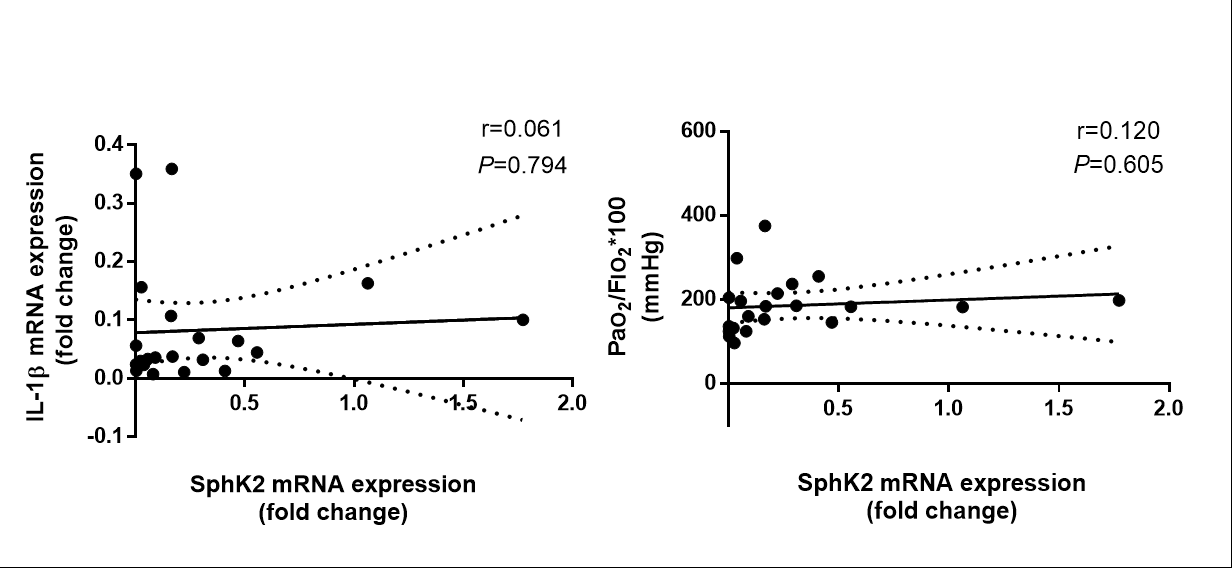
**Supplemental results**

**A B**

**Figure S1. *SphKs* mRNA expression in peripheral blood mononuclear cells of septic patients and non-septic patients.** *SphK1* (**A**) and *SphK2* (**B**) mRNA expression in peripheral blood mononuclear cells of septic patients (n=4) and non-septic patients (n=4) were assessed by real-time quantitative polymerase chain reaction. Dots represent data from individual subjects. Data are presented as means±SD with Mann-Whitney test*.* \**P<0.05.*

**A B**



**Figure S2. *SphK2* mRNA expression does not correlate with *IL-1 β* mRNA expression of peripheral blood mononuclear cells or** **PaO2/FIO2 ratios in septic patients.** Correlation of *SphK2* mRNA expression with *IL-1β* mRNA expression in peripheral blood mononuclear cells (**A**) and with PaO2/FIO2 ratios (**B**) in septic patients (n=21). Data were analyzed with linear regression analysis with a 95% confidence interval.



**Figure S3. Inhibition of SphK1 improves lung injury in septic mice.** Mice were subjected to sham surgery or cecal ligation and puncture. Immediately after surgery, mice were intraperitoneally injected with PF-543 (10mg/kg) or control. Lung tissues were harvested 24 hours after surgery for Hematoxylin and Eosin staining. Scare bar: 200um.

**A B**



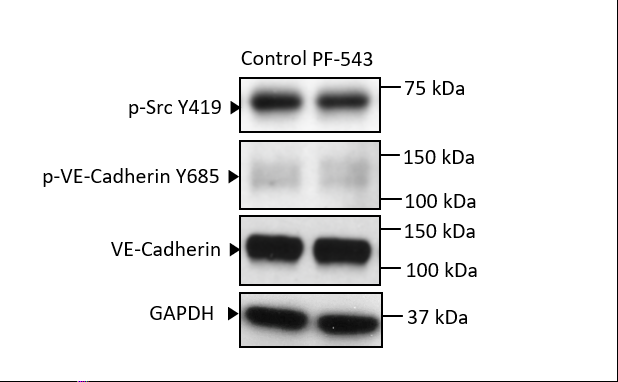
**Figure S4. *SphK1* deletionsuppresses NLRP3 inflammasome activation in macrophages.** Wild-type and *SphK1-/-* macrophages were primed with lipopolysaccharide (LPS, 1ug/ml) for 3 hours, and subsequently incubated with adenosine triphosphate (ATP, 5mM) for 30 min. (**A**) Representative immunoblot of caspase-1 p20 and IL-1β in cell lysates. (**B**) IL-1β release in supernatants was measured by enzyme linked immunosorbent assay. Data are presented as means±SD with Student‘s *t* test (n=3)*.* \*\*\**P<0.001.*

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**Figure S5. *SphK1*deletion inhibits NLRP3 inflammasome activation in lungs of septic mice.** Lung tissues were harvested from wild type and *SphK1-/-* mice 24 hours after surgery. Immunoblot of caspase-1 activation and IL-1β maturation in whole lung lysates.



**Figure S6. *SphK1* deletion improves lung injury in septic mice.** *SphK1-/-* and wild-type mice were subjected to sham surgery or cecal ligation and puncture. Lung tissues were harvested 24 hours after surgery for Hematoxylin and Eosin staining. Scare bar: 200um.



