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Supplementary Materials for

Pharmacological Inhibition of a MicroRNA Family in Nonhuman Primates by a Seed-Targeting 8-Mer AntimiR

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at
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Table S2. Raw data and *P* values for data with a sample size of $n < 20$ (provided separately in an Excel file).

Supplementary material for Rottiers et al.

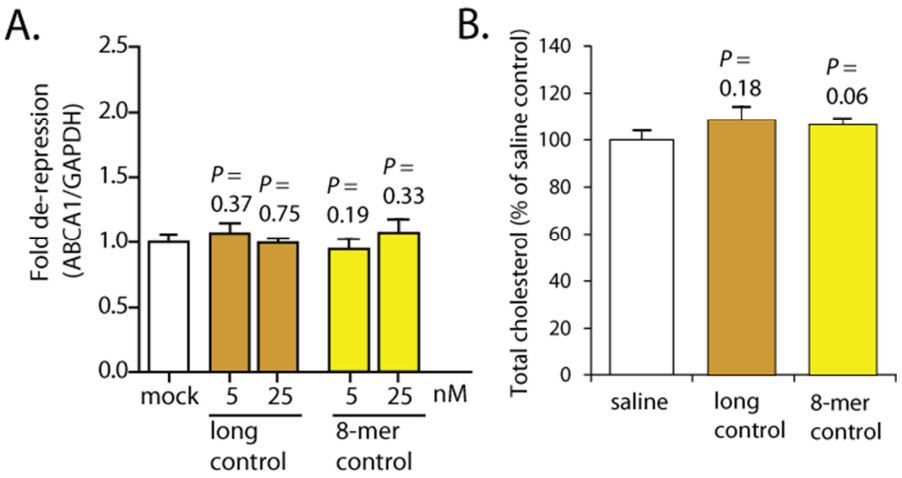

Fig. S1. ABCA1 mRNA in HepG2 cells and total cholesterol in mice after treatment with control oligonucleotides. (**A**) Transfection with scrambled long LNA/DNA or 8-mer LNA control oligonucleotides (5 nM or 25 nM) does not affect ABCA1 mRNA expression in HepG2 cells. (**B**) Treatment with scrambled control oligonucleotides does not alter circulating total cholesterol in mice.

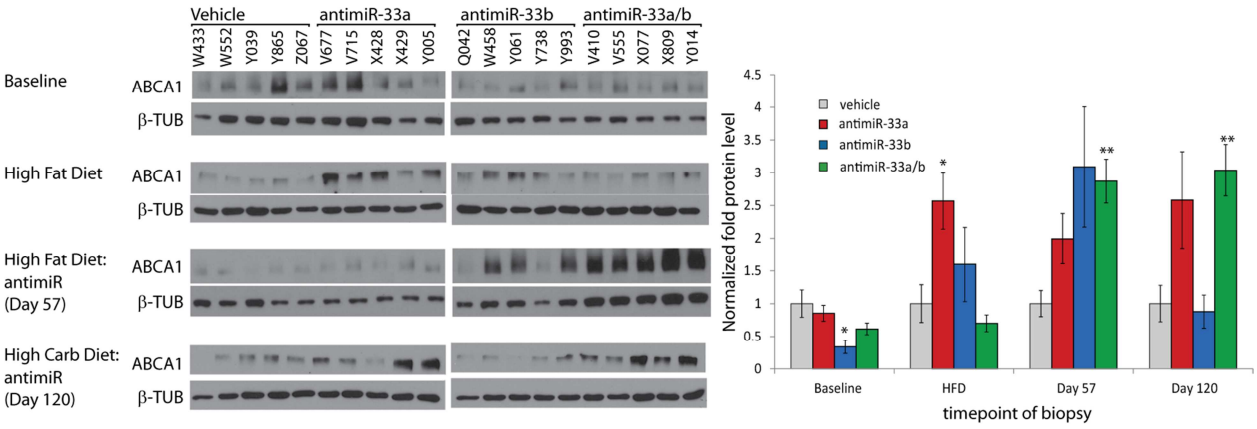

Fig. S2. Hepatic ABCA1 protein in African green monkeys after treatment with antimiR-33a, antimiR-33b or antimiR-33a/b.

