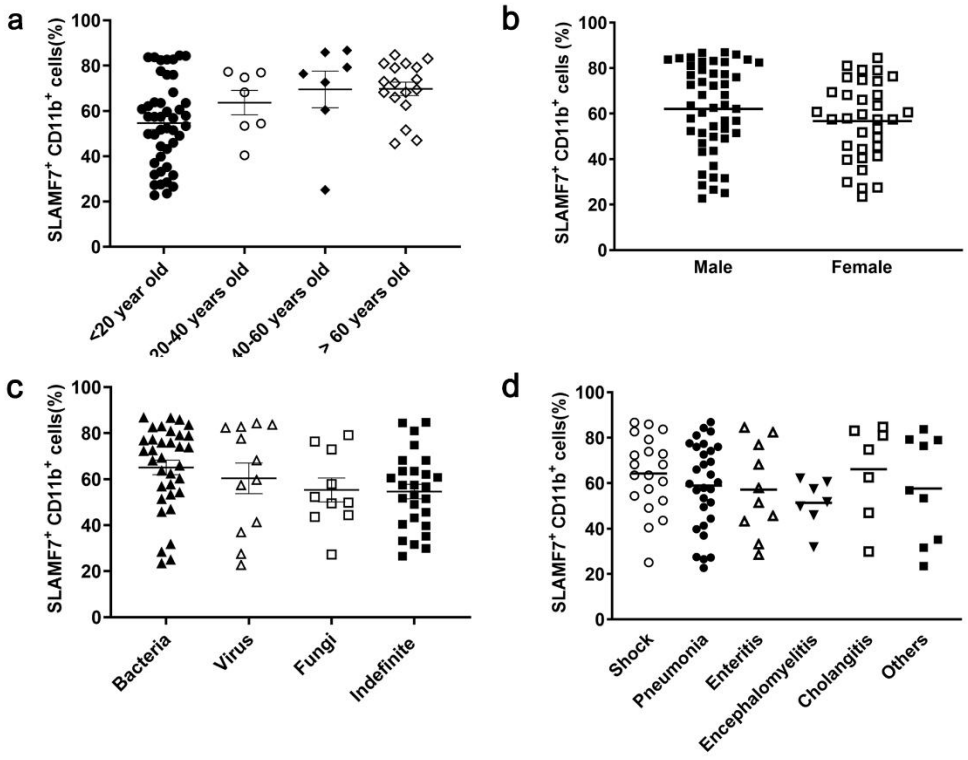
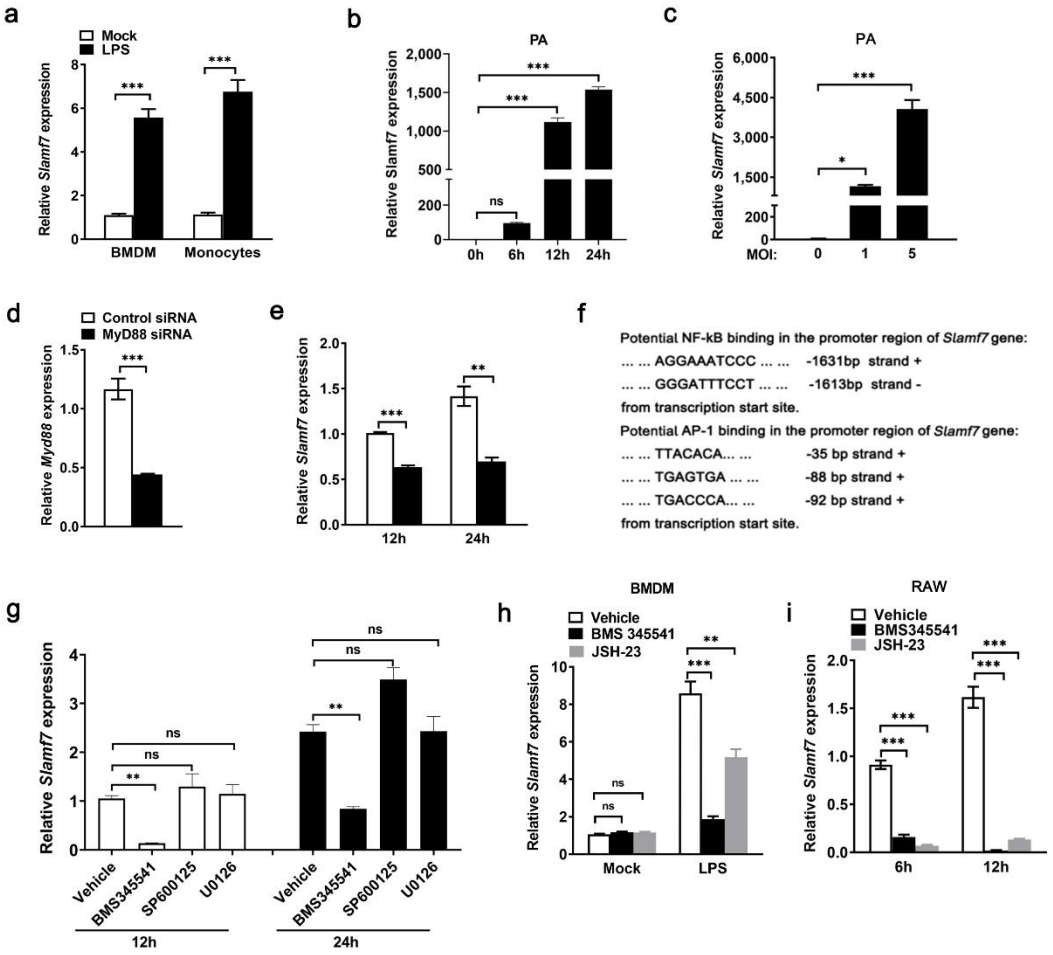
Supplementary Data



