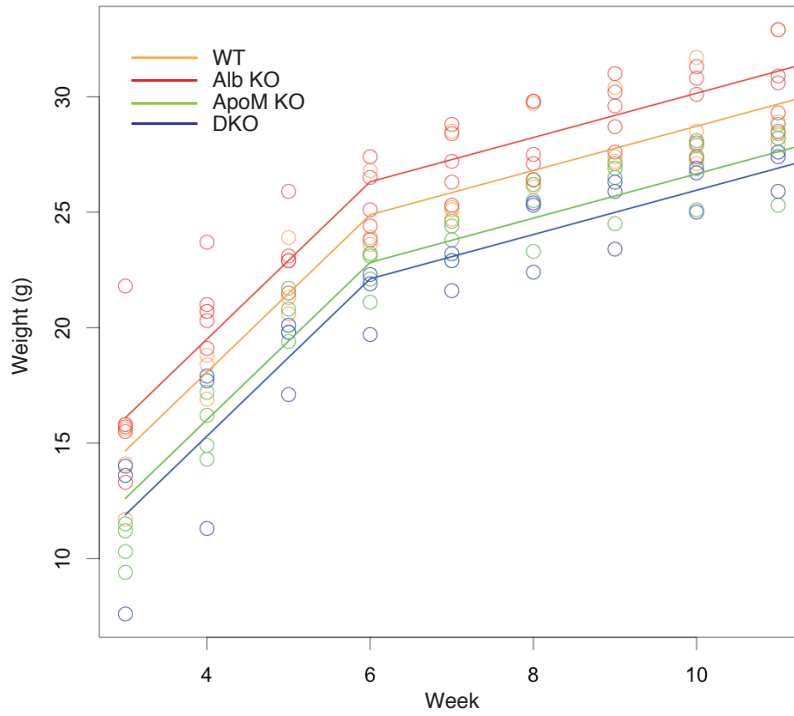
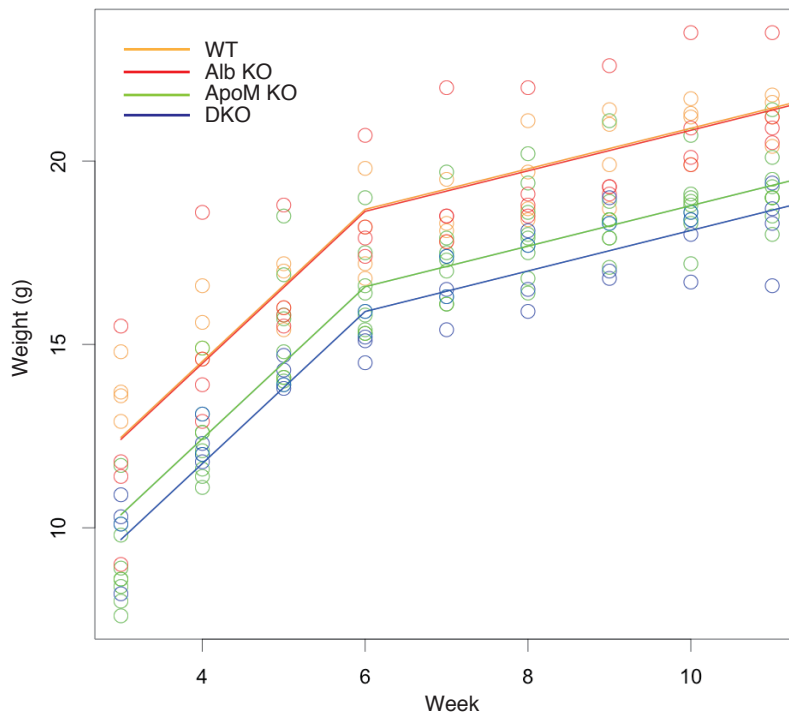