Error bars represent standard error of the mean (SEM). Statistical analysis was performed with

Student's t-test. *P* = 0.02 (Baseline; antimiR-33b), 0.02 (HFD; antimiR-33a), 0.001 (Day 57; antimiR-33a/b) and 0.003 (Day 120; antimiR-33a/b).

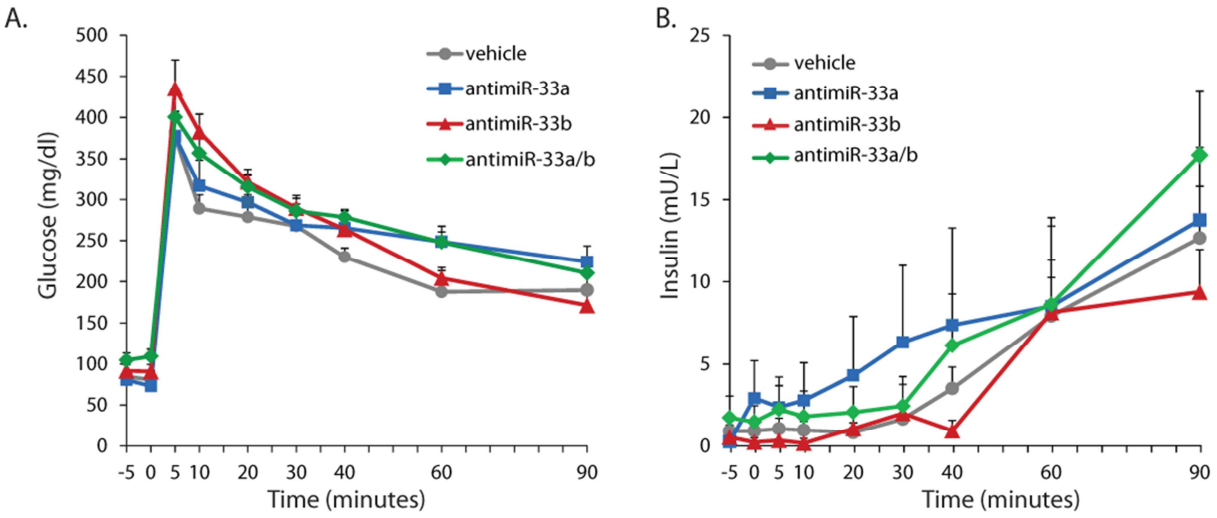

Fig. S3. Plasma glucose and insulin concentrations in vehicle- and antimiR-33–treated African green monkeys. The non-human primates in this study displayed a hyperglycemic and insulin-resistant phenotype. Intravenous Glucose Tolerance Test (IVGTT) to assess glucose regulation (**A**) and measurements of insulin (**B**) on study Day 120 indicate that the African green monkeys in our study demonstrated poor glucose and insulin responses. Error bars represent SEM.

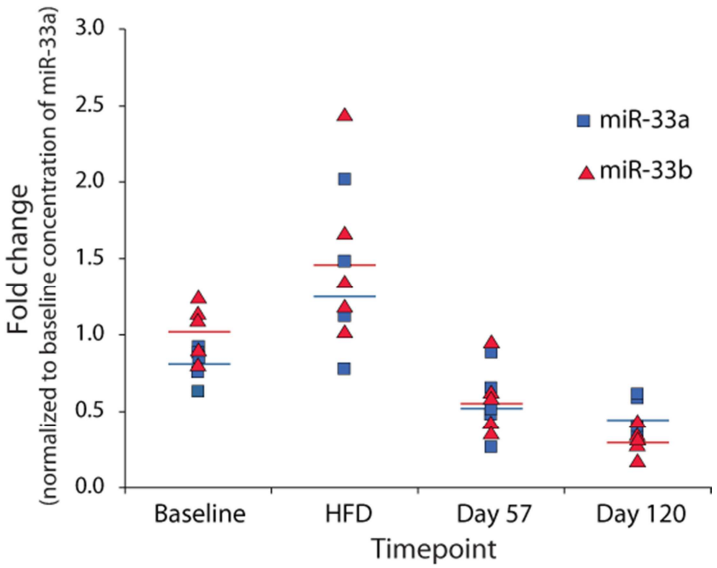

Fig. S4. **Quantification of hepatic miR-33a and miR-33b in control African green monkeys.** The lack of increase in miR-33b expression in response to the shift to a high-carbohydrate diet is consistent with low insulin levels. Lines represent the mean values of 5 monkeys per time-point.

