Supplemental Figure Legends

Figure S1. Western Blot of Total Plasma apoA-I. Related to Figure 1
(A-B) Plasma was collected from (A) chow-fed and (B) WTD-fed L-FoxO1,3,4 mice and littermate controls. Plasma was diluted by 1:200 with PBS, from which 20µl from each sample were run on a 4%-15% Tris-HCL gradient gel and blotted for apoA-I. Numbers denote relative apoA-I expression levels by densitometric scanning (n=4-7). Values are presented as mean ± SEM. Shown is a typical experiment. Independent experiments yielded qualitatively identical results.

Figure S2. Western Blot of Liver ABCA1 and SR-BI from Chow-Fed and WTD-Fed L-FoxO1,3,4 Mice. Related to Figure 2
Representative western blot of hepatic ABCA1 and SR-BI expression in both chow-fed and WTD-fed L-FoxO1,3,4 mice and littermate controls. ABCA1/Actin and SR-BI/Actin denote relative ABCA1 and SR-BI expression levels by densitometric scanning, respectively (n=4). ABCA1 antibody was commercially purchased from Novus (Novus – NB400-105). Data are presented as mean ± SEM.

Figure S3. Plasma Decay Kinetics of ^{125}I-TC-/[^{3}H]CEt-WT-HDL in WTD-Fed L-FoxO1,3,4 Mice. Related to Figure 3
(A-B) ^{125}I-TC-/[^{3}H]CEt-WT-HDL was injected intravenously in WTD-fed L-FoxO1,3,4 mice and littermate controls. Thereafter, during a 24-hr interval, periodic blood samples were harvested and plasma was analyzed for ^{125}I-TC (crosses) and [^{3}H]CEt (circles). The y-axis represents the fraction of the tracer in plasma (%). Shown is a trace from a representative mouse from each genotype, with (A) control on the left and (B) L-FoxO1,3,4 on the right. The experiment was carried out in 6 control and 5 L-FoxO1,3,4 mice.

Figure S4. Plasma-FCRs and Tissue Tracer Uptake Rates for ^{125}I-TC-/[^{3}H]CEt-WT-HDL in WTD-Fed L-FoxO1,3,4 Mice. Related to Figure 4
^{125}I-TC-/[^{3}H]CEt-WT-HDL was injected intravenously in WTD-fed L-FoxO1,3,4 mice and littermate controls. (A) During the subsequent 24-hr interval, blood was harvested periodically to determine the plasma decay of both tracers. ^{125}I-TC (^{125}I) and [^{3}H]CEt ([^{3}H]) were analyzed, and plasma-FCRs for ^{125}I-TC and [^{3}H]CEt were calculated. The difference in plasma-FCRs between [^{3}H]CEt and ^{125}I-TC was calculated. 24-hrs after tracer injection, the animals were euthanized, and tissues were analyzed for both tracers. (B) Liver, (C) adrenal and (D) kidney organ-FCRs for ^{125}I-TC (^{125}I), [^{3}H]CEt ([^{3}H]), and the difference in organ-FCRs between [^{3}H]CEt and ^{125}I-TC ([^{3}H]CEt - ^{125}I-TC) were calculated. All calculations were done as described in Materials and Methods. n = 6 control and n = 5 L-FoxO1,3,4 mice. An independent similar experiment with n=5 control mice and n=4 L-FoxO1,3,4 mice yielded qualitatively identical results as shown in the graphs. *$p < 0.05$, **$p < 0.01$, by Student's t-tests. Data are presented as mean ± SEM.

Figure S5. Adrenal Gene Expression in Chow-Fed L-FoxO1,3,4 Mice. Related to Figure 4
Relative adrenal *Scarb1* and *Foxo1* gene expression by qPCR in chow-fed L-FoxO1,3,4 mice (n=3) and littermate controls (n=3). Data are presented as mean ± SEM.

Figure S6. Kinetics of Uptake of ^{125}I-TC-/[^{3}H]CEt-WT-HDL by Hepatocytes Isolated from Chow-Fed L-FoxO1,3,4 Mice. Related to Figure 5
(A-B) Hepatocytes from chow-fed L-FoxO1,3,4 mice and littermate controls were incubated (37°C, 10, 30 or 120 minutes) in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL (20 µg HDL protein/ml). Finally, cells were harvested, and apparent HDL particle uptake was analyzed as outlined in Materials and Methods. Values are means of (A) n = 3 (control) or of (B) n = 3 (L-FoxO1,3,4) independent determinations. Shown is a typical experiment. An independent similar experiment yielded qualitatively identical results. §§ $p < 0.01$, §§§ $p < 0.001$ comparing [^{3}H]CEt between L-FoxO1,3,4 and control hepatocytes; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ comparing [^{3}H]CEt - ^{125}I-TC between L-FoxO1,3,4 and control hepatocytes by Student's t-tests. Data are presented as mean ± SEM. Where no error bars are visible, the error was smaller than the symbol.

