Supplemental Legends

Fig. S1. Ezetimibe reduces serum cholesterol and induces PCSK9 (liver mRNA and serum protein) in a mouse model with a human-like serum lipid profile. (A.) A significant reduction in serum non-HDL is observed following 7 days of ezetimibe treatment (10 mg/kg/day) in Apoa1-CETP +/-, LDLR +/- mice fed a low fat western diet (0.21% cholesterol, 9% fat). Data represented as group means with error bars ±S.D. non-HDL. The percent difference between vehicle and ezetimibe treatment is indicated. qRT-PCR revealed a 2-fold increase in *Pcsk9* mRNA expression **(B.)** and a 3-fold increase in serum PCSK9 protein levels **(C.)**. Individual animals (circles) and group mean (bars) are shown. P-values were calculated using a two-tailed t-test (** $p < 0.001$, *** $p < 0.0001$).

Fig. S2. siRNA mediated knockdown of *Pcsk9* led to reduced serum cholesterol in combination with ezetimibe. (A.) A 2-fold increase in *Pcsk9* mRNA expression and **(B.)** PCSK9 serum protein levels was observed following 7 days of ezetimibe treatment (10 mg/kg/day), which was reduced 4-fold following a 6 mg/kg dose (i.v.) of the *Pcsk9*(1035) siRNA on day 4, 3 days prior to analysis. **(C.)** siRNA mediated knockdown of *Pcsk9* resulted in a significant decrease in serum non-HDL levels in combination with the ezetimibe treatment. Vehicle, Vehicle/PBS, and Vehicle/cntrl siRNA groups serve as negative controls. Individual animals (circles) and group means (bars) are shown. P-values and the percent difference were calculated relative to the Vehicle/cntrl siRNA group unless otherwise indicated by a bar. P-values were calculated using a one-way ANOVA, Tukey post-test (* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$).

Fig. S3. Analysis of liver enzymes and histological analysis of haematoxylin and eosin (H&E) stained liver sections revealed similar levels of minimal to mild inflammation across all treatment groups. (A.-C.) No significant differences were observed in ALT (alanine aminotransferase), AST (aspartate aminotransferase), or LDH (lactate dehydrogenase) levels. ALT, AST, and LDH levels all fell within a range consistent with normal hepatic function. Individual animals (circles) and group means (bars) are shown. **(D.)** Hepatic sections from 59 mice divided into 6 groups were reviewed using H&E. **(E.)** A subjective scoring system was utilized: 0 = no apparent lesion; 1 = minimal lesion; 2 =mild; 3 = moderate; 4 = marked; and 5 = severe. Data represented as group means with error bars (±S.D.).

Fig. S4. Hepatic LDLr (mRNA and protein) levels increase following *Pcsk9*(1076) siRNA treatment. (A.) Hepatic LDLr protein levels are elevated for the the *Pcsk9*(1076) treatments relative to the negative controls (Veh/PBS and Veh/cntrl). **(B.)** Hepatic *Ldlr* expression levels increase 2-fold following rosuvastatin/ezetimibe treatments (RSV/EZ/cntrl and RSV/EZ/*Pcsk9*(1076). Data represented as group means (bars) with error bars (±S.E.M.).

Fig. S5. siRNA mediated knockdown of *Pcsk9* led to greater reductions in both serum cholesterol and serum APOB protein levels in combination with ezetimibe, rosuvastatin, and the ezetimibe/rosuvastatin combination treatment. *Pcsk9* mRNA expression **(A.)** and serum protein levels **(B.)** were induced following the rosuvastatin

(RSV)/ezetimibe (EZ) treatment (RSV/EZ/cntrl) following 14 days of daily dosing (p.o.) with ezetimibe (10 mg/kg/day) and rosuvastatin (20 mg/kg/day), which was reduced to control treatment levels following a 6 mg/kg dose (i.v.) of a second *Pcsk9* siRNA sequence (*Pcsk9*(1035)) on day 11, 3 days prior to analysis. **(C.)** siRNA mediated knockdown of *Pcsk9* resulted in a significant decrease in serum APOB protein and (**D.**) serum non-HDL levels in combination with rosuvastatin, ezetimibe, and ezetimibe/rosuvastatin treatments. Vehicle/PBS, and Vehicle/cntrl siRNA groups serve as negative controls. Individual animals (circles) and group means (bars) are shown.

