Supplementary Table 1

Viability is reduced in mice with S1pr2 mutation.

Genetic background	Age	Matings (F × M)	Number of postnatal mice	S1pr2 Genotype (percentage)		
				+/+	+/-	-/-
F1N3	4 weeks after birth	+/- × +/-	179	49(27)	102(57)	28(16)
F1N7	4 weeks after birth	+/- × +/-	122	45(37)	69(57)	8(6)

The total number and percentage of mice from *S1pr2*$^{+/-}$*Apoe*$^{-/-}$ crosses identified in each generation at week 4. In F1N7 generation, only 6% of the offspring were homozygous for S1pr2, which was much less than the expected value of 25%, while the percentage of *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ mice in F1N3 generation was 16%.

Supplementary Table 2

Plasma cholesterol, triglyceride, and S1P levels in *S1pr2*$^{+/+}$*Apoe*$^{-/-}$and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$mice

Genotype	n	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Sphingosine-1-phosphate (nM)
S1pr2$^{+/+}$ *Apoe*$^{-/-}$	6	1492±284	154±23	778±56
S1pr2$^{-/-}$ *Apoe*$^{-/-}$	5	1549±291	107±22	786±112

Mice (F1N3 background) were fed HCD for 12 weeks. All data are expressed as means±SEM.

Supplementary Table 3

Body weight and food intake

Genotype	n	Body Weight (6-week old / 18-week old)* (g)	Food Intake (g / 30g BW)#
S1pr2$^{+/+}$ *Apoe*$^{-/-}$	11	21±4 / 32±4	6.4 ±1.6
S1pr2$^{-/-}$ *Apoe*$^{-/-}$	8	19±4 / 29±5	5.9 ± 2.1

*HCD started at 6-week old and ended at 18-week old.

#BW, body weight; food intake was corrected for body weight.

All mice were from F1N3, and the results are presented as means±SEM.

Supplementary Table 4

Peripheral blood counts of erythrocytes and leukocytes

	n	rbc*	Leukocyte cell	Differential White Blood Cell Count(%)		
		Number ($\times 10^4/mm^3$)	Number ($\times 10^2/mm^3$)	Lymphocyte	Monocyte	Neutrophil
S1pr2$^{+/+}$ *Apoe*$^{-/-}$	8	691±50	35±3	78.5±2.4	2.5±0.7	18.1±2.0
S1pr2$^{-/-}$ *Apoe*$^{-/-}$	7	735±78	37±6	83.7±1.3	3.2±1.1	12.3±2.2

*rbc= red blood cell

All results are presented as means±SEM.

Mice (F1N3 background) were fed HCD for 12 weeks.

Supplementary Table 5

RT-PCR primers

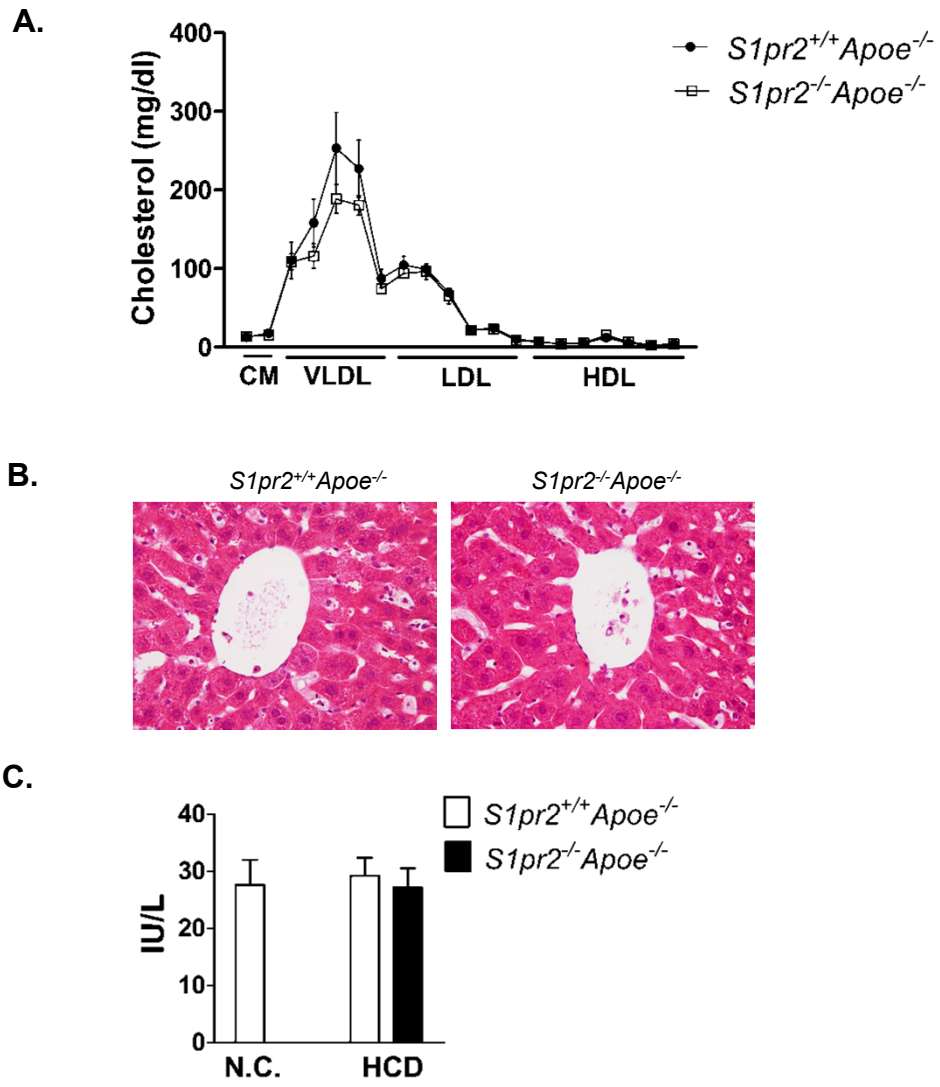
Gene symbol		Primer sequence (5' to 3')	Annealing temp.* (℃)	Product size (bp)
S1pr1	F	TCCATGTAAACTGGGTCAAG	60	315
	R	AAAGGTGCTGTAGGGGTTAG		
S1pr2	F	TTTTAAAATTGGGACAGGGT	60	258
	R	TTCTCCACAGGATTTAGCAA		
S1pr3	F	ATGGCATTTGCTCTTGTTTA	60	234
	R	TATTTTTCCCTTAACCCAGC		
S1pr4	F	AACTGTGGGTATGACTCTGG	60	190
	R	ATACAGTTGGAACAGTTGGG		
S1pr5	F	CTAGGTCTGGAAATTTGGCT	60	319
	R	AACTGAAGTTGCCAGAATCA		
Vcam1	F	GCAGAGACTTGAAATGCCTGTG	57	340
	R	CTTCGTTCCAGCTTCCCAGAGCC		
Icam1	F	TGCGTTTTGGAGCTAGCGGACCA	60	300
	R	CGAGGACCATACAGCACGTGCAG		
Gapdh	F	TGATGGGTGTGAACCACGAG	54	521
	R	GTCATTGAGAGCAATGCCAG		

