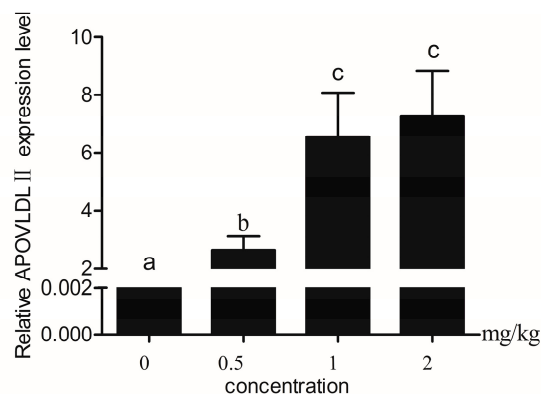
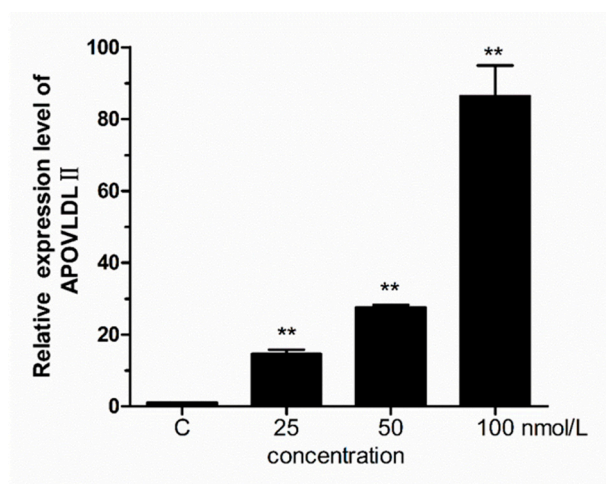


## Supplementary material



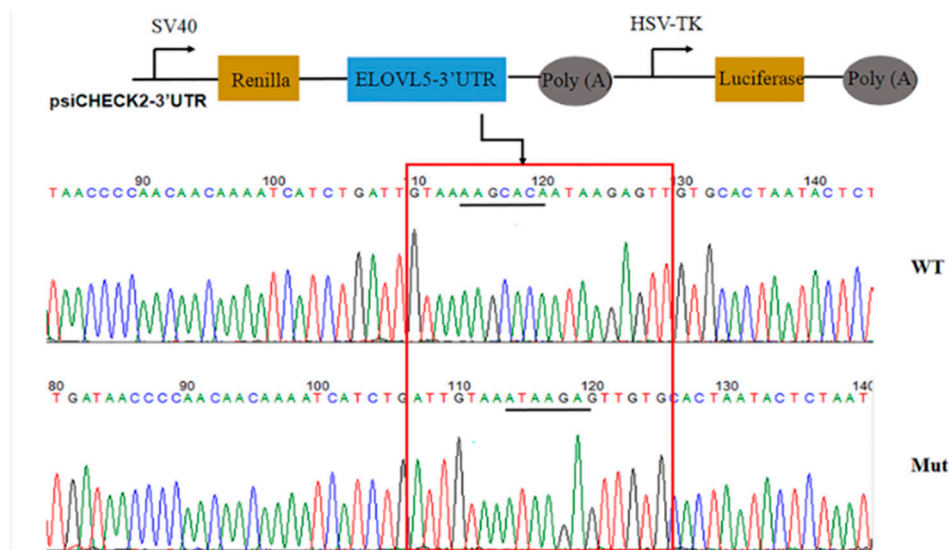
**Figure S1.** Expression level of *ApoVLDLII* mRNA in the liver of laying hens. The birds were injected intramuscularly with 0.5, 1, and 2 mg 17  $\beta$ -estradiol (dissolved in olive oil) per kg of body weight, or with the vehicle (the same amount of solvent olive oil).  $\beta$ -Actin was used as the referenced gene to estimate mRNA. Data are presented as Mean  $\pm$  SEM ( $n = 6$  for each group). Values with different superscripts indicate statistical difference ( $p < 0.05$ ).



