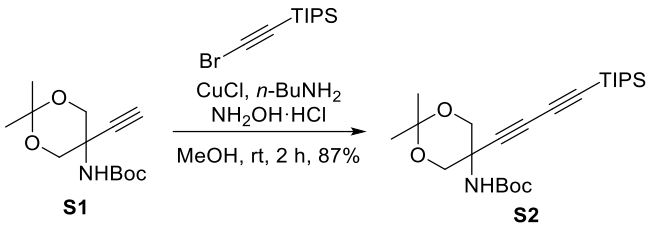
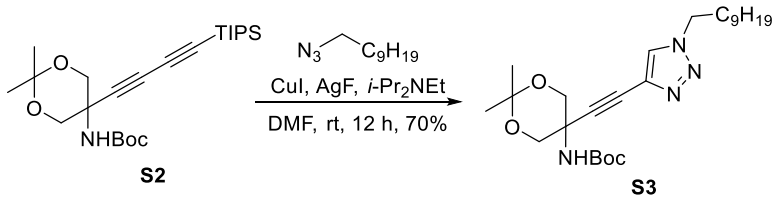
Supplementary Methods.

Synthesis of SLB736. ^{1}H and ^{13}C NMR spectra of SLB736 are depicted in Supplementary Fig. 2.



S1 S2

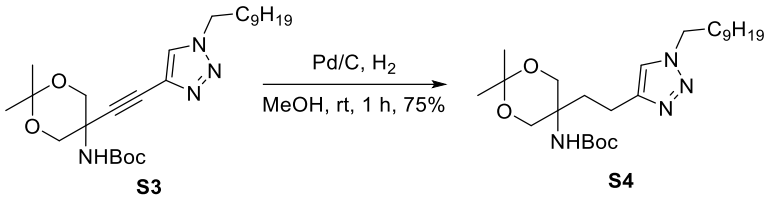
S2. To a solution of CuCl (10 mg, 0.10 mmol), $NH_2OH{\cdot}HCl$ (1.02 g, 14.7 mmol) and *n*-$BuNH_2$ (3.87 mL, 39.2 mmol) in MeOH (50 mL), **S1**[1] (1.25 g, 4.89 mmol) in MeOH (30 mL) was added under nitrogen. 2-Bromo-1-triisopropylsilyl acetylene (2.56 g, 9.78 mmol) in MeOH (20 mL) was added dropwise to the mixture and stirred at room temperature for 2 h. The reaction was quenched with H_2O and concentrated. The mixture was diluted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane/EtOAc, 7:1) to afford **S2** (1.85 g, 87%) as a white solid; m.p. 107–109 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.11 (s, 1H), 3.95 (s, 4H), 1.43–1.45 (m, 12H), 1.38 (s, 3H), 1.04 (s, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.3, 98.6, 88.9, 84.6, 80.4, 72.8, 70.5, 66.1 (2C), 48.0, 28.3 (3C), 28.2 (2C), 18.5 (6C), 11.2 (3C); IR (neat) υ_{max} = 3244, 2943, 2865, 1711, 1460, 1360, 1161, 1070, 879, 828 (cm^{-1}); HRMS (FAB) calcd for $C_{24}H_{42}NO_4Si$ ($[M+H]^+$) 436.2883, found 436.2884.



S2 S3

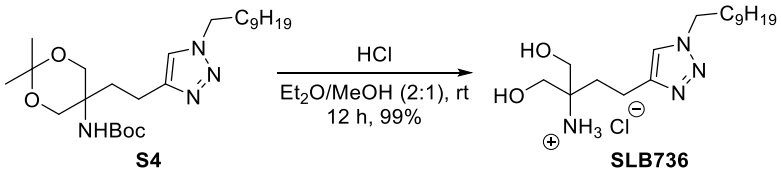
S3. To a solution of **S2** (2.69 g, 6.2 mmol) in anhydrous DMF (70 mL), 1-azidodecane (3.39 g, 18.6 mmol), CuI (1.18 g, 6.2 mmol), *i*-Pr_2NEt (3.2 mL, 18.6 mmol) and AgF (940 mg, 7.4 mmol) were added. The reaction mixture was stirred at room temperature for 12 h. The reaction was quenched with saturated NH_4Cl aqueous solution and extracted with EtOAc twice. The organic layers were washed with saturated NH_4Cl aqueous solution and brine, dried over $MgSO_4$, and then concentrated *in vacuo*. The residue was purified using flash column chromatography (hexane/EtOAc, 3:1) to afford **S3** as a