Figure S7. Effect of SR-BI Adenovirus on HL in WTD-fed L-FoxO1,3,4 Mice. Related to Figure 6
(A) Relative hepatic *Lipc* expression by qPCR in WTD-fed L-FoxO1,3,4 mice and littermate controls transduced with Adeno.SR-BI or control virus (Adeno.GFP). (n=3-4). **p<0.01 by two-way ANOVA. (B) Relative Plasma hepatic lipase activity in same mice as (A). **p<0.01 by two-way ANOVA.

Figure S8. Potential FoxO Binding Site Analysis of *Scarb1* and *Lipc* Using HOMER Motif Discovery Algorithm.
(A) Summary of potential FoxO binding motifs near *Scarb1* and *Lipc*. The entire sequences for mouse *Scarb1* and *Lipc*, along with 50kb of sequence upstream of their respective transcription start sites were taken from the UCSC Genome Browser (https://genome.ucsc.edu). The sequences were analyzed by the HOMER algorithm software, which provided the coordinates of all the potential FoxO binding motifs within the selected regions. Values are grouped by motifs within the gene sequence, motifs from the transcription start site to 5kb upstream of start site, 5kb-20kb upstream of start site, 20kb-50kb upstream of start site, and total number of motifs.
(B-C) Schematics from the UCSC Genome Browser highlighting positions of all motifs in selected region for (B) *Scarb1* and (C) *Lipc*. Highlighted in yellow are the relative coordinates of all potential FoxO binding motifs found though HOMER. Each motif is represented as a vertical line ("|"). Transcription start sites are indicated by the red arrow (both genes are transcribed in the reverse direction).

Figure S9. Cholesterol Levels in Plasma Fractionated by FPLC in Chow-fed Hepatic FoxO Floxed Mice Transduced with Liver Specific Adeno-Associated Virus Expressing Cre. **Related to Figure 7**
Plasma was collected 2 weeks after virus transduction and fractionated by FPLC in chow-fed, adult control mice containing all three FoxO alleles floxed (*Foxo1*$^{flox/flox}$, *Foxo3*$^{flox/flox}$, and *Foxo4*$^{flox/Y}$), and transduced with liver specific adeno-associated virus expressing either Cre (AAV8.Tbg.Cre) or control virus (AAV.GFP).

Figure S10. Western Blot of Liver PDZK1 from WTD-Fed L-FoxO1,3,4 Mice and Hepatocytes Isolated from L-FoxO1,3,4 mice. Related to Figure 7
(A-B) Representative western blot of hepatic PDZK1 expression from (A) WTD-fed L-FoxO1,3,4 mice and littermate controls *in vivo*, or (B) hepatocytes isolated from chow-fed L-FoxO1,3,4 mice and littermate controls *in vitro*. PDZK1 antibody was commercially purchased from Novus (Novus – NB400-149).

Table S1. L-FoxO1,3,4 Microarray From Liver Tissues. Microarrays were performed from liver tissues of chow-fed L-FoxO1,3,4 mice and littermate controls (Haeusler et al., 2014).

Gene	Control	L-FoxO1,3,4	*P* value
Scarb1	**1**	**0.727**	**0.043**
Lipc	**1**	**0.364**	**0.00091**
Abca1	1	1.132	0.27
Apoa1	1	0.950	0.17
Apoa2	1	0.96	0.56
Apoc3	1	0.86	0.28
Apoe	1	0.951	0.25
Lcat	1	1.021	0.86
Srebp2	1	0.80	0.47
Hmgcr	1	0.473	0.22
Pltp	1	1.89	0.11
Abcg5	1	0.928	0.76
Abcg8	1	0.825	0.532

Supplemental Experimental Procedures

Primer Sequences. Primer sequences for genes that were measured via qPCR. For liver and adrenal tissues, genes were normalized to *36b4*. For primary hepatocytes, genes were normalized to *B2m*.

