SUPPLEMENTAL MATERIAL

Reagents: Lipopolysaccharide (Cat No. L2630) and 5-Bromo-2′-deoxyuridine (Cat No. B5002) were obtained from Sigma Aldrich. Sphingosine kinase 1 (SPHK1) inhibitor, PF-543 (Cat No. 1415562-82-1) was purchased from Cayman Chemical, while STAT3 inhibitor, S3I-201 (Cat No. SML0330) was procured from MilliporeSigma. SPHK2 inhibitor, ABC294640 (Cat No. B-0025) was purchased from Echelon Biosciences. ERK inhibitor, SCH 772984 (Cat No. 19166) was acquired from Cayman Chemical. S1P lyase inhibitor was from Abcam (Cat No. ab144103). The solubility of inhibitors is listed in online Table III. Sphingosine-1-phosphate (Cat No. BML-SL140-001) was purchased from Enzo life Inc. S1P (1mM stock solution) was prepared by suspending in methanol. CYM 5442 (Cat No. 16925) was acquired from Cayman Chemical. In situ cell death detection kit (Cat No. 12156792910) was purchased from Roche Diagnostics GmbH. Anti-S1PR1 antibody (Cat No. ASR-011) was acquired from Alomone Labs while, FACS anti-mouse antibodies, APC-CD31 (Cat No. 102409), PECy7-CD45 (Cat No. 103114), PE-EpCAM (Cat No. 118205), CD90-APC (Cat No. 105311), anti-BrdU (Cat No. 339808) were purchased from BioLegend. Anti-ki-67 eFluor 450 was obtained from ThermoFisher Scientific (Cat No. 48-5698-82). FC block (CD16/32) from BioLegend (Cat No.101320). Anti-vWF (Cat. No. ab11713) and anti-GFP (Cat No. ab6556) antibodies were acquired from Abcam. Anti-Phospho-ERK (Cat No. 4370S), anti-ERK (Cat No. 4695T), anti-Phospho-STAT3 (Cat No. 9145T), anti-STAT3 (Cat No. 8768T), anti-EGR1 (Cat No. 4154S) antibodies were procured from Cell Signaling Technologies. Anti-SPHK1 antibody (Cat No. sc-365401) and anti-actin (Cat No. sc-47778) were purchased from Santa Cruz Biotechnology while anti-SPNS2 (Cat No. NBP1-54345) antibody was from Novus Biologicals.

Mouse models: All animals were approved by the Institutional Animal Care and Use Committee of the University of Illinois. S1PR1^{knockin} mice were generated as described.[22] S1PR1-GFP reporter mice were generated by crossing S1PR1^{knockin} mice with histone-GFP reporter (H2B-GFP) mice (Jackson laboratory Stock No. 027563). H2B-GFP mice were used as control. Endothelial cell specific, *EC-S1PR1*$^{-/-}$ mice were generated by crossing S1PR1$^{fl/fl}$ with mice expressing Cre under the control of tamoxifen-inducible 5'

enhancer stem cell leukemia (Scl-CreERT) promoter[8,23-26]. At the age of 4-5 weeks, mice were injected tamoxifen (80mg/kg, *i.p.*) for five consecutive days followed by a week of rest for drug wash out. Twenty milligrams of tamoxifen (Sigma, Cat No. T5648) was suspended in 1 ml of corn oil (Sigma, Cat No. C8267). The mixture was vortexed at full speed for 30 min after which it was wrapped in an aluminum foil in an orbital shaker overnight. The drug was filtered using a 0.2 µm filter before injecting into animal. All experiments were performed on 6-8 weeks old mice of weight 20-25 gm. Sex-matched groups of male and female mice were used for these studies. No animals were excluded from analysis. We calculated sample size using software G Power based on pre-designed effect size between the groups based on Cohen's principles. A power =0.80, significance level = 0.05 [27].

Cell culture treatment and transfection: Primary human lung microvascular endothelial cells (HLMVEC) were obtained from Lonza, Allendale, NJ, USA (Cat. No: CC-2527) and used for all cell studies. Cells were cultured in a humidified atmosphere with 5% CO_2 at 37°C in EGM-2 medium supplemented with growth factors (Lonza, Cat No. CC-3162), 10% FBS and penicillin-streptomycin antibiotics. All studies were conducted on HLMVEC that were between passage 6-8. HLMVEC were transfected at passage 4-6 using Santa Cruz transfection reagent or Amaxa electroporation as described previously.[28] For depletion of S1PR1, HLMVEC were transfected with custom designed siRNA (siS1PR1) against 3′UTR region of S1PR1, antisense sequence 5′-AAACCAUCUUCAUCUUCCCUU -3′ (Dharmacon Inc).[29] ON-TARGETplus STAT3 siRNA (siSTAT3) (D-003544-03-0002) from Dharmacon Inc was used in order to deplete STAT3. Ambion™ Silencer™ Pre-Designed siRNA (Cat No. AM16708) was used to knock down EGR1. The SPNS2 siRNA (siSPNS2) (ThermoFisher Scientific, Cat No. 230791) was used to deplete SPNS2. Custom designed Human SPHK1 siRNA: antisense sequence 5′-CGGCAAGGGCAAGGCCUUGCAGCUCUU -3′ (Dharmacon Inc) was used to knockdown SPHK1 expression in HLMVEC.[30] In all experiments, control siRNA (siSC) (ON-TARGETplus non-targeting pool (D-001810-10), Dharmacon Inc was used.

FACS analysis: Lung tissues were minced and enzymatically digested with 1mg/ml collagenase A (Roche, cat No. 10103578001) for 30 min at 37°C after which digested tissue was passed through a 75µm cell

strainer to obtain single-cell suspensions. All antibodies used for flow cytometry were anti-mouse antigens. 5-bromo-2'-deoxyuridine (BrdU) 80mg/kg *i.p.* was administered four hours before sacrificing the mice.[5] Samples preparation for FACS acquisition was performed as described previously.[26,31,32]

Immunofluorescence: Mice were sacrificed and lungs were perfused with normal saline followed by administration of ice-cold 4% paraformaldehyde (PFA) solution. Lungs were harvested and fixed in 4% PFA for 2h at 4°C followed by equilibration with 30% sucrose solution overnight[22]. Lungs were then embedded in Optimal cutting temperature (OCT) compound and fast frozen at -20°C. Lungs were sectioned (8 to 10µm) and immunostained using indicated antibodies. Antigen retrieval was performed in a citrate-based antigen unmasking solution (Vector Laboratories, Cat No. H-3300) following manufacturer's instructions with slide immersed in retrieval solution. Blocking was performed using 5% normal goat serum supplemented with 1% Bovine serum albumin in TBS (0.2M Tris base, 1.5M NaCl) for 2h at room temperature. For vWF staining, lung sections were incubated with dilution 1:50 anti-vWF antibody (Abcam, Cat No. ab11713) over night at 4^0C. Sections were washed and incubated with Alexa-Fluor 594 secondary Donkey anti-Sheep antibody at a dilution of 1:250 (ThermoFisher Scientifc, Cat No. A-11016) at Room temperature for 1h. Sections were also stained with anti-GFP antibody at a dilution of 1:50 (Abcam, rabbit polyclonal antibody Cat No. ab6556) followed by secondary goat anti-rabbit IgG, Alexa Flour-488 at dilution 1:200 (ThermoFisher Scientific, Cat No. R37116). Slides were mounted with Prolong Gold antifade reagent (Invitrogen, Cat No. P36934). Isotype control primary antibodies (ThermoFisher Scientifc, Cat No. 31243 & 02-6102) were used as negative control in order to validate specificity of antibodies and to eliminate the background signal. DAPI (ThermoFisher Scientific, Cat No. D-1306) at dilution 1:1000 was used for nuclei staining. The images were acquired with an inverted laser-scanning confocal microscope (Carl Zeiss Microscopy) using the Zeiss LSM software. Representative images shown in the figures were selected to most accurately match the quantitative analysis. Regions were selected randomly to avoid biasing.

Quantitative real time PCR analysis: Total RNA was isolated using Trizol reagent (ThermoFisher Scientific, Cat No. 15596026). RNA was quantified and quality was determined by using BioDrop

(Biochrom, UK). Total RNA (1µg) was reverse transcribed using High-Capacity RNA to cDNA Kit (Cat No. 4368814, Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The cDNA products were used for quantitative Real Time PCR analysis using Fast SYBR™ Green Master Mix (Applied Biosystems, Cat No. 4385612) and each measurement was carried out in duplicate using a CFX384 real Time on Applied Biosystems QuantStudio Real-Time PCR Systems. The PCR conditions were 95°C for 10 min followed by 40 cycles 95 °C for 15 sec and to 60 °C for 1 min. The quantitative real-time PCR data were analyzed by $2^{-\Delta\Delta CT}$ method. The expression level was normalized to GAPDH. The primer sequences were designed by NCBI's Primer-BLAST. The list of primers used has been provided in Table I as Online Supplementary data.