Supplementary Figure 1

SLAMF7 expression is not related to clinical parameters.

Percentage of SLAMF7$^+$ CD11b$^+$ cells in PBMC from sepsis patients were divided by (**a**) gender, (**b**) age, (**c**) infectious microbes or (**d**) diagnosis.

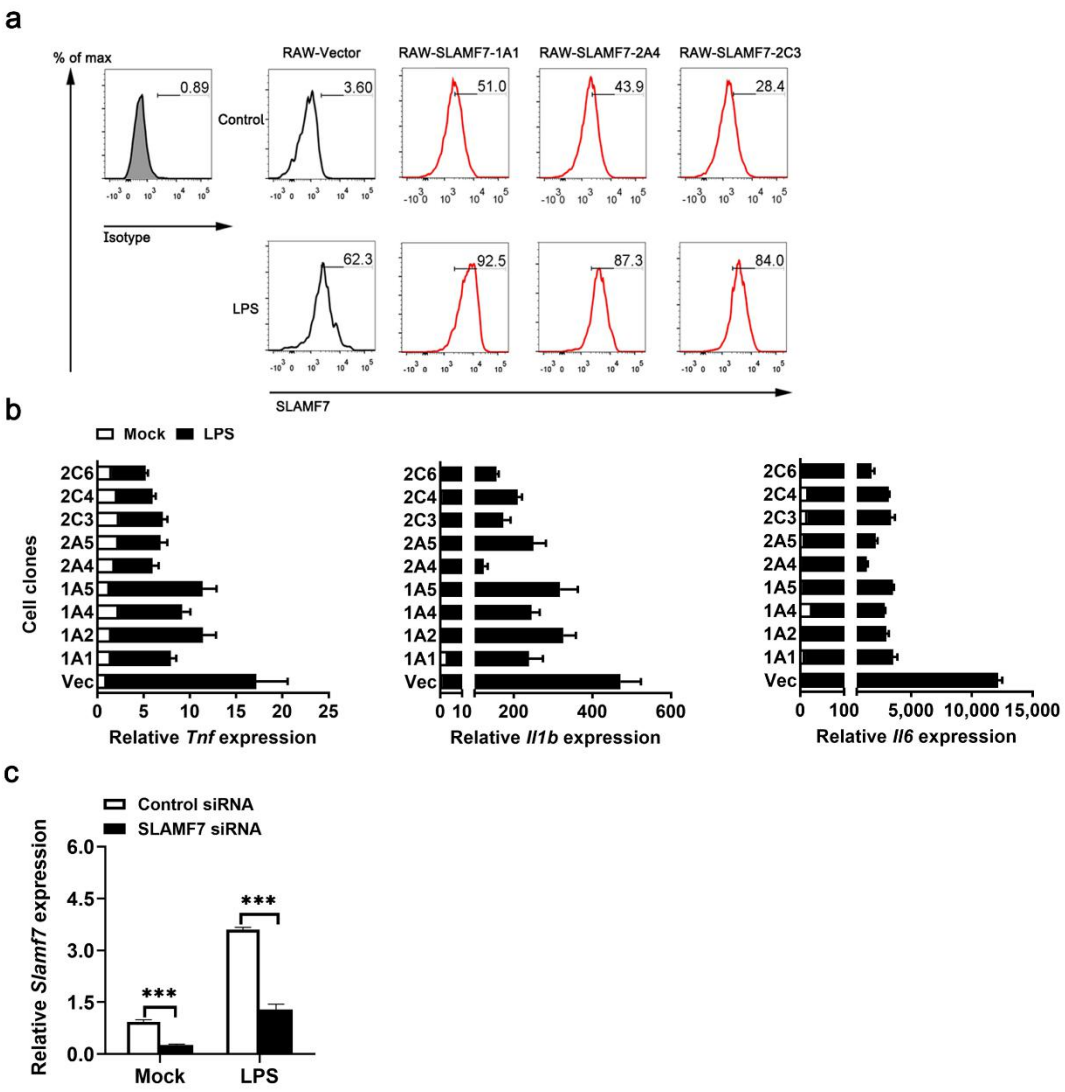


Supplementary Figure 2

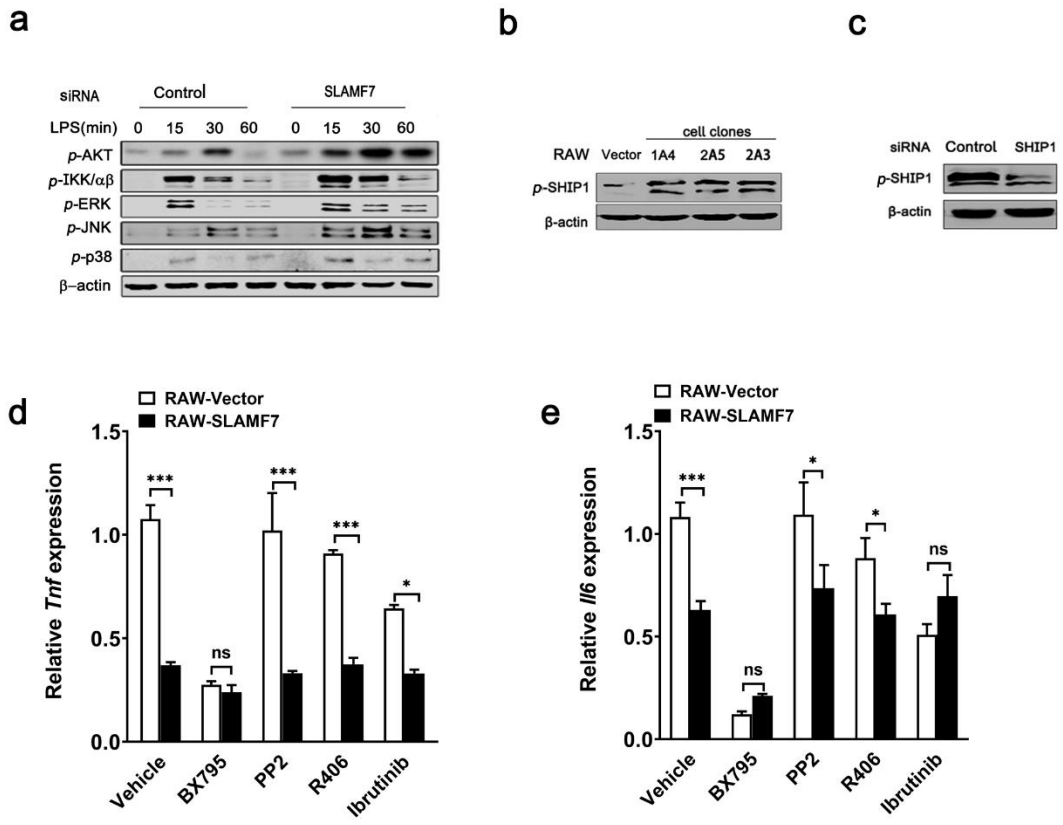
TLR/NF-κB signaling induces SLAMF7 expression in macrophages.

