

(43) International Publication Date
24 October 2013 (24.10.2013)(51) International Patent Classification:
C12N 15/113 (2010.01)(21) International Application Number:
PCT/US2013/037579(22) International Filing Date:
22 April 2013 (22.04.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/636,059	20 April 2012 (20.04.2012)	US
61/681,750	10 August 2012 (10.08.2012)	US
61/782,838	14 March 2013 (14.03.2013)	US

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(US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD,
ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI,
NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU,
RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ,
TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA,
ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Published:— without international search report and to be republished
upon receipt of that report (Rule 48.2(g))

(54) Title: MIRNA MODULATORS OF THERMOGENESIS

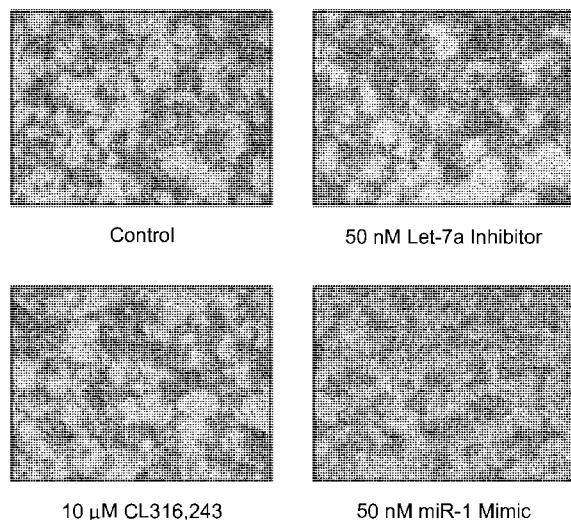


FIG. 22

(57) Abstract: Provided are novel methods and compositions for the modulation of thermogenesis. Such methods are particularly advantageous in that they allow for the reduction of body fat in a subject without the subject having to adjust their caloric intake through dieting, modify their physical activity or undergo bariatric surgery. Accordingly, the methods of the invention are particularly useful for treating or preventing obesity. Also provided are methods of screening for novel agents that modulate the activity of thermogenic regulators.

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 61/636,059, filed on April 20, 2012; U.S. Application Serial No. 61/681,750, filed on August 10, 2012; and U.S. Application Serial No. 61/782,838, filed on March 14, 2013, each of which is hereby
5 incorporated by reference.

BACKGROUND OF THE INVENTION

Obesity has reached pandemic proportions, affecting all ages and socioeconomic groups. The World Health Organization estimated that in 2008, 1.5 billion adults aged 20 years and older were overweight and over 200 million men and 300 million women were obese. These figures
10 are estimated to increase to 2.16 billion overweight and 1.12 billion obese individuals by 2030. Obesity is the source of lost earnings, restricted activity days, absenteeism, lower productivity at work (presenteeism), reduced quality of life, permanent disability, significant morbidity and mortality, and shortened lifespan. Indeed, the total annual economic cost of overweight and obesity in the United States and Canada caused by medical costs, excess mortality and disability
15 was estimated to be about \$300 billion in 2009. International studies on the economic costs of obesity have shown that they account for between 2% and 10% of total health care costs.

Obesity is the result of a chronic imbalance between energy intake and expenditure. This leads to storage of excess energy into adipocytes, which typically exhibit both hypertrophy (increase in cell size) and hyperplasia (increase in cell number or adipogenesis). The recent
20 worsening of obesity is due to the combination of excessive consumption of energy-dense foods high in saturated fats and sugars, and reduced physical activity.

The current symptomatic medical treatments of obesity fail to achieve their long-term therapeutic goals, largely due to limited drug efficacy and patients' poor adherence with lifestyle changes and therapies. Several obesity drugs have been removed from the market for safety
25 reasons and small molecules currently in development are struggling to gain regulatory approval because of their modest short-term efficacy and unknown safety profile. Presently, only restrictive and malabsorptive bariatric surgery can achieve significant long-term reduction of weight excess with some favorable cardiovascular benefits.

Accordingly, there is a need in the art for novel treatments for obesity.

SUMMARY OF THE INVENTION

Obesity is the consequence of a chronic imbalance of energy intake over expenditure, leading to the storage of excess energy inside white adipocytes. This disclosure features a novel treatment for obesity targeting peripheral adipocytes, including energy-storing lipid-filled white adipocytes (WAT), and energy-expendending mitochondria-rich brown adipocytes (BAT). In addition, the disclosure provides methods for the modulation of thermogenesis (the process of heat production in organisms) using microRNA (miRNAs) agents. The methods described herein generally involve the direct and/or indirect modulation of at least one thermogenic regulator (e.g., a mitochondrial uncoupler, such as Uncoupling Protein 1 (UCP1 also known as Thermogenin) or Uncoupling Protein 2 (UCP2)) in a cell, tissue and/or subject using an isolated miRNA agent. UCPs uncouple oxidative phosphorylation from ATP synthesis. In certain instances, this uncoupling reaction results in energy dissipated as heat. Such methods are particularly advantageous in that they allow for the reduction of body fat in a subject without the subject having to adjust their caloric intake through dieting, modify their physical activity or undergo bariatric surgery. Accordingly, the methods of the invention are particularly useful for treating or preventing obesity.

The invention also provides novel miRNA agent compositions (e.g., miRNA, agomirs, and antagomirs) that can modulate the activity of thermogenic regulators. Yet further, the invention provides methods of screening for novel miRNA agents that modulate the activity of thermogenic regulators. Further still, the invention provides novel agent compositions (e.g. aptamer-miRNA complexes or “aptamirs”) that provide cell/tissue-specific delivery of the miRNA agents.

Accordingly, in one aspect, the invention provides a method of modulating respiratory chain uncoupling in a cell, the method comprising contacting the cell with an isolated miRNA agent that modulates the expression level and/or activity of at least one mitochondrial uncoupler. In some embodiments, the method further comprises the step of selecting a subject in need of modulating respiratory chain uncoupling (e.g., an obese patient). In one embodiment, the miRNA agent increases the expression level and/or activity of the at least one mitochondrial uncoupler. In certain embodiments, the mitochondrial uncoupler is UCP1 or UCP2. In some embodiments, the method increases respiratory chain uncoupling in a cell in vivo. In other

embodiments, the method increases respiratory chain uncoupling in a cell *ex vivo*. In certain embodiments, the method further comprises determining the level of expression (mRNA or protein) or activity of the mitochondrial uncoupler. In certain embodiments, the cell is a pre-adipocyte, adipocyte, adipose tissue derived mesenchymal stem cell, hepatocyte, myocyte, or a precursor thereof. Optionally, adipocytes can be white fat or brown fat adipocytes.

In another aspect, the invention provides a method of modulating thermogenesis in a tissue, the method comprising contacting the tissue with an isolated miRNA agent that modulates the expression level and/or activity of at least one mitochondrial uncoupler. In some embodiments, the method further comprises the step of selecting a subject in need of modulating thermogenesis (e.g., an obese patient). In one embodiment, the miRNA agent increases the expression level and/or activity of the at least one mitochondrial uncoupler. In certain embodiments, the mitochondrial uncoupler is UCP1 or UCP2. In certain embodiments, the method involves increasing thermogenesis. In certain embodiments, the method further comprises determining the level of expression (mRNA or protein) or activity of the mitochondrial uncoupler. In certain embodiments, the tissue is brown fat, white fat, subcutaneous adipose tissue, liver or muscle. In certain embodiments, the tissue is contacted with the miRNA agent *ex vivo*.

In another aspect, the invention provides a method of treating obesity in human subject in need of treatment thereof, the method generally comprising administering to the human subject an effective amount of a miRNA agent that modulates activity of at least one mitochondrial uncoupler. In certain embodiments, the human subject selected for treatment has a genetic or epigenetic predisposition to obesity. In certain embodiments, the mitochondrial uncoupler is UCP1, UCP2 or UCP3.

In certain embodiments of all of the above aspects, the miRNA agent is an isolated miRNA selected from the group consisting of hsa-miR-1-1, hsa-miR-1-2, miR-19a-b, hsa-miR-105, hsa-miR-1283, hsa-mir-129, hsa-miR-133a-1, hsa-miR-133a-2, hsa-miR-143, hsa-mir-143-5p, hsa-mir-147, hsa-mir-149, hsa-mir-199a, hsa-mir-199b, hsa-mir-200c, hsa-mir-204, hsa-mir-205, hsa-miR-206, hsa-mir-21, hsa-mir-211, hsa-mir-218, hsa-mir-218-1, hsa-mir-218-2, hsa-mir-219-2, hsa-mir-219-2-3p, hsa-mir-22, hsa-mir-22-3p, hsa-mir-22-5p, hsa-mir-24-2, hsa-miR-30a-e, hsa-miR-3177-5p, hsa-mir-325, hsa-mir-331, hsa-mir-331-5p, hsa-miR-3613-3p, hsa-mir-362, hsa-mir-362-5p, hsa-miR-3658, hsa-mir-367, hsa-mir-371, hsa-mir-371-5p, hsa-mir-377,

hsa-mir-378, hsa-mir-378a-5p, hsa-mir-382, hsa-mir-383, hsa-mir-422a, hsa-mir-425, hsa-miR-455-3p, hsa-miR-455-5p, hsa-miR-491, hsa-mir-508, hsa-mir-508-5p, hsa-mir-512-1, hsa-mir-512-2, hsa-miR-515-3p, hsa-mir-519c, hsa-miR-520a, hsa-mir-543, hsa-mir-545, hsa-mir-549, hsa-mir-556, and hsa-miR-568, hsa-mir-620, hsa-mir-643, hsa-mir-654-3p, hsa-miR-7a-g, hsa-mir-765, hsa-mir-871, hsa-mir-888, hsa-mir-888-3p, hsa-mir-92b, hsa-mir-93, hsa-mir-96, and hsa-mir-99a. In certain embodiments of all of the above aspects, the miRNA agent is an isolated miRNA that is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of a miRNA listed above. In certain embodiments of all of the above aspects, the miRNA agent is a seed sequence of a miRNA listed above.

In certain embodiments of all of the above aspects, the miRNA agent is an isolated miRNA selected from the group consisting of the 536 miRNAs set forth in Table 1. In certain embodiments of all of the above aspects, the miRNA agent is an isolated miRNA that is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of a miRNA listed in Table 1. In certain embodiments of all of the above aspects, the miRNA agent is a seed sequence of a miRNA listed in Table 1.

Table 1.

Adipocyte miRNAs listed in ascending order (miRBase 19 nomenclature):

hsa-let-7a-3p	hsa-miR-185-5p	hsa-miR-329	hsa-miR-509-3p
hsa-let-7a-5p	hsa-miR-186-3p	hsa-miR-330-3p	hsa-miR-511
hsa-let-7b-3p	hsa-miR-186-5p	hsa-miR-330-5p	hsa-miR-513a-3p
hsa-let-7b-5p	hsa-miR-187-3p	hsa-miR-331-3p	hsa-miR-513a-5p
hsa-let-7c	hsa-miR-188-5p	hsa-miR-331-5p	hsa-miR-513b
hsa-let-7d-3p	hsa-miR-18a-3p	hsa-miR-335-3p	hsa-miR-514a-3p
hsa-let-7d-5p	hsa-miR-18a-5p	hsa-miR-335-5p	hsa-miR-515-3p
hsa-let-7e-5p	hsa-miR-18b-5p	hsa-miR-337-3p	hsa-miR-516b-3p
hsa-let-7f-1-3p	hsa-miR-1909-3p	hsa-miR-337-5p	hsa-miR-516b-5p
hsa-let-7f-5p	hsa-miR-190a	hsa-miR-338-3p	hsa-miR-518b
hsa-let-7g-3p	hsa-miR-190b	hsa-miR-338-5p	hsa-miR-518c-3p
hsa-let-7g-5p	hsa-miR-191-3p	hsa-miR-339-3p	hsa-miR-518c-5p
hsa-let-7i-3p	hsa-miR-191-5p	hsa-miR-339-5p	hsa-miR-518f-3p
hsa-let-7i-5p	hsa-miR-192-5p	hsa-miR-33a-5p	hsa-miR-519a-5p
hsa-miR-1	hsa-miR-193a-3p	hsa-miR-33b-5p	hsa-miR-519b-5p
hsa-miR-100-5p	hsa-miR-193a-5p	hsa-miR-340-3p	hsa-miR-519c-3p
hsa-miR-101-3p	hsa-miR-193b-3p	hsa-miR-340-5p	hsa-miR-519c-5p

hsa-miR-101-5p	hsa-miR-193b-5p	hsa-miR-342-3p	hsa-miR-519d
hsa-miR-103a-2-5p	hsa-miR-194-5p	hsa-miR-342-5p	hsa-miR-520c-3p
hsa-miR-103a-3p	hsa-miR-195-3p	hsa-miR-345-5p	hsa-miR-520e
hsa-miR-103b	hsa-miR-195-5p	hsa-miR-346	hsa-miR-520f
hsa-miR-105-5p	hsa-miR-196a-5p	hsa-miR-34a-5p	hsa-miR-520g
hsa-miR-106a-5p	hsa-miR-196b-5p	hsa-miR-34b-3p	hsa-miR-520h
hsa-miR-106b-3p	hsa-miR-197-3p	hsa-miR-34b-5p	hsa-miR-521
hsa-miR-106b-5p	hsa-miR-198	hsa-miR-34c-5p	hsa-miR-522-5p
hsa-miR-107	hsa-miR-199a-3p	hsa-miR-3545-5p	hsa-miR-523-5p
hsa-miR-10a-3p	hsa-miR-199a-5p	hsa-miR-3591-3p	hsa-miR-525-3p
hsa-miR-10a-5p	hsa-miR-199b-3p	hsa-miR-361-3p	hsa-miR-532-3p
hsa-miR-10b-3p	hsa-miR-199b-5p	hsa-miR-361-5p	hsa-miR-532-5p
hsa-miR-10b-5p	hsa-miR-19a-3p	hsa-miR-3613-5p	hsa-miR-539-5p
hsa-miR-1179	hsa-miR-19b-3p	hsa-miR-3615	hsa-miR-542-3p
hsa-miR-1185-5p	hsa-miR-200a-3p	hsa-miR-362-3p	hsa-miR-542-5p
hsa-miR-1208	hsa-miR-200a-5p	hsa-miR-362-5p	hsa-miR-545-3p
hsa-miR-122-5p	hsa-miR-200b-3p	hsa-miR-363-3p	hsa-miR-545-5p
hsa-miR-1227-3p	hsa-miR-200c-3p	hsa-miR-363-5p	hsa-miR-548d-3p
hsa-miR-1228-5p	hsa-miR-202-3p	hsa-mir-365a-3p	hsa-miR-548e
hsa-miR-1229-3p	hsa-miR-203a	hsa-mir-3653	hsa-miR-548i
hsa-miR-124-3p	hsa-miR-204-5p	hsa-miR-3656	hsa-miR-548m
hsa-miR-125a-3p	hsa-miR-205-5p	hsa-miR-365a-3p	hsa-miR-550a-5p
hsa-miR-125a-5p	hsa-miR-206	hsa-miR-365a-5p	hsa-miR-551b-3p
hsa-miR-125b-1-3p	hsa-miR-20a-3p	hsa-miR-367-3p	hsa-miR-552
hsa-miR-125b-2-3p	hsa-miR-20a-5p	hsa-mir-3676-3p	hsa-miR-553
hsa-miR-125b-5p	hsa-miR-20b-5p	hsa-miR-369-3p	hsa-miR-554
hsa-miR-126-3p	hsa-miR-21-3p	hsa-miR-369-5p	hsa-miR-557
hsa-miR-126-5p	hsa-miR-21-5p	hsa-miR-370	hsa-miR-563
hsa-miR-1260a	hsa-miR-210	hsa-miR-371a-3p	hsa-miR-564
hsa-miR-1260b	hsa-miR-211-5p	hsa-miR-373-3p	hsa-miR-567
hsa-miR-1268a	hsa-miR-2110	hsa-miR-373-5p	hsa-miR-569
hsa-miR-127-3p	hsa-miR-212-3p	hsa-miR-374a-3p	hsa-miR-570-3p
hsa-miR-127-5p	hsa-miR-214-3p	hsa-miR-374a-5p	hsa-miR-572
hsa-miR-1271-5p	hsa-miR-214-5p	hsa-miR-374b-3p	hsa-miR-574-3p
hsa-miR-1273a	hsa-miR-215	hsa-miR-374b-5p	hsa-miR-574-5p
hsa-miR-1277-3p	hsa-miR-216a-5p	hsa-miR-375	hsa-miR-575
hsa-miR-128	hsa-miR-217	hsa-mir-376a-2-5p	hsa-miR-576-3p
hsa-miR-128-2	hsa-miR-218-5p	hsa-miR-376a-3p	hsa-miR-576-5p
hsa-miR-1285-3p	hsa-miR-219-1-3p	hsa-miR-376a-5p	hsa-miR-582-3p
hsa-miR-1287	hsa-miR-219-5p	hsa-miR-376b-3p	hsa-miR-582-5p
hsa-miR-1288	hsa-miR-22-3p	hsa-miR-376c-3p	hsa-miR-583

hsa-miR-129-5p	hsa-miR-22-5p	hsa-miR-377-3p	hsa-miR-584-5p
hsa-miR-1290	hsa-miR-221-3p	hsa-miR-378a-3p	hsa-miR-585
hsa-miR-1292-5p	hsa-miR-221-5p	hsa-miR-378a-5p	hsa-miR-586
hsa-miR-1301	hsa-miR-222-3p	hsa-miR-378c	hsa-miR-589-5p
hsa-miR-1305	hsa-miR-222-5p	hsa-miR-378d	hsa-miR-590-3p
hsa-miR-1307-3p	hsa-miR-223-3p	hsa-miR-379-5p	hsa-miR-590-5p
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hsa-miR-132-5p	hsa-miR-23a-3p	hsa-miR-384	hsa-miR-603
hsa-miR-1323	hsa-miR-23b-3p	hsa-miR-3912	hsa-miR-605
hsa-miR-133a	hsa-miR-23b-5p	hsa-miR-3928	hsa-miR-606
hsa-miR-133b	hsa-miR-24-1-5p	hsa-miR-409-3p	hsa-miR-609
hsa-miR-134	hsa-miR-24-2-5p	hsa-miR-409-5p	hsa-miR-611
hsa-miR-135a-5p	hsa-miR-24-3p	hsa-miR-410	hsa-miR-615-3p
hsa-miR-135b-5p	hsa-miR-25-3p	hsa-miR-411-5p	hsa-miR-619
hsa-miR-136-3p	hsa-miR-26a-2-3p	hsa-miR-421	hsa-miR-625-5p
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hsa-miR-137	hsa-miR-26b-3p	hsa-miR-422b	hsa-miR-628-3p
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hsa-miR-144-3p	hsa-miR-299-5p	hsa-miR-449a	hsa-miR-649
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hsa-miR-146b-5p	hsa-miR-29c-5p	hsa-miR-452-3p	hsa-miR-663a
hsa-miR-147a	hsa-miR-301a-3p	hsa-miR-452-5p	hsa-miR-664a-3p
hsa-miR-148a-3p	hsa-miR-301b	hsa-miR-454-3p	hsa-miR-664a-5p

hsa-miR-148a-5p	hsa-miR-302a-5p	hsa-miR-454-5p	hsa-miR-668
hsa-miR-148b-3p	hsa-miR-302b-5p	hsa-miR-455-3p	hsa-miR-671-5p
hsa-miR-148b-5p	hsa-miR-302c-5p	hsa-miR-455-5p	hsa-miR-675-3p
hsa-miR-149-5p	hsa-miR-302d-3p	hsa-miR-4634	hsa-miR-675-5p
hsa-miR-150-3p	hsa-miR-3065-3p	hsa-miR-4732-5p	hsa-miR-7-2-3p
hsa-miR-150-5p	hsa-miR-3065-5p	hsa-miR-4792	hsa-miR-7-5p
hsa-miR-151a-3p	hsa-miR-3074-3p	hsa-miR-483-3p	hsa-miR-708-3p
hsa-miR-151a-5p	hsa-miR-3074-5p	hsa-miR-483-5p	hsa-miR-708-5p
hsa-miR-151b	hsa-miR-30a-3p	hsa-miR-484	hsa-miR-718
hsa-miR-152	hsa-miR-30a-5p	hsa-miR-485-5p	hsa-miR-744-5p
hsa-miR-153	hsa-miR-30b-3p	hsa-miR-486-3p	hsa-miR-765
hsa-miR-1539	hsa-miR-30b-5p	hsa-miR-486-5p	hsa-miR-769-5p
hsa-miR-154-3p	hsa-miR-30c-1-3p	hsa-miR-487b	hsa-miR-770-5p
hsa-miR-154-5p	hsa-miR-30c-2-3p	hsa-miR-488-3p	hsa-miR-874
hsa-miR-155-5p	hsa-miR-30c-5p	hsa-miR-489	hsa-miR-885-3p
hsa-miR-15a-3p	hsa-miR-30d-3p	hsa-miR-491-3p	hsa-miR-887
hsa-miR-15a-5p	hsa-miR-30d-5p	hsa-miR-491-5p	hsa-miR-889
hsa-miR-15b-3p	hsa-miR-30e-3p	hsa-miR-492	hsa-miR-890
hsa-miR-15b-5p	hsa-miR-30e-5p	hsa-miR-493-3p	hsa-miR-891a
hsa-miR-16-1-3p	hsa-miR-31-3p	hsa-miR-493-5p	hsa-miR-891b
hsa-miR-16-2-3p	hsa-miR-31-5p	hsa-miR-494	hsa-miR-9-5p
hsa-miR-16-5p	hsa-miR-3120-3p	hsa-miR-495-3p	hsa-miR-92a-3p
hsa-miR-17-3p	hsa-miR-3120-5p	hsa-miR-497-5p	hsa-miR-92b-3p
hsa-miR-17-5p	hsa-miR-3184-5p	hsa-miR-498	hsa-miR-93-3p
hsa-miR-181a-2-3p	hsa-miR-32-3p	hsa-miR-499a-5p	hsa-miR-93-5p
hsa-miR-181a-3p	hsa-miR-32-5p	hsa-miR-500a-3p	hsa-miR-935
hsa-miR-181a-5p	hsa-miR-320a	hsa-miR-501-3p	hsa-miR-942
hsa-miR-181b-5p	hsa-miR-320b	hsa-miR-501-5p	hsa-miR-95
hsa-miR-181c-3p	hsa-miR-320c	hsa-miR-502-3p	hsa-miR-96-3p
hsa-miR-181c-5p	hsa-miR-323a-3p	hsa-miR-502-5p	hsa-miR-96-5p
hsa-miR-181d	hsa-miR-324-3p	hsa-miR-503-5p	hsa-miR-98-5p
hsa-miR-182-5p	hsa-miR-324-5p	hsa-miR-504	hsa-miR-99a-3p
hsa-miR-183-5p	hsa-miR-325	hsa-miR-505-3p	hsa-miR-99a-5p
hsa-miR-184	hsa-miR-326	hsa-miR-505-5p	hsa-miR-99b-3p
hsa-miR-185-3p	hsa-miR-328	hsa-miR-506-3p	hsa-miR-99b-5p

In certain embodiments of all of the above aspects, the miRNA agent is a miRNA selected from the group consisting of the isolated miRNAs set forth in Tables 11, 13 and 14. In certain embodiments of all of the above aspects, the miRNA agent is an isolated miRNA that is

5 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence

of a miRNA listed in Tables 1, 11, 13 and 14. In certain embodiments of all of the above aspects, the miRNA agent is a seed sequence of a miRNA listed in Tables 11, 13 and 14.

In certain embodiments of all of the above aspects, the miRNA agent is an agomir or antagomir of a miRNA selected from the group consisting of the miRNAs set forth in Table 1.

5 In certain embodiments of all of the above aspects, the miRNA agent is an agomir or antagomir of a miRNA selected from the group consisting of hsa-miR-1-1, hsa-miR-1-2, miR-19a-b, hsa-miR-105, hsa-miR-1283, hsa-mir-129, hsa-miR-133a-1, hsa-miR-133a-2, hsa-miR-143, hsa-mir-143-5p, hsa-mir-147, hsa-mir-149, hsa-mir-199a, hsa-mir-199b, hsa-mir-200c, hsa-mir-204, hsa-mir-205, hsa-miR-206, hsa-mir-21, hsa-mir-211, hsa-mir-218, hsa-mir-218-1, hsa-mir-218-2, hsa-mir-219-2, hsa-mir-219-2-3p, hsa-mir-22, hsa-mir-22-3p, hsa-mir-22-5p, hsa-mir-24-2, hsa-miR-30a-e, hsa-miR-3177-5p, hsa-mir-325, hsa-mir-331, hsa-mir-331-5p, hsa-miR-3613-3p, hsa-mir-362, hsa-mir-362-5p, hsa-miR-3658, hsa-mir-367, hsa-mir-371, hsa-mir-371-5p, hsa-mir-377, hsa-mir-378, hsa-mir-378a-5p, hsa-mir-382, hsa-mir-383, hsa-mir-422a, hsa-mir-425, hsa-miR-455-3p, hsa-miR-455-5p, hsa-miR-491, hsa-mir-508, hsa-mir-508-5p, hsa-mir-512-1, hsa-mir-512-2, hsa-miR-515-3p, hsa-mir-519e, hsa-miR-520a, hsa-mir-543, hsa-mir-545, hsa-mir-549, hsa-mir-556, and hsa-miR-568, hsa-mir-620, hsa-mir-643, hsa-mir-654-3p, hsa-miR-7a-g, hsa-mir-765, hsa-mir-871, hsa-mir-888, hsa-mir-888-3p, hsa-mir-92b, hsa-mir-93, hsa-mir-96, and hsa-mir-99a.

20 In certain embodiments of all of the above aspects, the miRNA agent is an agomir or antagomir of a miRNA selected from the group consisting of the miRNAs set forth in Table 11.

In certain embodiments of all of the above aspects, the miRNA agent is an antagomir of a miRNA selected from the group consisting of hsa-miR-19b-2-5p, hsa-miR-21-5p, hsa-miR-130b-5p, hsa-miR-211, hsa-miR-325, hsa-miR-382-3p/5p, hsa-miR-543, hsa-miR-515-3p, and hsa-miR-545.

25 In certain embodiments of all of the above aspects, the miRNA agent is an antagomir of a miRNA selected from the group consisting of hsa-miR-331-5p, hsa-miR-552, hsa-miR-620, and hsa-miR-1179.

In certain embodiments of all of the above aspects, the miRNA agent is linked to a targeting moiety (e.g., an aptamer). In one embodiment, the targeting moiety delivers the
30 miRNA agent to a specific cell type or tissue.

In certain embodiments of all of the above aspects, the miRNA agent directly binds to the mRNA or promoter region of at least one mitochondrial uncoupler.

In certain embodiments of all of the above aspects, the miRNA agent directly binds to the 5'UTR or coding sequence of the mRNA of at least one mitochondrial uncoupler.

5 In certain embodiments of all of the above aspects, the miRNA agent directly binds to the 3'UTR of the mRNA of at least one mitochondrial uncoupler.

In certain embodiments of all of the above aspects, the miRNA agent modulates the activity of an activator or repressor of a mitochondrial uncoupling protein. In one embodiment, the miRNA agent directly binds to the mRNA or promoter region of the activator or repressor.

10 In one embodiment, the miRNA agent directly binds to the 5'UTR or coding sequence of the mRNA of the activator or repressor. In one embodiment, the miRNA agent directly binds to the 3'UTR of the mRNA of the activator or repressor. In one embodiment, the activator or repressor is selected from the group listed in Table 2.

15 In certain embodiments of all of the above aspects, the mRNA or protein expression of the mitochondrial uncoupling protein is upregulated.

In certain embodiments of all of the above aspects, the mitochondrial uncoupling activity of the mitochondrial uncoupling protein is upregulated.

In another aspect, the invention provides a method of screening for a miRNA agent that modulates thermogenesis, the method generally comprising: providing an indicator cell; 20 contacting the indicator cell with a test miRNA agent; and determining the cellular activity of at least one thermogenic regulator in the indicator cell in the presence and absence of the miRNA agent, wherein a change in the activity of the thermogenic regulator in the presence of the test miRNA agent identifies the test miRNA agent as a miRNA agent that modulates thermogenesis. The indicator cell can be a mammalian cell. In certain embodiments, the indicator cell is a 25 human cell comprising at least a portion of a human genome.

In certain embodiments, the cell is a pre-adipocyte, adipocyte, adipose tissue derived mesenchymal stem cell, hepatocyte, myocyte, or a precursor thereof.

In certain embodiments, the cellular activity of the thermogenic regulator determined in the method is the mRNA expression level, protein expression level or mitochondrial uncoupling 30 activity of the thermogenic regulator.

In certain embodiments, the test miRNA agent increases the activity of the thermogenic regulator compared to the level of activity of the thermogenic regulator in the absence of the test miRNA agent.

In certain embodiments, the thermogenic regulator is UCP1 or UCP2.

5 In another aspect, the invention provides an agomir or antagomir that modulates the activity of at least one thermogenic regulator in a cell.

In certain embodiments, the agomir or antagomir is an agomir or antagomir of a miRNA selected from the group consisting of the miRNAs set forth in Tables 11, 13 and 14.

10 In certain embodiments, the agomir or antagomir is an agomir or antagomir of a miRNA selected from the group consisting of the miRNAs set forth in Table 1.

In certain embodiments, the agomir or antagomir is an agomir or antagomir of a miRNA selected from the group consisting of hsa-miR-1-1, hsa-miR-1-2, miR-19a-b, hsa-miR-105, hsa-miR-1283, hsa-mir-129, hsa-miR-133a-1, hsa-miR-133a-2, hsa-miR-143, hsa-mir-143-5p, hsa-mir-147, hsa-mir-149, hsa-mir-199a, hsa-mir-199b, hsa-mir-200c, hsa-mir-204, hsa-mir-205, hsa-miR-206, hsa-mir-21, hsa-mir-211, hsa-mir-218, hsa-mir-218-1, hsa-mir-218-2, hsa-mir-219-2, hsa-mir-219-2-3p, hsa-mir-22, hsa-mir-22-3p, hsa-mir-22-5p, hsa-mir-24-2, hsa-miR-30a-e, hsa-miR-3177-5p, hsa-mir-325, hsa-mir-331, hsa-mir-331-5p, hsa-miR-3613-3p, hsa-mir-362, hsa-mir-362-5p, hsa-miR-3658, hsa-mir-367, hsa-mir-371, hsa-mir-371-5p, hsa-mir-377, hsa-mir-378, hsa-mir-378a-5p, hsa-mir-382, hsa-mir-383, hsa-mir-422a, hsa-mir-425, hsa-miR-455-3p, hsa-miR-455-5p, hsa-miR-491, hsa-mir-508, hsa-mir-508-5p, hsa-mir-512-1, hsa-mir-512-2, hsa-miR-515-3p, hsa-mir-519e, hsa-miR-520a, hsa-mir-543, hsa-mir-545, hsa-mir-549, hsa-mir-556, and hsa-miR-568, hsa-mir-620, hsa-mir-643, hsa-mir-654-3p, hsa-miR-7a-g, hsa-mir-765, hsa-mir-871, hsa-mir-888, hsa-mir-888-3p, hsa-mir-92b, hsa-mir-93, hsa-mir-96, and hsa-mir-99a.

25 In certain embodiments, the agomir or antagomir is an antagomir of a miRNA selected from the group consisting of hsa-miR-19b-2-5p, hsa-miR-21-5p, hsa-miR-130b-5p, hsa-miR-211, hsa-miR-325, hsa-miR-382-3p/5p, hsa-miR-543, hsa-miR-515-3p, and hsa-miR-545.

In certain embodiments, the agomir or antagomir is an antagomir of a miRNA selected from the group consisting of hsa-miR-331-5p, hsa-miR-552, hsa-miR-620, and hsa-miR-1179.

30 In certain embodiments, the agomir or antagomir is linked to a targeting moiety.

In certain embodiments, the targeting moiety is an aptamer.

In certain embodiments, the targeting moiety delivers the agomir or antagomir to a specific cell type or tissue.

In certain embodiments, the agomir or antagomir directly binds to the mRNA or promoter region of at least one mitochondrial uncoupler.

5 In certain embodiments, the agomir or antagomir directly binds to the 5'UTR or coding sequence of the mRNA of at least one mitochondrial uncoupler.

In certain embodiments, the agomir or antagomir directly binds to the 3'UTR of the mRNA of at least one mitochondrial uncoupler.

10 In certain embodiments, the agomir or antagomir modulates the activity of an activator or repressor of a mitochondrial uncoupling protein.

In certain embodiments, the activator or repressor is selected from the group listed in Table 2.

In certain embodiments, the agomir or antagomir directly binds to the mRNA or promoter region of the activator or repressor.

15 In certain embodiments, the agomir or antagomir directly binds to the 5'UTR or coding sequence of the mRNA of the activator or repressor. In other embodiments, the agomir or antagomir directly binds to the 3'UTR of the mRNA of the activator or repressor.

20 The disclosure also provides a pharmaceutical composition comprising two or more miRNAs selected from hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir and hsa-miR-30b antagomir. In certain embodiments the pharmaceutical composition also includes a pharmaceutically acceptable excipient. In certain embodiments, the two or more miRNAs are expressed from a recombinant vector. The recombinant vector can be selected from DNA plasmids, viral vectors and DNA minicircles.

25 The disclosure also provides a method of inducing pre-adipocytes to differentiate initially into white adipocytes and subsequently into brown adipocytes comprising administering to a population of pre-adipocytes one or more miRNAs selected from hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir and hsa-miR-30b antagomir. The one or more miRNAs can also
30 be selected from hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir and hsa-miR-30b antagomir.

antagomir. In certain embodiments, the induction of pre-adipocytes to differentiate into adipocytes is greater than the differentiation of pre-adipocytes to adipocytes when pre-adipocytes are exposed to 100 nM rosiglitazone for two days followed by maintenance medium. In certain embodiments, the adipocytes are brown adipocytes. In other embodiments, the adipocytes are white adipocytes. Additional criteria for differentiation can be found in the Examples, below.

The disclosure also provides a method for decreasing the lipid content of adipocytes comprising administering to a population of adipocytes one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir and hsa-miR-30b antagomir. In certain embodiments, the lipid content of the adipocytes is less than the lipid content of adipocytes exposed to 100 nM rosiglitazone for two days followed by maintenance medium or less than the fat content of adipocytes exposed to 100 nM rosiglitazone for the duration of culture. The duration of culture can be 8-16, 10-14 or 14 days. The duration of culture can also be 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 days. Additional criteria for lipid content of adipocytes can be found in the Examples, below.

The disclosure also provides a method for increasing insulin sensitivity in a subject in need thereof comprising administering the subject one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir and hsa-miR-30b antagomir.

In certain embodiments, the subject is a mammal.

The disclosure also provides a method of increasing expression or activity of one or more uncoupling proteins in a cell comprising administering to the cell one or more, two or more, or three or more miRNAs selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir and hsa-miR-30b agomir. In certain embodiments, the cell is selected from the group consisting of a brown adipocyte, a white adipocyte, a subcutaneous adipocyte, a liver cell or a muscle cell. In other embodiments, the one or more uncoupling proteins include UCP1 or UCP2. In certain embodiments, the method is an ex vivo method. In other embodiments, the method is an in vivo method. In certain embodiments, the method involves selecting a subject (e.g., a human) in need of increasing the level of expression or activity of one or more uncoupling proteins (e.g., UCP1, UCP2). In some embodiments, the subject has, or is at risk of developing, obesity. In certain embodiments, the subject has, or is at

risk of developing, diabetes. In certain embodiments, the method further comprises determining the expression level (mRNA or protein) or activity of the one or more uncoupling proteins.

The disclosure also provides a method of causing fat loss in a subject in need thereof comprising administering the subject one or more miRNAs selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir and hsa-miR-30b agomir. In certain embodiments, the subject is a mammal. In other embodiments, the mammal is a human.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B are schematic representations of the interactions of 83 thermogenic regulators determined using the STRING 9.0 database at two different levels of stringency.

Figure 2A is a schematic representation of the interaction of 83 thermogenic regulators determined using the Ingenuity Pathway Analysis Software program.

Figure 2B is a schematic representation of the interaction of 83 thermogenic regulators determined using the Reactome Functional Interaction Network program.

Figure 3 is a schematic representation of the overlap of results from multiple miRNA prediction programs predicting miRNA binding sites in the 5'UTR, promoter region, coding sequence and 3'UTR of the human UCP1 gene.

Figure 4 is a schematic representation of the overlap of results from multiple miRNA prediction programs predicting miRNA binding sites in the 5'UTR, promoter region, coding sequence and 3'UTR of the genes of 83 thermogenic regulators.

Figure 5 is a schematic representation of oxidative phosphorylation in mitochondria, illustrating the uncoupling of oxidative phosphorylation from ATP synthesis by UCP1 to generate heat.

Figure 6 depicts the transcriptional control of UCP1 by other exemplary thermogenic regulators.

Figure 7 depicts exemplary positive (a) and negative (b) transcriptional regulators of UCP1 gene transcription.

Figure 8A depicts the location of various regulatory elements in reference to the transcription start site (position 5,001) in the 15,910 base pair (bp) sequence of the human UCP1 gene (NCBI Reference Sequence: gi|237858805|ref|NG_012139.1| Homo sapiens uncoupling protein 1 (mitochondrial, proton carrier) (UCP1), RefSeqGene on chromosome 4).

Figure 8B depicts the location of various regulatory elements in reference to the transcription start site (position 5,001) in the 15,174 bp sequence of the human UCP2 gene (ENSG00000175567), including 5,000 bp 5'UTR and 2,000 bp 3'UTR on chromosome 11.

Figure 9 is a bar graph showing relative fluorescence in pre-adipocytes either unlabeled
5 or transfected with a Dy547-labeled non-targeting miRNA mimic or hairpin inhibitor.

Figure 10A is a bar graph showing the reduction of GAPDH gene expression in pre-adipocytes transfected with siRNA control and a GAPDH siRNA 4 days after transfection.

Figure 10B is a bar graph showing the reduction of GAPDH gene expression in pre-adipocytes transfected with siRNA control and a GAPDH siRNA 12 days after transfection.

10 Figure 11A is a light micrograph of pre-adipocytes stained with Oil Red O cultured for 2 weeks in maintenance medium alone.

Figure 11B is a light micrograph of pre-adipocytes stained with Oil Red O cultured in the presence of insulin, tri-iodothyronine, dexamethasone, isobutyl-methylxanthine and rosiglitazone for two days followed by maintenance medium for 12 days.

15 Figure 11C is a light micrograph of pre-adipocytes stained with Oil Red O cultured in the presence of insulin, tri-iodothyronine, dexamethasone, isobutyl-methylxanthine and rosiglitazone throughout the experiment.

Figure 11D is a light micrograph of pre-adipocytes stained with Oil Red O cultured in the presence of hsa-miR-30b mimic.

20 Figure 11E is a light micrograph of pre-adipocytes stained with Oil Red O cultured in the presence of a non-targeting miRNA mimic.

Figure 11F is a light micrograph of pre-adipocytes stained with Oil Red O cultured in the presence of a non-targeting miRNA inhibitor.

25 Figure 12A is a bar graph showing mRNA expression of thermogenesis targets in the presence of rosiglitazone.

Figure 12B is a bar graph showing mRNA expression of thermogenesis targets in the presence of hsa-let-7a inhibitor.

Figure 12C is a bar graph showing mRNA expression of thermogenesis targets in the presence of hsa-miR-1 mimic.

30 Figure 12D is a bar graph showing mRNA expression of thermogenesis targets in the presence of hsa-miR-19b mimic.

Figure 12E is a bar graph showing mRNA expression of thermogenesis targets in the presence of and hsa-miR-30b mimic.

Figure 12F is a bar graph showing mRNA expression of thermogenesis targets in untreated pre-adipocytes.

5 Figure 13 is an M-A plot showing the mean gene expression on the x-axis and the difference between pairs in logarithmic scale on the y-axis.

Figure 14 is a schematic showing a Venn Diagram showing that the numbers of genes significantly upregulated in the presence of the miRNA analogs hsa-let-7a inhibitor, hsa-miR-1 mimic, hsa-miR-19b mimic and hsa-miR-30b mimic were respectively 305, 247, 255 and 267. A
10 set of 127 genes was commonly upregulated by the listed miRNA analogs.

Figure 15 is a schematic showing a Venn diagram showing that the numbers of genes significantly downregulated in the presence of the miRNA analogs hsa-let-7a inhibitor, hsa-miR-1 mimic, hsa-miR-19b mimic and hsa-miR-30b mimic were respectively 143, 177, 115 and 165. A set of 60 genes was commonly downregulated by the listed miRNA analogs.

15 Figure 16 is a bar graph showing relative fluorescence in adipocytes either unlabeled or transfected with a Dy547 labeled non-targeting miRIDIAN mimic or hairpin inhibitor.

Figure 17A is a bar graph showing the reduction of GAPDH gene expression in cells transfected with siRNA control and a GAPDH siRNA 4 days after transfection.

20 Figure 17B is a bar graph showing the reduction of GAPDH gene expression in cells transfected with siRNA control and a GAPDH siRNA 12 days after transfection.

Figure 18A is a light micrograph of mature adipocytes stained with Oil Red O cultured for 2 weeks in maintenance medium alone.

Figure 18B is a light micrograph of mature adipocytes stained with Oil Red O cultured in the presence of rosiglitazone for two weeks.

25 Figure 18C is a light micrograph of mature adipocytes stained with Oil Red O cultured in the presence of a non-targeting miRNA.

Figure 18D is a light micrograph of mature adipocytes stained with Oil Red O cultured in the presence of hsa-miR-30b mimic.

30 Figure 19 is a bar graph showing the amount of lipids (Nile Red fluorescent dye) in mature adipocytes exposed to various miRNA analogs or rosiglitazone.

Figure 20 is a bar graph showing the amounts of total RNA extracted from mature adipocytes exposed to various transfecting agents.

Figure 21 is a bar graph showing reduction of GAPDH gene expression in mature adipocytes transfected with a GAPDH-specific miRNA mimic using various transfecting agents.

Figure 22 represents bright field micrographs of mature adipocytes cultured for 2 weeks in maintenance medium alone (control), 50 nM hsa-let-7a inhibitor, 10 μ M beta adrenergic receptor agonist CL316,243, 50 nM hsa-miR-1 mimic, 10 nM thyroid hormone tri-iodothyronine, 50 nM hsa-miR-19b mimic, 100 nM Rosiglitazone or 50 nM hsa-miR-30b mimic.

Figure 23 is a schematic representation of the Cell-SELEX process use to isolate aptamers specifically directed against unique targets at the surface of human cells.

Figure 24 depicts the results of a FACS experiment assessing binding of selected fluorescent aptamers to human hepatocytes and adipocytes.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application, including definitions, will control.

As used herein, the term "miRNA agent" refers to an oligonucleotide or oligonucleotide mimetic that directly or indirectly modulates the activity of a thermogenic regulator (e.g., a mitochondrial uncoupler or an activator or repressor thereof). miRNA agents can act on a target gene or on a target miRNA.

As used herein, the term "miRNA" refers to a single-stranded RNA molecule (or a synthetic derivative thereof), which is capable of binding to a target gene (either the mRNA or the DNA) and regulating expression of that gene. In certain embodiments, the miRNA is naturally expressed in an organism.

As used herein, the term "seed sequence" refers to a 6–8 nucleotide (nt) long substring within the first 8 nt at the 5'-end of the miRNA (i.e., seed sequence) that is an important determinant of target specificity.

As used herein, the term "agomir" refers to a synthetic oligonucleotide or oligonucleotide mimetic that functionally mimics a miRNA. An agomir can be an oligonucleotide with the same

or similar nucleic acid sequence to a miRNA or a portion of a miRNA. In certain embodiments, the agomir has 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotide differences from the miRNA that it mimics. Further, agomirs can have the same length, a longer length or a shorter length than the miRNA that it mimics. In certain embodiments, the agomir has the same sequence as 6-8
5 nucleotides at the 5' end of the miRNA it mimics. In other embodiments, an agomir can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides in length. In other embodiments, an agomir can be 5-10, 6-8, 10-20, 10-15 or 5-500 nucleotides in length. In certain embodiments, agomirs include any of the sequences shown in Tables 1, 11, 13 and 14.

10 These chemically modified synthetic RNA duplexes include a guide strand that is identical or substantially identical to the miRNA of interest to allow efficient loading into the miRISC complex, whereas the passenger strand is chemically modified to prevent its loading to the Argonaute protein in the miRISC complex (Thorsen SB et al., *Cancer J.*, 18(3):275-284 (2012); Broderick JA et al., *Gene Ther.*, 18(12):1104-1110 (2011)).

15 As used herein, the term "antagomir" refers to a synthetic oligonucleotide or oligonucleotide mimetic having complementarity to a specific microRNA, and which inhibits the activity of that miRNA. In certain embodiments, the antagomir has 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotide differences from the miRNA that it inhibits. Further, antagomirs can have the same length, a longer length or a shorter length than the miRNA that it inhibits. In certain
20 embodiments, the antagomir hybridizes to 6-8 nucleotides at the 5' end of the miRNA it inhibits. In other embodiments, an antagomir can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides in length. In other embodiments, an antagomir can be 5-10, 6-8, 10-20, 10-15 or 5-500 nucleotides in length. In certain embodiments, antagomirs include nucleotides
25 that are complementary to any of the sequences shown in Tables 1, 11, 13 and 14. The antagomirs are synthetic reverse complements that tightly bind to and inactivate a specific miRNA. Various chemical modifications are used to improve nuclease resistance and binding affinity. The most commonly used modifications to increase potency include various 2' sugar modifications, such as 2'-O-Me, 2'-O-methoxyethyl (2'-MOE), or 2'-fluoro(2'-F). The nucleic
30 acid structure of the miRNA can also be modified into a locked nucleic acid (LNA) with a methylene bridge between the 2' oxygen and the 4' carbon to lock the ribose in the 3'-endo

(North) conformation in the A-type conformation of nucleic acids (Lennox KA et al.. Gene Ther. Dec 2011;18(12):1111-1120; Bader AG et al. Gene Ther. Dec 2011;18(12):1121-1126). This modification significantly increases both target specificity and hybridization properties of the molecules.

5 As used herein, the term "aptamir" refers to the combination of an aptamer (oligonucleic acid or peptide molecule that bind to a specific target molecule) and an agomir or antagomir as defined above, which allows cell or tissue-specific delivery of the miRNA agents.

 As used herein, the term "interfering RNA" refers to any double stranded or single stranded RNA sequence capable of inhibiting or down-regulating gene expression by mediating
10 RNA interference. Interfering RNAs include, but are not limited to, small interfering RNA ("siRNA") and small hairpin RNA ("shRNA"). "RNA interference" refers to the selective degradation of a sequence-compatible messenger RNA transcript.

 As used herein, the term "small interfering RNA" or "siRNA" refers to any small RNA molecule capable of inhibiting or down regulating gene expression by mediating RNA
15 interference in a sequence specific manner. The small RNA can be, for example, about 16 to 21 nucleotides long.

 As used herein, the term "shRNA" (small hairpin RNA) refers to an RNA molecule comprising an antisense region, a loop portion and a sense region, wherein the sense region has complementary nucleotides that base pair with the antisense region to form a duplex stem.
20 Following post-transcriptional processing, the small hairpin RNA is converted into a small interfering RNA (siRNA) by a cleavage event mediated by the enzyme Dicer, which is a member of the RNase III family.

 As used herein, the term "antisense oligonucleotide" refers to a synthetic oligonucleotide or oligonucleotide mimetic that is complementary to a DNA or mRNA sequence (e.g., a
25 miRNA).

 As used herein, the term "miR-mask" refers to a single stranded antisense oligonucleotide that is complementary to a miRNA binding site in a target mRNA, and that serves to inhibit the binding of miRNA to the mRNA binding site. See, e.g., Xiao, et al. "Novel approaches for gene-specific interference via manipulating actions of microRNAs: examination on the pacemaker
30 channel genes HCN2 and HCN4," Journal of Cellular Physiology, vol. 212, no. 2, pp. 285-292, 2007, which is incorporated herein in its entirety.

As used herein, the term "miRNA sponge" refers to a synthetic nucleic acid (e.g. a mRNA transcript) that contains multiple tandem-binding sites for a miRNA of interest, and that serves to titrate out the endogenous miRNA of interest, thus inhibiting the binding of the miRNA of interest to its endogenous targets. See, e.g., Ebert et al., "MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells," Nature Methods, vol. 4, no. 9, pp. 721–726, 2007, which is incorporated herein in its entirety.

As used herein, the term "respiratory chain uncoupling" refers to the dissipation of the mitochondrial inner membrane proton gradient, thereby preventing the synthesis of ATP in the mitochondrion by oxidative phosphorylation.

As used herein, the term "mitochondrial uncoupler" refers to a protein (or the encoding nucleic acid) that can dissipate of the mitochondrial inner membrane proton gradient, thereby preventing the synthesis of ATP in the mitochondrion by oxidative phosphorylation. Exemplary mitochondrial uncouplers include UCP1 and UCP2.

As used herein, the terms "activator" or "repressor" of a mitochondrial uncoupler refers to a protein that serves to upregulate or downregulate, respectively, an activity of a mitochondrial uncoupler.

As used herein, the term "thermogenic regulator" refers to a protein (or the encoding nucleic acid) that regulates thermogenesis either directly or indirectly. The term encompasses mitochondrial uncouplers, and also activators and repressors of mitochondrial uncouplers.

Exemplary thermogenic regulators are set forth in Table 2 herein.

As used herein, the term "modulate" refers to increasing or decreasing a parameter. For example, to modulate the activity of a protein that protein's activity could be increased or decreased.

As used herein, the term "activity" of mitochondrial uncoupler or thermogenic regulator refers to any measurable biological activity including, without limitation, mRNA expression, protein expression, or respiratory chain uncoupling.

The "effective amount" of the miRNA agent composition is an amount sufficient to be effective in treating or preventing a disorder or to regulate a physiological condition in humans. In certain embodiments, this physiological condition is obesity.

A "subject" is a vertebrate, including any member of the class Mammalia, including humans, domestic and farm animals, zoo, sports or pet animals, such as mouse, rabbit, pig, sheep, goat, cattle and higher primates.

The term "mammal" refers to any species that is a member of the class Mammalia, including rodents, primates, dogs, cats, camelids and ungulates. The term "rodent" refers to any species that is a member of the order rodentia including mice, rats, hamsters, gerbils and rabbits. The term "primate" refers to any species that is a member of the order primates, including monkeys, apes and humans. The term "camelids" refers to any species that is a member of the family camelidae including camels and llamas. The term "ungulates" refers to any species that is a member of the superorder ungulata including cattle, horses and camelids. According to some embodiments, the mammal is a human.

"Treatment", or "treating" as used herein, is defined as the application or administration of a therapeutic agent (e.g., a miRNA agent or vector or transgene encoding same) to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has the disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease or disorder, the symptoms of the disease or disorder, or the predisposition toward disease.

"Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers to the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype").

The "effective amount" of the miRNA agent composition is an amount sufficient to be effective in treating or preventing a disorder or to regulate a physiological condition in humans.

II. Thermogenesis and Obesity

In certain embodiments, the invention provides methods for modulating thermogenesis. These methods generally involve contacting cells or tissue with a miRNA agent that modulates activity of at least one mitochondrial uncoupler (e.g., UCP1 and/or UCP2). Such methods and compositions are particularly useful for treating obesity.

Mammalian adipocytes can be categorized into two major categories based on their functional profiles: 1) energy-storing and releasing, lipid-filled white adipocytes (WAT) and; 2) energy-expenditure and heat producing, mitochondria-rich brown adipocytes (BAT). Until recently, it was believed that BAT underwent rapid involution in early childhood, leaving only vestigial amounts in adults. However, positron-emission tomography (PET) studies performed in humans with the tracer 18F-fluorodeoxyglucose (18F-FDG) demonstrated that: 1) multiple depots of BAT are still present in the cervical, supraclavicular, axillary and paravertebral regions in adult subjects; 2) BAT in adult humans can be rapidly activated by exposure to cold temperatures; 3) there is an inverse correlation between the activity of BAT and age, body-mass index (BMI), the percentage of body fat, fasting plasma glucose level, beta-blocker use and outdoor temperature; and 4) BAT expansion may drive the weight loss associated with catecholamine-producing pheochromocytomas, whereas beta3-adrenoreceptor polymorphisms leading to a reduction in receptor function have been linked to weight gain and early onset type 2 diabetes mellitus.

Although WAT and BAT are derived from mesenchymal stem cells, they have distinct lineages, with Myf5 (Myogenic Regulatory Factor 5) (shared with skeletal myocyte progenitors), PGC-1alpha and PRDM16 (PR-domain-containing 16) expression distinguishing the brown from white adipocyte precursors. In addition to the classic brown adipocytes, a different type of brown fat cells can be induced in tissues where WAT predominates. The termed "brite" (brown-in-white) adipocyte has been coined and the appearance of brown-like adipocytes within WAT depots is associated with improved metabolic phenotypes. Increasing BAT mass and/or activity offers a degree of protection from obesity. Heat production by BAT is 300 W/g compared to 1 W/g in all other tissues. Relatively limited amounts of BAT would be required to make significant impact on energy balance, since as little as 50 g of BAT would account for 20% of daily energy expenditure. It has been speculated that the estimated 63 g of BAT found in the supraclavicular/paracervical depot of one subject could combust the energy equivalent of 4.1 kg of WAT over 1 year.

Mitochondrial uncoupling proteins (UCP) are members of the family of mitochondrial anion carrier proteins (MACP). UCPs separate oxidative phosphorylation from ATP synthesis with energy dissipated as heat (also referred to as the "mitochondrial proton leak"). UCPs facilitate the transfer of anions from the inner to the outer mitochondrial membrane and the

return transfer of protons from the outer to the inner mitochondrial membrane generating heat in the process. UCPs are the primary proteins responsible for thermogenesis and heat dissipation. Uncoupling Protein 1 (UCP1), also named thermogenin, is a BAT specific protein responsible for thermogenesis and heat dissipation. UCP2 is another Uncoupling Protein also expressed in adipocytes. UCPs are part of network of thermogenic regulator proteins (see Figure 1). Exemplary thermogenic regulators are set forth in Table 2.

Modulation of thermogenic regulators to induce BAT differentiation and/or mitochondrial uncoupling proteins provides a method to induce thermogenesis in a subject and, hence, to treat obesity. However, chemical pharmacologic approaches cannot target these molecules, as they do not belong to the classic ‘target classes’ (kinases, ion channels, G-protein coupled receptors, etc.) that dominate the ‘druggable space’ of traditional drug discovery. Accordingly, the invention provides novel methods and compositions for modulating these thermogenic regulators using miRNA agents.

In certain embodiments, miRNA agents are employed to upregulate the activity of a mitochondrial uncoupler (e.g., the mRNA expression level, protein expression level, or mitochondrial uncoupling activity). Upregulation of a mitochondrial uncoupler can be achieved in several ways. In one embodiment, the miRNA agent directly inhibits the activity of a naturally occurring miRNA that is responsible for downregulation of the activity (e.g., the mRNA expression level, protein expression level) of the mitochondrial uncoupler. In another embodiment, the miRNA agent upregulates the activity (e.g., the mRNA expression level or the protein expression level) of an activator of the mitochondrial uncoupler. This upregulation can be achieved, for example, by directly inhibiting the activity of a naturally occurring miRNA that is responsible for downregulation of the expression of the activator. In yet another embodiment, the miRNA agent downregulates the activity (e.g., the mRNA expression level or the protein expression level) of a repressor of the mitochondrial uncoupler. This downregulation can be achieved, for example, by directly inhibiting the expression of a repressor of a mitochondrial uncoupler using a miRNA agent.

In certain embodiments, miRNA agents are employed that are capable of modulating the activity of multiple thermogenic regulators simultaneously (Pathway-specific miRNA agents as opposed to universal miRNA agents). For example, a single miRNA, agomir or antagomir that binds to multiple thermogenic regulators can be used. This approach is particularly

advantageous in that it allows for the modulation of multiple members of an entire signaling pathway using a single miRNA agent.

In certain embodiments, multiple inhibitory miRNA agents (e.g., antagomirs or miR-masks) are employed. These inhibitory miRNA agents can have the same or different miRNA targets.

III. miRNA Agents

In certain embodiments, the invention employs miRNA agents for the modulation of thermogenic regulators (e.g., mitochondrial uncouplers, such as UCP1 and/or UCP2). miRNA agents, suitable for use in the methods disclosed herein, included, without limitation, miRNA, agomirs, antagomirs, miR-masks, miRNA-sponges, siRNA (single- or double-stranded), shRNA, antisense oligonucleotides, ribozymes, or other oligonucleotide mimetics which hybridize to at least a portion of a target nucleic acid and modulate its function.

In certain embodiments, the miRNA agents are miRNA molecules or synthetic derivatives thereof (e.g., agomirs). In one particular embodiment, the miRNA agent is a miRNA. miRNAs are a class of small (e.g., 18-24 nucleotides) non-coding RNAs that exist in a variety of organisms, including mammals, and are conserved in evolution. miRNAs are processed from hairpin precursors of about 70 nucleotides which are derived from primary transcripts through sequential cleavage by the RNase III enzymes drosha and dicer. Many miRNAs can be encoded in intergenic regions, hosted within introns of pre-mRNAs or within ncRNA genes. Many miRNAs also tend to be clustered and transcribed as polycistrons and often have similar spatial temporal expression patterns. In general, miRNAs are post-transcriptional regulators that bind to complementary sequences on a target gene (mRNA or DNA), resulting in gene silencing by, e.g., translational repression or target degradation. One miRNA can target many different genes simultaneously. Exemplary miRNA molecules for use in the disclosed methods include without limitation: hsa-miR-1-1, hsa-miR-1-2, hsa-miR-7a-g, hsa-miR-105, hsa-miR-1283, hsa-mir-129, hsa-miR-133a-1, hsa-miR-133a-2, hsa-miR-143, hsa-mir-143-5p, hsa-mir-147, hsa-mir-149, hsa-miR-19a-b, hsa-mir-199a, hsa-mir-199b, hsa-mir-200c, hsa-mir-204, hsa-mir-205, hsa-miR-206, hsa-mir-21, hsa-mir-211, hsa-mir-218, hsa-mir-218-1, hsa-mir-218-2, hsa-mir-219-2, hsa-mir-219-2-3p, hsa-mir-22, hsa-mir-22-3p, hsa-mir-22-5p, hsa-mir-24-2, hsa-miR-30a-e, hsa-miR-3177-5p, hsa-mir-325, hsa-mir-331, hsa-mir-331-5p, hsa-miR-3613-3p, hsa-mir-362, hsa-mir-

362-5p, hsa-mir-367, hsa-mir-371, hsa-mir-371-5p, hsa-mir-377, hsa-mir-378, hsa-mir-378a-5p, hsa-mir-382, hsa-mir-383, hsa-miR-3658, hsa-mir-422a, hsa-mir-425, hsa-miR-455-3p, hsa-miR-455-5p, hsa-miR-491, hsa-mir-508, hsa-mir-508-5p, hsa-mir-512-1, hsa-mir-512-2, hsa-miR-515-3p, hsa-mir-519e, hsa-miR-520a, hsa-mir-543, hsa-mir-545, hsa-mir-549, hsa-mir-556, hsa-miR-568, hsa-mir-620, hsa-mir-643, hsa-mir-654-3p, hsa-mir-765, hsa-mir-871, hsa-mir-888, hsa-mir-888-3p, hsa-mir-92b, hsa-mir-93, hsa-mir-96, hsa-mir-99a. In other embodiments, exemplary miRNA molecules for use in the disclosed methods miRNA disclosed in Tables 1, 11, 13 and 14, herein. In one particular embodiment, the miRNA agent is human miR-22, or a functional derivative thereof.

In another particular embodiment, the miRNA agent is an agomir. Agomirs of a particular miRNA can be identified using the screening methods disclosed herein. In one particular embodiment, the agomir is a functional mimetic of human miR-22 (Davidson BL et al., *Nat. Rev. Genet.*, 12(5):329-340 (2011)).

In certain embodiments, the miRNA agents are oligonucleotide or oligonucleotide mimetics that inhibit the activity of one or more miRNA. Examples of such molecules include, without limitation, antagomirs, interfering RNA, antisense oligonucleotides, ribozymes, miRNA sponges and miR-masks. In one particular embodiment, the miRNA agent is an antagomir. In general, antagomirs are chemically modified antisense oligonucleotides that bind to a target miRNA and inhibit miRNA function by preventing binding of the miRNA to its cognate gene target. Antagomirs can include any base modification known in the art. In one particular embodiment, the antagomir inhibits the activity of human miR-22 (van Rooij E et al., *Circ. Res.*, 110(3):496-507 (2012); Snead NM et al., *Nucleic Acid Ther.*, 22(3):139-146 (2012); Czech MP et al., *Nat. Rev. Endocrinol.*, 7(8):473-484 (2011)).

In certain embodiments, the miRNA agents are 10 to 50 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies oligonucleotides having antisense portions of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length, or any range therewithin.

In certain embodiments, the miRNA agents are chimeric oligonucleotides that contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least one region of modified nucleotides that confers one or

more beneficial properties (such as, for example, increased nuclease resistance, increased uptake into cells, increased binding affinity for the target) and a region that is a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. Chimeric inhibitory nucleic acids of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures comprise, but are not limited to, US patent nos: 5,013,830; 5,149,797; 5, 220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference in its entirety.

In certain embodiments, the miRNA agents comprise at least one nucleotide modified at the 2' position of the sugar, most preferably a 2'-O-alkyl, 2'-O-alkyl-O-alkyl or 2'-fluoro-modified nucleotide. In other preferred embodiments, RNA modifications include 2'-fluoro, 2'-amino and 2' O-methyl modifications on the ribose of pyrimidines, a basic residue or an inverted base at the 3' end of the RNA. Such modifications are routinely incorporated into oligonucleotides and these oligonucleotides have been shown to have a higher T_m (i.e., higher target binding affinity) than 2'-deoxyoligonucleotides against a given target.

A number of nucleotide and nucleoside modifications have been shown to make an oligonucleotide more resistant to nuclease digestion, thereby prolonging *in vivo* half-life. Specific examples of modified oligonucleotides include those comprising backbones comprising, for example, phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Most preferred are oligonucleotides with phosphorothioate backbones and those with heteroatom backbones, particularly $\text{CH}_2\text{-NH-O-CH}_2$, $\text{CH}_2\text{-N(CH}_3\text{)-O-CH}_2$ (known as a methylene(methylimino) or MMI backbone), $\text{CH}_2\text{-O-N(CH}_3\text{)-CH}_2$, $\text{CH}_2\text{-N(CH}_3\text{)-N(CH}_3\text{)-CH}_2$ and $\text{O-N(CH}_3\text{)-CH}_2\text{-CH}_2$ backbones, wherein the native phosphodiester backbone is represented as O-P-O-CH_2); amide backbones (see De Mesmaeker et al. *Ace. Chem. Res.* 1995, 28:366-374); morpholino backbone structures (see Summerton and Weller, U.S. Pat. No. 5,034,506); peptide nucleic acid (PNA) backbone (wherein the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone, see Nielsen et al., *Science* 1991,

254, 1497), each of which is herein incorporated by reference in its entirety. Phosphorus-containing linkages include, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'alkylene phosphonates and chiral phosphonates, phosphinates, 5 phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs 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'; see US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5, 177,196; 5,188,897; 10 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321, 131; 5,399,676; 5,405,939; 5,453,496; 5,455, 233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563, 253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference in its entirety.

Morpholino-based oligomeric compounds are described in Dwaine A. Braasch and David R. Corey, *Biochemistry*, 2002, 41(14), 4503-4510; *Genesis*, volume 30, issue 3, 2001; Heasman, J., 15 *Dev. Biol.*, 2002, 243, 209-214; Nasevicius et al., *Nat. Genet.*, 2000, 26, 216-220; Lacerra et al., *Proc. Natl. Acad. Sci.*, 2000, 97, 9591-9596; and U.S. Pat. No. 5,034,506, issued Jul. 23, 1991, each of which is herein incorporated by reference in its entirety. Cyclohexenyl nucleic acid oligonucleotide mimetics are described in Wang et al., *J. Am. Chem. Soc.*, 2000, 122, 8595-8602, the contents of which is incorporated herein in its entirety.

20 Modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by 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. These comprise those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, 25 sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts; see US patent nos. 5,034,506; 5,166,315; 5, 185,444; 5,214,134; 5,216, 141; 5,235,033; 5,264,562; 5, 264,564; 30 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596, 086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623, 070; 5,663,312;

5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference in its entirety.

In certain embodiments, miRNA agents comprise one or more substituted sugar moieties, e.g., one of the following at the 2' position: OH, SH, SCH₃, F, OCN, OCH₃, OCH₃, O(CH₂)_n CH₃, O(CH₂)_n NH₂ or O(CH₂)_n CH₃ where n is from 1 to about 10; Ci to CIO lower alkyl, alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF₃; OCF₃; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; SOCH₃; SO₂ CH₃; ONO₂; NO₂; N₃; NH₂; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted 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 pharmacokinetic/pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy [2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl)] (Martin et al., *Helv. Chim. Acta*, 1995, 78, 486). Other preferred modifications include 2'-methoxy (2'-O-CH₃), 2'-propoxy (2'-OCH₂ CH₂CH₃) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group.

In certain embodiments, miRNA agents comprise one or more base modifications and/or substitutions. As used herein, "unmodified" or "natural" bases include adenine (A), guanine (G), thymine (T), cytosine (C) and uracil (U). Modified bases include, without limitation, bases found only infrequently or transiently in natural nucleic acids, e.g., hypoxanthine, 6-methyladenine, 5-Me pyrimidines, particularly 5-methylcytosine (also referred to as 5-methyl-2' deoxycytosine and often referred to in the art as 5-Me-C), 5-hydroxymethylcytosine (HMC), glycosyl HMC and gentobiosyl HMC, as well as synthetic bases, e.g., 2-aminoadenine, 2-(methylamino)adenine, 2-(imidazolylalkyl)adenine, 2-(aminoalkylamino)adenine or other heterosubstituted alkyladenines, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 8-azaguanine, 7-deazaguanine, N6 (6-aminohexyl)adenine and 2,6-diaminopurine. Kornberg, A., *DNA Replication*, W. H. Freeman & Co., San Francisco, 1980, pp75-77; Gebeyehu, G., et al. *Nucl. Acids Res.* 1987, 15:4513). A "universal" base known in the art, e.g., inosine, can also be included. 5-Me-C substitutions can also be included. These have been shown to increase nucleic acid duplex stability by 0.6-1.2OC. (Sanghvi, Y. S., in Crooke, S. T. and Lebleu, B., eds.,

Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278). Further suitable modified bases are described in US patent nos. 3,687,808, as well as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,596,091; 5,614,617; 5,750,692, and 5,681,941, each of which is herein incorporated by reference.

It is not necessary for all positions in a given oligonucleotide to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single oligonucleotide or even at a single nucleoside within an oligonucleotide.

In certain embodiments, both a sugar and an internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, for example, an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds comprise, but are not limited to, US patent nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., *Science*, 1991, 254, 1497-1500.

In certain embodiments, the miRNA agent is linked (covalently or non-covalently) to one or more moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide. Such moieties include, without limitation, lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N. Y. Acad. Sci.*, 1992, 660, 306-309; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Kabanov et al., *FEBS Lett.*, 1990, 259, 327-330; Svinarchuk et al., *Biochimie*, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Mancharan

et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-t oxysterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937), each of which is herein

5 incorporated by reference in its entirety. See also US patent nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552, 538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486, 603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762, 779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082, 830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 10 5,214,136; 5, 245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391, 723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5, 565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599, 928 and 5,688,941, each of which is herein incorporated by reference in its entirety.

In one particular embodiment, the miRNA agent is linked to (covalently or non- 15 covalently) to a nucleic acid aptamer. Aptamers are synthetic oligonucleotides or peptide molecules that bind to a specific target molecule. Aptamers appropriate for use with the miRNA agents provided herein are described in U.S. Provisional Patent Application No. 61/695,477 filed 8/31/2012 and incorporated by reference herein in its entirety.

Accordingly, in a first aspect, the invention provides an adipocyte-specific miRNA 20 modulator composition comprising: I) a targeting moiety that selectively binds to a cellular surface marker on an adipose target cell in a human and II) a thermogenic miRNA modulator moiety, wherein the targeting moiety facilitates uptake of the miRNA modulatory moiety by the target cell such that the miRNA is capable of targeting a thermogenic pathway and up regulating thermogenesis in the target cell.

25 In one embodiment, the composition comprises an aptamer comprising an aptamer as the targeting moiety.

In certain embodiments, the aptamers used with the miRNAs disclosed herein specifically bind to cell surface marker proteins on an adipose tissue mesenchymal stem cell (ATMSC), white adipose tissue (WAT) adipocytes and brown adipose tissue (BAT) adipocytes. Cell 30 surface markers for ATMSCs include CD9, CD10, CD13, CD29, CD36, CD44, CD49d, CD54, CD55, CD59, CD73, CD90, CD91, CD105, CD137, CD146, CD166, and HLA-ABC. Cell

surface markers for WAT adipocytes include Adiponectin, Caveolin-1, Caveolin-2, CD36 (FAT), CLH-22 (Clathrin Heavy Chain Chr 22), FABP4 (Adipocyte protein 2, aP2), SLC27A1 (FATP1), SLC27A2 (FATP2), GLUT4 (Glucose Transporter 4), Perilipin 2 or Resistin. Cell surface markers for all adipocytes include Neprilysin (CD10), FAT (CD36), Thy-1 (CD90), Low density lipoprotein receptor-related protein 1 (LRP1 or CD91), Caveolin-1, Caveolin-2, Fatty acid binding protein 4 (FABP4), Cell surface glycoprotein MUC18 (CD146), Activated leukocyte cell adhesion molecule (CD166) and Natriuretic peptide receptor A (NPR1).

According to other embodiments, the aptamers for use with the miRNAs disclosed herein can also specifically bind to markers of adipose tissue including adiponectin, leptin, resistin, FGF 17, FGF 19, BMP7, PYY, MetAP2, RBP4, endostatin, and angiostatin.

In certain embodiments, the aptamers are selected by the Cell-SELEX technology which uses whole living cells as the target, whereby aptamers that recognize specific molecules in their native conformation in their natural environment on the surface of intact cells are selected by repeated amplification and binding to living cells. In this cell-based selection, specific cell surface molecules or even unknown membrane receptors can be directly targeted within their native environment, allowing a straightforward enrichment of cell-specific aptamers.

In certain exemplary embodiments, the miRNA modulator is combined with an aptamer to create an "AptamiR" composition. There are many different ways to combine an aptamer and miRNA analog(s) to create an aptamir. They include, for example, aptamer-miRNA analog chimeras, aptamer-splice-switching oligonucleotide chimeras, and aptamer conjugated to nanoparticles or liposomes containing the miRNA analog(s). "Escort Aptamers" may be inserted at the surface of functional polymers, liposomes, and nanoparticles, each of which can carry many miRNA analogs. For instance, the size of thioaptamer-conjugated liposomes is about 120 nm. Nanoparticle approaches have several functional advantages, including, for example, cellular uptake, the ability to cross membranes, and triggered nanoparticle disassembly.

In one embodiment, an aptamiR composition comprises an aptamer that is directly linked or fused to a miRNA modulator. Such aptamiRs are entirely chemically synthesized, which provides more control over the composition of the conjugate. For instance, the stoichiometry (ratio of miRNA analog per aptamer) and site of attachment can be precisely defined. The linkage portion of the conjugate presents a plurality (2 or more) of nucleophilic and/or electrophilic moieties that serve as the reactive attachment point for the aptamers and miRNA

analogs. In addition, the aptamer may further comprise a linker between the aptamer and the miRNA analog. In some embodiments, the linker is a polyalkylene glycol, particularly a polyethylene glycol. In other embodiments, the linker is a liposome, exosome, dendrimer, or comb polymer. Other linkers can mediate the conjugation between the aptamer and the miRNA analog, including a biotin-streptavidin bridge, or a ribonucleic acid. Exemplary non-covalent linkers include linkers formed by base pairing a single stranded portion or overhang of the miRNA moiety and a complementary single-stranded portion or overhang of the aptamer moiety.

In another particular embodiment, an aptamer is combined with a miRNA analog in the form of a liposome-based aptamiR. Liposomes are spherical nanostructures made of a lipid bilayer that can be loaded with pharmaceuticals, such as miRNAs. Furthermore, the liposome surface can be loaded with different substances, such as polyethylene glycol (extending their systemic half-life) or molecular recognition moieties like aptamers for specific binding to targeted cells. For example, aptamer-modified liposomes have been developed, with each liposome displaying approximately 250 aptamers tethered to its surface to facilitate target binding. In a preferred embodiment, liposomes are created to encapsulate miRNA analog(s) and display at their surface aptamers that specifically bind with high affinity and specificity to molecules (e.g. lipid transporters) highly expressed at the surface of adipocytes and ATMSCs. The fusion of the liposomes with the targeted cells causes the release of the miRNA analog(s) into the cell cytoplasm, which then alter a specific intra-cellular pathway. Alternatively, stable thioaptamers may be inserted at the surface of liposomes to guide delivery of the liposome miRNA analog(s) load to targeted ATMSCs and adipocytes.

In a further particular embodiment, an aptamer is combined with a miRNA analog in the form of a carrier-based aptamiR. Exemplary carriers include nanoparticles, liposomes or exosomes. Such carrier-based aptamiR compositions have the capability of delivering a cargo of multiple miRNA modulators to the target cell in a single carrier. To accomplish targeting and accumulation, the carriers are formulated to present the targeting moiety on their external surface so they can react/bind with selected cell surface antigens or receptors on the adipose target cell. As an example, carriers may be created to encapsulate miRNA modulators while displaying at their surface aptamers that specifically bind with high affinity and specificity to molecules (e.g. lipid transporters) highly expressed at the surface of adipocytes and ATMSCs. The internalized exosomes release inside the cell cytoplasm their miRNA analog(s) load, which alters a specific

intra-cellular pathway.

In one embodiment, the carrier is an exosome. Exosomes, which originate from late endosomes, are naturally occurring nanoparticles that are specifically loaded with proteins, mRNAs, or miRNAs, and are secreted endogenously by cells. Exosomes are released from host
5 cells, are not cytotoxic, and can transfer information to specific cells based on their composition and the substance in/on the exosome. Because exosomes are particles of approximately 20–100 nm in diameter, the exosomes evade clearance by the mononuclear phagocyte system (which clears circulating particles >100 nm in size), and are very efficiently delivered to target tissues.

Moreover, synthetic exosomes may offer several advantages over other carriers. For
10 example, they may deliver their cargo directly into the cytosol, while their inertness avoids attack and clearance in the extracellular environment. The structural constituents of exosomes may include small molecules responsible for processes like signal transduction, membrane transport, antigen presentation, targeting/adhesion, among many others.

The miRNA agents must be sufficiently complementary to the target mRNA, i.e.,
15 hybridize sufficiently well and with sufficient specificity, to give the desired effect.

"Complementary" refers to the capacity for pairing, through hydrogen bonding, between two sequences comprising naturally or non-naturally occurring bases or analogs thereof. For example, if a base at one position of a miRNA agent is capable of hydrogen bonding with a base at the corresponding position of a target nucleic acid sequence, then the bases are considered to
20 be complementary to each other at that position. In certain embodiments, 100% complementarity is not required. In other embodiments, 100% complementarity is required.

miRNA agents for use in the methods disclosed herein can be designed using routine methods. While the specific sequences of certain exemplary target nucleic acid sequences and miRNA agents are set forth herein, one of skill in the art will recognize that these serve to
25 illustrate and describe particular embodiments within the scope of the present invention.

Additional target segments are readily identifiable by one having ordinary skill in the art in view of this disclosure. Target segments of 5, 6, 7, 8, 9, 10 or more nucleotides in length comprising a stretch of at least five (5) consecutive nucleotides within the seed sequence, or immediately adjacent thereto, are considered to be suitable for targeting a gene. In some embodiments, target
30 segments can include sequences that comprise at least the 5 consecutive nucleotides from the 5' terminus of one of the seed sequence (the remaining nucleotides being a consecutive stretch of

the same RNA beginning immediately upstream of the 5'-terminus of the seed sequence and continuing until the miRNA agent contains about 5 to about 30 nucleotides). In some embodiments, target segments are represented by RNA sequences that comprise at least the 5 consecutive nucleotides from the 3'-terminus of one of the seed sequence (the remaining
5 nucleotides being a consecutive stretch of the same miRNA beginning immediately downstream of the 3'-terminus of the target segment and continuing until the miRNA agent contains about 5 to about 30 nucleotides). One having skill in the art armed with the sequences provided herein will be able, without undue experimentation, to identify further preferred regions to target using miRNA agents. Once one or more target regions, segments or sites have been identified,
10 inhibitory nucleic acid compounds are chosen that are sufficiently complementary to the target, i.e., that hybridize sufficiently well and with sufficient specificity (i.e., do not substantially bind to other non-target nucleic acid sequences), to give the desired effect.

In certain embodiments, miRNA agents used to practice this invention are expressed from a recombinant vector. Suitable recombinant vectors include, without limitation, DNA
15 plasmids, viral vectors or DNA minicircles. Generation of the vector construct can be accomplished using any suitable genetic engineering techniques well known in the art, including, without limitation, the standard techniques of PCR, oligonucleotide synthesis, restriction endonuclease digestion, ligation, transformation, plasmid purification, and DNA sequencing, for example as described in Sambrook et al. *Molecular Cloning: A Laboratory Manual*. (1989),
20 Coffin et al. *(Retroviruses)*. (1997) and *"RNA Viruses: A Practical Approach"* (Alan J. Cann, Ed., Oxford University Press, (2000)). As will be apparent to one of ordinary skill in the art, a variety of suitable vectors are available for transferring nucleic acids of the invention into cells. The selection of an appropriate vector to deliver nucleic acids and optimization of the conditions for insertion of the selected expression vector into the cell, are within the scope of one of ordinary
25 skill in the art without the need for undue experimentation. Viral vectors comprise a nucleotide sequence having sequences for the production of recombinant virus in a packaging cell. Viral vectors expressing nucleic acids of the invention can be constructed based on viral backbones including, but not limited to, a retrovirus, lentivirus, adenovirus, adeno-associated virus, pox virus or alphavirus. The recombinant vectors can be delivered as described herein, and persist in
30 target cells (e.g., stable transformants).

In certain embodiments, miRNA agents used to practice this invention are synthesized *in vitro* using chemical synthesis techniques, as described in, e.g., Adams (1983) J. Am. Chem. Soc. 105:661; Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic. Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896; Narang (1979) Meth. Enzymol. 68:90; Brown (1979) Meth. Enzymol. 68: 109; Beaucage (1981) Tetra. Lett. 22: 1859; U.S. Patent No. 4,458,066, each of which is herein incorporated by reference in its entirety.

IV. Methods of Treatment

In one aspect, the invention provides a method of treating obesity in human subject. The method generally comprises administering to the human subject an effective amount of a miRNA agent that modulates activity of at least one thermogenic regulator, (e.g., a mitochondrial uncoupler, such as UCP1 and/or UCP2).

Such methods of treatment may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the target gene molecules of the present invention or target gene modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

miRNA agents can be tested in an appropriate animal model e.g., an obesity model including ob/ob mice (Lindstrom P., Scientific World Journal, 7:666-685 (2007) and db/db mice (Sharma K et al., Am J Physiol Renal Physiol., 284(6):F1138-1144 (2003)). For example, a miRNA agent (or expression vector or transgene encoding same) as described herein can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with said agent. Alternatively, a therapeutic agent can be used in an animal model to determine the mechanism of action of such an agent. For example, a miRNA agent can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent can be used in an animal model to determine the mechanism of action of such an agent.

The disclosure also provides a method of inducing pre-adipocytes to differentiate into white adipocytes and white adipocytes into brown adipocytes, comprising administering to a population of pre-adipocytes one or more miRNAs selected from hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir and hsa-miR-30b antagomir. In certain embodiments, the induction of pre-adipocytes to differentiate into adipocytes is greater than the differentiation of pre-adipocytes to adipocytes than when pre-adipocytes are exposed to 100 nM rosiglitazone for two days followed by maintenance medium. In certain embodiments, the adipocytes are brown adipocytes. In other embodiments, the adipocytes are white adipocytes.

The disclosure also provides a method for increasing insulin sensitivity in a subject in need thereof comprising administering the subject one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir and hsa-miR-30b antagomir. In certain embodiments, the subject is a mammal.

The disclosure also provides a method of causing fat loss in a subject in need thereof comprising administering the subject one or more miRNAs selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir and hsa-miR-30b agomir. In certain embodiments, the subject is a mammal. In other embodiments, the mammal is a human.

A miRNA agent modified for enhancing uptake into cells (e.g., adipose cells) can be administered at a unit dose less than about 15 mg per kg of bodyweight, or less than 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00005 or 0.00001 mg per kg of bodyweight, and less than 200 nmole of miRNA agent (e.g., about 4.4×10^{16} copies) per kg of bodyweight, or less than 1500, 750, 300, 150, 75, 15, 7.5, 1.5, 0.75, 0.15, 0.075, 0.015, 0.0075, 0.0015, 0.00075, 0.00015 nmole of RNA silencing agent per kg of bodyweight. The unit dose, for example, can be administered by injection (e.g., intravenous or intramuscular), an inhaled dose, or a topical application. Particularly preferred dosages are less than 2, 1, or 0.1 mg/kg of body weight.

Delivery of a miRNA agent directly to an organ or tissue (e.g., directly to adipose tissue) can be at a dosage on the order of about 0.00001 mg to about 3 mg per organ/tissue, or preferably about 0.0001-0.001 mg per organ/tissue, about 0.03-3.0 mg per organ/tissue, about 0.1-3.0 mg per organ/tissue or about 0.3-3.0 mg per organ/tissue. The dosage can be an amount effective to treat or prevent obesity or to increase insulin sensitivity. In one embodiment, the

unit dose is administered less frequently than once a day, e.g., less than every 2, 4, 8 or 30 days. In another embodiment, the unit dose is not administered with a frequency (e.g., not a regular frequency). For example, the unit dose may be administered a single time. In one embodiment, the effective dose is administered with other traditional therapeutic modalities.

5 In certain embodiment, a subject is administered an initial dose, and one or more maintenance doses of a miRNA agent. The maintenance dose or doses are generally lower than the initial dose, e.g., one-half less of the initial dose. A maintenance regimen can include treating the subject with a dose or doses ranging from 0.01 mg/kg to 1.4 mg/kg of body weight per day, e.g., 10, 1, 0.1, 0.01, 0.001, or 0.00001 mg per kg of bodyweight per day. The maintenance doses
10 are preferably administered no more than once every 5, 10, or 30 days. Further, the treatment regimen may last for a period of time which will vary depending upon the nature of the particular disease, its severity and the overall condition of the patient. In preferred embodiments the dosage may be delivered no more than once per day, e.g., no more than once per 24, 36, 48, or more hours, e.g., no more than once every 5 or 8 days. Following treatment, the patient can be
15 monitored for changes in condition, e.g., changes in percentage body fat. The dosage of the compound may either be increased in the event the patient does not respond significantly to current dosage levels, or the dose may be decreased if a decrease in body fat is observed, or if undesired side effects are observed.

 The effective dose can be administered in a single dose or in two or more doses, as
20 desired or considered appropriate under the specific circumstances. If desired to facilitate repeated or frequent infusions, implantation of a delivery device, e.g., a pump, semi-permanent stent (e.g., sub-cutaneous, intravenous, intraperitoneal, intracisternal or intracapsular), or reservoir may be advisable. In one embodiment, a pharmaceutical composition includes a plurality of miRNA agent species. In another embodiment, the miRNA agent species has
25 sequences that are non-overlapping and non-adjacent to another species with respect to a naturally occurring target sequence. In another embodiment, the plurality of miRNA agent species is specific for different naturally occurring target genes. In another embodiment, the miRNA agent is allele specific. In another embodiment, the plurality of miRNA agent species target two or more SNP alleles (e.g., two, three, four, five, six, or more SNP alleles).

30 Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the compound of the

invention is administered in maintenance doses, ranging from 0.01 mg per kg to 100 mg per kg of body weight (see U.S. Pat. No. 6,107,094).

The concentration or amount of miRNA agent administered will depend on the parameters determined for the agent and the method of administration, e.g. nasal, buccal, or pulmonary. For example, nasal formulations tend to require much lower concentrations of some ingredients in order to avoid irritation or burning of the nasal passages. It is sometimes desirable to dilute an oral formulation up to 10-100 times in order to provide a suitable nasal formulation.

Certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a miRNA agent can include a single treatment or, preferably, can include a series of treatments. It will also be appreciated that the effective dosage of a miRNA agent for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein. For example, the subject can be monitored after administering a miRNA agent composition. Based on information from the monitoring, an additional amount of the miRNA agent composition can be administered.

Dosing is dependent on severity and responsiveness of the disease condition to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual compounds, and can generally be estimated based on EC50s found to be effective in *in vitro* and *in vivo* animal models. In some embodiments, the animal models include transgenic animals that express a human gene, e.g., a gene that produces a target mRNA (e.g., a thermogenic regulator). The transgenic animal can be deficient for the corresponding endogenous mRNA. In another embodiment, the composition for testing includes a miRNA agent that is complementary, at least in an internal region, to a sequence that is conserved between a nucleic acid sequence in the animal model and the target nucleic acid sequence in a human.

Several studies have reported successful mammalian dosing using miRNA agents. For example, Esau C, et al., *Cell Metabolism*, 3(2): 87-98 (2006) reported dosing of normal mice with intraperitoneal doses of miR-122 antisense oligonucleotide ranging from 12.5 to 75 mg/kg twice weekly for 4 weeks. The mice appeared healthy and normal at the end of treatment, with
5 no loss of body weight or reduced food intake. Plasma transaminase levels were in the normal range (AST $\frac{3}{4}$ 45, ALT $\frac{3}{4}$ 35) for all doses with the exception of the 75 mg/kg dose of miR-122 ASO, which showed a very mild increase in ALT and AST levels. They concluded that 50mg/kg was an effective, nontoxic dose. Another study by Krutzfeldt J., et al., *Nature*, 438, 685-689 (2005), injected antagomirs to silence miR-122 in mice using a total dose of 80, 160 or 240 mg
10 per kg body weight. The highest dose resulted in a complete loss of miR-122 signal. In yet another study, locked nucleic acids ("LNAs") were successfully applied in primates to silence miR-122. Elmen J., et al., (2008) *Nature* 452, 896-899, report that efficient silencing of miR-122 was achieved in primates by three doses of 10 mg per kg LNA-antimiR, leading to a long-lasting and reversible decrease in total plasma cholesterol without any evidence for LNA-associated
15 toxicities or histopathological changes in the study animals.

In certain embodiments, miRNA agents used to practice this invention are administered through expression from a recombinant vector. Suitable recombinant vectors include, without limitation, DNA plasmids, viral vectors or DNA minicircles. Generation of the vector construct can be accomplished using any suitable genetic engineering techniques well known in the art,
20 including, without limitation, the standard techniques of PCR, oligonucleotide synthesis, restriction endonuclease digestion, ligation, transformation, plasmid purification, and DNA sequencing, for example as described in Sambrook et al. *Molecular Cloning: A Laboratory Manual*. (1989)), Coffin et al. (*Retroviruses*. (1997)) and "RNA Viruses: A Practical Approach" (Alan J. Cann, Ed., Oxford University Press, (2000)). As will be apparent to one of ordinary skill
25 in the art, a variety of suitable vectors are available for transferring nucleic acids of the invention into cells. The selection of an appropriate vector to deliver nucleic acids and optimization of the conditions for insertion of the selected expression vector into the cell, are within the scope of one of ordinary skill in the art without the need for undue experimentation. Viral vectors comprise a nucleotide sequence having sequences for the production of recombinant virus in a packaging
30 cell. Viral vectors expressing nucleic acids of the invention can be constructed based on viral backbones including, but not limited to, a retrovirus, lentivirus, adenovirus, adeno-associated

virus, pox virus or alphavirus. The recombinant vectors can be delivered as described herein, and persist in target cells (e.g., stable transformants).

miRNA agents may be directly introduced into a cell (e.g., an adipocyte); or introduced extracellularly into a cavity, interstitial space, into the circulation of an organism, introduced orally, or may be introduced by bathing a cell or organism in a solution containing the nucleic acid. Vascular or extravascular circulation, the blood or lymph system, and the cerebrospinal fluid are sites where the nucleic acid may be introduced.

The miRNA agents of the invention can be introduced using nucleic acid delivery methods known in art including injection of a solution containing the nucleic acid, bombardment by particles covered by the nucleic acid, soaking the cell or organism in a solution of the nucleic acid, or electroporation of cell membranes in the presence of the nucleic acid. Other methods known in the art for introducing nucleic acids to cells may be used, such as lipid-mediated carrier transport, chemical-mediated transport, and cationic liposome transfection such as calcium phosphate, and the like. The miRNA agents may be introduced along with other components e.g., compounds that enhance miRNA agent uptake by a cell.

In certain embodiments, the methods described herein include co-administration of miRNA agents with other drugs or pharmaceuticals, e.g., compositions for modulating thermogenesis, compositions for treating diabetes, compositions for treating obesity. Compositions for modulating thermogenesis include beta-3 adrenergic receptor agonists, thyroid hormones, PPARG agonists, leptin, adiponectin, and orexin.

V. Screening Methods

In another aspect, the invention provides a method of screening for a miRNA agent that modulates thermogenesis, decreases obesity, or improves insulin sensitivity. The method generally comprises the steps of: providing an indicator cell; contacting the indicator cell with a test miRNA agent; and determining the expression level and/or cellular activity of at least one thermogenic regulator in the indicator cell in the presence and absence of the miRNA agent, wherein a change in the activity of the thermogenic regulator in the presence of the test miRNA agent identifies the test miRNA agent as a miRNA agent that modulates thermogenesis, decreases obesity, or improves insulin sensitivity. In certain embodiments, the method involves determining an increase the expression level and/or activity of the thermogenic regulator (e.g.,

UCP1, UCP2). The indicator cell can be a mammalian cell. In certain embodiments, the mammalian cell is a human cell, which comprises at least a portion of a human genome.

Any thermogenic regulator can be assayed in the methods disclosed herein. Exemplary thermogenic regulators are set forth in Table 2. In a preferred embodiment, the thermogenic
5 regulator is a mitochondrial uncoupling protein e.g., UCP1 and/or UCP2.

Any cell in which the activity of a thermogenic regulator can be measured is suitable for use in the methods disclosed herein. Exemplary cells include pre-adipocytes, adipocytes, adipose tissue derived mesenchymal stem cells, hepatocytes, myocytes, or precursors thereof.

Any activity of a thermogenic regulator can be assayed, including, without limitation,
10 mRNA expression level, protein expression level or mitochondrial uncoupling activity of the thermogenic regulator. Methods for determining such activities are well known in the art.

Any miRNA agent can be screened, including, without limitation, miRNA, agomirs, antagomirs, aptamirs, miR-masks, miRNA sponges, siRNA (single- or double-stranded), shRNA, antisense oligonucleotides, ribozymes, or other oligonucleotide mimetics which
15 hybridize to at least a portion of a target nucleic acid and modulate its function.

VI. Pharmaceutical Compositions

In one aspect, the methods disclosed herein can include the administration of pharmaceutical compositions and formulations comprising miRNA agents capable of modulating
20 the activity of at least one thermogenic modulator.

In certain embodiments, the compositions are formulated with a pharmaceutically acceptable carrier. The pharmaceutical compositions and formulations can be administered parenterally, topically, by direct administration into the gastrointestinal tract (e.g., orally or rectally), or by local administration, such as by aerosol or transdermally. The pharmaceutical
25 compositions can be formulated in any way and can be administered in a variety of unit dosage forms depending upon the condition or disease and the degree of illness, the general medical condition of each patient, the resulting preferred method of administration and the like. Details on techniques for formulation and administration of pharmaceuticals are well described in the scientific and patent literature, see, e.g., Remington: The Science and Practice of Pharmacy, 21st
30 ed., 2005.

The miRNA agents can be administered alone or as a component of a pharmaceutical formulation (composition). The compounds may be formulated for administration, in any convenient way for use in human or veterinary medicine. Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Formulations of the compositions of the invention include those suitable for intradermal, inhalation, oral/ nasal, topical, parenteral, rectal, and/or intravaginal administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient (e.g., nucleic acid sequences of this invention) which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration, e.g., intradermal or inhalation. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect, e.g., an antigen specific T cell or humoral response.

Pharmaceutical formulations of the invention can be prepared according to any method known to the art for the manufacture of pharmaceuticals. Such drugs can contain sweetening agents, flavoring agents, coloring agents and preserving agents. A formulation can be admixed with nontoxic pharmaceutically acceptable excipients which are suitable for manufacture. Formulations may comprise one or more diluents, emulsifiers, preservatives, buffers, excipients, etc. and may be provided in such forms as liquids, powders, emulsions, lyophilized powders, sprays, creams, lotions, controlled release formulations, tablets, pills, gels, on patches, in implants, etc.

Pharmaceutical formulations for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in appropriate and suitable dosages. Such carriers enable the pharmaceuticals to be formulated in unit dosage forms as tablets, pills, powder, dragées, capsules, liquids, lozenges, gels, syrups, slurries, suspensions, etc., suitable for ingestion by the patient. Pharmaceutical preparations for oral use can be formulated as a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable additional compounds, if desired, to obtain tablets or dragée cores. Suitable solid

excipients are carbohydrate or protein fillers include, e.g., sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxy-methylcellulose; and gums including arabic and tragacanth; and proteins, e.g., gelatin and collagen. Disintegrating or
5 solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate. Push-fit capsules can contain active agents mixed with a filler or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active agents can be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without
10 stabilizers.

Aqueous suspensions can contain an active agent (e.g., nucleic acid sequences of the invention) in admixture with excipients suitable for the manufacture of aqueous suspensions, e.g., for aqueous intradermal injections. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium
15 alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethylene oxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol (e.g.,
20 polyoxyethylene sorbitol mono-oleate), or a condensation product of ethylene oxide with a partial ester derived from fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan mono-oleate). The aqueous suspension can also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, aspartame or saccharin. Formulations can be
25 adjusted for osmolarity.

In certain embodiments, oil-based pharmaceuticals are used for administration of the miRNA agents. Oil-based suspensions can be formulated by suspending an active agent in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin; or a mixture of these. See e.g., U.S. Patent No. 5,716,928 describing using
30 essential oils or essential oil components for increasing bioavailability and reducing inter- and intra-individual variability of orally administered hydrophobic pharmaceutical compounds (see

also U.S. Patent No. 5,858,401). The oil suspensions can contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents can be added to provide a palatable oral preparation, such as glycerol, sorbitol or sucrose. These formulations can be preserved by the addition of an antioxidant such as ascorbic acid. As an example of an injectable oil vehicle,
5 see Minto (1997) J. Pharmacol. Exp. Ther. 281:93-102.

In certain embodiments, the pharmaceutical compositions and formulations are in the form of oil-in- water emulsions. The oily phase can be a vegetable oil or a mineral oil, described above, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin,
10 esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan mono-oleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan mono-oleate. The emulsion can also contain sweetening agents and flavoring agents, as in the formulation of syrups and elixirs. Such formulations can also contain a demulcent, a preservative, or a coloring agent. In alternative embodiments, these injectable oil-
15 in-water emulsions of the invention comprise a paraffin oil, a sorbitan monooleate, an ethoxylated sorbitan monooleate and/or an ethoxylated sorbitan trioleate.

In certain embodiments, the pharmaceutical compositions and formulations are administered by in intranasal, intraocular and intravaginal routes including suppositories, insufflation, powders and aerosol formulations (for examples of steroid inhalants, see e.g.,
20 Rohatagi (1995) J. Clin. Pharmacol. 35: 1 187-1193; Tjwa (1995) Ann. Allergy Asthma Immunol. 75: 107-1 11). Suppositories formulations can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at body temperatures and will therefore melt in the body to release the drug. Such materials are cocoa butter and polyethylene glycols.

25 In certain embodiments, the pharmaceutical compositions and formulations are delivered transdermally, by a topical route, formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

In certain embodiments, the pharmaceutical compositions and formulations are delivered as microspheres for slow release in the body. For example, microspheres can be administered via
30 intradermal injection of drug which slowly release subcutaneously; see Rao (1995) J. Biomater Sci. Polym. Ed. 7:623-645; as biodegradable and injectable gel formulations, see, e.g., Gao

(1995) Pharm. Res. 12:857-863 (1995); or, as microspheres for oral administration, see, e.g., Eyles (1997) J. Pharm. Pharmacol. 49:669-674.

In certain embodiments, the pharmaceutical compositions and formulations are parenterally administered, such as by intravenous (IV) administration or administration into a body cavity or lumen of an organ. These formulations can comprise a solution of active agent dissolved in a pharmaceutically acceptable carrier. Acceptable vehicles and solvents that can be employed are water and Ringer's solution, an isotonic sodium chloride. In addition, sterile fixed oils can be employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can likewise be used in the preparation of injectables. These solutions are sterile and generally free of undesirable matter. These formulations may be sterilized by conventional, well-known sterilization techniques. The formulations may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight, and the like, in accordance with the particular mode of administration selected and the patient's needs. For IV administration, the formulation can be a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated using those suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a suspension in a nontoxic parenterally-acceptable diluent or solvent, such as a solution of 1,3-butanediol. The administration can be by bolus or continuous infusion (e.g., substantially uninterrupted introduction into a blood vessel for a specified period of time).

In certain embodiments, the pharmaceutical compounds and formulations are lyophilized. Stable lyophilized formulations comprising an inhibitory nucleic acid can be made by lyophilizing a solution comprising a pharmaceutical of the invention and a bulking agent, e.g., mannitol, trehalose, raffinose, and sucrose or mixtures thereof. A process for preparing a stable lyophilized formulation can include lyophilizing a solution about 2.5 mg/mL nucleic acid, about 15 mg/mL sucrose, about 19 mg/mL NaCl, and a sodium citrate buffer having a pH greater than 5.5 but less than 6.5. See, e.g., U.S. 20040028670.

In certain embodiments, the pharmaceutical compositions and formulations are delivered by the use of liposomes. By using liposomes, particularly where the liposome surface carries ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the active agent into target cells in vivo. See, e.g., U.S. Patent Nos. 6,063,400; 6,007,839; Al-Muhammed (1996) *J. Microencapsul.* 13 :293-306; Chonn (1995) *Curr. Opin. Biotechnol.* 6:698-708; Ostro (1989) *Am. J. Hosp. Pharm.* 46: 1576-1587.

The formulations of the invention can be administered for prophylactic and/or therapeutic treatments. In certain embodiments, for therapeutic applications, compositions are administered to a subject who is need of reduced triglyceride levels, or who is at risk of or has a disorder described herein, in an amount sufficient to cure, alleviate or partially arrest the clinical manifestations of the disorder or its complications; this can be called a therapeutically effective amount. For example, in certain embodiments, pharmaceutical compositions of the invention are administered in an amount sufficient to treat obesity in a subject.

The amount of pharmaceutical composition adequate to accomplish this is a therapeutically effective dose. The dosage schedule and amounts effective for this use, i.e., the dosing regimen, will depend upon a variety of factors, including the stage of the disease or condition, the severity of the disease or condition, the general state of the patient's health, the patient's physical status, age and the like. In calculating the dosage regimen for a patient, the mode of administration also is taken into consideration.

The dosage regimen also takes into consideration pharmacokinetics parameters well known in the art, i.e., the active agents' rate of absorption, bioavailability, metabolism, clearance, and the like (see, e.g., Hidalgo-Aragones (1996) *J. Steroid Biochem. Mol. Biol.* 58:611-617; Groning (1996) *Pharmazie* 51:337-341; Fotherby (1996) *Contraception* 54:59-69; Johnson (1995) *J. Pharm. Sci.* 84: 1 144-1 146; Rohatagi (1995) *Pharmazie* 50:610-613; Brophy (1983) *Eur. J. Clin. Pharmacol.* 24: 103-108; Remington: *The Science and Practice of Pharmacy*, 21st ed., 2005). The state of the art allows the clinician to determine the dosage regimen for each individual patient, active agent and disease or condition treated. Guidelines provided for similar compositions used as pharmaceuticals can be used as guidance to determine the dosage regiment, i.e., dose schedule and dosage levels, administered practicing the methods of the invention are correct and appropriate. Single or multiple administrations of formulations can be given depending on for example: the dosage and frequency as required and tolerated by the patient, the

degree and amount of cholesterol homeostasis generated after each administration, and the like. The formulations should provide a sufficient quantity of active agent to effectively treat, prevent or ameliorate conditions, diseases or symptoms, e.g., treat obesity.

In certain embodiments, pharmaceutical formulations for oral administration are in a daily amount of between about 1 to 100 or more mg per kilogram of body weight per day. Lower dosages can be used, in contrast to administration orally, into the blood stream, into a body cavity or into a lumen of an organ. Substantially higher dosages can be used in topical or oral administration or administering by powders, spray or inhalation. Actual methods for preparing parenterally or non-parenterally administrable formulations will be known or apparent to those skilled in the art and are described in more detail in such publications as Remington: The Science and Practice of Pharmacy, 21st ed., 2005.

VII. Exemplification

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of Sequence Listing, figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Furthermore, in accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, Volumes I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hybridization* [B.D. Hames & S.J. Higgins eds. (1985)]; *Transcription And Translation* [B.D. Hames & S.J. Higgins, Eds. (1984)]; *Animal Cell Culture* [R.I. Freshney, ed. (1986)]; *Immobilized Cells And Enzymes* [IRL Press, (1986)]; B. Perbal, *A Practical Guide To Molecular Cloning* (1984); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994).

Example 1. *In-silico* Analysis of Thermogenic Regulators

Eighty-three proteins that are involved in regulation of thermogenesis were selected based upon a critical assessment and review of the available scientific information and our own experimental data. These proteins were categorized as activators or repressors of thermogenesis based upon their functions. These thermogenic regulator proteins are set forth in Table 2.

5

Table 2.**Thermogenic regulator proteins:**

Activators			
	Name	Entrez Gene ID	Ensembl Gene ID
1	ALDH1A1	216	ENSG00000165092
2	ANP (NPPA)	4878	ENSG00000175206
3	AZGP1	563	ENSG00000160862
4	BMP7	655	ENSG00000101144
5	BMP8B	656	ENSG00000116985
6	CEBPA	1050	ENSG00000245848
7	CEBPB	1051	ENSG00000172216
8	CEBPD	1052	ENSG00000221869
9	CIDEA	1149	ENSG00000176194
10	COX7A1	1346	ENSG00000161281
11	CRAT	1384	ENSG00000095321
12	CREB1	1385	ENSG00000118260
13	CREBBP	1387	ENSG00000005339
14	CTBP1	1487	ENSG00000159692
15	CTBP2	1488	ENSG00000175029
16	DIO2	1734	ENSG00000211448
17	ELOVL3	83401	ENSG00000119915
18	FGF16	8823	ENSG00000196468
19	FGF19	9965	ENSG00000162344
20	FGF21	26291	ENSG00000105550
21	FNDC5	252995	ENSG00000160097
22	FOXC2	2303	ENSG00000176692
23	GDF3	9573	ENSG00000184344
24	HCRT (OREXIN)	3060	ENSG00000161610
25	HOXC8	3224	ENSG00000037965
26	INSR	3643	ENSG00000171105
27	IRS1	3667	ENSG00000169047
28	KDM3A (JMJD1A)	55818	ENSG00000115548
29	KLF5	688	ENSG00000102554
30	KLF11	8462	ENSG00000172059
31	KLF15	28999	ENSG00000163884

32	LRP6	4040	ENSG00000070018
33	MAPK14	1432	ENSG00000112062
34	MED13	9969	ENSG00000108510
35	NCOA1	8648	ENSG00000084676
36	NCOA2	10499	ENSG00000140396
37	NCOA3	8202	ENSG00000124151
38	NR4A3	8013	ENSG00000119508
39	NRF1	4899	ENSG00000106459
40	PLAC8	51316	ENSG00000145287
41	PPARA	5465	ENSG00000186951
42	PPARD	5467	ENSG00000112033
43	PPARG	5468	ENSG00000132170
44	PPARGC1A	10891	ENSG00000109819
45	PPARGC1B	133522	ENSG00000155846
46	PRDM16	63976	ENSG00000142611
47	PRDX3	10935	ENSG00000165672
48	PRKAA1 (AMPKA1)	5562	ENSG00000132356
49	PRKAA2 (AMPKA2)	5563	ENSG00000162409
50	PRKACA	5566	ENSG00000072062
51	PRKACB	5567	ENSG00000142875
52	PRKAR1A	5573	ENSG00000108946
53	SIRT1	23411	ENSG00000096717
54	SIRT3	23410	ENSG00000142082
55	SLC27A2 (FATP2)	11001	ENSG00000140284
56	SREBF1	6720	ENSG00000072310
58	SREBF2	6721	ENSG00000198911
58	STAT5A	6776	ENSG00000126561
59	TRPM8	79054	ENSG00000144481
60	UCP1 (SLC25A7)	7350	ENSG00000109424
61	UCP2 (SLC25A8)	7351	ENSG00000175567
62	UCP3 (SLC25A9)	7352	ENSG00000175564

Repressors

	Name	Entrez Gene ID	Ensembl Gene ID
1	ATG7	10533	ENSG00000197548
2	BMP2	650	ENSG00000125845
3	BMP4	652	ENSG00000125378
4	CIDEA	63924	ENSG00000187288
5	CTNNA1	1499	ENSG00000168036
6	DLK1 (Pref-1)	8788	ENSG00000185559
7	E2F4 (p107)	1874	ENSG00000205250
8	EIF4EBP1	1978	ENSG00000187840
9	ESRRA (NR3B1)	2101	ENSG00000173153
10	IKBKE	9641	ENSG00000143466
11	NR1H3 (LXRA)	10062	ENSG00000025434
12	NRIP1 (RIP140)	8204	ENSG00000180530

13	RB1 (pRb)	5925	ENSG00000139687
14	NR0B2 (SHP)	8431	ENSG00000131910
15	RPS6KB1	6198	ENSG00000108443
16	RUNX1T1	862	ENSG00000079102
17	RUNX2	860	ENSG00000124813
18	TNFRSF1A	7132	ENSG00000067182
19	TWIST1	7291	ENSG00000122691
20	WNT5A	7474	ENSG00000114251
21	WNT10B	7480	ENSG00000169884

The STRING 9.0 database of known and predicted protein interactions (string-db.org/) was used to test these 83 candidate molecules. The interactions include direct (physical) and indirect (functional) associations; they are derived from four sources: genomic context; high-throughput experiments; co-expression; and previous knowledge. STRING quantitatively integrates interaction data from these sources for a large number of organisms, and transfers information between these organisms where applicable. The database currently covers 5,214,234 proteins from 1,133 organisms. As an example, the relationships between the 83 thermogenic regulator molecules were centered on UCP1, and molecules having direct and indirect connections with UCP1 could be distinguished using the highest confidence score of 0.90. This relationship is set forth in schematic form in Figure 1A. From this analysis, it was discovered that nine molecules (CEBPB, CIDEA, KDM3A, NRIP1, PRDM16, PPARG, PPARGC1A, PPKAA2, and UCP2) are directly linked to UCP1, whereas many more molecules are connected to UCP1 on a second or higher degree order.

When the degree of confidence was set to high with a score of 0.70, eight additional proteins were found to be directly linked to UCP1 (AZGP1, DIO2, KLF11, KLF15, NR1H3, PPARG, PPARGC1B), Figure 1B.

Similarly, the interactions among these 83 thermogenic regulator molecules were independently assessed using other software programs. The interactions predicted by the Ingenuity Pathway Analysis (IPA) Software program (www.ingenuity.com) are shown in Figure 2A (UCP1 in yellow, activators in green and repressors in purple). The interactions predicted by the Reactome Functional Interaction (Reactome IF) Software program (<http://wiki.reactome.org>) are shown in Figure 2B (UCP1 in yellow, activators in green and repressors in purple). The IPA and Reactome IF networks differ from the ones set forth in Figures 1A and 1B, obtained with the

STRING program. It is not surprising that the results of these algorithms are different because they rely on different predefined parameters, sources of information and selection criteria.

Example 2. *In-silico* Selection of Relevant miRNA Targets

- 5 To select thermogenic regulators suitable as targets for miRNA agents, several internet-based resources were employed to match miRNAs and their targets (the “micronome”). Exemplary tools are set forth in Table 3.

Table 3.

10 **Exemplary bioinformatics tools used to select miRNAs and their targets:**

Field & Name	Web Address
Integrated Data Mining (8)	
BioCarta	http://www.biocarta.com/
Database for Annotation, Visualization and Integrated Discovery (DAVID)	http://david.abcc.ncifcrf.gov/home.jsp
GeneOntology	http://www.geneontology.org/
Gene Set Enrichment Analysis (GSEA)	http://www.broadinstitute.org/gsea/index.jsp
KEGG	http://www.genome.jp/kegg/
PubGene	http://www.pubgene.org/
Reactome	http://www.reactome.org/ReactomeGWT/entrypoint.html
STRING	http://string-db.org/
miRNA Mining & Mapping (8)	
deepBase	http://deepbase.sysu.edu.cn/
Human microRNA disease database (HMDD)	http://202.38.126.151/hmdd/mirna/md/

miRBase V19	http://www.mirbase.org/
miRGen 2.0	http://diana.cslab.ece.ntua.gr/mirgen/
miRNAmap	http://mirnamap.mbc.nctu.edu.tw/
miRSel	http://services.bio.ifi.lmu.de/mirsel/
miRStart	http://mirstart.mbc.nctu.edu.tw/home.php
miR2Disease	http://www.mir2disease.org
miRNA Targets & Expression (21)	
DIANA-microT 3.0	http://diana.cslab.ece.ntua.gr/microT/
DIANA-mirExTra	http://diana.cslab.ece.ntua.gr/hexamers/
GSEA Molecular Signatures Database v3.0	http://www.broadinstitute.org/gsea/index.jsp
MicroCosm Targets	http://www.ebi.ac.uk/enright-srv/microcosm/cgi-bin/targets/v5/download.pl
MicroInspector	http://bioinfo.uni-plovdiv.bg/microinspector/
microRNA.org (ex. miRanda)	http://www.microrna.org/microrna/home.do
miRDB	http://mirdb.org/miRDB/
miRTarBase	http://mirtarbase.mbc.nctu.edu.tw/index.html
miRTar.Human	http://mirtar.mbc.nctu.edu.tw/human/download.php
miRvestigator	http://mirvestigator.systemsbiology.net/
mirZ	http://www.mirz.unibas.ch/EIMMo2/
MultiMiTar	http://www.isical.ac.in/~bioinfo_miu/multimitar.htm
PhenomiR	http://mips.helmholtz-muenchen.de/phenomir/index.gsp
PicTar	http://pictar.mdc-berlin.de/
PITA	http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html
RepTar	http://bioinformatics.ekmd.huji.ac.il/reptar/

RNAhybrid	http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/
RNA22	http://cbcsrv.watson.ibm.com/rna22.html
Sylamer	http://www.ebi.ac.uk/enright/sylamer/
TarBase 6.0	http://diana.cslab.ece.ntua.gr/DianaToolsNew/index.php?r=tarbase/index
TargetScanHuman 6.2	http://www.targetscan.org/
Integrated miRNA Targets & Expression Tools (13)	
GOmir	http://www.bioacademy.gr/bioinformatics/projects/GOmir/
MAMI (MetaMiR:Target Inference)	http://mami.med.harvard.edu/
mimiRNA	http://mimirna.centenary.org.au/mep/formulaire.html
MMIA (microRNA and mRNA Integrated Analysis)	http://147.46.15.115/MMIA/index.html
mirDIP	http://ophid.utoronto.ca/mirDIP/
miRGator V3.0	http://mirgator.kobic.re.kr
miRecords	http://mirecords.biolead.org/
MIRNA-DISTILLER	http://www.ikp-stuttgart.de/content/language1/html/10415.asp
MiRonTop	http://www.microarray.fr:8080/miRonTop/index
miRror	http://www.proto.cs.huji.ac.il/mirror
miRSystem	http://mirsystem.cgm.ntu.edu.tw/
miRWalk	http://www.ma.uni-heidelberg.de/apps/zmf/mirwalk/index.html
StarBase	http://starbase.sysu.edu.cn/index.php
miRNA Secondary Structure (5)	

OligoWalk	http://rna.urmc.rochester.edu/cgi-bin/server_exe/oligowalk/oligowalk_form.cgi
PicTar RNA Studio	http://www.pictar.org/
RNA2D	http://protein3d.ncifcrf.gov/shuyun/rna2d.html
Vienna RNA Package	http://www.tbi.univie.ac.at/ivo/RNA/
Whitehead siRNA algorithm	http://jura.wi.mit.edu/bioc/siRNAext/
Network Searches & Analyses (8)	
ARIADNE Pathway Studio	http://www.ariadnegenomics.com/products/pathway-studio/
Cytoscape	http://www.cytoscape.org/
Database for Annotation, Visualization and Integrated Discovery (DAVID)	http://david.abcc.ncifcrf.gov/home.jsp
Genego MetaCore	http://www.genego.com/metacore.php
Ingenuity Systems IPA (Ingenuity Pathway Analysis)	http://www.ingenuity.com/products/IPA/microRNA.html
MATISSE (Module Analysis via Topology of Interactions and Similarity Sets)	http://acgt.cs.tau.ac.il/matisse/
MIR@NT@N	http://mironton.uni.lu
NAViGaTOR	http://ophid.utoronto.ca/navigator/index.html
Molecular Visualization (4)	
Foldit	http://fold.it/portal/info/science
PyMOL	http://www.pymol.org/
Qlucore Omics Explorer	http://www.qlucore.com/ProdOverviewmiRNA.aspx

WebMol	http://www.cmpfarm.ucsf.edu/cgi-bin/webmol.pl
Information Integration (1)	
TIBCO Spotfire	http://spotfire.tibco.com/

Specifically, these tools were used to perform: 1) Integrated Data Mining (8 tools); 2) miRNA Mining and Mapping (6 tools); 3) miRNA Target Targets and Expression (21 tools); 4) Integrated miRNA Targets and Expression (13 tools); 5) miRNA Secondary Structure Prediction and Comparison (5 tools); 6) Network Searches and Analyses (8 tools); 7) Molecular Visualization (4 tools); and 8) Information Integration and Exploitation (1 tool).

A single gene target can be controlled by several miRNAs whereas a single miRNA can control several gene targets. Sophisticated bioinformatics resources have been developed to select the most relevant miRNAs to target diseases (Gallagher IJ, et al. *Genome medicine*. 2010; Fujiki K, et al. *BMC Biol.* 2009; Okada Y, et al., *J Androl.* 2010; Hao T, et al., *Mol Biosyst.* 2012; Hao T, et al., *Mol Biosyst.* 2012). However, the results of these algorithms are acutely dependent on predefined parameters and the degree of convergence between these algorithms is rather limited. Therefore, there is a need to develop better performing bioinformatics tools with improved sensitivity, specificity and selectivity for the identification of miRNA/target relationships.

