

## Supplementary Material and Methods

### *RS-2982 and AntagoMIR122 Treatment of High Fat Diet- or Atherogenic Diet-Fed Mice*

Male C57BL/6J mice, 7–8 weeks old, were housed randomly in standard cages and were fed an HFD or atherogenic diet (consisting of 1% Chol and 0.5% cholic acid; see also [Supplementary Table 7](#)). All mice had free access to water during the experimental period. During the feeding period, body weight was monitored every 3 days. In the HFD experiment, after 4 or 6 weeks, the resultant obese mice were treated with antagoMIR122 (5  $\mu$ g/mouse once a week for 3 weeks) or intraperitoneally injected with RS-2982 (RORA agonist, 7.5 mg/kg twice a week for 3 weeks or 15 mg/kg 3 times a week for 3 weeks). The obese control (HFD) group was administered only saline with DMSO and antagomiR control. After 3 weeks of treatment, mice were killed, and livers were taken for RNA-sequencing analysis. Livers, gonadal white adipose, and skeletal muscle tissues were frozen in liquid nitrogen or in optimum cutting temperature-embedded frozen blocks for further RNA and histologic analysis. In the atherogenic diet experiment, mice were treated with 15 mg/kg RS-2982 after 3 weeks with the diet. After 3.5 weeks of treatment, mice were killed, and the livers were frozen in liquid nitrogen or in optimum cutting temperature-embedded frozen blocks. Plasma was collected from atherogenic diet-fed mice and stored at  $-20^{\circ}\text{C}$  for ALT and AST analysis with the Reflotron Analyzer and test strips (Roche, Basel, Switzerland).

### *Multiparameter Metabolic Assessment*

Metabolic and activity profiles of the mice were measured with the Promethion High-Definition Behavioral Phenotyping System (Sable Instruments, Inc, Las Vegas, NV), which is a multiparameter assessment incorporating subsystems for open-circuit indirect calorimetry, feeding, water intake, activity, running wheel, and body mass measurements in a conventional live-in home cage that minimizes stress. Data acquisition and instrument control were performed using MetaScreen software, version 2.2.18.0 (Promethion Software of Stable System International, Las Vegas NV), and the obtained raw data were processed with ExpeData, version 1.8.4, using an analysis script detailing all aspects of data transformation. C57BL/6 mice were fed for 6 weeks with HFD and then treated with 15 mg/kg RS-2982, 3 times a week for 2 weeks, and then were placed in metabolic chambers with free access to food and water and were subjected to a standard 12-hour dark/12-hour light cycle, which consisted of a 24-hour acclimation period followed by a 48-hour sampling duration. Respiratory gases were measured by using the GA-3 gas analyzer (Sable Systems) with a pull-mode negative-pressure system. Air flow was measured and controlled by the FR-8 (Sable Systems), with a set flow rate of 2000 mL/min. Water vapor was continuously measured, and its dilution effects on  $\text{O}_2$  and  $\text{CO}_2$  were mathematically compensated. Effective mass was calculated by analysis of covariance analysis. Respiratory quotient (RQ) was calculated as the ratio of  $V_{\text{CO}_2}/V_{\text{O}_2}$ .

Total energy expenditure was calculated as  $V_{\text{O}_2} \times (3.815 + 1.232 \times \text{RQ})$ , normalized to effective body mass, and expressed as kcal/h/kg<sup>eff.Mass</sup>. Fat oxidation (FO) and carbohydrate oxidation (CHO) (data not shown) were calculated as follows:  $\text{FO} = 1.69 \times V_{\text{O}_2} - 1.69 \times V_{\text{CO}_2}$  and  $\text{CHO} = 4.57 \times V_{\text{CO}_2} - 3.23 \times V_{\text{O}_2}$  and expressed as g/d/kg<sup>eff.Mass</sup>. Ambulatory activity and position were monitored simultaneously with the collection of the calorimetry data using the XYZ beam arrays with a beam spacing of 0.25 cm.

### *Oil Red O Staining*

Liver tissues were embedded in Optimal Cutting Temperature gel and cut into 10- $\mu$ m frozen sections. For Oil Red O staining, a stock solution of Oil Red O (Sigma-Aldrich) (1g/10 mL in propylene glycol) was prepared, filtered, and protected from light. Frozen sections were dipped in formalin and stained with Oil Red O for 15 minutes, followed by counterstaining with hematoxylin for 30 seconds.

### *Immunofluorescent Staining for Natural Killer Cells*

Paraffin-embedded sections were placed at  $60^{\circ}\text{C}$  for 20 minutes, incubated in xylene at room temperature for 15 minutes, and then transferred sequentially into 100% ethanol (EtOH), 95% EtOH, 70% EtOH, and 50% EtOH for 2 minutes at room temperature. Sections were rinsed in deionized water and stored in phosphate-buffered saline (PBS). To determine the optimal condition for antigen retrieval, we used buffer (10 mmol/L citrate, pH 6.0) that was previously shown to give optimal results with immunofluorescence. Tissues outlined with 100  $\mu$ L of liquid blocker CAS-Block (Termo Fisher Scientific) to minimize the volume of antibody solution needed for staining. As a marker of NK cells, mouse anti-NK1.1 (at a dilution of 1:30; NB100-77528ss, R&D Systems, Minneapolis, MN) was used and incubated overnight at  $4^{\circ}\text{C}$ . After washes with PBS, secondary antibodies conjugated with Cy-2 (Gout Anti Mouse IgG Alexa Fluor 488 at a dilution of 1:150; Ab 150117, Abcam, Cambridge, MA) were incubated for 1 hour at room temperature in the dark. To preserve staining, sections were stacked and covered with Fluoroshield Mounting Medium with DAPI (Abcam; ab104139) and then stored at  $4^{\circ}\text{C}$  while awaiting analysis. A Zeiss LSM 710 Confocal laser-scanning system attached to a Zeiss Axiovert 135M microscope used. Fluorescence images were collected by using a plan-apochromat Zeiss 63X lens. The system was equipped with an argon laser (488-nm excitation line) for green fluorescence and an Alexa Fluor laser (552 nm) for red fluorescence.