Male



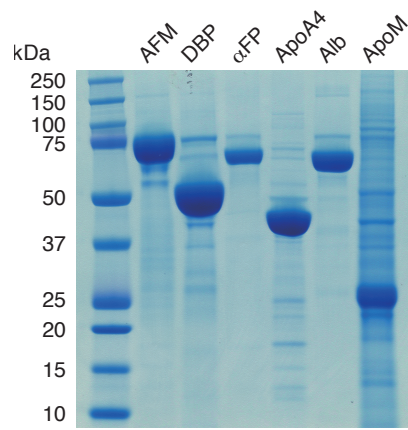
Female



Supplemental

Figure S1. Multiple linear regression model to examine the statistical differences of the growth curves between the four genotype groups

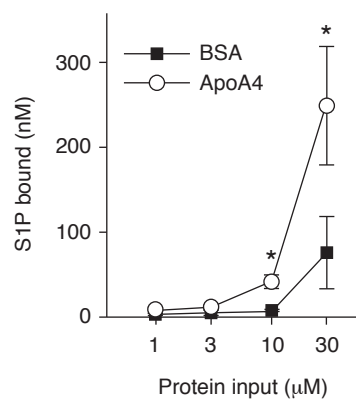
In order to express the non-linear time trend of the growth curves, a spline-based function was used, and the location of the knot was optimized for minimizing the residual standard errors, which resulted in the regression model with $R^2 = 0.907$ and 0.863 for the male and female group, respectively.



Supplemental

Figure S2. SDS-PAGE analysis of the recombinant proteins

Purified recombinant proteins were separated by SDS-PAGE with a 4-12% precast gel and revealed by Coomassie Brilliant Blue staining.

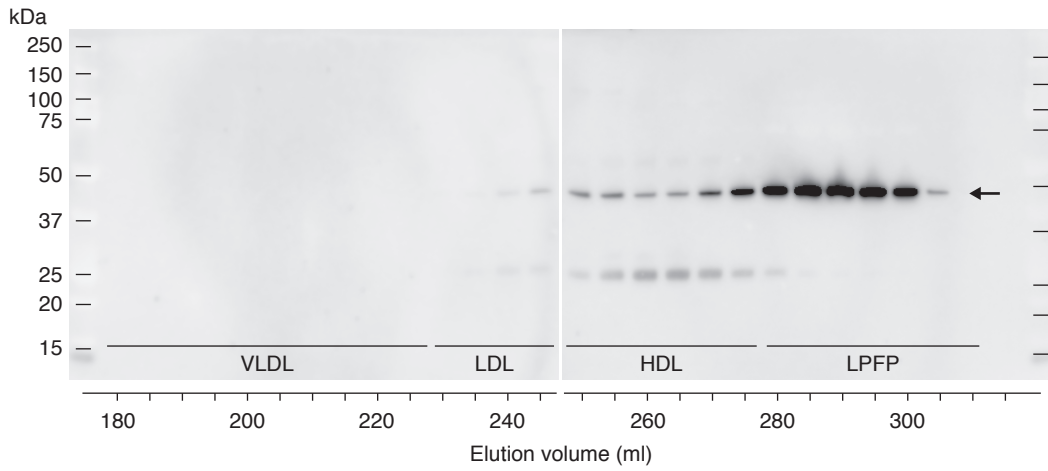


Supplemental

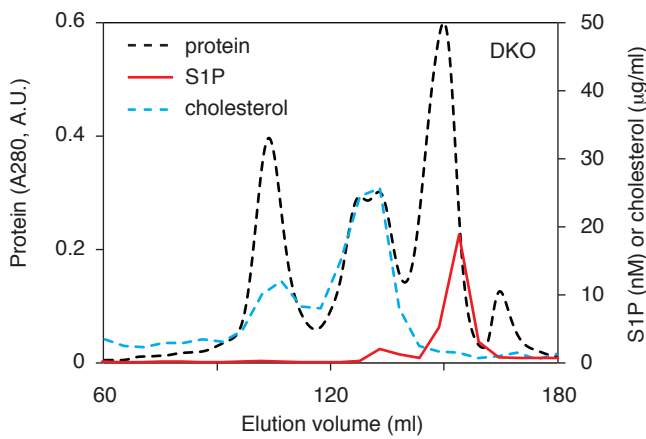
Figure S3. Close-up comparison between ApoA4 and albumin in the S1P-binding capacity