Fig. S6. Hepatic LDLr (mRNA and protein) levels increase following *Pcsk9*(1035) treatment. (A.) Hepatic LDLr protein levels are elevated for the *Pcsk9*(1035) treatments relative to the negative controls (Veh/PBS and Veh/cntrl). **(B.)** Hepatic *Ldlr* expression levels increase 2-fold following rosuvastatin/ezetimibe treatments (RSV/EZ/cntrl and RSV/EZ/*Pcsk9*(1035). Data represented as group means (bars) with error bars (±S.E.M.).

Fig. S7. siRNA mediated knockdown of *Pcsk9* led to reduced serum cholesterol in combination with rosuvastatin (**A.-B.**) Rosuvastatin (RSV) treatment (20 mg/kg/day) led to a significant increase in PCSK9 serum protein but not mRNA expression levels following 14 days of treatment. (**C.**) A small decrease in serum non-HDL was observed following rosuvastatin treatment and a slightly greater decrease was observed in combination with the *Pcsk9*(1035) siRNA, 3 days prior to analysis. Vehicle/PBS, and Vehicle/cntrl siRNA groups serve as negative controls. Individual animals (circles) and group means (bars) are shown. P-values and the percent difference were calculated relative to the Vehicle/cntrl siRNA group unless otherwise indicated by a bar. P-values were calculated using a one-way ANOVA, Tukey post-test (* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$).

Fig. S8. Induction of the SREBP-2 pathway does not translate into increased hepatic cholesterol esters (CE) and free cholesterol (FC). Individual animals (circles) and group means (bars) are shown. The percent difference and p-value between groups relative to Veh/cntrl are indicated. P-values calculated using a one-way ANOVA with a Tukey post-test (* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$).

Table S1. Summary of the changes in the hepatic lipid metabolic gene signature after ezetimibe and ezetimibe/ rosuvastatin treatments. 361 genes involved in hepatic lipid metabolism were analyzed using a PCR array (SA Biosciences). The fold regulation was calculated for the treatment group relative to the Veh/cntrl siRNA group. Red indicates a significant induction in expression ($p \leq 0.05$). P-values were calculated using a two-tailed t-test between control and treatment groups. The analysis software contains a 6-digit cut-off. Therefore, p-values below 6-digits are represented with a 0.

Table S2. siRNA sequences. siRNA sequences contained the following chemical modifications added to the 2' position of the ribose sugar when indicated: deoxy (d), 2' fluoro (f), or 2' O-methyl (o) (58). Modification abbreviations are given immediately

preceding the base to which they were applied. Passenger strands were capped with an inverted abasic nucleotide on the 5' and 3' ends (iB).