***** temp.=temperature. F, forward primer. R, reverse primer.

Supplementary Table 6

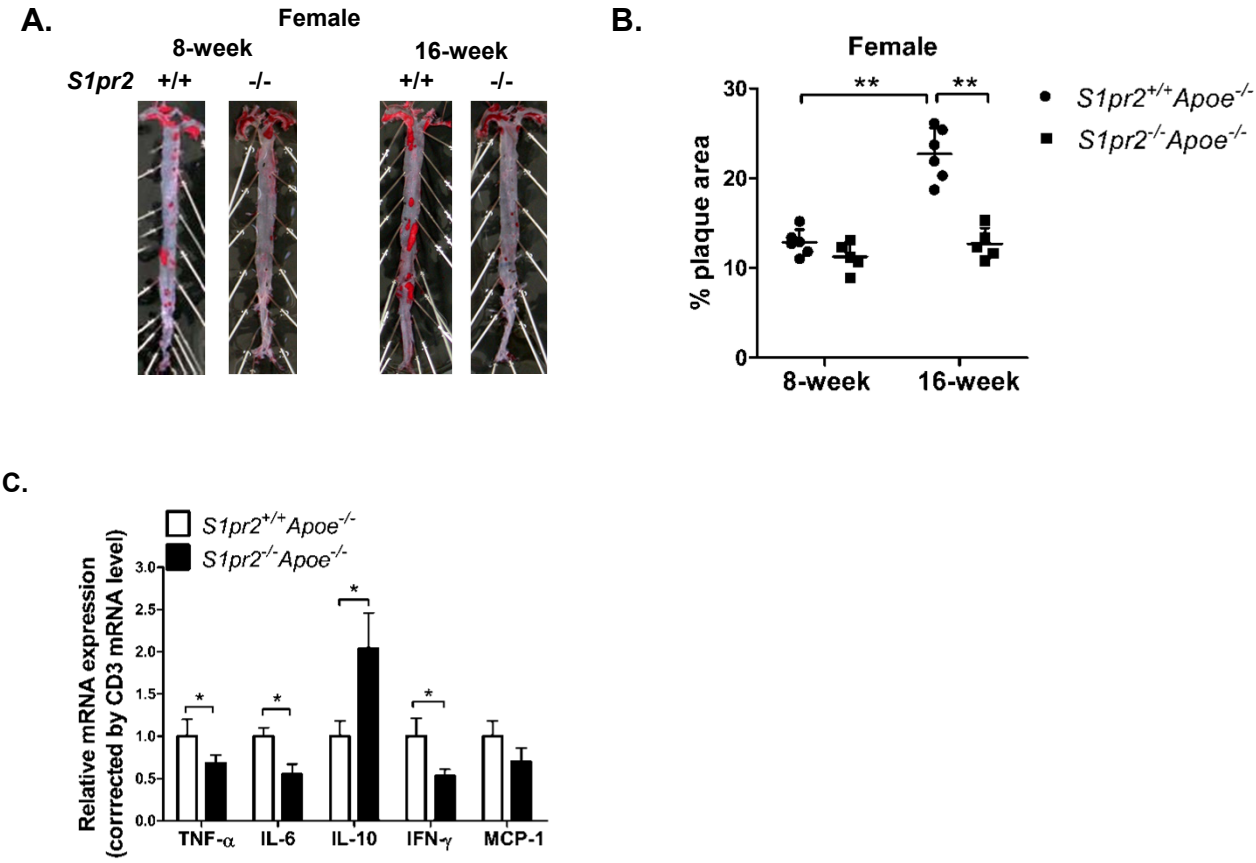
Real-time PCR primers

Gene symbol	ID number
Tnf	Mm00443258_m1
Il6	Mm00446190_m1
Cd36	Mm00432403_m1
Scara	Mm01313828_m1
Scarb1	Mm00450236_m1
S1pr1	Mm00514644_m1
S1pr2	Mm02620208_m1
S1pr3	Mm00515669_m1
Sphk1	Mm00448841_m1
Sphk2	Mm00445020_m1
Spp1	Mm00473016_m1
Spl1	Mm00486079_m1
Ccl2	Mm99999056_m1
Abca1	Mm00442663_m1
Abcg1	Mm00437390_m1
Ifng	Mm99999071_m1
Pparg	Mm01184323_m1
Vcam1	Mm01320970_m1
Icam1	Mm00516024_m1
Nr1h3(LXRα)	Mm00443454_m1
Nr1h2(LXRβ)	Mm00437265_m1
Csf2(GM-CSF)	Mm01290062_ m1
CD3e	Mm01179194_m1
IL10	Mm00439614_m1
18S rRNA	Hs99999901_s1



