Table S1 A list of antibodies used for Western blotting, IP, and immunofluorescence staining

Name of antibody	Catalog number	Company	Molecular weight (kD)	Dilution (Western blotting/IP/immunofluorescence)
CIRP	LS-C334809	LSBio	18	1:1000 (Western blotting); 1:50 (IP); 1:100 (immunofluorescence)
PERK	ab229912	Abcam	150	1:1000 (Western blotting)
p-PERK	PA5-40294	Invitrogen	150	1:1000 (Western blotting)
eIF2α	ab242148	Abcam	36	1:1000 (Western blotting)
p-eIF2α	ab32157	Abcam	38	1:1000 (Western blotting)
ATF4	#11815	CST	50	1:1000 (Western blotting)
CHOP	#2895	CST	27	1:1000 (Western blotting)
HSP70	ab2787	Abcam	70	1:1000 (Western blotting)
Histone H3	ab1791	Abcam	15	1:1000 (Western blotting)
Pan-lactate	SAB5701141	Sigma	35 − 100	1:1000 (Western blotting)
Klac	PTM1401RM	PTM BIO	15	1:1000 (Western blotting); 1:50 (IP); 1:100 (immunofluorescence)
CD31	#3528	CST	130	1:800 (immunofluorescence)
CD63	ab134045	Abcam	26	1:1000 (Western blotting)
CD81	ab109201	Abcam	26	1:1000 (Western blotting)
Calnexin	ab133615	Abcam	90	1:1000 (Western blotting)
CASP1	AG-20B-0042	Adipogen	45, 20	1:1000 (Western blotting); 1:100 (IP); 1:100 (immunofluorescence)
GSDMD	ab209845	Abcam	53, 34	1:1000 (Western blotting)
AIM2	# MA5-38442	Invitrogen	48	1:1000 (Western blotting); 1:100 (immunofluorescence)
F4/80	ab300421	Abcam	150	1:100 (immunofluorescence)
ASC	#67824	CST	22	1:1000 (Western blotting); 1:100 (immunofluorescence)
ZBP1	sc-271483	Santa Cruz	55	1:1000 (Western blotting); 1:50 (IP); 1:100 (immunofluorescence)
β-actin	#93473	CST	45	1:1000 (Western blotting)
GAPDH	5174	CST	37	1:1000 (Western blotting)
Tubulin	sc-32293	Santa Cruz	55	1:1000 (Western blotting)
TRIM32	NBP1-33737	Novus Biologicals	72	1:1000 (Western blotting); 1:50 (IP); 1:100 (immunofluorescence)

Name of antibody	Catalog number	Company	Molecular weight (kD)	Dilution (Western blotting/IP/immunofluorescence)
Ubiquitin	#43124	CST	-	1:1000 (Western blotting); 1:50 (IP)
MLKL	#70934	CST	54	1:1000 (Western blotting); 1:100 (immunofluorescence)
p-MLKL	#70934	CST	54	1:1000 (Western blotting)
Caspase-3	#70934	CST	35, 19, 17	1:1000 (Western blotting); 1:100 (immunofluorescence)
RIP3	#70934	CST	55	1:1000 (Western blotting); 1:100 (immunofluorescence)
p-RIP3	#70934	CST	55	1:1000 (Western blotting)
RIP1	ab300617	Abcam	75	1:1000 (Western blotting); 1:100 (immunofluorescence)
p-RIP1	ab316923	Abcam	75	1:1000 (Western blotting)
Cleaved caspase-8	#9496	CST	18	1:1000 (Western blotting); 1:100 (immunofluorescence)
Caspase-8	#4927	CST	55	1:1000 (Western blotting); 1:100 (immunofluorescence)

IP immunoprecipitation, *CIRP* cold-inducible RNA-binding protein, *PERK* protein kinase R (PKR)-like endoplasmic reticulum kinase, *p-PERK* phosphorylated protein kinase R (PKR)-like endoplasmic reticulum kinase, *eIF2α* eukaryotic initiation factor 2 alpha, *p-eIF2α* phosphorylated eukaryotic initiation factor 2 alpha, *ATF4* activating transcription factor 4, *CHOP* C/EBP homologous protein, *HSP70* heat shock protein 70, *CASP1* caspase-1, *GSDMD* gasdermin D, *AIM2* absent in melanoma 2, *ASC* apoptosis-associated speck-like protein containing a CARD, *ZBP1* Z-DNA binding protein 1, *TRIM32* tripartite motif-containing protein 32, *MLKL* mixed lineage kinase domain-like protein, *p-MLKL* phosphorylated mixed lineage kinase domain-like protein, *RIPK3* receptor-interacting protein kinase 3, *p-RIP3* phosphorylated receptor-interacting protein kinase 3, *RIP1* receptor-interacting protein kinase 1, *p-RIP1* phosphorylated receptor-interacting protein kinase 1