The interactions between miRNAs and their targets go beyond the original description of miRNAs as post-transcriptional regulators whose seed region of the driver strand (5' bases 2-7) bind to complementary sequences in the 3' UTR region of target mRNAs, usually resulting in translational repression or target degradation and gene silencing. The interactions can also involve various regions of the driver or passenger strands of the miRNAs as well as the 5'UTR, promoter, and coding regions of the mRNAs.

Upon analysis of the available data, it was decided to favor pathway-specific miRNAs which target multiple genes within one discrete signaling pathway, rather than universal miRNAs which are involved in many signaling pathways, functions or processes. Using 34 publicly available Internet tools predicting miRNA targets, specific human miRNAs were searched for that could potentially modulate several targets among the 83 thermogenic regulator molecules (which include 36 Transcription Factors) selected in Example 1.

Several paradigms were considered:

A) A one microRNA-multiple mRNAs pathway-specific paradigm.

A1. First example of one microRNA-multiple mRNAs pathway-specific paradigm

The methylation state of histones can be dynamically regulated by histone
5 methyltransferases and demethylases. The human lysine (K)-specific demethylase 3A (KDM3A)
is critically important in regulating the expression of metabolic genes. Its loss of function results
in obesity and hyperlipidemia in mice. Beta-adrenergic stimulation of KDM3A binding to
the PPAR responsive element (PPRE) of the UCP1 gene not only decreases levels of H3K9me2
(dimethylation of lysine 9 of histone H3) at the PPRE, but also facilitates the recruitment of
10 PPARG and RXRA and their co-activators PPARGC1A, CREBBP and NCOA1 to the PPRE.
The interrogation of the TargetScan Human database (release 6.0) revealed that the human
KDM3A 3' UTR 29-35 region is a conserved target for hsa-miR-22. Several other miRNA
Targets Databases also confirmed this match between hsa-miR-22 and KDM3A. Therefore,
increased production of the demethylase KDM3A by an hsa-miR-22 antagomir should lead to
15 demethylation of the UCP1 gene promoter region, thus facilitating binding of several regulatory
elements and increased UCP1 production.

In addition, we used the 34 miRNA Targets and Expression tools (Table 4) to identify the
mRNA targets of a given miRNA.

Applying the above in silico strategy, it was discovered that hsa-miR-22-3p and hsa-miR-22-5p interact respectively with a total of 42 and 8 of the chosen 83 thermogenic targets. This data is set forth in Table 5.

5 Table 5.

Thermogenic regulators identified as predicted and/or validated targets for hsa-miR-22:

hsa-miR-22-3p				
ALDH1A1	DIO2	NCOA1	PRKAA1	STAT5A
BMP4	FGF19	NPPA	PRKACA	TNFRSF1A
BMP7	FGF21	NRF1	PRKACB	TRPM8
CEBPA	FOXC2	NRIP1	PRKAR1A	UCP2
CEBPD	INSR	PPARA	RUNX1T1	WNT10B
CIDEA	KDM3A	PPARGC1A	RUNX2	WNT5A
CREB1	KLF11	PPARGC1B	SIRT1	
CREBBP	LRP6	PRDM16	SREBF1	
CTNNB1	MAPK14	PRDX3	SREBF2	
hsa-miR-22-5p				
BMP7	DIO2	FNDCC5	IKBKE	INSR
		MAPK14	NR1H3	PPARA

A2. Other examples of one microRNA-multiple mRNAs pathway-specific paradigm

We also utilized the 34 miRNA Targets and Expression tools (Table 4) to look for potential relations between any of the adipocyte 536 miRNAs (Table 1) and the 83 thermogenic targets (Table 2).

It appears that many adipocyte miRNAs interact (prediction and/or validation) with at least one of the 83 thermogenic targets. For example, miR-17-3p and hsa-miR-17-5p interact respectively with a total of 23 and 65 of the chosen thermogenic 83 targets. This data is set forth in Table 6.

Table 6.

Thermogenic regulators identified as predicted and/or validated targets for hsa-miR-17:

hsa-miR-17-3p				
ATG7	CTBP2	KLF11	PPARD	TNFRSF1A
BMP2	E2F4	MAPK14	PRDM16	TWIST1
BMP4	FGF19	NCOA3	RB1	WNT10B

CEBPB	IKBKE	PLAC8	RUNX1T1	
CREB1	IRS1	PPARA	STAT5A	
hsa-miR-17-5p				
ALDH1A1	CREB2	IKBKE	NRIP1	RB1
ATG7	CTNNB1	INSR	PLAC8	RPS6KB1
BMP2	CTBP1	IRS1	PPARA	RUNX1T1
BMP4	CTBP2	KLF11	PPARD	RUNX2
BMP7	DIO2	MAPK14	PPARG	SIRT1
BMP8b	ELOVL3	MED13	PPARGC1A	SIRT3
CEBPA	FGF19	NCOA1	PPARGC1B	SREBF1
CEBPB	FGF21	NCOA2	PRDX3	STAT5A
CEBPD	FNDC5	NCOA3	PRKAA1	TNFRSF1A
CIDEA	FOXC2	NPPA	PRKAA2	TWIST1
COX7A1	GDF3	NR1H3	PRKACA	UCP1
CRAT	HCRT	NR4A3	PRKACB	UCP3
CREB1	HOXC8	NRF1	PRKAR1A	WNT5A

Once the lists of miRNAs of interest and their mRNA targets were produced, the following filters were applied to refine the results:

Parameters

- 5 1 Expression of miRNAs in tissue/cell of interest
- 2 Number of algorithms predicting one miRNA for a given gene or set of genes
- 3 Score/percent from algorithms
- 4 Number of preferred genes targeted by one miRNA
- 5 Number of binding sites in a target gene for one miRNA
- 10 6 Number of binding sites in a target gene for several miRNAs
- 7 Over-representation of one miRNA seed complementary sequence among target genes (miRvestigator)
- 8 Validated miRNA-mRNA target couples
- 9 Genomic location of miRNA binding site (5'UTR-Promoter-CDS-3'UTR)
- 15 10 Intronic location of miRNA
- 11 Clustering of miRNAs
- 12 Abundance of miRNA in specific tissue/cell of interest

Applying the above parameters, it was discovered that 229 miRNAs met at least two of these criteria. This data is set forth in Table 7.

Table 7.

5 Ranking of miRNAs according to decreasing number of selection criteria:

hsa-miR-20b-5p	hsa-miR-155-5p	hsa-miR-15b-5p	hsa-let-7a-3p
hsa-miR-27b-3p	hsa-miR-181a-5p	hsa-miR-16-5p	hsa-miR-126-3p
hsa-miR-103a-3p	hsa-miR-519d	hsa-miR-195-5p	hsa-miR-20a-3p
hsa-miR-22-3p	hsa-miR-96-5p	hsa-miR-196b-5p	hsa-miR-499a-5p
hsa-miR-34a-5p	hsa-miR-212-3p	hsa-miR-23a-3p	hsa-let-7g-5p
hsa-miR-130b-3p	hsa-miR-29a-3p	hsa-miR-29c-3p	hsa-miR-152
hsa-miR-132-3p	hsa-miR-98-5p	hsa-miR-373-3p	hsa-miR-26a-5p
hsa-miR-181b-5p	hsa-let-7c	hsa-miR-7-5p	hsa-miR-124-3p
hsa-miR-211-5p	hsa-let-7d-5p	hsa-miR-214-3p	hsa-miR-203a
hsa-miR-148b-3p	hsa-miR-141-3p	hsa-miR-421	hsa-miR-24-3p
hsa-miR-17-5p	hsa-miR-183-5p	hsa-miR-15a-5p	hsa-miR-301b
hsa-miR-182-5p	hsa-miR-19a-3p	hsa-miR-193b-3p	hsa-miR-590-3p
hsa-miR-20a-5p	hsa-miR-196a-5p	hsa-miR-194-5p	hsa-miR-1179
hsa-miR-27a-3p	hsa-miR-30b-5p	hsa-miR-223-3p	hsa-miR-325
hsa-miR-301a-3p	hsa-miR-378a-3p	hsa-miR-30d-5p	hsa-miR-552
hsa-miR-204-5p	hsa-miR-302c-5p	hsa-miR-424-5p	hsa-miR-185-5p
hsa-miR-143-3p	hsa-miR-30e-5p	hsa-miR-454-3p	hsa-miR-455-3p
hsa-miR-1	hsa-miR-130a-3p	hsa-miR-545-3p	hsa-miR-583
hsa-miR-9-5p	hsa-let-7e-5p	hsa-miR-485-5p	hsa-miR-122-5p
hsa-miR-30a-5p	hsa-miR-216a-5p	hsa-miR-335-5p	hsa-miR-1305
hsa-miR-138-5p	hsa-miR-450a-5p	hsa-miR-133a	hsa-miR-139-5p
hsa-miR-217	hsa-let-7d-3p	hsa-miR-222-3p	hsa-miR-146a-5p
hsa-miR-19b-3p	hsa-miR-26b-5p	hsa-miR-494	hsa-miR-18a-5p
hsa-miR-382-5p	hsa-miR-181c-5p	hsa-miR-498	hsa-miR-18b-5p
hsa-miR-106a-5p	hsa-miR-186-5p	hsa-miR-513a-5p	hsa-miR-199b-5p
hsa-miR-107	hsa-miR-519c-3p	hsa-miR-92a-3p	hsa-miR-340-5p
hsa-miR-135a-5p	hsa-let-7b-5p	hsa-miR-495-3p	hsa-miR-34c-5p
hsa-miR-93-5p	hsa-miR-10b-5p	hsa-miR-503-5p	hsa-miR-423-3p
hsa-miR-21-5p	hsa-miR-125b-5p	hsa-miR-539-5p	hsa-miR-489
hsa-miR-515-3p	hsa-miR-134	hsa-miR-16-2-3p	hsa-miR-520f
hsa-miR-106b-3p	hsa-miR-137	hsa-miR-302b-5p	hsa-miR-520g
hsa-miR-125a-5p	hsa-miR-150-5p	hsa-miR-425-3p	hsa-miR-605
hsa-miR-148a-3p	hsa-miR-153	hsa-miR-99a-3p	hsa-miR-668

hsa-let-7a-5p	hsa-miR-30c-5p	hsa-miR-136-3p	hsa-miR-376a-5p
hsa-let-7f-5p	hsa-miR-335-3p	hsa-miR-150-3p	hsa-miR-378a-5p
hsa-miR-10a-3p	hsa-miR-374a-5p	hsa-miR-154-3p	hsa-miR-424-3p
hsa-miR-135b-5p	hsa-miR-410	hsa-miR-15a-3p	hsa-miR-451a
hsa-miR-144-3p	hsa-miR-429	hsa-miR-15b-3p	hsa-miR-452-3p
hsa-miR-181d	hsa-miR-497-5p	hsa-miR-16-1-3p	hsa-miR-487b
hsa-miR-200b-3p	hsa-miR-513a-3p	hsa-miR-181a-2-3p	hsa-miR-493-5p
hsa-miR-200c-3p	hsa-miR-542-3p	hsa-miR-181c-3p	hsa-miR-500a-3p
hsa-miR-218-5p	hsa-miR-653	hsa-miR-186-3p	hsa-miR-502-3p
hsa-miR-23b-3p	hsa-miR-122-3p	hsa-miR-195-3p	hsa-miR-516b-3p
hsa-miR-25-3p	hsa-miR-101-5p	hsa-miR-20b-3p	hsa-miR-518e-3p
hsa-miR-29b-3p	hsa-miR-1178-3p	hsa-miR-223-5p	hsa-miR-518f-3p
hsa-miR-383	hsa-miR-191-5p	hsa-miR-224-3p	hsa-miR-519a-5p
hsa-miR-202-3p	hsa-miR-214-5p	hsa-miR-24-1-5p	hsa-miR-519b-5p
hsa-miR-381-3p	hsa-miR-302d-5p	hsa-miR-24-2-5p	hsa-miR-521
hsa-miR-377-3p	hsa-miR-572	hsa-miR-27a-5p	hsa-miR-523-5p
hsa-miR-452-5p	hsa-miR-574-3p	hsa-miR-27b-5p	hsa-miR-545-5p
hsa-miR-501-3p	hsa-miR-26a-2-3p	hsa-miR-29b-1-5p	hsa-miR-585
hsa-miR-514a-3p	hsa-miR-611	hsa-miR-302a-5p	hsa-miR-7-2-3p
hsa-miR-654-3p	hsa-let-7f-1-3p	hsa-miR-3065-5p	hsa-miR-93-3p
hsa-let-7b-3p	hsa-let-7i-3p	hsa-miR-30d-3p	hsa-miR-96-3p
hsa-miR-125a-3p	hsa-miR-100-5p	hsa-miR-34a-3p	hsa-miR-99b-3p
hsa-miR-133b	hsa-miR-106b-5p	hsa-miR-371a-3p	
hsa-miR-192-5p	hsa-miR-132-5p	hsa-miR-373-5p	
hsa-miR-199a-3p	hsa-miR-135b-3p	hsa-miR-374a-3p	

B) A multiple microRNAs-one mRNA paradigm.

B1. One exemplary multiple miRNAs-one mRNA paradigm involves UCP1.

In adipocytes the key thermogenic regulator ultimately is UCP1 (also named thermogenin) and, thus, all thermogenic regulators must ultimately impact UCP1 activity. UCP1 is a mitochondrial transporter protein that creates proton leaks across the inner mitochondrial membrane, thus uncoupling oxidative phosphorylation from ATP synthesis. As a result, energy is dissipated in the form of heat (adaptive thermogenesis) (see Figure 5) Lowell et al., Nature (2000); Friedman et al., Bioinformatics (2010); Hsu et al., Nucleic acids research (2011); Rieger et al., Frontiers in Genetics (2011)).

UCP1 biosynthesis is mainly controlled at the transcription level. Figure 6 depicts the transcriptional control of UCP1 by other exemplary thermogenic regulators. The promoter's region of the UCP1 gene contains many distinct regulatory sites, allowing a wide range of proteins to influence its transcription, both positively (see Figure 7a) and negatively (see Figure 7b).

Mendelian randomization is a method of using measured variation in genes of known function to examine the causal effect of a modifiable exposure on disease in non-experimental studies. Mendelian randomization can be thought of as a "natural" Randomized Clinical Trial.

Genetic polymorphism of the UCP1 gene, such as the -3826 A/G single nucleotide polymorphism in the promoter in exon 2 of UCP1, has been reported to be associated with reduced mRNA expression and obesity. Healthy children with the G/G genotype had a lower capacity for thermogenesis in response to a high-fat meal and acute cold exposure. The same -3826 A/G UCP1 genetic polymorphism diminishes resting energy expenditure and thermoregulatory sympathetic nervous system activity in young females. In a study of 367 Korean women, the G allele of -3826A>G and the C allele of -412A>C were significantly associated with larger areas of abdominal subcutaneous fat in a dominant model ($p<0.001$ and $p<0.0004$, respectively); combining them together (ht2[GC]) enhanced this significance ($p<0.00005$). A study of 100 severe obese adults (BMI > 40 kg/m²) and 100 normal-weight control subjects (BMI range = 19–24.9 kg/m²) identified 7 variations in the promoter region, 4 in the intronic region and 4 in the exonic region of the UCP1 gene. These variations could contribute to the development of obesity, particularly, g.-451C>T, g.940G>A, and g.IVS4-208T>G could represent "thrifty" factors that promote energy storage. Finally, two polymorphisms (A-3826G and C-3740A), located in the upstream promoter region of the UCP1 gene affect gene expression and are correlated with human longevity.

All aforementioned information supports targeting UCP1 expression and activity as a meaningful way to alter adaptive thermogenesis and consequently treat human obesity. Many strategies could be implemented to achieve this goal, however, the one employed in the methods of the invention uses miRNA agents to modulate simultaneously several elements within the thermogenic pathways to increase UCP1 synthesis and activity. Both direct and indirect interactions between miRNAs and the UCP1 gene are considered. Direct interaction means the

direct binding of miRNAs to the various regions of the UCP1 gene, resulting in alterations of the transcription, translation, stability and/or degradation of the UCP1 mRNA. Indirect interaction means that miRNAs alter the transcription, translation, stability and/or degradation of thermogenic mRNAs, whose expressed proteins alter the transcription of the UCP1 gene.

- 5 Furthermore, indirect interaction means that miRNAs alter the transcription, translation, stability and/or degradation of other miRNAs that modify the transcription of the UCP1 gene.

The promoter region of the human UCP1 gene (gi|237858805|ref|NG_012139.1| Homo sapiens uncoupling protein 1 (mitochondrial, proton carrier) (UCP1), RefSeqGene on chromosome 4) is particularly rich in regulatory element motifs (Table 8).

10

Table 8.

UCP1 Gene Regulatory Elements:

	Name of regulatory element	Sequence	Number	Nucleotide Location
1	BRE1 (Brown Fat Response Element 1)	CCTCTCTGCTTCTTCT	1	1,129 to 1,144
2	BRE2 (Brown Fat Response Element 2)	CTCCTTGGAA	1	1,269 to 1,278
3	CRE2	ATTCTTTA	4	1,121 to 1,128; 3,631 to 3,638; 10,982 to 10,989; 15,881 to 15,888
4	CREB	ACGTCA	5	1,082 to 1,087; 1,345 to 1,350; 1,348 to 1,343; 11,439 to 11,434; 13,831 to 13,836
5	DR1	TTGCCCTTGCTCA	1	1,099 to 1,111
6	DR4	ACGTCATAAAGGGTCA	1	1,082 to 1,097
7	DR4 Type RARE	RGKTCANNNNRGKTCA	1	1,316 to 1,301
8	ERE	GCTCATACTGACCT	1	1,107 to 1,120
9	PRE	GTTAATGTGTTCT	1	1,009 to 1,021
10	RARE	TGACCACAGTTTGATCA	1	983 -> 999
11	RXR	AGGTCA	12	1,120 to 1,115; 1,316 to 1,311; 3,517 to 3,522; 3,560 to 3,555;

				3,813 to 3,808; 5,318 to 5,313; 6,233 to 6,238; 6,831 to 6,836; 8,122 to 8,127; 9,966 to 9,971; 11,339 to 11,334; 11,412 to 11,407
12	GC Box 1	CGCCC	7	4,593 to 4,589; 4,615 to 4,619; 4,615 to 4,619; 4,747 to 4,751; 4,765 to 4,769; 5,914 to 5,910; 13,715 to 13,711
13	GC Box 2	GCGGG	9	4,463 to 4,459; 4,585 to 4,589; 4,593 to 4,597; 4,639 to 4,643; 4,883 to 4,887; 5,176 to 5,172; 5,929 to 5,933; 5,940 to 5,944; 14,994 to 14,990
14	GT Box 1	CACCC	25	194 to 190; 452 to 448; 1,184 to 1,188; 1,803 to 1,807; 2,428 to 2,424; 3,037 to 3,041; 3,330 to 3,334; 4,137 to 4,141; 4,566 to 4,562; 4,599 to 4,595; 4,869 to 4,865; 5,104 to 5,108; 5,461 to 5,457; 6,237 to 6,241; 6,293 to 6,289; 8,096 to 8,092; 8,198 to 8,194; 9,649 to 9,645; 9,912 to 9,908; 12,962 to 12,958; 13,136 to 13,132;

				13,723 to 13,719; 14,404 to 14,400; 14,960 to 14,964; 15,576 to 15,572
15	GT Box 2	GTGGG	20	25 to 21; 1,805 to 1,801; 1,809 to 1,805; 2,119 to 2,123; 3,854 to 3,850; 4,310 to 4,314; 4,339 to 4,343; 4,765 to 4,761; 4,867 to 4,871; 6,291 to 6,295; 7,554 to 7,558; 8,280 to 8,284; 8,681 to 8,685; 9,615 to 9,619; 9,689 to 9,693; 9,906 to 9,910; 10,363 to 10,359; 13,074 to 13,070; 13,640 to 13,644; 13,941 to 13,945
16	CpG Methylation Island	CG	366	4,519 to 5,258; 5,639 to 6,694

Figure 8A depicts the location of these various regulatory elements in reference to the UCP1 transcription start site at nucleotide position 5,001 of the 15,910 base pair human UCP-1 gene (FASTA accession number: >gi|237858805|ref|NG_012139.1| Homo sapiens uncoupling protein 1 (mitochondrial, proton carrier) (UCP1), RefSeqGene on chromosome 4; NCBI Reference Sequence: NG_012139.1).

Direct or indirect activation or repression of these regulatory elements by miRNAs will result in alterations of UCP1 gene expression and activity. Under normal conditions, the UCP1 gene expression and activity are repressed by a rich network of regulatory elements, in order to avoid energy wasting. Under stress, such as exposure to a cold environment, the expression of the UCP1 gene is upregulated, via various activators and repressors which are under the control of several miRNAs.

An initial survey of miRNAs targeting the human UCP1 3'UTR with several programs, including microRNA.org, was negative. However, other programs, including MicroCosm Targets, using the UCP1 Ensembl 1,462 base pair transcript ENST00000262999 as a target revealed binding sites for 27 miRNAs at 28 locations in UCP1 3'UTR as shown in Table 9.

5

Table 9.

Binding sites for miRNAs in the 3'UTR of UCP1 (NCBI Reference Sequence NG_012139.1) determined using microCosm Targets:

Name	Sequence	From bp	To bp	Length
hsa-miR-21	AATGTAATGCAGATAAGCTA	14143	14162	20
hsa-miR-219-2-3p	ACATGTTTTAATTACAATTC	14217	14236	20
hsa-miR-22	GATTGGCAGCTT	14857	14868	12
hsa-miR-222a	GATTTTTTAATGTTTAGAGTCCAG	14500	14522	23
hsa-miR-290-3p	TTTAGAGCTGGAGGGTACTT	14621	14640	20
hsa-miR-292-3p	TTTAGAGCTGGAGGGTACTT	14621	14640	20
hsa-miR-292-5p	GACAGAGGAACAGTTTGAG	14648	14666	19
hsa-miR-325	ATTTTGGCAGGATTGCTACTAG	14568	14589	22
hsa-miR-331-5p	TTTGTGAGATCTATACCTGG	14383	14401	19
hsa-miR-362-5p	ATTTTAAGCTAAATCCAAGGATT	14838	14860	23
hsa-miR-367	TGACCATTTCTGGAGTGCAATT	14170	14191	22
hsa-miR-371-5p	ACAGTTTGAT	988	997	10
hsa-miR-371-5p	ACAGTTTGAG	14657	14666	10
hsa-miR-377	CTGGAGTGCAATTGTGTGA	14179	14197	19
hsa-miR-378	TTTAAATGTTTAGAGTCCAG	14503	14522	20
hsa-miR-382	TGATGACATCTCTAACAACCTC	14526	14547	22
hsa-miR-460	AGAAACTGAGTGAAATGCAG	14250	14269	20
hsa-miR-508-5p	TGACCATTTCTGGAGTG	14170	14186	17
hsa-miR-543	TACTCTGAATGTT	14478	14490	13
hsa-miR-549	TTAACCACAGTTGTCA	14321	14336	16
hsa-miR-643	CAAGTTCAGTAGAATAACAAG	14412	14431	20
hsa-miR-654-3p	AAGGTTACAGGCTGCCAGACAT	14880	14901	22
hsa-miR-664	GTGTGAATGAATG	14192	14204	13
hsa-miR-871	TAGGCATGAACCTACTCTGAATG	14466	14488	23
hsa-miR-883a-3p	AAACTGAGTGAAATGCAGTT	14252	14271	20
hsa-miR-883b-3p	AAACTGAGTGAAATGCAGTT	14252	14271	20
hsa-miR-888-3p	TTTATTAACCACAGTTGTCAGTT	14317	14339	23
hsa-miR-92b	GAGTGCAAT	14182	14190	9

Other programs, such as miRWalk, miRGen, miRGator-miRanda, and DIANA microT, using the UCP1 Ensembl 1,462 base pair transcript (ENST00000262999), the UCP1 Ensembl 9,371 base pair gene sequence (ENSG00000109424) or the 15,910 base pair UCP1 sequence (NCBI Reference Sequence: NG_012139.1) as targets, revealed binding sites for a total of 50 miRNAs at 69 locations in UCP1 3'UTR as shown in Table 10.

Table 10.

Binding sites for miRNAs in the 3'UTR of UCP1 (NCBI Reference Sequence.

NG_012139.1) according to several programs:

	Name	Sequence	From bp	To bp	Length
1	hsa-miR-1179	AAGTATCCTTT	15346	15356	11
2	hsa-miR-1302	ATGGGACACA	15021	15030	10
3	hsa-miR-130b	TTATTTTCCCT	15161	15171	11
4	hsa-miR-146a	TGACAACTGT	14327	14336	10
	hsa-miR-146a	AGGGAAGTGA	15231	15240	10
	hsa-miR-146a	TGTGAAGTGG	15679	15688	10
5	hsa-miR-181c	AACCATAGT	15304	15312	9
6	hsa-miR-19b-2	ACTTTTGCGG	14991	15000	10
7	hsa-miR-203	TTAAATGTT	15584	15592	9
8	hsa-miR-204-5p	TTCCTTTATC	14006	14015	10
	hsa-miR-204-5p	TTCCTCTGTC	14648	14657	10
9	hsa-miR-21-5p	TAGCTTATCT	14153	14162	10
10	hsa-miR-211-5p	TTCCCTATCTC	14779	14789	11
11	hsa-miR-214	CAGCAAGCA	15052	15060	9
12	hsa-miR-22-3p	AAGCTGCCAA	14859	14868	10
	hsa-miR-22-5p	AGTTCTTCACA	14203	14213	11
13	hsa-miR-26a-2-3p	CATTTTCTTG	13918	13927	10
	hsa-miR-26a-2-3p	CCAATCCTTG	14853	14862	10
	hsa-miR-26a-2-3p	CCTTTTCATG	15616	15625	10
14	hsa-miR-30b	GTAACCTTCC	14878	14887	10
15	hsa-miR-325	CAGAGTAGGT	14475	14484	10
	hsa-miR-325	CCTTGTAGGC	15378	15387	10
16	hsa-miR-328	CTGTTCCTCT	14651	14660	10
17	hsa-miR-362-5p	ATCCTTGGAT	14850	14859	10
18	hsa-miR-367-3p	AATTGCACTC	14182	14191	10
19	hsa-miR-371a-3p	AAGTGCCTGC	15435	15444	10

	hsa-miR-371a-5p	TCTCAAAGCTG	14658	14667	10
20	hsa-miR-378a-3p	ACTGGCCTTG	15816	15825	10
21	hsa-miR-382-3p	ATTCATTAC	14194	14203	10
22	hsa-miR-382-5p	GAAGTTGTTAGAGAT	14533	14547	15
23	hsa-miR-383	AGATTAGAA	14545	14553	9
24	hsa-miR-421	ATTAAGCTGAC	14333	14342	10
	hsa-miR-421	CTCAAAAGAC	14380	14389	10
25	hsa-miR-422a	ACTGGCCTT	15817	15825	9
26	hsa-miR-431	TGTCTGGCA	14892	14900	9
27	hsa-miR-452	TTATCTGC	14151	14158	8
	hsa-miR-452	TCTTCTGC	14773	14780	8
	hsa-miR-452	ACATCTGC	15009	15016	8
28	hsa-miR-455-3p	CAGTCCAT	13893	13900	8
	hsa-miR-455-5p	TGTGTGCCTT	15641	15650	10
29	hsa-miR-491-5p	AATGGGGAAG	14975	14984	10
30	hsa-miR-501-3p	ATGCATCAGG	15547	15556	10
31	hsa-miR-504	AGACCCTGT	15325	15333	9
32	hsa-miR-508-5p	TATTCTAGTGAAGTTGACTCTTA	14405	14427	23
33	hsa-miR-512-5p	CACTCAG	14255	14261	7
34	hsa-miR-514a-3p	TTGACTCTT	14406	14414	9
35	hsa-miR-515-3p	GACTGCCTT	15539	15547	9
	hsa-miR-515-3p	GTGTGCCTT	15641	15649	9
36	hsa-miR-517a-3p	ATGGTGCATT	15650	15659	10
37	hsa-miR-545	CAGCAAGCACT	15050	15060	11
38	hsa-miR-549	TGACAAGCTGT	14327	14336	10
39	hsa-miR-552	CACAGGTGA	15130	15138	9
40	hsa-miR-616-5p	ACTCTAAAC	14510	14518	9
41	hsa-miR-620	ATGAATATAG	14560	14569	10
42	hsa-miR-643	ACTGGTATGT	13933	13942	10
	hsa-miR-643	TCTTGTATTC	14423	14432	10
	hsa-miR-643	CCTTGTAGGC	15378	15387	10
	hsa-miR-643	ACATGCATGC	15553	15562	10
43	hsa-miR-651	TTAAAATAAG	13988	13997	10
	hsa-miR-651	TTAGGTAAA	13993	14002	10
	hsa-miR-651	TCATGATAAG	15700	15709	10
44	hsa-miR-654-3p	TATCTCTTCT	14775	14784	10
	hsa-miR-654-3p	TATGTATACT	15493	15502	10
45	hsa-miR-655	GTAATACAT	15593	15601	9
46	hsa-miR-767-3p	CCTGCTCAT	14871	14879	9
47	hsa-miR-888-3p	GACTGACTCC	15772	15781	10

48	hsa-miR-92b-3p	ATTGCACTCC	14181	14190	10
49	hsa-miR-941	CACCCAGGT	14396	14404	9
50	hsa-miR-99a-3p	AAGCTGGCTC	15117	15126	10

Alignment of the sequence of the human UCP1 gene with several miRNA sequences yielded matches in the 5'UTR, the promoter region and the coding regions of the UCP1 gene. Interrogation of the publicly available Internet tools predicting miRNAs targeting the various regions of the UCP1 gene elicited several hits. Surprisingly, the overlap between these prediction tools was zero, as shown in Figure 3.

Nevertheless, miRNA databases were screened using the alignment program Geneious. A total of 191 human microRNAs were found which have complementary 450 binding sites in the UCP1 gene sequence (Table 11). The length of the matches goes from 7 bases to 12 bases (e.g. hsa-miR-24-2-5p and hsa-miR-192-5p). The number of hits per miRNA varies from 1 to several (e.g. 9 for hsa-miR-19b2 (an abundant adipocyte miRNA), 14 for hsa-miR-26a-2-3p, 11 for hsa-miR-181c, and 12 for hsa-miR-620).

Table 11.

miRNAs with predicted binding sites in the UCP1 gene sequence (NCBI Reference Sequence: NG_012139.1):

miRNA	Sequence	From bp	To bp	Length
hsa-let-7c	TAGAGTTTC	5918	5926	9
hsa-let-7e	GGAGGTAGG	13283	13291	9
hsa-let-7e	TGAAGTAGG	7612	7620	9
hsa-let-7e	AGAGGTAGG	3306	3314	9
hsa-let-7i-3p	CTGTGCAAG	3588	3596	9
hsa-miR-17	CAAAGTGCT	12200	12208	9
hsa-miR-17	CAAAGTGCT	9931	9939	9
hsa-miR-17	CAAAGTGCT	218	226	9
hsa-miR-19a	TGTGCAAAT	3916	3924	9
hsa-miR-19a	TGTGCAAAT	834	842	9
hsa-miR-19b-2	ACTTTTGCGG	14991	15000	10
hsa-miR-19b-2	AGTTTTACAA	11998	12007	10
hsa-miR-19b-2	AGTTTTGTAT	10023	10032	10
hsa-miR-19b-2	AGTCTTGAAG	9399	9408	10
hsa-miR-19b-2	AGGTTTGTAG	7758	7767	10

hsa-miR-19b-2	AGTATTGAAG	7159	7168	10
hsa-miR-19b-2	AGGCTTGCAG	3546	3555	10
hsa-miR-19b-2	AATTTGGCAG	529	538	10
hsa-miR-19b-2	AGTTTTGGAA	312	321	10
hsa-miR-20b	CAAAGTGCT	12200	12208	9
hsa-miR-20b	CAAAGTGCT	9931	9939	9
hsa-miR-20b	CAAAGTGCT	218	226	9
hsa-miR-21-5p	TAGCTTATCT	14153	14162	10
hsa-miR-22-3p	AAGCTGCCAA	14859	14868	10
hsa-miR-22-3p	AAGCTTCCAG	1482	1491	10
hsa-miR-22-5p	AGTTCTTCACA	14203	14213	11
hsa-miR-22-5p	AATTCTTCAGG	8032	8042	11
hsa-miR-22-5p	GGTTCTTCAGC	5389	5399	11
hsa-miR-24-2-5p	TGCCTACTGGCC	8651	8662	12
hsa-miR-25-3p	CATTGCAC	11565	11572	8
hsa-miR-25-5p	AGGCGGAG	5963	5970	8
hsa-miR-26a-2-3p	CCTTTTCATG	15616	15625	10
hsa-miR-26a-2-3p	CCAATCCTTG	14853	14862	10
hsa-miR-26a-2-3p	CATTTTCTTG	13918	13927	10
hsa-miR-26a-2-3p	CCTACTCTTC	13505	13514	10
hsa-miR-26a-2-3p	ACGATTCTTG	13192	13201	10
hsa-miR-26a-2-3p	TCTATTCTTT	12883	12892	10
hsa-miR-26a-2-3p	CATATTTTGTG	10197	10206	10
hsa-miR-26a-2-3p	GCTAGTCTTG	9978	9987	10
hsa-miR-26a-2-3p	CATATTTTGTG	9890	9899	10
hsa-miR-26a-2-3p	CCTTTTCTTT	6631	6640	10
hsa-miR-26a-2-3p	CCCATTCTCG	4709	4718	10
hsa-miR-26a-2-3p	TTTATTCTTG	3893	3902	10
hsa-miR-26a-2-3p	CCTTTACTTG	1885	1894	10
hsa-miR-26a-2-3p	GCGATTCTTG	376	385	10
hsa-miR-27-5p	AGAGCTTAGG	2949	2958	10
hsa-miR-30b	GTAACCTTCC	14878	14887	10
hsa-miR-30b	GTAACCATCA	12991	13000	10
hsa-miR-30b	GTAATCATAC	12831	12840	10
hsa-miR-30b	GTCAACATCA	11401	11410	10
hsa-miR-30b	GTAAACATAA	9365	9374	10
hsa-miR-30b	GTACTCATCC	9016	9025	10
hsa-miR-30b	CTATACATCC	8586	8595	10
hsa-miR-30b	CTAAACATCT	7495	7504	10
hsa-miR-31	GGCTATGCC	7712	7720	9

hsa-miR-32	ATTGCACA	11564	11571	8
hsa-miR-92b	ATTGCACTCC	14181	14190	10
hsa-miR-92b	ATTGCACTAG	11282	11291	10
hsa-miR-93	CAAAGTGCTG	12199	12208	10
hsa-miR-93	CAAAGTGCTG	217	226	10
hsa-miR-93-3p	ACTCCTGGGCT	12356	12366	11
hsa-miR-93-3p	ACTGATAAGCT	11055	11065	11
hsa-miR-93-3p	ACTCCTGACCT	9966	9976	11
hsa-miR-96-3p	AATCATGTGCC	8659	8669	11
hsa-miR-99a-3p	AAGCTGGCTC	15117	15126	10
hsa-miR-99a-3p	AAACTCTTTC	13344	13353	10
hsa-miR-99a-3p	AATCTTGTTTC	11952	11961	10
hsa-miR-99a-3p	AAGCTCCTTT	11050	11059	10
hsa-miR-99a-3p	AAGCTCCTTT	8099	8108	10
hsa-miR-99a-3p	AAGCTCTGTC	7523	7532	10
hsa-miR-99b-3p	CAACCTCGAG	13666	13675	10
hsa-miR-99b-3p	CGAGCTCCTG	13660	13669	10
hsa-miR-99b-3p	GAAGCTTGTC	6436	6445	10
hsa-miR-99b-3p	CAAACCTCCTG	257	266	10
hsa-miR-100	TCCAGTAGAT	11866	11875	10
hsa-miR-100	ACGCGCAGAT	5634	5643	10
hsa-miR-106b-5p	CAAAGTGCTG	12199	12208	10
hsa-miR-106b-5p	CAAAGTGCTG	217	226	10
hsa-miR-126-3P	TCATACAGT	12828	12836	9
hsa-miR-126-3P	TTGTACTGT	11542	11550	9
hsa-miR-126-3P	TGGTCCCGT	7922	7930	9
hsa-miR-126-3P	TCATACAGT	932	940	9
hsa-miR-130b	TTATTTTCCCT	15161	15171	11
hsa-miR-130b	CTCTTTTCAGT	9670	9680	11
hsa-miR-130b	CTCTCTTCACT	8977	8987	11
hsa-miR-130b	CTCTTTTCCC	8444	8454	11
hsa-miR-130b	CTTTTCCCCT	6624	6634	11
hsa-miR-130b	CTATTTTCCGT	5742	5752	11
hsa-miR-130b	TTCCTTTCCCT	5007	5017	11
hsa-miR-130b	CTCTTTGCCCC	1845	1855	11
hsa-miR-130b	CTCCTTTCCTT	1033	1043	11
hsa-miR-133a-1	TTTGGTGCCC	7393	7402	10
hsa-miR-140-3p	TACCACAG	5893	5900	8
hsa-miR-141	TAACACTG	5852	5859	8
hsa-miR-143	GGTGCAGTG	4132	4140	9

hsa-miR-143-3p	TGAGATGAGG	13727	13736	10
hsa-miR-143-3p	TGAGATGGAG	10172	10181	10
hsa-miR-143-3p	TTAGATGAAG	9572	9581	10
hsa-miR-144-3p	TACAGTATT	12825	12833	9
hsa-miR-144-3p	TACAATATA	8859	8867	9
hsa-miR-144-3p	GACAGTATA	1491	1499	9
hsa-miR-146a	CCTCTGAAA	3499	3507	9
hsa-miR-146a	TGTGAACTGG	15679	15688	10
hsa-miR-146a	AGGGAAGTGA	15231	15240	10
hsa-miR-146a	TGACAACTGT	14327	14336	10
hsa-miR-146a	TAAGAACTAA	8935	8944	10
hsa-miR-146a	TTAGAACAGA	7908	7917	10
hsa-miR-146a	TGAGAAGTGC	6926	6935	10
hsa-miR-146a	TGAAAACCTTA	3883	3892	10
hsa-miR-146a	ACAGAACTGA	2259	2268	10
hsa-miR-146a	TGAGACCAGA	2235	2244	10
hsa-miR-146a	TGAGAAATAA	1614	1623	10
hsa-miR-147	TGTGTGGATAA	7223	7233	11
hsa-miR-147	TTTGTGCAAAT	3916	3926	11
hsa-miR-154	AATCATACA	12830	12838	9
hsa-miR-154	AATCATACA	934	942	9
hsa-miR-181c	AACCATAGT	15304	15312	9
hsa-miR-181c	AACCAAAGA	13244	13252	9
hsa-miR-181c	AACCATCAC	12990	12998	9
hsa-miR-181c	ATCCAGCGA	11466	11474	9
hsa-miR-181c	AAACATCTA	7494	7502	9
hsa-miR-181c	AAAAATCGA	6201	6209	9
hsa-miR-181c	AACCCCCGA	5540	5548	9
hsa-miR-181c	AACCCTCTA	3614	3622	9
hsa-miR-181c	AGCCAGCGA	3471	3479	9
hsa-miR-181c	AACCATAGG	2801	2809	9
hsa-miR-181c	AACCATCAC	194	202	9
hsa-miR-185	TGGAGAGAA	2979	2987	9
hsa-miR-192-5p	CTAACATATGAA	114	125	12
hsa-miR-194-1	TGTAACAGCA	1895	1904	10
hsa-miR-196a	AGGTAGTTT	12139	12147	9
hsa-miR-199a-5p	CCCTGTGTTC	5753	5762	10
hsa-miR-200a	TAACACTG	5852	5859	8
hsa-miR-200b	TAATAATGCC	11184	11193	10
hsa-miR-200b	GAATACTGCC	10340	10349	10

hsa-miR-200c-3p	TAATACTGT	12466	12474	9
hsa-miR-200c-3p	TAATAATGC	11185	11193	9
hsa-miR-200c-3p	GAATACTGC	10341	10349	9
hsa-miR-200c-3p	TAATACAGC	7594	7602	9
hsa-miR-203	TTAAATGTT	15584	15592	9
hsa-miR-203	TGAAATTTT	9782	9790	9
hsa-miR-203	TGAAAGGTT	4495	4503	9
hsa-miR-204-5p	TTCCTCTGTC	14648	14657	10
hsa-miR-204-5p	TTCCTTTATC	14006	14015	10
hsa-miR-205	TCCTTCATT	10659	10667	9
hsa-miR-208b	ATAAGAAGA	9493	9501	9
hsa-miR-208b	ATAAGAAGA	1770	1778	9
hsa-miR-211-5p	TTCCCTATCTC	14779	14789	11
hsa-miR-211-5p	TCCCCTCTGTC	5238	5248	11
hsa-miR-211-5p	TTCCCTTGCTC	5002	5012	11
hsa-miR-211-5p	TTCCCATTCTC	4710	4720	11
hsa-miR-214	CAGCAAGCA	15052	15060	9
hsa-miR-214	CAGAAGGCA	6918	6926	9
hsa-miR-214	CCGCAGGCA	5935	5943	9
hsa-miR-214	CACCAGGCA	2087	2095	9
hsa-miR-218	TGTGCTTGA	10385	10393	9
hsa-miR-302c	TTTAACATG	2932	2940	9
hsa-miR-324-5p	CGCGTCCCCT	4876	4885	10
hsa-miR-325	CCTTGTAGGC	15378	15387	10
hsa-miR-325	CAGAGTAGGT	14475	14484	10
hsa-miR-325	CCAAGTAGCT	10066	10075	10
hsa-miR-325	CCAAGTAGCT	354	363	10
hsa-miR-328	CTGTTCTCT	14651	14660	10
hsa-miR-328	CTGGCTCCCT	8215	8224	10
hsa-miR-328	CTGGCCCTTC	8062	8071	10
hsa-miR-328	CTGGCACTCA	6653	6662	10
hsa-miR-328	CTGGCTTTCT	6496	6505	10
hsa-miR-328	CTGCCCCTCC	6048	6057	10
hsa-miR-328	CTGGGCCGCT	4804	4813	10
hsa-miR-328	CTGGAGCTCT	4477	4486	10
hsa-miR-328	CTGACCCTTT	1089	1098	10
hsa-miR-330	CAAAGCACAC	13845	13854	10
hsa-miR-330	CAAAGCACAC	11657	11666	10
hsa-miR-331-5p	CTAGGTGTGG	7719	7728	10
hsa-miR-361-3p	CCCCCAGG	5112	5119	8

hsa-miR-362-5p	ATCCTTGGAT	14850	14859	10
hsa-miR-367-3p	AATTGCACTC	14182	14191	10
hsa-miR-367-3p	AAATGCACTT	999	1008	10
hsa-miR-369	AATAATACA	2266	2274	9
hsa-miR-371a-3p	AAGTGCCTGC	15435	15444	10
hsa-miR-371a-3p	AAGAGCCGAC	11455	11464	10
hsa-miR-371a-3p	ACGTGCCACC	10044	10053	10
hsa-miR-371a-3p	AAGTGCCTCT	7047	7056	10
hsa-miR-371a-3p	AAGTGCACCC	5457	5466	10
hsa-miR-371a-5p	TCTCAAACCTG	14658	14667	10
hsa-miR-372	AAAGTGCTG	12199	12207	9
hsa-miR-372	AAAGTGCTG	217	225	9
hsa-miR-374a-3p	TCATCAGATT	10606	10615	10
hsa-miR-377-3p	AGCACACAAA	13842	13851	10
hsa-miR-378a-3p	ACTGGCCTTG	15816	15825	10
hsa-miR-378a-3p	ACTGGTCTTG	11837	11846	10
hsa-miR-378a-5p	CTCCTGCCTC	12216	12225	10
hsa-miR-378a-5p	CTCCTGCCTC	10082	10091	10
hsa-miR-378a-5p	CTCCTGTCTC	8207	8216	10
hsa-miR-378a-5p	CTCCTAACTC	7650	7659	10
hsa-miR-382-3p	ATTCATTAC	14194	14203	10
hsa-miR-383	AGATTAGAA	14545	14553	9
hsa-miR-383	AGATTAGAA	7912	7920	9
hsa-miR-383	AGAACAGAA	5801	5809	9
hsa-miR-412	ACTTCACCT	737	745	9
hsa-miR-421	CTCAAAAGAC	14380	14389	10
hsa-miR-421	ATTAAGTAC	14333	14342	10
hsa-miR-421	AACATCAGAC	11398	11407	10
hsa-miR-421	ATCAACTGAG	3427	3436	10
hsa-miR-421	ATCAACAGGT	2443	2452	10
hsa-miR-421	ATCAAAAGAT	2333	2342	10
hsa-miR-422a	ACTGGCCTT	15817	15825	9
hsa-miR-422a	ACTGGTCTT	11838	11846	9
hsa-miR-422a	ACTGGACGT	5847	5855	9
hsa-miR-425	AGCGGGAAGGT	5167	5177	11
hsa-miR-431	TGTCTGGCA	14892	14900	9
hsa-miR-431	TGTCTAGCA	9218	9226	9
hsa-miR-432-5p	TCCTGGAGT	13624	13632	9
hsa-miR-432-5p	TATTGGAGT	10785	10793	9
hsa-miR-432-5p	TCTTAGAGT	9263	9271	9

hsa-miR-432-5p	TCTTAGAGT	6666	6674	9
hsa-miR-432-5p	TCTTGGAGC	2180	2188	9
hsa-miR-452	ACATCTGC	15009	15016	8
hsa-miR-452	TCTTCTGC	14773	14780	8
hsa-miR-452	TTATCTGC	14151	14158	8
hsa-miR-452	TCCTCTGC	13488	13495	8
hsa-miR-452	TCATGTGC	8660	8667	8
hsa-miR-452	TCATCTGG	8221	8228	8
hsa-miR-452	TCATGTGC	7945	7952	8
hsa-miR-452	ACATCTGC	7508	7515	8
hsa-miR-452	CCATCTGC	6787	6794	8
hsa-miR-452	TCATCCGC	5912	5919	8
hsa-miR-452	TCATCTGT	4053	4060	8
hsa-miR-452	TCATCTCC	3667	3674	8
hsa-miR-452	TCCTCTGC	3457	3464	8
hsa-miR-452	TCTTCTGC	2210	2217	8
hsa-miR-455-3p	CAGTCCAT	13893	13900	8
hsa-miR-455-5p	TGTGTGCCTT	15641	15650	10
hsa-miR-455-5p	TCTGTGCCTT	11203	11212	10
hsa-miR-455-5p	TATGTGCTTT	10522	10531	10
hsa-miR-483-3p	CACTCCTC	13536	13543	8
hsa-miR-483-3p	CACTCCTC	10333	10340	8
hsa-miR-483-3p	CACTCCTC	6101	6108	8
hsa-miR-486-5p	TCATGTACT	9835	9843	9
hsa-miR-486-5p	TCCTGTCCT	6526	6534	9
hsa-miR-487a	AATCATAACAG	12829	12838	10
hsa-miR-487a	AATCATAACAG	933	942	10
hsa-miR-491-5p	AATGGGGAAG	14975	14984	10
hsa-miR-491-5p	AGAGGGGACC	12315	12324	10
hsa-miR-491-5p	AGTTGGGCAC	11555	11564	10
hsa-miR-491-5p	AGTAGAGAAC	6909	6918	10
hsa-miR-491-5p	GGTGAGGAAC	6005	6014	10
hsa-miR-491-5p	AGCGGGGCAC	4455	4464	10
hsa-miR-491-5p	AGTGGGAAAT	3846	3855	10
hsa-miR-496	TTAGTATTA	10948	10956	9
hsa-miR-496	TGAGTATAA	10768	10776	9
hsa-miR-496	TCAGTATTA	9666	9674	9
hsa-miR-501-3p	ATGCATCAGG	15547	15556	10
hsa-miR-501-3p	ATCCACCGGG	11497	11506	10
hsa-miR-501-3p	AGGCACCAGG	2089	2098	10

hsa-miR-504	AGACCCTGT	15325	15333	9
hsa-miR-504	AGCCCCTGG	12898	12906	9
hsa-miR-504	AGTCCCTGG	10591	10599	9
hsa-miR-504	AGACCCGGG	4767	4775	9
hsa-miR-508-3p	TGATTATAGC	13565	13574	10
hsa-miR-508-3p	TGAGTGTAGC	3231	3240	10
hsa-miR-512-3p	CAGTGCTGTC	13211	13220	10
hsa-miR-512-3p	AAGTGCTCTC	7688	7697	10
hsa-miR-512-3p	AAGTGCTCTC	3184	3193	10
hsa-miR-512-5p	CACTCAG	14255	14261	7
hsa-miR-512-5p	CACTCAG	13591	13597	7
hsa-miR-512-5p	CACTCAG	12291	12297	7
hsa-miR-512-5p	CACTCAG	6652	6658	7
hsa-miR-512-5p	CACTCAG	5067	5073	7
hsa-miR-514a-3p	TTGACTCTT	14406	14414	9
hsa-miR-514a-3p	TTGACAGTT	13870	13878	9
hsa-miR-514a-3p	TTAACACTT	11237	11245	9
hsa-miR-514a-3p	ATGACACTT	10617	10625	9
hsa-miR-515-3p	GTGTGCCTT	15641	15649	9
hsa-miR-515-3p	GACTGCCTT	15539	15547	9
hsa-miR-515-3p	GAGTGACTT	1371	1379	9
hsa-miR-516a-3p	TGCTTCCT	10301	10308	8
hsa-miR-517a-3p	ATGGTGCATT	15650	15659	10
hsa-miR-517a-3p	ATCTTGCTTC	10303	10312	10
hsa-miR-519b-3p	AAAGTGCAT	13782	13790	9
hsa-miR-519e-3p	AAGTGCCTC	7048	7056	9
hsa-miR-520a-5p	CTCCAGATGG	6274	6283	10
hsa-miR-545	CAGCAAGCACT	15050	15060	11
hsa-miR-545	CAGAACACATT	11639	11649	11
hsa-miR-545	CTGCAAACACT	3450	3460	11
hsa-miR-549	TGACAACTGT	14327	14336	10
hsa-miR-551b-3p	GCTACCCAT	2411	2419	9
hsa-miR-552	CACAGGTGA	15130	15138	9
hsa-miR-552	AACAGGTCA	11407	11415	9
hsa-miR-552	AACATGTGA	9513	9521	9
hsa-miR-552	AACAGGTTA	2441	2449	9
hsa-miR-552	AACAGGTAA	1569	1577	9
hsa-miR-583	AAAAGAGGA	2921	2929	9
hsa-miR-583	CAAATAGGA	2833	2841	9
hsa-miR-583	CAACGAGGA	1824	1832	9

hsa-miR-583	CAAAGAAGA	1139	1147	9
hsa-miR-593-3p	TGTCTCTGT	8204	8212	9
hsa-miR-593-3p	TGGCTCTGC	6852	6860	9
hsa-miR-593-3p	TGCCTCTGC	231	239	9
hsa-miR-593-5p	AGGCACCAG	2090	2098	9
hsa-miR-593-5p	AGGCACCAG	2083	2091	9
hsa-miR-598	ACGTCATC	11432	11439	8
hsa-miR-611	GCGAGGTCTC	4779	4788	10
hsa-miR-611	GAGAGGCCCC	2121	2130	10
hsa-miR-611	GAGAGGACCT	1546	1555	10
hsa-miR-616-5p	ACTCTAAAC	14510	14518	9
hsa-miR-619	GACCTGGA	5824	5831	8
hsa-miR-620	ATGAATATAG	14560	14569	10
hsa-miR-620	ATGGAAATAT	12111	12120	10
hsa-miR-620	TTGGATATAG	11026	11035	10
hsa-miR-620	GTGGAGATGG	10397	10406	10
hsa-miR-620	ATGGAGATCC	6268	6277	10
hsa-miR-620	ATGGAGGGAG	5626	5635	10
hsa-miR-620	CTGGAGAAAG	3827	3836	10
hsa-miR-620	ATCCAGATAG	2959	2968	10
hsa-miR-620	ATGGGGCTAG	2843	2852	10
hsa-miR-620	AGGGAGAGAG	1551	1560	10
hsa-miR-620	CAGGAGATAG	1430	1439	10
hsa-miR-620	TTGGAGAGAG	1201	1210	10
hsa-miR-623	TCCCTTGC	8306	8313	8
hsa-miR-623	TCCCTTGC	5004	5011	8
hsa-miR-631	CACCTGGCC	9900	9908	9
hsa-miR-631	GACATGGCC	8632	8640	9
hsa-miR-634	AACCAGCAC	4520	4528	9
hsa-miR-636	TGTGCTTG	10386	10393	8
hsa-miR-638	ACGGAGCGCG	4905	4914	10
hsa-miR-638	AGGGAGGGCG	4615	4624	10
hsa-miR-642a-5p	ATCCCTCTC	8983	8991	9
hsa-miR-642a-5p	GTCCCTCCC	4722	4730	9
hsa-miR-643	ACATGCATGC	15553	15562	10
hsa-miR-643	CCTTG TAGGC	15378	15387	10
hsa-miR-643	TCTTG TATTC	14423	14432	10
hsa-miR-643	ACTGG TATGT	13933	13942	10
hsa-miR-643	ACTTCTATTC	12886	12895	10
hsa-miR-643	ACTTTTCTGC	12044	12053	10

hsa-miR-643	GCTTGTAAGC	11698	11707	10
hsa-miR-643	AGTTGTATGT	10531	10540	10
hsa-miR-643	ACTTGGAAGC	8105	8114	10
hsa-miR-643	ACTTGTGTGG	7227	7236	10
hsa-miR-643	ACTTGTTTGA	1880	1889	10
hsa-miR-643	ACATGTTTGC	1695	1704	10
hsa-miR-650	AGGAGGCAC	9647	9655	9
hsa-miR-650	AGAAGGCAG	6917	6925	9
hsa-miR-650	AGGAGCCAG	3474	3482	9
hsa-miR-650	ATGAGGCAG	3052	3060	9
hsa-miR-651	TCATGATAAG	15700	15709	10
hsa-miR-651	TTAGGTAAAA	13993	14002	10
hsa-miR-651	TTAAAATAAG	13988	13997	10
hsa-miR-651	TTAGCATAAC	12788	12797	10
hsa-miR-651	TTATGATGAG	12617	12626	10
hsa-miR-651	TTTGGATGAG	11069	11078	10
hsa-miR-651	TGAGTATAAG	10767	10776	10
hsa-miR-651	TTACAATAAG	10546	10555	10
hsa-miR-651	TAAGGATAAA	8265	8274	10
hsa-miR-651	TGTGGATAAG	7222	7231	10
hsa-miR-651	GTAGGATAGG	5553	5562	10
hsa-miR-651	CTAGGAAAAG	2823	2832	10
hsa-miR-651	CTATGATAAG	1635	1644	10
hsa-miR-651	TAAGGATAGG	1562	1571	10
hsa-miR-654-3p	TATGTATACT	15493	15502	10
hsa-miR-654-3p	TATCTCTTCT	14775	14784	10
hsa-miR-654-3p	TCTATCTGCT	8354	8363	10
hsa-miR-654-3p	AATGTCTGGT	6720	6729	10
hsa-miR-654-3p	TATGTTTCCT	6638	6647	10
hsa-miR-654-3p	TTTTTCTGCT	6586	6595	10
hsa-miR-654-3p	TATGTCTTTT	6534	6543	10
hsa-miR-654-3p	TATATCTGCA	6214	6223	10
hsa-miR-654-3p	TATGTAGGCT	97	106	10
hsa-miR-655	GTAATACAT	15593	15601	9
hsa-miR-655	ATAGTACAT	4200	4208	9
hsa-miR-655	ATAAGACAT	3642	3650	9
hsa-miR-655	ATAATACAG	2265	2273	9
hsa-miR-655	ACAATACAT	1757	1765	9
hsa-miR-656	AATATTATA	657	665	9
hsa-miR-664-3p	TATTCATTT	9385	9393	9

hsa-miR-765	TGGAGGA	5020	5026	7
hsa-miR-766	CTCCAGCCCC	12901	12910	10
hsa-miR-766	CTCCAGCCCC	5032	5041	10
hsa-miR-767-3p	CCTGCTCAT	14871	14879	9
hsa-miR-767-3p	TCTTCTCAT	9155	9163	9
hsa-miR-875	CCTGGAAATA	5820	5829	10
hsa-miR-875	CCTAGAAACA	5294	5303	10
hsa-miR-876	TGGATTCT	6366	6374	9
hsa-miR-876	TGGATTCT	142	150	9
hsa-miR-888-3p	GACTGACTCC	15772	15781	10
hsa-miR-888-3p	GACTGACAGC	9119	9128	10
hsa-miR-890	TACTTGGAAG	8106	8115	10
hsa-miR-940	AAGGCAGTG	1807	1815	9
hsa-miR-941	CACCCAGGT	14396	14404	9
hsa-miR-941	CACCCTGCC	13715	13723	9
hsa-miR-941	CACCCCTCT	13128	13136	9
hsa-miR-941	CACTCAGCT	12289	12297	9
hsa-miR-941	CTCCCGGGT	10102	10110	9
hsa-miR-941	CAGCCTGCT	10034	10042	9
hsa-miR-941	CACCCACCT	9904	9912	9
hsa-miR-941	CACCTGGCC	9900	9908	9
hsa-miR-941	CATCTGGCT	8219	8227	9
hsa-miR-941	CACTCGACT	8148	8156	9
hsa-miR-941	CTCCCAGCT	6840	6848	9
hsa-miR-941	CTCACGGCT	6031	6039	9
hsa-miR-941	CAGCCCGCT	5928	5936	9
hsa-miR-941	CACCTGACT	5510	5518	9
hsa-miR-941	CACGCCGCT	5142	5150	9
hsa-miR-941	CTCCCTGCT	3983	3991	9
hsa-miR-941	CACCAGGCA	2087	2095	9
hsa-miR-941	CTCCCGGGT	390	398	9
hsa-miR-941	CACCCAGCC	186	194	9
hsa-miR-941-2	ATCCGACTGT	9657	9666	10
hsa-miR-941-2	TCCCTGCTGT	8726	8735	10
hsa-miR-941-2	TCCCAGCTGT	6838	6847	10
hsa-miR-941-2	AGCCCGCTGT	5926	5935	10
hsa-miR-941-2	ACCCGGGCGT	4764	4773	10
hsa-miR-1179	AAGTATCCTTT	15346	15356	11
hsa-miR-1179	ATGCATTCTGT	3357	3367	11
hsa-miR-1179	ATGCATTCTCT	1854	1864	11

hsa-miR-1207-5p	TGGCAGGG	11441	11448	8
hsa-miR-1224-3p	CTCCACCTCC	399	408	10
hsa-miR-1228-3p	TCCCACCTG	13637	13645	9
hsa-miR-1228-3p	TCACGCCTG	4992	5000	9
hsa-miR-1231	GTGTCTGGC	12807	12815	9
hsa-miR-1231	GTGTCCGGG	4739	4747	9
hsa-miR-1245	AAGTGATCT	8341	8349	9
hsa-miR-1245	AAGTGATCT	2020	2028	9
hsa-miR-1249	CGCCCTTC	5907	5914	8
hsa-miR-1251	ACTCTAGGT	12854	12862	9
hsa-miR-1251	ACTCTATCT	8357	8365	9
hsa-miR-1251	ACTCCAGCT	4044	4052	9
hsa-miR-1251	AGTCTAGCT	457	465	9
hsa-miR-1252	AGAGGGAAAT	3819	3828	10
hsa-miR-1252	GGAAGGAAAT	1625	1634	10
hsa-miR-1268	CGGGCGTGG	4762	4770	9
hsa-miR-1270	CTGGAAATA	5820	5828	9
hsa-miR-1270	CTGGAGATG	5055	5063	9
hsa-miR-1270	CTGGAGAAA	3828	3836	9
hsa-miR-1270	CAGGAGATA	1431	1439	9
hsa-miR-1272	GATGATGA	10622	10629	8
hsa-miR-1275	GTAGGGGAGA	1189	1198	10
hsa-miR-1302	ATGGGACACA	15021	15030	10
hsa-miR-1302	TTTGGATATA	11027	11036	10
hsa-miR-1302	TTAGGGCATA	8421	8430	10
hsa-miR-1302	TTGGAACAGA	6076	6085	10
hsa-miR-1302	CTGGGACTTA	4819	4828	10
hsa-miR-1302	GTGGGAAATA	3845	3854	10
hsa-miR-1302	TTGTGAGATA	1944	1953	10
hsa-miR-1302	CTGGGAAATA	867	876	10
hsa-miR-1324	TCAAGACAGA	9426	9435	10
hsa-miR-1827	TGAGGCAGT	3051	3059	9
hsa-miR-1911-3p	CACCAGGCA	2087	2095	9
hsa-miR-1915	CCCCAGGG	5111	5118	8
hsa-miR-2909	TTTAGGGCC	3728	3736	9

B2. Another exemplary multiple miRNAs-one mRNA paradigm involves UCP2.

UCP2 is a mitochondrial transporter protein expressed in WAT, skeletal muscle, pancreatic islets and the central nervous system. Like UCP1, it creates proton leaks across the

inner mitochondrial membrane, thus uncoupling oxidative phosphorylation from ATP synthesis (adaptive thermogenesis, see Figure 5) (Lowell et al., Nature (2000)).

Two recent meta-analyses report an association between polymorphisms in the promoter region of UCP2 and obesity (Liu et al., Gene (2013); Andersen et al., Int. J. Obes. (2013)). The first meta-analysis included 14 studies (7,647 cases and 11,322 controls) and concluded that there is a significant association of the A allele of the UCP2 -866G/A polymorphism with reduced risk of obesity, especially in European populations. In the second meta-analysis including 12,984 subjects, the common UCP2 -866G allele is associated with obesity. The same UCP2 -866G allele is associated with decreased insulin sensitivity in 17,636 Danish subjects. In a study, UCP2 mRNA levels in visceral fat were decreased in subjects with the GG phenotype (Esterbauer et al., Nat. Genet. (2001)). A trend toward a negative correlation between subcutaneous adipocyte UCP2 mRNA and percent body fat was found in another study (Wang et al., American Journal of Physiol. (2004)). This information supports targeting UCP2 expression and activity as a meaningful way to alter adaptive thermogenesis and consequently treat human obesity. Many strategies could be implemented to achieve this goal, however, the one employed in the methods of the invention uses miRNA agents to modulate simultaneously several elements within the thermogenic pathways to increase UCP2 synthesis and activity. Both direct and indirect interactions between miRNAs and the UCP2 gene are considered. Direct interaction means the direct binding of miRNAs to the various regions of the UCP2 gene, resulting in alterations of the transcription, translation, stability and/or degradation of the UCP1 mRNA. Indirect interaction means that miRNAs alter the transcription, translation, stability and/or degradation of thermogenic mRNAs, whose expressed proteins alter the transcription of the UCP2 gene. Furthermore, indirect interaction means that miRNAs alter the transcription, translation, stability and/or degradation of other miRNAs that modify the transcription of the UCP2 gene.

The promoter region of the human UCP2 gene (ENSG00000175567, Homo sapiens uncoupling protein 2 (mitochondrial, proton carrier) (UCP2), RefSeqGene on chromosome 11) is rich in regulatory element motifs (Table 12).

Table 12.

UCP2 Gene Regulatory Elements:

	Name of regulatory element	Sequence	Number	Nucleotide Location
1	RXR/T3RE	AGGTCA	8	1,074 to 1,079; 3,083 to 3,088; 3,239 to 3,244; 4,304 to 4,309; 6,965 to 6,970; 7,420 to 7,425; 7,677 to 7,682; 13,319 to 13,324
2	GC Box 1	CGCCC	16	2,605 to 2,609; 4,323 to 4,327; 4,523 to 4,527; 4,933 to 4,937; 4,959 to 4,963; 5,048 to 5,052; 5,066 to 5,070; 5,146 to 5,150; 5,155 to 5,159; 5,387 to 5,391; 5,483 to 5,487; 6,067 to 6,071; 8,523 to 8,527; 9,790 to 9,794; 10,819 to 10,823; 11,754 to 11,758
3	GC Box 2	GCGGG	5	4,263 to 4,267; 4,757 to 4,761; 4,860 to 4,864; 7,619 to 7,623; 11,262 to 11,266
4	GT Box 1	CACCC	30	1,421 to 1,425; 1,677 to 1,681; 1,761 to 1,765; 1,825 to 1,829; 1,833 to 1,837; 2,036 to 2,040; 3,003 to 3,007; 4,903 to 4,907; 4,947 to 4,951; 5,210 to 5,214; 6,204 to 6,208; 6,247 to 6,251; 6,469 to 6,473; 6,828 to 6,832; 7,681 to 7,685; 8,048 to 8,052; 8,437 to 8,441; 8,572 to 8,576; 8,599 to 8,603; 8,702 to 8,706; 11,077 to 11,081; 11,235 to 11,239; 12,006 to 12,010; 12,374 to 12,378; 13,475 to 13,479; 13,666 to 13,670; 13,687 to 13,691; 13,838 to 13,842; 14,410 to 14,414; 14,545 to 14,549
5	GT Box 2	GTGGG	26	123 to 127; 1,006 to 1,010; 2,105 to 2,109; 4,562 to 4,566; 5,793 to 5,797; 6,029 to 6,033; 6,034 to 6,038; 6,040 to 6,044; 6,150 to 6,154; 7,271 to 7,275; 7,392 to 7,396; 9,040 to 9,044; 9,697 to 9,701; 10,227 to 10,231; 10,238 to 10,242; 10,247 to 10,251; 11,817 to 11,821; 12,410 to 12,414; 12,414 to 12,418; 12,678 to 12,682; 13,047 to 13,051; 13,238 to 13,742; 13,743 to 13,747; 14,252 to 14,256;

				14,969 to 14,973; 15,104 to 15,108
6	CpG Methylation Island	CG	295	4,071 to 5,212

Figure 8B depicts the location of these various regulatory elements in reference to the UCP2 transcription start site at nucleotide position 5,001 of the 15,174 base pair human UCP2 gene. Direct or indirect activation or repression of these regulatory elements by miRNAs will result in alterations of UCP2 gene expression and activity.

A survey of miRNAs targeting the human UCP2 3'UTR with several prediction programs, using the UCP2 Ensembl 2,113 base pair transcript ENST00000310473 as a target revealed binding sites for 161 miRNAs as shown in Table 13.

Table 13.

miRNAs with predicted binding sites in the 3'UTR of UCP2 transcript sequence:

hsa-miR-1	hsa-miR-1275	hsa-miR-149	hsa-miR-219-1
hsa-miR-1-2	hsa-miR-1276	hsa-miR-150-3p	hsa-miR-219-2
hsa-miR-101-1	hsa-miR-1278	hsa-miR-150-5p	hsa-miR-221-5p
hsa-miR-101-2	hsa-miR-1285-1	hsa-miR-1538	hsa-miR-23b
hsa-miR-103	hsa-miR-1286	hsa-miR-155	hsa-miR-24-1
hsa-miR-105-1	hsa-miR-1293	hsa-miR-15a	hsa-miR-24-2
hsa-miR-105-2	hsa-miR-1300	hsa-miR-15b	hsa-miR-27b-5p
hsa-miR-106b	hsa-miR-1302-1	hsa-miR-16-1	hsa-miR-28
hsa-miR-107	hsa-miR-1302-10	hsa-miR-16-2	hsa-miR-296-3p
hsa-miR-1204	hsa-miR-1302-11	hsa-miR-184	hsa-miR-296-5p
hsa-miR-1207	hsa-miR-1302-2	hsa-miR-185-3p	hsa-miR-3064
hsa-miR-1208	hsa-miR-1302-3	hsa-miR-185-5p	hsa-miR-323a
hsa-miR-1226	hsa-miR-1302-4	hsa-miR-186	hsa-miR-328
hsa-miR-1246	hsa-miR-1302-5	hsa-miR-188	hsa-miR-330
hsa-miR-1252	hsa-miR-1302-6	hsa-miR-18a	hsa-miR-331
hsa-miR-1253	hsa-miR-1302-7	hsa-miR-18b	hsa-miR-338
hsa-miR-1255a	hsa-miR-1302-8	hsa-miR-193a	hsa-miR-342
hsa-miR-1255b-1	hsa-miR-1302-9	hsa-miR-195	hsa-miR-3619
hsa-miR-1255b-2	hsa-miR-1303	hsa-miR-199b	hsa-miR-370
hsa-miR-1260a	hsa-miR-130a	hsa-miR-200a	hsa-miR-377
hsa-miR-1262	hsa-miR-1321	hsa-miR-203	hsa-miR-378a
hsa-miR-1263	hsa-miR-138-1	hsa-miR-206	hsa-miR-383
hsa-miR-1265	hsa-miR-138-2	hsa-miR-214	hsa-miR-411

hsa-miR-412
hsa-miR-422a
hsa-miR-424
hsa-miR-425
hsa-miR-4291
hsa-miR-432-3p
hsa-miR-4505
hsa-miR-450b
hsa-miR-453
hsa-miR-4533
hsa-miR-4539
hsa-miR-4745
hsa-miR-4747
hsa-miR-485-5p
hsa-miR-486
hsa-miR-490
hsa-miR-491
hsa-miR-493
hsa-miR-497
hsa-miR-498
hsa-miR-503w
hsa-miR-505
hsa-miR-508-3p
hsa-miR-532
hsa-miR-539
hsa-miR-541
hsa-miR-5481
hsa-miR-552
hsa-miR-563
hsa-miR-575
hsa-miR-577
hsa-miR-580
hsa-miR-583
hsa-miR-584
hsa-miR-608
hsa-miR-612
hsa-miR-613
hsa-miR-615-3p
hsa-miR-618
hsa-miR-625
hsa-miR-626
hsa-miR-634
hsa-miR-635

hsa-miR-638
hsa-miR-645
hsa-miR-646
hsa-miR-647
hsa-miR-652
hsa-miR-654
hsa-miR-658
hsa-miR-663a
hsa-miR-663b
hsa-miR-664-5p
hsa-miR-675
hsa-miR-7-1
hsa-miR-7-2
hsa-miR-7-3
hsa-miR-708
hsa-miR-761
hsa-miR-765
hsa-miR-769
hsa-miR-770
hsa-miR-876
hsa-miR-877
hsa-miR-921
hsa-miR-922
hsa-miR-92a-1
hsa-miR-92a-2-5p
hsa-miR-92b

Moreover, a survey of miRNAs targeting the human UCP2 5'UTR with several prediction programs, using the human UCP2 gene (ENSG00000175567, 15,174 base pair (bp), including 5,000 bp 5'UTR as a target revealed binding sites for 54 miRNAs in UCP2 5'UTR as shown in Table 14.

5

Table 14.

miRNAs with predicted binding sites in the 5'UTR of UCP2 gene sequence:

MicroRNA	Seed Length	Start	Sequence	End	P value
hsa-let-7c	9	3052	UAGAGUUAC	3044	0.0374
hsa-let-7i-3p	9	3051	CUGCGCAAG	3043	0.0374
hsa-miR-1228-5p	9	3419	UGGGCGGGG	3411	0.0374
hsa-miR-1229-3p	9	3419	UCUCACCAC	3411	0.0374
hsa-miR-129-1-3p	10	2784	AGCCCUUACC	2775	0.0095
hsa-miR-1302	9	4219	UGGGACAU	4211	0.0374
hsa-miR-1303	9	2159	UUAGAGACG	2151	0.0374
hsa-miR-136	9	4486	CUCCAUUUG	4478	0.0374
hsa-miR-155	9	2160	UUAAUGCUA	2152	0.0374
hsa-miR-16	10	3603	UAGCAGCACG	3594	0.0095
hsa-miR-18a-3p	10	3603	ACUGCCCUAA	3594	0.0095
hsa-miR-190	9	2428	UGAUAUGUU	2420	0.0374
hsa-miR-191	9	3052	CAACGGAAU	3044	0.0374
hsa-miR-192	9	4390	CUGACCUAU	4382	0.0374
hsa-miR-194	9	1643	UGUAAACAGC	1635	0.0374
hsa-miR-197	9	5001	UCACCACCU	4993	0.0374
hsa-miR-19b-2-5p	10	3052	AGUUUUGCAG	3043	0.0095
hsa-miR-203	9	3051	UGAAAUGUU	3043	0.0374
hsa-miR-218	10	3603	UUGUGCUUGA	3594	0.0095
hsa-miR-218-1-3p	9	5001	UGGUUCCGU	4993	0.0374
hsa-miR-219-1-3p	9	3614	AGAGUUGAG	3606	0.0374
hsa-miR-26a-2-3p	9	2163	CCUAUUCUU	2155	0.0374
hsa-miR-27a-3p	10	3603	UUCACAGUGG	3594	0.0095
hsa-miR-27a-5p	11	3336	AGGGCUUAGCU	3326	0.0024
hsa-miR-28-5p	10	3603	AAGGAGCUCA	3594	0.0095
hsa-miR-331-3p	9	4134	GCCCCUGGG	4126	0.0374
hsa-miR-337-5p	10	115	GAACGGGCUUC	106	0.0095
hsa-miR-340-3p	9	1872	CCGUCUCAG	1864	0.0374
hsa-miR-34c-3p	11	2162	AAUCACUAACC	2152	0.0024
hsa-miR-373-5p	11	530	ACUCAAAAUGG	520	0.0024

hsa-miR-425	9	1013	AAUGACACG	1005	0.0374
hsa-miR-497	9	3661	AGCAGCACA	3653	0.0374
hsa-miR-501-5p	9	4164	AUCCUUUGU	4156	0.0374
hsa-miR-505	9	1015	GUCAACACU	1007	0.0374
hsa-miR-508-3p	9	1274	GAUUGUAGC	1266	0.0374
hsa-miR-509-3p	12	2554	UGAUUGGUACGU	2543	0.0006
hsa-miR-512-5p	10	987	ACUCAGCCUU	978	0.0095
hsa-miR-514	9	5001	UUGACACUU	4993	0.0374
hsa-miR-515-5p	9	59	UUCUCCAAA	51	0.0374
hsa-miR-518a-3p	9	19	GAAAGCGCU	11	0.0374
hsa-miR-519e-5p	11	2525	UCUCCAAAAGG	2515	0.0024
hsa-miR-548a-3p	10	680	CAAAACUGGC	671	0.0095
hsa-miR-550a-3p	9	4312	GUCUUACUC	4304	0.0374
hsa-miR-571	9	739	UGAGUUGGC	731	0.0374
hsa-miR-578	9	1377	CUUCUUGUG	1369	0.0374
hsa-miR-606	9	4420	AACUACUGA	4412	0.0374
hsa-miR-615-5p	10	1140	GGGGGUCCCC	1131	0.0095
hsa-miR-638	9	2710	GGGAUCGCG	2702	0.0374
hsa-miR-657	12	1316	GCAGGUUCUCAC	1305	0.0006
hsa-miR-658	9	3673	GGCGGAGGG	3665	0.0374
hsa-miR-877-3p	9	4349	UCCUCUUCU	4341	0.0374
hsa-miR-93-3p	9	799	ACUGCUGAG	791	0.0374
hsa-miR-96-3p	9	799	AAUCAUGUG	791	0.0374
hsa-miR-99b-3p	9	2163	CAAGCUCGU	2155	0.0374

C) A multiple microRNAs-multiple mRNAs paradigm.

The 83 thermogenic regulator molecules selected in Table 2 were screened for high stringency Multiple miRNAs-Multiple mRNAs associations. The results of these analyses with 7 major prediction tools are shown in Figure 4. The union of these 7 tools produces 4439 miRNA-gene couples. Overlap between these tools decreases as the number of tools increases, reaching only 15 miRNA-gene couples when 7 tools are considered.

D) An over-representation of one microRNA seed sequence motif among co-regulated mRNA targets paradigm.

Several approaches can be used to identify pathway-specific miRNAs. For example, searching the 3'-UTRs of putatively co-regulated genes for an over-represented sequence from a miRNA seed region could identify a common regulatory miRNA. To determine if particular

miRNA seed sequences were overrepresented among the 3' UTR of the chosen 83 thermogenesis targets, the miRvestigator web application (miRvestigator. systemsbiology.net/) was employed. Using the following parameters (motif size of 8bp, default Weeder model, seed model of 8mer, 100% complementarity homology and 0.25 wobble base-pairing allowed), it was determined that

5 that the motif 5'-UUUGUACA-3' recognized by hsa-miR-19a/19b is overrepresented among 15 of the 83 thermogenesis targets with a complementarity p value of 1.7×10^{-04} as shown in Table 15. Of note is that hsa-miR-19 has been reported as an abundant human adipocyte miRNA.

Table 15.**Complementarity between the common motif UUUGUACA and hsa-miR-19a/19b:**

miRNA Name	miRNA Seed	Seed Model	Length of Complementarity	Complementary Base-Pairing	Complementarity P-Value
hsa-miR-19a hsa-miR-19b	UGUGCAAA	8mer	8	hsa-miR-19a 5' UUUGUACA 3' : 3' AAACGUGU 5' miRNA Seed	1.7×10^{-04}

The Minimum Free Energy levels of the hsa-miR-19 mRNA/miRNA duplexes identified by miRvestigator were quite low, favoring tight binding. Accordingly, the miRvestigator analysis was repeated with less stringent levels of complementarity. This analysis identified a

15 further 10 additional targets (CEBPD, PRKAA1, TWIST1, IRS1, NCOA1, NCOA2, NCOA3, KLF5, RPS6KB1, NRIP1) with 95% similarity to the consensus hsa-miR-19 motif. Interestingly, hsa-miR-19 is among the most abundant miRNAs in adipose tissue. The genes identified as containing a sequence complementary to hsa-miR-19 seed region are set forth in Table 16.

Table 16.**Thermogenic regulators identified as targets for hsa-miR-19:**

Gene	Gene symbol	Sequence of Site	Start Relative to Stop Codon (bp)	% Similarity to Consensus Motif (Position 1-700)	Minimum Free Energy (MFE) of miRNA-mRNA Duplex
950	BMP2	UUUGUACA	386	100.00	-6.80
1052	CESPD	UUUGUAAA	263	95.44	-3.40
7132	TNFRSF1A	UUUGUACA	510	100.00	-6.80
5582	PRKAA1	UUUGUAAA	2400	95.44	-3.40
5583	PRKAA2	UUUGUACA	542	100.00	-6.80
955	BMP7	UUUGUACA	1927	100.00	-6.80
952	BMP4	UUUGUACA	770	100.00	-6.80
133822	PFARGC1B	UUUGUACA	7199	100.00	-6.80
7474	WNT5A	UUUGUACA	1414	100.00	-6.80
6720	SREBF1	UUUGUACA	510	100.00	-6.80
7281	TWIST1	UUUGUAAA	649	95.44	-3.40
3687	IRS1	UUUGUAAA	992	95.44	-3.40
12422	NCOA2	UUUGUAAA	1381	95.44	-3.40
9204	NRIP1	UUUGUACA	1718	100.00	-6.80
9204	NRIP1	UUUGUAAA	1935	95.44	-3.40
9202	NCOA3	UUUGUAAA	965	95.44	-3.40
1366	CREB1	UUUGUAAA	1973	95.44	-3.40
1366	CREB1	UUUGUACA	2822	100.00	-6.80
1366	CREB1	UUUGUACA	2822	100.00	-6.80
1365	CREB1	UUUGUAAA	4175	95.44	-3.40
3843	INSR	UUUGUAAA	2105	95.44	-3.40
8012	NR4A3	UUUGUACA	2347	100.00	-6.80
950	RUNX2	UUUGUACA	2425	100.00	-6.80
6775	STAT5A	UUUGUACA	1214	100.00	-6.80
1874	E2F4	UUUGUACA	755	100.00	-6.80
555	KLF5	UUUGUAAA	549	95.44	-3.40
3843	NCOA1	UUUGUAAA	381	95.44	-3.40
8128	RPS6KB1	UUUGUAAA	2531	95.44	-3.40

Accordingly, the miRvestigator analysis was repeated with less stringent levels of
5 complementarity (motif size of 8bp, default Weeder model, seed model of 8mer, 95%
complementarity homology and 0.25 wobble base-pairing allowed). This analysis identified a

further 10-12 additional targets (CEBPD, CREB1, PRKAA1, TWIST1, INSR, IRS1, NCOA1, NCOA2, NCOA3, KLF5, RPS6KB1, NRIP1) with 95% similarity to the consensus hsa-miR-19 motif. Interestingly, hsa-miR-19 is among the most abundant miRNAs in adipose tissue. The genes identified as containing a sequence complementary to hsa-miR-19 seed region are set forth
5 in Table 17.

Table 17.

Thermogenic regulators identified as targets for hsa-miR-19a/b with 95% to 100% similarity to consensus motif:

Gene	Gene symbol	Sequence of Site	Start Relative to Stop Codon (bp)	% Similarity to Consensus Motif (Quality = [Medium] [Poor])	Minimum Free Energy (MFE) of mRNA-miRNA Duplex
<u>650</u>	BMP2	UUUGUACA	386	100.00	-6.80
<u>1052</u>	CEBPD	UUUGUAAA	263	95.42	-3.40
<u>7132</u>	TNFRSF1A	UUUGUACA	510	100.00	-6.80
<u>4040</u>	LRP6	UUUGUACA	151	100.00	-6.80
<u>4040</u>	LRP6	UUUGUAAA	4965	95.42	-3.40
<u>5562</u>	PRKAA1	UUUGUAAA	2400	95.42	-3.40
<u>5563</u>	PRKAA2	UUUGUACA	542	100.00	-6.80
<u>638</u>	BMP7	UUUGUACA	1927	100.00	-6.80
<u>652</u>	BMP4	UUUGUACA	770	100.00	-6.80
<u>133522</u>	PPARGC1B	UUUGUACA	7199	100.00	-6.80
<u>1874</u>	E2F4	UUUGUACA	755	100.00	-6.80
<u>7474</u>	WNT5A	UUUGUACA	1414	100.00	-6.80
<u>9720</u>	SREBF1	UUUGUACA	510	100.00	-6.80
<u>7291</u>	TWIST1	UUUGUAAA	649	95.42	-3.40
<u>3867</u>	IRS1	UUUGUAAA	992	95.42	-3.40
<u>10499</u>	NCOA2	UUUGUAAA	1981	95.42	-3.40
<u>8204</u>	NRIP1	UUUGUACA	1718	100.00	-6.80
<u>8204</u>	NRIP1	UUUGUAAA	1935	95.42	-3.40

8202	NCOA3	UUUGUAAA	965	95.42	-3.40
8543	INSR	UUUGUAAA	2105	95.42	-3.40
8213	NR4A3	UUUGUACA	2347	100.00	-6.80
8776	STAT5A	UUUGUACA	1214	100.00	-6.80
888	KLF5	UUUGUAAA	549	95.42	-3.40
8545	NCOA1	UUUGUAAA	381	95.42	-3.40
880	RUNX2	UUUGUACA	2425	100.00	-6.80
1385	CREB1	UUUGUAAA	1973	95.42	-3.40
1385	CREB1	UUUGUACA	2822	100.00	-6.80
1385	CREB1	UUUGUAAA	4175	95.42	-3.40
8198	RPS8KB1	UUUGUAAA	2531	95.42	-3.40

Without wobbling, the same motif 5'-UUUGUACA-3' is overrepresented among targets of hsa-miR-1283 with a complementarity p value of 1.4×10^{-4} . Furthermore, hsa-miR-1283 binds to other mRNAs of interest like ABCA1 (cholesterol transporter), the adiponectin receptor and the transcription factor TCF7L2 that is implicated in genetic human obesity.

Similarly, other miRNA over-represented seed sequences were identified for miRNAs expressed in adipocytes. They include the universal hsa-let-7 family (sequence CUAUACAA, p value = 7.5×10^{-4}) and the adipocyte-rich hsa-miR-30 family (sequence UGUAAACA, p value = 1.9×10^{-3}) to name a few.

With respect to PRDM16, CIDEA, NRIP1, KDM3A, CEPPB, PPARG, PPARGC1A, and PPKAA2, which according to the STRING software package are directly linked to UCP1, it appears that all of them share (at motif size 8bp, default Weeder model, seed model 8mer, 95% complementarity homology and 0.25 wobble base-pairing allowed) a consensus sequence with several miRNAs, including hsa-miR-3658 (p value = 1.9×10^{-3}) and the hsa-miR-30 family (p value = 6.3×10^{-3}) as follows:

hsa-miR-3658:

```

Notif 5' UUUUUUAC 3'
      ||*|||
3' ...AAGAAUUU 5' miRNA Seed

```

hsa-miR-30a/b/c/d/e:

```

Notif 5' UUUUUUAC 3'
      |||||
3' ...ACAAAUGU 5' miRNA Seed

```

E) An intronic miRNA-multiple mRNAs pathway-specific paradigm.

5 Many mammalian miRNAs are located within introns of protein-coding genes rather than in their own unique transcription units. Intronic miRNAs are typically expressed and processed with the precursor mRNA in which they reside. Although the intronic miRNAs and their host genes can be regulated independently, an intronic miRNA can down-regulate its own host protein-coding gene by targeting the host gene's UTR. Feedback regulation on host protein-coding genes could be achieved by selecting the transcription factors that are miRNA targets or
10 by protein-protein interactions between intronic miRNA host gene product and miRNA target gene products. As an example, miR-33 acts in concert with the SREBP host genes to control cholesterol homeostasis and the pharmacological inhibition of miR-33a and miR-33b is a promising therapeutic strategy to raise plasma HDL and lower VLDL triglyceride levels for the
15 treatment of dyslipidemias.

Examination of the 83 thermogenic target genes reveals two intronic miRNAs: miR-378 located in the PPARGC1B gene and miR-4251 located in the PRDM16 gene.

Mining of the Internet tools predicting miRNA targets indicates that miR-378 targets include BMP2, PPARA, PPARGC1A, PRDM16, STAT5 and WNT10A as well as ADIPOQ and IGFR1;
20 and that miR-4251 targets include BMP2, CTBP1, CTBP2, MAPK14, NCOA3, PLAC8, PPARA, PPARD, TRPM8, as well as ABCA5, ABCA13, ADIPOQR2, KDM5B, KLF-12, KLF-14 and TCF7L2.

Example 3. High-Content Cellular Phenotypic Screening

High-content screening methods are used to screen for novel miRNA agents that modulate the activity of thermogenic regulators (e.g., UCP1 and UCP2). High-content screening is a drug discovery method that uses images of living cells to facilitate molecule discovery. Such automated image based screening methods are particularly suitable for identifying agents which alter cellular phenotypes.

WAT cells contain large lipid droplets, whereas, in contrast, BAT cells contain numerous smaller droplets and a much higher number of mitochondria, which contain iron and make them appear brown. The large number of mitochondria in BAT leads to an increased oxygen consumption, when compared to WAT. Accordingly, it is possible to distinguish between BAT and WAT cells visually based on their cellular phenotype.

Accordingly, high-content screening methods were used to screen for novel miRNA agents that modulate the activity of thermogenic regulators. Specifically, the phenotypic appearance of cultured human adipocytes and adipose tissue derived mesenchymal stem cells grown in the presence and absence of miRNA agonists or antagonists was assessed over two weeks by phase contrast microscopy of the cultured cells, measurement of the cellular lipid content (using Oil Red O Staining or Nile Red fluorescence); mitochondrial content (e.g., using Life Technologies Mito-Tracker Red FM), and/or oxygen consumption *in vitro* (e.g., using the Seahorse Bioscience Extra-Cellular Flux Instrument). mRNA expression is measured by targeted q-RT-PCR, NanoString and universal RNA-Sequencing. Protein expression is measured by targeted Western Blotting and universal proteomic profiling.

A. Differentiation of human pre-adipocytes into adipocytes.

1. Differentiation Protocol.

In order to assess the effect of miRNA analogs on human pre-adipocytes differentiation into mature adipocytes, human subcutaneous pre-adipocytes (SuperLot 0048 from 8 female donors, ZenBio, NC) were plated on Day 0 into 96-well plates and allowed to attach overnight in preadipocyte medium (DMEM/Ham's F-12 (1:1, v/v), HEPES buffer, Fetal bovine serum and Antibiotics). The next day (Day 1), the medium was removed and replaced with differentiation medium (DMEM/Ham's F-12 (1:1, v/v), 100 μ M Ascorbic Acid, 0.85 μ M insulin, 20 nM sodium selenite, 0.2 nM, tri-iodothyronine, 1 μ M dexamethasone, 100 μ M isobutyl-methylxanthine, 100 nM Rosiglitazone and Antibiotics). The cells were allowed to incubate for 2 days at 37°, 5% CO₂. After 2 days (Day 3), the medium was removed and replaced with fresh

maintenance medium (DMEM/Ham's F-12 (1:1, v/v), 100 μ M Ascorbic Acid, 0.85 μ M insulin, 20 nM sodium selenite, 0.2 nM tri-iodothyronine, and Antibiotics). On Day 3, the cells were transfected with miRNA analogs (Dharmacon specific miRIDIAN Mimics and Hairpin Inhibitors) using the transfecting agent Dharmafect1. All treatments were in triplicate. Post
5 transfection, the negative control was maintenance medium only and the positive control was maintenance medium with 100 nM of the PPAR γ agonist rosiglitazone. After 2 days, medium was removed and replaced with fresh maintenance medium. The maintenance medium then changed every two days until the end of the treatment period (Day 15). At the end of the treatment (total of 15 days in culture) cells were processed for Phenotyping and Genotyping
10 Screening.

2. Transfection of pre-adipocytes.

Transfection reagents are used to facilitate the penetration of miRNA analogs into target cells. As an example, the extent of transfection efficiency we achieved in pre-adipocytes with the transfecting agent Dharmafect 1 (Dharmacon, CO) is depicted herein. Transfection efficiency
15 was assessed in two ways:

- a. Measurement of cellular epifluorescence after transfection with fluorescent miRNA analogs.

Fluorescence was measured on Day 15 (540 excitation/590 emission) in cells transfected on Day 3 with the Dy547-labeled non-targeting miRIDIAN Mimic and Hairpin Inhibitor (100 nM). As shown in Figure 9, there was a significantly greater fluorescence of cells
20 transfected with the fluorescent miRNA analogs, even 12 days after transfection:

- b. Reduction of control gene expression.

To confirm successful transfection of pre-adipocytes, the reduction of expression of the control gene GAPDH ("housekeeping gene") was measured 4 days (Day 7) (Figure 10A) and
25 12 days (Day 15) (Figure 10B) after transfection of pre-adipocytes with a GAPDH-specific siRNA. Cell lysates were obtained and RT-PCR was conducted using pure RNA obtained by Cells-to-Ct reagents. 91% and 86% knockdowns of the GAPDH mRNA expression were observed at Day 4 and Day 12 post transfection, both highly significant, as shown in Figures 10A and 10B.

3. Phenotypic changes during human pre-adipocytes differentiation into adipocytes.

At the end of treatment (15 days in culture) cells were stained with Oil Red O for assessment of lipid content. As shown in Figures 11A to F, in the presence of medium without rosiglitazone, the pre-adipocytes show little differentiation into lipid-loaded mature adipocytes.

5 In the presence of differentiation medium including 100 nM Rosiglitazone for 2 days followed by maintenance medium for 12 days (negative control), some differentiation into lipid-loaded mature adipocytes is noted. In the presence of 100 nM rosiglitazone throughout the experiment (positive control), most of the cells became lipid-loaded mature adipocytes. As an example, in the presence of 25 nM hsa-miR-30b mimic, about half of the cells became lipid-loaded mature
10 adipocytes. The non-targeting miRNA mimic and inhibitor showed patterns similar to the negative control.

4. Genotypic changes during human pre-adipocyte differentiation into adipocytes.

Profiling of mRNA changes occurring during the differentiation of human pre-adipocyte into mature adipocyte induced by rosiglitazone or miRNA analogs was performed by RNA-Seq
15 technology. Small RNA sequencing (RNA-Seq) is a high-throughput next-generation sequencing platform which now allows transcriptome-wide profiling of all small RNAs, known and unknown, with no need for prior sequence or secondary structure information.

RNA samples were extracted from pre-adipocytes (pre-adipocyte negative control) and from pre-adipocytes cultured in the presence of 100 nM rosiglitazone (differentiation positive
20 control) or 25 nM miRNA mimics or inhibitors for 12 days. RNA sequencing was performed on the Illumina Hi-Seq 2000 equipment. The results were mapped against Human Genome 19 (<http://genome.ucsc.edu/>). It appears that in the presence of a miRNA analog, between 313 and 449 mRNA are significantly differentially expressed in reference to pre-adipocytes. In reference to Rosiglitazone, the number of significantly differentially expressed genes is reduced between
25 111 and 216, thus suggesting common pathways of activation of adipocyte differentiation between miRNAs and the PPARG analog.

Regarding our 83 thermogenic activators and inhibitors, the expression of 73 of them is altered in the presence of rosiglitazone or miRNA analogs. The changes of mRNA expression of the thermogenesis targets in the presence of rosiglitazone (Figure 12A) or miRNA analogs hsa-
30 let-7a inhibitor, hsa-miR-1 mimic, hsa-miR-19b mimic, hsa-miR-30b mimic or control adipocytes are shown on Figures 12B-F, respectively).

Changes in mRNA expression of UCP1, 2 and 3 were also measured in the presence of rosiglitazone or miRNA analogs, as shown below in Table 18.

Table 18.

5 Changes in thermogenic mRNA expression:

Agent	mRNA Expression changes (log ratios)		
	UCP1	UCP2	UCP3
Rosiglitazone	15.70	263	0.26
hsa-let-7a inhibitor	2.23	173	0.65
hsa-miR-1 mimic	0.41	110	0.40
hsa-miR-19b mimic	0.18	33	0.26
hsa-miR-30b mimic	0.76	119	0.28
<i>Baseline level in pre-adipocytes</i>	0.02	1.35	0.30

The expression levels of the three Uncoupling Proteins were low in pre-adipocytes. The expression of UCP1 was significantly increased in the presence of rosiglitazone 100 nM which was renewed with the culture medium every other day. The magnitude of UCP1 mRNA rise with the miRNA analogs was lower than with rosiglitazone, but one has to keep in mind the miRNA analogs concentration used (25 nM) and the fact that only one transfection was performed 12 days before RNA extraction. A major finding is the dramatic increase of UCP2 expression in the presence of rosiglitazone as well as the miRNA analogs. The expression of UCP3 did not change in any condition, as expected for a gene that is mainly expressed in myocytes. This increase in UCP1 and UCP2 expression suggests that administration of these miRNA produces a cellular differentiation into adipocytes with greater potential for thermogenesis and thus are likely effective pharmaceuticals for the treatment of obesity and other metabolic diseases and disorders.

Furthermore, we looked at genes differentially expressed during pre-adipocyte culture in the presence of miRNA analogs. As an example shown on Figure 13, an M-A plot was created to visualize the differences of mRNA expression between pre-adipocytes grown in maintenance medium and pre-adipocytes grown in the presence of hsa-miR-19b mimic. The x-axis is the mean gene expression and the y-axis is the difference between pairs in logarithmic scale. The red dots are the differentially expressed genes (up regulated above zero and down regulated below zero). The gray dots are the genes not differentially expressed between control and hsa-miR-19b mimic (up regulated above zero and down regulated below zero).

As an example shown on Figure 14, in reference to pre-adipocytes cultured in maintenance medium only, the numbers of significantly differentially expressed genes in the presence of the miRNA analogs hsa-let-7a inhibitor, hsa-miR-1 mimic, hsa-miR-19b mimic and hsa-miR-30b mimic were respectively 406, 382, 370 and 433. A set of 127 genes was commonly upregulated by these 4 miRNA analogs (Venn Diagram, Figure 14).

They include not only some of our 83 thermogenic targets like ALDH1A1, AZGP1, CEBPA, PPARGC1A, UCP1 and UCP2, but also numerous genes involved in lipid metabolism and adipocyte differentiation (Table 19).

Table 19.

Set of 127 genes commonly upregulated by 4 miRNA analogs:

ABCC6	CHI3L2	KCNE3	PPL
ABCD2	CILP	KCNK3	PPP1R1A
ACACB	CKB	KIT	PRKAR2B
ACHE	CKMT1B	KLB	PTGDS
ACSF2	CLCA2	LBP	QPRT
ACSM5	CLMN	LEP	RASL12
ACSS2	COL14A1	LGALS12	RNF157
ADH1B	COL21A1	LIPE	S100B
AIF1L	CPB1	LPL	SDPR
AKR1C3	CYB5A	LRRC4C	SELENBP1
ALDH1A1	CYP4F12	LRRN4CL	SEMA3G
AOC3	CYP4F22	MAN1C1	SEPP1
AOC4	DARC	MAOA	SLC2A4
APCDD1	DGAT2	MAOB	SLC2A5
APOC1	DHCR24	MARCO	SLC40A1
AQP3	DPT	MCAM	SLCO4C1
AQP7	DTX4	METTL7A	SMOC2
AQP9	EPHB6	MGP	SNCG
AZGP1	FABP4	MLXIPL	SPARCL1
BBOX1	FADS2	MOBKL2B	SPRY1
BHLHE22	FAM65C	MOSC1	SVEP1
C11orf87	FMO1	MVD	TF
C14orf180	FMO2	NAT8L	TM7SF2
C1orf115	G0S2	NKD2	TMEM132C
C1orf95	GPD1	PCSK9	TMEM176B
C3	GPR109A	PFKFB1	TMEM37

CA2	GPR109B	PKD1L2	TNMD
CADM3	HAVCR2	PLA2G2A	TPRG1
CDO1	HRASLS5	PLIN1	TRIL
CEBPA	IGSF10	PLIN4	UCP1
CFD	ITIH1	PLXDC1	UCP2
CFHR1	ITIH5	PPARGC1A	

A set of 60 genes was commonly downregulated by these 4 miRNA analogs (Venn Diagram, Figure 15).

They include numerous chemokines genes and genes involved in cell proliferation and
5 (Table 20).

Table 20.

Set of 60 genes commonly downregulated by 4 miRNA analogs:

ACTC1	CENPF	ID1	KRTAP2-1
ANLN	CKAP2L	ID3	MALL
ARSI	CXCL1	IER3	MMP3
ATOH8	CXCL2	IL13RA2	NCAPH
AURKB	CXCL3	IL6	PHLDA1
BLM	CXCL5	IL8	PLK1
BRCA2	CXCL6	INHBA	PPAPDC1A
BUB1	E2F7	IQGAP3	PTGS2
BUB1B	ESCO2	KIAA1244	RELN
CASC5	FAM83D	KIF11	SHCBP1
CCL26	GABBR2	KIF14	SLC17A9
CDC6	GREM2	KIF18B	SLC6A17
CDCA5	GTSE1	KIF2C	THBD
CDCA8	HAS1	KIFC1	TMSL3
CDH15	HJURP	KRT34	TOP2A

10 **B. Differentiation of human white adipocytes into brown adipocytes.**

1. Differentiation Protocol.

In order to assess the effect of miRNA analogs on human white adipocytes differentiation into brown adipocytes, human subcutaneous pre-adipocytes (SuperLot 0048 from 8 female donors, ZenBio, NC) were plated on Day 0 into 96-well plates and allowed to attach overnight in

preadipocyte medium (DMEM/Ham's F-12 (1:1, v/v), HEPES buffer, Fetal bovine serum and Antibiotics). The next day (Day 1), the medium was removed and replaced with differentiation medium-2 (DMEM/Ham's F-12 (1:1, v/v), HEPES buffer, Fetal bovine serum, Biotin, Pantothenate, Human insulin, Dexamethasone, Isobutyl-methylxanthine, Proprietary PPARG agonist and Antibiotics. The cells were allowed to incubate for 7 days at 37° C, 5% CO₂. After 7 days (Day 7), a partial medium exchange was performed with AM-1 adipocyte maintenance medium (DMEM/Ham's F-12 (1:1, v/v), HEPES buffer, Fetal bovine serum, Biotin, Pantothenate, Human insulin, Dexamethasone and Antibiotics). The cells were allowed to incubate for an additional 7 days at 37° C, 5% CO₂. On Day 17, the cells were transfected with miRNA analogs (Dharmacon specific miRIDIAN Mimics and Hairpin Inhibitors) using the transfecting agent Dharmafect 3. All treatments were in triplicate. Post transfection, the negative control was maintenance medium only and the positive control was maintenance medium with 100 nM of the PPARG agonist rosiglitazone. After 2 days, medium was removed and replaced with fresh maintenance medium. The maintenance medium then changed every two to three days until the end of the treatment period (Day 30). At the end of the treatment (total of 30 days in culture) cells were processed for Phenotyping and Genotyping Screening.

2. Transfection of adipocytes.

Transfection reagents are used to facilitate the penetration of miRNA analogs into target cells.

As an example, the extent of transfection efficiency we achieved in adipocytes with the transfecting agent Dharmafect 3 (Dharmacon, CO) is depicted herein. Transfection efficiency was assessed in two ways:

- a. Measurement of cellular epifluorescence after transfection with fluorescent miRNA analogs.

Fluorescence was measured on Day 30 (540 excitation/590 emission) in cells transfected on Day 17 with the Dy547-labeled non-targeting miRIDIAN Mimic and Hairpin Inhibitor (100 nM). As shown in Figure 16, there was a significantly greater fluorescence of cells transfected with the fluorescent miRNA analogs, even 12 days after transfection.

- b. Reduction of control gene expression.

To confirm successful transfection of adipocytes, the reduction of expression of the control gene GAPDH (“housekeeping gene”) was measured 4 days (Day 22) and 12 days (Day 30) after Dharmafect 3 (Dharmacon, CO) mediated transfection of adipocytes with a GAPDH-specific siRNA. Cell lysates were obtained and RT-PCR was conducted using pure RNA obtained by Cells-to-Ct reagents. Efficient transfection of mature adipocytes (a cell type known to be difficult to transfect) was achieved with the transfecting agent Dharmafect 3. 54% and 73% knockdowns of the GAPDH mRNA expression were observed at Day 4 and Day 12 post transfection, both highly significant, as shown in Figure 17.

3. Phenotypic changes during maintenance of human adipocytes in culture for thirty days.

At the end of treatment (total of 30 days in culture) cells were stained with Oil Red O for assessment of lipid content (Figure 18). In the presence of maintenance medium only from Day 16 to Day 30 (control), the adipocytes appear loaded with large lipid droplets. In the presence of 100 nM rosiglitazone throughout the experiment (positive control), the intensity of the red staining seems reduced and the lipid droplets appear smaller. As an example, in the presence of 25 nM hsa-miR-30b mimic, the intensity of the red staining seems also reduced and the lipid droplets appear smaller. No such change was notice in the presence of a non targeting miRNA analog.

The amount of lipids present in the mature adipocytes at Day 30 was measured with the fluorescent Nile Red Dye. As shown in Figure 19, the highest fluorescence was noted in the adipocytes which were not exposed to rosiglitazone from Day 15 to day 30. A similar fluorescence level was noted in the cells which were transfected with the non-targeting miRNA mimic and inhibitor. When the cells were exposed to rosiglitazone for two days, the fluorescence dropped significantly and was further reduced in the presence of rosiglitazone from Day 15 to Day 30. It appears that in the presence of the miRNA inhibitors tested, the level of fluorescence is within the range observed with rosiglitazone 2 day to throughout. In the presence of miRNA mimics, the level of fluorescence appears lower, an indication of lower lipid content.

4. Optimization of human mature adipocyte transfection.

As efficient transfection of mature adipocytes is known to be difficult to achieve, we tested eleven different transfecting agents and assessed the degree of reduction of mRNA

expression of the control gene GAPDH. Human subcutaneous pre-adipocytes were plated in 6-well plates and differentiated for two weeks following the protocol described above.

Subsequently, a miRNA mimic (50 nM) targeting GAPDH was introduced into the differentiated adipocytes using transfecting agents following their manufacturers' protocol. The transfected cells were incubated for 72 hours with reagents and miRNA mimic, then switched to maintenance medium. Fourteen days post-transfection, RNA was isolated using RNeasy Mini kit and RT-PCR reactions for the control gene GAPDH and the reference gene 18S were performed in triplicate using 100 ng of cDNA per well.

The amounts of RNA extracted per well were very similar, except for the transfecting agents TransIT TKO and TransIT siQuest which may produce potential cellular toxicity in the conditions of the experiment (Figure 20).

The cells transfected with Dharmafect 1 and siPORT NeoFX had significantly reduced levels of 18S expression and were excluded from the RT-PCR experiment analysis.

Among the remaining 7 transfecting agents analyzed, the often-used transfecting agent Lipofectamine RNAiMAX led to a 66% reduction of GAPDH expression at day 14 post-transfection, Dharmafect 3 and Dharmafect 4 respectively produced 60% and 75% reduction of GAPDH expression (Figure 21).

5. Phenotypic changes of human mature adipocytes cultured for two weeks in the presence of miRNA analogs or known activators of adipogenesis and/or thermogenesis.

Human subcutaneous adipocytes were plated in 6-well plates at a density of 391,000 cells per well as described above. Using Dharmafect 4, these adipocytes were transfected at Day 14 with:

1. One of the following miRNA analogs (50 nM):

- hsa-let-7a inhibitor (hsa-let-7a is a universal miRNA reported to modulate adipogenesis)
- hsa-miR-1 mimic (hsa-miR-1 has been reported to modulate PRDM16 and UCP1)
- hsa-miR-19b mimic (hsa-miR-19b is an abundant adipocyte miRNA which according to our in silico work is predicted to interact with many of our 83 mRNA targets) or
- hsa-miR-30b mimic (hsa-miR-30b is a miRNA which according to our in silico work is predicted to interact with many of our 83 mRNA targets and whose over-expression stimulates adipogenesis)

2. A negative control (mock transfection)
3. Three positive controls (the PPARG agonist rosiglitazone (100 nM), the beta 3 adrenergic receptor agonist CL316,243 (10 μ M) or the thyroid hormone tri-iodothyronine (10 nM) known to alter adipogenesis and/or adaptive thermogenesis).

5 At day 17, the cells were switched to maintenance medium, which was then changed every two-three days until day 28 when bright field microscopy pictures of the cells were taken.

As shown on Figure 22, and in reference to the control condition, there is an increase in cell density and “browning” appearance in the presence of the positive controls CL316,243 and Rosiglitazone as well as in the presence of hsa-let-7a inhibitor, has-miR-19b and hsa-miR-30b mimic. The effects on cell density, lipid content, number and size of lipid droplets of the different agents are summarized in Table 21.

Table 21.

Agent	Picture field covered by adipocytes (% of control)	Cell area occupied by lipid droplets (%)	Number of lipid droplet per cell	Average size of lipid droplet
Control	Ref	42%	68	Ref
50 nM hsa-let-7a inhibitor	+ 147%	56%	103	-13%
10 μ M CL316,243	+ 24%	44%	124	-44%
50 nM hsa-miR-1 mimic	+ 15%	36%	69	-16%
10 nM T3	+ 13%	42%	94	-27%
50 nM hsa-miR-19b mimic	+ 145%	58%	91	+3%
100 nM Rosiglitazone	+ 198%	57%	113	-19%
50 nM hsa-miR-30b mimic	+ 246%	53%	140	-63%

Example 4. High-Throughput miRNA Target Screening by Luciferase Activity and qRT-PCR

High-throughput screening using luciferase reporter assay constructs are used to identify novel miRNA targets involved in thermogenesis.

Luciferase is commonly used as a reporter to assess the transcriptional activity in cells that

are transfected with a genetic construct containing the luciferase gene under the control of a promoter of interest. SwitchGear Genomics has created a genome-wide library of over 18,000 human promoters and 12,000 human 3' UTR regions cloned into an optimized luciferase reporter vector system containing SwitchGear's RenSP reporter cassette (GoClone™) as a component of the LightSwitch™ Luciferase Assay System. This modified form of luciferase greatly facilitates detailed kinetic studies, especially those focusing on repression, which might otherwise be obscured by reporter protein accumulation.

The multiple microRNAs-one mRNA paradigm was tested with the SwitchGear Genomic GoClone system, using UCP1 as the single thermogenic target gene. In order to explore the possible interactions between various human miRNAs and the 3'UTR region, the 5'UTR region and the promoter/enhancer region of the human UCP1 gene in Hela and HepG2 cells, three reporter constructs were made:

1. A human UCP1 3'UTR construct containing a reporter gene driven by a strong constitutive promoter (RPL10-prom) with a 2,218 bp 3'UTR fragment of the human UCP1 sequence cloned in the 3'UTR region of the reporter gene. The effects of a specific miRNA mimic, inhibitor, or non-targeting control on this reporter's activity are compared to those of an empty-3'UTR and an Actin Beta-3'UTR to identify effects that are specific to the putative UCP1 3'UTR construct.
2. A human UCP1 Promoter construct containing a reporter gene driven by a 4,147 bp 5'UTR fragment of the human UCP1 sequence that spans the Transcription Start Site and upstream region covering the methylation region and the enhancer region of the human UCP1 gene sequence. The effects of a specific miRNA mimic, inhibitor, or non-targeting control on this reporter's activity are compared to those of an Actin Beta-Promoter to identify effects that are specific to the putative UCP1 5'UTR construct.
3. A human UCP1 Enhancer Region construct containing a reporter gene driven by a short minimal promoter from the HSV-TK locus with a 601 bp 5'UTR fragment of the human UCP1 sequence that spans the Enhancer Region of the human UCP1 gene sequence. The effects of a specific miRNA mimic, inhibitor, or non-targeting control on this reporter's activity are compared to those of an empty 5'Enhancer Region to identify effects that are specific to the putative UCP1 5'Enhancer construct.

In addition, miRNAxxx_3'UTR constructs were made. They contain the reporter gene driven by a strong promoter (RPL10_prom) with a perfect match to the target sequence of miRNAxxx cloned into the 3'UTR region of the reporter gene. The effect of a miRNA mimic, inhibitor, or non-targeting control on this reporter's activity can be compared to EMPTY_3'UTR and Actin B_3'UTR to determine whether a miRNA mimic's or inhibitor's activity can be reasonably detected in the experimental cell type. If the cell type has no endogenous expression of the miRNA in question, the addition of a mimic should knock down the activity of this reporter, and the addition of an inhibitor should have no significant effect. If the cell type has high endogenous expression of the miRNA in question, the addition of an inhibitor should increase the activity of this reporter, and the addition of a mimic should have no significant effect. The range of endogenous miRNA expression in Hela and HepG2 cell types is broad, so the synthetic target activity changes are likely to reflect this variability.

For each miRNA candidate (38 in total), the following conditions were tested:

- miRNA mimic (specific) * 8 reporter constructs in Hela cells
- miRNA mimic (specific) * 8 reporter constructs in HepG2 cells
- miRNA mimic non-targeting control * 8 reporter constructs in Hela cells
- miRNA mimic non-targeting control * 8 reporter constructs in HepG2 cells
- miRNA inhibitor (specific) * 8 reporter constructs in Hela cells
- miRNA inhibitor (specific) * 8 reporter constructs in HepG2 cells
- miRNA inhibitor non-targeting control * 8 reporter constructs in Hela cells
- miRNA inhibitor non-targeting control * 8 reporter constructs in HepG2 cells

To the extensive list of miRNAs that may bind to the UCP1 sequence, 10 filters were applied (in addition to required binding to UCP1 3'UTR region) to reduce the number of miRNA candidates to be tested. These filters were length of binding sites, number of binding sites, binding to the 5'UTR region, chromosomal clustering with other miRNAs, intronic location, wobbling, expression across species, binding to the Enhancer Region, binding to the Methylation Region and proof of experimental evidence of a relation to UCP1. 38 miRNAs that met at least 3 of these criteria were tested (Table 22).

Table 22.

miRNA with putative binding sites in the UCP1 gene sequence:

	miRNA	# of criteria	Binding length	# of sites	3'UTR	5'UTR	Chr. Clusters	Intronic	Wobbling	Inter-species	Enhancer Region	Methylation Region	Exp. Evidence
1	hsa-miR-130b-5p	7	11	3	+	+	22				+	+	+
2	hsa-miR-328	6	10	4	+	+					+	+	+
3	hsa-miR-655	6	10	5	+	+	14					+	+
4	hsa-miR-19b-2-5p	5	10	4	+	+	X		+				+
5	hsa-miR-26a-2-3p	5	10	7	+	+						+	+
6	hsa-miR-367-3p	5	10 to 18	3	+	+	4		+	+			
7	hsa-miR-371a-5p	5	10 to 12	9	+	+	19			+			+
8	hsa-miR-377-3p	5	10 to 14	5	+	+	14			+			+
9	hsa-miR-378a-3p	5	7 to 13	19	+	+		+	+	+			+
10	hsa-miR-382-3p/5p	5	15	2	+	+	14		+	+			
11	hsa-miR-421	5	10	5	+	+	X						+
12	hsa-miR-515-3p	5	9	3	+	+	19				+		+
13	hsa-miR-620	5	10	7	+	+					+		+
14	hsa-miR-941/2	5	9	5	+	+	20					+	
15	hsa-miR-1179	4	11	3	+	+	15		+				
16	hsa-miR-1302	4	10	5	+	+						+	
17	hsa-miR-146a	4	9 to 10	8	+	+							+
18	hsa-miR-181c	4	9	5	+	+	19						+
19	hsa-miR-203	4	9	1	+	+	14					+	+
20	hsa-miR-331-5p	4	8 to 15	6	+	+	12		+	+			
21	hsa-miR-422a	4	7 to 14	6	+	+			+	+			+
22	hsa-miR-452	4	8	7	+	+	X						+
23	hsa-miR-491-5p	4	10	3	+	+							
24	hsa-miR-501-3p	4	10	2	+	+	X						+
25	hsa-miR-543	4	10 to 14	4	+	+	14		+	+			
26	hsa-miR-545	4	11	2	+	+	X						+
27	hsa-miR-549	4	13 to 14	3	+	+				+			+

28	hsa-miR-643	4		10 to 14	9	+	+	+		+								+
29	hsa-miR-651	4		10	6	+	+	+										+
30	hsa-miR-654-3p	4		8 to 10	11	+	+	+	14	+								
31	hsa-miR-21-5p	3		10 to 14	2	+	+	+		+	+				+			+
32	hsa-miR-211-5p	3		11	1	+	+	+									+	
33	hsa-miR-22-3p	3		9	5	+	+	+		+							+	
34	hsa-miR-30b-5p	3		10	1	+	+	+	8								+	
35	hsa-miR-325	3		7 to 8	11	+	+	+									+	
36	hsa-miR-362-5p	3		10	1	+	+	+	X								+	
37	hsa-miR-504	3		9	2	+	+	+									+	
38	hsa-miR-552	3		9	3	+	+	+									+	

In these Luciferase reporter gene assay experiments, a miRNA candidate was considered to interact with UCP1 if both the specific miRNA inhibitor increases the luciferase signal and the specific miRNA mimic decreases the luciferase signal with an Inhibitor/Mimic Ratio ≥ 1.5 and or/a p value < 0.05 . These selection criteria identify 9 miRNAs (hsa-miR-19b-2-5p, hsa-miR-21-5p, hsa-miR-130b-5p, hsa-miR-211, hsa-miR-325, hsa-miR-382-3p/5p, hsa-miR-543, hsa-miR-515-3p, and hsa-miR-545) (Table 23). A few more barely missed these selection criteria; they are hsa-miR-331-5p, hsa-miR-552, hsa-miR-620, and hsa-miR-1179.

Table 23.