white solid (2.00 g, 70%); m.p. 84–88 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 5.21 (br s, 1H), 4.31 (t, J = 7.1 Hz, 2H), 4.06 (dd, J = 11.5, 15.0 Hz, 4H), 1.83–1.87 (m, 2H), 1.42–1.49 (m, 15H), 1.23–1.27 (m, 14H), 0.85 (t, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.3, 130.1, 126.2, 98.6, 89.0, 80.1, 74.7, 66.1 (2C), 50.5, 47.9, 31.8, 30.9, 30.1, 29.4, 29.3, 29.2, 28.9, 28.4 (3C), 26.3, 22.6, 18.5, 14.1; IR (neat) υ_{max} = 3342, 2926, 2856, 1711, 1369, 1104, 1073, 829 (cm^{-1}); HRMS (FAB) calcd for $C_{25}H_{43}N_4O_4$ ([M+H]$^+$) 463.3284, found 463.3278.



S3 **S4**

S4. To a solution of **S3** (1.49 g, 3.2 mmol) in MeOH (300 mL) was added 10 % Pd/C (450 mg). The flask was evacuated, filled with H_2, and stirred at room temperature for 1 h. The mixture was then filtered through Celite. The filtrate was concentrated, and the residue was purified by flash column chromatography (hexane/EtOAc, 2:1) on silica gel to afford **S4** (1.11 g, 75%) as a white solid; m.p. 74–76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (s, 1H), 5.02 (br s, 1H), 4.26 (t, J = 7.2 Hz, 2H), 3.79 (dd, J = 11.7, 84.5 Hz, 4H), 2.68 (t, J = 8.3 Hz, 2H), 2.05 (t, J = 8.3 Hz, 2H), 1.84 (t, J = 6.6 Hz, 2H), 1.43 (s, 9H), 1.42 (s, 3H), 1.40 (s, 3H), 1.23–1.28 (m, 14H), 0.86 (t, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.9, 147.6, 120.4, 98.3, 79.3, 66.1 (2C), 51.5, 50.1, 31.8, 31.3, 30.2, 29.4, 29.3, 29.2, 28.9, 28.3 (3C), 27.1, 26.4, 22.6, 19.9, 19.1, 4.0; IR (neat) υ_{max} = 3334, 2925, 2857, 1711, 1368, 1248, 1104, 1074, 832 (cm^{-1}); HRMS (FAB) calcd. for $C_{25}H_{47}N_4O_4$ ([M+H]$^+$) 467.3597, found 467.3597.



