Supplemental Information

Methods & Protocols

1. RNA-seq and KEGG enrichment analysis

For RNA sequencing, RNA was isolated in triplicate from control and *GM47544*-overexpressed AML-12 cells. Each sample consisted of 3 μg RNA, which was used as input material for the preparation of RNA samples. Sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (New England Biolabs). Subsequently, these libraries were subjected to paired-end sequencing with 150 bp reads on the Novaseq 6000 platform (Illumina). The resulting sequencing reads were aligned to the mm10 (mouse) genome using STAR 2.7.9a. HTSeq v0.6.0 was utilized to determine the read numbers. For differential expression analysis, DESeq2 v.1.20 was applied to calculate the Fragments Per Kilobase Million (FPKM) of each gene and filter out the differentially expressed genes based on significance analysis and FDR analysis using the following criteria: i) |log2FC| > 1; ii) *p*-value < 0.05. To identify significant pathways associated with these differentially expressed genes, pathway analysis was performed utilizing data according to the KEGG database. Fisher's exact test was employed in selecting significant pathways while considering both *p*-value and FDR thresholds as indicators of significance. The RNA-Seq data has been deposited in the Gene Expression Omnibus under the accession number GSE232866.

2. RNA-fluorescence in situ hybridization (FISH) and subcellular fractionation

RNA-FISH was conducted using a digoxin-labeled RNA probe (Supplementary

Table S1) to investigate the subcellular localization of *GM47544* in AML-12 cells. Initially, cells were seeded onto a multi-chamber culture slide and allowed to fully adhere. Subsequently, fixation and permeabilization procedures were performed. The slides were then subjected to hybridization with DIG-labeled RNA probes, followed by digestion of excess RNA probes and four washes. Finally, DAPI staining was used to visualize cell nuclei, which were imaged using an Axio Imager.A2 (Carl Zeiss AG). To isolate nuclear and cytoplasmic fractions, RNA extraction from AML-12 cells was performed using the Nuc-Cyto-Mem Preparation Kit (Applygen), following the manufacturer's instructions. RT-qPCR analysis was employed to determine *GM47544* RNA expression levels in both nuclear and cytoplasmic fractions. U6 and GAPDH served as positive controls for nuclear and cytoplasmic fractions respectively. The primer sequences can be found in Supplementary Table S1.

3. Plasmid Construction and transfection

GM47544, *GM47544 SL2,* and *AP006216.5* were cloned into the pcDNA3.1, vector (AugCt Biotech). pcDNA3.1-ApoC3-Flag encodes mouse ApoC3, followed by a 3×FLAG epitope (AugCt Biotech). *GM47544* ORF1 and *GM47544* ORF2 were cloned into pEGFP-N1 vectors, in which the GFP start codon (ATGGTG) was mutated to ATTGTT (pGFPmut) (AugCt Biotech). Transfection assays were conducted using Lipofectamine 3000, following the guidelines provided by Thermo Fisher Scientific.

4. The assays of lipids and lipoproteins

Intracellular and liver lipid levels were assayed using triglyceride and

cholesterol assay kits (Zhongsheng Beikong), respectively, according to the manufacturer's instructions.

Blood was collected from the tips of the tails following a period of fasting. Plasma was isolated through centrifugation. Triglyceride and total cholesterol levels were determined using triglyceride and cholesterol assay kits (Zhongsheng Beijing Kong, China), respectively, according to the manufacturer's instructions.

To examine the lipid distribution, we performed fast protein liquid chromatography (FPLC) on 500 μl of pooled plasma samples from 4 animals in different groups. The samples were then subjected to Superose™ 6 Increase 10/300 GL columns (Cytiva). Triglyceride and cholesterol concentrations in the eluted fractions were measured using triglyceride and cholesterol assay kits, respectively (Zhongsheng Beikong).

5. Oral lipid tolerance assay

Adenoviruses are characterized by rapid expression of target genes. Therefore, we used them for short-term animal experiments. 8-week-old male WT mice were injected with Ad-*GM47544* or Ad-Ctrl recombinant adenovirus (Weizhen Biosciences). The mice were subjected to a 6-hour fasting period, followed by administration of 200 μl olive oil through oral gavage. Blood samples were collected from the tail vein immediately at various time intervals (0, 1, 2, 3, and 4 h) post-oral gavage. Plasma was isolated to measure TG levels using the Triglycerides assay kit (Zhongsheng Beijing Kong).