Fig.6B is enlarged to show the close-up comparison between ApoA4 and albumin. ApoA4 showed significantly higher S1P-binding capacity at 10 and 30 µM (Student's t-test, $p < 0.05$).

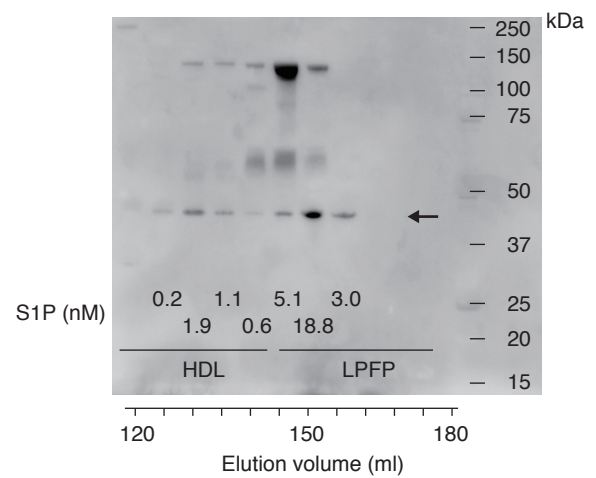
A.



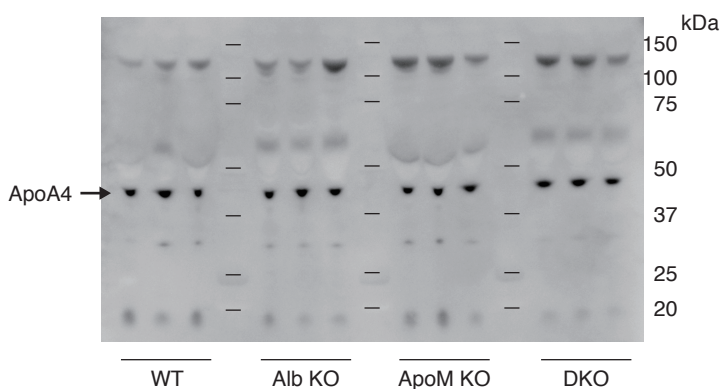
B.



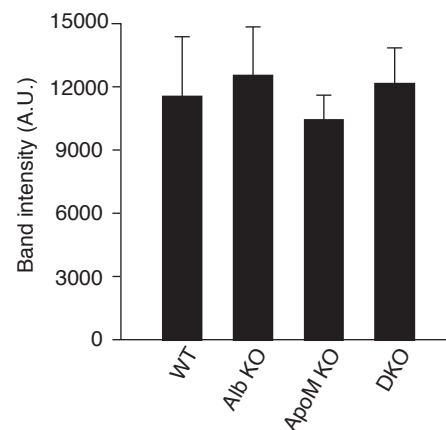
C.



D.



E.