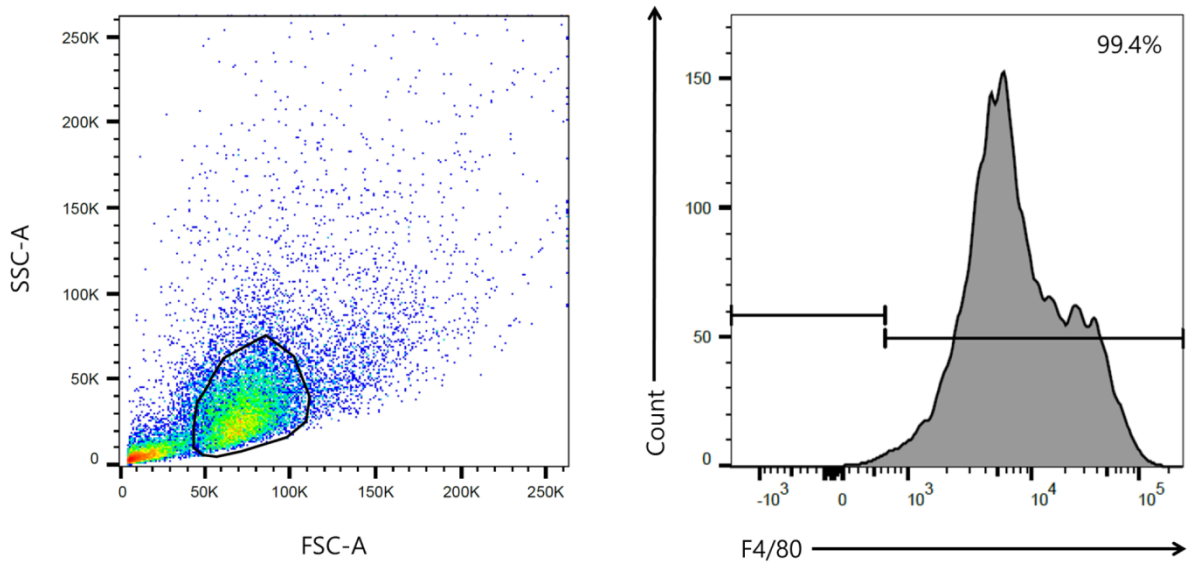
S4 **SLB736**

SLB736. A 2.0 M HCl in Et_2O (40 mL) was added to a solution of compound **S4** (1.25 g, 2.68 mmol) in MeOH (20 mL). The reaction mixture was stirred at room temperature for 12 h. Then, the mixture was concentrated under reduced pressure to afford **SLB736** (962 mg, 99%) as a white solid; m.p. 130 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.35 (s, 1H), 4.53 (t, J = 7.2 Hz, 2H), 3.69 (s, 4H), 2.97 (td, J = 4.3,

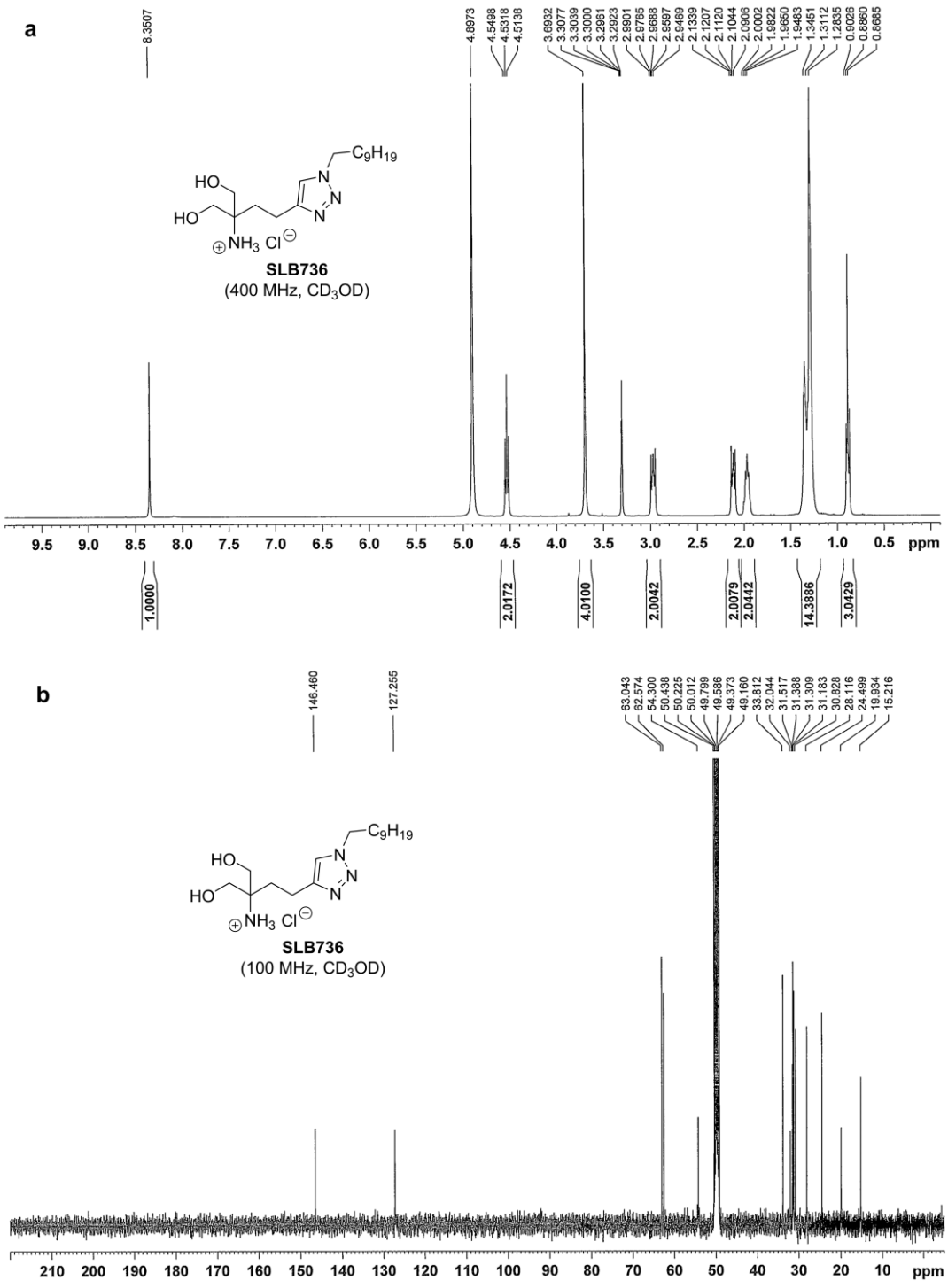
8.0 Hz, 2H), 2.11 (td, J = 4.3, 8.0 Hz, 2H), 2.00–1.95 (m, 2H), 1.35–1.28 (m, 14H), 0.89 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 146.5, 127.3, 63.0 (2C), 62.6, 54.3, 33.8, 32.0, 31.5, 31.4, 31.3, 31.2, 30.8, 28.1, 24.5, 19.9, 15.2; IR (neat) υ_{max} = 3180, 2918, 2851, 2421, 1599, 1454, 1080, 1063, 958, 715 (cm^{-1}); HRMS (FAB) calcd. for $C_{17}H_{35}N_4O_2$ $[M-Cl^-]^+$ 327.2760, found 327.2762.

