	0,94737634	0,498515772	1,82226153 5,1600099
14	hsa-let-7i	<u>hsa-let-7i</u>	1,897106069	0,58663358	0,309225506	1,48257927 5,1600099
68	hsa-miR-2	<u>hsa-miR-222</u>	1,886268385	0,55635819	0,294951766	1,45156733 5,1600099
97	hsa-miR-4	<u>hsa-miR-422b</u>	1,856882941	0,84621964	0,455720507	1,82463313 5,1600099
28	hsa-miR-1	<u>hsa-miR-130a</u>	1,84481411	0,67545637	0,366137906	1,58442701 5,1600099
46	hsa-miR-1	<u>hsa-miR-193a</u>	1,695817119	1,00563655	0,593010026	1,82514514 7,4820144
19	hsa-miR-1	<u>hsa-miR-106b</u>	1,507570917	0,50208526	0,333042547	1,45237131 10,391687
54	hsa-miR-1	<u>hsa-miR-199b</u>	1,42310625	0,67341997	0,473204279	1,58737508 14,599052
53	hsa-miR-1	<u>hsa-miR-199a*</u>	1,422388268	1,08449603	0,762447255	1,9419421 14,599052
130	hsa-miR-2	<u>hsa-miR-27b</u>	1,36183848	0,72696218	0,533809397	1,61158123 16,032888
Negative genes (28)						
Row	Gene ID	Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change q-value(%)
26	hsa-miR-1	<u>hsa-miR-125b</u>	-5,307902656	-0,87283027	0,164439766	0,54581789 0
120	hsa-miR-9	<u>hsa-miR-99b</u>	-3,319282075	-0,7876049	0,237281702	0,58239304 0
23	hsa-miR-1	<u>hsa-miR-122a</u>	-3,091017016	-2,29557408	0,742659799	0,17738672 0
50	hsa-miR-1	<u>hsa-miR-197</u>	-2,69162708	-0,62681495	0,232875852	0,64415248 4,676259
16	hsa-miR-1	<u>hsa-miR-100</u>	-2,621714944	-0,76668573	0,292436723	0,58706565 4,676259
94	hsa-miR-3	<u>hsa-miR-381</u>	-2,600891984	-0,487246	0,187338037	0,71214905 4,676259
114	hsa-miR-5	<u>hsa-miR-518b</u>	-2,545067041	-0,84176382	0,330743278	0,56394914 4,676259
10	hsa-miR-5	<u>hsa-miR-524*</u>	-2,397262458	-1,05228285	0,438951874	0,49914806 4,676259
126	hsa-let-7e	<u>hsa-let-7e</u>	-2,248140883	-0,75991931	0,338021214	0,60050376 5,1600099
82	hsa-miR-3	<u>hsa-miR-30c</u>	-1,982757588	-0,64015809	0,322862509	0,63160564 12,067765
90	hsa-miR-3	<u>hsa-miR-365</u>	-1,868138265	-0,6624109	0,354583442	0,61714601 12,067765
31	hsa-miR-1	<u>hsa-miR-133b</u>	-1,783954708	-1,69990138	0,952883707	0,22302786 12,067765
21	hsa-miR-1	<u>hsa-miR-10a</u>	-1,776839036	-0,58901933	0,331498419	0,66764029 12,067765
30	hsa-miR-1	<u>hsa-miR-133a-133b</u>	-1,757675811	-1,50215643	0,854626557	0,40459161 12,067765
66	hsa-miR-2	<u>hsa-miR-22</u>	-1,680288261	-0,66312364	0,394648737	0,61419675 12,067765
87	hsa-miR-3	<u>hsa-miR-326</u>	-1,610882797	-0,78233891	0,485658495	0,55702207 14,599052
65	hsa-miR-2	<u>hsa-miR-215</u>	-1,592738733	-0,87139451	0,547104491	0,56299509 14,599052
113	hsa-miR-5	<u>hsa-miR-516-5p</u>	-1,461293425	-0,37861039	0,259092652	0,76965012 18,705036
12	hsa-let-7c	<u>hsa-let-7c</u>	-1,439174915	-0,68241616	0,474171799	0,60965569 18,705036
13	hsa-let-7d	<u>hsa-let-7d</u>	-1,43845147	-0,37713491	0,262181187	0,76637288 18,705036
89	hsa-miR-3	<u>hsa-miR-335</u>	-1,411689579	-0,80761774	0,572093013	0,63146066 18,705036
103	hsa-miR-4	<u>hsa-miR-492</u>	-1,400491698	-0,38617242	0,275740601	0,75733855 18,705036
15	hsa-miR-1	<u>hsa-miR-1</u>	-1,386855692	-1,40056087	1,009882193	0,2602516 18,705036
115	hsa-miR-5	<u>hsa-miR-519d</u>	-1,278039174	-0,52748368	0,412728879	0,66876353 21,175512
22	hsa-miR-1	<u>hsa-miR-10b</u>	-1,275506037	-0,53978559	0,423193286	0,70088255 21,175512
132	hsa-miR-4	<u>hsa-miR-483</u>	-1,260685484	-0,35279829	0,279846397	0,77645168 21,175512
48	hsa-miR-1	<u>hsa-miR-194</u>	-1,250780026	-0,8234822	0,65837492	0,48999421 21,175512
117	hsa-miR-5	<u>hsa-miR-526b</u>	-1,214089964	-0,46597639	0,383807136	0,74282263 21,175512

Table 6

Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(%)
<u>hsa-miR-31-4373190</u>	11.76748	5.83201	0.495604	53.46021	0
<u>hsa-miR-146a-4373132</u>	6.157881	1.361142	0.221041	2.550485	0
<u>hsa-miR-21-4373090</u>	5.690929	1.950575	0.342752	3.77251	0
<u>hsa-miR-135b-4373139</u>	4.495819	2.897555	0.6445	6.784366	0
<u>hsa-miR-205-4373093</u>	3.249398	0.596745	0.183648	1.506377	1.230214
<u>hsa-miR-142-3p-4373136</u>	2.985761	0.690889	0.231395	1.600742	1.230214
<u>hsa-miR-155-4373124</u>	2.979155	1.345772	0.451729	2.436721	1.230214
<u>hsa-miR-223-4373075</u>	2.842751	0.966022	0.339819	1.907887	1.230214
<u>hsa-miR-17-5p-4373119</u>	2.791165	0.875423	0.313641	1.798112	1.230214
<u>hsa-miR-93-4373012</u>	2.68718	1.080648	0.402149	2.036204	1.230214
<u>hsa-miR-203-4373095</u>	2.368399	0.656687	0.27727	1.555213	1.230214
<u>hsa-miR-132-4373143</u>	2.322615	0.845088	0.363852	1.749608	1.230214
<u>hsa-miR-425-4373202</u>	2.30791	1.157098	0.501362	2.097308	1.230214
<u>hsa-miR-362-4378092</u>	2.272419	0.833846	0.366942	1.735073	1.230214
<u>hsa-miR-324-5p-4373052</u>	2.193747	0.361533	0.164802	1.281118	1.230214
<u>hsa-miR-224-4373187</u>	1.980246	1.188235	0.600044	2.081201	1.230214
<u>hsa-miR-432-4373280</u>	1.751434	1.16155	0.663199	2.032994	1.230214
<u>hsa-miR-301-4373064</u>	1.721598	0.4905	0.28491	1.385618	1.230214
<u>hsa-miR-20a-4373286</u>	1.699744	0.41699	0.245325	1.32226	1.230214

Table 7

Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(%)
<u>hsa-miR-100-4373160</u>	-12.7485	-1.71946	0.134875	0.303266	0
<u>hsa-miR-30a-3p-4373062</u>	-12.4543	-2.60401	0.209085	0.163481	0
<u>hsa-miR-195-4373105</u>	-10.4344	-1.77231	0.169853	0.291858	0
<u>hsa-miR-30a-5p-4373061</u>	-10.0142	-1.41049	0.14085	0.37559	0
<u>hsa-miR-30e-5p-4373058</u>	-8.79751	-1.41719	0.16109	0.373476	0
<u>hsa-miR-99a-4373008</u>	-7.87582	-1.55446	0.197371	0.338712	0
<u>hsa-miR-193b-4373185</u>	-7.7373	-1.6623	0.214842	0.313894	0
<u>hsa-miR-149-4373128</u>	-7.19907	-2.06626	0.287018	0.235224	0
<u>hsa-miR-26a-4373070</u>	-7.12656	-1.51078	0.211993	0.348681	0
<u>hsa-let-7c-4373167</u>	-6.77945	-1.46013	0.215376	0.361063	0
<u>hsa-miR-26b-4373069</u>	-6.63502	-1.13863	0.171609	0.452712	0
<u>hsa-miR-218-4373081</u>	-6.60577	-1.43134	0.21668	0.368235	0
<u>hsa-miR-30e-3p-4373057</u>	-6.49389	-1.4485	0.223057	0.363718	0
<u>hsa-miR-23b-4373073</u>	-6.44952	-1.24703	0.193352	0.419313	0
<u>hsa-let-7g-4373163</u>	-6.36312	-1.3551	0.212961	0.388355	0
<u>hsa-miR-411-4381013</u>	-6.23084	-1.32534	0.212707	0.396504	0
<u>hsa-miR-125b-4373148</u>	-6.13734	-1.6646	0.271224	0.311236	0
<u>hsa-miR-199b-4373100</u>	-6.08363	-1.19458	0.19636	0.434804	0
<u>hsa-miR-29c-4373289</u>	-5.46365	-1.11354	0.203809	0.459639	0
<u>hsa-miR-101-4373159</u>	-5.27585	-1.34222	0.254409	0.390295	0
<u>hsa-miR-197-4373102</u>	-5.18262	-1.37159	0.264651	0.381852	0
<u>hsa-miR-214-4373085</u>	-5.06303	-1.21827	0.240621	0.425845	0
<u>hsa-miR-375-4373027</u>	-4.99986	-2.55427	0.510869	0.160598	0
<u>hsa-miR-335-4373045</u>	-4.916	-2.21699	0.450974	0.205585	0
<u>hsa-miR-181d-4373180</u>	-4.83571	-0.58649	0.121284	0.665428	0
<u>hsa-miR-125a-4373149</u>	-4.59551	-1.44142	0.313659	0.361267	0
<u>hsa-miR-140-4373138</u>	-4.5163	-1.49654	0.331364	0.346769	0
<u>hsa-miR-30c-4373060</u>	-4.51423	-1.00186	0.221934	0.495636	0
<u>hsa-miR-30b-4373290</u>	-4.45057	-0.83052	0.186609	0.559952	0
<u>hsa-miR-152-4373126</u>	-4.37175	-1.24311	0.284351	0.416297	0
<u>hsa-miR-328-4373049</u>	-4.27844	-1.052	0.245885	0.477677	0
<u>hsa-miR-497-4373222</u>	-4.19971	-1.3895	0.330856	0.373094	0
<u>hsa-miR-130a-4373145</u>	-4.17585	-0.52426	0.125546	0.694672	0
<u>hsa-miR-127-4373147</u>	-4.09023	-0.93473	0.228529	0.518965	0
<u>hsa-miR-148b-4373129</u>	-3.92101	-0.8323	0.212266	0.558215	0
<u>hsa-miR-186-4373112</u>	-3.9143	-0.96048	0.245377	0.508719	0
<u>hsa-miR-143-4373134</u>	-3.75643	-1.8063	0.480855	0.269531	0
<u>hsa-miR-145-4373133</u>	-3.66864	-1.76478	0.481045	0.277357	0
<u>hsa-miR-30d-4373059</u>	-3.61196	-1.06998	0.296232	0.468453	0
<u>hsa-miR-126-4373269</u>	-3.59126	-1.26174	0.351338	0.407415	0
<u>hsa-miR-199a-4378068</u>	-3.58301	-1.15516	0.322399	0.439907	0
<u>hsa-miR-196b-4373103</u>	-3.48818	-0.46805	0.134181	0.722021	0
<u>hsa-miR-486-4378096</u>	-3.48593	-3.58037	1.02709	0.061813	0
<u>hsa-miR-99b-4373007</u>	-3.44326	-0.9523	0.276569	0.510162	0
<u>hsa-miR-365-4373194</u>	-3.40403	-0.97013	0.284994	0.502766	0
<u>hsa-miR-29a-4373065</u>	-3.35492	-1.15777	0.345095	0.436984	0
<u>hsa-miR-320-4373055</u>	-3.28942	-0.69838	0.212311	0.61238	0
<u>hsa-miR-361-4373035</u>	-3.24661	-0.95643	0.294594	0.507426	0

<u>hsa-miR-95-4373011</u>	-3.12399	-1.15348	0.369234	0.437066	0
<u>hsa-let-7d-4373166</u>	-3.05807	-1.21594	0.397615	0.417051	0
<u>hsa-miR-532-4380928</u>	-3.02082	-0.87273	0.288905	0.537995	0
<u>hsa-miR-331-4373046</u>	-2.96463	-1.17026	0.394742	0.428809	0
<u>hsa-miR-199a-4373272</u>	-2.86962	-0.85379	0.297526	0.544137	0
<u>hsa-miR-196a-4373104</u>	-2.78127	-1.04927	0.377263	0.468517	0
<u>hsa-miR-451-4373209</u>	-2.71471	-2.73461	1.007331	0.111021	0
<u>hsa-miR-193a-4373107</u>	-2.71086	-1.08375	0.399782	0.455581	0
<u>hsa-miR-126-4378064</u>	-2.64503	-0.93877	0.354917	0.509333	0
<u>hsa-miR-10a-4373153</u>	-2.46892	-1.36565	0.553138	0.359901	0
<u>hsa-miR-23a-4373074</u>	-2.41463	-0.74724	0.309464	0.585067	0
<u>hsa-miR-660-4380925</u>	-2.31775	-0.53945	0.232746	0.682082	0
<u>hsa-let-7e-4373165</u>	-2.24737	-0.99439	0.442466	0.479835	0
<u>hsa-let-7b-4373168</u>	-2.00893	-0.58168	0.289546	0.658749	0
<u>hsa-miR-16-4373121</u>	-1.97327	-0.99477	0.504123	0.470933	0
<u>hsa-miR-182-4373271</u>	-1.96757	-0.70994	0.36082	0.595922	0
<u>hsa-miR-1-4373161</u>	-1.91902	-1.60722	0.837523	0.264057	0
<u>hsa-miR-22-4373079</u>	-1.83572	-0.6217	0.338668	0.634454	0.521376
<u>hsa-miR-27a-4373287</u>	-1.79766	-0.45225	0.251576	0.723423	0.521376
<u>hsa-miR-383-4373018</u>	-1.76341	-1.26552	0.717654	0.358066	0.9999
<u>hsa-let-7a-4373169</u>	-1.75317	-0.52559	0.299796	0.682696	0.9999
<u>hsa-miR-191-4373109</u>	-1.66002	-0.65045	0.391831	0.614944	1.459854

Table 8

List of Significant Genes for Delta = 0.396

Significance Analysis of Microarray results:

The microRNA expression profile of healthy skin was compared with the united group of atopic eczema and psoriasis samples (inflammation).

Positive genes (36)						
Row	Gene ID	Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change q-value(%)
55	hsa-miR-20a	<u>hsa-miR-20a</u>	2,905315198	1,34053576	0,461408029	2,71452043 0
36	hsa-miR-146a	<u>hsa-miR-146a</u>	2,770957363	1,31199403	0,47348041	2,64393374 0
63	hsa-miR-20a	<u>hsa-miR-20a</u>	2,658656949	1,28401942	0,482957916	2,58040886 0
84	hsa-miR-31	<u>hsa-miR-31</u>	2,65571483	1,8038398	0,679229478	3,44146864 0
6	hsa-miR-21	<u>hsa-miR-21</u>	2,652995661	1,70939927	0,644327579	2,88447197 0
128	hsa-miR-17-5p	<u>hsa-miR-17-5p</u>	2,547591685	1,61621128	0,634407505	3,17696276 0
46	hsa-miR-193a	<u>hsa-miR-193a</u>	2,436138517	1,16673767	0,478929119	2,04619037 0
8	hsa-miR-146b	<u>hsa-miR-146b</u>	2,416960169	1,21513647	0,502754033	2,45394547 0
37	hsa-miR-146a	<u>hsa-miR-146a</u>	2,415597859	1,37355695	0,568619873	2,49229018 0
68	hsa-miR-222	<u>hsa-miR-222</u>	2,335412904	0,65566419	0,280748724	1,5601487 0
34	hsa-miR-142-3p	<u>hsa-miR-142-3p</u>	2,291851252	1,57441445	0,686961906	2,99187629 0
14	hsa-let-7i	<u>hsa-let-7i</u>	2,269720024	0,80231236	0,353485168	1,75891743 0
18	hsa-miR-106a	<u>hsa-miR-106a</u>	2,218340153	1,03354189	0,46590776	2,07247275 0
56	hsa-miR-200a	<u>hsa-miR-200a</u>	2,175172577	1,12640654	0,517846975	2,13854216 0
129	hsa-miR-30e-5p	<u>hsa-miR-30e-5p</u>	2,093551785	1,40340449	0,670346202	2,89347513 0
60	hsa-miR-203	<u>hsa-miR-203</u>	2,03328799	2,49095468	1,225086998	3,86565608 0
130	hsa-miR-27b	<u>hsa-miR-27b</u>	1,834318325	0,78477709	0,42783037	1,6582404 0
33	hsa-miR-141	<u>hsa-miR-141</u>	1,791636261	1,22331281	0,682790833	2,67991665 2,413553
28	hsa-miR-130a	<u>hsa-miR-130a</u>	1,769327429	0,57845065	0,326932508	1,48033218 2,413553
72	hsa-miR-24	<u>hsa-miR-24</u>	1,703628092	0,67452023	0,395931617	1,59498406 2,413553
54	hsa-miR-199b	<u>hsa-miR-199b</u>	1,693170981	0,72247169	0,42669742	1,64730197 2,413553
52	hsa-miR-199a	<u>hsa-miR-199a</u>	1,640866609	0,5632198	0,34324533	1,48094888 2,413553
19	hsa-miR-106b	<u>hsa-miR-106b</u>	1,640332115	0,52149369	0,317919577	1,4572494 2,413553
53	hsa-miR-199a*	<u>hsa-miR-199a*</u>	1,557072626	0,92345675	0,593072371	1,72309216 2,413553
41	hsa-miR-15a	<u>hsa-miR-15a</u>	1,475571462	0,66594758	0,45131503	1,52312451 6,235012
121	hsa-miR-451	<u>hsa-miR-451</u>	1,464462114	0,88975906	0,607567144	1,84810235 6,235012
77	hsa-miR-29a	<u>hsa-miR-29a</u>	1,426987269	0,56297049	0,394516826	1,50961726 6,235012
97	hsa-miR-422b	<u>hsa-miR-422b</u>	1,399326098	0,58233408	0,416153234	1,5207666 6,235012
61	hsa-miR-205	<u>hsa-miR-205</u>	1,378585252	1,1696045	0,848409264	1,65259237 6,235012
138	hsa-miR-361	<u>hsa-miR-361</u>	1,2210991	0,49461286	0,405055463	1,39879716 11,510791
135	hsa-miR-487b	<u>hsa-miR-487b</u>	1,218967281	0,53997635	0,442978541	1,49422845 11,510791
20	hsa-miR-107	<u>hsa-miR-107</u>	1,155915545	0,40506566	0,350428421	1,34219172 13,467626
100	hsa-miR-451	<u>hsa-miR-451</u>	1,146530723	0,55210614	0,481545001	1,51714886 13,467626
43	hsa-miR-16	<u>hsa-miR-16</u>	0,960909677	0,56733408	0,590413535	1,31307339 22,31478
75	hsa-miR-27a	<u>hsa-miR-27a</u>	0,938165609	0,4120479	0,439205933	1,36792156 22,31478
40	hsa-miR-155	<u>hsa-miR-155</u>	0,869422986	0,45825653	0,527081222	1,23399018 25,80005

Negative genes (22)						
Row	Gene ID	Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change q-value(%)
23	hsa-miR-122a	<u>hsa-miR-122a</u>	-3,683826842	-2,27197491	0,616743134	0,18259516 0
30	hsa-miR-133a-133b	<u>hsa-miR-133a-133b</u>	-2,496214028	-1,59884601	0,640508384	0,3417988 0
50	hsa-miR-197	<u>hsa-miR-197</u>	-2,431295211	-0,59229149	0,243611509	0,66162907 0
87	hsa-miR-326	<u>hsa-miR-326</u>	-2,372605166	-1,02946833	0,433897869	0,47503232 0
31	hsa-miR-133b	<u>hsa-miR-133b</u>	-2,326329928	-1,6790701	0,721767827	0,22502103 0
10	hsa-miR-524*	<u>hsa-miR-524*</u>	-2,281702632	-0,89378321	0,391717659	0,54933809 0
65	hsa-miR-215	<u>hsa-miR-215</u>	-2,25191431	-1,04481209	0,463966184	0,49169685 0
48	hsa-miR-194	<u>hsa-miR-194</u>	-1,823265304	-0,9350104	0,51282191	0,45462983 8,0886642
15	hsa-miR-1	<u>hsa-miR-1</u>	-1,670332657	-1,32505022	0,793285224	0,28201272 8,0886642
126	hsa-let-7e	<u>hsa-let-7e</u>	-1,598893279	-0,63719403	0,399021049	0,67031898 13,467626
94	hsa-miR-381	<u>hsa-miR-381</u>	-1,593699657	-0,40970867	0,257080226	0,75895339 13,467626
132	hsa-miR-483	<u>hsa-miR-483</u>	-1,543748501	-0,60908253	0,394547773	0,6729507 13,467626
21	hsa-miR-10a	<u>hsa-miR-10a</u>	-1,528933362	-0,52719213	0,344810404	0,703028 13,467626
90	hsa-miR-365	<u>hsa-miR-365</u>	-1,493215754	-0,49915012	0,334278632	0,69638992 13,467626
103	hsa-miR-492	<u>hsa-miR-492</u>	-1,46308005	-0,38342335	0,262065874	0,76032138 13,467626
120	hsa-miR-99b	<u>hsa-miR-99b</u>	-1,454017884	-0,48856399	0,336009614	0,73008171 13,467626
26	hsa-miR-125b	<u>hsa-miR-125b</u>	-1,442119416	-0,52412424	0,363440246	0,7200251 13,467626
16	hsa-miR-100	<u>hsa-miR-100</u>	-1,375492355	-0,54318835	0,394904663	0,71003751 14,388489
112	hsa-miR-515-5p	<u>hsa-miR-515-5p</u>	-1,363339572	-0,73965175	0,542529366	0,55193365 14,388489
89	hsa-miR-335	<u>hsa-miR-335</u>	-1,29589199	-0,64808235	0,500105224	0,68315652 14,388489
114	hsa-miR-518b	<u>hsa-miR-518b</u>	-1,228635772	-0,58333506	0,474782739	0,71938929 15,24114
12	hsa-let-7c	<u>hsa-let-7c</u>	-1,175642329	-0,5588639	0,475368987	0,68801083 22,31478

Table 9

Gene Symbol	Description
CEBPB	CCAAT/enhancer-binding protein beta (C/EBP beta) (Nuclear factor NF- IL6) (Transcription factor 5).
BCL11A	B-cell lymphoma/leukemia 11A (B-cell CLL/lymphoma 11A) (COUP-TF- interacting protein 1) (Ecotropic viral integration site 9 protein)
ETS1	C-ets-1 protein (p54)
ITK	Tyrosine-protein kinase ITK/TSK (EC 2.7.10.2) (T-cell-specific kinase) (Tyrosine-protein kinase Lck) (Kinase EMT).
SP3	Transcription factor Sp3 (SPR-2)
CSF1R	Macrophage colony-stimulating factor 1 receptor precursor (EC 2.7.10.1) (CSF-1-R) (Fms proto-oncogene) (c-fms) (CD115 antigen)
PCDH9	Protocadherin-9 precursor
SOCS6	Suppressor of cytokine signaling 6 (SOCS-6) (Suppressor of cytokine signaling 4) (SOCS-4)
RAPGEF4	Rap guanine nucleotide exchange factor 4 (cAMP-regulated guanine nucleotide exchange factor II) (cAMP-GEFII)
RAB6A	Ras-related protein Rab-6A (Rab-6). [Source: Uniprot/SWISSPROT;Acc:P20340]
SOCS1	Suppressor of cytokine signaling 1 (SOCS-1) (JAK-binding protein) (JAB) (STAT-induced STAT inhibitor 1) (SSI-1)
GDF6	Growth/differentiation factor 6 precursor (GDF-6) (Growth/differentiation factor 16)
FGF7	Keratinocyte growth factor precursor (KGF) (Fibroblast growth factor 7) (FGF-7) (HBGF-7)
CTLA4	Cytotoxic T-lymphocyte protein 4 precursor (Cytotoxic T-lymphocyte-associated antigen 4) (CTLA-4) (CD152 antigen)
NFAT5	Nuclear factor of activated T-cells 5 (T-cell transcription factor NFAT5) (NF-AT5)

Table 10

Claims

1. A method of diagnosing an inflammatory skin disorder in an individual comprising;

5 determining the expression of one or more of the miRNAs selected from the group consisting of miR-203, miR-155, miR-21, miR-125b, miR-31, miR-146a, miR-30c, miR-142-3p, miR-17-5p, miR-205, miR-99b, miR-193a, miR-133a, miR-326, let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-200a, miR-9, miR-10a, 10 miR-10b, miR-15a, miR-15b, miR-16, miR-20a, miR-22, miR-23a, miR-23b, miR-24, miR-26a, miR-26b, miR-27a, miR-27b, miR-29a, miR-29c, miR-30a-5p, miR-30a-3p, miR-30b, miR-30d, miR-30e-5p, miR-30e-3p, miR-92, miR-93, miR-95, miR-99a, miR-100, miR-101, miR-106a, miR-106b, miR-107, miR-122a, miR-125a, miR-126, miR-127, miR-130a, miR-15 130b, miR-132, miR-133b, miR-135b, miR-140, miR-141, miR-142-5p, miR-143, miR-145, miR-146b, miR-148a, miR-148b, miR-149, miR-152, miR-181d, miR-182, miR-186, miR-187, miR-191, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-199a, miR-199b, miR-200b, miR-200c, miR-204, miR-214, miR-218, miR-222, miR-223, miR-224, miR-20 301, miR-320, miR-324-5p, miR-328, miR-331, miR-335, miR-361, miR-362, miR-365, miR-375, miR-381, miR-383, miR-411, miR-422b, miR-425, miR-432, miR-451, miR-452, miR-483, miR-486, miR-487b, miR-492, miR-497, miR-501, miR-515-5p, miR-516-5p, miR-518b, miR-519d, miR-524* and miR-526b, miR-532, miR-615 and miR-660 in a sample of skin cells 25 obtained from the individual,

wherein a change in expression of the one or more miRNAs in the sample relative to controls is indicative that the individual has an inflammatory skin disorder.