a, mRNA levels of *Slamf7* in mouse bone marrow derived macrophages (BMDM) or human monocytes isolated from peripheral blood (monocytes) were examined after LPS stimulation for 24 h. **b**,**c**, mRNA levels of *Slamf7* were examined in RAW264.7 cells after PA infection (MOI=1) at indicated time points (**b**) or indicated concentrations at 24h post PA stimulation (**c**). **d**,**e**, BMDM were transiently transfected with MyD88 specific or control siRNA for 24h, followed by LPS stimulation for 24 h. mRNA levels of *Myd88* (**d**) and *Slamf7* (**e**) were examined by real-time PCR. **f**, The potential binding sites for transcription factors including NF-κB (-1631bp from the transcription start site) and AP-1 (-35bp, -88bp and -92bp from the transcription start site) were analyzed in the promoter region of *Slamf7* by JASPAR software. **g**, *Slamf7* expression was examined in RAW264.7 cells pre-treated with inhibitors

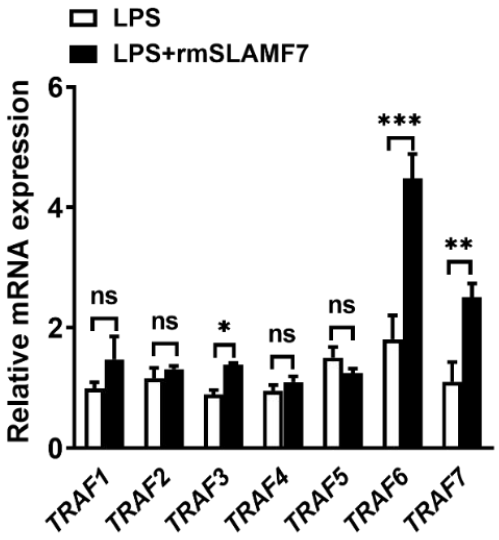
targeting IKKα/β (BMS345541), JNK (SP600125) or p38 (U0126) for 1 h, followed by LPS challenge for 24 h. **h**,**i**, *Slamf7* expression in BMDM (**h**) or RAW264.7 cells (**i**) after pretreatment with NF-κB signaling inhibitors (BMS345541 for IKKα/β and JSH-23 for NF-κB) and LPS stimulation. Data are the mean ± SEM and represent three individual experiments. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.



Supplementary Figure 3. SLAMF7 negatively regulates inflammatory cytokines production *in vitro*. a, SLAMF7 expression in RAW-Vector cells and multiple RAW-SLAMF7 overexpressing cell clones before and after LPS stimulation for 24 h. **b**, RAW264.7 cells stably expressing SLAMF7 (RAW-SLAMF7) and control vector (RAW-Vector) cells were constructed. mRNA levels of inflammatory cytokines in different RAW-SLAMF7 cell clones vs RAW-Vector cells were analyzed. **c**, The expression of *Slamf7* in BMDM transfected with SLAMF7 siRNA or Control siRNA, followed by LPS stimulation for 24 h. Data are the mean ± SEM and represent three individual experiments. ***, P <0.001.