Supplementary References.

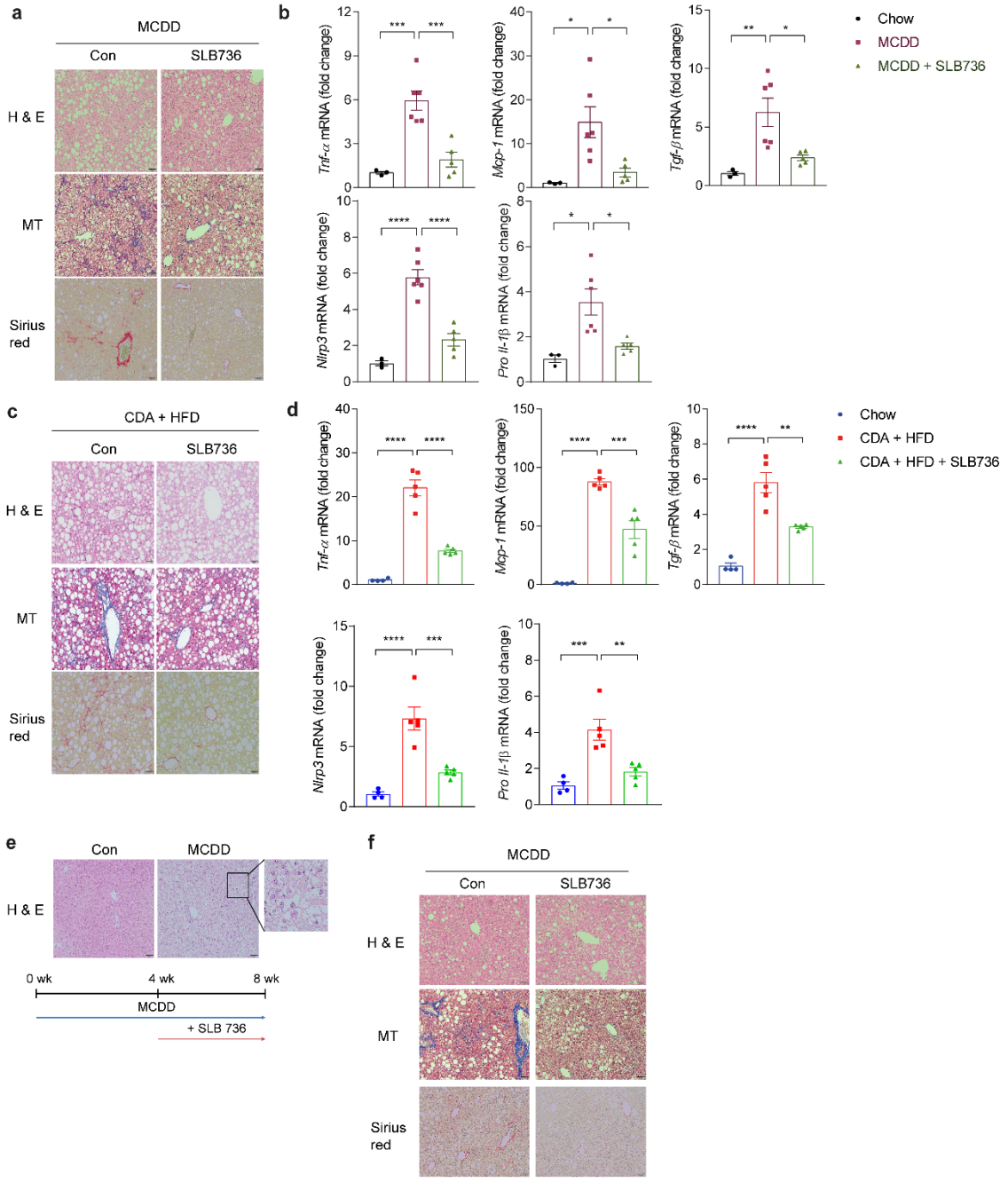
1. Kim, S., Lee, H., Lee, M. & Lee, T. Efficient synthesis of the immunosuppressive agent FTY720. *Synthesis* **5**, 753–755 (2006).



