Supplemental materials and methods

Animals.

Human cholesteryl ester transfer protein transgenic (hCETP tg) mice, expressing a human CETP minigene under the control of its natural flanking regions (1), originated from Dr. Alan Tall´s laboratory at Columbia University (New York, NY) and were bred at the Leiden University Medical Center, The Netherlands.

Western blotting.

Western blots for apolipoprotein (apo) A-I and apoB48/100 were carried out on plasma. Western blots for apoA-I and human apoE were carried out on fast protein liquid chromatography (FPLC) fractions. Equal amounts of plasma and individual FPLC fractions were resolved by SDS-PAGE electrophoresis and blotted onto nitrocellulose. ApoA-I was visualized using a commercially available rabbit anti-mouse apoA-I antibody (Calbiochem, San Diego, CA), apoB48/100 was detected using a commercially available rabbit anti-mouse apoB48/100 antibody (Biodesign, Memphis, TN) and human apoE was visualized using a mouse monoclonal anti-human apoE3 antibody (2) each followed by the appropriate HRP-conjugated secondary antibody. HRP was detected using chemiluminescence (ECL, GE Healthcare, Piscataway, NJ). For plasma apoA-I and apoB48/100 Western blots densitometry analysis of the bands was performed using ImageJ software (National Institutes of Health, Bethesda, MD). Results were normalized to the relative expression levels of the respective controls, i.e mice receiving the control adenovirus AdNull.

Western blots for SR-BI were carried out on total liver homogenates as well as on hepatic membrane fractions prepared essentially as described (3). Protein concentrations were determined with the bicinchoninic acid (BCA) assay (Pierce Biotechnology, Inc., Rockford, IL). Equal amounts of protein were resolved by SDS-PAGE electrophoresis and blotted onto nitrocellulose. SR-BI was visualized using a commercially available goat anti-mouse SR-BI antibody (Novus Biologicals, Littleton, CO), followed by the appropriate HRP-conjugated secondary antibody. Detection of HRP and densitometry analysis of the bands was performed as detailed above.

References

1. Jiang, X. C., L. B. Agellon, A. Walsh, J. L. Breslow, and A. Tall. 1992. Dietary cholesterol increases transcription of the human cholesteryl ester transfer protein gene in transgenic mice. Dependence on natural flanking sequences. *J Clin Invest* **90**: 1290-1295.

2. Tangirala, R. K., D. Pratico, G. A. FitzGerald, S. Chun, K. Tsukamoto, C. Maugeais, D. C. Usher, E. Pure, and D. J. Rader. 2001. Reduction of isoprostanes and regression of advanced atherosclerosis by apolipoprotein E. *J Biol Chem* **276**: 261-266.

3. Tietge, U. J., N. Nijstad, R. Havinga, J. F. Baller, F. H. van der Sluijs, V. W. Bloks, T. Gautier, and F. Kuipers. 2008. Secretory phospholipase A2 increases SR-BI-mediated selective uptake from HDL but not biliary cholesterol secretion. *J Lipid Res* **49**: 563-571.

Supplemental figure legends

Supplemental figure I: Apolipoprotein E overexpression decreases plasma protein levels of apoA-I and apoB100.

On day 4 following injection with either the control adenovirus AdNull or the human apolipoprotein E3 expressing adenovirus AdhApoE3 plasma samples were taken. Protein expression of (A) apoA-I, (B) apoB100, and (C) apoB48 in plasma was determined by Western blotting as described under "Supplemental materials and methods". The upper panel shows the Western blot and the lower panel its respective quantification. Data are presented as means ± SEM. n = 6 mice for each condition. -, AdNull-injected mice; +, AdhApoE3-injected mice. White bars, AdNull-injected mice; black bars, AdhApoE3-injected mice. * significantly different from the respective AdNull-injected controls as assessed by Mann-Whitney *U*-test (at least $P < 0.05$).

Supplemental figure II: Distribution of human apolipoprotein E over the different lipoprotein fractions.

Pooled plasma samples (n=6 mice per group) collected on day 4 following injection of wild-type mice with either the control adenovirus AdNull or the human apolipoprotein E3 expressing adenovirus AdhApoE3 were subjected to fast protein liquid chromatography (FPLC) analysis using a Superose 6 column, then cholesterol concentrations were determined. Protein expression of apoA-I and human apoE in individual fractions was determined by Western blotting as described under "Supplemental materials and

methods". Open circles, AdNull-injected controls; filled squares; AdhApoE3-injected mice.