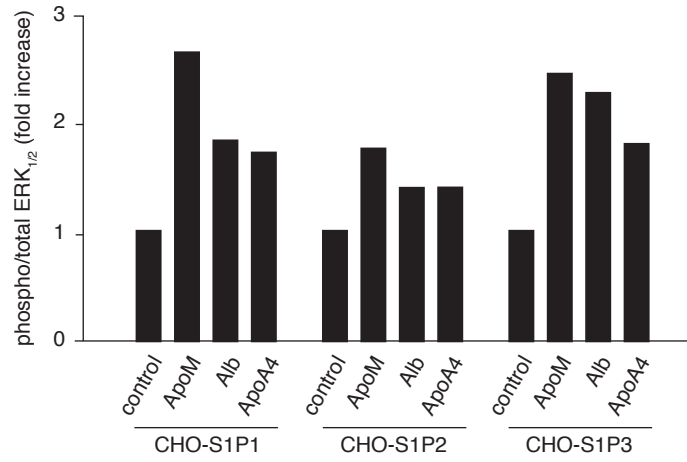
Supplemental

Figure S4. ApoA4 distribution in human and mouse plasma

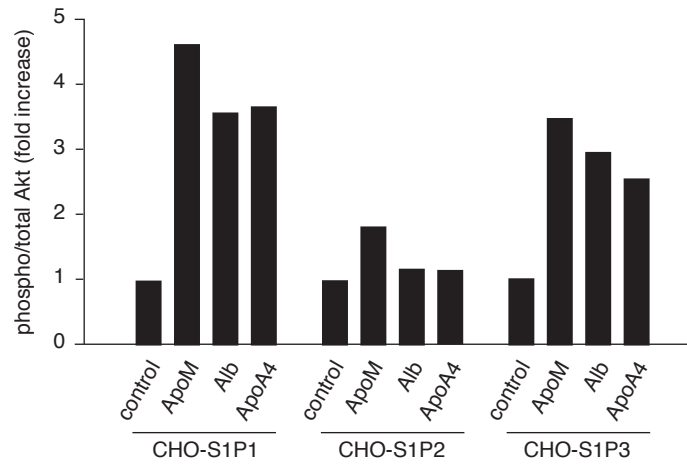
(A) Human plasma (1 ml) or (B) plasma from the DKO mice (0.5 ml) was separated by size exclusion chromatography with tandemly-connected Superose™6 and Superdex™200 columns (16 mm x 600 mm each). ApoA4 distribution was revealed by western blot analysis (A, C). S1P concentration in each fraction of the DKO plasma was determined and shown (B, C). Plasma (1 µl) from each genotype of mice was separated by SDS-PAGE with a 4-12% precast gel, and ApoA4 was revealed by western blot analysis (D). Intensity of each ApoA4 band was quantified by using ImageJ software (E).

A.

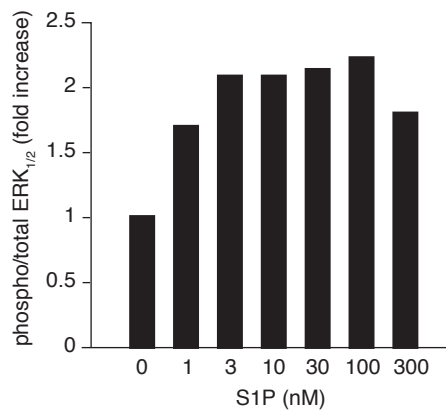
phospho/total ERK_{1/2}



phospho/total Akt



B.



Supplemental

Figure S5. Activation of S1P receptors by ApoA4-bound S1P

Intensity of each band in Fig.7A and 7B was quantified by using ImageJ software and shown in S5A and S5B, respectively.

Supplemental

Table S1. Protein list identified by the shot-gun proteomic analysis

The tryptic digests from Fig.5B were subjected to the shot-gun proteomic analysis by the nano LC-MS/MS system. The raw data were analyzed by Mascot search engine (Matrix Science) referring to the SwissProt Knowledgebase to identify the proteins. The identified proteins in each gel piece are listed. Proteins with more than 5 distinct peptides assigned are shown. Keratin species were omitted from the list. pep_#, number of distinct peptides assigned for each protein. cover_%, percentage of coverage of each protein assigned.