6. Quantitative real-time PCR

The RNA extraction was performed using the RNA isolator Total RNA Extraction Reagent (Vazyme), followed by quantification of mRNA levels through quantitative real-time PCR (RT-qPCR) on a 7900HT Fast Real-Time PCR System (Applied Biosystems) with Taq Pro Universal SYBR qPCR Master Mix (Vazyme). To normalize the mRNA levels, GAPDH was used as an internal control. The primer sequences utilized for qPCR can be found in Supplementary Table 1.

7. Immunoblotting assays

Hepatic tissues and cells were lysed and homogenized in cell lysis buffer for western blotting and IP (Beyotime). The protein content of the extracts was determined using a BCA protein quantification kit (Boster Bio), followed by separation on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) system. Subsequently, the proteins were transferred onto a PVDF membrane (Millipore). Membranes were blocked with 5% free-fat dry milk in TBST for 2h and then incubated overnight at 4°C with specific primary antibodies as indicated. After washing with TBST buffer, membranes were incubated with secondary antibodies at room temperature for 1-2 h. Finally, Meilunbio® fg super sensitive ECL luminescence reagent (Meilunbio) was used to visualize the protein bands on the membranes which were then captured using ChemiDoc Touch Imaging System (Bio-Rad). Supplementary Table 2 provides details about all the antibodies employed in this study.

8. LDL uptake assay

The control and *GM47544*-overexpressed AML-12 cells on glass coverslips

were incubated in serum-free medium at 37°C for 24 h. Cells were refed with DMEM containing 15 μg/ml DiI-LDL (Yeasen) and incubated at 37 °C for 4 h. After that, cells were covered with DMEM containing 5 μg/ml Hoechst (Yeasen) for 5 min. Cells were treated with 4% paraformaldehyde in PBS for a duration of 25 minutes under dark conditions. The cell fluorescence signals were examined with an Axio Imager.A2 (Carl Zeiss AG).