Gene	Direction	Sequence (5'-3')
36b4	Forward	AGATGCAGCAGATCCGCAT
36b4	Reverse	GTTCTTGCCCATCAGCACC
B2m	Forward	CTGGTGCTTGTCTCACTGAC
B2m	Reverse	GTTCAGTATGTTCGGCTTCC
Abca1	Forward	GGTTTGGAGATGGTTATACAATAGTTGT
Abca1	Reverse	CCCGGAAACGCAAGTCC
Abcg5	Forward	TGGATCCAACACCTCTATGCTAAA
Abcg5	Reverse	GGCAGGTTTTCTCGATGAACTG
Abcg8	Forward	GTAGCTGATGCCGATGACAA
Abcg8	Reverse	GGGGCTGATGCAGATTCA
Apoa1	Forward	TGTGTATGTGGATGCGGTCA
Apoa1	Reverse	ATCCCAGAAGTCCCGAGTCA
Apoc3	Forward	GCATCTGCCCGAGCTGAAGAG
Apoc3	Reverse	CTGAAGTGATTGTCCATCCAGC
Apoe	Forward	CCGGTGCTGTTGGTCACATTGCTGACAGGAT
Apoe	Reverse	GTTCTTGTGTGACTTGGGAGCTCTGCAGCT
Foxo1	Forward	TCCAGTTCCTTCATTCTGCACT
Foxo1	Reverse	GCGTGCCCTACTTCAAGGATAA
G6pc	Forward	GTCTGGATTCTACCTGCTAC
G6pc	Reverse	AAAGACTTCTTGTGTGTCTGTC
Gck	Forward	CTGTTAGCAGGATGGCAGCTT
Gck	Reverse	TTTCCTGGAGAGATGCTGTGG
Hmgcr	Forward	CTTGTGGAATGCCTTGTGATTG
Hmgcr	Reverse	AGCCGAAGCAGCACATGAT
Lcat	Forward	GCTGGCCTGGTAGAGGAGATG
Lcat	Reverse	CCAAGGCTATGCCCAATGA
Lipc	Forward	GACGGGAAGAACAAGATTGG
lipc	Reverse	GGCATCATCAGGAGAAAGG
Pltp	Forward	TGGGACGGTGTTGCTCAA
Pltp	Reverse	CCCACGAGATCATCCACAGA
Scarb1	Forward	GGCTGCTGTTTGCTGCG
Scarb1	Reverse	GCTGCTTGATGAGGGAGGG
Srebp2	Forward	GATGATCACCCCGACGTTCAG
Srebp2	Reverse	GTACCGTCTGCACCTGCTGCT

Supplemental Figure S1

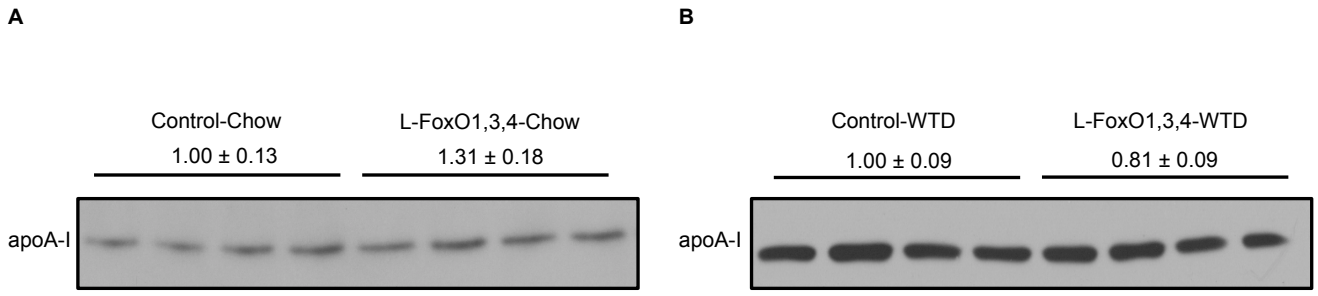


Figure S1. Western Blot of Total Plasma apoA-I. Related to Figure 1
(A-B) Plasma was collected from (A) chow-fed and (B) WTD-fed L-FoxO1,3,4 mice and littermate controls. Plasma was diluted by 1:200 with PBS, from which 20µl from each sample were run on a 4%-15% Tris-HCL gradient gel and blotted for apoA-I. Numbers denote relative apoA-I expression levels by densitometric scanning (n=4-7). Values are presented as mean ± SEM. Shown is a typical experiment. Independent experiments yielded qualitatively identical results.

Supplemental Figure S2

	Control		L-FoxO1,3,4	
Diet	Chow	WTD	Chow	WTD
ABCA1/Actin	1.00 ± 0.32	0.93 ± 0.32	1.39 ± 0.19	1.69 ± 0.29
SR-BI/Actin	1.00 ± 0.17	1.35 ± 0.42	0.24 ± 0.07	0.38 ± 0.13