Fig. S1

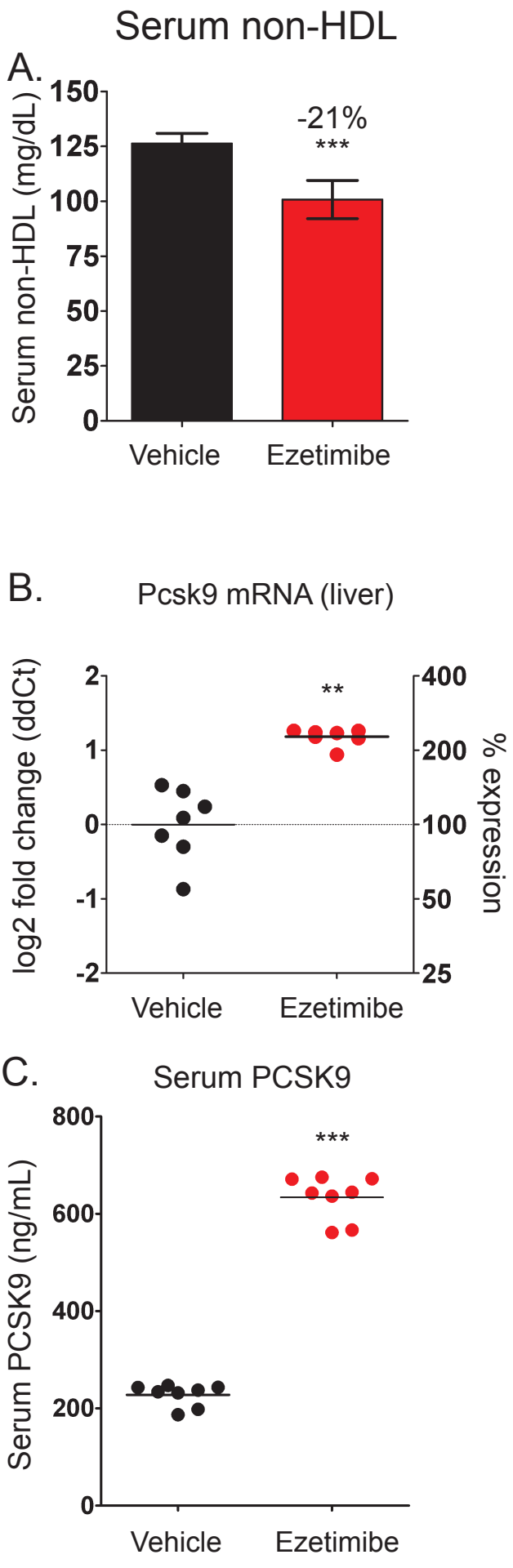

A.
Serum non-HDL
Serum non-HDL (mg/dL)
150
125
100
75
50
25
0
-21%
***
Vehicle
Ezetimibe
B.
Pcsk9 mRNA (liver)
log2 fold change (ddCt)
2
1
0
-1
-2
**
400
200
100
50
25
% expression
Vehicle
Ezetimibe
C.
Serum PCSK9
Serum PCSK9 (ng/mL)
800
600
400
200
0
***
Vehicle
Ezetimibe