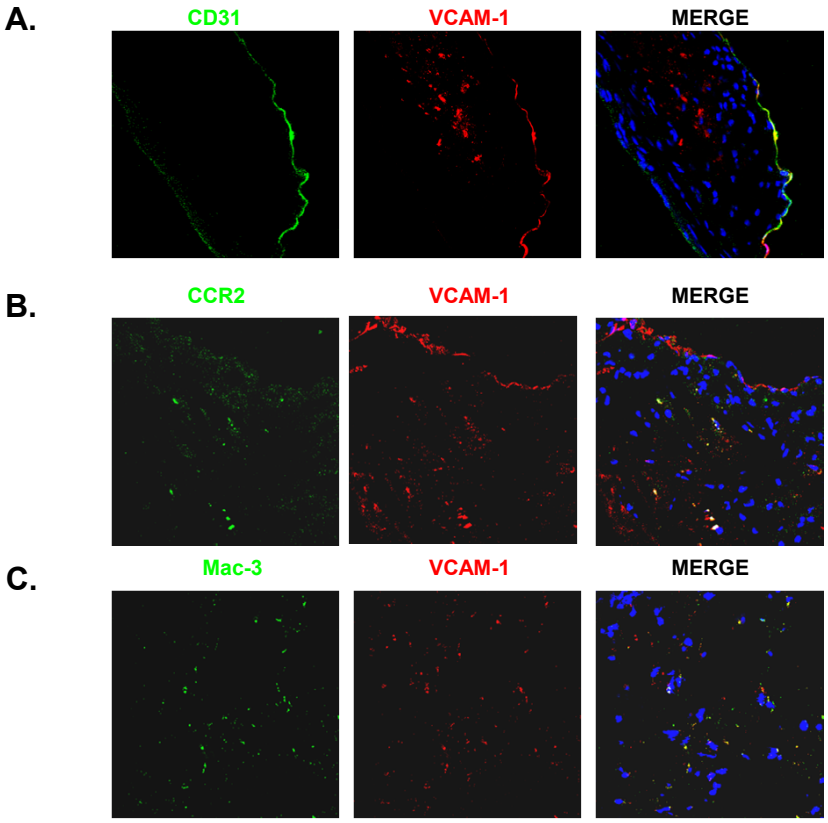
Supplementary Figure 1.

(A) Distribution of cholesterol in the plasma of *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ (closed circle) and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ (open square) mice fed with HCD for 12 weeks. No difference in the distribution of cholesterol in plasma lipoproteins was detected (CM: chylomicron). **(B)** H&E staining of sections of livers isolated from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ mice fed with HCD. **(C)** Serum ALT levels in *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ (closed circle) and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ (open square) mice fed with HCD and *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ fed with normal chow (N.C.). (n=5 each).



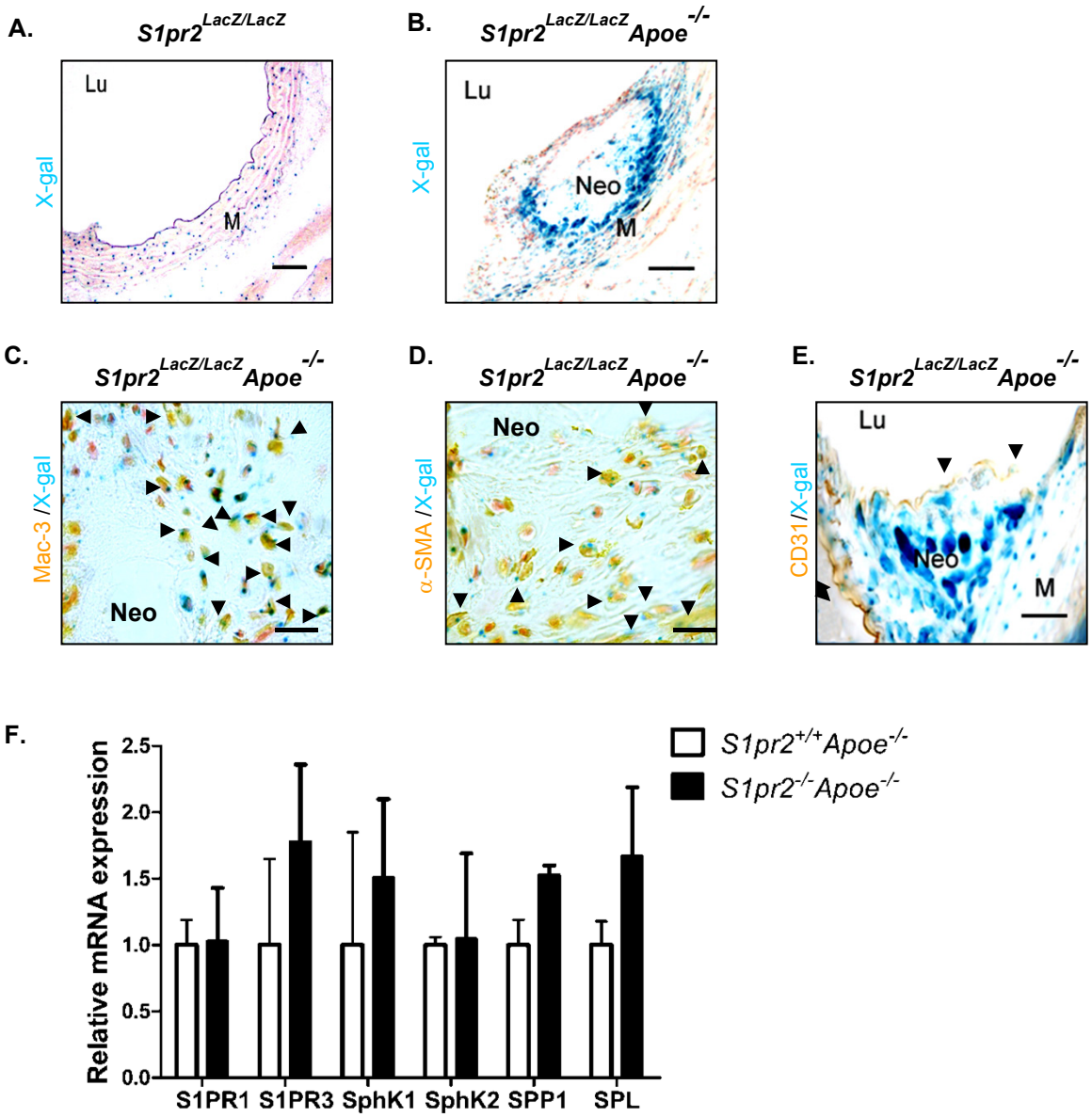
Supplementary Figure 2.