Gene expression profiling (RNA-seq) and bioinformatics analysis: S1PR1-GFP reporter mice or control mice received LPS (10mg/kg body weight *i.p.*) after which they were sacrificed at indicated times. Lungs were harvested, minced and subjected to single cell suspension following enzymatic digestion. RNA was isolated and RNA quality control (QC) was performed using bioanalyzer. RNA-Seq analysis was performed in Genomic Core facility, University of Chicago on samples with 7-10 RIN (RNA Integrity Number). Bioinformatics analysis was performed by the University of Illinois Research Informatics. Raw reads were aligned to the reference genome in a splice-aware manner using the STAR aligner.[33] Gene expression was quantified using FeatureCounts[34] against Ensembl gene annotations. Differential expression statistics (fold-change and p-value) were computed using edgeR[35] and p-values were adjusted for multiple testing using the false discovery rate (FDR) correction of Benjamini and Hochberg.[36] Raw data are deposited in GEO database (accession ID: GSE165000). Results from RNA-seq analysis were uploaded into Qiagen's Ingenuity Pathway Analysis system (Ingenuity Systems; Mountain View, CA, USA). Top 100 genes that depicted significant changes at 8 and 16h post LPS in $S1PR1^{+}$ EC than control samples were subjected to IPA core analysis based on IPA annotation databases.

Western blotting: HLMVEC were lyzed using radioimmunoprecipitation assay buffer (RIPA buffer) of components 10mM Tris-HCl, (pH 8.0), 1mM EDTA, 0.5mM EGTA, 1% Triton X-100, 0.1% sodium

deoxycholate, 0.1% SDS, 140mM NaCl, 1mM phenylmethylsulfonyl fluoride (PMSF). Western blot experiments were performed using indicated antibodies as described previously.[26]

In situ cell death assay: Cell death was determined using in situ cell death detection (Roche, Cat. No. 11684795910) following manufacture's protocol. Briefly, lung sections from S1PR1-GFP reporter mice or control mice were subjected to protease treatment followed by permeabilization and labelling with Tunel reaction mixture as described previously.[5]

Promoter Analysis: Putative EGR1 and STAT3 binding sites were identified within the proximal 2 kb promoter of SPHK1 and SPNS2, respectively using Eukaryotic Promoter Database.[37]

Plasmid construct transfection and luciferase assay: HLMVEC were transfected with either empty vector or GeneCopoeia GLuc-ON^{TM} human SPHK1 or SPNS2 wild-type or mutated promoter luciferase reporter (GeneCopoeia, Rockville, MD, USA). The Gaussia luciferase (GLuc) and secreted alkaline phosphatase (SEAP) activity were determined using secrete-pair dual luminescence assay kit (Cat No. LF061, GeneCopoeia, Rockville, Maryland) using the manufacturer's protocol. GLuc activity was normalized taking SEAP activity as an internal control.

Chromatin immunoprecipitation (*ChIP*) assay: Protein-DNA complex (100-120μg) was immunoprecipitated with the antibody against EGR1 and STAT3 as described previously.[38,39] Briefly, Protein-DNA complex (100–120μg) was immunoprecipitated with the anti-EGR1 or anti-STAT3 antibodies. DNA fragments were collected by phenol– chloroform–isoamyl alcohol extraction, followed by ethanol precipitation, and then resuspended in 14μl nuclease water for PCR. Promoter region of SPHK1 and SPNS2 targeted a 152bp fragment was quantified by Sybr green-based real time quantitative PCR (q-PCR) using ViiA7 (Applied Biosystem, Foster City, CA). Normal rabbit IgG was used as negative antibody control and DNA from the input (20–40 μg protein-DNA complexes) was used as an internal control. Primers used to amplify the EGR1 binding site were forward 5′-GCATCTGGAAGCTAAAAATCTGGC-3′ reverse 5′-AGTCCTCGAGGGCATTCTACTG-3′, while for STAT3 binding site were 5′-TTCGTAATGGGTCACGTCCG-3′ reverse 5′-ATCCCATCACGGTTTCACCC-3′.

S1P analysis: S1P was quantified in S1PR1^{+} EC or Non-GFP EC (EC) (~0.5x10^{6}) sorted under same conditions using liquid chromatography-mass spectrometry (LC-MS) as described previously.[6] Briefly, cells were washed two times with PBS and were resuspended in PBS along with 1μM of S1P lyase inhibitor. S1P was determined in Mass Spectrometry Core Facility, UIC using AB SCIEX QTRAP 6500 coupled to Agilent UPLC/HPLC system. All samples were analyzed by Phenomenex Kintex C8 column (2.1 x100mm, 1.7um) coupled to an Agilent UPLC/HPLC system, which was operated at a flow rate of 350 uL/min. A gradient of buffer A (30% MeOH in H2O, 0.1% Formic acid) and buffer B (MeOH:ACN:IPA:FA, 4:1:1:0.1 v/v/v/v) was applied as: 0 min, 25% of buffer B; increase buffer B to 100% in 2 min; kept B at 100% for 4 min. The column was equilibrated for 3 min at 25% B between the injections. The column temperature was 45°C and the autosampler was kept at 4°C. The quantification was achieved using area of monitored transitions.

Bone marrow transplantation: Mice were sacrificed and femur and tibia were isolated and subsequently flushed with sterile PBS containing 1% FBS using 10ml syringe connected with 27 guage needle. Flushed bone marrow cells were centrifuged at 300Xg for 10 min and pellet was washed with PBS. Bone marrow cells were dissolved in 1ml of PBS and ~1.5x10^{6} cells were injected retro-orbitally into sublethally irradiated (10cG) WT mice as described.[31, 39,32] The mice were monitored for 2 weeks daily. After 5th week of bone marrow transplantation, mice were challenged with LPS (10mg/kg, *i.p.*) and lungs were harvested for FACS analysis and immunohistochemistry.

Transplantation of S1PR1^{+} EC: S1PR1-GFP reporter mice were challenged with LPS (10mg/kg body weight, *i.p.*) and at 16h lungs were dissected as described above to obtain single cell suspension. S1PR1^{+} EC (GFP^{+}CD31^{+}CD45^{-}) and EC (GFP^{-}CD31^{+}CD45^{-}) were flow sorted and ~1.5x10^{6} EC were injected retro-orbitally into *EC-S1PR1*$^{-/-}$ and *S1PR1*$^{fl/fl}$ mice. PBS was used as vehicle control.

Assessment of lung vascular injury: Mice were exposed to LPS (10mg/kg body weight, *i.p.*). Extravasation of Evans blue albumin and lung wet-dry weight ratio were determined to quantify lung vascular injury as described previously.[6,29,31]

Trans-endothelial electrical resistance (TEER): HLMVEC were seeded on gelatin coated gold-plated eight-well electrodes (8W standard Array; 8W10E+) (Applied Biosciences, Carlsbad, CA). Cells were transfected with indicated siRNAs for 48 h. The smaller electrode and larger counter electrode were then connected to a phase-sensitive lock-in amplifier to monitor the voltage. A constant current of 1 μA was supplied by a 1 V, 4000 Hz AC signal connected serially to a 1 MΩ resistor between the smaller electrode and the larger counter electrode. EC monolayers were incubated in serum-free medium for 2 h following which S1P or LPS was added to assess dynamic change in TEER.[29,38]

Blinding Procedure: While data collection could not be completely blinded, authors ensure that the outcomes measured are as objective as possible. All experiments were done multiple times for rigor and reproducibility. The FACS analysis and western blotting were done by operator1 while in the same animals operator2 performed lung physiological measurements without being informed of the status of the animal. Operator3 performed the experiments to validate SPHK1 antibody used without involvement of the first author. RNAseq analysis was performed by the Informatics core facility at the UIC which was verified by Operator1.

Statistical analysis: The statistical analyses were performed using GraphPad Prism version 8.0. We assume normality for all the tests relying on the central limit theorem. Normally distributed data were expressed as mean ±SD. Statistical significance for individual experiment is described in the figure legends with their significance values. Online Table II includes detailed information about statistical tests performed for each figure. A value of $p<0.05$ was considered statistically significant.

Table I: Primers List

Genes	Mouse
SPHK1	Forward - 5′-GGCAGTCATGTCCGGTGATG-3′ Reverse - 5′-ACAGCAGTGTGCAGTTGATGA-3′
EGR1	Forward - 5′-TTACCCGCCATATCCGCATC-3′ Reverse - 5′-TGCCTCTTGCGTTCATCACT-3′

SPNS2	Forward - 5′-GCTGCTGCCATCCTGAGTTT-3′ Reverse - 5′-CAGGTAGCCAAAGATGGGGG-3′
GAPDH	Forward - 5′-AAGGTCATCCCAGAGCTGAA- 3′ Reverse - 5′-CTGCTTCACCACCTTCTTGA- 3′
S1PR1	Forward - 5′-AGCTCAGGGAACTTTGCGAG- 3′ Reverse - 5′- GAGAAACAGCAGCCTCGCTC- 3′
S1PR2	Forward - 5′-CAACTCCGGGACATAGACCG- 3′ Reverse - 5′-CATGGGGCTGAGCACTGG- 3′
S1PR3	Forward - 5′-CCTTGCAGAACGAGAGCCTAT- 3′ Reverse - 5′-CCCGGAGAGTGTCATTTCCC- 3′
	Human
S1PR1	Forward - 5′-ACTCTGACCAACAAGGAGATGCGTAC-3′ Reverse - 5′-GGCGATGATGGGTCGCTTGAATTT-3′
STAT3	Forward - 5′-GGAGAAACAGGATGGCCCAA-3′ Reverse - 5′ -TGGCAGATTAACTCTCACCCAG-3′
SPHK1	Forward - 5′-GCTGCGAAGTTGAGCGAAAA-3′ Reverse - 5′-GGCTGGACCCAGTCGG-3′
SPNS2	Forward - 5′-TGTGAAAGTCTGAGGTGGTGC-3′ Reverse - 5′-CAAACCTAGGGCTGGGAACC-3′
EGR1	Forward - 5′-CACCTGACCGCAGAGTCTTTT-3′ Reverse - 5′-GGCCAGTATAGGTGATGGGG-3′
SPHK2	Forward - 5′-CCCAGTGTTGGAGAGCTGAA-3′ Reverse - 5′-TGCTCCTCTGCTTCAAGGTG-3′
GAPDH	Forward - 5′-AATGGGCAGCCGTTAGGAAA-3′ Reverse - 5′-GCCCAATACGACCAAATCAGAG-3′

