

**New pyripyropene A derivatives, highly SOAT2-selective inhibitors, improve  
hypercholesterolemia and atherosclerosis in atherogenic mouse models**

Taichi Ohshiro, Masaki Ohtawa, Tohru Nagamitsu, Daisuke Matsuda, Hiroaki Yagyū,

Matthew A. Davis, Lawrence L. Rudel, Shun Ishibashi, Hiroshi Tomoda

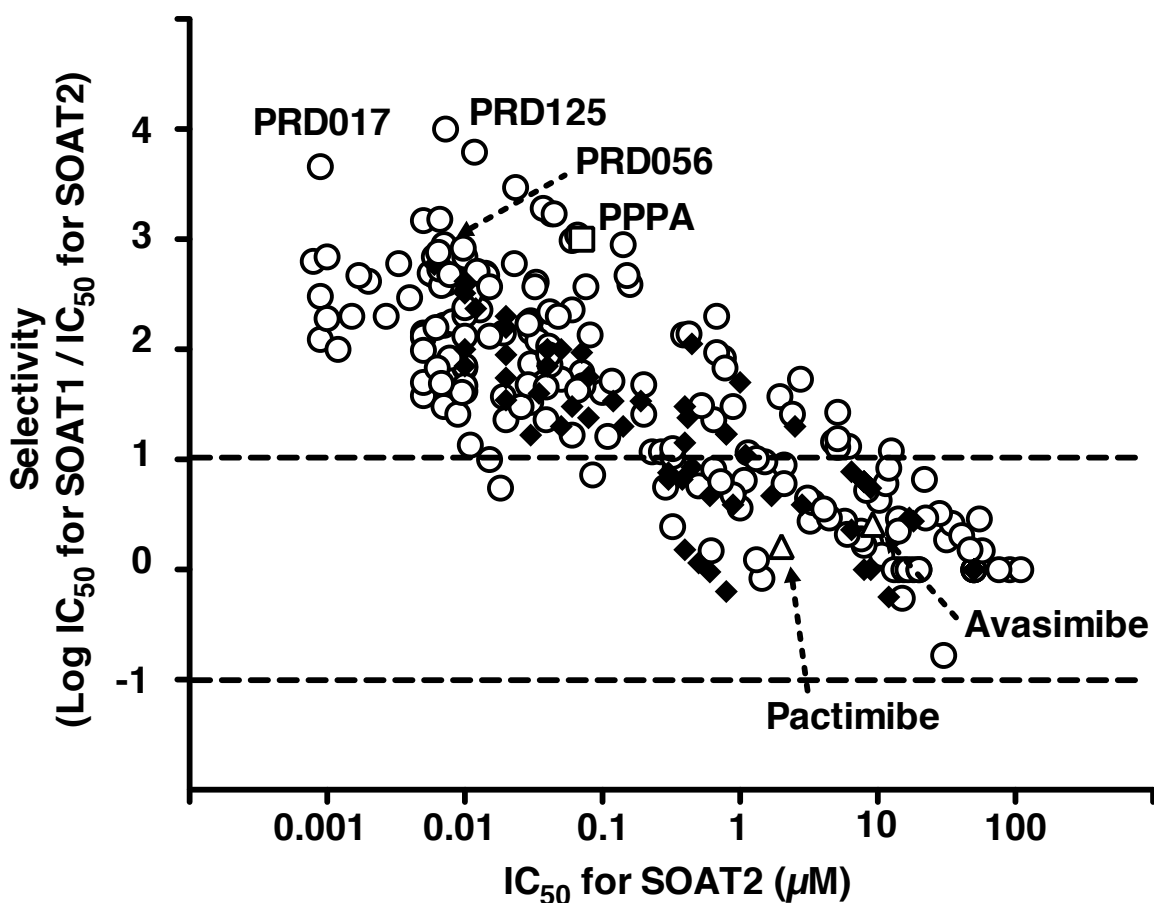
*From Graduate School of Pharmaceutical Sciences, Kitasato University, Japan (T.O.,*

*M.O., T.N., D.M., H.T.); Department of Medicine, Jichi Medical University, Japan*

*(T.O., H.Y., S.I.); Department of Internal Medicine, Section on Molecular Medicine,*