Supplemental figure III: Apolipoprotein E overexpression affects plasma cholesterol distribution over the different lipoprotein subclasses in human CETP transgenic mice.

Fast protein liquid chromatography (FPLC) profiles in response to apolipoprotein E overexpression in human CETP transgenic mice (hCETP tg). Pooled plasma samples collected on day 4 following injection with either the control adenovirus AdNull or the human apolipoprotein E3 expressing adenovirus AdhApoE3 were subjected to gel filtration chromatography analysis using a Superose 6 column. n = 6 mice for each condition. Open circles, AdNull-injected controls; filled squares; AdhApoE3-injected mice.

Supplemental figure IV: Apolipoprotein E overexpression does not affect hepatic protein expression of SR-BI.

On day 4 following injection with either the control adenovirus AdNull or the human apolipoprotein E3 expressing adenovirus AdhApoE3 livers were snap-frozen in liquid nitrogen. Hepatic protein expression for SR-BI was determined in total liver homogenates in (A) wild-type and (B) human CETP transgenic mice as well as in hepatic membrane fractions in (C) wild-type and (D) human CETP transgenic mice by Western blotting as described under "Supplemental materials and methods". The upper panel shows the Western blot and the lower panel its quantification. Data are presented as means ± SEM.

n = 6 mice for each condition. -, AdNull-injected mice; +, AdhApoE3-injected mice. White bars, AdNull-injected mice; black bars, AdhApoE3-injected mice. hCETP tg, human cholesteryl ester transfer protein transgenic mice.

Supplemental figure V: Apolipoprotein E overexpression decreases biliary cholesterol excretion in human CETP transgenic mice.

Bile flow (A) and biliary secretion rates of (B) bile acids (BA), (C) phospholipids (PL), and (D) cholesterol (TC) in response to hepatic apolipoprotein E overexpression in human CETP transgenic mice. On day 4 following injection with either the control adenovirus AdNull or the human apolipoprotein E3 expressing adenovirus AdhApoE3 bile was collected continuously for 30 minutes. Biliary output rates of bile acids, phospholipids, and cholesterol were determined as described under "Materials and Methods". Data are presented as means ± SEM. n = 6 mice for each condition. White bars, AdNull-injected mice; black bars, AdhApoE3-injected mice. * significantly different from the respective AdNull-injected controls as assessed by Mann-Whitney *U*-test (at least $P < 0.05$).

Supplemental figure VI: Apolipoprotein E overexpression does not affect *in vivo* macrophage-to-feces reverse cholesterol transport in human CETP transgenic mice.

On day 2 following injection with either the control adenovirus AdNull or the human apolipoprotein E3 expressing adenovirus AdhApoE3 mice received intraperitoneal injections with ^{3}H-cholesterol-loaded primary mouse macrophage foam cells as described under "Materials and Methods". (A) Time course of ^{3}H-cholesterol recovery in plasma.

(B) ^{3}H-cholesterol within liver 48 h after macrophage administration. (C) ^{3}H-cholesterol appearance in feces collected continuously from 0 to 48 h after macrophage administration and separated into bile acid and neutral sterol fractions as indicated. Data are expressed as percentage of the injected tracer dose and presented as means ± SEM. n = 8 mice for each condition. White bars, AdNull-injected mice; black bars, AdhApoE3-injected mice. * significantly different from the respective AdNull-injected controls as assessed by Mann-Whitney *U*-test (at least $P < 0.05$).

Supplemental tables

Supplemental table I. Plasma lipids in human CETP transgenic mice in response to hepatic apolipoprotein E overexpression.

	hCETP tg	
	AdNull	AdhApoE3
Total cholesterol (mg/dl)	54.5 ± 4.1	46.9 ± 3.5
Free cholesterol (mg/dl)	25.3 ± 3.9	28.0 ± 2.5
Esterified cholesterol (mg/dl)	29.2 ± 1.6	19.0 ± 1.4*
Phospholipids (mg/dl)	87.9 ± 10.1	87.1 ± 8.2
Triglycerides (mg/dl)	29.8 ± 5.3	38.7 ± 9.0

Samples were taken on day 4 following adenovirus injection, and plasma lipids were determined as described under "Materials and Methods". Values are means ± SEM; n = 6 mice for each condition. AdhApoE3, recombinant adenovirus expressing human apoE3; AdNull, empty control adenovirus; hCETP tg, human cholesteryl ester transfer protein transgenic mice.
* significantly different from the respective AdNull-injected controls as assessed by Mann-Whitney *U*-test (at least $P < 0.05$).