Table S2 For luciferase and mutant assay

Name	**Sequence (5’ – 3’)**	
pcDNA3.1ATF4 (1000 bp)	Forward	CGCGGATCCCGCAACATGACCGAAATGAGC
	Reverse	GGAATTCCAACTAGGGGACCCTTTTCTTCC
pGL4.1Cirp (1900 bp)	Forward	CGGGGTACCTCACAGAACCAGGGAACGATG
	Reverse	CTAGCTAGCCTCGGAGCCGGTGCAGCCAG
pGL4.1 Cirp-mutantA	Forward	CGGCAAGAAGCTTAGAATTATGCAG
	Reverse	CCTGCATAATTCTAAGCTTCTTGC
pGL4.1 Cirp-mutantB	Forward	CGTGAGTGGCATCGTGAGCAGC
	Reverse	CGGCTGCTCACGATGCCACTCAC
pGL4.1 Cirp-mutantC	Forward	GTCTTCCCGCAACGTACAGGGACC
	Reverse	AGGTCCCTGTACGTTGCGGGAAG
pGL4.1 Cirp-mutantABC	Forward	GCCTCCGATCGTTGTCAGAAG
	Reverse	GGTAGTCGGTCTTGCTATCCATG

Table S3 Baseline characteristics of healthy controls and sepsis-induced ALI patients for cohort

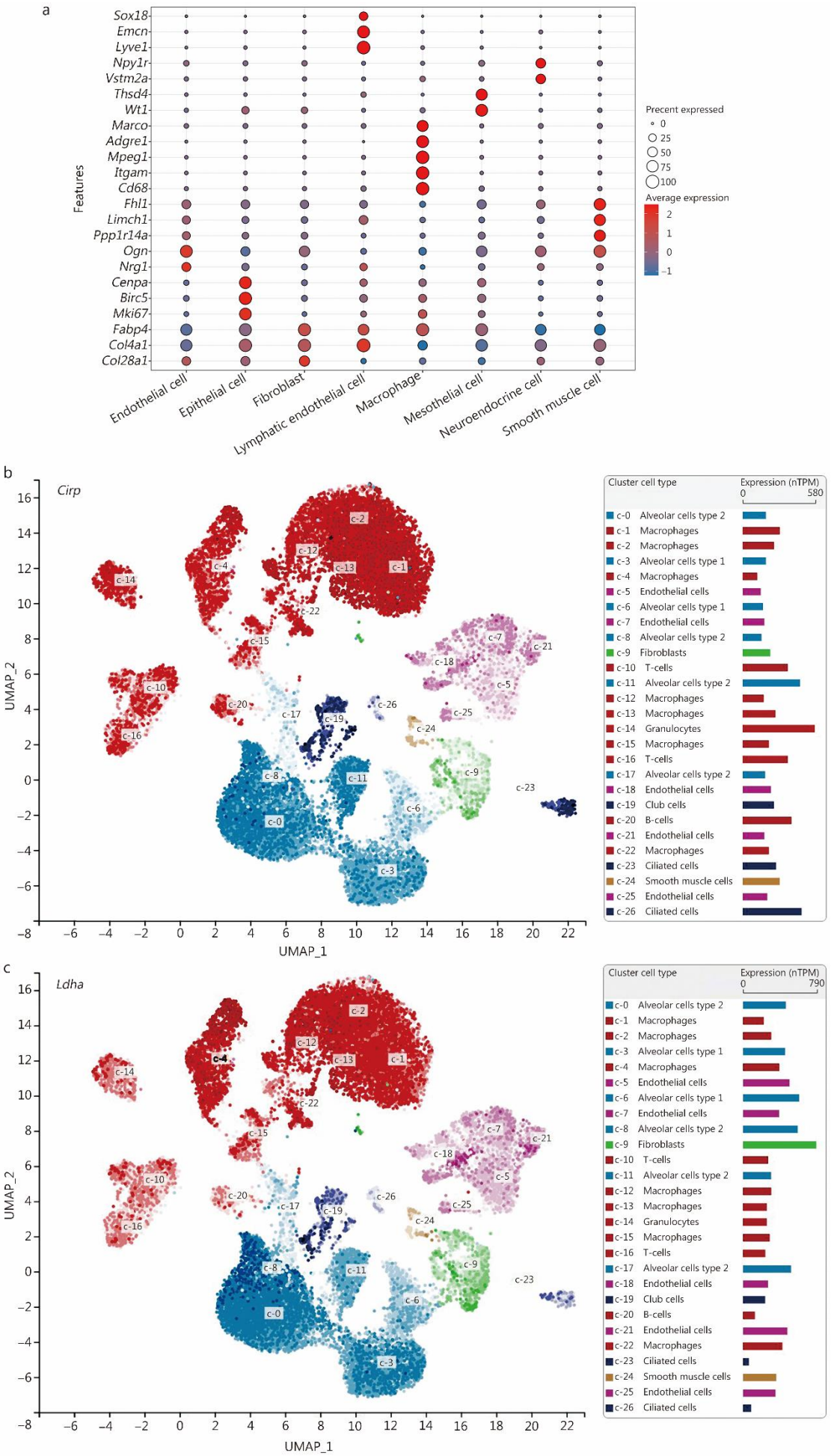
Characteristic	**Healthy controls[a] (*n* = 30)**	**Sepsis-induced ALI[a] (*n* = 60)**	***P*-value**
Age (years)	54 ± 3	55 ± 2	0.77
Gender [male, *n* (%)]	16 (53)	34 (57)	
Body mass index (kg/m^2)	22.3 ± 0.5	21.5 ± 0.4	0.215
Septic shock [*n* (%)]	-	32 (53)	
Source of infection [*n* (%)]			
Abdominal	-	44 (74)	
Urogenital	-	8 (13)	
Blood	-	5 (8)	
Wound	-	3 (5)	
Acute lung injury [*n* (%)]	-	60 (100)	
SOFA score, median (IQR)	-	8.0 (5.0 − 10.0)	
APACHE II score, median (IQR)	-	20.5 (16.0 − 25.8)	