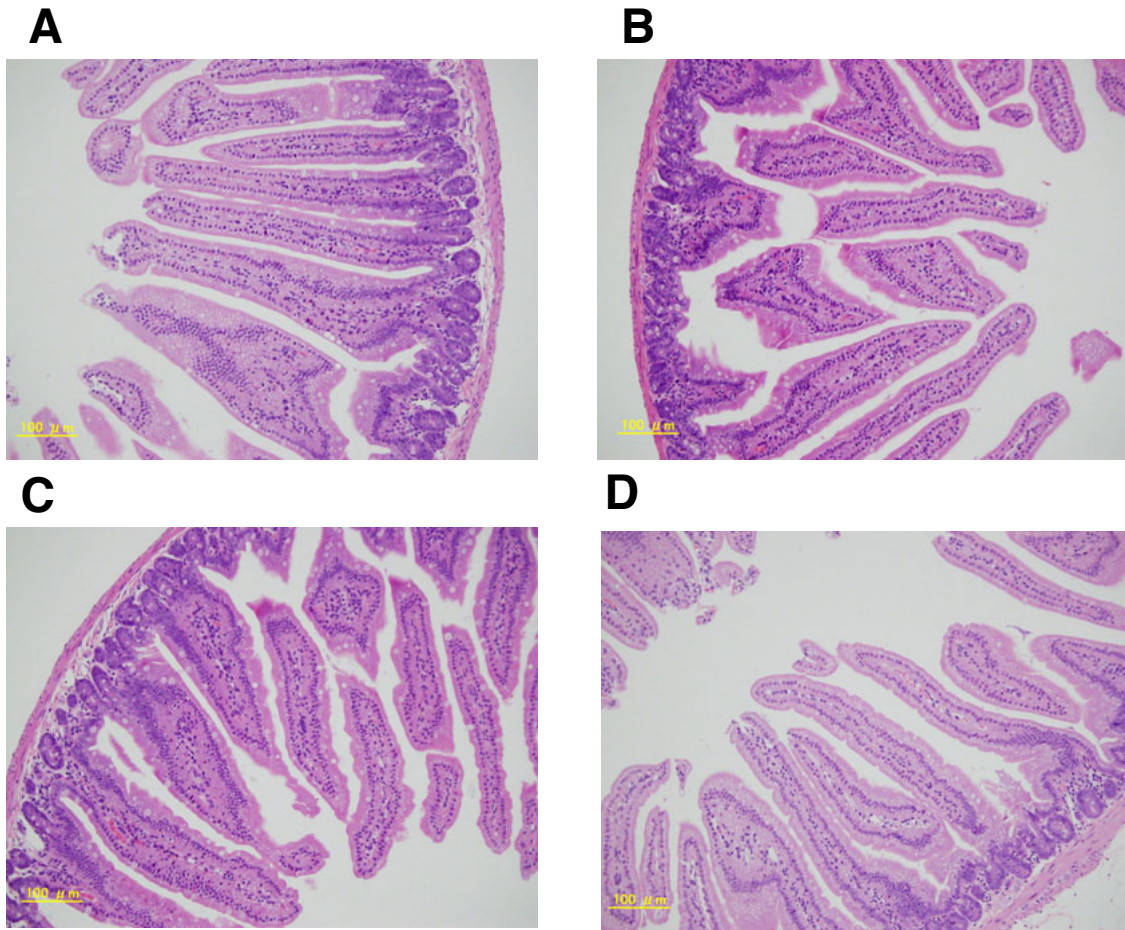
*Wake Forest School of Medicine, USA (T.O., M.D., L.R.).*

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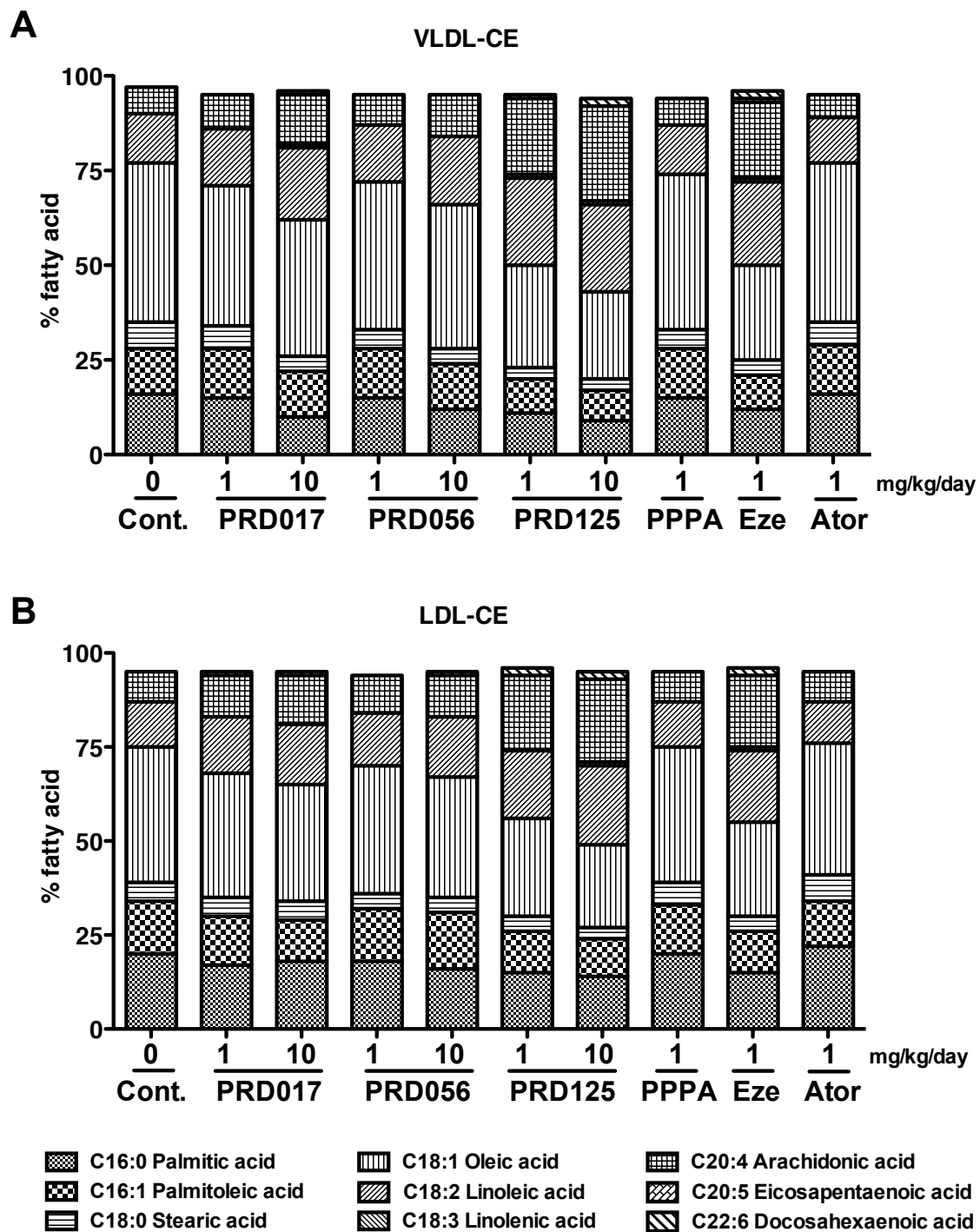
**Supplemental Figure 1 The selectivity of PPPA derivatives toward SOAT2.**

$IC_{50}$  values for SOAT2 ( $\mu M$ ) of PPPA derivatives and the selectivity index toward SOAT2 (SI values ( $\log IC_{50}$  for SOAT1/  $IC_{50}$  for SOAT2)) were plotted on X and Y axes, respectively; PPPA ( $\square$ ), the first ( $\blacklozenge$ ) and the second (O) generation PPPA derivatives, avasimibe ( $\triangle$ ) and pactimibe ( $\triangle$ ).