Online Table II: Statistical assessment

Figure	**Statistical test**	**Post hoc correction**	***p*-values**
1C	Two tailed, Unpaired t test	N/A	Raw
1D	Two tailed, Unpaired t test	N/A	Raw
1E	Two tailed, Unpaired t test	Welch’s correction	Raw
1F	Two tailed, Unpaired t test	Welch’s correction	Raw
2B	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
2C	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted

2E	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
3B	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
3D	Two tailed, Unpaired t test	N/A	Raw
4B	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
4D	Two tailed, Unpaired t test	N/A	Raw
4E	Two tailed, Unpaired t test	N/A	Raw
4F	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
4H	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
5A	Two tailed, Unpaired t test	N/A	Raw
5C	Two tailed, Unpaired t test	N/A	Raw
5D	Two tailed, Unpaired t test	N/A	Raw
5F	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
5H	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
5J	Two tailed, Unpaired t test	N/A	Raw
5K	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
6B	Two tailed, Unpaired t test	N/A	Raw
6C	Two tailed, Unpaired t test	N/A	Raw
6E	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
6G	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
7A	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
7C	Two tailed, Unpaired t test	N/A	Raw
8B	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
8D	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
8E	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted

Online ID	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online IVB	Two tailed, Unpaired t test	N/A	Raw
Online VIA	Repeated measures one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online VIC	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online VID	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online VIG	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online VII	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online VIJ	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online VIK	Repeated measures one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online VIL	Repeated measures one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online VIIB	Ordinary one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online VIIC	Repeated measures one-way ANOVA	Tukey, Bartlett's statistic	Adjusted
Online VIID	Repeated measures one-way ANOVA	Tukey, Bartlett's statistic	Adjusted

Table III. Inhibitors/chemicals solubility

S. No	Inhibitors/Chemicals	Cat. No.	Solubility
1	Sphingosine kinase 1 (SPHK1) inhibitor, PF-543	1415562-82-1 (Cayman Chemical)	DMSO (Sigma Aldrich, Cat. No. D2650)
2	Sphingosine kinase 2 (SPHK2) inhibitor, ABC294640 (Cat No.	B-0025 (Echelon Biosciences)	DMSO
3	STAT3 inhibitor, S3I-201	SML0330 (MilliporeSigma)	DMSO
4	Specific ERK inhibitor, SCH 772984	19166 (Cayman Chemical)	DMSO

5	CYM 5442	16925 (Cayman Chemical)	DMSO

Legends

Online Figure I-VII

Online Figure I: S1PR1 agonists rapidly activate S1PR1. A, Schematics of generation of S1PR1-GFP reporter mice. In the S1PR1^{knockin} reporter mouse, S1PR1 is fused with two fusion proteins, namely, a tetracycline-regulated transactivator (tTA) and tobacco etch virus (TEV) protease along with β-arrestin. The S1PR1^{knockin} reporter mouse is bred with H2B-GFP mouse to create the S1PR1-GFP reporter mouse where GFP expression reports S1PR1 activity. **B-D,** S1PR1-GFP reporter mice received either S1P (1mg/kg, *i.v.* **B**) or CYM-5442 (2.5 mg/kg; *i.p.* **C**) at indicated times, FACS analysis was performed in lung single cell suspension to quantify GFP$^+$ cells. **B-C** show the corresponding representative scatter dot plot of GFP$^+$ gated cells, while **D** shows quantification of GFP$^+$ cells in lungs of these mice at indicated time points calculated as percent of total lung cell population at each time (n=5 mice/group). **D** shows individual values with mean ±SD. Data were analyzed using one-way ANOVA followed by Post hoc Tukey's multiple comparisons test (See also Online Table II). D, p=0.016, p=5.79E-13, p=5.18E-13 (S1P); and p=5.66E-06, p=2.65E-09, p=1.51E-12, p=4.71E-13 (CYM-5442) indicate significance relative to uninjected mice. ns=not significant.

Online Figure II: Assessment of S1PR1 activation in lung endothelial cells. A, Gating strategy for analyzing S1PR1$^+$ EC (CD31$^+$CD45$^-$GFP$^+$). Lung cells were first gated for forward and side scatter to eliminate doublets. Cell population was then delineated for CD31$^+$CD45$^-$ to select all endothelial cells and then demarcated with GFP to assess S1PR1$^+$ EC. **B**, **C and D**, Single cell suspension obtained from lungs of S1PR1-GFP reporter mice following 16h LPS (10 mg/kg, *i.p.*) injection were immunostained with indicated antibodies after which FACS analysis was performed. A representative dot plot is shown (n=5 mice/group).

Online Figure III: S1PR1 activation occurs primarily in lung endothelial cells. A, FACS dot plot shows GFP expression in bone marrow cells isolated from S1PR1-GFP reporter mice (n=5 mice/group). **B**, Workflow depicts S1PR1-GFP reporter bone marrow transplantation into WT mice. Bone marrow cells

were isolated from S1PR1-GFP reporter mice and transplanted into sub-lethally irradiated WT mice. Five weeks later, these mice were challenged with LPS (10 mg/kg, *i.p.*) and FACS analysis was performed after 16h. **C-D**, FACS dot plot shows GFP^+ cells (*n*=7 mice/group) while, **D** shows immuno-histochemical detection of GFP^+ cells in lung sections of transplanted mice lungs after LPS exposure (n=3). Scale bar 50µm.

Online Figure IV: The generated $S1PR1^+$ endothelial cells show proliferative phenotype and $S1PR1^+$ EC do not undergo apoptosis. A, Endothelial cells were gated as $CD31^+CD45^-$, which were further delineated on GFP to assess $S1PR1^+$ EC. Next, $S1PR1^+$ EC were demarcated as $Ki\text{-}67^+$ cells to assess proliferating EC. **B**, quantification of $S1PR1^+$ EC proliferation using $CD31^+CD45^-Ki\text{-}67^+$ antibodies after without or with LPS challenge (10 mg/kg,*i.p*) (*n*=5 mice/group). Plot shows individual data with mean ±SD. Statistical significance was assessed by unpaired t test for **B** (See also Online Table II). *p*=0.0001, indicate significance relative to "No LPS". **C**, Immuno-histochemical analysis of TUNEL assay in lung sections of S1PR-GFP reporter mice post-LPS administration (10mg/kg, *i.p.*) (*n*=5 mice/group). Scale bar 50 µm. Arrowhead indicates TUNEL +ve cells, while the inset shows GFP expressing cell are TUNEL-ve. **D**, heatmap of differentially expressed transcriptomes in EC transitioning from $S1PR1^{lo}$ EC to $S1PR1^+$ EC. $S1PR1^+$ EC were flow sorted from unexposed or LPS administered (10mg/kg, *i.p*) S1PR1-GFP reporter mice at indicated time points and RNA-seq analysis was performed to generate heatmap.

Online Figure V: Pathway analysis in $S1PR1^+$ lung endothelial cells. A, Gene Ontology biological process terms show significantly enriched pathway with upregulated genes in $S1PR1^+$ EC versus $S1PR1^{lo}$ EC. The graph shows upregulated genes in each functional category. **B**, Ingenuity Pathway Analysis identified a major protein network associated with reparative signaling. Red and green symbols indicate upregulated and downregulated, respectively. Direct interaction is represented as solid lines, whereas indirect interactions appear as dotted lines. **C**, Volcano plot of transcriptomes in $S1PR1^+$ EC post without or with 8h LPS administration (10mg/kg, *i.p.*). **D-E,** HLMVEC transfected with control (siSC) or SPHK1

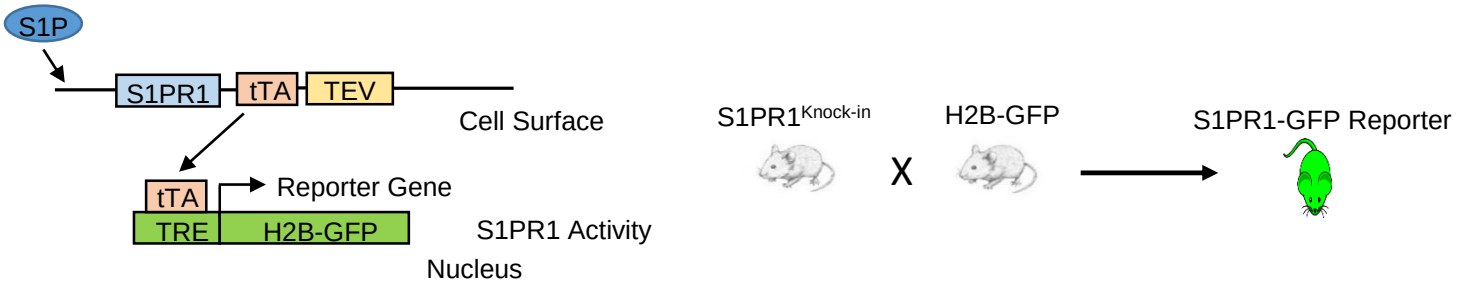
siRNA (siSPHK1) were lysed after 72h of transfection. Lysates were immunoblotted using anti-SPHK1 antibody. The experiment was repeated three times independently. Actin was used as loading control.