Supplemental table II. Liver lipid composition in human CETP transgenic mice in response to hepatic apolipoprotein E overexpression.

	hCETP tg	
	AdNull	AdhApoE3
Total cholesterol (nmol/mg liver)	7.7 ± 0.1	10.4 ± 0.6*
Free cholesterol (nmol/mg liver)	5.6 ± 0.1	8.0 ± 0.8*
Esterified cholesterol (nmol/mg liver)	2.0 ± 0.1	2.5 ± 0.4
Phospholipids (nmol/mg liver)	25.5 ± 1.3	26.4 ± 0.5
Triglycerides (nmol/mg liver)	38.8 ± 2.4	78.5 ± 9.2*

Livers of mice administered the respective adenoviruses were harvested on day 4 following adenovirus injection and snap-frozen in liquid nitrogen. Liver lipids were measured as described under "Materials and Methods". Values are means ± SEM; n = 6 mice for each condition. AdhApoE3, recombinant adenovirus expressing human apoE3; AdNull, empty control adenovirus; hCETP tg, human cholesteryl ester transfer protein transgenic mice.

* significantly different from the respective AdNull-injected controls as assessed by Mann-Whitney *U*-test (at least $P < 0.05$).

Supplemental table III. Hepatic mRNA expression in human CETP transgenic mice in response to hepatic apolipoprotein E overexpression.

	hCETP tg	
	AdNull	AdhApoE3
Sr-b1	1.00 ± 0.07	0.65 ± 0.06*
Abcb11	1.00 ± 0.06	0.60 ± 0.07*
Abcb4	1.00 ± 0.10	0.85 ± 0.03
Abcg5	1.00 ± 0.08	0.48 ± 0.06*
Abcg8	1.00 ± 0.07	0.62 ± 0.08*
Cyp7a1	1.00 ± 0.19	0.38 ± 0.07
Cyp27a1	1.00 ± 0.11	0.41 ± 0.03*
Cyp8b1	1.00 ± 0.20	0.48 ± 0.06*
Srebp2	1.00 ± 0.05	0.77 ± 0.04*
Ldlr	1.00 ± 0.04	0.77 ± 0.06*
Hmgcr	1.00 ± 0.10	0.74 ± 0.06
Abca1	1.00 ± 0.05	0.88 ± 0.07

Livers of mice administered the respective adenoviruses were harvested on day 4 following adenovirus injection and snap-frozen in liquid nitrogen. mRNA expression levels were determined by real-time quantitative PCR as described under "Materials and Methods". Values are means ± SEM; n = 6 mice for each condition. Within each set of experiments, gene expression levels are related to the respective AdNull-injected controls. AdhApoE3, recombinant adenovirus expressing human apoE3; AdNull, empty control adenovirus; hCETP tg, human cholesteryl ester transfer protein transgenic mice. * significantly different from the respective AdNull-injected controls as assessed by Mann-Whitney *U*-test (at least $P < 0.05$).

Supplemental table IV. Fecal excretion of neutral sterols in wild-type and human CETP transgenic mice in response to hepatic apoE overexpression.