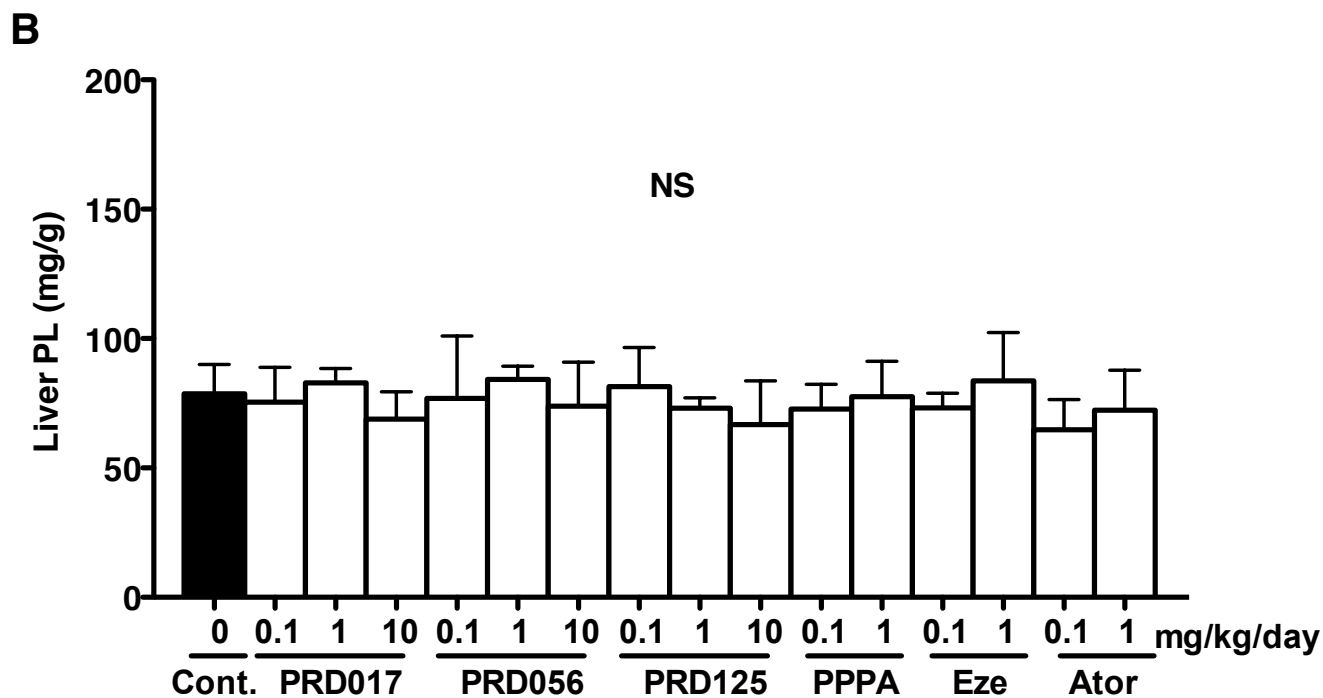
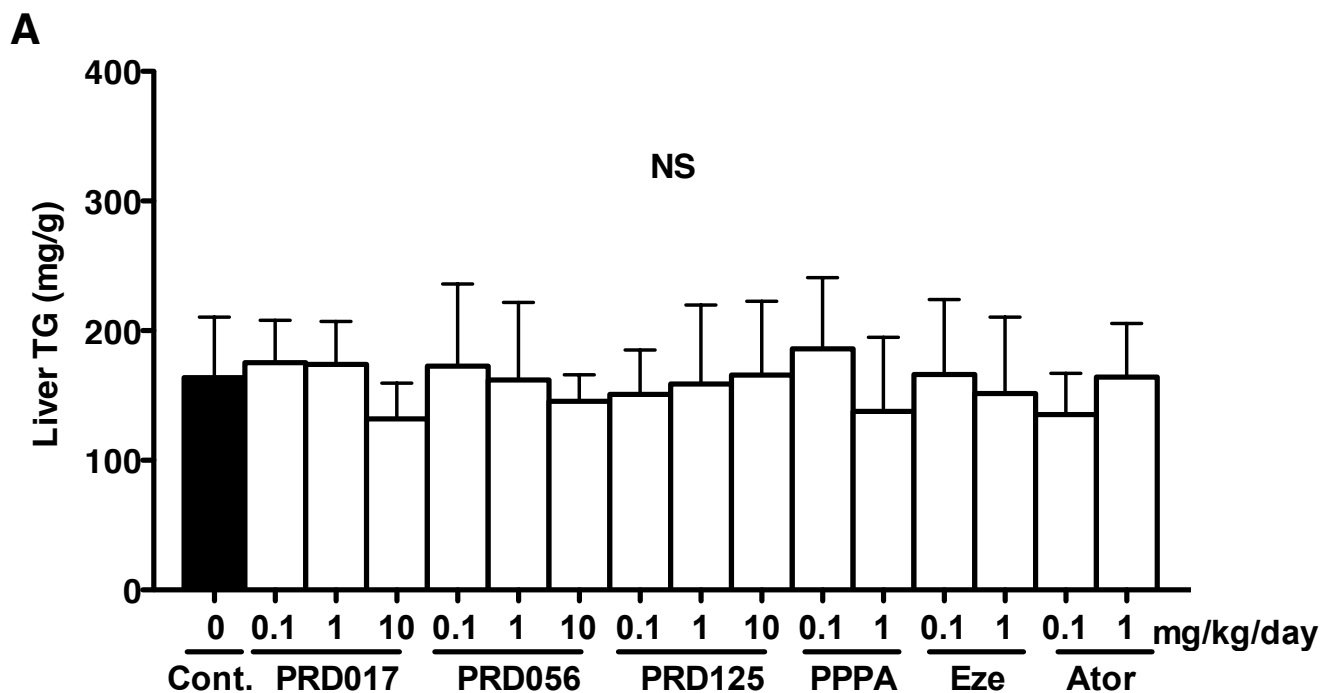
**Supplemental Figure 2 The histology of small intestine of PPPA derivative-treated *Apoe*<sup>-/-</sup> mice.**