Online Figure VI: EGR1 and STAT3 induction of S1PR1^{+} lung endothelial cell generation and enhanced endothelial barrier function. A, HLMVEC plated on transendothelial electrical resistance (TEER) electrodes were transfected with scrambled (siSC) or EGR1 siRNA (siEGR1) for 48h followed by stimulation with 1μM of S1P. TEER was determined in triplicate/condition to assess endothelial barrier function in real time. The assay was repeated three times independently. **B-D**, Immunoblot shows ERK and STAT3 phosphorylation in HLMVEC post LPS (1□g/ml) stimulation. A representative blot is shown from three independent experiments. **C-D** show densitometry of phosphorylated proteins expressed as fold increase over total proteins. **E,** Immunoblot shows changes in phosphorylation of STAT proteins in flow sorted S1PR1^{+} EC from lung of S1PR1-GFP reporter mice post 16h LPS (10 mg/kg, *i.p.*) stimulation. A representative immunoblot from three different experiments is shown. Actin was used as a loading control. **F-G,** Immunoblot showing STAT3 phosphorylation in HLMVEC pretreated without or with ERK inhibitor, SCH772984 and LPS treatment. HLMVEC were pretreated with 5μM of SCH772984 for 1h before stimulating with LPS (1μg/ml) for 8h. A representative immunoblot is shown from three independent experiments. **G** shows densitometry of P-STAT3 expressed as fold increase over total protein. **H-J,** S1PR1 depleted HLMVEC were stimulated with or without LPS (1μg/ml) and after 8h, phosphorylation of ERK and STAT3 was determined (n=3). **I and J**, densitometric analyses of ERK and STAT3 phosphorylation calculated as a fold increase over ERK or STAT3 total proteins. **K-L**, HLMVEC plated on TEER electrodes were transfected with scrambled (siSC) or STAT3 siRNA (siSTAT3) **(K)** or SPNS2 siRNA (siSPNS2) **(L)** for 48h, and subsequently, stimulated with 1μM of S1P. TEER was determined in triplicate/condition to assess endothelial barrier function in real time. The assay was repeated three times independently. **A**, **C, D, G, I, J, K and L** show mean ±SD. Data in plots **C, D, G, I and J** were analyzed using one-way ANOVA followed by Post hocTukey's multiple comparisons test, whereas, repeated measures one-way ANOVA and Post hoc hocTukey's multiple comparisons test were used for **A, K and L** (See also Online Table II).

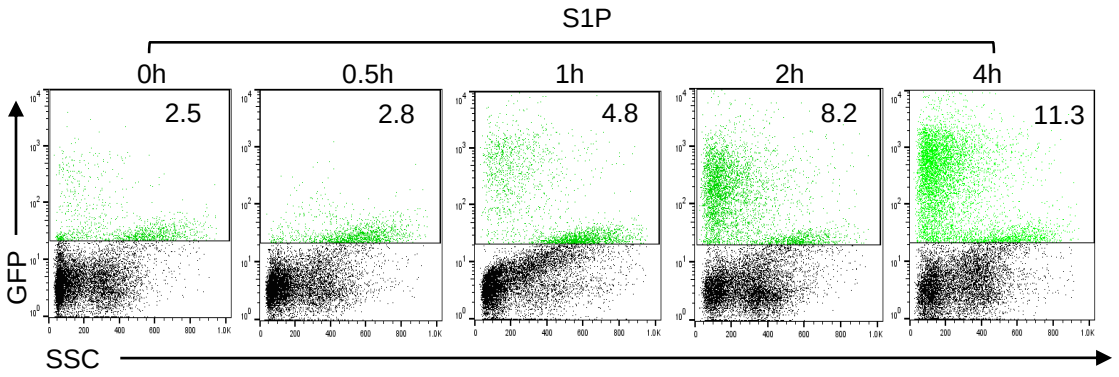
A, *p*=3.8E-06 indicates significance relative to siSC; C, *p*=0.0181, *p*=5.63E-07, *p*=2.1E-07, *p*=1.56E-07; D, *p*=0.0213, *p*=3.31E-06, *p*=1.17E-06, *p*=1.77-05 indicate significance relative to 0h LPS. G, *p*=0.0006 indicates significance relative to –LPS while *p*=0.0003 indicates significance relative to –LPS plus SCH772984; I, *p*=0.0004; J, *p*=0.0008 indicate significance relative to –LPS. K, *p*=2.24E-06, and L, *p*=2.36E-07 indicate significance relative to siSC. ns=not significant.

Online Figure VII: S1PR1^{+} lung endothelial cell generation is independent of SPHK2. A and B, GFP-gated EC (GFP^{+}CD31^{+}CD45^{-}) were analyzed from lungs of S1PR1-GFP reporter mice receiving SPHK2 inhibitor, ABC294640 (5mg/kg, *i.p*) and LPS (1h after SPHK2 inhibitor, 10 mg/kg, *i.p.*) administration. A representative FACS plot at indicated time points is shown in **A**, while quantification of GFP^{+}CD31^{+}CD45^{-} (S1PR1^{+} EC) as a percentage of total EC population (CD31^{+}CD45^{-}) in the lung at each time post injury is shown in **B** (n=5 mice/group). **C,** HLMVEC pretreated with SPHK1 inhibitor (1μM of PF-543), and STAT3 inhibitor (5μM of S3I-201) were stimulated with LPS (1□g/ml). TEER was determined in triplicate/condition to assess endothelial barrier function in real time. The assay was repeated three times. **D,** HLMVEC, plated on TEER electrodes were transfected with scrambled or SPNS2 siRNA for 48h, and subsequently, stimulated with 1μg/ml LPS. TEER was determined to assess endothelial barrier function in real time. The assay was repeated three times independently. Data are shown as mean ±SD. Data in **C and D** were analyzed using repeated measures one-way ANOVA and Post hoc Tukey's multiple comparisons test whereas one-way ANOVA followed by Tukey's multiple comparisons test was used for **B** (See also Online Table II). C, *p*=0.0005, *p*=5.14E-07, *p*=7.9E-07 indicate significance relative to No LPS; D, *p*=0.0003 and *p*=2.46E-05 indicate significance relative to values before LPS addition (arrow). ns=not significant.

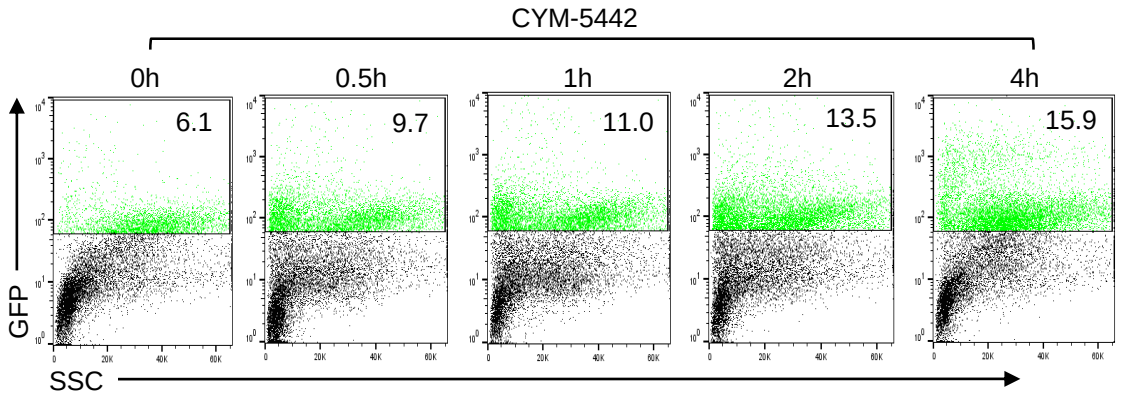
A


S1P
S1PR1
tTA
TEV
Cell Surface
tTA
Reporter Gene
TRE
H2B-GFP
S1PR1 Activity
Nucleus
S1PR1Knock-in
H2B-GFP
X
S1PR1-GFP Reporter