10 **miRNA identified as regulators of UCP1 gene expression by luciferase reporter assay in HeLa and/or HepG2 cells:**

Cell Line(s)	miRNA
HeLa	hsa-miR-130b-5p
HeLa + HepG2	hsa-miR-19b-2-5p
HepG2	hsa-miR-382-3p/5p
HeLa	hsa-miR-515-3p
HeLa	hsa-miR-543
HepG2	hsa-miR-545
HeLa + HepG2	hsa-miR-21-5p
HeLa	hsa-miR-211-5p
HeLa + HepG2	hsa-miR-325

Out of these 9 selected miRNAs, 3 appear to bind to the 3 regions of UCP1 which were studied (hsa-miR-21-5p, hsa-miR-211, and hsa-miR-515-3p); 3 appear to bind to 2 regions of UCP1 (hsa-miR-19b-2-5p, hsa-miR-130b-5p, and hsa-miR-325), and 3 bind to a single region of UCP1 (hsa-miR-331-5p, hsa-miR-543, and hsa-miR-545). All but hsa-miR-331-5p appear to bind to the 3'UTR region of UCP1 (Table 24).

Table 24.

20 **miRNA identified as regulators of UCP1 gene expression by luciferase reporter assay:**

	miRNA	UCP1 3' UTR	UCP1 Enhancer	UCP1 Promoter
1	hsa-miR-21-5p	X	X	X
2	hsa-miR-211	X	X	X
3	hsa-miR-515-3p	X	X	X
4	hsa-miR-19b-2-5p	X		X

5	hsa-miR-130b-5p	X	X	
6	hsa-miR-325	X	X	
7	hsa-miR-331-5p		X	
8	hsa-miR-543	X		
9	hsa-miR-545	X		

Further screening is performed by transfection of the promoter/3'UTR library into human adipocytes or adipose-derived mesenchymal stem cells in cell culture, followed by addition of miRNA agents (e.g., agomirs or antagomirs) to the cell culture. Measurement of luciferase activity and identification of mRNAs is performed 24 hours after transfection and addition of miRNA agents.

In order to confirm the results of the transfection experiments set forth above over a longer time frame, lentiviral transduction experiments are performed using lentiviral vectors containing the miRNA agents of interest (from System Biosciences (SBI) collection of miRNA precursors expressed in the pMIRNA1 SBI vectors allowing the expression of the copGFP fluorescent marker). Specifically, cells containing the promoter/3'UTR library are transduced with lentiviral particles at an MOI of 1:10 and GFP-positive cells are sorted by FACS, according to the supplier's instructions. The level of expression of the mature miRNAs and their targeted mRNAs is assessed at several time points (0, 3, and 6 hr.; 1, 4, and 7 days) by *Taqman* Quantitative Real-time PCR in control cells (HEK293 cells), Human Adipose-Derived Mesenchymal Stem Cells, Human Subcutaneous Pre-adipocytes, and Human Proliferating Subcutaneous Adipocytes. Pooling of RNAs from 5 different time points after transduction is optionally employed to reduce the complexity of the qRT-PCR based screening approach while preserving the detection sensitivity.

Example 5. Proteomic Profiling

Proteomic Profiling is also used to identify novel miRNA targets involved in thermogenesis.

Shotgun proteomics is a method of identifying proteins in complex mixtures using high performance liquid chromatography (HPLC) combined with mass spectrometry (MS). Transfected and transduced cells with miRNA agents and promoter/3'UTR library (as described in Example 4) are harvested and lysed to produce crude soluble (cytosolic) and insoluble (nuclear) fractions. Peptides from these fractions are then separated by HPLC and analyzed

using nanoelectrospray-ionization tandem MS using the isotopic labeling technique SILAC to quantify protein abundance. Spectra are searched against the Ensembl release 54 human protein-coding sequence database using Sequest (Bioworks version 3.3.1, Thermo Scientific).

To avoid missing low abundance proteins, a targeted proteomics approach is also employed to accurately quantify a set of proteins that are known regulators of adipogenesis, adipocyte differentiation and BAT function. Some examples include UCP1, KDM3A, PRDM16, PPARA, PPARGC1A, CEBPB, CIDEA, BMP7, COX7A1, SIRT1, SIRT3, DIO2, FABP4, and ADIPOQ. These proteins are analyzed via ELISA based or Luminex based immunoassays using commercially available antibodies.

Optionally, the protein fractions are analyzed using Multiple Reaction Monitoring-Mass Spectrometry on a proteomics platform, whereby only one protein (e.g. UCP1) of the thermogenic pathway is accurately quantified using LC-MS-MS.

Example 6. Development and characterization of clonal DNA aptamers specifically targeting human adipocytes

We used the Cell-SELEX technology to develop and characterize DNA aptamers that specifically recognize mature human subcutaneous adipocytes. With Cell-SELEX, aptamers recognizing specific molecules in their native conformation in their natural environment on the surface of intact cells are selected by repeated amplification and binding to living cells. In this cell-based selection illustrated in Figure 23, specific known and unknown cell surface markers or membrane receptors can be directly targeted within their native environment, allowing a straightforward enrichment of cell-specific aptamers. Cell-SELEX consists of a combination of positive selection with the target cells and negative selection with non-targeted cells. In the present case, negative selection was performed with freshly isolated human hepatocytes and positive selection utilized primary cultures of human subcutaneous adipocytes. Two rounds of negative selection and five rounds of positive selection from a 32 mer library were completed. Isolated aptamers were sequenced, synthesized and labeled with 6-fluorescein amidite (FAM) for binding studies. Human hepatocytes (negative cells) and adipocytes (positive cells) were labeled for 15 minutes at room temperature with a saturating concentration (1 μ M) of FAM conjugated aptamers and analyzed by fluorescence-activated cell sorting (FACS). As shown on Figure 24, some aptamers (e.g. aptamer 974) do not bind to adipocytes nor hepatocytes, some aptamers (e.g. aptamer 975 bind to both adipocytes and hepatocytes, ratio: 2.69) and other aptamers bind

preferentially to adipocytes (e.g. aptamers 972 and 973, ratio: 4.76 and 5.40, respectively). Further characterization of these adipocyte-specific aptamers is in progress.

Example 7. Reconciliation of the Phenotypic, Genotyping, and Proteomic datasets

- 5 The results of the *in vitro* experiments set forth in Examples 3-5, herein, are reconciled. Specifically, to narrow further the initial set of microRNAs, mRNAs and target proteins and pathways to a relevant yet manageable number of targets, the experimental data is integrated with Network Searches and Analyses Packages (DAVID, Ingenuity Systems IPA and ARIADNE Pathway Studio.
- 10 Global analysis of the results of the *in vitro* experiments set forth in Examples 3-5, herein, is performed the Business Intelligence tool TIBCO Spotfire. This allows for a visualization of the relationships between the miRNA agents and target gene.

Example 8. Animal Models of Obesity

- 15 Several animal models of obesity have been developed and validated (Kanasaki K et al., J. Biomed. Biotechnol., 2011:197636 (2011); Speakman J et al., Obesity reviews : an official journal of the International Association for the Study of Obesity, 8 Suppl 1:55-61 (2007)). The most commonly used are the Leptin Signaling Defects Lep^{ob/ob} and Lepr^{db/db} Mouse Models as well as the High-Fat Diet model in C57BL/6J mice (Wang CY et al., Methods in molecular
- 20 biology, 821:421-433 (2012). This diet-induced obesity (DIO) model closely mimics the increased availability of the high-fat/high-density foods in modern society.

A DIO mouse model is used for *in vivo* validation of the effectiveness of the miRNA analogs described herein for the increase in thermogenesis and/or the treatment of obesity and other metabolic disorders (Yin H et al., Cell Metab., 17(2):210-224 (2013)).

- 25 DIO mice are administered one or more of an hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir. Rosiglitazone is used as a positive control. Food intake, blood metabolic parameters, body composition (body weight,, body fat, bone mineral and lean mass, body fat distribution, body temperature, O₂ consumption and CO₂ production,
- 30 exercise induced thermogenesis, cold induced thermogenesis and resting thermogenesis are measured in the mice prior to and after treatment. A reduction in body mass or body fat or an

increase in body temperature or any kind of thermogenesis indicate the in vivo effectiveness of the administered composition.

Example 9. Nucleic acid sequences of human UCP1 and UCP2 genes and transcripts

5

Table 25.

Nucleic acid sequence of the 1,462 base pair (bp) transcript ENST00000262999 of the human UCP1 gene (Six Exons are in capital letters):

No.	Exon / Intron	Start	End	Length	Sequence
	5' upstream sequence				gtcggttcaaaaaacagaaatcgggttgctg cccggcggacaggcgtga
1	ENSE00001081761	141,489,959	141,489,758	202	AGAGCAAGGGAAAGGAACCTTCC TCCACCTTCGGGGCTGGAGCCCT TTTCCTCTGCATCTCCAGTCTCTG AGTGAAGATGGGGGGCCTGACA GCCTCGGACGTACACCCGACCCT GGGGGTCCAGCTCTTCTCAGCTG GAATAGCGGCGTGCTTGGCGGAC GTGATCACCTTCCCGCTGGACAC GGCCAAAGTCCGGCTCCAG
	Intron 1-2	141,489,757	141,489,132	626	gtagctaggcagaggggtaagacaa.....tgttct gcaccttcttattccag
2	ENSE00001009006	141,489,131	141,488,933	199	GTCCAAGGTGAATGCCCGACGTC CAGTGTTATTAGGTATAAAGGTG TCCTGGGAACAATCACCGCTGTG GTAAAAACAGAAGGGCGGATGA AACTCTACAGCGGGCTGCCTGCG GGGCTTCAGCGGCAAATCAGCTC CGCCTCTCTCAGGATCGGCCTCT ACGACACGGTCCAGGAGTTCCTC ACCGCAGGGAAAGAAA
	Intron 2-3	141,488,932	141,484,673	4,260	gtaagccgtgagcgttctctgggagg.....aataat tttttctctctggatag
3	ENSE00001081759	141,484,672	141,484,472	201	CAGCACCTAGTTTAGGAAGCAAG ATTTTAGCTGGTCTAACGACTGG AGGAGTGGCAGTATTCATTGGGC AACCCACAGAGGTCGTGAAAGTC AGACTTCAAGCACAGAGCCATCT CCACGGAATCAAACCTCGCTACA CGGGGACTTATAATGCGTACAGA ATAATAGCAACAACCGAAGGCTT GACGGGTCTTTGGAAAG
	Intron 3-4	141,484,471	141,484,366	106	gtaactaacttcaaatgggttta.....acattttctt

					ttttttttccccag
4	ENSE00001081762	141,484,365	141,484,264	102	GGACTACTCCCAATCTGATGAGA AGTGTCATCATCAATTGTACAGA GCTAGTAACATATGATCTAATGA AGGAGGCCTTTGTGAAAAACAAC ATATTAGCAG
	Intron 4-5	141,484,263	141,483,528	736	gtaacttcccatttcataatacaaa.....gacctgtt catcgatccatttag
5	ENSE00001081763	141,483,527	141,483,347	181	ATGACGTCCCCTGCCACTTGGTG TCGGCTCTTATCGCTGGATTTTGC GCAACAGCTATGTCTCCCCGGT GGATGTAGTAAAAACCAGATTTA TTAATTCTCCACCAGGACAGTAC AAAAGTGTGCCCAACTGTGCAAT GAAAGTGTTCACTAACGAAGGAC CAACGGCTTTCTTCAAGGG
	Intron 5-6	141,483,346	141,481,165	2,182	gtaagatatgatctgtgtatctgt.....cgaacgat gacatgcacttttctag
6	ENSE00001081760	141,481,164	141,480,588	577	GTTGGTACCTTCTTCTTGCGACT TGGATCCTGGAACGTCATTATGT TTGTGTGCTTTGAACAACCTGAAA CGAGAACTGTCAAAGTCAAGGC AGACTATGGACTGTGCCACATAA TCAGCTTCAAGAAAATGATGTAA CATACCAGTGGGAATCTTGCTGA CTGGATCATAAAAAACAAACAAA ACTTATTCACCTTATTTTAACCTAA AAAGATAAAGGAATTTTGGCAG AGAATTTTGGACTTTTTTATATAA AAAAGAGGAAAATTAATGCCTAT TTCATATAACTTTTTTTTTTCTC AGTGTCTTAAGAAGGGGAAAGC AAAACATTCAGCATATACCCTGG CAAATGTAATGCAGATAAGCTAC TGCATTTGACCATTCTGAGTG CAATTGTGTGAATGAATGTGAAG AACTTTAACATGTTTTAATTACA ATTCCAACCTGGTGGAAAAGAAAC TGAGTGAAATGCAGTTTATATTT ATAAATACTTAAAAATGAAGTTA TTAAAAATATTAGTTTTTATTAAAC CACAGTTGTCAGTTAATATATTC AATAAAGTATTGCTAATACCTTT T
	3' downstream sequence				aaagtttgtcttttgagatctataacctgggtgtaagagtc aagttcacta.....

Table 26.

Nucleic acid sequence of 9,371 base pair (bp) of the human UCP1 gene

(ENSG00000109424), (Exons are in bold letters):

>chromosome:GRCh37:4:141479988:141490559:-1

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5  AGAGAAGGCCGCAAGGTGCCTGCAAGATGTCTGGGGAGTTGGAGGAATGGAAGAG
   TGCCCCGCTCTTCCTTCTGGGAGAGCTCCAGCTAGGCAGAACCTTTCACCAAGGCTC
   TGATATCGTGCTGGTTTCCGAAAGCCCCAGCCGAAGGTGTGCAGCCAAAGGGTGAC
   AGAAGGTGAGGCACGTGCGGGGGCGCGGGTGCTGACCGCCGCGGTGCGCCCTCCCT
   CCGACGTGCGTGTGCGGGGGCGCAGACAACCAGCGGCCGCCCCAGGGCTTTCGGGGA
10  GCGAAGCAGGGCTCCCGAGGCACCGAGCGAGAATGGGAATGGGAGGGACCCGGTG
   CTCCCGGACACGCCCCCGGCAGGTCCCACGCCCGGGTCTTCTGAGACCTCGCGCGGC
   CCAGCCCCGGGAGCGGCCCAGCTATATAAGTCCCAGCGGAAGACCGGAACGCAGAG
   GGTCTTGCTGGCGCGAGGGTGGGTAGGAGGGGACGCGGGGACTCGGCCCCCAACAC
   CGCGCTCCGTCTGCAGCCGCGCCTCTGCACCGCCGCTGCCCGGCGGTTCGTTCAAA
15  AAACAGAAATCGGGTTTGCTGCCCGGCGGACAGGCGTGAAGAGCAAGGGAAAGGA
   ACTTCCTCCACCTTCGGGGGCTGGAGCCCTTTTCCTCTGCATCTCCAGTCTCTGA
   GTGAAGATGGGGGGCCTGACAGCCTCGGACGTACACCCGACCCTGGGGGTCC
   AGCTCTTCTCAGCTGGAATAGCGGCGTGCTTGGCGGACGTGATCACCTTCCCG
   CTGGACACGGCCAAAGTCCGGCTCCAGGTAGCTAGGCAGAGGGGTAAGACAAGG
20  GGTCTCAGGACAGAGGGGACGCTGTTGCGTGCATTCCATTTATTCTCTGCTTTGGTGT
   AACCACTGTTTCTAGGTAGGGTAGGTGACCTTCCAAAGCAGTCTGGCCTTGTCCAG
   GGCTGGTGCTTTAGGATGGGAAACTGGAACTTTTTCTGGGATTAGCTGAAGAACCAC
   CAGGGCCACAGAGAATGGGTTGACCATGACTACTACCAAATTCTCCCAAATTTAG
   GGTGCACTTAGTATTTTAAGAGCTGAGAATATTGGCCTCTCCTGAGTTTACTAGTCA
25  GGTGCTTTTTTCCTTTCTTTGATTCTTCGGGGGTTCTGTCTATCCTACTGCCCTAGGGG
   TTCTGGAGAGTTCCTGGGGAGGGGGATATTCAAAATGTGCATTGTAGCCAGCCTCCC
   TCCATCTGCGCGTGAGCGAACACACACACACACACACACACACACACACACACA
   CACACACACACGGTAGAGGGAGGTGGATGGAAGAGGAATGTTGCTGAGAAAAGAA
   ACGGAAAATAGGAACACAGGGGGAAATCTTGGCTTAAGAGTGAACCTCAATTTGCT
30  CCCTTCTGTTCTGCACCTTTCTTATTTCCAGGTCCAAGGTGAATGCCCGACGTCCA
   GTGTTATTAGGTATAAAGGTGTCCTGGGAACAATCACCGCTGTGGTAAAAACA
   GAAGGGCGGATGAACTCTACAGCGGGCTGCCTGCGGGGCTTCAGCGGCAAT
   CAGCTCCGCCTCTCTCAGGATCGGCCTCTACGACACGGTCCAGGAGTTCCTCA
   CCGCAGGGAAAGAAAGTAAGCCGTGAGCGTTCCTGGGAGGGGCAGAAAAGCCTTG
35  GGCTCCGCTCTGTTCCAAAAAGTGTAACACACAGAGGAGTGGTTTTTCATAACAAATT
   GCGGAGAAAACATTCATATTTGAACTCTCCCTTCCCCAACATTAGCTCATTGTTTCAT
   AGAAAAAAGTATGCAAAATCGATTTTTTTAGATGCAGATATATACTTGTAAGGTCAC
   CCAGTCATGGAAGTTTTGTGCCAGTTTGGATCTCCATCTGGAGAATATGGGTGGGC
   TACAGAAAAATGTTTAACTTAAAGTTCTCCAAAGAGGGAAGTATATCAGAAACATC
40  TATGGAGCTTGTGAGAAATCCAAACGAGGACTACCATGGTCCTCTGAGTCTGAATCC
   TCAGGCTAGAGACCAGAGTGTCTTTCCACAAGCTTCCCTCATCATTTGTGTATGCAA
   CAAAGTTCAAAGCCTTCTGTTTGAAGCAAAGAAAGCCAGACTTTGTGAAGAGAGTT
   GAAAGGACAGGAAAAGACATATTTCTCTTAAGAGGTTCTCATCAGGTCCAGGAA
   AGACCAGAGCAGAAAAAGTGGACGAATGCTGCAGGGAGTTTGTTTAGGGGAAAAAA
45  GAAAAGGAAACATATTTCTGAGTGCCAGTGCACCTCTAAGAATTCCTGTCACTTTAG
   GTAGCATTTATTTGAGGGCTTAACTATGAACCAGACATTGTTCTAAGTGCTTCAGAT

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ACATTATAACTGGAAGGGTATTAGTACCATTATCCCTTGGCAGATGGGAAAACCTGAA
CACAGAGCAGATTCATCACTTGCCCAAGGTCACACAGCTGGGAGGGGGCAGAGCCA
GGGTTCAAACCCAGGCAGTCTGGCCTCGGACTCCAGGCTCCTAACCTGTTCTCTAC
TGCCTTCTGCACTTCTCATATGATTCTGCCCATCATTCAAACCGCACAACTGCTGT
5 GAGTAAAAAGTGTTAGCCGAATATCAGGGTAGTTAAGTAACATGCACAAAATCACA
CAGCTAATCAACATCAGAGGCACTTTTCATGTGGAGTAGACAAGCCAGAGAGAAGAT
GTGCTGATGGCACAATGAATACATTAAGTGAAATCCACCTTGTAGATTTTCATCATTT
CTGCTGTGAGTAACCTTCAATACTATAATTTTATGGGATAATTTATAAATGTTGTCTA
TACAAATATATAAGTTATACTTATCCACACAAGTACTTTCAAAGTGAAGATAAAGTC
10 TGGATGTTACTAGATCAAAACTGCATTTTTTTTATTTATAGATGTAGCAAGAGAGGAA
ACACAAAGGAGGTAAAGCTGCCCCGTTTCAGGTGGTTTTCTTCACAGATTGACTGTTCT
ACCAATTGTTGTGGACTTTGGGCACCAAATTAATAGGATATATGTTGGCAGTGTTCT
ATGTTATATAGATTCAGTTTATTTAGTAGGCTTTATTGAACTGCCATGTGCCAGTAAC
TATGTTAGATGTTTAGATGGCAGATGTGTCTCTAGACAGAGCTTACAGTTGAGAGTA
15 TGGGTTGTGTGGGGAGAAGTGAATAGATGACTATATCCATGATACATGCTGTATTA
CAATACAGTCCTACTTCACTTAACGATGGGGATACATTCTCAGAAATGAGTTAGGAG
GCAAATTGGTTGTTGAATGAACATCACAGAGAGCACTTACACAAACCTAGATGGCA
TAGCCACACCTAGGCTATATGGTATAATCTATTGCTCCTAGGCTACAAACCTGTGCA
GCATGTTGGTATTGAATACTACAGGCAATTGTTACATAAAGTTAAGTGTTTGTGTAC
20 CTA AAAATAGAAAAGGTAATGCATTACACTACAGTCTTATGGGGCTGGGATGTCACT
AGGTGATAGGAATTTTTTCAGCTCTGTTCTAATCTTACGGGACCACCATCATGTATGC
AGCACATGACTAACTGTAATTACAAGATGGTGGCTATATTA AACAGAACTACTTAAG
CTAGCCATGGAGGTATGGTCCGTGAGATTTTCCTGAAGAATTAACGTCTGGATCAAT
TCTGGAAGGGCCAGCAGGAGTACTCCAGGCAAAGGGGTGAGAAAGGAGCTTCCAA
25 GTAGAGTGAAGGTCATGTGCAAAGACTCAGTGAGGAGTCGAGTGAACATAGCACAG
GGAGGACATGTTGGTGAGGAAGGAGGGGTGAAGCCACAGAGACAGGAGGGAGCCA
GATGACAGAAGGCCTTGCAGGCGGTGCTAAGGAGTTTGGATTTTATCCTTACAGTGG
TGGGAAGTCATTGTAAAAATATTAAGCAAGGGAGTGGCATAAACAATTTACATTTTC
AAAAGATCACTTTGGCAGCAGATAGAGTATATATGTAAAAGGAGTAAGAAAGAGGT
30 AAGTTAGAAAGCAAGAAATGATCAGGGTATGCCCTAAACACTGGCAATAGGGAAA
AAGAGATGTCAATCAGAAAGATTGAGAAAGTATAATTGAATTGACTTGGTGAACAA
ATAGAAGTAAGGCATAAGGGACAGGTAGAAATATGAGATGACTTCCAAGTTTCTGT
TTAAAGATAACCCTTTATTGAGAGAGGATGTATAGAAGCTGTCTTAGGGGGAAGACA
AGAAATTTGGTTTAGGCCATGTCAACAGGTAATGGCCAGTAGGCACATGATTCAGTT
35 TATTTAGTGGGCTCCTTTTAGGAGAAAATCTGAGCCAGATTCCAGGAAGTCACAGCA
GGGACTACCAATAGGGTCAAACAGCAGAGAGTGTGGAAAGGACTGAAAAGTGATC
ATTGTACATAACAAATAGAAGCTCACTGATTTTCTAGCAAAAACATCTTCAGCAGAG
TAGCGTGGTATAAGCTATATTGTAGGGGACTGAGGAAGAAATGGGCTCTGAGAAGT
AAAGACAAACAATATGTTTTGTAAATAAATTTCTTTTAGTTCTTAAAAAAAAGCCT
40 CTTTTCCAGCTTGATTGGGAAGTGAAGAGAGGGATTTGAAAGTTGGAGATTGGAGG
ATAGGATGAGTACATCAAGATACACTACGTTGTAGTGCAGTGCATTACAAATGTGA
GCTAAAAGTGAAGGCATTTGTAATCATATGATATTGCTAATTA AAAGACAGCTGTCA
GTCATATGCCCAGCTCCTGGTAAAGCATGATGAGAAGAGTACAATCATGGTAGTGA
TTTAAAAATTGCTGCCAGTTTTGTGGATTTTCTTTATGCTAGACAGTGTAAGCTCTTT
45 ATCAATATTATTTAACTCACACAACTCTAAGAGGTAGATATTATTATCCCTTTTTGAC
AAATTAGGAAACAGAATTATAATGACTGAGAAAGTCTCTGCTGAGTAAATGTTACT
GAACCTTAATTTTATGTTTACTTAATGATAGAAATGAATATTGGGCTTCAAGACTATT
TGTA CTTAATGAAATCTGTCTTGAGCAACATAAGCTATTTTTTTTCAA AATTTTAAGAC

AAAAATCACTTTCTTCTCTCCTGTCTTCTTATTTTTGTTCCTTCACATGTTGTAGCCT
AACACTACTTGATGGCCCATTTTGGTGCAGTTTGTCCACTGGGCTTCATCTAAGGCC
ACCAAGTCCCATAATTAACATGATCATTCGTGGGAGAAAGATCAAGCCTCATTGGTG
ATGGGTGCCTCCTCACAGTCGGATAATACTGAAAAGAGAGCTAAATGTGGGAAAGA
5 ACCAAGTTGAACACAGGAAAGAATCAGGCCACTGTGAAAATAAGCATTGTGTTTTT
TTGTTCCCTTGAAAGTCTTCATTTTTTAAAAAATTTTCAGACACCTGAAGTTTTCTAGCCT
TACTCTGAGTTGACGCACATTTAGTACATGATCAACACATAAACAAGCATTAGAGAA
ATAGAAAAGCTGTAAGAATACAAAAATATGGGCCAGGTGGGTGGCTCATACCTGTA
ATCCTAGCACTTTGGGAGGCCGAGGCAGACGGATCACCTGAGGTCAGGAGTTCAAG
10 ACTAGCCTGGCCAATATAGTGAAACCCTGTCTCTACTAAAAATACAAAACCTTAGCAG
GCTGTGGTGGCACGTGCCTATAATCCCAGCTACTTGGGAGGCTGAGGCAGGAGAAT
CTCTTGAACCCGGGAGGCGGAGATTGCAGTGAGCCAAGATCACACCACTGCACTCT
AGCCTAGATAACAGAGCAAGACTCCATCTCAAAAAAAAAAAAAAAAAATACAAAAATATG
AACCCTGAAAATTA AAAAGACATGCATGCATTCTAGGTCTTTAATTTTTTTTTCTTAA
15 TAATTTTTTTTTCTCTCTGGATAGCAGCACCTAGTTTAGGAAGCAAGATTTTAGCTG
GTCTAACGACTGGAGGAGTGGCAGTATTCATTGGGCAACCCACAGAGGTCGTG
AAAGTCAGACTTCAAGCACAGAGCCATCTCCACGGAATCAAACCTCGCTACAC
GGGGA CTTATAATGCGTACAGAATAATAGCAACAACCGAAGGCTTGACGGGTC
TTTGGAAGGTA ACTA ACTTCAAAAATGGGTTTTATAACCACCAAAGCACATACATA
20 CAACTAGCAACTTATTGTAAAGTAGAGTTAATAAACATTTTTCTTTTTTTTTTCCCCA
GGGACTACTCCCAATCTGATGAGAAGTGTCA TCATCAATTGTACAGAGCTAGTA
ACATATGATCTAATGAAGGAGGCCTTTGTGAAAAACAACATATTAGCAGGTAAC
TTCCCATTTTCATATAACAAACAGGTCGCACCTTTAGAAGTTCATCTTGGAGCTTCTGC
AGCCACCTTATACTCAATCTCTTAACTCCAATAGTTTTCTCTTTTTTAAAAATTAAGTA
25 ATTTTGAACCATATATAACTTTGTGAGAAGCAGGAAAAGACCAAATATTAAGTTA
AGAAGTTTTGCCACAACAAAAATATTTTGCAACAAAAATAACAGGCAATTTTCATGTC
AGCATTATTCTCATTTAATACTAATATATGGGACTTTTGTTAGAACTTATTCTTTAT
ACAGCAGAATTCAGGAGGTAA GTCCATCCTGCATACTATATCCAAAAGATCTAGTTA
TAAAAGGAGCTTATCAGTGGTCTCATCCAAAAAGTAATACCATAAGATAGGTTCTTA
30 AAAATAATATTCTAACAACTTCTAGAGACATTGAAATTTCCCTTATTTCAATAAAAA
AGTATTAGATGCTCATATATTAGGCATTATTACAGGCCTTAAAGGCACAGAGGAAAC
TAACAGTTTACTTTCCCTAAAGTGTTAACAATCTATTAAGCCATTTACTCTTTACCTTC
TTTTTCTAGTGCAATACCTTTCTTATTTTTATTTTATTTTATAAGACATCTTCATTG
ACCTACTGTTATCAATAGGTTTATAAAGATATGACAGATAACTAAATTGCAAGCCCC
35 CAAAAGTCTGATGTTGACCTGTTTCATCGATCCATTTTAGATGACGTCCCCCTGCCA
CTTGGTGTCGGCTCTTATCGCTGGATTTTGCGCAACAGCTATGTCCTCCCCGGT
GGATGTAGTAAAAACCAGATTTATTAATTCTCCACCAGGACAGTACAAAAGTGT
GCCCCA ACTGTGCAATGAAAGTGTTCACTAACGAAGGACCAACGGCTTTCTTCAA
GGGGTAAGATATGATCTTGTGTATCTGTAATGTGTTCTGGCTGTCTGTGTGCTTTGG
40 GACACTCTCATGTCAAGCAACCGACATTTAGCTTACAAGCCTTAGTATATTCATATA
CTTAGTATTGACTTTTCCCTTGCCACAGATTTCTCCAATCCACCAATTCCACTGTGCCA
GAAAGTAAAAAGCCATGATATTCAAATTTTCTCAACTTTGATCAAAGGCTCATTCAA
GACCAGTGCCTTTTCCACTGGTCCCAATCTACTGGAAATGCAGACAGTATTTTGCCTT
CTCTGGGCAAGAAAGTTATAAAGTAGAGGGAAATCATAATAGAGAGCTATGAGAGA
45 ACAAGATTTGATTTGATTTAATTTGATGGACTCAAGTTTTAACATTGTAAAAC TAGA
GATAAGACATCACCACCAATCTAGAAAAGTGATGCAGAAAAGTATTTGATTTGGGT
AATTATTACACTCACCTAGAAACAAGTGTTGTGTAATAGATTACATATTTCCATAAT
GCAATGTTGTATCAGAACTACCTTCCTAAGAAAATATAGTATGGGCTCGGCGTGGT

GGCTCGCACCTGTAATCCCAGCACTTTGGGAGATGGAGGCAGGAGGATCACTTGAG
CCCAGACTGGGCAACAAAGCGAGACCCTGTCTCAACAAAAAATTTAAAAATTAGCT
GAGTGTGGTGGCACGCACTGATGGTCCCCTCTACTTGGGAAGCTGAGGCAAGAGGA
TCTCCTGAGCCCAGGAGTTCAAGGTTTCAGCGAGCTATGATTGTGCCACTGCACTCC
5 AGCCTGGGAGACAGAGCAAGTCCCTGTCTCAAAAAAGAAGAAGGAGAAGGAGGAG
AAAATACAGTATTAAGTAATCTGTCAATATATTCCACAAGGATTACACTAGTGGTTT
AATAATAAAATTATATTACCTTTTTTAAATTGTAAGGCCATTCTCTCAAGCTTTATAAAT
TAAGCATGAATGCATCATACACATTTTATAAAAAAGTTCCAACATCATATAATCTGTA
CTTATGATACATTAATACAAATGAAGTTCATTATAAAATTAACCTTAAATGGATATA
10 CCAGTTATTAACCATTAAACCTTAATAATTTTATTTTTTTCAAATTTAAAAACCTT
TTGGGGAAGAAATACTACAACATGGATGAACCTTGAAAACGTTATGCTAAGTGAAA
TAAGCCAGACACAAAAGGACAAATACTGTATGATTACACTTAAATGAGGTACCTAG
AGTAGTCAAATTCATAGAGACAGAAAGAATAGAAGTTACCAGGGGCTGGAGGTAGG
AAAAAATGGAGAGCTGTTTAATGGGTAGAGAGTTTCTTTTTGGGGTGACAAAAGG
15 TTCTAGAGATGGATAGTGGTGTGTTACACACAATGTGTGTGTACTTAATGCTACT
GAAATGTAATTTTATGATTTTTTTTTTTTGCAGCAAAATACCCACATTGGGAAGTGA
AGAGAAACATGTAAAGAGACTTGAAGGAAAAAAATTGGGGCAGAGGGGTGTTTTTT
ATAGGTTAAACAATAAAAGCCATTTAAACAGTAACAATTTCTCTAAGGACAAGAAT
CGTCAAGATTGAGACAGCACTGATTTCTTGACTCTACTCAATACTTCTTTGGTTTCTC
20 TTCTTCCTTCCCCCTTCTAATAGTTTCCTACCTCCCATTCAGAAAGCAAAGCAAAACA
AGCAAAAATTCCCCCTTCCCTCAAAAAAGGAAAGAGTTTTTGAAAAAGTTCATGTCA
GTGAAGAAAAGACATGTTTTGGGAGTGAAGGATATTTGTGGATTTGTATAGATGTGA
TCATCAGGGCTGTGTTGTTTTGAAGTAATATAGGACATCTAGAGGAAAATTTATTTT
CAGCAGAGGAGGGAAAGATGAAGAGTAGGTACTTTTAAGCATCTTCACTTGAGGAG
25 TGGCAAAATGAGAAGCATAACCTGCTATAATCACTTTAAGAATTTCAAGGCTGAGTGT
GGTGGTGCAGTCTCTAGTCCCAGTTACTCCAGGAGGCTCAGGTGGGAGGATCACTTA
AGCCCAGGAGCTCGAGGTTGCAGTGAGCTATGATTACACTACTGCATTCCAGCCTGG
GCGGCAGGGTGAAGCCTCATCTCAAAAATTAAAAAAAAAAAAAAAAAATCAAACAAATTA
ATCGAACGATGACATGCACCTTTTCTAGGTTGGTACCTTCCTTCTTGCGACTTGGAT
30 CCTGGAACGTCATTATGTTTGTGTGCTTTGAACAACCTGAAACGAGAACTGTCAA
AGTCAAGGCAGACTATGGACTGTGCCACATAATCAGCTTCAAGAAAATGATGT
AACATAACCAGTGGGAATCTTGCTGACTGGATCATAAAAACAAACAAAACCTTATT
CACTTATTTTAACCTAAAAAGATAAAGGAATTTTGGCAGAGAATTTTGGACTTT
TTTATATAAAAAAGAGGAAAATTAATGCCTATTTTCATATAACTTTTTTTTTTTCT
35 CAGTGTCTTAAGAAGGGGAAAGCAAAACATTCAGCATATAACCCTGGCAAATGT
AATGCAGATAAGCTACTGCATTTGACCATTTCTGGAGTGCAATTGTGTGAATGA
ATGTGAAGAACTTTAACATGTTTTAATTACAATTCCAACCTGGTGGAAAAGAAAC
TGAGTGAAATGCAGTTTATATTTATAAATACTTAAAAATGAAGTTATTAATAAT
ATTAGTTTTTATTAACCACAGTTGTCAGTTAATATATTCAATAAAGTATTGCTAA
40 TACCTTTTAAAGTTTGTCTTTTGAGATCTATACCTGGGTGTAAGAGTCAAGTTCCT
AGAATACAAGACTGCCCAATAGCAAATGCAGGTCTTTAGAATCATAGGCATGAACC
TACTCTGAATGTTATTAGTATAGATTTTTTAATGTTTAGAGTCCAGATTTGATGACATC
TCTAACAACCTTCTAATCTAAGACACTATATTCATTTTGGCAGGATTGCTACTAGAGTC
TTGGTATCTGTGCTAGCATCACATAATTTTAGAGCTGGAGGGTACTTCTGGGAAGAC
45 AGAGGAACAGTTTGAGATTCTACTGAGATGAAAACGAATCTTCATGGAATCTTTCA
GCAAAGCCAAATTCAAATTCATCATTAGCACCTGTAGTAACCTTTTCAATGCCTACA
AACTGCATGCAGAAGAGATAGGGAAACAGTAAAACAGATATTAAGAAGTTTTTA
AGACAAAGCCCAGCCTGATTTTAAGCTAAATCCAAGGATTGGCAGCTTGGATGAGC

AGGAAGGTTACAGGCTGCCAGACATCATTCTAGTTCTGTTTTAATCAACTCCATGTT
ACATTTACTATCAGGGATTCTCACCTC ACCCTCATGCAT

Table 27.

5 Nucleic acid sequence of 15,910 base pair (bp) of the human UCP1 gene (NCBI Reference Sequence: NG_012139.1, RefSeqGene on chromosome 4):

	CTGTACAGCT	CTCCGACAAT	CCCACATCTA	GATGCCAAGC	TGAGGTTGGC	ATTCTCACTA	61
	ATTTGCTGTT	ATAAATATTA	AGCTATCATA	AGCGTTAGCC	TACATATGAC	TCTTTCATAT	121
	GTTAGTTAAT	TATTTTAGGG	TAGAAATCCA	AAAGTGGAGT	TACCAGAAGT	GGATATAGAC	181
10	ATTCTGGCTG	GGTGTGATGG	TTCATGCCTG	TAATCCCAGC	ACTTTGGGAG	GCAGAGGCAG	241
	GCGGATCACT	TGAGGCCAGG	AGTTTGAGAT	CAGCCTGGGC	CAACACAGCG	AAACCCCATC	301
	TCTACTAAAA	ATTCCAAAAC	TAGCCAGGCA	TAGTGGCACA	TGCCTGTACT	CCCAGCTACT	361
	TGGGAGGCTA	AGACACAAGA	ATCGCTTGAA	CCCGGGAGGG	AGGTGGAGGT	TGCGGTGAGC	421
	TGAGATTGTG	CCACCGTACT	CCAGCCTGGG	TGACACAGCT	AGACTCTGTT	TCAAAAAAAAA	481
15	AAAGAAAAAG	AAAAGAAAAA	AATAGACTTT	CTCTTGGCTC	AGTGTATACT	GCCAAATTGT	541
	TTTCCAAAAA	AATTGTGTCA	ATGTATAACA	CCATCACTAA	TATAGTATTG	ATATTATGGT	601
	TATTACATTT	TAAAAATCAT	AATTTGTAAT	TATAACATTC	ATAATTTATT	ACTATTTATA	661
	ATATTAATGT	AAATGTATAT	TATATATAAA	TGTTATAGTA	ATTATAACTT	TGGTAGTGAC	721
	AAAGTATTAA	TTTATTAGGT	GAAGTATATG	CTTTTTTATT	AGTGATAATA	AATATATCCT	781
20	CTCTCCCATT	ATAAAAGTTT	GTATTTCTTC	TTTTAGAAAT	TGATTCTTCT	GTCATTTGCA	841
	CATTTATCTG	TATAATTATA	ACAGGGTATT	TCCCAGTGGT	GGCTAATGAG	AGAATTATGG	901
	GAAAGTATAG	AACACTATTC	AAATGCAAAG	CACTGTATGA	TTTTTATTTA	ATAGGAAGAC	961
	ATTTTGTGCA	GCGATTTCTG	ATTGACCACA	GTTTGATCAA	GTGCATTTGT	TAATGTGTTC	1021
	TACATTTTCA	AAAAGGAAAG	GAGAATTTGT	TACATTCAGA	ACTTGCTGCC	ACTCCTTTGC	1081
25	TACGTCATAA	AGGGTCAGTT	GCCCTTGCTC	ATACTGACCT	ATTCTTTACC	TCTCTGCTTC	1141
	TTCTTTGTGC	CAGAAGAGTA	GAAATCTGAC	CCTTTGGGGA	TACCACCCTC	TCCCCTACTG	1201
	CTCTCTCCAA	CCTGAGGCAA	ACTTTCTCCT	ACTTCCGAGA	GCCTGTCAGA	AGTGGTGAAG	1261
	CCAGCCTGCT	CCTTGGAATC	CAGAACTACT	TTCAGAATCT	TGAACCTTCTG	TGACCTCTCA	1321
	GGGTCCCCTT	GTGTGAAGTT	TTTGACGTCA	GCTTCTCCTG	TGACCCTTAG	AAGTCACTCT	1381
30	TGTGTCTAGC	ACATCCCAGG	TGCTCAGTCA	CCATTGAACT	ACAGTCATAC	TATCTCCTGG	1441
	CAAAGGCTCT	TAAGTGTCCA	TGTTAGCCTG	ATATTAATAT	CCTGGAAGCT	TATACTGTCTG	1501
	TTCTTCCTTC	CAGGTTTAAA	TAAGGCAGCC	CCTTTATCCT	GTCACAGGTC	CTCTCTCCCT	1561
	ACCTATCCTT	ACCTGTTTTG	GATAACAACC	TTTCTTATTT	CTAATAGATT	TATTTATTTT	1621
	TCACATTTCC	TTCCCTTATC	ATAGTTTTCC	TCTCACTTTT	TCCTCTAGTT	TGCTATACCT	1681
35	TGGCTTTTAA	ACATGCAAAC	ATGTGCCTTA	TGGGGAAAAA	AAGACAATTT	TAATTTACCT	1741
	TGCTTCTTCT	TTACAAATGT	ATTGTGGCTT	CTTCTTATAG	TCCAAATCTA	AAACTCTTTA	1801
	CCCACCCACT	GCCTTGAACT	CCTTCCTCGT	TGTGAAAGTA	GGATGGGGCA	AAGAGAGAAT	1861
	GCATGCCCTT	CCCAACTGCT	CAAACAAGTA	AAGGTGCTGT	TACAGTTATC	TTTTTGCTACC	1921
	TTAATACAAT	AATTATTTTA	TTATATCTCA	CAATTTTATG	GATCAGGAAT	TTAGACTGGG	1981
40	CTCAGCTAGG	CGATTCTTCT	GCTTTACTGA	CATCATAGGA	GATCACTTGG	TGGTATTCAA	2041
	CTGTCAGGTA	GGCTTATCTG	GAGGGTCCAA	GATAGCTGTA	CTCTGGTGCC	TGGTGCCTTG	2101
	GTAAAGAGGG	ATGATGATGT	GGGGCCTCTC	CAGCATGAAC	AGCCTCAGAG	AAGTTTGCTT	2161
	TCTTACATGC	TGGCCCAGGG	CTCCAAGAGC	AAATGTTGCA	GTGAGTAAAG	CAGAAGATAC	2221
	AAGGACTTTT	ATAATCTGGT	CTCAGAAGCC	ACATGGCATC	AGTTCTGTAT	TATTCTATTG	2281
45	GTCAAAACAT	TCATAAGCCT	GCCAGATGCA	AGGGGAAGGC	ATATGTACCC	TCATCTTTTG	2341
	ATGGGAGGAA	TGTGATGGAT	TTGCAATTAT	GTTTTTAAAC	TACTACAGAC	AGAACCACTG	2401
	AGAAAGATTC	ATGGGTAGCT	TTGGGGTGAG	GACTGGGAAT	TAACCTGTTG	ATAGCAGAGG	2461
	TTCACTAGAG	TCAACAAGGA	ATAAGGTCTC	CTCTTGTTACA	CTTTAGTCAT	ACTATACCAA	2521
	CATTCTTAAC	CACTGCTTAG	CCATCAGCCT	CACAACATAA	CAACTCCATC	ATAGTTGTAC	2581
50	TCCCTAAGAT	CACCAACAAT	GTTAGAGTCA	AATCCGGTAG	GTTTTTCTTT	GTTTTTGTCC	2641
	TCCTGACATT	TTTTCTAAAC	TTGACACTGG	TCAGACCCAA	TCCTTCTTTA	ATCATATTCT	2701
	TAAATACCAG	TTCTATCACT	GGATATGTTA	CTGTTTCTTG	TTCTCACTCT	ACCTTTGACA	2761
	AAGCCATTCT	TTCCAGACTA	TAACCTCTGG	TCTGGGTCCC	CCTATGGTTT	GGCCCTTGAA	2821
	TTCTTTTCTT	AGTCCTATTT	GACTAGCCCC	ATTTTCCCGT	GAAAAGCATG	CCCCTTTCAT	2881
55	TGCATCCATA	TCATGACTAC	CAAATACCTC	CTCTATTTCT	TCCTCTTTTA	GCATGTTAAA	2941

	TGCAGCTTCC	TAAGCTCTCT	ATCTGGATAT	CAACAGTATT	CTCTCCAAAT	AATTCTAAGA	3001
	CTTTAAAAAT	TGGTTTAAATC	TTCTTACCCC	TAAAATCACC	CCCCTTACCA	ACTGCCTCAT	3061
	GACAATCATT	GGTACTGTCA	CTGAGCTTGC	AACCCATGTT	CTTAAACATA	GAGTAATCTT	3121
	TGACTCCACA	TCTAATCATT	CATAAAGCTG	TATTGTCTAT	CAAATTAAAT	CTGACATTTA	3181
5	TGTGAGAGCA	CTTCATAGTC	TGTAAAGCAC	TACACAGGTG	ATAACATGAA	GCTACACTCA	3241
	TAATGGATTT	GCAGGCTCTG	CTTCTCATTT	GGCTTCTACA	GCCTCATCCC	TCACCAACTT	3301
	CTTGCCCTAC	CTCTCTCTTT	CTTCCCCATC	ACCCAATTTT	CCAGTCAGTC	AGGCCAACAG	3361
	AATGCATTCT	ATATACGCGA	CTTGCTTTTC	CCAACATCTT	TGCCTGTATG	CATGCCACTT	3421
	ATTTGCCTCA	GTTGATCTTT	ATTTCAACAA	GTGTTTGCAG	AGGAGAAACC	TCGCTGGCTC	3481
10	CTTCTCCTTT	CTATTTTTTT	TCAGAGGCTA	CCCGTCAGGT	CAACATTGCC	TTTTTCAGGG	3541
	AAGCTCTGCA	AGCCTGACCT	CCCTTGGAAG	TGCCTTAGGA	CTGGCTTCTT	GCACAGTACA	3601
	CAACCTTTAC	TTATAGAGGG	TTTGGAGATT	ATTCTTTATT	CATGTCTTAT	TTCTCCTGCT	3661
	CCTGGAGGAG	ATGACTCTGA	CTTCCACTGA	CTCTTTTGGG	GGGCTTAAGT	CAGGGTTGAG	3721
	TACCAGAGGC	CCTAAATAGC	TGGACGTGGA	TTCTGGTAAT	ATCAAATCCA	TCTTTGGCTT	3781
15	AACTGAGAGG	TTCTGAAAGC	TGGGACCTGA	CCTTGTCAT	TTCCCTCTTT	CTCCAGTTTC	3841
	CTATTATTTT	CCACTGTTTT	TTTTAAAAGT	TTTTTGTTTT	CTTAAGTTTT	CACAAGAATA	3901
	AACATTGAAA	ATAAAATTTG	CACAAAGATC	GAAGTAGGAA	AGGCCACACA	ACCAACACAT	3961
	ATTACATCAT	TATAGGTAAG	TTAGCAGGGA	GATTTTCAGAC	CTGGGCTAGC	TCTGGAACCA	4021
	CATTTTACAC	TGTTGAAAAT	AAAAGCTGGA	GTACAGATGA	CTTTCCCAGG	TTCACAGAGT	4081
20	TGGTAAGCTG	GAGAGCTGCA	CCTGGAGCCA	AGCAACCTGC	CCTGTCCTTT	CCACTGCACC	4141
	CTCTAAGAAA	TCTAATTAGA	AGGAACAGGT	GGTATCTCAT	TTTGTACGGT	GCTTTAGCAA	4201
	TGTACTATTT	GCTTTCTAGT	GTGTCTATTG	TCTCGTTTGA	CATCTTCTCT	CAAAAAGTGA	4261
	TGAAACGAAA	CGCTCTTTTT	GACAAGTTCA	GAGTGCTCTT	GGTTCCTGTG	TGGGATTCTT	4321
	CCAAGTCTGA	ATTTGGTAGT	GGGAAGAGAA	GGAATCCGGA	GGAAGGAGGA	TGAGAAGTTT	4381
25	AAAGGAGAGG	AAAGGGAAGC	AGAGAAGGCC	GCAAGGTGCC	TGCAAGATGT	CTGGGGAGTT	4441
	GGAGGAATGG	AAGAGTGCCC	CGCTCTTCCT	TCTGGGAGAG	CTCCAGCTAG	GCAGAACCTT	4501
	TCACCAAGGC	TCTGATATCG	TGCTGGTTTT	CGAAAGCCCC	AGCCGAAGGT	GTGCAGCCAA	4561
	AGGGTGACAG	AAGGTGAGGC	ACGTGCGGGG	GCGCGGGTGC	TGACCGCCGC	GGTGCGCCCT	4621
	CCCTCCGACG	TGCGGTGTGC	GGGGCGCAGA	CAACCAGCGG	CCGGCCCAGG	GCTTTCGGGG	4681
30	AGCGAAGCAG	GGCTCCCAGG	GCACCGAGCG	AGAATGGGAA	TGGGAGGGAC	CCGGTGCTCC	4741
	CGGACACGCC	CCCGGCAGGT	CCCACGCCCC	GGCTCTTCTGA	GACCTCGCGC	GGCCCCCCCC	4801
	GGGAGCGGCC	CAGCTATATA	AGTCCCAGCG	GAAGACCGGA	ACGCAGAGGG	TCCTGTCTGGC	4861
	GCGAGGGTGG	GTAGGAGGGG	ACGCGGGGAC	TGCGCCCCCA	ACACCGCGCT	CCGTCTGCAG	4921
	CCGCCGCCTC	TGCACCGCCG	CTGCCCGGCG	GTCGGTTCAA	AAAACAGAAA	TCGGGTTTGC	4981
35	TGCCCGGCGG	ACAGGCGTGA	AGAGCAAGGG	AAAGGAACTT	CCTCCACCTT	CGGGGCTGGA	5041
	GCCCTTTTTC	TCTGCATCTC	CAGTCTCTGA	GTGAAGATGG	GGGGCCTGAC	AGCCTCGGAC	5101
	GTACACCCGA	CCCTGGGGGT	CCAGCTCTTC	TCAGCTGGAA	TAGCGGCGTG	CTTGCGGAC	5161
	GTGATCACCT	TCCCCTGGA	CACGGCCAAA	GTCCGGCTCC	AGGTAGCTAG	GCAGAGGGGT	5221
	AAGACAAGGG	GTCTCAGGAC	AGAGGGGACG	CTGTTGCGTG	CATTCCATTT	ATTCTCTGCT	5281
40	TTGGTGTAAC	CACCTGTTTCT	AGGTAGGGTA	GGTGACCTTC	CAAAGCAGTC	TGGCCTTGTC	5341
	CCAGGGCTGG	TGCTTTAGGA	TGGGAAACTG	GAACCTTTTTC	TGGGATTAGC	TGAAGAACCA	5401
	CCAGGGCCAC	AGAGAATGGG	TTGACCATGA	CTACTACCAA	ATTCTCCCAA	AATTTAGGGT	5461
	GCACTTAGTA	TTTTAAGAGC	TGAGAATATT	GGCCTCTCCT	GAGTTTACTA	GTCAGGTGCT	5521
	TTTTCTTTTC	TTTGATTCTT	CGGGGGTTCT	GTCCTATCCT	ACTGCCCTAG	GGGTTCTGGA	5581
45	GAGTTCCTGG	GGAGGGGGAT	ATTCAAAATG	TGCATTGTAG	CCAGCCTCCC	TCCATCTGCG	5641
	CGTGAGCGAA	CACACACACA	CACACACACA	CACACACACA	CACACACACA	CACACACGGT	5701
	AGAGGGAGGT	GGATGGAAGA	GGAATGTTGC	TGAGAAAAGA	AACGAAAAAT	AGGAACACAG	5761
	GGGGAAATCT	TGGCTTAAGA	GTGAACCTCA	TTTCGCTCCC	TTCTGTTCTG	CACCTTCTCT	5821
	ATTTCCAGGT	CCAAGGTGAA	TGCCCCGACG	CCAGTGTTAT	TAGGTATAAA	GGTGTCTCTG	5881
50	GAACAATCAC	CGCTGTGGTA	AAAACAGAAG	GGCGGATGAA	ACTCTACAGC	GGGCTGCCTG	5941
	CGGGGCTTCA	GCGGCAAATC	AGCTCCGCCT	CTCTCAGGAT	CGGCCTCTAC	GACACGGTCC	6001
	AGGAGTTCCT	CACCGCAGGG	AAAGAAAGTA	AGCCGTGAGC	GTTCTCTGGG	GGGGCAGAAA	6061
	AGCCTTGGGC	TCCGCTCTGT	TCCAAAAAGT	GTAACACACA	GAGGAGTGGT	TTTCATAACA	6121
	AATTGGCGAG	AAAACATTCA	TATTTGAACT	CTCCCTTCCC	CAAACATTAG	CTCATTGTTC	6181
55	ATAGAAAAAA	GTATGCAAAA	TCGATTTTTT	AGATGCAGAT	ATATACTTGT	AAAGGTCACC	6241
	CAGTCATGGA	AGTTTTGTGC	CCAGTTTGGA	TCTCCATCTG	GAGAATATGG	GTGGGCTACA	6301
	GAAAAATGTT	TAACTTAAAG	TTCTCCAAAG	AGGGAAGTAT	ATCAGAAACA	TCTATGGAGC	6361
	TTGTCAGAAA	TCCAAACGAG	GACTACCATG	GTCCTCTGAG	TCTGAATCCT	CAGGCTAGAG	6421

	ACCAGAGTGT	CTTTCCACAA	GCTTCCCTCA	TCATTTGTGT	ATGCAACAAA	GTTCAAAGCC	6481
	TTCTGTTTGA	AGCAAAGAAA	GCCAGACTTT	GTGAAGAGAG	TTGAAAGGAC	AGGAAAAGAC	6541
	ATATTTCCCTC	TTAAGAGGTT	CCTCATCAGG	TCCAGGAAAG	ACCAGAGCAG	AAAAAGTGGA	6601
	CGAATGCTGC	AGGGAGTTTG	TTTAGGGGAA	AAAGAAAAGG	AAACATATTT	CCTGAGTGCC	6661
5	AGTGCACCTCT	AAGAATTCCT	GTCACTTTAG	GTAGCATTTA	TTTGAGGGCT	TAACATATGAA	6721
	CCAGACATTG	TTCTAAGTGC	TTCAGATACA	TTATAACTGG	AAGGGTATTA	GTACCATTAT	6781
	CCCTTGGCAG	ATGGGAAAAC	TGAACACAGA	GCAGATTTCAT	CACTTGCCCA	AGGTCACACA	6841
	GCTGGGAGGG	GGCAGAGCCA	GGGTTCAAAC	CCAGGCAGTC	TGGCCTCGGA	CTCCAGGCTC	6901
	CTAACCCTGT	TCTCTACTGC	CTTCTGCACT	TCTCATATGA	TTCTGCCCAT	CATTCAAACC	6961
10	GCACAACACT	GCTGTGAGTA	AAAAGTGTTA	GCCGAATATC	AGGGTAGTTA	AGTAACATGC	7021
	ACAAAATCAC	ACAGCTAATC	AACATCAGAG	GCACTTTTCAT	GTGGAGTAGA	CAAGCCAGAG	7081
	AGAAGATGTG	CTGATGGCAC	AATGAATACA	TTAAGTGAAA	TCCACCTTGT	AGATTTTCATC	7141
	ATTTCTGCTG	TGAGTAACCT	TCAATACTAT	AATTTTATGG	GATAATTTAT	AAATGTTGTC	7201
	TATACAAATA	TATAAGTTAT	ACTTATCCAC	ACAAGTACTT	TCAAAGTGAA	GATAAAGTCT	7261
15	GGATGTTACT	AGATCAAAAAC	TGCATTTTTT	TATTTATAGA	TGTAGCAAGA	GAGGAAAACAC	7321
	AAAGGAGGTA	AAGCTGCCCCG	TTCAGGTGGT	TTTCTTCACA	GATTGACTGT	TCTACCAATT	7381
	GTTGTGGACT	TTGGGCACCA	AATTAATAGG	ATATATGTTG	GCAGTGTTCT	ATGTTATATA	7441
	GATTCAGTTT	ATTTAGTAGG	CTTTATTGAA	CTGCCATGTG	CCAGTAACTA	TGTTAGATGT	7501
	TTAGATGGCA	GATGTGTCTC	TAGACAGAGC	TTACAGTTGA	GAGTATGGGT	TGTGTGGGGA	7561
20	GAAGTGAATA	GATGACTATA	TTCCATGATA	CATGCTGTAT	TACAATACAG	TCCTACTTCA	7621
	CTTAACGATG	GGGATACATT	CTCAGAAATG	AGTTAGGAGG	CAAATTGGTT	GTTGAATGAA	7681
	CATCACAGAG	AGCACTTACA	CAAACCTAGA	TGGCATAGCC	ACACCTAGGC	TATATGGTAT	7741
	AATCTATTGC	TCCTAGGCTA	CAAACCTGTG	CAGCATGTTG	GTATTGAATA	CTACAGGCAA	7801
	TTGTTACATA	AAGTTAAGTG	TTTGTGTACC	TAAAAATAGA	AAAGGTAATG	CATTACACTA	7861
25	CAGTCTTATG	GGGCTGGGAT	GTCACTAGGT	GATAGGAATT	TTTCAGCTCT	GTTCTAATCT	7921
	TACGGGACCA	CCATCATGTA	TGCAGCACAT	GACTAACTGT	AATTACAAGA	TGGTGGCTAT	7981
	ATTAAACAGA	ACTACTTAAG	CTAGCCATGG	AGGTATGGTC	CGTGAGATTT	TCCTGAAGAA	8041
	TTAACGTCTG	GATCAATTCT	GGAAGGGCCA	GCAGGAGTAC	TCCAGGCAAA	GGGGTGAGAA	8101
	AGGAGCTTCC	AAGTAGAGTG	AAGGTCATGT	GCAAAGACTC	AGTGAGGAGT	CGAGTGAACA	8161
30	TAGCACAGGG	AGGACATGTT	GGTGAGGAAG	GAGGGGTGAA	GCCACAGAGA	CAGGAGGGAG	8221
	CCAGATGACA	GAAGGCCTTG	CAGGCGGTGC	TAAGGAGTTT	GGATTTTATC	CTTACAGTGG	8281
	TGGGAAGTCA	TTGTAAAAAT	ATTAAGCAAG	GGAGTGGCAT	AAACAATTTA	CATTTTCAAA	8341
	AGATCACTTT	GGCAGCAGAT	AGAGTATATA	TGTAAAAGGA	GTAAGAAAGA	GGTAAGTTAG	8401
	AAAGCAAGAA	ATGATCAGGG	TATGCCCTAA	AACACTGGCA	ATAGGGAAAA	AGAGATGTCA	8461
35	ATCAGAAAGA	TTGAGAAAGT	ATAATTGAAT	TGACTTGGTG	AACAAATAGA	AGTAAGGCAT	8521
	AAGGGACAGG	TAGAAATATG	AGATGACTTC	CAAGTTTCTG	TTTAAAGATA	CCCTTTATTG	8581
	AGAGAGGATG	TATAGAAGCT	GTCTTAGGGG	GAAGACAAGA	AATTTGGTTT	AGGCCATGTC	8641
	AACAGGTAAT	GGCCAGTAGG	CACATGATTC	AGTTTATTTA	GTGGGCTCCT	TTTAGGAGAA	8701
	AATCTGAGCC	AGATTCCAGG	AAGTCACAGC	AGGGACTACC	AATAGGGTCA	AACAGCAGAG	8761
40	AGTGTGGAAA	GGACTGAAAA	GTGATCATTG	TACATAACAA	ATAGAAGCTC	ACTGATTTTC	8821
	TAGCAAAAAC	ATCTTCAGCA	GAGTAGCGTG	GTATAAGCTA	TATTGTAGGG	GACTGAGGAA	8881
	GAAATGGGCT	CTGAGAAGTA	AAGACAAACA	ATATGTTTTG	TAAATAAAAT	TCTTTTAGTT	8941
	CTTAAAAAAA	AAGCCTCTTT	TCCAGCTTGA	TTGGGAAGTG	AAGAGAGGGA	TTTGAAAGTT	9001
	GGAGATTGGA	GGATAGGATG	AGTACATCAA	GATACACTAC	GTTGTAGTGC	AGTGCATTAC	9061
45	AAATGTGAGC	TAAAAGTGAA	GGCATTGTGA	ATCATATGAT	ATTGCTAATT	AAAAGACAGC	9121
	TGTCAGTCAT	ATGCCAGCT	CCTGGTAAAG	CATGATGAGA	AGAGTACAAT	CATGGTAGTG	9181
	ATTTAAAAAT	TGCTGCCAGT	TTTGTGGATT	TTCTTTATGC	TAGACAGTGT	AAGCTCTTTA	9241
	TCAATATTAT	TTAACTCACA	CAACTCTAAG	AGGTAGATAT	TATTATCCCT	TTTTGACAAA	9301
	TTAGGAAACA	GAATTATAAT	GA CTGAGAAA	GTCTCTGCTG	AGTAAATGTT	ACTGAACCTT	9361
50	AATTTTATGT	TTACTTAATG	ATAGAAATGA	ATATTGGGCT	TCAAGACTAT	TTGTACTTAA	9421
	TGAAATCTGT	CTTGAGCAAC	ATAAGCTATT	TTTTTCAAAA	TTTTAAGACA	AAAATCACTT	9481
	TCTTCTCTCC	TGTCTTCTTA	TTTTTGTTC	CTTCACATGT	TGTAGCCTAA	CACTACTTGA	9541
	TGGCCCATTT	TGGTGCAGTT	TGTCCACTGG	GCTTCATCTA	AGGCCACCAA	GTCCCATAA	9601
	TAACATGATC	ATTCTGTGGG	GAAAGATCAA	GCCTCATTGG	TGATGGGTGC	CTCCTCACAG	9661
55	TCGGATAATA	CTGAAAAGAG	AGCTAAATGT	GGGAAAGAAC	CAAGTTGAAC	ACAGGAAAGA	9721
	ATCAGGCCAC	TGTGAAAATA	AGCATTGTGT	TTTCTTGTTT	CTTGAAAGTC	TTCATTTTTTA	9781
	AAAAATTTCA	GACACCTGAA	GTTTTCTAGC	CTTACTCTGA	GTTGACGCAC	ATTTAGTACA	9841
	TGATCAACAC	ATAAACAAGC	ATTAGAGAAA	TAGAAAAGCT	GTAAGAATAC	AAAAATATGG	9901

	GCCAGGTGGG	TGGCTCATAC	CTGTAATCCT	AGCACTTTGG	GAGGCCGAGG	CAGACGGATC	9961
	ACCTGAGGTC	AGGAGTTCAA	GACTAGCCTG	GCCAATATAG	TGAAACCCTG	TCTCTACTAA	10021
	AAATACAAAA	CTTAGCAGGC	TGTGGTGGCA	CGTGCCTATA	ATCCCAGCTA	CTTGGGAGGC	10081
	TGAGGCAGGA	GAATCTCTTG	AACCCGGGAG	GCGGAGATTG	CAGTGAGCCA	AGATCACACC	10141
5	ACTGCACTCT	AGCCTAGATA	ACAGAGCAAG	ACTCCATCTC	AAAAAAAAAA	AAAATACAAA	10201
	AATATGAACC	ACTGAAAATT	AAAAAGACAT	GCATGCATTC	TAGGTCTTTA	ATTTTTTTTC	10261
	TTAATAATTT	TTTTTCTCTC	TGGATAGCAG	CACCTAGTTT	AGGAAGCAAG	ATTTTtagCTG	10321
	GTCTAACGAC	TGGAGGAGTG	GCAGTATTCA	TTGGGCAACC	CACAGAGGTC	GTGAAAGTCA	10381
	GACTTCAAGC	ACAGAGCCAT	CTCCACGGAA	TCAAACCTCG	CTACACGGGG	ACTTATAATG	10441
10	CGTACAGAAT	AATAGCAACA	ACCGAAGGCT	TGACGGGTCT	TTGGAAAGGT	AACTAACTTC	10501
	AAAATGGGTT	TTATAACCAC	CAAAGCACAT	ACATACAAC	AGCAACTTAT	TGTAAAGTAG	10561
	AGTTAATAAA	CATTTTCTTT	TTTTTTTTTCC	CCAGGGACTA	CTCCCAATCT	GATGAGAAGT	10621
	GTCAATCATCA	ATTGTACAGA	GCTAGTAACA	TATGATCTAA	TGAAGGAGGC	CTTTGTGAAA	10681
	AACAACATAT	TAGCAGGTAA	CTTCCCATT	CATATAACAA	ACAGGTCTGC	ACCTTTAGAA	10741
15	GTTTCATCTTG	GAGCTTCTGC	AGCCACCTTA	TACTCAATCT	CTTAACCTCA	ATAGTTTTCT	10801
	CTTTTTAAAA	ATTAAGTAAT	TTTGAACCAT	ATATAACTTT	GTGAGAAGCA	GGAAAAAGACC	10861
	AAAATATTAA	GTTTAAGAAG	TTTTGCCACA	ACAAAAATAT	TTTGCAACAA	AAATAACAGG	10921
	CAATTTTCATG	TCAGCATTAT	TCTCATTTAA	TACTAATATA	TGGGACTTTT	GTTAGAATCT	10981
	TATTCTTTAT	ACAGCAGAAT	TCAGGAGGTA	AGTCCATCCT	GCATACTATA	TCCAAAAGAT	11041
20	CTAGTTATAA	AAGGAGCTTA	TCAGTGGTCT	CATCCAAAAA	GTAATACCAT	AAGATAGGTT	11101
	CTTAAAAATA	ATATTCTAAC	AACTTCTAGA	GACATTGAAA	TTTCCCTTAT	TTCAATAAAA	11161
	AAGTATTAGA	TGCTCATATA	TTAGGCATTA	TTACAGGCCT	TAAAGGCACA	GAGGAAACTA	11221
	ACAGTTTACT	TTCCTAAAGT	GTTAACAATC	TATTAAGCCA	TTTACTCTTT	ACCTTCTTTT	11281
	TCTAGTGCAA	TACCTTTCTT	ATTTTATTTT	ATTTATTTAT	AAGACATCTT	CATTGACCTA	11341
25	CTGTTATCAA	TAGGTTTATA	AAGATATGAC	AGATAACTAA	ATTGCAAGCC	CCCAAAAAGTC	11401
	TGATGTTGAC	CTGTTTCATC	GATCCATTTT	AGATGACGTC	CCCTGCCACT	TGGTGTCTGGC	11461
	TCTTATCGCT	GGATTTTGCG	CAACAGCTAT	GTCCTCCCCG	GTGGATGTAG	TAAAAACCAG	11521
	ATTTATTAAT	TCTCCACCAG	GACAGTACAA	AAGTGTGCCC	AACTGTGCAA	TGAAAGTGTT	11581
	CACATAACGAA	GGACCAACGG	CTTTCTTCAA	GGGGTAAGAT	ATGATCTTGT	GTATCTGTAA	11641
30	TGTGTTCTGG	CTGTCTGTGT	GCTTTGGGAC	ACTCTCATGT	CAAGCAACCG	ACATTCTAGCT	11701
	TACAAGCCTT	AGTATATTCA	TATACTTAGT	ATTGACTTTT	CCTTGCCACA	GATTTCTCCA	11761
	ATCCACCAAT	TCCACTGTGC	CAGAAAGTAA	AAAGCCATGA	TATTCAAATT	TTCTCAACTT	11821
	TGATCAAAGG	CTCATTCAAG	ACCAGTGCC	TTTCCACTGG	TCCCAATCTA	CTGGAAATGC	11881
	AGACAGTATT	TTGCCTTCTC	TGGGCAAGAA	AGTTATAAAG	TAGAGGGAAA	TCATAATAGA	11941
35	GAGCTATGAG	AGAACAAGAT	TTGATTTGAT	TTAATTTGAT	GGACTCAAGT	TTTAACATTG	12001
	TAAAACTAGA	GATAAGACAT	CACCACCAAT	CTAGAAAAGT	GATGCAGAAA	AGTATTTGAT	12061
	TTGGGTAAAT	ATTACACTCA	CCTAGAAACA	AGTGTGTGTG	AATAGATTAC	ATATTTCCAT	12121
	AATGCAATGT	TGTATCAGAA	ACTACCTTCC	TAAGAAAATA	TAGTATGGGC	TCGGCGTGGT	12181
	GGCTCGCACC	TGTAATCCCA	GCACTTTGGG	AGATGGAGGC	AGGAGGATCA	CTTGAGCCCA	12241
40	GACTGGGCAA	CAAAGCGAGA	CCCTGTCTCA	ACAAAAAATT	TAAAAATTAG	CTGAGTGTGG	12301
	TGGCACGCAC	TGATGGTCCC	CTCTACTTGG	GAAGCTGAGG	CAAGAGGATC	TCCTGAGCCC	12361
	AGGAGTTCAA	GGTTTCAGCG	AGCTATGATT	GTGCCACTGC	ACTCCAGCCT	GGGAGACAGA	12421
	GCAAGTCCCT	GTCTCAAAAA	AGAAGAAGGA	GAAGGAGGAG	AAAATACAGT	ATTAAGTAAT	12481
	CTGTCAATAT	ATTCCACAAG	GATTACACTA	GTGGTTTAA	AATAAAATTA	TATTACCTTT	12541
45	TTAAATTGTA	AGGCCATTCC	TCAAGCTTTA	TAAATTAAGC	ATGAATGCAT	CATACACATT	12601
	TTATAAAAAG	TTCCAACTCA	TCATAATCTG	TACTTATGAT	ACATTAATAC	AAATGAAGTT	12661
	CATTATAAAA	TTAACTTTAA	ATGGATATAC	CAGTTATTAA	ACCATTAACC	ATTTAATAAT	12721
	TTTATTTTTT	TCAAATTTAA	AAACCTTTTG	GGGAAGAAAT	ACTACAACAT	GGATGAACCT	12781
	TGAAAACGTT	ATGCTAAGTG	AAATAAGCCA	GACACAAAAG	GACAAATACT	GTATGATTAC	12841
50	ACTTAAATGA	GGTACCTAGA	GTAGTCAAA	TCATAGAGAC	AGAAAGAATA	GAAGTTACCA	12901
	GGGGCTGGAG	GTAGGAAAAA	ATGGAGAGCT	GTTTAAATGGG	TAGAGAGTTT	CTTTTTGGGG	12961
	TGACAAAAAG	GTTCTAGAGA	TGGATAGTGG	TGATGGTTAC	ACACAATGTG	TGTGTACTTA	13021
	ATGCTACTGA	AATGTAATTT	TATGATTTTT	TTTTTTTTGCA	GCAAAATACC	CCACATTGGG	13081
	AAGTGAAGAG	AAACATGTTA	AGAGACTTGA	AGGAAAAAAA	TTGGGGCAGA	GGGGTGTTTT	13141
55	TTATAGGTTA	AACAATAAAA	GCCATTTAAA	CAGTAACAAT	TTCTCTAAGG	ACAAGAATCG	13201
	TCAAGATTGA	GACAGCACTG	ATTTCTTGAC	TCTACTCAAT	ACTTCTTTGG	TTTCTCTTCT	13261
	TCCTTCCCCC	TTCTAATAGT	TTCTTACCTC	CCATTTCAGAA	AGCAAAGCAA	AACAAGCAAA	13321
	AATTCCCCCT	TCCCTCAAAA	AAGGAAAGAG	TTTTTGAAAA	AGTTCATGTC	AGTGAAGAAA	13381

	AGACATGTTT	TGGGAGTGAA	GGATATTTGT	GGATTTGTAT	AGATGTGATC	ATCAGGGCTG	13441
	TGTTGTTTTG	AAGTAATATA	GGACATCTAG	AGGAAAATTT	ATTTTCAGCA	GAGGAGGGAA	13501
	AGATGAAGAG	TAGGTACTTT	TAAGCATCTT	CACTTGAGGA	GTGGCAAAAT	GAGAAGCATA	13561
	ACCTGCTATA	ATCACTTTAA	GAATTTTCAGG	CTGAGTGTGG	TGGTGCAGTC	TCTAGTCCCA	13621
5	GTTACTCCAG	GAGGCTCAGG	TGGGAGGATC	ACTTAAGCCC	AGGAGCTCGA	GGTTGCAGTG	13681
	AGCTATGATT	ACACTACTGC	ATTCCAGCCT	GGCGGCGAGG	GTGAAGCCTC	ATCTCAAAAA	13741
	TTAAAAAATA	AAAAAATCAA	ACAAATTAAT	CGAACGATGA	CATGCACTTT	TCTAGGTTGG	13801
	TACCTTCCTT	CTTGCGACTT	GGATCCTGGA	ACGTCATTAT	GTTTGTGTGC	TTTGAACAAC	13861
	TGAAACGAGA	ACTGTCAAAG	TCAAGGCAGA	CTATGGACTG	TGCCACATAA	TCAGCTTCAA	13921
10	GAAAATGATG	TAACATACCA	GTGGGAATCT	TGCTGACTGG	ATCATAAAAA	CAAACAAAAC	13981
	TTATTCACCT	ATTTTAACTT	AAAAAGATAA	AGGAATTTTG	GCAGAGAATT	TTGGACTTTT	14041
	TTATATAAAA	AAGAGGAAAA	TTAATGCCTA	TTTCATATAA	CTTTTTTTTT	TTCTCAGTGT	14101
	CTTAAGAAGG	GGAAAGCAAA	ACATTCAGCA	TATACCCTGG	CAAATGTAAT	GCAGATAAGC	14161
	TACTGCATTT	GACCATTTCT	GGAGTGCAAT	TGTGTGAATG	AATGTGAAGA	ACTTTAACAT	14221
15	GTTTTAATTA	CAATTCCAAC	TGGTGGAAAA	GAAACTGAGT	GAAATGCAGT	TTATATTTAT	14281
	AAATACTTAA	AAATGAAGTT	ATTAAAAATA	TTAGTTTTTA	TTAACCACAG	TTGTCAGTTA	14341
	ATATATTCAA	TAAAGTATTG	CTAATACCTT	TTAAAGTTTG	TCTTTTGAGA	TCTATACCTG	14401
	GGTGTAAGAG	TCAAGTTCAC	TAGAATACAA	GACTGCCCAA	TAGCAAATGC	AGGTCTTTAG	14461
	AATCATAGGC	ATGAACCTAC	TCTGAATGTT	ATTAGTATAG	ATTTTTAATG	TTTAGAGTCC	14521
20	AGATTTGATG	ACATCTCTAA	CAACTTCTAA	TCTAAGACAC	TATATTCATT	TTGGCAGGAT	14581
	TGCTACTAGA	GTCTTGGTAT	CTGTGCTAGC	ATCACATAAT	TTTAGAGCTG	GAGGGTACTT	14641
	CTGGGAAGAC	AGAGGAACAG	TTTGAGATTC	CTACTGAGAT	GAAAACGAAT	CTTCATGGAA	14701
	TCTTTTCAGCA	AAGCCAAATT	CAAATTCATC	ATTAGCACCT	GATGTAACCT	TTTCAATGCC	14761
	TACAAACTGC	ATGCAGAAGA	GATAGGGAAA	CAGTAAACAA	GATATTAAAA	GAAGTTTTTA	14821
25	AGACAAAGCC	CAGCCTGATT	TTAAGCTAAA	TCCAAGGATT	GGCAGCTTGG	ATGAGCAGGA	14881
	AGGTTACAGG	CTGCCAGACA	TCATTCTAGT	TCTGTTTTTA	TCAACTCCAT	GTTACATTTA	14941
	CTATCAGGGA	TTCTCACCTC	ACCCTCATGC	ATGTCTTCCC	CATTCATTAC	CCGCAAAAGT	15001
	GTCTTGTAGC	AGATGTCTTC	TGTGTCCCAT	ACATACCATT	TTGCTCTTTA	GTGCTTGCTG	15061
	GCCTGACTTC	CTATTGTCTC	GTGAGCATCT	GCCCTTTTTA	GGGTCTCTGG	CCACCAGAGC	15121
30	CAGCTTTACT	AACCTGTGCA	TGGCATTCTA	GAAGAGCAGC	AGGGAAAATA	ACACAGCCCC	15181
	AGTGCAGCCC	TTAACCACCA	ATAACTGGTA	GTAGTTGGTG	TACAAATATC	TCAGTTCCCT	15241
	CAACTGTCAG	GTGGAATACC	GCTGAGGGAT	CAAACCTAG	TAACACACAG	TAGTGTTTTG	15301
	CTTACTATGG	TTAACTAAAA	AATCACAGGG	TCTTCATGCA	TTTGGAAGG	ATACTTTATT	15361
	TCTTACAAAG	GGTTACAGCC	TACAAGGTGG	TCATTCTGCA	GGCTAGAAAG	CGTAACCTCC	15421
35	AGCAAAGACC	GGAGGCAGGC	ACTTCTAGGG	AAGGAAGAGT	AAGACAGAAA	TTTAAATTGA	15481
	ATGGGTTGGC	CAAGTATACA	TATTCAACAG	GCTACAGGTG	GATTCATGAA	TATTTCATGAA	15541
	GGCAGTCCCTG	ATGCATGCAT	GTTACACCTT	GGGGTGGAGG	CTTAACATTT	AAATGTATTA	15601
	CAGTTAGGCC	CTATACATGA	AAAGGTGAAG	CAGTAACACG	AAGGCACACA	ATGCACCATT	15661
	TCTGTAAACA	GGCCAGAGCC	AGTTCACAGT	GGTTGGTCTC	TTATCATGAG	AAAGCTACTA	15721
40	AAATCCTCTT	GTCCAGTTAA	AAGTGTAGTT	ATGGCTGGTG	GAAAATGGGC	TGGAGTCAGT	15781
	CAACACTTGG	TGAAGCTGCA	GTTGCTTCAG	ACACTCAAGG	CCAGTGTTTG	TTTAGCTGCT	15841
	CGAGAAAAAG	AAAAATCTTG	TGGCAGTTAG	AACATAGTTT	ATTCTTTAAG	TGTAGGAGTG	15901
	TGTGACTTAA	//					

45 **Table 28.**

Nucleic acid sequence of the 2,113 base pair (bp) transcript ENST00000310473 of the human UCP2 gene (Eight Coding Exons are in capital letters):

No.	Exon / Intron	Start	End	Length	Sequence
	5' upstream sequence				aatcgacagcggaggccggtcgagggccc cagtcccgccctgcaggagcc
1	ENSE00002287650	73,694,352	73,693,766	587	AGCCGCGCGCTCGCTCGCAGGAG GGTGGGTAGTTTGCCAGCGTAG GGGGGCTGGGCCCATAAAAGAG GAAGTGCACTTAAGACACGCCCC

					CGCTGGACGCTGTTAGAAACCGT CCTGGCTGGGAAGGCAAGAGGT GTGTGACTGGACAAGACTTGTTT CTGGCGGTCAGTCTTGCCATCCT CACAGAGGTTGGCGGCCCGAGA GAGTGTGAGGCAGAGGCGGGGA GTGGCAAGGGAGTGACCATCTCG GGGAACGAAGGAGTAAACGCGG TGATGGGACGCACGGAACGGG AGTGGAGAAAGTCATGGAGAGA ACCTAGGCGGGGCGGTCCCCGC GGAAAGGCGGCTGCTCCAGGGT CTCCGCACCCAAGTAGGAGCTGG CAGGCCCCGGCCCCGCCCGCAGG CCCCACCCCGGGCCCCGCCCGG AGGCTTAAGCCGCGCCGCCGCT GCGCGGAGCCCCACTGCGAAGCC CAGCTGCGCGCGCCTTGGGATTG ACTGTCCACGCTCGCCCGGCTCG TCCGACGCGCCCTCCGCCAGCCG ACAGACACAGCCGCACGCACTGC CGTGTTCTCCCTGCGGCTCG
	Intron 1-2	73,693,765	73,692,678	1,088	gtgagcctggccccagccctgcgcc.....actctc tgcctttgctcaccacag
2	ENSE00001184362	73,692,677	73,692,521	157	GACACATAGTATGACCATTAGGT GTTTCGTCTCCCACCCATTTTCTA TGGAAAACCAAGGGGATCGGGC CATGATAGCCACTGGCAGCTTTG AAGAACGGGACACCTTTAGAGA AGCTTGATCTTGGAGGCCTCACC GTGAGACCTTACAAAGCCGG
	Intron 2-3	73,692,520	73,689,523	2,998	gtaagagtcagtcgaaggaagagg.....tgggg cttttctctcttgcttag
3	ENSE00001184370	73,689,522	73,689,298	225	ATTCCGGCAGAGTTCCTCTATCT CGTCTTGTTGCTGATTAAAGGTG CCCCTGTCTCCAGTTTTTCTCCAT CTCCTGGGACGTAGCAGGAAATC AGCATCATGGTTGGGTTCAAGGC CACAGATGTGCCCCCTACTGCCA CTGTGAAGTTTCTTGGGGCTGGC ACAGCTGCCTGCATCGCAGATCT CATCACCTTTCCTCTGGATACTGC TAAAGTCCGGTTACAG
	Intron 3-4	73,689,297	73,689,142	156	gtgaggggatgaagcctgggagctct.....tagcta ccctgtcttgccctgcag
4	ENSE00001252503	73,689,141	73,688,931	211	ATCCAAGGAGAAAGTCAGGGGC CAGTGCGCGCTACAGCCAGCGCC

					CAGTACCGCGGTGTGATGGGCAC CATTCTGACCATGGTGCGTACTG AGGGCCCCCGAAGCCTCTACAAT GGGCTGGTTGCCGGCCTGCAGCG CCAAATGAGCTTTGCCTCTGTCC GCATCGGCCTGTATGATTCTGTC AAACAGTTCTACACCAAGGGCTC TGAGC
	Intron 4-5	73,688,930	73,688,063	868	gtgagtatggagcaagggtgtaggc.....cactg accccatggctcgccacag
5	ENSE00001184355	73,688,062	73,687,868	195	ATGCCAGCATTGGGAGCCGCCTC CTAGCAGGCAGCACCACAGGTGC CCTGGCTGTGGCTGTGGCCCAGC CCACGGATGTGGTAAAGGTCCGA TTCCAAGCTCAGGCCCCGGCTGG AGGTGGTCGGAGATACCAAAGC ACCGTCAATGCCTACAAGACCAT TGCCCGAGAGGAAGGGTTCCGG GGCCTCTGGAAAG
	Intron 5-6	73,687,867	73,687,788	80	gtgtgtaccagtgttttcccttc.....accagga tcttctctctctacag
6	ENSE00003361285	73,687,787	73,687,686	102	GGACCTCTCCAATGTTGCTCGT AATGCCATTGTCAACTGTGCTGA GCTGGTGACCTATGACCTCATCA AGGATGCCCTCCTGAAAGCCAAC CTCATGACAG
	Intron 6-7	73,687,685	73,686,717	969	gtgagtcagaggtagacggtgctg.....tgcttg cctgtctctcttgag
7	ENSE00001184349	73,686,716	73,686,536	181	ATGACCTCCCTTGCCACTTCACTT CTGCCTTTGGGGCAGGCTTCTGC ACCACTGTCATCGCCTCCCCTGT AGACGTGGTCAAGACGAGATAC ATGAACTCTGCCCTGGGCCAGTA CAGTAGCGCTGGCCACTGTGCCC TTACCATGCTCCAGAAGGAGGGG CCCCGAGCCTTCTACAAAGG
	Intron 7-8	73,686,535	73,686,167	369	gtgagcctctggtctctccaccca.....atgacct gtgattttctctctag
8	ENSE00001184368	73,686,166	73,685,712	455	GTTTCATGCCCTCCTTTCTCCGCTT GGGTTCTTGGAACGTGGTGATGT TCGTCACCTATGAGCAGCTGAAA CGAGCCCTCATGGCTGCCTGCAC TTCCCGAGAGGCTCCCTTCTGAG CCTCTCCTGCTGCTGACCTGATC ACCTCTGGCTTTGTCTCTAGCCG GGCCATGCTTTCCCTTTTCTTCTT CTTCTCTTCCCTCCTTCCCTTCT

					CTCCTTCCCTCTTTCCCCACCTCT TCCTTCCGCTCCTTTACCTACCAC CTTCCCTCTTTCTACATTCTCATC TACTCATTGTCTCAGTGCTGGTG GAGTTGACATTTGACAGTGTGGG AGGCCTCGTACCAGCCAGGATCC CAAGCGTCCCGTCCCTTGGAAG TTCAGCCAGAATCTTCGTCCTGC CCCCGACAGCCCAGCCTAGCCCA CTTGTCATCCATAAAGCAAGCTC AACCTTGGCGTC
	3' downstream sequence				tctctctctctttagctcttaccagaggtcttggtcca atggcctttt.....

Table 29.

Nucleic acid sequence of the 15,174 base pair (bp) of the human UCP2 gene (ENSG00000175567), including 5,000 bp 5'UTR and 2,000 bp 3'UTR, (Eight exons are

5 highlighted):

TCCAGCCTGGGCAACAAGAGTGAACTCGGTCTCAAAAAAAAAAAAAAAAAAGAGAAGA
AGAAGAAAGAAAAGTAGGTGGAGTGTGGTGGCTTGCACCTATAATCCCAGCACTTT
GGGAGGCCGAGGTGGGTGGATCTATTGAGGCTAGGAGTTCAAGATCAACCTGCCAA
CATGACGAAACCCACCTCTACTAAAAATACAAAAAATTAGCACGGCGTGGTGTGT
10 GTGCCTGTAATCCTAGCTACTTGGGAAGGCTGAGGCAGGAATCGCTTGAACCTGGGG
GGCAGAGGTTGCAGTGAGCCAAGATCTTGCCACTGCACTCCAGGCTGGGCGACACA
GCACAACTCTATCTCAAAAAAAAAAAGAAAAACAAAAGAAAAGAACTAATATATCAA
AATAATTTCTAGTTAGTTGGATTCTCTACTTATTCAATTCAATGACTTATTGAATTATC
ATATATTACTAGTGCTTTTAAATACATACCTTCTACAATTTTCAACTGAAAATTACT
15 TCATTGATCAGGGCTCTTTAAACTGATCTCCATTTGCATTGTTTTACTAACTATAGTT
ATTATTCATGTATTAGCACTCTGAGCCTACTGTAATGATGTGTACCTTAATAAGAA
CTGAATATTTGTAATGGCTGGCAGTGAATTTAGTAGTTCTTGAATTTAGAGCTCAAA
ATATGGGAGTAATTTGCTGCTTTATTTCCCTTTGAGAGGTAATAGAGGAAAAACAGAA
TCTAATAACAATCACAGATTTTCGGGAAAGCACTGTAAAACCATATGATCAATTCTA
20 GCTTCTTATGTAAACATGGAAAGATTGCCAGCTGAACACCTGTCATGCTCTAAGAAG
TTGGGGAGAATTTGCATTTTGTAGAACTGTGAGCAAAATGAGAACGACTGCTATGTTT
ATGCTTTGTGAATTTAGCTTTATTTCAATTCACACAATTCATGGGAAAAAATGCATCTT
TTAACTCGGTGTTTTTCAATTCAACTTTTAAAATACAGGAGTGGGCCAGACCCGGTG
GCTCACACCTGTAATCTCATCACTTTGGGAGGCCGAGGCAGGTGGACCACAAGGTC
25 AAGAGATAGACACCATCCTGGCCAACATGGTGAAACCCCATCTCCACTAAAAATAC
AAAAATTAGCTGGGCATGTTGGCACGTACCTGTAATCCCAGCTACTCGGGAGACTGA
GGCAGGAGAATCGCTTGAACCTGGGAGATGGAGGTTACAGTAAGCCGAGATCGCGC
CACTGCACTCCAGCCTGGCGACAGAGCAAGACTCCATCTCAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAACCAGGATGTGTTACCAAGGAAAATTCATTTAC
30 AATGGTTAATTATGTGACAAACATGTCAAGTAATTCATCTGGCTTTGTGTACCAATT
TCCCCACCCTTTTTTCAGAAACCAAAACCAAGAAGAACAACATCAAAATGGA
CATGGAAATTAACAAATATATGATTCAATTTAATCTCCTAAGAGGTTTTTTAAAATT

ATTTTATTTTGAGACGGAGTCTTGCTCTGTGCGCCAGGCTGGAGTGCAGTGGCAGGAT
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AAG

CLAIMS

What is claimed is:

1. A method of modulating respiratory chain uncoupling in a cell, the method comprising contacting the cell with a miRNA agent that modulates activity of at least one mitochondrial uncoupler.
5
2. The method of claim 1, wherein the cell is a pre-adipocyte, adipocyte, adipose tissue derived mesenchymal stem cell, hepatocyte, myocyte, or a precursor thereof.
- 10 3. A method of modulating thermogenesis in a tissue, the method comprising contacting the tissue with a miRNA agent that modulates activity of at least one mitochondrial uncoupler.
4. The method of claim 3, wherein the tissue is brown fat, white fat, subcutaneous adipose tissue, liver or muscle.
15
5. The method of claim 4, wherein the tissue is contacted with the miRNA agent ex vivo.
6. A method of treating obesity in human subject in need of treatment thereof, the method comprising administering to the human subject an effective amount of a miRNA agent that
20 modulates activity or expression of at least one mitochondrial uncoupler.
7. The method of claim 6, wherein the human subject selected for treatment has a genetic or epigenetic predisposition to obesity.
- 25 8. The method of any one of the preceding claims, wherein the mitochondrial uncoupler is UCP1 or UCP2.
9. The method of any one of the preceding claims, wherein the miRNA agent is a miRNA selected from the group consisting of hsa-miR-1-1, hsa-miR-1-2, miR-19a-b, hsa-miR-105, hsa-miR-1283, hsa-mir-129, hsa-miR-133a-1, hsa-miR-133a-2, hsa-miR-143, hsa-mir-143-
30 5p, hsa-mir-147, hsa-mir-149, hsa-mir-199a, hsa-mir-199b, hsa-mir-200c, hsa-mir-204, hsa-

mir-205, hsa-miR-206, hsa-mir-21, hsa-mir-211, hsa-mir-218, hsa-mir-218-1, hsa-mir-218-2, hsa-mir-219-2, hsa-mir-219-2-3p, hsa-mir-22, hsa-mir-22-3p, hsa-mir-22-5p, hsa-mir-24-2, hsa-miR-30a-e, hsa-miR-3177-5p, hsa-mir-325, hsa-mir-331, hsa-mir-331-5p, hsa-miR-3613-3p, hsa-mir-362, hsa-mir-362-5p, hsa-miR-3658, hsa-mir-367, hsa-mir-371, hsa-mir-371-5p, hsa-mir-377, hsa-mir-378, hsa-mir-378a-5p, hsa-mir-382, hsa-mir-383, hsa-mir-422a, hsa-mir-425, hsa-miR-455-3p, hsa-miR-455-5p, hsa-miR-491, hsa-mir-508, hsa-mir-508-5p, hsa-mir-512-1, hsa-mir-512-2, hsa-miR-515-3p, hsa-mir-519e, hsa-miR-520a, hsa-mir-543, hsa-mir-545, hsa-mir-549, hsa-mir-556, and hsa-miR-568, hsa-mir-620, hsa-mir-643, hsa-mir-654-3p, hsa-miR-7a-g, hsa-mir-765, hsa-mir-871, hsa-mir-888, hsa-mir-888-3p, hsa-mir-92b, hsa-mir-93, hsa-mir-96, and hsa-mir-99a.

10. The method of any one of the preceding claims, wherein the miRNA agent is a miRNA selected from the group consisting of the miRNAs set forth in Tables 1, 11, 13 and 14.

11. The method of any one of the preceding claims, wherein the miRNA agent is an agomir or antagomir of a miRNA selected from the group consisting of hsa-miR-1-1, hsa-miR-1-2, miR-19a-b, hsa-miR-105, hsa-miR-1283, hsa-mir-129, hsa-miR-133a-1, hsa-miR-133a-2, hsa-miR-143, hsa-mir-143-5p, hsa-mir-147, hsa-mir-149, hsa-mir-199a, hsa-mir-199b, hsa-mir-200c, hsa-mir-204, hsa-mir-205, hsa-miR-206, hsa-mir-21, hsa-mir-211, hsa-mir-218, hsa-mir-218-1, hsa-mir-218-2, hsa-mir-219-2, hsa-mir-219-2-3p, hsa-mir-22, hsa-mir-22-3p, hsa-mir-22-5p, hsa-mir-24-2, hsa-miR-30a-e, hsa-miR-3177-5p, hsa-mir-325, hsa-mir-331, hsa-mir-331-5p, hsa-miR-3613-3p, hsa-mir-362, hsa-mir-362-5p, hsa-miR-3658, hsa-mir-367, hsa-mir-371, hsa-mir-371-5p, hsa-mir-377, hsa-mir-378, hsa-mir-378a-5p, hsa-mir-382, hsa-mir-383, hsa-mir-422a, hsa-mir-425, hsa-miR-455-3p, hsa-miR-455-5p, hsa-miR-491, hsa-mir-508, hsa-mir-508-5p, hsa-mir-512-1, hsa-mir-512-2, hsa-miR-515-3p, hsa-mir-519e, hsa-miR-520a, hsa-mir-543, hsa-mir-545, hsa-mir-549, hsa-mir-556, and hsa-miR-568, hsa-mir-620, hsa-mir-643, hsa-mir-654-3p, hsa-miR-7a-g, hsa-mir-765, hsa-mir-871, hsa-mir-888, hsa-mir-888-3p, hsa-mir-92b, hsa-mir-93, hsa-mir-96, and hsa-mir-99a.

12. The method of any one of the preceding claims, wherein the miRNA agent is an agomir or antagomir of a miRNA selected from the group consisting of the miRNA set forth in Tables 1, 11, 13 and 14.

13. The method of any one of the preceding claims wherein the miRNA agent is an antagomir of a miRNA selected from the group consisting of hsa-miR-19b-2-5p, hsa-miR-21-5p, hsa-miR-130b-5p, hsa-miR-211, hsa-miR-325, hsa-miR-382-3p/5p, hsa-miR-543, hsa-miR-515-3p, and hsa-miR-545.
14. The method of any one of the preceding claims wherein the miRNA agent is an antagomir of a miRNA selected from the group consisting of hsa-miR-331-5p, hsa-miR-552, hsa-miR-620, and hsa-miR-1179.
15. The method of any one of the preceding claims, wherein the miRNA agent is linked to targeting moiety.
16. The method of any one of the preceding claims, wherein the targeting moiety is an aptamer.
17. The method of any one of the preceding claims, wherein the targeting moiety delivers the miRNA agent to a specific cell type or tissue.
18. The method of any one of the preceding claims, wherein the miRNA agent directly binds to the mRNA or promoter region of at least one mitochondrial uncoupler.
19. The method of any one of the preceding claims, wherein the miRNA agent directly binds to the 5'UTR or coding sequence of the mRNA of at least one mitochondrial uncoupler.
20. The method of any one of the preceding claims, wherein the miRNA agent modulates the activity of an activator or repressor of a mitochondrial uncoupling protein.
21. The method of claim 18, wherein the activator or repressor is selected from the group consisting of the activators or repressors set forth in Table 2.
22. The method of claim 20 or 21, wherein the miRNA agent directly binds to the mRNA or promoter region of the activator or repressor.

23. The method of claim 20 or 21, wherein the miRNA agent directly binds to the 5'UTR or coding sequence of the mRNA of the activator or repressor.
- 5 24. The method of any one of the preceding claims, wherein the mRNA or protein expression of the mitochondrial uncoupling protein is upregulated.
25. The method of any one of the preceding claims, wherein the mitochondrial uncoupling activity of the mitochondrial uncoupling protein is upregulated.
- 10 26. A method of screening for a miRNA agent that modulates thermogenesis, the method comprising:
- a) providing an indicator cell comprising a human genome;
 - b) contacting the indicator cell with a test miRNA agent; and
 - 15 c) determining the cellular activity of at least one thermogenic regulator in the indicator cell in the presence and absence of the miRNA agent, wherein a change in the activity of the thermogenic regulator in the presence of the test miRNA agent identifies the test miRNA agent as a miRNA agent that modulates thermogenesis.
- 20 27. The method of claim 26, wherein the cell is an adipocyte, adipose tissue derived mesenchymal stem cell, hepatocyte, myocyte, or a precursor thereof.
28. The method of claim 26, wherein the cellular activity of the thermogenic regulator determined in step (c) is the mRNA expression level, protein expression level or
- 25 mitochondrial uncoupling activity of the thermogenic regulator.
29. The method of any one of the preceding claims, wherein the thermogenic regulator is UCP1.
30. An agomir or antagomir that modulates the activity of at least one thermogenic regulator in
- 30 a cell.

31. The agomir or antagomir of claim 30, which is an agomir or antagomir of a miRNA selected from the group consisting of the miRNA set forth in Tables 1, 11, 13 and 14.

32. The agomir or antagomir of claim 30, which is an agomir or antagomir of a miRNA
5 selected from the group consisting of hsa-miR-1-1, hsa-miR-1-2, miR-19a-b, hsa-miR-105, hsa-miR-1283, hsa-mir-129, hsa-miR-133a-1, hsa-miR-133a-2, hsa-miR-143, hsa-mir-143-5p, hsa-mir-147, hsa-mir-149, hsa-mir-199a, hsa-mir-199b, hsa-mir-200c, hsa-mir-204, hsa-mir-205, hsa-miR-206, hsa-mir-21, hsa-mir-211, hsa-mir-218, hsa-mir-218-1, hsa-mir-218-2, hsa-mir-219-2, hsa-mir-219-2-3p, hsa-mir-22, hsa-mir-22-3p, hsa-mir-22-5p, hsa-mir-24-
10 2, hsa-miR-30a-e, hsa-miR-3177-5p, hsa-mir-325, hsa-mir-331, hsa-mir-331-5p, hsa-miR-3613-3p, hsa-mir-362, hsa-mir-362-5p, hsa-miR-3658, hsa-mir-367, hsa-mir-371, hsa-mir-371-5p, hsa-mir-377, hsa-mir-378, hsa-mir-378a-5p, hsa-mir-382, hsa-mir-383, hsa-mir-422a, hsa-mir-425, hsa-miR-455-3p, hsa-miR-455-5p, hsa-miR-491, hsa-mir-508, hsa-mir-508-5p, hsa-mir-512-1, hsa-mir-512-2, hsa-miR-515-3p, hsa-mir-519e, hsa-miR-520a, hsa-
15 mir-543, hsa-mir-545, hsa-mir-549, hsa-mir-556, and hsa-miR-568, hsa-mir-620, hsa-mir-643, hsa-mir-654-3p, hsa-miR-7a-g, hsa-mir-765, hsa-mir-871, hsa-mir-888, hsa-mir-888-3p, hsa-mir-92b, hsa-mir-93, hsa-mir-96, and hsa-mir-99a.

33. The agomir or antagomir of claim 30, which is an antagomir of a miRNA selected from the
20 group consisting of hsa-miR-19b-2-5p, hsa-miR-21-5p, hsa-miR-130b-5p, hsa-miR-211, hsa-miR-325, hsa-miR-382-3p/5p, hsa-miR-543, hsa-miR-515-3p, and hsa-miR-545.

34. The agomir or antagomir of claim 30, which is an antagomir of a miRNA selected from the
25 group consisting of hsa-miR-331-5p, hsa-miR-552, hsa-miR-620, and hsa-miR-1179.

35. The agomir or antagomir of any one of claims 30-34, wherein the agomir or antagomir is linked to targeting moiety.

36. The agomir or antagomir of claim 35, wherein the targeting moiety is an aptamer.

37. The agomir or antagomir of claim 35 or 36, wherein the targeting moiety delivers the
30 agomir or antagomir to a specific cell type or tissue.

38. The agomir or antagomir of any one of claims 28-33, wherein the agomir or antagomir directly binds to the mRNA or promoter region of at least one mitochondrial uncoupler.
- 5 39. The agomir or antagomir of any one of claims 30-37, wherein the agomir or antagomir directly binds to the 5'UTR or coding sequence of the mRNA of at least one mitochondrial uncoupler.
40. The agomir or antagomir of any one of claims 30-37, wherein the agomir or antagomir
10 modulates the activity of an activator or repressor of a mitochondrial uncoupling protein.
41. The agomir or antagomir of any one of claims 30-37, wherein the activator or repressor is selected from the group consisting of the activators or repressors set forth in Table 2.
- 15 42. The agomir or antagomir of any one of claims 30-37, wherein the agomir or antagomir directly binds to the mRNA or region promoter of the activator or repressor.
43. The agomir or antagomir of any one of claims 30-37, wherein the agomir or antagomir directly binds to the 5'UTR or coding sequence of the mRNA of the activator or repressor.
20
44. A pharmaceutical composition comprising two or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir.
25
45. The pharmaceutical composition of claim 44, further comprising a pharmaceutically acceptable excipient.
46. The pharmaceutical composition of claim 44, wherein the two or more miRNAs are
30 expressed from a recombinant vector.

47. The pharmaceutical composition of claim 47, wherein the recombinant vector is selected from the group consisting of DNA plasmids, viral vectors and DNA minicircles.
- 5 48. The pharmaceutical composition of claim 44, comprising two or more miRNAs selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir, and hsa-miR-30b agomir.
49. The pharmaceutical composition of claim 48, further comprising a pharmaceutically acceptable excipient.
- 10 50. The pharmaceutical composition of claim 48, wherein the two or more miRNAs are expressed from a recombinant vector.
- 15 51. The pharmaceutical composition of claim 48, wherein the recombinant vector is selected from the group consisting of DNA plasmids, viral vectors and DNA minicircles.
52. A method of inducing pre-adipocytes to differentiate into adipocytes comprising administering to a population of pre-adipocytes one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir.
- 20 53. The method of claim 52, wherein the induction of pre-adipocytes to differentiate into adipocytes is greater than the differentiation of pre-adipocytes to adipocytes than when pre-adipocytes are exposed to 100 nM rosiglitazone for two days followed by maintenance medium.
- 25 54. The method of claim 52, wherein the one or more miRNAs are selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir, and hsa-miR-30b agomir.
- 30

55. A method of decreasing the lipid content of adipocytes comprising administering to a population of adipocytes one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir.

5

56. The method of claim 55, wherein the lipid content of the adipocytes is less than the fat content of adipocytes exposed to 100 nM rosiglitazone for two days followed by maintenance medium.

10 57. The method of claim 55, wherein the lipid content of the adipocytes is less than the fat content of adipocytes exposed to 100 nM rosiglitazone for the duration of culture.

58. The method of claim 57, wherein the duration of culture is 8-16 days.

15 59. The method of claim 58, wherein the duration of culture is 10-14 days.

60. The method of claim 59, wherein the duration of culture is 14 days.

20 61. The method of claim 55, wherein the one or more miRNAs are selected from the group consisting of hsa-miR-1 agomir, hsa-miR-19b agomir, and hsa-miR-30b agomir.

25 62. A method for increasing insulin sensitivity in a subject in need thereof comprising administering the subject one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir and hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir.

63. The method of claim 62, wherein the subject is a mammal.

64. The method of claim 63, wherein the mammal is a human.

30

65. The method of claim 62, wherein the one or more miRNAs are selected from the group consisting of hsa-miR-1 agomir, hsa-miR-19b agomir, and hsa-miR-30b agomir.

- 5 66. A method of increasing expression or activity of one or more uncoupling proteins in a cell comprising administering to the cell one or more miRNAs selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir and hsa-miR-30b agomir.
67. The method of claim 66, wherein the cell is selected from the group consisting of a brown fat cell, a white fat cell, a subcutaneous adipocyte, a liver cell or a muscle cell.
- 10 68. The method of claim 66, wherein the one or more uncoupling proteins include UCP-1 or UCP-2.
69. A method of causing fat loss in a subject in need thereof comprising administering the subject one or more miRNAs selected from the group consisting of hsa-let-7a antagomir, 15 hsa-miR-1 agomir, hsa-miR-19b agomir and hsa-miR-30b agomir.
70. The method of claim 69, wherein the subject is a mammal.
71. The method of claim 70, wherein the mammal is a human.
- 20 72. Use of an agomir or antagomir of one or more miRNAs selected from the group consisting of the miRNA set forth in Tables 1, 11, 13 and 14 in the manufacture of a medicament for the treatment of obesity.
- 25 73. The use of claim 72, wherein the one or more miRNAs are selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir, and hsa-miR-30b agomir.
74. The use of claim 72, wherein the agomir or antagomir is linked to targeting moiety.
- 30 75. The use of claim 74, wherein the targeting moiety is an aptamer.

76. A composition comprising an agomir or antagomir of one or more miRNAs selected from the group consisting of the miRNA set forth in Tables 1, 11, 13 and 14 for the treatment of obesity.
- 5 77. The composition of claim 76, wherein the one or more miRNAs are selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir, and hsa-miR-30b agomir.
78. The composition of claim 76, wherein the agomir or antagomir is linked to targeting moiety.
- 10 79. The composition of claim 76, wherein the targeting moiety is an aptamer.