### *Triglycerides, Free Fatty Acids, and $\beta$ -Hydroxybutyrate Quantification*

To determine the liver and muscle lipid content, muscle and liver tissues (40–80 mg) were homogenized in 0.5 mL of chloroform: Tris solution (volume/volume, 1:1), the homogenate was transferred to 1 mL of chloroform: methanol solution (volume/volume, 2:1), centrifuged at 3000g (at  $-2^{\circ}\text{C}$ ) for 10 minutes (Heraeus [Hanau, Germany] Megafuge 16R centrifuge). The organic phase was mixed with 5% Triton X100 in chloroform, dried, and redissolved in water. After lipid

extraction, TG concentration in samples was measured with TG Quantification Kit (BioVision, Milpitas, CA), according to the manufacturer's instructions. Plasma FFAs and  $\beta$ -hydroxybutyrate were determined using commercial colorimetric kits (BioVision) directly from plasma samples.

### Human Blood Samples and Heparin Elimination

For the measurement of MIR122, FFA, and human Fgf21 (Abcam, Cambridge, UK) analysis in blood samples collected from patients undergoing major blood vessel cardiovascular surgery with the usage of the cardiopulmonary machine and systemic body cooling. This was performed under the approval of the Hadassah Hospital institutional review board committee approval number 0025-18-HMO. Informed consent and permission to use biological materials for research were obtained from all participants. Tube 2 indicates the time during the surgery before cooling the patient, and tube 3 represents the time when the body temperature was the lowest during the surgery. Heparin elimination from RNA solutions isolated from plasma samples of patients was performed according to the protocol described previously,<sup>1,2</sup> briefly, a 5- $\mu$ L RNA sample in water was mixed with 5  $\mu$ L of heparinase working solution (0.085 IU/mL of Heparinase I [Sigma-Aldrich; catalog no. H2519], 2000 units/mL of Ribo-Lock RNase Inhibitor [Life Technologies, Rockville, MD; catalog no. E00381], 10 mmol/L Tris HCl pH 7.5, 2 mmol/L CaCl<sub>2</sub>, and 25 mmol/L NaCl) and incubated at 25°C for 3 hours. After reaction, the samples were directly used in reverse-transcription reactions as RNA templates.

### RNA-Sequencing and Bioinformatics Analysis

Each sample represents RNA obtained from 1 mouse. RNA-sequencing libraries were constructed using TruSeq RNA Library preparation kit according to Illumina (San Diego, CA) protocol and sequenced with the Illumina NextSeq 500 System. The NextSeq base-calls files were converted to fastq files by using the bcl2fastq (version 2.15.0.4) program. Raw reads were quality trimmed; then adapter sequences were removed with cutadapt (version 1.7.1). The processed fastq files were mapped to the mouse transcriptome and genome by using TopHat (version 2.0.13). The genome version was GRCm38, with annotations from the Ensembl release. Quantification, normalization, and differential expression were done with the Cufflinks package (version 2.2.1). Results were visualized in R, by using the cummeRbund package (version 2.8.2) and in-house R scripts. Differentially expressed genes were defined as ones with at least 0.5 FPKM level of expression in at least 1 of the conditions and a *Q* value less than .05.

### Insulin Tolerance Test

At 4 months of age, the insulin tolerance test was performed on fasted C57BL/6 male mice fed for 6 weeks with HFD and injected with 15 mg/kg RS-2982 (or saline + DMSO) 3 times a week for 3 weeks. Mice received an intraperitoneal injection of human insulin (Actrapid, Novo

Nordisk A/S, Copenhagen, Denmark) at a dosage of 0.88 units/kg followed by glucose checks every 20 minutes. Glucose levels were measured at 0, 20, 40, 60, 80, and 100 minutes after insulin injection. Glucose measurements were assessed on blood from the tail after a tail snip at indicated timepoints by using a blood glucometer (Accu-Chek, Roche, Indianapolis, IN) and test strips (Accu-Chek).

### Tissue Histology and Immunohistochemistry

Livers and adipose samples were placed in 4% buffered formaldehyde for 24 hours, followed by 80% ethanol, and then embedded in paraffin blocks. Liver and adipose tissues were cut into 5-mm sections, deparaffinized with xylene, and hydrated through graded ethanol. For the H&E staining, tissue sections were stained with hematoxylin (Emmonya Biotech, Sofia, Bulgaria) and eosin (Surgipath, Leica, Wetzlar, Germany). Liver macrophages were stained using rat anti-mouse F4/80 antigen (Serotec, Oxford, UK), followed by anti-rat HRP (Histofine, Nichirei Bio, Tokyo, Japan) and developed with a 3,3'-diaminobenzidine tetra hydrochloride (DAB) kit (Zymed, San Francisco, CA). Liver sections were stained for Masson trichrome (Sigma-Aldrich). Liver CD3<sup>+</sup> T cells were stained using rat anti-human-CD3-antibody (Bio-Rad, Hercules, CA), followed by anti-rat HRP (Histofine) and developed with aminoethyl carbazole (Invitrogen).  $\alpha$ -SMA-positive cells were stained using mouse anti-human smooth muscle actin antibody (Dako), followed by anti-mouse HRP (Dako, Glostrup, Denmark) and developed with DAB. The percent area stained positively per high-power field was calculated by ImageJ software in 5–10 random fields.

### General Chemical Procedures

Anhydrous solvents were purchased from Aldrich Chemical (Milwaukee, WI). All commercially available reagents were used without further purifications. Reagents were purchased from commercial sources. All reactions were carried out under nitrogen in oven-dried glassware unless otherwise noted. Thin-layer chromatography was performed on Analtech GHLF silica gel plates (Analtech, Inc, Newark, DE). Column chromatography was accomplished on Combiflash Rf200. <sup>1</sup>H, <sup>13</sup>C, <sup>19</sup>F, and <sup>31</sup>P nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Ascend 400 spectrometer at 25°C (400, 101, 377, and 162 MHz) as noted, and residual proton solvent signals were used as internal standards. Deuterium exchange and decoupling experiments were used to confirm proton assignments. NMR processing was performed with MestReNova, version 10.0.2-15465. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), br (broad), bs (broad singlet), and m (multiplet). Coupling constants (*J*) are in hertz. Mass spectra were determined on a Waters Acuity SQ spectrometer (Milford, MA) by using electrospray ionization. Purity of the final compounds was determined to be >95%, using ultraperformance liquid chromatography analyses performed on a Waters Acuity Ultraperformance Liquid Chromatography System with a Kinetex LC column