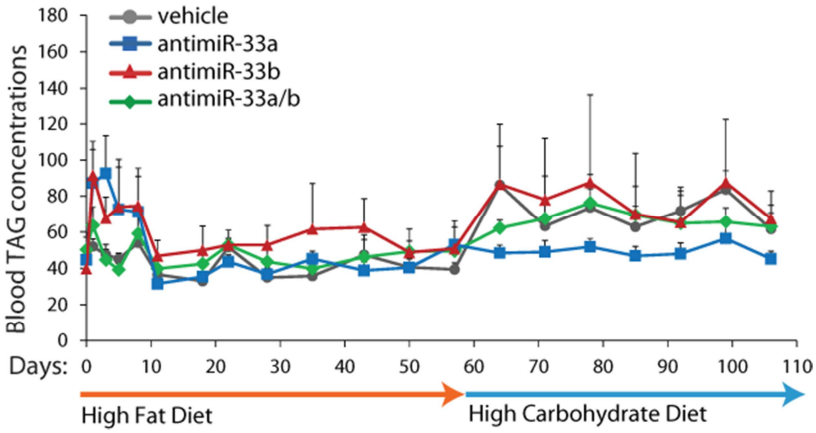

Fig. S5: **Plasma triglyceride concentrations in antimiR-33–treated African green monkeys.** Triglyceride (TAG) concentrations were not significantly changed by the antimiR compounds targeting miR-33 family members in this non-human primate study. Error bars represent SEM.