□□

30

2. A method according to claim 1 comprising

determining the expression of one or more of the miRNAs selected from the group consisting of miR-155, miR-21, miR-31, miR-20a, miR-146a, miR-20a, miR-17-5p, miR-193a, miR-146b, miR-146a, 35 miR-222, miR-142-3p, let-7i, miR-106a, miR-200a, miR-30e-5p, miR-203, miR-27b, miR-141, miR-130a, miR-24, miR-199b, miR-199a, miR-

106b, miR-199a*, miR-15a, miR-451, miR-29a, miR-422b, miR-205, miR-361, miR-487b, miR-107, miR-451, miR-16, and miR-27a in the sample,

wherein an increase in the expression of one or more of these miRNAs in the sample relative to controls is indicative that the

5 individual has an inflammatory skin disorder

3. A method according to claim 2 comprising determining the expression of miR-155 and/or miR-21.

10 4. A method according to any one of claims 1 to 3 comprising determining the expression of one or more of the miRNAs selected from the group consisting of miR-122a, miR-133a-133b, miR-197, miR-326, miR-133b, miR-524*, miR-215, miR-194, miR-1, let-7e, miR-381, miR-483, miR-10a, miR-365, miR-492, miR-99b, miR-125b, miR-15 100, miR-515-5p, miR-335, miR-518b and let-7c in the sample,

wherein a decrease in the expression of one or more of these miRNAs in the sample relative to controls is indicative that the individual has an inflammatory skin disorder.

20 5. A method according to claim 4 comprising determining the expression of miR-125b.

6. A method according to any one of the preceding claims wherein the inflammatory skin disorder is psoriasis or atopic eczema.

25

7. A method according to claim 1 wherein inflammatory skin disorder is psoriasis.

8. A method according to claim 7 comprising
30 determining the expression of one or more miRNAs selected from the group consisting of miR-203, miR-20a, miR-146a, miR-146b, miR-31, miR-146a, miR-20a, miR-200a, miR-17-5p, miR-30e-5p, miR-141, miR-142-3p, miR-21, miR-106a, miR-487b, miR-15a, let-7i, miR-222, miR-422b, miR-130a, miR-193a, miR-106b, miR-199b, miR-199a*, miR-

27b, miR-135b, miR-205, miR-155, miR-223, miR-93, miR-132, miR-425, miR-362, miR-324-5p, miR-224, miR-432 and miR-301 in the sample,

wherein an increase in expression of the one or more miRNAs in the sample relative to controls is indicative that the individual has psoriasis.

9. A method according to claim 8 comprising determining the expression of miR-203 in the sample.

10. A method according to any one of claims 7 to 9 comprising; determining the expression of one or more miRNAs selected from the group consisting of miR-125b, miR-99b, miR-122a, miR-197, miR-100, miR-381, miR-518b, miR-524*, let-7e, miR-30c, miR-365, miR-133b, miR-10a, miR-133a-133b, miR-22, miR-326, miR-215, miR-516-5p, let-7c, let-7d, miR-335, miR-492, miR-1, miR-519d, miR-10b, miR-483, miR-194, miR-526b, miR-30a-3p, miR-195, miR-30a-5p, miR-30e-5p, miR-99a, miR-193b, miR-149, miR-26a, miR-26b, miR-218, miR-30e-3p, miR-23b, let-7g, miR-411, miR-199b, miR-29c, miR-101, miR-375, miR-214, miR-375, miR-181d, miR-125a, miR-140, miR-30b, miR-152, miR-328, miR-497, miR-130a, miR-127, miR-148b, miR-186, miR-143, miR-145, miR-30d, miR-126, miR-199a, miR-196b, miR-486, miR-365, miR-29a, miR-320, miR-361, miR-95, miR-532, miR-331, miR-199a, miR-196a, miR-451, miR-193a, miR-126, miR-23a, miR-660, let-7b, miR-16, miR-182, miR-27a, miR-383, let-7a and miR-191 in the sample,

wherein a decrease in the expression of the one or more miRNAs in the sample relative to controls is indicative that the individual has psoriasis.

11. A method according to claim 1 wherein inflammatory skin disorder is atopic eczema.

12. A method according to claim 11 comprising determining the expression of one or more miRNAs selected from the group consisting of miR-203, miR-146a, let-7i, miR-29a, miR-222, miR-24, miR-193a, miR-199a, miR-27a, miR-21, miR-20a, miR-17-5p,

miR-106b, miR-142-3p, miR-30e-5p, miR-107, miR-200a, miR-146b, miR-27b, miR-199b, miR-106a, miR-101, miR-451, miR-130a, miR-451, miR-141, miR-31, miR-223, miR-199a*, miR-200c, miR-155, miR-16, miR-361, miR-205, miR-143, miR-422b, miR-200b, miR-15b, miR-135b, miR-187,
 5 miR-142-5p, miR-501, miR-487b, miR-362, miR-301, miR-130b, and miR-146b,

wherein an increase in expression of the one or more miRNAs in the sample relative to controls is indicative that the individual has atopic eczema.

10

13. A method according to claim 11 or claim 12 comprising

determining the expression of one or more miRNAs selected from the group consisting of miR-122a, miR-133a-133b, miR-326, miR-215, miR-483, miR-519d, miR-335, miR-133b, miR-515-5p, miR-194, miR-524*,
 15 miR-197 miR-99a, miR-383, miR-125b, miR-193b, miR-10a, miR-30a, miR-100, miR-328, miR-375, let-7c, miR-149, let-7a, miR-130a, miR-196b, miR-197, miR-26b, miR-30e-3p, miR-214, let-7b, miR-26a, miR-101, miR-30c, miR-195, let-7f, miR-148a, miR-125a, miR-30e-5p, miR-29c, miR-199b, miR-365, let-7d, miR-204, miR-23a, miR-196a, miR-452, miR-
 20 143, miR-199a, miR-145, miR-23b, miR-486, miR-92, miR-615, miR-497, miR-99b, miR-9, miR-10b, miR-1 and miR-451,

wherein a decrease in expression relative to controls is indicative that the individual has atopic eczema.

25 14. A method of treatment of an inflammatory skin disorder in an individual comprising;

increasing or reducing the expression or activity of one or more miRNAs selected from the group consisting of of miR-203, miR-155, miR-21, miR-125b, miR-31, miR-146a, miR-30c, miR-142-3p, miR-
 30 17-5p, miR-205, miR-99b, miR-193a, miR-133a, miR-326, let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-9, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-20a, miR-22, miR-23a, miR-23b, miR-24, miR-26a, miR-26b, miR-27a, miR-27b, miR-29a, miR-29c, miR-30a-5p, miR-30a-3p, miR-30b, miR-30d, miR-30e-5p, miR-
 35 30e-3p, miR-92, miR-93, miR-95, miR-99a, miR-100, miR-101, miR-106a,

miR-106b, miR-107, miR-122a, miR-125a, miR-126, miR-127, miR-130a, miR-130b, miR-132, miR-133b, miR-135b, miR-140, miR-141, miR-142-5p, miR-143, miR-145, miR-146b, miR-148a, miR-148b, miR-149, miR-152, miR-181d, miR-182, miR-186, miR-187, miR-191, miR-193b, miR-194, 5 miR-195, miR-196a, miR-196b, miR-197, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-204, miR-214, miR-218, miR-222, miR-223, miR-224, miR-301, miR-320, miR-324-5p, miR-328, miR-331, miR-335, miR-361, miR-362, miR-365, miR-375, miR-381, miR-383, miR-411, miR-422b, miR-425, miR-432, miR-451, miR-452, miR-483, miR-486, miR-10 487b, miR-492, miR-497, miR-501, miR-515-5p, miR-516-5p, miR-518b, miR-519d, miR-524*, miR-526b, miR-532, miR-615 and miR-660 in cells of the individual.

15. A method according to claim 14 comprising;
15 reducing the expression or activity of one or more target miRNAs selected from the group consisting of miR-203, miR-20a, miR-146a, miR-20a, miR-31, miR-21, miR-17-5p, miR-193a, miR-146b, miR-146a, miR-222, miR-142-3p, let-7i, miR-106a, miR-200a, miR-30e-5p, miR-27b, miR-141, miR-130a, miR-24, miR-199b, miR-199a, miR-106b, 20 miR-199a*, miR-15a, miR-451, miR-29a, miR-422b, miR-205, miR-361, miR-487b, miR-107, miR-451, miR-16, miR-27a and miR-155 in the cells. □□

16. A method according to claim 15 comprising reducing the
25 expression or activity of miR-21.

17. A method according to claim 15 or claim 16 wherein the inflammatory skin disorder is psoriasis or atopic eczema.

30 18. A method according to claim 14 wherein inflammatory skin disorder is psoriasis.

19. A method according to claim 18 comprising;
reducing the expression or activity of one or more target
35 miRNAs selected from the group consisting of miR-203, miR-20a, miR-

146a, miR-146b, miR-31, miR-146a, miR-20a, miR-200a, miR-17-5p, miR-30e-5p, miR-141, miR-142-3p, miR-21, miR-106a, miR-487b, miR-15a, let-7i, miR-222, miR-422b, miR-130a, miR-193a, miR-106b, miR-199b, miR-199a*, miR-27b, miR-135b, miR-205, miR-155, miR-223, miR-93, 5 miR-132, miR-425, miR-362, miR-324-5p, miR-224, miR-432 and miR-301 in the cells.

20. A method according to claim 19 comprising
reducing the expression or activity of miR-203 and/or miR-10 146a.

21. A method according to claim 14 wherein inflammatory skin disorder is atopic eczema.

22. A method according to claim 21 comprising;
reducing the expression or activity of one or more target miRNAs selected from the group consisting of miR-203, miR-146a, let-7i, miR-29a, miR-222, miR-24, miR-193a, miR-199a, miR-27a, miR-21, miR-20a, miR-17-5p, miR-106b, miR-142-3p, miR-30e-5p, miR-107, miR-20 200a, miR-146b, miR-27b, miR-199b, miR-106a, miR-101, miR-451, miR-130a, miR-451, miR-141, miR-31, miR-223, miR-199a*, miR-200c, miR-155, miR-16, miR-361, miR-205, miR-143, miR-422b, miR-200b, miR-15b miR-135b, miR-187, miR-142-5p, miR-501, miR-487b, miR-362, miR-301, miR-130b, and miR-146b.

23. A method according to any one of claims 14 to 22 wherein the expression or activity of one or more target miRNAs is reduced by administering a therapeutically effective amount of an miRNA inhibitor to the individual.

24. A method according to claim 23 wherein the miRNA inhibitor is a sense or anti-sense oligonucleotide which inhibits the activity or production of the target miRNA or increase its rate of depletion

25. A method according to claim 14 comprising;

increasing the expression or activity of one or more target miRNAs selected from the group consisting of miR-122a, miR-133a-133b, miR-197, miR-326, miR-133b, miR-524*, miR-215, miR-194, miR-1, let-7e, miR-381, miR-483, miR-10a, miR-365, miR-492, miR-99b, miR-125b, miR-100, miR-515-5p, miR-335, miR-518b and let-7c in the cells.

26. A method according to claim 25 comprising increasing the expression or activity of miR-125b.

27. A method according to any one of claims 25 to 26 wherein the inflammatory skin disorder is psoriasis or atopic eczema.

28. A method according to claim 14 wherein the inflammatory skin disorder is psoriasis.

29. A method according to claim 28 comprising increasing the amount or activity of one or target miRNAs selected from the group consisting of miR-125b, miR-99b, miR-122a, miR-197, miR-100, miR-381, miR-518b, miR-524*, let-7e, miR-30c, miR-365, miR-133b, miR-10a, miR-133a-133b, miR-22, miR-326, miR-215, miR-516-5p, let-7c, let-7d, miR-335, miR-492, miR-1, miR-519d, miR-10b, miR-483, miR-194, miR-526b, miR-30a-3p, miR-195, miR-30a-5p, miR-30e-5p, miR-99a, miR-193b, miR-149, miR-26a, miR-26b, miR-218, miR-30e-3p, miR-23b, let-7g, miR-411, miR-199b, miR-29c, miR-101, miR-375, miR-214, miR-375, miR-181d, miR-125a, miR-140, miR-30b, miR-152, miR-328, miR-497, miR-130a, miR-127, miR-148b, miR-186, miR-143, miR-145, miR-30d, miR-126, miR-199a, miR-196b, miR-486, miR-365, miR-29a, miR-320, miR-361, miR-95, miR-532, miR-331, miR-199a, miR-196a, miR-451, miR-193a, miR-126, miR-23a, miR-660, let-7b, miR-16, miR-182, miR-27a, miR-383, let-7a and miR-191 in the cells.

30. A method according to claim 14 wherein the inflammatory skin disorder is atopic eczema.

31. A method according to claim 30 comprising increasing the amount or activity of one or more target miRNAs selected from the group consisting of miR-122a, miR-133a-133b, miR-326, miR-215, miR-483, miR-519d, miR-335, miR-133b, miR-515-5p, miR-194, miR-524*,
5 miR-197, miR-99a, miR-383, miR-125b, miR-193b, miR-10a, miR-30a, miR-100, miR-328, miR-375, let-7c, miR-149, let-7a, miR-130a, miR-196b, miR-197, miR-26b, miR-30e-3p, miR-214, let-7b, miR-26a, miR-101, miR-30c, miR-195, let-7f, miR-148a, miR-125a, miR-30e-5p, miR-29c, miR-199b, miR-365, let-7d, miR-204, miR-23a, miR-196a, miR-452,
10 miR-143, miR-199a, miR-145, miR-23b, miR-486, miR-92, miR-615, miR-497, miR-99b, miR-9, miR-10b, miR-1 and miR-451 in the cells.

32. A method according to any one of claims 25 to 31 wherein the expression or activity of the target miRNA is increased by
15 administering to an individual in need thereof a therapeutically effective amount of;

- (i) the target miRNA or a precursor thereof,
- (ii) a nucleic acid encoding the target miRNA or a precursor thereof,
- 20 (iii) an analogue, derivative or modified form of the target miRNA which retains activity.

33. Use of an inhibitor of an miRNA selected from the group consisting of miR-203, miR-20a, miR-146a, miR-20a, miR-31, miR-21,
25 miR-17-5p, miR-193a, miR-146b, miR-146a, miR-222, miR-142-3p, let-7i, miR-106a, miR-200a, miR-30e-5p, miR-27b, miR-141, miR-130a, miR-24, miR-199b, miR-199a, miR-106b, miR-199a*, miR-15a, miR-451, miR-29a, miR-422b, miR-205, miR-361, miR-487b, miR-107, miR-451, miR-16, miR-27a and miR-155 in the manufacture of a medicament for use a
30 method of treatment of an inflammatory skin disorder.

34. Use according to claim 33 wherein the method is a method according to any one of claims 14 to 24.

35. Use according to claim 33 or claim 34 wherein the miRNA inhibitor is a sense or anti-sense oligonucleotide which inhibits the activity or production of the target miRNA or increase its rate of depletion.

5

36. Use of an miRNA selected from the group consisting of miR-122a, miR-133a-133b, miR-197, miR-326, miR-133b, miR-524*, miR-215, miR-194, miR-1, let-7e, miR-381, miR-483, miR-10a, miR-365, miR-492, miR-99b, miR-125b, miR-100, miR-515-5p, miR-335, miR-518b and let-7c
10 or a precursor thereof, a nucleic acid encoding the miRNA or precursor thereof, or an analogue, derivative or modified form of the miRNA in the manufacture of a medicament for use a method of treatment of an inflammatory skin disorder.

15 37. Use according to claim 36 wherein the method is a method according to claim 14 or any one of claims 25 to 32.

38. A method of screening for a compound useful in the treatment of an inflammatory skin disorder comprising;

20 contacting a cell with a test compound and;

determining the expression of one or more miRNAs selected from the group consisting of of miR-203, miR-155, miR-21, miR-125b, miR-31, miR-146a, miR-30c, miR-142-3p, miR-17-5p, miR-205, miR-99b, miR-193a, miR-133a, miR-326, let-7a, let-7b, let-7c, let-7d, let-7e,
25 let-7f, let-7g, let-7i, miR-1, miR-9, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-20a, miR-22, miR-23a, miR-23b, miR-24, miR-26a, miR-26b, miR-27a, miR-27b, miR-29a, miR-29c, miR-30a-5p, miR-30a-3p, miR-30b, miR-30d, miR-30e-5p, miR-30e-3p, miR-92, miR-93, miR-95, miR-99a, miR-100, miR-101, miR-106a, miR-106b, miR-107, miR-122a,
30 miR-125a, miR-126, miR-127, miR-130a, miR-130b, miR-132, miR-133b, miR-135b, miR-140, miR-141, miR-142-5p, miR-143, miR-145, miR-146b, miR-148a, miR-148b, miR-149, miR-152, miR-181d, miR-182, miR-186, miR-187, miR-191, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-204,
35 miR-214, miR-218, miR-222, miR-223, miR-224, miR-301, miR-320, miR-

324-5p, miR-328, miR-331, miR-335, miR-361, miR-362, miR-365, miR-375, miR-381, miR-383, miR-411, miR-422b, miR-425, miR-432, miR-451, miR-452, miR-483, miR-486, miR-487b, miR-492, miR-497, miR-501, miR-515-5p, miR-516-5p, miR-518b, miR-519d, miR-524*, miR-526b, miR-532, miR-615, and miR-660 relative to controls,

wherein an increase or decrease in expression in the presence of the test compound is indicative that the compound is useful in the treatment of an inflammatory skin disorder.

39. A method according to claim 38 comprising

determining the expression of one or more miRNAs selected from the group consisting of miR-203, miR-20a, miR-146a, miR-20a, miR-31, miR-21, miR-17-5p, miR-193a, miR-146b, miR-146a, miR-222, miR-142-3p, let-7i, miR-106a, miR-200a, miR-30e-5p, miR-27b, miR-141, miR-130a, miR-24, miR-199b, miR-199a, miR-106b, miR-199a*, miR-15a, miR-451, miR-29a, miR-422b, miR-205, miR-361, miR-487b, miR-107, miR-451, miR-16, miR-27a and miR-155 in the cell,

wherein a decrease in expression in the presence of the test compound relative to its absence is indicative that the compound is useful in the treatment of an inflammatory skin disorder.

40. A method according to claim 39 comprising determining the expression of miR-21 and/or miR-155,

wherein a decrease in expression in the presence of the test compound relative to its absence is indicative that the compound is useful in the treatment of an inflammatory skin disorder.

41. A method according to claim 38 comprising

determining the expression of one or more miRNAs selected from the group consisting of miR-122a, miR-133a-133b, miR-197, miR-326, miR-133b, miR-524*, miR-215, miR-194, miR-1, let-7e, miR-381, miR-483, miR-10a, miR-365, miR-492, miR-99b, miR-125b, miR-100, miR-515-5p, miR-335, miR-518b and let-7c in the cell,

wherein an increase in expression of the one or more miRNAs in the presence of the test compound relative to its absence is

indicative that the compound is useful in the treatment of an inflammatory skin disorder.□□

42. A method according to claim 41 comprising
5 determining the expression of miR-125b,
wherein an increase in expression in the presence of the test compound relative to its absence is indicative that the compound is useful in the treatment of an inflammatory skin disorder.□□

10 43. A method according to any one of claims 38 to 42 wherein the inflammatory skin disorder is psoriasis or atopic eczema.

15 44. A method according to claim 38 wherein inflammatory skin disorder is psoriasis.

45. A method according to claim 44 comprising
determining the expression of one or more miRNAs selected from the group consisting of miR-203, miR-20a, miR-146a, miR-146b, miR-
20 31, miR-146a, miR-20a, miR-200a, miR-17-5p, miR-30e-5p, miR-141, miR-142-3p, miR-21, miR-106a, miR-487b, miR-15a, let-7i, miR-222, miR-422b, miR-130a, miR-193a, miR-106b, miR-199b, miR-199a*, miR-27b, miR-135b, miR-205, miR-155, miR-223, miR-93, miR-132, miR-425, miR-362, miR-324-5p, miR-224, miR-432 and miR-301 in the cell,
25 wherein a decrease in expression in the presence of the test compound relative to its absence is indicative that the compound is useful in the treatment of psoriasis.□□

□□

30 46. A method according to claim 44 or claim 45 comprising
determining the expression of one or more miRNAs selected from the group consisting of miR-125b, miR-99b, miR-122a, miR-197, miR-100, miR-381, miR-518b, miR-524*, let-7e, miR-30c, miR-365, miR-133b, miR-10a, miR-133a-133b, miR-22, miR-326, miR-215, miR-516-5p,
35 let-7c, let-7d, miR-335, miR-492, miR-1, miR-519d, miR-10b, miR-483, miR-194, miR-526b, miR-30a-3p, miR-195, miR-30a-5p, miR-30e-5p, miR-

99a, miR-193b, miR-149, miR-26a, miR-26b, miR-218, miR-30e-3p, miR-23b, let-7g, miR-411, miR-199b, miR-29c, miR-101, miR-375, miR-214, miR-375, miR-181d, miR-125a, miR-140, miR-30b, miR-152, miR-328, miR-497, miR-130a, miR-127, miR-148b, miR-186, miR-143, miR-145, miR-30d, miR-126, miR-199a, miR-196b, miR-486, miR-365, miR-29a, miR-320, miR-361, miR-95, miR-532, miR-331, miR-199a, miR-196a, miR-451, miR-193a, miR-126, miR-23a, miR-660, let-7b, miR-16, miR-182, miR-27a, miR-383, let-7a and miR-191 in the cell,

wherein an increase in expression in the presence of the test compound relative to its absence is indicative that the compound is useful in the treatment of treatment of psoriasis.

47. A method according to claim 38 wherein inflammatory skin disorder is atopic eczema.

48. A method according to claim 47 comprising;

determining the expression of one or more microRNAs selected from the group consisting of miR-203, miR-146a, let-7i, miR-29a, miR-222, miR-24, miR-193a, miR-199a, miR-27a, miR-21, miR-20a, miR-17-5p, miR-106b, miR-142-3p, miR-30e-5p, miR-107, miR-200a, miR-146b, miR-27b, miR-199b, miR-106a, miR-101, miR-451, miR-130a, miR-451, miR-141, miR-31, miR-223, miR-199a*, miR-200c, miR-155, miR-16, miR-361, miR-205, miR-143, miR-422b, miR-200b, miR-15b, miR-135b, miR-187, miR-142-5p, miR-501, miR-487b, miR-362, miR-301, miR-130b, and miR-146b in the cell,

wherein a decrease in expression in the presence of the test compound relative to its absence is indicative that the compound is useful in the treatment of atopic eczema.