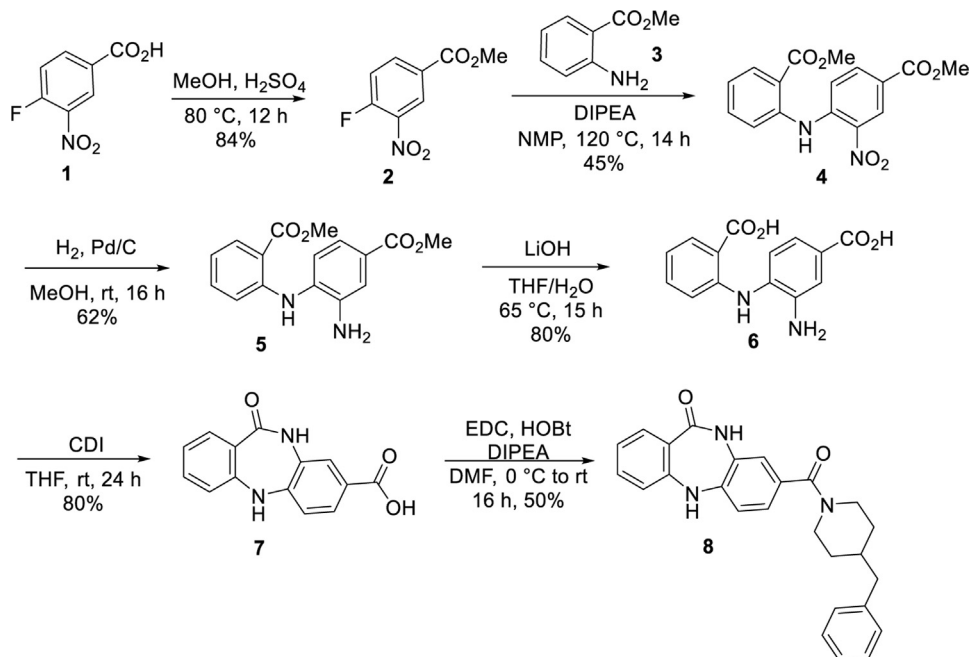
(2.1 mm Å, 50 mm, 1.7 μm, C18, 100 Å) and further supported by clean NMR spectra. Mobile phase flow was 0.4 mL/min with a 1.20-minute gradient from 95% aqueous media (0.05% formic acid) to 95% CH<sub>3</sub>CN (0.05% formic acid) and a 4.5-minute total acquisition time. Photodiode array detection was from 190 to 360 nm.

Scheme 1. Synthesis of RS-2982.

**Methyl 4-fluoro-3-nitrobenzoate (2).** 4-Fluoro-3-nitrobenzoic acid **1** (10 g, 54 mmol) was dissolved in methanol (200 mL) and concentrated H<sub>2</sub>SO<sub>4</sub> (1 mL) at room temperature. The reaction mixture was stirred overnight at

afford compound **4** as a yellow solid (996 mg, 45%); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.15 (s, 1H), 8.66 (d, *J* = 2.1 Hz, 1H), 8.09–7.96 (m, 2H), 7.71–7.61 (m, 2H), 7.59 (d, *J* = 9.0 Hz, 1H), 7.29 (ddd, *J* = 8.2, 5.9, 2.1 Hz, 1H), 3.87 (s, 6H); <sup>13</sup>C NMR (101 MHz, DMSO): δ 166.6, 164.5, 142.7, 139.7, 135.4, 134.7, 133.9, 131.5, 128.0, 124.2, 121.9, 120.4, 120.0, 117.8, 52.5, 52.3; SM (IS): *m/z*: 331.4 [M + 1].

**Methyl 3-amino-4-((2-(methoxycarbonyl)phenyl)amino)benzoate (5).** A solution of methyl 4-((2-(methoxycarbonyl)phenyl)amino)-3-nitrobenzoate **4** (2.5 g, 7.5 mmol) and 10% Pd/C (1.25 g, 50% wet) in methanol



80°C. After completion of the reaction, the solvent was evaporated under reduced pressure. The crude mixture was diluted with H<sub>2</sub>O (200 mL) and basified with a saturated solution of NaHCO<sub>3</sub>. The precipitated solid was filtered, washed with water (2 × 100 mL) and dried under vacuum to afford compound **2** as a white solid (10.75 g, 84%); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.54 (dd, *J* = 7.3, 2.3 Hz, 1H), 8.31 (ddd, *J* = 8.8, 4.3, 2.3 Hz, 1H), 7.73 (dd, *J* = 11.1, 8.7 Hz, 1H), 3.90 (s, 3H); <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>): 158.7, 156.0, 136.9, 136.8 (d, *J* = 10.8 Hz), 127.1 (d, *J* = 1.6 Hz), 126.7 (d, *J* = 3.9 Hz), 119.4 (d, *J* = 21.7 Hz), 52.9; <sup>19</sup>F NMR (377 MHz, DMSO) δ -111.97.

**Methyl 4-((2-(methoxycarbonyl)phenyl)amino)-3-nitrobenzoate (4).** To a solution of methyl 4-fluoro-3-nitrobenzoate **2** (1 g, 6.61 mmol) in N-methyl-2-pyrrolidone (20 mL) were added diisopropylethylamine (0.76 mL, 19.83 mmol) and methyl 4-fluoro-3-nitrobenzoate **3** (1.5 g, 9.92 mmol) at room temperature, under inert atmosphere. The mixture was stirred at 120°C for 14 hours, and after completion of the reaction, the mixture was cooled down to room temperature, diluted with diethyl ether (20 mL), and stirred for 1 hour. The obtained solid was filtered, washed with ethyl ethanoate (20 mL), and dried under vacuum to

(MeOH) was stirred under hydrogen atmosphere for 16 hours at room temperature. After completion of the reaction, the mixture was filtered through Celite (Oakwood Products, Inc Estill, SC) and washed with 20% MeOH/dichloromethane (250 mL). The filtrate was concentrated, and the crude residue was purified by flash column chromatography (EtOAc/hexanes 3/7) to afford compound **5** as a yellow solid (1.4 g, 62%); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.93 (s, 1H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.52–7.36 (m, 2H), 7.21 (s, 2H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.88–6.72 (m, 1H), 5.19 (s, 2H), 3.85 (s, 3H), 3.80 (s, 3H); <sup>13</sup>C NMR (101 MHz, DMSO) δ 167.9, 166.3, 146.7, 142.2, 134.3, 131.2, 130.6, 125.5, 121.9, 118.1, 117.7, 116.2, 114.9, 112.3, 51.9, 51.8; SM (IS): *m/z*: 301.4 [M + 1].