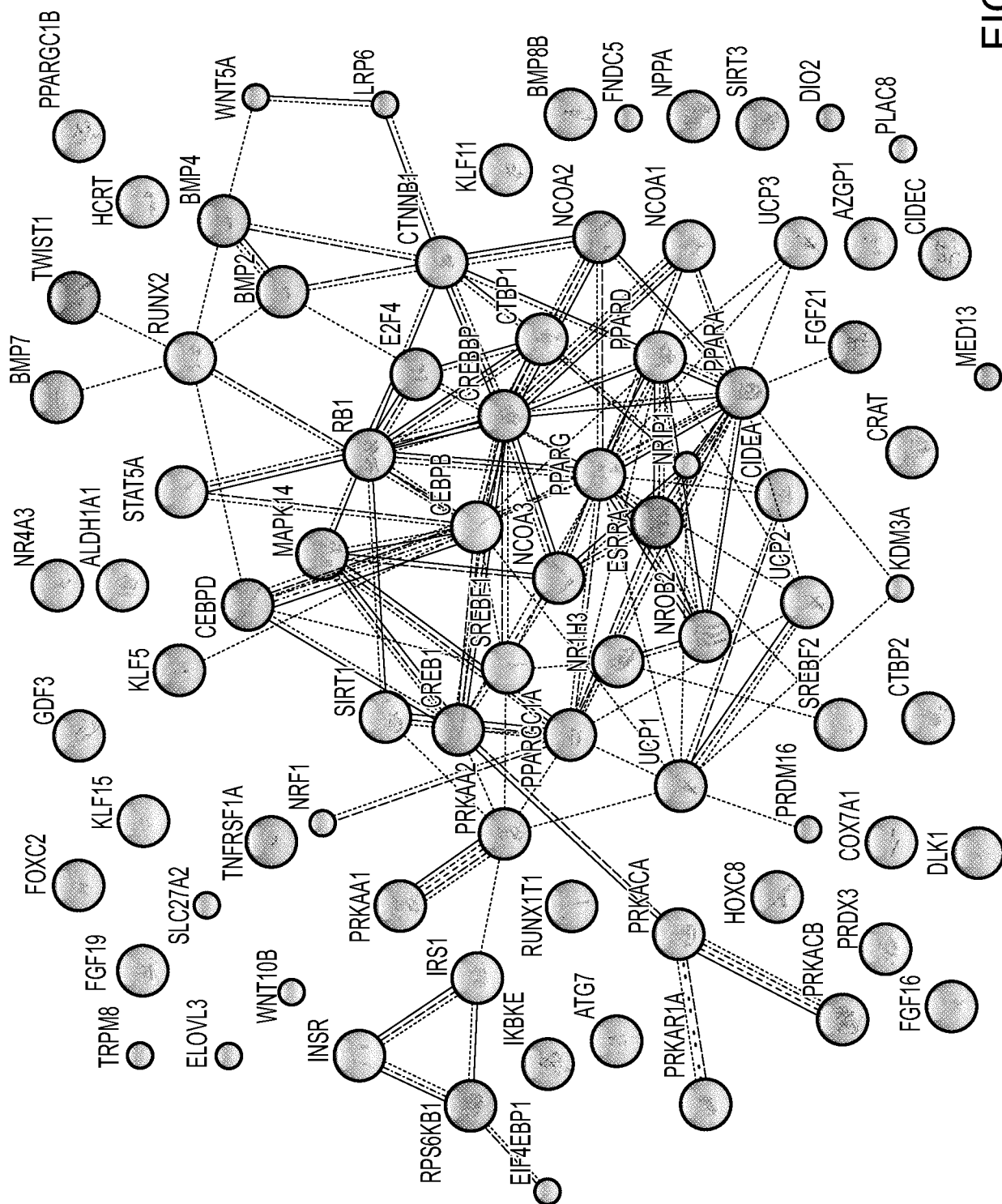


FIG. 1A

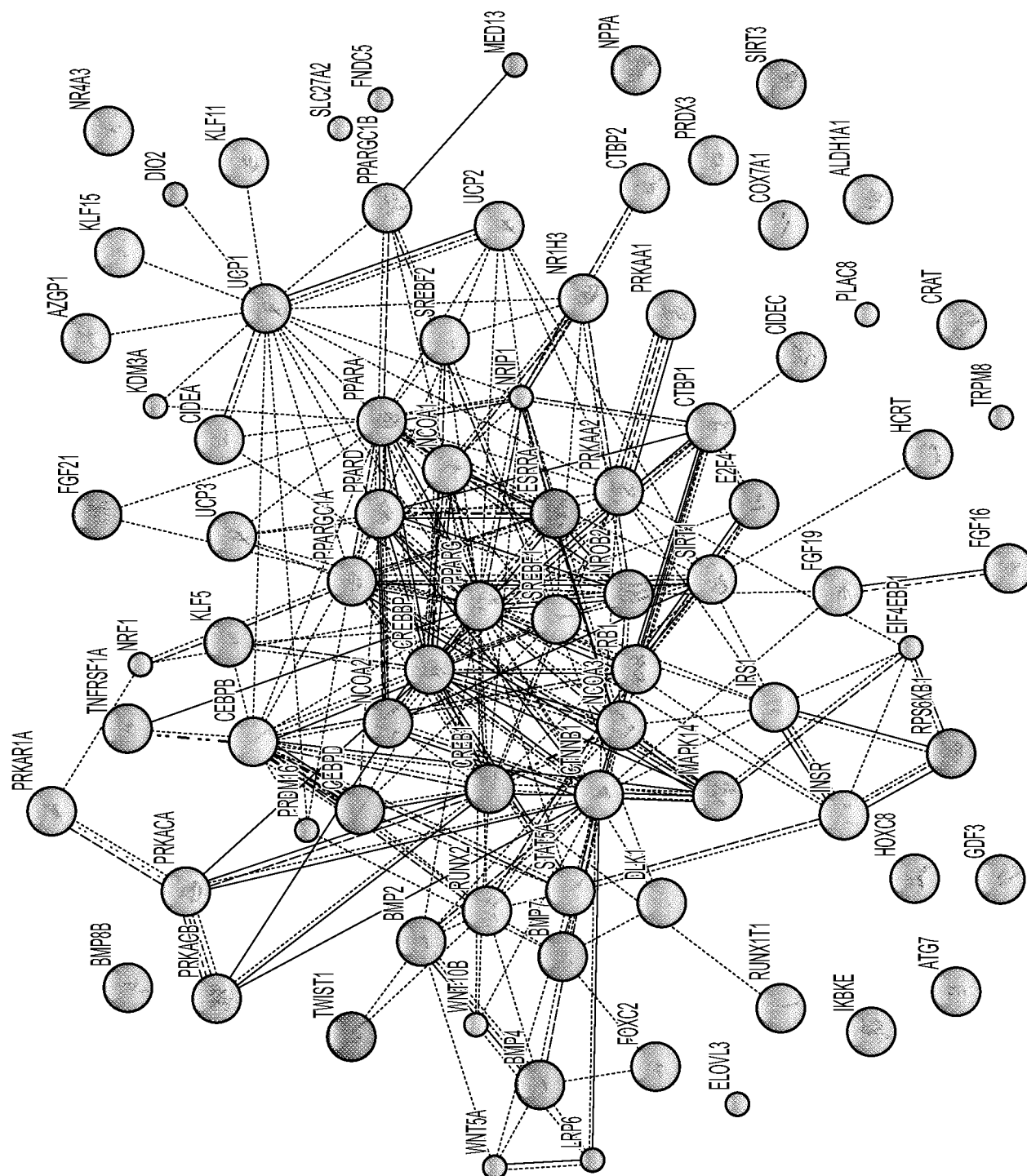


FIG. 1B

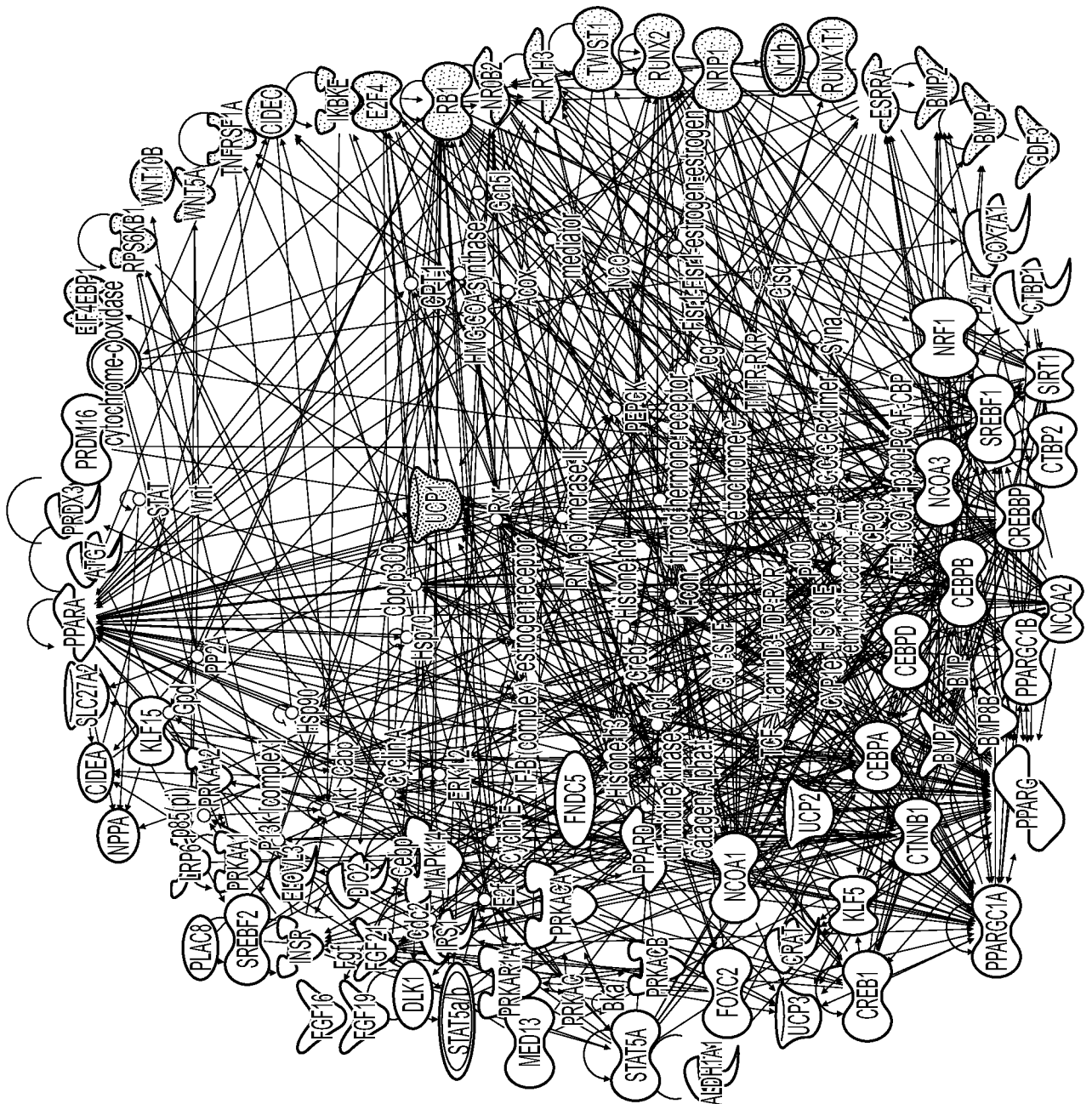


FIG. 2A

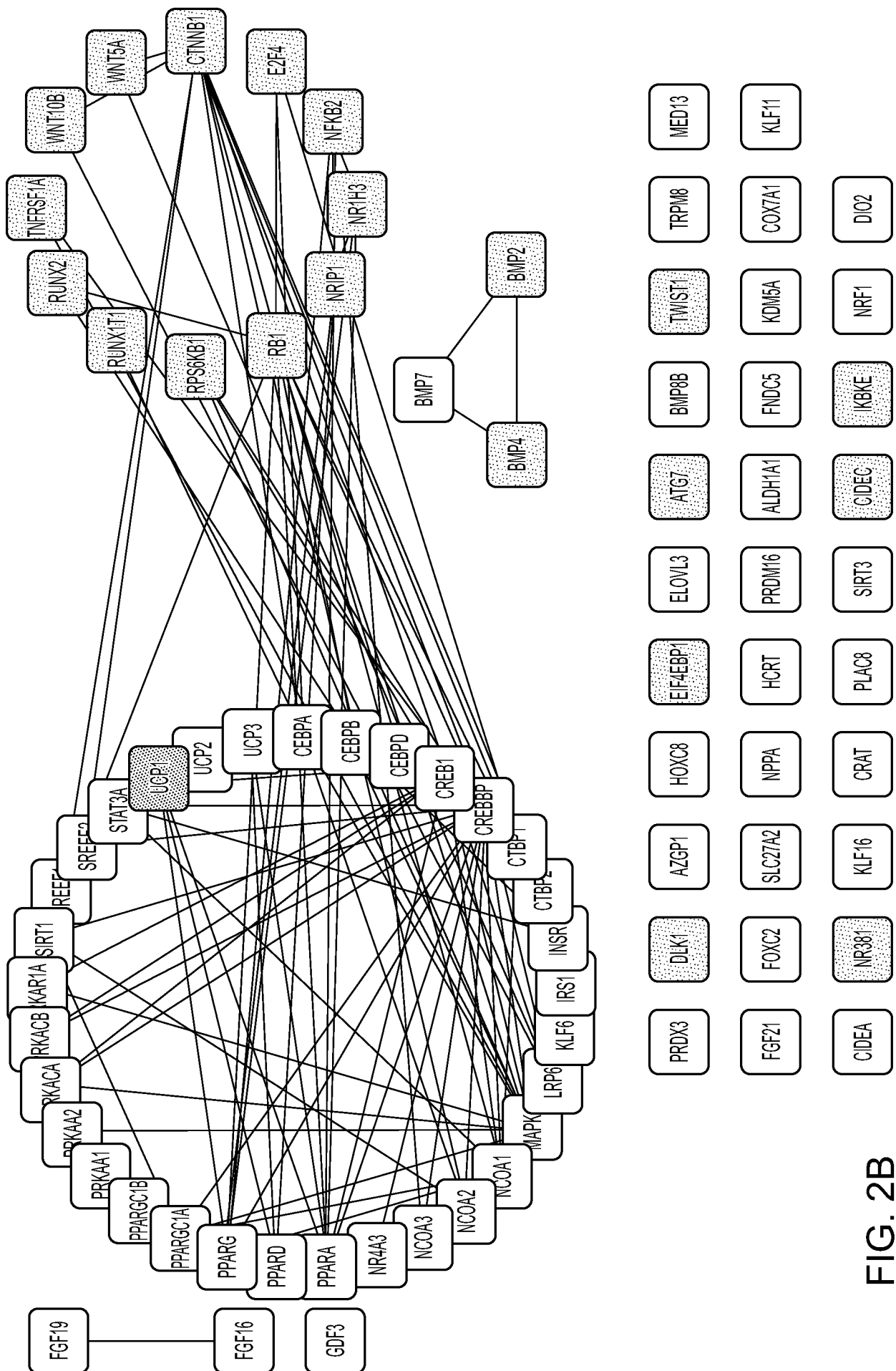


FIG. 2B

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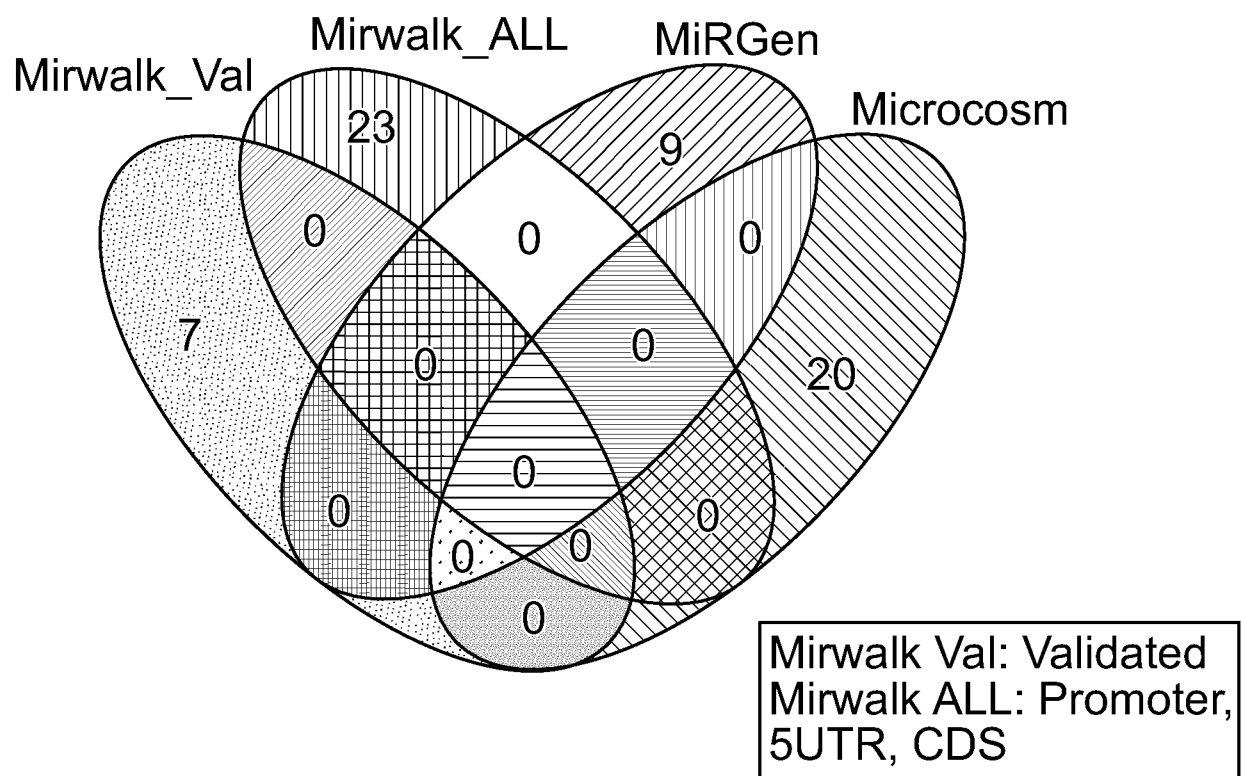


FIG. 3

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Common miR_gene Predictions Between 7 Major Prediction Tools
(Targetscan, Mirwalk, RNA22, PITA, Pictar, Miranda, Diana)

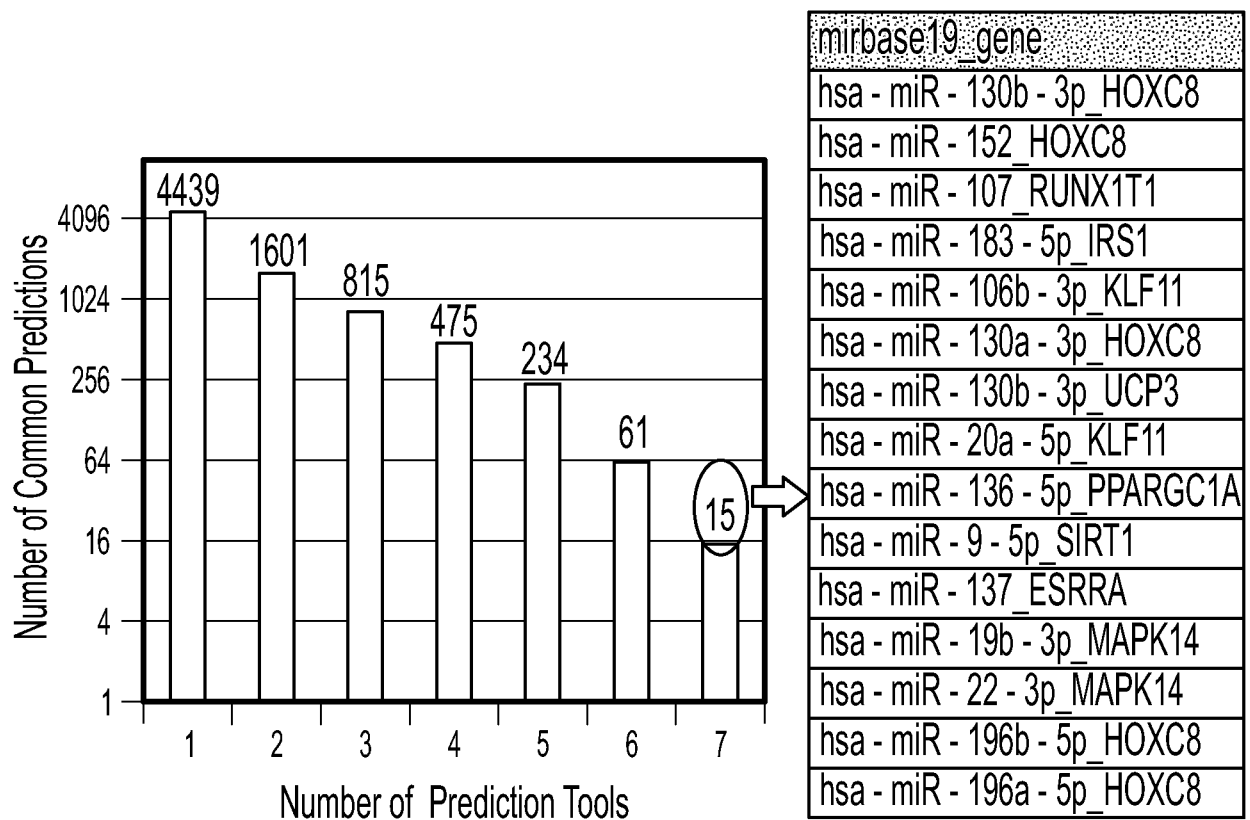


FIG. 4

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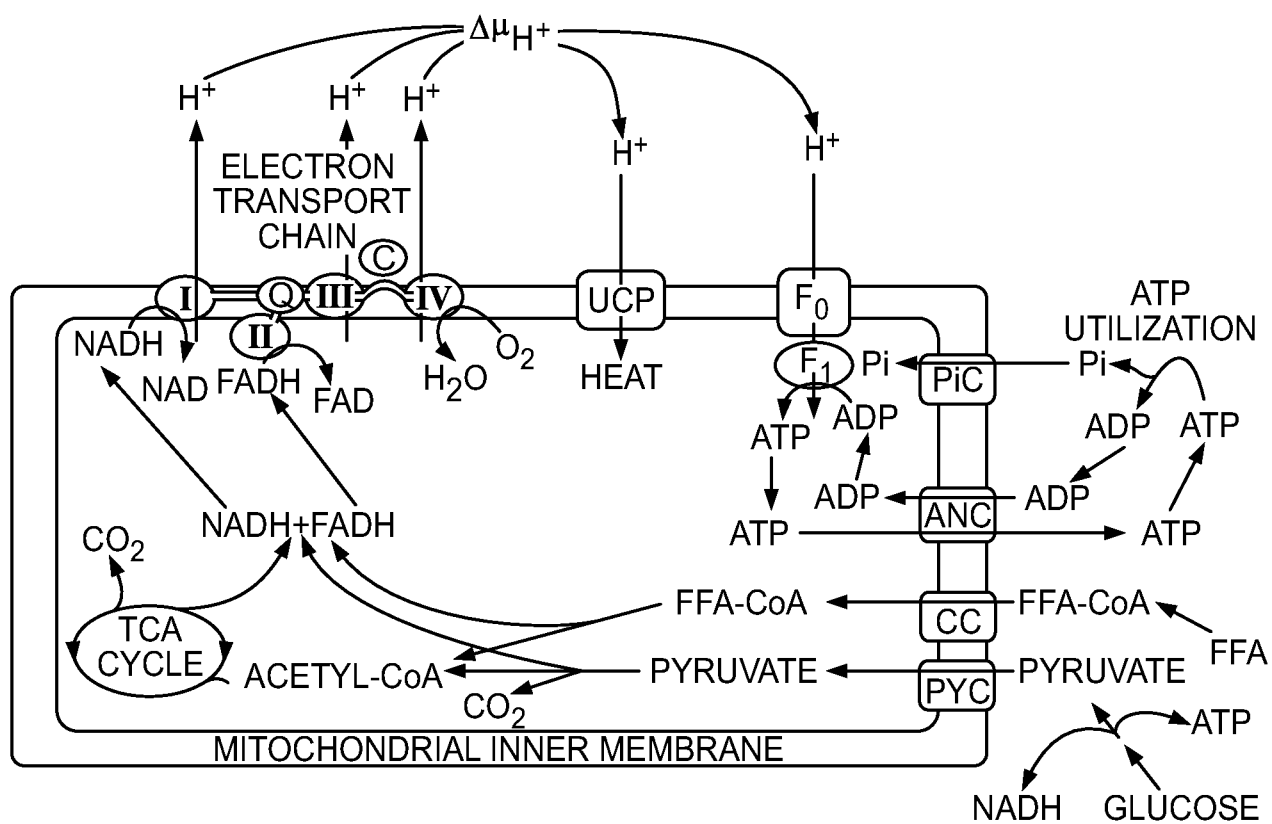


FIG. 5

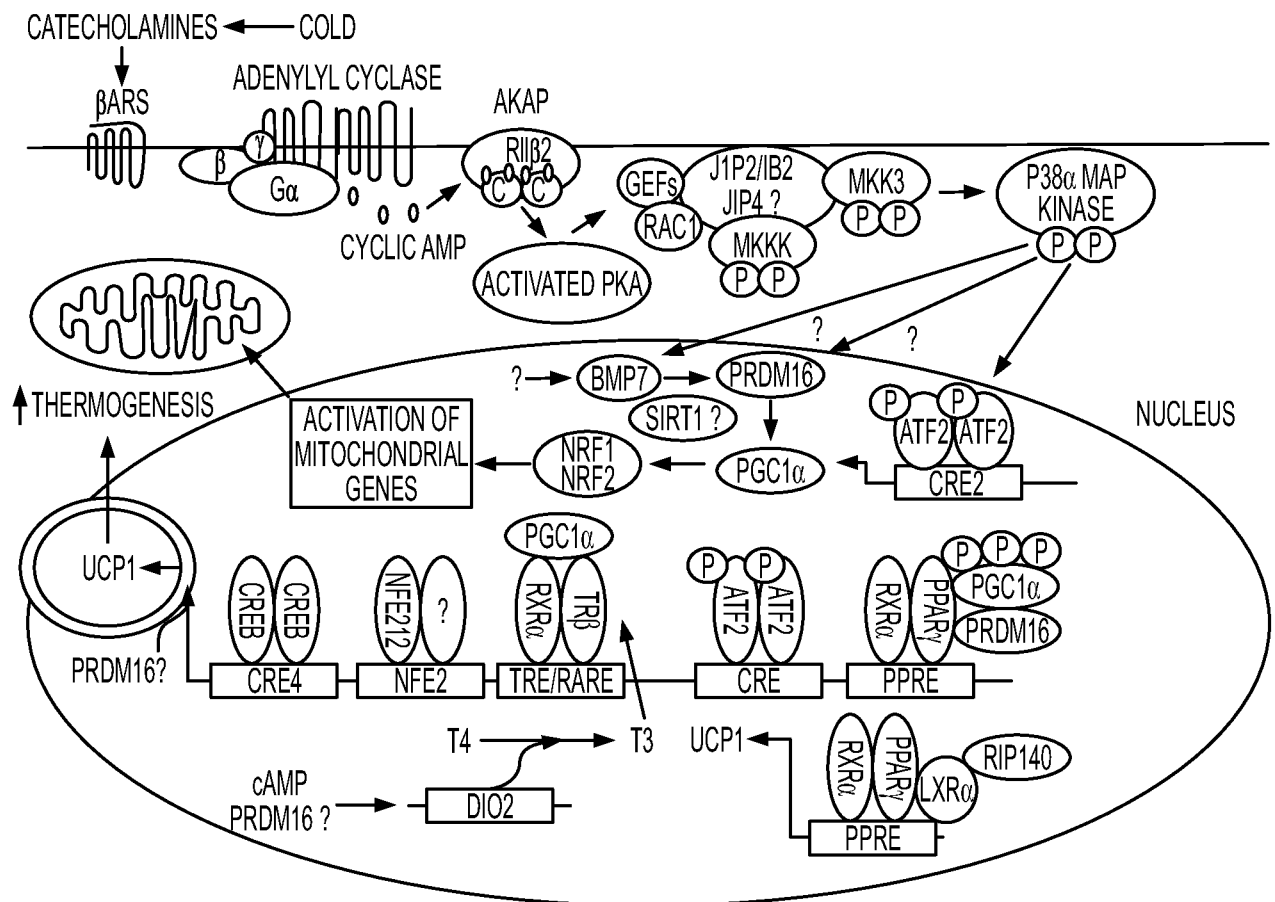


FIG. 6

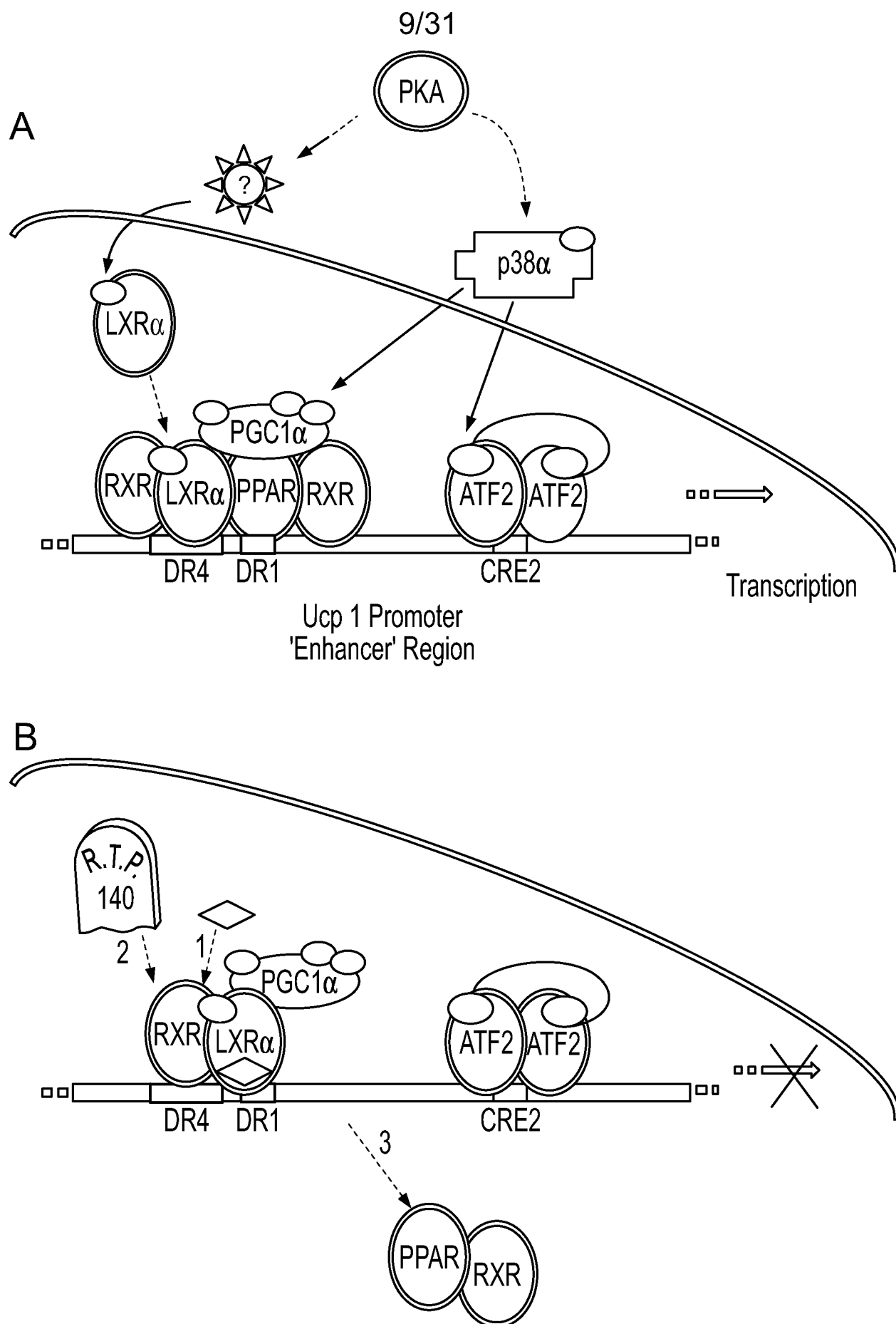


FIG. 7

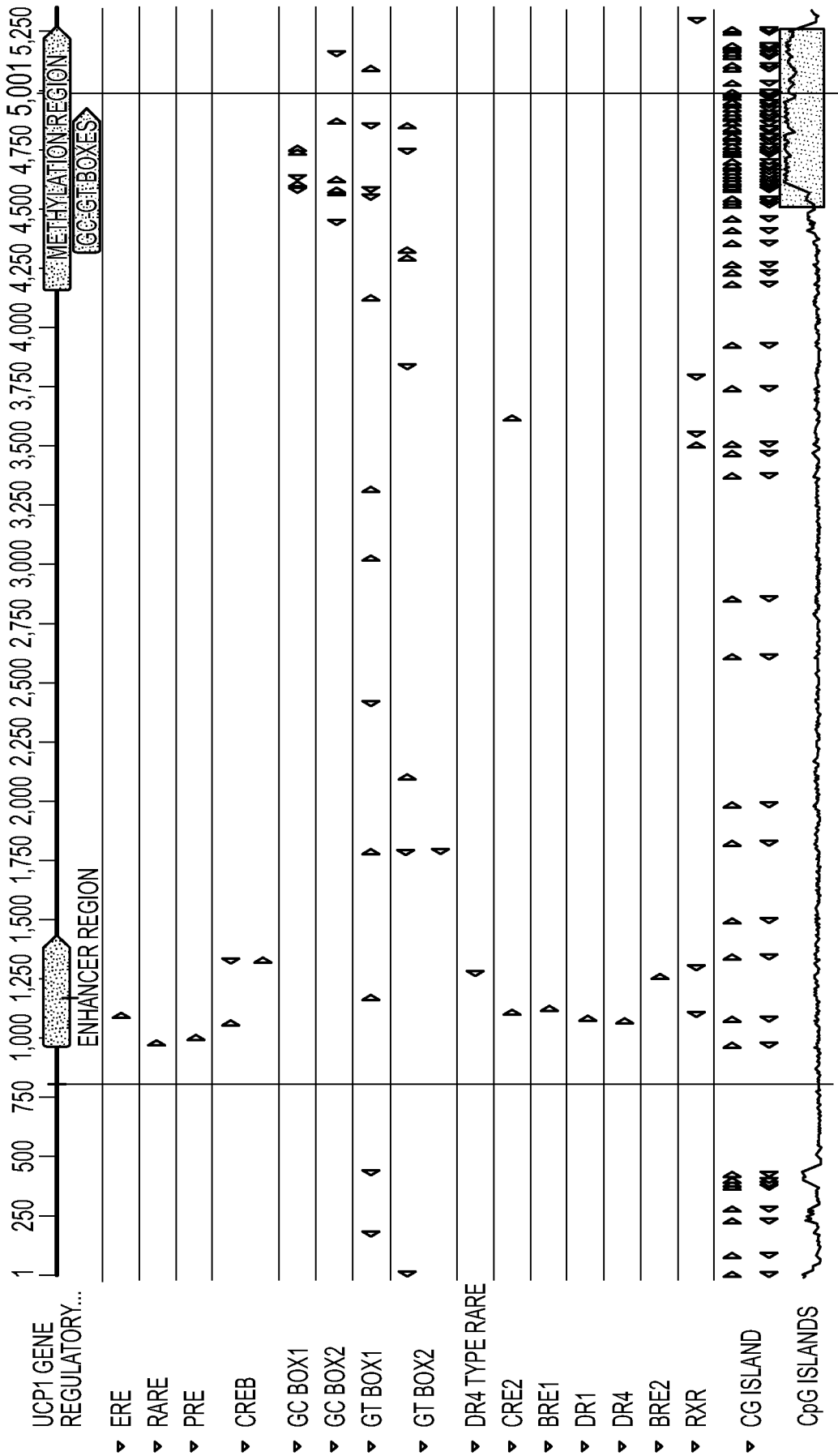


FIG. 8A

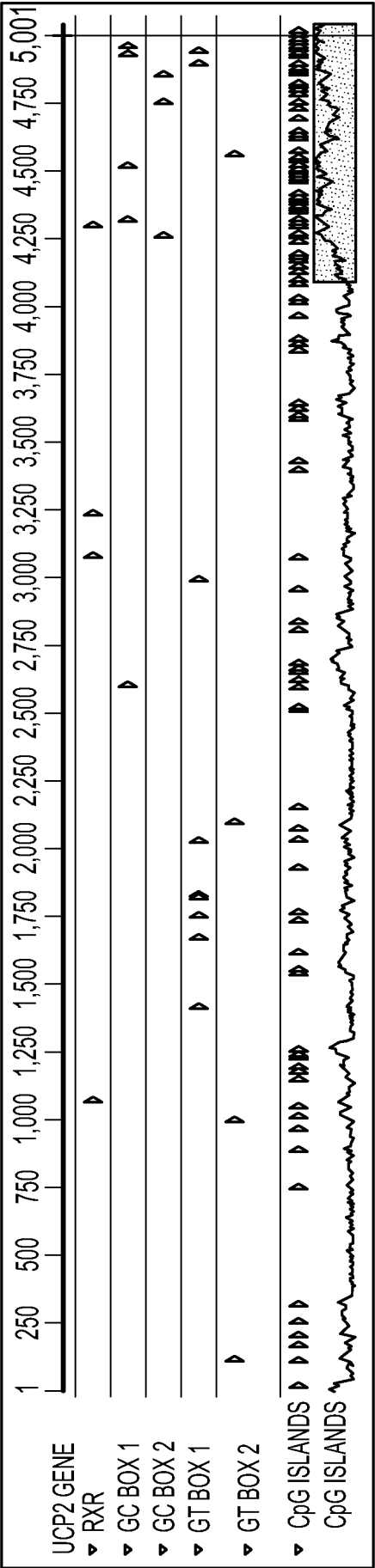
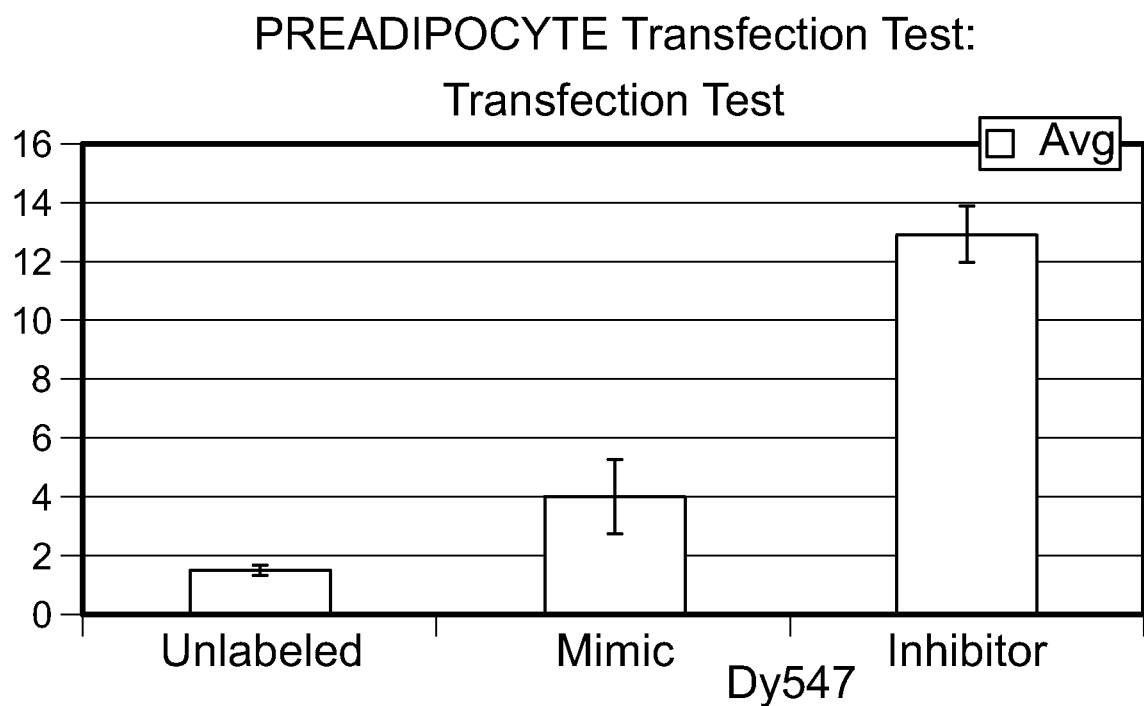


FIG. 8B

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The Dy547 Transfections were Significantly Higher than Unlabeled Transfectants $p < .0001$.

FIG. 9

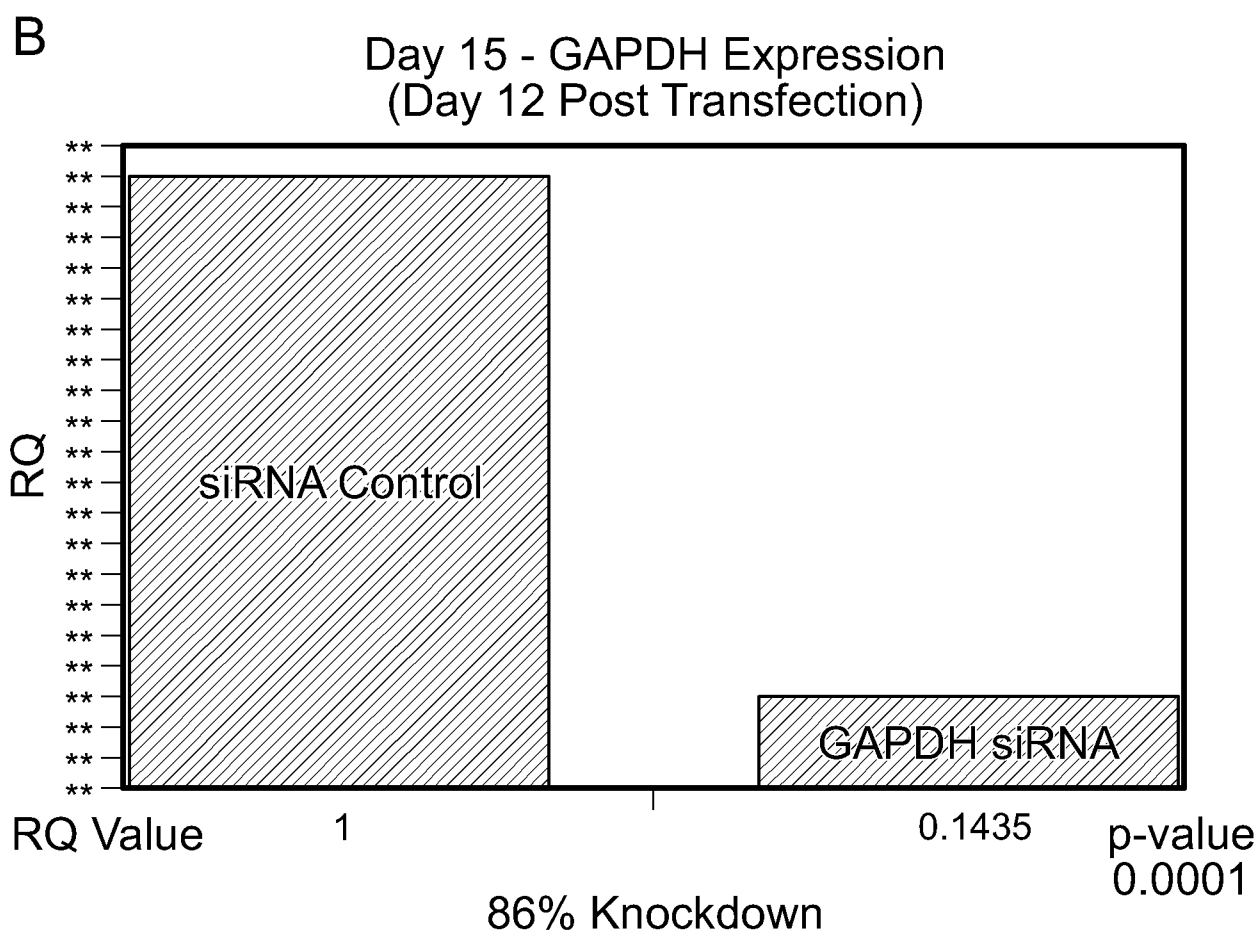
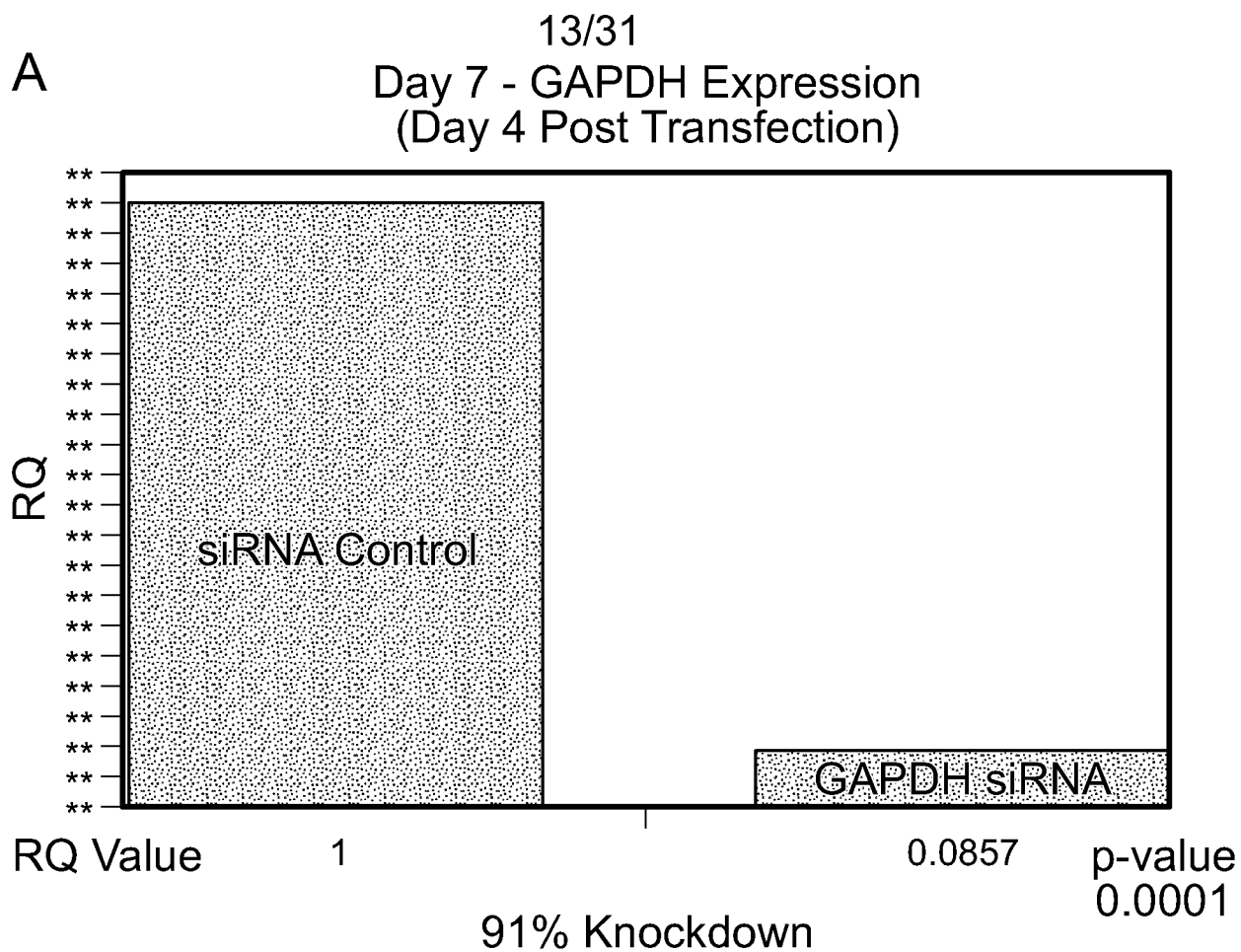
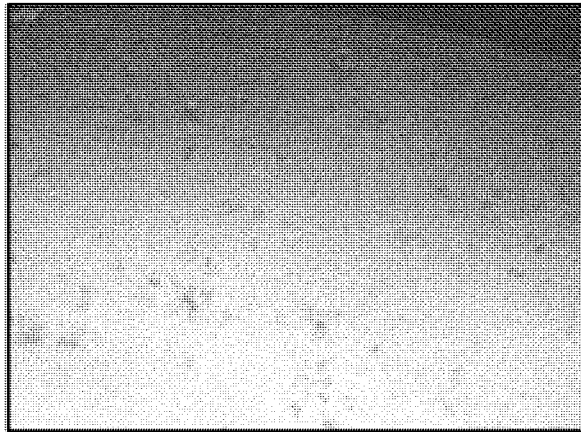


FIG. 10

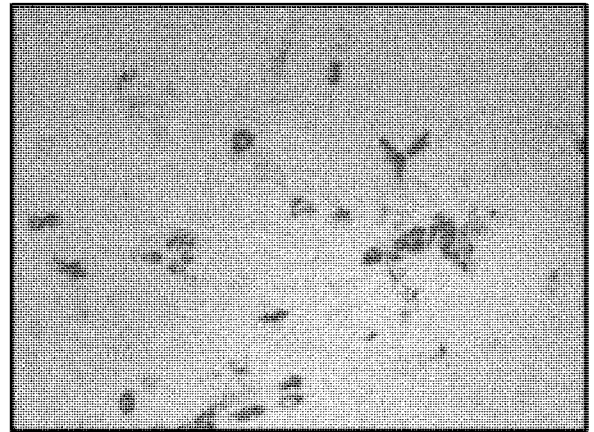
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A

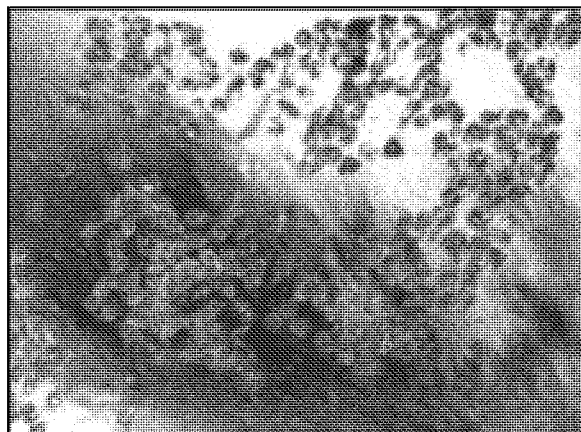


Control

B

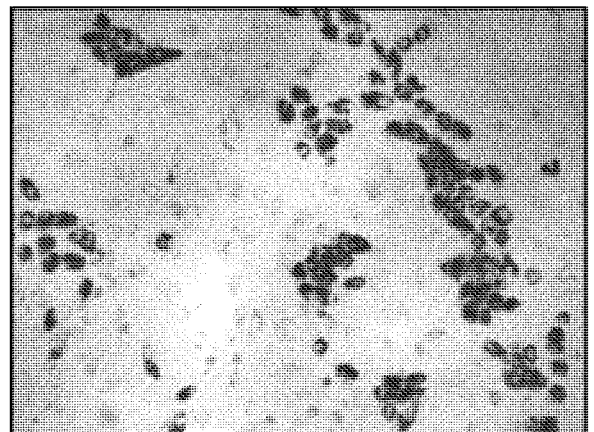
Insulin + T3 + DXM +
IBMX + Rosi for 2 Days

C



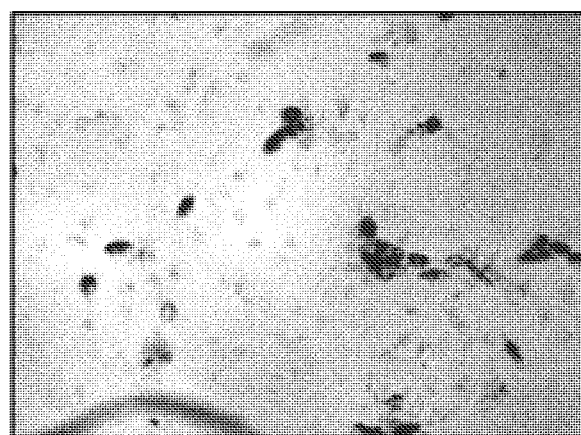
100 nM Rosiglitazone

D



25 nM miR-30b Mimic

E

Non Targeting miRNA
Mimic

F

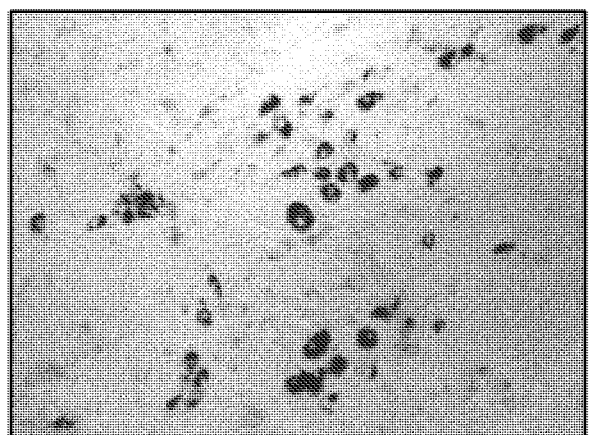
Non Targeting miRNA
Inhibitor

FIG. 11

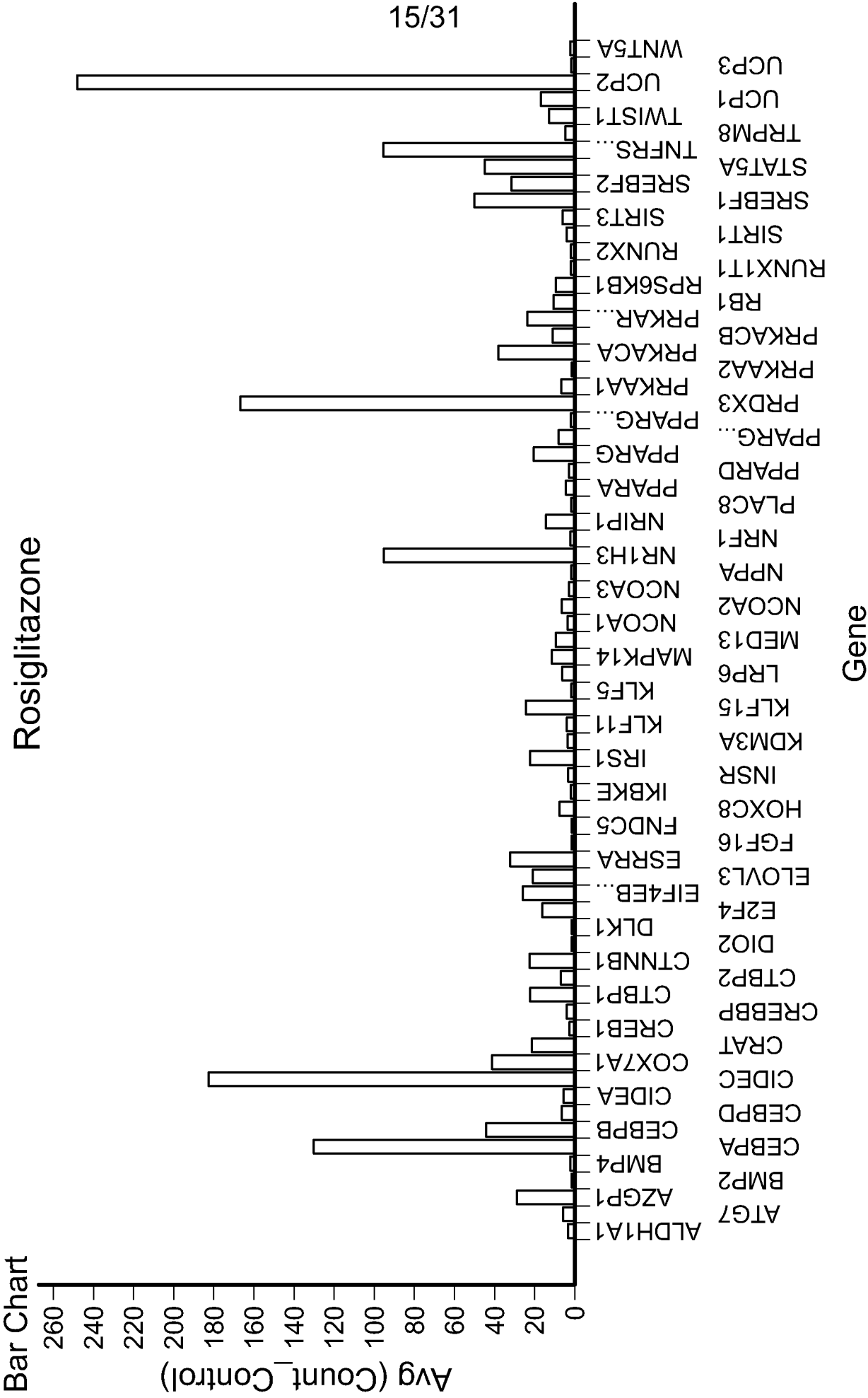


FIG. 12A

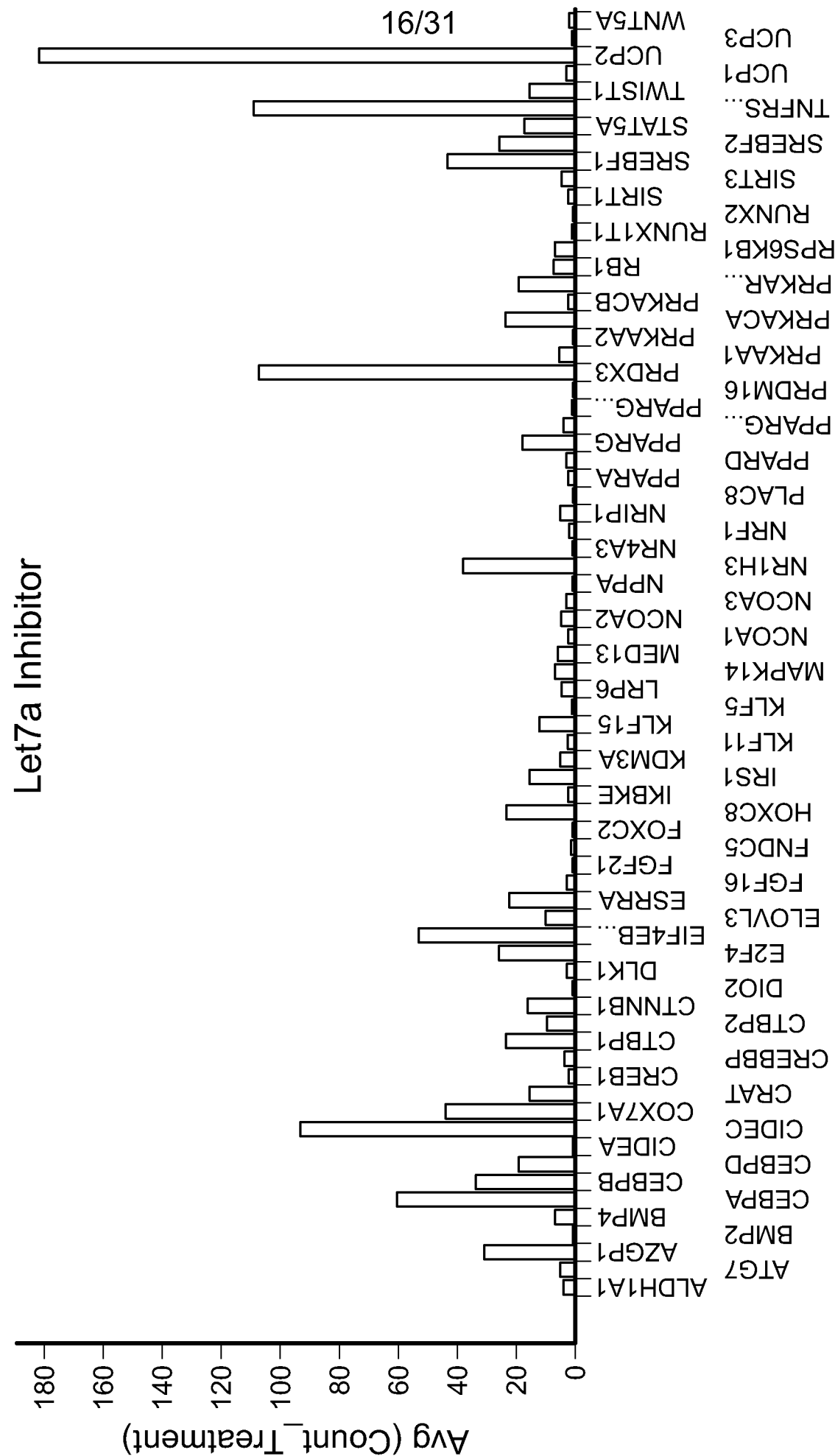


FIG. 12B

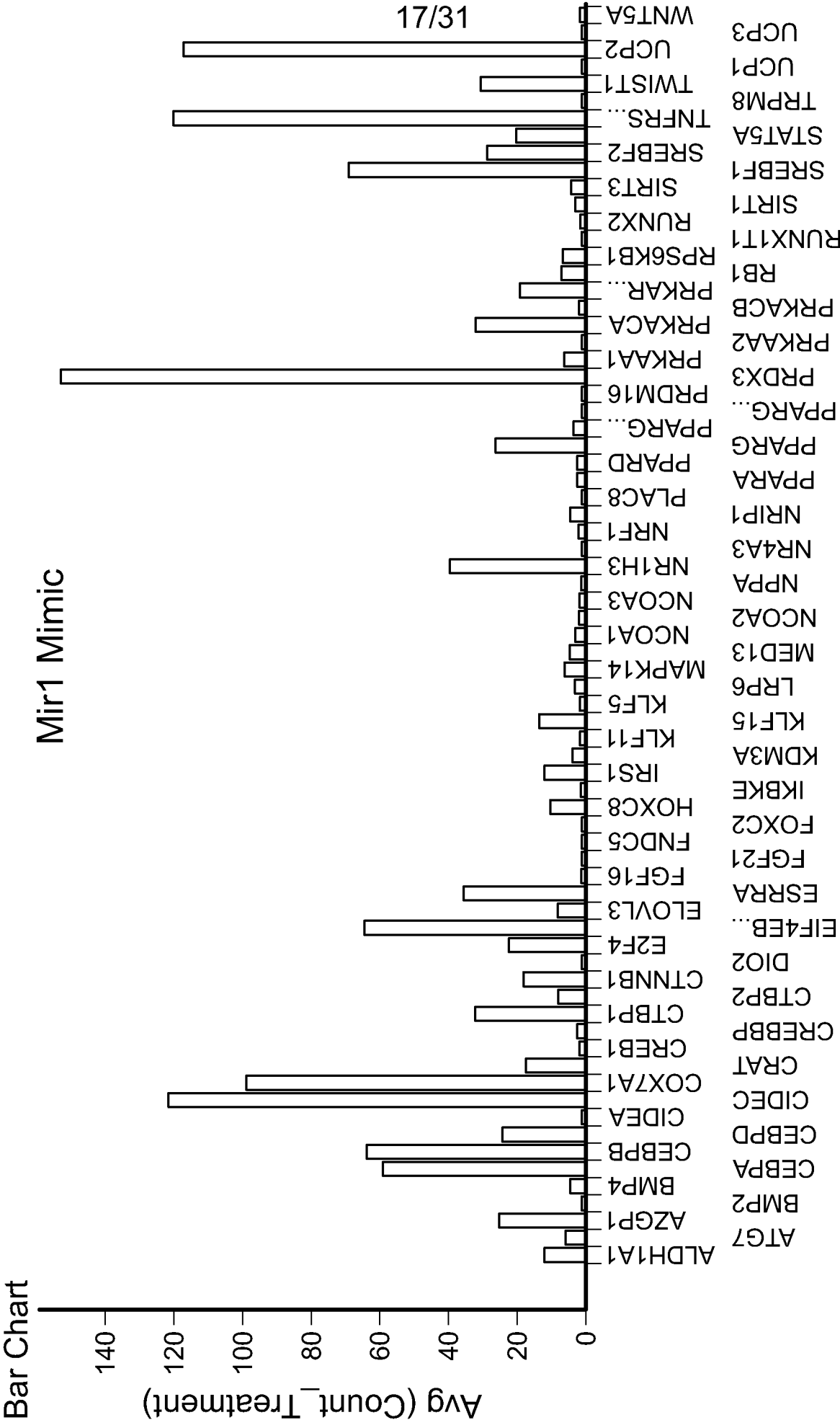


FIG. 12C

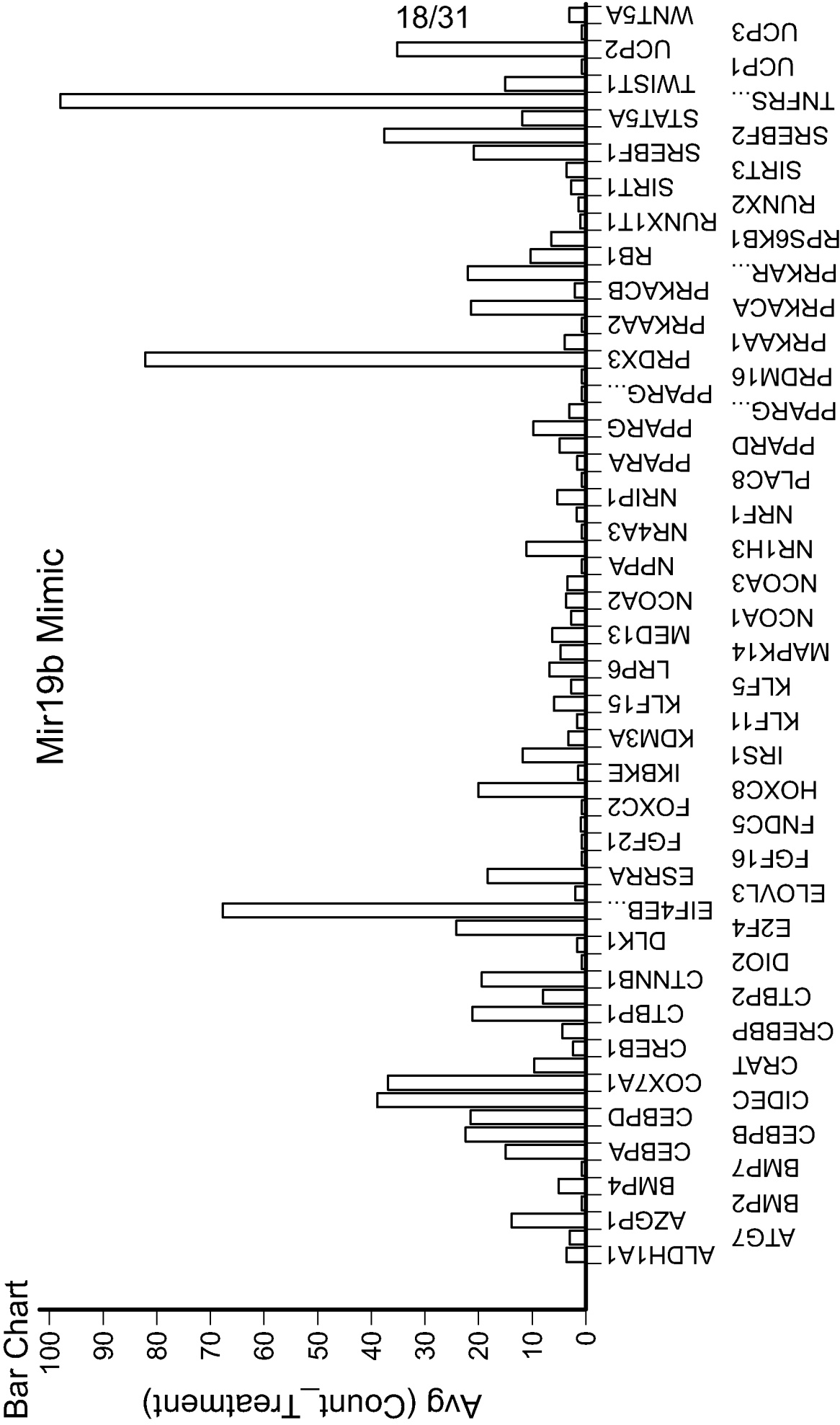
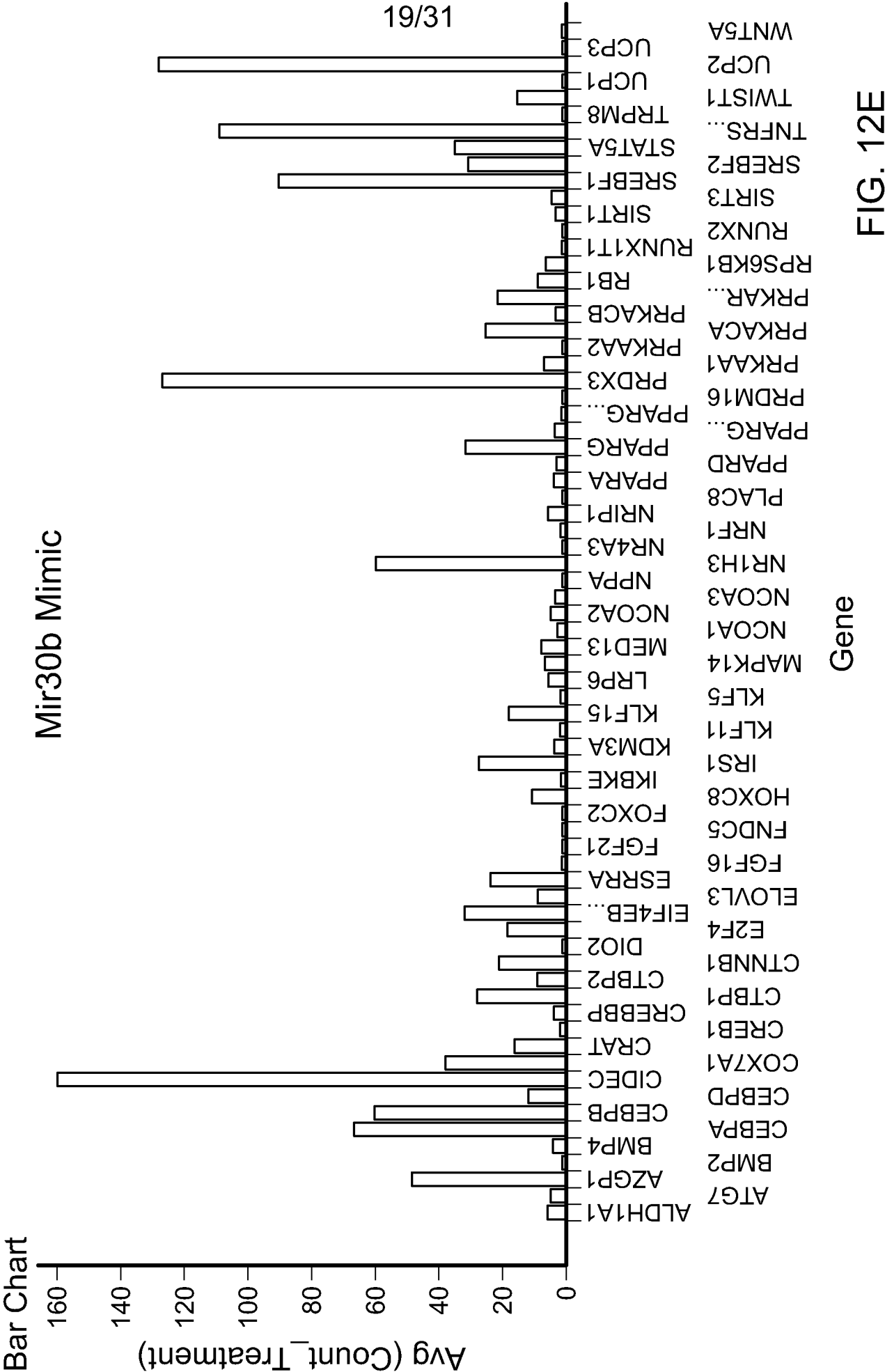
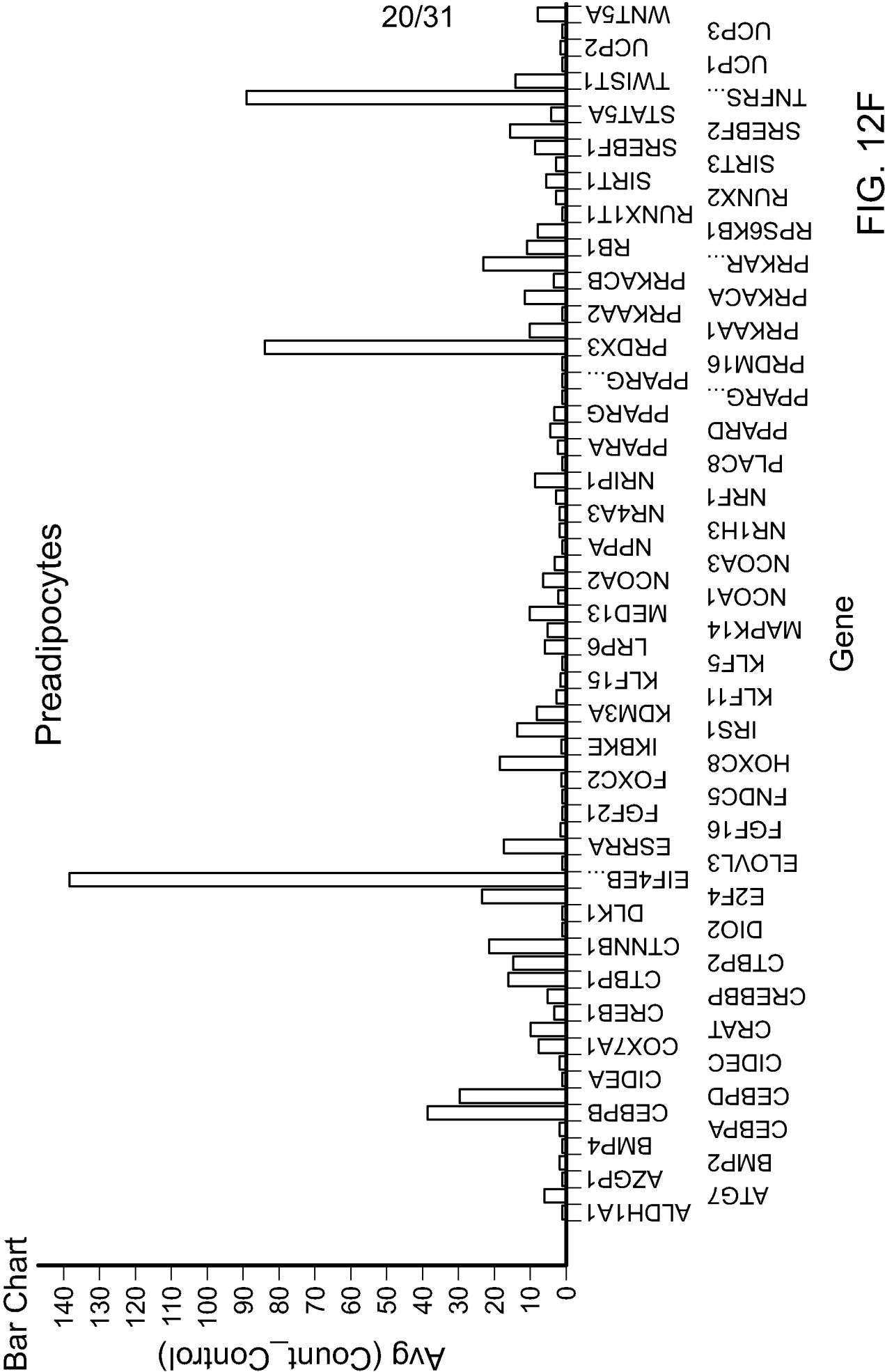


FIG. 12D





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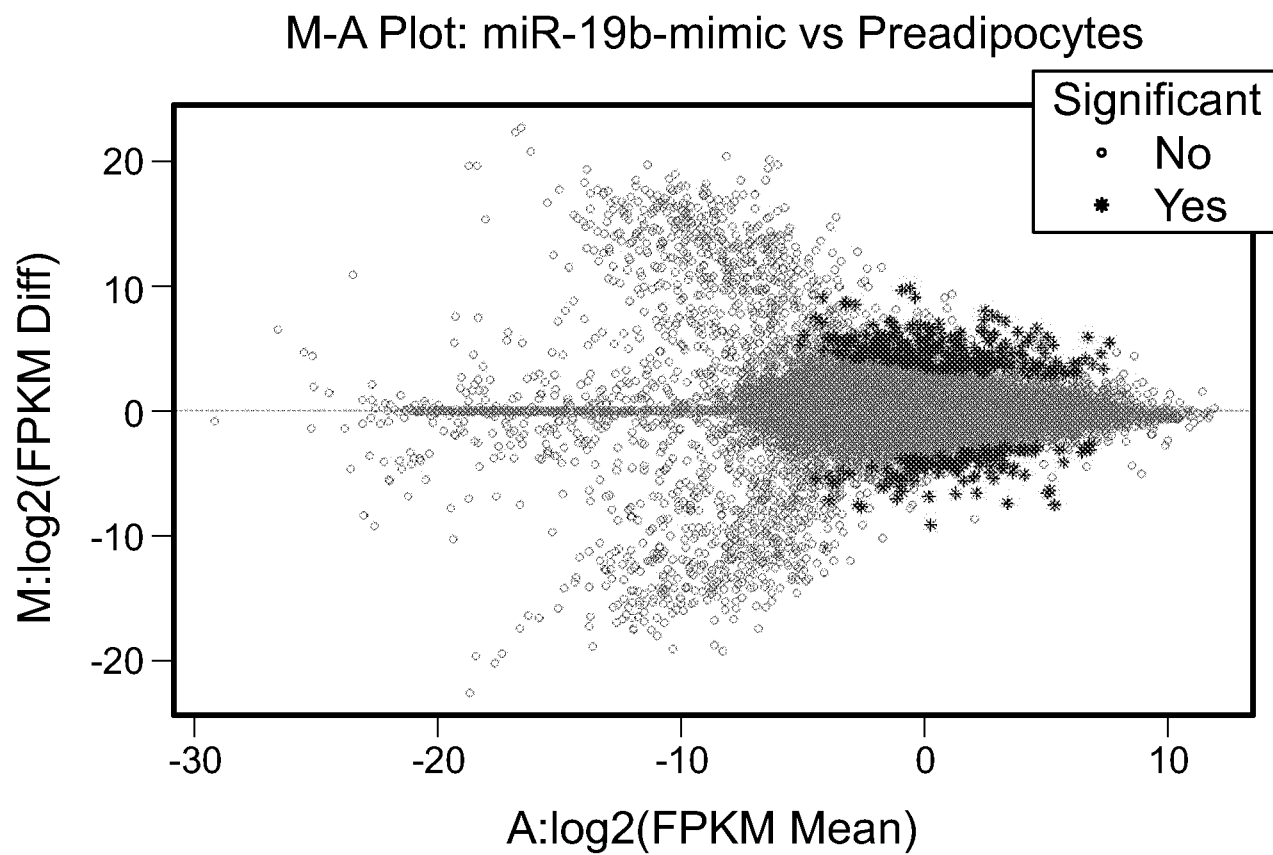


FIG. 13

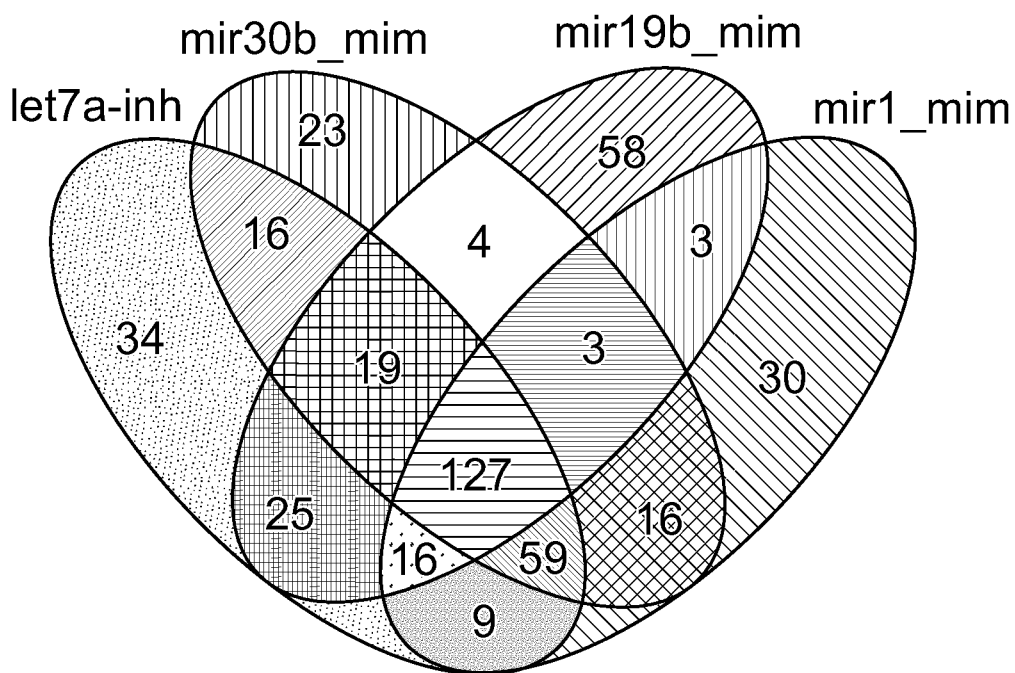


FIG. 14

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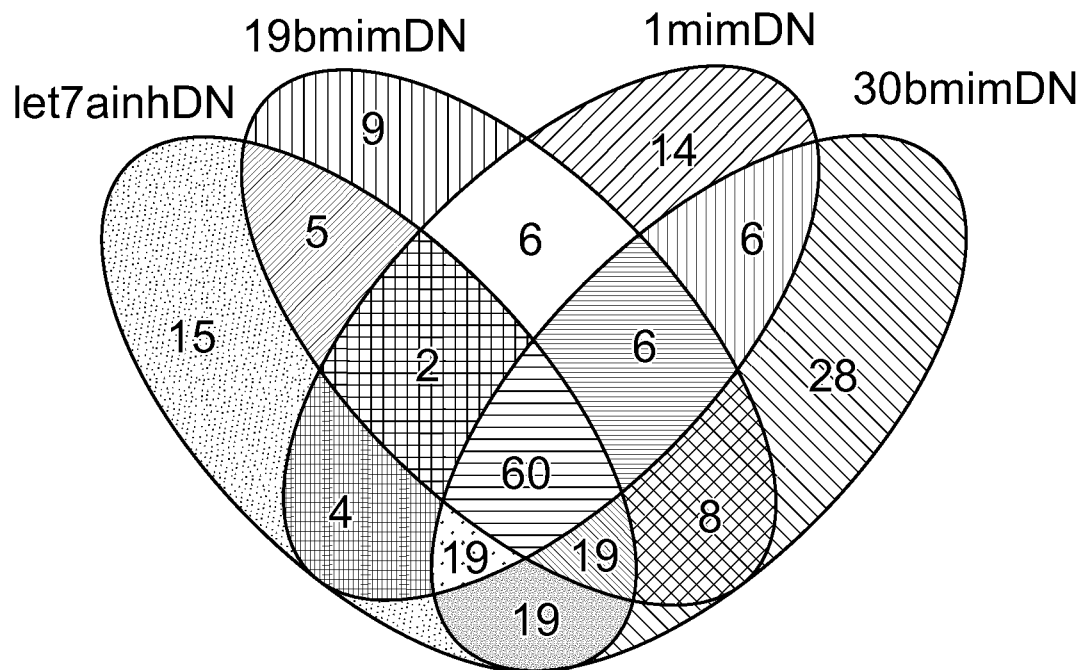
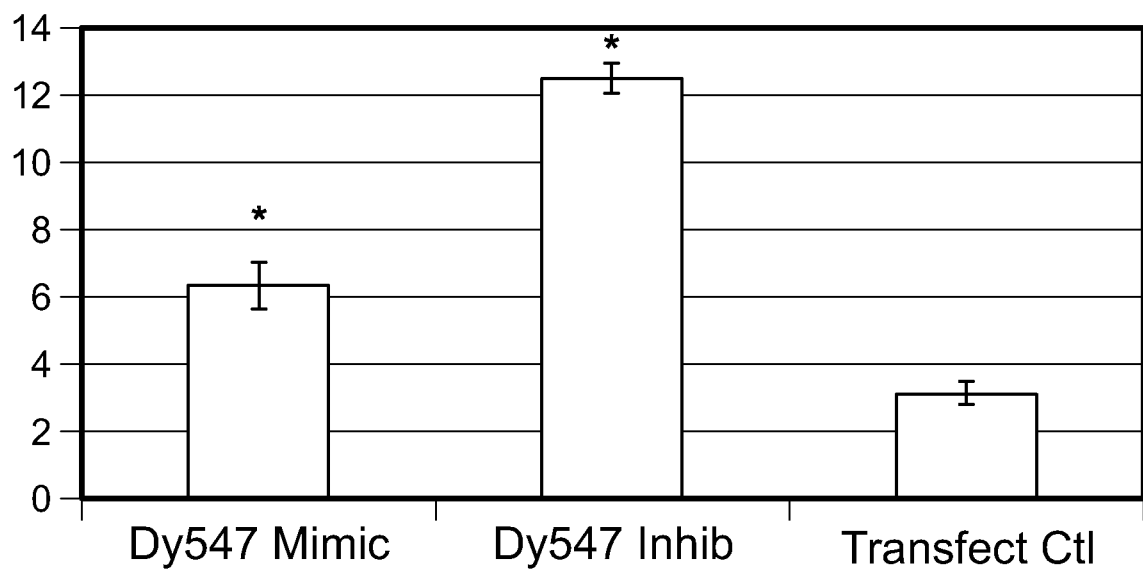


FIG. 15

ADIPOCYTE Transfection Test:

540_{ex} 590_{em} 570_{cutoff}

The Dy547 Transfections were Significantly Higher than Unlabeled Transfected Controls. p-values are < 0.01.

FIG. 16

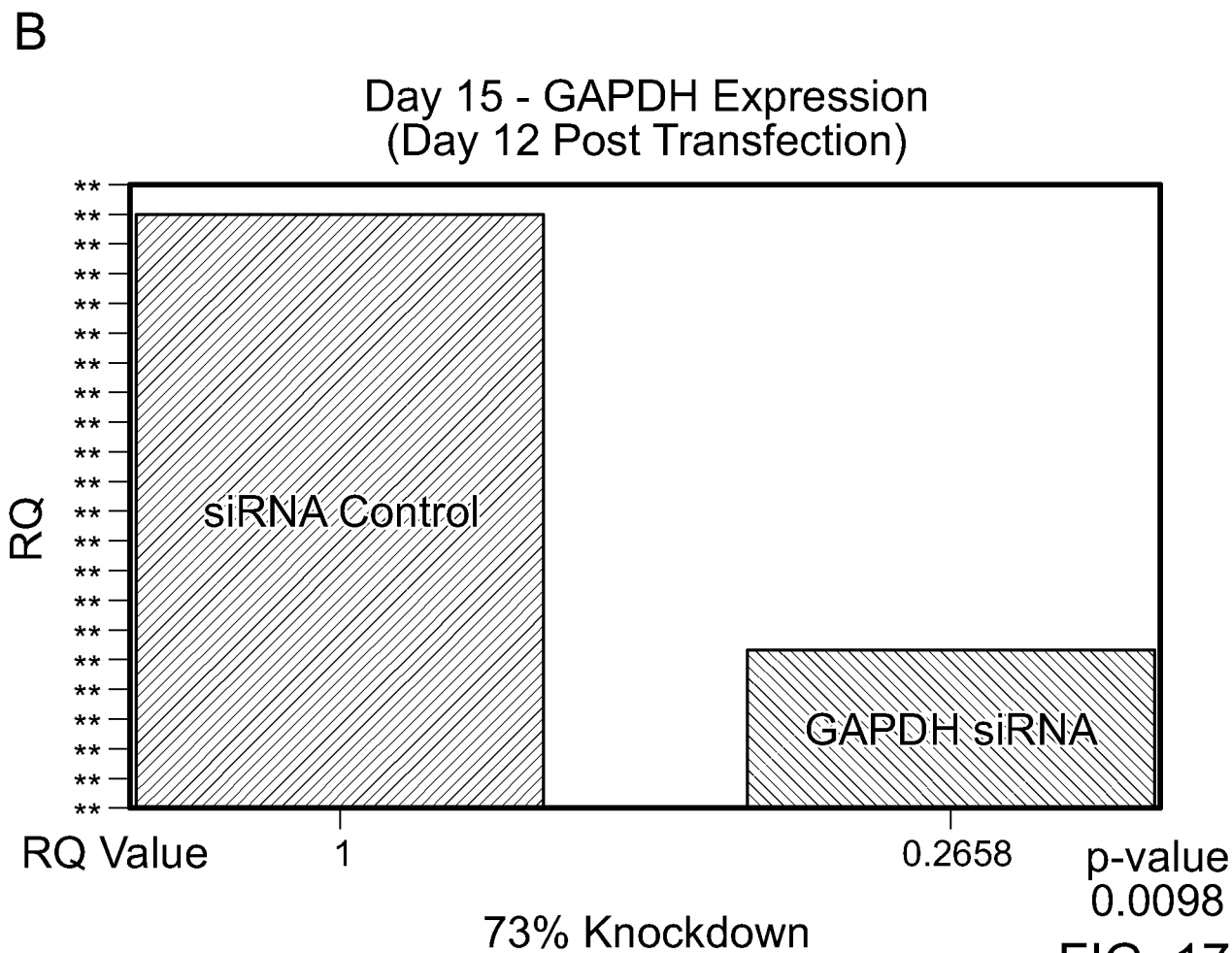
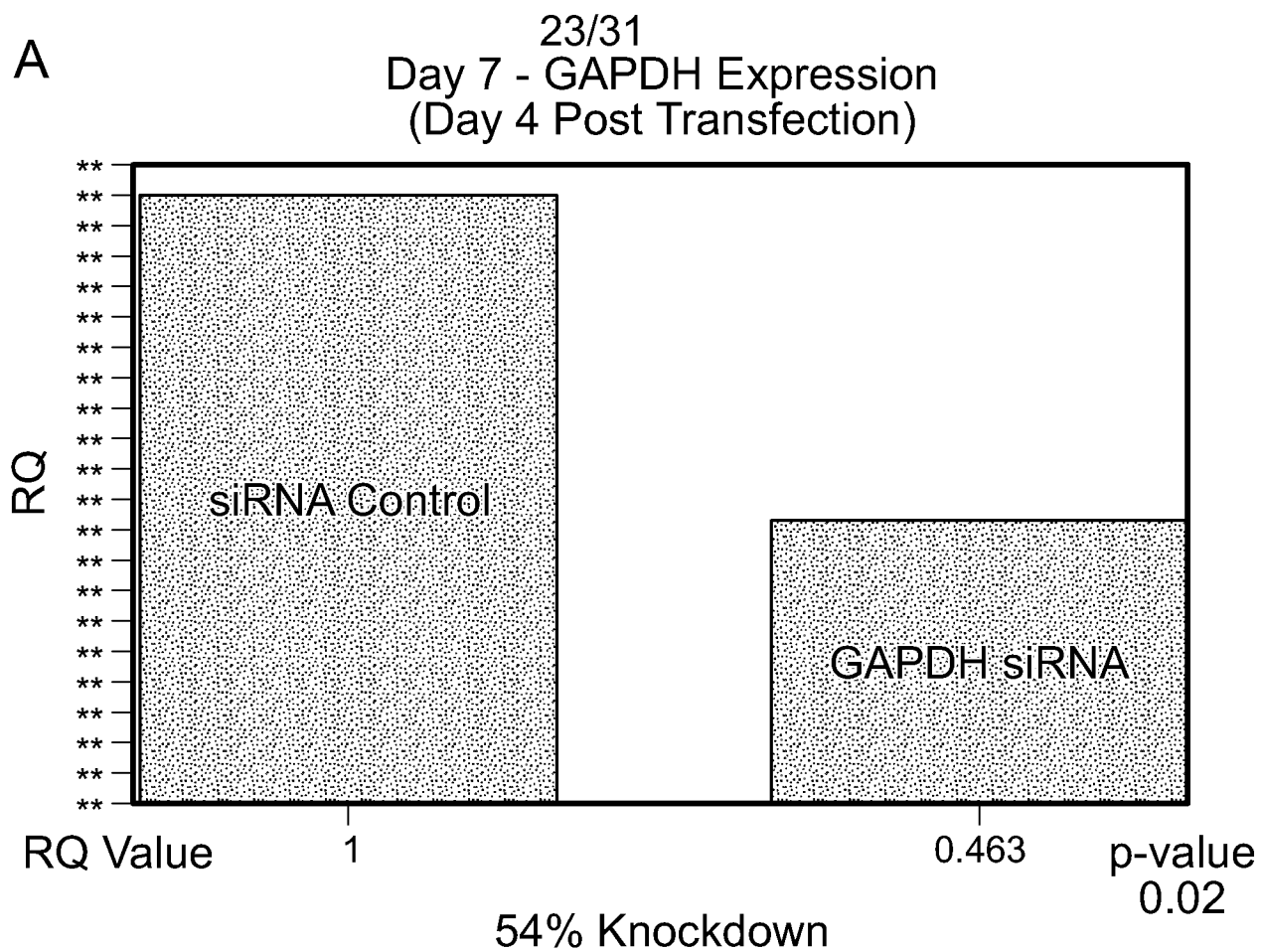
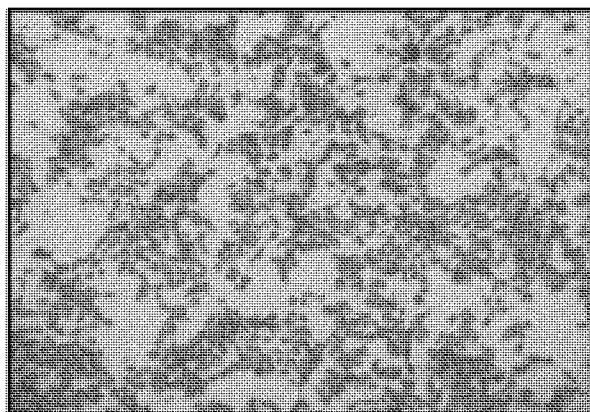


FIG. 17

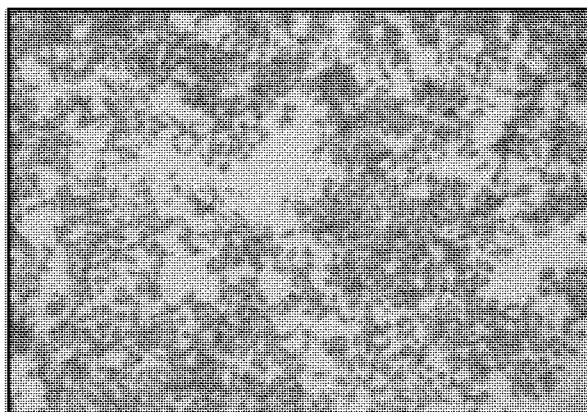
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A



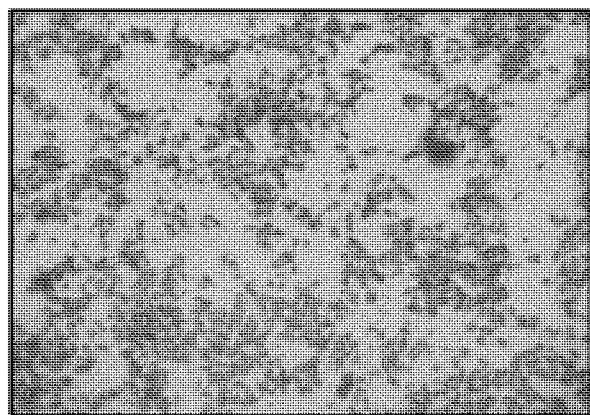
Control

B



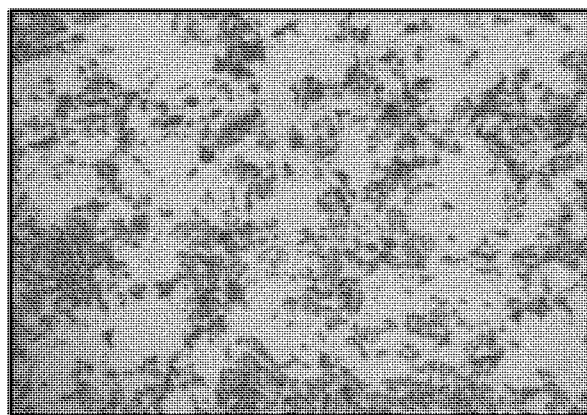
100 nM Rosiglitazone

C



Non Targeting miRNA

D



25 nM miR-30b Mimic

FIG. 18

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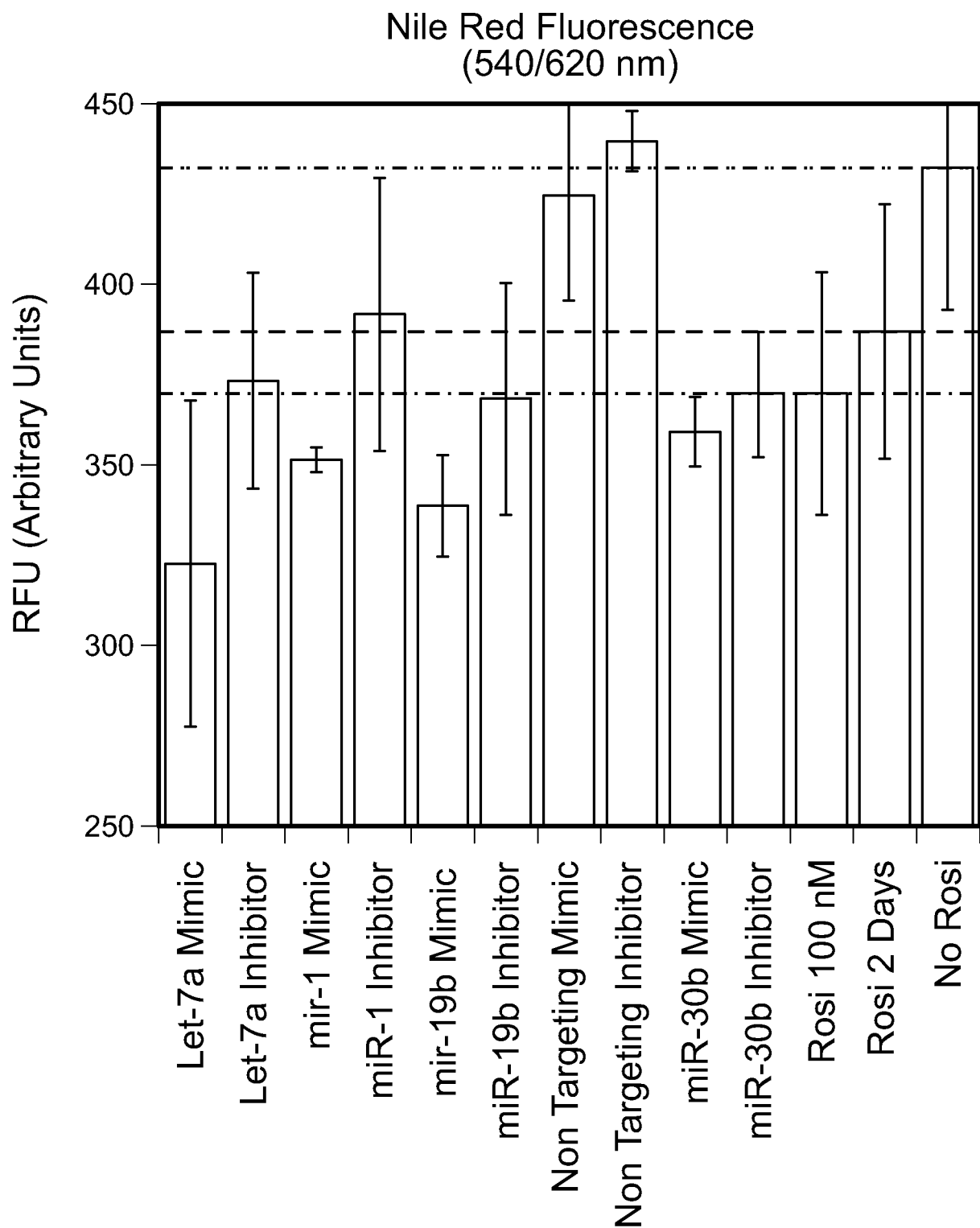


FIG. 19

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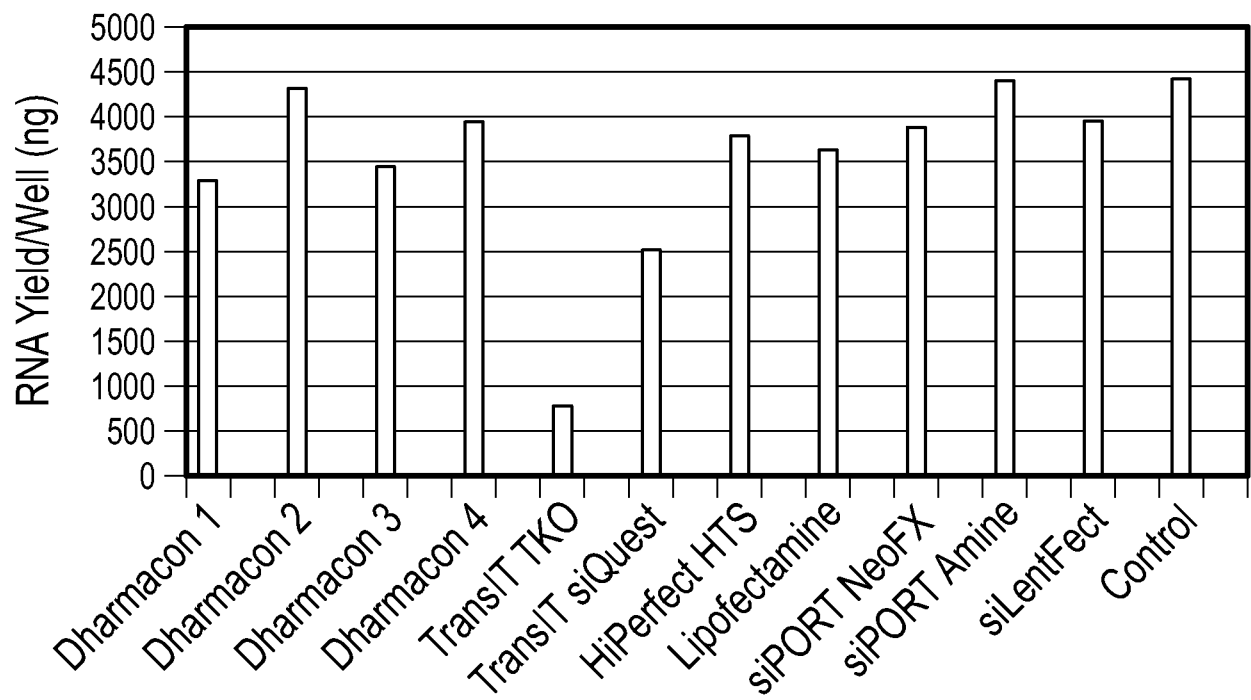


FIG. 20

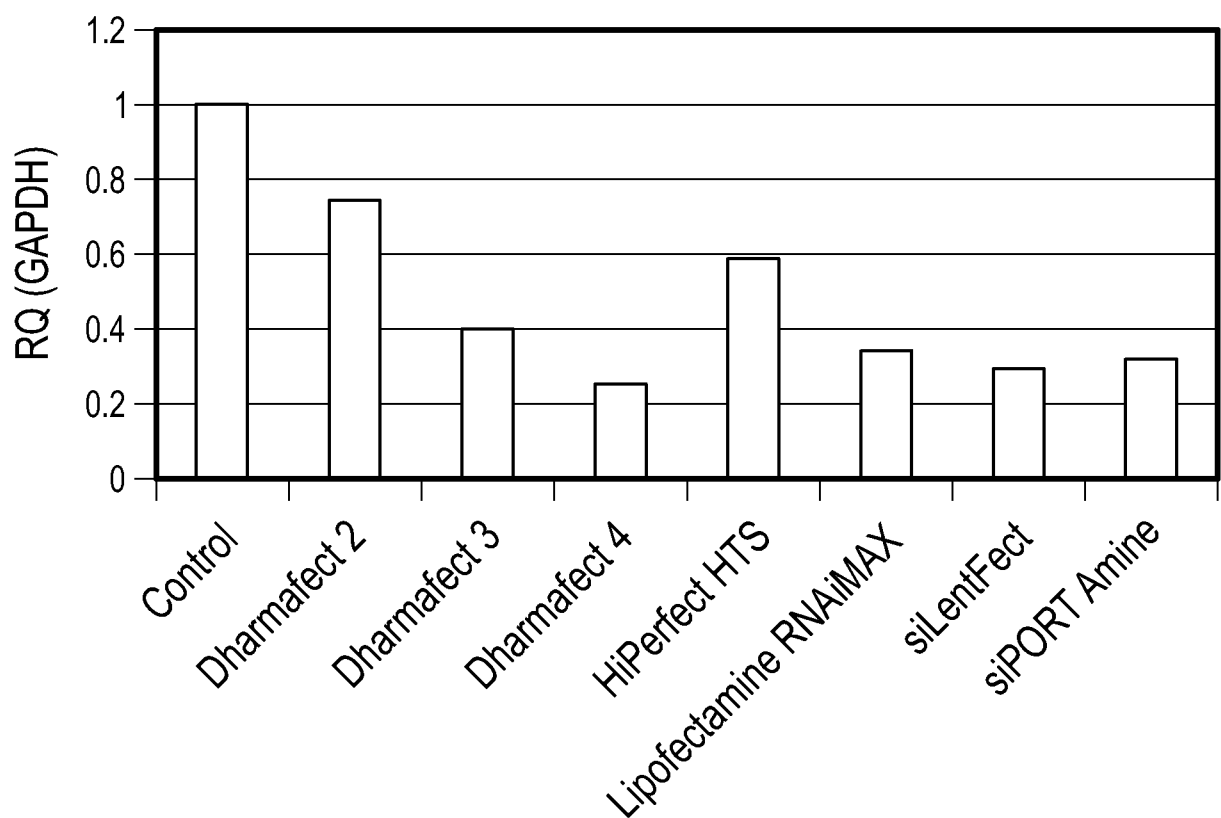
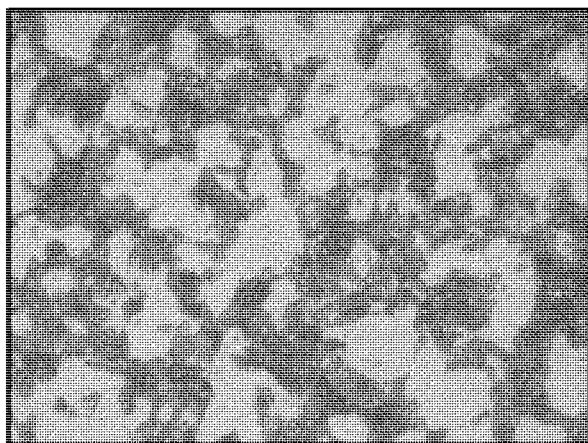
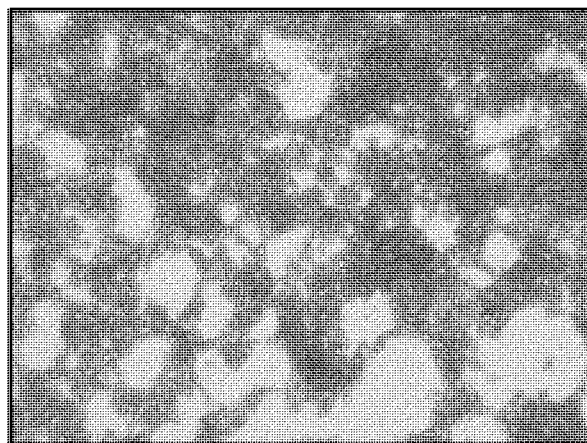


FIG. 21

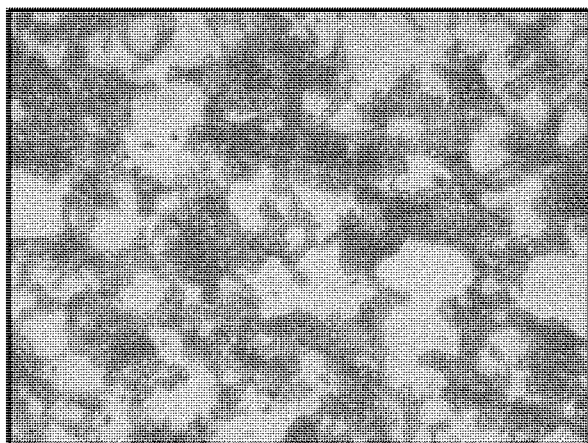
27/31



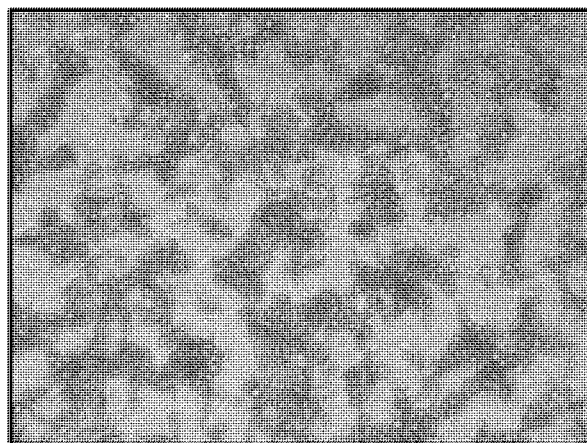
Control



50 nM Let-7a Inhibitor



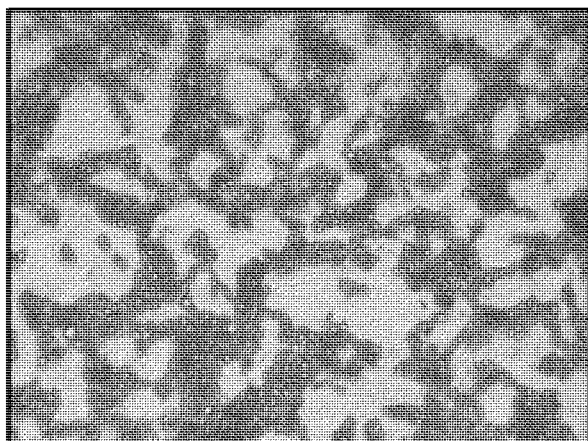
10 μ M CL316,243



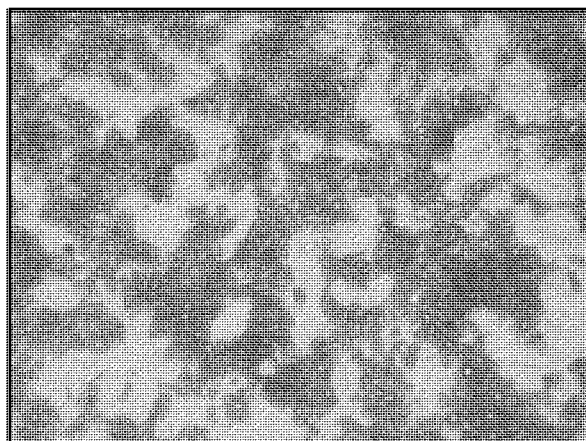
50 nM miR-1 Mimic

FIG. 22

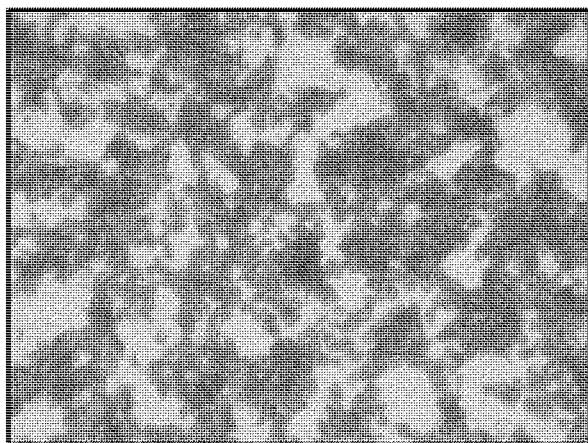
28/31



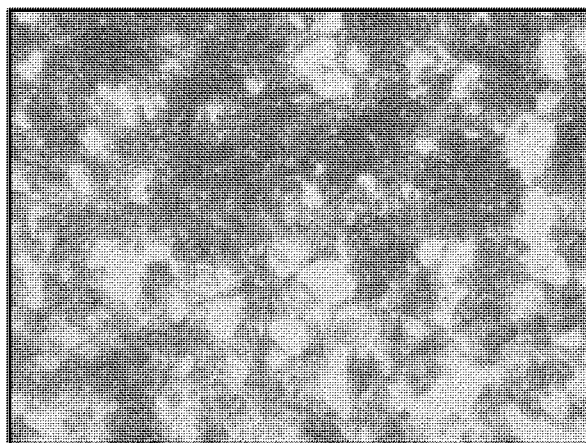
10 nM T3



50 nM miR-19b Mimic



100 nM Rosiglitazone



50 nM miR-30b Mimic

FIG. 22 (Cont.)

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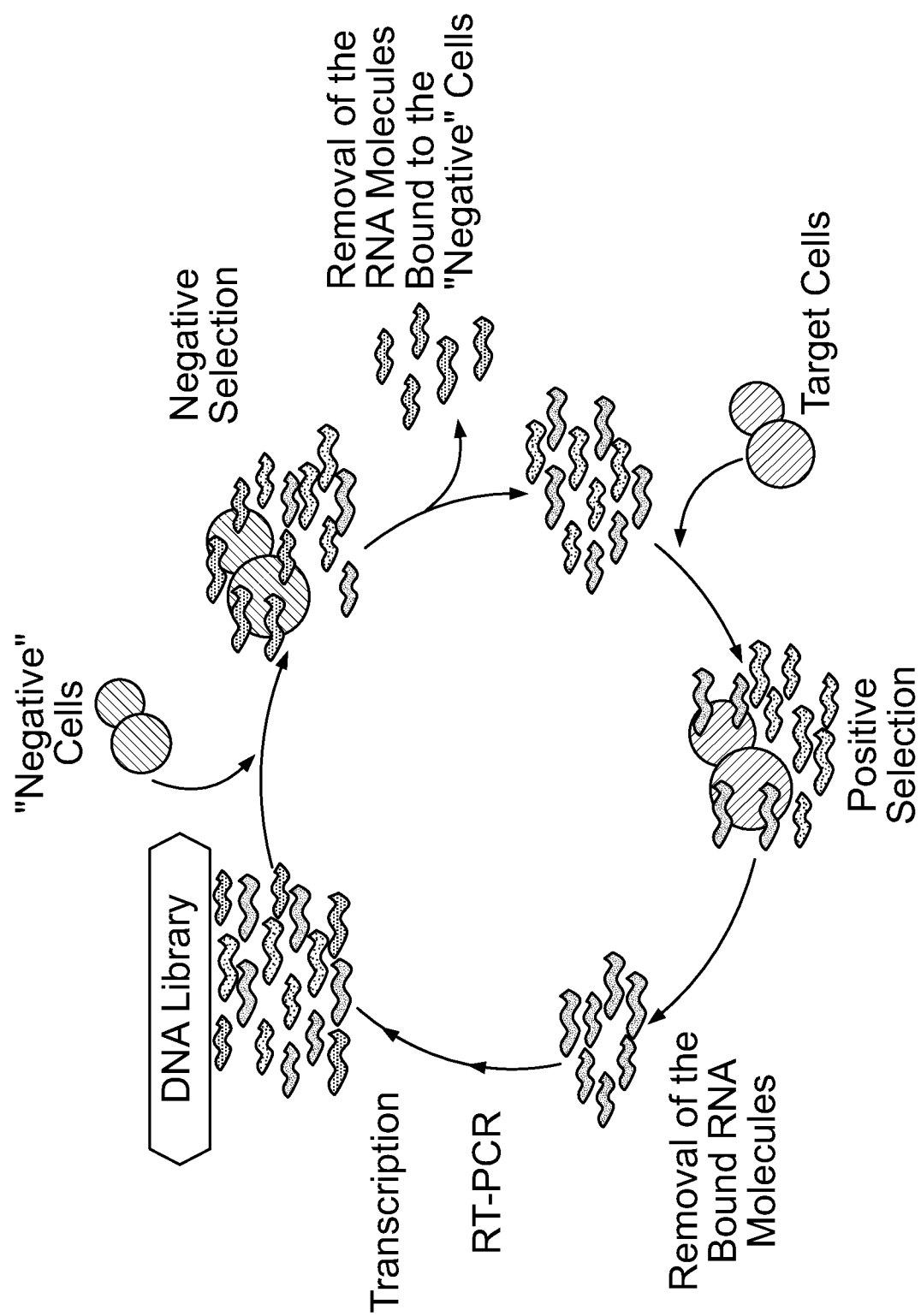


FIG. 23

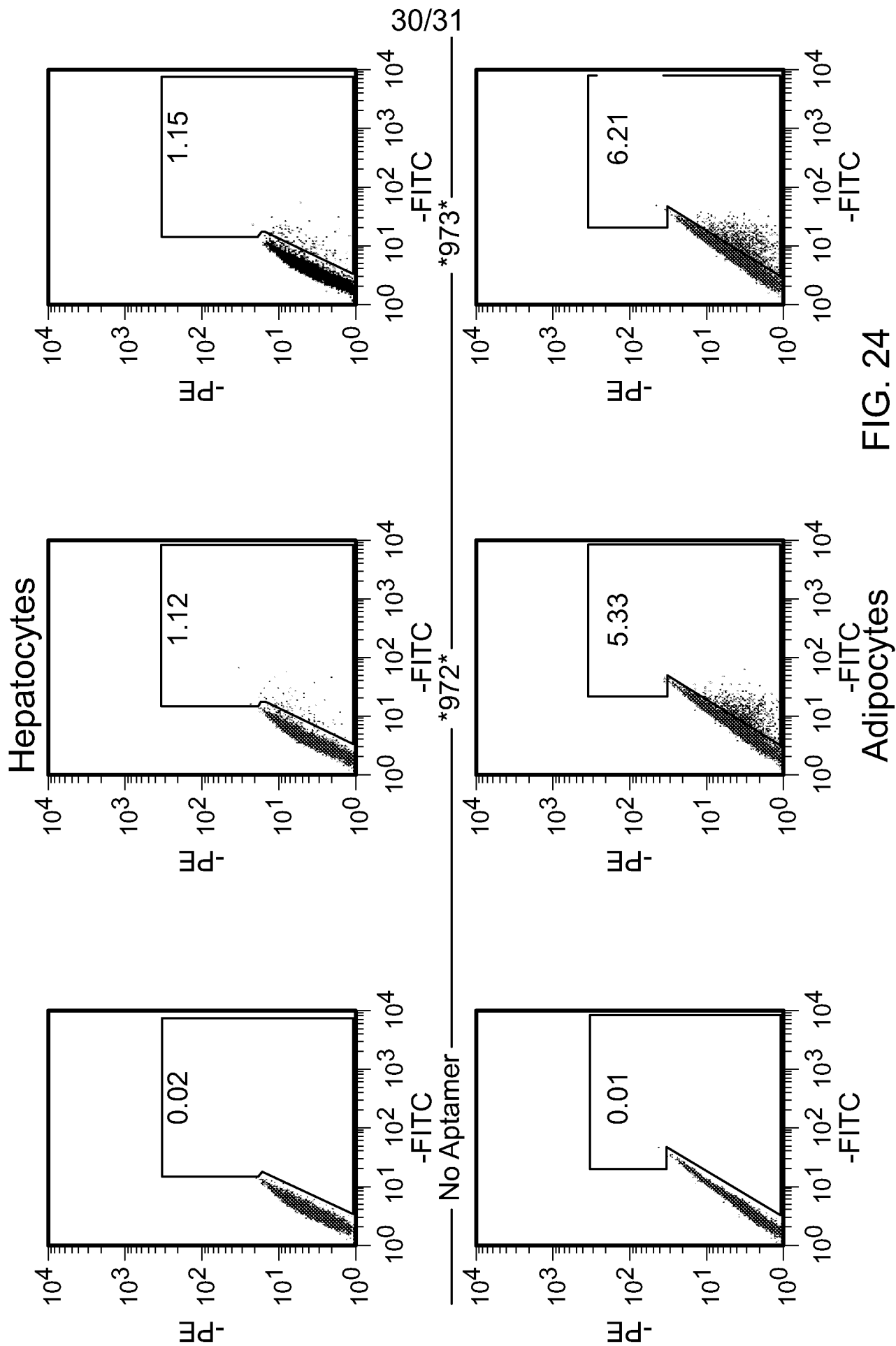


FIG. 24

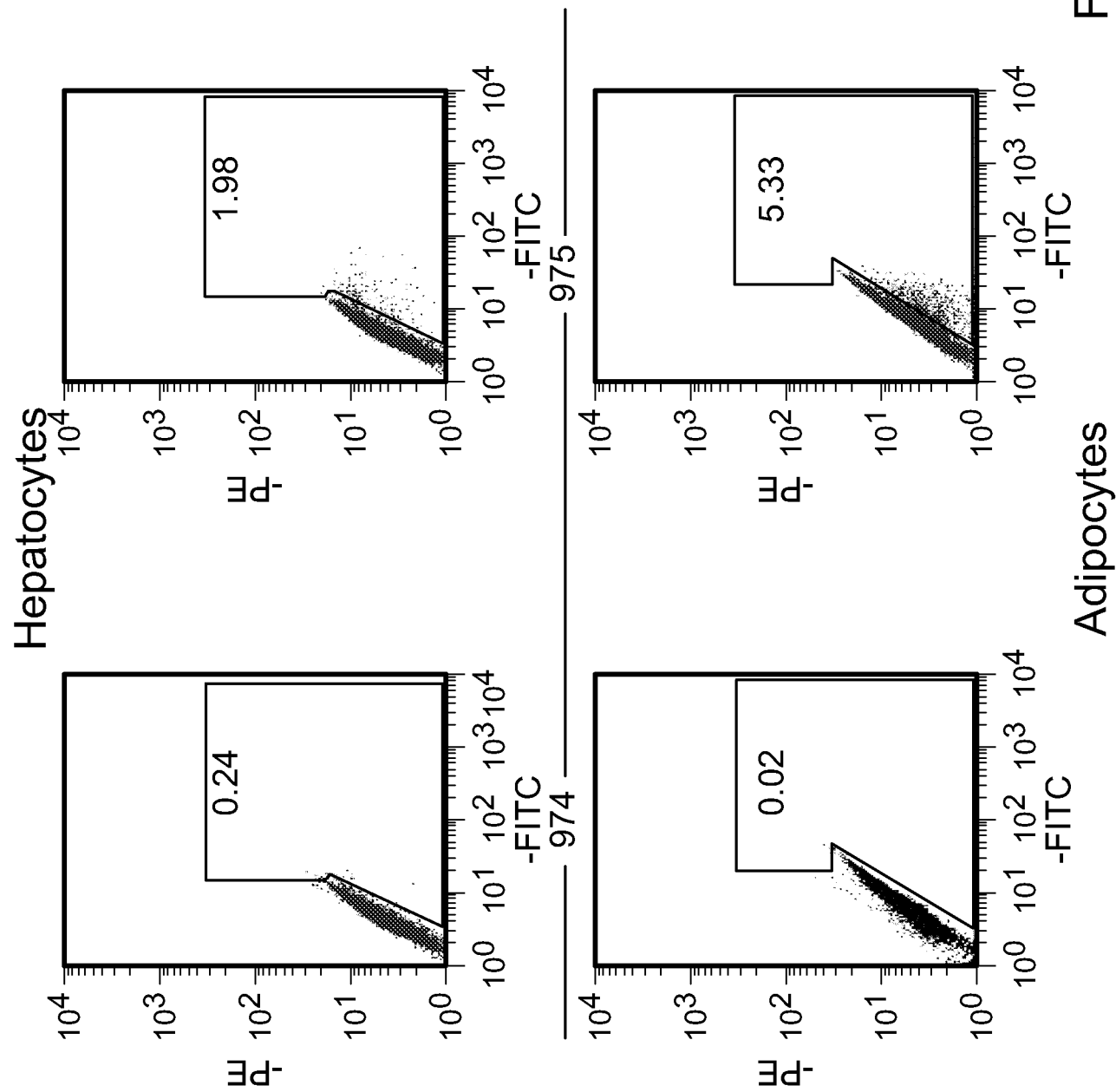


FIG. 24 (Cont.)

(43) International Publication Date
24 October 2013 (24.10.2013)

(51) International Patent Classification:

C12N 15/113 (2010.01) C07H 21/02 (2006.01)
A61K 48/00 (2006.01)

(21) International Application Number:

PCT/US2013/037579

(22) International Filing Date:

22 April 2013 (22.04.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/636,059	20 April 2012 (20.04.2012)	US
61/681,750	10 August 2012 (10.08.2012)	US
61/782,838	14 March 2013 (14.03.2013)	US

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WORSKI LLP, 98 San Jacinto Blvd., Suite 1100, Austin,
TX 78701 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD,
ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI,
NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU,
RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ,
TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA,
ZM, ZW.(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

[Continued on next page]

(54) Title: MIRNA MODULATORS OF THERMOGENESIS

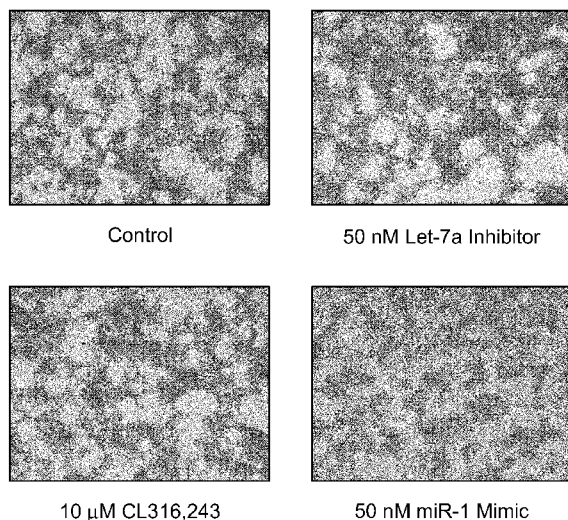


FIG. 22

(57) Abstract: Provided are novel methods and composi-
tions for the modulation of thermogenesis. Such methods are
particularly advantageous in that they allow for the reduction
of body fat in a subject without the subject having to adjust
their caloric intake through dieting, modify their physical
activity or undergo bariatric surgery. Accordingly, the meth-
ods of the invention are particularly useful for treating or
preventing obesity. Also provided are methods of screening
for novel agents that modulate the activity of thermogenic
regulators.