(**A**) Representative Oil red O staining of spread aortas from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ (*n*=12) and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ (*n* =10) female mice (N3 generation) fed HCD for 8 and 16 weeks. (**B**) Quantified plaque areas over total aortic areas are shown. ***P*<0.01. (**C**) The mRNA expression levels of TNF-α, IL-6, IL-10, IFN-γ and MCP-1 that were corrected by CD3 mRNA levels (n=5 each).



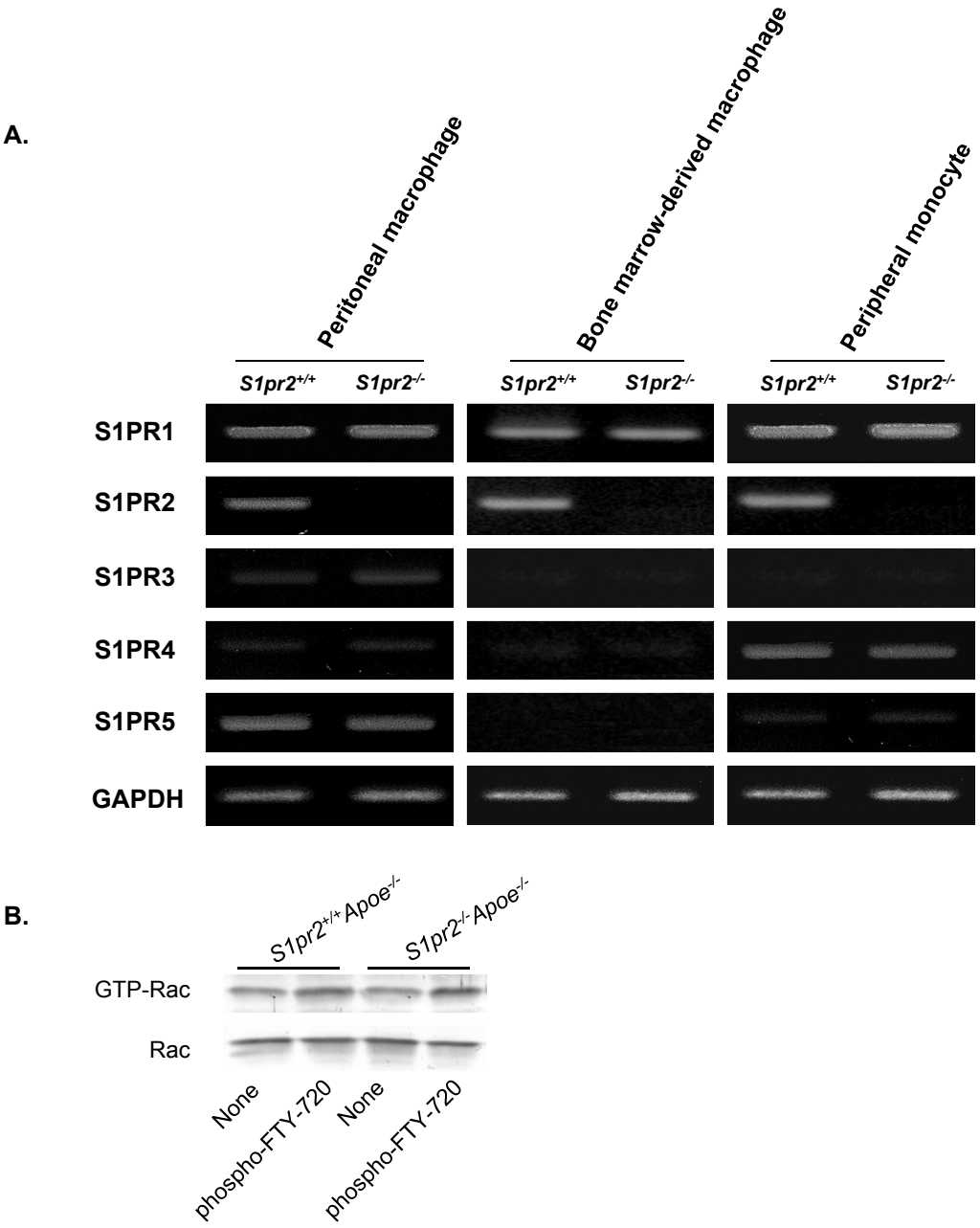
Supplementary Figure 3.

(A-C) Colocalization staining of ECs, Macrophages**. (A)** CD31(green), VCAM-1(red) stainings colocalized ECs.**(B)** Freshly migrated macrophages are shown by costaining CCR2(green) and VCAM-1(red).**(C)** Macrophages are shown by staining Mac-3(green) and VCAM-1(red).



Supplementary Figure 4.