**3-Amino-4-((2-(methoxycarbonyl)phenyl)amino)benzoic acid (6).** To a solution of methyl 3-amino-4-((2-(methoxycarbonyl)phenyl)amino)benzoate **5** (1.4 g, 4.66 mmol) in a mixture of tetrahydrofuran:H<sub>2</sub>O (2.5/L, 105 mL) was added lithium hydroxide monohydrate (1.75 g, 41.9 mmol) at room temperature. The reaction mixture was stirred at 65°C for 5 hours, and the volatiles were removed under vacuum. The pH of the residue was acidified to 4 with 2N HCl. The precipitated solid was filtered, washed with water (10 mL) and dried under vacuum to afford

compound 6 as a white solid (1 g, 80%).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.64 (s, 2H), 9.20 (s, 1H), 7.89 (d,  $J = 7.9$  Hz, 1H), 7.44 – 7.31 (m, 2H), 7.18 (s, 2H), 6.91 (d,  $J = 8.5$  Hz, 1H), 6.76 (t,  $J = 7.5$  Hz, 1H), 5.06 (s, 2H);  $^{13}\text{C}$  NMR (101 MHz, DMSO)  $\delta$  169.8, 167.4, 147.3, 142.1, 134.0, 131.7, 130.4, 126.6, 121.9, 118.4, 117.3, 116.5, 114.5, 112.8; SM (IS):  $m/z$ : 273.4 [M + 1].

**11-Oxo-10,11-dihydro-5H-dibenzo[b,e][1,4]diazepine-8-carboxylic acid (7).** A solution of compound 6 (1 g, 3.67 mmol) and 1,1'-carbonyldiimidazole (2.39 g, 14.6 mmol) in THF (40 mL) was stirred at room temperature for 24 hours under inert atmosphere. After completion of the reaction, the volatiles were removed under vacuum. The pH of the residue was adjusted to 2 using 2N HCl. The precipitated solid was filtered, washed with pentane (10 mL), and dried under vacuum to afford compound 7 as a pale green solid (746 mg, 80%).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ) 12.66 (s, 1H), 9.93 (s, 1H), 8.28 (s, 1H), 7.70 (dd,  $J = 7.9, 1.7$  Hz, 1H), 7.57 – 7.49 (m, 2H), 7.36 (td,  $J = 7.9, 1.7$  Hz, 1H), 7.02 (dd,  $J = 17.0, 8.1$  Hz, 2H), 6.91 (t,  $J = 7.4$  Hz, 1H);  $^{13}\text{C}$  NMR (101 MHz, DMSO)  $\delta$  167.4, 166.7, 148.8, 143.8, 133.5, 132.3, 129.2, 126.0, 125.0, 122.5, 122.2, 121.1, 119.4, 119.2, SM (IS):  $m/z$ : 255.4 [M + 1].

**8-(4-Benzylpiperidine-1-carbonyl)-10,11a-dihydro-4aH-dibenzo[b,e][1,4]diazepin-11(5H)-one (8).** To a solution of compound 7 (100 mg, 0.393 mmol) in dimethylformamide (5 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HCl (121 mg, 0.629 mmol), hydroxybenzotriazole (85 mg, 6.29 mmol), and 4-benzylpiperidine (91  $\mu\text{L}$ , 0.511 mmol) and DIPEA (205  $\mu\text{L}$ , 0.117 mmol) at 0°C. The reaction mixture was stirred for 16 hours at room temperature. After completion of the reaction, water was added (30 mL). The precipitated solid was filtered and washed with water (10 mL). The crude residue was purified by flash column chromatography (dichloromethane/MeOH, 95/5) to afford compound 8 as a light yellow solid (111 mg, 68 %);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  9.91 (s, 1H), 8.06 (s, 1H), 7.68 (d,  $J = 7.8$  Hz, 1H), 7.34 (t,  $J = 7.8$  Hz, 1H), 7.27 (t,  $J = 7.8$  Hz, 2H), 7.16 (d,  $J = 7.8$  Hz, 3H), 7.05–6.99 (m, 4H), 6.89 (d,  $J = 7.8$  Hz, 1H), 4.34 (s, 1H), 3.67 (s, 1H), 2.78 (s, 2H), 2.52 (s, 2H), 1.75 (s, 1H), 1.56 (s, 2H), 1.17 – 1.02 (m, 2H);  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  168.2, 167.6, 149.6, 140.7, 140.0, 133.4, 132.2, 130.6, 129.3, 129.0, 128.1, 125.8, 123.4, 122.5, 120.9, 120.0, 119.4, 119.1, 42.1, 37.5, 31.6; HRMS (ESI) [M + H] $^+$  calculated for C<sub>26</sub>H<sub>26</sub>N<sub>3</sub>O<sub>2</sub>: 412.1947, found: 412.2018.

### Lipidomic Analysis

Serum lipidomic profiles were semiquantified by ultra-high performance liquid chromatography coupled to mass spectrometry, as previously described.<sup>3</sup> Briefly, 2 separate ultra-high performance liquid chromatography coupled to mass spectrometry-based platforms analyzing methanol and chloroform/methanol serum extracts were used. Identified ion features in the methanol extract platform included fatty acids, oxidized fatty acids, acyl carnitines, lysoglycerophospholipids (monoacylglycerophospholipids and monoetherglycerophospholipids), free sphingoid bases, bile acids, and steroid sulfates. The chloroform/methanol