After 12-week treatment, the cross-section of the small intestine (A~D) of *Apoe*<sup>-/-</sup> mice was performed with hematoxylin-eosin. (A) control; (B) PRD017 at 10 mg/kg/day; (C) PRD056 at 10 mg/kg/day; (D) PRD125 at 10 mg/kg/day.



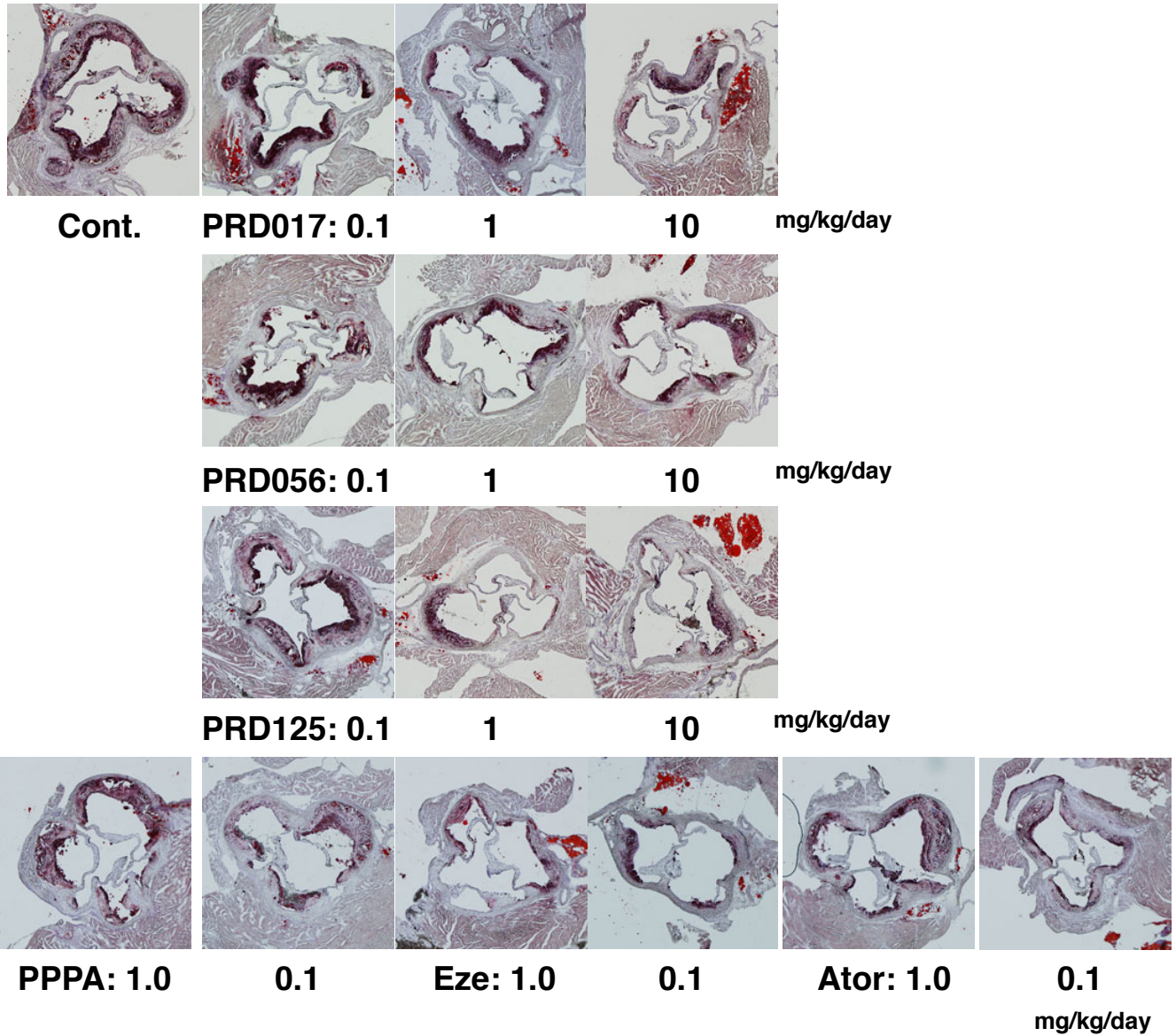
**Supplemental Figure 3 Fatty acids of cholesteryl ester in 12-week drug-treated *ApoE*<sup>-/-</sup> mice.**

After 12-week treatment, the cholesteryl ester fatty acid composition in VLDL (A) and LDL (B) of *ApoE*<sup>-/-</sup> mice was measured with mass-spec.