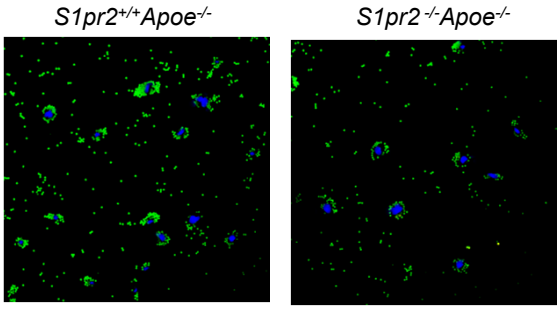
Expression of S1PR2 in normal and atherosclerotic aortas detected by LacZ activity in *S1pr2*$^{LacZ/LacZ}$*Apoe*$^{+/+}$ and *S1pr2*$^{LacZ/LacZ}$*Apoe*$^{-/-}$ mice. (**A**) X-gal (blue) staining of a cross section of the normal abdominal aorta from *S1pr2*$^{LacZ/LacZ}$*Apoe*$^{+/+}$ mice showing that S1PR2 is expressed in both ECs and SMCs. (**B-D**) Histological staining of the atherosclerotic aortas from *S1pr2*$^{LacZ/LacZ}$*Apoe*$^{-/-}$ mice fed HCD for 12 weeks. X-gal staining of a cross section of the atherosclerotic aorta (**B**), X-gal and anti-Mac-3 (brown) double staining of the aortic sinus (**C**), X-gal and anti-α-SMA (brown) double staining of the aortic sinus (**D**), and X-gal and anti-CD31 (brown) double staining of the aortic sinus (**E**). Lu: lumen; M: media; Neo: neointima. Black arrowheads in (**C-E**) show LacZ-positive macrophages, smooth muscle cells and ECs, respectively. Cell nuclei are counterstained by nuclear fast red (pink). Scale bars: 50 μm. **(F)** The mRNA expression of S1P receptors, S1P synthesizing and degrading enzymes in the aortas from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ (open bar) and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ (closed bar) mice. The mRNA expression levels were determined by real-time PCR. 18S rRNA was used as an internal control. Data are expressed as the ratio of the values in *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ mice over *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ mice (*n*=3 each).



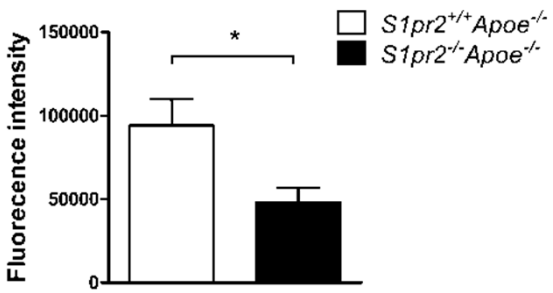
Supplementary Figure 5.

The expression of S1P receptor mRNA in monocytes/macrophages and the Rac response to FTY-720 phosphate in macrophages. (**A**) The expression of S1P receptor mRNA in peritoneal macrophages, BM-derived macrophages, and peripheral blood monocytes from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ mice. The mRNA expression levels of S1P receptors were determined by semi-quantitative RT-PCR. GAPDH was used as an internal control. Similar results were obtained in three different experiments. (**B**) S1PR2 deletion does not alter S1PR1-dependent Rac activation by phosphorylated FTY-720. Serum-starved peritoneal macrophages isolated from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ mice were stimulated with phosphorylated FTY-720 (1 μM) for 10 min and subjected to pulldown assay for GTP-Rac.

A.

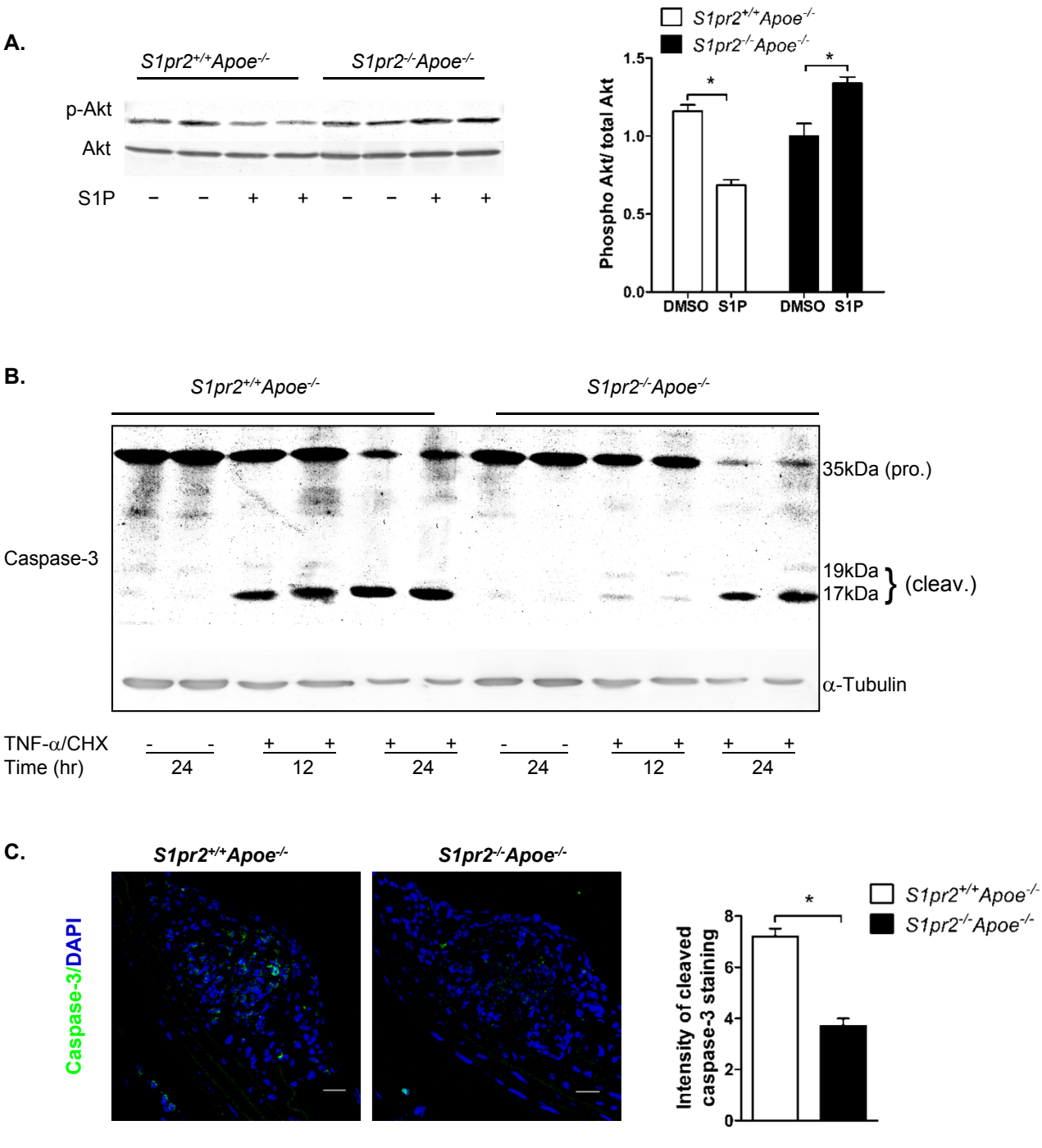


B.