**Figure S2.** Expression level of *ApoVLDLII* mRNA in chicken primary hepatocytes. The cells were incubated with 25, 50, and 100 nM of 17  $\beta$ -estradiol, or vehicle (0.1% ethanol).  $\beta$ -Actin was used as the reference gene to estimate mRNA. Data are presented as Mean  $\pm$  SEM ( $n = 6$  for each group). \*  $p < 0.05$ , \*\*  $p < 0.01$ .

Position 57-64 of ELOVL5 3' UTR	5' ... AUCAUCUGAUUGUAAAAGCACAA ...	3' UGUACCAAUUAGUUCGUGUU
<b>gga-miR-218-5p</b>		
Position 271-277 of ELOVL5 3' UTR	5' ... AUACGACACUGCUGUUGCCUUA ...	3' ACCGUAAGUGACGC--ACGGAUU
<b>gga-miR-124b</b>		
Position 271-277 of ELOVL5 3' UTR	5' ... AUACGACACUGCUGUUGCCUUA ...	3' ACCGUAAGUGGCGC--ACGGAUU
<b>gga-miR-124a-3p</b>		
Position 413-420 of ELOVL5 3' UTR	5' ... GUAGUAAAACACACA-UGCCUUA ...	3' ACCGUAAGUGGCGCACGGAUU
<b>gga-miR-124a-3p</b>		
Position 413-420 of ELOVL5 3' UTR	5' ... GUAGUAAAACACACA-UGCCUUA ...	3' ACCGUAAGUGACGCACGGAUU
<b>gga-miR-124b</b>		
Position 1374-1381 of ELOVL5 3' UTR	5' ... AUCUUUUUAAAAUUUUUUGCACA ...	3' AGUCAAACGUAACCUAAACGUGU
<b>gga-miR-19b-3p</b>		
Position 1374-1381 of ELOVL5 3' UTR	5' ... AUCUUUUUAAAAUUUUUUGCACA ...	3' AGUCAAACGUAUCUAAACGUGU
<b>gga-miR-19a-3p</b>		
Position 1460-1467 of ELOVL5 3' UTR	5' ... AGACAGUGUCCGUCAGUUUACA ...	3' UCGACUCACAUCCU--ACAAUGU
<b>gga-miR-30c-5p</b>		
Position 1460-1467 of ELOVL5 3' UTR	5' ... AGACAGUGUCCGUCAGUUUACA ...	3' UCGACUCACAUCCU--ACAAUGU
<b>gga-miR-30b-5p</b>		
Position 1460-1467 of ELOVL5 3' UTR	5' ... AGACAGUGUCCGUCAGUUUACA ...	3' GGUCAGUCCUACAAUGU
<b>gga-miR-30e-5p</b>		
Position 1460-1467 of ELOVL5 3' UTR	5' ... AGACAGUGUCCGUCAGUUUACA ...	3' GAAGGUCAGCUCCUACAAUGU
<b>gga-miR-30a-5p</b>		
Position 1460-1467 of ELOVL5 3' UTR	5' ... AGACAGUGUCCGUCAGUUUACA ...	3' GAAGGUCAGCCCCUACAAUGU
<b>gga-miR-30d</b>		

**Figure S3.** The binding sites in the 3'UTRs of target gene *ELOVL5* complemented with seed regions of miRNAs.



**Figure S4.** Construction and verification of plasmids. The 3'UTRs of target gene *ELOVL5* were cloned into psiCHECK-2 Dual Luciferase Reporter vectors for validation in vitro. WT, Wild type vector; Mut, Mutated vector.