Fig. S2

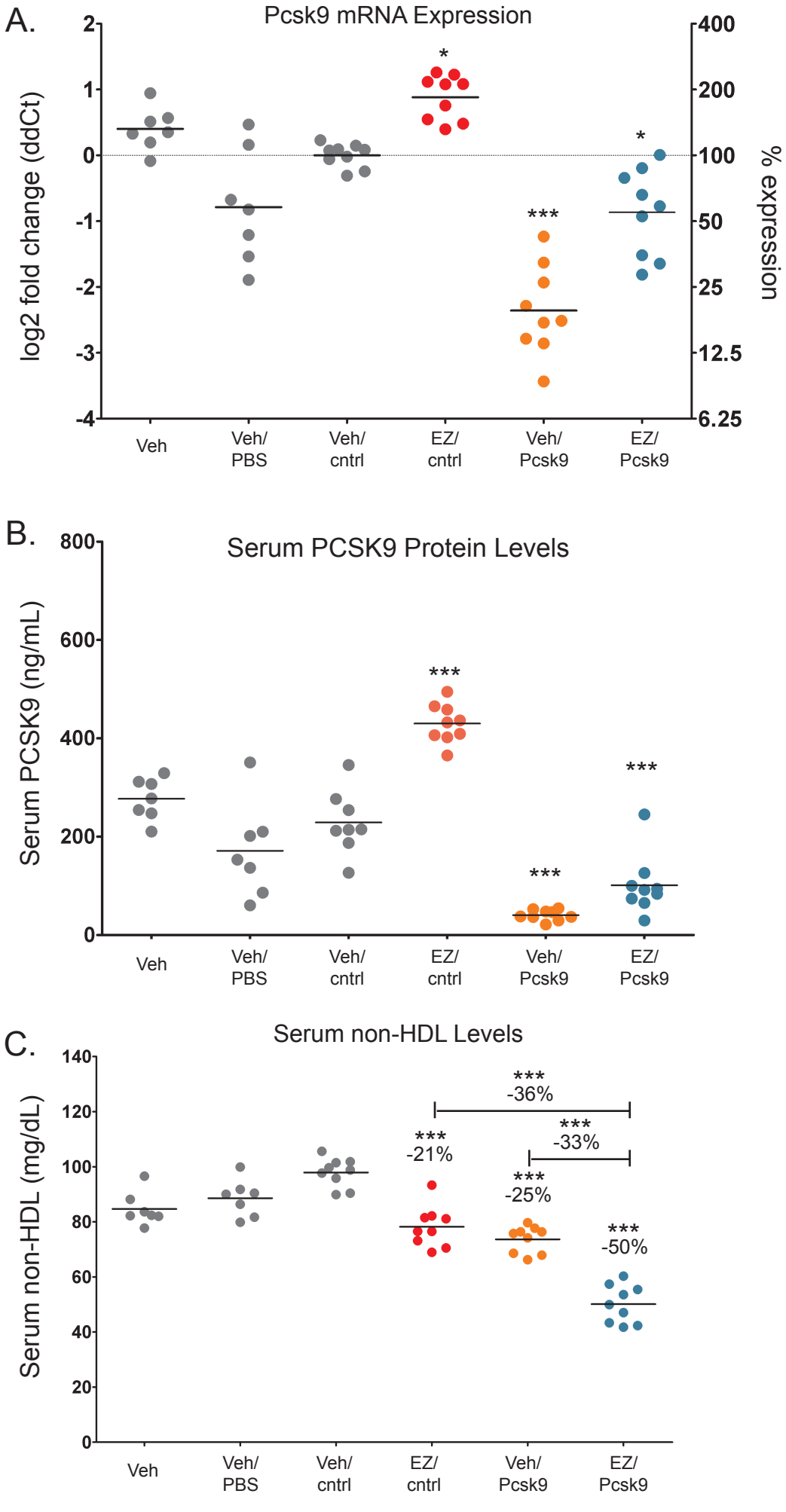

A.
Pcsk9 mRNA Expression
log2 fold change (ddCt)
% expression
2
1
0
-1
-2
-3
-4
400
200
100
50
25
12.5
6.25
*
***
*
Veh
Veh/ PBS
Veh/ cntrl
EZ/ cntrl
Veh/ Pcsk9
EZ/ Pcsk9
B.
Serum PCSK9 Protein Levels
Serum PCSK9 (ng/mL)
800
600
400
200
0
***
***
***
Veh
Veh/ PBS
Veh/ cntrl
EZ/ cntrl
Veh/ Pcsk9
EZ/ Pcsk9
C.
Serum non-HDL Levels
Serum non-HDL (mg/dL)
140
120
100
80
60
40
20
0
***
-36%
***
-33%
***
-21%
***
-25%
***
-50%
Veh
Veh/ PBS
Veh/ cntrl
EZ/ cntrl
Veh/ Pcsk9
EZ/ Pcsk9