49. A method according to claim 47 comprising

determining the expression of one or more miRNAs selected from the group consisting of miR-122a, miR-133a-133b, miR-326, miR-215, miR-483, miR-519d, miR-335, miR-133b, miR-515-5p, miR-194, miR-524*, miR-197, miR-99a, miR-383, miR-125b, miR-193b, miR-10a, miR-30a, miR-100, miR-328, miR-375, let-7c, miR-149, let-7a, miR-130a, miR-

196b, miR-197, miR-26b, miR-30e-3p, miR-214, let-7b, miR-26a, miR-101, miR-30c, miR-195, let-7f, miR-148a, miR-125a, miR-30e-5p, miR-29c, miR-199b, miR-365, let-7d, miR-204, miR-23a, miR-196a, miR-452, miR-143, miR-199a, miR-145, miR-23b, miR-486, miR-92, miR-615, miR-497, miR-99b, miR-9, miR-10b, miR-1 and miR-451 in the cell,

wherein an increase in expression in the presence of the test compound relative to its absence is indicative that the compound is useful in the treatment of treatment of atopic eczema.

50. A method according to any one of claims 38 to 49 comprising identifying said test compound as a compound useful in the treatment of an inflammatory skin disorder.

51. A method according to claim 50 further comprising determining the ability of said test compound to ameliorate the symptoms of an inflammatory skin disorder in a non-human animal model.

52. A method according to claim 50 or claim 51 comprising isolating and/or purifying said test compound.

53. A method according to any one of claims 50 to 53 comprising producing and/or synthesising said test compound.

54. A method according to any one of claims 50 to 53 comprising formulating said test compound in a pharmaceutical composition with a pharmaceutically acceptable excipient, vehicle or carrier.

55. A method for monitoring the treatment of individual with a DNA damaging therapy comprising:

(a) subjecting the individual to a regimen of therapy for an inflammatory skin disorder; and

(b) monitoring in one or more cells of the individual the expression of one or more of the miRNAs selected from the group consisting of miR-203, miR-155, miR-21, miR-125b, miR-31, miR-146a, miR-30c, miR-142-3p, miR-17-5p, miR-205, miR-99b, miR-193a, miR-

133a, miR-326, let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-200a, miR-9, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-20a, miR-22, miR-23a, miR-23b, miR-24, miR-26a, miR-26b, miR-27a, miR-27b, miR-29a, miR-29c, miR-30a-5p, miR-30a-3p, miR-30b, miR-30d, miR-30e-5p, miR-30e-3p, miR-92, miR-93, miR-95, miR-99a, miR-100, miR-101, miR-106a, miR-106b, miR-107, miR-122a, miR-125a, miR-126, miR-127, miR-130a, miR-130b, miR-132, miR-133b, miR-135b, miR-140, miR-141, miR-142-5p, miR-143, miR-145, miR-146b, miR-148a, miR-148b, miR-149, miR-152, miR-181d, miR-182, miR-186, miR-187, miR-191, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-199a, miR-199b, miR-200b, miR-200c, miR-204, miR-214, miR-218, miR-222, miR-223, miR-224, miR-301, miR-320, miR-324-5p, miR-328, miR-331, miR-335, miR-361, miR-362, miR-365, miR-375, miR-381, miR-383, miR-411, miR-422b, miR-425, miR-432, miR-451, miR-452, miR-483, miR-486, miR-487b, miR-492, miR-497, miR-501, miR-515-5p, miR-516-5p, miR-518b, miR-519d, miR-524* and miR-526b, miR-532, miR-615 and miR-660 during said treatment

wherein a change in expression is indicative that the regimen is efficacious in the individual.

56. A method according to claim 54 wherein inflammatory skin disorder is psoriasis.

57. A method according to claim 55 comprising monitoring in one or more cells of the individual the expression of one or more of the miRNAs selected from the group consisting of miR-203, miR-20a, miR-146a, miR-146b, miR-31, miR-146a, miR-20a, miR-200a, miR-17-5p, miR-30e-5p, miR-141, miR-142-3p, miR-21, miR-106a, miR-487b, miR-15a, let-7i, miR-222, miR-422b, miR-130a, miR-193a, miR-106b, miR-199b, miR-199a*, miR-27b, miR-135b, miR-205, miR-155, miR-223, miR-93, miR-132, miR-425, miR-362, miR-324-5p, miR-224, miR-432 and miR-301 in the sample,

wherein an decrease in expression of the one or more miRNAs in the sample after initiation of the regimen is indicative that the regimen is efficacious in the individual.

58. A method according to claim 55 comprising;

monitoring in one or more cells of the individual the expression of one or more of the miRNAs selected from the group consisting of miR-125b, miR-99b, miR-122a, miR-197, miR-100, miR-381, miR-518b, miR-524*, let-7e, miR-30c, miR-365, miR-133b, miR-10a, miR-133a-133b, miR-22, miR-326, miR-215, miR-516-5p, let-7c, let-7d, miR-335, miR-492, miR-1, miR-519d, miR-10b, miR-483, miR-194, miR-526b, miR-30a-3p, miR-195, miR-30a-5p, miR-30e-5p, miR-99a, miR-193b, miR-149, miR-26a, miR-26b, miR-218, miR-30e-3p, miR-23b, let-7g, miR-411, miR-199b, miR-29c, miR-101, miR-375, miR-214, miR-375, miR-181d, miR-125a, miR-140, miR-30b, miR-152, miR-328, miR-497, miR-130a, miR-127, miR-148b, miR-186, miR-143, miR-145, miR-30d, miR-126, miR-199a, miR-196b, miR-486, miR-365, miR-29a, miR-320, miR-361, miR-95, miR-532, miR-331, miR-199a, miR-196a, miR-451, miR-193a, miR-126, miR-23a, miR-660, let-7b, miR-16, miR-182, miR-27a, miR-383, let-7a and miR-191 in the sample,

wherein an increase in the expression of the one or more miRNAs in the sample after initiation of the regimen is indicative that the regimen is efficacious in the individual.

59. A method according to claim 55 wherein inflammatory skin disorder is atopic eczema.

60. A method according to claim 58 comprising

monitoring in one or more cells of the individual the expression of one or more of the miRNAs selected from the group consisting of miR-203, miR-146a, let-7i, miR-29a, miR-222, miR-24, miR-193a, miR-199a, miR-27a, miR-21, miR-20a, miR-17-5p, miR-106b, miR-142-3p, miR-30e-5p, miR-107, miR-200a, miR-146b, miR-27b, miR-199b, miR-106a, miR-101, miR-451, miR-130a, miR-451, miR-141, miR-31, miR-223, miR-199a*, miR-200c, miR-155, miR-16, miR-361, miR-205, miR-143, miR-422b,

miR-200b, miR-15b, miR-135b, miR-187, miR-142-5p, miR-501, miR-487b, miR-362, miR-301, miR-130b, and miR-146b,

wherein an decrease in expression of the one or more miRNAs in the sample after initiation of the regimen is indicative that the regimen is efficacious in the individual.

61. A method according to claim 58 or claim 59 comprising monitoring in one or more cells of the individual the expression of one or more of the miRNAs selected from the group consisting of miR-122a, miR-133a-133b, miR-326, miR-215, miR-483, miR-519d, miR-335, miR-133b, miR-515-5p, miR-194, miR-524*, miR-197 miR-99a, miR-383, miR-125b, miR-193b, miR-10a, miR-30a, miR-100, miR-328, miR-375, let-7c, miR-149, let-7a, miR-130a, miR-196b, miR-197, miR-26b, miR-30e-3p, miR-214, let-7b, miR-26a, miR-101, miR-30c, miR-195, let-7f, miR-148a, miR-125a, miR-30e-5p, miR-29c, miR-199b, miR-365, let-7d, miR-204, miR-23a, miR-196a, miR-452, miR-143, miR-199a, miR-145, miR-23b, miR-486, miR-92, miR-615, miR-497, miR-99b, miR-9, miR-10b, miR-1 and miR-451,

wherein an increase in expression of the one or more miRNAs in the sample after initiation of the regimen is indicative that the regimen is efficacious in the individual.

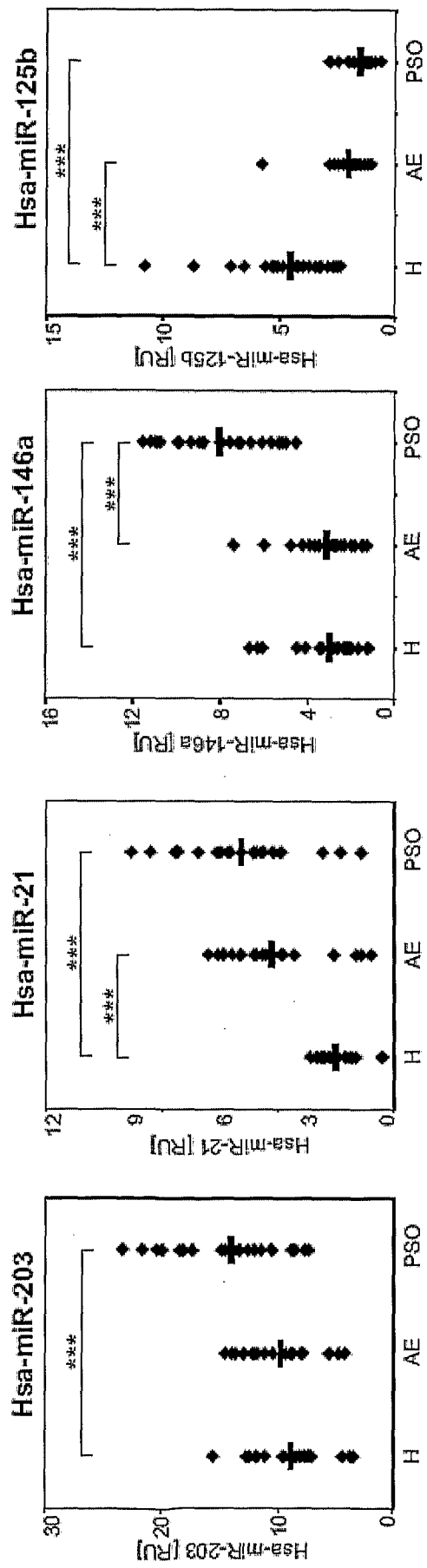


Figure 1

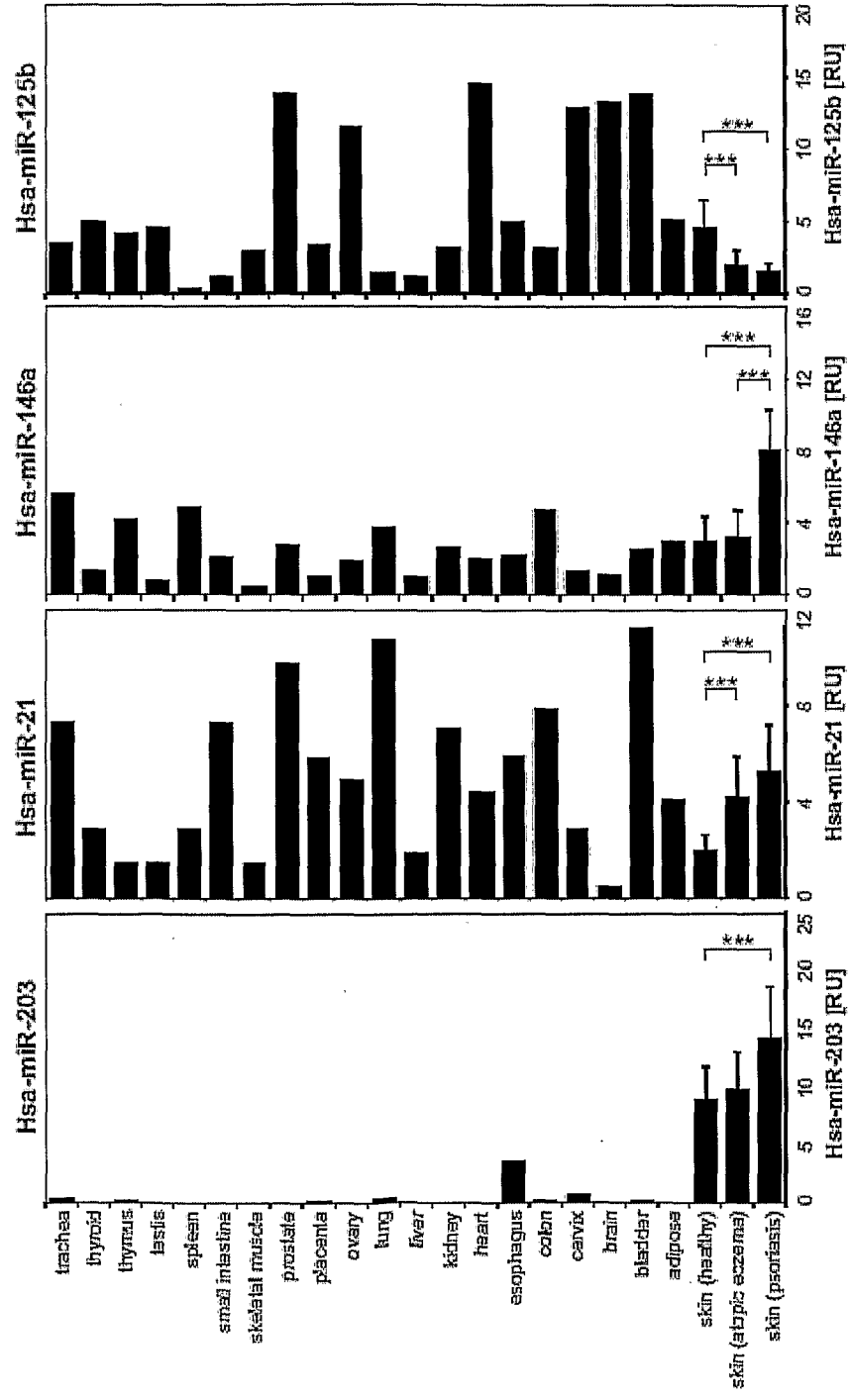


Figure 2

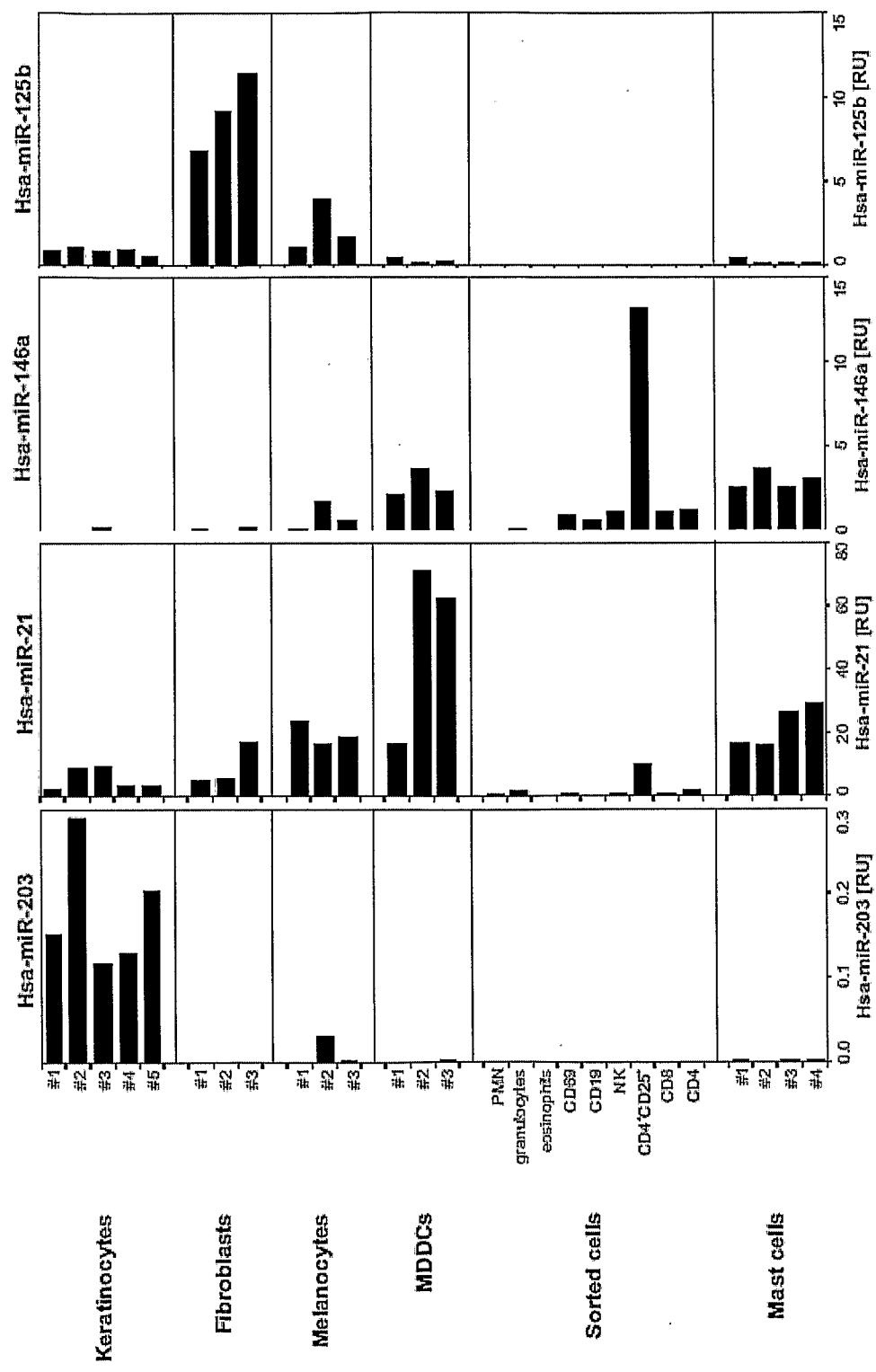


Figure 3

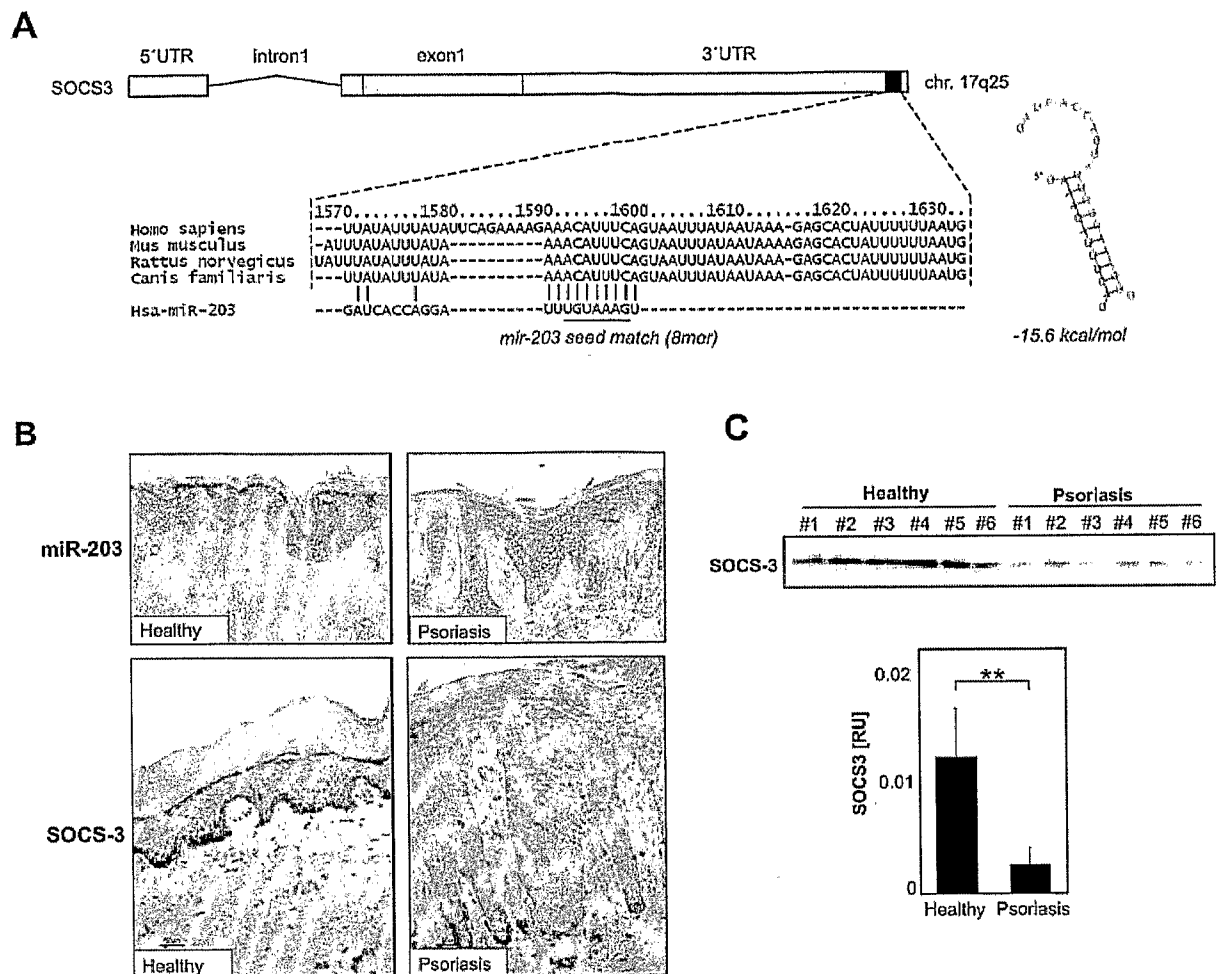


Figure 4

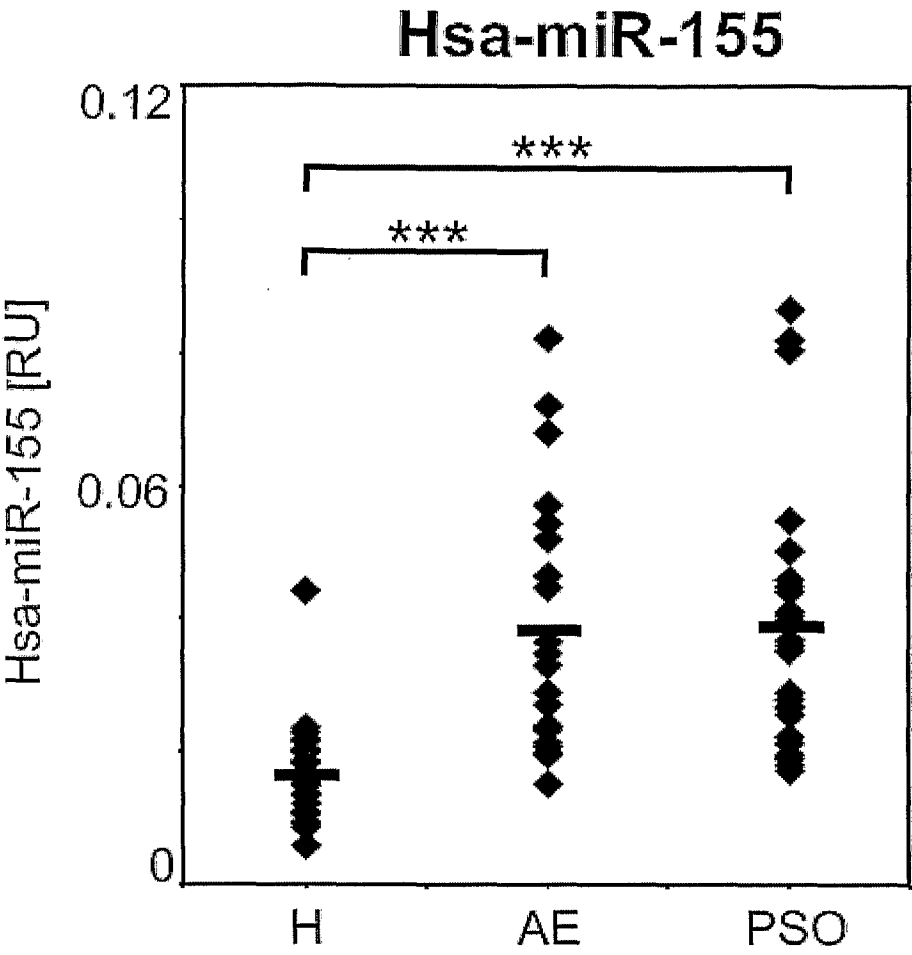


Figure 5

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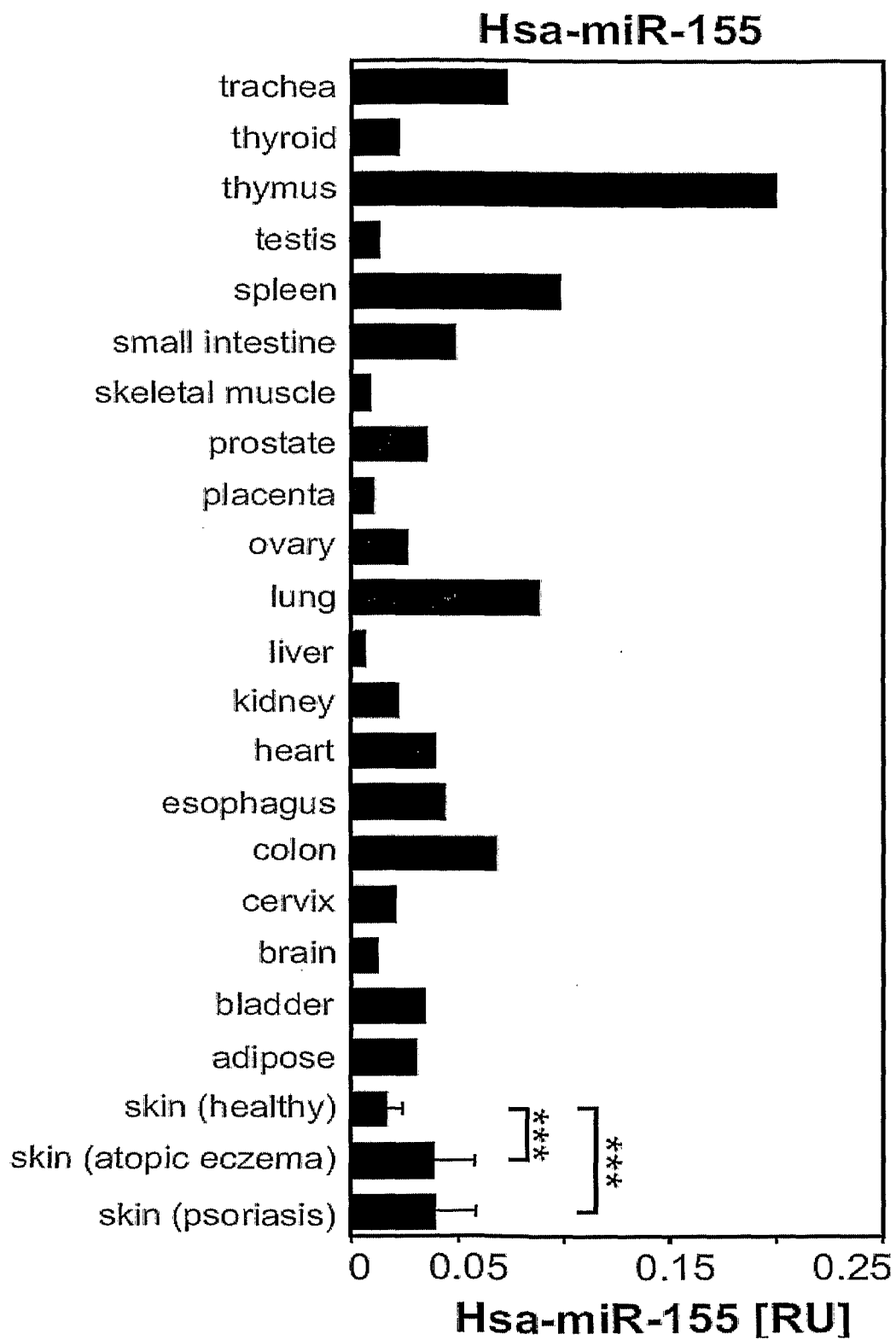