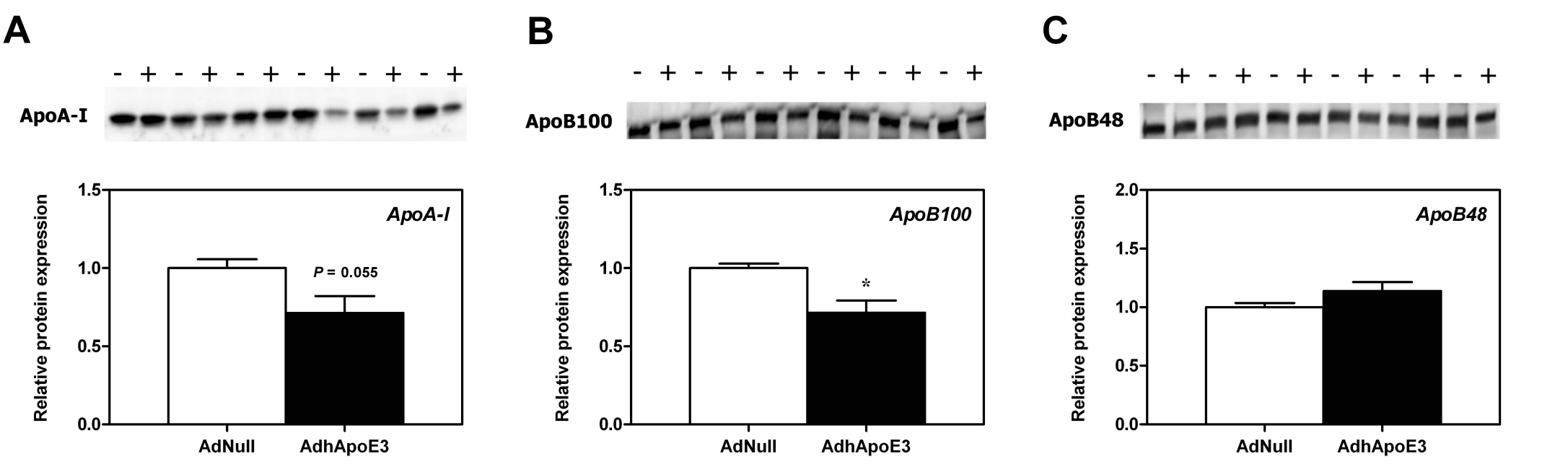
	Wild-type		hCETP tg	
	AdNull	AdhApoE3	AdNull	AdhApoE3
Coprostanol (μmol/day)	0.55 ± 0.15	0.36 ± 0.09	0.78 ± 0.08	0.83 ± 0.09
Cholesterol (μmol/day)	2.30 ± 0.09	1.94 ± 0.27	2.77 ± 0.27	2.62 ± 0.30
Dihydrocholesterol (μmol/day)	0.25 ± 0.03	0.19 ± 0.02*	0.26 ± 0.01	0.23 ± 0.01
Total neutral sterols (μmol/day)	3.11 ± 0.22	2.49 ± 0.34	3.80 ± 0.34	3.67 ± 0.40

Mice of the indicated genotypes administered the respective adenoviruses were individually housed and feces were collected over a period of 24 h starting on day 3 after adenovirus injection. Fecal samples were separated from the bedding, dried, weighed, and ground. Aliquots were used to determine the content of different neutral sterol species by gas liquid chromatography as described under "Materials and Methods". Values are means ± SEM; n = 6 mice for each condition. AdhApoE3, recombinant adenovirus expressing human apoE3; AdNull, empty control adenovirus; hCETP tg, human cholesteryl ester transfer protein transgenic mice. * significantly different from the respective AdNull-injected controls as assessed by Mann-Whitney *U*-test (at least $P < 0.05$).

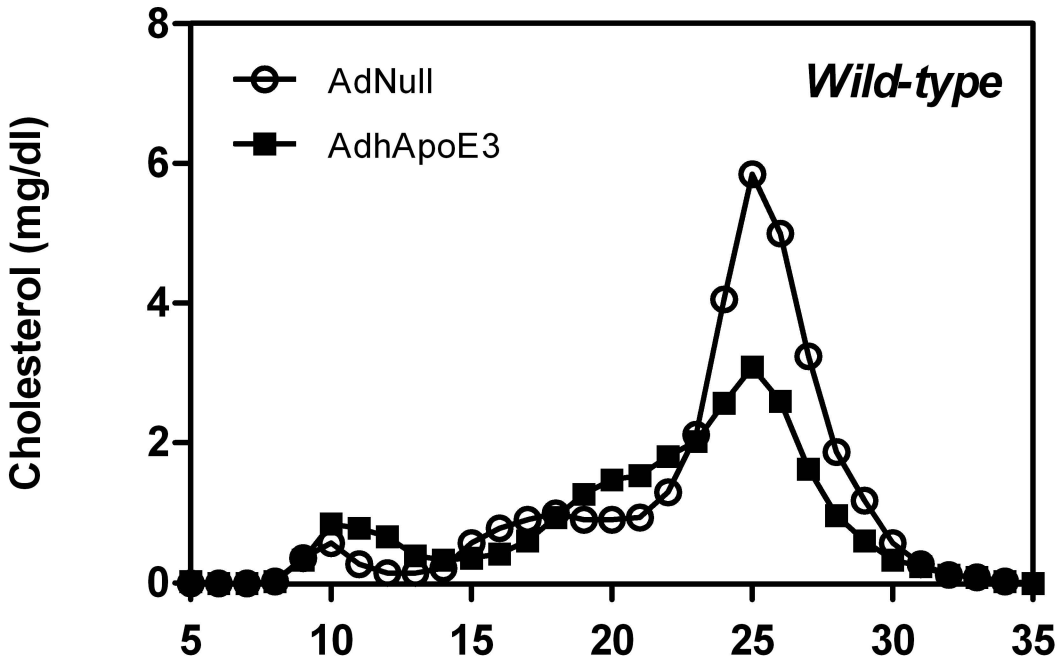
Supplemental table V. Fecal excretion of bile acids in wild-type and human CETP transgenic mice in response to hepatic apoE overexpression.