[a]Confirmatory sample for ELISA. The values are presented as mean ± SD, median (IQR), or *n* (%). *APACHE II* Acute Physiology and Chronic Health Evaluation score II, SOFA Sequential Organ Failure Assessment, ALI acute lung injury

Table S4 Demographic and clinical characteristics of survivors and non-survivors

Characteristic	**Survivor (*n* = 22)**	**Non-survivor (*n* = 16)**	***P*-value**
Age (years)	55.4 ± 3.1	60.0 ± 2.9	0.31
Gender [male, *n* (%)]	10 (45)	10 (63)	
Body mass index (kg/m^2)	21.9 ± 0.6	20.4 ± 0.6	0.11
Septic shock [*n* (%)]	11 (50)	10 (63)	0.52
Source of infection [*n* (%)]			
Abdominal	16 (73)	12 (75)	
Urogenital	3 (13)	2 (13)	
Blood	2 (9)	1 (6)	
Wound	1 (5)	1 (6)	
Acute lung injury [*n* (%)]	22 (100)	16 (100)	
SOFA score, median (IQR)	7 (5 − 9)	11(9 − 13)	< 0.001
APACHE II score, median (IQR)	18 (12 − 25)	22 (19 − 26)	0.04

The values are presented as mean ± SD, median (IQR), or *n* (%). *APACHE II* Acute Physiology and Chronic Health Evaluation score II, *SOFA* Sequential Organ Failure Assessment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Sox18
Emcn
Lyve1
Npy1r
Vstm2a
Thsd4
Wt1
Marco
Adgre1
Mpeg1
Itgam
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Fhl1
Limch1
Ppp1r14a
Ogn
Nrg1
Cenpa
Birc5
Mki67
Fabp4
Col4a1
Col28a1
Features
Endothelial cell
Epithelial cell
Fibroblast
Lymphatic endothelial cell
Macrophage
Mesothelial cell
Neuroendocrine cell
Smooth muscle cell
Precent expressed
0
25
50
75
100
Average expression
2
1
0
−1
b
Cirp
UMAP_1
UMAP_2
Cluster cell type
Expression (nTPM)
0
580
c-0 Alveolar cells type 2
c-1 Macrophages
c-2 Macrophages
c-3 Alveolar cells type 1
c-4 Macrophages
c-5 Endothelial cells
c-6 Alveolar cells type 1
c-7 Endothelial cells
c-8 Alveolar cells type 2
c-9 Fibroblasts
c-10 T-cells
c-11 Alveolar cells type 2
c-12 Macrophages
c-13 Macrophages
c-14 Granulocytes
c-15 Macrophages
c-16 T-cells
c-17 Alveolar cells type 2
c-18 Endothelial cells
c-19 Club cells
c-20 B-cells
c-21 Endothelial cells
c-22 Macrophages
c-23 Ciliated cells
c-24 Smooth muscle cells
c-25 Endothelial cells
c-26 Ciliated cells
c
Ldha
UMAP_1
UMAP_2
Cluster cell type
Expression (nTPM)
0
790
c-0 Alveolar cells type 2
c-1 Macrophages
c-2 Macrophages
c-3 Alveolar cells type 1
c-4 Macrophages
c-5 Endothelial cells
c-6 Alveolar cells type 1
c-7 Endothelial cells
c-8 Alveolar cells type 2
c-9 Fibroblasts
c-10 T-cells
c-11 Alveolar cells type 2
c-12 Macrophages
c-13 Macrophages
c-14 Granulocytes
c-15 Macrophages
c-16 T-cells
c-17 Alveolar cells type 2
c-18 Endothelial cells
c-19 Club cells
c-20 B-cells
c-21 Endothelial cells
c-22 Macrophages
c-23 Ciliated cells
c-24 Smooth muscle cells
c-25 Endothelial cells
c-26 Ciliated cells

