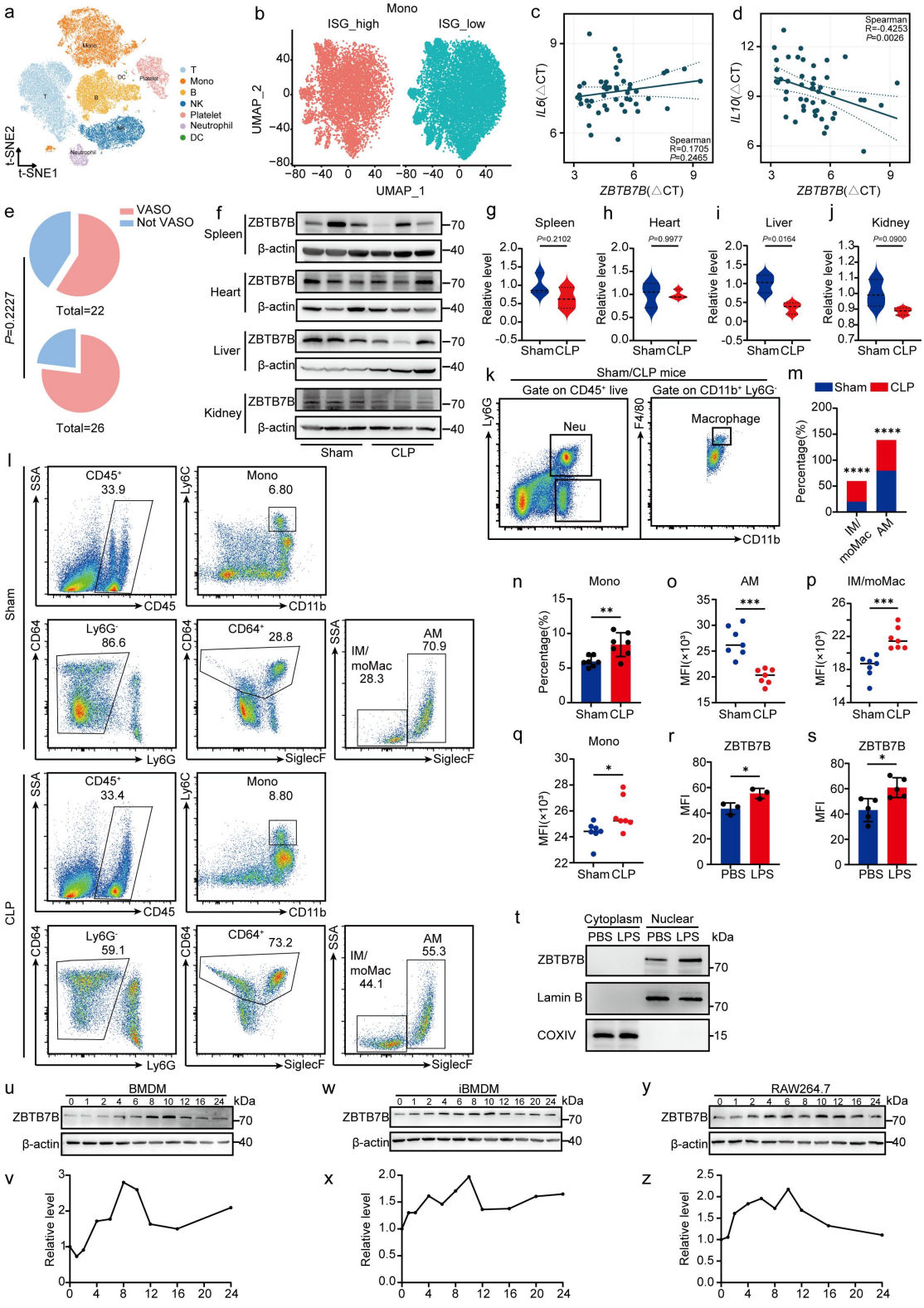
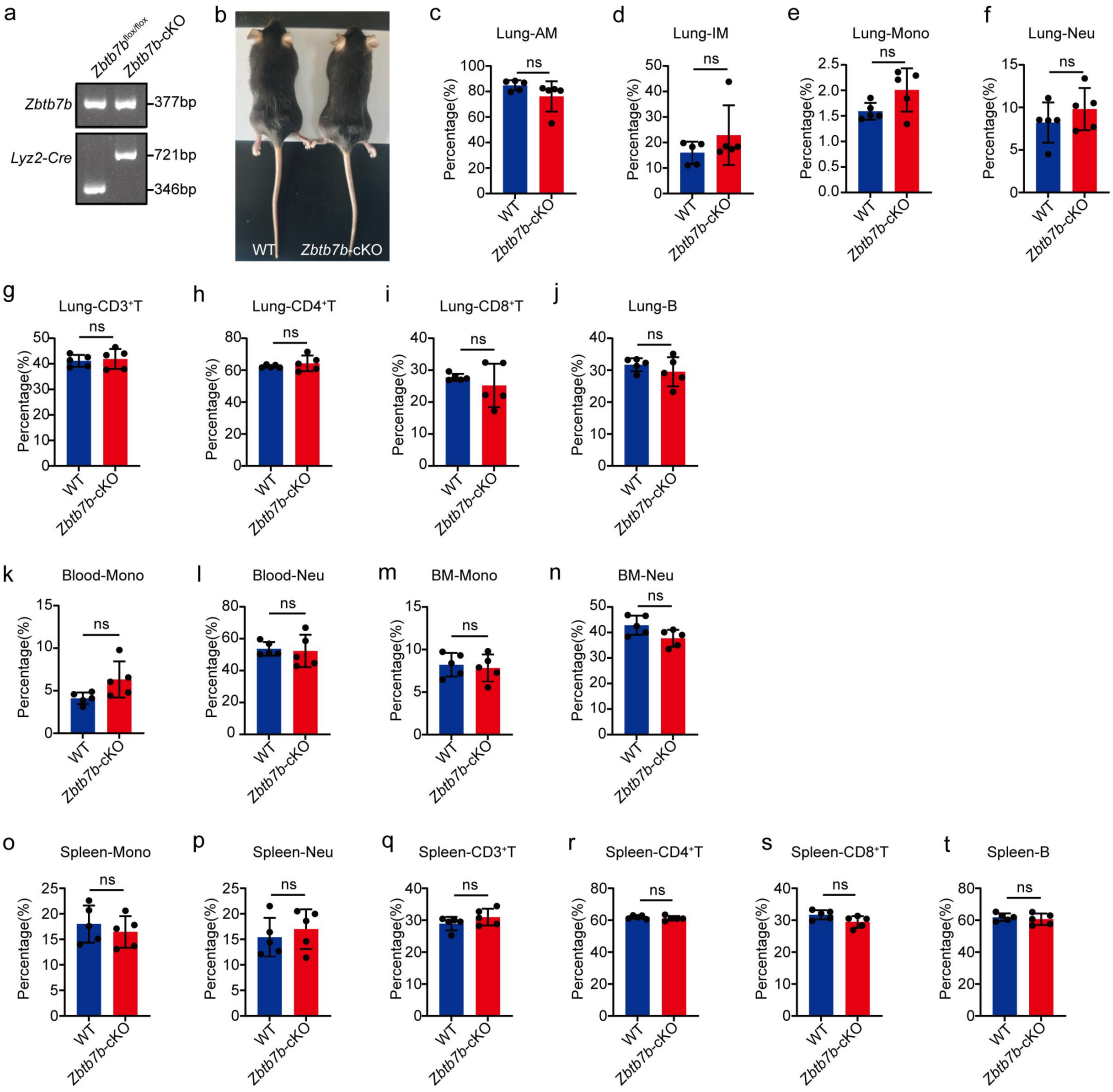
Extended Data Fig.1