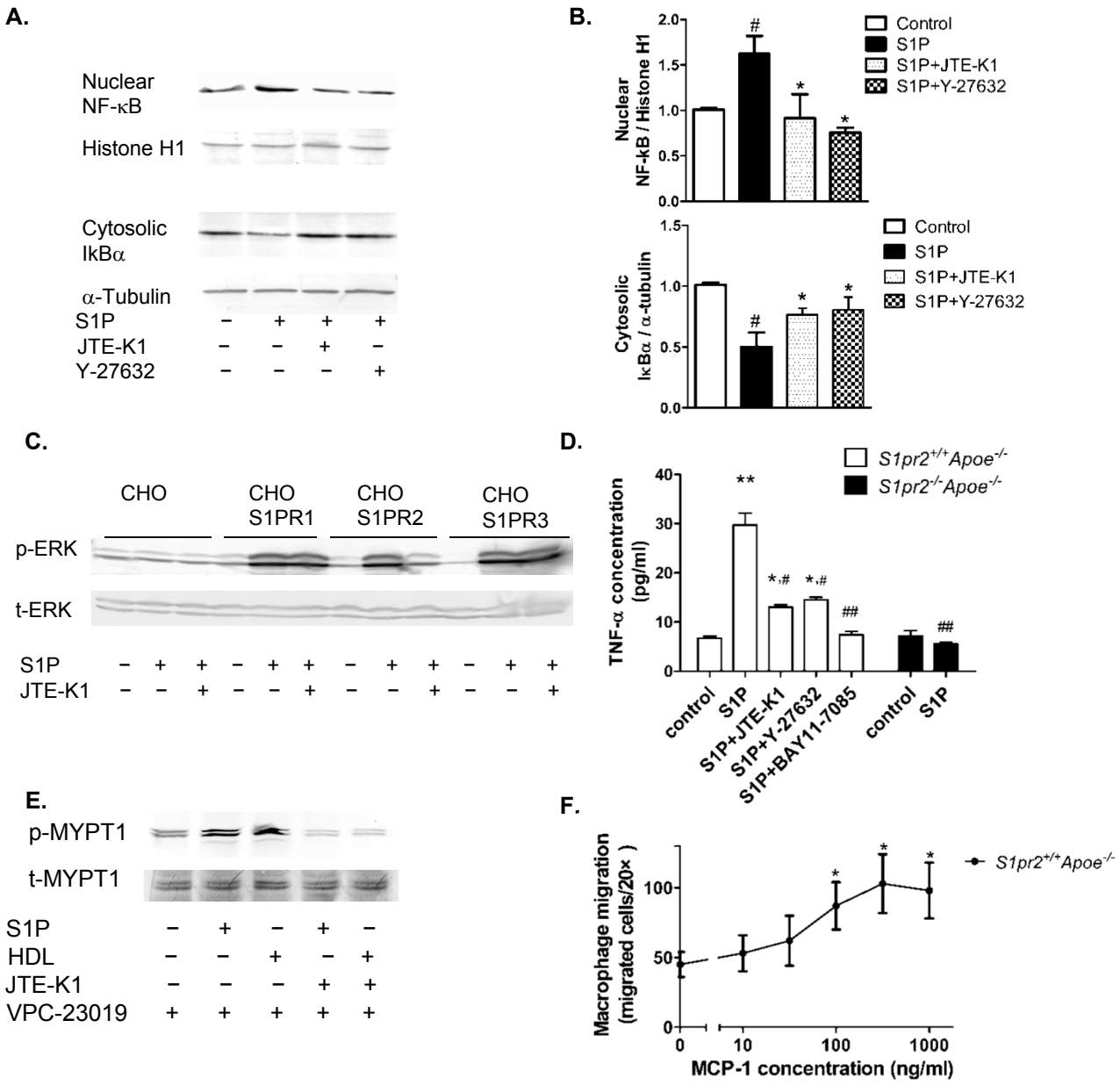
Supplementary Figure 6.

(**A**) Decreased phagocytosis in *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ macrophage. Macrophages from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$, and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ mice were incubated with fluorescent polystyrene microspheres, fluoresbrite carboxylate, for 1 hour at 37℃. Scale bars: 20 μm. **(B)** Quantified data of beads uptake is shown. (n=3 each). **P*<0.05.



Supplementary Figure 7.