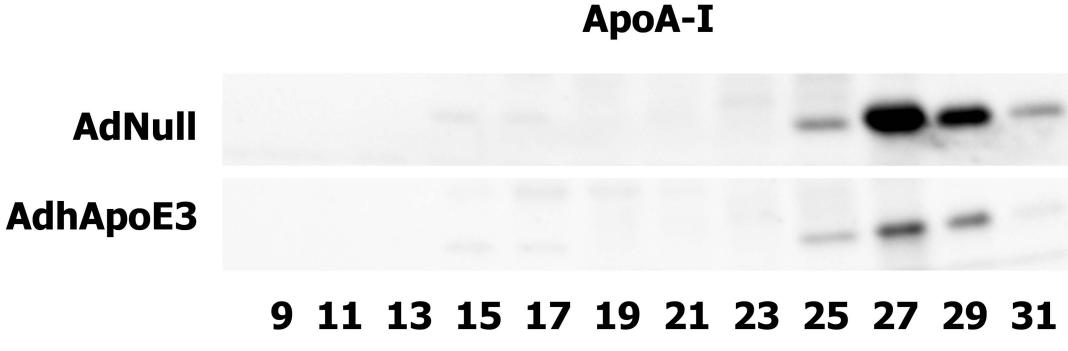
	Wild-type		hCETP tg	
	AdNull	AdhApoE3	AdNull	AdhApoE3
Allocholic acid (μmol/day)	0.067 ± 0.008	0.088 ± 0.006	ND	ND
α-Muricholic acid (μmol/day)	0.124 ± 0.016	0.124 ± 0.008	0.332 ± 0.046	0.246 ± 0.032
Deoxycholic acid (μmol/day)	0.717 ± 0.056	0.923 ± 0.060*	1.204 ± 0.163	1.198 ± 0.205
Cholic acid (μmol/day)	0.175 ± 0.037	0.286 ± 0.043	0.195 ± 0.041	0.247 ± 0.037
Chenodeoxycholic acid (μmol/day)	0.050 ± 0.006	0.051 ± 0.008	ND	ND
Hyodeoxycholic acid (μmol/day)	0.043 ± 0.003	0.057 ± 0.007	0.160 ± 0.018	0.135 ± 0.026
β-Muricholic acid (μmol/day)	0.240 ± 0.028	0.124 ± 0.009*	0.479 ± 0.070	0.568 ± 0.064
ω-Muricholic acid (μmol/day)	0.910 ± 0.069	1.021 ± 0.056	2.054 ± 0.204	1.864 ± 0.276
Total bile acids (μmol/day)	2.33 ± 0.19	2.65 ± 0.11	4.42 ± 0.48	4.26 ± 0.58

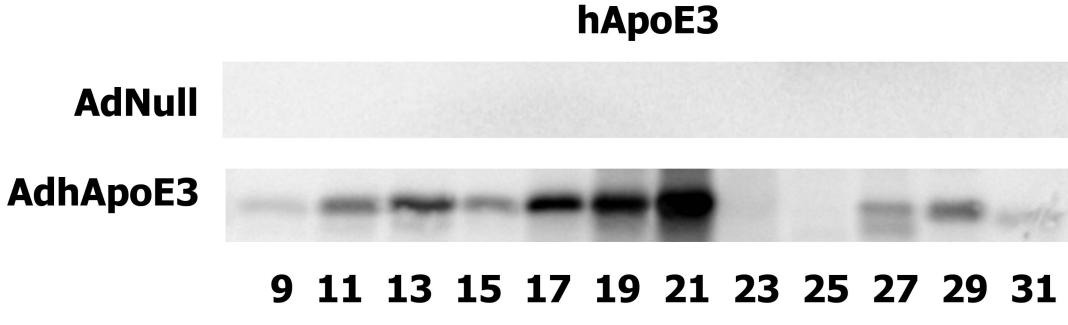
Mice of the indicated genotypes administered the respective adenoviruses were individually housed and feces were collected over a period of 24 h starting on day 3 after adenovirus injection. Fecal samples were separated from the bedding, dried, weighed, and ground. Aliquots were used to determine the content of different bile acid species by gas liquid chromatography as described under "Materials and Methods". Values are means ± SEM; n = 6 mice for each condition. AdhApoE3, recombinant adenovirus expressing human apoE3; AdNull, empty control adenovirus; hCETP tg, human cholesteryl ester transfer protein transgenic mice; ND = not detectable. * significantly different from the respective AdNull-injected controls as assessed by Mann-Whitney *U*-test (at least $P < 0.05$).