Extended Data Fig. 1| ZBTB7B expression in monocytes/macrophages correlates with sepsis severity. a, t-SNE plot of single-cell transcriptomic profiles of PBMCs from healthy controls and sepsis patients. Cells are colored by annotated cell type. Each dot represents one cell. **b,** UMAP plot of monocytes showing two

subpopulations defined by ISG expression levels. **c, d,** Correlation of *ZBTB7B* with *IL6* and *IL10* mRNA level in peripheral blood $CD14^+$ monocytes of patients with septic ARDS (n = 48). **e,** Proportion of patients requiring vasopressor usage segmented by the median of *ZBTB7B* mRNA level in patients with septic ARDS (n = 24 per group). **f–j,** Immunoblotting and quantification of ZBTB7B expression in spleen, heart, liver or kidney from mice subjected to CLP or Sham (n = 3 per group). **k,** Flow cytometric gating strategy for the identification of pulmonary $F4/80^+$ macrophages from mice. **l,** Flow cytometric gating strategy for alveolar macrophages (AMs), interstitial macrophages (IMs)/monocyte-derived macrophages (moMacs) and monocytes of lung tissue from mice subjected to CLP or Sham. **m, n,** Quantification of the percentages of AMs, IMs/moMacs or monocytes in lung tissue from mice subjected to CLP or Sham (n = 7 per group). **o–q,** Mean fluorescence intensity (MFI) of ZBTB7B in AMs, IMs/moMacs or monocytes in lung tissue from mice subjected to CLP or Sham (n = 7 per group). **r,** Quantification of MFI of ZBTB7B from three randomly selected immunofluorescence images in PBS or LPS-stimulated (200 ng/mL) BMDMs (n = 3 images per group). **s,** Quantification of MFI of ZBTB7B in five randomly selected regions of interest (ROIs) from one representative immunofluorescence image in PBS or LPS-stimulated BMDMs. **t,** Immunoblot analysis of ZBTB7B level and location in PBS or LPS-stimulated BMDMs. **u–z,** Immunoblot analysis and quantification of ZBTB7B expression at indicated time points post-stimulation of LPS in BMDMs, immortalized BMDMs or RAW264.7 cells. P values were calculated by Spearman's correlation analysis (**c, d**), a two-sided Fisher's exact test (**e**) and unpaired two-tailed Student's t-test (**g–j, m–s**). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns = not significant. Data are shown as mean ± SD.