Fig. S1 Expression distribution and single cell analysis. **a** The single-cell RNA sequencing analysis demonstrated distinct cell clustering within lung tissue. Single-cell sequencing data from the Protein Atlas database indicates that the *Cirp* (**b**) and *Ldha* (**c**) genes are predominantly expressed in macrophages within lung tissue. Cirp cold-inducible RNA-binding protein, Ldha lactate dehydrogenase A

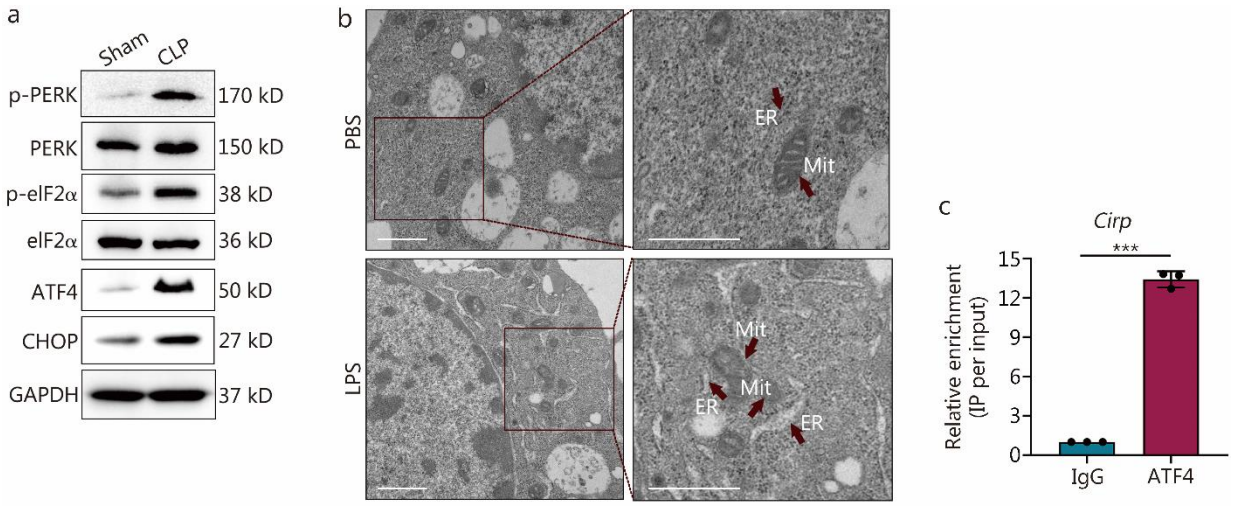

Fig. S2 Evident endoplasmic reticulum (ER) stress in sepsis mice and cells. **a** Immunoblotting was used to assess the expression of ER stress proteins p-PERK, PERK, p-eIF2α, eIF2α, ATF4, and CHOP in lung macrophages from CLP mice compared to the sham group. **b** Changes in mitochondrial morphology in macrophages after LPS stimulation were observed through electron microscopy (scale bar = 1 μm). The upper panel shows normal mitochondria and ER in the control group, indicated by arrows. The lower panel shows ER swelling and mitochondria vacuolation after LPS stimulation, indicated by arrows. **c** ChIP-qPCR analysis demonstrated that ATF4 binding to the *Cirp* promoter was significantly enriched compared to the IgG control. The results represent 3 independent experiments. The data are presented as the mean ± SD. $^{***}P < 0.001$. p-PERK phosphorylated protein kinase RNA-like endoplasmic reticulum kinase, PERK protein kinase RNA-like endoplasmic reticulum kinase, p-eIF2α phosphorylated eukaryotic initiation factor 2α, eIF2α eukaryotic initiation factor 2α, ATF4 activating transcription factor 4, CHOP C/EBP homologous protein, CLP cecal ligation and puncture, LPS lipopolysaccharide, ChIP-qPCR chromatin immunoprecipitation quantitative polymerase chain reaction, Mit mitochondria