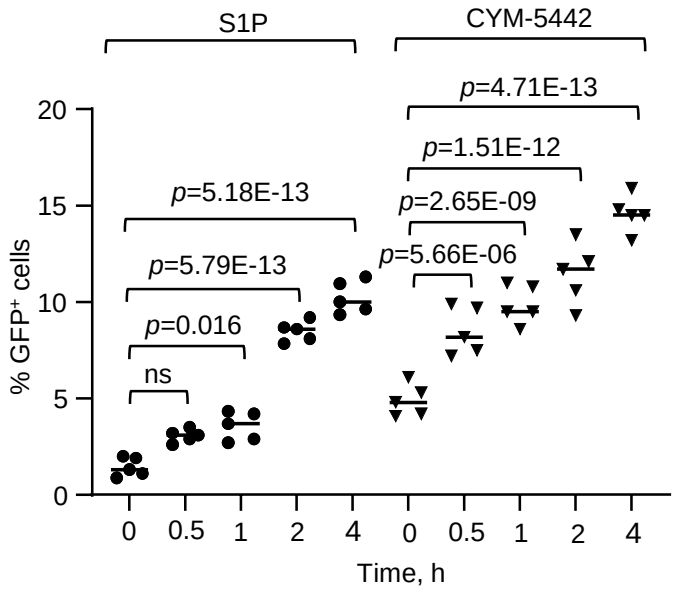
B


S1P
0h
0.5h
1h
2h
4h
2.5
2.8
4.8
8.2
11.3
GFP
SSC

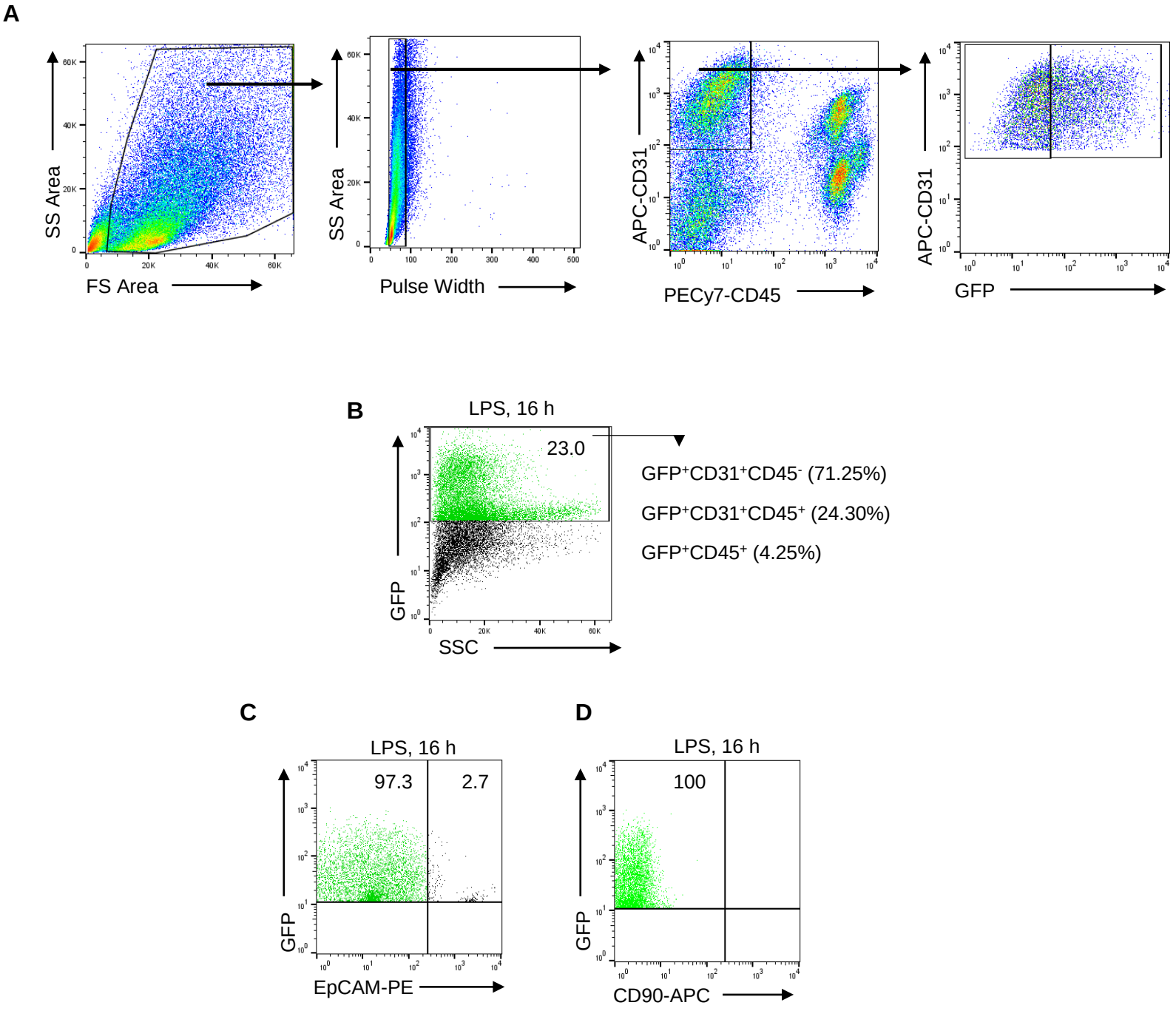
C


CYM-5442
0h
0.5h
1h
2h
4h
6.1
9.7
11.0
13.5
15.9
GFP
SSC

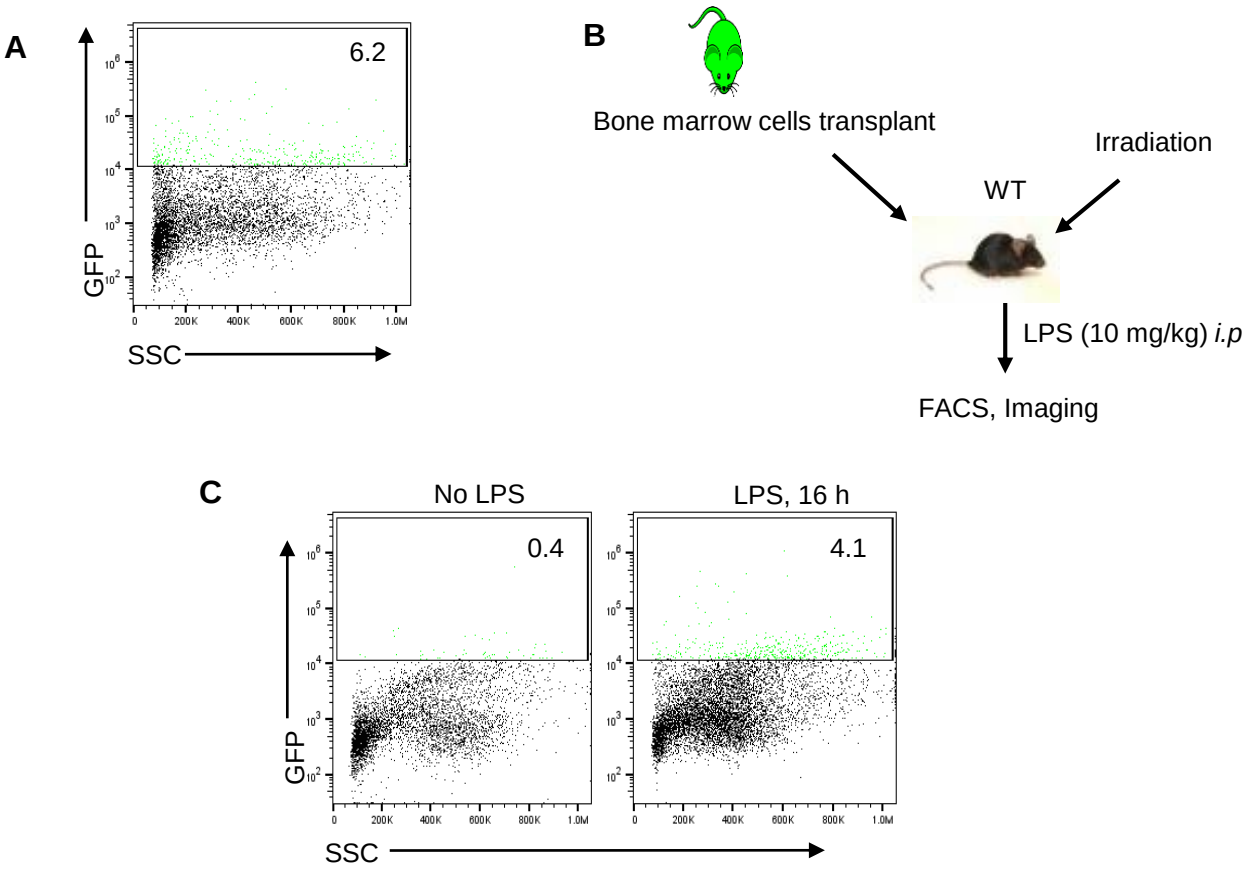
D

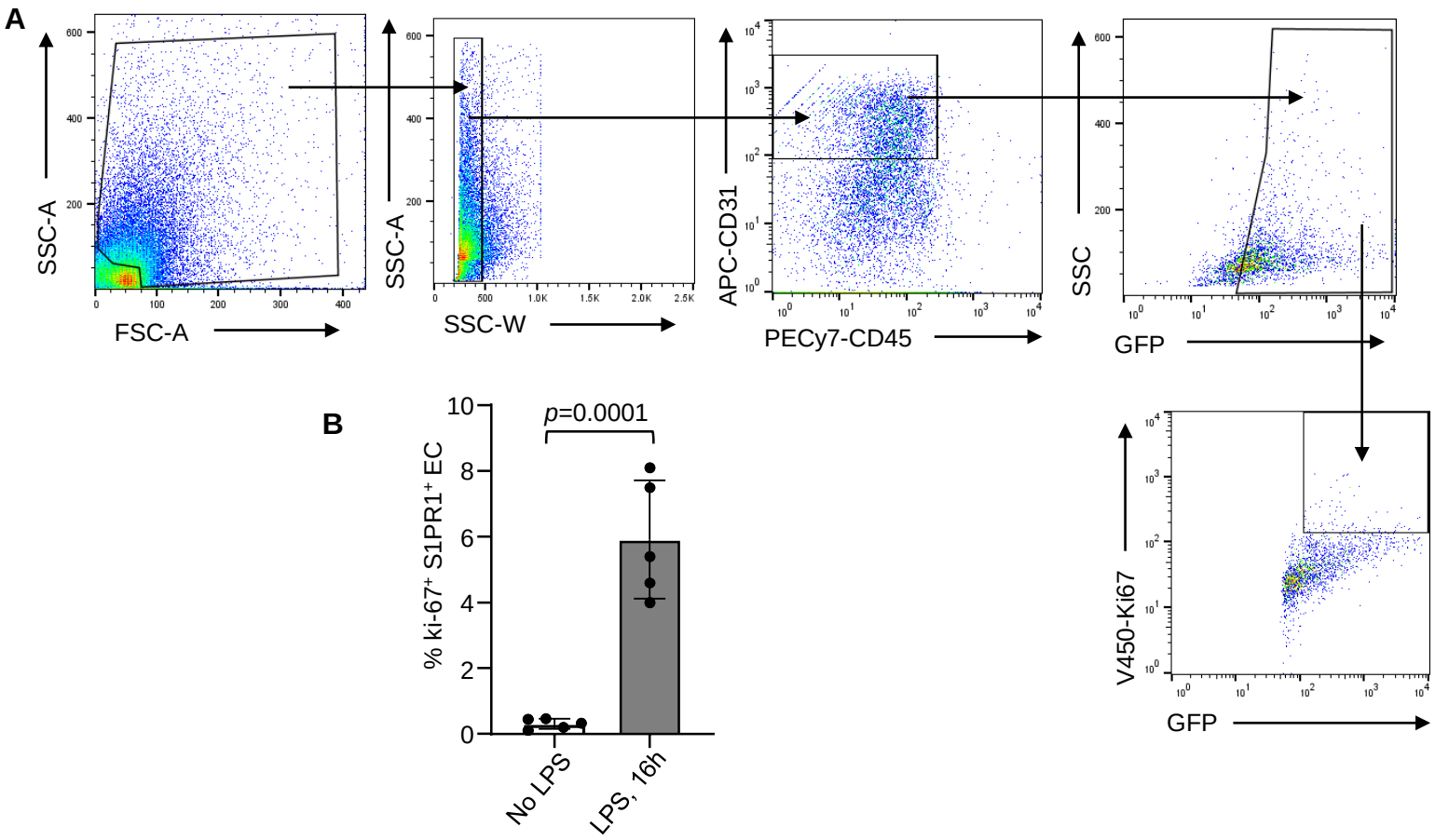

S1P
CYM-5442
p=4.71E-13
p=1.51E-12
p=5.18E-13
p=2.65E-09
p=5.66E-06
p=5.79E-13
p=0.016
ns
% GFP+ cells
20
15
10
5
0
0 0.5 1 2 4 0 0.5 1 2 4
Time, h