Fig. S3

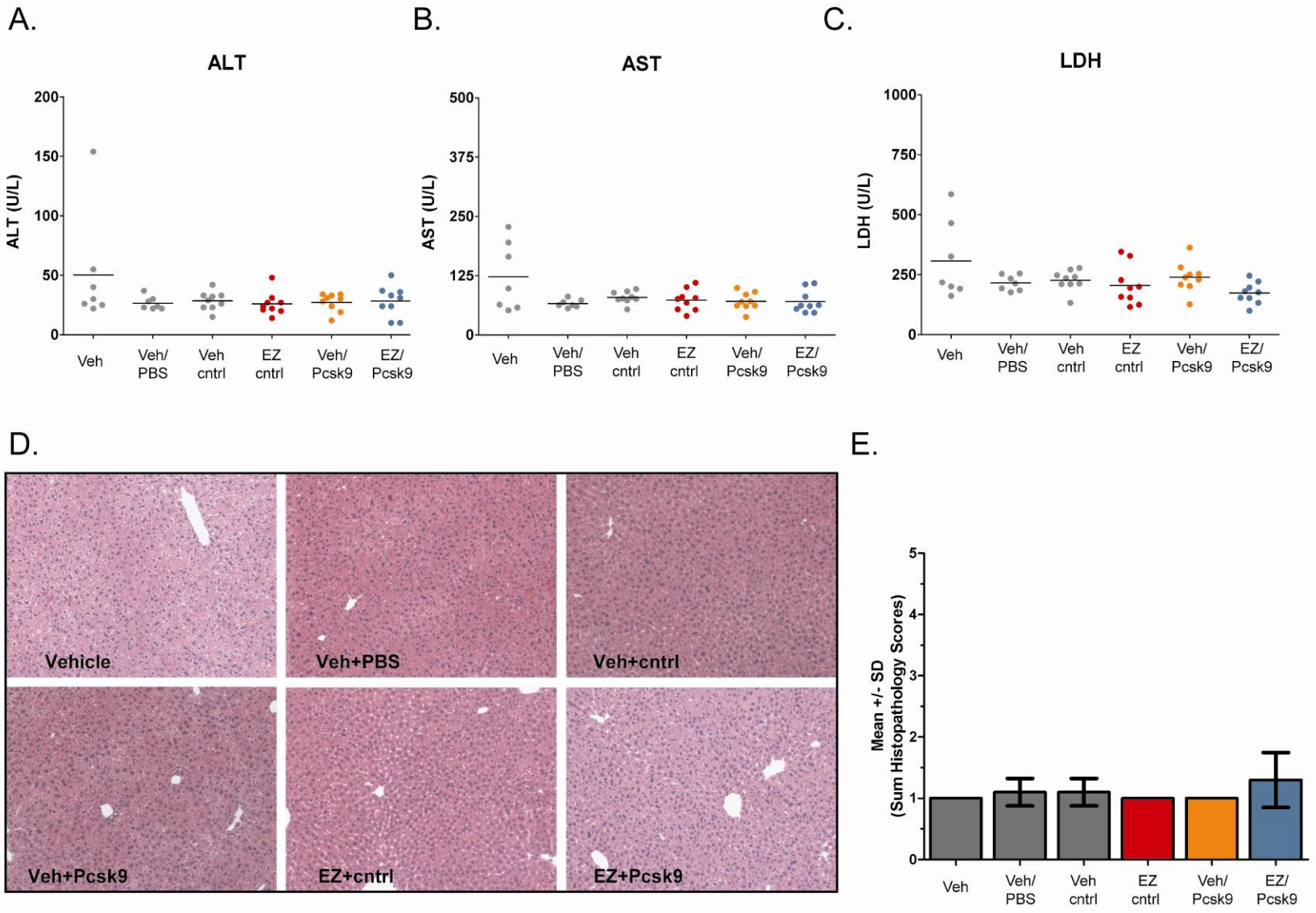
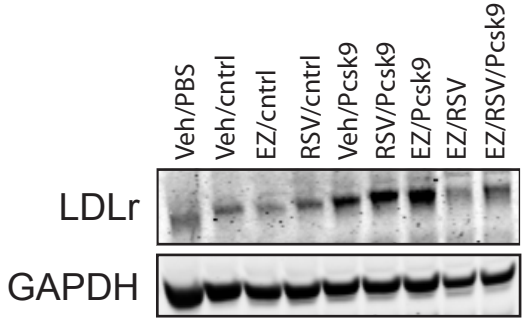

A.
ALT
ALT (U/L)
200
150
100
50
0
Veh
Veh/ PBS
Veh cntrl
EZ cntrl
Veh/ Pcsk9
EZ/ Pcsk9
B.
AST
AST (U/L)
500
375
250
125
0
Veh
Veh/ PBS
Veh cntrl
EZ cntrl
Veh/ Pcsk9
EZ/ Pcsk9
C.
LDH
LDH (U/L)
1000
750
500
250
0
Veh
Veh/ PBS
Veh cntrl
EZ cntrl
Veh/ Pcsk9
EZ/ Pcsk9
D.
Vehicle
Veh+PBS
Veh+cntrl
Veh+Pcsk9
EZ+cntrl
EZ+Pcsk9
E.
Mean +/- SD
(Sum Histopathology Scores)
5
4
3
2
1
0
Veh
Veh/ PBS
Veh cntrl
EZ cntrl
Veh/ Pcsk9
EZ/ Pcsk9

Fig. S4

Pcsk9(1076)

A.