**Figure S7. PF-543 does not affect VE-Cadherin and Src activation in endothelial cells.** Human lung microvascular endothelial cells were incubated with PF-543 or control for 24 hours. The cell lysates were immunoblotted with antibodies as indicated.



**Figure S8. IL-1β induces VE-Cadherin internalization on endothelial cells.** Human lung microvascular endothelial cells were challenged with recombinant human IL-1β (200ng/ml) or control for 24 hours. Cell membrane fraction and intracellular fraction were immunoblotted with VE-Cadherin.

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**Figure S9. Inhibition of sphingosine kinase 1 attenuates sepsis-induced microvascular leakage via inhibiting macrophage NLRP3 inflammasome activation.** NLRP3 inflammasome activation includes initial transcriptional upregulation of NLRP3 and pro-IL-β via stimulation of Toll-like receptor by lipopolysaccharide (LPS), and secondary activation of NLPR3 by damage-associated molecular patterns such as ATP, resulting in activation of caspase-1, which cleaves pro-IL-1β into bioactive pro-inflammatory mediator. Cleaved IL-1β released from macrophages activates Src in endothelial cells. VE-Cadherin in endothelial cell membrane is phosphorylated by activated Src and internalized into cytosol, leading to disassembly of adherens junctions and increased vascular permeability. Selective inhibition of SphK1 with PF-543 suppresses NLRP3 inflammasome activation, sequentially blocks release of IL-1β from macrophages, and thereby improves sepsis-induced microvascular leakage.

**Table S1. Primers**

|  |  |  |
| --- | --- | --- |
| Gene | Forward Primer | Reverse Primer |
| GAPDH | 5’- GGAGCGAGATCCCTCCAAAAT -3’ | 5’- GGCTGTTGTCATACTTCTCATGG -3’ |
| SPHK1 | 5’- GGCTGCTGTCACCCATGAA-3’ | 5’-TCACTCTCTAGGTCCACATCAG-3’ |
| SPHK2 | 5’- AGCGTGGTAGCCACTTCAG-3’ | 5’- GAGCAGTGTACCGATGCCA-3’ |
| IL-1β | 5’- ATGATGGCTTATTACAGTGGCAA-3’ | 5’-GTCGGAGATTCGTAGCTGGA-3’ |

**Table S2. Key Reagents and Antibodies**

|  |  |  |
| --- | --- | --- |
| Reagents or Antibodies | Manufacturer | Model Number |
| Reagents | | |
| Ficoll-Hypaque | GE Healthcare | 17544202 |
| TRIzol reagent | Invitrogen | 15596026 |
| Reverse Transcription Master Mix | EZbioscience | A0010 |
| SYBR Green qPCR Master Mix | EZbioscience | A0001 |
| Evan Blue Dye | Sigma | E2129-10G |
| PF543 | Sigma | PZ0234-5MG |
| ATP | Sigma | A2383-1G |
| Recombinant human IL-1β | Sino Biological | 10139-HNAE |
| Protein A/G PLUS Agarose beards | Santa Cruz | sc-2003 |
| Primary antibodies | | |
| anti-NLRP3 | AdipoGen | AG-20B-0014-C100 |
| anti-Caspase-1 (p20) | AdipoGen | AG-20B-0042-C100 |
| anti-IL-1β | R&D systems | AF-401-NA |
| anti-VE-Cadherin | Santa Cruz | sc6458 |
| anti-p-VE-Cadherin Y685 | Abcam | ab119785 |
| anti-c-Src Tyr416 | Cell signaling | 2109S |
| anti-p-Src | Cell Signaling | 6943S |
| anti-Na+-K+ATPase | Abcam | ab58475 |
| anti-β-actin | Santa Cruz | sc47778 |
| anti-GAPDH | Proteintech | 60004 |

**Table S3. Clinical Characteristics of Septic Patients**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Patients  number | Sex | Age | SOFA  24h | PaO2/FiO2 ratio | Site of infection | Diagnosis |
| 1 | Male | 31 | 7 | 145 | Acute suppurative appendicitis | Septic shock |
| 2 | Female | 70 | 4 | 184 | Acute cholangitis | Sepsis |
| 3 | Male | 46 | 4 | 237 | Acute peritonitis | Sepsis |
| 4 | Male | 61 | 12 | 255 | Acute peritonitis | Sepsis |
| 5 | Male | 77 | 11 | 375 | Acute peritonitis | Sepsis |
| 6 | Male | 67 | 17 | 124 | Acute suppurative cholangitis | Septic shock |
| 7 | Male | 68 | 4 | 185 | Pyothorax | Sepsis |
| 8 | Male | 52 | 5 | 182.5 | Pyothorax | Sepsis |
| 9 | Male | 72 | 5 | 182 | Pyothorax | Sepsis |
| 10 | Male | 68 | 11 | 160 | Acute peritonitis | Sepsis |
| 11 | Male | 73 | 5 | 124 | Pyothorax | Sepsis |
| 12 | Male | 64 | 11 | 136 | Pyothorax | Sepsis |
| 13 | Male | 68 | 5 | 204 | Pyothorax | Sepsis |
| 14 | Male | 66 | 5 | 153 | Peumonia | Sepsis |
| 15 | Male | 71 | 4 | 112 | Pyothorax | Sepsis |
| 16 | Male | 19 | 4 | 196 | Peumonia | Sepsis |
| 17 | Female | 34 | 5 | 298 | Acute cholangitis | Sepsis |
| 18 | Male | 59 | 4 | 214 | Peumonia | Sepsis |
| 19 | Male | 61 | 3 | 132 | Pyothorax | Sepsis |
| 20 | Male | 77 | 11 | 96 | Acute peritonitis | Sepsis |
| 21 | Female | 73 | 10 | 198 | Acute peritonitis | Septic shock |