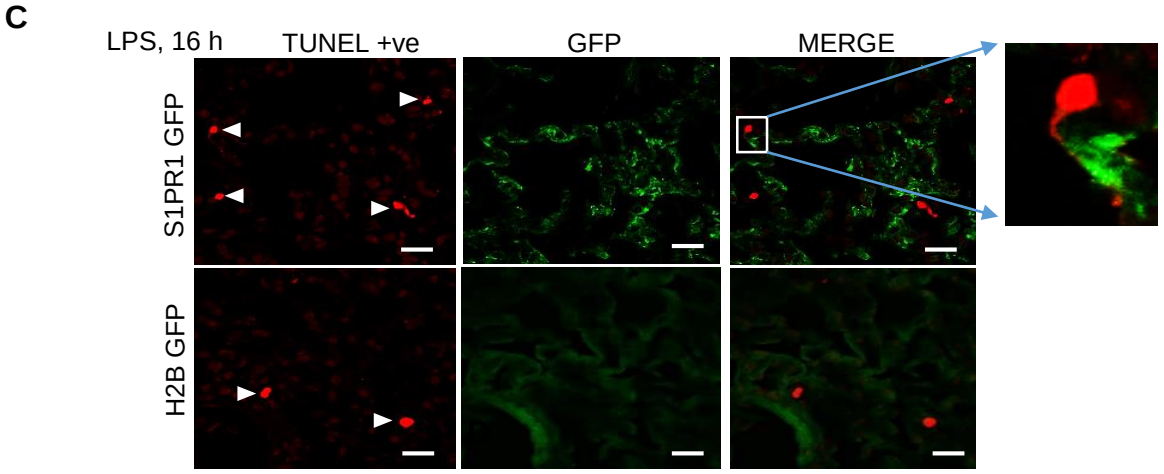
Online Figure II

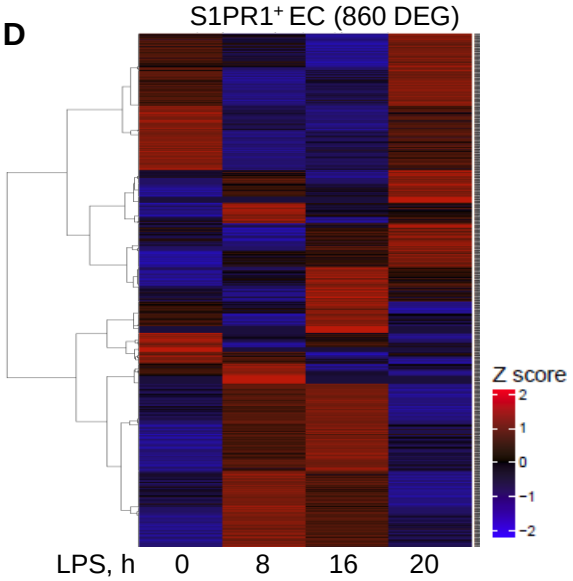


Online Figure III



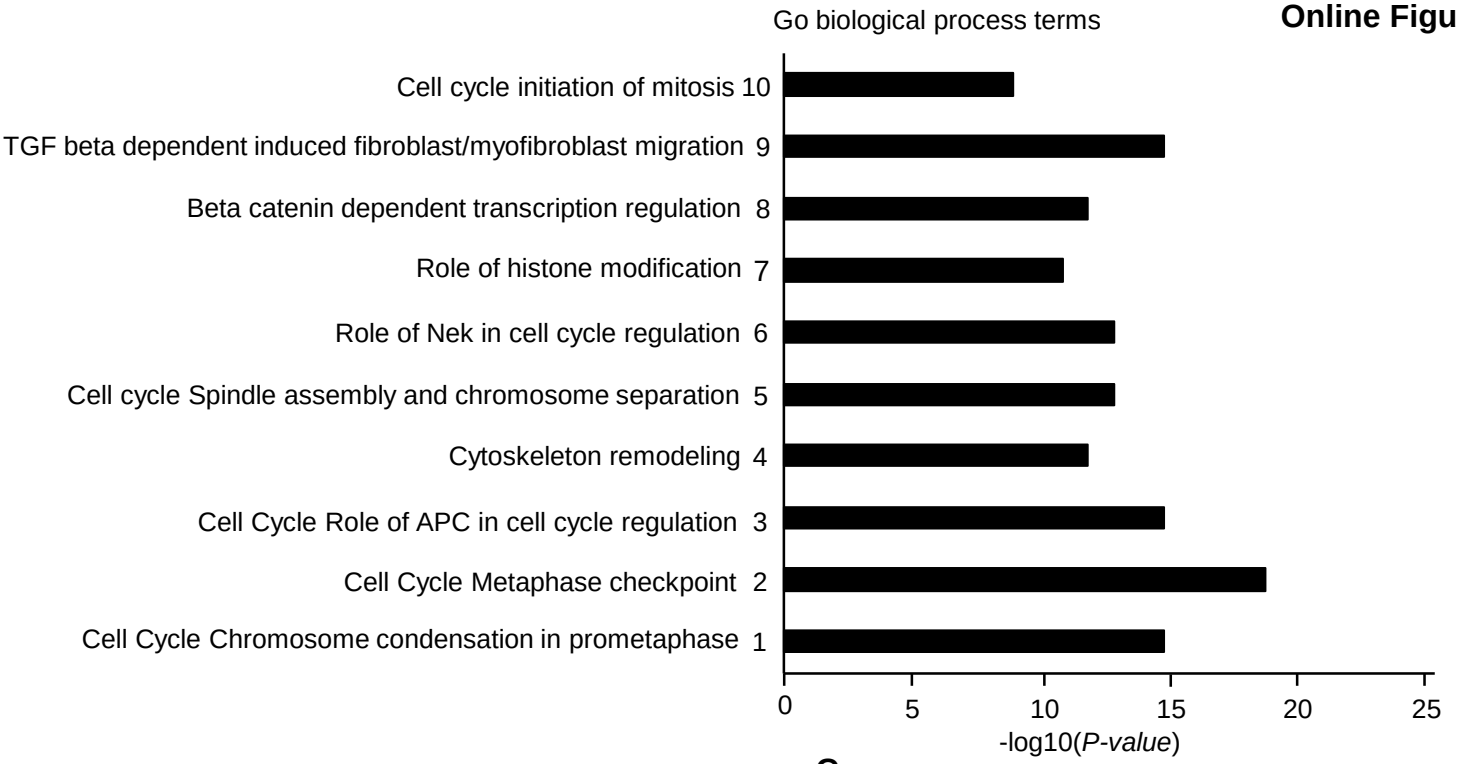

A
SSC-A
FSC-A
SSC-A
SSC-W
APC-CD31
PECy7-CD45
SSC
GFP
V450-Ki67
GFP
B
p=0.0001
% ki-67+ S1PR1+ EC
No LPS
LPS, 16h


C
LPS, 16 h
TUNEL +ve
GFP
MERGE
S1PR1 GFP
H2B GFP

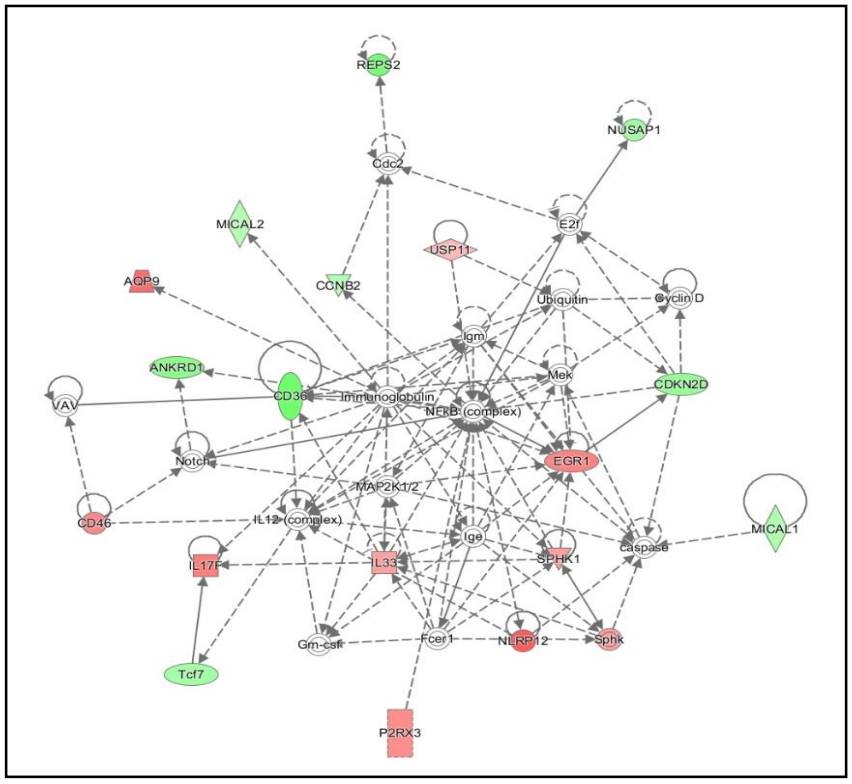

D
S1PR1+ EC (860 DEG)
Z score
LPS, h 0 8 16 20

Online Figure V

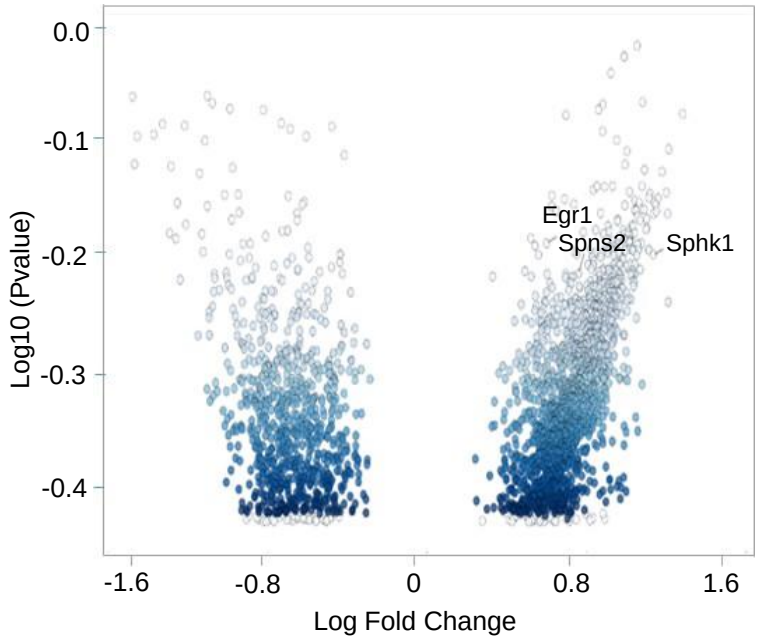
A



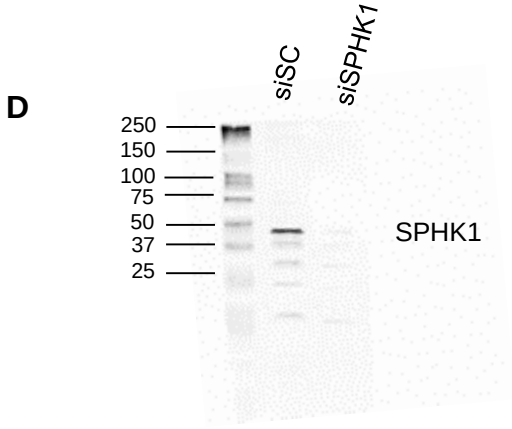
B



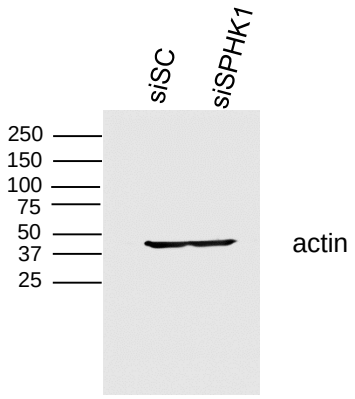
C

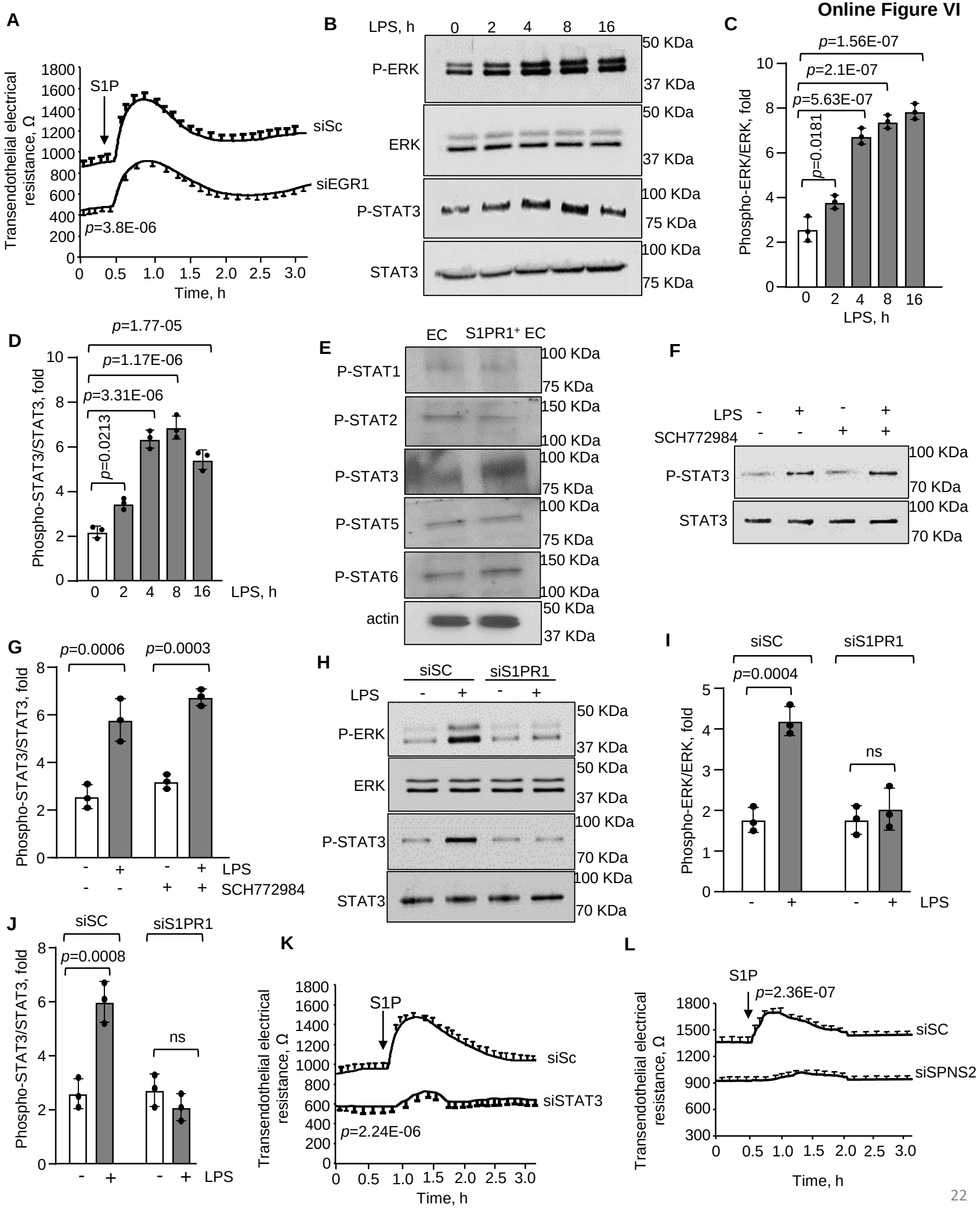


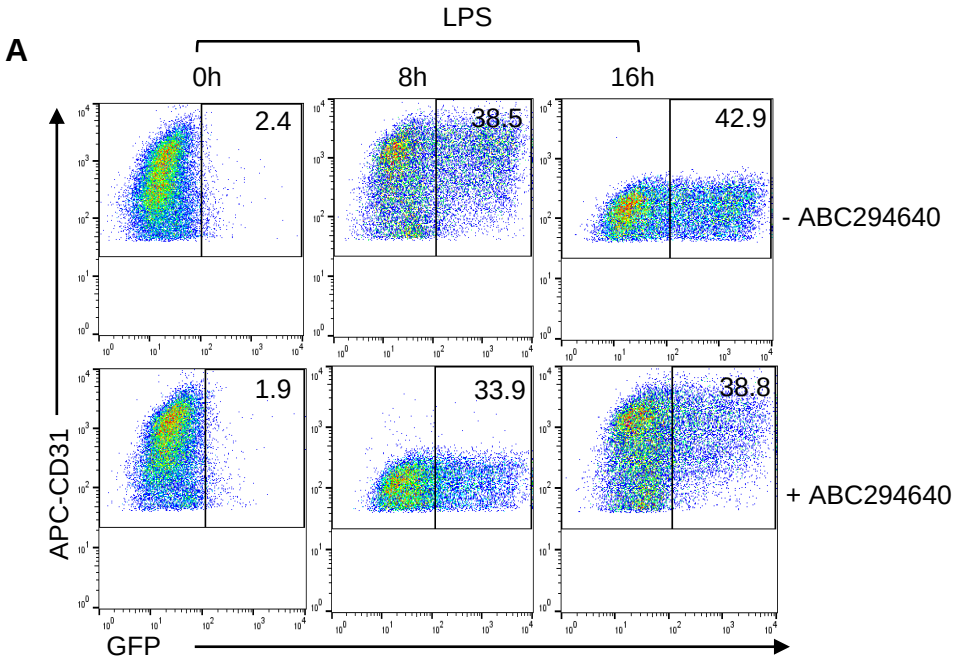
D