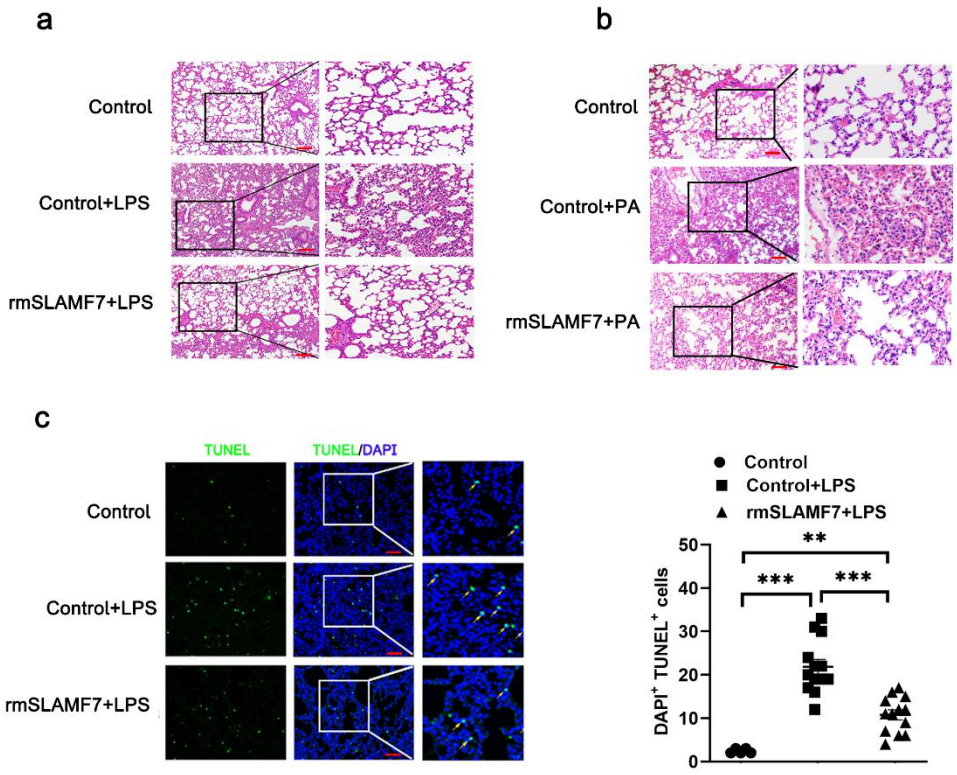


Supplementary Figure 4. SLAMF7 inhibits MAPKs/NF-kB signaling pathways requiring TBK1 kinases. a, Immunoblot analysis of phosphorylated signaling molecules in MAPKs and AKT signaling pathways of BMDM transfected with SLAMF7 siRNA and Control siRNA after stimulating for 0min,15min,30min,60 min. **b**, **c**, Phosphorylation of SHIP1 in RAW-SLAMF7 cell clones compared to RAW-Vector (**b**) or after transfected with siRNA targeting SHIP1 (**c**). **d**, **e,** The expression of *Tnf* (**d**) and *Il6* (**e**) of RAW264.7 cells after pre-transfected with inhibitors of TBK1 (BX795), Src (PP2), Syk (R406) and Btk (Ibrutinib), followed by LPS stimulation for 6 h. Data are the mean ± SEM and represent three individual experiments. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.



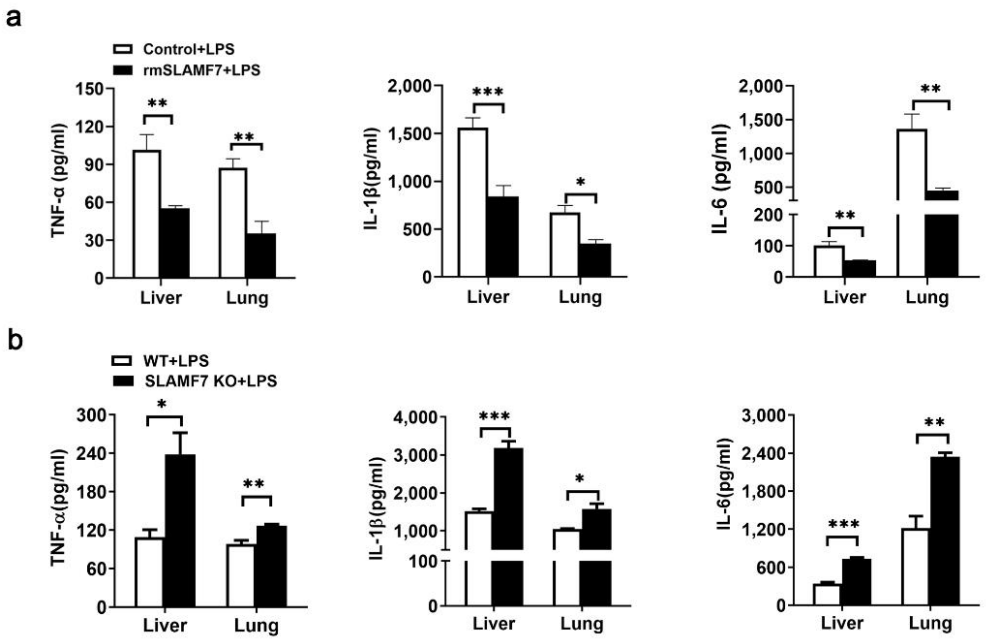
Supplementary Figure 5.