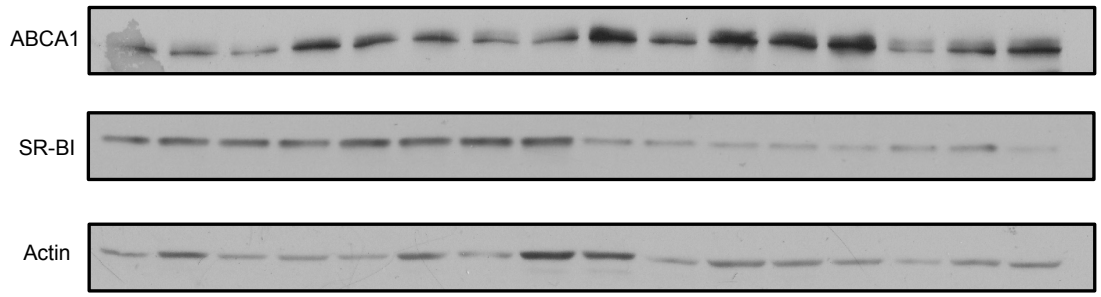
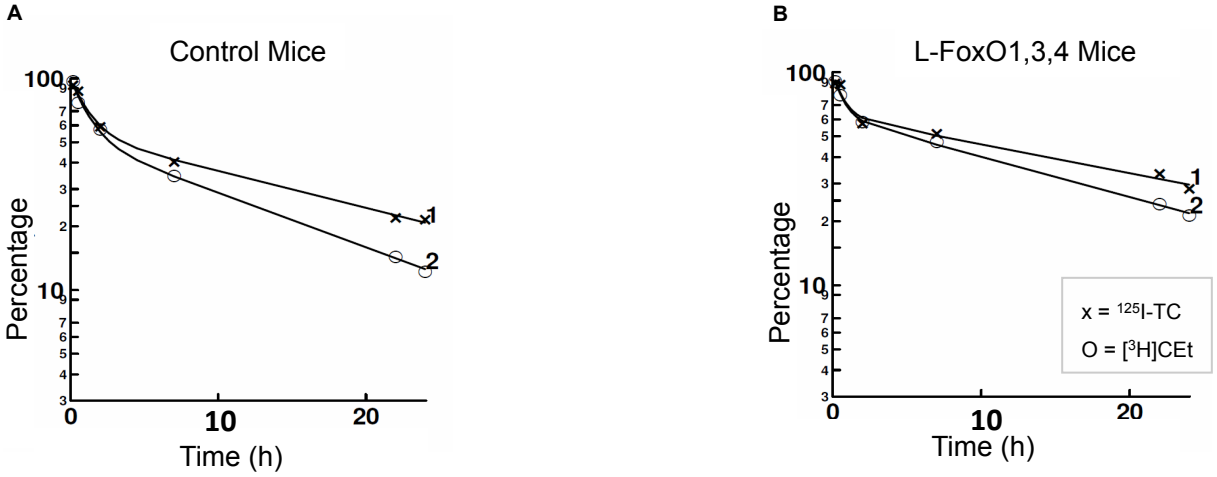


Figure S2. Western Blot of Liver ABCA1 and SR-BI from Chow-Fed and WTD-Fed L-FoxO1,3,4 Mice. Related to Figure 2
Representative western blot of hepatic ABCA1 and SR-BI expression in both chow-fed and WTD-fed L-FoxO1,3,4 mice and littermate controls. ABCA1/Actin and SR-BI/Actin denote relative ABCA1 and SR-BI expression levels by densitometric scanning, respectively (n=4). ABCA1 antibody was commercially purchased from Novus (Novus – NB400-105). Data are presented as mean ± SEM.