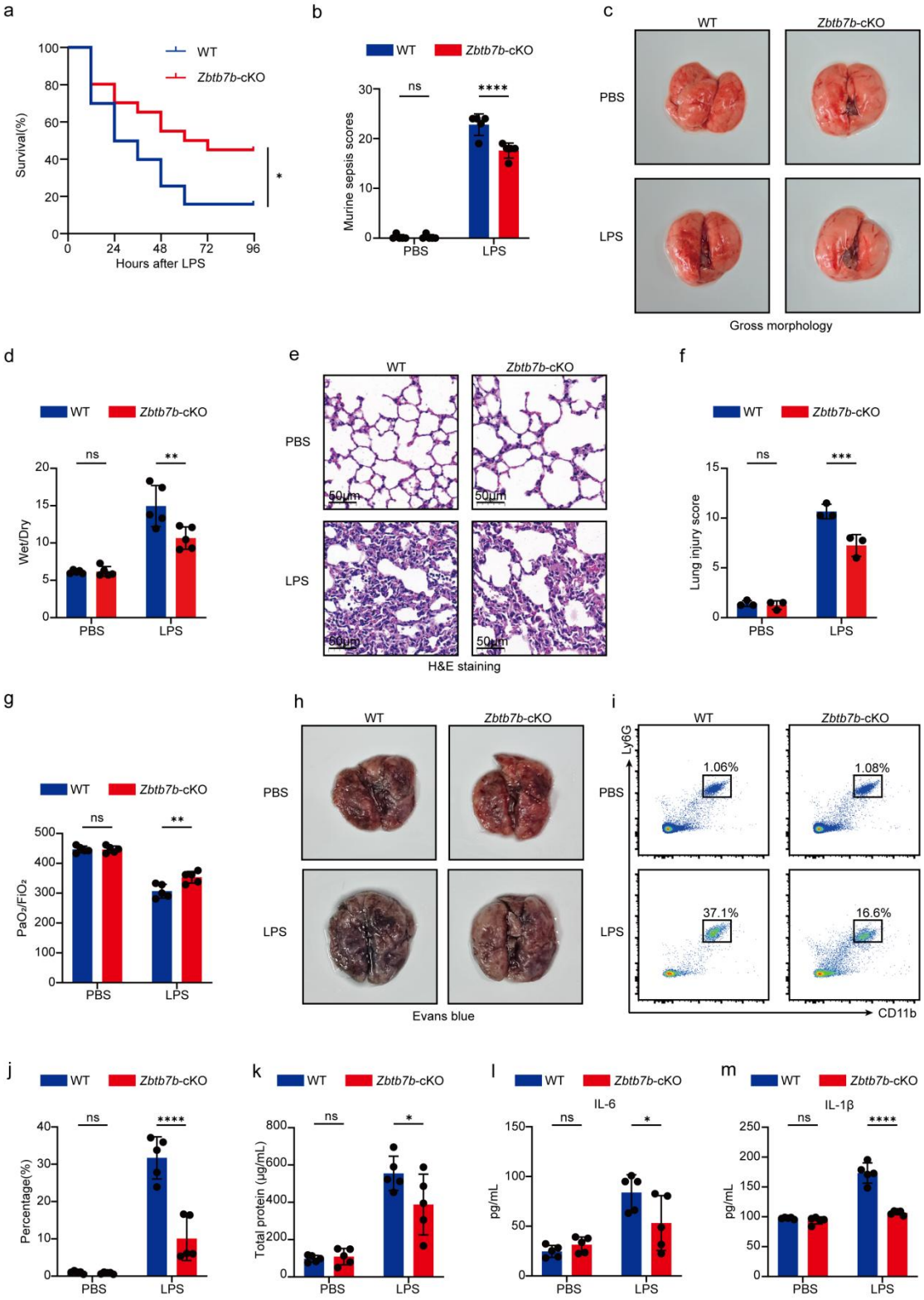
Extended Data Fig.2



Extended Data Fig. 2| Generation and Baseline Characterization of Myeloid cell-specific *Zbtb7b* deficiency mice. a, Genotype analysis of *Zbtb7b*$^{flox/flox}$ (WT) and *Zbtb7b*$^{flox/flox}$*Lyz2*-Cre (*Zbtb7b*-cKO) mice by PCR. **b,** Gross phenotype of WT and *Zbtb7b*-cKO mice. **c–j,** Flow cytometric quantification of the percentages of AMs (**c**), IMs (**d**), monocytes (**e**), neutrophils (**f**), T cells (**g–i**) and B cells (**j**) in the lung tissues of WT and *Zbtb7b*-cKO mice (n = 5 per group). **k, l,** Flow cytometric quantification of the percentages of monocytes and neutrophils in peripheral blood of WT and *Zbtb7b*-cKO mice (n = 5 per group). **m, n,** Flow cytometric quantification of the percentages of monocytes and neutrophils in bone marrows of WT and *Zbtb7b*-cKO mice (n = 5 per group). **o–t,** Flow cytometric quantification of the percentages of monocytes (**o**), neutrophils (**p**), T cells (**q–s**) or B cells (**t**) in spleen tissues of WT and

Zbtb7b-cKO mice (n = 5 per group). P values were calculated by unpaired two-tailed Student's t-test. ns = not significant. Data are shown as mean ± SD.