(88) Date of publication of the international search report:

30 April 2015

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/37579

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/113; A61K 48/00; C07H 21/02 (2013.01)

USPC - 514/44A; 536/24.1, 24.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C12N 15/113, 11, 00, 5/00, 02; A61K 48/00; C07H 21/02; A61P 3/00, 21/00 (2013.01)

USPC: 514/44A, 44R, 43, 42, 23, 1; 435/375, 325; 536/23.1, 22.1, 18.7, 1.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; DialogPRO; PubMed; 'UCP1,' 'UCP2,' 'UCP3,' uncoupled, protein, mitochondria*, thermogenin, 'miRNA,' 'microRNA,' micro, 'RNA,' mir, hsa*mir*, thermogenesis, agomir, antagomir, anti*mir, blockmir, adipocyte, adipogenesis, pre*adipocyte, rosiglitazone

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/079263 A2 (COLLARD J et al.), June 30, 2011; abstract; paragraphs [0030], [0060], [0072], [00105], [00119], [00120], [00178]-[00180]	1-7, 8/1-8/7
X	CHEN, X et al. RNA-Mediated Regulation And Noncoding RNAs: In Vitro Evidence Suggests That miR-133a-mediated Regulation Of Uncoupling Protein 2 (UCP2) Is An Indispensable Step In Myogenic Differentiation. The Journal of Biological Chemistry. 2009, Vol. 284, pp 5362-5369; page 5362, column 2, paragraph 2 to page 5363, column 1, paragraph 1; page 5364, column 1, paragraph 3.	30-32
Y		-----
		35/30-35/32,
		36/35/30-36/35/32
X	ALEXANDER, R et al. MicroRNAs In Adipogenesis And As Therapeutic Targets For Obesity. Expert Opinion in Therapeutic Targets. May 2011, Vol. 15, No. 5, pp 623-636 (Author Manuscript, October 7, 2011, pp 1-21); page 4, section 2.1, paragraph 1; page 9, section 3.2, paragraph 3 to page 10, section 3.2, paragraph 2.	30, 33
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Y		35/33, 36/35/33, 62-65,
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A		-----
		34, 35/34, 36/35/34
Y	WO 2010/108126 A2 (MENDLEIN, JD et al.), September 23, 2010; page 133, line 3 to page 135, line 36; page 143, lines 20-27; page 145 line 14 to page 147, line 20; page 264, lines 24-31; page 267, line 20 to page 268, line 5; page 276, lines 10-15; page 321, line 30 to page 322, line 2.	35/30-35/33,
		36/35/30-36/35/33, 52-54,
		61-79



Further documents are listed in the continuation of Box C.



* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 October 2013 (18.10.2013)

Date of mailing of the international search report

25 OCT 2013

Name and mailing address of the ISA/US

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P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 9-25, 37-43
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

****-Please See Supplemental Page-****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Group I: Claims 1-8, 30-36 and 44-79

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

international application no.

PCT/US13/37579

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUN, T et al. MicroRNA let-7 Regulates 3T3-L1 Adipogenesis. Molecular Endocrinology, 2009, Vol. 23, pp 925 to 931; page 928, column 1, paragraph 1; 929, column 2, paragraph 1; page 930, column 2, paragraph 9 to page 931, column 1, paragraph 1.	55
Y		56-61
Y	WO 2011/138457 A1 (SCHEIDELER, M et al.), November 10, 2011; abstract; page 14, line 32 to page 15, line 21; page 16, lines 20-33; page 46, lines 1-10	66-68
Y	WO 2012/007725 A2 (CHOO, Y et al.), January 19, 2012; page 17, lines 23-33; page 19, lines 1-6; page 23, lines 13-20; page 27, lines 25-28; page 47, lines 1-14; page 47, lines 22-27	53, 56-60
X	US 2011/0224286 A1 (YU, X et al.), September 15, 2011; paragraphs [0081], [0082], [0084], [0104], [0105], [0192]	44-51
Y	ZARAGOSI, LE et al. Small RNA Sequencing Reveals miR-642-3p As A Novel Adipocyte-Specific microRNA And miR-30 As A Key Regulator Of Human Adipogenesis. Genome Biology. 2011, Vol. 12, pp 1-13; page 2, column 2, paragraph 4; figure 2, Table 1.	52-54
A	RANTALAINEN, M et al. MicroRNA Expression In Abdominal And Gluteal Adipose Tissue Is Associated With mRNA Expression Levels And Partly Genetically Driven. PLoS ONE. 2011, Vol. 6, No. 11, pp 1-12; e27338. doi:10.1371/journal.pone.0027338; page 2, column 2, paragraph 3; page 8, column 1, paragraph 6-column 2, paragraph 1.	34, 35/34, 36/35/34

-Continued from Box No. III: Observations Where Unity Of Invention Is Lacking

Group I: Claims 1-8, 30-36, 44-79

Group II: Claims 26-29

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-8, 30-36 and 44-79 are directed toward a method of modulating respiratory chain uncoupling in a cell, the method comprising contacting the cell with a miRNA agent that modulates activity of at least one mitochondrial uncoupler; a method of modulating thermogenesis in a tissue, the method comprising contacting the tissue with a miRNA agent that modulates activity of at least one mitochondrial uncoupler; a method of treating obesity in a human subject in need of treatment thereof, the method comprising administering to the human subject an effective amount of a miRNA agent that modulates activity or expression of at least one mitochondrial uncoupler; an agomir or antagomir that modulates the activity of at least one thermogenic regulator in a cell; a pharmaceutical composition comprising two or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir; a method of inducing pre-adipocytes to differentiate into adipocytes comprising administering to a population of pre-adipocytes one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-a agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir; a method of decreasing the lipid content of adipocytes comprising administering to a population of adipocytes one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir, hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir; a method for increasing insulin sensitivity in a subject in need thereof comprising administering to the subject one or more miRNAs selected from the group consisting of hsa-let-7a agomir, hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-1 antagomir, hsa-miR-19b agomir and hsa-miR-19b antagomir, hsa-miR-30b agomir, and hsa-miR-30b antagomir; a method of increasing expression or activity of one or more uncoupling proteins in a cell comprising administering to the cell one or more miRNAs selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir and hsa-miR-30b agomir; a method of causing fat loss in a subject in need thereof comprising administering to the subject one or more miRNAs selected from the group consisting of hsa-let-7a antagomir, hsa-miR-1 agomir, hsa-miR-19b agomir and hsa-miR-30b agomir; use of an agomir or antagomir of one or more miRNAs selected from the group consisting of the miRNA set forth in Tables 1, 11, 13 and 14 in the manufacture of a medicament for the treatment of obesity; and a composition comprising an agomir or antagomir of one or more miRNAs selected from the group consisting of the miRNA set forth in Tables 1, 11, 13 and 14 for the treatment of obesity.

Group II: Claims 26-29 are directed toward a method of screening for a miRNA agent that modulates thermogenesis, the method comprising: a) providing an indicator cell comprising a human genome; b) contacting the indicator cell with a test miRNA agent; and c) determining the cellular activity of at least one thermogenic regulator in the indicator cell in the presence and absence of the miRNA agent, wherein a change in the activity of the thermogenic regulator in the presence of the test miRNA agent identifies the test miRNA agent as a miRNA agent that modulates thermogenesis.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a method of modulating respiratory chain uncoupling in a cell, the method comprising contacting the cell with a miRNA agent that modulates activity of at least one mitochondrial uncoupler, which is not present in Group II; Group II having special technical features including a method of screening for a miRNA agent that modulates thermogenesis, the method comprising: a) providing an indicator cell comprising a human genome; b) contacting the indicator cell with a test miRNA agent; and c) determining the cellular activity of at least one thermogenic regulator in the indicator cell in the presence and absence of the miRNA agent, wherein a change in the activity of the thermogenic regulator in the presence of the test miRNA agent identifies the test miRNA agent as a miRNA agent that modulates thermogenesis.

Groups I-II share the technical features including a method comprising a miRNA agent that modulates thermogenesis, the method comprising: a miRNA agent that modulates thermogenesis.

However, these shared technical features are previously disclosed by WO 2011/079263 A2 to Collard, et al. (hereinafter 'Collard'). Collard discloses a method (Claim 1) comprising a miRNA agent (paragraphs [00105], [00107]) that modulates thermogenesis (paragraph [0060]), the method (Claim 1) comprising: a miRNA agent (paragraphs [00105], [00107]) that modulates thermogenesis (paragraph [0060]).

Since none of the special technical features of the Groups I-II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Collard reference, unity of invention is lacking.



(12) 发明专利申请

(10) 申请公布号 CN 104704122 A

(43) 申请公布日 2015. 06. 10

(21) 申请号 201380030602. 5

(51) Int. Cl.

(22) 申请日 2013. 04. 22

C12N 15/113(2006. 01)

(30) 优先权数据

61/636, 059 2012. 04. 20 US

61/681, 750 2012. 08. 10 US

61/782, 838 2013. 03. 14 US

(85) PCT国际申请进入国家阶段日

2014. 12. 10

(86) PCT国际申请的申请数据

PCT/US2013/037579 2013. 04. 22

(87) PCT国际申请的公布数据

W02013/159091 EN 2013. 10. 24

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代理人 郑斌 彭鲲鹏

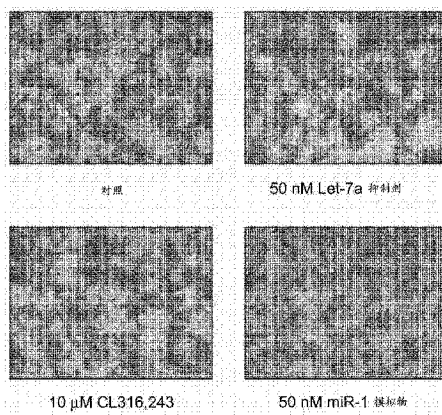
权利要求书5页 说明书119页 附图31页

(54) 发明名称

产热的 miRNA 调节剂

(57) 摘要

本发明提供了用于调节产热的新方法和组合物。这样的方法是特别有利的,因为它们能够减少对象的身体脂肪,而无需所述对象通过节食调节其热量摄取、改变身体活动或进行肥胖治疗手术。因此,本发明的方法对治疗或预防肥胖特别有用。本发明还提供了筛选调节产热调节子活性的新试剂的方法。



1. 一种调节细胞中的呼吸链解偶联的方法,所述方法包括使所述细胞与调节至少一种线粒体解偶联剂之活性的 miRNA 试剂接触。

2. 根据权利要求 1 所述的方法,其中所述细胞是前脂肪细胞、脂肪细胞、脂肪组织来源的间充质干细胞、肝细胞、肌细胞或其前体。

3. 一种调节组织中的产热的方法,所述方法包括使所述组织与调节至少一种线粒体解偶联剂之活性的 miRNA 试剂接触。

4. 根据权利要求 3 所述的方法,其中所述组织是褐色脂肪、白色脂肪、皮下脂肪组织、肝或肌肉。

5. 根据权利要求 4 所述的方法,其中使所述组织与所述 miRNA 试剂离体接触。

6. 一种治疗有此治疗需要的人对象之肥胖的方法,所述方法包括向所述人对象施用有效量的调节至少一种线粒体解偶联剂之活性或表达的 miRNA 试剂。

7. 根据权利要求 6 所述的方法,其中所选进行治疗的所述人对象具有肥胖的遗传素质或表观遗传素质。

8. 根据前述权利要求中任一项所述的方法,其中所述线粒体解偶联剂是 UCP1 或 UCP2。

9. 根据前述权利要求中任一项所述的方法,其中所述 miRNA 试剂是选自以下的 miRNA: hsa-miR-1-1、hsa-miR-1-2、miR-19a-b、hsa-miR-105、hsa-miR-1283、hsa-mir-129、hsa-miR-133a-1、hsa-miR-133a-2、hsa-miR-143、hsa-mir-143-5p、hsa-mir-147、hsa-mir-149、hsa-mir-199a、hsa-mir-199b、hsa-mir-200c、hsa-mir-204、hsa-mir-205、hsa-miR-206、hsa-mir-21、hsa-mir-211、hsa-mir-218、hsa-mir-218-1、hsa-mir-218-2、hsa-mir-219-2、hsa-mir-219-2-3p、hsa-mir-22、hsa-mir-22-3p、hsa-mir-22-5p、hsa-mir-24-2、hsa-miR-30a-e、hsa-miR-3177-5p、hsa-mir-325、hsa-mir-331、hsa-mir-331-5p、hsa-miR-3613-3p、hsa-mir-362、hsa-mir-362-5p、hsa-miR-3658、hsa-mir-367、hsa-mir-371、hsa-mir-371-5p、hsa-mir-377、hsa-mir-378、hsa-mir-378a-5p、hsa-mir-382、hsa-mir-383、hsa-mir-422a、hsa-mir-425、hsa-miR-455-3p、hsa-miR-455-5p、hsa-miR-491、hsa-mir-508、hsa-mir-508-5p、hsa-mir-512-1、hsa-mir-512-2、hsa-miR-515-3p、hsa-mir-519e、hsa-miR-520a、hsa-mir-543、hsa-mir-545、hsa-mir-549、hsa-mir-556 和 hsa-miR-568、hsa-mir-620、hsa-mir-643、hsa-mir-654-3p、hsa-miR-7a-g、hsa-mir-765、hsa-mir-871、hsa-mir-888、hsa-mir-888-3p、hsa-mir-92b、hsa-mir-93、hsa-mir-96 和 hsa-mir-99a。

10. 根据前述权利要求中任一项所述的方法,其中所述 miRNA 试剂是选自表 1、11、13 和 14 中记载的 miRNA 中的 miRNA。

11. 根据前述权利要求中任一项所述的方法,其中所述 miRNA 试剂是选自以下的 miRNA 的 agomir 或 antagomir: hsa-miR-1-1、hsa-miR-1-2、miR-19a-b、hsa-miR-105、hsa-miR-1283、hsa-mir-129、hsa-miR-133a-1、hsa-miR-133a-2、hsa-miR-143、hsa-mir-143-5p、hsa-mir-147、hsa-mir-149、hsa-mir-199a、hsa-mir-199b、hsa-mir-200c、hsa-mir-204、hsa-mir-205、hsa-miR-206、hsa-mir-21、hsa-mir-211、hsa-mir-218、hsa-mir-218-1、hsa-mir-218-2、hsa-mir-219-2、hsa-mir-219-2-3p、hsa-mir-22、hsa-mir-22-3p、hsa-mir-22-5p、hsa-mir-24-2、hsa-miR-30a-e、hsa-miR-3177-5p、hsa-mir-325、hsa-mir-331、hsa-mir-331-5p、hsa-miR-3613-3p、

hsa-mir-362、hsa-mir-362-5p、hsa-miR-3658、hsa-mir-367、hsa-mir-371、hsa-mir-371-5p、hsa-mir-377、hsa-mir-378、hsa-mir-378a-5p、hsa-mir-382、hsa-mir-383、hsa-mir-422a、hsa-mir-425、hsa-miR-455-3p、hsa-miR-455-5p、hsa-miR-491、hsa-mir-508、hsa-mir-508-5p、hsa-mir-512-1、hsa-mir-512-2、hsa-miR-515-3p、hsa-mir-519e、hsa-miR-520a、hsa-mir-543、hsa-mir-545、hsa-mir-549、hsa-mir-556、和 hsa-miR-568、hsa-mir-620、hsa-mir-643、hsa-mir-654-3p、hsa-miR-7a-g、hsa-mir-765、hsa-mir-871、hsa-mir-888、hsa-mir-888-3p、hsa-mir-92b、hsa-mir-93、hsa-mir-96 以及 hsa-mir-99a。

12. 根据前述权利要求中任一项所述的方法,其中所述 miRNA 试剂是选自表 1、11、13 和 14 中记载的 miRNA 的 agomir 或 antagomir。

13. 根据前述权利要求中任一项所述的方法,其中所述 miRNA 试剂是选自以下的 miRNA 的 antagomir:hsa-miR-19b-2-5p、hsa-miR-21-5p、hsa-miR-130b-5p、hsa-miR-211、hsa-miR-325、hsa-miR-382-3p/5p、hsa-miR-543、hsa-miR-515-3p 和 hsa-miR-545。

14. 根据前述权利要求中任一项所述的方法,其中所述 miRNA 试剂是选自以下的 miRNA 的 antagomir:hsa-miR-331-5p、hsa-miR-552、hsa-miR-620 和 hsa-miR-1179。

15. 根据前述权利要求中任一项所述的方法,其中所述 miRNA 试剂与靶向部分连接。

16. 根据前述权利要求中任一项所述的方法,其中所述靶向部分是适配体。

17. 根据前述权利要求中任一项所述的方法,其中所述靶向部分将所述 miRNA 试剂递送至特定细胞类型或组织。

18. 根据前述权利要求中任一项所述的方法,其中所述 miRNA 试剂直接与至少一种线粒体解偶联剂的 mRNA 或启动子区结合。

19. 根据前述权利要求中任一项所述的方法,其中所述 miRNA 试剂直接与至少一种线粒体解偶联剂之 mRNA 的 5' UTR 或编码序列结合。

20. 根据前述权利要求中任一项所述的方法,其中所述 miRNA 试剂调节线粒体解偶联蛋白的激活蛋白或阻抑蛋白的活性。

21. 根据权利要求 18 所述的方法,其中所述激活蛋白或阻抑蛋白选自表 2 中记载的激活蛋白或阻抑蛋白。

22. 根据权利要求 20 或 21 所述的方法,其中所述 miRNA 试剂直接与所述激活蛋白或阻抑蛋白的 mRNA 或启动子区结合。

23. 根据权利要求 20 或 21 所述的方法,其中所述 miRNA 试剂直接与所述激活蛋白或阻抑蛋白之 mRNA 的 5' UTR 或编码序列结合。

24. 根据前述权利要求中任一项所述的方法,其中上调所述线粒体解偶联蛋白的 mRNA 或蛋白质表达。

25. 根据前述权利要求中任一项所述的方法,其中上调所述线粒体解偶联蛋白的线粒体解偶联活性。

26. 一种筛选调节产热的 miRNA 试剂的方法,所述方法包括:

a) 提供包含人基因组的指示细胞;

b) 使所述指示细胞与受试 miRNA 试剂接触;以及

c) 在存在和不存在所述 miRNA 试剂的情况下,测定所述指示细胞中的至少一种产热调

节子的细胞活性,其中,在存在所述受试 miRNA 试剂的情况下所述产热调节子活性的改变将所述受试 miRNA 试剂鉴定为调节产热的 miRNA 试剂。

27. 根据权利要求 26 所述的方法,其中所述细胞是脂肪细胞、脂肪组织来源的间充质干细胞、肝细胞、肌细胞或其前体。

28. 根据权利要求 26 所述的方法,其中在步骤 (c) 中测定的所述产热调节子的细胞活性是所述产热调节子的 mRNA 表达水平、蛋白质表达水平或线粒体解偶联活性。

29. 根据前述权利要求中任一项所述的方法,其中所述产热调节子是 UCP1。

30. 一种调节细胞中至少一种产热调节子之活性的 agomir 或 antagomir。

31. 根据权利要求 30 所述的 agomir 或 antagomir,其为选自表 1、11、13 和 14 中记载的 miRNA 中的 miRNA 的 agomir 或 antagomir。

32. 根据权利要求 30 所述的 agomir 或 antagomir,其为选自以下的 miRNA 的 agomir 或 antagomir: hsa-miR-1-1、hsa-miR-1-2、miR-19a-b、hsa-miR-105、hsa-miR-1283、hsa-miR-129、hsa-miR-133a-1、hsa-miR-133a-2、hsa-miR-143、hsa-miR-143-5p、hsa-miR-147、hsa-miR-149、hsa-miR-199a、hsa-miR-199b、hsa-miR-200c、hsa-miR-204、hsa-miR-205、hsa-miR-206、hsa-miR-21、hsa-miR-211、hsa-miR-218、hsa-miR-218-1、hsa-miR-218-2、hsa-miR-219-2、hsa-miR-219-2-3p、hsa-miR-22、hsa-miR-22-3p、hsa-miR-22-5p、hsa-miR-24-2、hsa-miR-30a-e、hsa-miR-3177-5p、hsa-miR-325、hsa-miR-331、hsa-miR-331-5p、hsa-miR-3613-3p、hsa-miR-362、hsa-miR-362-5p、hsa-miR-3658、hsa-miR-367、hsa-miR-371、hsa-miR-371-5p、hsa-miR-377、hsa-miR-378、hsa-miR-378a-5p、hsa-miR-382、hsa-miR-383、hsa-miR-422a、hsa-miR-425、hsa-miR-455-3p、hsa-miR-455-5p、hsa-miR-491、hsa-miR-508、hsa-miR-508-5p、hsa-miR-512-1、hsa-miR-512-2、hsa-miR-515-3p、hsa-miR-519e、hsa-miR-520a、hsa-miR-543、hsa-miR-545、hsa-miR-549、hsa-miR-556 和 hsa-miR-568、hsa-miR-620、hsa-miR-643、hsa-miR-654-3p、hsa-miR-7a-g、hsa-miR-765、hsa-miR-871、hsa-miR-888、hsa-miR-888-3p、hsa-miR-92b、hsa-miR-93、hsa-miR-96 和 hsa-miR-99a。

33. 根据权利要求 30 所述的 agomir 或 antagomir,其为选自以下的 miRNA 的 antagomir: hsa-miR-19b-2-5p、hsa-miR-21-5p、hsa-miR-130b-5p、hsa-miR-211、hsa-miR-325、hsa-miR-382-3p/5p、hsa-miR-543、hsa-miR-515-3p 和 hsa-miR-545。

34. 根据权利要求 30 所述的 agomir 或 antagomir,其为选自以下的 miRNA 的 antagomir: hsa-miR-331-5p、hsa-miR-552、hsa-miR-620 和 hsa-miR-1179。

35. 根据权利要求 30 至 34 中任一项所述的 agomir 或 antagomir,其中所述 agomir 或 antagomir 与靶向部分连接。

36. 根据权利要求 35 所述的 agomir 或 antagomir,其中所述靶向部分是适配体。

37. 根据权利要求 35 或 36 所述的 agomir 或 antagomir,其中所述靶向部分将所述 agomir 或 antagomir 递送至特定细胞类型或组织。

38. 根据权利要求 28 至 33 中任一项所述的 agomir 或 antagomir,其中所述 agomir 或 antagomir 直接与至少一种线粒体解偶联剂的 mRNA 或启动子区结合。

39. 根据权利要求 30 至 37 中任一项所述的 agomir 或 antagomir,其中所述 agomir 或 antagomir 直接与至少一种线粒体解偶联剂之 mRNA 的 5' UTR 或编码序列结合。

40. 根据权利要求 30 至 37 中任一项所述的 agomir 或 antagomir, 其中所述 agomir 或 antagomir 调节线粒体解偶联蛋白的激活蛋白或阻抑蛋白的活性。

41. 根据权利要求 30 至 37 中任一项所述的 agomir 或 antagomir, 其中所述激活蛋白或阻抑蛋白选自表 2 中记载的激活蛋白或阻抑蛋白。

42. 根据权利要求 30 至 37 中任一项所述的 agomir 或 antagomir, 其中所述 agomir 或 antagomir 直接与所述激活蛋白或阻抑蛋白的 mRNA 或启动子区结合。

43. 根据权利要求 30 至 37 中任一项所述的 agomir 或 antagomir, 其中所述 agomir 或 antagomir 直接与所述激活蛋白或阻抑蛋白之 mRNA 的 5' UTR 或编码序列结合。

44. 一种药物组合物, 其包含选自以下的两种或更多种 miRNA : hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-1 agomir、hsa-miR-1 antagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。

45. 根据权利要求 44 所述的药物组合物, 其还包含可药用赋形剂。

46. 根据权利要求 44 所述的药物组合物, 其中所述两种或更多种 miRNA 由重组载体表达。

47. 根据权利要求 44 所述的药物组合物, 其中所述重组载体选自 DNA 质粒、病毒载体和 DNA 微环。

48. 根据权利要求 44 所述的药物组合物, 其包含选自以下的两种或更多种 miRNA : hsa-let-7a antagomir、hsa-miR-1 agomir、hsa-miR-19b agomir 和 hsa-miR-30b agomir。

49. 根据权利要求 48 所述的药物组合物, 其还包含可药用赋形剂。

50. 根据权利要求 48 所述的药物组合物, 其中所述两种或更多种 miRNA 由重组载体表达。

51. 根据权利要求 48 所述的药物组合物, 其中所述重组载体选自 DNA 质粒、病毒载体和 DNA 微环。

52. 一种诱导前脂肪细胞分化成脂肪细胞的方法, 其包括向前脂肪细胞群施用选自以下的一种或更多种 miRNA : hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-1 agomir、hsa-miR-1 antagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。

53. 根据权利要求 52 所述的方法, 其中使前脂肪细胞分化成脂肪细胞的诱导大于当将前脂肪细胞暴露于 100nM 罗格列酮两天接着暴露于维持培养基时前脂肪细胞向脂肪细胞的分化。

54. 根据权利要求 52 所述的方法, 其中所述一种或更多种 miRNA 选自 hsa-let-7a antagomir、hsa-miR-1 agomir、hsa-miR-19b agomir 和 hsa-miR-30b agomir。

55. 一种降低脂肪细胞之脂质含量的方法, 其包括向脂肪细胞群施用选自以下的一种或更多种 miRNA : hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-1 agomir、hsa-miR-1 antagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。

56. 根据权利要求 55 所述的方法, 其中所述脂肪细胞的脂质含量低于暴露于 100nM 罗格列酮两天随后暴露于维持培养基的脂肪细胞的脂肪含量。

57. 根据权利要求 55 所述的方法, 其中所述脂肪细胞的脂质含量低于在培养持续时间

内暴露于 100nM 罗格列酮之脂肪细胞的脂质含量。

58. 根据权利要求 57 所述的方法,其中所述培养持续时间是 8 天至 16 天。

59. 根据权利要求 58 所述的方法,其中所述培养持续时间是 10 天至 14 天。

60. 根据权利要求 59 所述的方法,其中所述培养持续时间是 14 天。

61. 根据权利要求 55 所述的方法,其中所述一种或更多种 miRNA 选自 hsa-miR-1 agomir、hsa-miR-19b agomir 和 hsa-miR-30b agomir。

62. 一种提高有此需要的对象之胰岛素敏感性的方法,其包括向所述对象施用选自以下的一种或更多种 miRNA :hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-1 agomir、hsa-miR-1 antagomir、hsa-miR-19b agomir 和 hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。

63. 根据权利要求 62 所述的方法,其中所述对象是哺乳动物。

64. 根据权利要求 63 所述的方法,其中所述哺乳动物是人。

65. 根据权利要求 62 所述的方法,其中所述一种或更多种 miRNA 选自 :hsa-miR-1 agomir、hsa-miR-19b agomir 和 hsa-miR-30b agomir。

66. 一种提高细胞中的一种或更多种解偶联蛋白表达或活性的方法,其包括向所述细胞施用选自以下的一种或更多种 miRNA :hsa-let-7a antagomir、hsa-miR-1 agomir、hsa-miR-19b agomir 和 hsa-miR-30b agomir。

67. 根据权利要求 66 所述的方法,其中所述细胞选自褐色脂肪细胞、白色脂肪细胞、皮下脂肪细胞、肝细胞或者肌肉细胞。

68. 根据权利要求 66 所述的方法,其中所述一种或更多种解偶联蛋白包括 UCP-1 或 UCP-2。

69. 一种引起有此需要的对象之脂肪消耗的方法,其包括向所述对象施用选自以下的一种或更多种 miRNA :hsa-let-7a antagomir、hsa-miR-1 agomir、hsa-miR-19b agomir 和 hsa-miR-30b agomir。

70. 根据权利要求 69 所述的方法,其中所述对象是哺乳动物。

71. 根据权利要求 70 所述的方法,其中所述哺乳动物是人。

72. 选自表 1、11、13 和 14 中记载的 miRNA 中的一种或更多种 miRNA 的 agomir 或 antagomir 在制造用于治疗肥胖的药物中的用途。

73. 根据权利要求 72 所述的用途,其中所述一种或更多种 miRNA 选自 has-let-7a antagomir、has-miR-1 agomir、has-miR-19b agomir 和 has-miR-30b agomir。

74. 根据权利要求 72 所述的用途,其中所述 agomir 或 antagomir 与靶向部分连接。

75. 根据权利要求 74 所述的用途,其中所述靶向部分是适配体。

76. 一种用于治疗肥胖的组合物,其包含选自表 1、11、13 和 14 中记载的 miRNA 之一或更多种 miRNA 的 agomir 或 antagomir。

77. 根据权利要求 76 所述的组合物,其中所述一种或更多种 miRNA 选自 has-let-7a antagomir、has-miR-1 agomir、has-miR-19b agomir 和 has-miR-30b agomir。

78. 根据权利要求 76 所述的组合物,其中所述 agomir 或 antagomir 与靶向部分连接。

79. 根据权利要求 76 所述的组合物,其中所述靶向部分是适配体。

产热的 miRNA 调节剂

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[0075] 本申请要求对以下文件的优先权：于 2012 年 4 月 20 日提交的美国申请序列号 61/636,059；于 2012 年 8 月 10 日提交的美国申请序列号 61/681,750；和于 2013 年 3 月 14 日提交的美国申请序列号 61/782,838，每个文件都通过引用并入本文。

背景技术

[0076] 肥胖已经达到了流行病的比例，影响到所有年龄段和社会经济群体。世界卫生组织估计，在 2008 年，有 15 亿 20 岁及以上的成年人超重，并且有超过 2 亿的男性和超过 3 亿的女性肥胖。据估计，到 2030 年，超重和肥胖个体的数据将分别增长至 21.6 亿和 11.2 亿。肥胖导致收入损失、活动天数受到限制、缺勤、工作效率低下（出勤）、生活质量下降、永久性失能、显著的发病率和死亡率，以及寿命缩短。事实上，据估计，2009 年美国 and 加拿大因医疗费用、过高的死亡率以及失能导致的超重和肥胖的年总经济费用为约 3000 亿美元。国际研究显示，肥胖症的经济费用占总医疗费用的 2% 至 10%。

[0077] 肥胖是由能量摄取与消耗之间的长期失衡造成的。这导致过多的能量储存在脂肪细胞中，这样的脂肪细胞通常表现出肥大（细胞体积增大）和增生（细胞数目或脂肪生成增加）二者。最近，肥胖的恶化源于过度消费含饱和脂肪和糖类的高能量密度食物结合体力活动的减少。

[0078] 现有的肥胖的症状性医药治疗不能达到长期治疗的目的，这很大程度上是由于有限的药物效力以及患者很少能坚持生活方式改变和治疗所导致的。数种肥胖药物已经由于安全因素而被移出市场，而一些正在研发的小分子由于其不理想的短期疗效和未知的安全性能 (profile) 正挣扎于获取审批。目前，仅限制性肥胖治疗 (bariatric) 手术和吸收不良性肥胖治疗手术可实现显著的长期减重并伴有一些有利的心血管益处。

[0079] 因此，本领域中需要新的治疗肥胖的方法。

发明内容

[0080] 肥胖是能量摄取与消耗长期失衡的结果，导致过多的能量储存进白色脂肪细胞中。本公开内容涉及肥胖的新治疗方法，其靶向周围脂肪细胞，包括储存能量的填充有脂质的白色脂肪细胞 (white adipocytes, WAT)，以及消耗能量的富含线粒体的褐色脂肪细胞 (brown adipocyte, BAT)。此外，本公开内容提供了通过使用微小 RNA (miRNA, miRNA) 试剂来调节产热（生物体中的热产生过程）的方法。本文所描述的方法一般包括使用经分离的 miRNA 试剂直接和 / 或间接地调节细胞、组织和 / 或对象中的至少一种产热调节子 (regulator)（例如，线粒体解偶联剂 (uncoupler)，如解偶联蛋白 1 (Uncoupling Protein 1, UCP1 还被称为产热素) 或解偶联蛋白 2 (UCP2)）。UCP 使氧化磷酸化与 ATP 合成解偶联。在某些情况下，该解偶联反应导致能量作为热消散。这样的方法是特别有利的，因为它们允许减少对象身体中的脂肪，而无需所述对象必须通过节食调节其热量摄入、改变身体活动或进行肥胖治疗手术。因此，本发明的方法对治疗或预防肥胖特别有用。

[0081] 本发明还提供了可调节产热调节子活性的新 miRNA 试剂组合物（例如 miRNA、agomir 和 antagomir）。此外，本发明提供了筛选调节产热调节子活性的新 miRNA 试剂的方法。此外，本发明还提供了提供 miRNA 试剂的细胞 / 组织特异性递送的新试剂组合物（例如，适配体 (aptamer)-miRNA 复合物或“aptamir”）。

[0082] 因此,在一个方面,本发明提供了调节细胞中的呼吸链解偶联的方法,所述方法包括使细胞与调节至少一种线粒体解偶联剂的表达水平和/或活性的经分离的miRNA试剂接触。在一些实施方案中,所述方法还包括选择需要调节呼吸链解偶联的对象(例如,肥胖患者)的步骤。在一个实施方案中,所述miRNA试剂提高至少一种线粒体解偶联剂的表达水平和/或活性。在某些实施方案中,所述线粒体解偶联剂是UCP1或UCP2。在一些实施方案中,所述方法在体内提高细胞中的呼吸链解偶联。在另一些实施方案中,所述方法离体提高细胞中的呼吸链解偶联。在某些实施方案中,所述方法还包括测定线粒体解偶联剂的表达水平(mRNA或蛋白质)或活性。在某些实施方案中,所述细胞是前脂肪细胞、脂肪细胞、脂肪组织来源的间充质干细胞、肝细胞、肌细胞或其前体。任选地,脂肪细胞可为白色脂肪细胞或褐色脂肪细胞。

[0083] 在另一个方面,本发明提供了调节组织中的产热的方法,所述方法包括使组织与调节至少一种线粒体解偶联剂的表达水平和/或活性的经分离的miRNA试剂接触。在一些实施方案中,所述方法还包括选择需要调节产热的对象(例如,肥胖患者)的步骤。在一个实施方案中,所述miRNA试剂提高至少一种线粒体解偶联剂的表达水平和/或活性。在某些实施方案中,所述线粒体解偶联剂是UCP1或UCP2。在某些实施方案中,所述方法包括提高产热。在某些实施方案中,所述方法还包括测定线粒体解偶联剂的表达水平(mRNA或蛋白质)或活性。在某些实施方案中,所述组织是褐色脂肪组织、白色脂肪组织、皮下脂肪组织、肝或肌肉。在某些实施方案中,使所述组织与所述miRNA试剂离体接触。

[0084] 在另一个方面,本发明提供了治疗需要此治疗的人对象的肥胖的方法,所述方法一般包括向所述人对象施用有效量的调节至少一种线粒体解偶联剂活性的miRNA试剂。在某些实施方案中,所选择用于治疗的人对象具有肥胖的遗传素质或表观遗传素质。在某些实施方案中,所述线粒体解偶联剂是UCP1、UCP2或UCP3。

[0085] 在所有以上方面的某些实施方案中,所述miRNA试剂是选自以下的经分离的miRNA:hsa-miR-1-1、hsa-miR-1-2、miR-19a-b、hsa-miR-105、hsa-miR-1283、hsa-mir-129、hsa-miR-133a-1、hsa-miR-133a-2、hsa-miR-143、hsa-mir-143-5p、hsa-mir-147、hsa-mir-149、hsa-mir-199a、hsa-mir-199b、hsa-mir-200c、hsa-mir-204、hsa-mir-205、hsa-miR-206、hsa-mir-21、hsa-mir-211、hsa-mir-218、hsa-mir-218-1、hsa-mir-218-2、hsa-mir-219-2、hsa-mir-219-2-3p、hsa-mir-22、hsa-mir-22-3p、hsa-mir-22-5p、hsa-mir-24-2、hsa-miR-30a-e、hsa-miR-3177-5p、hsa-mir-325、hsa-mir-331、hsa-mir-331-5p、hsa-miR-3613-3p、hsa-mir-362、hsa-mir-362-5p、hsa-miR-3658、hsa-mir-367、hsa-mir-371、hsa-mir-371-5p、hsa-mir-377、hsa-mir-378、hsa-mir-378a-5p、hsa-mir-382、hsa-mir-383、hsa-mir-422a、hsa-mir-425、hsa-miR-455-3p、hsa-miR-455-5p、hsa-miR-491、hsa-mir-508、hsa-mir-508-5p、hsa-mir-512-1、hsa-mir-512-2、hsa-miR-515-3p、hsa-mir-519e、hsa-miR-520a、hsa-mir-543、hsa-mir-545、hsa-mir-549、hsa-mir-556、和hsa-miR-568、hsa-mir-620、hsa-mir-643、hsa-mir-654-3p、hsa-miR-7a-g、hsa-mir-765、hsa-mir-871、hsa-mir-888、hsa-mir-888-3p、hsa-mir-92b、hsa-mir-93、hsa-mir-96以及hsa-mir-99a。在所有以上方面的某些实施方案中,所述miRNA试剂是与以上列出的miRNA序列有80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%同一性的经分离的miRNA试剂。在

所有以上方面的某些实施方案中,所述 miRNA 试剂是上文列出的 miRNA 的种子序列 (seed sequence)。

[0086] 在所有以上方面的某些实施方案中,miRNA 试剂是选自表 1 中记载的 536 个 miRNA 中的经分离的 miRNA。在所有以上方面的某些实施方案中,miRNA 试剂是与表 1 中列出的 miRNA 序列有 80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98% 或 99% 同一性的经分离的 miRNA 试剂。在所有以上方面的某些实施方案中,所述 miRNA 试剂是表 1 中列出的 miRNA 的种子序列。

[0087] 表 1.

[0088] 以升序列出的脂肪细胞 miRNA (miRBase 19 命名法) :

[0089]

hsa-let-7a-3p	hsa-miR-185-5p	hsa-miR-329	hsa-miR-509-3p
hsa-let-7a-5p	hsa-miR-186-3p	hsa-miR-330-3p	hsa-miR-511
hsa-let-7b-3p	hsa-miR-186-5p	hsa-miR-330-5p	hsa-miR-513a-3p
hsa-let-7b-5p	hsa-miR-187-3p	hsa-miR-331-3p	hsa-miR-513a-5p
hsa-let-7c	hsa-miR-188-5p	hsa-miR-331-5p	hsa-miR-513b
hsa-let-7d-3p	hsa-miR-18a-3p	hsa-miR-335-3p	hsa-miR-514a-3p
hsa-let-7d-5p	hsa-miR-18a-5p	hsa-miR-335-5p	hsa-miR-515-3p
hsa-let-7e-5p	hsa-miR-18b-5p	hsa-miR-337-3p	hsa-miR-516b-3p
hsa-let-7f-1-3p	hsa-miR-1909-3p	hsa-miR-337-5p	hsa-miR-516b-5p
hsa-let-7f-5p	hsa-miR-190a	hsa-miR-338-3p	hsa-miR-518b
hsa-let-7g-3p	hsa-miR-190b	hsa-miR-338-5p	hsa-miR-518e-3p
hsa-let-7g-5p	hsa-miR-191-3p	hsa-miR-339-3p	hsa-miR-518e-5p
hsa-let-7i-3p	hsa-miR-191-5p	hsa-miR-339-5p	hsa-miR-518f-3p
hsa-let-7i-5p	hsa-miR-192-5p	hsa-miR-33a-5p	hsa-miR-519a-5p
hsa-miR-1	hsa-miR-193a-3p	hsa-miR-33b-5p	hsa-miR-519b-5p
hsa-miR-100-5p	hsa-miR-193a-5p	hsa-miR-340-3p	hsa-miR-519c-3p
hsa-miR-101-3p	hsa-miR-193b-3p	hsa-miR-340-5p	hsa-miR-519c-5p

[0090]

hsa-miR-101-5p	hsa-miR-193b-5p	hsa-miR-342-3p	hsa-miR-519d
hsa-miR-103a-2-5p	hsa-miR-194-5p	hsa-miR-342-5p	hsa-miR-520c-3p
hsa-miR-103a-3p	hsa-miR-195-3p	hsa-miR-345-5p	hsa-miR-520e
hsa-miR-103b	hsa-miR-195-5p	hsa-miR-346	hsa-miR-520f
hsa-miR-105-5p	hsa-miR-196a-5p	hsa-miR-34a-5p	hsa-miR-520g
hsa-miR-106a-5p	hsa-miR-196b-5p	hsa-miR-34b-3p	hsa-miR-520h

hsa-miR-106b-3p	hsa-miR-197-3p	hsa-miR-34b-5p	hsa-miR-521
hsa-miR-106b-5p	hsa-miR-198	hsa-miR-34c-5p	hsa-miR-522-5p
hsa-miR-107	hsa-miR-199a-3p	hsa-miR-3545-5p	hsa-miR-523-5p
hsa-miR-10a-3p	hsa-miR-199a-5p	hsa-miR-3591-3p	hsa-miR-525-3p
hsa-miR-10a-5p	hsa-miR-199b-3p	hsa-miR-361-3p	hsa-miR-532-3p
hsa-miR-10b-3p	hsa-miR-199b-5p	hsa-miR-361-5p	hsa-miR-532-5p
hsa-miR-10b-5p	hsa-miR-19a-3p	hsa-miR-3613-5p	hsa-miR-539-5p
hsa-miR-1179	hsa-miR-19b-3p	hsa-miR-3615	hsa-miR-542-3p
hsa-miR-1185-5p	hsa-miR-200a-3p	hsa-miR-362-3p	hsa-miR-542-5p
hsa-miR-1208	hsa-miR-200a-5p	hsa-miR-362-5p	hsa-miR-545-3p
hsa-miR-122-5p	hsa-miR-200b-3p	hsa-miR-363-3p	hsa-miR-545-5p
hsa-miR-1227-3p	hsa-miR-200c-3p	hsa-miR-363-5p	hsa-miR-548d-3p
hsa-miR-1228-5p	hsa-miR-202-3p	hsa-mir-365a-3p	hsa-miR-548e
hsa-miR-1229-3p	hsa-miR-203a	hsa-mir-3653	hsa-miR-548i
hsa-miR-124-3p	hsa-miR-204-5p	hsa-miR-3656	hsa-miR-548m
hsa-miR-125a-3p	hsa-miR-205-5p	hsa-miR-365a-3p	hsa-miR-550a-5p
hsa-miR-125a-5p	hsa-miR-206	hsa-miR-365a-5p	hsa-miR-551b-3p
hsa-miR-125b-1-3p	hsa-miR-20a-3p	hsa-miR-367-3p	hsa-miR-552
hsa-miR-125b-2-3p	hsa-miR-20a-5p	hsa-mir-3676-3p	hsa-miR-553
hsa-miR-125b-5p	hsa-miR-20b-5p	hsa-miR-369-3p	hsa-miR-554
hsa-miR-126-3p	hsa-miR-21-3p	hsa-miR-369-5p	hsa-miR-557
hsa-miR-126-5p	hsa-miR-21-5p	hsa-miR-370	hsa-miR-563
hsa-miR-1260a	hsa-miR-210	hsa-miR-371a-3p	hsa-miR-564
hsa-miR-1260b	hsa-miR-211-5p	hsa-miR-373-3p	hsa-miR-567

hsa-miR-1268a	hsa-miR-2110	hsa-miR-373-5p	hsa-miR-569
hsa-miR-127-3p	hsa-miR-212-3p	hsa-miR-374a-3p	hsa-miR-570-3p
hsa-miR-127-5p	hsa-miR-214-3p	hsa-miR-374a-5p	hsa-miR-572
hsa-miR-1271-5p	hsa-miR-214-5p	hsa-miR-374b-3p	hsa-miR-574-3p
hsa-miR-1273a	hsa-miR-215	hsa-miR-374b-5p	hsa-miR-574-5p
hsa-miR-1277-3p	hsa-miR-216a-5p	hsa-miR-375	hsa-miR-575
hsa-miR-128	hsa-miR-217	hsa-miR-376a-2-5p	hsa-miR-576-3p
hsa-miR-128-2	hsa-miR-218-5p	hsa-miR-376a-3p	hsa-miR-576-5p
hsa-miR-1285-3p	hsa-miR-219-1-3p	hsa-miR-376a-5p	hsa-miR-582-3p
hsa-miR-1287	hsa-miR-219-5p	hsa-miR-376b-3p	hsa-miR-582-5p
hsa-miR-1288	hsa-miR-22-3p	hsa-miR-376c-3p	hsa-miR-583

[0091]

hsa-miR-129-5p	hsa-miR-22-5p	hsa-miR-377-3p	hsa-miR-584-5p
hsa-miR-1290	hsa-miR-221-3p	hsa-miR-378a-3p	hsa-miR-585
hsa-miR-1292-5p	hsa-miR-221-5p	hsa-miR-378a-5p	hsa-miR-586
hsa-miR-1301	hsa-miR-222-3p	hsa-miR-378c	hsa-miR-589-5p
hsa-miR-1305	hsa-miR-222-5p	hsa-miR-378d	hsa-miR-590-3p
hsa-miR-1307-3p	hsa-miR-223-3p	hsa-miR-379-5p	hsa-miR-590-5p
hsa-miR-130a-3p	hsa-miR-223-5p	hsa-miR-380-3p	hsa-miR-595
hsa-miR-130b-3p	hsa-miR-224-3p	hsa-miR-381-3p	hsa-miR-598
hsa-miR-130b-5p	hsa-miR-224-5p	hsa-miR-382-5p	hsa-miR-601
hsa-miR-132-3p	hsa-miR-2355-3p	hsa-miR-383	hsa-miR-602
hsa-miR-132-5p	hsa-miR-23a-3p	hsa-miR-384	hsa-miR-603
hsa-miR-1323	hsa-miR-23b-3p	hsa-miR-3912	hsa-miR-605

hsa-miR-133a	hsa-miR-23b-5p	hsa-miR-3928	hsa-miR-606
hsa-miR-133b	hsa-miR-24-1-5p	hsa-miR-409-3p	hsa-miR-609
hsa-miR-134	hsa-miR-24-2-5p	hsa-miR-409-5p	hsa-miR-611
hsa-miR-135a-5p	hsa-miR-24-3p	hsa-miR-410	hsa-miR-615-3p
hsa-miR-135b-5p	hsa-miR-25-3p	hsa-miR-411-5p	hsa-miR-619
hsa-miR-136-3p	hsa-miR-26a-2-3p	hsa-miR-421	hsa-miR-625-5p
hsa-miR-136-5p	hsa-miR-26a-5p	hsa-miR-422a	hsa-miR-627
hsa-miR-137	hsa-miR-26b-3p	hsa-miR-422b	hsa-miR-628-3p
hsa-miR-138-1-3p	hsa-miR-26b-5p	hsa-miR-423-3p	hsa-miR-628-5p
hsa-miR-138-5p	hsa-miR-27a-3p	hsa-miR-423-5p	hsa-miR-629-3p
hsa-miR-139-3p	hsa-miR-27a-5p	hsa-miR-424-3p	hsa-miR-629-5p
hsa-miR-139-5p	hsa-miR-27b-3p	hsa-miR-424-5p	hsa-miR-630
hsa-miR-140-3p	hsa-miR-27b-5p	hsa-miR-425-3p	hsa-miR-636
hsa-miR-140-5p	hsa-miR-28-3p	hsa-miR-425-5p	hsa-miR-638
hsa-miR-141-3p	hsa-miR-28-5p	hsa-miR-429	hsa-miR-639
hsa-miR-142-3p	hsa-miR-296-5p	hsa-miR-431-5p	hsa-miR-641
hsa-miR-142-5p	hsa-miR-297	hsa-miR-432-5p	hsa-miR-642a-3p
hsa-miR-143-3p	hsa-miR-298	hsa-miR-433	hsa-miR-642a-5p
hsa-miR-143-5p	hsa-miR-299-3p	hsa-miR-4421	hsa-miR-646
hsa-miR-144-3p	hsa-miR-299-5p	hsa-miR-449a	hsa-miR-649
hsa-miR-144-5p	hsa-miR-29a-3p	hsa-miR-450a-5p	hsa-miR-651
hsa-miR-145-3p	hsa-miR-29a-5p	hsa-miR-450b-3p	hsa-miR-652-3p
hsa-miR-145-5p	hsa-miR-29b-1-5p	hsa-miR-450b-5p	hsa-miR-653
hsa-miR-1468	hsa-miR-29b-2-5p	hsa-miR-4510	hsa-miR-654-3p

hsa-miR-146a-5p	hsa-miR-29b-3p	hsa-miR-4516	hsa-miR-659-3p
hsa-miR-146b-3p	hsa-miR-29c-3p	hsa-miR-451a	hsa-miR-660-5p
hsa-miR-146b-5p	hsa-miR-29c-5p	hsa-miR-452-3p	hsa-miR-663a
hsa-miR-147a	hsa-miR-301a-3p	hsa-miR-452-5p	hsa-miR-664a-3p
hsa-miR-148a-3p	hsa-miR-301b	hsa-miR-454-3p	hsa-miR-664a-5p

[0092]

hsa-miR-148a-5p	hsa-miR-302a-5p	hsa-miR-454-5p	hsa-miR-668
hsa-miR-148b-3p	hsa-miR-302b-5p	hsa-miR-455-3p	hsa-miR-671-5p
hsa-miR-148b-5p	hsa-miR-302c-5p	hsa-miR-455-5p	hsa-miR-675-3p
hsa-miR-149-5p	hsa-miR-302d-3p	hsa-miR-4634	hsa-miR-675-5p
hsa-miR-150-3p	hsa-miR-3065-3p	hsa-miR-4732-5p	hsa-miR-7-2-3p
hsa-miR-150-5p	hsa-miR-3065-5p	hsa-miR-4792	hsa-miR-7-5p
hsa-miR-151a-3p	hsa-miR-3074-3p	hsa-miR-483-3p	hsa-miR-708-3p
hsa-miR-151a-5p	hsa-miR-3074-5p	hsa-miR-483-5p	hsa-miR-708-5p
hsa-miR-151b	hsa-miR-30a-3p	hsa-miR-484	hsa-miR-718
hsa-miR-152	hsa-miR-30a-5p	hsa-miR-485-5p	hsa-miR-744-5p
hsa-miR-153	hsa-miR-30b-3p	hsa-miR-486-3p	hsa-miR-765
hsa-miR-1539	hsa-miR-30b-5p	hsa-miR-486-5p	hsa-miR-769-5p
hsa-miR-154-3p	hsa-miR-30c-1-3p	hsa-miR-487b	hsa-miR-770-5p
hsa-miR-154-5p	hsa-miR-30c-2-3p	hsa-miR-488-3p	hsa-miR-874
hsa-miR-155-5p	hsa-miR-30c-5p	hsa-miR-489	hsa-miR-885-3p
hsa-miR-15a-3p	hsa-miR-30d-3p	hsa-miR-491-3p	hsa-miR-887
hsa-miR-15a-5p	hsa-miR-30d-5p	hsa-miR-491-5p	hsa-miR-889
hsa-miR-15b-3p	hsa-miR-30e-3p	hsa-miR-492	hsa-miR-890

hsa-miR-15b-5p	hsa-miR-30e-5p	hsa-miR-493-3p	hsa-miR-891a
hsa-miR-16-1-3p	hsa-miR-31-3p	hsa-miR-493-5p	hsa-miR-891b
hsa-miR-16-2-3p	hsa-miR-31-5p	hsa-miR-494	hsa-miR-9-5p
hsa-miR-16-5p	hsa-miR-3120-3p	hsa-miR-495-3p	hsa-miR-92a-3p
hsa-miR-17-3p	hsa-miR-3120-5p	hsa-miR-497-5p	hsa-miR-92b-3p
hsa-miR-17-5p	hsa-miR-3184-5p	hsa-miR-498	hsa-miR-93-3p
hsa-miR-181a-2-3p	hsa-miR-32-3p	hsa-miR-499a-5p	hsa-miR-93-5p
hsa-miR-181a-3p	hsa-miR-32-5p	hsa-miR-500a-3p	hsa-miR-935
hsa-miR-181a-5p	hsa-miR-320a	hsa-miR-501-3p	hsa-miR-942
hsa-miR-181b-5p	hsa-miR-320b	hsa-miR-501-5p	hsa-miR-95
hsa-miR-181c-3p	hsa-miR-320c	hsa-miR-502-3p	hsa-miR-96-3p
hsa-miR-181c-5p	hsa-miR-323a-3p	hsa-miR-502-5p	hsa-miR-96-5p
hsa-miR-181d	hsa-miR-324-3p	hsa-miR-503-5p	hsa-miR-98-5p
hsa-miR-182-5p	hsa-miR-324-5p	hsa-miR-504	hsa-miR-99a-3p
hsa-miR-183-5p	hsa-miR-325	hsa-miR-505-3p	hsa-miR-99a-5p
hsa-miR-184	hsa-miR-326	hsa-miR-505-5p	hsa-miR-99b-3p
hsa-miR-185-3p	hsa-miR-328	hsa-miR-506-3p	hsa-miR-99b-5p

[0093] 在所有以上方面的某些实施方案中,miRNA 试剂是选自表 11、13 和 14 中记载的经分离的 miRNA 中的 miRNA。在所有以上方面的某些实施方案中,所述 miRNA 试剂是与表 1、11、13 和 14 中列出的 miRNA 序列有 80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98% 或 99% 同一性的经分离的 miRNA。在所有以上方面的某些实施方案中,所述 miRNA 试剂是表 11、13 和 14 中列出的 miRNA 的种子序列。

[0094] 在所有以上方面的某些实施方案中,miRNA 试剂是选自表 1 中记载的 miRNA 中的 miRNA 的 agomir 或 antagomir。

[0095] 在所有以上方面的某些实施方案中,miRNA 试剂是选自以下的 miRNA 的 agomir 或 antagomir:hsa-miR-1-1、hsa-miR-1-2、miR-19a-b、hsa-miR-105、hsa-miR-1283、hsa-miR-129、hsa-miR-133a-1、hsa-miR-133a-2、hsa-miR-143、hsa-miR-143-5p、hsa-miR-147、hsa-miR-149、hsa-miR-199a、hsa-miR-199b、hsa-miR-200c、hsa-miR-204、

hsa-mir-205、hsa-miR-206、hsa-mir-21、hsa-mir-211、hsa-mir-218、hsa-mir-218-1、hsa-mir-218-2、hsa-mir-219-2、hsa-mir-219-2-3p、hsa-mir-22、hsa-mir-22-3p、hsa-mir-22-5p、hsa-mir-24-2、hsa-miR-30a-e、hsa-miR-3177-5p、hsa-mir-325、hsa-mir-331、hsa-mir-331-5p、hsa-miR-3613-3p、hsa-mir-362、hsa-mir-362-5p、hsa-miR-3658、hsa-mir-367、hsa-mir-371、hsa-mir-371-5p、hsa-mir-377、hsa-mir-378、hsa-mir-378a-5p、hsa-mir-382、hsa-mir-383、hsa-mir-422a、hsa-mir-425、hsa-miR-455-3p、hsa-miR-455-5p、hsa-miR-491、hsa-mir-508、hsa-mir-508-5p、hsa-mir-512-1、hsa-mir-512-2、hsa-miR-515-3p、hsa-mir-519e、hsa-miR-520a、hsa-mir-543、hsa-mir-545、hsa-mir-549、hsa-mir-556、and hsa-miR-568、hsa-mir-620、hsa-mir-643、hsa-mir-654-3p、hsa-miR-7a-g、hsa-mir-765、hsa-mir-871、hsa-mir-888、hsa-mir-888-3p、hsa-mir-92b、hsa-mir-93、hsa-mir-96 和 hsa-mir-99a。

[0096] 在所有以上方面的某些实施方案中，miRNA 试剂是选自表 11 中记载的 miRNA 中的 miRNA 的 agomir 或 antagomir。

[0097] 在所有上述方面的某些实施方案中，miRNA 试剂是选自以下的 miRNA 的 antagomir：hsa-miR-19b-2-5p、hsa-miR-21-5p、hsa-miR-130b-5p、hsa-miR-211、hsa-miR-325、hsa-miR-382-3p/5p、hsa-miR-543、hsa-miR-515-3p 和 hsa-miR-545。

[0098] 在所有上述方面的某些实施方案中，miRNA 试剂是选自以下的 miRNA 的 antagomir：hsa-miR-331-5p、hsa-miR-552、hsa-miR-620 和 hsa-miR-1179。

[0099] 在所有以上方面的某些实施方案中，miRNA 试剂与靶向部分（例如适配体）连接。在一个实施方案中，所述靶向部分将 miRNA 试剂递送至特定细胞类型或组织。

[0100] 在所有以上方面的某些实施方案中，miRNA 试剂直接与至少一种线粒体解偶联剂的 mRNA 或启动子区结合。

[0101] 在所有以上方面的某些实施方案中，miRNA 试剂直接与至少一种线粒体解偶联剂之 mRNA 的 5' UTR 或编码序列结合。

[0102] 在所有以上方面的某些实施方案中，miRNA 试剂直接与至少一种线粒体解偶联剂之 mRNA 的 3' UTR 结合。

[0103] 在所有以上方面的某些实施方案中，miRNA 试剂调节线粒体解偶联蛋白的激活蛋白 (activator) 或阻抑蛋白 (repressor) 的活性。在一个实施方案中，所述 miRNA 试剂直接与激活蛋白或阻抑蛋白的 mRNA 或启动子区结合。在一个实施方案中，miRNA 试剂直接与激活蛋白或阻抑蛋白之 mRNA 的 5' UTR 或编码序列结合。在一个实施方案中，miRNA 试剂直接与激活蛋白或阻抑蛋白之 mRNA 的 3' UTR 结合。在一个实施方案中，激活蛋白或阻抑蛋白选自列于表 2 中的组。

[0104] 在所有以上方面的某些实施方案中，上调线粒体解偶联蛋白的 mRNA 或蛋白质表达。

[0105] 在所有以上方面的某些实施方案中，上调线粒体解偶联蛋白的线粒体解偶联活性。

[0106] 在另一个方面，本发明提供了筛选调节产热的 miRNA 试剂的方法，所述方法一般包括：提供指示细胞 (indicator cell)；使所述指示细胞与受试 miRNA 试剂接触；以及在存在及不存在所述 miRNA 试剂的情况下，测定所述指示细胞中的至少一种产热调节子的细胞

活性,其中,所述产热调节子活性在存在所述受试 miRNA 试剂的情况下的改变将所述受试 miRNA 试剂鉴定为调节产热的 miRNA 试剂。所述指示细胞可为哺乳动物细胞。在某些实施方案中,所述指示细胞是包含至少部分人基因组的人细胞。

[0107] 在某些实施方案中,所述细胞是前脂肪细胞、脂肪细胞、脂肪组织来源的间充质干细胞、肝细胞、肌细胞或其前体。

[0108] 在某些实施方案中,在该方法中测定的产热调节子细胞活性是所述产热调节子的 mRNA 表达水平、蛋白质表达水平或线粒体解偶联活性。

[0109] 在某些实施方案中,与不存在受试 miRNA 试剂的条件下产热调节子的活性水平相比,受试 miRNA 试剂提高了产热调节子的活性。

[0110] 在某些实施方案中,产热调节子是 UCP1 或 UCP2。

[0111] 在另一个方面,本发明提供了调节细胞中的至少一种产热调节子活性的 agomir 或 antagomir。

[0112] 在某些实施方案中,agomir 或 antagomir 是选自表 11、13 和 14 中所记载 miRNA 的 miRNA 的 agomir 或 antagomir。

[0113] 在某些实施方案中,agomir 或 antagomir 是选自表 1 中所记载 miRNA 的 miRNA 的 agomir 或 antagomir。

[0114] 在某些实施方案中,agomir 或 antagomir 是选自以下的 miRNA 的 agomir 或 antagomir:hsa-miR-1-1、hsa-miR-1-2、miR-19a-b、hsa-miR-105、hsa-miR-1283、hsa-mir-129、hsa-miR-133a-1、hsa-miR-133a-2、hsa-miR-143、hsa-mir-143-5p、hsa-mir-147、hsa-mir-149、hsa-mir-199a、hsa-mir-199b、hsa-mir-200c、hsa-mir-204、hsa-mir-205、hsa-miR-206、hsa-mir-21、hsa-mir-211、hsa-mir-218、hsa-mir-218-1、hsa-mir-218-2、hsa-mir-219-2、hsa-mir-219-2-3p、hsa-mir-22、hsa-mir-22-3p、hsa-mir-22-5p、hsa-mir-24-2、hsa-miR-30a-e、hsa-miR-3177-5p、hsa-mir-325、hsa-mir-331、hsa-mir-331-5p、hsa-miR-3613-3p、hsa-mir-362、hsa-mir-362-5p、hsa-miR-3658、hsa-mir-367、hsa-mir-371、hsa-mir-371-5p、hsa-mir-377、hsa-mir-378、hsa-mir-378a-5p、hsa-mir-382、hsa-mir-383、hsa-mir-422a、hsa-mir-425、hsa-miR-455-3p、hsa-miR-455-5p、hsa-miR-491、hsa-mir-508、hsa-mir-508-5p、hsa-mir-512-1、hsa-mir-512-2、hsa-miR-515-3p、hsa-mir-519e、hsa-miR-520a、hsa-mir-543、hsa-mir-545、hsa-mir-549、hsa-mir-556、和 hsa-miR-568、hsa-mir-620、hsa-mir-643、hsa-mir-654-3p、hsa-miR-7a-g、hsa-mir-765、hsa-mir-871、hsa-mir-888、hsa-mir-888-3p、hsa-mir-92b、hsa-mir-93、hsa-mir-96 以及 hsa-mir-99a。

[0115] 在某些实施方案中,agomir 或 antagomir 是选自以下的 miRNA 的 agomir 或 antagomir:hsa-miR-19b-2-5p、hsa-miR-21-5p、hsa-miR-130b-5p、hsa-miR-211、hsa-miR-325、hsa-miR-382-3p/5p、hsa-miR-543、hsa-miR-515-3p 和 hsa-miR-545。

[0116] 在某些实施方案中,agomir 或 antagomir 是选自以下的 miRNA 的 antagomir:hsa-miR-331-5p、hsa-miR-552、hsa-miR-620 和 hsa-miR-1179。

[0117] 在某些实施方案中,agomir 或 antagomir 与靶向部分连接。

[0118] 在某些实施方案中,靶向部分是适配体。

[0119] 在某些实施方案中,靶向部分将所述 agomir 或 antagomir 递送至特定细胞类型或

组织。

[0120] 在某些实施方案中, agomir 或 antagomir 直接与至少一种线粒体解偶联剂的 mRNA 或启动子区结合。

[0121] 在某些实施方案中, agomir 或 antagomir 直接与至少一种线粒体解偶联剂之 mRNA 的 5' UTR 或编码序列结合。

[0122] 在某些实施方案中, agomir 或 antagomir 直接与至少一种线粒体解偶联剂之 mRNA 的 3' UTR 结合。

[0123] 在某些实施方案中, agomir 或 antagomir 调节线粒体解偶联蛋白的激活蛋白或阻抑蛋白的活性。

[0124] 在某些实施方案中, 激活蛋白或阻抑蛋白选自列于表 2 中的组。

[0125] 在某些实施方案中, agomir 或 antagomir 直接与激活蛋白或阻抑蛋白的 mRNA 或启动子区结合。

[0126] 在某些实施方案中, agomir 或 antagomir 直接与激活蛋白或阻抑蛋白之 mRNA 的 5' UTR 或编码序列结合。在另一些实施方案中, agomir 或 antagomir 直接与激活蛋白或阻抑蛋白之 mRNA 的 3' UTR 结合。

[0127] 本公开内容还提供了药物组合物, 其包含选自以下的两种或更多种 miRNA: hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-lagomir、hsa-miR-lantagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。在某些实施方案中, 所述药物组合物还包含可药用赋形剂。在某些实施方案中, 所述两种或更多种 miRNA 由重组载体表达。所述重组载体可选自 DNA 质粒、病毒载体和 DNA 微环。

[0128] 本公开内容还提供了诱导前脂肪细胞先分化成白色脂肪细胞并且随后分化成褐色脂肪细胞的方法, 其包括向前体脂肪细胞群施用选自以下的一种或更多种 miRNA: hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-lagomir、hsa-miR-lantagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。所述一种或更多种 miRNA 还可选自 hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-lagomir、hsa-miR-lantagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。在某些实施方案中, 使前脂肪细胞分化成脂肪细胞的诱导大于当将前脂肪细胞暴露于 100nM 罗格列酮两天随后暴露于维持培养基中时得到的前脂肪细胞向脂肪细胞的分化。在某些实施方案中, 所述脂肪细胞是褐色脂肪细胞。在另一些实施方案中, 所述脂肪细胞是白色脂肪细胞。其他分化标准可在下文的实施例中找到。

[0129] 本公开内容还提供了用于降低脂肪细胞的脂质含量的方法, 其包括向脂肪细胞群施用选自以下的一种或更多种 miRNA: hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-lagomir、hsa-miR-lantagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。在某些实施方案中, 所述脂肪细胞中的脂质含量小于暴露于 100nM 罗格列酮两天随后暴露于维持培养基中的脂肪细胞中的脂质含量, 或者小于在培养持续时间内暴露于 100nM 罗格列酮的脂肪细胞的脂肪含量。培养持续时间可为 8 天至 16 天、10 天至 14 天或者 14 天。培养持续时间还可为 8 天、9 天、10 天、

11 天、12 天、13 天、14 天、15 天、16 天、17 天、18 天、19 天或 20 天。脂肪细胞之脂质含量的另外的标准可在下文的实施例中找到。

[0130] 本公开内容还提供了用于在有此需要的对象中提高胰岛素敏感性的方法,其包括向所述对象施用选自以下的一种或更多种 miRNA :hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-lagomir、hsa-miR-lantagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。

[0131] 在某些实施方案中,所述对象是哺乳动物。

[0132] 本公开内容还提供了提高细胞中的一种或更多种解偶联蛋白之表达或活性的方法,其包括向所述细胞施用选自 hsa-let-7a antagomir、hsa-miR-lagomir、hsa-miR-19b agomir 和 hsa-miR-30b agomir 的一种或更多种、两种或多于两种或者三种或多于三种 miRNA。在某些实施方案中,所述细胞选自褐色脂肪细胞、白色脂肪细胞、皮下脂肪细胞、肝细胞或者肌肉细胞。在另一些实施方案中,所述一种或更多种解偶联蛋白包括 UCP1 或者 UCP2。在某些实施方案中,所述方法是离体方法。在另一些实施方案中,所述方法是体内方法。在某些实施方案中,所述方法包括选择需要提高一种或更多种解偶联蛋白(例如 UCP1、UCP2)之表达水平或活性水平的对象(例如,人)。在一些实施方案中,所述对象患有肥胖或者有发展成肥胖的风险。在某些实施方案中,所述对象患有糖尿病或者有发展成糖尿病的风险。在某些实施方案中,所述方法还包括测定一种或更多种解偶联蛋白的表达水平(mRNA 或蛋白质)或活性。

[0133] 本公开内容还提供了引起有此需要之对象中的脂肪消耗的方法,其包括向所述对象施用选自以下的一种或更多种 miRNA :hsa-let-7a antagomir、hsa-miR-lagomir、hsa-miR-19b agomir 和 hsa-miR-30b agomir。在某些实施方案中,所述对象是哺乳动物。在另一些实施方案中,所述哺乳动物是人。

附图说明

[0134] 图 1A 和 1B 是使用 STRING 9.0 数据库在两种不同严格(stringency)水平上确定的 83 种产热调节子相互作用的示意图。

[0135] 图 2A 是使用 Ingenuity Pathway Analysis Software 程序确定的 83 种产热调节子相互作用的示意图。

[0136] 图 2B 是使用 Reactome Functional Interaction Network 程序确定的 83 种产热调节子相互作用的示意图。

[0137] 图 3 是多种 miRNA 预测程序预测的人 UCP1 基因的 5' UTR、启动子区、编码序列和 3' UTR 的 miRNA 结合位点的结果的重叠示意图。

[0138] 图 4 是多种 miRNA 预测程序预测的 83 种产热调节子基因的 5' UTR、启动子区、编码序列和 3' UTR 的 miRNA 结合位点的结果的重叠示意图。

[0139] 图 5 是线粒体中氧化磷酸化的示意图,描述通过 UCP1 使氧化磷酸化与 ATP 合成解偶联以产生热。

[0140] 图 6 描述了由另一些示例性产热调节子对 UCP1 进行的转录控制。

[0141] 图 7 描述了 UCP1 基因转录的示例性正性(a)转录调节子和负性(b)转录调节子。

[0142] 图 8A 描述了人 UCP1 基因的 15,910 个碱基对(bp)序列(NCBI 参考序列:

gi|237858805|ref|NG_012139.1| 人解偶联蛋白 1(线粒体、质子载体)(UCP1),4 号染色体上的 RefSeqGene) 中多种调控元件相对于 UCP1 转录起始位点(第 5,001 位)的位置。

[0143] 图 8B 描述了人 UCP2 基因的 15,174bp 序列(ENSG00000175567)(包含 11 号染色体上的 5,000bp 5' UTR 和 2,000bp 3' UTR) 中多种调控元件相对于转录起始位点(第 5,001 位)的位置。

[0144] 图 9 是示出未标记的前脂肪细胞或者经 Dy547- 标记的非靶向 miRNA 模拟物或发夹抑制剂转染的前脂肪细胞中的相对荧光的条形图。

[0145] 图 10A 是示出经 siRNA 对照和 GAPDH siRNA 转染的前脂肪细胞在转染 4 天后 GAPDH 基因表达降低的条形图。

[0146] 图 10B 是示出经 siRNA 对照和 GAPDH siRNA 转染的前脂肪细胞在转染 12 天后 GAPDH 基因表达降低的条形图。

[0147] 图 11A 是单独在维持培养基中培养两周后的前脂肪细胞用油红 O 染色的光学显微照片(micrograph)。

[0148] 图 11b 是在胰岛素、三-碘甲状腺原氨酸(tri-iodothyronine)、地塞米松、异丁基-甲基黄嘌呤和罗格列酮的存在下培养两天随后在维持培养基中培养 12 天的前脂肪细胞用油红 O 染色的光学显微照片。

[0149] 图 11C 是实验中始终在胰岛素、三-碘甲状腺原氨酸、地塞米松、异丁基-甲基黄嘌呤和罗格列酮的存在下培养的前脂肪细胞用油红 O 染色的光学显微照片。

[0150] 图 11D 是在 hsa-miR-30b 模拟物的存在下培养的前脂肪细胞用油红 O 染色的光学显微照片。

[0151] 图 11E 是在非靶向 miRNA 模拟物的存在下培养的前脂肪细胞用油红 O 染色的光学显微照片。

[0152] 图 11F 是在非靶向 miRNA 抑制剂的存在下培养的前脂肪细胞用油红 O 染色的光学显微照片。

[0153] 图 12A 是示出在罗格列酮的存在下产热靶标的 mRNA 表达的条形图。

[0154] 图 12B 是示出在 hsa-let-7a 抑制剂的存在下产热靶标的 mRNA 表达的条形图。

[0155] 图 12C 是示出在 hsa-miR-1 模拟物的存在下产热靶标的 mRNA 表达的条形图。

[0156] 图 12D 是示出在 hsa-miR-19b 模拟物的存在下产热靶标的 mRNA 表达的条形图。

[0157] 图 12E 是示出在 hsa-miR-30b 模拟物的存在下产热靶标的 mRNA 表达的条形图。

[0158] 图 12F 是示出未经处理的前脂肪细胞中产热靶标的 mRNA 表达的条形图。

[0159] 图 13 是示出 x 轴上的平均基因表达和 y 轴上的碱基对之间的对数级差异的 M-A 图(plot)。

[0160] 图 14 是示出维恩图的示意图,其示出在 miRNA 类似物 hsa-let-7a 抑制剂、hsa-miR-1 模拟物、hsa-miR-19b 模拟物和 hsa-miR-30b 模拟物的存在下,显著上调的基因数分别是 305、247、255 和 267。127 个基因的组通常被所列的 miRNA 类似物上调。

[0161] 图 15 是示出维恩图的示意图,其示出在 miRNA 类似物 hsa-let-7a 抑制剂、hsa-miR-1 模拟物、hsa-miR-19b 模拟物和 hsa-miR-30b 模拟物的存在下,显著下调的基因数分别是 143、177、115 和 165。60 个基因的组通常被所列的 miRNA 类似物下调。

[0162] 图 16 是示出未标记的脂肪细胞或者经 Dy547- 标记的非靶向 miRIDIAN 模拟物或

发夹抑制剂转染的脂肪细胞中的相对荧光的条形图。

[0163] 图 17A 是示出经 siRNA 对照和 GAPDH siRNA 转染的细胞在转染 4 天后 GAPDH 基因表达降低的条形图。

[0164] 图 17B 是示出经 siRNA 对照和 GAPDH siRNA 转染的细胞在转染 12 天后 GAPDH 基因表达降低的条形图。

[0165] 图 18A 是单独在维持培养基中培养两周后的成熟脂肪细胞用油红 O 染色的光学显微照片。

[0166] 图 18B 是在罗格列酮的存在下培养两周的成熟脂肪细胞用油红 O 染色的光学显微照片。

[0167] 图 18C 是在非靶向 miRNA 的存在下培养的成熟脂肪细胞用油红 O 染色的光学显微照片。

[0168] 图 18D 是在 hsa-miR-30b 模拟物的存在下培养的成熟脂肪细胞用油红 O 染色的光学显微照片。

[0169] 图 19 是示出暴露于多种 miRNA 类似物或罗格列酮的成熟脂肪细胞中脂质（尼罗红 (Nile Red) 荧光染料）的量的条形图。

[0170] 图 20 是示出从暴露于多种转染试剂的成熟脂肪细胞中提取的 RNA 总量的条形图。

[0171] 图 21 是示出使用多种转染试剂用 GAPDH 特异性的 miRNA 模拟物转染的成熟脂肪细胞中 GAPDH 基因表达降低的条形图。

[0172] 图 22 示出在单独的维持培养基（对照）、50nM hsa-let-7a 抑制剂、10 μ M β 肾上腺素能受体激动剂 CL 316, 243、50nM hsa-miR-1 模拟物、10nM 甲状腺激素三 - 碘甲状腺原氨酸、50nM hsa-miR-19b 模拟物、100nM 罗格列酮或 50nM hsa-miR-30b 模拟物中培养两周的成熟脂肪细胞的明场显微照片。

[0173] 图 23 是用于分离特异性针对人细胞表面独特靶标的适配体的 Cell-SELEX 方法的示意图。

[0174] 图 24 描述了评价所选的荧光适配体与人肝细胞和脂肪细胞结合的 FACS 实验的结果。

具体实施方式

[0175] I. 定义

[0176] 除非另有定义，否则本文所用的所有技术术语和科学术语都具有由本发明所属领域的普通技术人员所通常理解的相同的含义。在相抵触的情况下，以本申请（包括定义）为准。

[0177] 本文所用术语“miRNA 试剂”是指直接或间接调节产热调节子（例如线粒体解偶联剂或其或激活蛋白或阻抑蛋白）活性的寡核苷酸或寡核苷酸模拟物。miRNA 试剂可作用于靶基因或靶 miRNA。

[0178] 本文所用术语“miRNA”是指能够与靶基因（mRNA 或 DNA）结合并调节该基因表达的单链 RNA 分子（或其合成衍生物）。在某些实施方案中，miRNA 天然表达于生物体中。

[0179] 本文所用术语“种子序列”是指 miRNA 5' 端的前 8 个核苷酸（nt）内的 6 至 8 个核苷酸至个核苷酸长的子串（substring）（即，种子序列），其为靶标特异性的重要决定因素。

[0180] 本文所用术语“agomir”是指功能上模拟 miRNA 的合成寡核苷酸或寡核苷酸模拟物 (oligonucleotide mimetic)。agomir 可为与 miRNA 或 miRNA 的一部分具有相同或相似的核酸序列的寡核苷酸。在某些实施方案中 agomir 与其所模拟的 miRNA 具有 1、2、3、4、5、6、7、8、9 或 10 个核苷酸差异。此外,与其所模拟的 miRNA 相比, agomir 还可具有相同的长度、更长的长度或更短的长度。在某些实施方案中, agomir 具有与其所模拟的 miRNA 5' 端的 6 至 8 个核苷酸相同的序列。在另一些实施方案中, agomir 的长度可为 5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48、49 或 50 个核苷酸。在另一些实施方案中, agomir 的长度可为 5 至 10 个核苷酸、6 至 8 个核苷酸、10 至 20 个核苷酸、10 至 15 个核苷酸或 5 至 500 个核苷酸。在某些实施方案中, agomir 包含表 1、11、13 和 14 中所示的任意序列。这些经化学修饰的合成 RNA 双链包含与目的 miRNA 相同或基本相同的引导链,以允许有效地加载进 miRISC 复合物中,而过客链 (passenger strand) 经化学修饰以防止其加载到 miRISC 复合物的 Argonaute 蛋白中 (Thorsen SB 等, Cancer J., 18(3):275-284(2012); Broderick JA 等, Gene Ther., 18(12):1104-1110(2011))。

[0181] 本文所用术语“antagomir”是指具有与特定的微小 RNA 互补并且抑制该 miRNA 活性的合成寡核苷酸或寡核苷酸模拟物。在某些实施方案中, antagomir 与其所抑制的 miRNA 具有 1、2、3、4、5、6、7、8、9 或 10 个核苷酸差异。此外,与其所抑制的 miRNA 相比, antagomir 还可具有相同的长度、更长的长度或更短的长度。在某些实施方案中,所述 antagomir 与其所抑制的 miRNA 5' 端的 6 至 8 个核苷酸杂交。在另一些实施方案中, antagomir 的长度可为 5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48、49 或 50 个核苷酸。在另一些实施方案中, antagomir 的长度可为 5 至 10 个核苷酸、6 至 8 个核苷酸、10 至 20 个核苷酸、10 至 15 个核苷酸或 5 至 500 个核苷酸。在某些实施方案中, antagomir 包含与表 1、11、13 和 14 中所示的任意序列互补的核苷酸。antagomir 是与特定 miRNA 紧密结合并使其失活的合成的反向互补。使用多种化学修饰以改进核酸酶抗性和结合亲和力。最常用的提高效价的修饰包括多种 2' 糖修饰,例如 2'-O-Me、2'-O- 甲氧基乙基 (2'-MOE) 或 2'- 氟代 (2'-F)。miRNA 的核酸结构还可修饰成锁核酸 (LNA),其中在 2' 氧和 4' 碳之间具有亚甲基桥从而将核糖锁定为 A 型核酸构象的 3' - 内 (North) 构象 (Lennox KA 等, Gene Ther. Dec 2011; 18(12):1111-1120; Bader AG 等, Gene Ther. Dec 2011; 18(12):1121-1126)。该修饰显著提高分子的靶特异性和杂交性质二者。

[0182] 本文所用术语“aptamir”是指适配体 (与特定靶分子结合的寡核苷酸或肽分子) 与如上文定义的 agomir 或 antagomir 的组合,其允许 miRNA 试剂的细胞特异性递送或组织特异性递送。

[0183] 本文所用术语“干扰 RNA”是指能够通过介导 RNA 干扰来抑制或下调基因表达的任何双链 RNA 序列或单链 RNA 序列。干扰 RNA 包括但不限于小干扰 RNA (“siRNA”) 和小发夹 RNA (“shRNA”)。“RNA 干扰”是指序列匹配 (compatible) 的信使 RNA 转录物的选择性降解。

[0184] 本文所用术语“小干扰 RNA”或“siRNA”是指能够通过以序列特异性的方式介导 RNA 干扰来抑制或下调基因表达的任何小 RNA 分子。所述小 RNA 的长度可为例如约 16 至

21 个核苷酸。

[0185] 本文所用术语“shRNA”(小发夹 RNA)是指包含反义区、环部分和正义区的 RNA 分子,其中所述正义区具有与所述反义区进行碱基配对的互补核苷酸以形成双链的茎。在转录后加工后,小发夹 RNA 通过由 RNA 酶 III 家族成员酶 Dicer 介导的切割事件转变成小干扰 RNA (siRNA)。

[0186] 本文所用术语“反义寡核苷酸”是指与 DNA 序列或 mRNA 序列(例如 miRNA)互补的合成寡核苷酸或寡核苷酸模拟物。

[0187] 本文所用术语“miR-掩蔽物(mask)”是指与靶 mRNA 中的 miRNA 结合位点互补并且用于抑制 miRNA 与该 mRNA 结合位点结合的单链反义寡核苷酸。参见例如,Xiao 等,“Novel approaches for gene-specific interference via manipulating actions of microRNAs :examination on the pacemaker channel genes HCN2and HCN4,”Journal of Cellular Physiology,第 212 卷,第 2 期,第 285-292 页,2007,其整体并入本文。

[0188] 本文所用术语“miRNA 海绵”是指包含目的 miRNA 的多个串联结合位点并且用于去除(titrate out)内源性目的 miRNA 以抑制目的 miRNA 与其内源靶标结合的合成核酸(例如 mRNA 转录物)。参见例如,Ebert 等,“MicroRNA sponges :competitive inhibitors of small RNAs in mammalian cells,”Nature Methods,第 4 卷,第 9 期,第 721-726 页,2007,其整体并入本文。

[0189] 本文所用术语“呼吸链解偶联”是指使线粒体的内膜质子梯度消失从而阻止线粒体中通过氧化磷酸化来合成 ATP。

[0190] 本文所用术语“线粒体解偶联剂”是指可使线粒体内膜质子梯度消失从而阻止线粒体中通过氧化磷酸化来合成 ATP 的蛋白质(或编码核酸)。示例性的线粒体解偶联剂包括 UCP1 和 UCP2。

[0191] 本文所用术语线粒体解偶联剂的“激活蛋白”或“阻抑蛋白”是指分别用于上调或下调线粒体解偶联剂活性的蛋白质。

[0192] 本文所用术语“产热调节子”是指直接或间接调控产热的蛋白质(或编码核酸)。该术语涵盖线粒体线粒体解偶联剂,以及还涵盖线粒体解偶联剂的激活蛋白和阻抑蛋白。示例性的产热调节子记载于本文的表 2 中。

[0193] 本文所用术语“调节”是指使参数提高或降低。例如,调节蛋白质的活性可为使蛋白质的活性提高或降低。

[0194] 本文所用术语线粒体解偶联剂或产热调节子的“活性”是指任何可测量的生物学活性,包括但不限于,mRNA 表达、蛋白质表达或呼吸链解偶联。

[0195] miRNA 试剂组合物的“有效量”是足以在人中有效治疗或预防病症或控制生理学症状(condition)的量。在某些实施方案中,该生理学症状是肥胖。

[0196] “对象”是脊椎动物,包括哺乳纲的任何成员,包括人、家养动物和农场动物、动物园动物、运动场动物或宠物,例如小鼠、兔子、猪、绵羊、山羊、牛和高等灵长类。

[0197] 术语“哺乳动物”是指哺乳纲的任何成员,包括啮齿类、灵长类、犬、猫、骆驼科动物(camelid)和有蹄类。术语“啮齿类”是指作为啮齿目成员的任何种,包括小鼠、大鼠、仓鼠、沙鼠和兔子。术语“灵长类”是指作为灵长目成员的任何种,包括猴子、猿和人。术语“骆驼科动物”是指作为骆驼科家族成员的任何种,包括骆驼和美洲驼(1lama)。术语“有蹄类”是

指作为有蹄超目成员的任何种,包括牛、马和骆驼科动物。根据一些实施方案,哺乳动物是人类。

[0198] 本文所用的“治疗”定义为以治疗、治愈、缓解、减轻、改变、补救、改善、改良或影响该疾病或病症、疾病或病症的症状或对疾病的易感性为目的,将治疗剂(例如 miRNA 试剂或载体或编码它们的转基因)应用或施用于患者,或将治疗剂应用或施用于从患有疾病或病症、疾病或病症的症状或者具有对疾病或病症的素质的患者分离的组织或细胞系。

[0199] 本文所用的“药物基因组学”是指基因组学技术例如基因测序、统计遗传学和基因表达分析在对临床开发中和市售的药物的应用。更具体地,该术语是指患者的基因如何决定他或她对药物的响应的研究(例如,患者的“药物响应表型”,或“药物响应基因型”)。

[0200] miRNA 试剂组合物的“有效量”是足以在人中有效治疗或预防疾病或控制生理学病症的量。

[0201] II. 产热和肥胖

[0202] 在某些实施方案中,本发明提供了用于调节产热的方法。这些方法一般包括使细胞或组织与调节至少一种线粒体解偶联剂(例如 UCP1 和 / 或 UCP2)活性的 miRNA 试剂接触。这样的方法和组合物对治疗肥胖特别有用。

[0203] 哺乳动物脂肪细胞可基于其功能性质分为两大类:1) 储存和释放能量的填充有脂质的白色脂肪细胞(WAT),以及;2) 消耗能量并产生热的富含线粒体的褐色脂肪细胞(BAT)。直至最近,认为 BAT 在幼儿期经历了快速的退化,在成年人中仅留下残余的量。然而,在人体中用示踪剂 18F- 氟脱氧葡萄糖(18F-FDG) 进行的正电子发射断层成像(PET) 研究证实:1) 成年对象的颈部、锁骨上、腋部和椎旁区域中仍然存在多个 BAT 储库;2) 成年人的 BAT 可通过暴露于冷温度而被迅速激活;3) BAT 的活性与年龄、体重指数(BMI)、身体脂肪百分比、空腹血浆葡萄糖水平、 β - 阻滞剂的使用以及室外温度反相关;并且 4) BAT 的增加(expansion) 可驱动与产生儿茶酚胺的嗜铬细胞瘤相关的重量减轻,但是已经将导致受体功能降低的 β 3- 肾上腺素能受体多态性与体重增加和 2 型糖尿病的早期发作联系到一起。

[0204] 虽然 WAT 和 BAT 来源于间充质干细胞,但是它们具有不同的谱系,其中 Myf5(肌源性调节因子 5)(也在骨骼肌祖细胞中表达)、PGC-1 α 和 PRDM16(含 PR- 结构域的 16) 的表达将褐色脂肪细胞前体与白色脂肪细胞前体区分开来。除了典型的褐色脂肪细胞外,在 WAT 占优势的组织中还可诱导不同类型的褐色脂肪细胞。创造了术语“白中褐(brite)”(白色中的褐色)脂肪细胞,并且 WAT 储库中的褐色样脂肪细胞的外观与改进的代谢表型有关。BAT 质量和 / 或活性的提高提供了对肥胖一定程度的预防。BAT 的产热量为 300W/g,相比之下,所有其他组织中的产热量为 1W/g。因为少至 50g BAT 就将占有 20% 的日常能量消耗,所以对能量平衡产生显著影响仅需要相对有限量的 BAT。据推测,在一个对象的锁骨上 / 颈旁的储库中发现的大约 63g BAT 在 1 年内可消耗掉的能量等于 4.1kg WAT。

[0205] 线粒体解偶联蛋白(UCP) 是线粒体阴离子载体蛋白质(MACP) 家族的成员。UCP 使氧化磷酸化与 ATP 合成分开,使能量作为热散失(也称为“线粒体质子泄漏”)。UCP 促进阴离子从线粒体的内膜向外膜转移和从线粒体外膜向内膜的返回传递,在该过程中产生热。UCP 是负责产热和热耗散的主要蛋白质。解偶联蛋白 1(UCP1) 也称为产热素,是负责产热和热耗散的 BAT 特异性蛋白质。UCP2 是同样在脂肪细胞中表达的另一种解偶联蛋白。UCP 是产热调节蛋白网络的一部分(参见图 1)。示例性的产热调节子记载于表 2 中。

[0206] 调节产热调节子以诱导 BAT 分化和 / 或线粒体解偶联蛋白提供了在对象中诱导产热从而治疗肥胖的方法。然而,化学药理学方法不能靶向这些分子,因为它们不属于传统药物发现的“可用药空间 (druggable space)”占主导地位的典型“靶标类型”(激酶、离子通道、G 蛋白偶联受体等)。因此,本发明提供了使用 miRNA 试剂来调节这些产热调节子的新方法和组合物。

[0207] 在某些实施方案中,使用 miRNA 试剂来上调线粒体解偶联剂的活性(例如,mRNA 表达水平、蛋白质表达水平或线粒体解偶联活性)。线粒体解偶联剂的上调可通过几种方式实现。在一个实施方案中,miRNA 试剂直接抑制负责下调线粒体解偶联剂之活性(例如,mRNA 表达水平、蛋白质表达水平)的天然 miRNA 的活性。在另一个实施方案中,miRNA 试剂上调线粒体解偶联剂之激活蛋白的活性(例如,mRNA 表达水平或蛋白质表达水平)。该上调可例如通过直接抑制负责下调该激活蛋白表达的天然 miRNA 的活性来实现。在另一个实施方案中,miRNA 试剂下调线粒体解偶联剂之阻抑蛋白的活性(例如,mRNA 表达水平或蛋白质表达水平)。该下调可例如通过使用 miRNA 试剂直接抑制线粒体解偶联剂之阻抑蛋白的表达来实现。

[0208] 在某些实施方案中,使用能够同时调节多种产热调节子活性的 miRNA 试剂(与通用 miRNA 试剂相反的通路特异性 miRNA 试剂)。例如,可使用与多种产热调节子结合的单一 miRNA、agomir 或 antagomir。该方法是特别有利的,因为其允许使用单一 miRNA 试剂来调节整个信号通路中的多个成员。

[0209] 在某些实施方案中,使用多种抑制性 miRNA 试剂(例如,antagomir 或 miR 掩蔽物)。这些抑制性 miRNA 试剂可具有相同的 miRNA 靶标或不同的 miRNA 靶标。

[0210] III. miRNA 试剂

[0211] 在某些实施方案中,本发明使用 miRNA 试剂来调节产热调节子(例如,线粒体解偶联剂,如 UCP1 和 / 或 UCP2)。适用于本文所公开方法的 miRNA 试剂包括但不限于:miRNA、agomir、antagomir、miR- 掩蔽物、miRNA- 海绵、siRNA(单链或双链的)、shRNA、反义寡核苷酸、核酶或与靶核酸的至少一部分杂交并调节其功能的其他寡核苷酸模拟物。

[0212] 在某些实施方案中,所述 miRNA 试剂是 miRNA 分子或其合成衍生物(例如 agomir)。在一个具体实施方案中,所述 miRNA 试剂是 miRNA。miRNA 是存在于包括哺乳动物在内的多种生物体中的一类非编码的小 RNA(例如,18 至 24 个核苷酸),并且其在进化上是保守的。miRNA 通过由 RNA 酶 III 酶 drosha 和 dicer 的顺序切割来加工来源于初级转录物的约 70 个核苷酸的发夹前体而得到。许多 miRNA 可在基因间区编码,位于前 mRNA 的内含子中或位于 ncRNA 基因内。许多 miRNA 还倾向于成簇分布并转录为多顺反子,并且经常具有相似的时空表达模式。一般而言,miRNA 是转录后调节子,其与靶基因(mRNA 或 DNA)上的互补序列结合,通过例如转录抑制或靶向降解来导致基因沉默。一种 miRNA 可同时靶向多个不同基因。用于所公开方法的示例性 miRNA 分子包括但不限于:hsa-miR-1-1、hsa-miR-1-2、hsa-miR-7a-g、hsa-miR-105、hsa-miR-1283、hsa-mir-129、hsa-miR-133a-1、hsa-miR-133a-2、hsa-miR-143、hsa-mir-143-5p、hsa-mir-147、hsa-mir-149、hsa-mir-199a、hsa-mir-19a-b、hsa-mir-199b、hsa-mir-200c、hsa-mir-204、hsa-mir-205、hsa-miR-206、hsa-mir-21、hsa-mir-211、hsa-mir-218、hsa-mir-218-1、hsa-mir-218-2、hsa-mir-219-2、hsa-mir-219-2-3p、hsa-mir-22、hsa-mir-22-3p、hsa-mir-22-5p、

hsa-mir-24-2、hsa-miR-30a-e、hsa-miR-3177-5p、hsa-mir-325、hsa-mir-331、hsa-mir-331-5p、hsa-miR-3613-3p、hsa-mir-362、hsa-mir-362-5p、hsa-mir-367、hsa-mir-371、hsa-mir-371-5p、hsa-mir-377、hsa-mir-378、hsa-mir-378a-5p、hsa-mir-382、hsa-mir-383、hsa-miR-3658、hsa-mir-422a、hsa-mir-425、hsa-miR-455-3p、hsa-miR-455-5p、hsa-miR-491、hsa-mir-508、hsa-mir-508-5p、hsa-mir-512-1、hsa-mir-512-2、hsa-miR-515-3p、hsa-mir-519e、hsa-miR-520a、hsa-mir-543、hsa-mir-545、hsa-mir-549、hsa-mir-556、and hsa-miR-568、hsa-mir-620、hsa-mir-643、hsa-mir-654-3p、hsa-mir-765、hsa-mir-871、hsa-mir-888、hsa-mir-888-3p、hsa-mir-92b、hsa-mir-93、hsa-mir-96 和 hsa-mir-99a。在另一些实施方案中,用于所公开方法的示例性 miRNA 分子是本文的表 1、11、13 和 14 中所公开的 miRNA。在一个特定实施方案中,所述 miRNA 试剂是人 miR-22 或其功能性衍生物。

[0213] 在另一个特定实施方案中,所述 miRNA 试剂是 agomir。可使用本文公开的筛选方法来鉴定特定 miRNA 的 agomir。在一个特定实施方案中,agomir 是人 miR-22 的功能模拟物 (Davidson BL 等, *Nat. Rev. Genet.*, 12(5):329-340(2011))。

[0214] 在某些实施方案中,所述 miRNA 试剂是抑制一种或更多种 miRNA 活性的寡核苷酸或寡核苷酸模拟物。这样的分子实例包括但不限于 antagomir、干扰 RNA、反义寡核苷酸、核酶、miRNA 海绵和 miR- 掩蔽物。在一个特定实施方案中,所述 miRNA 试剂是 antagomir。一般而言,antagomir 是经化学修饰的反义寡核苷酸,其与靶 miRNA 结合并通过阻止该 miRNA 与其同源基因靶标结合来抑制 miRNA 的功能。antagomir 可包含本领域已知的任何碱基修饰。在一个特定实施方案中,antagomir 抑制人 miR-22 的活性 (van Rooij E 等, *Circ. Res.*, 110(3):496-507(2012); Snead NM 等, *Nucleic Acid Ther.*, 22(3):139-146(2012); Czech MP 等, *Nat. Rev. Endocrinol.*, 7(8):473-484(2011))。

[0215] 在某些实施方案中,所述 miRNA 试剂的长度为 10 至 50 个核苷酸。本领域普通技术人员应理解,这体现在寡核苷酸具有长度为 10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48、49 或 50 个或其中任意范围个核苷酸的反义部分。

[0216] 在某些实施方案中,所述 miRNA 试剂是嵌合寡核苷酸,其包含两个或更多个化学上不同的区域,每个区域由至少一个核苷酸构成。这些寡核苷酸通常包含赋予一种或更多种有益性质(例如,提高核酸酶抗性、提高进入细胞的摄取、提高与靶标的结合亲和力)的至少一个经修饰的核苷酸区域,以及作为使酶能够切割 RNA:DNA 或 RNA:RNA 杂合体(hybrid)的底物的区域。本发明的嵌合抑制性核酸可形成为由上述两种或更多种寡核苷酸、经修饰的寡核苷酸、寡聚核苷(oligonucleoside)和/或寡核苷酸模拟物组成的复合结构。这样的化合物在本领域也被称为杂合体或缺口体(gapmer),教导制备这样的杂合体结构的代表性美国专利包括但不限于以下美国专利 no:5,013,830;5,149,797;5,220,007;5,256,775;5,366,878;5,403,711;5,491,133;5,565,350;5,623,065;5,652,355;5,652,356;以及 5,700,922,其分别通过引用整体并入本文。

[0217] 在某些实施方案中,所述 miRNA 试剂包含至少一种在糖的 2' 位经修饰的核苷酸,最优选经 2' -O-烷基、2' -O-烷基-O-烷基或 2' -氟代修饰的核苷酸。在另一些优选实施方案中,RNA 修饰包括嘧啶的核糖、碱基残基或 RNA 3' 端的反向碱基上的 2' -氟代、

2' - 氨基以及 2' -O- 甲基修饰。寡核苷酸中常规地并入这样的修饰,并且这些寡核苷酸比 2' - 脱氧寡核苷酸示出了针对指定靶标的更高 T_m (即,更高的靶向结合亲和力)。

[0218] 已经示出许多核苷酸和核苷修饰以得到更加抗核酸酶消化的寡核苷酸,从而延长体内半衰期。经修饰寡核苷酸的具体实例包括包含含有例如以下之骨架的那些:硫代磷酸酯、磷酸三酯、磷酸甲酯、短链烷基或环烷基糖间键(intersugar linkage)或短链杂原子或杂环糖间键。最优选的是具有硫代磷酸酯骨架的寡核苷酸和具有杂原子骨架的寡核苷酸,所述杂原子骨架特别是: $\text{CH}_2\text{-NH-O-CH}_2$ 、 $\text{CH}_2\sim\text{N}(\text{CH}_3)\sim\text{O}\sim\text{CH}_2$ (称为亚甲基(甲基亚氨基)或 MMI 骨架)、 $\text{CH}_2\text{-O-N}(\text{CH}_3)\text{-CH}_2$ 、 $\text{CH}_2\text{-N}(\text{CH}_3)\text{-N}(\text{CH}_3)\text{-CH}_2$ 和 $\text{O-N}(\text{CH}_3)\text{-CH}_2\text{-CH}_2$ 骨架,其中天然的磷酸二酯骨架表示为 O-P-O-CH ; 酰胺骨架(参见 De Mesmaeker 等, *Ace. Chem. Res.* 1995, 28:366-374); 吗啉基骨架结构(参见 Summerton 和 Weller, 美国专利 No. 5, 034, 506); 肽核酸(PNA)骨架(其中,寡核苷酸的磷酸二酯骨架由聚酰胺骨架替代,核苷酸直接或间接与聚酰胺骨架的氮杂氮原子结合,参见 Nielsen 等, *Science* 1991, 254, 1497),各自通过引用整体并入本文。含磷连接包括但不限于,硫代磷酸酯、手性硫代磷酸酯、二硫代磷酸酯、磷酸三酯、氨基烷基磷酸三酯、包括 3' 亚烷基磷酸酯和手性磷酸酯在内的甲基磷酸酯或其他烷基磷酸酯、次磷酸酯、包括 3' - 氨基氨基磷酸酯和氨基烷基氨基磷酸酯在内的氨基磷酸酯、硫羰氨基磷酸酯、硫羰烷基磷酸酯、硫羰烷基磷酸三酯、硼烷磷酸酯、以及这些物质的具有正常 3' -5' 连接、2' -5' 连接的类似物,以及具有反转极性的那些(其中相邻核苷单元对连接从 3' -5' 变成 5' -3' 或者从 2' -5' 变成 5' -2') ; 参见美国专利 no. 3, 687, 808、4, 469, 863、4, 476, 301、5, 023, 243、5, 177, 196、5, 188, 897、5, 264, 423、5, 276, 019、5, 278, 302、5, 286, 717、5, 321, 131、5, 399, 676、5, 405, 939、5, 453, 496、5, 455, 233、5, 466, 677、5, 476, 925、5, 519, 126、5, 536, 821、5, 541, 306、5, 550, 111、5, 563, 253、5, 571, 799、5, 587, 361 和 5, 625, 050, 其各自通过引用整体并入本文。基于吗啉基的寡聚化合物描述在 Dwaine A. Braasch 和 David R. Corey, *Biochemistry*, 2002, 41(14), 4503-4510; *Genesis*, 2001 年第 30 卷第 3 期; Heasman, J., *Dev. Biol.*, 2002, 243, 209-214; Nasevicius 等, *Nat. Genet.*, 2000, 26, 216-220; Lacerra 等, *Proc. Natl. Acad. Sci.*, 2000, 97, 9591-9596; 以及 1991 年 7 月 23 日出版的美国专利 No. 5, 034, 506 中, 其各自通过引用整体并入本文。环己基核苷酸寡核苷酸模拟物描述在 Wang 等, *J. Am. Chem. Soc.*, 2000, 122, 8595-8602 中, 其内容通过引用整体并入本文。

[0219] 其中不包含磷原子的经修饰的寡核苷酸骨架具有由短链烷基或环烷基核苷酸间连接、混合的杂原子和烷基或环烷基核苷酸间连接或者一种或更多种短链杂原子或杂环核苷酸间连接形成的骨架。这些包含具有以下结构的那些: 吗啉基连接(部分地由核苷的糖部分形成); 硅氧烷骨架; 硫醚、亚砷和砷骨架; 甲酰乙酰基(formacetyl)和硫代甲酰乙酰基骨架; 亚甲基甲酰乙酰基和亚甲基硫代甲酰乙酰基骨架; 含烯烃骨架; 氨基磺酸酯骨架、亚甲基亚氨基和亚甲基胍基骨架; 磺酸酯和磺酰胺骨架; 酰胺骨架; 以及混合了 N、O、S 和 CH_2 组成部分的其他骨架; 参见美国专利 no. 5, 034, 506、5, 166, 315、5, 185, 444、5, 214, 134、5, 216, 141、5, 235, 033、5, 264, 562、5, 264, 564、5, 405, 938、5, 434, 257、5, 466, 677、5, 470, 967、5, 489, 677、5, 541, 307、5, 561, 225、5, 596, 086、5, 602, 240、5, 610, 289、5, 602, 240、5, 608, 046、5, 610, 289、5, 618, 704、5, 623, 070、5, 663, 312、5, 633, 360、5, 677, 437 以及 5, 677, 439, 其各自通过引用整体并入本文。

[0220] 在某些实施方案中, miRNA 试剂包含一种或更多种经取代的糖部分, 例如在 2' 位置的以下的一种: OH、SH、SCH₃、F、OCN、OCH₃OCH₃、OCH₃O(CH₂)_nCH₃、O(CH₂)_nNH₂或 O(CH₂)_nCH₃, 其中 n 为 1 至约 10; Ci 至 C10 低级烷基、烷氧基烷氧基、经取代的低级烷基、烷芳基或芳烷基; Cl; Br; CN; CF₃; OCF₃; O- 烷基、S- 烷基或 N- 烷基; O- 烯基、S- 烯基或 N- 烯基; SOCH₃; SO₂CH₃; ONO₂; NO₂; N₃; NH₂; 杂环烷基; 杂环烷基芳基; 氨基烷基氨基; 聚烷基氨基; 经取代的甲硅烷基; RNA 切割基团; 报告子基团; 嵌入子 (intercalator) 基团; 用于改进寡核苷酸药代动力学性质的基团; 或者用于改进寡核苷酸的药代动力学 / 药效学性质的基团以及具有类似性质的其他取代基。优选的修饰包括 2' - 甲氧基乙氧基 [2' -O-CH₂CH₂OCH₃, 也称为 2' -O-(2- 甲氧基乙基)] (Martin 等, *Helv. Chim. Acta*, 1995, 78, 486)。其他优选的修饰包括 2' - 甲氧基 (2' -O-CH₃)、2' - 丙氧基 (2' -OCH₂CH₂CH₃) 和 2' - 氟代 (2' -F)。类似的修饰还可在寡核苷酸的另一些位置上发生, 特别是 3' 端核苷酸上糖的 3' 位置和 5' 端核苷酸的 5' 位置。寡核苷酸还可含有糖模拟物, 例如环丁基替代戊呋喃基。

[0221] 在某些实施方案中, miRNA 试剂包含一种或更多种碱基修饰和 / 或替换。本文所用的“未经修饰的”或“天然的”碱基包括腺嘌呤 (A)、鸟嘌呤 (G)、胸腺嘧啶 (T)、胞嘧啶 (C) 和尿嘧啶 (U)。经修饰的碱基包括但不限于天然核酸中发现的仅稀有的或瞬变的碱基, 例如, 次黄嘌呤、6- 甲基腺嘌呤、5-Me 嘧啶, 特别是 5- 甲基胞嘧啶 (也称为 5- 甲基-2' 脱氧胞嘧啶, 并且在本领域经常称为 5-Me-C)、5- 羟甲基胞嘧啶 (HMC)、糖基 HMC 和龙胆二糖基 (gentobiosyl)HMC; 以及合成碱基, 例如 2- 氨基腺嘌呤、2- (甲基氨基) 腺嘌呤、2- (咪唑基烷基) 腺嘌呤、2- (氨基烷基氨基) 腺嘌呤或其他杂原子取代的烷基腺嘌呤、2- 硫尿嘧啶、2- 硫代胸腺嘧啶、5- 溴尿嘧啶、5- 羟甲基尿嘧啶、8- 氮杂鸟嘌呤、7- 脱氮鸟嘌呤、N6 (6- 氨基己基) 腺嘌呤和 2,6- 二氨基嘌呤。(Kornberg, A., *DNA Replication*, W. H. Freeman & Co., San Francisco, 1980, 第 75-77 页; Gebeyehu, G. 等, *Nucl. Acids Res.* 1987, 15: 4513)。还可包括本领域已知的“通用”碱基, 例如肌苷。还可包括 5-Me-C 替换。已显示, 这些修饰和 / 或替换使核酸双链的稳定性提高了 0.6 °C 至 1.2 °C。(Sanghvi, Y. S., Crooke, S. T 和 Lebleu, B. 编写, *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, 第 276-278 页)。另一些合适的经修饰的碱基在美国专利 no. 3, 687, 808, 以及 4, 845, 205; 5, 130, 302; 5, 134, 066; 5, 175, 273; 5, 367, 066; 5, 432, 272; 5, 457, 187; 5, 459, 255; 5, 484, 908; 5, 502, 177; 5, 525, 711; 5, 552, 540; 5, 587, 469; 5, 596, 091; 5, 614, 617; 5, 750, 692 中有描述, 其各自通过引用并入本文。

[0222] 不需要在指定的寡核苷酸中的所有位置进行统一修饰, 并且事实上, 在单一的寡核苷酸中可并入多于一种前述修饰, 甚至在寡核苷酸内的单一核苷酸上并入多于一种前述修饰。

[0223] 在某些实施方案中, 核苷酸单元的糖和核苷间连接 (即, 骨架) 两者都被新基团替换。碱基单元维持与合适的核酸靶标化合物杂交。一种这样的寡聚化合物, 已示出具有优异杂交性质的寡核苷酸模拟物, 被称为肽核酸 (PNA)。在 PNA 化合物中, 寡核苷酸的糖骨架被含酰胺的骨架例如氨基乙基甘氨酸骨架替代。核碱基 (nucleobase) 被保留, 并直接或间接与骨架酰胺部分的氮杂氮原子结合。教导 PNA 化合物之制备的代表性的美国专利包括但不限于美国专利 no. 5, 539, 082; 5, 714, 331; 以及 5, 719, 262, 其各自通过引用并入本文。PNA 化合物的另一些教导还可见于 Nielsen 等, *Science*, 1991, 254, 1497-1500 中。

[0224] 在某些实施方案中, miRNA 试剂(共价或非共价地)与一种或更多种增强寡核苷酸活性、细胞分布或细胞摄取的部分或缀合物连接。这样的部分包括但不限于, 脂质部分, 例如胆固醇部分 (Letsinger 等, Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556)、胆酸 (Manoharan 等, Bioorg. Med. Chem. Lett., 1994, 4, 1053-1060); 硫醚, 例如己基-S-三苯甲基硫醇 (Manoharan 等, Ann. N. Y. Acad. Sci., 1992, 660, 306-309; Manoharan 等, Bioorg. Med. Chem. Lett., 1993, 3, 2765-2770); 巯基胆固醇 (Oberhauser 等, Nucl. Acids Res., 1992, 20, 533-538); 脂肪族链, 例如十二烷基二醇或十一烷基残基 (Kabanov 等, FEBS Lett., 1990, 259, 327-330; Svinarchuk 等, Biochimie, 1993, 75, 49-54); 磷脂, 例如二-十六烷基-外消旋-甘油或三乙铵 1,2-二-O-十六烷基-外消旋-甘油基-3-H-磷酸酯 (Manoharan 等, Tetrahedron Lett., 1995, 36, 3651-3654; Shea 等, Nucl. Acids Res., 1990, 18, 3777-3783); 聚胺链或聚乙二醇链 (Mancharan 等, Nucleosides&Nucleotides, 1995, 14, 969-973); 或金刚烷乙酸 (Manoharan 等, Tetrahedron Lett., 1995, 36, 3651-3654); 棕榈基部分 (Mishra 等, Biochim. Biophys. Acta, 1995, 1264, 229-237); 或十八胺或己基氨基-羰基-叔氧胆固醇部分 (Crooke 等, J. Pharmacol. Exp. Ther., 1996, 277, 923-937), 其各自通过引用整体并入本文。还参见美国专利 no. 4, 828, 979、4, 948, 882、5, 218, 105、5, 525, 465、5, 541, 313、5, 545, 730、5, 552, 538、5, 578, 717、5, 580, 731、5, 580, 731、5, 591, 584、5, 109, 124、5, 118, 802、5, 138, 045、5, 414, 077、5, 486, 603、5, 512, 439、5, 578, 718、5, 608, 046、4, 587, 044、4, 605, 735、4, 667, 025、4, 762, 779、4, 789, 737、4, 824, 941、4, 835, 263、4, 876, 335、4, 904, 582、4, 958, 013、5, 082, 830、5, 112, 963、5, 214, 136、5, 082, 830、5, 112, 963、5, 214, 136、5, 245, 022、5, 254, 469、5, 258, 506、5, 262, 536、5, 272, 250、5, 292, 873、5, 317, 098、5, 371, 241、5, 391, 723、5, 416, 203、5, 451, 463、5, 510, 475、5, 512, 667、5, 514, 785、5, 565, 552、5, 567, 810、5, 574, 142、5, 585, 481、5, 587, 371、5, 595, 726、5, 597, 696、5, 599, 923、5, 599, 928 和 5, 688, 941, 其各自通过引用整体并入本文。

[0225] 在一个特定实施方案中, miRNA 试剂(共价或非共价地)与核酸适配体连接。适配体是与特定靶分子结合的合成的寡核苷酸分子或肽分子。适于与本文提供的 miRNA 试剂一起使用的适配体在 2012 年 8 月 31 日提交的美国临时专利申请 No. 61/695, 477 中有描述, 其通过引用整体并入本文。

[0226] 因此, 在第一方面, 本发明提供了脂肪细胞特异性的 miRNA 调节剂(modulator) 组合物, 其包含: I) 与人脂肪靶细胞上的细胞表面标记选择性结合的靶向部分, 以及 II) 产热的 miRNA 调节剂部分, 其中所述靶向部分促进所述靶细胞对所述 miRNA 调节部分的摄取以使所述 miRNA 能够靶向产热通路并上调所述靶细胞中的产热。

[0227] 在一个实施方案中, 所述组合物包含含有作为靶向部分之适配体的 agomir。

[0228] 在某些实施方案中, 与本文公开的 miRNA 一起使用的适配体与脂肪组织间充质干细胞(ATMSC)、白色脂肪组织(WAT) 脂肪细胞以及褐色脂肪组织(BAT) 脂肪细胞上的细胞表面标记蛋白特异性结合。ATMSC 的细胞表面标记包括: CD9、CD10、CD13、CD29、CD36、CD44、CD49d、CD54、CD55、CD59、CD73、CD90、CD91、CD105、CD137、CD146、CD166 和 HLA-ABC。WAT 脂肪细胞的细胞表面标记包括: 脂联蛋白、窖蛋白-1、窖蛋白-2、CD36(FAT)、CLH-22(网格蛋白重链 Chr22)、FABP4(脂肪细胞蛋白 2, aP2)、SLC27A1(FATP1)、SLC27A2(FATP2)、

GLUT4(葡萄糖转运子 4)、围脂滴蛋白 2 或抵抗素。所有脂肪细胞的细胞表面标记包括中性溶酶 (CD10)、FAT (CD36)、Thy-1 (CD90)、低密度脂蛋白受体相关蛋白 1 (LRP1 或 CD91)、窖蛋白 -1、窖蛋白 -2、脂肪酸结合蛋白 4 (FABP4)、细胞表面糖蛋白 MUC18 (CD146)、激活的白细胞细胞粘分子 (CD166) 和利尿钠肽受体 A (NPR1)。根据另一些实施方案,与本文公开的 miRNA 一起使用的适配体还可与包括以下的脂肪组织标记特异性结合:脂联蛋白、瘦素、抵抗素、FGF 17、FGF 19、BMP7、PYY、MetAP2、RBP4、内皮抑制素和制管张素。

[0229] 在某些实施方案中,通过使用整个活细胞作为靶标的 Cell-SELEX 技术来选择适配体,借此,通过重复扩增和与活细胞结合来选择识别完整细胞表面上的天然环境中处于其天然构象的特定分子的适配体。在该基于细胞的选择中,可在其天然环境中直接靶向特定的细胞表面分子或甚至未知的膜受体,从而允许对细胞特异性适配体的直接富集。

[0230] 在某些示例性实施方案中,所述 miRNA 调节剂与适配体组合,产生“aptamir”组合物。有许多方法来将适配体和 miRNA 类似物组合成产生 aptamir。它们包括例如,适配体-miRNA 类似物嵌合体、适配体-剪接-转换寡核苷酸嵌合体以及与含 miRNA 类似物的纳米颗粒或脂质体缀合的适配体。“护送适配体 (Escort Aptamer)”可插入至可携带多种 miRNA 类似物的功能性聚合物、脂质体和纳米颗粒的表面。例如,硫代适配体缀合的脂质体的大小为约 120nm。纳米颗粒方法有几个功能优点,包括例如,细胞摄取、跨膜能力以及经触发的纳米颗粒解体。

[0231] 在一个实施方案中,aptamir 组合物包含直接与 miRNA 调节剂连接或融合的适配体。这样的 aptamir 完全是化学合成的,这为缀合物的组合物提供了更多的控制。例如,化学计量学(miRNA 类似物/适配体的比率)和附着位点可如前文所定义。缀合物的连接部分存在充当适配体和 miRNA 类似物之反应附着点的多个(两个或多于两个)亲核部分和/或亲电部分。此外,所述 aptamir 还可包含处于所述适配体和所述 miRNA 类似物之间的接头。在一些实施方案中,所述接头是聚亚烷基二醇,特别是聚乙二醇。在另一些实施方案中,所述接头是脂质体、外来体、树状聚体或梳形聚合物(comb polymer)。另一些接头可介导适配体与 miRNA 类似物之间的缀合,包括生物素链霉素桥或核糖核酸。示例性的非共价接头包括由 miRNA 部分的单链部分或突出端(overhang)与适配体部分的互补性单链部分或突出端碱基配对形成的接头。