Figure 6

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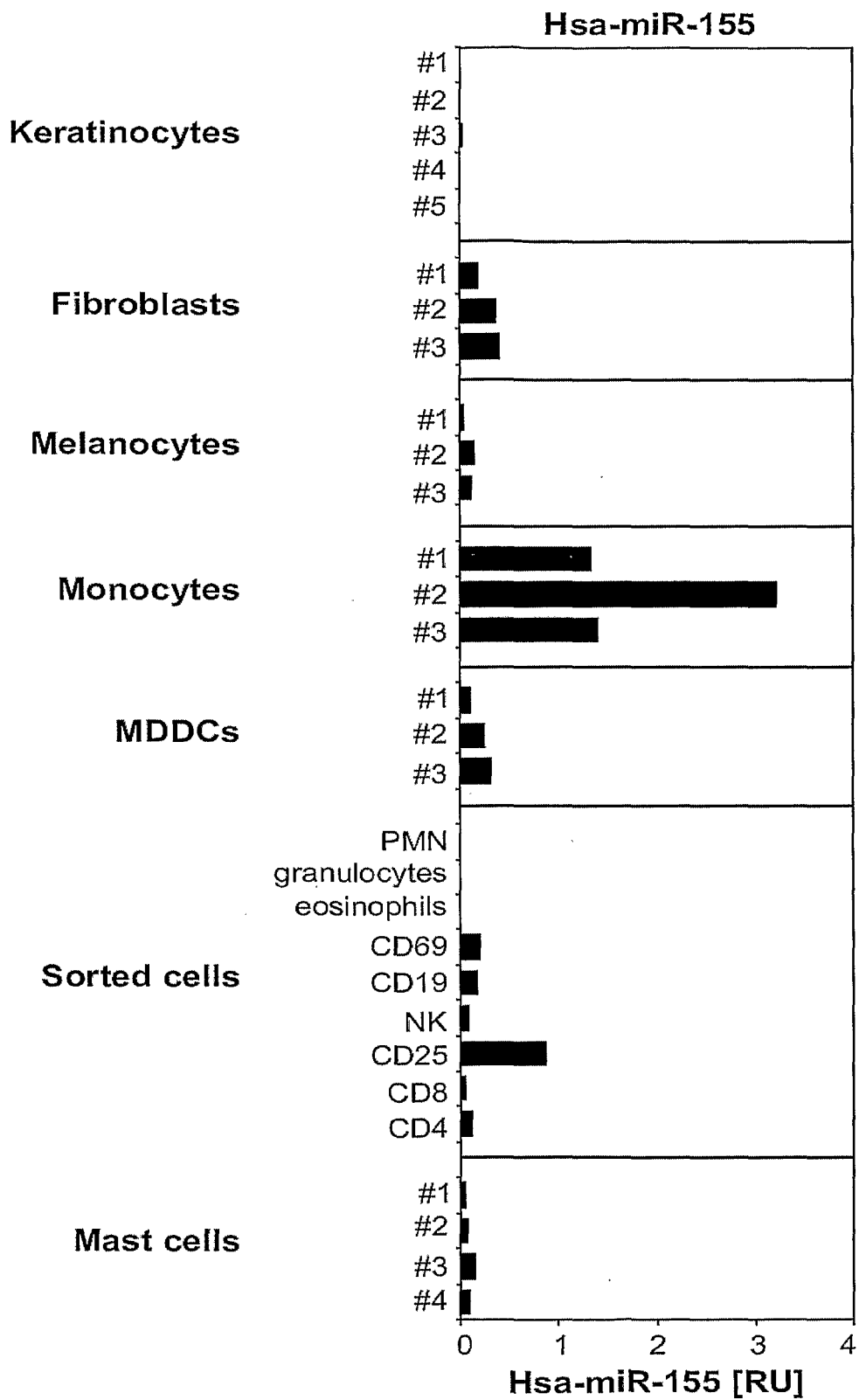


Figure 7

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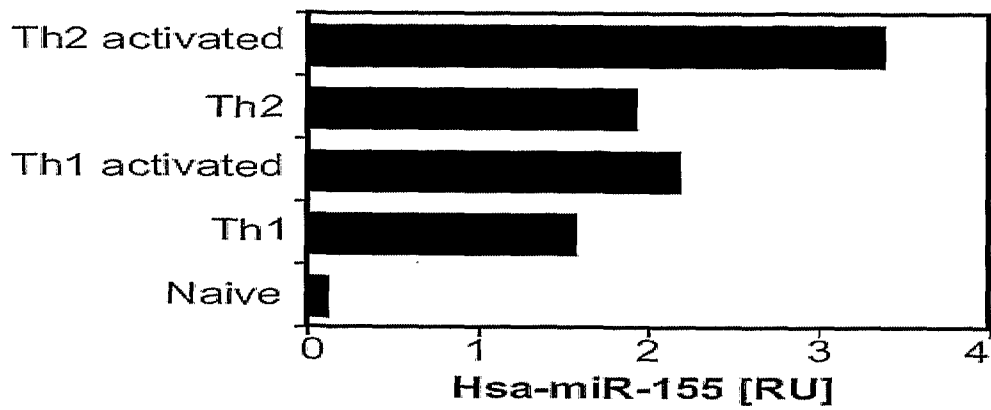


Figure 8

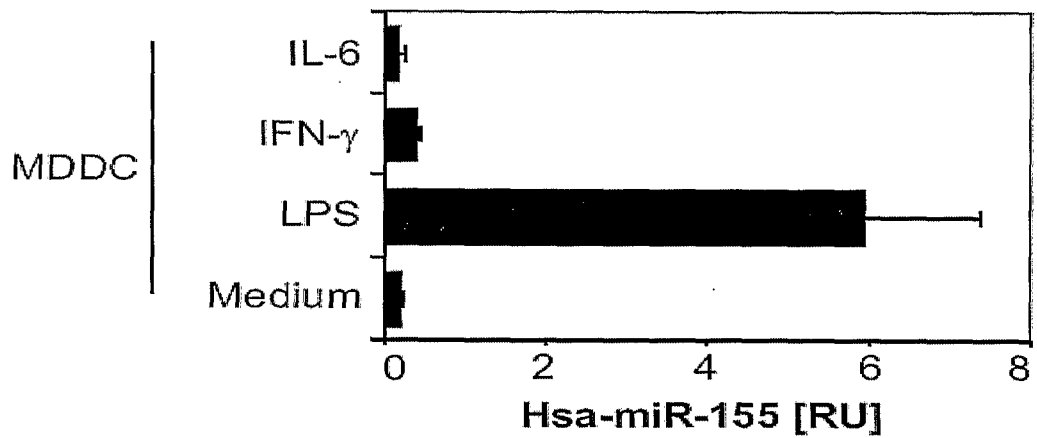


Figure 9

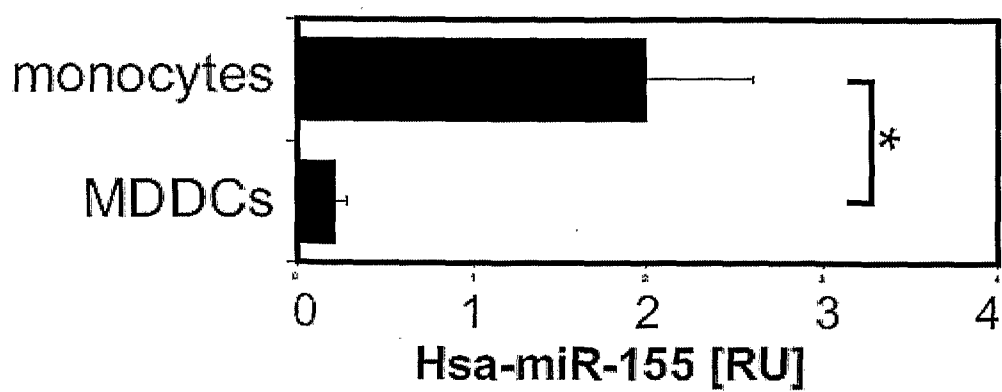


Figure 10

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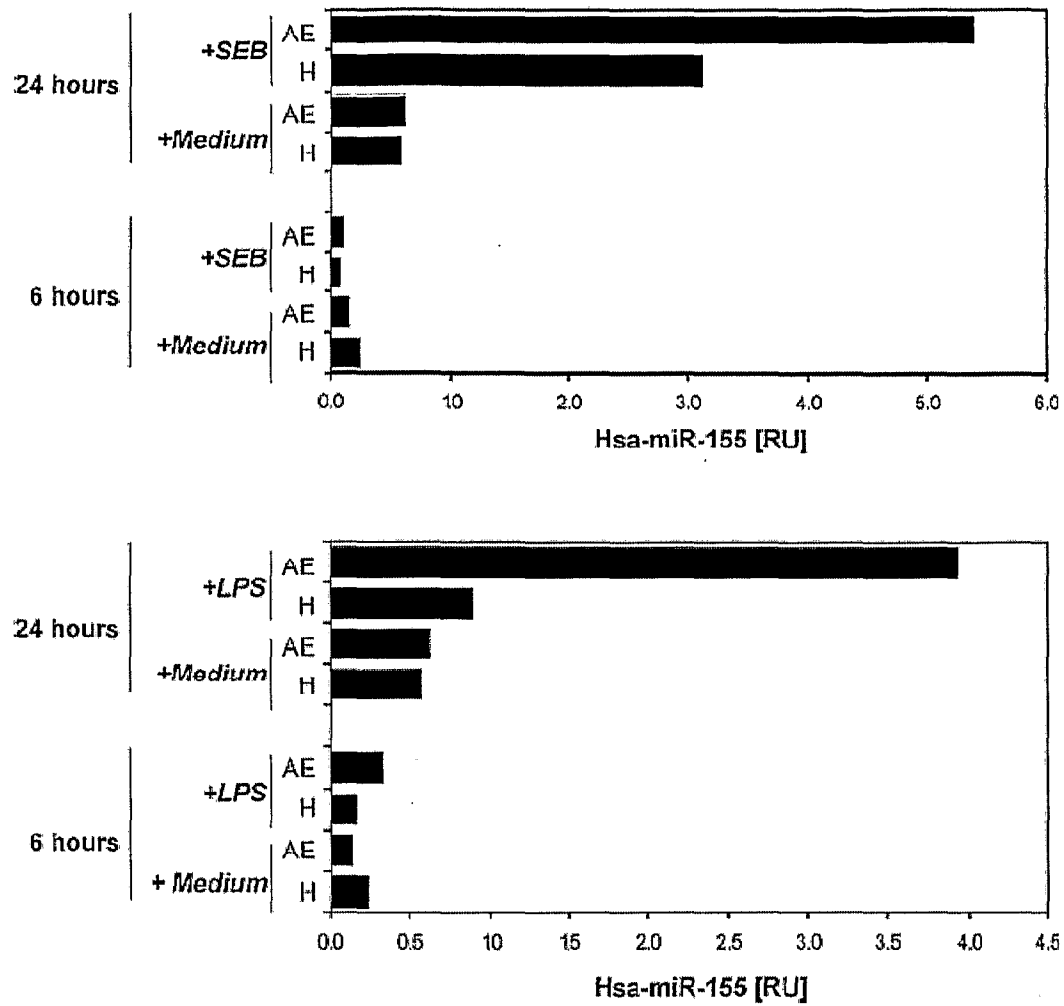


Figure 11

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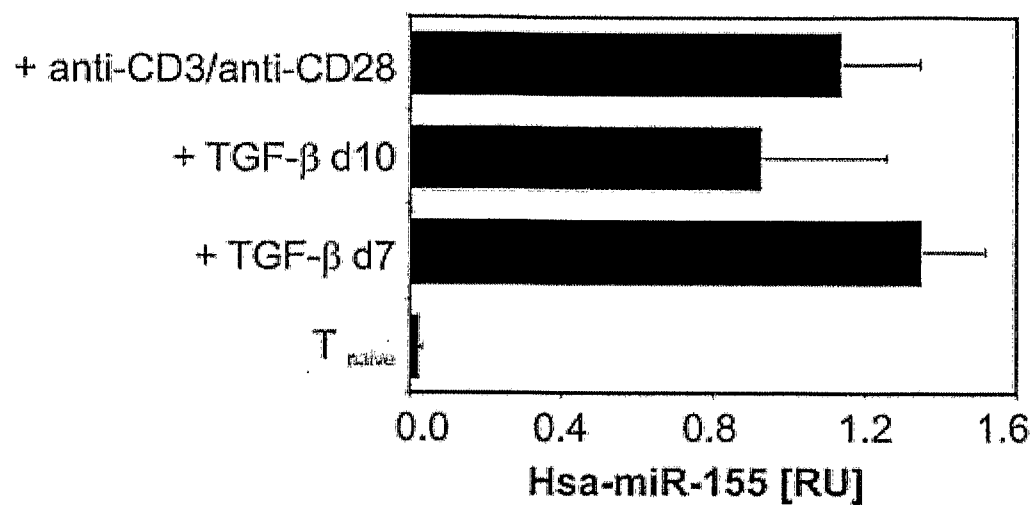
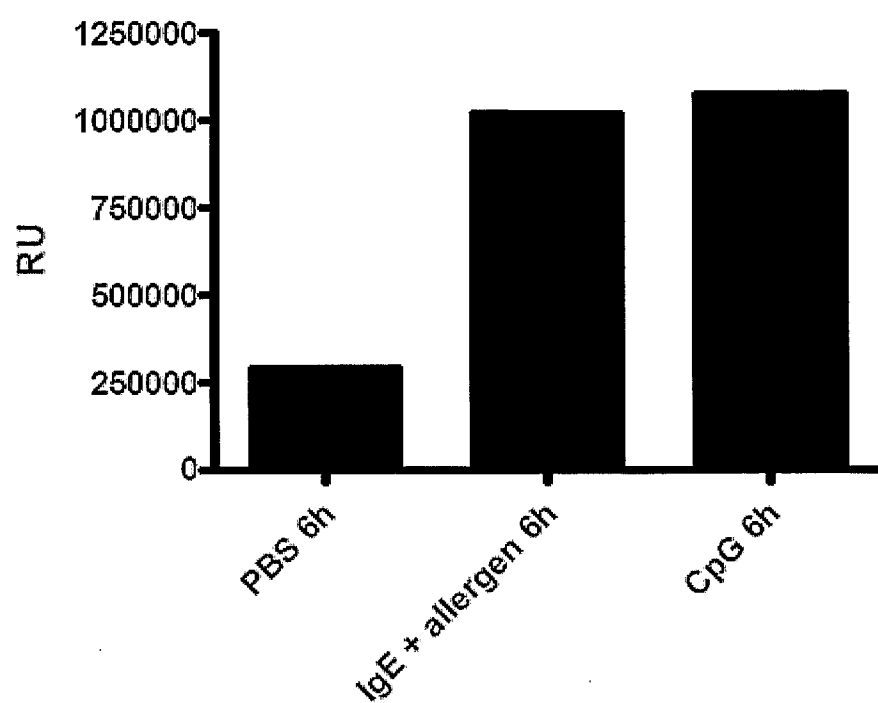


Figure 12

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Endogenous control: 5S

Figure 13

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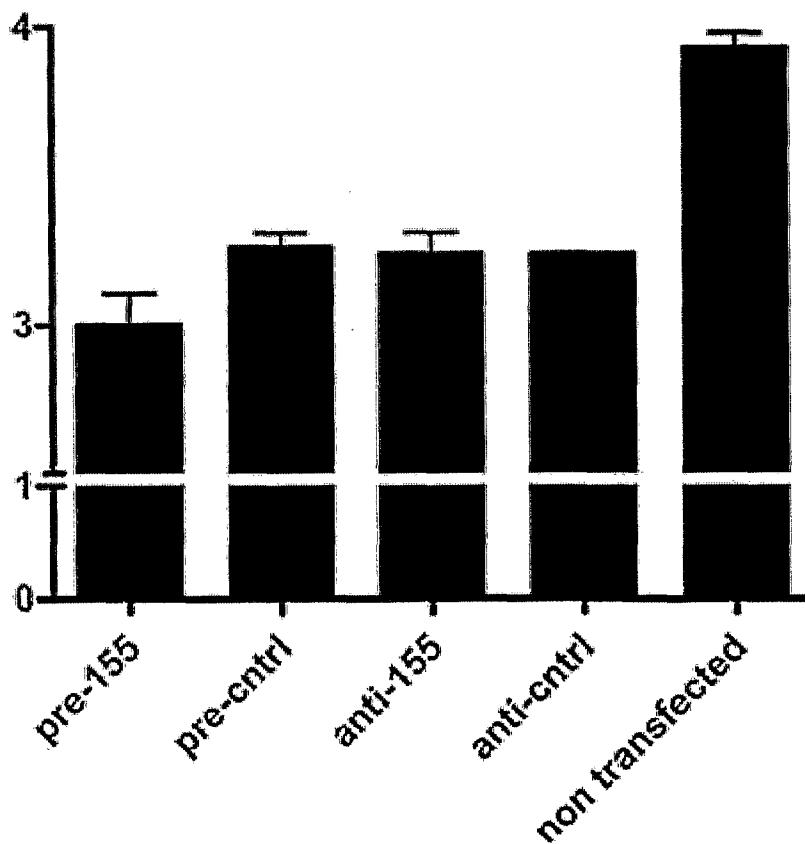
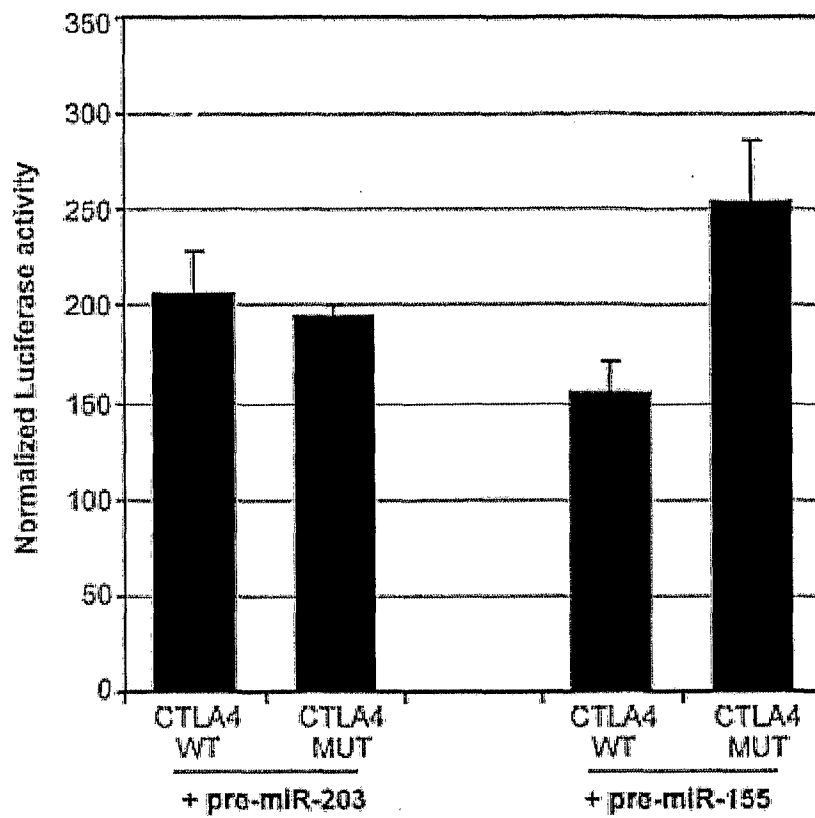


Figure 14

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Groups: Saline (32) OVA (32)