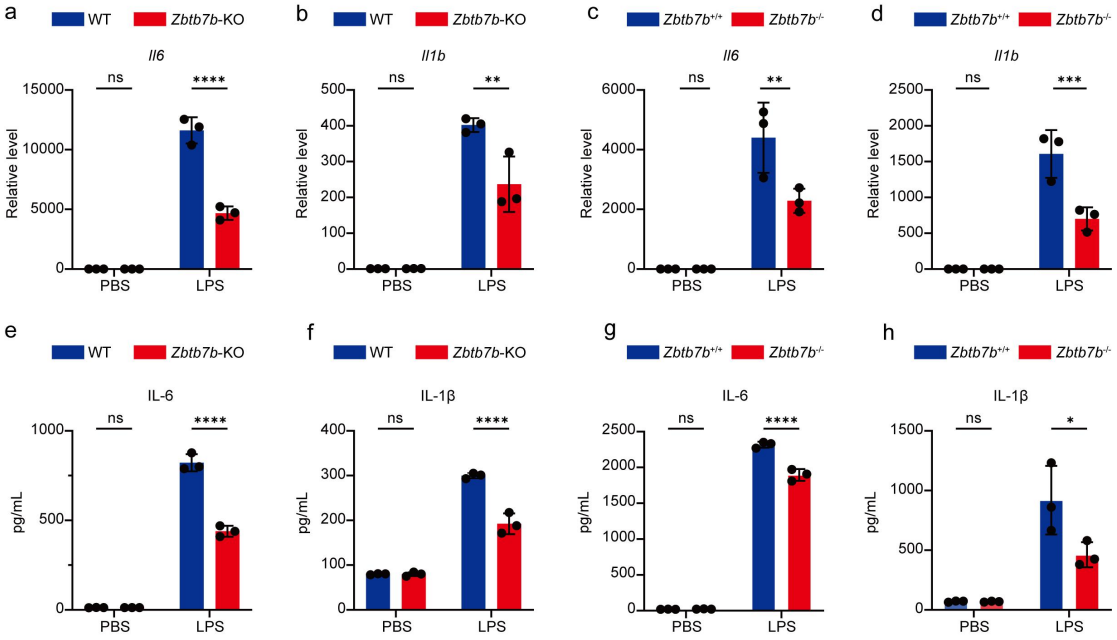
Extended Data Fig.3



Extended Data Fig. 3| Myeloid cell-specific *Zbtb7b* deficiency attenuates septic ALI. a–m, WT or *Zbtb7b*-cKO mice were intraperitoneally (i.p.) injected with lipopolysaccharide (LPS, 10 mg/kg, 12 hrs). **a,** The survival rates (n = 20 per group). **b,** The murine sepsis scores (n = 5 per group). **c,** Gross morphology of lung tissue. **d,**

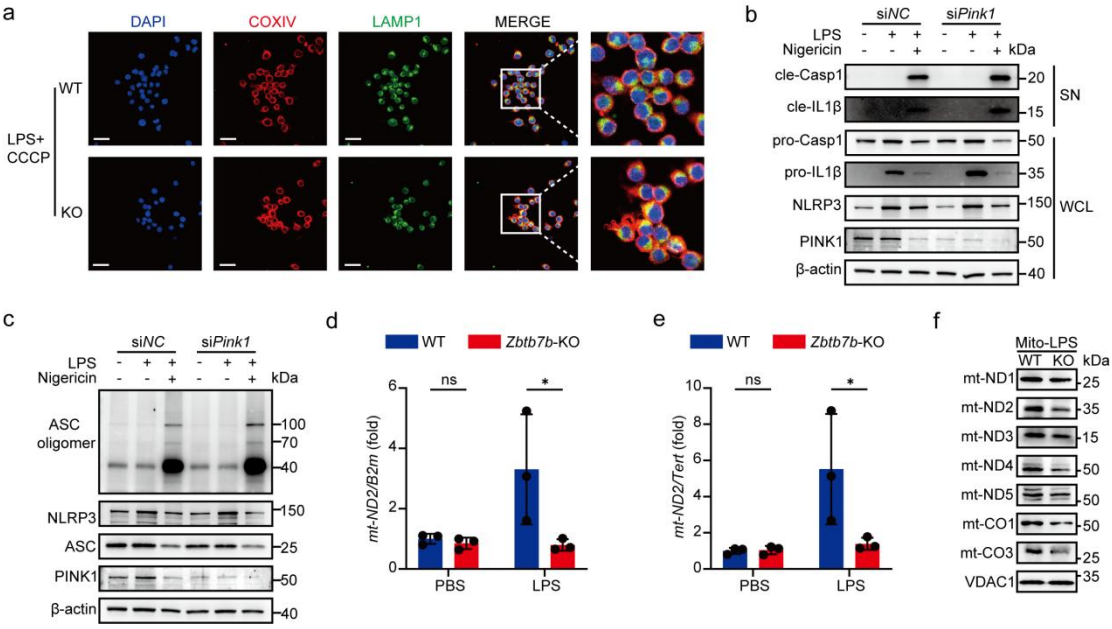
Lung W/D ratio analysis (n = 5 per group). **e, f,** Representative images (**e**) and lung injury scores (**f**) of H&E staining of lung tissues (Scale bar, 50 μm). **g,** Arterial blood PaO_2/FiO_2 (n = 5 per group). **h,** Evans blue dye leakage of lung tissue. **i, j,** Representative images (**i**) and quantification (**j**) of the percentages of neutrophils in BALFs (n = 5 per group). **k–m,** Concentration of total protein (**k**), IL-6(**l**) and IL-1β (**m**) in BALFs (n = 5 per group). P values were calculated by Kaplan-Meier analysis (**a**) and two-way ANOVA followed by Fisher's LSD post hoc test (**b, d, f, g, j–m**). *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$; ns = not significant. Data are shown as mean ± SD.