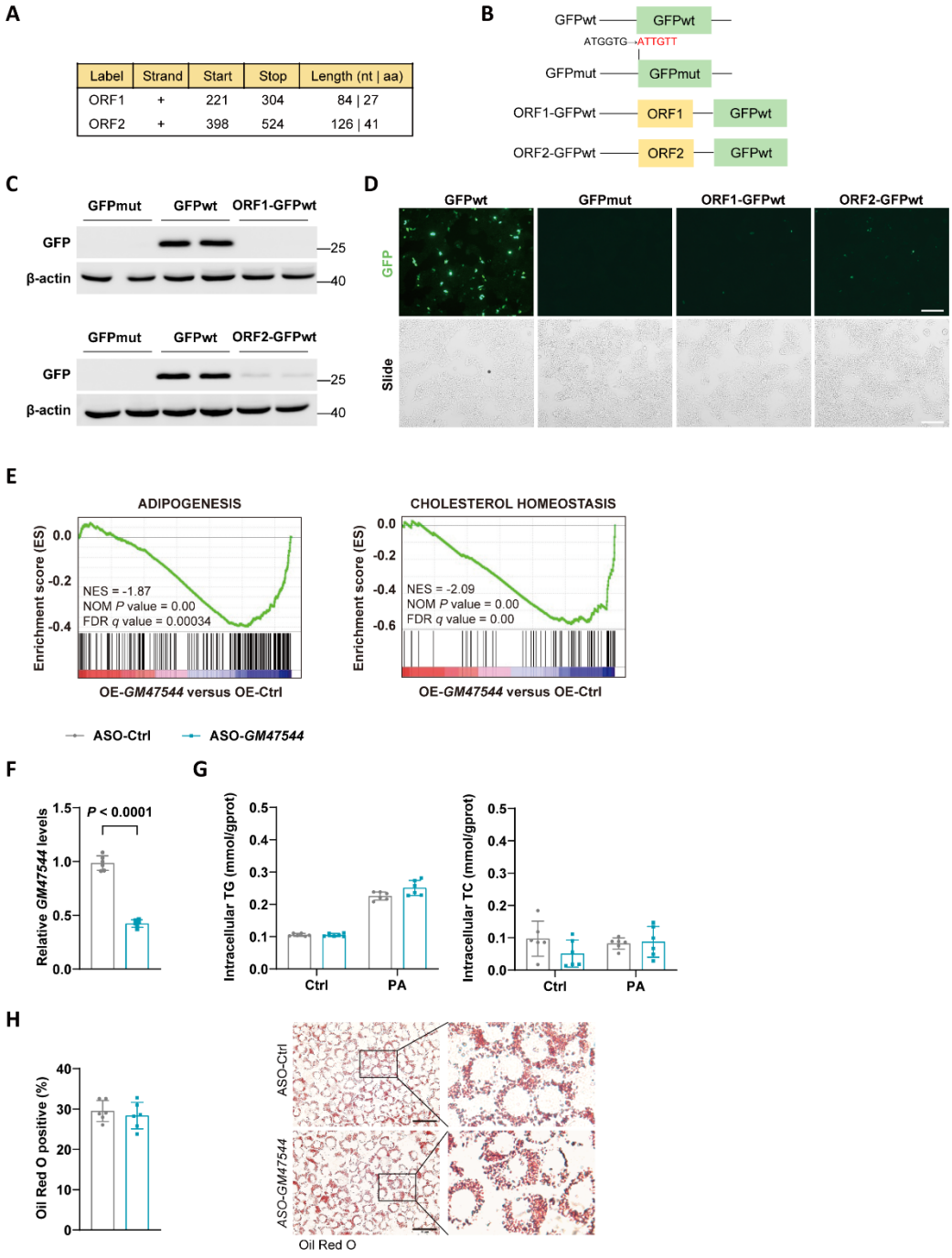
9. RNA Immunoprecipitation

AML-12 cells were transfected with pcDNA3.1-ApoC3-Flag for 24 h and lysed with cell lysis buffer for western blotting and IP (Beyotime) supplemented with an RNase Inhibitor (Thermo Fisher Scientific) and protease inhibitor cocktail (Abclonal). The lysate was centrifuged at 12,000 rpm for 15 minutes, and the supernatant was carefully transferred to an RNase-free tube. Total protein quantification was performed using a bicinchoninic acid protein quantification kit, and then immunoprecipitation was carried out on the cytoplasmic lysate protein (2.5 mg). Anti-Flag magnetic beads (MedChemExpress) or Protein A/G Magnetic Beads (MedChemExpress) were added to the samples and allowed to hybridize overnight at a temperature of 4°C. Following six washes with RIP buffer containing 150 mM KCl, 25 mM Tris (pH7.4), 5 mM EDTA, and 0.5% NP-40, the RNA bound to the beads was isolated using the RNA isolator Total RNA Extraction Reagent (Vazyme). The RNA fraction was subjected to RT-qPCR.

10. RNA pull-down assay

The RNA pull-down assay was conducted using a Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific) following the manufacturer's

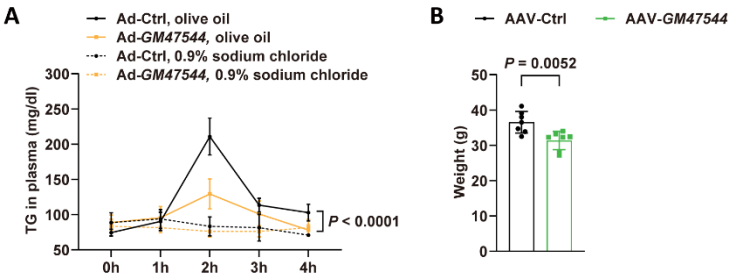
instructions. In brief, we synthesized full-length *GM47544* and its stem-loop region sequence through in vitro transcription with biotin RNA-labeling mix and T7 RNA polymerase (Beyotime). Biotinylated *GM47544*, *GM47544 SL1, GM47544 SL2,* and *GM47544 SL3* were then incubated with streptavidin-conjugated magnetic beads at room temperature for 1 h. Following three washes with Tris buffer to remove non-specific binding, the complexes were incubated with total AML-12 cell lysates at 4℃ for 2 h. The proteins that co-precipitated with the complexes were collected, separated by SDS-PAGE, and detected via immunoblotting.



Supplementary Figure 1 (Related to Figure 1).