Supplemental Figure S3



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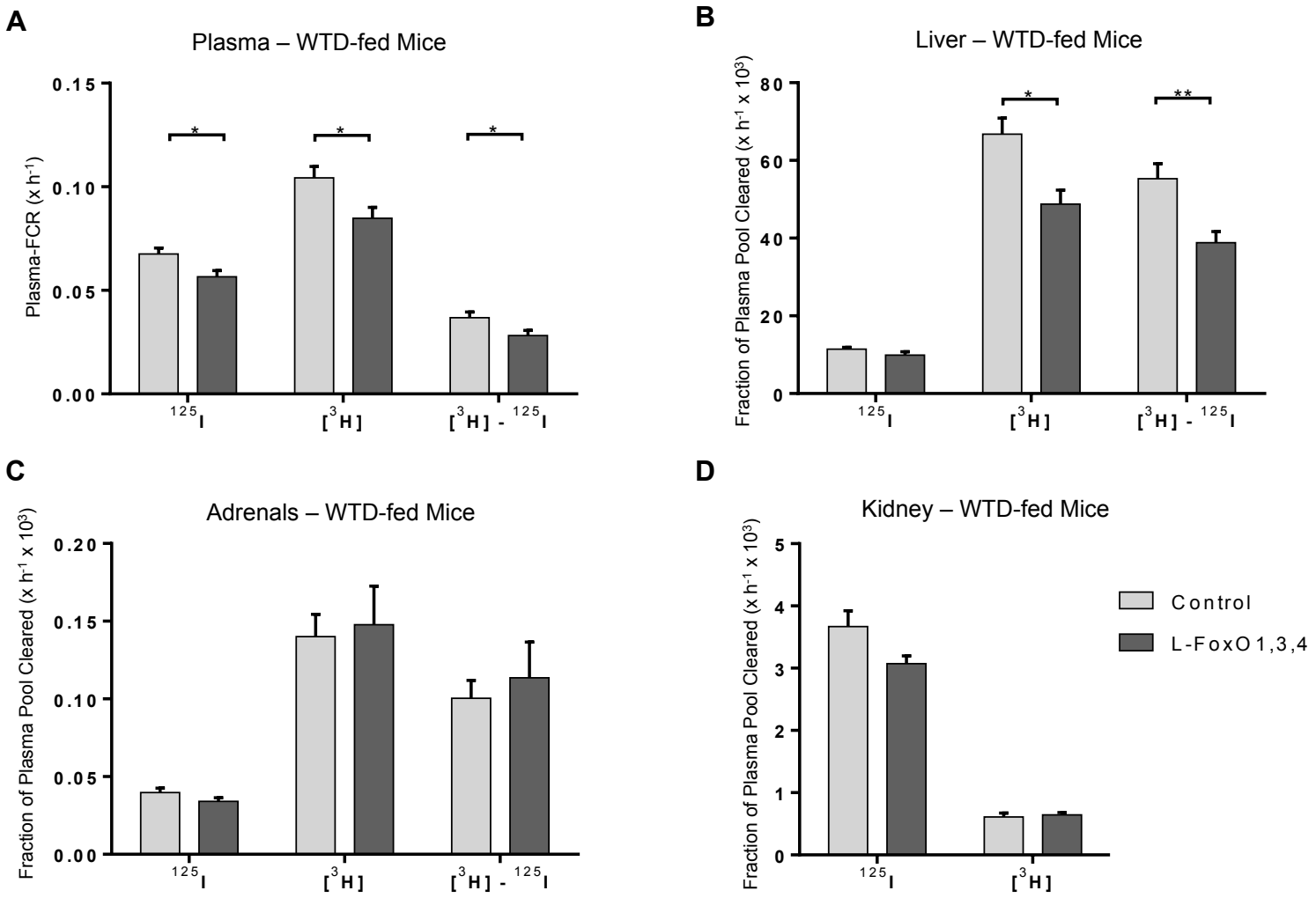
(A-B) ^{125}I-TC-/[^{3}H]CEt-WT-HDL was injected intravenously in WTD-fed L-FoxO1,3,4 mice and littermate controls. Thereafter, during a 24-hr interval, periodic blood samples were harvested and plasma was analyzed for ^{125}I-TC (crosses) and [^{3}H]CEt (circles). The y-axis represents the fraction of the tracer in plasma (%). Shown is a trace from a representative mouse from each genotype, with (A) control on the left and (B) L-FoxO1,3,4 on the right. The experiment was carried out in 6 control and 5 L-FoxO1,3,4 mice.

Supplemental Figure S4



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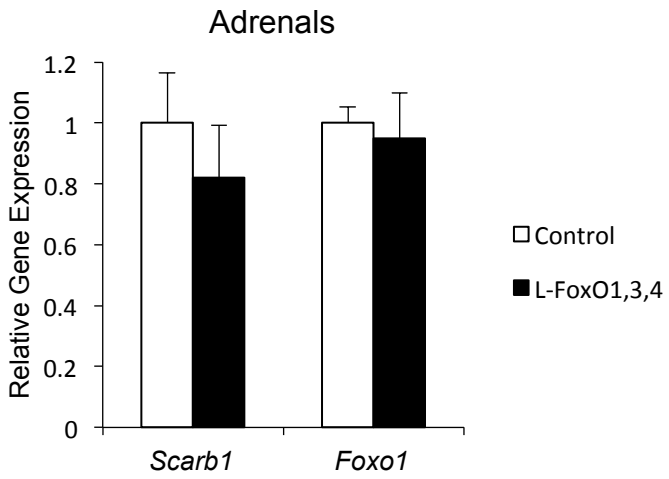
^{125}I-TC-/[^{3}H]CEt-WT-HDL was injected intravenously in WTD-fed L-FoxO1,3,4 mice and littermate controls. (A) During the subsequent 24-hr interval, blood was harvested periodically to determine the plasma decay of both tracers. ^{125}I-TC (^{125}I) and [^{3}H]CEt ([^{3}H]) were analyzed, and plasma-FCRs for ^{125}I-TC and [^{3}H]CEt were calculated. The difference in plasma-FCRs between [^{3}H]CEt and ^{125}I-TC was calculated. 24-hrs after tracer injection, the animals were euthanized, and tissues were analyzed for both tracers. (B) Liver, (C) adrenal and (D) kidney organ-FCRs for ^{125}I-TC (^{125}I), [^{3}H]CEt ([^{3}H]), and the difference in organ-FCRs between [^{3}H]CEt and ^{125}I-TC ([^{3}H]CEt - ^{125}I-TC) were calculated. All calculations were done as described in Materials and Methods. n = 6 control and n = 5 L-FoxO1,3,4 mice. An independent similar experiment with n=5 control mice and n=4 L-FoxO1,3,4 mice yielded qualitatively identical results as shown in the graphs. *$p < 0.05$, **$p < 0.01$, by Student's t-tests. Data are presented as mean ± SEM.