Extended Data Fig.4



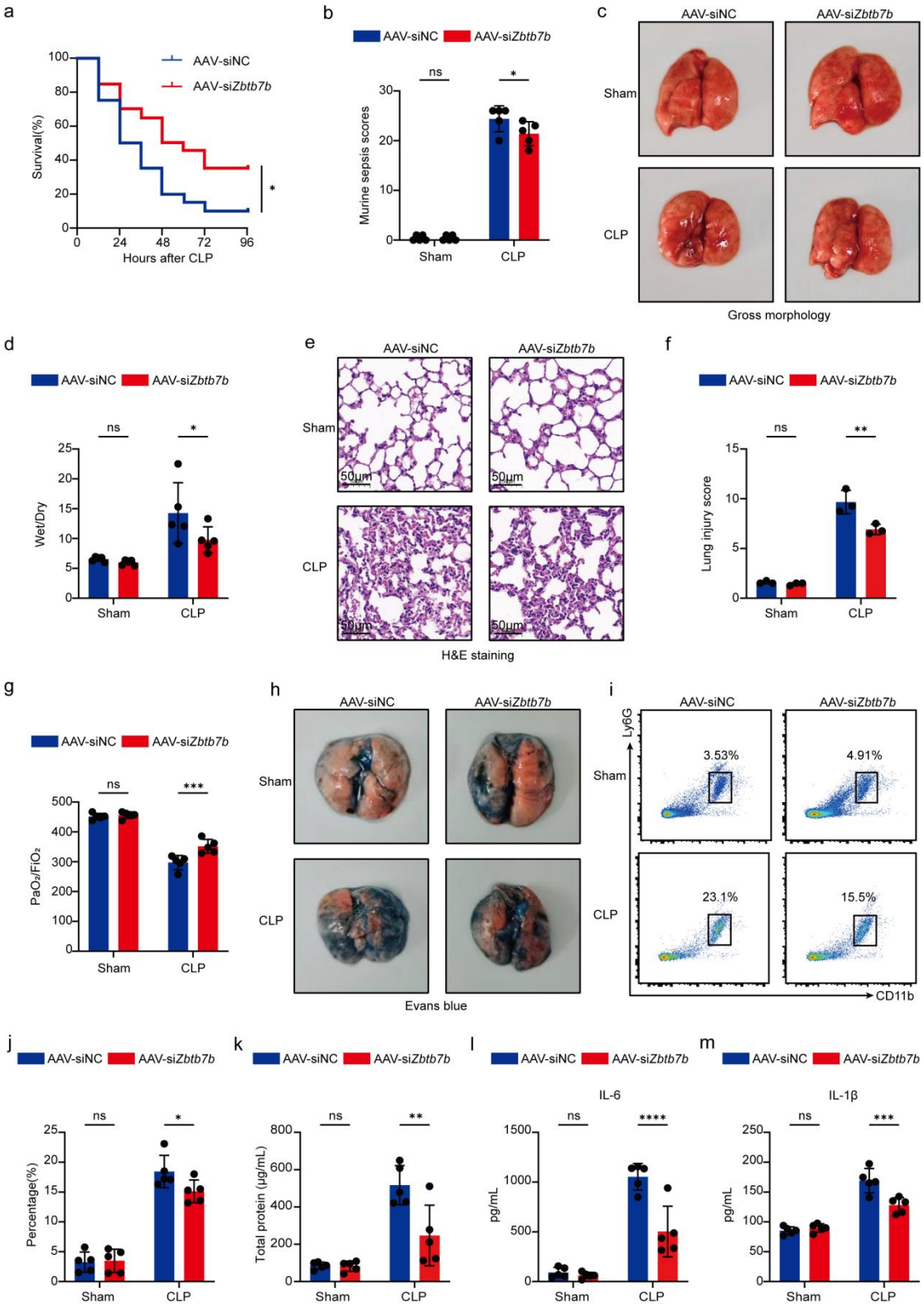
Extended Data Fig. 4| *Zbtb7b* deficiency attenuates inflammatory responses in macrophages after LPS stimulation. a, b, qPCR analysis of *Il6* (**a**) and *Il1b* (**b**) mRNA levels in WT or *Zbtb7b*-KO BMDMs treated with PBS or LPS (200 ng/mL). **c, d,** qPCR analysis of *Il6* (**c**) or *Il1b* (**d**) mRNA levels in *Zbtb7b*$^{+/+}$ or *Zbtb7b*$^{-/-}$ iBMDMs treated with PBS or LPS (200 ng/mL). **e, f,** ELISA of IL-6 (**e**) or IL-1β (**f**) release by WT or *Zbtb7b*-KO BMDMs treated with PBS or LPS (200 ng/mL). **g, h,** ELISA of IL-6 (**g**) or IL-1β (**h**) release by *Zbtb7b*$^{+/+}$ or *Zbtb7b*$^{-/-}$ iBMDMs treated with PBS or LPS (200 ng/mL). *P* values were calculated by two-way ANOVA followed by Fisher's LSD post hoc test (**a–h**). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns = not significant. Data are shown as mean ± SD.