[0232] 在另一个特定实施方案中,适配体与 miRNA 类似物组合形成基于脂质体的 aptamiR。脂质体是由脂质双层形成的可加载有药物例如 miRNA 的球形纳米结构。此外,脂质体表面可加载有不同物质,例如,聚乙二醇(延长其系统半衰期)或分子识别部分如与靶细胞特异性结合的适配体。例如,已开发出经适配体修饰的脂质体,其中每个脂质体展示与其表面结合的约 250 个适配体,以促进靶向结合。在一个优选实施方案中,制造了脂质体来包封 miRNA 类似物并在其表面展示以高亲和力和特异性与在脂肪细胞和 ATMSC 表面高表达的分子(例如脂质转运蛋白)特异性结合的适配体。所述脂质体与靶细胞的融合导致 miRNA 类似物释放至细胞质中,然后其改变特定的细胞内通路。或者,可在脂质体表面插入稳定的硫代适配体,以引导脂质体 miRNA 类似物递送并加载至靶向的 ATMSC 和脂肪细胞中。

[0233] 在另一个特定实施方案中,适配体与 miRNA 类似物组合形成基于载体的 aptamiR。示例性的载体包括纳米颗粒、脂质体或外来体。这样的基于载体之 aptamir 组合物具有在单一的载体中将多种 miRNA 调节剂货物递送至靶细胞的能力。为了完成靶向和积

累,将所述载体配制成在其外表面存在靶向部分以使其可与所选的靶脂肪细胞上的细胞表面抗原或受体反应/结合。例如,可将载体制造为包封 miRNA 调节剂同时在其表面展示以高亲和力和特异性与在脂肪细胞和 ATMSC 表面高表达的分子(例如脂质转运蛋白)特异性结合的适配体。内化的外来体在细胞的细胞质中释放其 miRNA 类似物负载,这改变了特定的细胞内通路。

[0234] 在一个实施方案中,所述载体是外来体。外来体起源于晚期内体,是天然的特异性加载蛋白质、mRNA 或 miRNA 的纳米颗粒,其由细胞进行内源性分泌。外来体从宿主细胞释放,不具有毒性,并且可基于其组分和外来体中/上的物质向特定细胞传递信息。因为外来体是直径为约 20nm 至 100nm 的颗粒,所以,外来体避过了单核巨噬细胞系统(其清除大小 > 100nm 的循环颗粒)的清除,并且被非常有效地递送至靶组织。

[0235] 此外,相比于其他载体,合成的外来体可提供数个优点。例如,它们直接将其货物递送至细胞溶胶中,同时,其惰性避过了细胞外环境的攻击和清除。外来体的结构组分可包括负责例如信号转导、膜转运、抗原呈递、靶向/附着等过程的小分子。

[0236] 所述 miRNA 试剂必须充分地、与靶 mRNA 互补,即,充分杂交并且具有足够的特异性,以得到期望的效果。“互补”是指两条包含天然或非天然碱基或其类似物的序列之间通过氢键进行配对的能力。例如,如果 miRNA 试剂的一个位置上的碱基能够与靶核苷酸序列的相应位置上的碱基进行氢键结合,则认为在该位置上的碱基是彼此互补的。在某些实施方案中,不要求 100% 的互补性。在另一些实施方案中,要求 100% 的互补性。

[0237] 用于本文公开的方法的 miRNA 试剂可使用常规方法设计。虽然本文记载了某些示例性靶核酸序列和 miRNA 试剂的具体序列,但是本领域技术人员应意识到,这些序列仅用于举例说明和描述在本发明范围内的具体实施方案。从本公开内容的角度来看,另外的靶区段对于本领域普通技术人员来说是容易鉴定的。包含在种子序列中或与其紧邻的至少五个(5)连续核苷酸延伸的长度为 5、6、7、8、9、10 或更多个核苷酸的靶区段被认为适于靶向基因。在一些实施方案中,靶区段可包括包含来自种子序列之一的 5' 端的至少 5 个连续核苷酸的序列(其余核苷酸为同一 RNA 从种子序列 5' 端上游紧接着开始延伸并且延续直至 miRNA 试剂包含约 5 至约 30 个核苷酸)。在一些实施方案中,靶区段可由包含来自种子序列之一的 3' 端的至少 5 个连续核苷酸的 RNA 序列来代表(其余核苷酸为同一 RNA 从靶区段 3' 端下游紧接着开始延伸并且延续直至 miRNA 试剂包含约 5 至约 30 个核苷酸)。通过掌握本文提供的序列,本领域技术人员将能够在不进行过度实验的情况下即可鉴定其他优选区域以靶向使用 miRNA 试剂。当鉴定了一个或更多个靶标区域、区段或位点后,选择与靶标充分互补——即,充分杂交并且具有足够的特异性(即,基本不与其他非靶核酸序列结合)——的抑制性核酸化合物以得到期望的效果。

[0238] 在某些实施方案中,由重组载体表达用于实施本发明的 miRNA 试剂。合适的重组载体包括但不限于 DNA 质粒、病毒载体或 DNA 微环。可使用本领域公知的任何合适的基因工程技术来完成载体构建体的生成,包括但不限于 PCR 标准技术、寡核苷酸合成、限制核酸内切酶消化、连接、转化、质粒纯化和 DNA 测序,例如,如 Sambrook 等, *Molecular Cloning: A Laboratory Manual*. (1989), Coffin 等 (*Retroviruses*. (1997)), 以及“*RNA Viruses: A Practical Approach*”(Alan J. Cann 编写, Oxford University 出版社 (2000)) 中所述。如对本领域普通技术人员显而易见的,用于将本发明的核酸转运至细胞中的多种合适的载体

是可得到的。递送核酸的合适载体的选择以及将选择的表达载体插入至细胞中的条件优化在本领域普通技术人员能力范围内,无需进行过度实验。病毒载体包含具有用于在包封细胞中生产重组病毒之序列的核苷酸序列。表达本发明核酸的病毒载体可基于包括但不限于以下的病毒骨架来构建:逆转录病毒、慢病毒、腺病毒、腺相关病毒、天花病毒或甲病毒。可如本文所描述的来递送重组载体,并且其可存在于靶细胞(例如稳定的转化体)中。

[0239] 在某些实施方案中,使用化学合成技术在体外合成用于实施本发明的 miRNA 试剂,如在例如以下文献中所述,Adams(1983)J. Am. Chem. Soc. 105 :661 ;Belousov(1997) Nucleic Acids Res. 25 :3440-3444 ;Frenkel(1995)Free Radic. Biol. Med. 19 :373-380 ;Blommers(1994)Biochemistry 33 :7886-7896 ;Narang(1979)Meth. Enzymol. 68 :90 ;Brown(1979)Meth. Enzymol. 68 :109 ;Beaucage(1981)Tetra. Lett. 22 :1859 ;美国专利 No. 4, 458, 066, 其各自通过引用整体并入本文。

[0240] IV. 治疗方法

[0241] 在一个方面,本发明提供了治疗人对象的肥胖的方法。所述方法一般包括向人对象施用有效量的调节至少一种产热调节子(例如线粒体解偶联剂,如 UCP1 和 / 或 UCP2) 活性的 miRNA 试剂。

[0242] 这样的治疗方法可基于从药物基因组领域得到的知识来具体调整和修改。因此,本发明的另一个方面提供了使用本发明的靶基因分子或根据个体的药物响应基因型的靶基因调节剂来调整个体预防性或治疗性治疗的方法。药物基因组学使得临床医生或医师能够将预防性或治疗性治疗靶向从治疗中受到最大益处的患者,并避免治疗将经历药物相关毒性副作用的患者。

[0243] 可在合适的动物模型例如肥胖模型(包括 ob/ob 小鼠(Lindstrom P., Scientific World Journal, 7 :666-685(2007) 和 db/db 小鼠(Sharma K 等, Am J Physiol Renal Physiol., 284(6) :F1138-1144(2003)) 中测试 miRNA 试剂。例如,可将本文所述的 miRNA 试剂(或编码所述 miRNA 试剂的表达载体或转基因)用于动物模型中,以确定使用所述试剂治疗的效力、毒性或副作用。或者,可将治疗剂用于动物模型中以确定这样的试剂的作用机制。例如,可将 miRNA 试剂用于动物模型中,以确定使用此试剂治疗的效力、毒性或副作用。或者,可将试剂用于动物模型中以确定此试剂的作用机制。

[0244] 本公开内容还提供了诱导前脂肪细胞分化成白色脂肪细胞和将白色脂肪细胞分化成褐色脂肪细胞的方法,其包括向前体脂肪细胞群施用选自以下的一种或更多种 miRNA : hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-lagomir、hsa-miR-lantagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。在某些实施方案中,使前脂肪细胞分化成脂肪细胞的诱导大于当将前脂肪细胞暴露于 100nM 罗格列酮两天随后暴露于维持培养基中时得到的前脂肪细胞向脂肪细胞的分化。在某些实施方案中,所述脂肪细胞是褐色脂肪细胞。在另一些实施方案中,所述脂肪细胞是白色脂肪细胞。

[0245] 本公开内容还提供了用于在有此需要的对象中提高胰岛素敏感性的方法,其包括向所述对象施用选自以下的一种或更多种 miRNA : hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-lagomir、hsa-miR-lantagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir。在某些实施方案中,所述对

象是哺乳动物。

[0246] 本公开内容还提供了引起有此需要的对象中脂肪消耗的方法,其包括向所述对象施用选自以下的一种或更多种 miRNA :hsa-let-7a antagomir、hsa-miR-lagomir、hsa-miR-19b agomir 和 hsa-miR-30b agomir。在某些实施方案中,所述对象是哺乳动物。在另一些实施方案中,所述哺乳动物是人。

[0247] 经修饰以增强进入细胞(例如,脂肪细胞)之摄取的 miRNA 试剂可以单位剂量施用,所述单位剂量低于约每 kg 体重 15mg,或低于每 kg 体重 10、5、2、1、0.5、0.1、0.05、0.01、0.005、0.001、0.0005、0.0001、0.00005 或 0.00001mg,并且低于每 kg 体重 200 纳摩尔 miRNA 试剂(例如约 4.4×10^{16} 个拷贝),或低于每 kg 体重 1500、750、300、150、75、15、7.5、1.5、0.75、0.15、0.075、0.015、0.0075、0.0015、0.00075、0.00015 纳摩尔 RNA 沉默试剂。单位剂量例如可通过注射(例如静脉内或肌内)、吸入剂量或局部施用来施用。特别优选的剂量为低于 2、1 或 0.1mg/kg 体重。

[0248] 可以大约 0.00001mg 至约 3mg 每器官/组织,或优选约 0.0001mg 至 0.001mg 每器官/组织,约 0.03mg 至 3.0mg 每器官/组织,约 0.1mg 至 3.0mg 每器官/组织或约 0.3mg 至 3.0mg 每器官/组织的剂量直接向器官或组织(例如,直接向脂肪组织)递送 miRNA 试剂。所述剂量可为对治疗或预防肥胖或提高胰岛素敏感性有效的量。在一个实施方案中,以低于一天一次的频率来施用单位剂量,例如低于每 2 天、4 天、8 天或 30 天一次。在另一个实施方案中,不以一定频率的方式来施用单位剂量(例如,不以规律的频率)。例如,可一次施用单位剂量。在一个实施方案中,以另一些传统治疗形式来施用有效剂量。

[0249] 在某些实施方案中,向对象施用 miRNA 试剂的初始剂量,以及一次或更多次维持剂量。维持剂量一般低于初始剂量,例如,是初始剂量的一半。维持方案可包括用每天 0.01mg/kg 体重至 1.4mg/kg 体重的剂量来治疗对象,例如,每天 10、1、0.1、0.01、0.001 或 0.00001mg/kg 体重。维持剂量优选施用不多于每 5 天、10 天或 30 天一次。此外,治疗方案的持续时间根据具体疾病的性质、其严重性以及的总体状况(condition)而不同。在一些优选实施方案中,剂量可以不高于一日一次递送,例如不高于一日 24 小时、36 小时、48 小时或更多个小时一次,例如不高于一日 5 天或 8 天一次。治疗后,可监测患者的状况改变,例如体脂肪百分比的改变。在患者对现有剂量水平不显著响应的情况下可增加所述化合物的剂量,或者如果观察到体脂肪降低或观察到不期望的副作用,则可降低剂量。

[0250] 如在具体情况下适当期望或考虑,有效剂量可以单次给药(dose)或以两次或更多次给药来施用。如果期望有助于反复输注或频繁输注,可建议植入递送装置,例如泵、半永久支架(例如,皮下、静脉内、腹膜内、脑池内(intracisternal)或囊内),或储库。在一个实施方案中,药物组合物包含多个 miRNA 试剂种类。在另一个实施方案中,相对于天然靶序列,miRNA 试剂种类具有与另一个试剂种类不重叠或不相邻的序列。在另一个实施方案中,多个 miRNA 试剂种类对不同的天然靶基因具有特异性。在另一个实施方案中,所述 miRNA 试剂是等位基因特异性的。在另一个实施方案中,多个 miRNA 试剂种类靶向两种或更多种 SNP 等位基因(例如,两个、三个、四个、五个、六个或更多个 SNP 等位基因)。

[0251] 在成功的治疗后,可期望使患者经历维持治疗以预防疾病状态的复发,其中,以 0.01mg/kg 至 100mg/kg 体重的维持剂量来施用本发明的化合物(参见美国专利 No. 6, 107, 094)。

[0252] 所施用的 miRNA 试剂的浓度或量将取决于为试剂和施用方法（例如经鼻、经口腔或经肺）所确定的参数。例如，经鼻制剂倾向于需要一些组分的浓度低得多以避免刺激或灼伤鼻通道。有时期望将经口制剂稀释多达 10 至 100 倍，以提供合适的经鼻制剂。

[0253] 某些因素可影响有效治疗对象所需要的剂量，这些因素包括但不限于，疾病或病症的严重程度、先前的治疗、对象的一般健康和 / 或年龄，以及存在的其他疾病。此外，用治疗有效量的 miRNA 试剂给对象治疗可包括单次治疗，或者优选可包括系列治疗。还应理解，在具体的疗程中用于治疗 miRNA 试剂的有效剂量可提高或降低。剂量的改变可从本文描述的诊断测定结果得出并变得显而易见。例如，可在施用 miRNA 试剂组合物后监测对象。基于监测信息，可施用额外量的 miRNA 试剂组合物。

[0254] 给药取决于待治疗疾病病状的严重性和响应性，治疗疗程持续数天到数月或直至实现治愈，或实现疾病状态的缓解。可通过药物在患者体内的药物积累测量值来计算优化的给药方案。本领域技术人员可容易地确定最佳剂量、给药方法学和重复速率。最佳剂量可根据单种化合物的相对效力而变化，并且可一般基于在动物模型体外和体内发现的有效 EC50 来估计。在一些实施方案中，所述动物模型包括表达人基因（例如产生靶 mRNA 的基因（例如产热调节子））的转基因动物。所述转基因动物可为相应内源 mRNA 缺陷的。在另一个实施方案中，用于测试的组合物包含至少在一个内部区域与在所述动物模型的核酸序列和人靶核酸序列之间保守的序列互补的 miRNA 试剂。

[0255] 一些研究已经报道了使用 miRNA 试剂对哺乳动物进行的成功给药。例如，Esau C, 等, *Cell Metabolism*, 3(2):87-98 (2006) 报道了用 miR-122 反义寡核苷酸的腹膜内剂量对正常小鼠进行给药，12.5mg/kg 至 75mg/kg，每周两次，持续四周。小鼠在治疗结束时表现健康并且正常，未出现体重损失或摄食减少。除了 75mg/kg 剂量 miR-122ASO 显示非常轻微的 ALT 和 AST 水平增加以外，所有剂量的血浆转氨酶水平处于正常范围内（AST 3/4 45，ALT 3/4 35）。这可推断，50mg/kg 是有效的无毒性的剂量。由 Krutzfeldt J., 等, *Nature*, 438, 685-689 (2005) 进行的另一个研究采用每 kg 体重 80mg、160mg 或 240mg 的总剂量将 antagomir 注射至小鼠中以沉默 miR-122。最高剂量导致完全失去 miR-122 信号。在另一个研究中，成功地将锁核酸（“LNA”）施用至灵长类动物中以沉默 miR-122。（Elmen J., 等, (2008) *Nature* 452, 896-899 报道，通过三次 10mg/kg LNA-antimiR 的给药实现了在灵长类动物中有效沉默 miR-122，导致总血浆胆固醇长时间并且可逆地降低，并且研究动物不显示任何 LNA 相关的毒性或组织病理学改变。

[0256] 在某些实施方案中，通过从重组载体表达来施用用于实施本发明的 miRNA 试剂。合适的重组载体包括但不限于 DNA 质粒、病毒载体或 DNA 微环。可使用本领域公知的任何合适的基因工程技术来完成载体构建体的产生，包括但不限于 PCR 标准技术、寡核苷酸合成、限制核酸内切酶消化、连接、转化、质粒纯化和 DNA 测序，例如，如 Sambrook 等, *Molecular Cloning: A Laboratory Manual*. (1989), Coffin 等 (*Retroviruses*. (1997)，以及“*RNA Viruses: A Practical Approach*” (Alan J. Caun, Ed., Oxford University Press (2000)) 中所述。如对本领域技术人员显而易见的，用于将本发明的核酸转运至细胞中的多种合适的载体是可用的。递送核酸的合适载体的选择以及将选择的表达载体插入至细胞中的条件优化在本领域技术普通人员的能力范围内，无需进行过度实验。病毒载体包含具有用于在包封细胞中产生重组病毒之序列的核苷酸序列。表达本发明核酸的病毒载体可基于包括但

不限于以下的病毒骨架来构建：逆转录病毒、慢病毒、腺病毒、腺相关病毒、天花病毒或甲病毒。重组载体可如本文所描述的来递送，并且其可存在于靶细胞（例如稳定的转化体）中。

[0257] miRNA 试剂可直接引入细胞（例如脂肪细胞）中；或以细胞外的方式引入腔、间隙（interstitial）空间中，引入生物体的循环中，经口引入，或者可通过将细胞或生物体浴在含有核酸的溶液中来引入。血管或血管外循环、血液系统或淋巴系统以及脑脊液都是可引入核酸的位点。

[0258] 可使用本领域已知的递送方法来引入本发明的 miRNA 试剂，所述方法包括：注射含核酸的溶液，用被核酸覆盖的颗粒进行轰击，将细胞或生物体浸渍于核酸溶液中，或在核酸的存在下对细胞膜进行电穿孔。可使用本领域已知的另外的方法来将核酸引入细胞中，例如脂质介导的载体转运、化学介导的转运和阳离子脂质体转染例如磷酸钙等。miRNA 试剂可与另一些组分（例如增强细胞摄取 miRNA 试剂的化合物）一起引入。

[0259] 在某些实施方案中，本文描述的方法包括将 miRNA 试剂与另一些药剂或药物（例如用于调节产热的组合物、用于治疗糖尿病的组合物、用于治疗肥胖的组合物）共施用。用于调节产热的组合物包括 β -3 肾上腺素能受体激动剂、甲状腺激素、PPARG 激动剂、瘦素、脂联蛋白和食欲肽。

[0260] V. 筛选方法

[0261] 在另一个方面，本发明提供了筛选调节产热、减少肥胖或提高胰岛素敏感性的 miRNA 试剂的方法。所述方法一般包括以下步骤：提供指示细胞；使所述指示细胞与受试 miRNA 试剂接触；以及在存在和不存在所述 miRNA 试剂的情况下，测定所述指示细胞中的至少一种产热调节子的表达水平和 / 或细胞活性，其中，所述产热调节子活性在存在所述受试 miRNA 试剂的情况下的改变将所述受试 miRNA 试剂鉴定为调节产热、减少肥胖或提高胰岛素敏感性的 miRNA 试剂。在某些实施方案中，所述方法包括测定产热调节子（例如 UCP1、UCP2）的表达水平和 / 或活性的提高。所述指示细胞可为哺乳动物细胞。在某些实施方案中，所述哺乳动物细胞是包含至少部分人基因组的人细胞。

[0262] 在本文公开的方法中可测定任何产热调节子。示例性的产热调节子记载于表 2 中。在一个优选实施方案中，产热调节子是线粒体解偶联蛋白，例如 UCP1 和 / 或 UCP2。

[0263] 其中可测量产热调节子之活性的任何细胞都适用于本文公开的方法。示例性的细胞包括前脂肪细胞、脂肪细胞、脂肪组织来源的间充质干细胞、肝细胞、肌细胞或其前体。

[0264] 可测定产热调节子的任何活性，包括但不限于，产热调节子的 mRNA 表达水平、蛋白质表达水平或线粒体解偶联活性。用于测定这些活性的方法是本领域公知的。

[0265] 可筛选任何 miRNA 试剂，包括但不限于：miRNA、agomir、antagomir、aptamir、miR- 掩蔽物、miRNA- 海绵、siRNA（单链或双链的）、shRNA、反义寡核苷酸、核酶或与至少靶核酸的一部分杂交并调节其功能的另外的寡核苷酸模拟物。

[0266] VI. 药物组合物

[0267] 在一个方面，本文公开的方法可包括施用包含能够调节至少一种产热调节子活性的 miRNA 试剂的药物组合物和制剂。

[0268] 在某些实施方案中，用可药用载体来配制所述组合物。所述药物组合物和制剂可经肠胃外施用、经局部施用、直接施用至胃肠道（例如，经口或经直肠），或局部施用例如经气雾剂或经皮。所述药物组合物可以任何方式配制，并且可以多种单位剂型来施用，这

取决于症状或疾病以及疾病的程度、每个患者的一般用药情况、产生优选结果的施用方法等。药物的配制和施用技术上的细节在科学文献和专利文献中有很好的描述,参见例如 Remington: The Science and Practice of Pharmacy, 第 21 版, 2005。

[0269] miRNA 试剂可单独施用, 或作为药物制剂(组合物)的组分来施用。所述化合物可以任何方便用于人医药或兽医医药施用的方式进行配制。所述组合物中还可存在润湿剂、乳化剂和润滑剂, 例如十二烷基硫酸钠和硬脂酸镁, 以及着色剂、释放剂、涂层剂、甜味剂、调味剂和香味剂、防腐剂和抗氧化剂。

[0270] 本发明的组合物制剂包括适于皮内施用、吸入施用、经口/经鼻施用、经局部施用、经肠胃外施用、经直肠施用和/或经阴道内施用的那些。所述制剂可适宜地以单位剂型存在, 并且可通过任何药学领域公知的方法来制备。可与载剂材料组合以生产单一剂型的活性成分(例如本发明的核酸序列)的量可根据待治疗的宿主、具体的施用方式例如皮内或吸入而不同。可与载剂材料组合以生产单一剂型的活性成分的量一般为产生治疗效果例如抗原特定 T 细胞应答或体液应答的化合物的量。

[0271] 本发明的药物制剂可根据本领域已知的制造药物的任何方法来制备。这样的药物可包含甜味剂、调味剂、着色剂和防腐剂。制剂可与适合于制造的无毒可药用赋形剂掺合。制剂可包含一种或更多种稀释剂、乳化剂、防腐剂、缓冲剂、赋形剂等, 并且可以这样的形式来提供: 液体剂、散剂、乳剂、冻干散剂、喷雾剂、霜剂、洗剂、受控释放的制剂、片剂、丸剂、凝胶剂、贴剂、植入物等。

[0272] 用于经口施用的药物制剂可使用本领域公知的可药用载剂以适当和合适的剂量来配制。这样的载剂使药物能够配制成适于患者摄取的以下单位剂型: 片剂、丸剂、散剂、糖衣剂、胶囊剂、液体剂、锭剂、凝胶剂、糖浆剂、浆料剂、混悬剂等。用于经口使用的药物制备物可配制成固体赋形剂, 任选经研磨得到混合物, 并加工成混合物颗粒, 在按需要添加合适的额外化合物后得到片剂或糖衣剂芯。合适的固体赋形剂是碳水化合物或蛋白质填料, 包括例如: 糖, 包括乳糖、蔗糖、甘露醇或山梨醇; 来自玉米、小麦、水稻、马铃薯或其他植物的淀粉; 纤维素例如甲基纤维素、羟丙甲基纤维素或羧甲基纤维素钠; 以及树胶, 包括阿拉伯胶和西黄蓍胶; 以及蛋白质, 例如明胶和骨胶原。可添加崩解剂或增溶剂, 例如交联的聚乙烯吡咯烷酮、琼脂、海藻酸或其盐, 例如海藻酸钠。适于推送(push-fit)的胶囊剂可包含混合有填料或粘合剂(例如乳糖或淀粉), 润滑剂(例如滑石或硬脂酸镁), 以及任选地, 稳定剂。在软胶囊剂中, 活性剂可溶解于或悬浮于含有或不含稳定剂的合适液体中, 例如, 脂肪油、液体石蜡或液体聚乙二醇。

[0273] 水性混悬剂可包含混合有适于制造水性混悬剂(例如水性皮内注射剂)的活性剂(例如, 本发明的核酸序列)。这样的赋形剂包括悬浮试剂, 例如羧基甲基纤维素钠、甲基纤维素、羟丙甲基纤维素、藻酸钠、聚乙烯吡咯烷酮、西黄蓍胶和阿拉伯胶, 以及分散剂或润湿剂, 例如天然磷脂(例如卵磷脂)、氧化烯与脂肪酸的缩合物(例如, 聚氧乙烯硬脂酸酯)、氧化乙烯与长链脂肪族醇的缩合物(例如, 十七乙烯氧基十六醇)、氧化乙烯与衍生自脂肪酸和己糖醇的偏酯的缩合物(例如, 聚氧乙烯山梨醇单油酸酯), 或氧化乙烯与衍生自脂肪酸和己糖醇酐的偏酯的缩合物(例如, 聚氧乙烯脱水山梨糖醇单油酸酯)。所述水性混悬剂还可包含一种或更多种防腐剂, 例如对羟基苯甲酸乙酯或对基苯甲酸正丙酯; 一种或更多种着色剂; 一种或更多种调味剂和一种或更多种甜味剂, 例如蔗糖、阿斯巴甜或糖精。可调

节制剂的摩尔渗透压浓度。

[0274] 在某些实施方案中,使用基于油的药物来施用 miRNA 试剂。可通过将活性剂混悬于以下物质中来配制基于油的混悬剂:植物油,例如花生油、橄榄油、芝麻油或椰子油;或矿物油,例如液体石蜡;或这些物质的混合物。参见例如,美国专利 No. 5, 716, 928 描述了使用精油或精油组分来提高生物利用度并降低经口施用的疏水性药物化合物的个体间差异和个体内差异(另参见美国专利 No. 5, 858, 401)。油混悬剂可包含增稠剂,例如蜂蜡、硬石蜡或十六醇。可添加甜味剂以提供适口的经口制剂,例如甘油、山梨醇或蔗糖。这些制剂可通过添加抗氧化剂例如抗坏血酸来保存。可注射油载剂的实例参见 Minto(1997) J. Pharmacol. Exp. Ther. 281 :93-102。

[0275] 在某些实施方案中,所述药物组合物和制剂是水包油乳剂的形式。油相可为上文描述的植物油或矿物油,或其混合物。合适的乳化剂包括天然树胶,例如阿拉伯胶和西黄蓍胶;天然磷脂,例如大豆卵磷脂;衍生自脂肪酸和己糖醇酸酐的酯或偏酯,例如脱水山梨糖醇单油酸酯;以及这些偏酯与氧化乙烯的缩合物,例如聚氧乙烯脱水山梨糖醇单油酸酯。所述乳剂还可包含甜味剂和调味剂,如在糖浆剂和酏剂制剂中。这样的制剂还可包含缓和剂(demulcent)、防腐剂或着色剂。在一些替代实施方案中,本发明的这些可注射水包油乳剂包含石蜡油、脱水山梨糖醇单油酸酯、乙氧基化脱水山梨糖醇单油酸酯和/或乙氧基化山梨糖醇三油酸酯。

[0276] 在某些实施方案中,药物组合物和制剂通过以鼻内、眼内和阴道内的途径来施用,包括栓剂、吹入剂、散剂和气雾剂制剂(类固醇吸入剂例如参见 Rohatagi (1995) J. Clin. Pharmacol. 35 :1 187-1193 ;Tjwa(1995) Ann. Allergy Asthma Immunol. 75 :107-111)。栓剂制剂可通过将药物与合适的非刺激性赋形剂混合来制备,所述非刺激性赋形剂在常温下为固体但是在体温下为液体,因而将在体内熔化以释放药物。这样的材料为可可油和聚乙二醇。

[0277] 在某些实施方案中,通过局部途径经皮递送的药物组合物和制剂配制为涂敷棒(applicator stick)、溶液剂、混悬剂、乳剂、凝胶剂、霜剂、软膏剂、糊剂、胶凝剂(jully)、涂剂(paint)、散剂和喷雾剂。

[0278] 在某些实施方案中,所述药物组合物和制剂作为微球递送,以在体内缓慢释放。例如,微球可经药物的皮内注射来施用,所述药物缓慢地经皮下释放;参见 Rao(1995) J. Biomater Sci. Polym. Ed. 7 :623-645;作为生物可降解和可注射凝胶制剂,参见例如, Gao(1995) Pharm. Res. 12 :857-863(1995);或者作为经口施用的微球,参见例如, Eyles(1997) J. Pharm. Pharmacol. 49 :669-674。

[0279] 在某些实施方案中,所述药物组合物和制剂经肠胃外施用,例如通过静脉内(Iv)施用,或施用至体腔或器官的管腔中。这些制剂可包括溶解于可药用载剂中的活性剂溶液。可使用的可接受的载剂和溶剂有水和作为等渗氯化钠的林格液(Ringer's solution)。此外,可使用无菌的固定油作为溶剂或混悬培养基。为此目的,可使用任何温和的固定油,包括合成的甘油单酯和甘油二酯。此外,还可在可注射剂的制备中使用脂肪酸,例如油酸。这些溶液是无菌的,并且一般不含不期望的物质。这些制剂可通过常规的公知的灭菌技术来灭菌。所述制剂可按需要包含可药用辅料物质以接近生理学状态,例如 pH 调节剂(adjusting)和缓冲剂,毒性调节剂,例如醋酸钠、氯化钠、氯化钾、氯化钙、乳酸钠等。这些

制剂中活性剂的浓度可大范围地不等,并且将根据所选的具体施用方式和患者的需要,主要基于流体体积、粘度、体重等来选择。对于 IV 施用,所述制剂可为无菌的可注射制备物,例如无菌的可注射水性混悬剂或油性混悬剂。该混悬剂可使用那些合适的分散剂或润湿剂或助悬剂来配制。所述无菌的可注射制备物还可为在无毒的肠胃外可接受的稀释剂或溶剂中的混悬剂,例如 1,3-丁二醇溶液。所述施用可为推注输注(bolus infusion)或连续输注(例如,在指定时间段内基本无打断地引入血管中)。

[0280] 在某些实施方案中,冻干所述药物化合物和制剂。包含抑制性核酸的稳定冻干制剂可通过冻干包含本发明药物和填充剂例如甘露醇、海藻糖、棉籽糖和蔗糖或其混合物的溶液来得到。用于制备稳定的冻干制剂的方法可包括冻干这样的溶液,其含有约 2.5mg/mL 核酸、约 15mg/mL 蔗糖、约 19mg/mL NaCl 和 pH 大于 5.5 但小于 6.5 的柠檬酸钠缓冲剂。参见例如美国 20040028670。

[0281] 在某些实施方案中,所述药物组合物和制剂通过使用脂质体来递送。通过使用脂质体,特别是其中脂质体表面携带对靶细胞具有特异性之配体的脂质体,或以其他方式优选指向特定器官的脂质体,我们可以着重于在体内将活性剂递送至靶细胞中。参见例如,美国专利 No. 6,063,400 ;6,007,839 ;Al-Muhammed(1996) J. Microencapsul. 13 :293-306 ; Chonn(1995) Curr. Opin. Biotechnol. 6 :698-708 ;Ostro(1989) Am. J. Hosp. Pharm. 46 :1576-1587。

[0282] 可施用本发明制剂用于预防性和/或治疗性治疗。在某些实施方案中,对于治疗应用,可以足以治愈、缓解或部分阻止病症或其并发症的临床表现的量将组合物施用至需要降低甘油三酯水平的对象,或有罹患本文所描述的病症风险或患有所述病症的对象;该量可称为治疗有效量。例如,在某些实施方案中,以足以治疗对象的肥胖的量来施用本发明的药物组合物。

[0283] 足以实现该效果的药物组合物的量是治疗有效剂量。对该应用有效的给药时间表和量(即,给药方案)将取决于多种因素,包括疾病或症状的阶段、疾病或症状的严重程度、患者健康的一般状态(state)、患者的身体状态、年龄等。在为患者计算给药方案的过程中,还将施用方式纳入考虑。

[0284] 给药方案将本领域公知的药代动力学参数纳入考虑,即,活性剂的吸收速率、生物利用度、代谢、清除率等(参见例如,Hidalgo-Aragones(1996) J. Steroid Biochem. Mol. Biol. 58 :611-617 ;Groning(1996) Pharmazie 51 :337-341 ;Fotherby(1996) Contraception 54 :59-69 ;Johnson(1995) J. Pharm. Sci. 84 :1144-1146 ; Rohatagi(1995) Pharmazie 50 :610-613 ;Brophy(1983) Eur. J. Clin. Pharmacol. 24 :103-108 ;Remington :The Science and Practice of Pharmacy,第21版,2005)。本领域现有技术使临床医生能够为每位个体患者、活性剂和被治疗的疾病或病症来确定给药方案。为用作药物的相似组合物提供的原则可用作指导来确定施用的给药方案(即给药时间表和剂量水平)实施本发明之方法是正确的并且是适当的。制剂的单独施用或多重使用可根据例如以下来给定:所需要的剂量和频率,以及患者的耐受,每次施用后所生成的胆固醇稳态的程度和量等。制剂应当提供足够量的活性剂以有效治疗、预防或缓解病症、疾病或症状,例如治疗肥胖。

[0285] 在某些实施方案中,用于经口施用的药物制剂的每日量为每千克体重约 1mg 至

100mg 每天。与经口施用相比,可使用更低的剂量施用至血流、体腔或器官的管腔中。可使用充分更高的剂量用于经局部施用或经口施用或通过散剂、喷雾剂或吸入剂来施用。用于制备能够经肠胃外或经非肠胃外施用的制剂的实际方法对本领域技术人员是已知或是显而易见的,并且这些方法在出版物例如 Remington:The Science and Practice of Pharmacy,第 21 版,2005 中有着更详细的描述。

[0286] VII. 例证

[0287] 进一步通过以下实施例对本发明进行举例说明,这些实施例不应当解读为进一步的限制。贯穿于本申请中的序列表内容、附图以及引用的所有文献、专利和已公开的专利申请都通过引用清楚地并入本文。

[0288] 此外,根据本发明,可采用本领域范围内的常规分子生物学、微生物学和重组 DNA 技术。这些技术在文献中有充分的解释。参见例如, Sambrook, Fritsch&Maniatis, Molecular Cloning:A Laboratory Manual, 第二版 (1989) Cold Spring Harbor Laboratory 出版社 Cold Spring Harbor, New York(本文中写为“Sambrook 等, 1989”); DNA Cloning:A Practical Approach, I 卷和 II 卷 (D.N.Glover 编写, 1985); Oligonucleotide Synthesis (M.J.Gait 编写, 1984); Nucleic Acid Hybridization [B. D.Hames 和 S.J.Higgins 编写 (1985)]; Transcription And Translation [B. D.Hames 和 S.J.Higgins 编写 (1984)]; Animal Cell Culture [R. I.Freshney 编写 (1986)]; Immobilized Cells And Enzymes [IRL 出版社, (1986)]; B.Perbal, A Practical Guide To Molecular Cloning (1984); F.M.Ausubel 等 (编写), Current Protocols in Molecular Biology, John Wiley&Sons, Inc. (1994)。

[0289] 实施例 1. 产热调节子的计算机分析

[0290] 基于对有效的科学信息和我们自己的实验数据的重要评估和回顾,选择了八十三种在产热调控中涉及到的蛋白质。基于其功能,将这些蛋白质划分为产热的激活蛋白或阻抑蛋白。这些产热调节蛋白记载于表 2 中。

[0291] 表 2.

[0292] 产热调节蛋白:

[0293]

激活蛋白			
	名称	Entrez基因ID	Ensembl基因ID
1	ALDH1A1	216	ENSG00000165092
2	ANP (NPPA)	4878	ENSG00000175206
3	AZGP1	563	ENSG00000160862
4	BMP7	655	ENSG00000101144
5	BMP8B	656	ENSG00000116985
6	CEBPA	1050	ENSG00000245848
7	CEBPB	1051	ENSG00000172216
8	CEBPD	1052	ENSG00000221869
9	CIDEA	1149	ENSG00000176194
10	COX7A1	1346	ENSG00000161281
11	CRAT	1384	ENSG00000095321
12	CREB1	1385	ENSG00000118260
13	CREBBP	1387	ENSG00000005339
14	CTBP1	1487	ENSG00000159692
15	CTBP2	1488	ENSG00000175029
16	DIO2	1734	ENSG00000211448
17	ELOVL3	83401	ENSG00000119915
18	FGF16	8823	ENSG00000196468
19	FGF19	9965	ENSG00000162344
20	FGF21	26291	ENSG00000105550
21	FNDC5	252995	ENSG00000160097
22	FOXC2	2303	ENSG00000176692
23	GDF3	9573	ENSG00000184344
24	HCRT (OREXIN)	3060	ENSG00000161610
25	HOXC8	3224	ENSG00000037965
26	INSR	3643	ENSG00000171105
27	IRS1	3667	ENSG00000169047
28	KDM3A (JMJD1A)	55818	ENSG00000115548
29	KLF5	688	ENSG00000102554
30	KLF11	8462	ENSG00000172059
31	KLF15	28999	ENSG00000163884

[0294]

32	LRP6	4040	ENSG00000070018
33	MAPK14	1432	ENSG00000112062
34	MED13	9969	ENSG00000108510
35	NCOA1	8648	ENSG00000084676
36	NCOA2	10499	ENSG00000140396
37	NCOA3	8202	ENSG00000124151
38	NR4A3	8013	ENSG00000119508
39	NRF1	4899	ENSG00000106459
40	PLAC8	51316	ENSG00000145287
41	PPARA	5465	ENSG00000186951
42	PPARD	5467	ENSG00000112033
43	PPARG	5468	ENSG00000132170
44	PPARGC1A	10891	ENSG00000109819
45	PPARGC1B	133522	ENSG00000155846
46	PRDM16	63976	ENSG00000142611
47	PRDX3	10935	ENSG00000165672
48	PRKAA1 (AMPKA1)	5562	ENSG00000132356
49	PRKAA2 (AMPKA2)	5563	ENSG00000162409
50	PRKACA	5566	ENSG00000072062
51	PRKACB	5567	ENSG00000142875
52	PRKARIA	5573	ENSG00000108946
53	SIRT1	23411	ENSG00000096717
54	SIRT3	23410	ENSG00000142082
55	SLC27A2 (FATP2)	11001	ENSG00000140284
56	SREBF1	6720	ENSG00000072310
58	SREBF2	6721	ENSG00000198911
58	STAT5A	6776	ENSG00000126561
59	TRPM8	79054	ENSG00000144481
60	UCP1 (SLC25A7)	7350	ENSG00000109424
61	UCP2 (SLC25A8)	7351	ENSG00000175567
62	UCP3 (SLC25A9)	7352	ENSG00000175564
阻抑蛋白			
	名称	Entrez基因ID	Ensembl基因ID
1	ATG7	10533	ENSG00000197548
2	BMP2	650	ENSG00000125845
3	BMP4	652	ENSG00000125378
4	CIDEA	63924	ENSG00000187288
5	CTNNA1	1499	ENSG00000168036
6	DLK1 (Pref-1)	8788	ENSG00000185559
7	E2F4 (p107)	1874	ENSG00000205250
8	EIF4EBP1	1978	ENSG00000187840
9	ESRRA (NR3B1)	2101	ENSG00000173153
10	IKBKE	9641	ENSG00000143466
11	NR1H3 (LXRA)	10062	ENSG00000025434
12	NRIP1 (RIP140)	8204	ENSG00000180530

[0295]

13	RB1 (pRb)	5925	ENSG00000139687
14	NR0B2 (SHP)	8431	ENSG00000131910
15	RPS6KB1	6198	ENSG00000108443
16	RUNX1T1	862	ENSG00000079102
17	RUNX2	860	ENSG00000124813
18	TNFRSF1A	7132	ENSG00000067182
19	TWIST1	7291	ENSG00000122691
20	WNT5A	7474	ENSG00000114251
21	WNT10B	7480	ENSG00000169884

[0296] 使用已知的 STRING 9.0 数据库和预测的蛋白质相互作用 (string-db.org/) 来测试这 83 个候选分子。该相互作用包括直接 (物理的) 缔合和间接 (功能性的) 缔合; 它们来自四个来源: 基因组背景; 高通量实验; 共表达; 以及现有知识。STRING 定量地整合了来自这些来源的大量生物体中的相互作用数据, 在可适用的情况下在这些生物体之间转移信息。所述数据库目前覆盖了来自 1,133 种生物体中的 5,214,234 个蛋白质。例如, 83 种产热调节子分子之间的关系以 UCP1 为中心, 与 UCP1 具有直接联系或间接联系的分子可通过使用最高为 0.90 的置信度分数来鉴别。该关系记载于图 1A 的示意图中。根据该分析发现, 九个分子 (CEBPB、CIDEA、KDM3A、NR1P1、PRDM16、PPARG、PPARGC1A、PPKAA2 和 UCP2) 直接与 UCP1 关联, 而更多分子在第二级或更高级别的级上与 UCP1 关联。

[0297] 当置信度设置在 0.70 分时, 发现另外八个蛋白质直接与 UCP1 关联 (AZGP1、DI02、KIF11、KLF15、NR1H3、PPARA、PPARD 和 PPARGC1B), 图 1B。

[0298] 类似地, 使用其他软件程序独立地评估这 83 种产热调控分子之间的相互作用。由 Ingenuity Pathway Analysis (IPA) Software 程序 (www.ingenuity.com) 预测的相互作用在图 2A 中示出 (UCP1 为黄色, 激活蛋白为绿色并且阻抑蛋白为紫色)。由 Reactome Functional Interaction (Reactome IF) Software 程序 (<http://wiki.reactome.org>) 预测的相互作用在图 2B 中示出 (UCP1 为黄色, 激活蛋白为绿色并且阻抑蛋白为紫色)。IPA 和 Reactome IF 网络与图 1A 和 1B 中记载的使用 STRING 程序得到的网络不同。这些算法的结果不同并不令人意外, 因为它们基于不同的预设参数、信息来源和选择标准。

[0299] 实施例 2. 相关 miRNA 靶标的计算机选择

[0300] 为了选择适合作为 miRNA 试剂靶标的产热调节子, 采用了数种基于互联网的资源来使 miRNA 与其靶标匹配 (“micronome”)。示例性的工具记载于表 3 中。

[0301] 表 3.

[0302] 用于选择 miRNA 及其靶标的示例性生物信息学工具:

[0303]

领域和名称	网址
整合的数据挖掘 (8)	
BioCarta	http://www.biocarta.com/
Database for Annotation, Visualization and Integrated Discovery (DAVID)	http://david.abcc.ncifcrf.gov/home.jsp
GeneOntology	http://www.geneontology.org/
Gene Set Enrichment Analysis (GSEA)	http://www.broadinstitute.org/gsea/index.jsp
KEGG	http://www.genome.jp/kegg/
PubGene	http://www.pubgene.org/
Reactome	http://www.reactome.org/ReactomeGWT/entrypoint.html
STRING	http://string-db.org/
miRNA挖掘和作图 (8)	
deepBase	http://deepbase.sysu.edu.cn/
Human microRNA disease database (HMDD)	http://202.38.126.151/hmdd/mirna/md/

[0304]

miRBase V19	http://www.mirbase.org/
miRGen 2.0	http://diana.cslab.ece.ntua.gr/mirgen/
miRNAMap	http://mirnamap.mbc.nctu.edu.tw/
miRSet	http://services.bio.ifi.lmu.de/mirsel/
miRStart	http://mirstart.mbc.nctu.edu.tw/home.php
miR2Disease	http://www.mir2disease.org
miRNA靶标和表达 (21)	
DIANA-microT 3.0	http://diana.cslab.ece.ntua.gr/microT/
DIANA-mirExTra	http://diana.cslab.ece.ntua.gr/hexamers/
GSEA Molecular Signatures Database v3.0	http://www.broadinstitute.org/gsea/index.jsp
MicroCosm Targets	http://www.ebi.ac.uk/enright-srv/microcosm/cgi-bin/targets/v5/download.pl
MicroInspector	http://bioinfo.uni-plovdiv.bg/microinspector/
microRNA.org (ex. miRanda)	http://www.microrna.org/microrna/home.do
miRDB	http://mirdb.org/miRDB/
miRTarBase	http://mirtarbase.mbc.nctu.edu.tw/index.html
miRTar.Human	http://mirtar.mbc.nctu.edu.tw/human/download.php
miRvestigator	http://mirvestigator.systemsbiology.net/
mirZ	http://www.mirz.unibas.ch/ElMMo2/
MultiMiTar	http://www.isical.ac.in/~bioinfo_miu/multimitar.htm
PhenomiR	http://mips.helmholtz-muenchen.de/phenomir/index.gsp
PicTar	http://pictar.mdc-berlin.de/
PITA	http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html
RepTar	http://bioinformatics.ekmd.huji.ac.il/reptar/

[0305]

RNAhybrid	http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/
RNA22	http://cbcsrv.watson.ibm.com/rna22.html
Sylamer	http://www.ebi.ac.uk/enright/sylamer/
TarBase 6.0	http://diana.cslab.ece.ntua.gr/DianaToolsNew/index.php?r=tarbase/index
TargetScanHuman 6.2	http://www.targetscan.org/
整合的miRNA靶标和表达工具 (13)	
GOmir	http://www.bioacademy.gr/bioinformatics/projects/GOmir/
MAMI (MetaMiR:Target Inference)	http://mami.med.harvard.edu/
mimiRNA	http://mimirna.centenary.org.au/mep/formulaire.html
MMIA (microRNA and mRNA Integrated Analysis)	http://147.46.15.115/MMIA/index.html
mirDIP	http://ophid.utoronto.ca/mirDIP/
miRGator V3.0	http://mirgator.kobic.re.kr
miRecords	http://mirecords.biolead.org/
MIRNA-DISTILLER	http://www.ikp-stuttgart.de/content/language1/html/10415.asp
MiRonTop	http://www.microarray.fr:8080/miRonTop/index
miRror	http://www.proto.cs.huji.ac.il/mirror
miRSystem	http://mirsystem.cgm.ntu.edu.tw/
miRWalk	http://www.ma.uni-heidelberg.de/apps/zmf/mirwalk/index.html
StarBase	http://starbase.sysu.edu.cn/index.php
miRNA二级结构 (5)	

[0306]

OligoWalk	http://rna.urmc.rochester.edu/cgi-bin/server_exe/oligowalk/oligowalk_form.cgi
PicTar RNA Studio	http://www.pictar.org/
RNA2D	http://protein3d.ncifcrf.gov/shuyun/rna2d.html
Vienna RNA Package	http://www.tbi.univie.ac.at/ivo/RNA/
Whitehead siRNA algorithm	http://jura.wi.mit.edu/bioc/siRNAext/
网络检索和分析 (8)	
ARIADNE Pathway Studio	http://www.ariadnegenomics.com/products/pathway-studio/
Cytoscape	http://www.cytoscape.org/
Database for Annotation, Visualization and Integrated Discovery (DAVID)	http://david.abcc.ncifcrf.gov/home.jsp
Genego MetaCore	http://www.genego.com/metacore.php
Ingenuity Systems IPA (Ingenuity Pathway Analysis)	http://www.ingenuity.com/products/IPA/microRNA.html
MATISSE (Module Analysis via Topology of Interactions and Similarity Sets)	http://acgt.cs.tau.ac.il/matisse/
MIR@NT@N	http://mironton.uni.lu
NAVIGATOR	http://ophid.utoronto.ca/navigator/index.html
分子可视化 (4)	
Foldit	http://fold.it/portal/info/science
PyMOL	http://www.pymol.org/
Qlucore Omics Explorer	http://www.qlucore.com/ProdOverviewmiRNA.aspx

[0307]

WebMol	http://www.cmpfarm.ucsf.edu/cgi-bin/webmol.pl
信息整合 (1)	
TIBCO Spotfire	http://spotfire.tibco.com/

[0308] 具体而言,这些工具用于进行:1) 整合的数据挖掘(8个工具);2) miRNA 挖掘和绘图(6个工具);3) miRNA 靶向的靶标和表达(21个工具);4) 整合的 miRNA 靶标和表达(13个工具);5) miRNA 二级结构的预测和比较(5个工具);6) 网络搜索和分析(8个工具);7) 分子显示(4个工具);以及 8) 信息整合以及开发(1个工具)。

[0309] 单个基因靶标可被几种 miRNA 来控制,然而单个 miRNA 可控制数个基因靶标。已经开发了庞杂的生物信息学资源来选择与靶疾病最相关的 miRNA (Gallagher IJ 等, Genome medicine. 2010; Fujiki K 等, BMC Biol. 2009; Okada Y, 等, J Androl. 2010; Hao T, 等, Mol Biosyst. 2012; Hao T, 等, Mol Biosyst. 2012)。然而这些算法的结果严重依赖于预设参数,并且这些算法之间的会聚程度十分有限。因此,需要开发出更好的对鉴定 miRNA/ 靶标关系具有改进的灵敏性、特异性和选择性的进行生物信息学分析的工具。

[0310] miRNA 与其靶标之间的相互作用超出了作为转录后调节子的 miRNA 的初始描述,所述转录后调节子的驱动链(driver strand)的种子区域(5' 碱基 2 位至 7 位)与靶 mRNA 的 3' UTR 区的互补序列结合,通常导致翻译抑制、靶标降解和基因沉默。所述相互作用还可包含 miRNA 的驱动链或过客链的多个区域以及 mRNA 的 5' UTR、启动子和编码区。

[0311] 根据对可用数据的分析,决定选择靶向一个离散的信号通路中多个基因的通路特异性 miRNA,而不是在许多信号通路、功能或过程中涉及到的通用 miRNA。通过使用 34 种可公开获取的互联网工具预测 miRNA 靶标,检索可潜在地调节实施例 1 中所选的 83 个产热调控分子(其中包括 36 个转录因子)中的数个靶标的特异性人 miRNA。

[0312] 考虑的数个范例:

[0313] A) 一种微小 RNA- 多种 mRNA 通路特异性的范例

[0314] A1. 一种小 RNA- 多种 RNA 通路特异性的范例的第一个实施例

[0315] 组蛋白的甲基化状态可通过组蛋白甲基化转移酶和去甲基化酶来动态调控。人赖氨酸(K) 特异性去甲基化酶 3A (KDM3A) 在代谢基因的表达调控中是至关重要的。其功能的缺失导致小鼠的肥胖和高脂血症。与 UCP1 基因的 PPAR 应答元件 (PPRE) 结合的 KDM3A 的 β -肾上腺素能刺激不仅降低在 PPRE 的 H3K9me2 (对组蛋白 H3 的第 9 位赖氨酸进行二甲基化) 水平,而且还促使 PPARG 和 RXRA 以及其共激活蛋白 PPARGC1A、CREBBP 和 NCOA1 募集至 PPRE。TargetScan Human 数据库 (release 6.0) 的查询显示,人 KDM3A 3' UTR 的 29 位至 35 位区域是 hsa-miR-22 的保守靶标。数种其他 miRNA 靶标数据库也确认了 hsa-miR-22 与 KDM3A 之间的该匹配。因此,通过 hsa-miR-22 antagomir 使去甲基化酶 KDM3A 的产量提高将导致 UCP1 基因启动子区的去甲基化,从而促进数种调控元件的结合,并提高 UCP1 的产量。

[0316] 此外,我们使用了 34 种 miRNA 靶标和表达工具(表 4) 来鉴定指定 miRNA 的 mRNA 靶标。

[0317] 表 4.

[0318] 用于选择 miRNA 及其靶标的生物信息学工具:

[0319]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
<i>DIANA-microT 3.0</i>	X																																	
<i>DIANA-microT 3a</i>		X																																
<i>GOmr</i>			X																															
<i>GSEA MSID v3.0</i>				X																														
<i>MAmI</i>					X																													
<i>MicroCosm Targets</i>					X																													
<i>MicroPhospho</i>								X																										
<i>microRNA.org</i>									X																									
<i>miRNA</i>										X																								
<i>miRDE</i>											X																							
<i>miRDP</i>												X																						
<i>miRGen V3</i>													X																					
<i>miRcofold</i>														X																				
<i>miRNA Database</i>															X																			
<i>MIRNATop</i>																X																		
<i>miRNet</i>																	X																	
<i>MIRSVect</i>																		X																
<i>miRTarget</i>																			X															
<i>miRTargetPlus</i>																				X														
<i>miRvestigator</i>																					X													
<i>miRWalk</i>																						X												
<i>miZ</i>																							X											
<i>MultiMiT</i>																								X										
<i>PhosphoR</i>																									X									
<i>PicTar</i>																										X								
<i>PITA</i>																											X							
<i>RegTar</i>																												X						
<i>RNA22</i>																													X					
<i>RNAfold</i>																														X				
<i>StarBase</i>																															X			
<i>StimBase</i>																																X		
<i>SymRank</i>																																	X	
<i>Target 6.0</i>																																	X	
<i>TargetScanHuman</i>																																	X	

元工具 (Meta tool) 以黑体字示出 [13]

元工具调用的引擎以斜体字示出 [13]

[0320] 通过应用上述计算机策略发现, hsa-miR-22-3p 和 hsa-miR-22-5p 分别与所选 83

种产热靶标中的总共 42 种和 8 种相互作用。该数据记载于表 5 中。

[0321] 表 5.

[0322] 鉴定为 hsa-miR-22 的预测靶标和 / 或已证实靶标的产热调节子：

[0323]

hsa-miR-22-3p				
ALDH1A1	DIO2	NCOA1	PRKAA1	STAT5A
BMP4	FGF19	NPPA	PRKACA	TNFRSF1A
BMP7	FGF21	NRF1	PRKACB	TRPM8
CEBPA	FOXC2	NRIP1	PRKAR1A	UCP2
CEBPD	INSR	PPARA	RUNX1T1	WNT10B
CIDEA	KDM3A	PPARGC1A	RUNX2	WNT5A
CREB1	KLF11	PPARGC1B	SIRT1	
CREBBP	LRP6	PRDM16	SREBF1	
CTNNB1	MAPK14	PRDX3	SREBF2	
hsa-miR-22-5p				
BMP7	DIO2	FNDCC5	IKBKE	INSR
			MAPK14	NR1H3
				PPARA

[0324] A2. 一种微小 RNA- 多 mRNA 通路特异性的范例的另一些实施例

[0325] 我们还利用 34 种 miRNA 靶标和表达工具 (表 4) 来寻找脂肪细胞的 536 种 miRNA (表 1) 中的任意一种与 83 种产热靶标 (表 2) 之间的潜在关系。

[0326] 显示许多脂肪细胞 miRNA 与所述 83 种产热靶标中的至少一种相互作用 (预测和 / 或证实)。例如 miR-17-3p 和 hsa-miR-17-5p 分别与所选 83 种产热靶标中的总共 23 种和 65 种相互作用。该数据记载于表 6 中。

[0327] 表 6.

[0328] 鉴定为 hsa-miR-17 的预测和 / 或已证实的靶标的产热调节子：

[0329]

hsa-miR-17-3p				
ATG7	CTBP2	KLF11	PPARD	TNFRSF1A
BMP2	E2F4	MAPK14	PRDM16	TWIST1
BMP4	FGF19	NCOA3	RB1	WNT10B
CEBPA	IKBKE	PLAC8	RUNX1T1	
CREB1	IRS1	PPARA	STAT5A	
hsa-miR-17-5p				
ALDH1A1	CREB2	IKBKE	NRIP1	RB1
ATG7	CTNNB1	INSR	PLAC8	RPS6KB1
BMP2	CTBP1	IRS1	PPARA	RUNX1T1
BMP4	CTBP2	KLF11	PPARD	RUNX2
BMP7	DIO2	MAPK14	PPARG	SIRT1
BMP8b	ELOVL3	MED13	PPARGC1A	SIRT3
CEBPA	FGF19	NCOA1	PPARGC1B	SREBF1
CEBPA	FGF21	NCOA2	PRDX3	STAT5A
CEBPD	FNDCC5	NCOA3	PRKAA1	TNFRSF1A
CIDEA	FOXC2	NPPA	PRKAA2	TWIST1
COX7A1	GDF3	NR1H3	PRKACA	UCP1
CRAT	HCRT	NR4A3	PRKACB	UCP3
CREB1	HOXC8	NRF1	PRKAR1A	WNT5A

- [0330] 当制作出目的 miRNA 及其 mRNA 靶标的清单后,使用以下过滤条件来提炼结果:
- [0331] 参数
- [0332] 1 目的组织 / 细胞中 miRNA 的表达
- [0333] 2 预测指定基因或基因组的一种 miRNA 的算法数量
- [0334] 3 来自算法的评分 / 百分比
- [0335] 4 由一种 miRNA 靶向的优选基因数量
- [0336] 5 靶基因中一种 miRNA 的结合位点数量
- [0337] 6 靶基因中数种 miRNA 的结合位点数量
- [0338] 7 靶基因中一种 miRNA 种子互补序列的过呈现 (over-representation) (miRvestigator)
- [0339] 8 经证实的 miRNA-mRNA 靶标对
- [0340] 9 miRNA 结合位点的基因位置 (5' UTR- 启动子 -CDS-3' UTR)
- [0341] 10 miRNA 的内含子位置
- [0342] 11 miRNA 的聚类
- [0343] 12 在目的特异性组织 / 细胞中 miRNA 的丰度
- [0344] 使用以上参数发现,229 种 miRNA 满足这些标准中的至少两种。该数据记载于表 7 中。
- [0345] 表 7.
- [0346] 根据选择标准数目减少的 miRNA 排序:
- [0347]

hsa-miR-20b-5p
hsa-miR-27b-3p
hsa-miR-103a-3p
hsa-miR-22-3p
hsa-miR-34a-5p
hsa-miR-130b-3p
hsa-miR-132-3p
hsa-miR-181b-5p
hsa-miR-211-5p
hsa-miR-148b-3p
hsa-miR-17-5p
hsa-miR-182-5p
hsa-miR-20a-5p
hsa-miR-27a-3p
hsa-miR-301a-3p
hsa-miR-204-5p
hsa-miR-143-3p
hsa-miR-1
hsa-miR-9-5p
hsa-miR-30a-5p
hsa-miR-138-5p
hsa-miR-217
hsa-miR-19b-3p
hsa-miR-382-5p
hsa-miR-106a-5p
hsa-miR-107
hsa-miR-135a-5p
hsa-miR-93-5p
hsa-miR-21-5p
hsa-miR-515-3p
hsa-miR-106b-3p
hsa-miR-125a-5p
hsa-miR-148a-3p

hsa-miR-155-5p
hsa-miR-181a-5p
hsa-miR-519d
hsa-miR-96-5p
hsa-miR-212-3p
hsa-miR-29a-3p
hsa-miR-98-5p
hsa-let-7c
hsa-let-7d-5p
hsa-miR-141-3p
hsa-miR-183-5p
hsa-miR-19a-3p
hsa-miR-196a-5p
hsa-miR-30b-5p
hsa-miR-378a-3p
hsa-miR-302c-5p
hsa-miR-30c-5p
hsa-miR-130a-3p
hsa-let-7e-5p
hsa-miR-216a-5p
hsa-miR-450a-5p
hsa-let-7d-3p
hsa-miR-26b-5p
hsa-miR-181c-5p
hsa-miR-186-5p
hsa-miR-519c-3p
hsa-let-7b-5p
hsa-miR-10b-5p
hsa-miR-125b-5p
hsa-miR-134
hsa-miR-137
hsa-miR-150-5p
hsa-miR-153

hsa-miR-15b-5p
hsa-miR-16-5p
hsa-miR-195-5p
hsa-miR-196b-5p
hsa-miR-23a-3p
hsa-miR-29c-3p
hsa-miR-373-3p
hsa-miR-7-5p
hsa-miR-214-3p
hsa-miR-421
hsa-miR-15a-5p
hsa-miR-193b-3p
hsa-miR-194-5p
hsa-miR-223-3p
hsa-miR-30d-5p
hsa-miR-424-5p
hsa-miR-454-3p
hsa-miR-545-3p
hsa-miR-485-5p
hsa-miR-335-5p
hsa-miR-133a
hsa-miR-222-3p
hsa-miR-494
hsa-miR-498
hsa-miR-513a-5p
hsa-miR-92a-3p
hsa-miR-495-3p
hsa-miR-503-5p
hsa-miR-539-5p
hsa-miR-16-2-3p
hsa-miR-302b-5p
hsa-miR-425-3p
hsa-miR-99a-3p

hsa-let-7a-3p
hsa-miR-126-3p
hsa-miR-20a-3p
hsa-miR-499a-5p
hsa-let-7g-5p
hsa-miR-152
hsa-miR-26a-5p
hsa-miR-124-3p
hsa-miR-203a
hsa-miR-24-3p
hsa-miR-301b
hsa-miR-590-3p
hsa-miR-1179
hsa-miR-325
hsa-miR-552
hsa-miR-185-5p
hsa-miR-455-3p
hsa-miR-583
hsa-miR-122-5p
hsa-miR-1305
hsa-miR-139-5p
hsa-miR-146a-5p
hsa-miR-18a-5p
hsa-miR-18b-5p
hsa-miR-199b-5p
hsa-miR-340-5p
hsa-miR-34c-5p
hsa-miR-423-3p
hsa-miR-489
hsa-miR-520f
hsa-miR-520g
hsa-miR-605
hsa-miR-668

[0348]

hsa-let-7a-5p	hsa-miR-30c-5p	hsa-miR-136-3p	hsa-miR-376a-5p
hsa-let-7f-5p	hsa-miR-335-3p	hsa-miR-150-3p	hsa-miR-378a-5p
hsa-miR-10a-3p	hsa-miR-374a-5p	hsa-miR-154-3p	hsa-miR-424-3p
hsa-miR-135b-5p	hsa-miR-410	hsa-miR-15a-3p	hsa-miR-451a
hsa-miR-144-3p	hsa-miR-429	hsa-miR-15b-3p	hsa-miR-452-3p
hsa-miR-181d	hsa-miR-497-5p	hsa-miR-16-1-3p	hsa-miR-487b
hsa-miR-200b-3p	hsa-miR-513a-3p	hsa-miR-181a-2-3p	hsa-miR-493-5p
hsa-miR-200c-3p	hsa-miR-542-3p	hsa-miR-181c-3p	hsa-miR-500a-3p
hsa-miR-218-5p	hsa-miR-653	hsa-miR-186-3p	hsa-miR-502-3p
hsa-miR-23b-3p	hsa-miR-122-3p	hsa-miR-195-3p	hsa-miR-516b-3p
hsa-miR-25-3p	hsa-miR-101-5p	hsa-miR-20b-3p	hsa-miR-518c-3p
hsa-miR-29b-3p	hsa-miR-1178-3p	hsa-miR-223-5p	hsa-miR-518f-3p
hsa-miR-383	hsa-miR-191-5p	hsa-miR-224-3p	hsa-miR-519a-5p
hsa-miR-202-3p	hsa-miR-214-5p	hsa-miR-24-1-5p	hsa-miR-519b-5p
hsa-miR-381-3p	hsa-miR-302d-5p	hsa-miR-24-2-5p	hsa-miR-521
hsa-miR-377-3p	hsa-miR-572	hsa-miR-27a-5p	hsa-miR-523-5p
hsa-miR-452-5p	hsa-miR-574-3p	hsa-miR-27b-5p	hsa-miR-545-5p
hsa-miR-501-3p	hsa-miR-26a-2-3p	hsa-miR-29b-1-5p	hsa-miR-585
hsa-miR-514a-3p	hsa-miR-611	hsa-miR-302a-5p	hsa-miR-7-2-3p
hsa-miR-654-3p	hsa-let-7f-1-3p	hsa-miR-3065-5p	hsa-miR-93-3p
hsa-let-7b-3p	hsa-let-7i-3p	hsa-miR-30d-3p	hsa-miR-96-3p
hsa-miR-125a-3p	hsa-miR-100-5p	hsa-miR-34a-3p	hsa-miR-99b-3p
hsa-miR-133b	hsa-miR-106b-5p	hsa-miR-371a-3p	
hsa-miR-192-5p	hsa-miR-132-5p	hsa-miR-373-5p	
hsa-miR-199a-3p	hsa-miR-135b-3p	hsa-miR-374a-3p	

[0349] B) 多种微小 RNA- 一种 mRNA 范例。

[0350] B1. 包括 UCP1 的一个示例性的多种 miRNA- 一种 mRNA 范例。

[0351] 在脂肪细胞中, 最关键的产热调节子是 UCP1 (也称为产热素), 并且因此, 所有产热调节子必须最终影响 UCP1 活性。UCP1 是产生质子跨线粒体内膜泄漏从而使氧化磷酸化与 ATP 合成解偶联的线粒体转运蛋白。因此, 能量以热的形式散失 (适应性产热) (参见图 5) Lowell 等, Nature (2000); Friedman 等, Bioinformatics (2010); Hsu 等, Nucleic acids research (2011); Rieger 等, Frontiers in Genetics (2011)。

[0352] UCP1 生物合成主要受到转录水平的控制。图 6 描述了由其他示例性产热调节子对 UCP1 的转录调控。UCP1 基因的启动子区包含许多不同的调控位点, 允许多种蛋白质正性 (参见图 7a) 和负性 (参见图 7b) 地影响其转录。

[0353] 孟德尔随机化是一种在非实验研究中使用已知功能基因中测量的变化来检查可变的暴露对疾病的因果效应。孟德尔随机化可视为“天然的”随机化临床试验。

[0354] 据报道, UCP1 基因的遗传多态性 (例如 UCP1 的外显子 2 的启动子中 -3826A/G 单核苷酸多态性) 与 mRNA 的表达降低及肥胖有关。基因型为 G/G 的健康儿童对高脂肪膳食和急性冷暴露具有较低的产热能力。年轻女性中, 相同的 -3826A/G UCP1 遗传多态性降低静息能力消耗, 并降低热调控交感神经系统的活性。在 367 位韩国女性的研究中, 在优势模型 (dominant model) 中 -3826A > G 的 G 等位基因和 -412A > C 的 C 等位基因与大面积的腹部皮下脂肪显著相关 (分别为, $p < 0.001$ 和 $p < 0.0004$); 它们组合到一起 (ht2[GC]) 提

高了该显著性 ($p < 0.00005$)。针对 100 名重度肥胖 ($BMI > 40\text{kg/m}^2$) 的成年人和 100 名正常体重的对照对象 (BMI 范围 = 19kg/m^2 至 24.9kg/m^2) 的研究鉴定了 UCP1 基因启动子区的 7 个突变、内含子区的 4 个突变和外显子区的 4 个突变。这些突变可促进肥胖的产生, 具体而言, $g. -451C > T$, $g. 940G > A$, 以及 $g. IVS4-208T > G$ 可表示促进能量储存的“节约 (thrifty)”因子。最后, 位于 UCP1 基因上游启动子区的两种多态性 (A-3826G 和 C-3740A) 影响基因表达, 并且与人类的长寿相关。

[0355] 所有前述信息都支持将靶向 UCP1 的表达和活性作为改变适应性产热并从而治疗人类肥胖的有意义的方法。可实施许多策略来达到实现该目的, 但是, 本发明方法采用的一种策略中使用 miRNA 试剂来同时调节产热通路中的数个元件以提高 UCP1 的合成和活性。miRNA 与 UCP1 基因之间的直接相互作用和间接相互作用都被考虑。直接相互作用意为, miRNA 与 UCP1 基因的多个区域直接结合, 导致 UCP1 mRNA 的转录、翻译、稳定性和 / 或降解发生改变。间接相互作用意为, miRNA 改变产热 mRNA 的转录、翻译、稳定性和 / 或降解, 所述产热 mRNA 表达的蛋白质改变 UCP1 基因的转录。另外, 间接相互作用意为, miRNA 改变修饰 UCP1 基因转录的其他 miRNA 的转录、翻译、稳定性和 / 或降解。

[0356] 人 UCP1 基因 (gi|237858805|ref|NG_012139.1| 人解偶联蛋白 1 (线粒体, 质子载体) (UCP1), 4 号染色体上的 RefSeqGene) 的启动子区特别富含调控元件基序 (表 8)。

[0357] 表 8.

[0358] UCP1 基因调控元件:

[0359]

	调控元件的名称	序列	数量	核苷酸位置
1	BRE1 (褐色脂肪应答元件1)	CCTCTCTGCTTCTTCT	1	1,129 至 1,144
2	BRE2 (褐色脂肪应答元件2)	CTCCTTGGA	1	1,269 至 1,278
3	CRE2	ATTCTTTA	4	1,121 至 1,128; 3,631 至 3,638; 10,982 至 10,989; 15,881 至 15,888
4	CREB	ACGTCA	5	1,082 至 1,087; 1,345 至 1,350; 1,348 至 1,343; 11,439 至 11,434; 13,831 至 13,836
5	DR1	TTGCCCTTGCTCA	1	1,099 至 1,111
6	DR4	ACGTCATAAAGGGTCA	1	1,082 至 1,097
7	DR4 型 RARE	RGKTCANNNNRGKTCA	1	1,316 至 1,301
8	ERE	GCTCATACTGACCT	1	1,107 至 1,120
9	PRE	GTTAATGTGTTCT	1	1,009 至 1,021
10	RARE	TGACCACAGTTTGATCA	1	983 -> 999
11	RXR	AGGTCA	12	1,120 至 1,115; 1,316 至 1,311; 3,517 至 3,522; 3,560 至 3,555;

[0360]

				3,813 至 3,808; 5,318 至 5,313; 6,233 至 6,238; 6,831 至 6,836; 8,122 至 8,127; 9,966 至 9,971; 11,339 至 11,334; 11,412 至 11,407
12	GC 盒 1	CGCCC	7	4,593 至 4,589; 4,615 至 4,619; 4,615 至 4,619; 4,747 至 4,751; 4,765 至 4,769; 5,914 至 5,910; 13,715 至 13,711
13	GC 盒 2	GCGGG	9	4,463 至 4,459; 4,585 至 4,589; 4,593 至 4,597; 4,639 至 4,643; 4,883 至 4,887; 5,176 至 5,172; 5,929 至 5,933; 5,940 至 5,944; 14,994 至 14,990
14	GT 盒 1	CACCC	25	194 至 190; 452 至 448; 1,184 至 1,188; 1,803 至 1,807; 2,428 至 2,424; 3,037 至 3,041; 3,330 至 3,334; 4,137 至 4,141; 4,566 至 4,562; 4,599 至 4,595; 4,869 至 4,865; 5,104 至 5,108; 5,461 至 5,457; 6,237 至 6,241; 6,293 至 6,289; 8,096 至 8,092; 8,198 至 8,194; 9,649 至 9,645; 9,912 至 9,908; 12,962 至 12,958; 13,136 至 13,132;

[0361]

				13,723 至 13,719; 14,404 至 14,400; 14,960 至 14,964; 15,576 至 15,572
15	GT 盒 2	GTGGG	20	25 至 21; 1,805 至 1,801; 1,809 至 1,805; 2,119 至 2,123; 3,854 至 3,850; 4,310 至 4,314; 4,339 至 4,343; 4,765 至 4,761; 4,867 至 4,871; 6,291 至 6,295; 7,554 至 7,558; 8,280 至 8,284; 8,681 至 8,685; 9,615 至 9,619; 9,689 至 9,693; 9,906 至 9,910; 10,363 至 10,359; 13,074 至 13,070; 13,640 至 13,644; 13,941 至 13,945
16	CpG 甲基化岛	CG	366	4,519 至 5,258; 5,639 至 6,694

[0362] 表 8A 描述了人 UCP1 基因的 15,910 碱基对 (bp) (FASTA 登录号: >gi|237858805|ref|NG_012139.1| 人解偶联蛋白 1 (线粒体、质子载体) (UCP1), 4 号染色体上的 RefSeqGene; NCBI 参考序列: NG_012139.1) 中这些多种调控元件相对于第 5,001 位核酸的 UCP1 转录起始位点的位置。

[0363] 由 miRNA 直接或间接激活或抑制这些调控元件将导致 UCP1 基因表达和活性的改变。在正常条件下, UCP1 基因表达和活性受到丰富的调控元件网络抑制, 以避免能量浪费。在胁迫条件下, 例如暴露于冷环境, UCP1 基因表达通过处于数种 miRNA 调控下的多种激活蛋白和阻抑蛋白上调。

[0364] 用数种程序 (包括 microRNA.org) 进行的靶向人 UCP1 3' UTR 之 miRNA 的初始调查是阴性的。然而, 另一些程序, 包括 MicroCosm Targets, 使用 UCP1Ensembl 的 1,462 碱基对的转录物 ENST00000262999 作为靶标揭示出在 UCP1 3' UTR 中的 28 个位置上的 27 种 miRNA 的结合位点, 其在表 9 中示出。

[0365] 表 9.

[0366] 使用 microCosm Targets 确定的 UCP1 (NCBI 参考序列 NG_012139.1) 的 3' UTR 中 miRNA 的结合位点:

[0367]

名称	序列	起始 bp	终止 bp	长度
hsa-miR-21	AATGTAATGCAGATAAGCTA	14143	14162	20

hsa-miR-219-2-3p	ACATGTTTAAATTACAATTC	14217	14236	20
hsa-miR-22	GATTGGCAGCTT	14857	14868	12
hsa-miR-222a	GATTTTAAATGTTTAGAGTCCAG	14500	14522	23
hsa-miR-290-3p	TTTAGAGCTGGAGGGTACTT	14621	14640	20
hsa-miR-292-3p	TTTAGAGCTGGAGGGTACTT	14621	14640	20
hsa-miR-292-5p	GACAGAGGAACAGTTTGAG	14648	14666	19
hsa-miR-325	ATTTTGGCAGGATTGCTACTAG	14568	14589	22
hsa-miR-331-5p	TTTTGAGATCTATACCTGG	14383	14401	19
hsa-miR-362-5p	ATTTTAAGCTAAATCCAAGGATT	14838	14860	23
hsa-miR-367	TGACCATTCTGGAGTGCAATT	14170	14191	22
hsa-miR-371-5p	ACAGTTTGAT	988	997	10
hsa-miR-371-5p	ACAGTTTGAG	14657	14666	10
hsa-miR-377	CTGGAGTGCAATTGTGTGA	14179	14197	19
hsa-miR-378	TTTTAATGTTTAGAGTCCAG	14503	14522	20
hsa-miR-382	TGATGACATCTCTAACAACCTC	14526	14547	22
hsa-miR-460	AGAACTGAGTGAAATGCAG	14250	14269	20
hsa-miR-508-5p	TGACCATTCTGGAGTG	14170	14186	17
hsa-miR-543	TACTCTGAATGTT	14478	14490	13
hsa-miR-549	TTAACCACAGTTGTCA	14321	14336	16
hsa-miR-643	CAAGTTCAGTAGAATACAAG	14412	14431	20
hsa-miR-654-3p	AAGGTTACAGGCTGCCAGACAT	14880	14901	22
hsa-miR-664	GTGTGAATGAATG	14192	14204	13
hsa-miR-871	TAGGCATGAACCTACTCTGAATG	14466	14488	23
hsa-miR-883a-3p	AAACTGAGTGAAATGCAGTT	14252	14271	20

hsa-miR-883b-3p	AAACTGAGTGAAATGCAGTT	14252	14271	20
hsa-miR-888-3p	TTTATTAACCACAGTTGTCAGTT	14317	14339	23
hsa-miR-92b	GAGTGCAAT	14182	14190	9

[0368] 另一些程序,例如 miRWalk、miRGen、miRator-miRanda 和 DIANA microT,使用 UCP1Ensembl 的 1,462 碱基对转录物 (ENST00000262999)、UCP1Ensembl 的 9,371 碱基对基因序列 (ENSG00000109424) 或所述 15,910 碱基对 UCP1 序列 (NCBI 引用序列 :NG_012139.1) 作为靶标揭示出在 UCP1 3' UTR 的 69 个位置上总共 50 种 miRNA 的结合位点,其在表 10 中示出。

[0369] 表 10.

[0370] 根据数种程序的 UCP1 (NCBI 引用序列 NG_012139.1) 的 3' UTR 中 miRNA 的结合位点 :

[0371]

	名称	序列	起始 bp	终止 bp	长度
1	hsa-miR-1179	AAGTATCCTTT	15346	15356	11
2	hsa-miR-1302	ATGGGACACA	15021	15030	10
3	hsa-miR-130b	TTATTTTCCCT	15161	15171	11
4	hsa-miR-146a	TGACAACTGT	14327	14336	10
	hsa-miR-146a	AGGGAAGTGA	15231	15240	10
	hsa-miR-146a	TGTGAAGTGG	15679	15688	10
5	hsa-miR-181c	AACCATAGT	15304	15312	9
6	hsa-miR-19b-2	ACTTTTGCGG	14991	15000	10
7	hsa-miR-203	TTAAATGTT	15584	15592	9
8	hsa-miR-204-5p	TTCCTTTATC	14006	14015	10
	hsa-miR-204-5p	TTCCTCTGTC	14648	14657	10
9	hsa-miR-21-5p	TAGCTTATCT	14153	14162	10
10	hsa-miR-211-5p	TTCCCTATCTC	14779	14789	11
11	hsa-miR-214	CAGCAAGCA	15052	15060	9

12	hsa-miR-22-3p	AAGCTGCCAA	14859	14868	10
	hsa-miR-22-5p	AGTTCTTCACA	14203	14213	11
13	hsa-miR-26a-2-3p	CATTTTCTTG	13918	13927	10
	hsa-miR-26a-2-3p	CCAATCCTTG	14853	14862	10
	hsa-miR-26a-2-3p	CCTTTTCATG	15616	15625	10
14	hsa-miR-30b	GTAACCTTCC	14878	14887	10
15	hsa-miR-325	CAGAGTAGGT	14475	14484	10
	hsa-miR-325	CCTTGTAGGC	15378	15387	10
16	hsa-miR-328	CTGTTCTCT	14651	14660	10
17	hsa-miR-362-5p	ATCCTTGGAT	14850	14859	10
18	hsa-miR-367-3p	AATTGCACTC	14182	14191	10
19	hsa-miR-371a-3p	AAGTGCCTGC	15435	15444	10

[0372]

	hsa-miR-371a-5p	TCTCAAACCTG	14658	14667	10
20	hsa-miR-378a-3p	ACTGGCCTTG	15816	15825	10
21	hsa-miR-382-3p	ATTCATTAC	14194	14203	10
22	hsa-miR-382-5p	GAAGTTGTTAGAGAT	14533	14547	15
23	hsa-miR-383	AGATTAGAA	14545	14553	9
24	hsa-miR-421	ATTAACCTGAC	14333	14342	10
	hsa-miR-421	CTCAAAAGAC	14380	14389	10
25	hsa-miR-422a	ACTGGCCTT	15817	15825	9
26	hsa-miR-431	TGTCTGGCA	14892	14900	9
27	hsa-miR-452	TTATCTGC	14151	14158	8
	hsa-miR-452	TCTTCTGC	14773	14780	8

	hsa-miR-452	ACATCTGC	15009	15016	8
28	hsa-miR-455-3p	CAGTCCAT	13893	13900	8
	hsa-miR-455-5p	TGTGTGCCTT	15641	15650	10
29	hsa-miR-491-5p	AATGGGGAAG	14975	14984	10
30	hsa-miR-501-3p	ATGCATCAGG	15547	15556	10
31	hsa-miR-504	AGACCCTGT	15325	15333	9
32	hsa-miR-508-5p	TATTCTAGTGAAGTTGACTCTTA	14405	14427	23
33	hsa-miR-512-5p	CACTCAG	14255	14261	7
34	hsa-miR-514a-3p	TTGACTCTT	14406	14414	9
35	hsa-miR-515-3p	GACTGCCTT	15539	15547	9
	hsa-miR-515-3p	GTGTGCCTT	15641	15649	9
36	hsa-miR-517a-3p	ATGGTGCATT	15650	15659	10
37	hsa-miR-545	CAGCAAGCACT	15050	15060	11
38	hsa-miR-549	TGACAACTGT	14327	14336	10
39	hsa-miR-552	CACAGGTGA	15130	15138	9
40	hsa-miR-616-5p	ACTCTAAAC	14510	14518	9
41	hsa-miR-620	ATGAATATAG	14560	14569	10
42	hsa-miR-643	ACTGGTATGT	13933	13942	10
	hsa-miR-643	TCTTGTATTC	14423	14432	10
	hsa-miR-643	CCTTGTAGGC	15378	15387	10
	hsa-miR-643	ACATGCATGC	15553	15562	10
43	hsa-miR-651	TTAAAATAAG	13988	13997	10
	hsa-miR-651	TTAGGTTAAA	13993	14002	10
	hsa-miR-651	TCATGATAAG	15700	15709	10

44	hsa-miR-654-3p	TATCTCTTCT	14775	14784	10
	hsa-miR-654-3p	TATGTATACT	15493	15502	10
45	hsa-miR-655	GTAATACAT	15593	15601	9
46	hsa-miR-767-3p	CCTGCTCAT	14871	14879	9
47	hsa-miR-888-3p	GACTGACTCC	15772	15781	10

[0373]

48	hsa-miR-92b-3p	ATTGCACTCC	14181	14190	10
49	hsa-miR-941	CACCCAGGT	14396	14404	9
50	hsa-miR-99a-3p	AAGCTGGCTC	15117	15126	10

[0374] 将人 UCP1 基因与数种 miRNA 序列的序列比对得到在 UCP1 基因的 5' UTR、启动子区和编码区中的匹配。集成可公开获取的互联网工具预测靶向 UCP1 基因多个区域之 miRNA 诱发的数个命中 (hit)。出乎意料的是,这些预测工具之间的重叠为零,如图 3 所示。

[0375] 然而,使用比对程序 Geneious 筛选 miRNA 数据库。发现了总共 191 个人微小 RNA,其在 UCP1 基因序列中具有 450 个互补结合位点(表 11)。匹配的长度为 7 个碱基至 12 个碱基(例如,hsa-miR-24-2-5p 和 hsa-miR-192-5p)。每个 miRNA 的命中数为 1 至若干个不等(例如,hsa-miR-19b2 有 9 个(丰富的脂肪细胞 miRNA),hsa-miR-26a-2-3p 有 14 个,hsa-miR-181c 有 11 个,并且 hsa-miR-620 有 12 个)。

[0376] 表 11.

[0377] 在 UCP1 基因序列 (NCBI 参考序列:NG_012139.1) 中具有预测的结合位点的 miRNA:

[0378]

miRNA	序列	起始 bp	终止 bp	长度
hsa-let-7c	TAGAGTTTC	5918	5926	9
hsa-let-7e	GGAGGTAGG	13283	13291	9
hsa-let-7e	TGAAGTAGG	7612	7620	9
hsa-let-7e	AGAGGTAGG	3306	3314	9
hsa-let-7i-3p	CTGTGCAAG	3588	3596	9
hsa-miR-17	CAAAGTGCT	12200	12208	9

hsa-miR-17	CAAAGTGCT	9931	9939	9
hsa-miR-17	CAAAGTGCT	218	226	9
hsa-miR-19a	TGTGCAAAT	3916	3924	9
hsa-miR-19a	TGTGCAAAT	834	842	9
hsa-miR-19b-2	ACTTTTGCGG	14991	15000	10
hsa-miR-19b-2	AGTTTACAA	11998	12007	10
hsa-miR-19b-2	AGTTTGTAT	10023	10032	10
hsa-miR-19b-2	AGTCTGAAG	9399	9408	10
hsa-miR-19b-2	AGGTTGTAG	7758	7767	10

[0379]

hsa-miR-19b-2	AGTATTGAAG	7159	7168	10
hsa-miR-19b-2	AGGCTGCAG	3546	3555	10
hsa-miR-19b-2	AATTTGGCAG	529	538	10
hsa-miR-19b-2	AGTTTTGGAA	312	321	10
h sa-miR-20b	CAAAGTGCT	12200	12208	9
hsa-miR-20b	CAAAGTGCT	9931	9939	9
hsa-miR-20b	CAAAGTGCT	218	226	9
hsa-miR-21-5p	TAGCTTATCT	14153	14162	10
hsa-miR-22-3p	AAGCTGCCAA	14859	14868	10
hsa-miR-22-3p	AAGCTTCAG	1482	1491	10
hsa-miR-22-5p	AGTTCTTCACA	14203	14213	11
hsa-miR-22-5p	AATTCTTCAGG	8032	8042	11
hsa-miR-22-5p	GGTTCTTCAGC	5389	5399	11
hsa-miR-24-2-5p	TGCCTACTGGCC	8651	8662	12

hsa-miR-25-3p	CATTGCAC	11565	11572	8
hsa-miR-25-5p	AGGCGGAG	5963	5970	8
hsa-miR-26a-2-3p	CCTTTTCATG	15616	15625	10
hsa-miR-26a-2-3p	CCAATCCTTG	14853	14862	10
hsa-miR-26a-2-3p	CATTTTCTTG	13918	13927	10
hsa-miR-26a-2-3p	CCTACTCTTC	13505	13514	10
hsa-miR-26a-2-3p	ACGATTCTTG	13192	13201	10
hsa-miR-26a-2-3p	TCTATTCTTT	12883	12892	10
hsa-miR-26a-2-3p	CATATTTTGT	10197	10206	10
hsa-miR-26a-2-3p	GCTAGTCTTG	9978	9987	10
hsa-miR-26a-2-3p	CATATTTTGT	9890	9899	10
hsa-miR-26a-2-3p	CCTTTTCTTT	6631	6640	10
hsa-miR-26a-2-3p	CCCATTCTCG	4709	4718	10
hsa-miR-26a-2-3p	TTTATTCTTG	3893	3902	10
hsa-miR-26a-2-3p	CCTTTACTTG	1885	1894	10
hsa-miR-26a-2-3p	GCGATTCTTG	376	385	10
hsa-miR-27-5p	AGAGCTTAGG	2949	2958	10
hsa-miR-30b	GTAACCTTCC	14878	14887	10
hsa-miR-30b	GTAACCATCA	12991	13000	10
hsa-miR-30b	GTAATCATAC	12831	12840	10
hsa-miR-30b	GTCAACATCA	11401	11410	10
hsa-miR-30b	GTAAACATAA	9365	9374	10
hsa-miR-30b	GTACTCATCC	9016	9025	10
hsa-miR-30b	CTATACATCC	8586	8595	10

hsa-miR-30b	CTAAACATCT	7495	7504	10
hsa-miR-31	GGCTATGCC	7712	7720	9

[0380]

hsa-miR-32	ATTGCACA	11564	11571	8
hsa-miR-92b	ATTGCACTCC	14181	14190	10
hsa-miR-92b	ATTGCACTAG	11282	11291	10
h sa-miR-93	CAAAGTGCTG	12199	12208	10
hsa-miR-93	CAAAGTGCTG	217	226	10
hsa-miR-93-3p	ACTCCTGGGCT	12356	12366	11
hsa-miR-93-3p	ACTGATAAGCT	11055	11065	11
hsa-miR-93-3p	ACTCCTGACCT	9966	9976	11
hsa-miR-96-3p	AATCATGTGCC	8659	8669	11
hsa-miR-99a-3p	AAGCTGGCTC	15117	15126	10
hsa-miR-99a-3p	AAACTCTTTC	13344	13353	10
hsa-miR-99a-3p	AATCTTGTTT	11952	11961	10
hsa-miR-99a-3p	AAGCTCCTTT	11050	11059	10
hsa-miR-99a-3p	AAGCTCCTTT	8099	8108	10
hsa-miR-99a-3p	AAGCTCTGTC	7523	7532	10
hsa-miR-99b-3p	CAACCTCGAG	13666	13675	10
hsa-miR-99b-3p	CGAGCTCCTG	13660	13669	10
hsa-miR-99b-3p	GAAGCTTGTG	6436	6445	10
hsa-miR-99b-3p	CAAACCTCTG	257	266	10
hsa-miR-100	TCCAGTAGAT	11866	11875	10
hsa-miR-100	ACGCGCAGAT	5634	5643	10

hsa-miR-106b-5p	CAAAGTGCTG	12199	12208	10
hsa-miR-106b-5p	CAAAGTGCTG	217	226	10
hsa-miR-126-3P	TCATACAGT	12828	12836	9
hsa-miR-126-3P	TTGTACTGT	11542	11550	9
hsa-miR-126-3P	TGGTCCCGT	7922	7930	9
hsa-miR-126-3P	TCATACAGT	932	940	9
hsa-miR-130b	TTATTTTCCCT	15161	15171	11
hsa-miR-130b	CTCTTTTCAGT	9670	9680	11
hsa-miR-130b	CTCTCTTCACT	8977	8987	11
hsa-miR-130b	CTCTTTTCCC	8444	8454	11
hsa-miR-130b	CTTTTCCCCT	6624	6634	11
hsa-miR-130b	CTATTTTCCGT	5742	5752	11
hsa-miR-130b	TTCCCTTCCCT	5007	5017	11
hsa-miR-130b	CTCTTTGCCCC	1845	1855	11
hsa-miR-130b	CTCCTTTCCTT	1033	1043	11
hsa-miR-133a-1	TTTGGTGCCC	7393	7402	10
hsa-miR-140-3p	TACCACAG	5893	5900	8
hsa-miR-141	TAACACTG	5852	5859	8
hsa-miR-143	GGTGCAGTG	4132	4140	9

[0381]

hsa-miR-143-3p	TGAGATGAGG	13727	13736	10
hsa-miR-143-3p	TGAGATGGAG	10172	10181	10
hsa-miR-143-3p	TTAGATGAAG	9572	9581	10
hsa-miR-144-3p	TACAGTATT	12825	12833	9

hsa-miR-144-3p	TACAATATA	8859	8867	9
hsa-miR-144-3p	GACAGTATA	1491	1499	9
hsa-miR-146a	CCTCTGAAA	3499	3507	9
hsa-miR-146a	TGTGAACTGG	15679	15688	10
hsa-miR-146a	AGGGAAGTGA	15231	15240	10
hsa-miR-146a	TGACAACTGT	14327	14336	10
hsa-miR-146a	TAAGAACTAA	8935	8944	10
hsa-miR-146a	TTAGAACAGA	7908	7917	10
hsa-miR-146a	TGAGAAGTGC	6926	6935	10
hsa-miR-146a	TGAAAACCTA	3883	3892	10
hsa-miR-146a	ACAGAACTGA	2259	2268	10
hsa-miR-146a	TGAGACCAGA	2235	2244	10
hsa-miR-146a	TGAGAAATAA	1614	1623	10
hsa-miR-147	TGTGTGGATAA	7223	7233	11
hsa-miR-147	TTTGTGCAAAT	3916	3926	11
hsa-miR-154	AATCATACA	12830	12838	9
hsa-miR-154	AATCATACA	934	942	9
hsa-miR-181c	AACCATAGT	15304	15312	9
hsa-miR-181c	AACCAAAGA	13244	13252	9
hsa-miR-181c	AACCATCAC	12990	12998	9
hsa-miR-181c	ATCCAGCGA	11466	11474	9
hsa-miR-181c	AAACATCTA	7494	7502	9
hsa-miR-181c	AAAAATCGA	6201	6209	9
hsa-miR-181c	AACCCCCGA	5540	5548	9

hsa-miR-181c	AACCCCTCTA	3614	3622	9
hsa-miR-181c	AGCCAGCGA	3471	3479	9
hsa-miR-181e	AACCATAGG	2801	2809	9
hsa-miR-181c	AACCATCAC	194	202	9
hsa-miR-185	TGGAGAGAA	2979	2987	9
hsa-miR-192-5p	CTAACATATGAA	114	125	12
hsa-miR-194-1	TGTAACAGCA	1895	1904	10
hsa-miR-196a	AGGTAGTTT	12139	12147	9
hsa-miR-199a-5p	CCCTGTGTTC	5753	5762	10
hsa-miR-200a	TAACACTG	5852	5859	8
hsa-miR-200b	TAATAATGCC	11184	11193	10
hsa-miR-200b	GAATACTGCC	10340	10349	10

[0382]

hsa-miR-200c-3p	TAATACTGT	12466	12474	9
hsa-miR-200c-3p	TAATAATGC	11185	11193	9
hsa-miR-200c-3p	GAATACTGC	10341	10349	9
hsa-miR-200c-3p	TAATACAGC	7594	7602	9
hsa-miR-203	TTAAATGTT	15584	15592	9
hsa-miR-203	TGAAATTTT	9782	9790	9
hsa-miR-203	TGAAAGGTT	4495	4503	9
hsa-miR-204-5p	TTCTCTGTC	14648	14657	10
hsa-miR-204-5p	TTCTTTATC	14006	14015	10
hsa-miR-205	TCCTTCATT	10659	10667	9
hsa-miR-208b	ATAAGAAGA	9493	9501	9

hsa-miR-208b	ATAAGAAGA	1770	1778	9
hsa-miR-211-5p	TTCCCTATCTC	14779	14789	11
hsa-miR-211-5p	TCCCCTCTGTC	5238	5248	11
hsa-miR-211-5p	TTCCCTTGCTC	5002	5012	11
hsa-miR-211-5p	TTCCCATCTC	4710	4720	11
hsa-miR-214	CAGCAAGCA	15052	15060	9
hsa-miR-214	CAGAAGGCA	6918	6926	9
hsa-miR-214	CCGCAGGCA	5935	5943	9
hsa-miR-214	CACCAGGCA	2087	2095	9
hsa-miR-218	TGTGCTTGA	10385	10393	9
hsa-miR-302c	TTTAACATG	2932	2940	9
hsa-miR-324-5p	CGCGTCCCCT	4876	4885	10
hsa-miR-325	CCTTGTAGGC	15378	15387	10
hsa-miR-325	CAGAGTAGGT	14475	14484	10
hsa-miR-325	CCAAGTAGCT	10066	10075	10
hsa-miR-325	CCAAGTAGCT	354	363	10
hsa-miR-328	CTGTTCTCT	14651	14660	10
hsa-miR-328	CTGGCTCCCT	8215	8224	10
hsa-miR-328	CTGGCCCTTC	8062	8071	10
hsa-miR-328	CTGGCACTCA	6653	6662	10
hsa-miR-328	CTGGCTTTCT	6496	6505	10
hsa-miR-328	CTGCCCCCTC	6048	6057	10
hsa-miR-328	CTGGGCCGCT	4804	4813	10
hsa-miR-328	CTGGAGCTCT	4477	4486	10

hsa-miR-328	CTGACCCTTT	1089	1098	10
hsa-miR-330	CAAAGCACAC	13845	13854	10
hsa-miR-330	CAAAGCACAC	11657	11666	10
hsa-miR-331-5p	CTAGGTGTGG	7719	7728	10
hsa-miR-361-3p	CCCCCAGG	5112	5119	8

[0383]

hsa-miR-362-5p	ATCCTTGAT	14850	14859	10
hsa-miR-367-3p	AATTGCACTC	14182	14191	10
hsa-miR-367-3p	AAATGCACTT	999	1008	10
hsa-miR-369	AATAATACA	2266	2274	9
hsa-miR-371a-3p	AAGTGCCTGC	15435	15444	10
hsa-miR-371a-3p	AAGAGCCGAC	11455	11464	10
hsa-miR-371a-3p	ACGTGCCACC	10044	10053	10
hsa-miR-371a-3p	AAGTGCCTCT	7047	7056	10
hsa-miR-371a-3p	AAGTGCACCC	5457	5466	10
hsa-miR-371a-5p	TCTCAAACGT	14658	14667	10
hsa-miR-372	AAAGTGCTG	12199	12207	9
hsa-miR-372	AAAGTGCTG	217	225	9
hsa-miR-374a-3p	TCATCAGATT	10606	10615	10
hsa-miR-377-3p	AGCACACAAA	13842	13851	10
hsa-miR-378a-3p	ACTGGCCTTG	15816	15825	10
hsa-miR-378a-3p	ACTGGTCTTG	11837	11846	10
hsa-miR-378a-5p	CTCCTGCCTC	12216	12225	10
hsa-miR-378a-5p	CTCCTGCCTC	10082	10091	10

hsa-miR-378a-5p	CTCCTGTCTC	8207	8216	10
hsa-miR-378a-5p	CTCCTAACTC	7650	7659	10
hsa-miR-382-3p	ATTCATTAC	14194	14203	10
hsa-miR-383	AGATTAGAA	14545	14553	9
hsa-miR-383	AGATTAGAA	7912	7920	9
hsa-miR-383	AGAACAGAA	5801	5809	9
hsa-miR-412	ACTTCACCT	737	745	9
hsa-miR-421	CTCAAAAGAC	14380	14389	10
hsa-miR-421	ATTAAGTGAC	14333	14342	10
hsa-miR-421	AACATCAGAC	11398	11407	10
hsa-miR-421	ATCAAGTGAG	3427	3436	10
hsa-miR-421	ATCAACAGGT	2443	2452	10
hsa-miR-421	ATCAAAAGAT	2333	2342	10
hsa-miR-422a	ACTGGCCTT	15817	15825	9
hsa-miR-422a	ACTGGTCTT	11838	11846	9
hsa-miR-422a	ACTGGACGT	5847	5855	9
hsa-miR-425	AGCGGAAGGT	5167	5177	11
hsa-miR-431	TGTCTGGCA	14892	14900	9
hsa-miR-431	TGTCTAGCA	9218	9226	9
hsa-miR-432-5p	TCCTGGAGT	13624	13632	9
hsa-miR-432-5p	TATTGGAGT	10785	10793	9
hsa-miR-432-5p	TCTTAGAGT	9263	9271	9
[0384]				
hsa-miR-432-5p	TCTTAGAGT	6666	6674	9

hsa-miR-432-5p	TCTTGGAGC	2180	2188	9
hsa-miR-452	ACATCTGC	15009	15016	8
hsa-miR-452	TCTTCTGC	14773	14780	8
hsa-miR-452	TTATCTGC	14151	14158	8
hsa-miR-452	TCCTCTGC	13488	13495	8
hsa-miR-452	TCATGTGC	8660	8667	8
hsa-miR-452	TCATCTGG	8221	8228	8
hsa-miR-452	TCATGTGC	7945	7952	8
hsa-miR-452	ACATCTGC	7508	7515	8
hsa-miR-452	CCATCTGC	6787	6794	8
hsa-miR-452	TCATCCGC	5912	5919	8
hsa-miR-452	TCATCTGT	4053	4060	8
hsa-miR-452	TCATCTCC	3667	3674	8
hsa-miR-452	TCCTCTGC	3457	3464	8
hsa-miR-452	TCTTCTGC	2210	2217	8
hsa-miR-455-3p	CAGTCCAT	13893	13900	8
hsa-miR-455-5p	TGTGTGCCTT	15641	15650	10
hsa-miR-455-5p	TCTGTGCCTT	11203	11212	10
hsa-miR-455-5p	TATGTGCTTT	10522	10531	10
hsa-miR-483-3p	CACTCCTC	13536	13543	8
hsa-miR-483-3p	CACTCCTC	10333	10340	8
hsa-miR-483-3p	CACTCCTC	6101	6108	8
hsa-miR-486-5p	TCATGTACT	9835	9843	9
hsa-miR-486-5p	TCCTGTCTT	6526	6534	9

hsa-miR-487a	AATCATACAG	12829	12838	10
hsa-miR-487a	AATCATACAG	933	942	10
hsa-miR-491-5p	AATGGGGAAG	14975	14984	10
hsa-miR-491-5p	AGAGGGGACC	12315	12324	10
hsa-miR-491-5p	AGTTGGGCAC	11555	11564	10
hsa-miR-491-5p	AGTAGAGAAC	6909	6918	10
hsa-miR-491-5p	GGTGAGGAAC	6005	6014	10
hsa-miR-491-5p	AGCGGGGCAC	4455	4464	10
hsa-miR-491-5p	AGTGGGAAAT	3846	3855	10
hsa-miR-496	TTAGTATTA	10948	10956	9
hsa-miR-496	TGAGTATAA	10768	10776	9
hsa-miR-496	TCAGTATTA	9666	9674	9
hsa-miR-501-3p	ATGCATCAGG	15547	15556	10
hsa-miR-501-3p	ATCCACCGGG	11497	11506	10
hsa-miR-501-3p	AGGCACCAGG	2089	2098	10

[0385]

hsa-miR-504	AGACCCTGT	15325	15333	9
hsa-miR-504	AGCCCCTGG	12898	12906	9
hsa-miR-504	AGTCCCTGG	10591	10599	9
hsa-miR-504	AGACCCGGG	4767	4775	9
hsa-miR-508-3p	TGATTATAGC	13565	13574	10
hsa-miR-508-3p	TGAGTGTAGC	3231	3240	10
hsa-miR-512-3p	CAGTGCTGTC	13211	13220	10
hsa-miR-512-3p	AAGTGCTCTC	7688	7697	10

hsa-miR-512-3p	AAGTGCTCTC	3184	3193	10
hsa-miR-512-5p	CACTCAG	14255	14261	7
hsa-miR-512-5p	CACTCAG	13591	13597	7
hsa-miR-512-5p	CACTCAG	12291	12297	7
hsa-miR-512-5p	CACTCAG	6652	6658	7
hsa-miR-512-5p	CACTCAG	5067	5073	7
hsa-miR-514a-3p	TTGACTCTT	14406	14414	9
hsa-miR-514a-3p	TTGACAGTT	13870	13878	9
hsa-miR-514a-3p	TTAACACTT	11237	11245	9
hsa-miR-514a-3p	ATGACACTT	10617	10625	9
hsa-miR-515-3p	GTGTGCCTT	15641	15649	9
hsa-miR-515-3p	GACTGCCTT	15539	15547	9
hsa-miR-515-3p	GAGTGACTT	1371	1379	9
hsa-miR-516a-3p	TGCTTCCT	10301	10308	8
hsa-miR-517a-3p	ATGGTGCATT	15650	15659	10
hsa-miR-517a-3p	ATCTTGCTTC	10303	10312	10
hsa-miR-519b-3p	AAAGTGCAT	13782	13790	9
hsa-miR-519e-3p	AAGTGCCTC	7048	7056	9
hsa-miR-520a-5p	CTCCAGATGG	6274	6283	10
hsa-miR-545	CAGCAAGCACT	15050	15060	11
hsa-miR-545	CAGAACACATT	11639	11649	11
hsa-miR-545	CTGCAAACACT	3450	3460	11
hsa-miR-549	TGACAACTGT	14327	14336	10
hsa-miR-551b-3p	GCTACCCAT	2411	2419	9

hsa-miR-552	CACAGGTGA	15130	15138	9
hsa-miR-552	AACAGGTCA	11407	11415	9
hsa-miR-552	AACATGTGA	9513	9521	9
hsa-miR-552	AACAGGTTA	2441	2449	9
hsa-miR-552	AACAGGTAA	1569	1577	9
hsa-miR-583	AAAAGAGGA	2921	2929	9
hsa-miR-583	CAAATAGGA	2833	2841	9
hsa-miR-583	CAACGAGGA	1824	1832	9

[0386]

hsa-miR-583	CAAAGAAGA	1139	1147	9
hsa-miR-593-3p	TGTCTCTGT	8204	8212	9
hsa-miR-593-3p	TGGCTCTGC	6852	6860	9
hsa-miR-593-3p	TGCCTCTGC	231	239	9
hsa-miR-593-5p	AGGCACCAG	2090	2098	9
hsa-miR-593-5p	AGGCACCAG	2083	2091	9
hsa-miR-598	ACGTCATC	11432	11439	8
hsa-miR-611	GCGAGGTCTC	4779	4788	10
hsa-miR-611	GAGAGGCCCC	2121	2130	10
hsa-miR-611	GAGAGGACCT	1546	1555	10
hsa-miR-616-5p	ACTCTAAAC	14510	14518	9
hsa-miR-619	GACCTGGA	5824	5831	8
hsa-miR-620	ATGAATATAG	14560	14569	10
hsa-miR-620	ATGGAAATAT	12111	12120	10
hsa-miR-620	TTGGATATAG	11026	11035	10

hsa-miR-620	GTGGAGATGG	10397	10406	10
hsa-miR-620	ATGGAGATCC	6268	6277	10
hsa-miR-620	ATGGAGGGAG	5626	5635	10
hsa-miR-620	CTGGAGAAAG	3827	3836	10
hsa-miR-620	ATCCAGATAG	2959	2968	10
hsa-miR-620	ATGGGGCTAG	2843	2852	10
hsa-miR-620	AGGGAGAGAG	1551	1560	10
hsa-miR-620	CAGGAGATAG	1430	1439	10
hsa-miR-620	TTGGAGAGAG	1201	1210	10
hsa-miR-623	TCCCTTGC	8306	8313	8
hsa-miR-623	TCCCTTGC	5004	5011	8
hsa-miR-631	CACCTGGCC	9900	9908	9
hsa-miR-631	GACATGGCC	8632	8640	9
hsa-miR-634	AACCAGCAC	4520	4528	9
hsa-miR-636	TGTGCTTG	10386	10393	8
hsa-miR-638	ACGGAGCGCG	4905	4914	10
hsa-miR-638	AGGGAGGGCG	4615	4624	10
hsa-miR-642a-5p	ATCCCTCTC	8983	8991	9
hsa-miR-642a-5p	GTCCCTCCC	4722	4730	9
hsa-miR-643	ACATGCATGC	15553	15562	10
hsa-miR-643	CCTTGTAGGC	15378	15387	10
hsa-miR-643	TCTTGATTC	14423	14432	10
hsa-miR-643	ACTGGTATGT	13933	13942	10
hsa-miR-643	ACTTCTATTC	12886	12895	10

hsa-miR-643	ACTTTTCTGC	12044	12053	10
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[0387]

hsa-miR-643	GCTTGTAAGC	11698	11707	10
hsa-miR-643	AGTTGTATGT	10531	10540	10
hsa-miR-643	ACTTGGAAGC	8105	8114	10
hsa-miR-643	ACTTGTTGG	7227	7236	10
hsa-miR-643	ACTTGTTTGA	1880	1889	10
hsa-miR-643	ACATGTTTGC	1695	1704	10
hsa-miR-650	AGGAGGCAC	9647	9655	9
hsa-miR-650	AGAAGGCAG	6917	6925	9
hsa-miR-650	AGGAGCCAG	3474	3482	9
hsa-miR-650	ATGAGGCAG	3052	3060	9
hsa-miR-651	TCATGATAAG	15700	15709	10
hsa-miR-651	TTAGGTAAA	13993	14002	10
hsa-miR-651	TAAAATAAG	13988	13997	10
hsa-miR-651	TTAGCATAAC	12788	12797	10
hsa-miR-651	TTATGATGAG	12617	12626	10
hsa-miR-651	TTGGATGAG	11069	11078	10
hsa-miR-651	TGAGTATAAG	10767	10776	10
hsa-miR-651	TTACAATAAG	10546	10555	10
hsa-miR-651	TAAGGATAAA	8265	8274	10
hsa-miR-651	TGTGGATAAG	7222	7231	10
hsa-miR-651	GTAGGATAGG	5553	5562	10
hsa-miR-651	CTAGGAAAAG	2823	2832	10

hsa-miR-651	CTATGATAAG	1635	1644	10
hsa-miR-651	TAAGGATAGG	1562	1571	10
hsa-miR-654-3p	TATGTATACT	15493	15502	10
hsa-miR-654-3p	TATCTCTTCT	14775	14784	10
hsa-miR-654-3p	TCTATCTGCT	8354	8363	10
hsa-miR-654-3p	AATGTCTGGT	6720	6729	10
hsa-miR-654-3p	TATGTTTCCT	6638	6647	10
hsa-miR-654-3p	TTTTTCTGCT	6586	6595	10
hsa-miR-654-3p	TATGTCTTTT	6534	6543	10
hsa-miR-654-3p	TATATCTGCA	6214	6223	10
hsa-miR-654-3p	TATGTAGGCT	97	106	10
hsa-miR-655	GTAATACAT	15593	15601	9
hsa-miR-655	ATAGTACAT	4200	4208	9
hsa-miR-655	ATAAGACAT	3642	3650	9
hsa-miR-655	ATAATACAG	2265	2273	9
hsa-miR-655	ACAATACAT	1757	1765	9
hsa-miR-656	AATATTATA	657	665	9
hsa-miR-664-3p	TATTCATTT	9385	9393	9

[0388]

hsa-miR-765	TGGAGGA	5020	5026	7
hsa-miR-766	CTCCAGCCCC	12901	12910	10
hsa-miR-766	CTCCAGCCCC	5032	5041	10
hsa-miR-767-3p	CCTGCTCAT	14871	14879	9
hsa-miR-767-3p	TCTTCTCAT	9155	9163	9

hsa-miR-875	CCTGGAAATA	5820	5829	10
hsa-miR-875	CCTAGAAACA	5294	5303	10
hsa-miR-876	TGGATTCT	6366	6374	9
hsa-miR-876	TGGATTCT	142	150	9
hsa-miR-888-3p	GACTGACTCC	15772	15781	10
hsa-miR-888-3p	GACTGACAGC	9119	9128	10
hsa-miR-890	TACTTGAAG	8106	8115	10
hsa-miR-940	AAGGCAGTG	1807	1815	9
hsa-miR-941	CACCCAGGT	14396	14404	9
hsa-miR-941	CACCCTGCC	13715	13723	9
hsa-miR-941	CACCCCTCT	13128	13136	9
hsa-miR-941	CACTCAGCT	12289	12297	9
hsa-miR-941	CTCCCGGT	10102	10110	9
hsa-miR-941	CAGCCTGCT	10034	10042	9
hsa-miR-941	CACCCACCT	9904	9912	9
hsa-miR-941	CACCTGGCC	9900	9908	9
hsa-miR-941	CATCTGGCT	8219	8227	9
hsa-miR-941	CACTCGACT	8148	8156	9
hsa-miR-941	CTCCCAGCT	6840	6848	9
hsa-miR-941	CTCACGGCT	6031	6039	9
hsa-miR-941	CAGCCCGCT	5928	5936	9
hsa-miR-941	CACCTGACT	5510	5518	9
hsa-miR-941	CACGCCGCT	5142	5150	9
hsa-miR-941	CTCCCTGCT	3983	3991	9

hsa-miR-941	CACCAGGCA	2087	2095	9
hsa-miR-941	CTCCCGGGT	390	398	9
hsa-miR-941	CACCCAGCC	186	194	9
hsa-miR-941-2	ATCCGACTGT	9657	9666	10
hsa-miR-941-2	TCCCTGCTGT	8726	8735	10
hsa-miR-941-2	TCCCAGCTGT	6838	6847	10
hsa-miR-941-2	AGCCCGCTGT	5926	5935	10
hsa-miR-941-2	ACCCGGGCGT	4764	4773	10
hsa-miR-1179	AAGTATCCTTT	15346	15356	11
hsa-miR-1179	ATGCATTCTGT	3357	3367	11
hsa-miR-1179	ATGCATTCTCT	1854	1864	11

[0389]

hsa-miR-1207-5p	TGGCAGGG	11441	11448	8
hsa-miR-1224-3p	CTCCACCTCC	399	408	10
hsa-miR-1228-3p	TCCCACCTG	13637	13645	9
hsa-miR-1228-3p	TCACGCCTG	4992	5000	9
hsa-miR-1231	GTGTCTGGC	12807	12815	9
hsa-miR-1231	GTGTCCGGG	4739	4747	9
hsa-miR-1245	AAGTGATCT	8341	8349	9
hsa-miR-1245	AAGTGATCT	2020	2028	9
hsa-miR-1249	CGCCCTTC	5907	5914	8
hsa-miR-1251	ACTCTAGGT	12854	12862	9
hsa-miR-1251	ACTCTATCT	8357	8365	9
hsa-miR-1251	ACTCCAGCT	4044	4052	9

hsa-miR-1251	AGTCTAGCT	457	465	9
hsa-miR-1252	AGAGGGAAAT	3819	3828	10
hsa-miR-1252	GGAAGGAAAT	1625	1634	10
hsa-miR-1268	CGGGCGTGG	4762	4770	9
hsa-miR-1270	CTGGAAATA	5820	5828	9
hsa-miR-1270	CTGGAGATG	5055	5063	9
hsa-miR-1270	CTGGAGAAA	3828	3836	9
hsa-miR-1270	CAGGAGATA	1431	1439	9
hsa-miR-1272	GATGATGA	10622	10629	8
hsa-miR-1275	GTAGGGGAGA	1189	1198	10
hsa-miR-1302	ATGGGACACA	15021	15030	10
hsa-miR-1302	TTTGATATA	11027	11036	10
hsa-miR-1302	TTAGGGCATA	8421	8430	10
hsa-miR-1302	TTGGAACAGA	6076	6085	10
hsa-miR-1302	CTGGGACTTA	4819	4828	10
hsa-miR-1302	GTGGGAAATA	3845	3854	10
hsa-miR-1302	TTGTGAGATA	1944	1953	10
hsa-miR-1302	CTGGGAAATA	867	876	10
hsa-miR-1324	TCAAGACAGA	9426	9435	10
hsa-miR-1827	TGAGGCAGT	3051	3059	9
hsa-miR-1911-3p	CACCAGGCA	2087	2095	9
hsa-miR-1915	CCCCAGGG	5111	5118	8
hsa-miR-2909	TTTAGGGCC	3728	3736	9

[0390] B2. 包括 UCP2 的另一个示例性的多种 miRNA- 一种 mRNA 范例。

[0391] UCP2 是在 wAT、骨骼肌、胰岛和中枢神经系统中表达的线粒体转运蛋白。与 UCP1 一

样,其制造跨线粒体内膜的质子泄漏,从而使氧化磷酸化与 ATP 合成解偶联(适应性产热,参见图 5)(Lowell 等, Nature (2000))。

[0392] 两个最近的元-分析报道了 UCP2 启动子区的多态性与肥胖之间的关联(Liu 等, Gene(2013); Andersen 等, Int. J. Obes. (2013))。第一个元-分析包括 14 个研究(7,647 个案例和 11,322 个对照),并得出以下结论,在 UCP2-866G/A 多态性的 A 等位基因与肥胖风险的降低之间有显著的关联,特别是在欧洲人群中。在第二个元-分析中包括 12,984 位对象,常见的 UCP2-866G 等位基因与肥胖有关。在 17,636 位丹麦对象中,同样的 UCP2-866G 等位基因与胰岛素敏感性的降低有关。在一个研究中,在具有 GG 表型的对象中,内脏脂肪中 UCP2 的 mRNA 水平降低(Esterbauer 等, Nat. Genet. (2001))。在另一个研究中发现了皮下脂肪细胞 UCP2mRNA 与体脂肪百分比之间的负相关趋势(Wang 等, American Journal of Physiol. (2004))。该信息支持将靶向 UCP2 的表达和活性作为改变适应性产热并从而治疗人肥胖的有意义的方法。可实施许多策略来达到实现该目的,但是,本发明方法采用的一种策略中使用 miRNA 试剂来同时调节产热通路中的数个元件以提高 UCP2 的合成和活性。考虑 miRNA 与 UCP2 基因之间的直接相互作用和间接相互作用二者。直接相互作用意为,miRNA 与 UCP2 基因的多个区域直接结合,导致 UCP1mRNA 的转录、翻译、稳定性和/或降解发生改变。间接相互作用意为,miRNA 改变产热 mRNA 的转录、翻译、稳定性和/或降解,所述产热 mRNA 表达的蛋白质改变 UCP2 基因的转录。此外,间接相互作用意为,miRNA 改变修饰 UCP2 基因之转录的其他 miRNA 转录、翻译、稳定性和/或降解。

[0393] 人 UCP2 基因(ENSG00000175567,人解偶联蛋白 2(线粒体,质子载体)(UCP2),11 号染色体上的 RefSeqGene)的启动子区富含调控元件基序(表 12)。

[0394] 表 12.