Supplemental Figure S5



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Relative adrenal *Scarb1* and *Foxo1* gene expression by qPCR in chow-fed L-FoxO1,3,4 mice (n=3) and littermate controls (n=3). Data are presented as mean ± SEM.

Supplemental Figure S6

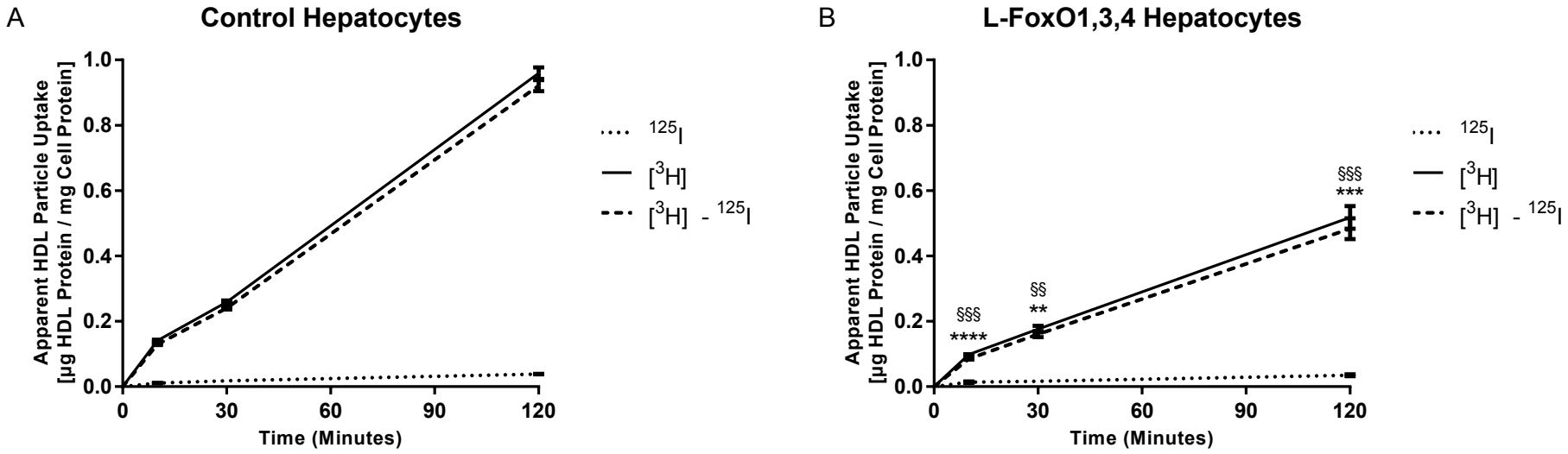


Figure S6. Kinetics of Uptake of ^{125}I-TC-/[^{3}H]CEt-WT-HDL by Hepatocytes Isolated from Chow-Fed L-FoxO1,3,4 Mice. Related to Figure 5

(A-B) Hepatocytes from chow-fed L-FoxO1,3,4 mice and littermate controls were incubated (37°C, 10, 30 or 120 minutes) in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL (20 µg HDL protein/ml). Finally, cells were harvested, and apparent HDL particle uptake was analyzed as outlined in Materials and Methods. Values are means of (A) n = 3 (control) or of (B) n = 3 (L-FoxO1,3,4) independent determinations. Shown is a typical experiment. An independent similar experiment yielded qualitatively identical results. §§ $p < 0.01$, §§§ $p < 0.001$ comparing [^{3}H]CEt between L-FoxO1,3,4 and control hepatocytes; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ comparing [^{3}H]CEt - ^{125}I-TC between L-FoxO1,3,4 and control hepatocytes by Student's t-tests. Data are presented as mean ± SEM. Where no error bars are visible, the error was smaller than the symbol.