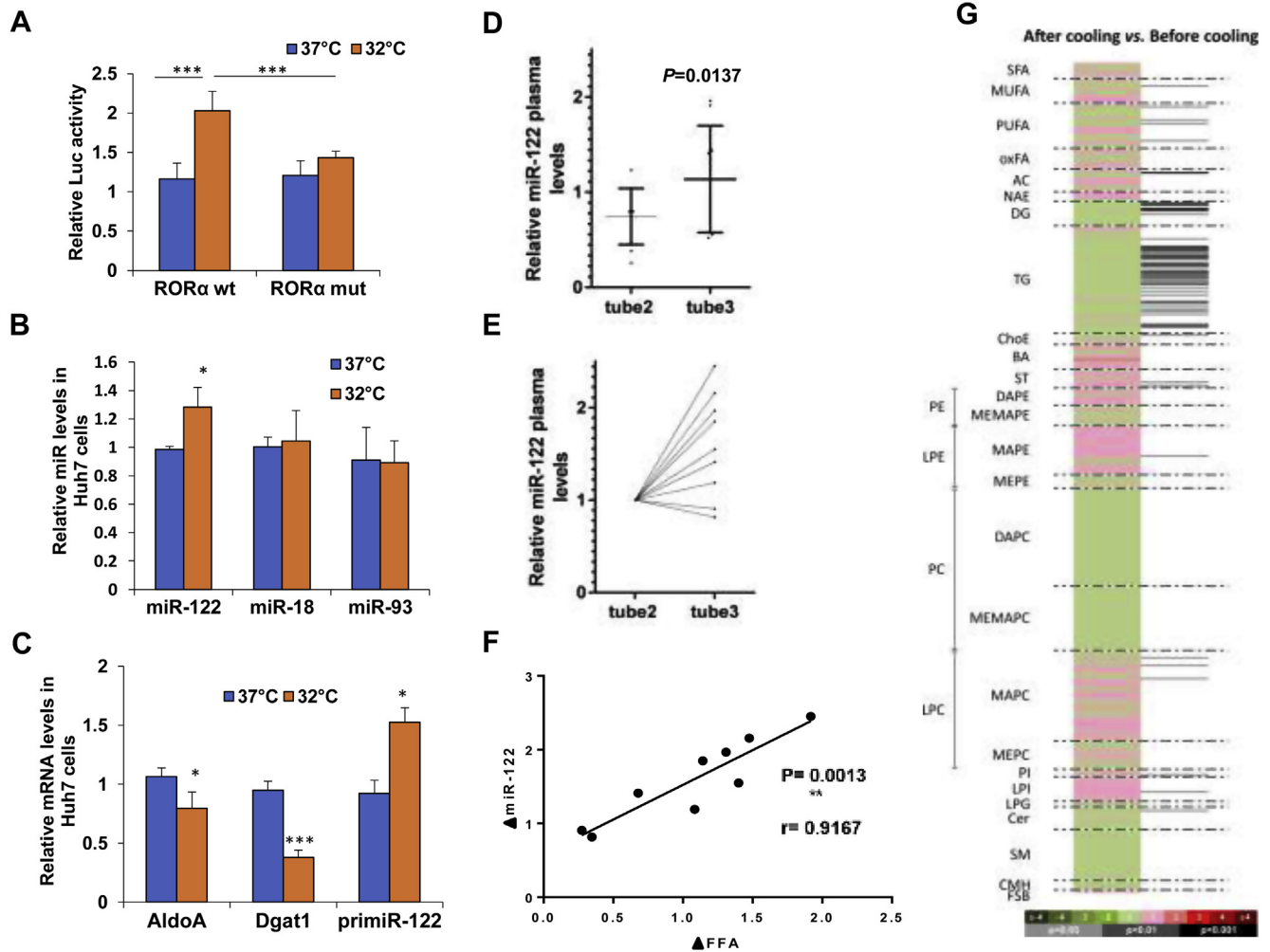
extract platform provided coverage over glycerolipids (di and triglycerides), cholesterol esters, sphingolipids (ceramides and sphingomyelins), and glycerophospholipids (diacylglycerophospholipids and 1-ether, 2-acylglycerophospholipids). Specific metabolite extraction procedures performed for each platform, chromatographic separation conditions and mass spectrometric detection conditions are also detailed in Barr et al.<sup>3</sup> Lipidomics data were preprocessed using the TargetLynx application manager for MassLynx 4.1 (Waters Corp, Milford, MA). Data normalization was performed by following the procedure described previously by us.<sup>4</sup>

**Immunofluorescent Staining for Natural Killer Cells.** Paraffin-embedded sections were placed at 60°C for 20 minutes, incubated in xylene at room temperature for 15 minutes and then transferred sequentially into 100% EtOH, 95% EtOH, 70% EtOH, and 50% EtOH for 2 minutes at room temperature. Sections were rinsed in deionized water and stored in PBS. To determine the optimal condition for antigen retrieval, we used buffer (10 mmol/L citrate, pH 6.0) that was previously shown to give optimal results with immunofluorescence. Tissues were outlined with 100  $\mu\text{L}$  of liquid Blocker CAS-Block to minimize the volume of antibody solution needed for staining. As a marker of NK cells, mouse anti-NK1.1 (at a dilution of 1:30; R&D, NB100-77528ss) was used and incubated overnight at 4°C. After washes with PBS, secondary antibodies conjugated with Cy-2 (Gout Anti Mouse IgG Alexa Fluor 488, at a dilution of 1:150; Abcam, Ab 150117) were incubated for 1 hour at room temperature in the dark. To preserve staining, sections were stacked and covered with Fluoroshield Mounting Medium with DAPI (Abcam; ab104139) and then stored at 4°C while awaiting analysis. A Zeiss LSM 710 Confocal laser-scanning system (Zeiss, Germany) attached to a Zeiss Axiovert 135M microscope used. Fluorescence images were collected by using plan-apochromat Zeiss 63X lens. The system was equipped with an argon laser (488-nm excitation line) for green fluorescence and an Alexa Fluor laser (552 nm) for red fluorescence.