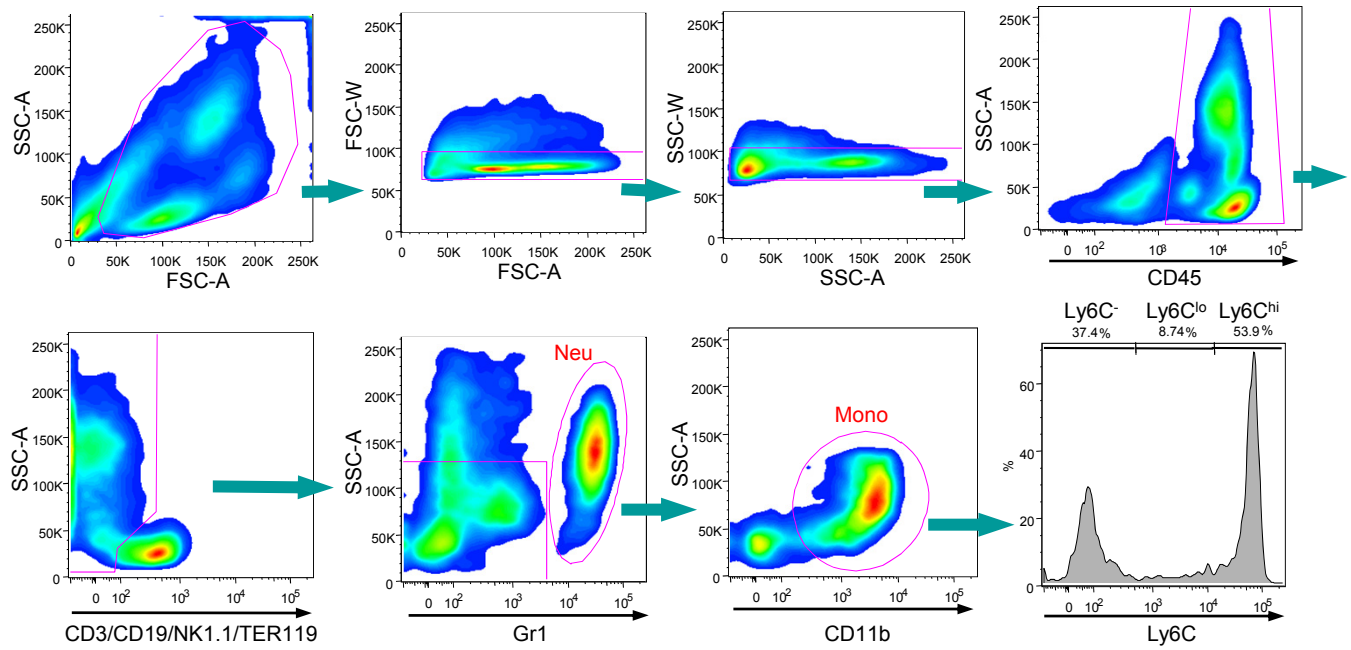
Effects of S1PR2 deficiency on macrophage apoptosis in peritoneal macrophages and plaque lesions in the aorta. (**A**) Phosphorylation of Akt in peritoneal macrophages from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ (open bar) and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ (closed bar) mice. The cells were serum-starved for 15 hours and then stimulated with S1P (0.1 μM) for 15 minutes. Cell lysates were subjected to Western blotting using antibodies against phospho-Akt and total Akt. Quantified data of band density are shown (right). (n=3 each). **P*<0.05. (**B**) Caspase-3 activation in peritoneal macrophages from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ (left panel) and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ (right panel) mice. The cells were treated with TNF-α(10 ng/ml) and cycloheximide (10 μg/ml) for 12 or 24 hours. Caspase-3 activation was determined by Western blotting using anti-caspase-3 antibody that recognizes both pro-caspase-3 and cleaved caspase-3. α-Tubulin was used as an internal control. The similar observations as those in (**A**) and (**B**) were also made in bone marrow-derived macrophages. (**C**) Immunostaining using anti-cleaved caspase-3 of the atherosclerotic aortas from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ (left panel) and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ (right panel) mice (*n*=3 each). Quantified data of cleaved caspase-3-positive cells are shown (left). Scale bars: 50 μm. **P*<0.05.



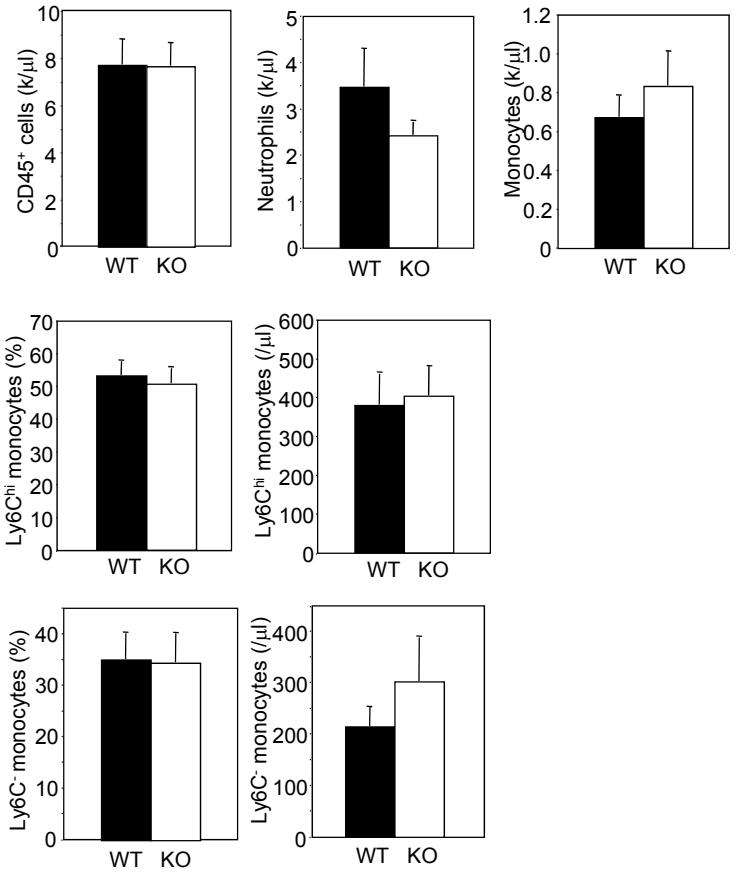
Supplementary Figure 8.