The expression levels of *TRAF1-7* in BMDM after treatment with rmSLAMF7, followed by LPS stimulation. Data are the mean ± SEM and represent three individual experiments. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.



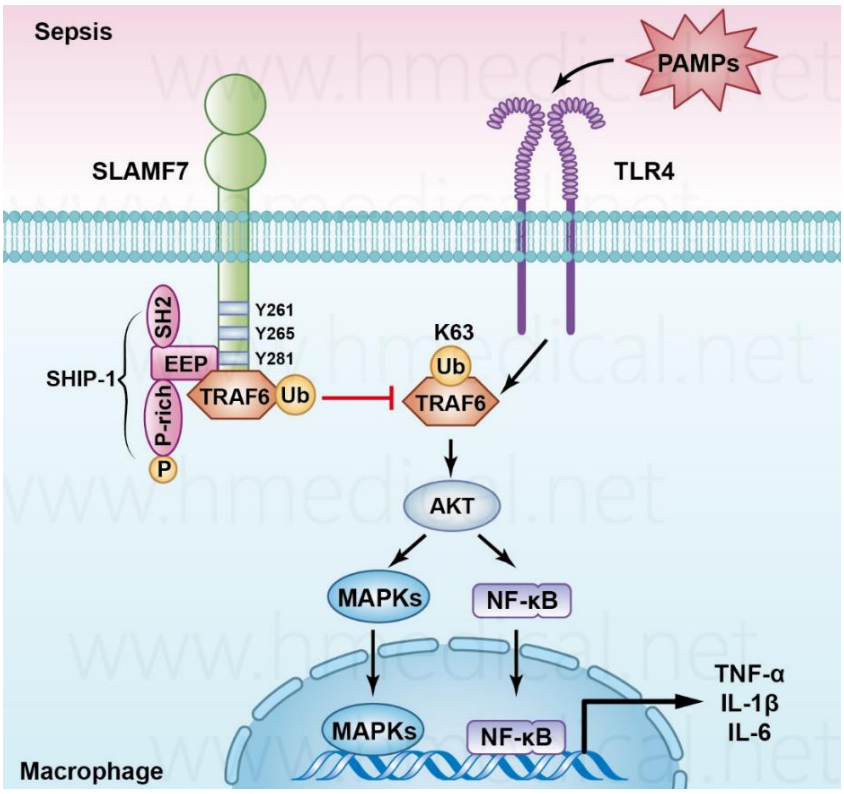
Supplementary Figure 6. Activating SLAMF7 attenuated lung injury in septic mice.

a,**b**, Sections from mice challenged with LPS (**a**) or PA (**b**) for 1h, followed by intraperitoneal injection of rmSLAMF7 or Control (0.9% NaCl) for 6 h. **c**, The TUNEL and DAPI co-localization cells of lung section from endotoxin mice. White square showed the area to zoom in. Yellow arrows point the co-localization of DAPI and TUNEL. Scale bars, 20 μm. Data are the mean ± SEM and represent three independent experiments. **, $P < 0.01$; ***, $P < 0.001$.



Supplementary Figure 7. SLAMF7 negatively regulates inflammatory cytokines production *in vivo*.

a, The protein levels of TNF-α, IL-1β and IL-6 in the supernatant of liver and lung from WT mice pre-challenged with LPS, followed by injection with rmSLAMF7 or vehicle control (0.9% NaCl) for 24h later. **b**, The protein levels of TNF-α, IL-1β and IL-6 in the supernatant of liver and lung from WT and SLAMF7 KO mice 24h after LPS treatment. Data are the mean ± SEM and represent three individual experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.