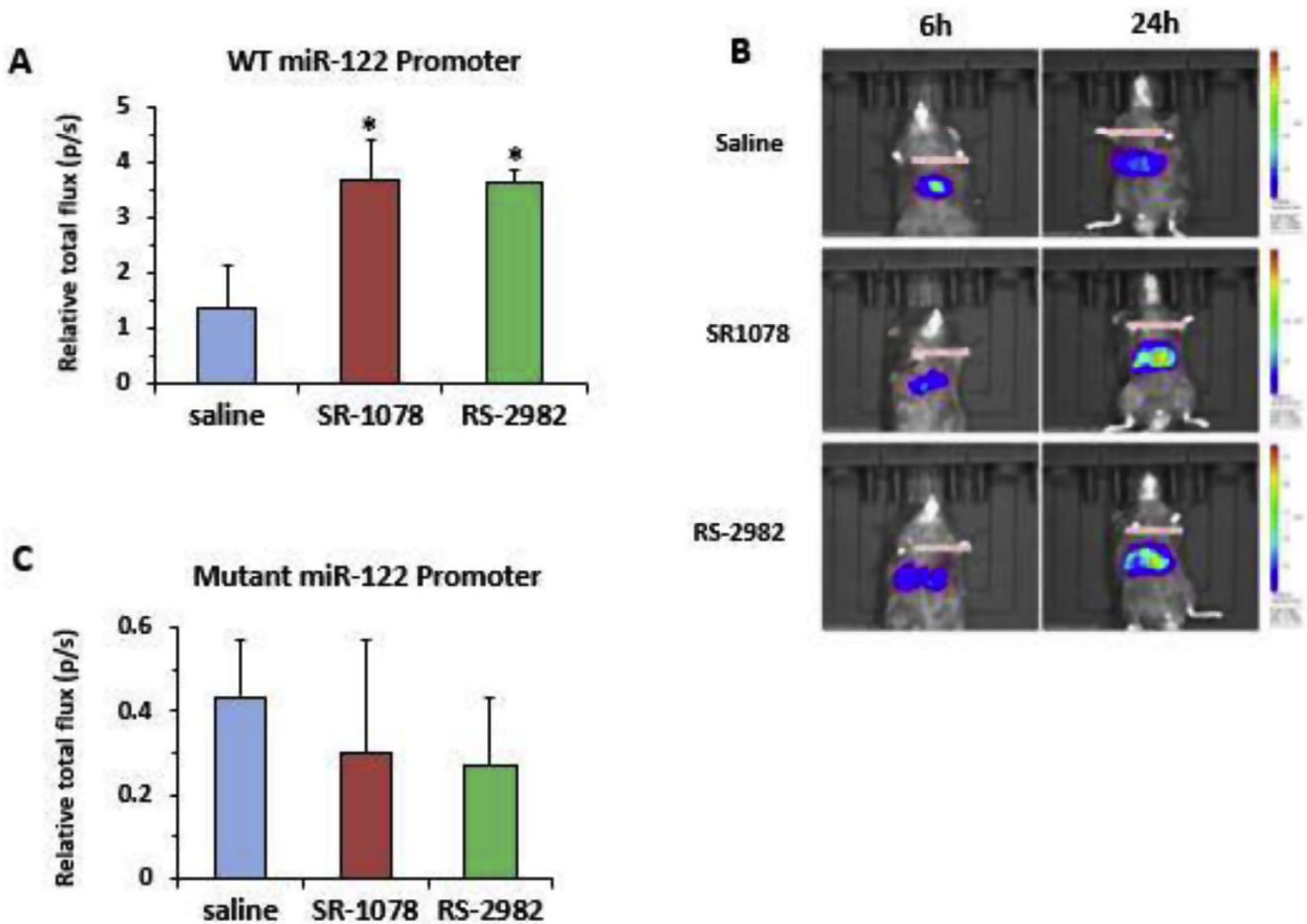
### Supplementary References

1. Kondratov K, Kurapeev D, Popov M, et al. Heparinase treatment of heparin-contaminated plasma from coronary artery bypass grafting patients enables reliable quantification of microRNAs. *Biomol Detect Quantif* 2016;8:9–14.
2. Izraeli S, Pfliegerer C, Lion T. Detection of gene expression by PCR amplification of RNA derived from frozen heparinized whole blood. *Nucleic Acids Res* 1991;19:6051.
3. Barr J, Caballería J, Martínez-Arranz I, et al. Obesity-dependent metabolic signatures associated with nonalcoholic fatty liver disease progression. *J Proteome Res* 2012;11:2521–2532.
4. Martínez-Arranz I, Mayo R, Pérez-Cormenzana M, et al. Enhancing metabolomics research through data mining. *J Proteomics* 2015;127(Pt B):275–288.





**Supplementary Figure 1.** Cold exposure increases MIR122 expression in a RORa-dependent manner. (A–C) Huh7 cells were incubated overnight at 32°C or 37°C. (A) Relative luciferase activity in Huh7 cells were transfected with pMIR122 carrying a wild type (wt) or mutated (mut) RORa binding site and incubated 1 day later at 32°C or 37°C. Luciferase activity was measured 48 hours after transfection and normalized to *Renilla* luciferase activity expressed from a cotransfected pRL plasmid. (B) qRT-PCR analysis of microRNAs. (C) qRT-PCR analysis of MIR122 target genes and pri-MIR122. (D) qRT-PCR analysis of MIR122 levels in plasma from patients undergoing major blood vessel cardiovascular surgery. Tube 2 represent the time during surgery before body cooling, and tube 3 represents the time when the body temperature was the lowest. (E) Relative changes of MIR122 plasma levels between tube 2 and tube 3 of each patient (n = 9). See also [Supplementary Table 3](#), which shows the reduced temperature for each patient. (F) Positive correlation between ΔMIR122 and ΔFFA in the plasma of hypothermic patients. ΔMIR122 and ΔFFA were calculated as relative changes between tube 2 and tube 3. The positive correlation coefficient (r) was calculated by Spearman correlation test. Data are represented as mean ± SD. \*P < .05, \*\*P < .01, \*\*\*P < .001. (G) Heatmap representing individual metabolic features from human plasma in a comparison between after versus before cooling. Green sections of the heatmap denote reduced metabolites (negative log2 pairwise fold changes), and red sections denote metabolites increased (positive log2 pairwise fold-changes). Gray/black bars indicate significant P values of the paired Student t test (light gray, P < .05; dark gray, P < .01; black, P < .001). Abbreviations from top to bottom are as follows: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; oxFA, oxidized fatty acid; AC, acylcarnitines; NAE, N-acylethanolamines; DG, diglycerides; TG, triglycerides; ChoE, cholesteryl ester; BA, bile acids; ST, steroids; phosphatidylethanolamines (PE); DAPE, diacyl-phosphatidylethanolamines; MEMAPE, 1-ether;2-acyl-phosphatidylethanolamines; LPE, lyso-phosphatidylethanolamines; MAPE, monoacyl-phosphatidylethanolamines; MEPE, monoether-phosphatidylethanolamines; PC, phosphatidylcholines; PI, phosphatidylinositols; LPI, lysoPI; LPG, lysophosphatidylglycerols; cer, ceramides; SM, sphingomyelins; CMH, monohexosylceramides; FSB, free sphingoid bases; DAPC, 1,2-Diarachidoyl-sn-glycero-3-phosphocholine; MEMAPC, 1-ether,2-acylglycerophosphocholine; MAPC, monoacylglycerophosphocholine; MEPC, monoetherglycerophosphocholine; LPC, lysophosphatidylcholine.