[0395] UCP2 基因调控元件:

[0396]

	调控元件名称	序列	数量	核苷酸位置
1	RXR/T3RE	AGGTCA	8	1,074 至 1,079; 3,083 至 3,088; 3,239 至 3,244; 4,304 至 4,309; 6,965 至 6,970; 7,420 至 7,425; 7,677 至 7,682; 13,319 至 13,324
2	GC 盒 1	CGCCC	16	2,605 至 2,609; 4,323 至 4,327; 4,523 至 4,527; 4,933 至 4,937; 4,959 至 4,963; 5,048 至 5,052; 5,066 至 5,070; 5,146 至 5,150; 5,155 至 5,159; 5,387 至 5,391; 5,483 至 5,487; 6,067 至 6,071; 8,523 至 8,527; 9,790 至 9,794; 10,819 至 10,823; 11,754 至 11,758
3	GC 盒 2	GCGGG	5	4,263 至 4,267; 4,757 至 4,761; 4,860 至 4,864; 7,619 至 7,623; 11,262 至 11,266
4	GT 盒 1	CACCC	30	1,421 至 1,425; 1,677 至 1,681; 1,761 至 1,765; 1,825 至 1,829; 1,833 至 1,837; 2,036 至 2,040; 3,003 至 3,007; 4,903 至 4,907; 4,947 至 4,951; 5,210 至 5,214; 6,204 至 6,208; 6,247 至 6,251; 6,469 至 6,473; 6,828 至 6,832; 7,681 至 7,685; 8,048 至 8,052; 8,437 至 8,441; 8,572 至 8,576; 8,599 至 8,603; 8,702 至 8,706; 11,077 至 11,081; 11,235 至 11,239; 12,006 至 12,010; 12,374 至 12,378; 13,475 至 13,479; 13,666 至 13,670; 13,687 至 13,691; 13,838 至 13,842; 14,410 至 14,414; 14,545 至 14,549
5	GT 盒 2	GTGGG	26	123 至 127; 1,006 至 1,010; 2,105 至 2,109; 4,562 至 4,566; 5,793 至 5,797; 6,029 至 6,033; 6,034 至 6,038; 6,040 至 6,044; 6,150 至 6,154; 7,271 至 7,275; 7,392 至 7,396; 9,040 至 9,044; 9,697 至 9,701; 10,227 至 10,231; 10,238 至 10,242; 10,247 至 10,251; 11,817 至 11,821; 12,410 至 12,414; 12,414 至 12,418; 12,678 至 12,682; 13,047 至 13,051; 13,238 至 13,242; 13,743 至 13,747; 14,252 至 14,256;

				14,969 至 14,973; 15,104 至 15,108
6	CpG 甲基化岛	CG	295	4,071 至 5,212

[0397] 表 8B 描述了人 UCP2 基因的 15,174 碱基对 (bp) 中这些多种调控元件相对于第 5,001 位核酸的 UCP2 转录起始位点的位置。由 miRNA 直接或间接激活或抑制这些调控元件将导致 UCP2 基因表达和活性的改变。

[0398] 用数个预测程序使用 UCP2Ensembl 2, 113 碱基对转录物 ENST00000310473 作为靶标进行的 miRNA 靶向人 UCP2 3'UTR 的调查显示了 161 个 miRNAs 的结合位点, 其在表 13 中示出。

[0399] 表 13.

[0400] UCP2 转录物序列 3' UTR 中具有预测的结合位点的 miRNA :

[0401]

hsa-miR-1	hsa-miR-1275	hsa-miR-149	hsa-miR-219-1
hsa-miR-1-2	hsa-miR-1276	hsa-miR-150-3p	hsa-miR-219-2
hsa-miR-101-1	hsa-miR-1278	hsa-miR-150-5p	hsa-miR-221-5p
hsa-miR-101-2	hsa-miR-1285-1	hsa-miR-1538	hsa-miR-23b
hsa-miR-103	hsa-miR-1286	hsa-miR-155	hsa-miR-24-1
hsa-miR-105-1	hsa-miR-1293	hsa-miR-15a	hsa-miR-24-2
hsa-miR-105-2	hsa-miR-1300	hsa-miR-15b	hsa-miR-27b-5p
hsa-miR-106b	hsa-miR-1302-1	hsa-miR-16-1	hsa-miR-28
hsa-miR-107	hsa-miR-1302-10	hsa-miR-16-2	hsa-miR-296-3p
hsa-miR-1204	hsa-miR-1302-11	hsa-miR-184	hsa-miR-296-5p
hsa-miR-1207	hsa-miR-1302-2	hsa-miR-185-3p	hsa-miR-3064
hsa-miR-1208	hsa-miR-1302-3	hsa-miR-185-5p	hsa-miR-323a
hsa-miR-1226	hsa-miR-1302-4	hsa-miR-186	hsa-miR-328
hsa-miR-1246	hsa-miR-1302-5	hsa-miR-188	hsa-miR-330
hsa-miR-1252	hsa-miR-1302-6	hsa-miR-18a	hsa-miR-331
hsa-miR-1253	hsa-miR-1302-7	hsa-miR-18b	hsa-miR-338
hsa-miR-1255a	hsa-miR-1302-8	hsa-miR-193a	hsa-miR-342
hsa-miR-1255b-1	hsa-miR-1302-9	hsa-miR-195	hsa-miR-3619
hsa-miR-1255b-2	hsa-miR-1303	hsa-miR-199b	hsa-miR-370
hsa-miR-1260a	hsa-miR-130a	hsa-miR-200a	hsa-miR-377
hsa-miR-1262	hsa-miR-1321	hsa-miR-203	hsa-miR-378a
hsa-miR-1263	hsa-miR-138-1	hsa-miR-206	hsa-miR-383
hsa-miR-1265	hsa-miR-138-2	hsa-miR-214	hsa-miR-411

[0402]

hsa-miR-412	hsa-miR-638
hsa-miR-422a	hsa-miR-645
hsa-miR-424	hsa-miR-646
hsa-miR-425	hsa-miR-647
hsa-miR-4291	hsa-miR-652
hsa-miR-432-3p	hsa-miR-654
hsa-miR-4505	hsa-miR-658
hsa-miR-450b	hsa-miR-663a
hsa-miR-453	hsa-miR-663b
hsa-miR-4533	hsa-miR-664-5p
hsa-miR-4539	hsa-miR-675
hsa-miR-4745	hsa-miR-7-1
hsa-miR-4747	hsa-miR-7-2
hsa-miR-485-5p	hsa-miR-7-3
hsa-miR-486	hsa-miR-708
hsa-miR-490	hsa-miR-761
hsa-miR-491	hsa-miR-765
hsa-miR-493	hsa-miR-769
hsa-miR-497	hsa-miR-770
hsa-miR-498	hsa-miR-876
hsa-miR-503w	hsa-miR-877
hsa-miR-505	hsa-miR-921
hsa-miR-508-3p	hsa-miR-922
hsa-miR-532	hsa-miR-92a-1
hsa-miR-539	hsa-miR-92a-2-5p
hsa-miR-541	hsa-miR-92b
hsa-miR-548l	
hsa-miR-552	
hsa-miR-563	
hsa-miR-575	
hsa-miR-577	
hsa-miR-580	
hsa-miR-583	
hsa-miR-584	
hsa-miR-608	
hsa-miR-612	
hsa-miR-613	
hsa-miR-615-3p	
hsa-miR-618	
hsa-miR-625	
hsa-miR-626	
hsa-miR-634	
hsa-miR-635	

[0403] 此外,用数个预测程序使用包含 5,000bp 5' UTR 的人 UCP2 基因 (ENSG00000175567, 15, 174 碱基对 (bp)) 作为靶标进行的 miRNA 靶向人 UCP25'UTR 的调查

揭示了在 UCP2 的 5' UTR 中的 54 个 miRNA 结合位点,其在表 14 中示出。

[0404] 表 14.

[0405] 在 UCP2 基因序列之 5' UTR 中具有预测的结合位点的 miRNA :

[0406]

微小 RNA	种子长度	起始	序列	终止	P 值
hsa-let-7c	9	3052	UAGAGUUAC	3044	0.0374
hsa-let-7i-3p	9	3051	CUGCGCAAG	3043	0.0374
hsa-miR-1228-5p	9	3419	UGGGCGGGG	3411	0.0374
hsa-miR-1229-3p	9	3419	UCUCACCAC	3411	0.0374
hsa-miR-129-1-3p	10	2784	AGCCCUUACC	2775	0.0095
hsa-miR-1302	9	4219	UGGGACAU	4211	0.0374
hsa-miR-1303	9	2159	UUAGAGACG	2151	0.0374
hsa-miR-136	9	4486	CUCCAUUUG	4478	0.0374
hsa-miR-155	9	2160	UUAAUGCUA	2152	0.0374
hsa-miR-16	10	3603	UAGCAGCACG	3594	0.0095
hsa-miR-18a-3p	10	3603	ACUGCCCUAA	3594	0.0095
hsa-miR-190	9	2428	UGAUAUGUU	2420	0.0374
hsa-miR-191	9	3052	CAACGGAU	3044	0.0374
hsa-miR-192	9	4390	CUGACCUAU	4382	0.0374
hsa-miR-194	9	1643	UGUACAGC	1635	0.0374
hsa-miR-197	9	5001	UCACCACCU	4993	0.0374
hsa-miR-19b-2-5p	10	3052	AGUUUUGCAG	3043	0.0095
hsa-miR-203	9	3051	UGAAAUGUU	3043	0.0374
hsa-miR-218	10	3603	UUGUGCUUGA	3594	0.0095
hsa-miR-218-1-3p	9	5001	UGGUUCCGU	4993	0.0374

hsa-miR-219-1-3p	9	3614	AGAGUUGAG	3606	0.0374
hsa-miR-26a-2-3p	9	2163	CCUAUUCUU	2155	0.0374
hsa-miR-27a-3p	10	3603	UUCACAGUGG	3594	0.0095
hsa-miR-27a-5p	11	3336	AGGGCUUAGCU	3326	0.0024
hsa-miR-28-5p	10	3603	AAGGAGCUCA	3594	0.0095
hsa-miR-331-3p	9	4134	GCCCCUGGG	4126	0.0374
hsa-miR-337-5p	10	115	GAACGGCUUC	106	0.0095
hsa-miR-340-3p	9	1872	CCGUCUCAG	1864	0.0374
hsa-miR-34c-3p	11	2162	AAUCACUAACC	2152	0.0024
hsa-miR-373-5p	11	530	ACUCAAAAUGG	520	0.0024

[0407]

hsa-miR-425	9	1013	AAUGACACG	1005	0.0374
hsa-miR-497	9	3661	AGCAGACA	3653	0.0374
hsa-miR-501-5p	9	4164	AUCCUUUGU	4156	0.0374
hsa-miR-505	9	1015	GUCAACACU	1007	0.0374
hsa-miR-508-3p	9	1274	GAUUGUAGC	1266	0.0374
hsa-miR-509-3p	12	2554	UGAUUGGUACGU	2543	0.0006
hsa-miR-512-5p	10	987	ACUCAGCCUU	978	0.0095
hsa-miR-514	9	5001	UUGACACUU	4993	0.0374
hsa-miR-515-5p	9	59	UUCUCCAAA	51	0.0374
hsa-miR-518a-3p	9	19	GAAAGCGCU	11	0.0374
hsa-miR-519e-5p	11	2525	UCUCCAAAAGG	2515	0.0024
hsa-miR-548a-3p	10	680	CAAAACUGGC	671	0.0095
hsa-miR-550a-3p	9	4312	GUCUUACUC	4304	0.0374

hsa-miR-571	9	739	UGAGUUGGC	731	0.0374
hsa-miR-578	9	1377	CUUCUUGUG	1369	0.0374
hsa-miR-606	9	4420	AACUACUGA	4412	0.0374
hsa-miR-615-5p	10	1140	GGGGGUCCCC	1131	0.0095
hsa-miR-638	9	2710	GGGAUCGCG	2702	0.0374
hsa-miR-657	12	1316	GCAGGUUCUCAC	1305	0.0006
hsa-miR-658	9	3673	GGCGGAGGG	3665	0.0374
hsa-miR-877-3p	9	4349	UCCUCUUCU	4341	0.0374
hsa-miR-93-3p	9	799	ACUGCUGAG	791	0.0374
hsa-miR-96-3p	9	799	AAUCAUGUG	791	0.0374
hsa-miR-99b-3p	9	2163	CAAGCUCGU	2155	0.0374

[0408] C) 多种微小 RNA- 多种 mRNA 范例。

[0409] 在从表 2 中选择的 83 种产热调控分子中筛选高严谨性的多种 miRNA- 多种 mRNA 关联。用 7 种主要的预测工具进行的这些分析的结果在图 4 中示出。这 7 种工具的联合产生了 4439 个 miRNA- 基因对。随着工具数量的增加, 这些工具之间的重叠降低, 当考虑 7 种工具时, 达到仅 15 个 miRNA- 基因对。

[0410] D) 共调控的 mRNA 靶标范例中的一个微小 RNA 种子序列基序的过呈现。

[0411] 可使用数种方法来鉴定通路特异性的 miRNA。例如, 检索推定为共调控基因的 3'-UTR 的来自 miRNA 种子区的过呈现序列可鉴定共有的调控 miRNA。为了确定特定的 miRNA 种子序列是否在所选择的 83 种产热靶标的 3'UTR 中过呈现, 使用 miRvestigator 网络应用 (miRvestigator.systemsbioology.net/)。使用以下参数 (基序大小为 8bp, 默认 Weeder 模式, 种子模式为 8 聚体, 100% 的互补同源性, 以及允许 0.25 的摆动碱基配对), 其确定, 如在表 15 中所示, 由 hsa-miR-19a/19b 识别的基序 5' -UUUGUACA-3' 在 83 种产热靶标的 15 种中过呈现, 互补性 p 值为 1.7×10^{-04} 。应注意, 据报道, hsa-miR-19 是丰富的人脂肪细胞 miRNA。

[0412] 表 15.

[0413] 共有基序 UUUGUACA 与 hsa-miR-19a/19b 之间的互补性:

[0414]

miRNA名称	miRNA种子	种子模式	互补性长度	互补碱基配对	互补性P值
<u>hsa-miR-19a</u> <u>hsa-miR-19b</u>	UGUGCAA	8聚体	8	基序 5' UUGUGACA 3' 3' AAACGUGU 5'miRNA种子	1.7e-04

[0415] 通过 miRvestigator 鉴定, hsa-miR-19mRNA/miRNA 双链的最小自由能水平十分低, 倾向于紧密结合。因此, 使用较低的互补性严谨水平来重复 miRvestigator 分析。该分析还鉴定了与 hsa-miR-19 基序具有 95% 相似度至一致的 10 种另外的靶标 (CEBPD、PRKAA1、TWIST1、IRS1、NCOA1、NCOA2、NCOA3、KLF5、RPS6KB1、NRIP1)。有趣的是, hsa-miR-19 是脂肪组织组织中最丰富的 miRNA 之一。鉴定为包含 hsa-miR-19 种子区域的互补序列的基因记载于表 16 中。

[0416] 表 16.

[0417] 鉴定为 hsa-miR-19 之靶标的产热调节子:

[0418]

基因	基因符号	位点序列	起始相对于终止密码子 (bp)	与共有基序的%相似度 (质量 = 100000000)	mRNA-miRNA双链的 最小自由能 (MFE)
850	BMP2	UUUGUACA	386	100.00	-6.80
1052	CEBPD	UUUGUAAA	283	95.44	-3.40
2132	TNFRSF1A	UUUGUACA	510	100.00	-6.80
5562	PRKAA1	UUUGUAAA	2400	95.44	-3.40
5563	PRKAA2	UUUGUACA	542	100.00	-6.80
935	BMP7	UUUGUACA	1927	100.00	-6.80
952	BMP4	UUUGUACA	770	100.00	-6.80
133822	PFARGC1B	UUUGUACA	7199	100.00	-6.80
2574	WNT5A	UUUGUACA	1414	100.00	-6.80
5722	SREBF1	UUUGUACA	510	100.00	-6.80
7251	TWIST1	UUUGUAAA	649	95.44	-3.40
9687	IRS1	UUUGUAAA	992	95.44	-3.40
10499	NCOA2	UUUGUAAA	1381	95.44	-3.40
2204	NRIP1	UUUGUACA	1716	100.00	-6.80
2204	NRIP1	UUUGUAAA	1995	95.44	-3.40
2202	NCOA3	UUUGUAAA	965	95.44	-3.40
1365	CREB1	UUUGUAAA	1973	95.44	-3.40
1365	CREB1	UUUGUACA	2822	100.00	-6.80
1365	CREB1	UUUGUACA	2822	100.00	-6.80
1365	CREB1	UUUGUAAA	4175	95.44	-3.40
2843	INSR	UUUGUAAA	2105	95.44	-3.40
2912	NR4A3	UUUGUACA	2347	100.00	-6.80
909	RUNX2	UUUGUACA	2425	100.00	-6.80
5778	STAT5A	UUUGUACA	1214	100.00	-6.80
1874	E2F4	UUUGUACA	755	100.00	-6.80
999	KLF5	UUUGUAAA	549	95.44	-3.40
3545	NCOA1	UUUGUAAA	381	95.44	-3.40
8128	RPS6KB1	UUUGUAAA	2531	95.44	-3.40

[0419] 因此,以更低的互补性严谨水平(基序大小为 8bp,默认 Weeder 模式,种子模式为 8 聚体,95%的互补同源性,以及允许 0.25 的摆动碱基配对)来重复 miRvestigator 分析。该分析还鉴定了与 hsa-miR-19 基序具有 95%相似度至一致的 10 至 12 种另外的靶标(CEBPD、CREB1、PRKAA1、TWIST1、INSR、IRS1、NCOA1、NCOA2、NCOA3、KLF5、RPS6KB1、NRIP1)。有趣的是,hsa-miR-19 是脂肪组织组织中最丰富的 miRNA 之一。鉴定为包含 hsa-miR-19 种

子区域之互补序列的基因记载于表 17 中。

[0420] 表 17.

[0421] 鉴定为 hsa-miR-19a/b 的靶标,与共有基序有 95%至 100%相似度的产热调节子:

[0422]

基因	基因符号	位点序列	起始相对于终止密码子 (bp)	与共有基序的%相似度 (质量=100 中 1%)	mRNA-miRNA 双链的 最小自由能 (MFE)
650	BMP2	UUUGUACA	386	100.00	-6.80
1092	CEBPD	UUUGUAAA	263	95.42	-3.40
7132	TNFRSF1A	UUUGUACA	510	100.00	-6.80
4040	LRP6	UUUGUACA	151	100.00	-6.80
4040	LRP6	UUUGUAAA	4965	95.42	-3.40
5582	PRKAA1	UUUGUAAA	2400	95.42	-3.40
5582	PRKAA2	UUUGUACA	542	100.00	-6.80
655	BMP7	UUUGUACA	1927	100.00	-6.80
692	BMP4	UUUGUACA	770	100.00	-6.80
133522	PPARGC1B	UUUGUACA	7199	100.00	-6.80
1874	E2F4	UUUGUACA	755	100.00	-6.80
7474	WNT5A	UUUGUACA	1414	100.00	-6.80
9720	SREBF1	UUUGUACA	510	100.00	-6.80
7321	TWIST1	UUUGUAAA	649	95.42	-3.40
9587	IRS1	UUUGUAAA	992	95.42	-3.40
10499	NCOA2	UUUGUAAA	1381	95.42	-3.40
8204	NRIP1	UUUGUACA	1715	100.00	-6.80
9204	NRIP1	UUUGUAAA	1935	95.42	-3.40

[0423]

8202	NCOA3	UUUGUAAA	955	95.42	-3.40
8543	INSR	UUUGUAAA	2105	95.42	-3.40
8913	NR4A3	UUUGUACA	2347	100.00	-6.80
9775	STAT5A	UUUGUACA	1214	100.00	-6.80
988	KLF5	UUUGUAAA	549	95.42	-3.40
9549	NCOA1	UUUGUAAA	381	95.42	-3.40
860	RUNX2	UUUGUACA	2425	100.00	-6.80
1385	CREB1	UUUGUAAA	1973	95.42	-3.40
1385	CREB1	UUUGUACA	2822	100.00	-6.80
1385	CREB1	UUUGUAAA	4175	95.42	-3.40
8198	RPS8KB1	UUUGUAAA	2531	95.42	-3.40

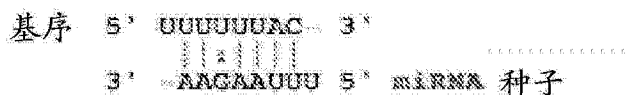
[0424] 在没有摆动的情況下,相同的基序 5' -UUUGUACA-3' 在 hsa-miR-1283 的靶标中过呈现,互补性 p 值为 1.4×10^{-4} 。此外, hsa-miR-1283 与和遗传性的人类肥胖有关的另一些目的 mRNA 如 ABCA1 (胆固醇转运蛋白)、脂联素受体以及转录因子 TCF7L2 结合。

[0425] 类似地,对在脂肪细胞中表达的 miRNA 鉴定了过呈现种子序列的另一些 miRNA。它们包括通用 hsa-let-7 家族 (序列 CUAUACAA, p 值 = 7.5×10^{-4}), 以及脂肪细胞中富含的 hsa-miR-30 家族 (序列 UGUAAACA, p 值 = 1.9×10^{-3}) 等。

[0426] 相对于根据 STRING 软件包与 UCP1 直接相连的 PRDM16、CIDEA、NRIP1、KDM3A、CEPPB、PPARG、PPARGC1A 和 PPKAA2, 似乎它们都和数个 miRNA 共享 (基序大小 8bp, 默认 Weeder 模式, 晶种模式 8 聚体, 95% 的互补同源性, 以及 0.25 的摆动碱基配对允许) 一个共有序列, 所述 miRNA 包括 hsa-miR-3658 (p 值 = 1.9×10^{-3}) 和 hsa-miR30 家族 (p 值 = 6.3×10^{-3}), 如下所示:

[0427] hsa-miR-3658:

[0428]



[0429] hsa-miR-30a/b/c/d/e:

[0430]



[0431] E) 内含子 miRNA- 多种 mRNA 通路特异性的范例。

[0432] 许多哺乳动物 miRNA 位于蛋白编码基因的内含子中, 而不是其本身的特有的转录单元中。内含子 miRNA 通常与它们所处在的前体 mRNA 一起表达和加工。虽然可对内含子

miRNA 和它们的宿主基因进行独立调控,但内含子 miRNA 可通过靶向宿主基因的 UTR 来下调其本身的宿主蛋白质编码基因。通过选择作为 miRNA 靶向的转录因子或通过内含子 miRNA 宿主基因产物与 miRNA 靶基因产品之间的蛋白质-蛋白质相互作用可实现宿主蛋白编码基因的反馈调控。例如,miR-33 与 SREBP 宿主基因配合作用以控制胆固醇稳态,并且 miR-33a 和 miR-33b 的药物抑制是提高血浆 HDL 和低级 VLDL 甘油三酯水平以治疗血脂异常的有前景的治疗策略。

[0433] 83 种产热靶基因的检验揭示出两种内含子 miRNA:位于 PPARGC1B 基因中的 miR-378,和位于 PRDM16 基因中的 miR-4251。

[0434] 预测 miRNA 靶标的互联网工具的挖掘显示,miR-378 靶标包括 BMP2、PPARA、PPARGC1A、PRDM16、STAT5 和 WNT10A,以及 ADIPOQ 和 IGFR1;并且 miR-4251 靶标包括 BMP2、CTBP1、CTBP2、MAPK14、NCOA3、PLAC8、PPARA、PPARD、TRPM8 以及 ABCA5、ABCA13、ADIPOQR2、KDM5B、KLF-12、KLF-14 和 TCF7L2。

[0435] 实施例 3. 高内涵细胞表型筛选

[0436] 使用高内涵筛选方法来筛选调节产热调节子(例如 UCP1 和 UCP2)活性的新 miRNA 试剂。高内涵筛选是使用活细胞图像以辅助分子发现的药物发现方法。这样的基于自动成像的筛选方法特别适用于鉴定改变细胞表型的试剂。

[0437] WAT 细胞包含大脂滴,而相反地,BAT 细胞包含许多较小的滴和较多数目的线粒体,所述线粒体包含铁并使其呈现棕色。与 WAT 相比,BAT 中的大量线粒体导致氧消耗的增加。因此,可基于其细胞表型来视觉地区分 BAT 细胞和 WAT 细胞。

[0438] 因此,高内涵筛选方法用于筛选调节产热调节子活性的新 miRNA。具体地,通过经培养的细胞的相差显微术、测量细胞脂质含量(使用油红 O 染色或尼罗红荧光染色);线粒体含量(例如,使用 Life Technologies Mito-Tracker Red FM),和/或体外氧消耗(例如,使用 Seahorse Bioscience Extra-Cellular Flux Instrument)来评估在存在或不存在 miRNA 激动剂或拮抗剂的情况下生长的经培养的人脂肪细胞和来源于间充质干细胞的脂肪组织,持续两周。通过靶向的 q-RT-PCR、NanoString 和通用 RNA 测序来测量 mRNA 表达。由通过靶向的 Western 印迹和通用蛋白质组学分析(proteomic profiling)来测量蛋白质表达。

[0439] A. 人前脂肪细胞分化成脂肪细胞。

[0440] 1. 分化方案。

[0441] 为了评估 miRNA 类似物对人前脂肪细胞分化成成熟脂肪细胞的影响,在第 0 天将人皮下的前脂肪细胞(来自 8 名女性供体的 SuperLot 0048, ZenBio, NC)平板接种于 96 孔板上,并使其在前脂肪细胞培养基(DMEM/Ham's F-12(1:1, v/v)、HEPES 缓冲剂、胎牛血清和抗生素)中附着过夜。次日(第 1 天),去除该培养基,并用分化培养基(DMEM/Ham's F-12(1:1, v/v), 100 μ M 抗坏血酸, 0.85 μ M 胰岛素, 20nM 亚硒酸钠, 0.2nM 三-碘甲状腺原氨酸, 1 μ M 地塞米松, 100 μ M 异丁基-甲基黄嘌呤, 100nM 罗格列酮和抗生素)更换。将细胞于 37°C, 5% CO₂ 中孵育 2 天。两天后(第 3 天),去除该培养基,并用新鲜维持培养基(DMEM/Ham's F-12(1:1, v/v), 100 μ M 抗坏血酸, 0.85 μ M 胰岛素, 20nM 亚硒酸钠, 0.2nM 三-碘甲状腺原氨酸和抗生素)更换。在第 3 天,用 miRNA 类似物(Dharmacon 特异性 miRIDIAN 模拟物和发夹抑制剂),使用转染试剂 Dharmafect1 转染细胞。所有处理为一

式三份。在转染后,阴性对照为仅维持培养基,并且阳性对照为具有 100nMPPARG 激动剂罗格列酮的维持培养基。2 天后,去除培养基,并用新鲜的维持培养基替换。然后,每两天更换维持培养基,直至处理时间结束(第 15 天)。在处理结束时(总共培养 15 天),将细胞加工用于表型筛选和基因型筛选。

[0442] 2. 前脂肪细胞的转染。

[0443] 使用转染试剂促进 miRNA 类似物渗透进靶细胞中。例如,本文中描述了我们用转染试剂 Dharmafect 1 (Dharmacon, CO) 在前脂肪细胞中实现的转染效率的范围。通过两种方式来评估转染效率:

[0444] a. 用荧光 miRNA 类似物转染后测量细胞的表面荧光。

[0445] 在第 15 天测量第 3 天用 Dy547 标记的非靶向 miRIDIAN 模拟物和发夹抑制剂 (100nM) 转染的细胞中的荧光 (540 激发 /590 发射)。如图 9 所示,用荧光 miRNA 类似物转染的细胞具有显著更强的荧光,甚至在转染后第 12 天亦如此:

[0446] b. 对照基因表达的降低。

[0447] 为了确认前脂肪细胞的成功转染,在用 GAPDH 特异性 siRNA 转染前脂肪细胞后 4 天(第 7 天)(图 10A)和 12 天(第 15 天)(图 10B)测量对照基因 GAPDH (“管家基因”)的表达降低。得到细胞裂解物,并使用通过细胞-至 -Ct 试剂得到的纯 RNA 来进行 RT-PCR。在转染后第 4 天和第 12 天观察到 GAPDH mRNA 表达的 91%和 86%敲除,这两者都是高度显著的,如图 10A 和 10B 所示。

[0448] 3. 在人前脂肪细胞分化成脂肪细胞过程中的表型变化。

[0449] 在处理结束时(培养的第 15 天),用油红 O 对细胞进行染色来评估脂质含量。如图 11A 至 11F 所示,在不含罗格列酮的培养基存在的情况下,前脂肪细胞显示基本没有分化成加载有脂质的成熟脂肪细胞。在含有 100nM 罗格列酮的分化培养基的存在下保持两天,随后在维持培养基中保持 12 天(阴性对照),观察到一些分化成加载有脂质的成熟脂肪细胞。在整个实验中 100nM 罗格列酮都存在的情况下(阳性对照),大多数细胞变成加载有脂质的成熟脂肪细胞。例如,在 25nM hsa-miR-30b 模拟物存在的情况下,约一半的细胞变成加载有脂质的成熟脂肪细胞。非靶向 miRNA 模拟物和抑制剂示出与阴性对照相似的模式。

[0450] 4. 在人前脂肪细胞分化成脂肪细胞过程中的基因型变化。

[0451] 通过 RNA-Seq 技术来进行在人前脂肪细胞由罗格列酮或 miRNA 类似物诱导分化成熟脂肪细胞期间发生的 mRNA 变化的作图。小 RNA 测序 (RNA-Seq) 是一种高通量的新一代测序平台,其现在允许在不需要在先的序列或二级结构信息的情况下对所有小 RNA (已知或未知) 进行转录组宽度的作图。

[0452] 从前脂肪细胞(前脂肪细胞阴性对照)和在 100nM 罗格列酮(分化阳性对照)或 25nM miRNA 模拟物或抑制剂的存在下培养 12 天的前脂肪细胞中提取 RNA 样品。使用 Illumina Hi-Seq 2000 设备进行 RNA 测序。将结果针对人基因组 19 进行作图 (<http://genome.ucsc.edu/>)。似乎在 miRNA 类似物的存在下,相对于前脂肪细胞,313 至 449 个 mRNA 存在显著差异的表达。相对于罗格列酮,显著差异表达的基因数减少 111 至 216 个,从而提示 miRNA 和 PPARG 类似物之间激活脂肪细胞分化的共同通路。

[0453] 关于我们的 83 种产热激活蛋白和抑制剂,其中 73 种的表达在罗格列酮或 miRNA 类似物的存在下改变。在罗格列酮的存在下(图 12A),或在 miRNA 类似物 hsa-let-7a 抑

制剂、hsa-miR-1 模拟物、hsa-miR-19b 模拟物、hsa-miR-30b 模拟物或对照脂肪细胞的存在下,产热靶标 mRNA 表达的改变分别在图 12B 至 12F 中示出。

[0454] 在罗格列酮或 miRNA 类似物的存在下,还测量了 UCP1、UCP2 和 UCP3 的 mRNA 表达的改变,如下文表 18 所示。

[0455] 表 18.

[0456] 产热 mRNA 表达的变化:

[0457]

试剂	mRNA 表达变化 (log 比率)		
	UCP1	UCP2	UCP3
罗格列酮	15.70	263	0.26
hsa-let-7a 抑制剂	2.23	173	0.65
hsa-miR-1 模拟物	0.41	110	0.40
hsa-miR-19b 模拟物	0.18	33	0.26
hsa-miR-30b 模拟物	0.76	119	0.28
前脂肪细胞中的基线水平	0.02	1.35	0.30

[0458] 这三种解偶联蛋白的表达水平在前脂肪细胞中低。在隔天随培养基更换的 100nM 罗格列酮的存在下,UCP1 的表达显著升高。随 miRNA 类似物的 UCP1mRNA 的升高幅度低于罗格列酮,但是我们必须记住所使用的 miRNA 类似物浓度 (25nM),以及仅在 RNA 提取 12 天前进行了一次转染的事实。主要发现的是,在罗格列酮以及 miRNA 类似物的存在下 UCP2 表达大幅升高。UCP3 的表达在任何条件下都没有变化,如所预期的,其是主要在肌细胞中表达的基因。UCP1 和 UCP2 表达的该升高说明,施用这些 miRNA 使得细胞分化成具有更大产热潜力的脂肪细胞,因此这些 miRNA 很可能是用于治疗肥胖和另一些代谢疾病或病症的有效药物。

[0459] 此外,我们观察了在 miRNA 类似物的存在下前脂肪细胞培养期间差异表达的基因。例如在图 13 中所示,作了 M-A 图以显示在维持培养基中生长的前脂肪细胞和在 hsa-miR-19b 模拟物的存在下生长的前脂肪细胞之间 mRNA 表达的差异。x 轴是平均基因表达,并且 y 轴是成对之间在对数级上的差值。红点是差异表达的基因 (上调大于 0 并且下调小于 0)。灰点是对照和 hsa-miR-19b 模拟物之间没有差异表达的基因 (上调大于 0 并且下调小于 0)。

[0460] 例如在图 14 中所示,相对于在仅维持培养基培养的前脂肪细胞,在 miRNA 类似物 hsa-let-7a 抑制剂、hsa-miR-1 模拟物、hsa-miR-19b 模拟物和 hsa-miR-30b 模拟物的存在下显著差异表达的基因数目分别是 406、382、370 和 433。127 个基因的组被这 4 种 miRNA 类似物共同上调 (维恩图,图 14)。

[0461] 它们不仅包括我们的 83 种产热靶标中的一些如 ALDH1A1、AZGP1、CEBPA、PPARGC1A、UCP1 和 UCP2,还包括脂类代谢和脂肪细胞分化中涉及的许多基因 (表 19)。

[0462] 表 19.

[0463] 被 4 种 miRNA 类似物共同上调的 127 个基因的组:

[0464]

ABCC6	CHI3L2	KCNE3	PPL
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ABCD2	CILP	KCNK3	PPP1R1A
ACACB	CKB	KIT	PRKAR2B
ACHE	CKMT1B	KLB	PTGDS
ACSF2	CLCA2	LBP	QPRT
ACSM5	CLMN	LEP	RASL12
ACSS2	COL14A1	LGALS12	RNF157
ADH1B	COL21A1	LIPE	S100B
AIF1L	CPB1	LPL	SDPR
AKR1C3	CYB5A	LRRC4C	SELENBP1
ALDH1A1	CYP4F12	LRRN4CL	SEMA3G
AOC3	CYP4F22	MAN1C1	SEPP1
AOC4	DARC	MAOA	SLC2A4
APCDD1	DGAT2	MAOB	SLC2A5
APOC1	DHCR24	MARCO	SLC40A1
AQP3	DPT	MCAM	SLC04C1
AQP7	DTX4	METTL7A	SMOC2
AQP9	EPHB6	MGP	SNCG
AZGP1	FABP4	MLXIPL	SPARCL1
BBOX1	FADS2	MOBKL2B	SPRY1
BHLHE22	FAM65C	MOSC1	SVEP1
C11orf87	FM01	MVD	TF
C14orf180	FM02	NAT8L	TM7SF2
C1orf1 15	GOS2	NKD2	TMEM132C
C1orf95	GPD1	PCSK9	TMEM176B

C3	GPR109A	PFKFB1	TMEM37
CA2	GPR109B	PKD1L2	TNMD
CADM3	HAVCR2	PLA2G2A	TPRG1
CD01	HRASLS5	PLIN1	TRIL
CEBPA	IGSF10	PLIN4	UCP1
CFD	ITIH1	PLXDC1	UCP2
CFHR1	ITIH5	PPARGC1A	

[0465]

[0466] 60 个基因的组被这 4 种 miRNA 类似物共同下调（维恩图，图 15）。

[0467] 它们包括许多趋化因子基因以及细胞增殖中涉及的基因（表 20）。

[0468] 表 20.

[0469] 被 4 种 miRNA 类似物共同下调的 60 个基因的组：

[0470]

ACTC1	CFNPF	ID1	KRTAP2-1
ANLN	CKAP2L	ID3	MALL
ARSI	CXCL1	IER3	MMP3
ATOX8	CXCL2	IL13RA2	NCAPH
AURKB	CXCL3	IL6	PHLDA1
BLM	CXCL5	IL8	PLK1
BRCA2	CXCL6	INHBA	PPAPDC1A
BUB1	E2F7	IQGAP3	PTGS2
BUB1B	ESCO2	KIAA1244	RELN
CASC5	FAM83D	KIF11	SHCBP1
CCL26	GABBR2	KIF14	SLC17A9
CDC6	GREM2	KIF18B	SLC6A17
CDCA5	GTSE1	KIF2C	THBD

CDC48	HAS1	KIFC1	TMSL3
CDH15	HJURP	KRT34	TOP2A

[0471] B. 人白色脂肪细胞分化成褐色脂肪细胞。

[0472] 1. 分化方案。

[0473] 为了评估 miRNA 类似物对人白色脂肪细胞分化成褐色脂肪细胞的影响,在第 0 天将人皮下的前脂肪细胞(来自 8 名女性供体的 SuperLot 0048,ZenBio,NC)平板接种于 96 孔板上,并使其在前脂肪细胞培养基(DMEM/Ham's F-12(1 : 1, v/v)、HEPES 缓冲剂、胎牛血清和抗生素)中附着过夜。在下一天(第 1 天),去除该培养基,并用分化培养基-2(DMEM/Ham's F-12(1 : 1, v/v)、HEPES 缓冲剂、胎牛血清、生物素、泛酸盐 / 酯、人胰岛素、地塞米松、异丁基-甲基黄嘌呤、专利的 PPARG 激动剂和抗生素)更换。使细胞于 37℃,5% CO₂ 中孵育 7 天。7 天后(第 7 天),用 AM-1 脂肪细胞维持培养基(DMEM/Ham's F-12(1 : 1, v/v)、HEPES 缓冲剂、胎牛血清、生物素、泛酸盐 / 酯、人胰岛素、地塞米松和抗生素)更换部分培养基。使细胞于 37℃,5% CO₂ 中再孵育 7 天。在第 17 天,用 miRNA 类似物(Dharmacon 特异性 miRIDIAN 模拟物和抑制剂),使用转染试剂 Dharmafect 3 转染细胞。所有处理一式三份进行。在转染后,阴性对照为仅维持培养基,并且阳性对照为具有 100nM PPARG 激动剂罗格列酮的维持培养基。2 天后,去除培养基,并用新鲜的维持培养基替换。然后,每 2 至 3 天更换维持培养基,直至处理持续时间结束(第 30 天)。在处理结束时(总共培养 30 天),将细胞加工用于表型筛选和基因型筛选。

[0474] 2. 脂肪细胞的转染。

[0475] 使用转染试剂以促进 miRNA 类似物渗透进靶细胞中。

[0476] 例如,本文中描述了我们用转染试剂 Dharmafect 3(Dharmacon, CO) 在脂肪细胞中实现的转染效率的范围。通过两种方式来评估转染效率:

[0477] a. 用荧光 miRNA 类似物转染后测量细胞表面荧光。

[0478] 在第 30 天测量第 17 天用 Dy547 标记的非靶向 miRIDIAN 模拟物和发夹抑制剂(100nM)转染的细胞中的荧光(540 激发 / 590 发射)。如图 16 所示,用荧光 miRNA 类似物转染的细胞具有显著更强的荧光,甚至在转染后第 12 天亦如此。

[0479] b. 对照基因表达的降低。

[0480] 为了确认脂肪细胞的成功转染,在用 GAPDH 特异性 siRNA 进行的 Dharmafect3(Dharmacon, CO) 介导的脂肪细胞转染后 4 天(第 22 天)和 12 天(第 30 天)测量对照基因 GAPDH(“管家基因”)的表达降低。得到细胞裂解物,并使用通过细胞-至 -Ct 试剂得到的纯 RNA 来进行 RT-PCR。用转染试剂 Dharmafect 3 实现了对成熟脂肪细胞(已知难以进行转染的细胞类型)的有效转染,在转染后第 4 天和第 12 天观察到 GAPDH mRNA 表达的 54% 和 71% 敲除,这两者都是高度显著的,如图 17 所示。

[0481] 3. 将人脂肪细胞维持在培养物中 30 天的过程中表型的变化。

[0482] 在处理结束时(总培养 30 天),用油红 O 对细胞进行染色来评估脂质含量(图 18)。在第 16 天至第 30 天仅维持培养基的存在下(对照),脂肪细胞表现出加载有大脂滴。在 100nM 罗格列酮整个实验过程中都存在的情况下(阳性对照),红色染色的强度似乎降低了,并且脂滴看起来更小。例如,在 25nM hsa-miR-30b 模拟物存在的情况下,红色染色似乎

也降低了,并且脂滴看起来更小。在非靶向 miRNA 类似物存在的情况下,未观察到这样的变化。

[0483] 用荧光尼罗红染料测量第 30 天存在于成熟脂肪细胞中的脂质的量。如图 19 所示,在第 15 天至第 30 天不暴露于罗格列酮的脂肪细胞中观察到最高荧光。在用非靶向 miRNA 模拟物和抑制剂转染的细胞中观察到相似的荧光水平。当将细胞暴露于罗格列酮两天时,荧光显著下降,并且在第 15 天至第 30 天存在罗格列酮的情况下,荧光进一步降低。看起来在受试的 miRNA 抑制剂存在的情况下,荧光水平处于用罗格列酮 2 天至贯穿实验所观察到的范围内。在 miRNA 模拟物存在的情况下,荧光水平看起来更低,指示更低的脂质含量。

[0484] 4. 人成熟脂肪细胞转染的优化。

[0485] 已知成熟脂肪细胞有效转染是难以实现的,我们测试了 11 种不同转染试剂,并且评估了对照基因 GAPDH 的 mRNA 表达的降低程度。将人皮下前脂肪细胞平板接种于 6 孔板中,并根据上文描述的方案分化两周。然后,根据其制造商的操作方案使用转染试剂将靶向 GAPDH 的 miRNA 模拟物 (50nM) 引入分化的脂肪细胞。将经转染的细胞用试剂和 miRNA 模拟物孵育 72 小时,然后转移至维持培养基。转染 14 天后,使用 GAPDHRNeasy Mini 试剂盒分离 RNA,并且在使用每孔 100ng cDNA 一式三份对对照基因 GAPDH 和参考基因 18S 进行 RT-PCR 反应。

[0486] 除了在实验条件下可产生潜在细胞毒性的转染试剂 TransIT TKO 和 TransIT siQuest 外,每个孔提取的 RNA 的量非常相似 (图 20)。

[0487] 用 Dharmafect 1 和 siPORT NeoFX 转染的细胞具有显著降低的 18S 表达水平,并且从 RT-PCR 实验分析中排除。在余下的 7 种转染试剂分析中,经常使用的转染试剂 Lipofectamine RNAiMAX 导致在转染后的第 14 天 GAPDH 表达降低 66%, Dharmafect 3 和 Dharmafect 4 分别导致 GAPDH 表达降低 60% 和 75% (图 21)。

[0488] 5. 在 miRNA 类似物或已知的脂肪生成和 / 或产热之激活蛋白的存在下培养两周的人成熟脂肪细胞的表型变化。

[0489] 如上文所述,将人皮下脂肪细胞以每孔 391,000 个细胞的密度平板接种于 6 孔板中。使用 Dharmafect 4,在第 14 天用以下物质转染这些脂肪细胞:

[0490] 1. 以下 miRNA 类似物中的一种 (50nM):

[0491] -hsa-let-7a 抑制剂 (hsa-let-7a 是报道的调节脂肪生成的通用 miRNA)

[0492] -hsa-miR-1 模拟物 (据报道 hsa-miR-1 调节 PRDM16 和 UCP1)

[0493] -hsa-miR-19b 模拟物 (hsa-miR-19b 是丰富的脂肪细胞 miRNA,根据我们的计算机分析,预测其与我们的 83 种靶标中的许多相互作用) 或者

[0494] -hsa-miR-30b 模拟物 (根据我们的计算机分析,预测 hsa-miR-30b 与我们的 83 种靶标中的许多相互作用)

[0495] 2. 阴性对照 (模拟转染)

[0496] 3. 三种阳性对照 (已知改变脂肪生成和 / 或适应性产热的 PPARG 激动剂罗格列酮 (100nM), β 3 肾上腺素能受体激动剂 CL 316,243 (10 μ M) 或甲状腺激素三 - 碘甲状腺原氨酸 (10nM))。

[0497] 在第 17 天,将细胞转移到维持培养基中,然后每 2 至 3 天更换培养基,直至第 28 天,拍摄细胞的明场显微镜图片。

[0498] 如图 22 所示,并对比于对照条件,在阳性对照 CL 316,243 和罗格列酮的存在下,以及在 hsa-let-7a 抑制剂、has-miR-19b 和 hsa-miR-30b 模拟物的存在下,细胞密度增加,并且外观“变褐”。不同试剂对细胞密度、脂质含量、脂滴数量和大小影响总结于表 21 中。

[0499] 表 21.

[0500]

试剂	脂肪细胞覆盖的图像范围 (对照%)	脂滴占的细胞面积 (%)	每个细胞的脂滴数	脂滴的平均大小
对照	参考	42%	68	参考
50 nM hsa-let-7a 抑制剂	+ 147%	56%	103	-13%
10 μ M CL316,243	+ 24%	44%	124	-44%
50 nM hsa-miR-1 模拟物	+ 15%	36%	69	-16%
10 nM T3	+ 13%	42%	94	-27%
50 nM hsa-miR-19b 模拟物	+ 145%	58%	91	+3%
100 nM 罗格列酮	+ 198%	57%	113	-19%
50 nM hsa-miR-30b 模拟物	+ 246%	53%	140	-63%

[0501] 实施例 4. 通过荧光素酶活性和 qRT-PCR 进行的高通量 miRNA 靶标筛选

[0502] 使用荧光素酶报告子测定构建体的高通量筛选用于鉴定产热中涉及的新 miRNA 靶标。

[0503] 荧光素酶通常用作报告子,以评估用包含在目的启动子控制下的荧光素酶基因的基因构建体转染的细胞中的转录活性。SwitchGear Genomics 已经建立了全基因组文库,将超过 18,000 种人启动子和 12,000 种人 3' UTR 区克隆至包含 SwitchGear's RenSP 报告子盒 (GoClone™) 作为 LightSwitch™ 荧光素酶测定系统组分的优化的荧光素酶报告基因载体系统中。该荧光素酶的改进形式极大地促进了详细的动力学研究,特别是那些聚焦于抑制上的研究,否则这样的抑制可能被报告蛋白积累而掩盖。

[0504] 用 SwitchGear Genomic GoClone 系统,使用 UCP1 作为单一的产热靶基因测试了多种微小 RNAs- 一种 mRNA 范例。为了探索多种人 miRNA 与 HeLa 细胞和 HepG2 细胞中的人 UCP1 基因的 3' UTR 区、5' UTR 区和启动子 / 增强子区的可能相互作用,制备了三种报告基因构建体:

[0505] 1. 人 UCP1 3' UTR 构建体,其包含被强组成型启动子 (RPL10-prom) 驱动的报告基因以及克隆于报告基因 3' UTR 区中的人 UCP1 序列 2,218bp 3' UTR 片段。将特异性 miRNA 模拟物、抑制剂或非靶向对照对该报告基因之活性的影响与由空的 3' UTR 和肌动蛋白 β -3' UTR 的那些进行对比,以鉴定对假定的 UCP1 3' UTR 构建体具有特异性的影响。

[0506] 2. 人 UCP1 启动子构建体,其包含由人 UCP1 序列的 4,147bp 5' UTR 片段驱动的报告基因,所述人 UCP1 序列跨越转录起始位点和覆盖人 UCP1 基因序列之甲基化区和增强子区的上游区域。将特异性 miRNA 模拟物、抑制剂或非靶向对照对该报告基因之活性的影响与肌动蛋白 β 启动子的那些进行对比,以鉴定对假定的 UCP1 5' UTR 构建体具有特异性的影响。

[0507] 3. 人 UCP1 增强子区构建体,其包含由来自 HSV-TK 基因座的短的最小启动子驱动的报告基因以及跨越人 UCP1 基因序列之增强子区的人 UCP1 序列的 601bp 5' UTR 片段的。将特异性 miRNA 模拟物、抑制剂或非靶向对照对该报告基因活性的影响与由空的 5' 增强子区的那些进行对比,以鉴定对假定的 UCP1 5' 增强子构建体具有特异性的影响。

[0508] 此外,制备了 miRNAxxx_3' UTR 构建体。它们包含由与克隆于报告基因 3' UTR 区的 miRNAxxx 靶序列完全匹配之强启动子 (RPL10_prom) 驱动的报告基因。将 miRNA 模拟物、抑制剂或非靶向对照对该报告基因活性的影响与 EMPTY_3' UTR 和肌动蛋白 B_3' UTR 进行对比,以确定 miRNA 模拟物或抑制剂的活性是否可以在实验细胞类型中进行被合理地检测到。如果该细胞类型没有目的 miRNA 的内源性表达,则模拟物的添加应敲低了该报告子的活性,并且抑制剂的添加应没有显著效果。如果该细胞类型具有目的 miRNA 的内源性高表达,则添加抑制剂应提高该报告基因的活性,并且添加模拟物没有显著效果。在 HeLa 和 HepG2 细胞类型中,内源 miRNA 的表达是宽范围的,因此,合成靶标活性的变化很可能影响可变性。

[0509] 对于每种 miRNA 候选物 (总共 38 种),测试以下条件:

[0510] -HeLa 细胞中的 miRNA 模拟物 (特异性) * 8 种报告基因构建体

[0511] -HepG2 细胞中的 miRNA 模拟物 (特异性) * 8 种报告基因构建体

[0512] -HeLa 细胞中的 miRNA 模拟物非靶向对照 * 8 种报告基因构建体

[0513] -HepG2 细胞中的 miRNA 模拟物非靶向对照 * 8 种报告基因构建体

[0514] -HeLa 细胞中的 miRNA 抑制剂 (特异性) * 8 种报告基因构建体

[0515] -HepG2 细胞中的 miRNA 抑制剂 (特异性) * 8 种报告基因构建体

[0516] -HeLa 细胞中的 miRNA 抑制剂非靶向对照 * 8 种报告基因构建体

[0517] -HepG2 细胞中的 miRNA 抑制剂非靶向对照 * 8 种报告基因构建体

[0518] 对可以与 UCP1 序列结合的庞大的 miRNA 列表施加 10 种过滤条件 (除了需要与 UCP1 3' UTR 区结合之外的),以减少待测试 miRNA 候选物的数量。这些过滤条件是,结合位点长度、结合位点数目、与 5' UTR 区的结合、具有其他 miRNA 的染色体群、内含子位置、摆动、跨物种表达、与增强子区的结合、与甲基化区的结合以及证明与 UCP1 有关的实验证据。对满足这些标准中至少 3 个的 38 种 miRNA 中进行测试 (表 22)。

[0519] 表 22.

[0520] 在 UCP1 基因序列中具有假定的结合位点的 miRNA:

[0521]

	miRNA	标准的#	结合长度	位点的#	3'UTR	5'UTR	染色体群	内含子	摆动	物种间	增强子区	甲基化区	实验证据
1	hsa-miR-130b-5p	7	11	3	+	+	22				+	+	+
2	hsa-miR-328	6	10	4	+	+					+	+	+
3	hsa-miR-655	6	10	5	+	+	14				+	+	+
4	hsa-miR-19b-2-5p	5	10	4	+	+	X		+			+	+
5	hsa-miR-26a-2-3p	5	10	7	+	+					+	+	+
6	hsa-miR-367-3p	5	10 to 18	3	+	+	4		+	+			+
7	hsa-miR-371a-5p	5	10 to 12	9	+	+	19			+			+
8	hsa-miR-377-3p	5	10 to 14	5	+	+	14			+			+
9	hsa-miR-378a-3p	5	7 to 13	19	+	+		+	+	+			+
10	hsa-miR-382-3p/5p	5	15	2	+	+	14		+	+			+
11	hsa-miR-421	5	10	5	+	+	X						+
12	hsa-miR-515-3p	5	9	3	+	+	19			+			+
13	hsa-miR-620	5	10	7	+	+				+			+
14	hsa-miR-941/2	5	9	5	+	+	20				+		+
15	hsa-miR-1179	4	11	3	+	+	15		+				+
16	hsa-miR-1302	4	10	5	+	+					+		+
17	hsa-miR-146a	4	9 to 10	8	+	+							+
18	hsa-miR-181c	4	9	5	+	+	19						+
19	hsa-miR-203	4	9	1	+	+	14				+		+
20	hsa-miR-331-5p	4	8 to 15	6	+	+	12		+	+			+
21	hsa-miR-422a	4	7 to 14	6	+	+			+	+			+
22	hsa-miR-452	4	8	7	+	+	X						+
23	hsa-miR-491-5p	4	10	3	+	+							+
24	hsa-miR-501-3p	4	10	2	+	+	X						+
25	hsa-miR-543	4	10 to 14	4	+	+	14		+	+			+
26	hsa-miR-545	4	11	2	+	+	X						+
27	hsa-miR-549	4	13 to 14	3	+	+				+			+

[0522]

	28	29	30	31	32	33	34	35	36	37	38
hsa-miR-643	4	4	4	3	3	3	3	3	3	3	3
hsa-miR-651	4	4	4	3	3	3	3	3	3	3	3
hsa-miR-654-3p	4	4	4	3	3	3	3	3	3	3	3
hsa-miR-21-5p	4	4	4	3	3	3	3	3	3	3	3
hsa-miR-211-5p	4	4	4	3	3	3	3	3	3	3	3
hsa-miR-22-3p	4	4	4	3	3	3	3	3	3	3	3
hsa-miR-30b-5p	4	4	4	3	3	3	3	3	3	3	3
hsa-miR-325	4	4	4	3	3	3	3	3	3	3	3
hsa-miR-362-5p	4	4	4	3	3	3	3	3	3	3	3
hsa-miR-504	4	4	4	3	3	3	3	3	3	3	3
hsa-miR-552	4	4	4	3	3	3	3	3	3	3	3

[0523] 在这些荧光素酶报告基因测定实验中,如果满足特定 miRNA 抑制剂使荧光素酶信号升高以及特定 miRNA 模拟物使荧光素酶信号降低二者,并且抑制剂 / 模拟物比率 ≥ 1.5

和或 /p 值 < 0.05, 则 miRNA 候选物视为与 UCP1 相互作用。这些选择标准鉴定出 9 种 miRNA (hsa-miR-19b-2-5p、hsa-miR-21-5p、hsa-miR-130b-5p、hsa-miR-211、hsa-miR-325、hsa-miR-382-3p/5p、hsa-miR-543、hsa-miR-515-3p 和 hsa-miR-545) (表 23)。还有一些勉强符合这些选择标准; 它们是 hsa-miR-331-5p、hsa-miR-552、hsa-miR-620 和 hsa-miR-1179。

[0524] 表 23.

[0525] 通过 HeLa 和 / 或 HepG2 细胞中的荧光素酶测定鉴定为 UCP1 基因表达调节子的 miRNA :

[0526]

细胞系	miRNA
HeLa	hsa-miR-130b-5p
HeLa+HepG2	hsa-miR-19b-2-5p
HepG2	hsa-miR-382-3p/5p
HeLa	hsa-miR-515-3p
HeLa	hsa-miR-543
HepG2	hsa-miR-545
HeLa+HepG2	hsa-miR-21-5p
HeLa	hsa-miR-211-5p
HeLa+HepG2	hsa-miR-325

[0527] 这 9 种选择的 miRNA 中, 3 种呈现与被研究的 UCP1 的 3 个区域结合 (hsa-miR-21-5p、hsa-miR-211 和 hsa-miR-515-3p); 3 种呈现与 UCP1 的 2 个区域结合 (hsa-miR-19b-2-5p、hsa-miR-130b-5p 和 hsa-miR-325), 并且 3 种与 UCP1 的单个区域结合 (hsa-miR-331-5p、hsa-miR-543 和 hsa-miR-545)。除 hsa-miR-331-5p 以外的所有 miRNA 都显示与 UCP1 的 3' UTR 区结合 (表 24)。

[0528] 表 24.

[0529] 通过荧光素酶测定鉴定为 UCP1 基因表达调节子的 miRNA :

[0530]

	miRNA	UCP1 3' UTR	UCP1 增强子	UCP1 启动子
1	hsa-miR-21-5p	X	X	X
2	hsa-miR-211	X	X	X

3	hsa-miR-515-3p	X	X	X
4	hsa-miR-19b-2-5p	X		X
5	hsa-miR-130b-5p	X	X	
6	hsa-miR-325	X	X	
7	hsa-miR-331-5p		X	
8	hsa-miR-543	X		
9	hsa-miR-545	X		

[0531] 通过以下来进行进一步筛选：将启动子 /3' UTR 文库转染至细胞培养物中的人脂肪细胞或脂肪来源的间充质干细胞中，然后向细胞培养物中添加 miRNA 试剂（例如 agomir 或 antagomir）。在转染并添加了 miRNA 试剂 24 小时后进行荧光素酶活性测量和 mRNA 鉴定。

[0532] 为了在更长的时间框架中验证上文记载的转染实验结果，使用包含目的 miRNA 试剂（来自在允许 copGFP 荧光标记表达的 pMIRNA1SBI 载体中表达的 miRNA 前体的 System Biosciences (SBI) 收集物）的慢病毒载体进行慢病毒转导实验。具体地，根据供应商的使用说明，用慢病毒颗粒以 1 : 10 的 MOI 转导包含启动子 /3' UTR 文库的细胞，并且通过 FACS 分选 GFP 阳性细胞。在几个时间点（0 小时、3 小时和 6 小时；1 天、4 天和 7 天）通过 Taqman 实时定量 PCR 评估对照细胞（HEK293 细胞）、人脂肪来源的间充质干细胞、人皮下前脂肪细胞和人增殖皮下脂肪细胞中成熟的 miRNA 以及它们靶向的 mRNA 的表达水平。汇集来自转导后 5 个不同时间点的 RNA，任选地用于降低基于 qRT-PCR 的筛选方法的复杂性，同时保持检测灵敏性。

[0533] 实施例 5. 蛋白质组学分析

[0534] 还使用蛋白质组学分析来鉴定产热涉及的新的 miRNA 靶标。

[0535] 鸟枪法蛋白质组学是使用高效液相色谱 (HPLC) 联合质谱 (MS) 在复杂混合物中鉴定蛋白质的方法。收获经 miRNA 试剂和启动子 /3' UTR 文库转染和转导的细胞（如实施例 4 中所描述），并使其裂解以产生粗制的可溶（胞质）级分和不溶（核）级分。然后通过 HPLC 从这些级分中分离肽，并使用纳电喷雾-离子化串联 MS 使用同位素标记技术 SILAC 进行分析，以对蛋白质丰度进行定量。针对 Ensembl 公开的 (release) 54 种人蛋白质编码序列数据库，使用 Sequest (Bioworks 版本 3.3.1, Thermo Scientific) 来检索谱。

[0536] 为了避免错过低丰度蛋白质，还使用了靶向蛋白质组学方法以对作为已知的脂肪生成、脂肪细胞分化和 BAT 功能之调节子的一系列蛋白质进行精确定量。一些实例包括 UCP1、KDM3A、PRDM16、PPARA、PPARGC1A、CEBPB、CIDEA、BMP7、COX7A1、SIRT1、SIRT3、DI02、FABP4 和 ADIPOQ。经基于 ELISA 或基于 Luminex 的免疫测定使用市售抗体来分析这些蛋白质。

[0537] 任选地，在蛋白质组学平台上使用多反应监测-质谱来分析蛋白质级分，借此使

用 LC-MS-MS 精确定量产热通路的仅一种蛋白质（例如 UCP1）。

[0538] 实施例 6. 特异性靶向人脂肪细胞的克隆 DNA 适配体的开发和表征

[0539] 我们使用了 Cell-SELEX 技术来开发和表征特异性识别成熟的人皮下脂肪细胞的 DNA 适配体。使用 Cell-SELEX, 通过重复扩增和与活细胞结合来选择识别完整细胞表面上的天然环境中处于其天然构象的特定分子的适配体。在图 23 描述的基于细胞的选择中, 可在其天然环境中直接靶向特定的已知或未知的细胞表面标志物或膜受体, 从而允许对细胞特异性适配体的直接富集。Cell-SELEX 由靶细胞的正选择和非靶标细胞的负选择的组合组成。在本发明的情况下, 用新鲜分离的人肝细胞进行负选择, 用人皮下脂肪细胞的初级培养物来进行正选择。完成了从 32 聚体文库中的两轮负选择和五轮正选择。对分离的适配体进行测序、合成并用 6- 荧光素酰胺 (FAM) 标记, 用于结合研究。用饱和浓度 (1 μ M) 的 FAM 缀合的适配体在室温标记人肝细胞 (阴性细胞) 和脂肪细胞 (阳性细胞) 15 分钟, 并通过荧光活化细胞分选 (FACS) 来分析。如图 24 所示, 一些适配体 (例如, 适配体 974) 既不与脂肪细胞结合也不与肝细胞结合, 一些适配体 (例如, 适配体 975 与脂肪细胞和肝细胞二者都结合, 比率: 2.69), 并且另一些适配体优选与脂肪细胞结合 (例如 aptamers 972 和 973, 比率分别为: 4.76 和 5.40)。这些脂肪细胞特异性的适配体的进一步表征还在进行中。

[0540] 实施例 7. 表型、基因型和蛋白质组学数据集的统一 (reconciliation)

[0541] 在此处将实施例 3 至 5 记载的体外实验结果进行统一。具体地, 为了将微小 RNA、mRNA 和靶蛋白质以及通路的初始集合进一步精选为相关的但是可管理的靶标数目, 用网络检索和分析压缩包 (DAVID, Ingenuity Systems IPA 和 ARIADNE Pathway Studio) 整合实验数据。

[0542] 本文中, 用 Business Intelligence 工具 TIBCO Spotfire 来对实施例 3 至 5 中记载的体外实验结果进行全局性分析。这使得能够使 miRNA 试剂和靶基因之间的关系可视化。

[0543] 实施例 8. 肥胖动物模型

[0544] 已经开发并验证了数种肥胖动物模型 (Kanasaki K 等, J. Biomed. Biotechnol., 2011:197636(2011); Speakman J 等, Obesity reviews: an official journal of the International Association for the Study of Obesity, 8 Suppl 1:55-61(2007))。最常用的是瘦素信号缺陷的 Lep^{ob/ob} 小鼠模型和 Lepr^{db/db} 小鼠模型, 以及 C57BL/6J 小鼠中的高脂肪膳食模型 (Wang CY 等, Methods in molecular biology, 821:421-433(2012))。这种膳食诱导的肥胖 (diet-induced obesity, DIO) 模式近似地模仿了现代社会中的高脂肪 / 高密度食物的提高的可获得性。

[0545] DIO 小鼠模型用于体内验证本文描述的 miRNA 类似物在提高产热和 / 或治疗肥胖和另一些代谢疾病的有效性 (Yin H 等, Cell Metab., 17(2):210-224(2013))。

[0546] 向 DIO 小鼠施用以下的一种或更多种: hsa-let-7a agomir、hsa-let-7a antagomir、hsa-miR-140 agomir、hsa-miR-140 antagomir、hsa-miR-19b agomir、hsa-miR-19b antagomir、hsa-miR-30b agomir 和 hsa-miR-30b antagomir 中。使用罗格列酮作为阳性对照。在处理前和处理后测量小鼠的食物摄取、血液代谢参数、身体组成 (体重、体脂肪、矿物质和瘦体重 (lean mass)、体脂分布、体温、O₂ 消耗和 CO₂ 产生、运动诱导的产热、冷诱导的产热和静息产热。体重或体脂肪的降低或者体温升高或者任何类型的产热都指示所使用

组合物的体内有效性。

[0547] 实施例 9. 人 UCP1 和 UCP2 基因的核酸序列和转录物

[0548] 表 25.

[0549] 人 UCP1 基因的 1,462 碱基对 (bp) 转录物 ENST00000262999 的核酸序列 (六个外显子以大写字母示出) :

[0550]

编号	外显子/内含子	起始	终止	长度	序列
	5' 上游序列				gtcgggtcaaaaaacagaaatcgggttgctg cccgccggacagcgctga
1	ENSE00001081761	141,489,959	141,489,758	202	AGAGCAAGGGAAAGGAACCTCC TCCACCTTCGGGGCTGGAGCCCT TTTCCTCTGCATCTCCAGTCTCTG AGTGAAGATGGGGGGCCTGACA GCCTCGGACGTACACCCGACCCT GGGGGTCCAGCTCTTCTCAGCTG GAATAGCGGCGTGCTTGGCGGAC GTGATCACCTTCCCGCTGGACAC GGCCAAAGTCCGGCTCCAG
	内含子1-2	141,489,757	141,489,132	626	gtagctaggcagaggggtaagacaa.....tggtct gcaccttcttattccag
2	ENSE00001009006	141,489,131	141,488,933	199	GTCCAAGGTGAATGCCCGACGTC CAGTGTTATTAGGTATAAAGGTG TCCTGGGAACAATCACCGCTGTG GTAAAAACAGAAGGGCGGATGA AACTCTACAGCGGGCTGCCTGCG GGGCTTCAGCGGCAAATCAGCTC CGCCTCTCTCAGGATCGGCCTCT ACGACACGGTCCAGGAGTTCCTC ACCGCAGGGAAAGAAA
	内含子2-3	141,488,932	141,484,673	4,260	gtaagccgtgagcgttctctggagg.....aataat ttttttctctctggatag
3	ENSE00001081759	141,484,672	141,484,472	201	CAGCACCTAGTTTAGGAAGCAAG ATTTTAGCTGGTCTAACGACTGG AGGAGTGGCAGTATTCATTGGGC AACCCACAGAGGTCTGTGAAAGTC AGACTTCAAGCACAGAGCCATCT CCACGGAATCAAACCTCGCTACA CGGGGACTTATAATGCGTACAGA ATAATAGCAACAACCGAAGGCTT GACGGGTCTTTGGAAAG
	内含子3-4	141,484,471	141,484,366	106	gtaactaacticaaaatgggttta.....acattttctt

[0551]

					tttttttccccag
4	ENSE00001081762	141,484,365	141,484,264	102	GGACTACTCCCAATCTGATGAGA AGTGTCATCATCAATTGTACAGA GCTAGTAACATATGATCTAATGA AGGAGGCCTTTGTGAAAAACAAC ATATTAGCAG
	内含子4-5	141,484,263	141,483,528	736	gtaacttcccatttcataaaca.....gacctgttt catcgatccatttag
5	ENSE00001081763	141,483,527	141,483,347	181	ATGACGTCCCCTGCCACTTGGTG TCGGCTCTTATCGCTGGATTTTGC GCAACAGCTATGTCCTCCCCGGT GGATGTAGTAAAAACCAGATTTA TTAATTCTCCACCAGGACAGTAC AAAAGTGTGCCCAACTGTGCAAT GAAAGTGTTCACATAACGAAGGAC CAACGGCTTCTTCAAGGG
	内含子5-6	141,483,346	141,481,165	2,182	gtaagatatgatcttgtgtatctgt.....cgaacgat gacatgcacttttctag
6	ENSE00001081760	141,481,164	141,480,588	577	GTTGGTACCTTCCTTCTTGCGACT TGGATCCTGGAACGTCATTATGT TTGTGTGCTTTGAACAACGTGAAA CGAGAACTGTCAAAGTCAAGGC AGACTATGGACTGTGCCACATAA TCAGCTTCAAGAAAATGATGTAA CATACCAGTGGGAATCTTGCTGA CTGGATCATAAAAAACAACAAA ACTTATTCACCTTATTTTAACCTAA AAAGATAAAGGAATTTTGGCAG AGAATTTTGGACTTTTATATATAA AAAAGAGGAAAATTAATGCCTAT TTCATATAACTTTTTTTTTTCTC AGTGTCTTAAGAAGGGGAAAGC AAAACATTGAGCATATACCCTGG CAAATGTAATGCAGATAAGCTAC TGCATTTGACCATTTCTGGAGTG CAATTGTGTGAATGAATGTGAAG AACTTTAACATGTTTAATTACA ATTCCAACCTGGTGGAAAAGAAAC TGAGTGAAATGCAGTTTATATTT ATAAATACTTAAAAATGAAGTTA TTAAAAATATTAGTTTATTATTAAC CACAGTTGTCAGTTAATATATTC AATAAAGTATTGCTAATACCTTT T
	3' 下游序列				aaagtttgtcttttgagatctatacctgggtgtaagagtc aagttcacta.....

[0552] 表 26.

[0553] 人 UCP1 基因 (ENSG00000109424) 的 9,371 碱基对 (bp) 核酸序列 (外显子以黑体字示出) :

[0554] >染色体:GRCh37:4:141479988:141490559:-1

[0555] AGAGAAGGCCGCAAGGTGCCTGCAAGATGTCTGGGGAGTTGGAGGAATGGAAGAGTGCCCCGCTCTT
CCTTCTGGGAGAGCTCCAGCTAGGCAGAACCTTTACCAAGGCTCTGATATCGTGCTGGTTTCCGAAAGCCCCAG
CCGAAGGTGTGCAGCCAAAGGGTGACAGAAGGTGAGGCACGTGCGGGGGCGGGGTGCTGACCGCCGCGGTGCGC
CCTCCCTCCGACGTGCGTGTGCGGGGCGCAGACAACCAGCGGCCGCCAGGGCTTTCGGGGAGCGAAGCAGGGC

TCCCGAGGCACCGAGCGAGAATGGGAATGGGAGGGACCCGGTGCTCCCGGACACGCCCCGGCAGGTCCCACGCC
CGGGTCTTCTGAGACCTCGCGCGGCCAGCCCCGGGAGCGGCCAGCTATATAAGTCCCAGCGGAAGACCGGAACG
CAGAGGGTCTGCTGGCGCGAGGGTGGGTAGGAGGGGACGCGGGGACTCGGCCCCAACACCGCGCTCCGTCTGC
AGCCGCCGCCTCTGCACCGCCGCTGCCCCGGCGGTTCGGTTCAAAAAACAGAAATCGGGTTGCTGCCCCGGCGGACA
GGCGTGA **AGAGCAAGGGAAAGGA**

ACTTCCTCCACCTTCGGGGCTGGAGCCCTTTTCCTCTGCATCTCCAGTCTCTGA
GTGAAGATGGGGGGCCTGACAGCCTCGGACGTACACCCGACCCTGGGGGTCC
AGCTCTTCTCAGCTGGAATAGCGGGCGTGCTTGGCGGACGTGATCACCTTCCCG
CTGGACACGGCCAAAGTCCGGCTCCAG GTAGCTAGGCAGAGGGGTAAGACAAGGGGTCT
TCAGGACAGAGGGGACGCTGTTGCGTGCATTCCATTTATTCTCTGCTTTGGTGTAACTACTGTTTCTAGGT
AGGGTAGGTGACCTTCCAAAGCAGTCTGGCCTTGTCCCAGGGCTGGTGCTTTAGGATGGGAACTGGA
TTTTCTGGGATTAGCTGAAGAACCACCGGGCCACAGAGAATGGGTTGACCATGACTACTACCAAATTCTC
CCAAAATTTAGGGTGCACCTTAGTATTTAAGAGCTGAGAATATTGGCCTCTCCTGAGTTTACTAGTCAGGT
GCTTTTTCTTTCTTTGATTCTTCGGGGGTCTGTCTATCCTACTGCCCTAGGGGTCTGGAGAGTTCCT
GGGGAGGGGATATTCAAATGTGCATTGTAGCCAGCCTCCCTCCATCTGCGCGTGAGCGAACACACACAC
ACACACACACACACACACACACACACACACACACAGGTAGAGGGAGGTGGATGGAAGAGGAATGTTGC
TGAGAAAAGAAACGGAAAATAGGAACACAGGGGAAATCTTGGCTTAAGAGTGAATCAATTTGCTCCC-
TTCTGTTCTGCACCTTTCTTATTTCCAG **GTCCAAGGTGAATGCCCGACGTCCA**

GTGTTATTAGGTATAAAGGTGTCTGGGAACAATCACCGCTGTGGTAAAAACA
GAAGGGCGGATGAACTCTACAGCGGGCTGCCTGCGGGGCTTCAGCGGCAAT
CAGCTCCGCCTCTCTCAGGATCGGCCTCTACGACACGGTCCAGGAGTTCTCA
CCGCAGGGAAAGAAA GTAAGCCGTGAGCGTTCCTGGGAGGGGAGAAAAGCCTTGGGCTCCGCTCTGTT
CCAAAAAGTGTAACACACAGAGGAGTGGTTTTTCATAACAAATTGGCGAGAAAACATTCATATTTGAACTCTCCCTT
CCCCAACATTAGCTCATTGTTCATAGAAAAAGTATGCAAAATCGATTTTTTAGATGCAGATATATACTTGTA
GGTCACCCAGTCATGGAAGTTTGTGCCAGTTTGGATCTCCATCTGGAGAATATGGGTGGGCTACAGAAAAATGT
TTAACTTAAAGTTCTCCAAAGAGGGAAGTATATCAGAAACATCTATGGAGCTTGTGAGAAATCCAAACGAGGACTA
CCATGGTCTCTGAGTCTGAATCCTCAGGCTAGAGACCAGAGTGTCTTTCCACAAGCTTCCCTCATCTTTGTGTA
TGCAACAAAGTTCAAAGCCTTCTGTTTGAAGCAAAGAAAGCCAGACTTTGTGAAGAGAGTTGAAAGGACAGGAAAA
GACATATTTCTCTTAAGAGGTTCCCTCATCAGGTCCAGGAAAGACCAGAGCAGAAAAAGTGGACGAATGCTGCAGG
GAGTTTGTGTTAGGGGAAAAAGAAAGGAAACATATTTCTGAGTGCCAGTGCACTCTAAGAATTCCTGTCACTTTA
GGTAGCATTTATTTGAGGGCTTAACTATGAACCAGACATTGTTCTAAGTGCTTCAGATACATTATAACTGGAAGGG
TATTAGTACCATTATCCCTTGGCAGATGGGAAAAGTGAACACAGAGCAGATTCATCACTTGCCCAAGGTACACAG
CTGGGAGGGGGCAGAGCCAGGGTTCAAACCCAGGCAGTCTGGCCTCGGACTCCAGGCTCCTAACCTGTTCTCTAC
TGCCTTCTGCACTTCTCATATGATTCTGCCCATCATTCAAACCGCACAACACTGCTGTGAGTAAAAAGTGTTAGCC
GAATATCAGGGTAGTTAAGTAACATGCACAAAATCACACAGCTAATCAACATCAGAGGCACTTTCATGTGGAGTAG
ACAAGCCAGAGAGAAGATGTGCTGATGGCACAATGAATACATTAAGTGAAATCCACCTTGATAGATTTTCATCTTTT
TGCTGTGAGTAACCTTCAATACTATAATTTTATGGGATAATTTATAAATGTTGTCTATACAAATATATAAGTTATA
CTTATCCACACAAGTACTTTCAAAGTGAAGATAAAGTCTGGATGTTACTAGATCAAACTGCATTTTTTTATTTAT
AGATGTAGCAAGAGAGGAAACACAAAGGAGGTAAAGCTGCCGTTTCAGGTGGTTTTCTTCACAGATTGACTGTTCT

ACCAATTGTTGTGGACTTTGGGCACCAAAATTAATAGGATATATGTTGGCAGTGTTCTATGTTATATAGATTTCAGTTT
ATTTAGTAGGCTTTATTGAACTGCCATGTGCCAGTAACTATGTTAGATGTTTAGATGGCAGATGTGTCTCTAGACAG
AGCTTACAGTTGAGAGTATGGGTTGTGTGGGGAGAAGTGAATAGATGACTATATCCATGATACATGCTGTATTACA
ATACAGTCCTACTTCACTTAACGATGGGGATACATTCTCAGAAATGAGTTAGGAGGCAAATTGGTTGTTGAATGAAC
ATCACAGAGAGCACTTACACAAACCTAGATGGCATAGCCACACCTAGGCTATATGGTATAATCTATTGCTCCTAGGC
TACAAACCTGTGCAGCATGTTGGTATTGAATACTACAGGCAATTGTTACATAAAGTTAAGTGTGTTGTGTACCTAAAA
ATAGAAAAGGTAATGCATTACACTACAGTCTTATGGGGCTGGGATGTCAGTGGTGATAGGAATTTTTCAGCTCTGT
TCTAATCTTACGGGACCACCATCATGTATGCAGCACATGACTAACTGTAATTACAAGATGGTGGCTATATTAACAG
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CCAGATGACAGAAGGCCTTGCAGGCGGTGCTAAGGAGTTTGGATTTTATCCTTACAGTGGTGGGAAGTCATTGTAAA
AATATTAAGCAAGGGAGTGGCATAAAACAATTTACATTTTCAAAAGATCACTTTGGCAGCAGATAGAGTATATATGTA
AAAGGAGTAAGAAAGAGGTAAGTTAGAAAAGCAAGAAATGATCAGGGTATGCCCTAAACACTGGCAATAGGGAAAAA
GAGATGTCAATCAGAAAGATTGAGAAAAGTATAATTGAATTGACTTGGTGAACAAATAGAAGTAAGGCATAAGGGACA
GGTAGAAATATGAGATGACTTCCAAGTTTCTGTTTAAAGATACCCTTTATTGAGAGAGGATGTATAGAAGCTGTCTT
AGGGGGAAGACAAGAAATTTGGTTTAGGCCATGTCAACAGGTAATGGCCAGTAGGCACATGATTCAGTTTATTTAGT
GGGCTCCTTTTAGGAGAAAAATCTGAGCCAGATTCCAGGAAGTCACAGCAGGGACTACCAATAGGGTCAAACAGCAGA
GAGTGTGGAAGGACTGAAAAGTGATCATTGTACATAACAAATAGAAGCTCACTGATTTTCTAGCAAAAACATCTTC
AGCAGAGTAGCGTGGTATAAGCTATATTGTAGGGGACTGAGGAAGAAATGGGCTCTGAGAAGTAAAGACAAACAATA
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AGCTAAAAGTGAAGGCATTTGTAATCATATGATATTGCTAATTAAAAGACAGCTGTCAGTCATATGCCAGCTCCTG
GTAAAGCATGATGAGAAGAGTACAATCATGGTAGTGATTTAAAAATTGCTGCCAGTTTTGTGGATTTTCTTTATGCT
AGACAGTGTAAGCTCTTTATCAATATTATTTAACTCACACAACCTCTAAGAGGTAGATATTATTATCCCTTTTTGAC
AAATTAGGAAACAGAATTATAATGACTGAGAAAGTCTCTGCTGAGTAAATGTTACTGAACCTTAATTTTATGTTTA
CTTAATGATAGAAATGAATATTGGGCTTCAAGACTATTTGTACTTAATGAAATCTGTCTTGAGCAACATAAGCTAT
TTTTTTCAAAATTTTAAGACAAAAATCACTTTCTCTCTCCTGTCTTCTTATTTTGTTCCTTCACATGTTGTAG
CCTAACACTACTTGATGGCCCATTTTGGTGCAGTTTGTCCACTGGGCTTCATCTAAGGCCACCAAGTCCCATAATT
AACATGATCATTGCTGGGAGAAAGATCAAGCCTCATTGGTGATGGGTGCCTCCTCACAGTCGGATAATACTGAAAA
GAGAGCTAAATGTGGGAAAGAACCAAGTTGAACACAGGAAAGAATCAGGCCACTGTGAAAATAAGCATTGTGTTTT
CTTGTTCCCTGAAAGTCTTCATTTTTAAAAAATTTTCAAGACACCTGAAGTTTTCTAGCCTTACTCTGAGTTGACGCA
CATTTAGTACATGATCAACACATAAACAAGCATTAGAGAAATAGAAAAGCTGTAAGAATACAAAAATATGGGCCAG
GTGGGTGGCTCATACCTGTAATCCTAGCACTTTGGGAGGCCGAGGCAGACGGATCACCTGAGGTCAGGAGTTCAAG
ACTAGCCTGGCCAATATAGTGAAACCCTGTCTCTACTAAAAATACAAAACTTAGCAGGCTGTGGTGGCACGTGCCT
ATAATCCCAGCTACTTGGGAGGCTGAGGCAGGAGAATCTCTTGAACCCGGGAGGCGGAGATTGCAGTGAGCCAAGA
TCACACCACTGCACTCTAGCCTAGATAACAGAGCAAGACTCCATCTCAAAAAAAAAAAAAATACAAAAATATGAAC
CACTGAAAATTA AAAAGACATGCATGCATTCTAGGTCTTTAATTTTTTTTCTTAATAATTTTTTTTCTCTCTGGAT
AG **CAGCACCTAGTTTAGGAAGCAAGATTTTAGCTG**

GTCTAACGACTGGAGGAGTGGCAGTATTCATTGGGCAACCCACAGAGGTCTGTG
AAAGTCAGACTTCAAGCACAGAGCCATCTCCACGGAATCAAACCTCGCTACAC
GGGGACTTATAATGCGTACAGAATAATAGCAACAACCGAAGGCTTGACGGGTC
TTTGGAAG GTAACCTAACTTCAAAATGGGTTTTATAACCACCAAAGCACATACATACTAGCAACTTATTG
TAAAGTAGAGTTAATAAACATTTTCTTTTTTTTTTTTCCCCAG
GGACTACTCCCAATCTGATGAGAAGTGTTCATCATCAATTGTACAGAGCTAGTA
ACATATGATCTAATGAAGGAGGCCCTTTGTGAAAAACAACATATTAGCAG GTAAC
TCCCATTTTATATAACAAACAGGTGCGACCTTTAGAAAGTTCATCTTGGAGCTTCTGCAGCCACCTTATACTCAATCT
CTTAACCTCAATAGTTTTCTTTTTTAAAAATTAAGTAATTTTGAACCATATATAACTTTGTGAGAAGCAGGAAAAG
ACAAAAATATTAAGTTTAAGAAGTTTTGCCACAACAAAAATATTTTGCAACAAAAATAACAGGCAATTTTATGTCAG
CATTATTCTCATTTAATACTAATATATGGGACTTTGTAGAACTTATTCTTTATACAGCAGAATTCAGGAGGTAA
GTCCATCCTGCATACTATATCCAAAAGATCTAGTTATAAAAGGAGCTTATCAGTGGTCTCATCCAAAAGTAATACC
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TAACAATCTATTAAGCCATTTACTCTTTACCTTCTTTTTCTAGTGCAATACCTTTCTTATTTTATTTTATTTATTTA
TAAGACATCTTCATTGACCTACTGTTATCAATAGGTTTATAAAGATATGACAGATACTAAATTGCAAGCCCCCAA
AGTCTGATGTTGACCTGTTTCATCGATCCATTTTAG **ATGACGTCCCCTGCCA**
CTTGGTGTCGGCTCTTATCGCTGGATTTTGCGCAACAGCTATGTCCTCCCCGGT
GGATGTAGTAAAAACCAGATTTATTAATTCTCCACCAGGACAGTACAAAAGTGT
GCCCAACTGTGCAATGAAAGTGTTCACTAACGAAGGACCAACGGCTTTCTTCAA
GGG GTAAGATATGATCTTGTGTATCTGTAATGTGTTCTGGCTGTCTGTGTGCTTTGGGACACTCTCATGTCAA
GCAACCGACATTTAGCTTACAAGCCTTAGTATATTCATATACTTAGTATTGACTTTTCCCTTGCCACAGATTTCTCC
AATCCACCAATTCCTGTGCCAGAAAGTAAAAAGCCATGATATTCAAATTTTCTCAACTTTGATCAAAGGCTCATT
CAAGACCAGTGCTTTTCCACTGGTCCCAATCTACTGGAAATGCAGACAGTATTTTGCCTTCTCTGGGCAAGAAAGT
TATAAAGTAGAGGGAAATCATAATAGAGAGCTATGAGAGAAACAAGATTTGATTTGATTTAATTTGATGGACTCAAGT
TTTAACATTGTAAACTAGAGATAAGACATCACCACCAATCTAGAAAAGTGATGCAGAAAAGTATTTGATTTGGGTA
ATTATTACACTCACCTAGAAACAAGTGTGTGTAAAGATTACATATTTCCATAATGCAATGTTGTATCAGAACTA
CCTTCCTAAGAAAATATAGTATGGGCTCGGCTGGTGGCTCGCACCTGTAATCCAGCACTTTGGGAGATGGAGGCA
GGAGGATCACTTGAGCCCAGACTGGGCAACAAAGCGAGACCCTGTCTCAACAAAAAATTTAAAAATTAGCTGAGTGT
GGTGGCACGCACTGATGGTCCCTCTACTTGGGAAGCTGAGGCAAGAGGATCTCCTGAGCCCAGGAGTTCAAGGTTT
CAGCGAGCTATGATTGTGCCACTGCACTCCAGCCTGGGAGACAGAGCAAGTCCCTGTCTCAAAAAAGAAGAAGGAGA
AGGAGGAGAAAAATACAGTATTAAGTAATCTGTCAATATATTCCACAAGGATTACACTAGTGGTTTAATAATAAAATT
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AAAGTTCCAACCTCATATAATCTGTACTTATGATACATTAATACAAATGAAGTTCATTATAAAATTAACCTAAAAATG
GATATACCAGTTATTAACCATTAAACATTTAATAATTTTATTTTTTTCAAATTTAAAAACCTTTTGGGGAAGAAAT
ACTACAACATGGATGAACCTTGAAAACGTTATGCTAAGTGAATAAGCCAGACACAAAAGGACAAATACTGTATGAT
TACACTTAAATGAGGTACCTAGAGTAGTCAAATTCATAGAGACAGAAAGAATAGAAGTTACCAGGGGCTGGAGGTAG
GAAAAAATGGAGAGCTGTTAATGGGTAGAGAGTTTCTTTTTGGGGTGACAAAAGGTTCTAGAGATGGATAGTGGT
GATGGTTACACACAATGTGTGTGTACTTAATGCTACTGAAATGTAATTTTATGATTTTTTTTTTTTTTGCAGCAAAATA

CCCCACATTGGGAAGTGAAGAGAAACATGTTAAGAGACTTGAAGGAAAAAATTGGGGCAGAGGGGTGTTTTTTATA
GGTTAAACAATAAAAGCCATTTAAACAGTAACAATTTCTCTAAGGACAAGAATCGTCAAGATTGAGACAGCACTGAT
TTCTTGACTCTACTCAATACTTCTTTGGTTTCTCTTCTTCCCTCCCCCTTCTAATAGTTTCCTACCTCCCATTGAGA
AAGCAAAGCAAAACAAGCAAAAATTTCCCCCTTCCCTCAAAAAAGGAAAGAGTTTTTGAAAAAGTTCATGTCAGTGAA
GAAAAGACATGTTTTGGGAGTGAAGGATATTTGTGGATTGTATAGATGTGATCATCAGGGCTGTGTTGTTTTGAAG
TAATATAGGACATCTAGAGGAAAAATTTATTTTCAGCAGAGGAGGGAAAGATGAAGAGTAGGTACTTTTAAGCATCTT
CACTTGAGGAGTGGCAAAATGAGAAGCATAACCTGCTATAATCACTTTAAGAATTTTCAGGCTGAGTGTGGTGGTGCA
GTCTCTAGTCCCAGTTACTCCAGGAGGCTCAGGTGGGAGGATCACTTAAGCCCAGGAGCTCGAGGTTGCAGTGAGCT
ATGATTACACTACTGCATTCCAGCCTGGGCGGCAGGGTGAAGCCTCATCTCAAAAATTAAAAAAAAAAAAAATCAAA
CAAATTAATCGAACGATGACATGCACCTTTCTAG **GTTGGTACCTTCCTTCTTGCGACTTGGAT**
CCTGGAACGTCATTATGTTTTGTGTGCTTTGAACAACCTGAAACGAGAACTGTCAA
AGTCAAGGCAGACTATGGACTGTGCCACATAATCAGCTTCAAGAAAATGATGT
AACATACCAGTGGGAATCTTGCTGACTGGATCATAAAAAACAAACAAAACCTTATT
CACTTATTTTAACCTAAAAAGATAAAGGAATTTTGGCAGAGAATTTTGGACTTT
TTTATATAAAAAAGAGGAAAATTAATGCCTATTTTCATATAACTTTTTTTTTTTCT
CAGTGTCTTAAGAAGGGGAAAGCAAAACATTCAGCATATAACCCTGGCAAATGT
AATGCAGATAAGCTACTGCATTGACCATTCTGGAGTGAATTTGTTGTAATGA
ATGTGAAGAACTTTAACATGTTTTAATTACAATTCCAACTGGTGGAAAAGAAAC
TGAGTGAAATGCAGTTTATATTTATAAATACTTAAAAATGAAGTTATTAATAAT
ATTAGTTTTTATTAACCACAGTTGTCAGTTAATATATTCAATAAAGTATTGCTAA
TACCTTTT AAAGTTTGTCTTTTGAGATCTATACCTGGGTGTAAGAGTCAAGTTCACTAGAATACAAGACTGCC
CAATAGCAAATGCAGGTCTTTAGAATCATAGGCATGAACCTACTCTGAATGTTATTAGTATAGATTTTTTAATGTTTA
GAGTCCAGATTTGATGACATCTCTAACAACCTTCTAATCTAAGACACTATATTCATTTTGGCAGGATTGCTACTAGAG
TCTTGGTATCTGTGCTAGCATCACATAATTTTAGAGCTGGAGGGTACTTCTGGGAAGACAGAGGAACAGTTTGAGAT
TCCTACTGAGATGAAAACGAATCTTCATGGAATCTTTCAGCAAAGCCAAATTCAAATTCATCATTAGCACCTGTAGT
AACCTTTTCAATGCCTACAACTGCATGCAGAAGAGATAGGGAAACAGTAAAACAGATATTAAGAAGTTTTTAAG
ACAAAGCCCAGCCTGATTTTAAGCTAAATCCAAGGATTGGCAGCTTGGATGAGCAGGAAGGTTACAGGCTGCCAGAC
ATCATTCTAGTTCTGTTTTAATCAACTCCATGTTACATTTACTATCAGGGATTCTCACCTCACCTCATGCAT

[0556] 表 27.

[0557] 人 UCP1 基因的 15,910 碱基对 (bp) 核酸序列 (NCBI 参考序列 :NG_012139.1,4 号染色体上的 RefSeqGene) :

[0558]

CTGTACAGCT	CTCCGACAAT	CCCACATCTA	GATGCCAAGC	TGAGGTTGGC	ATTCTCACTA	61
ATTTGCTGTT	ATAAATATTA	AGCTATCATA	AGCGTTAGCC	TACATATGAC	TCTTTCATAT	121
GTTAGTTAAT	TATTTTAGGG	TAGAAATCCA	AAAGTGGAGT	TACCAGAAGT	GGATATAGAC	181
ATTCTGGCTG	GGTGTGATGG	TTCATGCCTG	TAATCCCAGC	ACTTTGGGAG	GCAGAGGCAG	241
GCGGATCACT	TGAGGCCAGG	AGTTTGAGAT	CAGCCTGGGC	CAACACAGCG	AAACCCCATC	301
TCTACTAAAA	ATTCCAAAAC	TAGCCAGGCA	TAGTGGCACA	TGCCTGTACT	CCCAGCTACT	361
TGGGAGGCTA	AGACACAAGA	ATCGCTTGAA	CCCGGGAGGG	AGGTGGAGGT	TGCGGTGAGC	421
TGAGATTGTG	CCACCGTACT	CCAGCCTGGG	TGACACAGCT	AGACTCTGTT	TCAAAAAAAA	481
AAAGAAAAAG	AAAAGAAAAA	AATAGACTTT	CTCTTGGCTC	AGTGTATACT	GCCAAATTGT	541
TTTCCAAAAA	AATTGTGTCA	ATGTATAACA	CCATCACTAA	TATAGTATTG	ATATTATGGT	601
TATTACATTT	TAAAATTTCAT	AATTTGTAAT	TATAACATTC	ATAATTTATT	ACTATTTATA	661
ATATTAATGT	AAATGTATAT	TATATATAAA	TGTTATAGTA	ATTATAACTT	TGGTAGTGAC	721
AAAGTATTAA	TTTATTAGGT	GAAGTATATG	CTTTTTTATT	AGTGATAATA	AATATATCCT	781
CTCTCCCATT	ATAAAAGTTT	GTATTTCTTC	TTTGTAGAAAT	TGATTCTTCT	GTCATTTGCA	841
CATTTATCTG	TATAATTATA	ACAGGGTATT	TCCCAGTGGT	GGCTAATGAG	AGAATTATGG	901
GAAAGTATAG	AACACTATTC	AAATGCAAAG	CACTGTATGA	TTTTTATTTA	ATAGGAAGAC	961
ATTTTGTGCA	GCGATTTCTG	ATTGACCACA	GTTTGATCAA	GTGCATTTGT	TAATGTGTTC	1021
TACATTTTCA	AAAAGGAAAG	GAGAATTTGT	TACATTCAGA	ACTTGCTGCC	ACTCCTTTGC	1081
TACGTCATAA	AGGGTCAGTT	GCCCTTGCTC	ATACTGACCT	ATTCTTTACC	TCTCTGCTTC	1141
TTCTTTGTGC	CAGAAGAGTA	GAAATCTGAC	CCTTTGGGGA	TACCACCCTC	TCCCCTACTG	1201
CTCTCTCCAA	CCTGAGGCAA	ACTTTCTCCT	ACTTCCCAGA	GCCTGTCAGA	AGTGGTGAAG	1261
CCAGCCTGCT	CCTTGGAATC	CAGAACTACT	TTCAGAATCT	TGAACCTCTG	TGACCTCTCA	1321
GGGTCCCCTT	GTGTGAAGTT	TTTGACGTCA	GCTTCTCCTG	TGACCCTTAG	AAGTCACTCT	1381
TGTGCTTAGC	ACATCCCAGG	TGCTCAGTCA	CCATTGAAC	ACAGTCATAC	TATCTCCTGG	1441
CAAAGGCTCT	TAACGTGCCA	TGTTAGCCTG	ATATTAATAT	CCTGGAAGCT	TATACCTGTCG	1501
TTCTTCCCTC	CAGGTTTAAA	TAAGGCAGCC	CCTTTATCCT	GTCACAGGTC	CTCTCTCCCT	1561
ACCTATCCTT	ACCTGTTTTG	GATAACAACC	TTTCTTATTT	CTAATAGATT	TATTTATTTTC	1621
TCACATTTCC	TTCCCTTATC	ATAGTTTTCC	TCTCACTTTC	TCCTCTAGTT	TGTCATACTC	1681
TGGCTTTAAA	ACATGCAAAC	ATGTGCCTTA	TGGGGAAAAA	AAGACAATTT	TAATTTACCT	1741
TGCTTCTTCT	TTACAAATGT	ATTGTGGCTT	CTTCTTATAG	TCCAAATCTA	AAACTCTTTA	1801
CCCACCCACT	GCCTTGAAC	CCTTCTCCTG	TGTGAAAGTA	GGATGGGGCA	AAGAGAGAAT	1861
GCATGCCCCCT	CCCACTGCT	CAAACAAGTA	AAGGTGCTGT	TACAGTTATC	TTTTTGCTACC	1921
TTAATACAAT	AATTATTTTA	TTATATCTCA	CAATTTTATG	GATCAGGAAT	TTAGACTGGG	1981
CTCAGCTAGG	CGATTCTTCT	GCTTTACTGA	CATCATAGGA	GATCACTTGG	TGGTATTCAA	2041
CTGTCAGGTA	GGCTTATCTG	GAGGGTCCAA	GATAGCTGTA	CTCTGGTGCC	TGGTGCCTTG	2101
GTAAAGAGGG	ATGATGATGT	GGGGCCTCTC	CAGCATGAAC	AGCCTCAGAG	AAGTTTGCTT	2161
TCTTACATGC	TGGCCCAGGG	CTCCAAGAGC	AAATGTTGCA	GTGAGTAAAG	CAGAAGATAC	2221
AAGGACTTTT	ATAATCTGGT	CTCAGAAGCC	ACATGGCATC	AGTTCTGTAT	TATTCTATTG	2281
GTCAAAACAT	TCATAAGCCT	GCCAGATGCA	AGGGGAAGGC	ATATGTACCC	TCATCTTTTG	2341
ATGGGAGGAA	TGTGATGGAT	TTGCAATTAT	GTTTTTAAAC	TACTACAGAC	AGAACCACTG	2401
AGAAAGATTC	ATGGGTAGCT	TTGGGGTGAG	GACTGGGAAT	TAACCTGTTG	ATAGCAGAGG	2461
TTCACTAGAG	TCAACAAGGA	ATAAGGTCTC	CTCTTGTAACA	CTTTAGTCAT	ACTATACCAA	2521
CATTCTTAAC	CACTGCTTAG	CCATCAGCCT	CACAACATAA	CAACTCCATC	ATAGTTGTAC	2581
TCCCTAAGAT	CACCAACAAT	GTTAGAGTCA	AATCCGGTAG	GTTTTTCTTT	GTTTTTGTCC	2641
TCCTGACATT	TTTTCTAAAC	TTGACACTGG	TCAGACCCAA	TCTTTCTTTA	ATCATATTCT	2701
TAAATACCAG	TTCTATCACT	GGATATGTTA	CTGTTTCTTG	TTCTCACTCT	ACCTTTGACA	2761
AAGCCATTCT	TTCCAGACTA	TAACCTCTGG	TCTGGGTCCC	CCTATGGTTT	GGCCCTTGAA	2821
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TGCATCCATA	TCATGACTAC	CAAATACCTC	CTCTATTTCT	TCCTCTTTTA	GCATGTTAAA	2941

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TGCAGCTTCC	TAAGCTCTCT	ATCTGGATAT	CAACAGTATT	CTCTCCAAAT	AATTCTAAGA	3001
CTTTAAAAAT	TGGTTTAATC	TTCTTACCCC	TAAAAACACC	CCCCTTACCA	ACTGCCTCAT	3061
GACAATCATT	GGTACTGTCA	CTGAGCTTGC	AACCCATGTT	CTTAAACATA	GAGTAATCTT	3121
TGACTCCACA	TCTAATCATT	CATAAAGCTG	TATTGTCTAT	CAAATTAAAT	CTGACATTTA	3181
TGTGAGAGCA	CTTCATAGTC	TGTAAAGCAC	TACACAGGTG	ATAACATGAA	GCTACACTCA	3241
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AAGCTCTGCA	AGCCTGACCT	CCCTTGGAAG	TGCCTTAGGA	CTGGCTTCTT	GCACAGTACA	3601
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CCTGGAGGAG	ATGACTCTGA	CTTCCACTGA	CTCTTTTGGG	GGGCTTAAGT	CAGGGTTGAG	3721
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AACTGAGAGG	TTCTGAAAGC	TGGGACCTGA	CCTTGTCCTT	TTCCCTCTTT	CTCCAGTTTC	3841
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TGTACTATTT	GCTTTCTAGT	GTGTCTATTG	TCTCGTTTGA	CATCTTCTCT	CAAAAAGTGA	4261
TGAAACGAAA	CGCTCTTTTT	GACAAGTTCA	GAGTGCTCTT	GGTTCCTGTG	TGGGATTCTT	4321
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TCACCAAGGC	TCTGATATCG	TGCTGGTTTC	CGAAAGCCCC	AGCCGAAGGT	GTGCAGCCAA	4561
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CCCTCCGACG	TGCGGTGTGC	GGGGCGCAGA	CAACCAGCGG	CCGGCCCAGG	GCTTTCGGGG	4681
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CGGACACGCC	CCCGGCAGGT	CCCACGCCCG	GGTCTTCTGA	GACCTCGCGC	GGCCCAGCCC	4801
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GCCCTTTTCC	TCTGCATCTC	CAGTCTCTGA	GTGAAGATGG	GGGGCCTGAC	AGCCTCGGAC	5101
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GCACTTAGTA	TTTTAAGAGC	TGAGAATATT	GGCTCTCCT	GAGTTTACTA	GTGAGGTGCT	5521
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CGGGGCTTCA	CGCGCAAATC	AGCTCCGCCT	CTCTCAGGAT	CGGCCTCTAC	GACACGGTCC	6001
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TCGGATAATA	CTGAAAAGAG	AGCTAAATGT	GGGAAAGAAC	CAAGTTGAAC	ACAGGAAAGA	9721
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CAGTTAGGCC CTATACATGA AAAGGTGAAG CAGTAACACG AAGGCACACA ATGCACCATT 15661
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AAATCCTCTT GTCCAGTTAA AACTGTAGTT ATGGCTGGTG GAAAATGGGC TGGAGTCAGT 15781
CAACACTTGG TGAAGCTGCA GTTGCTTCAG AACTCAAGG CCAGTGTTTG TTTAGCTGCT 15841
CGAGAAAAAG AAAAACTTG TGGCAGTTAG AACATAGTTT ATTCTTTAA TGTAGGAGTG 15901
TGTGACTTAA //

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[0563] 表 28.

[0564] 人 UCP2 基因的 2,113 碱基对 (bp) 转录物 ENST00000310473 的核酸序列 (八个编码外显子以大写字母示出):

[0565]

编号	外显子/内含子	起始	终止	长度	序列
	5' 上游序列				aatcgacagcagagccggctcgcgagggccc cagtcgccgcccgcaggagcc
1	ENSE00002287650	73,694,352	73,693,766	587	AGCCGCGCGCTCGCTCGCAGGAG GGTGGGTAGTTTGGCCAGCGTAG GGGGGCTGGGCCCATAAAAGAG GAAGTGCACTTAAGACACGGCCC

[0566]

					CGCTGGACGCTGTTAGAAACCGT CCTGGCTGGGAAGGCAAGAGGT GTGTGACTGGACAAGACTTGT CTGGCGGTCAGTCTTGCCATCCT CACAGAGGTTGGCGGCCCGAGA GAGTGTGAGGCAGAGGCGGGGA GTGGCAAGGGAGTGACCATCTCG GGGAACGAAGGAGTAAACGCGG TGATGGGACGCACGGAAACGGG AGTGGAGAAAGTCATGGAGAGA ACCCTAGGCGGGGCGGTCCCCGC GGAAAGGCGGCTGCTCCAGGGT CTCCGCACCCAAGTAGGAGCTGG CAGGCCCCGGCCCCGCCCGCAGG CCCCACCCCGGGCCCCGCCCGCG AGGCTTAAGCCGCGCCGCCGCT GCGCGGAGCCCCACTGCGAAGCC CAGCTGCGCGCGCCTTGGGATTG ACTGTCCACGCTCGCCCGGCTCG TCCGACGCGCCCTCCGCCAGCCG ACAGACACAGCCGCACGCACTGC CGTGTCTCCCTGCGGCTCG
	内含子1-2	73,693,765	73,692,678	1,088	gtgagcctgccccagccctgccc.....actctc tgccttgcctcaccacag
2	ENSE00001184362	73,692,677	73,692,521	157	GACACATAGTATGACCATTAGGT GTTTCGTCTCCACCCATTTTCTA TGGAACCAAGGGGATCGGGC CATGATAGCCACTGGCAGCTTTG AAGAACGGGACACCTTTAGAGA AGCTTGATCTTGGAGGCCTCACC GTGAGACCTTACAAAGCCGG
	内含子2-3	73,692,520	73,689,523	2,998	gtaagagtcagtcgaaggaagagg.....tgggg ctttctcctcttggcttag
3	ENSE00001184370	73,689,522	73,689,298	225	ATTCCGGCAGAGTTCCTCTATCT CGTCTTGTTGCTGATTAAGGTG CCCCTGTCTCCAGTTTTTCTCCAT CTCCTGGGACGTAGCAGGAAATC AGCATCATGGTTGGGTTCAAGGC CACAGATGTGCCCCCTACTGCCA CTGTGAAGTTTCTTGGGGCTGGC ACAGCTGCCTGCATCGCAGATCT CATCACCTTTCCTCTGGATACTGC TAAAGTCCGGTTACAG
	内含子3-4	73,689,297	73,689,142	156	gtgaggggaatgaagcctgggagtct.....tagcta ccctgtcttggccttgag
4	ENSE00001252503	73,689,141	73,688,931	211	ATCCAAGGAGAAAGTCAGGGGC CAGTGCGCGCTACAGCCAGCGCC

[0567]

					CAGTACCGCGGTGTGATGGGCAC CATTCTGACCATGCTGCGTACTG AGGGCCCCCGAAGCCTCTACAAT GGGCTGGTTGCCGGCCTGCAGCG CCAAATGAGCTTTGCCTCTGTCC GCATCGGCCTGTATGATTCTGTC AAACAGTTCTACACCAAGGGCTC TGAGC
	内含子4-5	73,688,930	73,688,063	868	gtgagtatggagcaagggttaggc.....cactg accccatggctgcccacag
5	ENSE00001184355	73,688,062	73,687,868	195	ATGCCAGCATTGGGAGCCGCCTC CTAGCAGGCAGCACCACAGGTGC CCTGGCTGTGGCTGTGGCCACGC CCACGGATGTGGTAAAGGTCCGA TTCCAAGCTCAGGCCCGGGCTGG AGGTGGTCGGAGATACCAAAGC ACCGTCAATGCCTACAAGACCAT TGCCCGAGAGGAAGGGTTCCGG GGCCTCTGGAAAG
	内含子5-6	73,687,867	73,687,788	80	gtgtgtaccagttgtttcccttcc.....accagga tcttctectctctacag
6	ENSE00003361285	73,687,787	73,687,686	102	GGACCTCTCCCAATGTTGCTCGT AATGCCATTGTCAACTGTGCTGA GCTGGTGACCTATGACCTCATCA AGGATGCCCTCCTGAAAGCCAAC CTCATGACAG
	内含子6-7	73,687,685	73,686,717	969	gtgagtcatgaggtagacggtgctg.....tgccittg cctgcicctccttggcag
7	ENSE00001184349	73,686,716	73,686,536	181	ATGACCTCCCTTGCCACTTCACTT CTGCCTTTGGGGCAGGCTTCTGC ACCACTGTCATCGCCTCCCCTGT AGACGTGGTCAAGACGAGATAC ATGAACTCTGCCCTGGGCCAGTA CAGTAGCGCTGGCCACTGTGCC TTACCATGCTCCAGAAGGAGGGG CCCCGAGCCTTCTACAAAGG
	内含子7-8	73,686,535	73,686,167	369	gtgagctctgtgtctctcccccaccca.....atgacct gtgatttttctctctag
8	ENSE00001184368	73,686,166	73,685,712	455	GTTTCATGCCCTCCTTTCTCCGCTT GGGTTCTTGGAACGTGGTGATGT TCGTACCTATGAGCAGCTGAAA CGAGCCCTCATGGCTGCCTGCAC TTCCCGAGAGGCTCCCTTCTGAG CCTCTCCTGCTGCTGACCTGATC ACCTCTGGCTTTGTCTCTAGCCG GGCCATGCTTTCTCTTTCTTCTT CTTCTCTTCCCTCCTTCCCTTCT

[0568]

					CTCCTTCCCTCTTTCCCCACCTCT TCCTTCCGCTCCTTTACCTACCAC CTTCCCTCTTTCTACATTCTCATC TACTCATTGTCTCAGTGCTGGTG GAGTTGACATTTGACAGTGTTGGG AGGCCTCGTACCAGCCAGGATCC CAAGCGTCCCGTCCCTTGGAAG TTCAGCCAGAATCTTCGTCTCTGC CCCCGACAGCCCAGCCTAGCCCA CTTGTCATCCATAAAGCAAGCTC AACCTTGGCGTC
	3' 下游序列				tctccctctctttagctcttaccagaggcttggcca atggcctttt.....

[0569] 表 29.