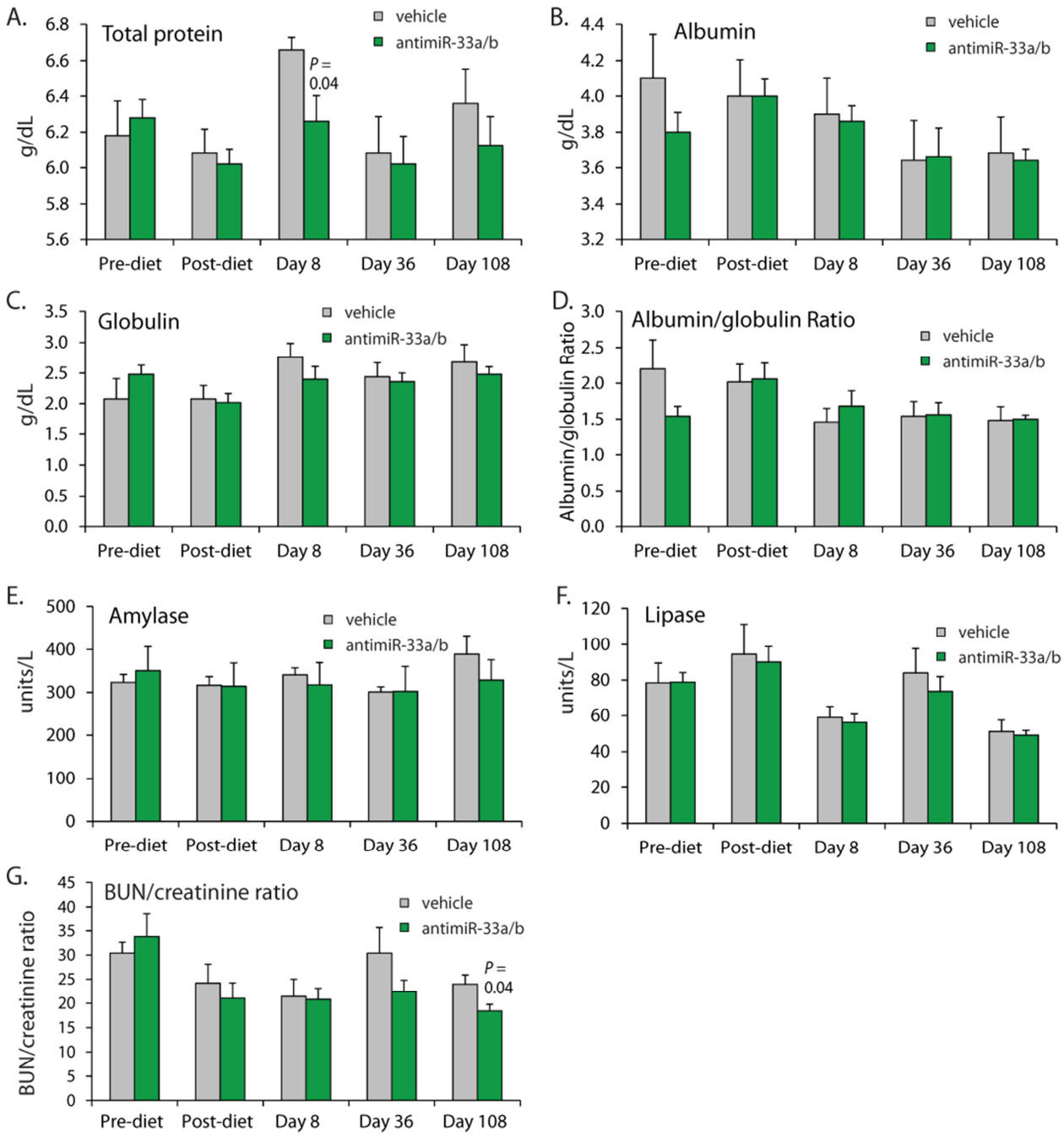

Fig. S6. **Clinical chemistry measurements in antimiR-33a/b– and vehicle-treated African green monkeys.** (**A**) Total protein. (**B**) Albumin. (**C**) Globulin. (**D**) Albumin/globulin ratio. (**E**) Amylase. (**F**) Lipase. (**G**) BUN/creatinine ratio. Error bars represent SEM. Statistical analysis was performed with Student's t-test comparing treatment and vehicle control.

Table S1. Characteristics of the LNA-modified antimiRs used in this study.

name	sequence	length (nt)	Tm (°C) miR-33a	Tm (°C) miR-33b	ΔTm	IC_{50} (nM) miR-33a	IC_{50} (nM) miR-33b	ΔIC_{50}
antimiR-33a	5' atgcaactacaatgca 3'	16	72	51	21	0.04	9.9	248x
antimiR-33b	5' atgcaacagcaatgca 3'	16	54	76	22	8.4	1.3	6.5x
antimiR-33a/b	5' acaatgca 3'	8	66	58	8	0.54	0.47	1.1x

Table S2. Raw data and *P* values for data with a sample size of $n < 20$ (provided separately in an Excel file).

Table S3. Weight of the African green monkeys at the start of the study.

Animal ID	Treatment Group	Baseline (kg)	% weight over normal
W433	Vehicle	4.46	49
W552	Vehicle	4.10	37
Y039	Vehicle	3.71	24
Y865	Vehicle	5.38	79
Z067	Vehicle	4.78	59
V410	antimiR-33a/b	4.74	58
V555	antimiR-33a/b	4.18	39
X077	antimiR-33a/b	4.32	44
X809	antimiR-33a/b	5.27	76
Y014	antimiR-33a/b	4.61	54
Q042	antimiR-33b	5.39	80
W458	antimiR-33b	6.04	101
Y061	antimiR-33b	3.51	17
Y738	antimiR-33b	5.35	78
Y993	antimiR-33b	5.41	80
V677	antimiR-33a	4.36	45
V715	antimiR-33a	4.69	56
X428	antimiR-33a	5.29	76
X429	antimiR-33a	5.06	69
Y005	antimiR-33a	4.84	61
	Average	4.77	59
	Stdev	0.6	21

Weight of all animals at the start of the study (Baseline) sorted by the cohort group assigned after the HFD period. Percentage weight over normal weight was calculated as % weight of the

monkey over 3 kg (=normal female African green monkey weight as determined by Liddie et al. (*27*)).

Table S4. Predicted miR-33 targets with increased expression after antimiR-33a/b treatment.