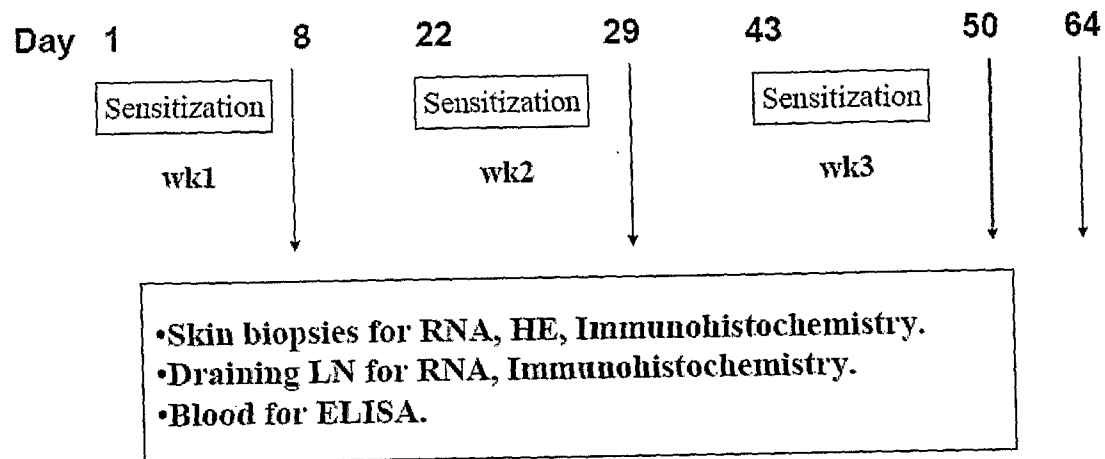


Figure 15

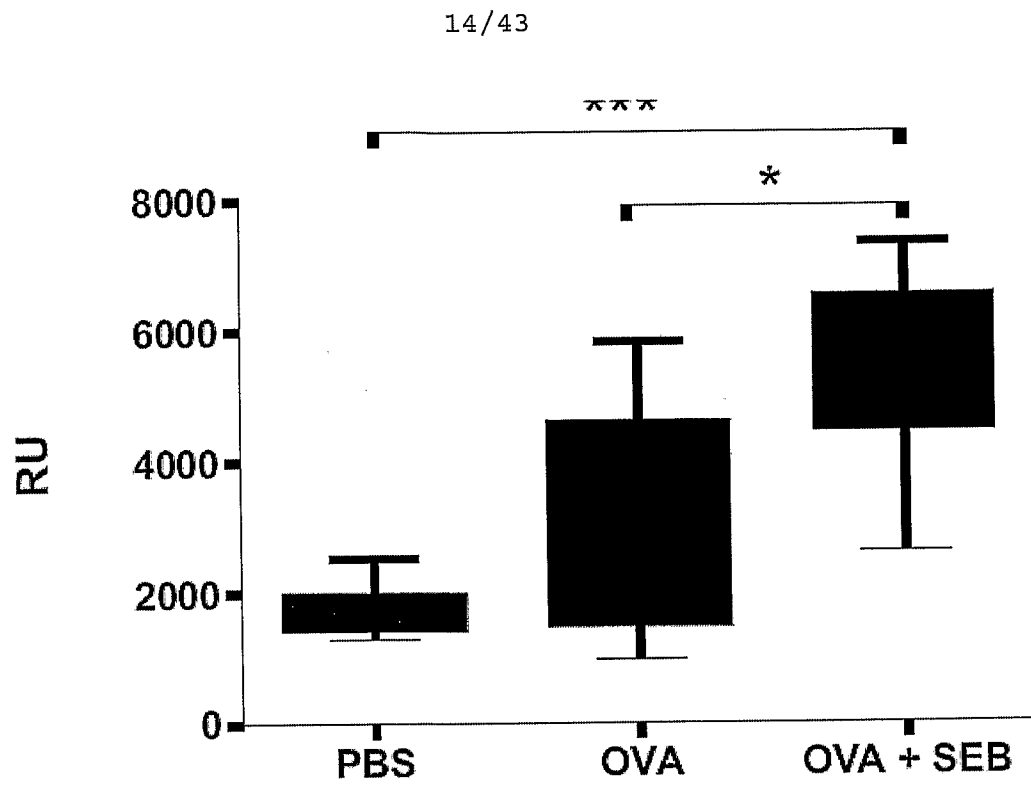


Figure 16

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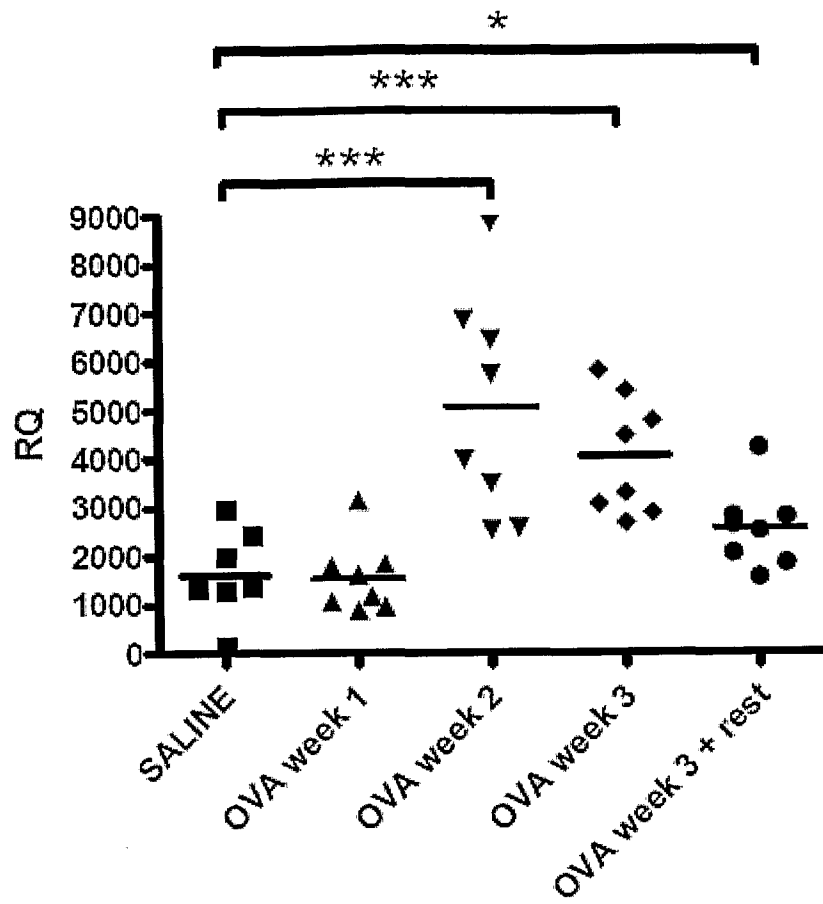


Figure 17

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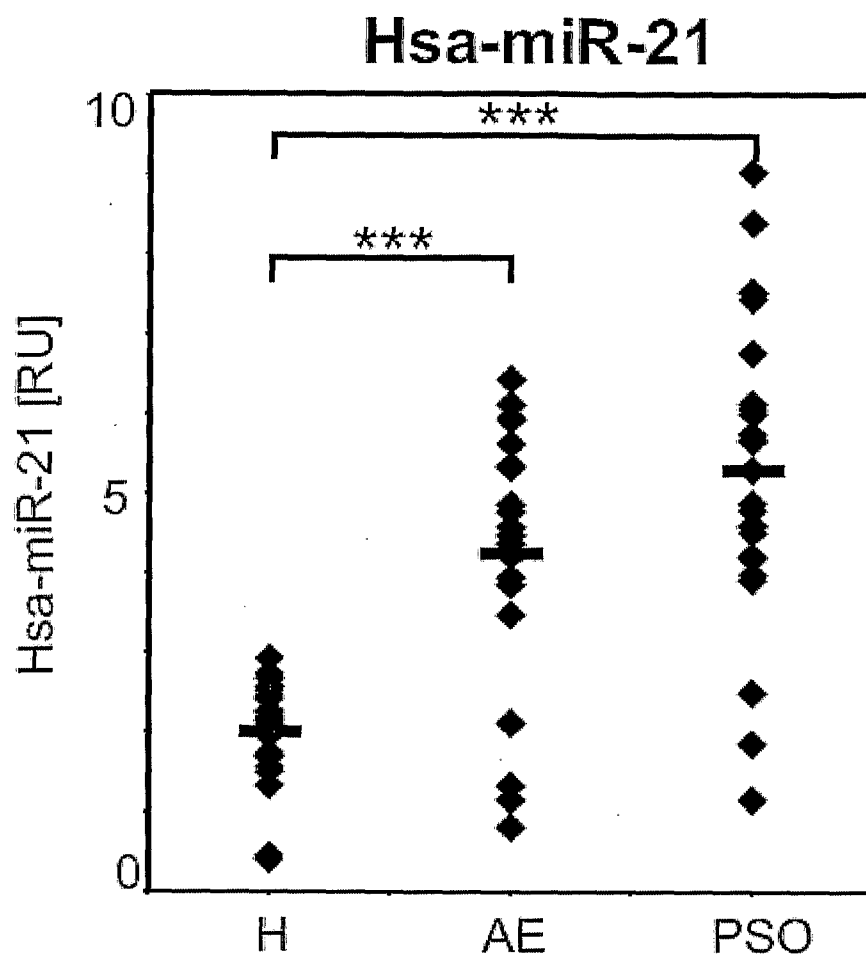


Figure 18

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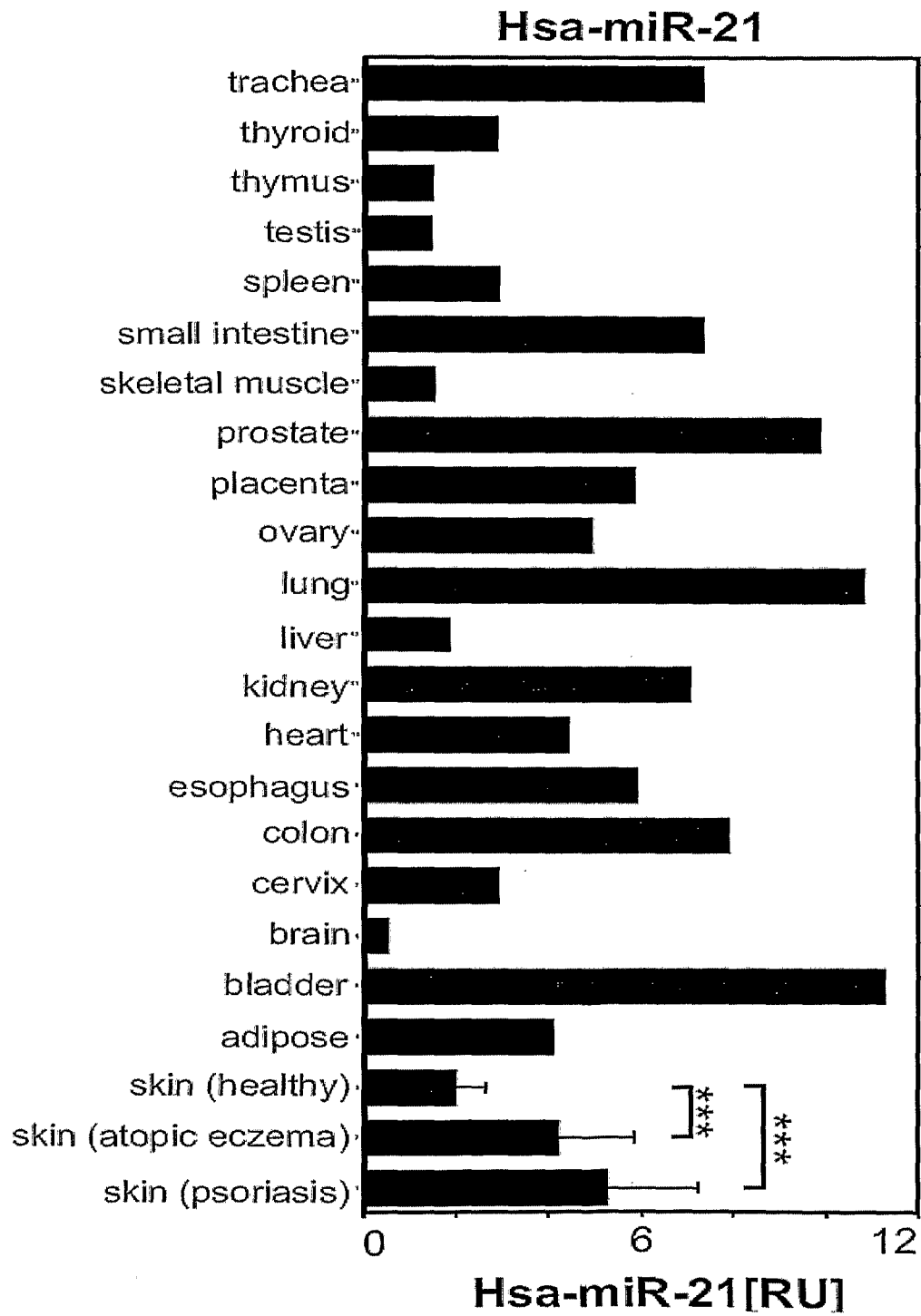


Figure 19

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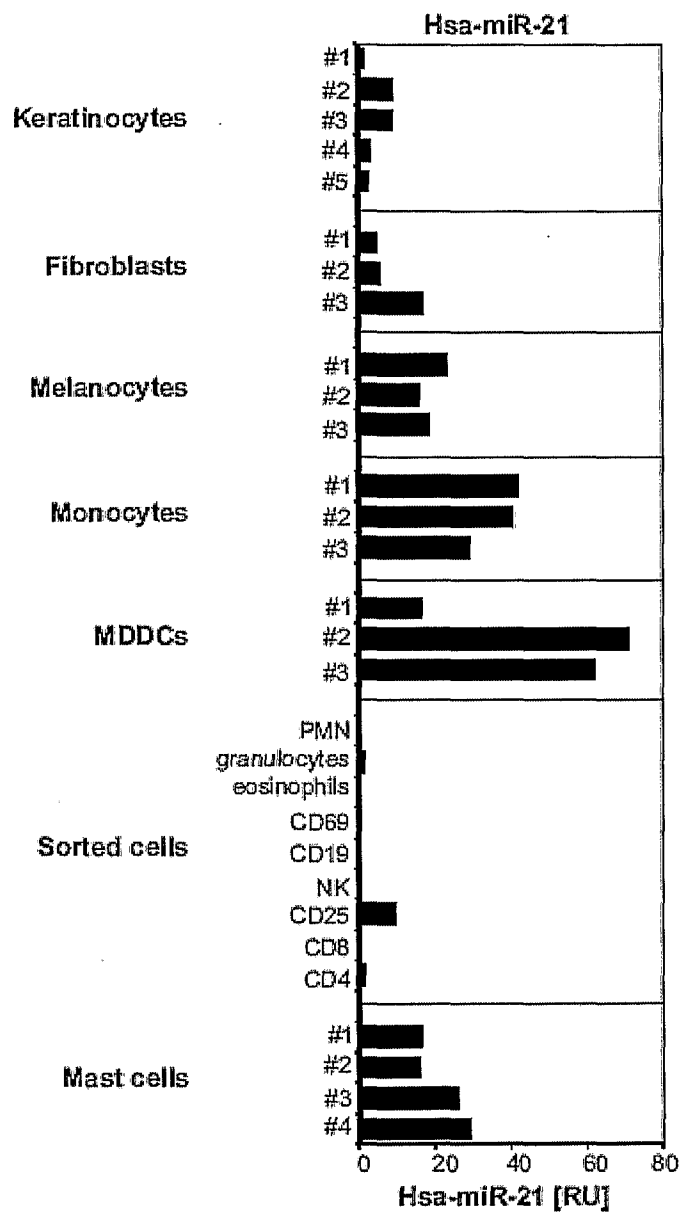


Figure 20

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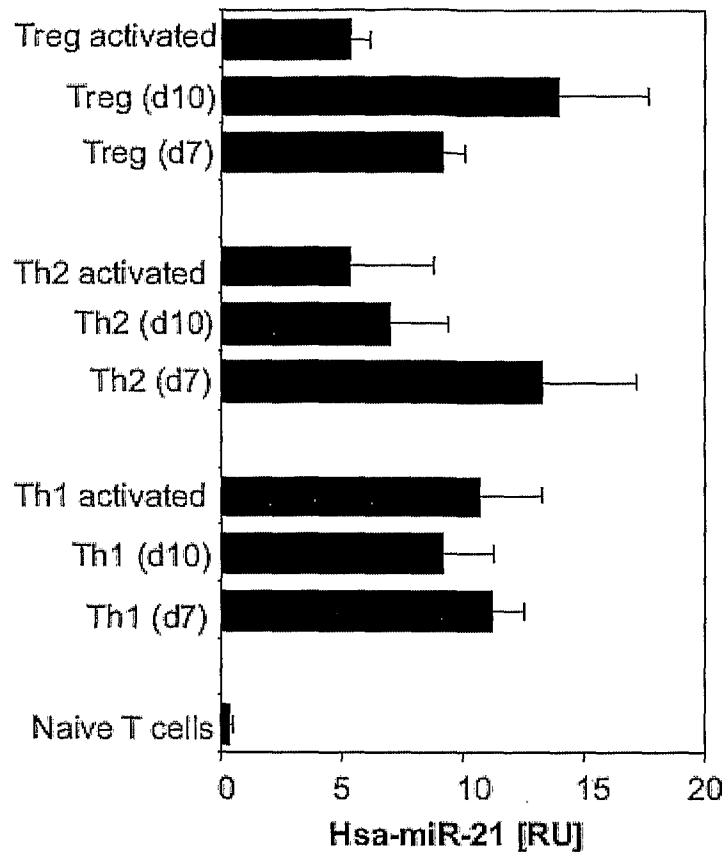


Figure 21

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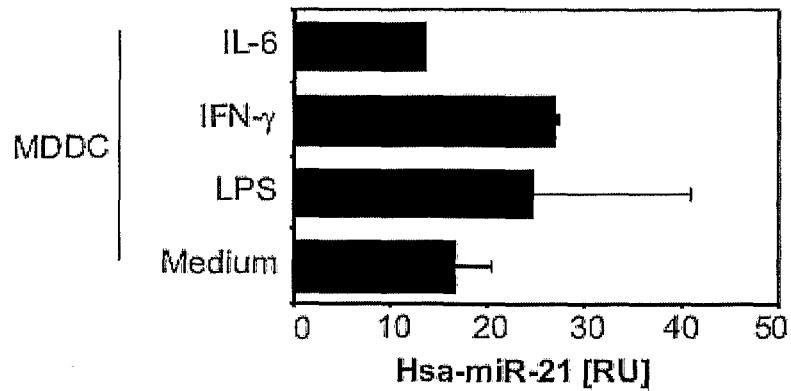


Figure 22

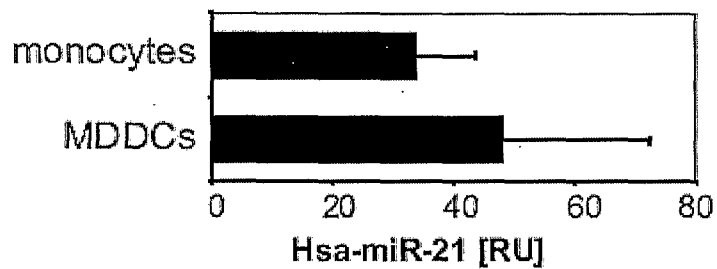


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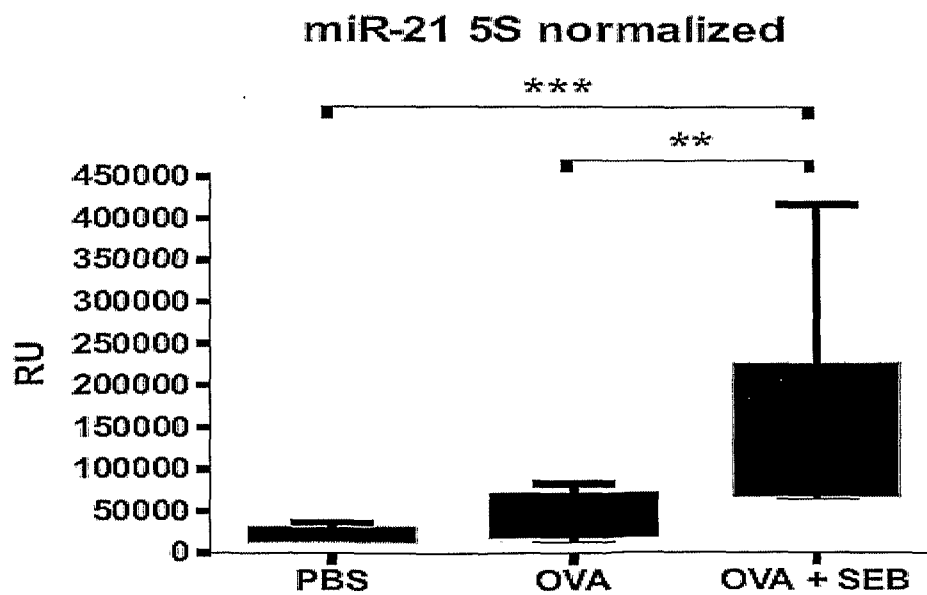


Figure 24

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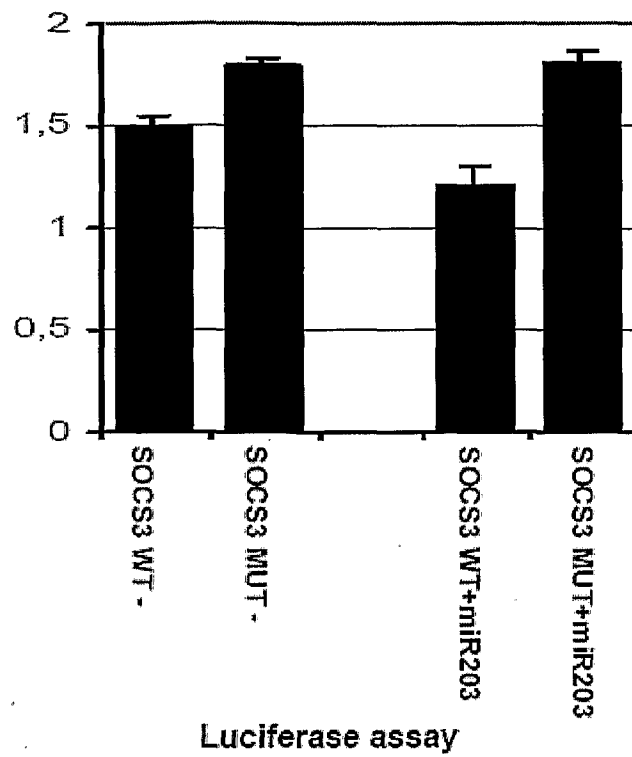


Figure 25

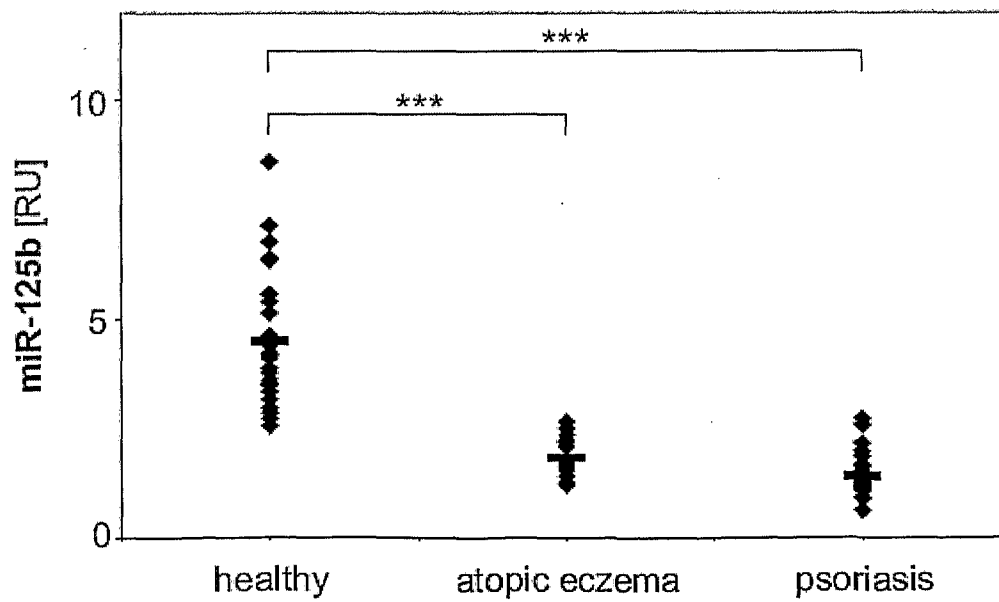


Figure 26

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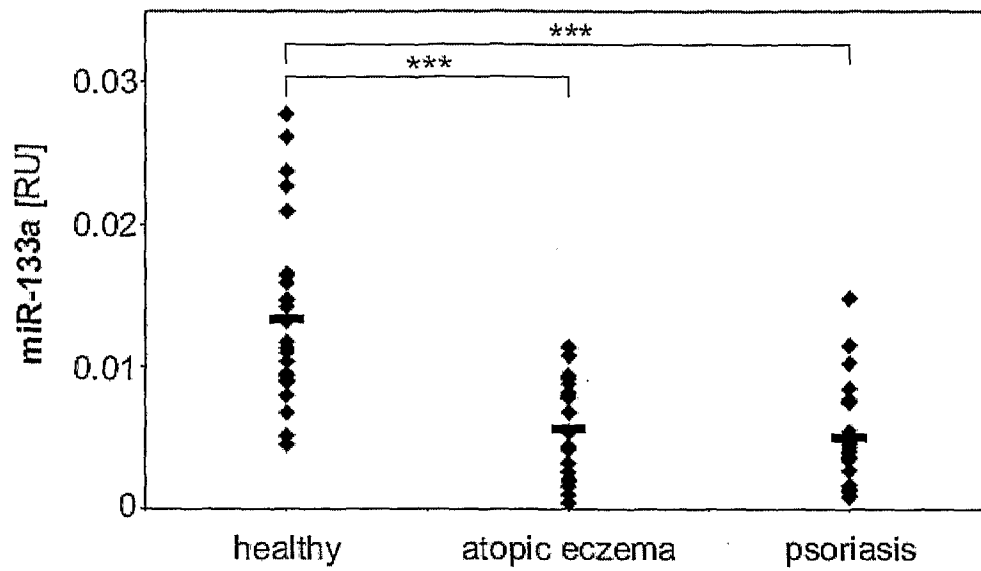