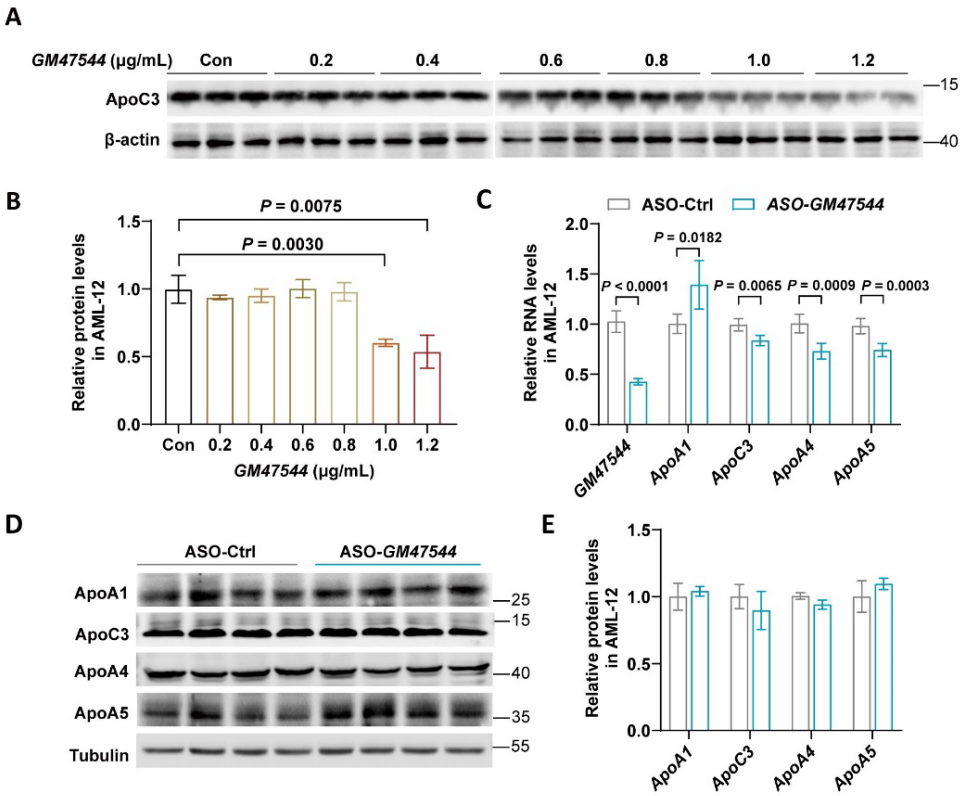
(A) Prediction of putative proteins encoded by *GM47544* using ORF finder. (B) Diagram of the GFP fusion constructs used for transfection. The start codon ATGGTG of the GFP (GFPwt) gene is mutated to ATTGTT (GFPmut). (C-D) The indicated constructs were transfected into AML-12 cells for 24 hr. GFP fluorescence was detected and *GM47544*-GFP fusion protein levels were

determined by western blotting with anti-GFP antibodies. (E) Gene Set Enrichment Analysis (GSEA) plots for pathways involved in adipogenesis and cholesterol homeostasis. GSEA was performed based on the average of GM47544 expression levels (OE-*GM47544* versus OE-Ctrl). (F-H) The following assays were performed in the AML-12 cell line expressing ASO-*GM47544* or ASO-NC. qPCR analysis showed efficient knockdown of *GM47544* in AML-12 cells (F). Intracellular TG and TC (G) levels of cells with or without PA treatment (250μM, 24h). Oil Red O staining of lipid droplets in cells (H). Scale bar, 50 μm. Data are presented as mean ± SD in F, G, and H, with statistical significance determined with unpaired two-tailed Student's *t*-test.



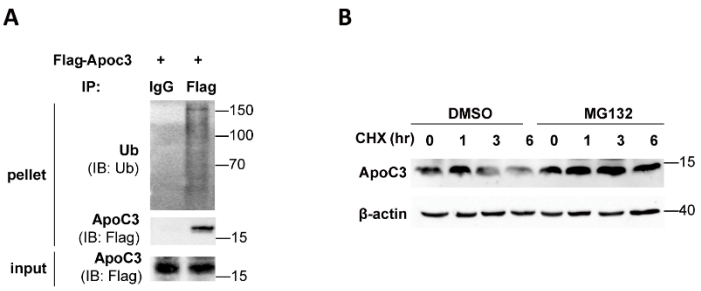
Supplementary Figure 2 (Related to Figure 2).

(A) 8-week WT male mice were injected with adenovirus harbored GFP or *GM47544* (Ad-Ctrl or Ad-*GM47544*) via the tail vein. After 5 days of chow diet feeding, the mice orally received olive oil or sodium chloride. Blood samples were collected each hour for four hours post-oil administration. Plasma TG levels at indicated times in control and *GM47544*-overexpressed mice (n=6). (B) Body weight of control and *GM47544*-overexpressed mice. Data are presented as mean ± SD in A, with statistical significance determined with two-way ANOVA. Data are presented as mean ± SD in B, with statistical significance determined with unpaired two-tailed Student's *t*-test.