Supplementary Fig. 1 Isolation and identification of Kupffer cells. Kupffer cells were obtained by collagenase perfusion, differential centrifugation, and selective adherence, as described in the Methods. FACS analysis using F4/80 antibody revealed that attached Kupffer cells were of high purity (> 95 %) and the average yield was about 1×10^5 cells/mouse.



Supplementary Fig. 2 a ^{1}H NMR **b** ^{13}C NMR spectra of SLB736.



Supplementary Fig. 3 SLB736 prevents NASH in other animal models and retards the progression to NASH and fibrosis. **a** Representative H&E, MT, and Sirius Red staining of the livers of MCDD-fed mice with or without SLB736 treatment. Scale bars, 50 μm. **b** Relative mRNA expression levels of inflammation, fibrosis, and inflammasome markers in the livers of chow-fed mice and MCDD-fed mice with or without SLB736 (n = 5–6). **c** Representative H&E, MT, and Sirius Red staining of the livers of CDA+HFD-fed mice with or without SLB736 treatment. Scale bars, 50 μm. **d** Relative mRNA

expression levels of markers for inflammation, fibrosis, and inflammasome in the livers of chow-fed mice and CDA+HFD-fed mice with or without SLB736 treatment (n = 4–5). **e** Representative H & E of livers of MCDD-fed mice for 4 weeks. Scale bars, 50 μm. Inlet shows the lipid accumulation in hepatocytes. The lower panel describes the schematic schedule for observing the therapeutic effect of SBL736 in MCDD-fed mice. **f** Representative H&E, MT, and Sirius Red staining of the livers. Scale bars, 50 μm. After feeding MCDD for 4 weeks, SLB736 (1 mg/kg/day) was administrated for 4 weeks with MCDD. All data are shown as mean ± SEM. Data in **b** and **d** were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Supplementary Fig. 4 Original Western blot gel images. This is the source data for the images shown in Figures 3, 4, 5, and 7.