LDLr protein (liver)



B.

Ldlr mRNA (liver)

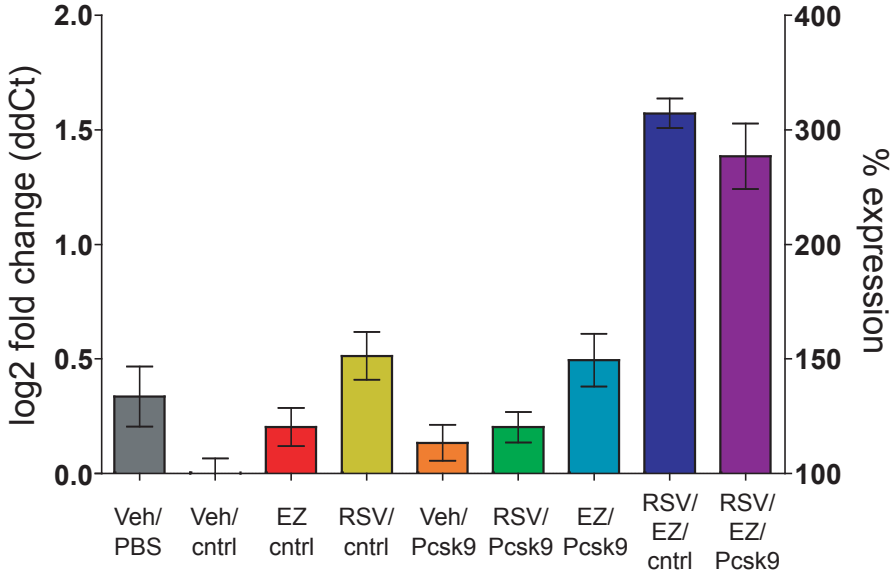


Fig. S5

Pcsk9(1035)