**Supplementary Figure 2.** Mutating the RORA binding site in the MIR122 promoter abolishes activation by RS-2982 in vivo. (A, B) C57BL/6 male mice were hydrodynamically tail vein injected with 5  $\mu$ g of (A, B) wild-type or (C) mutant MIR122 promoter reporter plasmid. Two days later, mice were injected with saline (n = 3), RS-2982 (n = 2), or SR1078 (positive control) (n = 2) at 10 mg/kg. Mice were imaged by IVIS Lumina LT at 6 and 24 hours after treatment. Images shown are of mice injected with the wild-type MIR122 promoter plasmid. The graphs exhibit the average values of the 24-hour/6-hour total flux ratio of each mouse group. Data are represented as mean  $\pm$  SD. \* $P < .05$ .

**Supplementary Table 1.** Primers Used for Real-Time PCR

Primer number	Direction	Sequence (5' to 3')
Mouse DGAT1	Forward	GTGGTTTCAGCAATTATCGTGG
	Reverse	GGGTCCCTTCAGAAACAGAGAC
Mouse HPRT	Forward	GCGATGATGAACCAGTTATGA
	Reverse	ATCTCGAGCAAGTCTTTCAGTCCT
Mouse pri-miR-122	Forward	TTCGGGAACTATGTGGAGTCACTTTG
	Reverse	CGCAAGGCTGCCCTCAAACCCCTCAG
Mouse pre-miR-122	Forward	CCATCAAACGCCATTATCACACTA
	Reverse	CACACAATGGAGAAGTCTAGCACAA
Mouse ALDOA	Forward	GGGTGATCCTCTTCCACGAGA
	Reverse	AGGGGCACCACACCCTTATC
Mouse AGPAT1	Forward	GGGCGCAATGTGAGAACATG
	Reverse	CTGGCAGGACCTCCATCATT
Mouse CPT1 $\alpha$	Forward	AGACAAGAACCCCAACATCC
	Reverse	CAAAGGTGTCAAATGGGAAGG
Mouse TGFB2	Forward	CAAGTTTTGCGATGTGAGACTG
	Reverse	CCGTCTCCAGAGTAATGTTCTTG
Mouse TGFB2	Forward	CTCTGTGGGTACCTTGATGCC
	Reverse	GGAAGACCCTGAACTCTGCC
Mouse Fgf21	Forward	CAAATCCTGGGTGTCAAAGC
	Reverse	CATGGGCTTCAGACTGGTAC
Mouse IFN $\beta$	Forward	TCCGAGCAGAGATCTTCAGGAA
	Reverse	TGCAACCACCACTCATTCTGAG
mouse ACTA2 (alpha sma)	Forward	GTGAAGAGGAAGACAGCACAG
	Reverse	GCCCATTCCAACCATTACTCC
Mouse RORA	Forward	CATGGTTCCTAAGGGATGAGAG
	Reverse	CATGGTTCCTAAGGGATGAGAG
Mouse ADGRE1 (F4/80)	Forward	CCCCAGTGCCTTACAGAGTG
	Reverse	GTGCCCAGAGTGATGTCT
Mouse COLA1	Forward	CATAAAGGGTCATCGTGGCT
	Reverse	TTGAGTCCGTCTTTGCCAG
Mouse COL3A1	Forward	GAAGTCTCTGAAGCTGATGGG
	Reverse	TTGCCTTGCGTGTGTTGATATC
Mouse G6PC	Forward	TCACTTCTACTCTTGCTATCTTTG
	Reverse	CCCAGAATCCCAACCACAAG
Mouse TNF $\alpha$	Forward	CTGTAGCCCACGTCGTAGCAA
	Reverse	CTGGCACCCTAGTTGGTTGT
miR-122	Forward	TGGAGTGTGACAAATGGTGTGTTG
miR-34	Forward	TGGCAGTGTCTTAGCTGGTTGT
miR-18a	Forward	TAAGGTGCATCTAGTGCAGATAG
miR-21	Forward	TAGCTTATCAGACTGATGTTGA
miR-126-5p	Forward	CATTATTACTTTTGGTACGCG
miR-93-5p	Forward	CAAAGTGTCTGTTGCTGCAGGTAG
RNU-6	Forward	CGCAAGGATGACACGCAAATTC
Cel-miR-39-3P	Forward	TCACCGGGTGAAATCAGCTTG

**Supplementary Table 2.** AntagomiR Sequences Used in the Study

Name	Sequence
AntagomiR-122	5'-mAsmCsmAmAmAmCmAmCmAmUmUmGmUmCmAmCmAmCmUsmCsmCsmAs-Chol-3'
AntagomiR-control	5'-mCsmAsmCmCmAmCmAmUmAmCmCmGmCmAsmCsmGsmGs-Chol-3'

NOTE. All oligonucleotides were synthesized by IDT (Coralville, IA). Chemical modifications of the antisense oligos: Subscript *m* represents 2'-O-methylation-modified nucleotides; subscript *s* represents a phosphorothioate linkage, and *Chol* represents cholesterol linked through a hydroxyprolinol linkage.