Supplementary Figure 3 (Related to Figure 4).

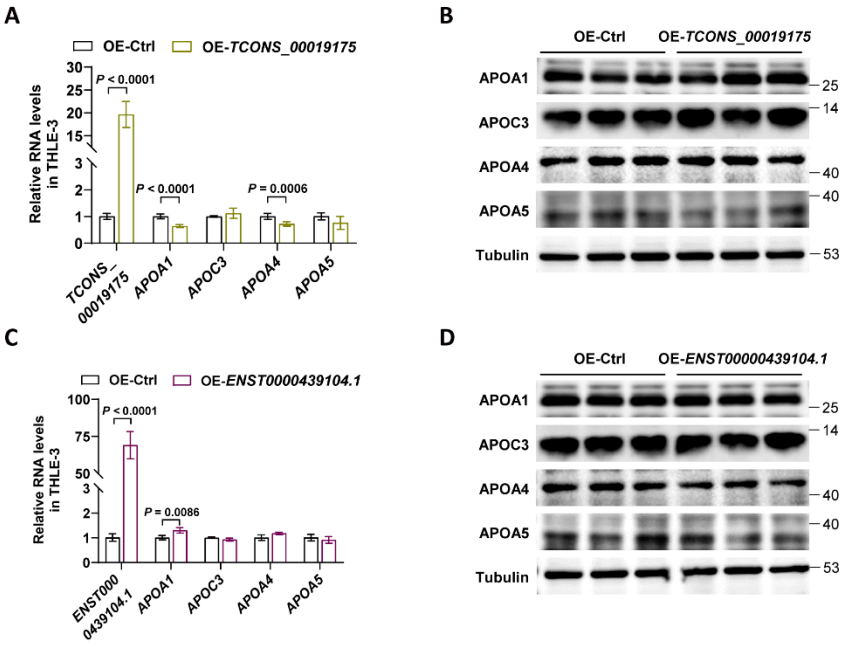
(A) AML-12 cells were treated with *GM47544* at the indicated amounts, followed by western blotting analysis with antibodies against ApoC3. (B) Relative protein levels of ApoC3 were quantified. (C) Relative expression of *GM47544* and ApoA1/C3/A4/A5 were measured by qRT-PCR in AML-12 cells expressing ASO-*GM47544* or ASO-NC. (D-E) ApoA1/C3/A4/A5 protein levels were measured by western blot in AML-12 cell line expressing ASO-*GM47544* or ASO-NC. Data are presented as mean ± SD in A and B, with statistical significance determined with unpaired two-tailed Student's *t*-test.



Supplementary Figure 4 (Related to Figure 5).

(A) IP assays were performed using anti-FLAG antibodies in AML-12 transfected with vectors expressing the FLAG-ApoC3, followed by western blotting analysis with antibodies against ubiquitin and FLAG.

(B) AML-12 cells were treated with MG132 (20 mM, 6 hours) in the presence of cycloheximide (CHX, 20μM) with the indicated time, followed by western blotting analysis with antibodies against ApoC3.



Supplementary Figure 5 (Related to Figure 7).

(A) Relative expression of APOA1/C3/A4/A5 in control and *TCONS_00019175*-overexpressed THLE-3 cells were measured by RT-qPCR. (B) APOA1/C3/A4/A5 protein levels in control and *TCONS_00019175*-overexpressed THLE-3 cells were measured by western blot. (C) Relative expression of APOA1/C3/A4/A5 in control and *ENST00000439104.1*-overexpressed THLE-3 cells were measured by RT-qPCR. (D) APOA1/C3/A4/A5 protein levels in control and *ENST00000439104.1*-overexpressed THLE-3 cells were measured by western blot. Data are presented as mean ± SD in A and C, with statistical significance determined with unpaired two-tailed Student's *t*-test.

Supplementary Table 1: List of primer sequences.