Fig 3d

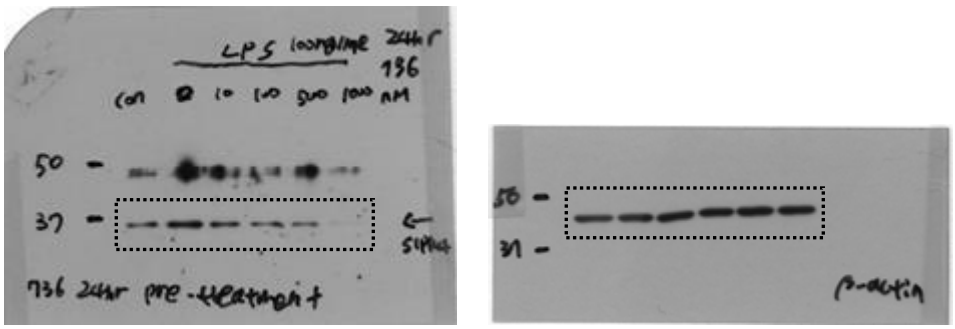


Fig 4f

Fig 5c

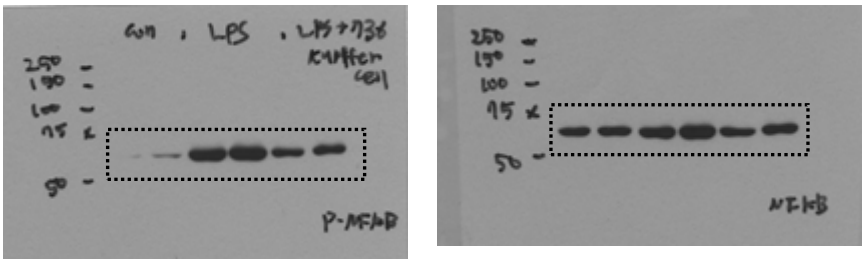


Fig 7f

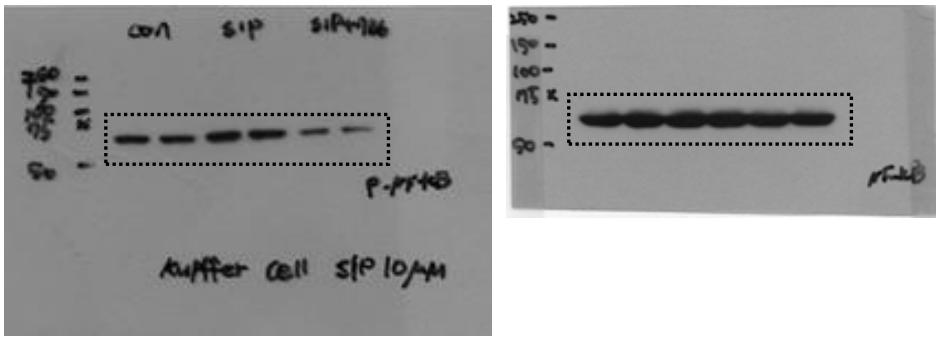
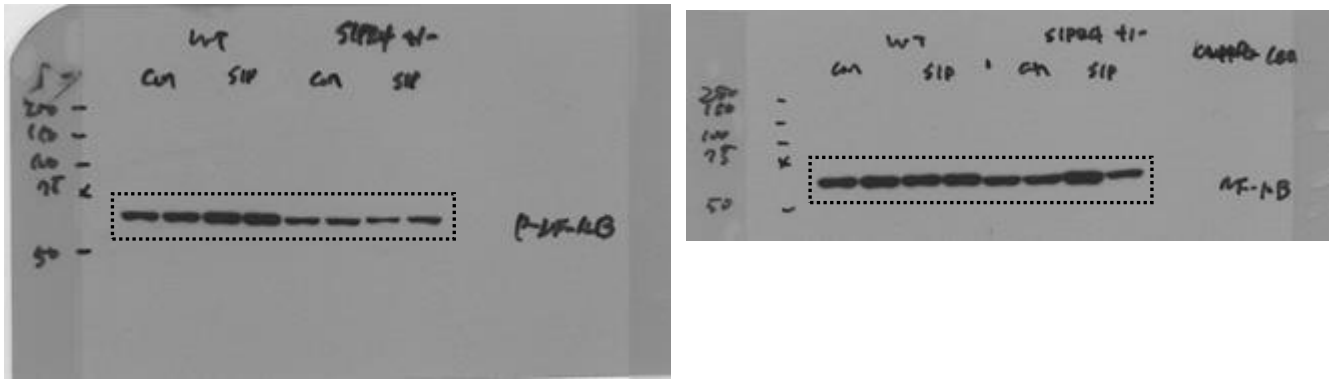