Extended Data Fig.5



Extended Data Fig. 5| ZBTB7B-induced CMPK2 promotes NLRP3 inflammasome activation through suppressing mitophagy. a, Representative immunofluorescence images of *Zbtb7b*$^{+/+}$ (WT) or *Zbtb7b*$^{-/-}$ (KO) iBMDMs co-stained for COXIV, LAMP1 and DAPI following stimulation with LPS and CCCP (Scale bar, 30 μm). **b, c,** Immunoblot analysis of SN and WCL(**b**), and ASC oligomerization in pellets(**c**) from *Zbtb7b*$^{-/-}$ (KO) iBMDMs transfected with scramble sequence (si-NC) or specific sequences targeting *Pink1* (si-*Pink1*) following stimulation with LPS and nigericin. **d, e,** Relative total mtDNA content in WT and *Zbtb7b*-KO BMDMs, pre- and post- LPS priming. **f,** Immunoblot analysis of mitochondrial protein in *Zbtb7b*$^{+/+}$ (WT) or *Zbtb7b*$^{-/-}$ (KO) iBMDMs following LPS stimulation. *P* values were calculated by two-way ANOVA followed by Fisher's LSD post hoc test. *, $P < 0.05$; ns = not significant. Data are shown as mean ± SD.

Extended Data Fig.6



Extended Data Fig. 6| Inhibition of ZBTB7B suppresses systemic inflammation and protects against lung injury after sepsis. a–m, WT mice were intratracheal injected AAV-siNC or AAV-si*Zbtb7b* 7 days before CLP. **a,** The survival rates (*n* = 20 per group). **b,** The murine sepsis scores (*n* = 5 per group). **c,** Gross morphology of

lung tissue. **d,** Lung W/D ratio (n = 5 per group). **e, f,** Representative images (**e**) and lung injury score (**f**) of H&E staining of lung tissues (Scale bar, 50 μm). **g,** Arterial blood PaO_2/FiO_2 (n = 5 per group). **h,** Evans blue dye leakage of lung tissue. **i, j,** Representative images (**i**) and quantification (**j**) of the percentages of neutrophils in BALFs (n = 5 per group). **k–m,** Concentration of total protein (**k**), IL-6 (**l**) and IL-1β (**m**) in BALFs (n = 5 per group). P values were calculated by Kaplan-Meier analysis (**a**) and two-way ANOVA with Fisher's LSD post hoc analysis (**b, d, f, g, j–m**). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns = not significant. Data are shown as mean ± SD.

Extended Data Table 1| Baseline Characteristics of Study Groups in the Validation Cohort.

Baseline Characteristics	Sepsis without ARDS patients **Group 1(n = 55)**	septic ARDS patients **Group 2(n=48)**	healthy control **Group 3(n = 20)**	P value in first two group	P value in all groups
Male sex(%)	32(58.18%)	26(54.17%)	9(45%)	0.6819	0.5973
Age(years)	70(65,75)	72.5(67,77)	72.5(68,78)	0.198	0.294
Comorbidities[n](%)					
Chronic Pulmonary Disease	13(23.6%)	9(18.75%)	NA	-	-
Chronic Kidney Disease	11(20.00%)	8(16.70%)	NA	-	-
Cardio-cerebrovascular	21(38.18%)	26(54.17%)	NA	-	-
Diabetes	25(45.46%)	28(58.33%)	NA	-	-
Hypertension	38(69.10%)	36(75.00%)	NA	-	-
Focus of infection[n](%)					
Gastrointestinal	13(23.64%)	16(33.33%)	NA	-	-
Pulmonary	20(36.36%)	19(39.58%)	NA	-	-
Urinary tract	16(29.09%)	7(14.58%)	NA	-	-
Skin or soft tissue	6(10.91%)	6(12.50%)	NA	-	-
Distribution of pathogens[n](%)					
Gram positive	20(36.36%)	12(25.00%)	NA	-	-
Gram negative	19(34.55%)	19(39.58%)	NA	-	-
Fungus	4(7.27%)	5(10.42%)	NA	-	-
Miscellaneous	7(12.73%)	9(18.75%)	NA	-	-
Unclear	5(9.09%)	3(6.25%)	NA	-	-
WBC,10^9/L	13.19(8.98,17.60)	17.00(12.74,20.96)	6.19(4.73,7.66)	0.0061	<.0001
CRP,mg/L	95.14(48.06,148.6)	126.7(68.25,166.6)	4.75(2.25,6.88)	0.08	<.0001
Procalcitonin,ng/mL	3.73(0.90,10.50)	6.15(2.15,36.28)	0.03(0.02,0.04)	0.063	<.0001
IL-6,pg/mL	70.31(25.50,201.6)	120.50(52.39,339.1)	3.00(1.83,4.68)	0.094	<.0001
Lactate,mmol/L	1.60(1.20,2.60)	2.05(1.33,3.65)	0.50(0.20,0.70)	0.083	<.0001
Vasopressor use(%)	18(32.73%)	33(68.75%)	NA	<.001	-
Mechanical Ventilation(%)	8(14.55%)	40(83.33%)	NA	<.0001	-
PaO_2/FiO_2,mmHg	375(342,422)	191(137,221)	NA	<.0001	-
SOFA	5(3,6)	8(6,10)	NA	<.0001	-
APACHII	14(11,18)	18(13,22)	NA	0.006	-
Length of Hospital stay(days)	15(12,19)	12(8,18)	NA	0.089	-
Length of ICU stay(days)	5(3,9)	8(5,12)	NA	0.017	-

Footnotes:Continuous variables are presented as median (interquartile range), and categorical variables as n (%). Comparisons between groups were performed using the Mann-Whitney U test for non-normally distributed continuous variables, Student's t-test for normally distributed variables, and χ^2 or Fisher's exact test for categorical variables. One-way ANOVA was used for comparisons across all groups. P values < 0.05 were considered statistically significant. Focus of infection and pathogen distribution are reported only for infected groups (Group 1, 2). Mechanical ventilation was defined as ongoing invasive ventilatory support at the time of study enrollment. SOFA scores were calculated 24 hours after admission.
Abbreviations: NA, not applicable; WBC, white blood cell count; CRP, C-reactive protein; IL-6, interleukin-6; PaO_2/FiO_2, partial pressure of oxygen to fraction of inspired oxygen ratio; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; ICU, intensive care unit.