Figure 27

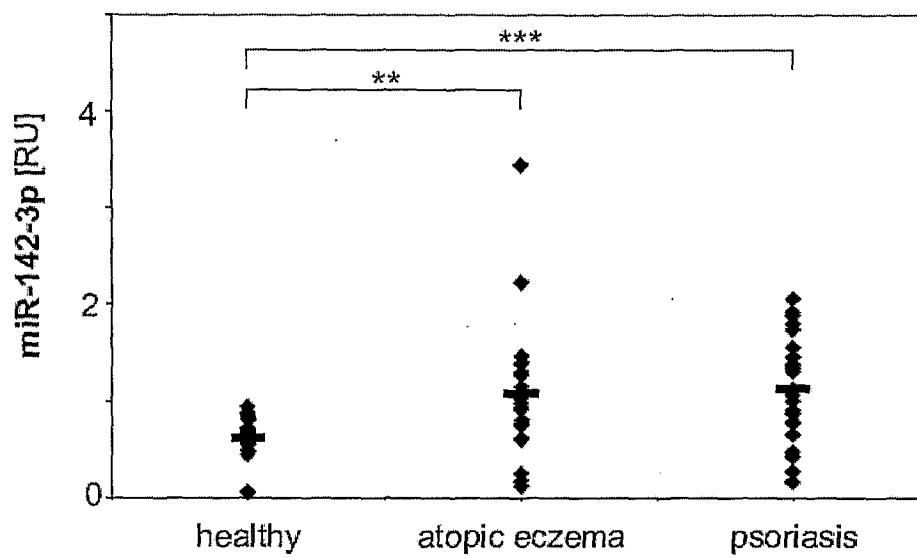


Figure 28

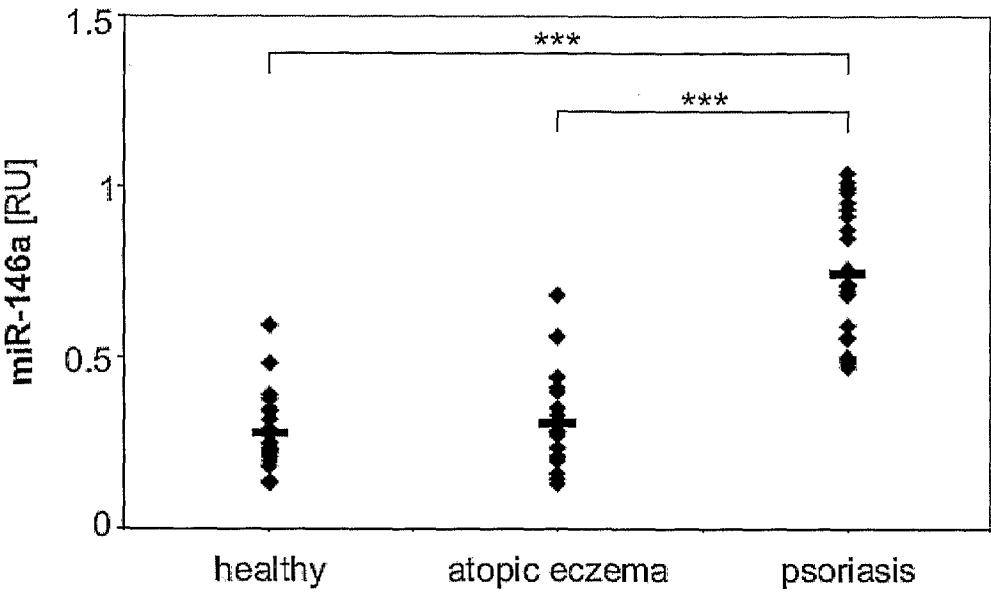


Figure 29

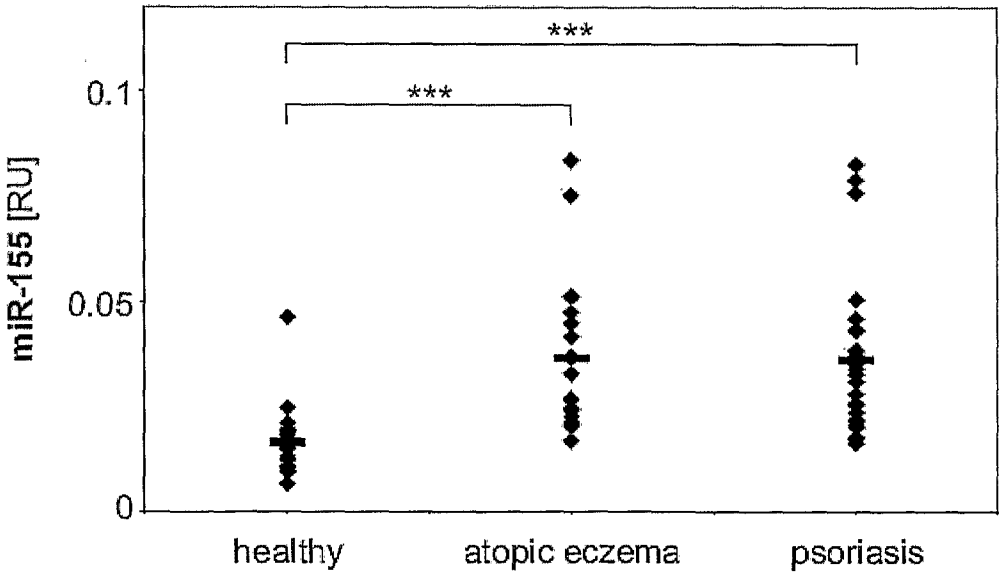


Figure 30

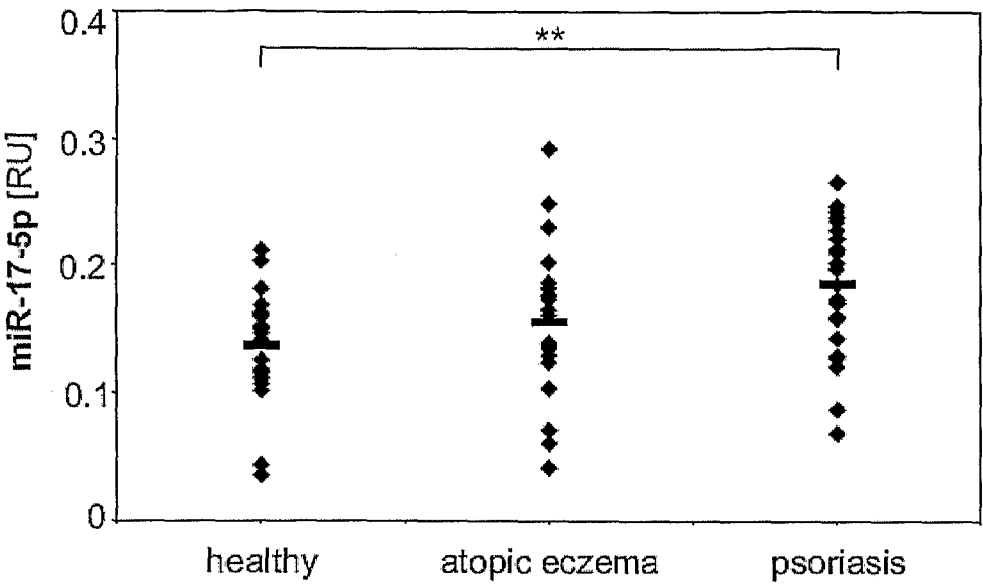


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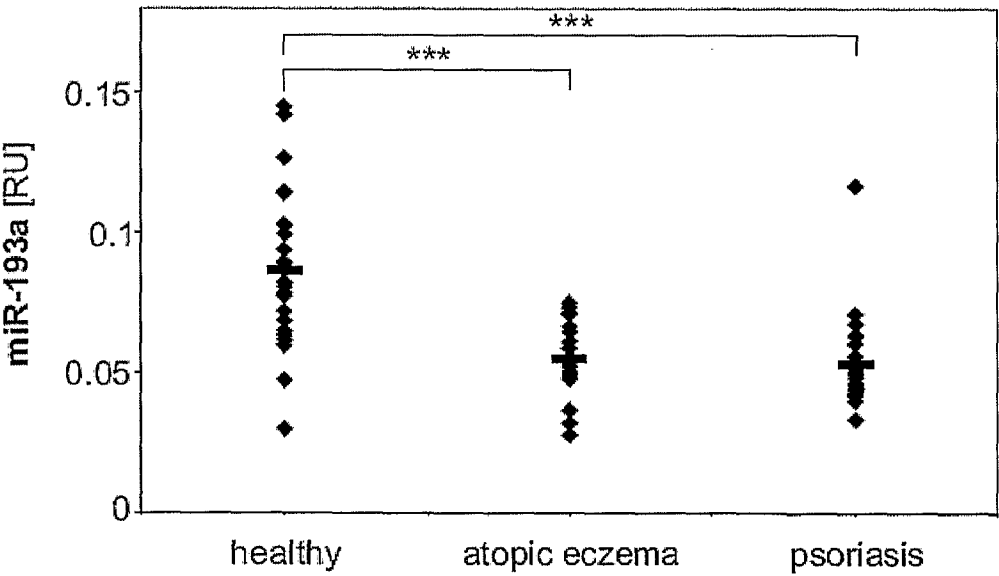


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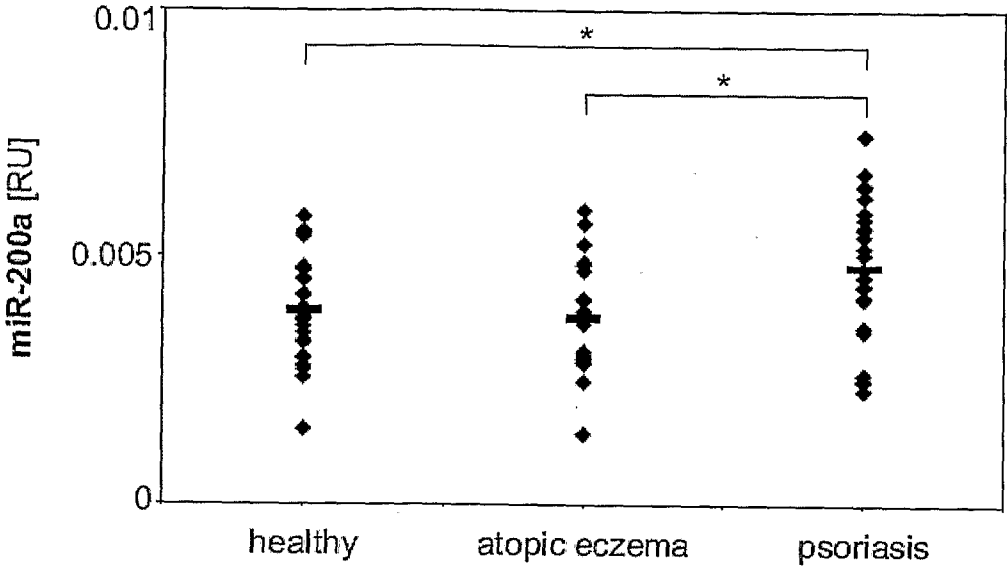


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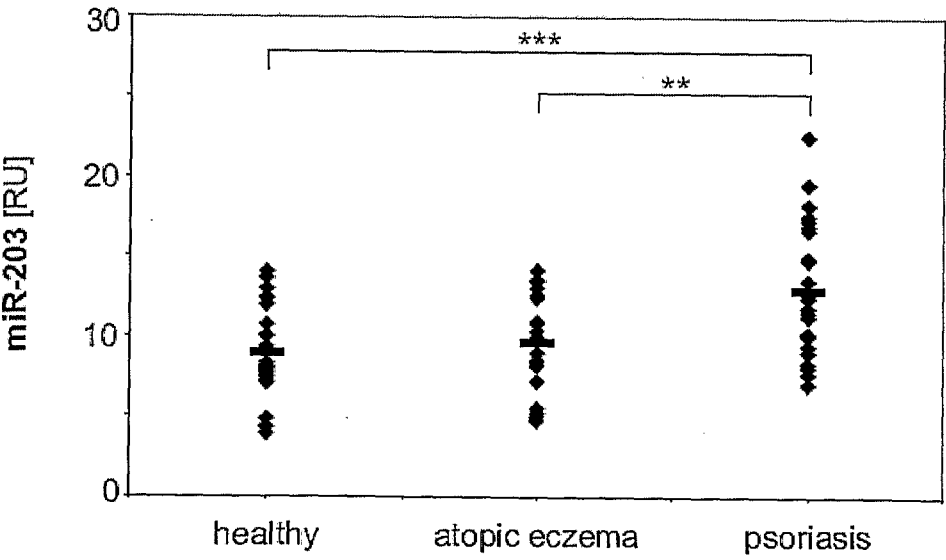


Figure 34

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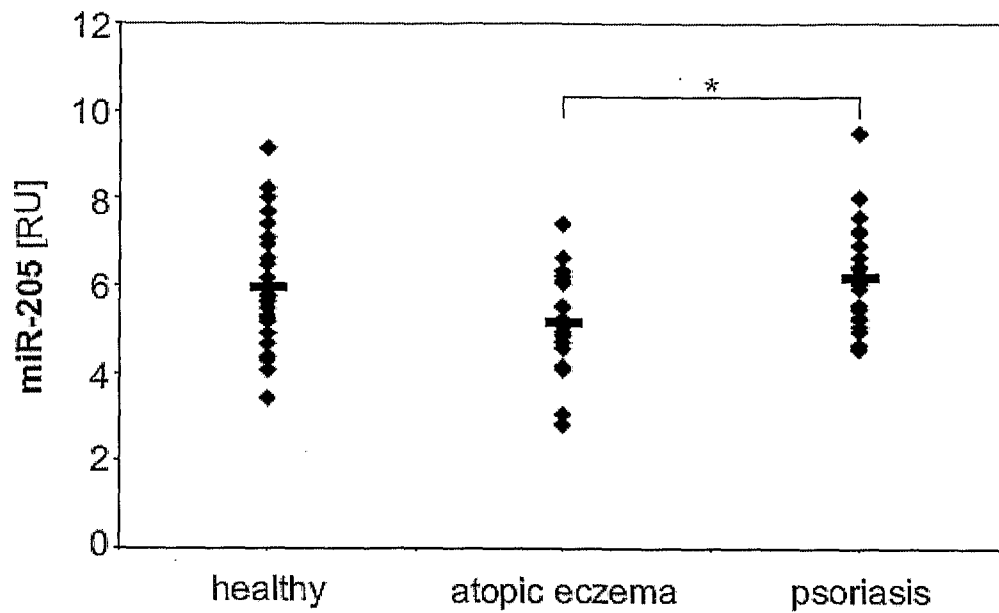


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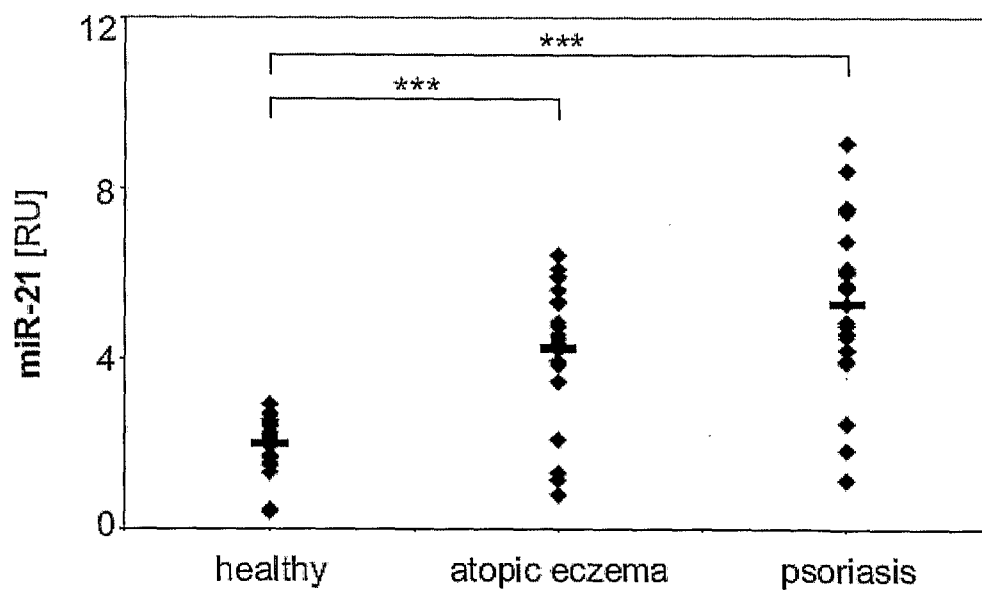


Figure 36

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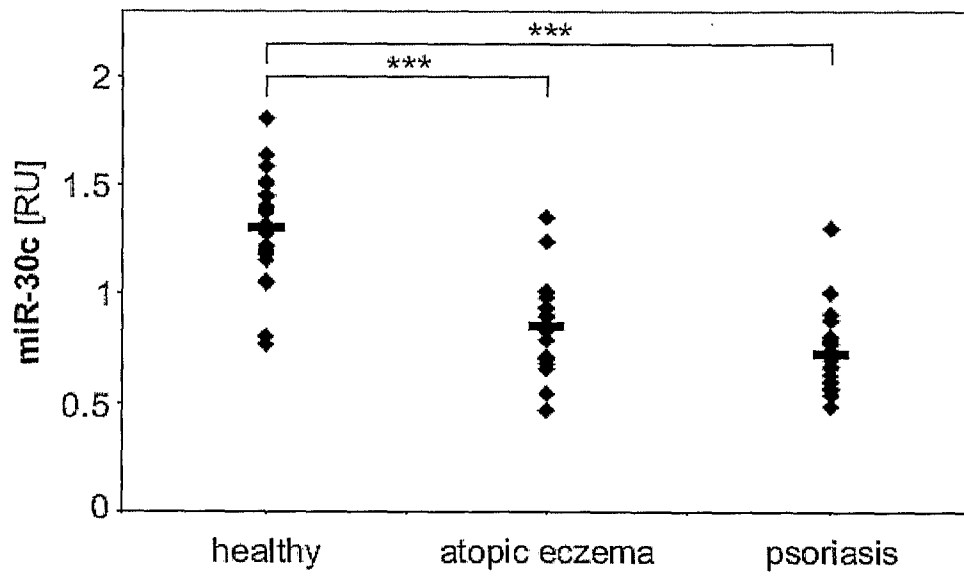


Figure 37

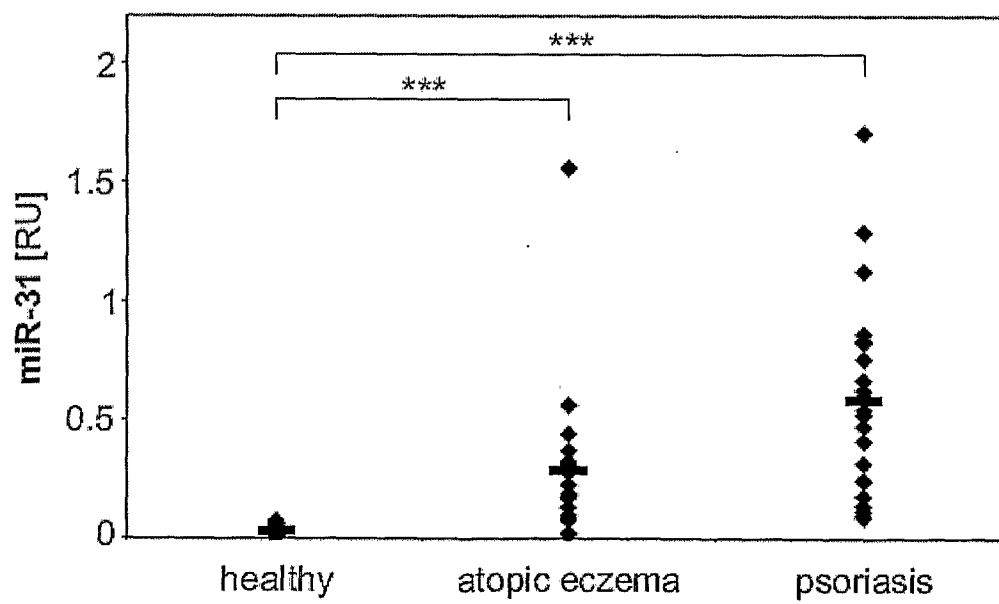


Figure 38

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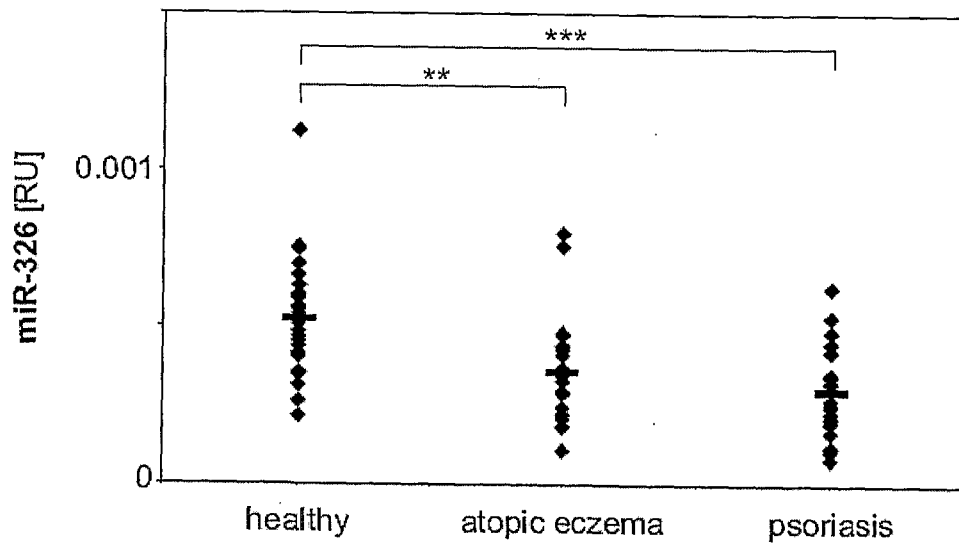


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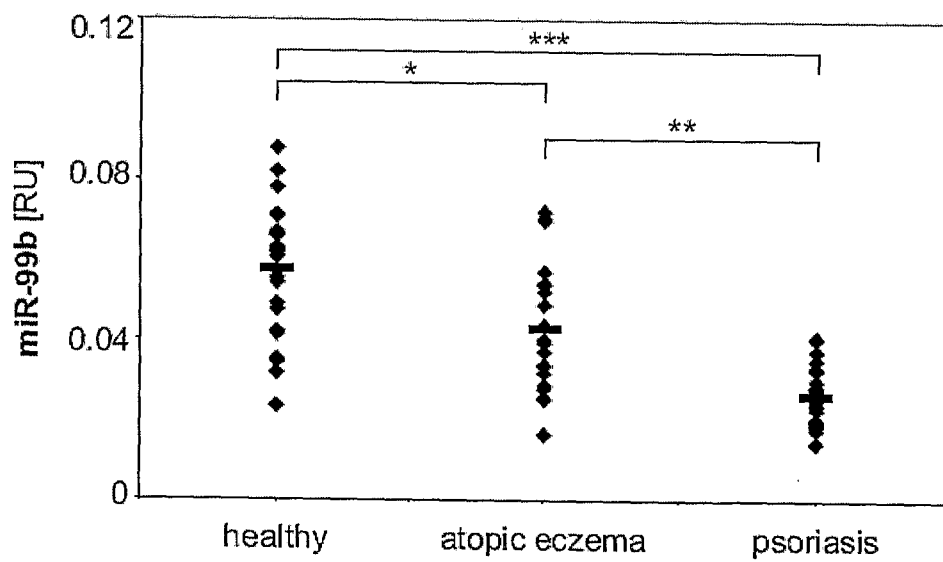