Gene Symbol	p-value	Fold	Description
SEC63	0.003	1.74	SEC63 homolog (S. cerevisiae)
HNRPA2B1	0.001	1.66	heterogeneous nuclear ribonucleoprotein A2/B1
CROT	0.013	1.60	carnitine O-octanoyltransferase
SNRK	0.000	1.52	SNF related kinase
PJA2	0.002	1.49	praja ring finger 2, E3 ubiquitin protein ligase
CD302	0.003	1.47	CD302 molecule
EIF2S2	0.003	1.45	eukaryotic translation initiation factor 2, subunit 2 beta, 38kDa
AHCYL1	0.004	1.44	adenosylhomocysteinase-like 1
MTDH	0.005	1.42	metadherin
STS	0.040	1.42	steroid sulfatase (microsomal), isozyme S
HNRPA3	0.000	1.42	heterogeneous nuclear ribonucleoprotein A3
NFIA	0.005	1.42	nuclear factor I/A
DUSP6	0.024	1.41	dual specificity phosphatase 6
REXO2	0.012	1.40	REX2, RNA exonuclease 2 homolog (S. cerevisiae)
EEF1A1	0.000	1.40	eukaryotic translation elongation factor 1 alpha 1
CROP	0.002	1.38	LUC7-like 3 (S. cerevisiae)
MORF4L1	0.005	1.38	mortality factor 4 like 1
NDN	0.007	1.38	necdin homolog (mouse)
QKI	0.005	1.38	QKI, KH domain containing, RNA binding
SMAD2	0.001	1.38	SMAD family member 2
PRKAA1	0.035	1.38	protein kinase, AMP-activated, alpha 1 catalytic subunit
SFRS12	0.000	1.37	splicing regulatory glutamine/lysine-rich protein 1
ABCA1	0.008	1.37	ATP-binding cassette, sub-family A (ABC1), member 1
BACH1	0.001	1.36	BTB and CNC homology 1, basic leucine zipper transcription factor 1
ROBO2	0.001	1.36	roundabout, axon guidance receptor, homolog 2 (Drosophila)
C9orf10	0.001	1.36	family with sequence similarity 120A
SMARCA5	0.001	1.36	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5
TBC1D15	0.034	1.35	TBC1 domain family, member 15
ACF	0.000	1.35	APOBEC1 complementation factor
ZFP36L1	0.044	1.34	zinc finger protein 36, C3H type-like 1

TPM3	0.000	1.34	tropomyosin 3
TNRC6C	0.000	1.33	trinucleotide repeat containing 6C
YTHDF3	0.003	1.33	YTH domain family, member 3
KIAA1432	0.000	1.32	KIAA1432
AXL	0.034	1.32	AXL receptor tyrosine kinase
NRIP1	0.003	1.31	nuclear receptor interacting protein 1
CHES1	0.003	1.30	forkhead box N3
DDX5	0.029	1.30	DEAD (Asp-Glu-Ala-Asp) box helicase 5
TFDP2	0.000	1.30	transcription factor Dp-2 (E2F dimerization partner 2)
NAALADL2	0.002	1.30	N-acetylated alpha-linked acidic dipeptidase-like 2
CDC2L6	0.002	1.29	cyclin-dependent kinase 19
UBE2V2	0.001	1.29	ubiquitin-conjugating enzyme E2 variant 2
NCOA3	0.007	1.28	nuclear receptor coactivator 3
EDD1	0.004	1.28	ubiquitin protein ligase E3 component n-recognin 5
HSPA8	0.022	1.28	heat shock 70kDa protein 8
SBNO1	0.000	1.28	strawberry notch homolog 1 (Drosophila)
SPIN	0.010	1.28	spindlin 1
HAS3	0.002	1.27	hyaluronan synthase 3
DDX3X	0.001	1.27	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked
ANKRD28	0.003	1.27	ankyrin repeat domain 28
PCDH7	0.000	1.26	protocadherin 7
WDR42A	0.007	1.26	DDB1 and CUL4 associated factor 8
PAPOLG	0.000	1.26	poly(A) polymerase gamma
NAP1L4	0.002	1.25	nucleosome assembly protein 1-like 4

Expression changes of predicted miR-33 targets in the livers of African green monkeys treated with the 8-mer antimiR-33a/b versus vehicle at the HFD:antimiR time point. Gene expression data were obtained from the NimbleGen DNA microarray analysis, 5 liver samples per group. Analysis was performed with Genespring GX 11.0 software. The gene list of predicted miR-33a/b human targets was obtained using TargetScan 6.2 (http://www.targetscan.org/) and imported into the Genespring GX 11.0 software. *P*-values are uncorrected. Previously described targets are marked in yellow (*14, 15, 18, 19, 26*).