Extended Data Table 2| Reagents used in this study.

Reagent	Source	Catalog number
Protease inhibitor cocktail	ABclonal	RM02916
Sonication ChIP Kit	ABclonal	RK20258
Mouse IL-6 ELISA Kit	ABclonal	RK00008
Evo M-MLV RT Premix	Accurate Biology	AG11706
SYBR Green Premix Pro Taq HS qPCR Kit	Accurate Biology	AG11701
Phenylmethylsulfonyl fluoride (PMSF)	Beyotime	ST506
Enhanced ATP Assay Kit	Beyotime	S0027
BeyoMag™ Protein A+G Magnetic Beads	Beyotime	P2108
Cell Lysis Buffer	Cell Signaling Technology	9803
Ficoll-Paque solution	Cytiva	17544602
Omni-ECL™Femto Light Chemiluminescence Kit	Epizyme	SQ201
Omni-Easy™ Ready-to-use BCA Protein Assay Kit	Epizyme	ZJ102
TRIzol	Thermo Fisher Scientific	15596018
MitoProbe™ TMRM	Thermo Fisher Scientific	M20036
Mouse IL-1 beta Uncoated ELISA Kit	Thermo Fisher Scientific	BMS6002-2TEN
eBioscience Foxp3 / Transcription Factor Staining Buffer Set	Thermo Fisher Scientific	00-5523-00
OPTI-MEM(TM) I (1X)	Thermo Fisher Scientific	3248487
Mitochondria Isolation Kit for Cultured Cells	Thermo Fisher Scientific	89874
Lipofectamine™RNAiMAX Transfection Reagent	Thermo Fisher Scientific	13778100
Evans blue	Merck	E2129
Lung Dissociation Kit	Miltenyi Biotec	130-095-0927
CD14 MicroBeads, human	Miltenyi Biotec	130-050-201
Anti-F4/80 MicroBeads UltraPure, mouse	Miltenyi Biotec	130-110-443
Murine macrophage colony-stimulating factor (M-CSF)	PeproTech	315-02
5X Protein Loading Dye	Sangon Biotech	C508320
Lipopolysaccharides	Sigma-Aldrich	L2630
FastPure Cell/Tissue DNA Isolation Mini Kit	Vazyme	DC102
CytoTox 96$^{@}$Non-Radioactive Cytotoxicity Assay	Promega	G1780
jetPRIME transfection reagent	Polyplus	101000046

Extended Data Table 3| Antibodies used in this study

Antibody	Source	Catalog number
ThPOK (D9V5T) Rabbit Monoclonal Antibody	Cell Signaling Technology	13205
β-Actin (13E5) Rabbit mAb	Cell Signaling Technology	4970
Lamin B1 (D9V6H) Rabbit mAb	Cell Signaling Technology	13435
COX IV(3E11) Rabbit mAb	Cell Signaling Technology	4850
LC3B Antibody	Cell Signaling Technology	2775
ASC/TMS1 (D2W8U) Rabbit Monoclonal Antibody	Cell Signaling Technology	67824
Tom20 (D8T4N) Rabbit Monoclonal Antibody	Cell Signaling Technology	42406
Stat1 Antibody	Cell Signaling Technology	9172
MT-ND1 Rabbit mAb	ABclonal	A9743
MT-ND2 Rabbit pAb	ABclonal	A17968
MT-ND3 Rabbit pAb	ABclonal	A17969
MT-ND4 Rabbit pAb	ABclonal	A17970
MT-ND5 Rabbit pAb	ABclonal	A8135
MT-CO1 Rabbit pAb	ABclonal	A17889
MT-CO3 Rabbit pAb	ABclonal	A9939
VDAC1 Rabbit mAb	ABclonal	A19707
KIF5B Rabbit mAb	ABclonal	A20928
DRP1 Rabbit mAb	ABclonal	A21968
Mitofusin-1/MFN1 Rabbit mAb	ABclonal	A21293
Mitofusin 2 Rabbit mAb	ABclonal	A19678
LAMP1/CD107a Rabbit mAb	ABclonal	A23947
DDDDK/Flag	ABclonal	AE005
Myc-Tag Rabbit mAb	ABclonal	AE070
anti-ASC antibody	Santa Cruz	sc-514414
THPOK Antibody(A-4)	Santa Cruz	sc-376250
anti-NLRP3/NALP3, mAb (Cryo-2)	AdipoGen Life Science	AG-20B-0014-C100
anti-Caspase-1 (p20) (mouse), mAb (Casper-1)	AdipoGen Life Science	AG-20B-0042
Mouse IL-1 beta /IL-1F2 Antibody	R&D Systems	AF-401-NA
Anti-CMPK2 Antibody	Abcam	ab194567
Stat1 Antibody [J19A4]	Selleckchem.com	F0263
Parkin Antibody [H15P18]	Selleckchem.com	F0296
PINK1 Antibody	Affinity Biosciences	AB_2841209
COXIV Monoclonal antibody	Proteintech	66110-1-lg
Zombie Aqua™ Fixable Viability Kit	BioLegend	423101
APC/Cyanine7 anti-mouse CD45	BioLegend	144718
FITC anti-mouse/human CD11b	BioLegend	101205
APC anti-mouse Ly-6C Antibody	BioLegend	128015
PE/Cyanine7 anti-mouse Ly-6G	BioLegend	127617
Brilliant Violet 421™ anti-mouse F4/80	BioLegend	123131
PerCP/Cyanine5.5 anti-mouse CD3	BioLegend	100127
APC anti-mouse CD19	BioLegend	152409
PE anti-Th-POK (ZFP-67) Antibody	BioLegend	656403
FITC anti-mouse CD4 Antibody	BioLegend	100405
PE/Cyanine7 anti-mouse CD8a Antibody	BioLegend	100721