S1P induces nuclear translocation of NF-κB (p65RelA). (**A**) Peritoneal macrophages from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ mice were stimulated with 0.1 μM S1P in the presence and absence of 1 μM JTE-K1 or 10 μM Y-27632 for 2 hours. Nuclear and cytosolic extracts were subjected to Western blotting using anti-p65RelA antibody and anti-IκBα antibody. Histone H1 and α-tubulin were used as nuclear and cytosolic internal controls, respectively. (**B**) Quantified data of NF-κB protein in nucleus and IκBα in cytosol. *n*=3 each. **P*<0.05 compared with S1P-treated macrophages, $^{\#}$*P*<0.05 compared with non-treated macrophages. (**C**) JTE-K1 specifically inhibits S1PR2-, but not S1PR1- or S1PR3- mediated ERK activation. The CHO cells expressing each receptor were pretreated with JTE-K1 (1 μM) for 10 minutes and stimulated with S1P (0.1 μM) for 5 minutes. **(D)** TNF-α secretion by *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ macrophages. Macrophages pretreated with either JTE-K1(1 μM), Y-27632(10 μM), or BAY11-7085(10 μM) or untreated were stimulated with S1P for 12 hours. TNF-α protein levels were measured by using ELISA. (n=3 each). **P*<0.05 compared with non-treated macrophages, $^{\#}$*P*<0.05 compared with S1P-treated macrophages. (**E**) Stimulation of MYPT1 phosphorylation by HDL via S1PR2 and ROCK. *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ macrophage were pretreated with VPC23019 (1 μM) or JTE-K1 (1 μM) for 10 minutes before the addition of S1P (0.1 μM) or HDL (0.45 mg/ml), which contains the equivalent concentration of S1P, for 5 minutes. **(F)** MCP-1 stimulates chemotaxis of *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ macrophages in the presence of various concentrations of MCP-1 in the lower well of the modified Boyden chamber. (*n*=3 each). **P*<0.05.

A.

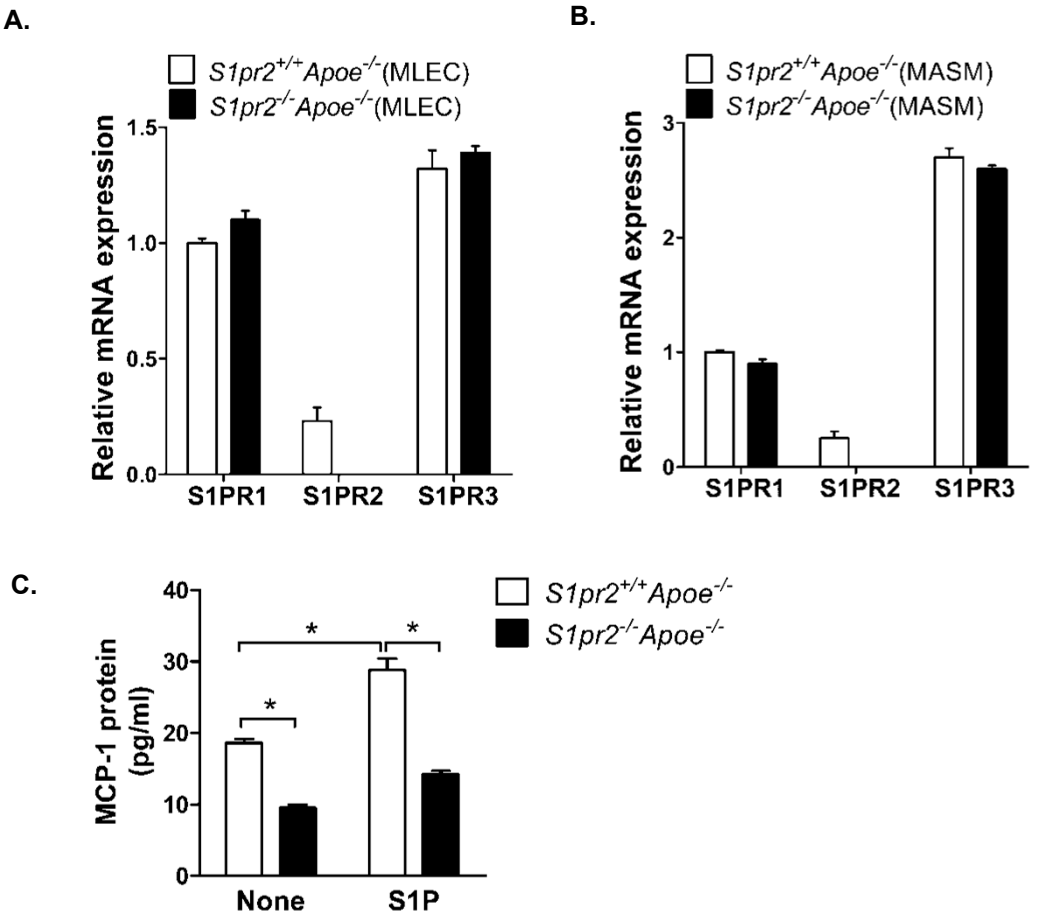


B.



Supplementary Figure 9.

CD11b^{+}Ly6C^{hi} proinflammatory monocyte subset is not different between *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ mice. We examined surface markers in peripheral blood monocyte to examine the activation state by flow cytometry. Peripheral blood neutrophils and monocytes were defined as CD45^{+}CD11b^{+}Gr1^{+}CD3^{-}CD19^{-}NK1.1^{-} and CD45^{+}CD11b^{+}Gr1^{-}CD3^{-}CD19^{-}NK1.1^{-}, respectively. (**A**) Scheme of CD11b^{+}Ly6C^{hi} monocyte subset selection processes. (**B**) Quantified data of each population characterized of the indicated surface markers.



Supplementary Figure 10.

The expression of S1P receptor mRNA in MLECs (**A**) and SMCs (**B**) from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ mice (*n*=3 each). The mRNA expression of S1P receptors was determined by real-time PCR. 18S rRNA was used as an internal control. Data are expressed as the ratio of the values in *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ mice over S1PR1 mRNA level in *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ mice. **(C)** MCP-1 secretion by MLECs. MLECs isloated from *S1pr2*$^{+/+}$*Apoe*$^{-/-}$ and *S1pr2*$^{-/-}$*Apoe*$^{-/-}$ mice, treated with or without S1P(0.1 μM) for 12 hours. MCP-1 protein levels were measured using ELISA. (n=3 each). **P*<0.05.