Figure 40

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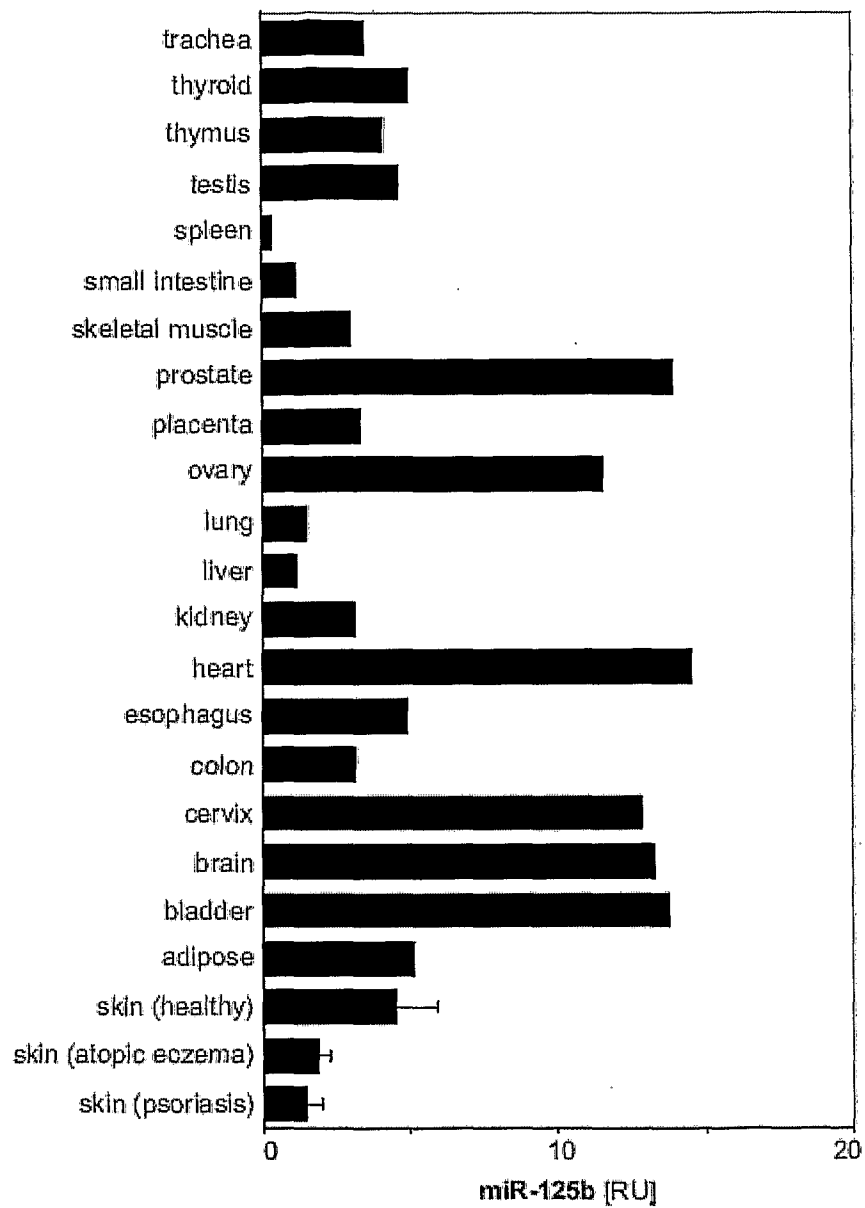


Figure 41

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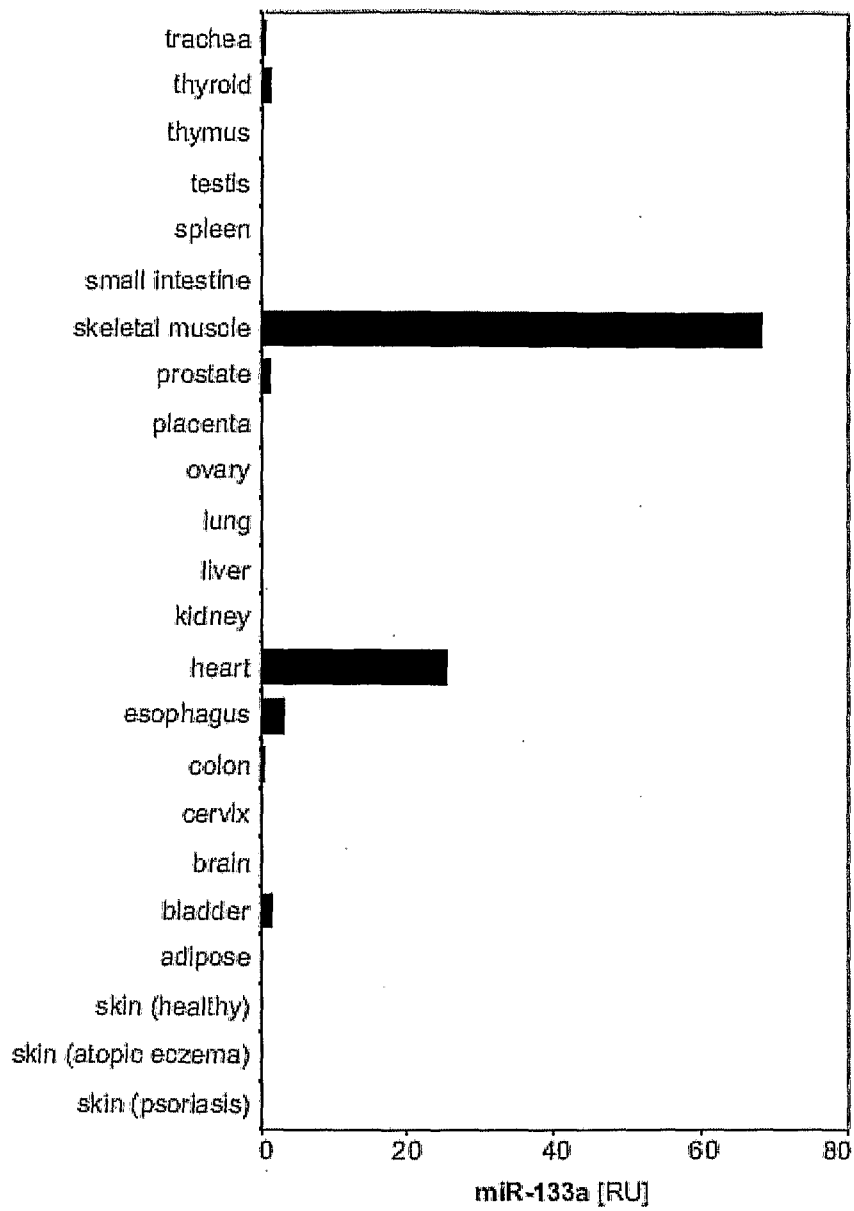


Figure 42

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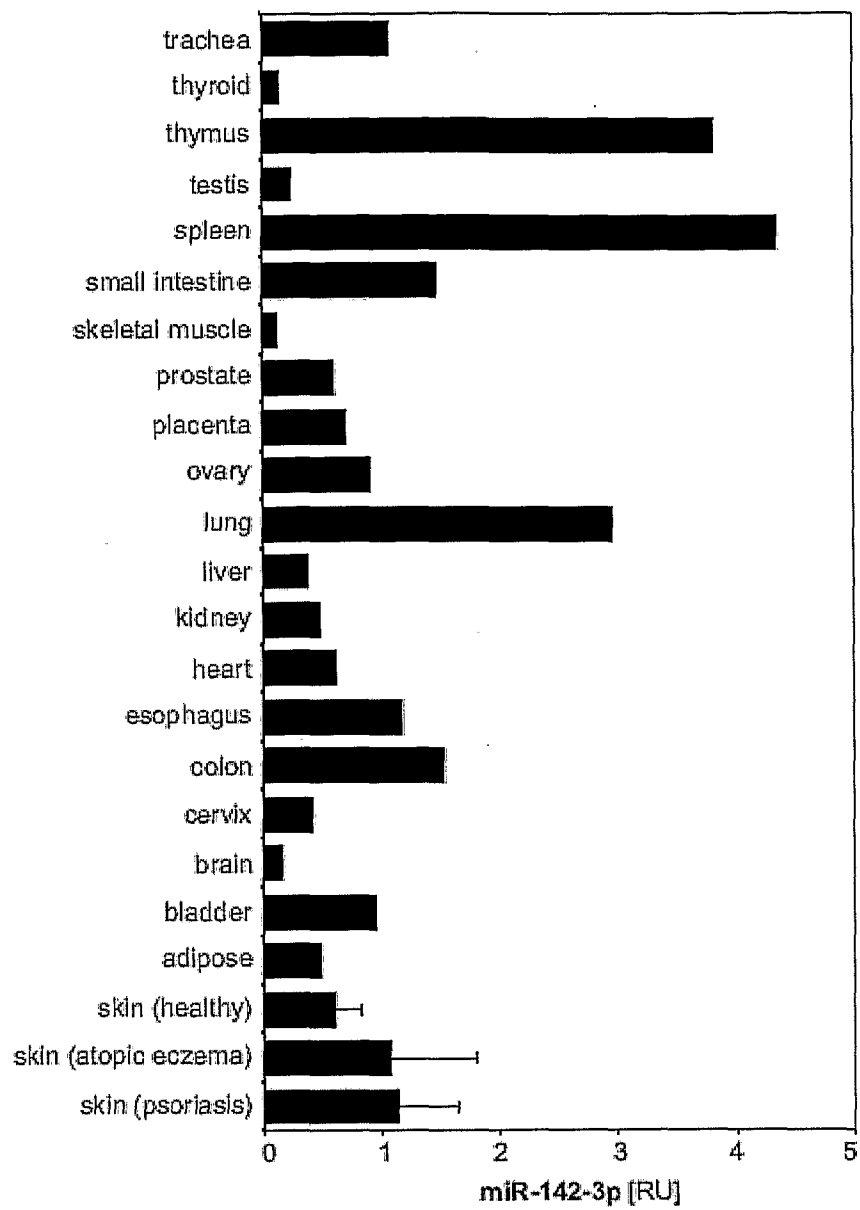


Figure 43

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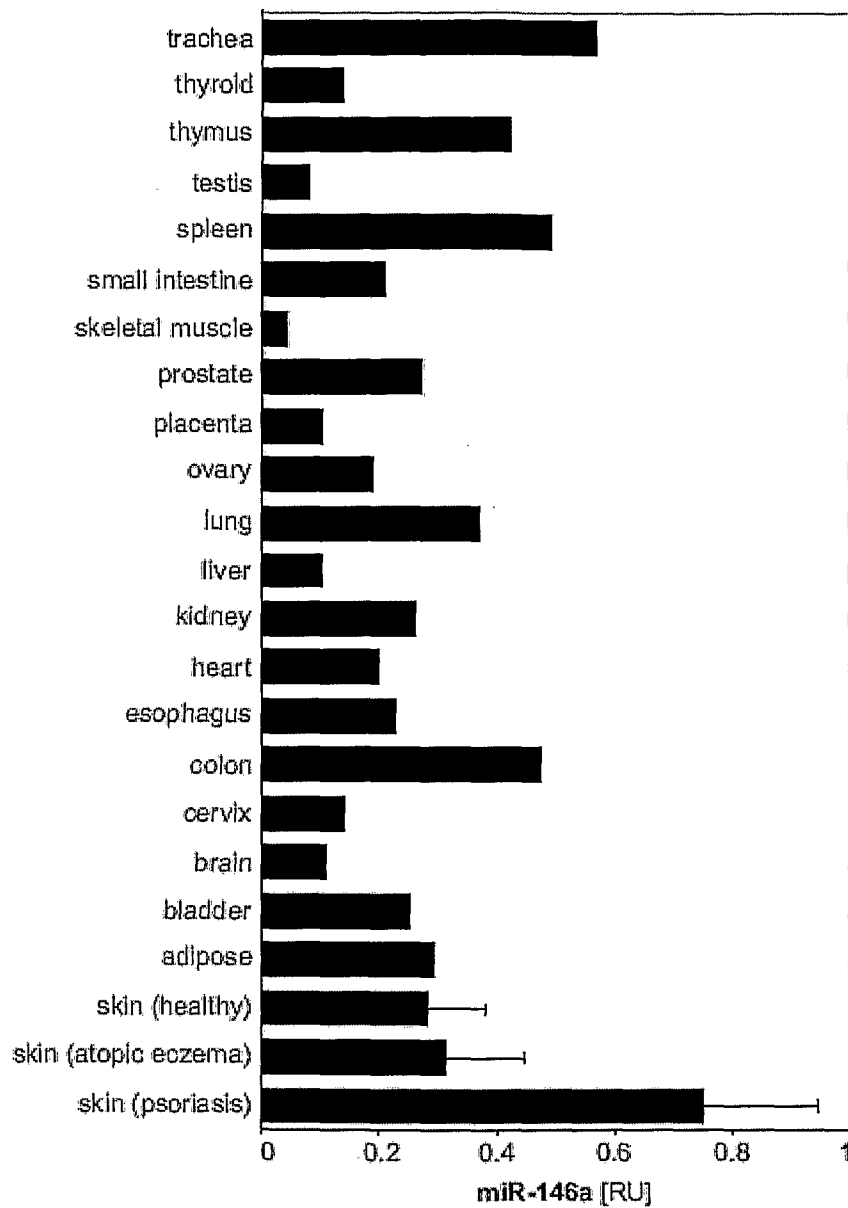


Figure 44

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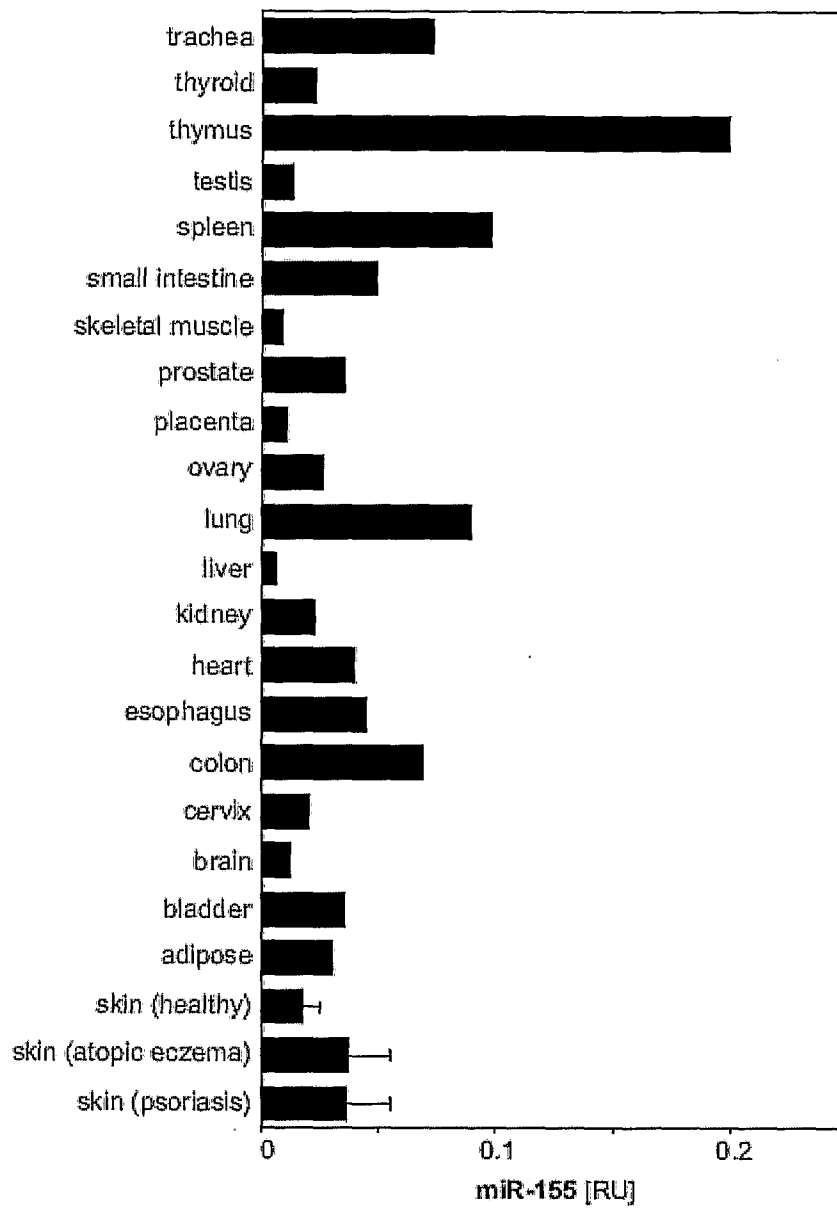


Figure 45

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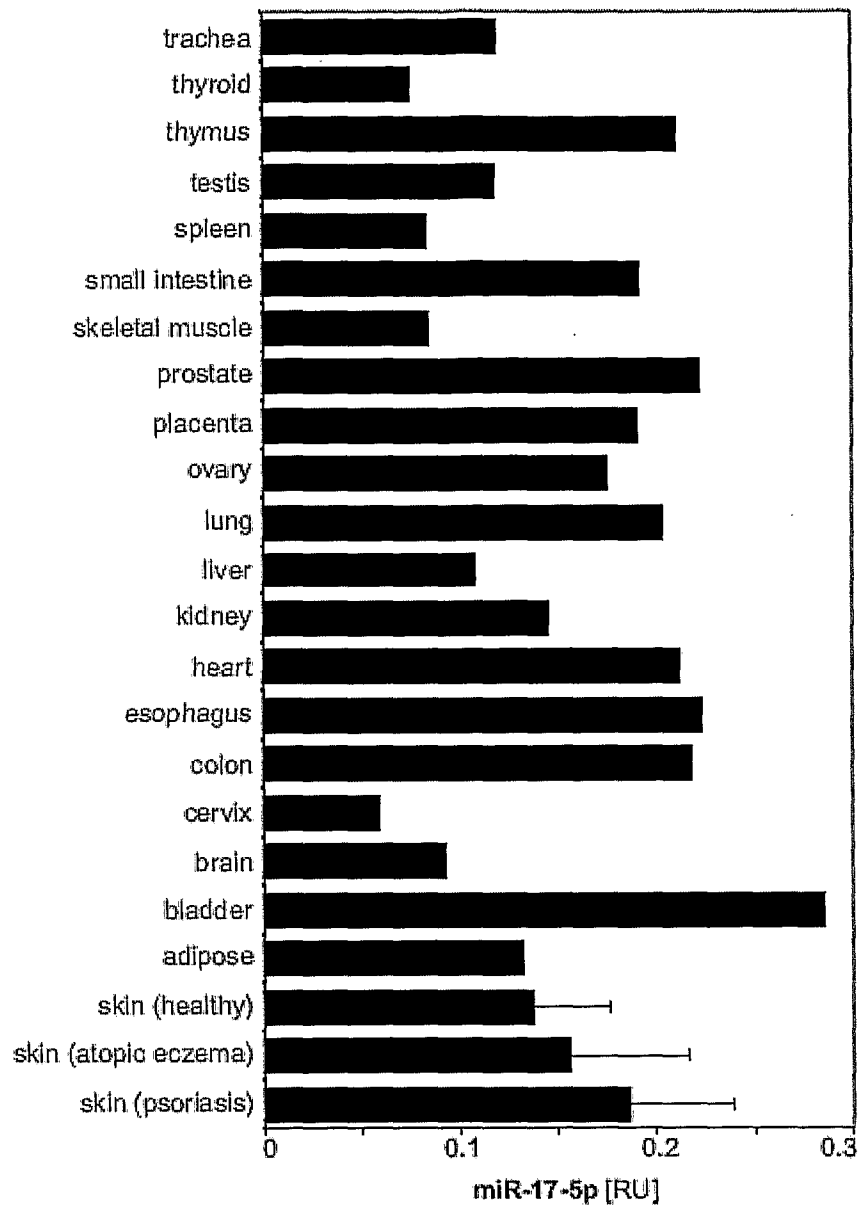


Figure 46

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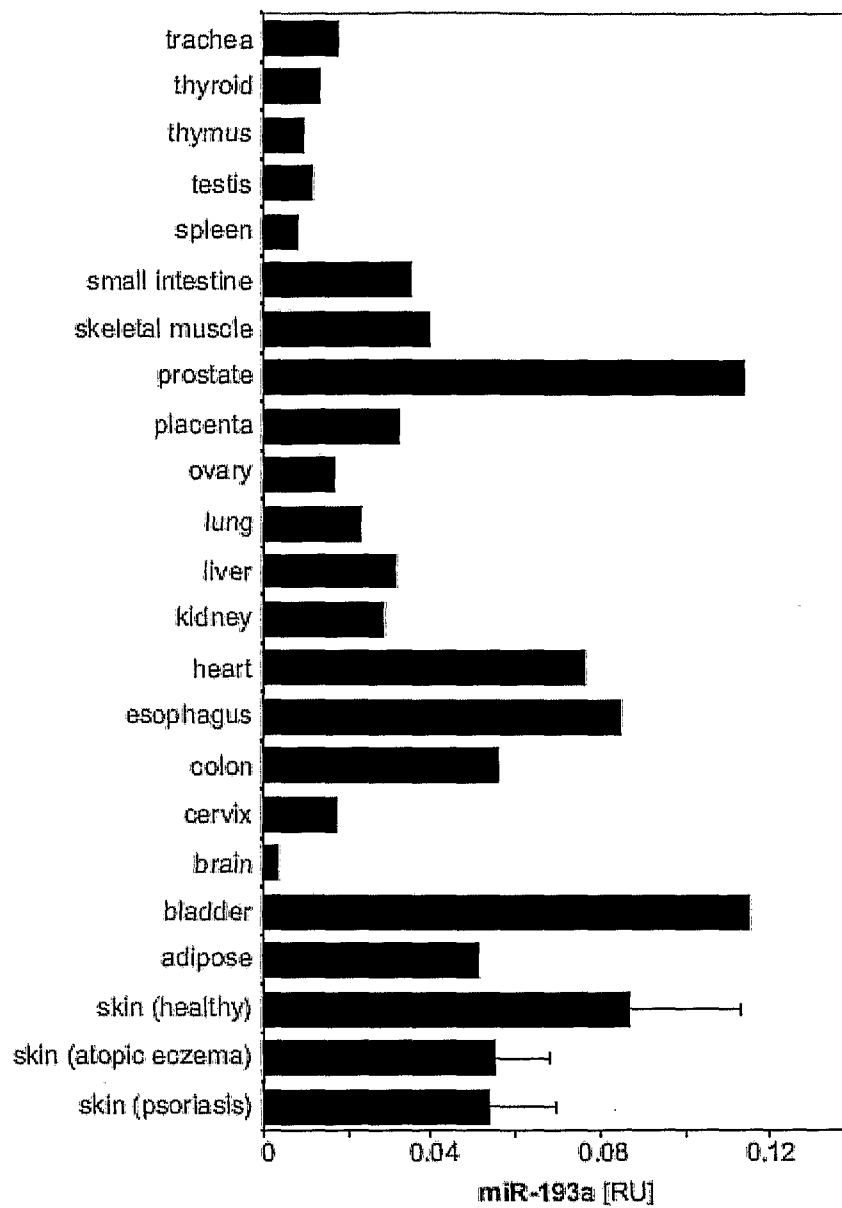


Figure 47

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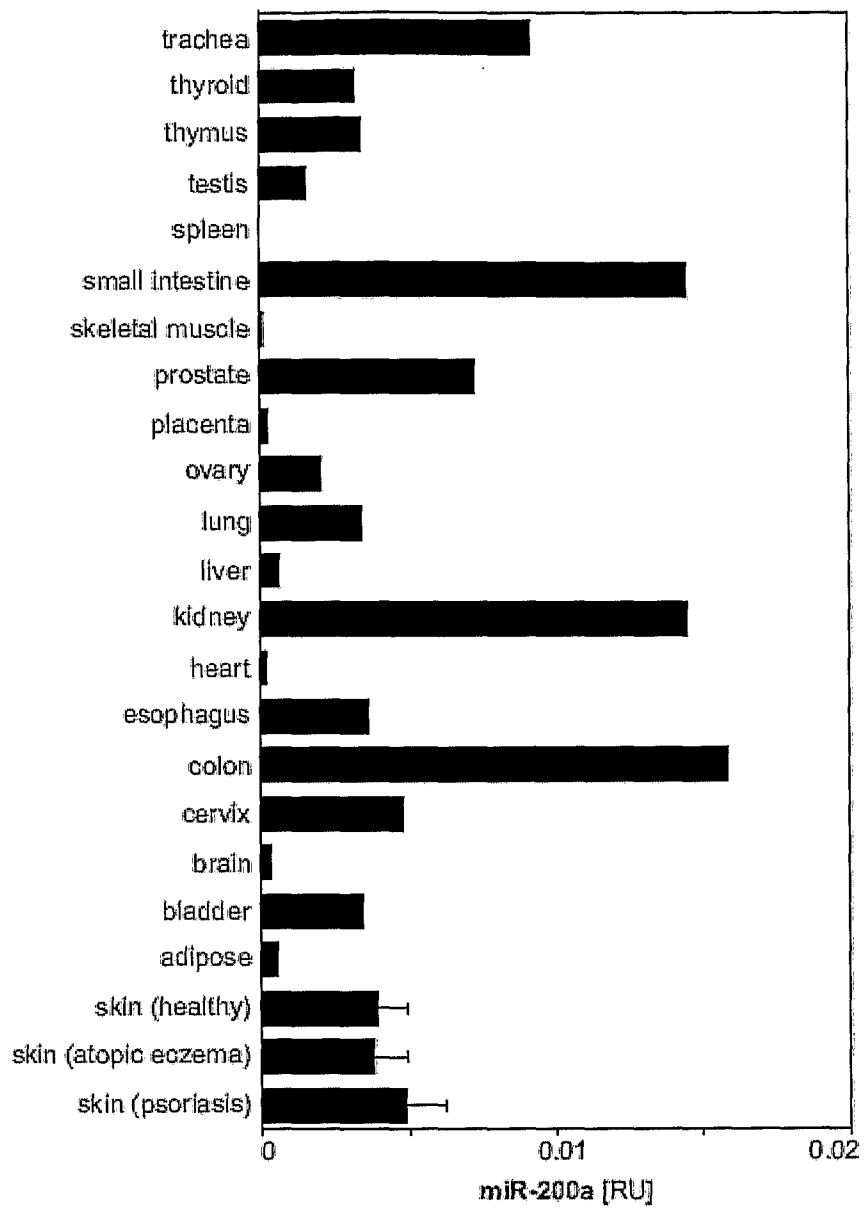