Supplemental Figure S7

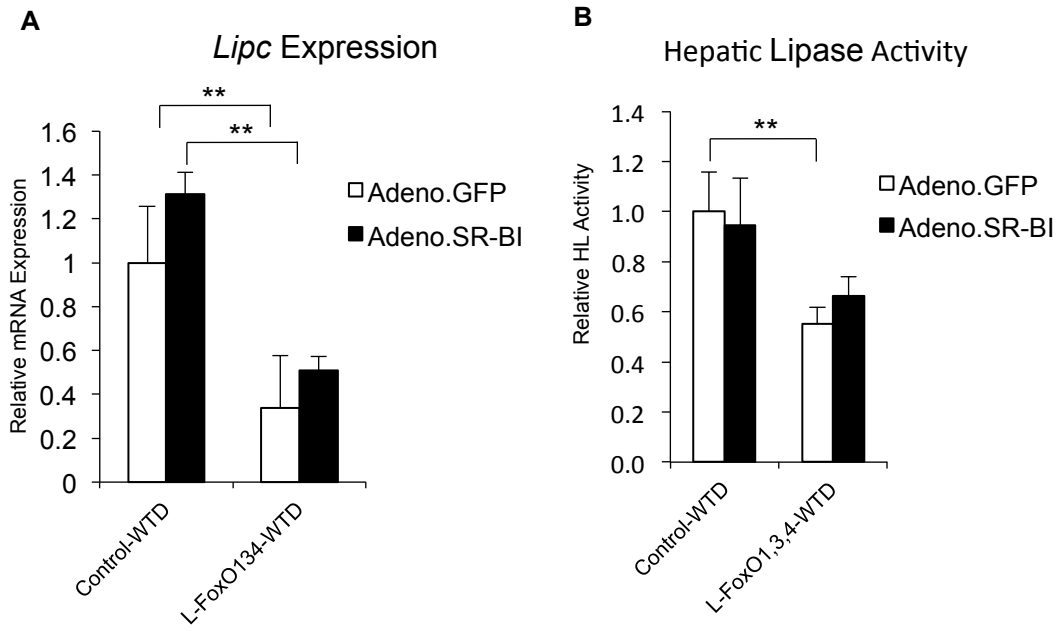


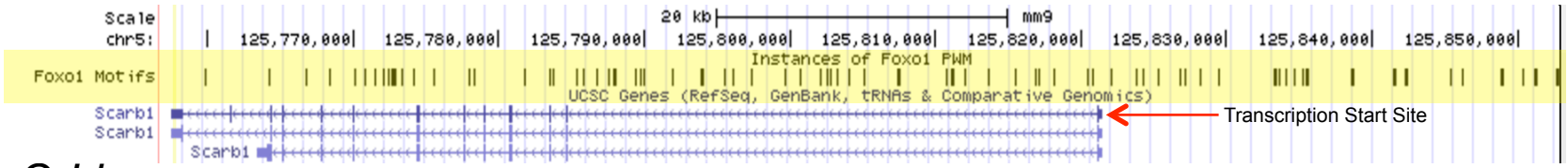
Figure S7. Effect of SR-BI Adenovirus on HL in WTD-fed L-FoxO1,3,4 Mice. Related to Figure 6 (A) Relative hepatic *Lipc* expression by qPCR in WTD-fed L-FoxO1,3,4 mice and littermate controls transduced with Adeno.SR-BI or control virus (Adeno.GFP). (n=3-4). **p<0.01 by two-way ANOVA. (B) Relative Plasma hepatic lipase activity in same mice as (A). **p<0.01 by two-way ANOVA.

Supplemental Figure S8

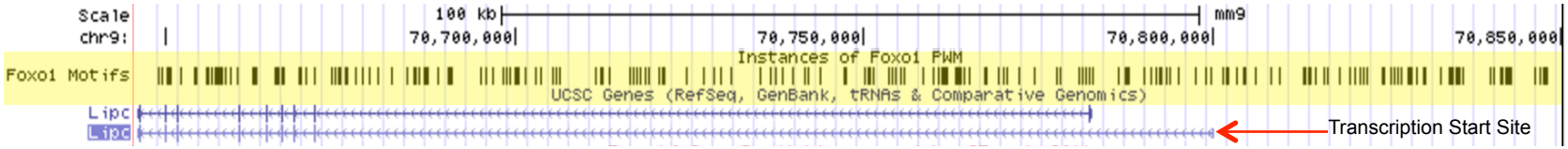
A

Gene Name	Motifs within gene sequence	Motifs from transcription start site to 5kb upstream	Motifs from 5kb-20kb upstream	Motifs from 20kb-50kb upstream	Total Motifs from end of gene to 50kb upstream
Scarb1	59	4	16	31	**=110**
Lipc	174	7	14	42	**=237**