Supplementary Figure 8 Graphical Abstract

In macrophage, Toll-Like Receptor 4 (TLR4) is activated by Pathogen-Associated Molecular Patterns (PAMPs), resulting in pro-inflammatory responses during the acute phase of sepsis. In this process, the expression of SLAMF7 is obviously upregulated. The activation of SLAMF7 negatively regulates MAPKs and NF-kB signaling pathways by inhibiting K63 ubiquitination of TRAF6 alone or co-operation with SHIP1, reducing the secretion of pro-inflammatory cytokines including TNF-α, IL-1β and IL-6.

Supplementary Table 1. Characteristics and blood parameters of healthy donors and sepsis patients

	Healthy control subjects	Patients with sepsis	*p* value*
Sample size (no.)	81	83	NA
Age (years)	35 (1,80)	42(1, 94)	NS
Sex (M/F)	(40/41)	(49/34)	NS
Hemoglobin (g/L)	118.26±2.54	90.09±2.72	<0.0001
Platelets ($\times 10^9$/ L)	312(181, 493)	246.23 (12,675)	NS
WBC ($\times 10^9$/ L)	7.70 (3.7, 11.8)	11.71 (0.2,78.5)	<0.0001
WBC (%)			
Neutrophil (%)	38 (21, 69)	59.9 (6,94)	<0.0001
Monocyte (%)	5.09 (3, 8)	6.66 (0,22)	NS
Lymphocyte (%)	53.74 (21, 72)	31.28 (6,81)	<0.0001
Eosinophil (%)	3.04 (0, 12)	0.72 (0,4)	<0.0001
Basophil (%)	0 .09 (0, 1)	0.33 (0,6)	NS

F, Female; M, male; WBC: White blood cell; NA, not applicable; NS, not significant. Data were shown by mean ± SD or median (25% percentile, 75% percentile).*The level of significance was evaluated by unpaired student *t* test or Mann-Whitney 2-tailed *U* test. *p* value <0.05 was considered statistically significant.

Supplementary Table 2. The primers and sequences of genes used in assays of siRNA knockdown.

Target Gene Name	siRNA sequences
SLAMF7-si-001	CCAGGAATCCAGTCAGCAA
SLAMF7-si-002	CCATGAAGCTCAGCCAATT
SLAMF7-si-003	GCAGAGATTTACAGTACAT
SHIP1-si-001	GCAGATGAAGAACAAGCAT
SHIP1-si-002	GCATATCCTGATCAGCATT
SHIP1-si-003	CCAGTGGAATGAAATGCTT

Supplementary Table 3. The primers and sequences of genes used in quantitative PCR.

Gene Name	Quantitative Primers（5'-3'）
SLAMF7 (human)	CGGGACCTGCACCTTGATACR-GTT GCTGATAGGGTTGCTCAC
β-actin (mouse)	GATTACTGCTCTGGCTCCTAGC GACTCATCGTACTCCTGCTTGC
SLAMF7 (mouse)	ACAAGAATGGCACCTGCGTA AGATCCGTGGCAGCATCTTC
SHIP1 (mouse)	CCAGGGCAAGATGAGGGAGA GGACCTCGGTTGGCAATGTA
TNF (mouse)	CACAGAAAGCATGATCCGCGAC TGCCACAAGCAGGAATGAGAAGAG
IL-6 (mouse)	TTCCTCTCTGCAAGAGACTTCCATC GCCTCCGACTTGTGAAGTGGTATAG
IL-1β (mouse)	AGATCCGTGGCAGCATCTTC CGCAGCAGCACATCAACAAGAGC
IL-10 (mouse)	AGATCCGTGGCAGCATCTTC AGATCCGTGGCAGCATCTTC
SAP (mouse)	ATGCAGTGACTGTGTACCACG AGGGACACTCTCGCTGTCT
EAT-2 (mouse)	CTGCCTGACCAAGCGAGAG TCTGAAGATTCGGTAGCTGTAGA