gel #	score	protein name	accession #	pep_#	cover_%
1	1435	Ceruloplasmin	Q61147	24	26.6
	1019	Serine protease inhibitor A3K	P07759	10	27.8
2	2119	Serine protease inhibitor A3K	P07759	11	25.8
	1137	Serine protease inhibitor A3M	Q03734	10	20.8
	93	Alpha-1-antitrypsin1-4	Q00897	6	15.3
	78	Alpha-1-antitrypsin1-1	P07758	7	17.2
	52	Ceruloplasmin	Q61147	6	6.1
3	1247	Serine protease inhibitor A3K	P07759	8	15.3
	709	Serine protease inhibitor A3M	Q03734	9	18.7
4	924	Serine protease inhibitor A3K	P07759	7	16.5
	62	Plasminogen	P20918	8	11.3
5	1371	Gelsolin	P13020	22	27.3
	1096	Serine protease inhibitor A3K	P07759	9	20.3
	350	Plasminogen	P20918	18	23.5
6	999	Serine protease inhibitor A3K	P07759	6	13.9
	125	Afamin	O89020	12	17.9
	34	Ceruloplasmin	Q61147	6	6.9
	28	Heparin cofactor 2	P49182	6	10.5
7	1253	Serine protease inhibitor A3K	P07759	7	14.1
	402	Carboxylesterase 1C	P23953	6	10.5
	294	Heparin cofactor 2	P49182	8	13.8
	181	Serotransferrin	Q92111	17	25.8
	146	Afamin	O89020	8	10.9
8	1673	Serine protease inhibitor A3K	P07759	9	16.0
	673	Heparin cofactor 2	P49182	15	28.7
	353	Alpha-1-antitrypsin1-1	P07758	6	12.6
	345	Alpha-1-antitrypsin1-4	Q00897	7	15.5
	161	Alpha-2-antiplasmin	Q61247	8	16.7
	100	Complement component C9	P06683	6	9.3
	77	Antithrombin-III	P32261	10	22.4
	35	Corticosteroid-binding globulin	Q06770	6	13.6

Supplemental
Table S1. (continued)

gel #	score	protein name	accession #	pep_#	cover_%
9	3368	Alpha-1-antitrypsin1-1	P07758	17	39.5
	3267	Alpha-1-antitrypsin1-4	Q00897	17	33.7
	2960	Alpha-1-antitrypsin1-2	P22599	16	37.0
	2561	Serine protease inhibitor A3K	P07759	13	32.1
	2262	Alpha-1-antitrypsin1-5	Q00898	14	36.1
	1170	Serine protease inhibitor A3M	Q03734	9	22.2
	513	Serine protease inhibitor A3N	Q91WP6	6	14.1
	134	Antithrombin-III	P32261	7	18.5
10	3246	Alpha-1-antitrypsin1-2	P22599	16	37.0
	3067	Alpha-1-antitrypsin1-1	P07758	17	39.5
	2616	Alpha-1-antitrypsin1-5	Q00898	15	39.2
	2592	Alpha-1-antitrypsin1-4	Q00897	16	33.7
	1525	Serine protease inhibitor A3K	P07759	12	31.8
	797	Serine protease inhibitor A3M	Q03734	8	22.0
	385	Serine protease inhibitor A3N	Q91WP6	7	20.6
11	718	Serine protease inhibitor A3K	P07759	10	23.0
	593	Serine protease inhibitor A3M	Q03734	13	27.3
	520	Alpha-1-antitrypsin1-1	P07758	11	29.1
	390	Alpha-1-antitrypsin1-4	Q00897	9	26.4
	374	Alpha-1-antitrypsin1-2	P22599	8	22.5
	246	Alpha-1-antitrypsin1-5	Q00898	6	13.6
	126	Apolipoprotein A-IV	P06728	8	22.3
	105	Pigment epithelium-derived factor	P97298	7	18.5
	79	Vitamin D-binding protein	P21614	6	12.4
12	1182	Alpha-1-antitrypsin1-2	P22599	11	23.0
	1141	Alpha-1-antitrypsin1-1	P07758	11	20.1
	834	Alpha-1-antitrypsin1-4	Q00897	8	17.9
	542	Alpha-1-antitrypsin1-5	Q00898	8	15.0