Supplemental figure I







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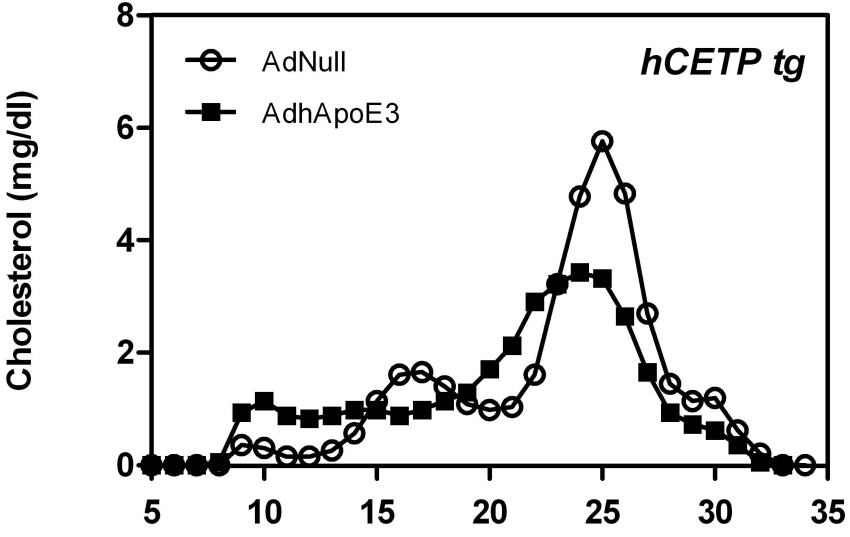