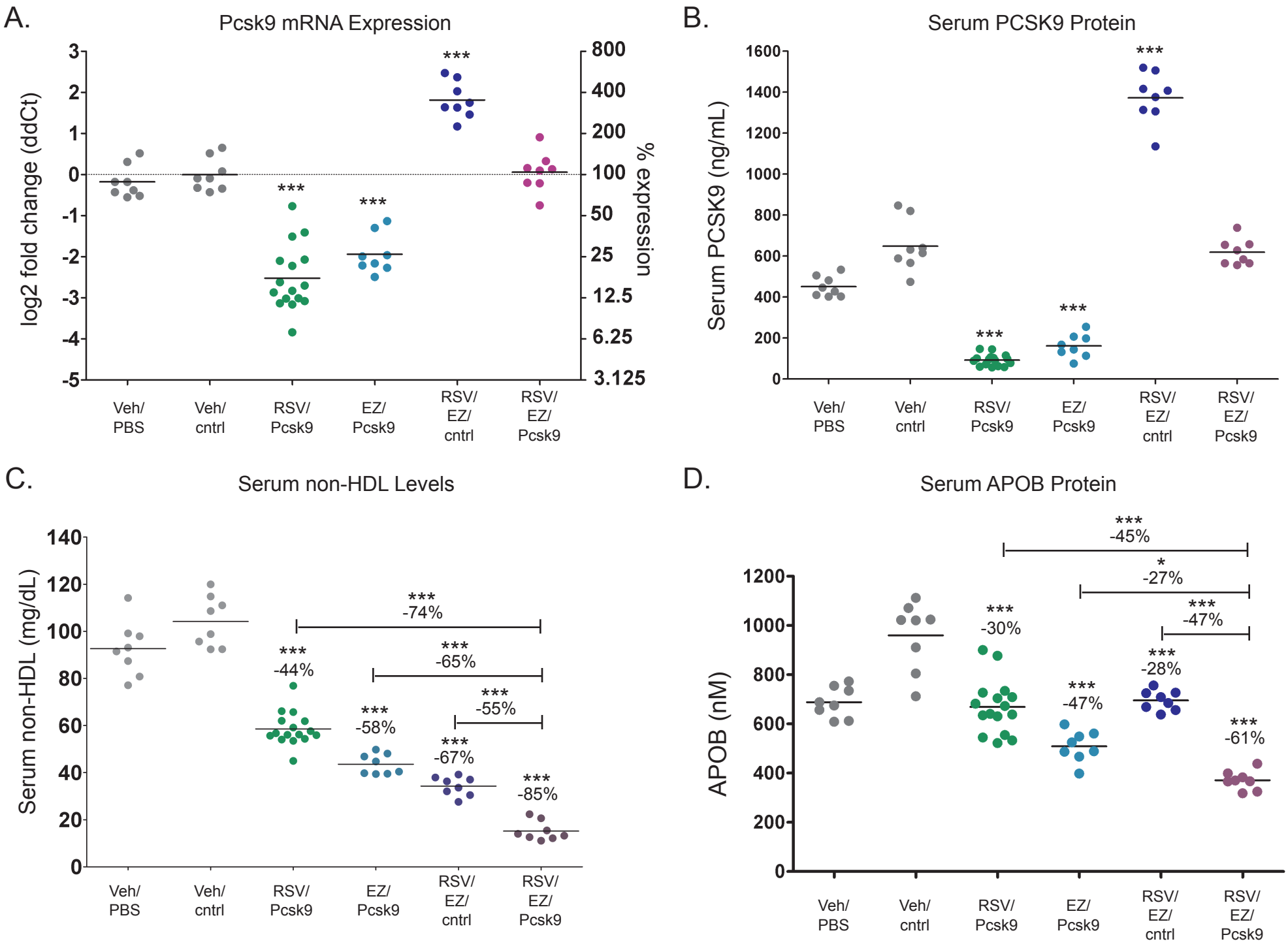
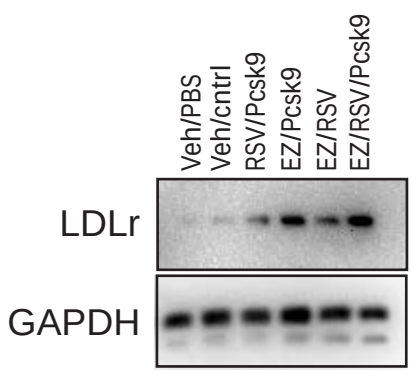


Fig. S6

Pcsk9(1035)

A.

LDLr protein (liver)



B.

Ldlr mRNA (liver)

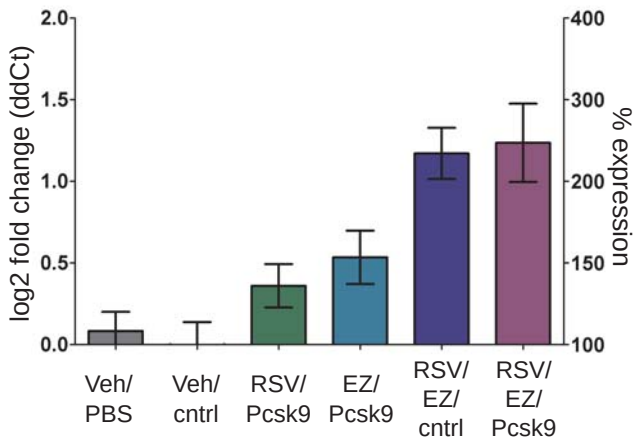


Fig. S7

Pcsk9(1035)