[0570] 人 UCP2 基因的 15,174 碱基对 (bp) 核酸序列 (ENSG00000175567), 包括 5,000bp 5' UTR 和 2,000bp 3' UTR (八个外显子突出显示):

[0571] TCCAGCCTGGGCAACAAGAGTGAAACTCGGTCTCAAAAAAAAAAAAAAGAGAAGAAGAAGAAAGAAAAC
TAGGTGGAGTGTGGTGGCTTGACCTATAATCCAGCACTTTGGGAGGCCGAGGTGGGTGGATCTATTGAGGCTAGG
AGTTCAAGATCAACCTGCCAACATGACGAAACCCACCTCTACTAAAAATACAAAAATTAGCACGGCGTGGTGTGT
GTGCCTGTAATCCTAGCTACTTGGAAGGCTGAGGCAGGAATCGCTTGAACCTGGGGGGCAGAGGTTGCAGTGAGCCA
AGATCTTGCCACTGCACTCCAGGCTGGGCGACACAGCACAACTCTATCTCAAAAAAAAAAAGAAAAACAAAAGAAA
ACTAATATATCAAAATAATTTCTAGTTAGTTGGATTCCCTCACTTATTCATTCAATGACTTATTGAATTATCATATAT
TACTAGTGCTTTTTAATACATACCTTCTACAATTTTTCAACTGAAAATTACTTCATTGATCAGGGCTCTTTAACTG
ATCTCCATTTGCATTGTTTTACTAACTATAGTTATTATTCATGTATTAGCACTCTGAGCCTACTGTAATGATGTGTA
CCTTAATAAAGAACTGAATATTTGTAATGGCTGGCAGTGAATTTAGTAGTTCTTGAATTTAGAGCTCAAAATATGGG
AGTAATTTGCTGCTTTATTTCTTTGAGAGGTAATAGAGGAAAAACAGAATCTAATAACAATCACAGATTTTCGGGA
AAGCACTGTAAACCATATGATCAATTCTAGCTTCTTATGTAAACATGGAAAGATTGCCAGCTGAACACCTGTCATG
CTCTAAGAAGTTGGGGAGAATTTGCATTTTTAGAACTGTGAGCAAAATGAGAACGACTGCTATGTTTCATGCTTTGTG
AATTTAGCTTTATTTCAATTCACACAATTCATGGGAAAAAATGCATCTTTTAACTCGGTGTTTTTCAATTCACTTTT
AAAATACAGGAGTGGGCCAGACCCGGTGGCTCACACCTGTAATCTCATCACTTTGGGAGGCCGAGGCAGGTGGACCA
CAAGGTCAAGAGATAGACACCATCCTGGCCAACATGGTGAAACCCCATCTCCACTAAAAATACAAAAATTAGCTGGG
CATGTTGGCAGTACCTGTAATCCCAGCTACTCGGGAGACTGAGGCAGGAGAATCGCTTGAACCTGGGAGATGGAGG
TTACAGTAAGCCGAGATCGCGCCACTGCACTCCAGCCTGGCGACAGAGCAAGACTCCATCTCAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAACCAGGATGTGTTACCAAGGAAAAATTCATTTACAATGGTTAATTATGTGACAAA
CATGTCAAGTAATTCATCTGGCTTTGTGTCAACATTTCCCCACCCTTTTTTCAGAAACCAAAACCAAGAAGAAGAA
CAAACATCAAAATGGACATGGAATTAACAAATATATGATTCAATTTAATCTCCTAAGAGGTTTTTTAAAATTATTT
TATTTTGAGACGGAGTCTTGCTCTGTGCGCCAGGCTGGAGTGCAGTGGCAGGATCTCAGCTCACTGCAACCTCCATCT
CCCAGGTTCAAGCGATTCTCCTGCTTCAGCCTCCCAAGTAGCTGGAACCTACAGGCAAGCACCACCACCCAGCTAA
TGTTTGTATTTTTGGTAGAGATGGGGTTTACCATGTTGGCCAGGATGGTCTCGATCTCTTGACCTCATGATCCACC
CGCCTTGGCCTCCCAAAGTGCTGGGATTACAGGTATTTTTTTATTTTTTTTGTAGACAGGGTCACCCTGTCACCAGGC
TGGAGTGTAGTGGCACAATCATGGCTCACTGCAGCCTCAACCTCCCAGGCTCAGGTGATCCTCCATGTCAGCCTCCC
AAGTAGCTGGAACATATAGGCGTGCAACACCATGCCAGCTAATTTTTGTATTTTTTGTAGAGACAGGGATTTGCCAT
GTTGGCCAGGCTGGTCTTCAACTCCTGGCCTCAAGTGATCCACCCGTCTCAACCTCCCAAACCTGCTAGGATTACAGG

TGTGAGCCACCGTGCCCATCTCTGCTAAGTGGGTTTAAAGAAATTCAGTTTCATGTCAATTTTAAAAATGTATG
GTTATCAAATTCGACTTCTTTTTAAAAATGCAATCAGATAACTGTATGCTTGTGATGAGGGGAGGAAAGTTAATA
TAGCCAATCTACTCAATATTTTAGCAGAAAATATCAGAGACTAAGGAAATGTTTAAGTTTTCTCATGTTGGTTTT
AATTACCTAATGTTTTAGTTTTCTTTCTTTCTTTGTTGTTCTTTTTTTCATTTTCAGTGTTTCAAATACAGTTTGTA
TTTAAAGATTTAGAAGTTCCAAAACTGTAAGCACAGTGGATTGTTTCCTGGGATGATGTTAAATATACAACAAAA
TATATGAAACTTTGTCAATTTGGTTATTGGCACATACAAAATATTTACAAATAAACGTGTGTGTGTGTGCGTGTACA
CACAATTCAATGAAATAGATGTGAAACAAGTTTTCTTTTTTTTTTTTTTGAGACAGAGTCTTGCTCTGTGCGCCAGG
CTGGAGTGCAATGTGCGAGTCTCAGCTCACTGCAACCTCTGCCTCCCGGGTTCAAGCGATTCTCCTGCCTCAGCCTC
CCGAGTAGCTGGGACTACAGGCACCTACCACCCTCCAGCTAATTTTTGTGTTTTTAGTAGAGACAGGGTTTCACC
ATGTTAGCCAGGCTAGTCTCCAACTCCTGACCTCAGGTGATCTGCCCCTCAGCCTCCCAAAGTGCTGGGATTGC
AGGCGTGAGCCACCTCACCTGGCTACAAGTTTTCAAATACATTTATCTGTACCCATACATTCTCCAGTTTGTCCA
CAGGACATCTTATGACTTGAGCAAGCTGCTAAAAATCCAAGGGTGACGCTTGTATGTCTATAGGATTGCTCAGA
TCTGCCCCCACCCTGAAAGAATTTAAGAGAATTTCTTGAGGCCAGGCACAGTGGCTCACACCTGTAATTCCAGTACT
GTGAGAGTCCGAGGTCAGAGGACTGCTTGAGGCCAGGAGTTCAAGAGCAGCCTGGACAACATAGGGAGACCTGTCAC
TACAAAGAATAAAATAAATTAGCCAGGCTTAGTGGCTCATCCCTGTGGTCCCAGCTACTAGGGAGGCAGAAGTAGGAC
TGCTTGTCCCAGGAGGTCAAGACTGCAGTGAGCTGAGACCCAGCCACCTGCATTCCAGCCTGGGCAACAAAAAGAGA
CCCTGTCTCAAAAAATAAGTTAAATAAAATAAATAAATAAAGTTTAAACCCTAAACACATCTTCTTTTTCAAAGA
GGACTTCTTAAGGACTTCATGCTGCGTCTGTGATCTCACTTCCCTTTTTCAGCGTCCACACTTTTAACAGTCTCT
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GTCTGCAGGCCTTTGCATCTGTTCTGCTGTTGCTCAGCAATCTCACAGCAAATTTGCCGAGCCTCTCCGGAATGCAC
AGCCAGACAGAGCTCAGCGCAAAAGCTAGAGAACCTGGCGGAGGGAGACTCACAGTGCCACAAAAAACTTTATCTT
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AGGACGGGTGAGCCAATTAAGGCGAACCAGGCCCGGTCCATCTCCTGACGCCTTTTCTCATCCCAGGGCTGGACAG
GCAGCTGGCCTGGGCCCGGCTCTGCCTTGTACGTGCGGGGGCCGGCCCGTTTGCTTGTCTGTGTGTAGGAGCGTGA
GGTCACGCTGGGTGCTCCCGCCCCGCCGGGGCCTTTAGTGTCTTGGTCCCTAAACGCCAGGCCGCTCCACCGGGGA
GAAGGCGCGAACCAGCCGAGCCCAACGGCTGTTGTGCGTTGCCGGGGCCACCTGTTGCTGCAGTTCTGATTGGTTCT
CTTCCCCGACAACGCGGCGGCTGTAACCAATCGACAGCGAGGCCGGTCCGAGGCCCCAGTCCCGCCCTGCAGGAG
CCAGCCGCGCGCTCGCTCGCAGGAGGGTGGGTAGTTTGGCCAGCGTAGGGGGGCTGGGGCCATAAAAGAGGAAGTGC
ACTTAAGACACGGCCCCGCTGGACGCTGTTAGAAAACCGTCTGGCTGGGAAGGCAAGAGGTGTGTGACTGGACAAGA
CTTGTTTTCTGGCGGTGAGTCTTGCCATCCTCACAGAGGTTGGCGGCCGAGAGAGTGTGAGGCAGAGGCGGGGAGTG
GCAAGGGAGTGACCATCTCGGGGAACGAAGGAGTAAACGCGGTGATGGGACGCACGGAACGGGAGTGGAGAAAGTC
ATGGAGAGAACCCTAGGCGGGGCGTCCCCGCGAAAGGCGGTGCTCCAGGGTCTCCGCACCCAAGTAGGAGCTGG
CAGGCCCGGGCCCCGCCCGCAGGCCCAACCCGGGCCCCCGCCCCGAGGCTTAAGCCGCGCCGCGCCTGCGCGGAG
CCC **C**ACTGCGAAGC

CCAGCTGCGCGCGCCTTGGGATTGACTGTCCACGCTCGCCCGGCTCGTCCGAC
GCGCCCTCCGCCAGCCGACAGACACAGCCGCACGCACTGCCGTGTTCTCCCTG
CGGCTCG GTGAGCCTGGCCCCAGCCCTGCGCCCTTTGCGCCCCCACGCTTGTCTGCGTGCGCTGCCCGCTC
TTCCATTTACCTTCTCTCCACCCAAAGTTTGACTCTTTTCTTTCTCTCGGTTTTATTTTTGTTTTGTTTTGTTTTG
TTTGAGACAGGCTTTTCGCTCTGTCTCCAGGCTGGAGTGCACTGGCGCGATCTCGGCTCACTGCAGCCTCCACCTCC
CAGGTTCAAGCGATCCGCCTGCCGAGTAGCTGGGATTACAGGCGCCCGCCACCACGCTGGCTAATTTTTGTGTTT
TGTAGAGATGGGGTTTCGCCATGTTGGCCAGGCTGGCCTCGAACTGCTGAGCTCAAGCAATCCGCCCGCCTCGGCCCT
CACAAAGTCCTAGAATTTTAGGCATGAGCCTCCGGGTCCGGCCTGTGCTAATCCTTTCTGTCCTTGGTTCTTTATTT
CTCTTCTCTCTTTTTCTTAGTCCCTTTTGTTCTTTCCCTCTCCCGTTCACTTGGCTGTGCTTTGAGCCTCCACCTTT
TCACTCCCTCCTTTCCACCACGATGCCGAGCCCTGCCCTTGATGGGGACCATCAGCGATGACCACAATGACCTCTCC
CTTACCAGGCAGCTCCAGGCAGTGTTCCTGCACCGCCTTTCCAGGGCTTGGGGGCTTTTTCTAGTGGGCTTTGAGC
TGCTCAATCTGGCCTCTGCAGGGCCGGCTCCAGCCCTTCCAACCTCCTCACAGCCCGACCTGGGACCTAGCCAATT
CCCGGAGAGTCTCTGTCCCATCGTGACCCCTCACAACTCTCCCACTCACCAAAGTCTGATGACTGTGCTAGGGGT
GCTTATATAGAGTACTGAGTGTTACAAAAGCAGAAGTCTGGATGAGAACCAATTTGTGATATTAAGCAGGTGGGGTG
GGGGTGGGGAGTGACCTAGGTTCATTTTCCGCCCTGCTTTTCCCTTTCCAGTGTGTGCACTTAACCAGTCCCTGG
GCCCTGTTCCCATCCCCCTCCAAGGCATGGATTGGGTGGGCTTGTGTGCTTGGGGCAGGTGGCCCTTTCTAAACT
CTCTGCCTTTGCTCACCCACAG

GACACATAGTATGACCATTAGGTGTTTCGCTCTCCACCCATTTTCTATGGAAAA
CCAAGGGGATCGGGCCATGATAGCCACTGGCAGCTTTGAAGAACGGGACACCT
TTAGAGAAGCTTGATCTTGGAGGCCTCACCGTGAGACCTTACAAAGCCGG GTA
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CAAAAATAATTACCATCCCCACCCCGACAGTGAAAGTGGCTCTTTCCAGTTCACAGAGCACTCTCACACCTCCCCGC
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TGAAGGACCTGACTTGGCAAGCCCAAGGCAACATTCAGAATATGGCAGCTGAACCTCTGTGCACTTGTCTTTGGGCA
GCAGCTGGGTCTTATTCTTCTCTGGCCTTCACAACATCCTGCAACCCAGCTCAAGGTCAGGAATGTGACAGACTCAT
GTCATCATATCTCTGATGCCAGAGAAGGGATACCATTTGCCTGAGCCTTCTCAGTACTGTTAATCAGCCTGTGAG
AACTTTCCTTGTGAAAGGCCCTGTCTGTGCCTGGGGCTGATAAACAGCAAGAACGAAGTGAAGGAGCTGGGCAGCAG
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ACCTGTAGCAACCATTCCTGCTTAGACATCCAGAACATAAGCCTATGGGTCTGTGCCTGTTGGGTCTTAGTCTGGG
TGAAACTTTTCTCTACTTCTGTCTCAGCTCTCCAGATGAACCACAGAAGCAGGAATGTGGGCATCATCAGTGAAATCT
CTGCATACAGCAGACAAAGGGCTGGTCCAGTGGCTGTTTATGAGGCAGCGCTAGGAGAGCTCTGATCCAGACTCTC
CCTGCAGTGAAAGGGAGGGAGCCCTTCATGAAGTATTGACTGCTTGAGCAGGAATTGCTTCACCAGCACCTAACTG
AGTGCCTCTCGAGCTCACATCGTTTTCCCTCATGAGGCCACTTGAGTCTTGCTGAGGGACTTGTTCTATTAGG
GAAGGTGAGTTTGGGGATGGTGAGCAGGGAGGGCCTGGGGACATTGTGGCTAATGGGGCTTTTCTCCTCTTGGCTT
AG ATTCCGGCAGAGTTCCTCTATCTC

GTCTTGTTGCTGATTAAAGGTGCCCTGTCTCCAGTTTTTCTCCATCTCCTGGG
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TAAACAGGCTGGGTGGGCATGAGCAAGTCAGCTGGTTCATTTCAGCAGCCTTAATAGACACGAGGCTACCCAATTCA
CTGTGGTTCTGGGTGTGGCCTTAGGACAATGAGCTGGGAACAGTGGTAGGAACCACTGGAAAACATACCAGTGGGTC
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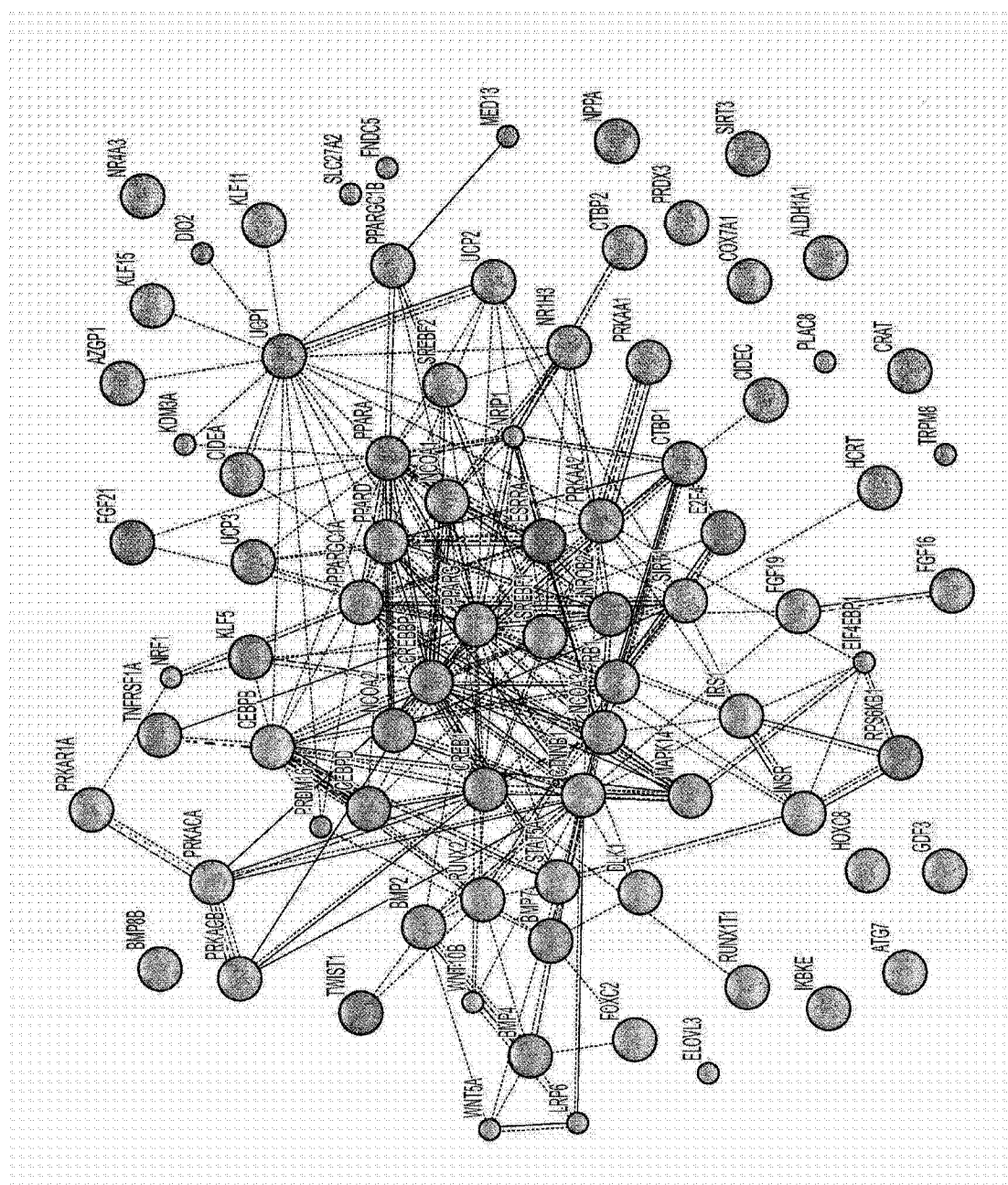
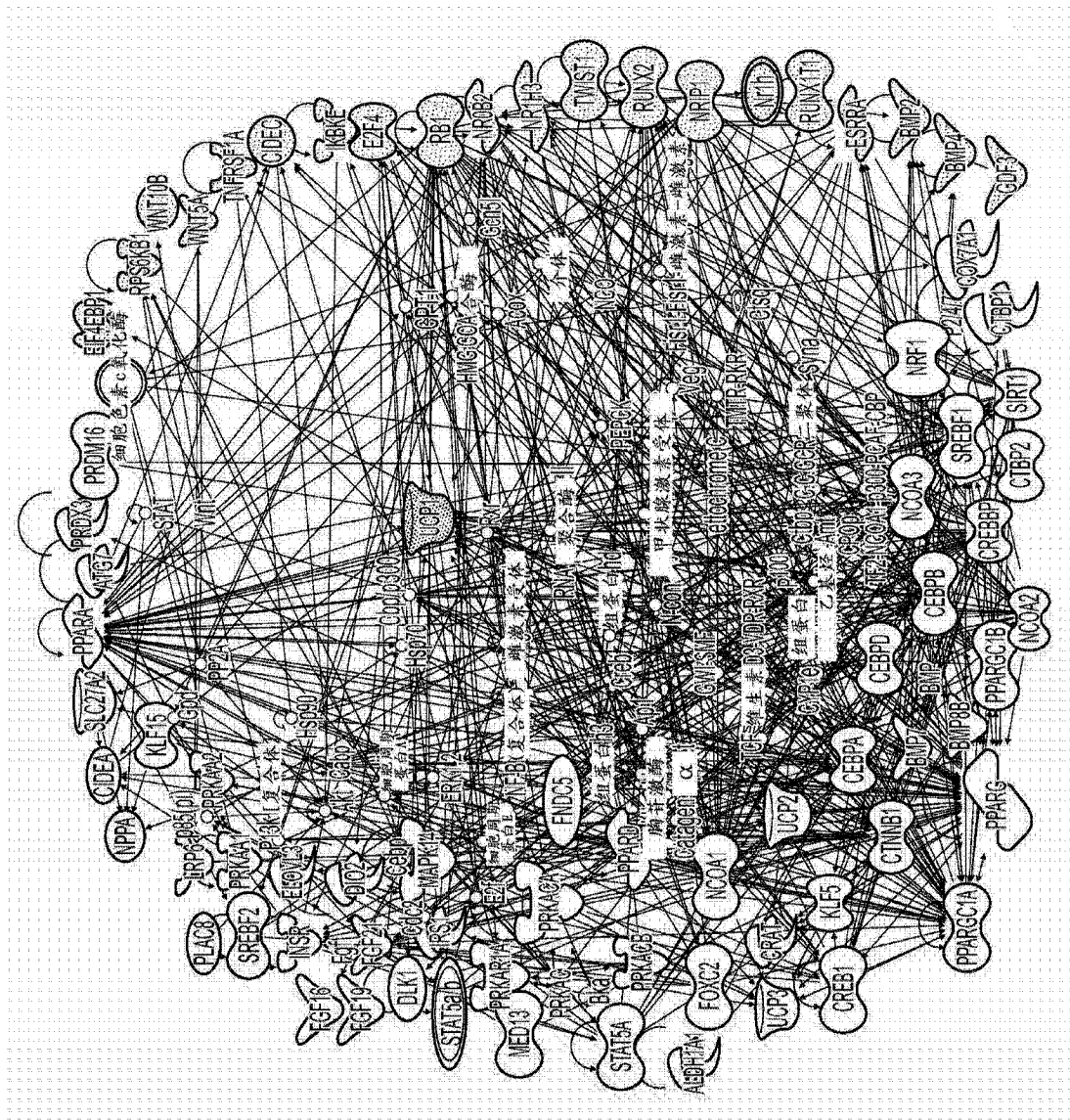


图 1B



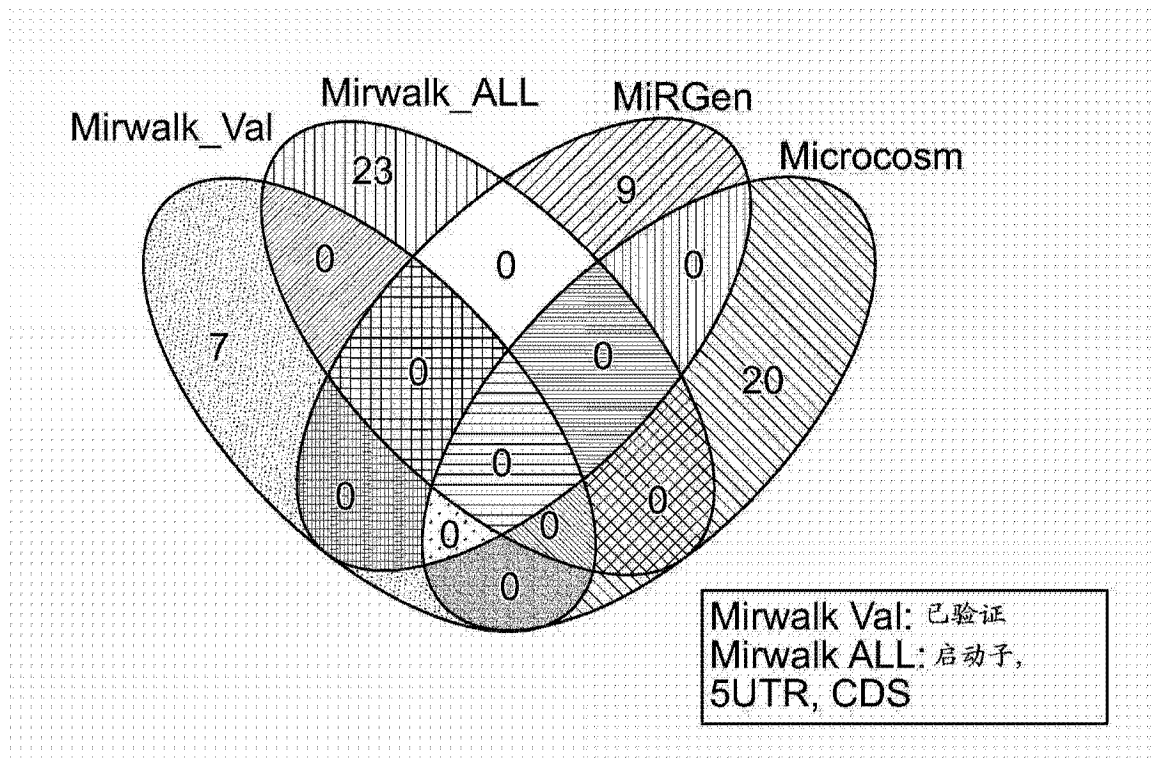


图 3

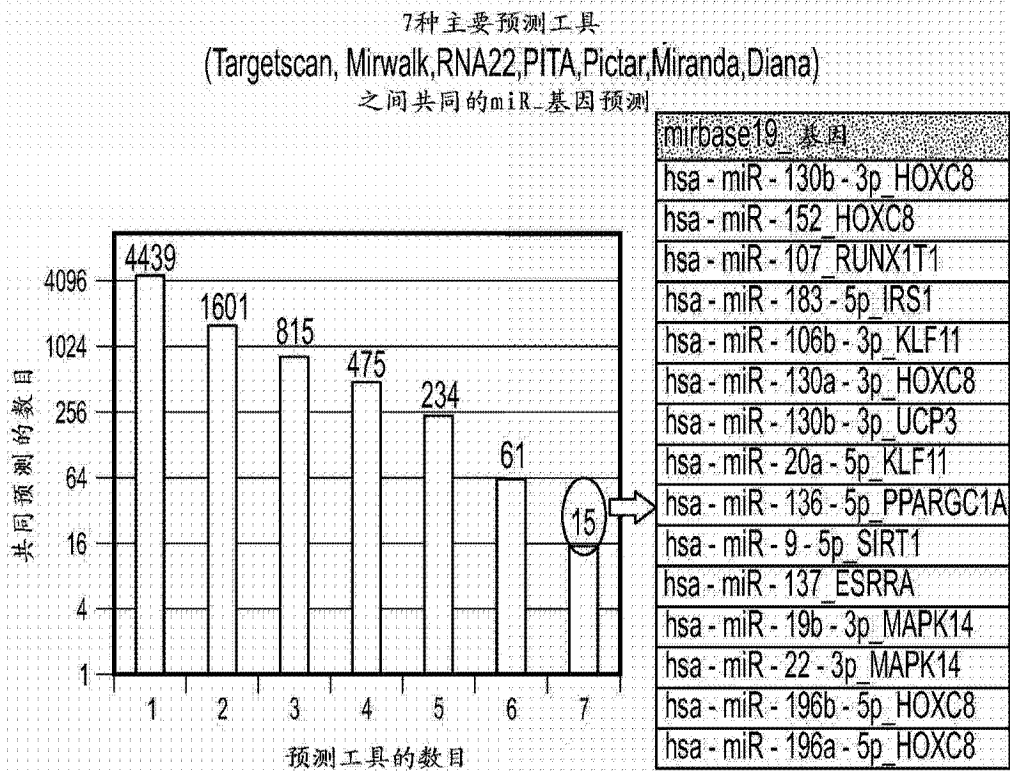


图 4

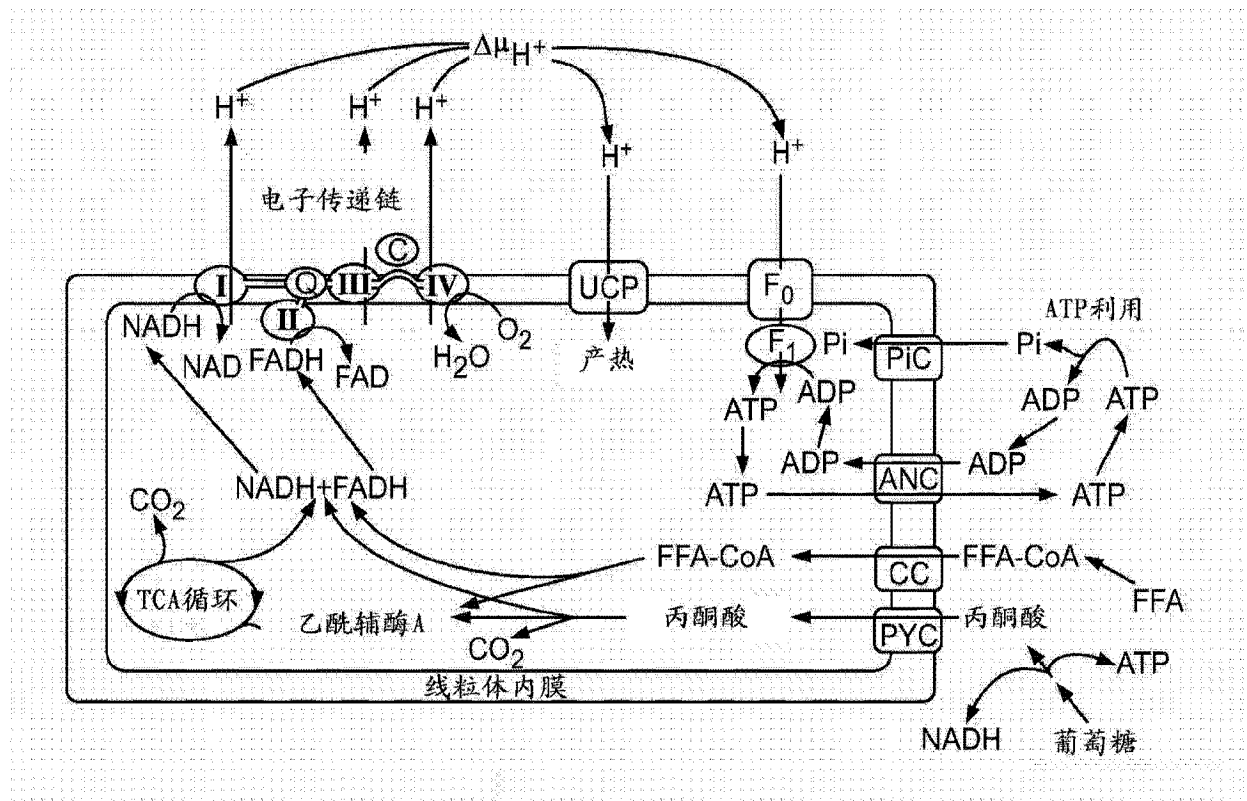


图 5

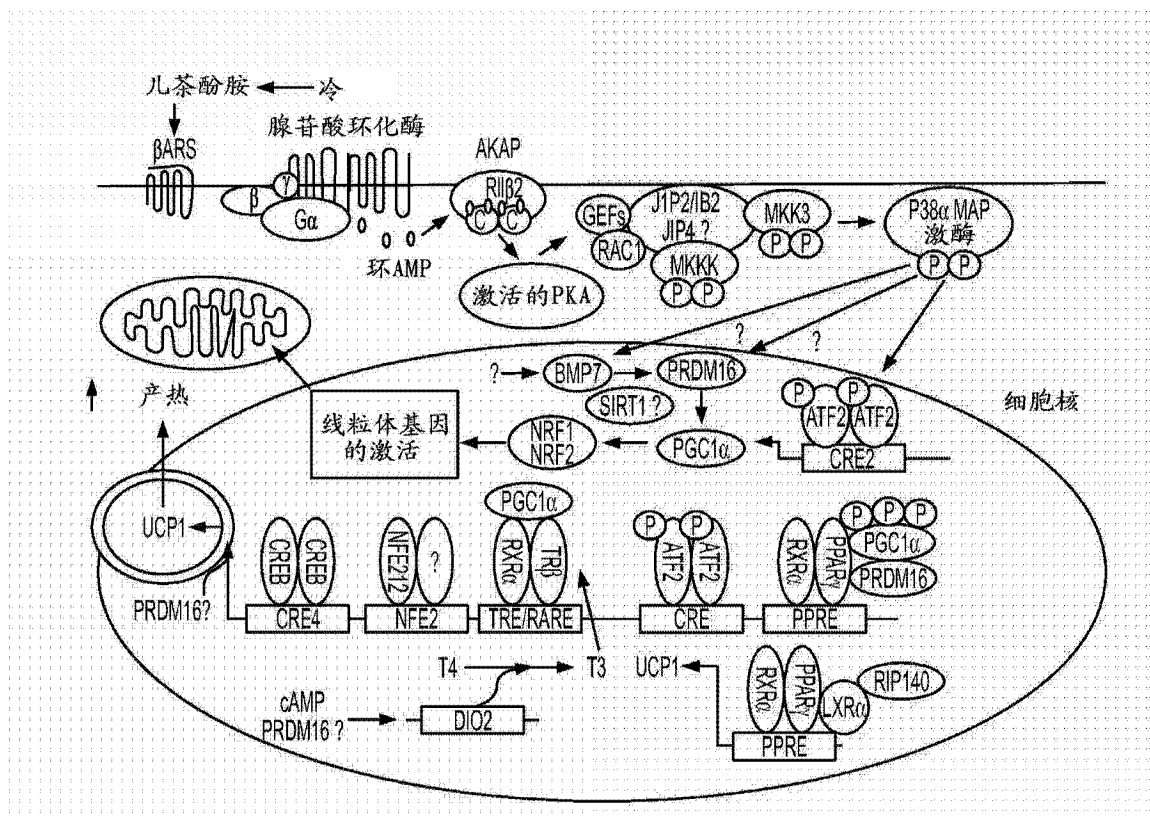


图 6

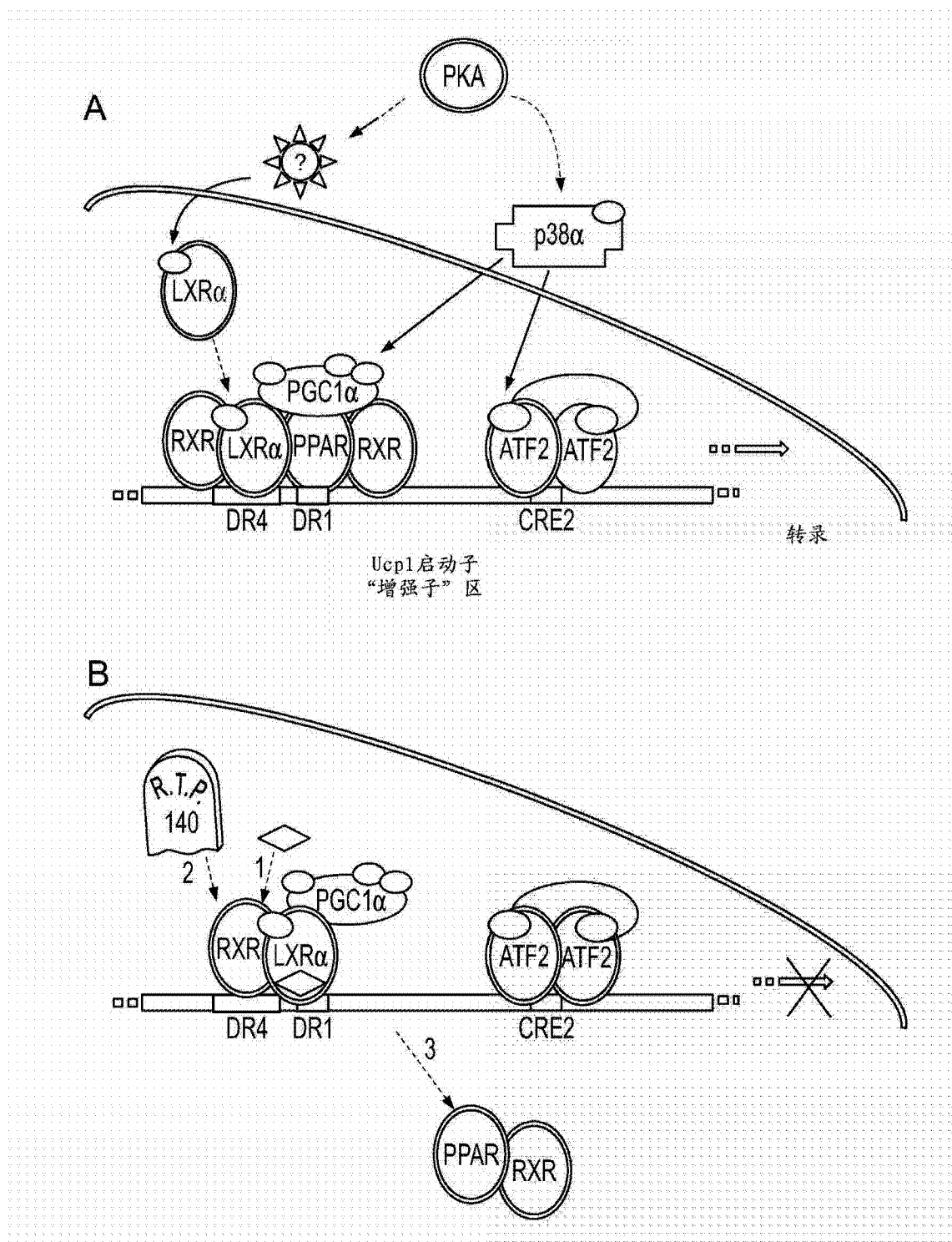


图 7

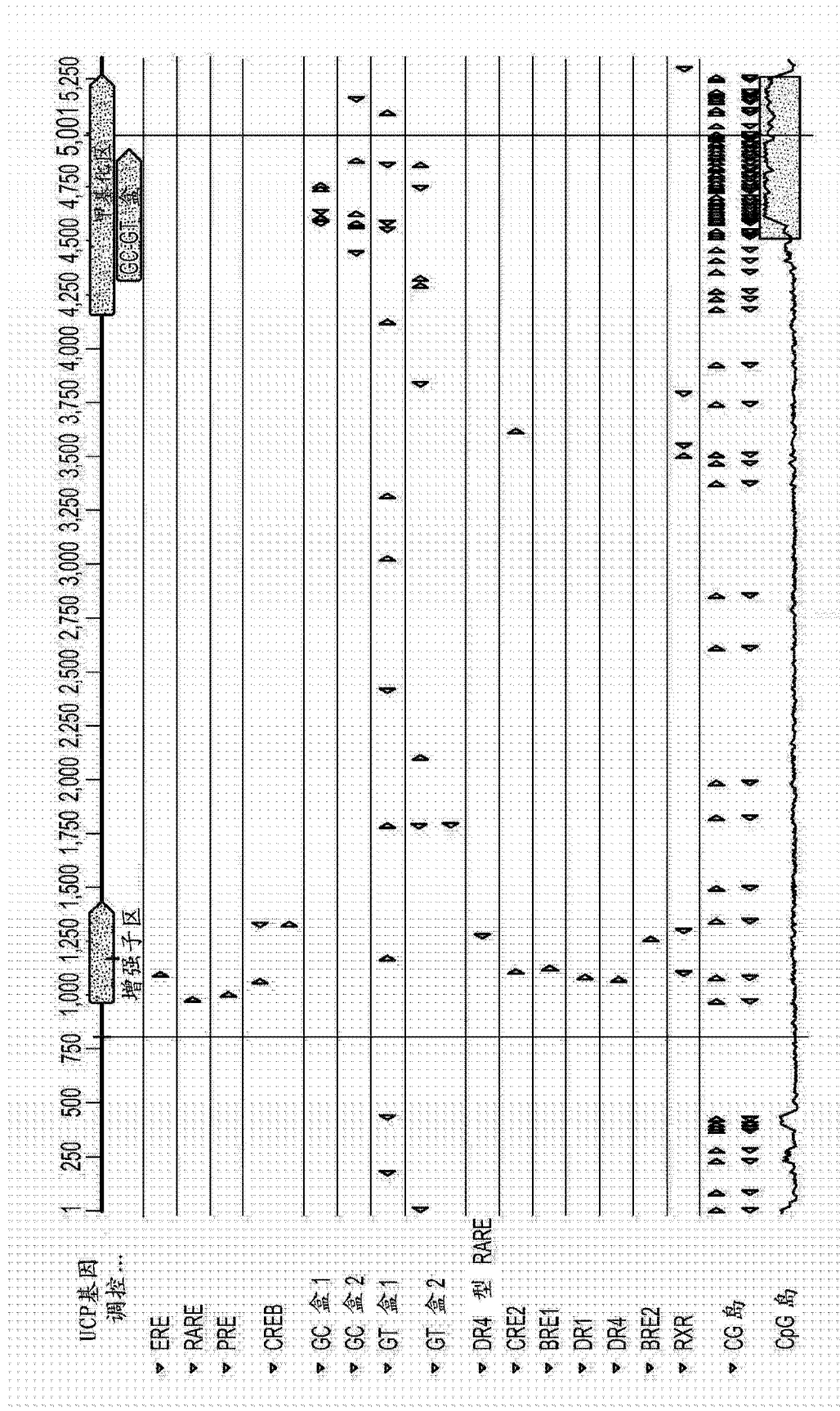


图 8A

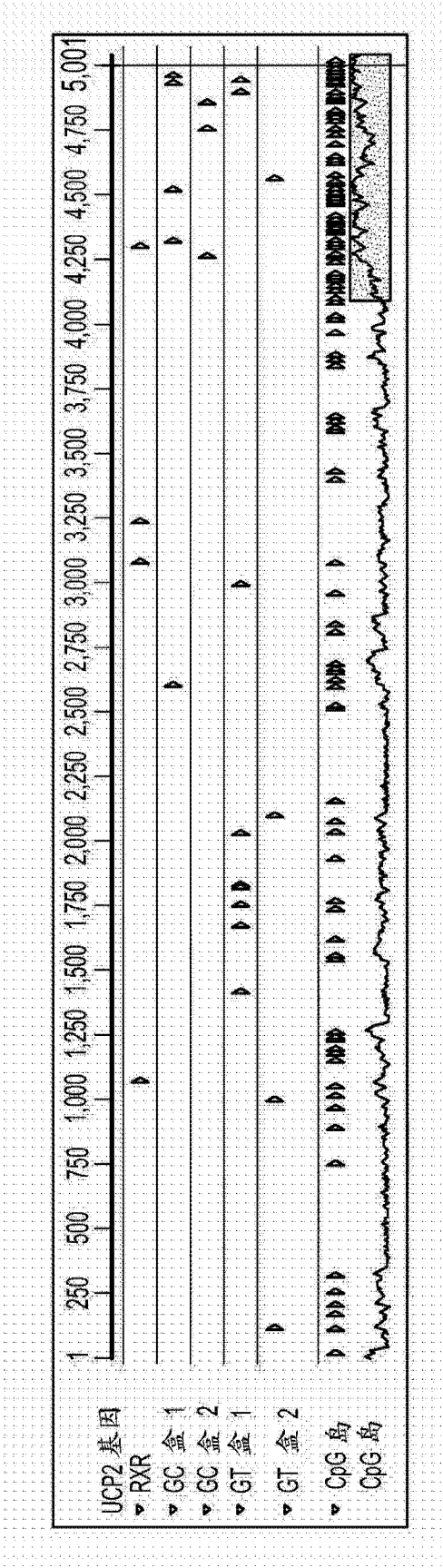


图 8B

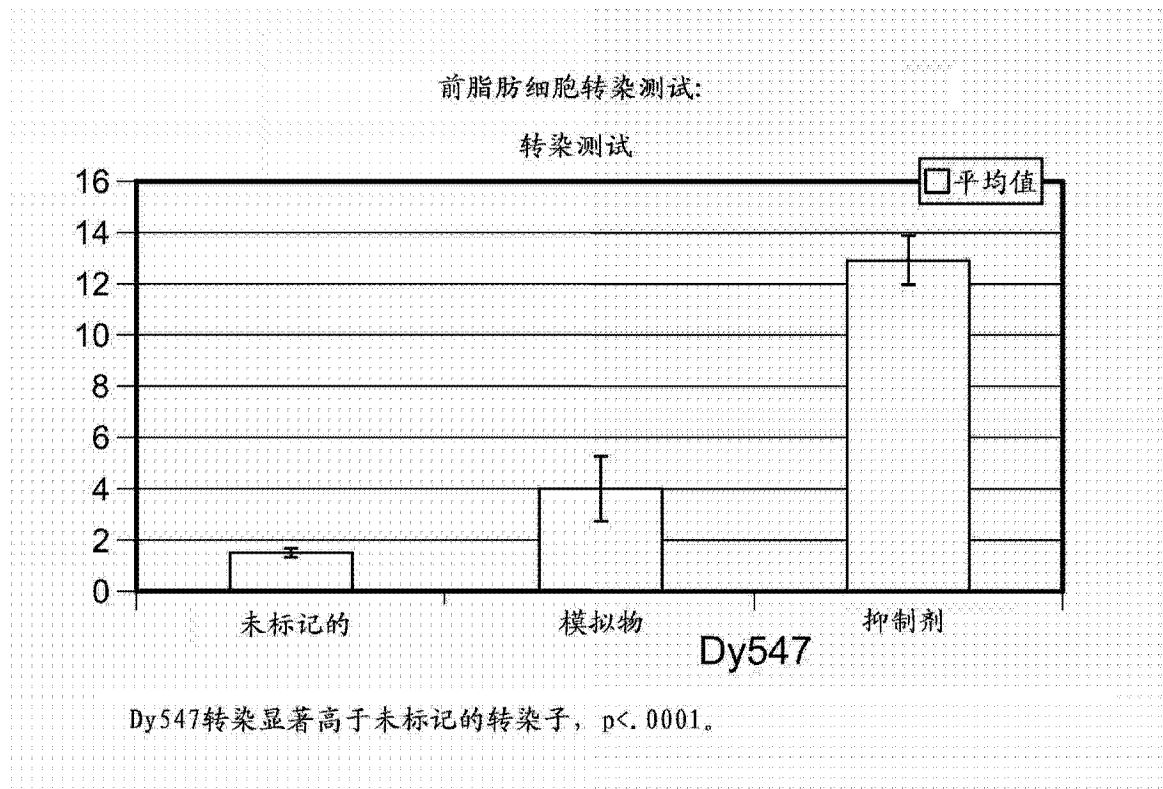


图 9

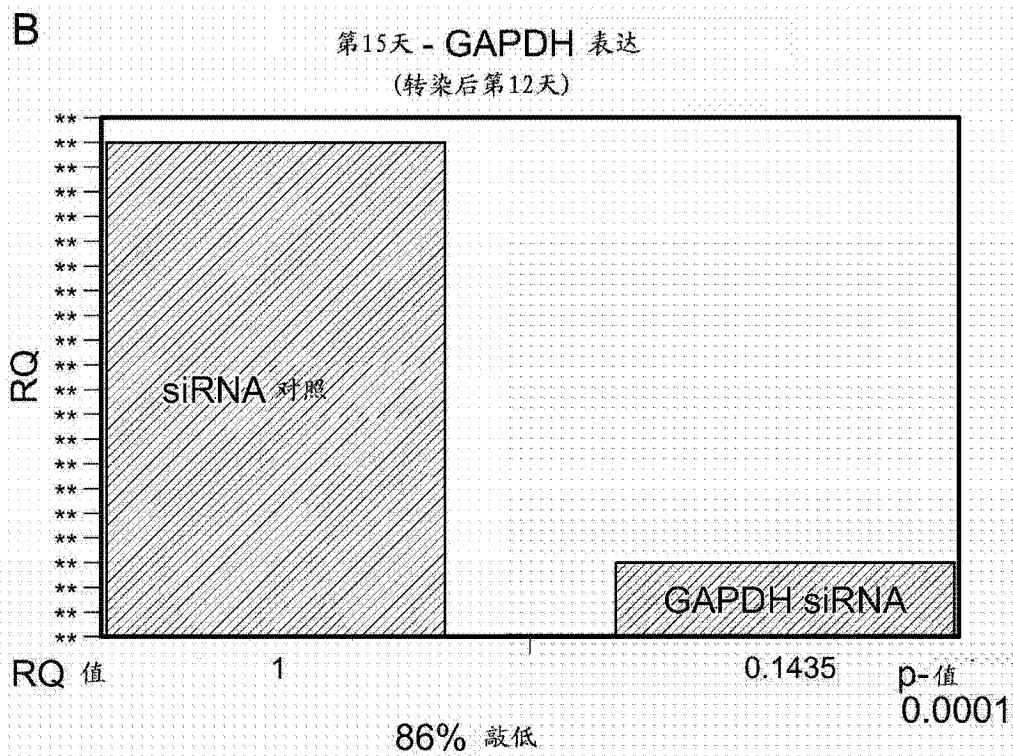
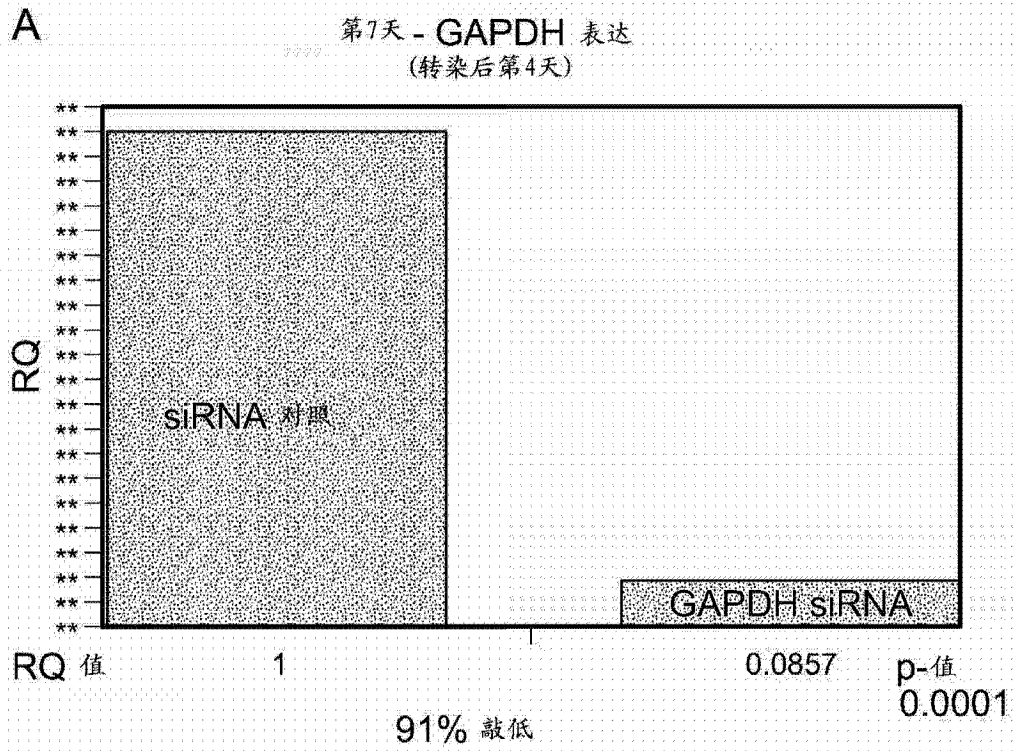


图 10

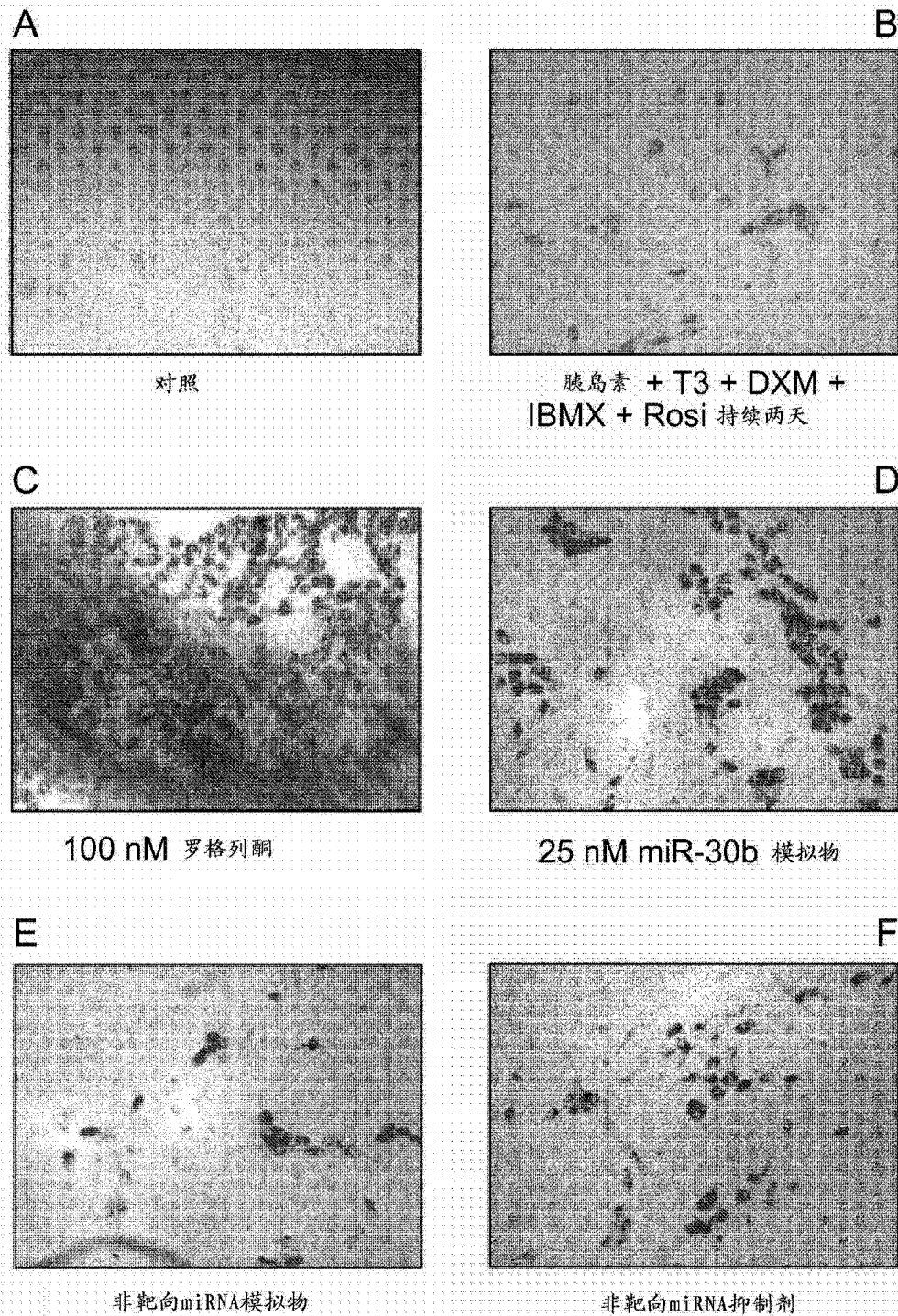


图 11

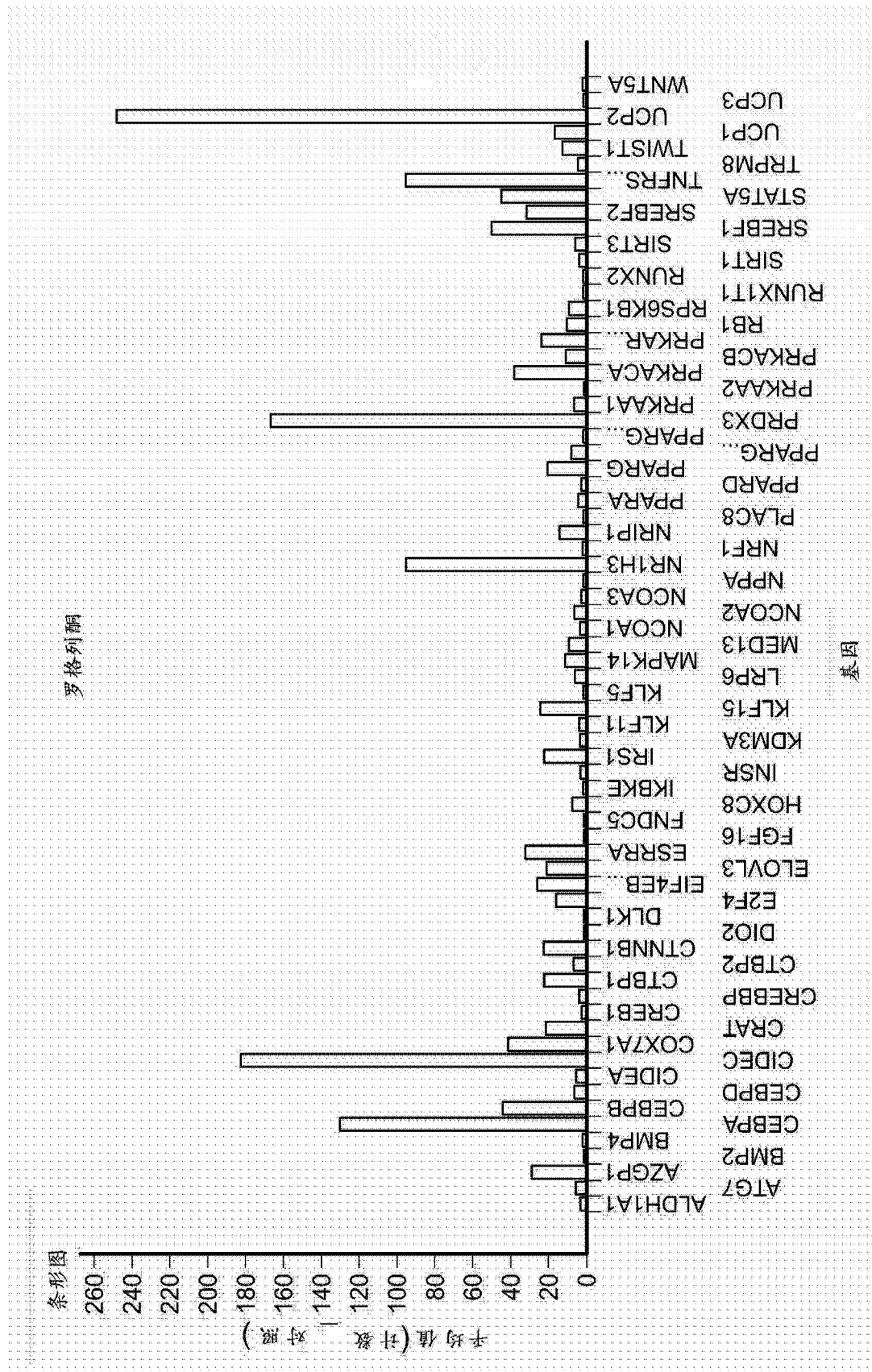


图 12A

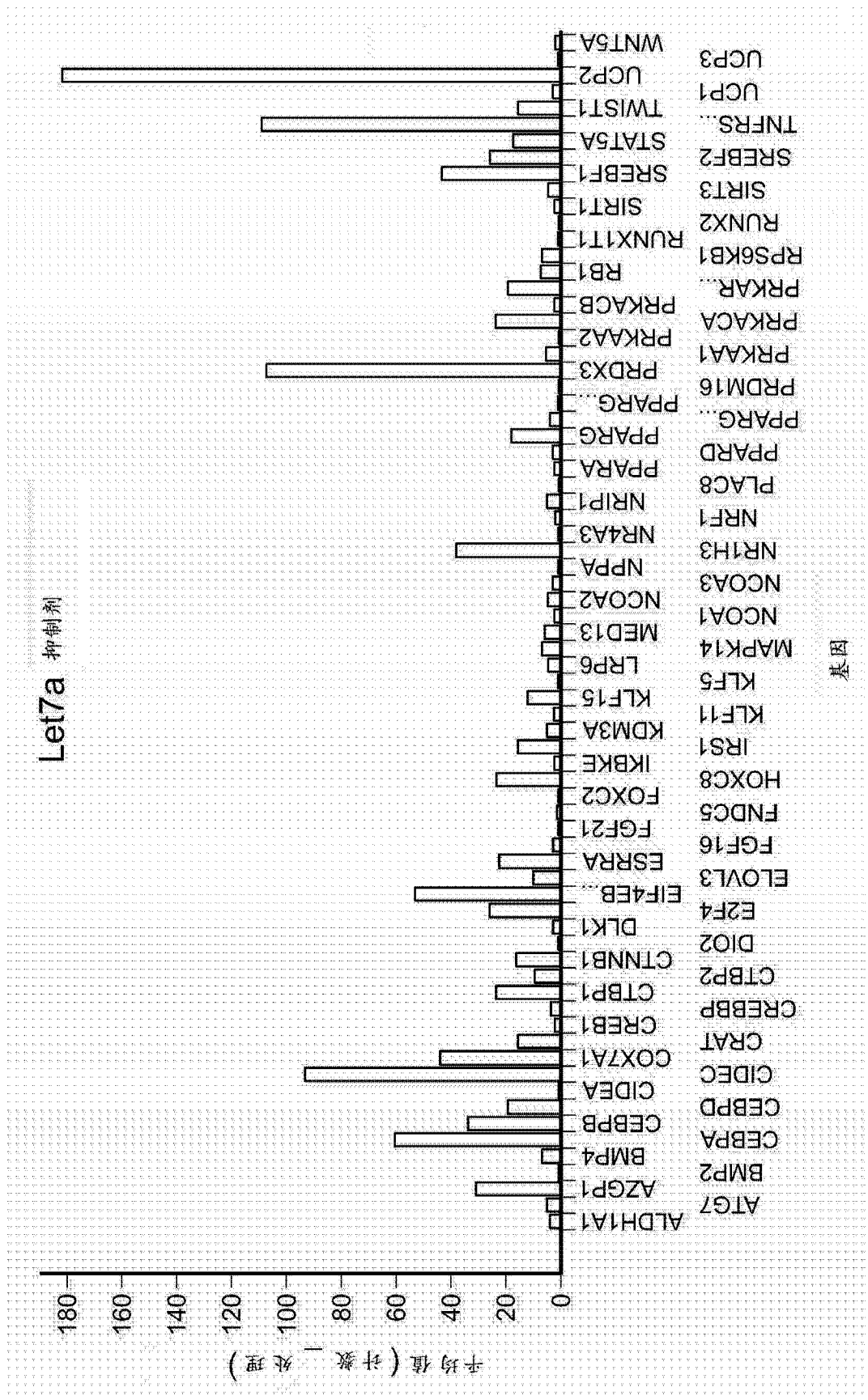


图 12B

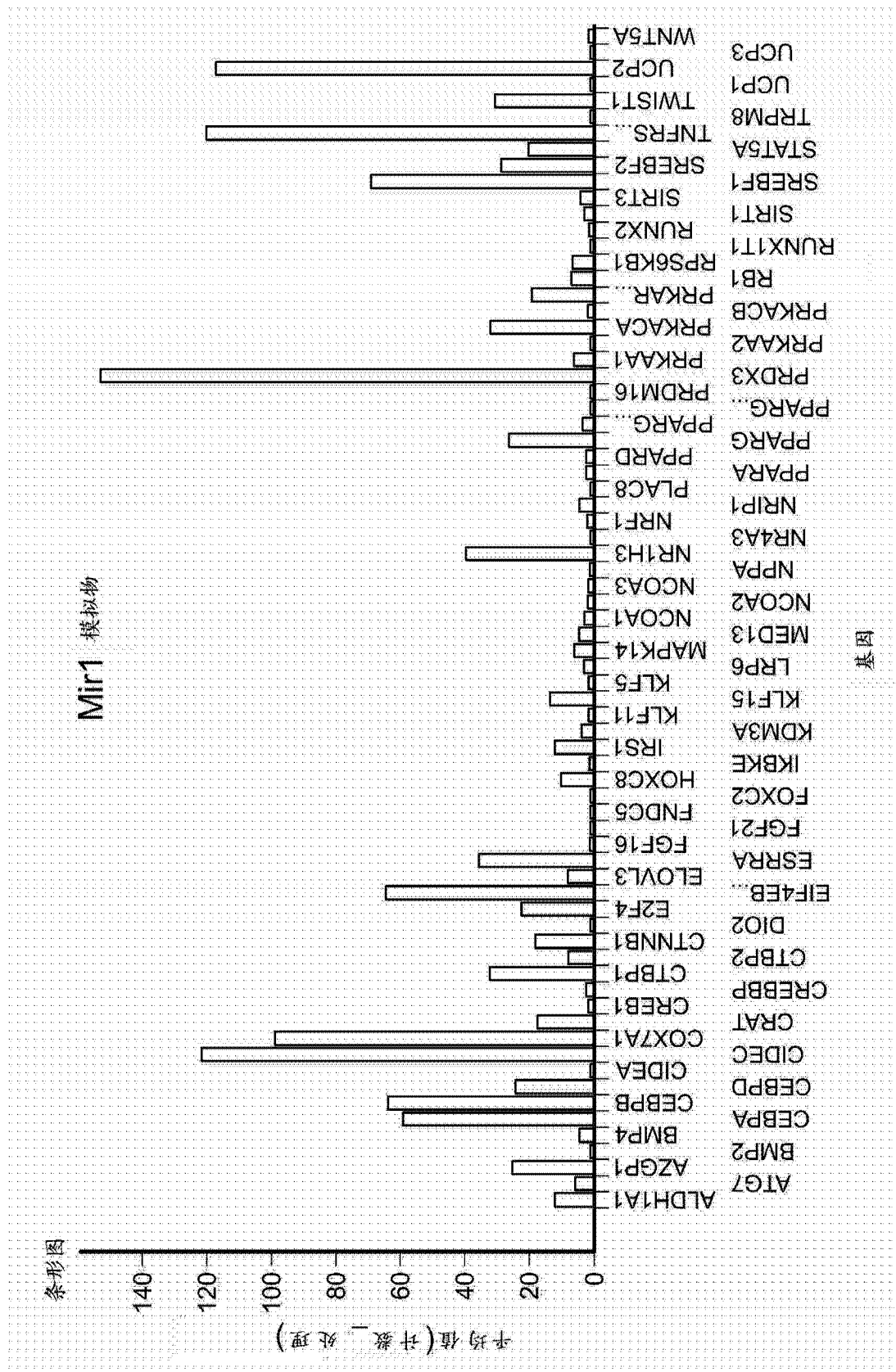


图 12C

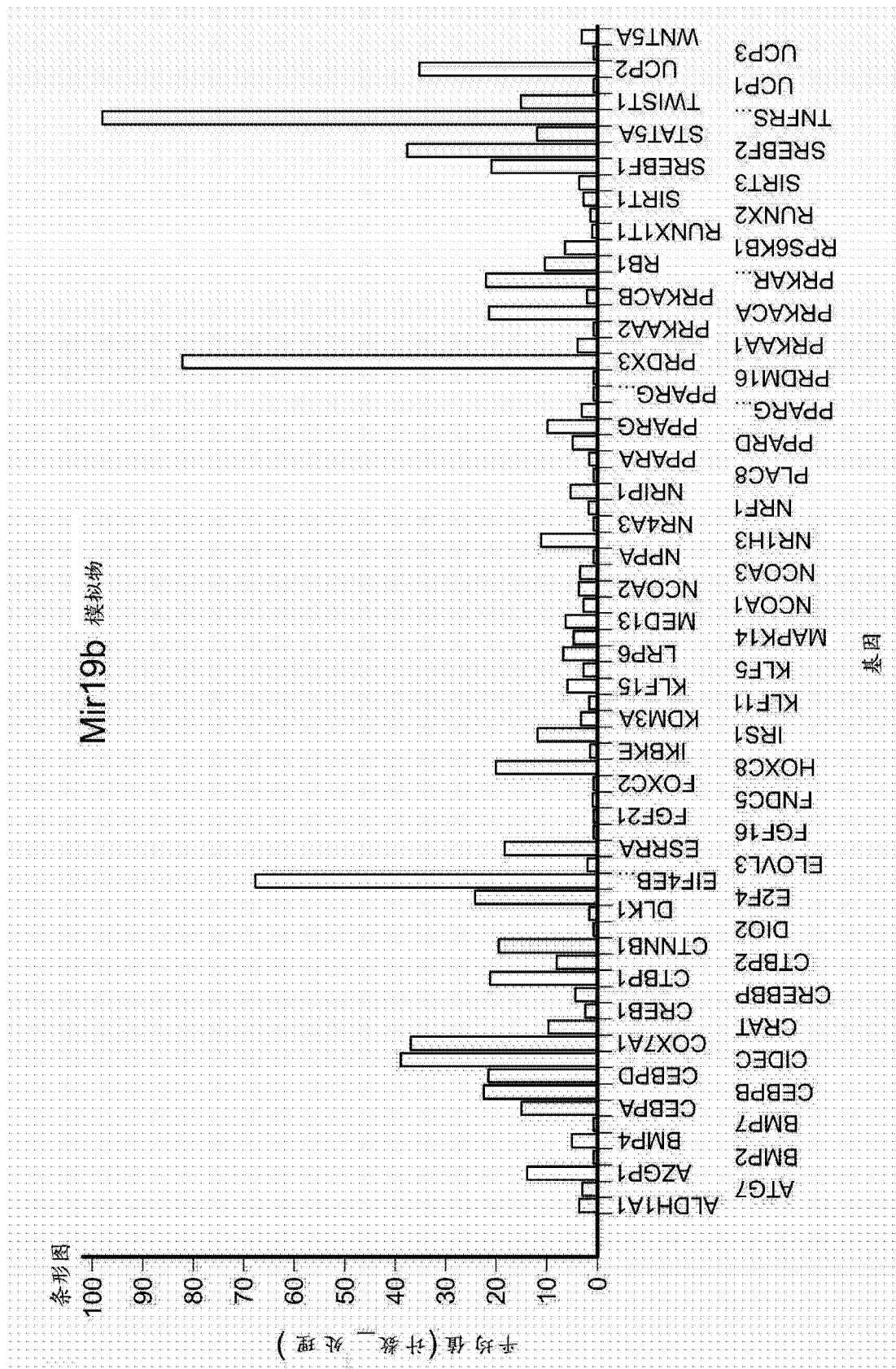


图 12D

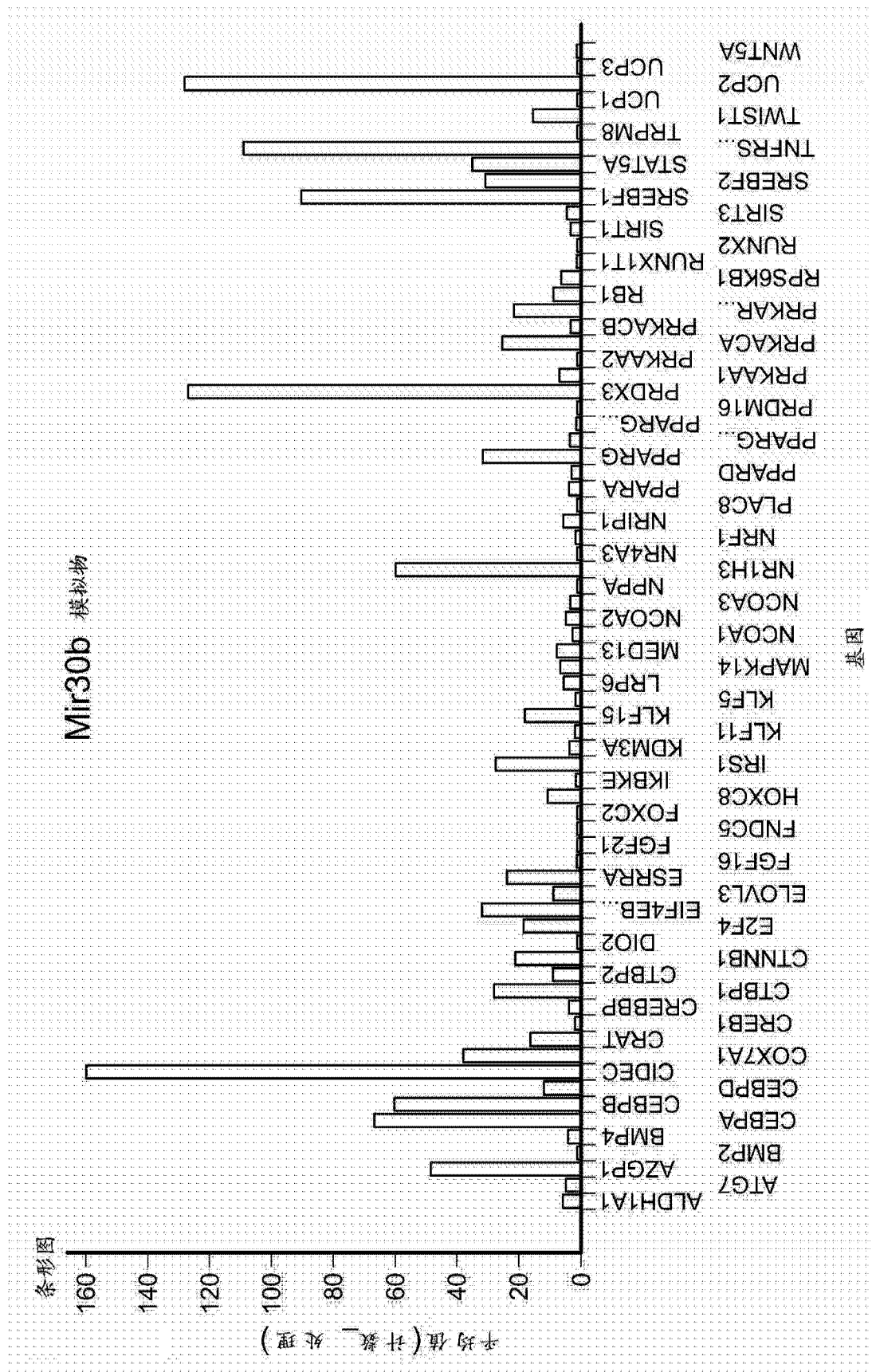


图 12E

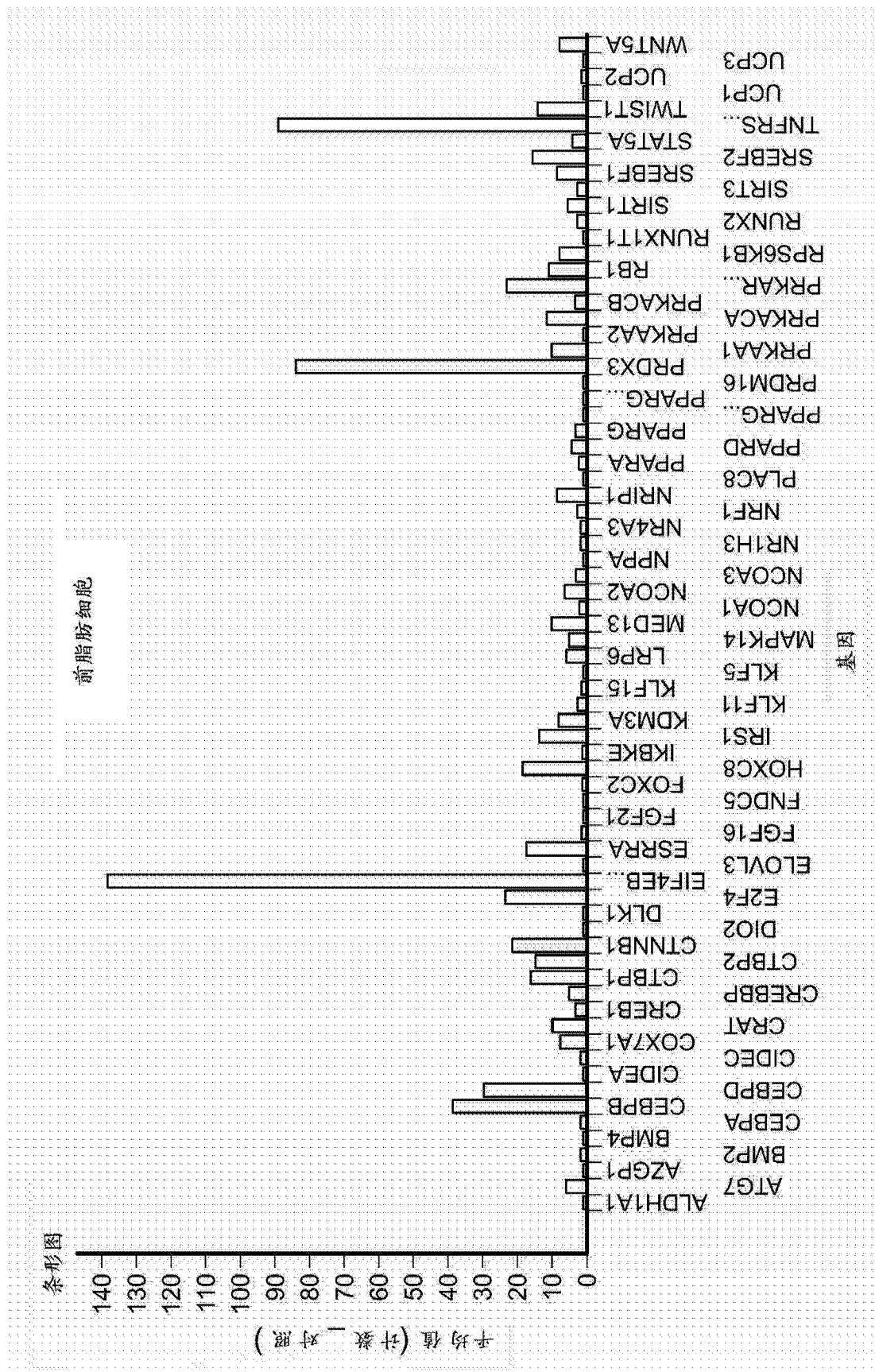


图 12F

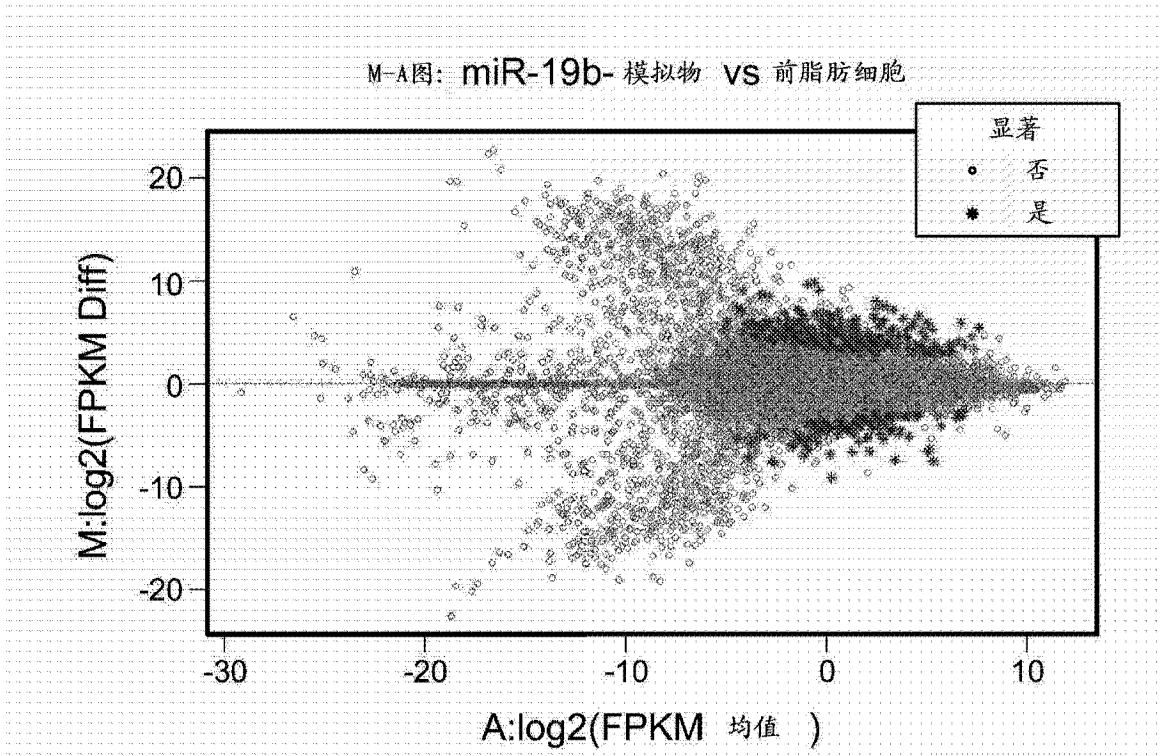


图 13

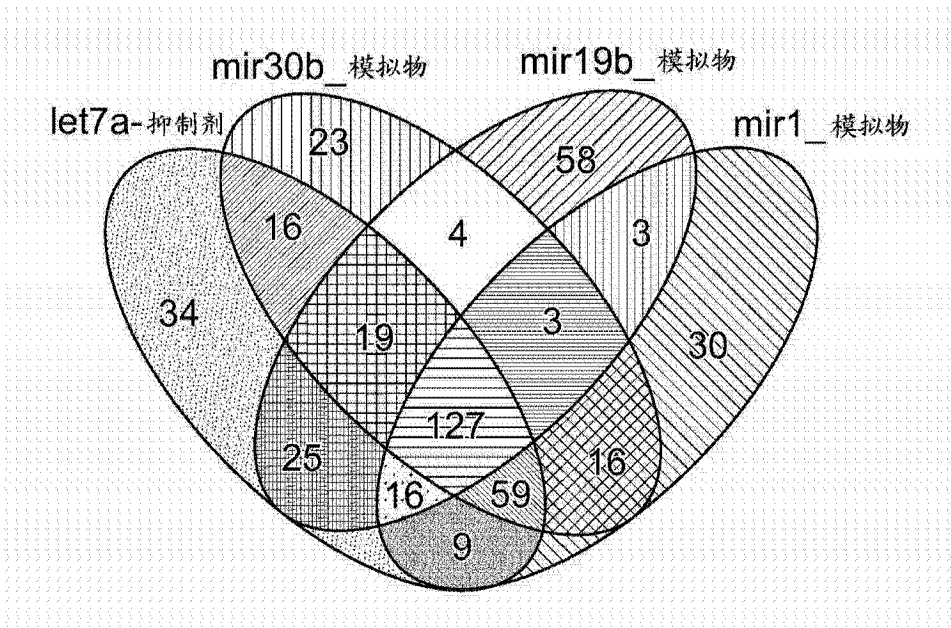


图 14

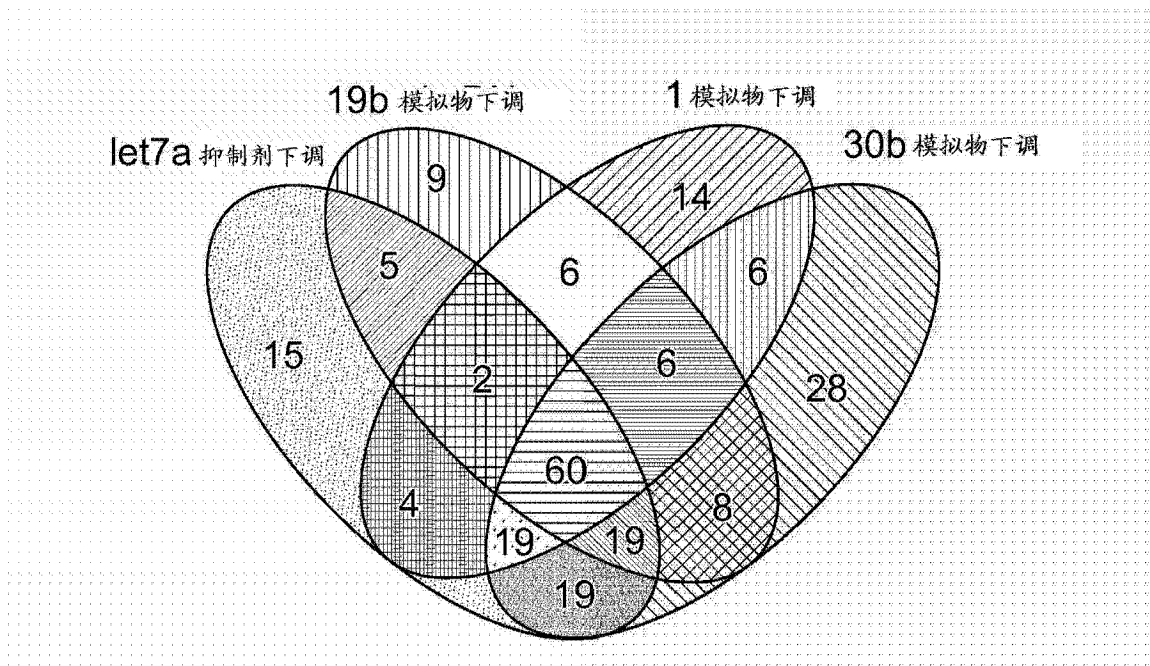


图 15

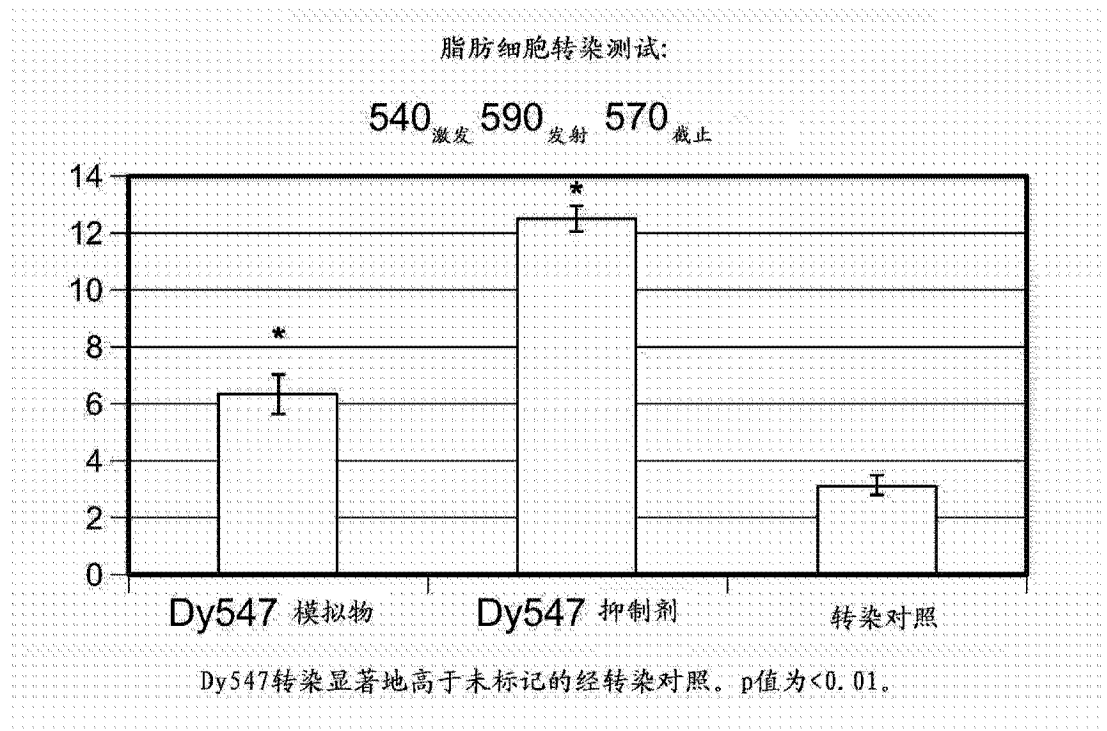


图 16

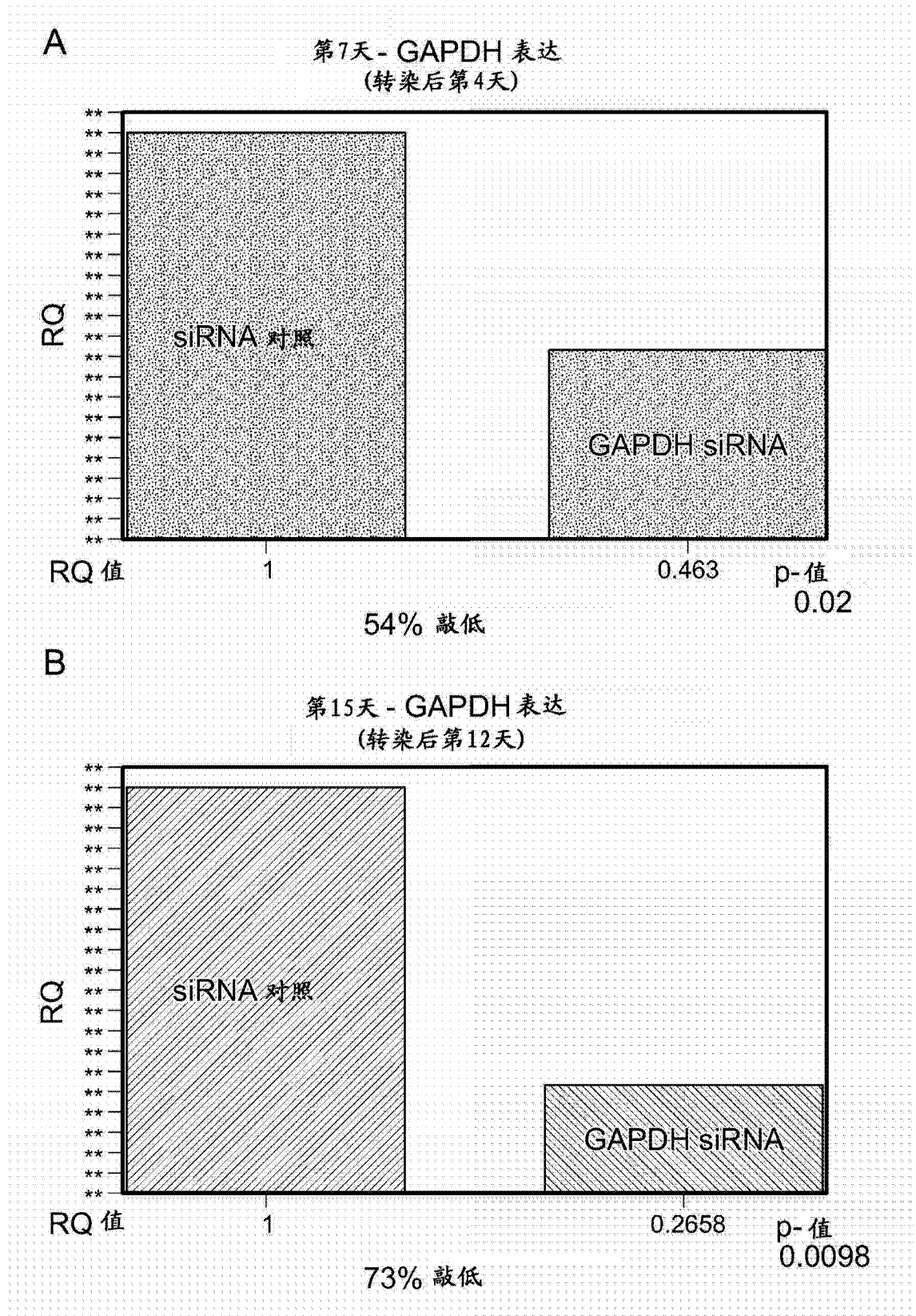


图 17

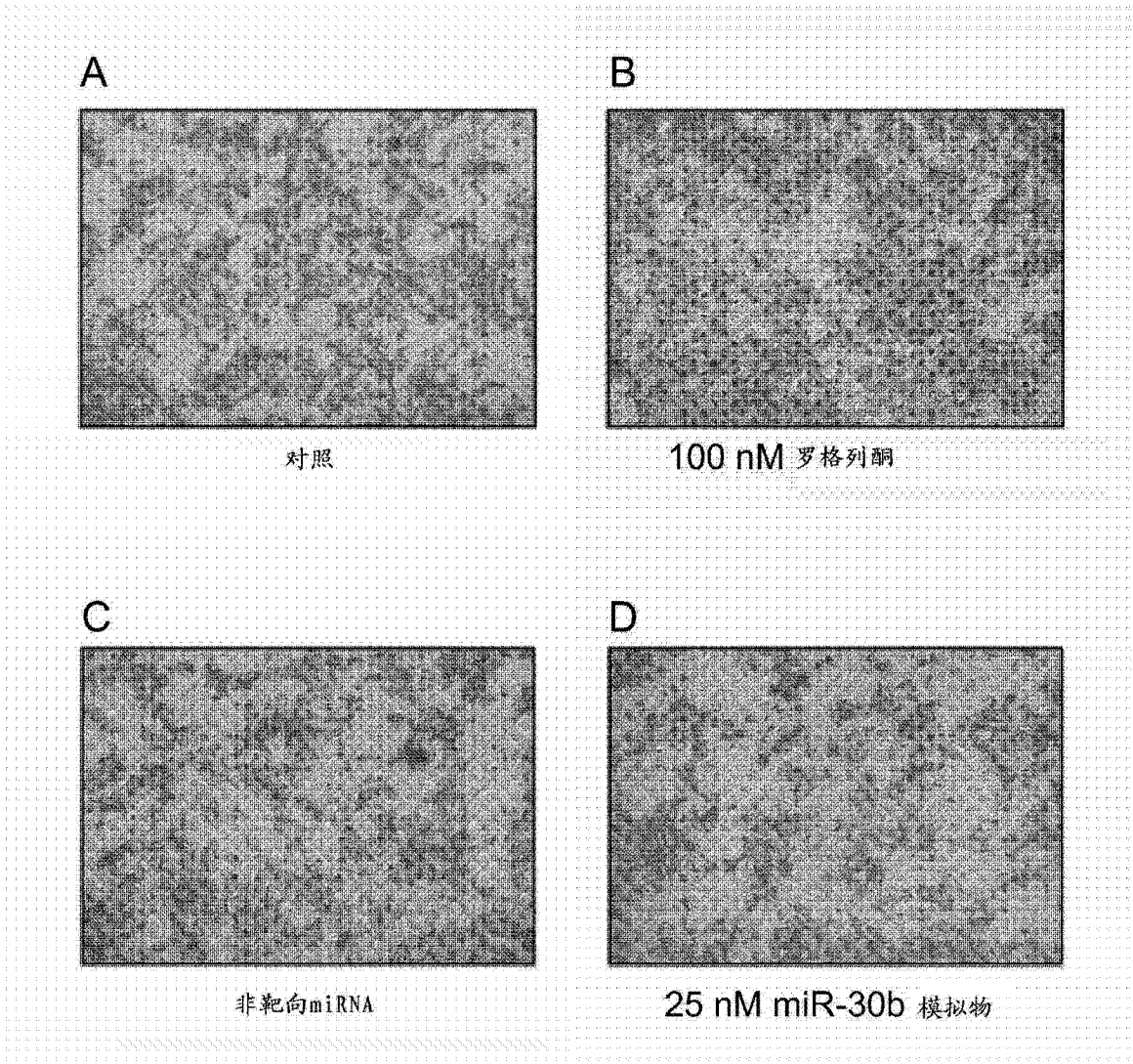


图 18

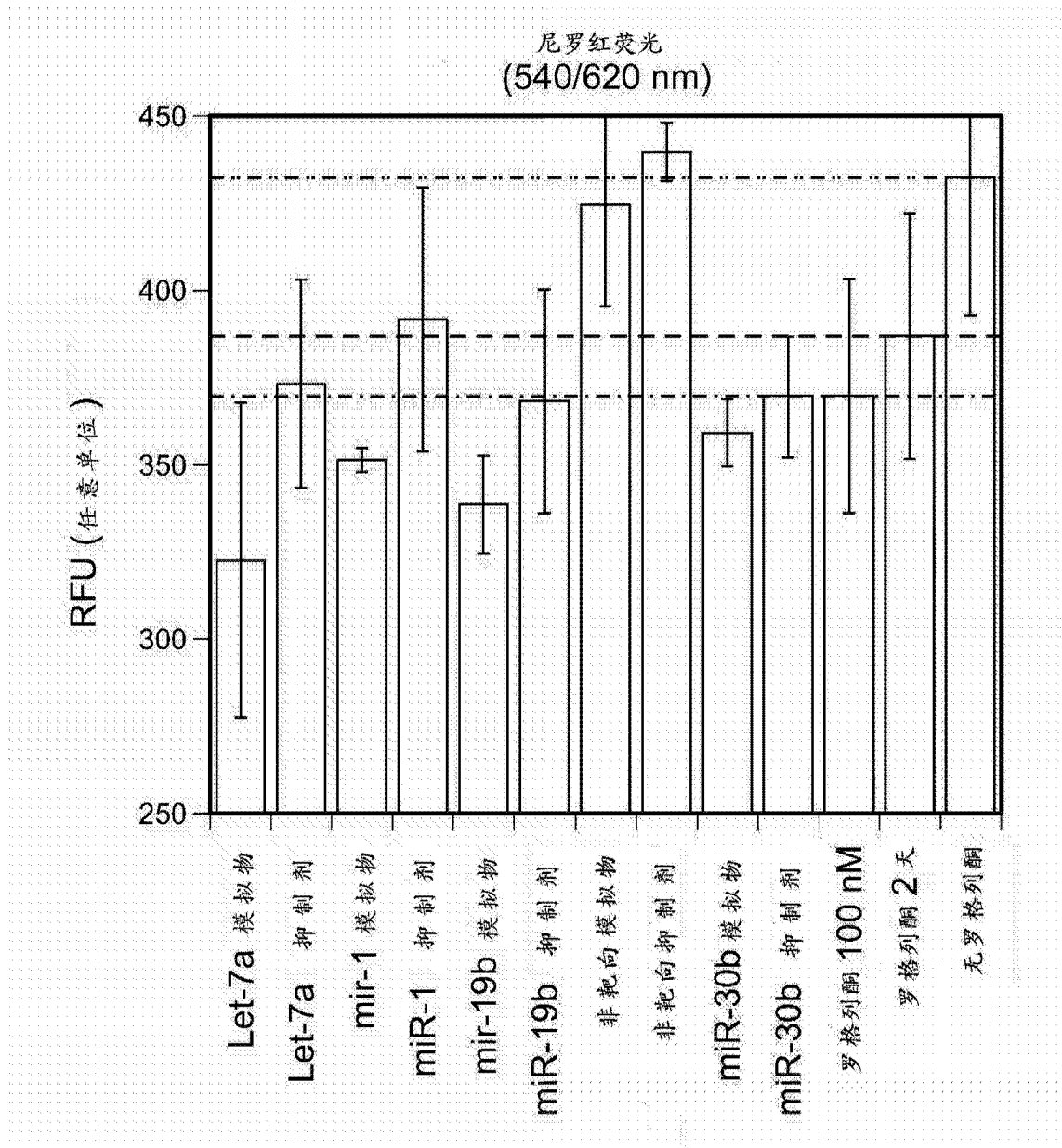


图 19

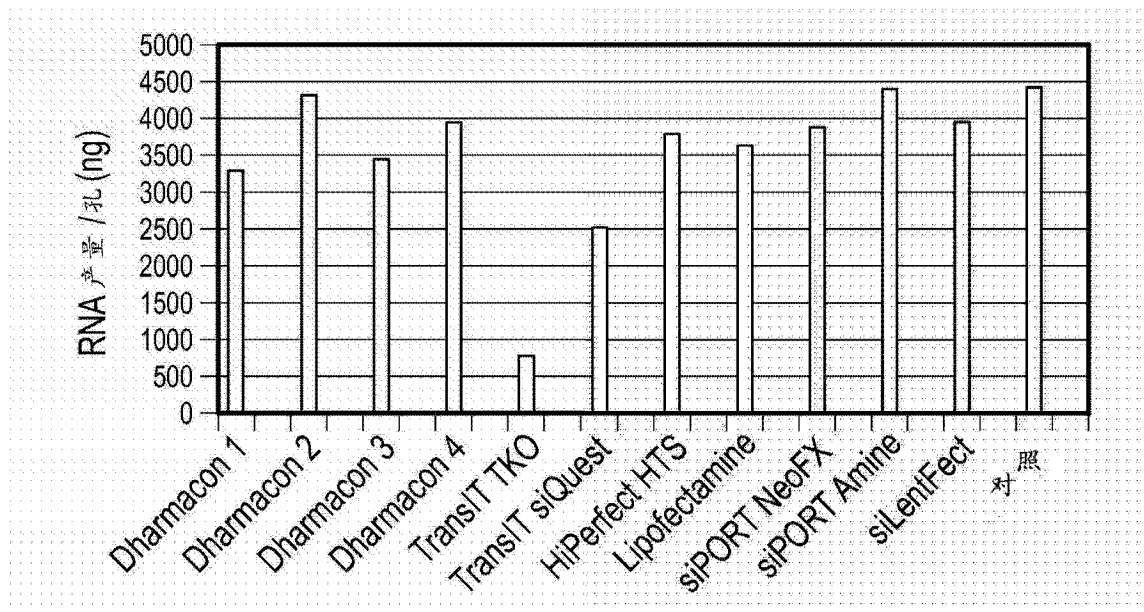


图 20

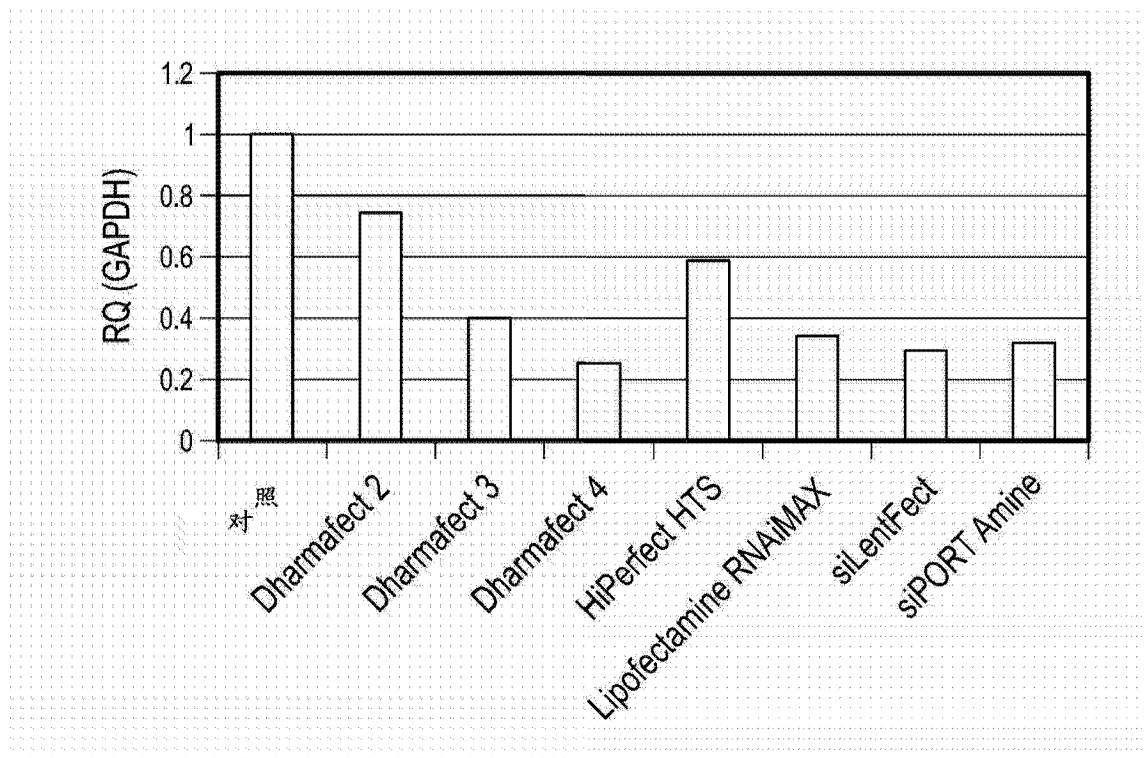


图 21

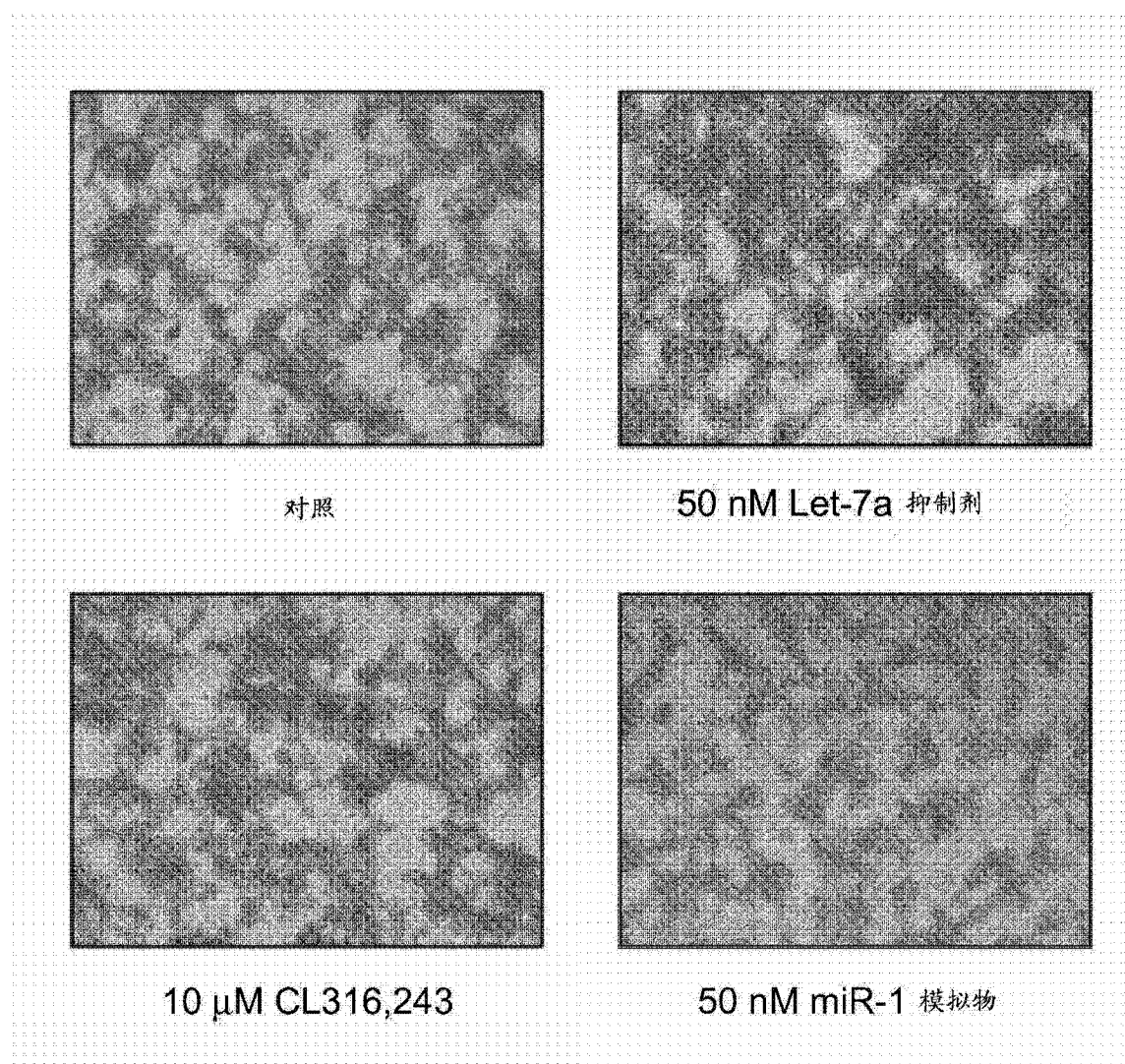


图 22

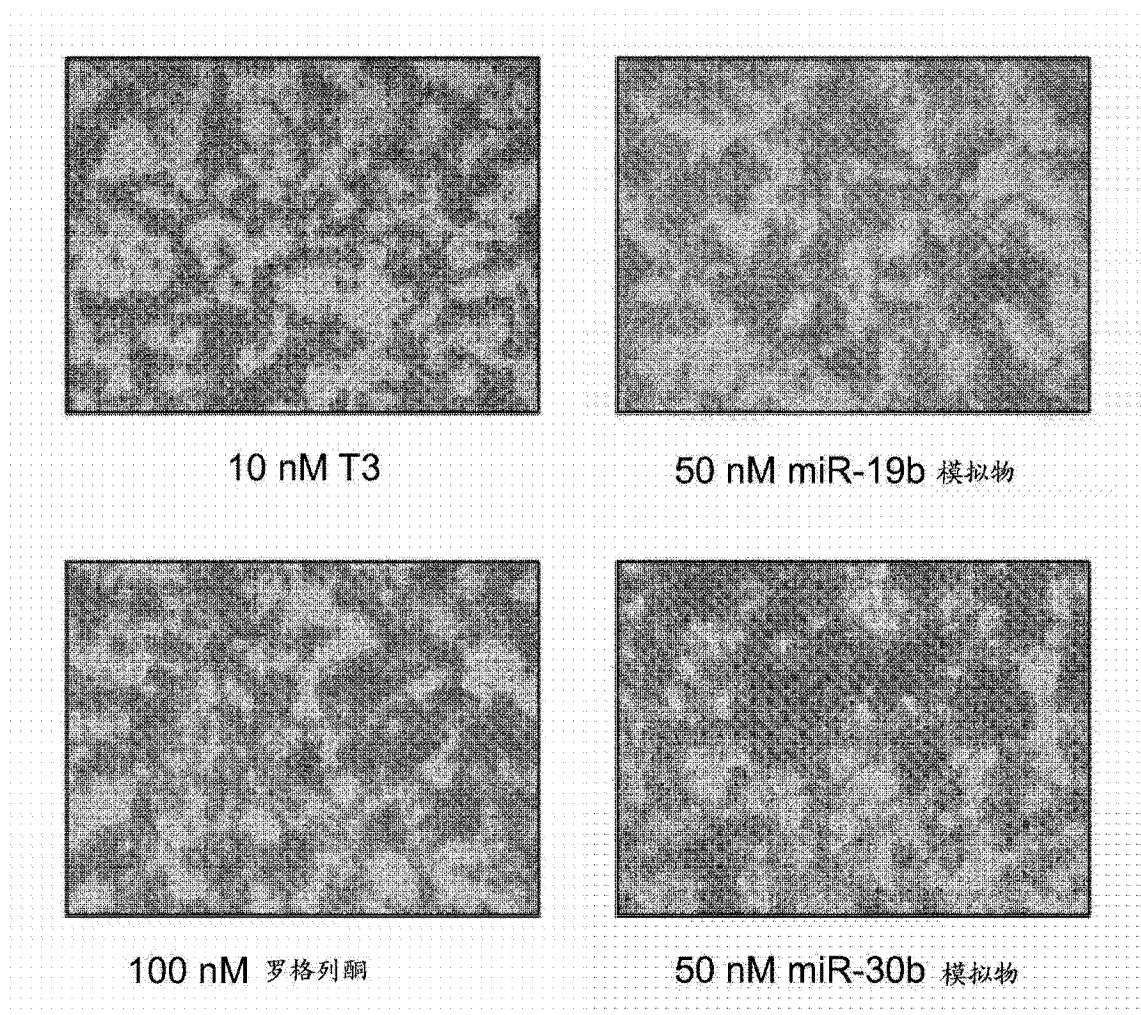


图 22(续)

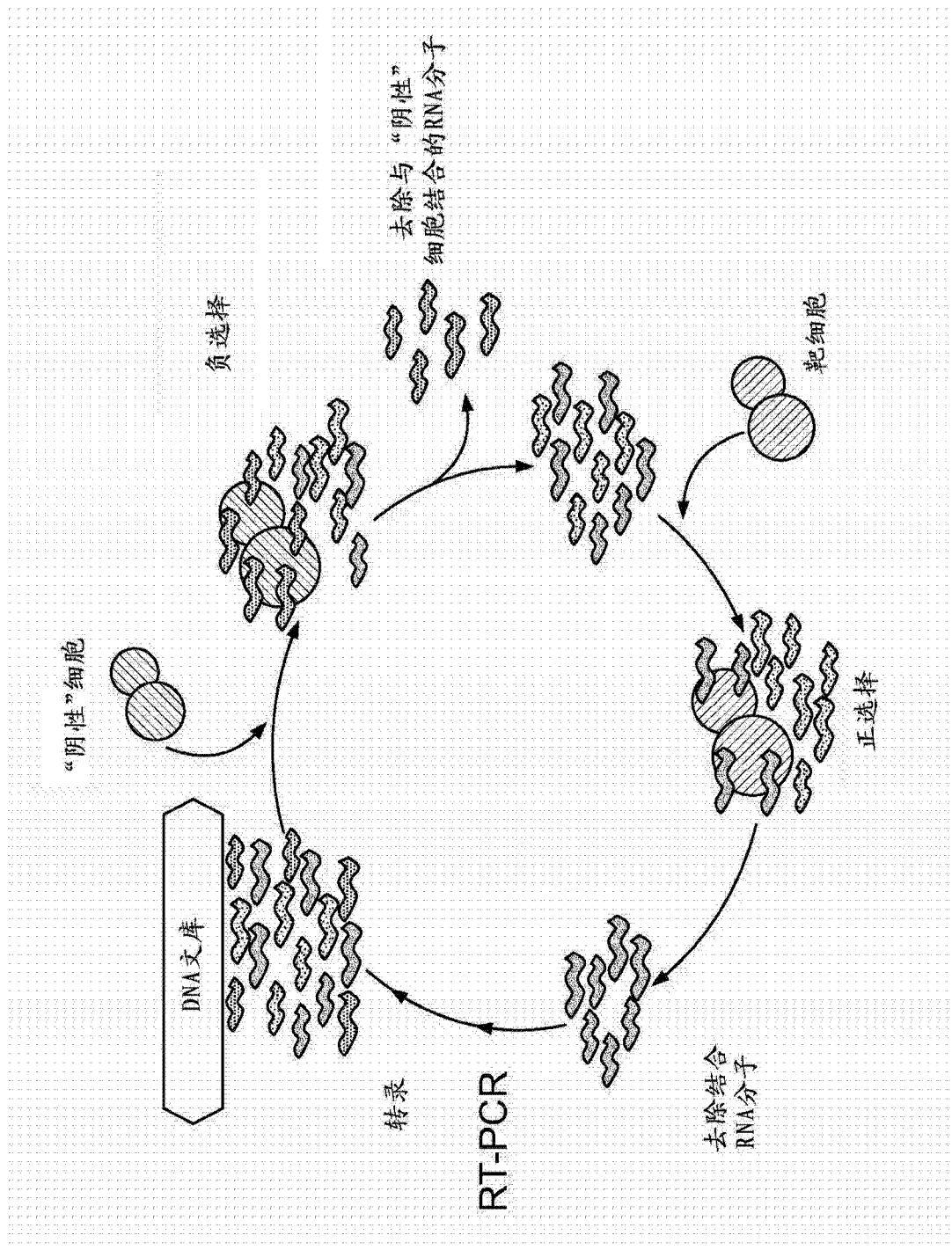


图 23

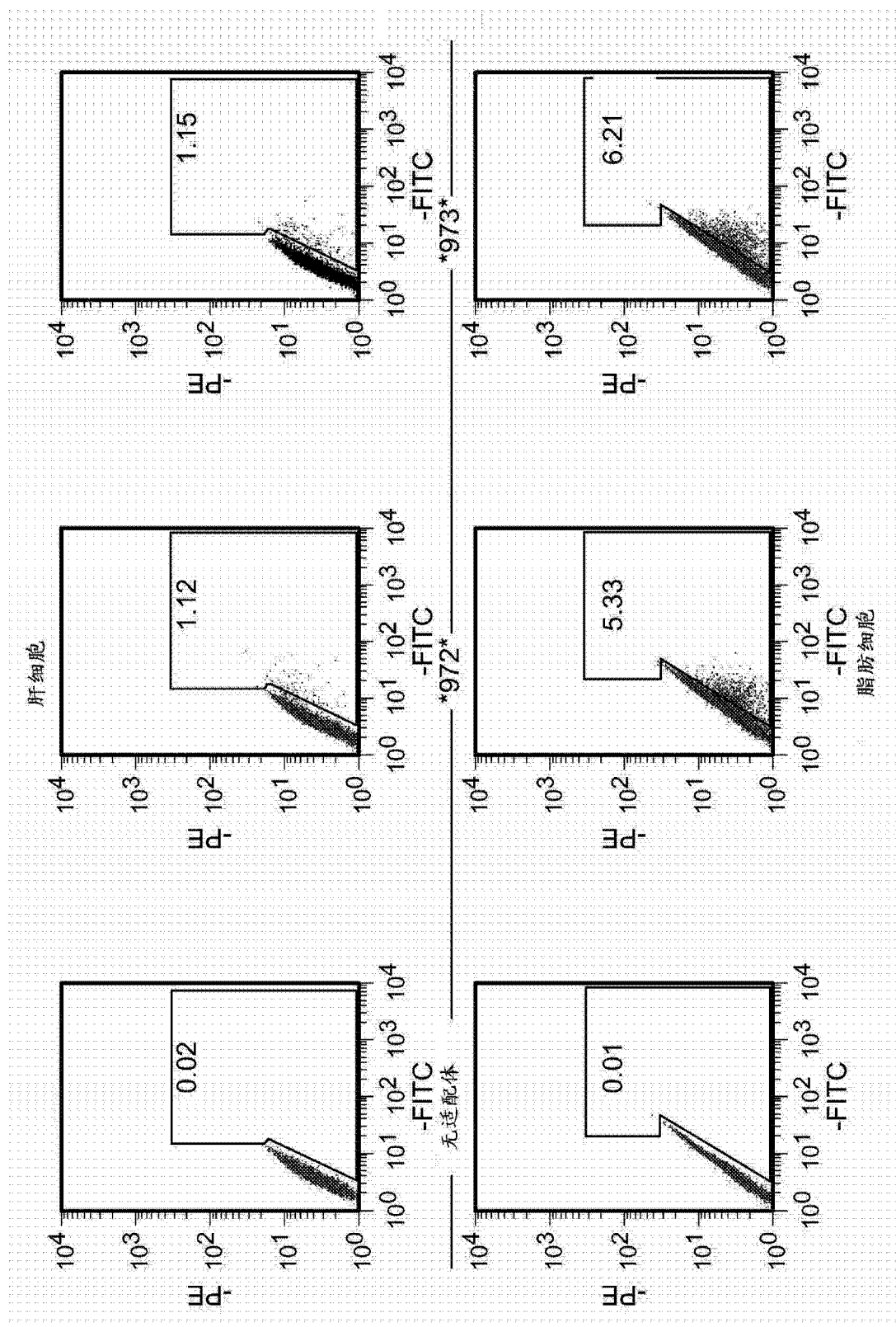


图 24

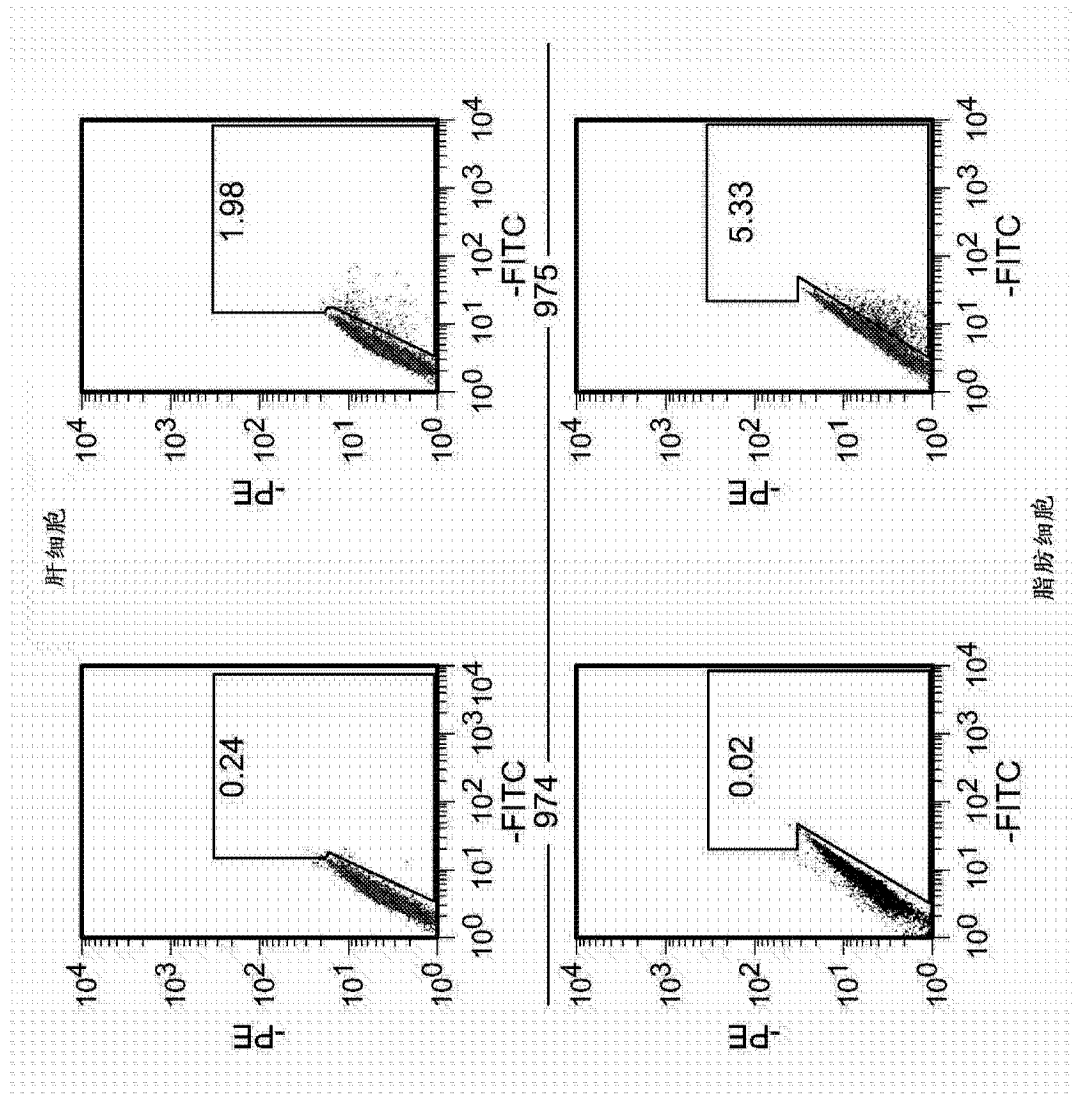


图 24(续)

Abstract

Provided are novel methods and compositions for the modulation of thermogenesis. Such methods are particularly advantageous in that they allow for the reduction of body fat in a subject without the subject having to adjust their caloric intake through dieting, modify their physical activity or undergo bariatric surgery. Accordingly, the methods of the invention are particularly useful for treating or preventing obesity. Also provided are methods of screening for novel agents that modulate the activity of thermogenic regulators.