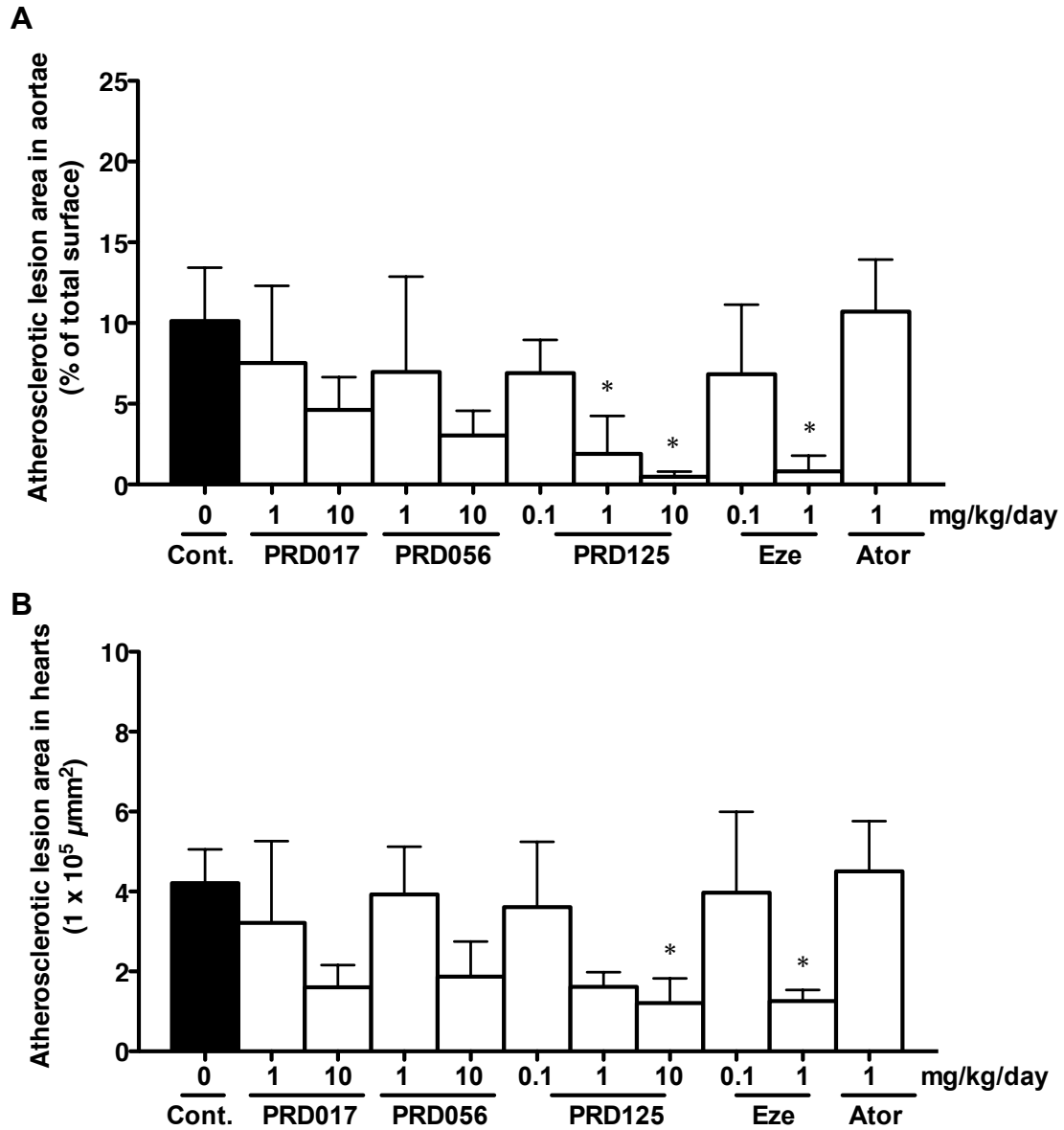
**Supplemental Figure 4 Triglyceride and phospholipid in the livers of 12-week drug-treated *Apoe*<sup>-/-</sup> mice.**

After 12-week treatment, the hepatic triglyceride (A) and phospholipid (B) of *Apoe*<sup>-/-</sup> mice were measured. All data are expressed as mean values  $\pm$  SD. NS indicates no significant difference (Kruskal-Wallis test followed by Dunn's post hoc test).



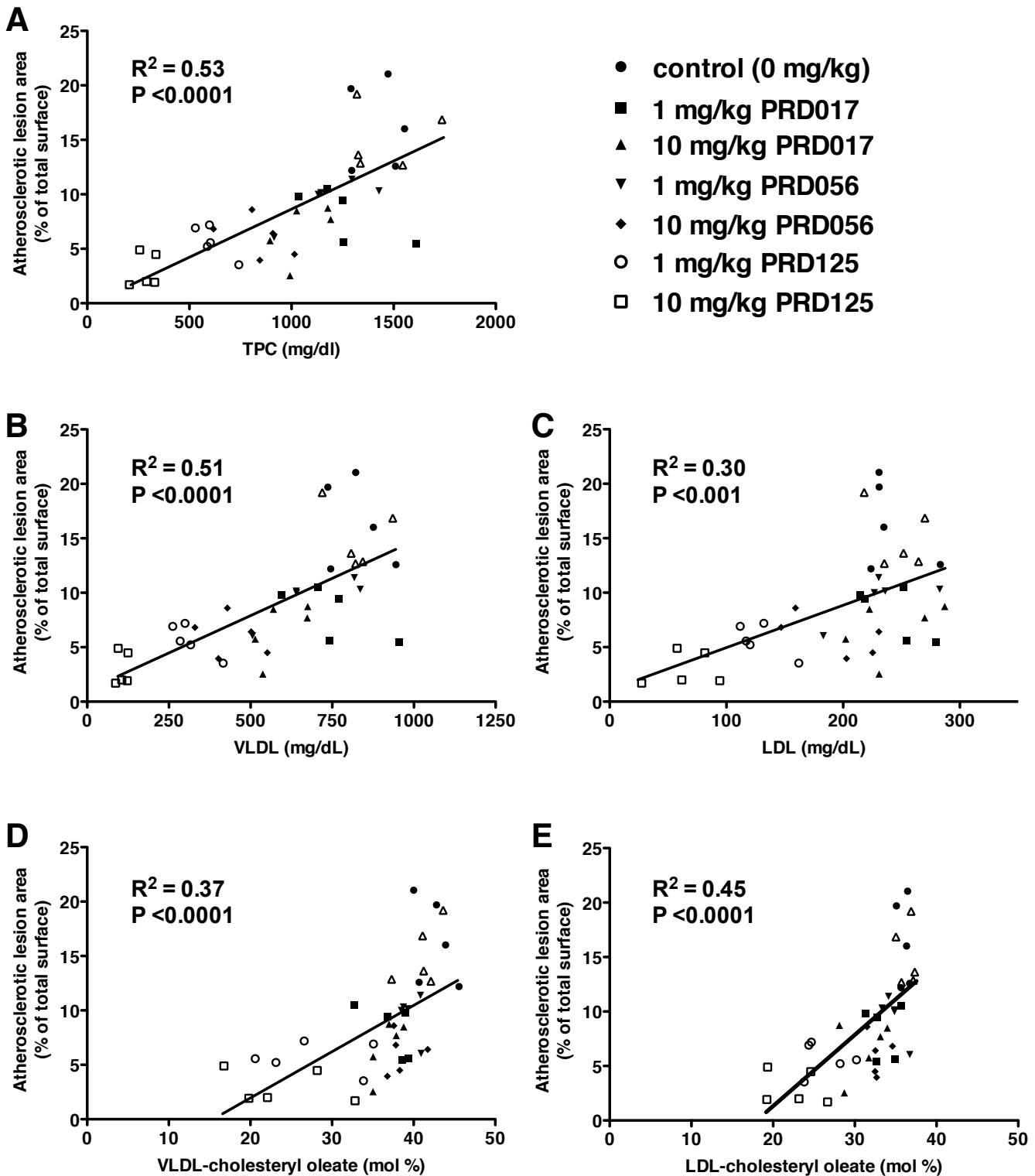
**Supplemental Figure 5 *In vivo* atheroprotective activity in hearts from 12-week drug-treated *Apoe*<sup>-/-</sup> mice.**