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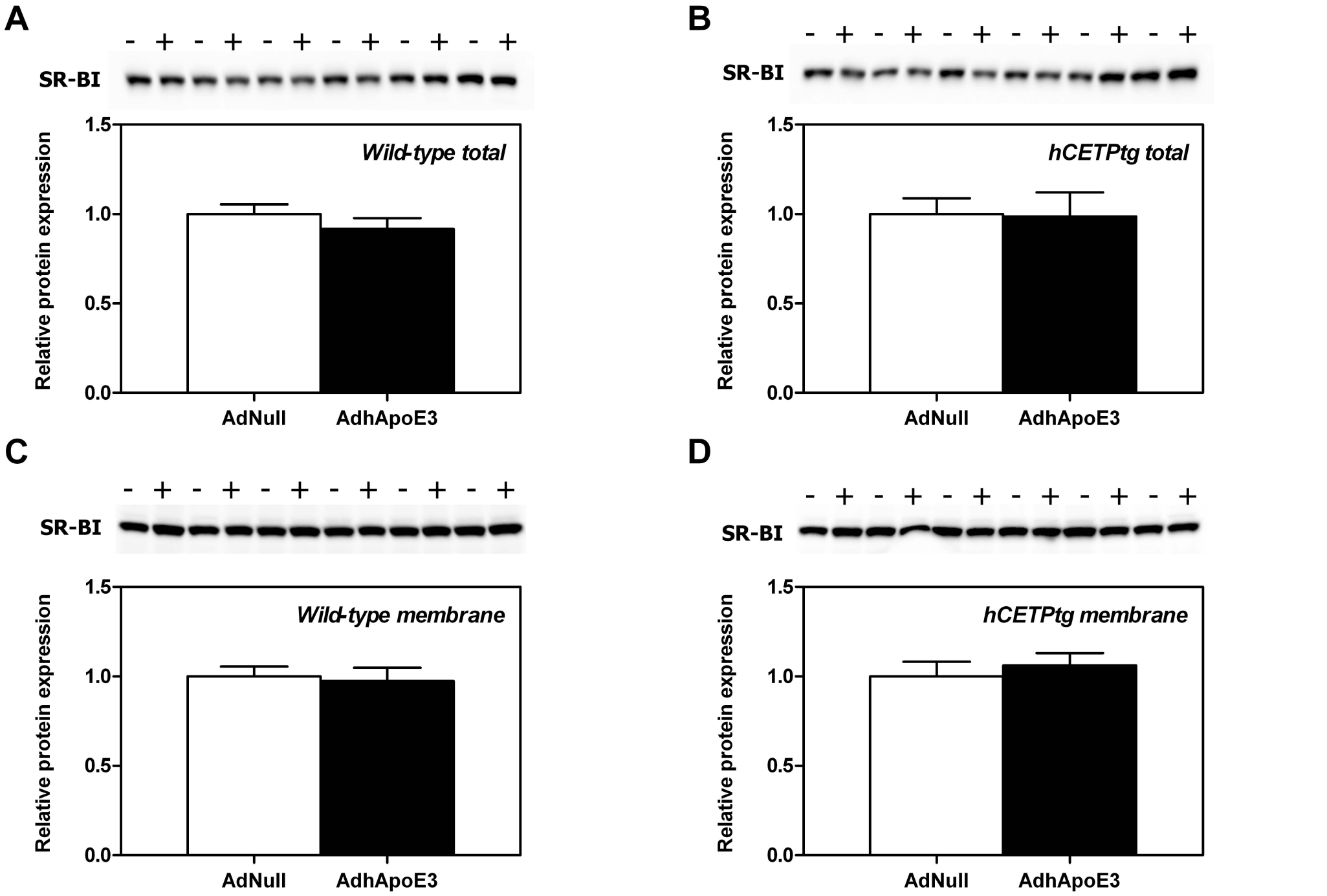
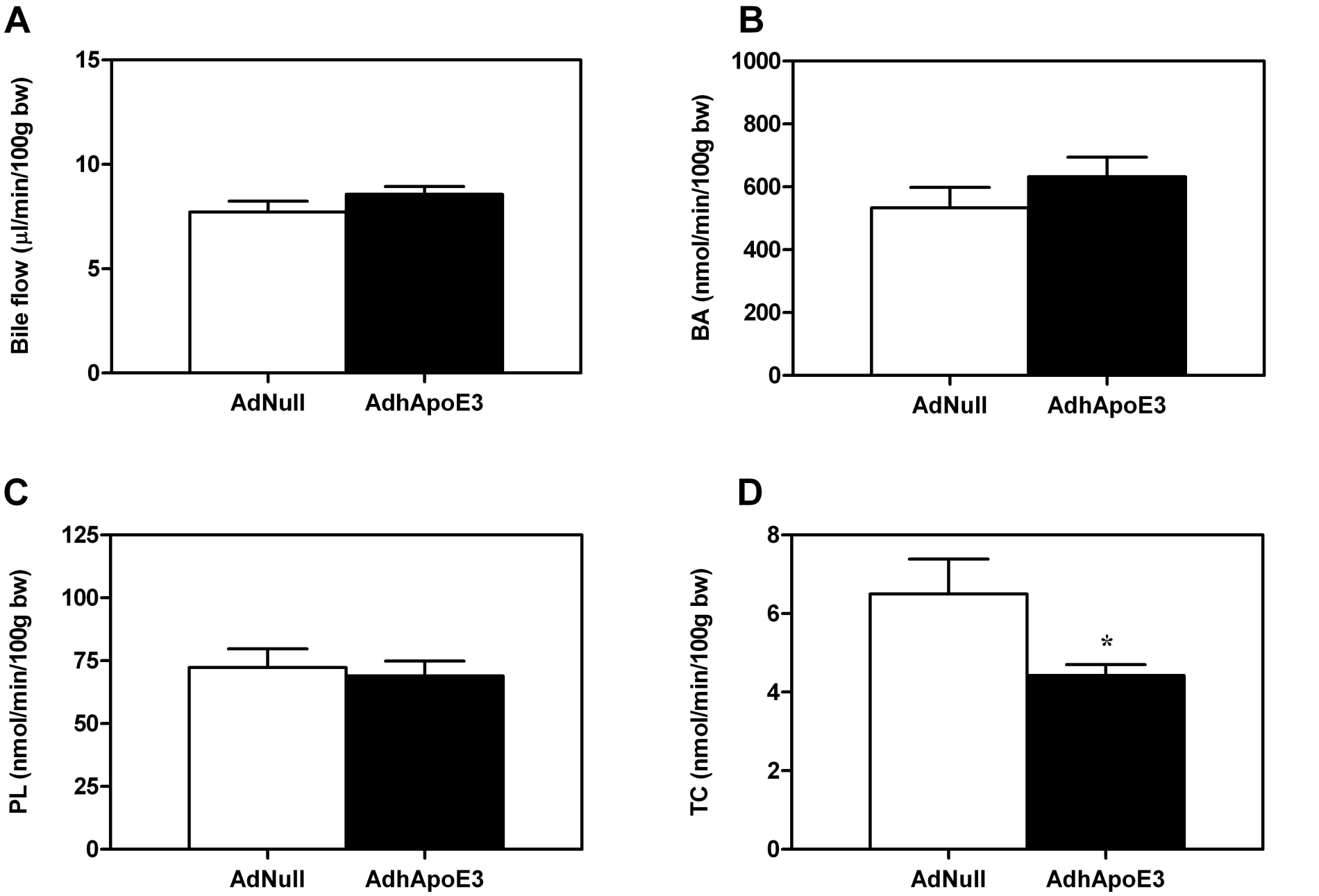