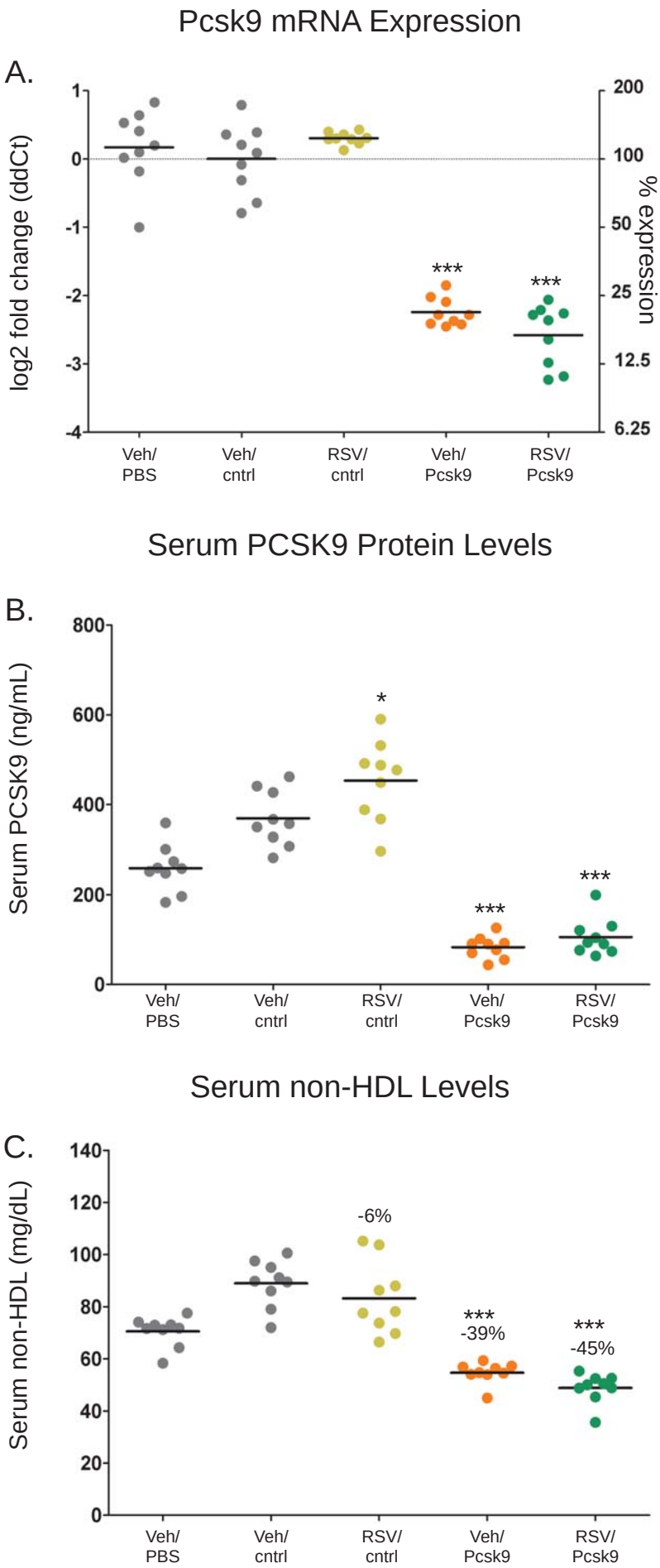
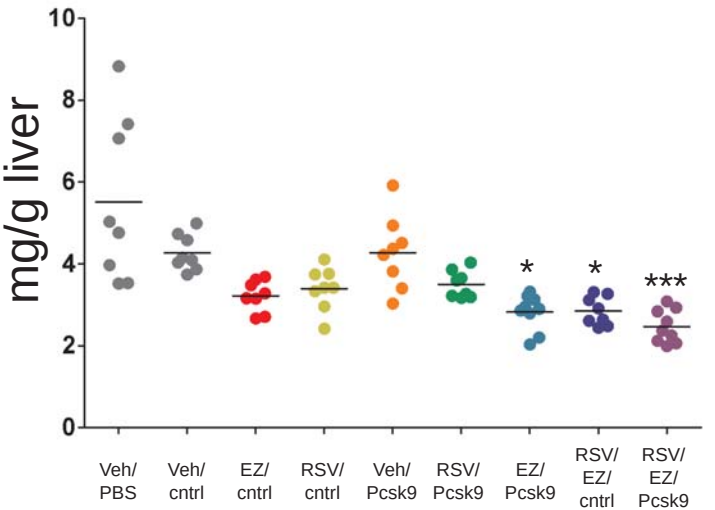


Fig. S8

Pcsk9(1076)

A. cholesterol esters



B. free cholesterol