Representative photographs showing atherosclerotic lesions in the aortic sinus of hearts from 12-week drug-treated *Apoe*<sup>-/-</sup> mice. The cross sections of each heart were stained with Oil red O.



**Supplemental Figure 6 *In vivo* atheroprotective activity in aortae and hearts from 12-week drug-treated *Ldlr*<sup>-/-</sup> mice.**

After 12-week treatment, the atherosclerotic lesion area in the aortae (A) and aortic sinus of heart (B) were quantified. All data are expressed as mean values  $\pm$  SD. Statistically significant differences among groups are analyzed with Kruskal-Wallis test followed by Dunn's post hoc test. \* denotes  $p < 0.05$  compared with control (0 mg/kg/day).



**Supplemental Figure 7 Relationship between atherosclerotic lesion area and plasma parameters in 12-week drug-treated *Apoe*<sup>-/-</sup> mice.**

Regression analysis was used to show the strength of the association among plasma parameters TPC (A), VLDL-C (B), LDL-C (C), VLDL-cholesteryl oleate (D) and LDL-cholesteryl oleate (E) and atherosclerotic lesion area using the least-squares best fit regression model.



Supplemental Table 1 Body weight in drug-treated *ApoE*<sup>-/-</sup> mice

Compound	Dosage (mg/ kg/day)	Body weight (g)		
		week 0	week 6	week 12
Control (non-treatment)		22.0 ± 2.6	29.5 ± 1.8	34.6 ± 3.4
PRD017	0.1	23.2 ± 2.4	30.1 ± 3.9	33.4 ± 5.6
	1	21.7 ± 2.8	28.0 ± 4.0	31.1 ± 5.4
	10	24.6 ± 1.5	28.5 ± 3.4	32.1 ± 5.5
PRD056	0.1	24.1 ± 2.3	31.0 ± 3.8	33.0 ± 4.5
	1	23.4 ± 1.8	29.0 ± 2.1	31.9 ± 3.2
	10	21.8 ± 3.3	25.6 ± 3.0	27.8 ± 5.3
PRD125	0.1	22.0 ± 1.6	27.5 ± 2.1	30.9 ± 3.7
	1	20.9 ± 2.9	29.0 ± 3.4	33.4 ± 5.5
	10	23.1 ± 1.3	28.1 ± 2.7	31.7 ± 3.6
PPPA	0.1	23.0 ± 3.4	28.4 ± 4.5	32.2 ± 5.4
	1	23.2 ± 2.7	27.2 ± 2.4	30.6 ± 3.4
Ezetimibe	0.1	21.0 ± 3.5	26.1 ± 4.3	28.0 ± 6.3
	1	22.7 ± 2.4	28.9 ± 3.6	31.8 ± 4.2
Atorvastatin	0.1	21.8 ± 1.5	29.3 ± 2.4	33.8 ± 3.6
	1	22.7 ± 2.6	28.4 ± 3.0	32.0 ± 4.6

All data are expressed as mean values ± SD. All data within each treatment period (week 0, week 6 or week 12) show no significant difference with  $p > 0.05$  (Kruskal-Wallis test followed by Dunn's post hoc test).

Supplemental Table 2 ALT in drug-treated *Apoe*<sup>-/-</sup> mice

Compound	Dosage (mg/ kg/day)	ALT (IU/L)	
		week 0	week 12
Control (non-treatment)		24.9 ± 5.9	54.8 ± 13.3
PRD017	0.1	26.1 ± 6.6	56.6 ± 15.6
	1	24.7 ± 6.9	42.5 ± 14.4
	10	21.3 ± 4.4	45.8 ± 28.8
PRD056	0.1	28.1 ± 21.1	47.8 ± 18.5
	1	21.6 ± 5.7	50.9 ± 16.5
	10	21.5 ± 6.8	38.1 ± 9.4
PRD125	0.1	19.8 ± 8.4	68.6 ± 57.6
	1	23.6 ± 6.4	51.5 ± 24.0
	10	25.3 ± 10.4	31.6 ± 6.8
PPPA	0.1	31.9 ± 8.6	53.4 ± 18.9
	1	25.5 ± 13.7	75.1 ± 20.9
Ezetimibe	0.1	24.4 ± 15.4	45.4 ± 27.2
	1	23.2 ± 7.3	39.4 ± 19.4
Atorvastatin	0.1	30.4 ± 12.6	43.8 ± 7.5
	1	32.0 ± 11.2	45.4 ± 15.8

All data are expressed as mean values ± SD. All data within each treatment period (week 0 or week 12) show no significant difference with  $p > 0.05$  (Kruskal-Wallis test followed by Dunn's post hoc test).