**Table S1.** Downregulated miRNAs in the liver of peak-laying period hens.

miRNA	Fold Change (L20/L30)	FDR
miR-146b-5p	8.5	0
miR-24-3p	7.4	1.696E-27
miR-146a-5p	6.0	3.81E-122
miR-221-5p	5.8	0.0244488
miR-7b	5.4	0.0001006
miR-147	5.1	0.0415756
miR-106-5p	5.0	0.088337
miR-20a-5p	4.6	4.977E-19
miR-140-5p	4.6	1.655E-08
<b>miR-218-5p</b>	<b>4.3</b>	<b>0.0385142</b>
<b>miR-19b-3p</b>	<b>4.3</b>	<b>3.687E-21</b>
miR-33-3p	4.2	6.707E-06
miR-16c-5p	4.0	0.000481
miR-221-3p	4.0	9.691E-12
<b>miR-19a-3p</b>	<b>3.9</b>	<b>6.599E-05</b>
miR-16-5p	3.8	1.116E-57
miR-146c-5p	3.7	0
miR-223	3.6	6.169E-06
miR-451	3.6	2.422E-62
miR-126-3p	3.6	3.22E-115
miR-10a-5p	3.5	0
miR-142-3p	3.5	3.956E-06
miR-126-5p	3.4	0
miR-17-5p	3.4	1.676E-09
miR-2188-5p	3.3	1.092E-13
miR-181a-3p	3.3	0.0106489
miR-23b-3p	3.3	0.0388315
miR-155	3.2	0.0428774
miR-193b-3p	3.2	2.564E-19
miR-200a-3p	3.2	6.113E-06
miR-21-3p	3.0	0.0094551
miR-122-3p	3.0	9.133E-18
miR-215-5p	2.9	0.0104972
<b>miR-30a-5p</b>	<b>1.7</b>	<b>0.000341357</b>
miR-34a-5p	2.8	0.0001375
miR-21-5p	2.5	0
miR-214	2.5	0.0399318
<b>miR-30e-5p</b>	<b>2.5</b>	<b>0.0020146</b>
miR-140-3p	2.4	1.579E-37
miR-199-5p	2.4	5.453E-06
<b>miR-30b-5p</b>	<b>2.4</b>	<b>0.0014809</b>
miR-29a-3p	2.3	0.0165375
miR-142-5p	2.3	2.285E-21
miR-122-5p	2.2	4.38E-207
miR-130b-3p	2.1	0.0461745
miR-101-3p	2.1	1.257E-16

Note: miRNA with binding sites at the 3'UTR of *ELOVL5* are in bold. L20/L30, miRNA expression level (Tpm) of 20 and 30-week-old Lushi chicken. FDR, False Discovery Rate.

**Table S2.** List of cloning and qPCR primers used in the present study.

Gene name	Primer sequences(5'-3')	Product size (bp)	Tann (°C)	Purpose
<i>ELOVL5-F</i>	ATTGGGTGCCTTGTGGTCA	180	60	qPCR
<i>ELOVL5-R</i>	AGCTGGTCTGGAAGATTGTCA			
<i>APVLDLII-F</i>	CAATGAAACGGCTAGACTCA	108	60	qPCR
<i>APVLDLII-R</i>	AACACCGACTTTTCTTCCAA			
<i>SLIT2-F</i>	CGTCTGGCAAACAAAAGGAT	191	60	qPCR
<i>SLIT2-R</i>	CTGTGGGATGTGATCAGGAA			
<i>β-actin-F</i>	GGACACCAAGCTCAGAGACT	112	60	qPCR
<i>β-actin-R</i>	GGACACCAAGCTCAGAGACT			
<i>Pre-miR-218-1-F</i>	CCGCTCGAGTCCCTCACTGTTACCCTTGG	174	60	Cloning
<i>Pre-miR-218-1-R</i>	TGCTCTAGATGACTTAAGCCTCAGCAGCA			
<i>ELOVL5-F</i>	CCGCTCGAGTGCCTGAAAGACCAAACTGA	230	59	Cloning
<i>ELOVL5-R</i>	ATTGCGGCCGCGGTCAGTGACAGCCCAGAAT			
<i>ELOVL5-Fm</i>	ACAAAATCATCTGATTGTAAATAAGAGTTGTGCACTAATA	230	59	Cloning
<i>ELOVL5-Rm</i>	TATTAGTGCACAACTCTTATTACAATCAGATGATTTTGT			

Note: F, forward primer for qPCR; R, reverse primer for qPCR and cloning. Fm, forward primer for cloning mut vector; Rm, reverse primer for cloning mut vector. Tann, annealing temperature for PCR program.