RT-qPCR primer	
GM47544-F	TCCTCCTGCTCCCTTCCAACAAA
GM47544-R	TCCACCCTTACGCAGAACGCTTG
m-APOA5-F	ACTCACACGTAAGGCGAAGG
m-APOA5-R	GTGTCATGCCGAAAAGCCTG
m-APOA1-F	GCTCAAGACAACCCTACCTT
m-APOA1-R	GCTTTCTCGCCAAGTTCTTC
m-APOC3-F	AAGACGGTCCAGGATGCGCTAA
m-APOC3-R	GTTGGTCCTCAGGGTTAGAATCC
m-APOA4-F	CAGAAGACGGATGTCACTCAGC
m-APOA4-R	AGCTGTACGACAAAGGGCACCA
m-actin-F	CATTGCTGACAGGATGCAGAAGG
m-actin-R	TGCTGGAAGGTGGACAGTGAGG
GM39329-F	TTGATTAGCGTGTGGAGGGG
GM39329-R	CCATCGGACTTATCGCTCGT
GM47141-F	GTGAGGCTGGGCTTCTTTGA
GM47141-R	TGGGTTTACTGCGTTCAGCA
4930448E22Rik-F	GGCACAGTATATGGCGCTCT
4930448E22Rik-R	TGTACCTGCGGGACCATAGA
GM31374-F	CGTGTTGAAGGAATGGCGAC
GM31374-R	AGCCTATGCCTTTCCTGGAT
4931429L15RIK-F	CATGTCCCAGAAGGGCGATT
4931429L15RIK-R	GTGGCTTGAGCAGAGGCTAA
GM10680-F	AGCTGCCTGTTCAGGTCTTC
GM10680-R	CTCCGCAAAAATCGACCAGC
GM47528-F	AAATACAAGGCCACCCCGAG
GM47528-R	GTGTAGCCGAAACTGTCCCA

GM31432-F	GATGAGCACATTCATGCCCG
GM31432-R	CTGTATCCACGCAGAGTCGG
GM22659-F	GCCCCTAGAGGTGTTGTAGG
GM22659-R	CCATAGGCCAGAACCCTAAGC
GM48945-F	TGTGACTATGCAAGGGGCTG
GM48945-R	CAAGCAGGGCCCATCACTTA
GM31557-F	AGCGTGAAAACCATGCTCAC
GM31557-R	GGAGAGCCTGCCTAAACCAG
AP006216.5-F	GCAGGATCAAAAGCAGCTCC
AP006216.5-R	GTTGCATCTGAAGGCTTGGC
TCONS_00019175-F	GGCAGTGTTTCCAACTGTGG
TCONS_00019175-R	GGGTTTGACACCTGCCTCTT
ENST00000439104.1-F	AGAACTGAAGCGTGGCAAGA
ENST00000439104.1-R	TTGGATGACCTCTTGCACCC
h-APOA1-F	GTGGATGTGCTCAAAGACAGCG
h-APOA1-R	GCTTGCTGAAGGTGGAGGTCAC
h-APOC3-F	CCGCCAAGGATGCACTGAG
h-APOC3-R	CTCCAGTAGTCTTTCAGGGAACT
h-APOA4-F	CCCAGCAACTCAATGCCCT
h-APOA4-R	CCTTCAGTTTCTCCGAGTCCT
h-APOA5-F	ACGGAAAGGCTTCTGGGACT
h-APOA5-R	GAGGTCTTGCTCAAGGCTGTCT

T7 primer	
T7-GM47544-F	TAATACGACTCACTATAGGGAGACCTG AGAGTCAGGAGGTAGGGAC
T7-GM47544-R	CCTGAGTGTGCAGGAGCTGC
T7-SL1-F	TAATACGACTCACTATAGGGAGACCTG AGAGTCAGGAGGTAGGGAC
T7-SL1-R	GCCCCTATTTCCTTAGAGAACTCTG
T7-SL2-F	TAATACGACTCACTATAGGGAGATCTG CCCGGTCCCATCACTGGACCC

T7-SL2-R	CCTGTCTCCCCAGGGTTGTCATGAG
T7-SL3-F	TAATACGACTCACTATAGGGAGAAGTT CCTCTCCATGGACCAACTGTA
T7-SL3-R	CCTGAGTGTGCAGGAGCTGC

Digoxin probe	
GM47544	CCCAACTCCAGTCACCAA

Supplementary Table 2: List of primary antibodies.

Primary antibody	Dilution	Source	Catalog number
β-actin	1/2000	Abclonal	AC026
β-Tubulin	1/2000	Abclonal	AC008
GFP	1/1000	Abclonal	AE012
GAPDH	1/2000	Servicebio	GB12002
ApoA5	1/1000	Abcam	Ab239579
ApoC3	1/1000	Invitrogen	PA5-78802
ApoA4	1/1000	Proteintech	17996
ApoA1	1/1000	Abclonal	A14211
Ub	1/1000	Proteintech	10201-2-AP
HA	1/2000	Abclonal	AE008
LDLR	1/1000	Abclonal	A14996
Flag	1/2000	Abclonal	AE092