Supplemental Table 3 BUN in drug-treated *ApoE*<sup>-/-</sup> mice

Compound	Dosage (mg/ kg/day)	BUN (mg/dL)	
		week 0	week 12
Control (non-treatment)		28.2 ± 5.4	24.4 ± 4.0
PRD017	0.1	26.7 ± 6.0	25.8 ± 5.1
	1	34.6 ± 4.4	27.1 ± 6.2
	10	33.3 ± 3.9	24.9 ± 2.7
PRD056	0.1	28.7 ± 7.5	24.0 ± 3.1
	1	26.6 ± 7.5	24.3 ± 2.6
	10	30.2 ± 6.4	22.3 ± 3.9
PRD125	0.1	30.9 ± 5.3	25.7 ± 5.7
	1	29.1 ± 4.7	22.3 ± 3.6
	10	31.8 ± 5.6	20.5 ± 2.9
PPPA	0.1	32.9 ± 8.3	19.1 ± 2.9
	1	31.6 ± 8.8	30.6 ± 3.4
Ezetimibe	0.1	32.6 ± 8.3	23.0 ± 3.3
	1	32.1 ± 5.0	22.5 ± 4.0
Atorvastatin	0.1	35.8 ± 6.9	28.0 ± 4.4
	1	31.8 ± 5.8	25.2 ± 4.0

All data are expressed as mean values ± SD. All data within each treatment period (week 0 or week 12) show no significant difference with  $p > 0.05$  (Kruskal-Wallis test followed by Dunn's post hoc test).

Supplemental Table 4 Blood glucose in drug-treated *ApoE*<sup>-/-</sup> mice

Compound	Dosage (mg/ kg/day)	Blood glucose (mg/dL)		
		week 0	week 6	week 12
Control (non-treatment)		55.8 ± 3.9	61.7 ± 10.2	79.0 ± 8.0
PRD017	0.1	55.2 ± 3.5	61.3 ± 13.9	84.5 ± 30.4
	1	52.8 ± 4.2	54.0 ± 11.8	69.3 ± 11.5
	10	53.4 ± 4.6	59.9 ± 6.1	81.7 ± 11.3
PRD056	0.1	60.1 ± 3.6	55.9 ± 6.3	72.9 ± 20.7
	1	58.8 ± 3.2	53.6 ± 6.1	74.6 ± 20.9
	10	52.6 ± 6.5	60.7 ± 7.8	74.8 ± 16.1
PRD125	0.1	56.8 ± 7.1	62.1 ± 9.0	69.0 ± 12.5
	1	49.6 ± 3.1	57.4 ± 6.2	71.6 ± 13.0
	10	53.0 ± 4.3	56.1 ± 5.0	68.8 ± 9.7
PPPA	0.1	46.1 ± 4.1	49.8 ± 8.9	75.8 ± 13.0
	1	57.0 ± 4.8	64.0 ± 16.9	85.0 ± 17.5
Ezetimibe	0.1	45.7 ± 3.0	65.5 ± 17.0	62.3 ± 21.6
	1	54.9 ± 2.5	64.3 ± 7.6	64.1 ± 16.9
Atorvastatin	0.1	47.6 ± 2.7	61.2 ± 7.1	86.2 ± 10.8
	1	51.7 ± 2.2	65.6 ± 6.7	81.4 ± 25.7

All data are expressed as mean values ± SD. All data within each treatment period (week 0, week 6 or week 12) show no significant difference with  $p > 0.05$  (Kruskal-Wallis test followed by Dunn's post hoc test).

Supplemental Table 5 Food intake in drug-treated *ApoE*<sup>-/-</sup> mice

Compound	Dosage (mg/ kg/day)	Food intake (g/day)		
		week 0	week 6	week 12
Control (non-treatment)		2.5 ± 0.8	3.7 ± 1.2	3.3 ± 0.2
PRD017	0.1	3.0 ± 1.3	4.3 ± 0.5	3.3 ± 0.9
	1	2.5 ± 1.0	4.0 ± 0.6	3.8 ± 0.3
	10	2.7 ± 1.4	4.3 ± 0.4	4.0 ± 0.4
PRD056	0.1	2.6 ± 1.1	4.5 ± 0.3	3.9 ± 0.6
	1	2.6 ± 0.4	4.5 ± 0.2	3.6 ± 0.6
	10	2.8 ± 0.7	3.8 ± 0.3	3.7 ± 0.3
PRD125	0.1	2.5 ± 0.8	4.3 ± 0.2	4.0 ± 0.6
	1	2.7 ± 1.3	4.7 ± 0.5	3.5 ± 0.4
	10	2.9 ± 1.3	4.2 ± 0.3	3.8 ± 0.5
PPPA	0.1	3.7 ± 1.4	5.0 ± 0.3	3.4 ± 0.6
	1	2.8 ± 0.9	4.0 ± 0.7	3.5 ± 0.6
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Ezetimibe	0.1	2.7 ± 1.0	4.3 ± 0.4	2.8 ± 0.4
	1	2.6 ± 1.1	4.2 ± 0.1	3.2 ± 0.8
Atorvastatin	0.1	2.4 ± 0.9	4.0 ± 0.2	3.9 ± 0.5
	1	2.5 ± 1.1	4.2 ± 0.5	3.8 ± 0.7

All data are expressed as mean values ± SD. All data within each treatment period (week 0, week 6 or week 12) show no significant difference with  $p > 0.05$  (Kruskal-Wallis test followed by Dunn's post hoc test).