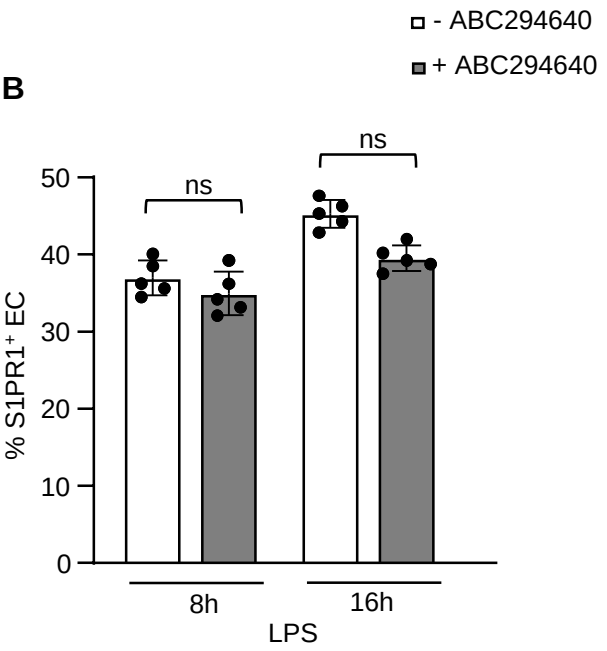


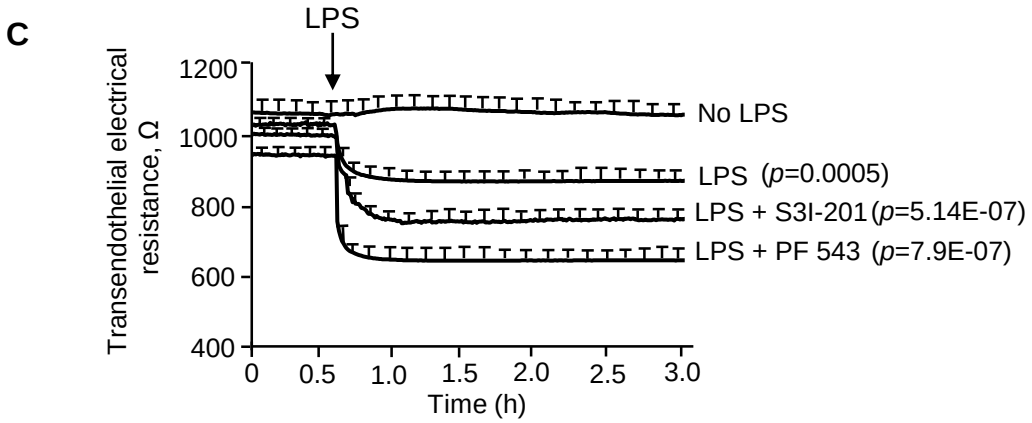
E

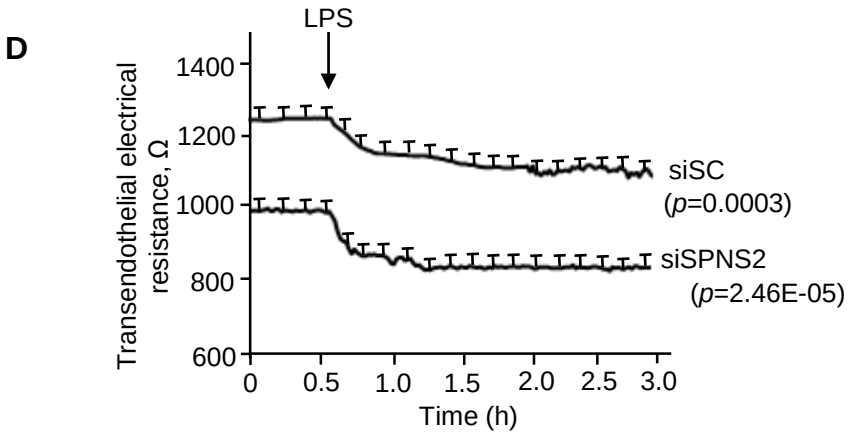



A
Transendothelial electrical resistance, Ω
S1P
siSc
siEGR1
p=3.8E-06
Time, h
B
LPS, h
0 2 4 8 16
P-ERK
ERK
P-STAT3
STAT3
50 KDa
37 KDa
100 KDa
75 KDa
C
Phospho-ERK/ERK, fold
p=1.56E-07
p=2.1E-07
p=5.63E-07
p=0.0181
LPS, h
D
Phospho-STAT3/STAT3, fold
p=1.77-05
p=1.17E-06
p=3.31E-06
p=0.0213
LPS, h
E
EC
S1PR1+ EC
P-STAT1
P-STAT2
P-STAT3
P-STAT5
P-STAT6
actin
100 KDa
75 KDa
150 KDa
50 KDa
37 KDa
F
LPS
SCH772984
P-STAT3
STAT3
100 KDa
70 KDa
G
Phospho-STAT3/STAT3, fold
p=0.0006
p=0.0003
LPS
SCH772984
H
siSC
siS1PR1
LPS
P-ERK
ERK
P-STAT3
STAT3
50 KDa
37 KDa
100 KDa
70 KDa
I
siSC
siS1PR1
Phospho-ERK/ERK, fold
p=0.0004
ns
LPS
J
siSC
siS1PR1
Phospho-STAT3/STAT3, fold
p=0.0008
ns
LPS
K
Transendothelial electrical resistance, Ω
S1P
siSc
siSTAT3
p=2.24E-06
Time, h
L
Transendothelial electrical resistance, Ω
S1P
p=2.36E-07
siSC
siSPNS2
Time, h


A
LPS
0h
8h
16h
2.4
38.5
42.9
1.9
33.9
38.8
- ABC294640
+ ABC294640
APC-CD31
GFP


B
- ABC294640
+ ABC294640
% S1PR1+ EC
ns
ns
8h
16h
LPS


C
LPS
Transendothelial electrical resistance, Ω
No LPS
LPS (p=0.0005)
LPS + S3I-201 (p=5.14E-07)
LPS + PF 543 (p=7.9E-07)
Time (h)


D
LPS
Transendothelial electrical resistance, Ω
siSC
(p=0.0003)
siSPNS2
(p=2.46E-05)
Time (h)