PerCP/Cyanine5.5 anti-mouse CD170 (Siglec-F) Antibody	BioLegend	155525
Brilliant Violet 785™ anti-mouse CD64 (FcγRI) Antibody	BioLegend	139340

Extended Data Table 4| siRNA used in this study

Gene	Primers	Seqience (5' - 3')	Application
Pink1	sense	CUGGCUGACUAUCCUGAUATT	RNA interference
Pink1	antisense	UAUCAGGAUAGUCAGCCAGTT	RNA interference

Extended Data Table 5| Oligos used in this study

Gene	Primers	Seqience (5' - 3')	Application
Gapdh (mouse)	Forward	AGAAGGTGGTGAAGCAGGCATC	RT-qPCR
	Reverse	CGAAGGTGGAAGAGTGGGAGTTG	RT-qPCR
Zbtb7b (mouse)	Forward	TGAAGGTGAGGAAGAAGAGGAGGAG	RT-qPCR
	Reverse	GCGAACTTAGGTAAGCCATCAGGTC	RT-qPCR
Cmpk2 (mouse)	Forward	CCAGTGGAGGAAGATCTTTGAT	RT-qPCR
	Reverse	TTAGCTATTTCAGAAGCCACGA	RT-qPCR
Il6 (mouse)	Forward	TAGTCCTTCCTACCCCAATTTCC	RT-qPCR
	Reverse	TTGGTCCTTAGCCACTCCTTC	RT-qPCR
Il1b (mouse)	Forward	GCAACTGTTCCTGAACTCAACT	RT-qPCR
	Reverse	ATCTTTTGGGGTCCGTCAACT	RT-qPCR
mt-Nd2 (mouse)	Forward	TCACAATATCCAGCACCAACCT	RT-qPCR
	Reverse	GCTGTTGCTTGTGTGACGAA	RT-qPCR
B2m (mouse)	Forward	ATGGGAAGCCGAACATACTG	RT-qPCR
	Reverse	CAGTCTCAGTGGGGGTGAAT	RT-qPCR
Tert (mouse)	Forward	CTAGCTCATGTGTCAAGACCCTCTT	RT-qPCR
	Reverse	GCCAGCACGTTTCTCTCGTT	RT-qPCR
GAPDH (human)	Forward	CAGTCCATGCCATCACTGCCACCCAG	RT-qPCR
	Reverse	CAGTGTAGCCCAGGATGCCCTTGAG	RT-qPCR
ZBTB7B (human)	Forward	TCGCTGCTTGCATGGAGATTCTG	RT-qPCR
	Reverse	GGACTGTCTTCACCATTGGGAACTC	RT-qPCR
CMPK2 (human)	Forward	ACCTTATCCTGCTGCTCACTGTG	RT-qPCR
	Reverse	CTGCTTCTTCCCTGGTCTTCTCC	RT-qPCR
RSAD2 (human)	Forward	CGTGAGCATCGTGAGCAATG	RT-qPCR
	Reverse	TCTTCTTTCCTTGGCCACGG	RT-qPCR
EPSTI1 (human)	Forward	ACCGCTGAGTTCTTGAGCAA	RT-qPCR
	Reverse	CAGCTGTGATCCCTAGGCAG	RT-qPCR
IL6 (human)	Forward	GTAAATGGGATTCCAACACGAACAA	RT-qPCR
	Reverse	TGTCGTGCAGTAACAACCAACTC	RT-qPCR
IL1B (human)	Forward	ATGATGGCTTATTACAGTGGCAA	RT-qPCR
	Reverse	GTCGGAGATTCGTAGCTGGA	RT-qPCR
IL10 (human)	Forward	CCCAAGCTGAGAACCAAGAC	RT-qPCR
	Reverse	AAGGCATTCTTCACCTGCTC	RT-qPCR
Zbtb7b-flox (mouse)	Forward	AAAGTATCAGGACTGACCTGCCT	Genotyping
	Reverse	TCCTGTTACACAAGAAGGGGT	Genotyping
Lyz2-cre wild type (mouse)	Forward	CTTGGGCTGCCAGAATTTCTC	Genotyping
	Reverse	TTACAGTCGGCCAGGCTGAC	Genotyping
Lyz2-cre mutant (mouse)	Forward	CTTGGGCTGCCAGAATTTCTC	Genotyping
	Reverse	CCCAGAAATGCCAGATTACG	Genotyping
Cmpk2 promoter	Forward	TTGATGCAGGAAATTTTGATTGGC	ChIP-qPCR
	Reverse	GCTCACGTGGGATGCAAAC	ChIP-qPCR