Supplemental Table 6 TPC concentrations in drug-treated *ApoE*<sup>-/-</sup> mice

Compound	Dosage (mg/ kg/day)	TPC (mg/dL)		
		week 0	week 6	week 12
Control (non-treatment)		559 ± 177	1256 ± 231	1439 ± 151
PRD017	0.1	569 ± 157	1224 ± 493	1302 ± 209
	1	565 ± 58	965 ± 423	1223 ± 236
	10	567 ± 115	903 ± 187	1031 ± 115
PRD056	0.1	548 ± 107	1007 ± 312	1312 ± 440
	1	555 ± 79	1041 ± 376	1212 ± 380
	10	564 ± 122	850 ± 324	839 ± 146* <sup>#</sup>
PRD125	0.1	558 ± 169	1037 ± 312	1155 ± 247
	1	562 ± 173	603 ± 116 <sup>#</sup>	606 ± 134* <sup>#</sup>
	10	575 ± 115	433 ± 108* <sup>#</sup>	295 ± 52* <sup>#</sup>
PPPA	0.1	552 ± 85	1041 ± 286	1353 ± 236
	1	531 ± 200	1281 ± 296	1507 ± 199
Ezetimibe	0.1	561 ± 135	970 ± 259	967 ± 257
	1	558 ± 148	507 ± 202 <sup>#</sup>	732 ± 187* <sup>#</sup>
Atorvastatin	0.1	569 ± 102	1394 ± 123	1420 ± 141
	1	568 ± 111	1268 ± 426	1254 ± 285 <sup>ab</sup>

All data are expressed as mean values ± SD. Statistically significant differences among groups are analyzed with Kruskal-Wallis test followed by Dunn's post hoc test. \* and # denotes  $p < 0.05$  compared with control (0 mg/kg/day) and PPPA (1 mg/kg/day), respectively.

Supplemental Table 7 Triglyceride in plasma of drug-treated *ApoE*<sup>-/-</sup> mice

Compound	Dosage (mg/ kg/day)	Triglyceride (mg/dL)		
		week 0	week 6	week 12
Control (non-treatment)		135 ± 47	126 ± 37	127 ± 26
PRD017	0.1	141 ± 62	104 ± 39	99 ± 25
	1	146 ± 63	85 ± 42	102 ± 32
	10	193 ± 24	147 ± 16	137 ± 34
PRD056	0.1	171 ± 93	103 ± 46	98 ± 34
	1	145 ± 89	114 ± 45	105 ± 44
	10	164 ± 65	170 ± 38	159 ± 46
PRD125	0.1	182 ± 133	135 ± 51	113 ± 44
	1	127 ± 49	178 ± 102	115 ± 72
	10	163 ± 54	180 ± 82	172 ± 42
PPPA	0.1	162 ± 59	98 ± 34	110 ± 21
	1	130 ± 41	112 ± 35	134 ± 40
Ezetimibe	0.1	142 ± 50	86 ± 34	81 ± 30
	1	151 ± 62	150 ± 80	112 ± 51
Atorvastatin	0.1	159 ± 46	93 ± 28	110 ± 42
	1	150 ± 53	103 ± 18	132 ± 53

All data are expressed as mean values ± SD. All data within each treatment period (week 0, week 6 or week 12) show no significant difference with  $p > 0.05$  (Kruskal-Wallis test followed by Dunn's post hoc test).

Supplemental Table 8 TPC concentrations in drug-treated *Ldlr*<sup>-/-</sup> mice

Compound	Dosage (mg/ kg/day)	TPC (mg/dL)		
		week 0	week 6	week 12
Control (non-treatment)		267 ± 89	1038 ± 263	1200 ± 174
PRD017	1	245 ± 53	809 ± 284	953 ± 299
	10	237 ± 22	634 ± 227	726 ± 174*
PRD056	1	293 ± 82	755 ± 347	941 ± 198
	10	266 ± 72	591 ± 197	680 ± 245
PRD125	0.1	279 ± 49	872 ± 165	816 ± 237
	1	272 ± 92	571 ± 149	564 ± 98*
	10	298 ± 94	536 ± 201*	348 ± 77*
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ezetimibe	0.1	279 ± 26	780 ± 250	1053 ± 351
	1	277 ± 71	564 ± 86	571 ± 78
atorvastatin	1	322 ± 47	921 ± 197	1266 ± 258

All data are expressed as mean values ± SD. Statistically significant differences among groups are analyzed with Kruskal-Wallis test followed by Dunn's post hoc test. \* denotes  $p < 0.05$  compared with control (0 mg/kg/day).



Supplemental Table 9 Cholesterol concentrations in lipoprotein from 12-week drug-treated *Ldlr*<sup>-/-</sup> mice

Compound	Dosage (mg/kg/day)	Cholesterol (mg/dL)			
		Chiomicron	VLDL	LDL	HDL
Control (non-treatment)		86 ± 54	631 ± 126	388 ± 40	94 ± 15
PRD017	1	72 ± 59	507 ± 101	393 ± 74	85 ± 21
	10	30 ± 11	356 ± 79	423 ± 61	101 ± 10
PRD056	1	65 ± 42	428 ± 102	366 ± 100	82 ± 30
	10	35 ± 19	231 ± 100	318 ± 116	96 ± 40
PRD125	0.1	59 ± 20	369 ± 125	293 ± 88	96 ± 32
	1	25 ± 7	190 ± 56	248 ± 47	100 ± 11
	10	17 ± 21*	93 ± 27*	159 ± 48*	79 ± 30
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ezetimibe	0.1	66 ± 53	522 ± 274	356 ± 80	109 ± 23
	1	44 ± 49	227 ± 79	223 ± 92	77 ± 41
atorvastatin	1	81 ± 36	639 ± 158	441 ± 119	106 ± 26

All data are expressed as mean values ± SD. Statistically significant differences among groups are analyzed with Kruskal-Wallis test followed by Dunn's post hoc test. \* denotes  $p < 0.05$  compared with control (0 mg/kg/day).