Supplemental figure IV



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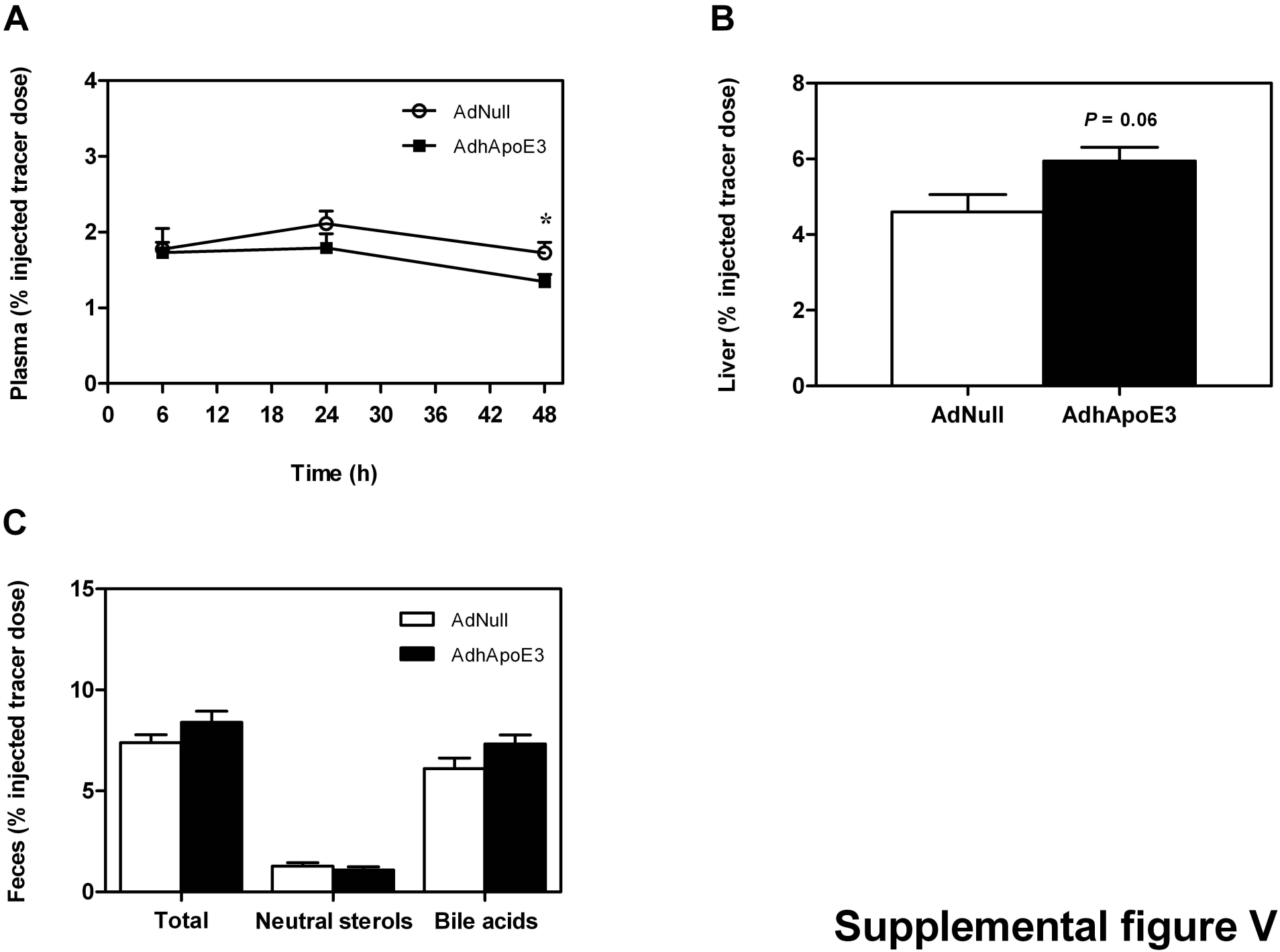

Supplemental figure VI