Figure 48

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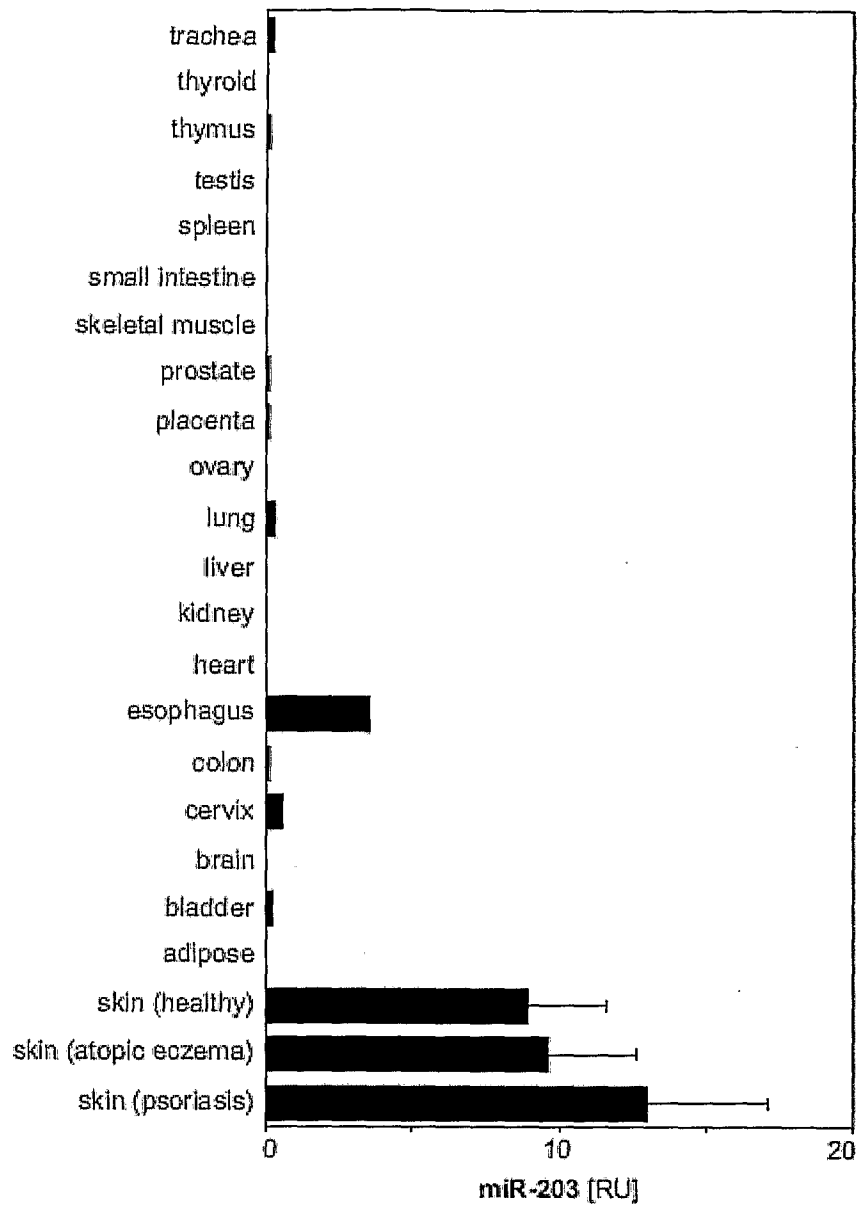


Figure 49

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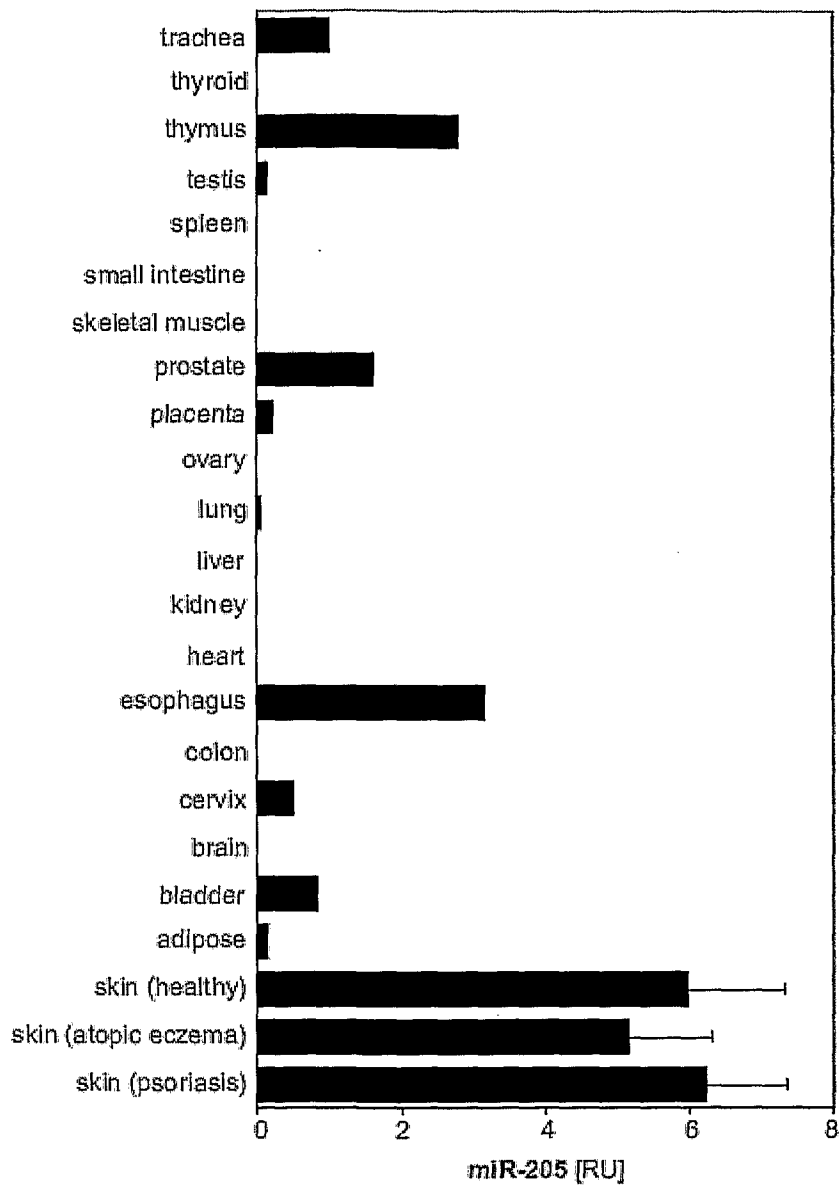


Figure 50

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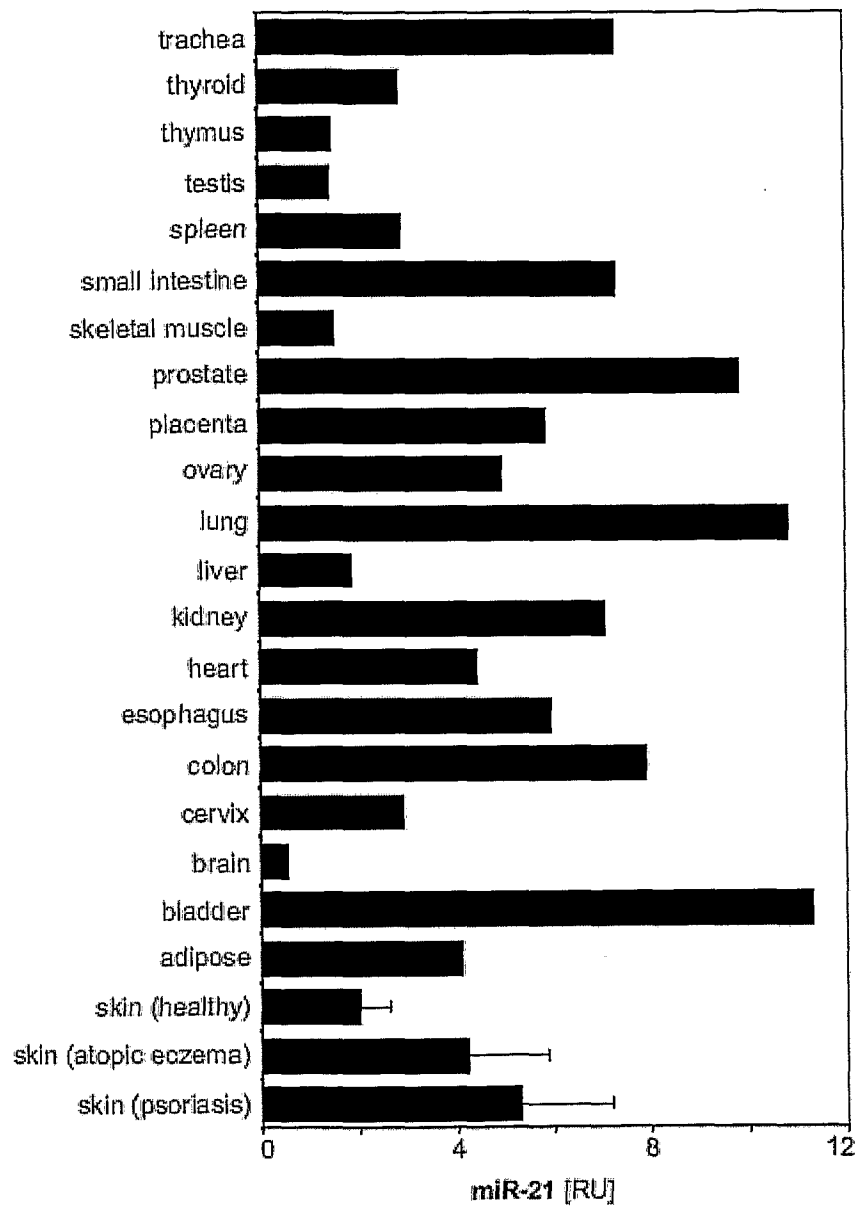


Figure 51

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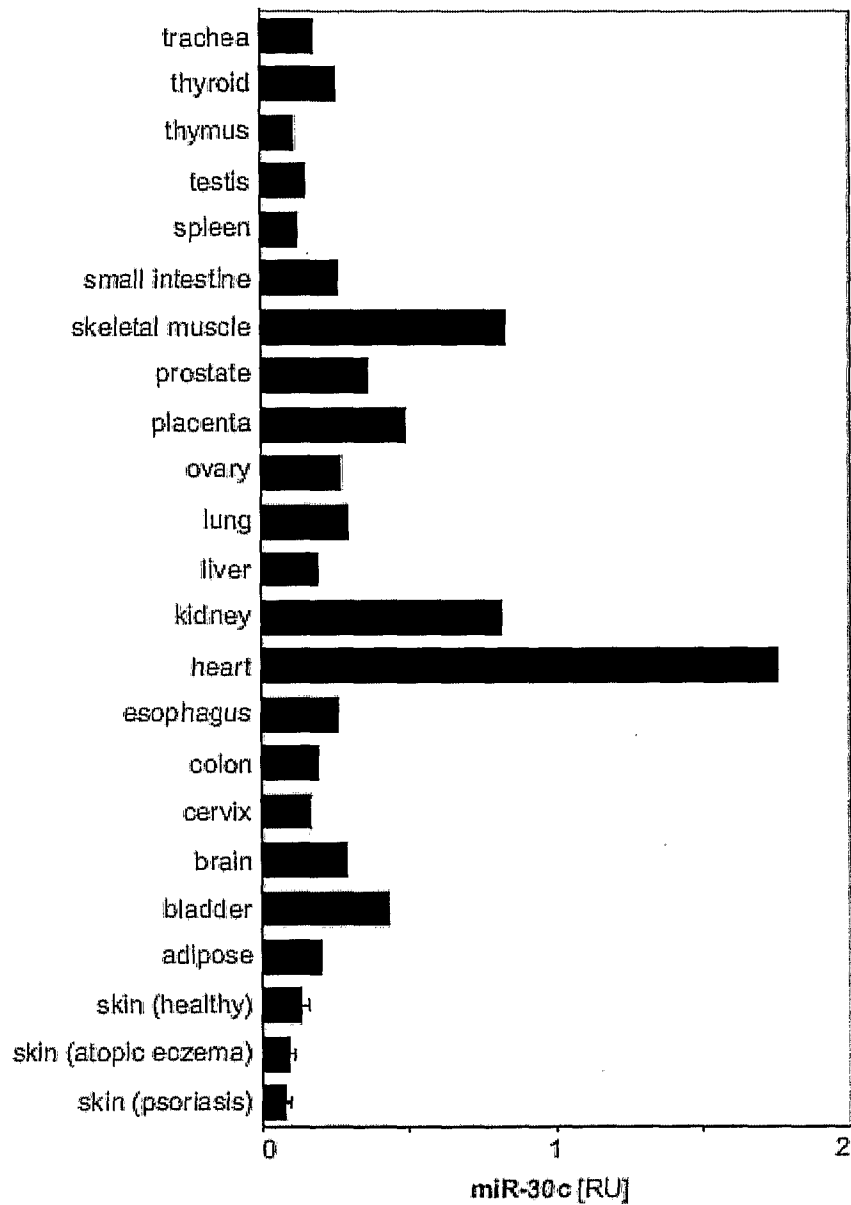


Figure 52

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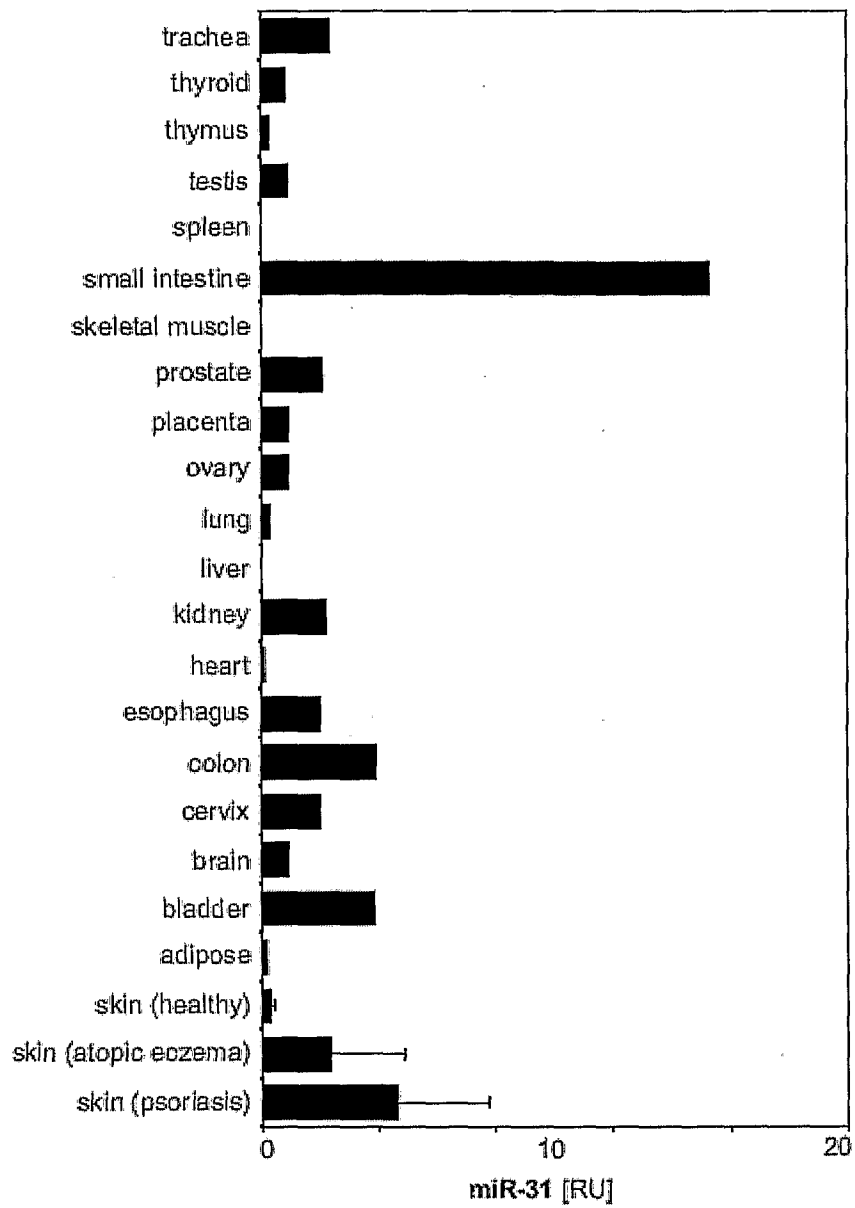


Figure 53

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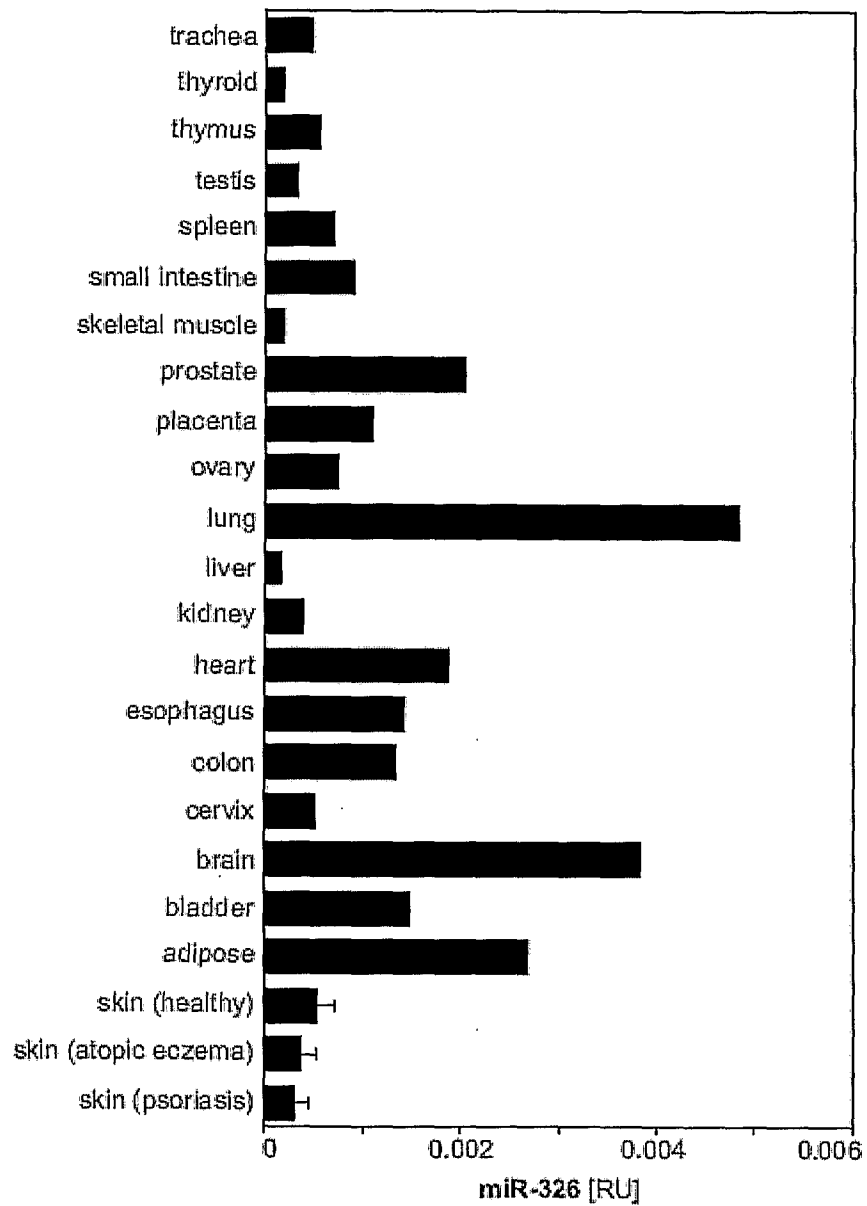


Figure 54

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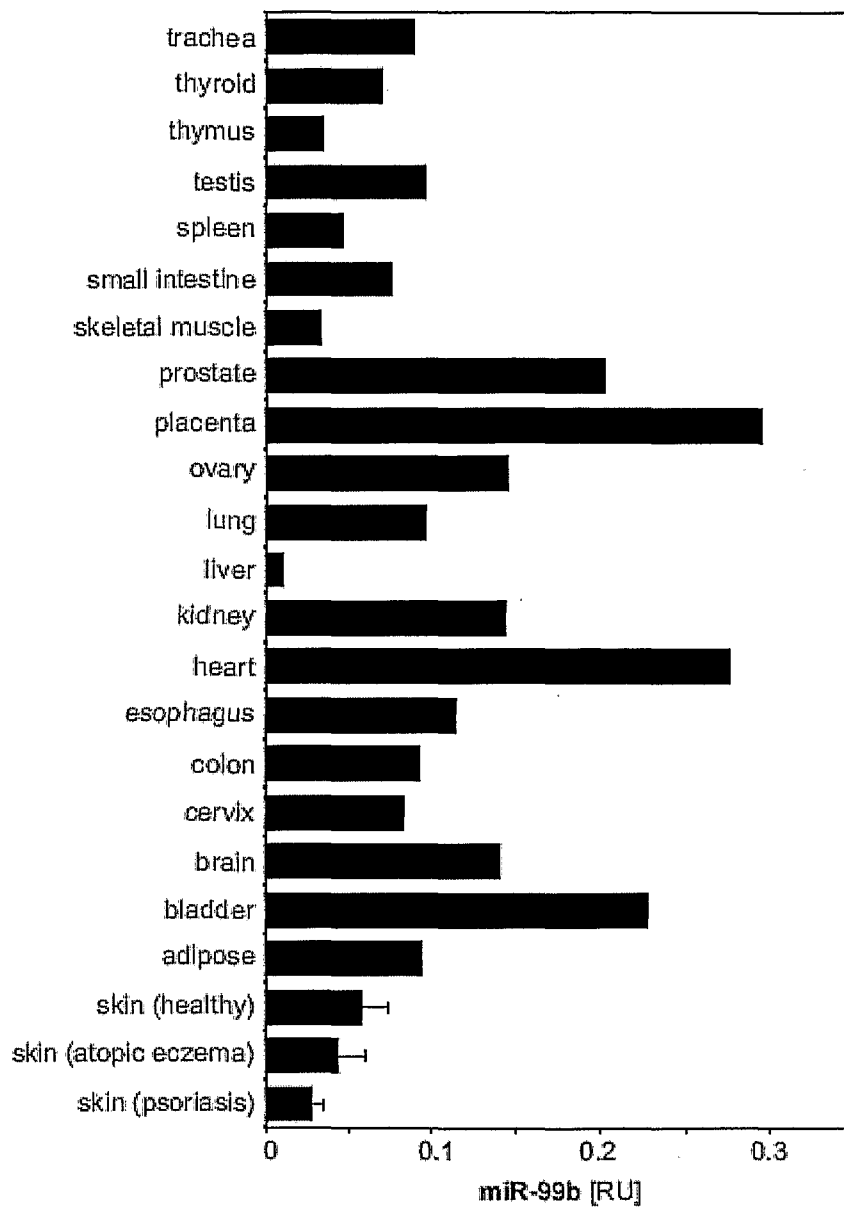


Figure 55