con
S1P
S1P+W146
P-AKT-NB
Kupffer Cell S1P 10μM

Fig 7g


WT
S1PR4 -/-
Con
S1P
Con
S1P
P-AKT-NB
WT
S1PR4 -/-
Con
S1P
Con
S1P
AKT-NB

Supplementary Table 1. List of primers

Primer	Forward	Reverse
RT-PCR primers		
Tbp	CCTTCACCAATGACTCCTATGAC	CAAGTTTACAGCCAAGATTCAC
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGC
Tnf-α	GAGAAAGTCAACCTCCTCTCTG	GAAGACTCCTCCCAGGTATATG
Mcp-1	ACGGCATGGATCTCAAAGAC	AGATAGCAAATCGGCTGACG
Tgf- β	TATAGCAACAATTCCTGGCG	CCTGTATTCCGTCTCCTTG
α-SMA	ACTGGGACGACATGGAAAAG	GTTCAGTGGTGCCTCTGTCA
Nlrp3	ATTACCCGCCCGAGAAAGG	TCGCAGCAAAGATCCACACAG
Il-1β	TCTTTGAAGTTGACGGACCC	TGAGTGATACTGCCTGCCTG
S1pr1	ATGGTGTCCACTAGCATCCC	CGATGTTCAACTTGCCTGTGTAG
S1pr2	ATGGGCGGCTTATACTCAGAG	GCGCAGCACAAGATGATGAT
S1pr3	ACTCTCCGGGAACATTACGAT	CAAGACGATGAAGCTACAGGTG
S1pr4	GGGTGTACTACTGCCTGCTG	AGCAGACTGAAGGTGGATGC
S1pr5	GCTTTGGTTTGCGCGTGAG	GGCGTCCTAAGCAGTTCCAG
Sk1	CCATCCAGAAACCCCTGTGT	ACCTGCTCGTACCCAGCATAGT
Sk2	AGACGGGCTGCTTTACGAG	CAGGGGAGGACACCAATG

Supplementary Table 2. Antibodies

NF-kB	Cell signaling Technology	Cat# 6956, RRID:AB_10828935
Phospho NF-kB	Cell signaling Technology	Cat# 3033, RRID:AB_331284
S1PR4	NOVUS	Cat# NBP1-00795, RRID:AB_1503063
β-actin	Sigma–Aldrich	Cat#A5441, RRID:AB_476744
HRP Goat anti-mouse IgG	BioLegend	Cat# 405306, RRID:AB_315009
HRP Donkey anti-rabbit IgG	BioLegend	Cat# 406401, RRID:AB_2099368
F4/80	Thermo Fisher Scientific	Cat# 12-4801-82, RRID:AB_465923

Supplementary Video

Supplementary Video 1. This video shows that LPS induces increases in intracellular Ca^{++} in Kupffer cells. Images of untreated cells were acquired (t = 0), then LPS (1 μg/ml) was added and the cells were imaged for 5 min at 5-sec intervals. After 5 min, ionomycin (1 μM) was added to the medium.

Supplementary Video 2. This video shows the intracellular Ca^{++}-induced increases of Kupffer cells treated with ATP. Images of untreated cells were acquired (t = 0), then 1 mM ATP was added in the LPS-primed Kupffer cells and the cells were imaged for 5 min at 5-sec intervals. After 5 min, ionomycin (1 μM) was added to the medium.

Supplementary Video 3. This video shows that LPS-induced intracellular Ca^{++} increases are blocked by SLB736 in Kupffer cells. Images of cells treated with SLB736 were acquired (t = 0), then LPS (1 μg/ml) was added and the cells were imaged for 5 min at 5-sec intervals. After 5 min, ionomycin (1 μM) was added to the medium.

Supplementary Video 4. This video shows that ATP-induced intracellular Ca^{++} increases are not blocked by SLB736. Images of cells treated with SLB736 were acquired (t = 0), then 1 mM ATP was added in the LPS-primed Kupffer cells and the cells were imaged for 5 min at 5-sec intervals. After 5 min, ionomycin (1 μM) was added to the medium.

Supplementary video 5. To compare with *S1pr4*$^{+/-}$ Kupffer cells, WT cells were treated with LPS. Images of untreated cells were acquired (t = 0), then LPS (1 μg/ml) was added and the cells were imaged for 5 min at 5-sec intervals.

Supplementary Video 6. LPS-induced intracellular Ca^{++} increases were substantially diminished in *S1pr4*$^{+/-}$ Kupffer cells. Images of untreated cells were acquired (t = 0), then LPS (1 μg/ml) was added in *S1pr4*$^{+/-}$ Kupffer cells and the cells were imaged for 5 min at 5-sec intervals.

Supplementary video 7. ATP-induced intracellular Ca^{++} increases in Kupffer cells. Images of untreated cells were acquired (t = 0), then 1 mM ATP was added in the LPS-primed Kupffer cells and the cells were imaged for 5 min at 5-sec intervals.

Supplementary Video 8. Unlike LPS stimulation, ATP exposure resulted in the elevation of intracellular Ca^{++} levels in *S1pr4*$^{+/-}$ Kupffer cells. Images of untreated cells were acquired (t = 0), then 1 mM ATP was added in the LPS-primed *S1pr4*$^{+/-}$ Kupffer cells and the cells were imaged for 5 min at 5-sec intervals.