Table S5. **Pharmacokinetic properties of the 8-mer antimiR-33a/b oligonucleotide.**

C_{max} [ng/mL]	16.6
AUC_t [µg·h/mL]	5.90
Terminal half-life, $t_{½}$ [days]	17.5
V_z [L/kg]	408
Cl [mL/h/kg]	674

Pharmacokinetic properties of subcutaneously administered antimiR-33a/b in obese African green monkeys. C_{max}, maximum observed plasma concentration; AUC_t, area under concentration versus time curve from time 0 to 50 days after the last dose; V_z, apparent volume of distribution during the terminal phase; Cl, total body clearance. Data are from times after the last dose on Day 106 and show mean values for 5 animals.

Table S6. **Assessment of liver steatosis after treatment with the 8-mer LNA-modified antimiR-33a/b.**

	Vehicle	SEM	antimiR-33a/b	SEM
Baseline	0.60	0.21	1.01	0.37
High Fat Diet	0.79	0.61	1.46	0.73
HFD:antimiR	2.32	0.84	3.30	1.06
HCD:antimiR	1.05	0.47	2.13	0.38

No increase in hepatic steatosis was observed after treatment with the antimiR-33a/b compound. Average percentages of steatosis for the vehicle or antimiR-33a/b-treated animals are indicated for the four liver biopsy time points (Baseline, High Fat Diet, High Fat Diet:antimiR, High Carb Diet:antimiR).

Table S7. GO term analysis of genes with changed expression after antimiR-33a/b treatment.

GO ID	GO Term	p-value	corrected p-value
5226	metabolic process	3.27E-16	0.000
18751	cellular metabolic process	1.83E-14	0.000
18752	primary metabolic process	6.58E-13	0.000
11078	carboxylic acid metabolic process	1.81E-10	0.000
3813	organic acid metabolic process	3.38E-10	0.000
18763	cellular biosynthetic process	2.34E-09	0.000
5965	biosynthetic process	7.69E-09	0.000
8587	mRNA metabolic process	2.25E-08	0.000
18112	biopolymer metabolic process	1.63E-07	0.000
4073	translation	2.02E-07	0.000
18762	cellular catabolic process	2.70E-07	0.001
14603	monocarboxylic acid metabolic process	3.65E-07	0.001
6776	cellular process	7.57E-07	0.001
5963	catabolic process	1.19E-06	0.002
7239	gene expression	1.25E-06	0.002
18002	macromolecule metabolic process	1.46E-06	0.002
13152	regulation of cellular biosynthetic process	3.54E-06	0.004
24161	establishment of localization in cell	3.40E-06	0.004
8586	RNA metabolic process	3.73E-06	0.004
4077	regulation of translation	6.48E-06	0.006
3851	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	1.29E-05	0.011
4238	protein targeting	1.24E-05	0.011
16446	cellular macromolecule biosynthetic process	1.29E-05	0.011
7380	posttranscriptional regulation of gene expression	1.50E-05	0.012
4153	amino acid and derivative metabolic process	2.37E-05	0.016
4154	amino acid metabolic process	2.34E-05	0.016
18774	cellular macromolecule metabolic process	2.25E-05	0.016
14086	regulation of cellular protein metabolic process	2.44E-05	0.016
4101	regulation of translational elongation	2.72E-05	0.018
4260	lipid metabolic process	2.98E-05	0.018
8573	organic acid biosynthetic process	3.02E-05	0.018
20132	carboxylic acid biosynthetic process	3.02E-05	0.018
20622	intracellular transport	2.87E-05	0.018
4261	fatty acid metabolic process	3.36E-05	0.019
4477	intracellular protein transport	3.34E-05	0.019
18769	cellular lipid metabolic process	4.33E-05	0.023
24153	cellular localization	4.33E-05	0.023
23765	regulation of protein metabolic process	5.77E-05	0.029
4074	translational initiation	6.26E-05	0.031
5578	lipid biosynthetic process	6.43E-05	0.031
5225	biological_process	6.65E-05	0.032

17103	response to chemical stimulus	8.18E-05	0.038
4415	nitrogen compound metabolic process	8.45E-05	0.038
5970	amino acid catabolic process	8.56E-05	0.038
18113	biopolymer biosynthetic process	8.64E-05	0.038

Gene ontology (GO) term analysis of differentially expressed genes at the HFD:antimiR time point performed with Genespring GX 11.0. No enrichment in “adverse effect-associated GO terms” was detected.