B *Scarb1*



C *Lipc*



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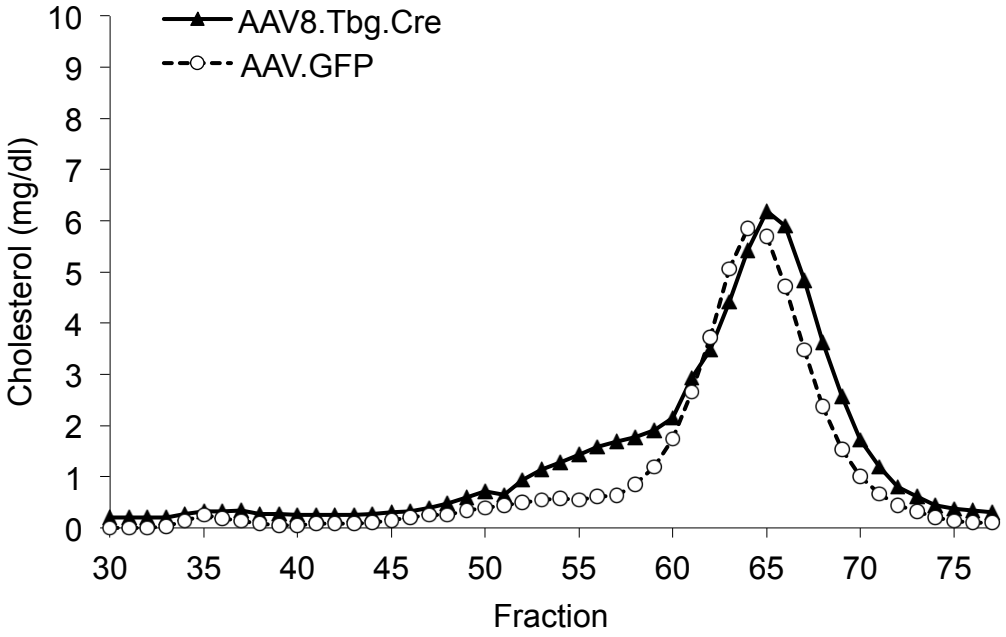
(A) Summary of potential FoxO binding motifs near *Scarb1* and *Lipc*. The entire sequences for mouse *Scarb1* and *Lipc*, along with 50kb of sequence upstream of their respective transcription start sites were taken from the UCSC Genome Browser (https://genome.ucsc.edu). The sequences were analyzed by the HOMER algorithm software, which provided the coordinates of all the potential FoxO binding motifs within the selected regions. Values are grouped by motifs within the gene sequence, motifs from the transcription start site to 5kb upstream of start site, 5kb-20kb upstream of start site, 20kb-50kb upstream of start site, and total number of motifs.
(B-C) Schematics from the UCSC Genome Browser highlighting positions of all motifs in selected region for (B) *Scarb1* and (C) *Lipc*. Highlighted in yellow are the relative coordinates of all potential FoxO binding motifs found though HOMER. Each motif is represented as a vertical line ("|"). Transcription start sites are indicated by the red arrow (both genes are transcribed in the reverse direction).

Supplemental Figure S9



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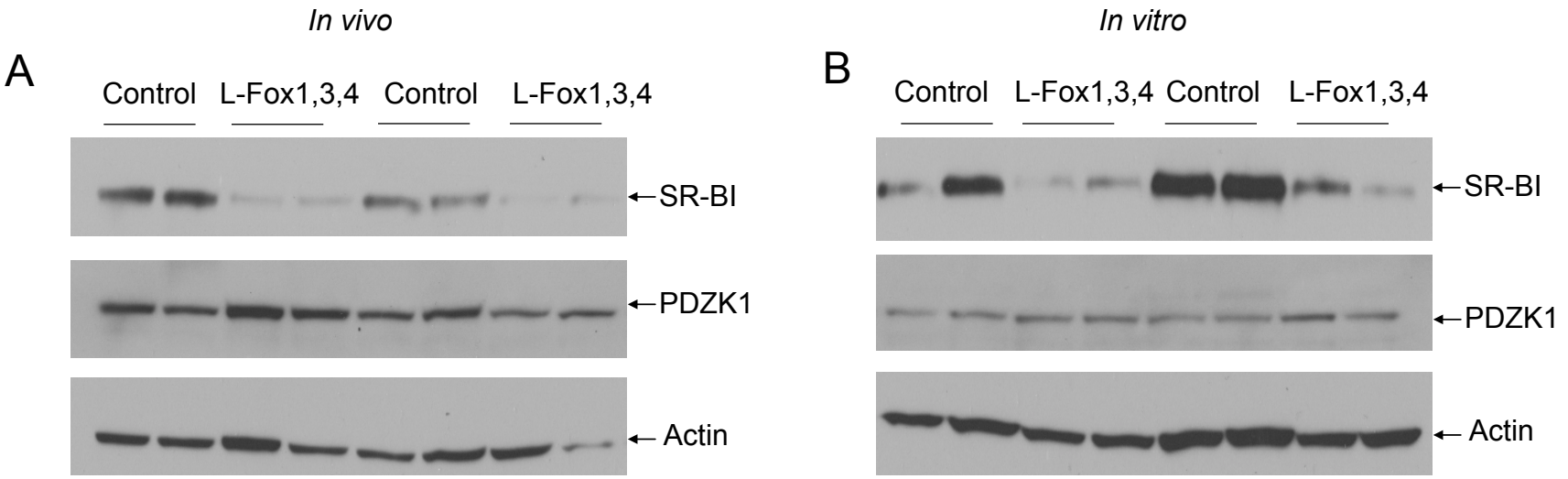
Supplemental Figure S10



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(A-B) Representative western blot of hepatic PDZK1 expression from (A) WTD-fed L-FoxO1,3,4 mice and littermate controls *in vivo*, or (B) hepatocytes isolated from chow-fed L-FoxO1,3,4 mice and littermate controls *in vitro*. PDZK1 antibody was commercially purchased from Novus (Novus – NB400-149).