**Supplementary Table 3.** Reduced Body Temperature of Patients Undergoing Major Blood Vessel Cardiovascular Surgery

No.	Gender	Type of surgery	Age	Tube 2 temp (°C)	Tube3 temp (°C)	Delta temp	Delta miR-122
1	M	Coronary artery bypass grafts	59	36	35	1	2.15604
2	M	Mitral valve replacement	69	36.3	33.9	2.4	1.41163
3	M	Aortic root replacement	43	35.7	23	12.7	2.45114
4	M	Coronary bypass grafts	67	36.5	34	2.5	1.54989
5	M	Coronary bypass grafts	72	35.5	34	1.5	1.84856
6	M	Coronary artery bypass grafts and aortic valve replacement	71	34.5	34.1	0.4	1.19047
7	M	Aortic root replacement and repair of aortic valve	33	35.8	25.3	10.5	1.96694
8	F	Mitral valve replacement	74	35.4	34.2	1.2	0.81637
9	F	Mitral valve replacement and tricuspid valve annuloplasty	68	35.5	34	1.5	0.90662

NOTE. Tube 2 represents the temperature (temp) during surgery before body cooling, and tube 3 represents the temperature when the body temperature was the lowest; blood was taken at both temperatures. Delta MIR122 levels were determined using qRT-PCR analysis of plasma MIR122 extracted from tube 2 and tube 3 plasma samples. n = 9. M, male; F, female.

**Supplementary Table 4.** Significantly Altered Triglycerides in Human Plasma Samples From Comparison After Cooling vs Before Cooling

Metabolite	Log <sub>2</sub> (fold-change)	Student's <i>t</i> -test ( <i>p</i> -value)
TG(48:2)	-0.19	2.15E-02
TG(50:1)	-0.31	3.95E-03
TG(50:2)	-0.35	1.86E-03
TG(50:3)	-0.45	1.91E-03
TG(50:4)	-0.63	2.14E-02
TG(51:1)	-0.21	1.88E-02
TG(51:2)	-0.36	2.81E-03
TG(51:3)	-0.37	4.71E-03
TG(51:4)	-0.37	1.65E-02
TG(52:1)	-0.42	2.29E-03
TG(52:4)	-0.45	5.19E-03
TG(52:5)	-0.51	2.21E-02
TG(53:2)	-0.37	2.73E-03
TG(53:3)	-0.46	5.80E-03
TG(53:4)	-0.46	2.29E-02
TG(54:2)	-0.38	7.35E-03
TG(54:3)	-0.30	1.28E-02
TG(54:4)	-0.33	1.87E-02
TG(54:5)	-0.42	3.83E-02
TG(54:5)	-0.34	9.99E-03
TG(54:6)	-0.33	2.29E-02
TG(54:7)	-0.30	4.25E-02
TG(55:4)	-0.24	3.91E-02
TG(56:3)	-0.35	2.05E-02
TG(56:5)	-0.46	4.01E-03
TG(56:6)	-0.34	1.74E-02
TG(56:7)	-0.41	2.25E-03
TG(56:8)	-0.44	1.42E-02
TG(58:5)	-0.42	2.40E-02
TG(58:6)	-0.45	5.01E-03
TG(58:8)	-0.27	4.66E-02

NOTE. Color codes for log<sub>2</sub> (fold-change) and *P* values follow the criterion of the heatmap.

**Supplementary Table 5.** Cytotoxicity for Compounds Identified by Virtual Screening

Compound #	PBM	CEM	Vero	HepG2
1	92.4	11.0	48.4	66.7
2	>100	100.0	85.1	>100
3	>100	21.9	<1.0	>100
4	9.5	2.9	6.3	13.6
5	≥100	39.6	14.5	31.6
6	>100	100.0	40.4	>100
7	>100	100.0	>100	>100
8	>100	100.0	33.1	69.3
9	>100	1.8	1.5	10.4
10	>100	14.6	4.0	68.7

NOTE. Values are CC<sub>50</sub> in μmol/L for compounds identified by virtual screening. Values are means of at least 3 replicates. Compound 1 is RS-2982. PBM indicates peripheral blood mononuclear cells. CEM is a cell line derived from human T cells. Vero cells are kidney epithelial cells extracted from an African green monkey, and HepG2 cells are human hepatoblastoma cells. See Figure 3.



**Supplementary Table 6.** Pri-miR-122 Is Not Detected in Muscle Tissue

gene	sample	CT
Pri-miR-122	Antagol22+saline1	44.84
Pri-miR-122	Antagol22+saline2	41.64
Pri-miR-122	Antagol22+saline3	41.79
Pri-miR-122	Antagol22+saline4	39.64
Pri-miR-122	Antagol22+saline5	38.97
Pri-miR-122	Antagol22+saline6	40.71
Pri-miR-122	Antagol22+saline7	39.71
Pri-miR-122	Control+saline1	41.16
Pri-miR-122	Control+saline2	40.59
Pri-miR-122	Control+saline3	40.98
Pri-miR-122	Control+saline4	41.53
Pri-miR-122	Control+saline5	36.45
Pri-miR-122	Antagol22+RS2982 1	41.94
Pri-miR-122	Antagol22+RS2982 2	39.15
Pri-miR-122	Antagol22+RS2982 3	40.48
Pri-miR-122	Antagol22+RS2982 4	35.11
Pri-miR-122	Antagol22+RS2982 5	36.48
Pri-miR-122	Control+RS2982 1	41.74
Pri-miR-122	Control+RS2982 2	40.1
Pri-miR-122	Control+RS2982 3	42.78
Pri-miR-122	Control+RS2982 4	37.02
Pri-miR-122	Control+RS2982 5	39.47

NOTE. qRT-PCR cycle threshold (CT) values of muscle pri-miR-122 mRNA extracted from C57BL/6 mice fed for 4 weeks with 50% HFD and injected with antagomiR-122 (Antago122) or antagomiR-control (control) once a week, together with 7.5mg/kg RS2982 (or saline) twice a week for 3 weeks. See [Figure 4](#).

**Supplementary Table 7.** Normal and Atherogenic Diet Compositions<sup>a</sup>

Dietary ingredients	Normal Diet, g/kg	Atherogenic Diet, g/kg
Cornstarch	397.5	397.5
Maltodextrin	132.0	132.0
Sucrose	100.0	100.0
Casein	200.0	200.0
Soybean oil	70.0	70.0
Cellulose	50.0	50.0
Mineral mix	35.0	35.0
Vitamin mix	10.0	10.0
L-methionine	3.0	3.0
Choline bitartrate	2.5	2.5
BTH	0.014	0.014
Cholesterol	0.0	10.0
Cholic acid	0.0	5.0
Total weight	1000.0	1015.0

BTH, butylated hydroxytoluene

<sup>a</sup>That were used in the experiment described in [Figure 6](#).