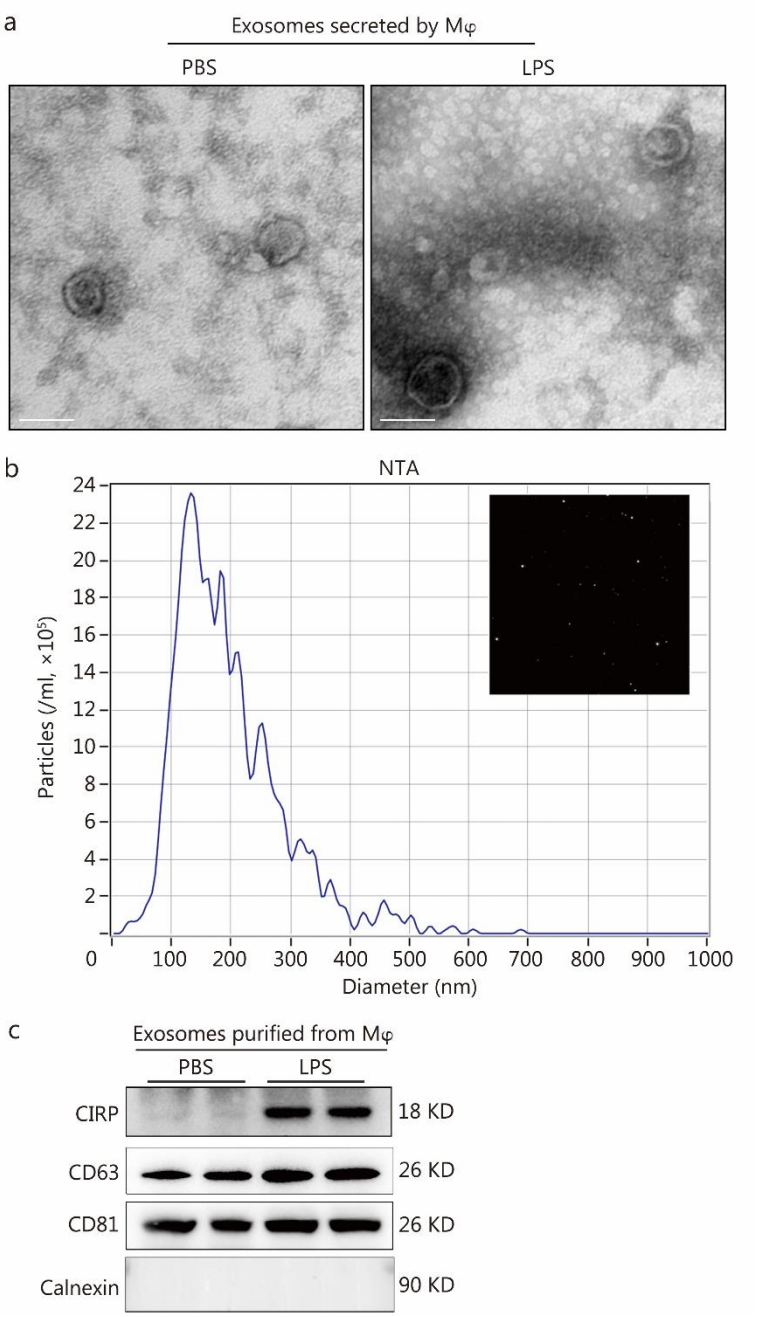

Fig. S3 CIRP is released through the exosome pathway. **a** Transmission electron microscopy images depict the morphology of exosomes isolated from macrophage supernatants (scale bar = 100 nm). **b** Concentration (particles/ml) and modal size (nm) of exosome samples were determined by NTA. **c** Western blotting analysis was used to detect CIRP and exosome markers, including CD63 and CD81, in exosomes purified from macrophages. CIRP cold-inducible RNA-binding protein, NTA nanoparticle tracking analysis, CD63 cluster of differentiation 63, CD81 cluster of differentiation 81, PBS phosphate-buffered saline, LPS lipopolysaccharide

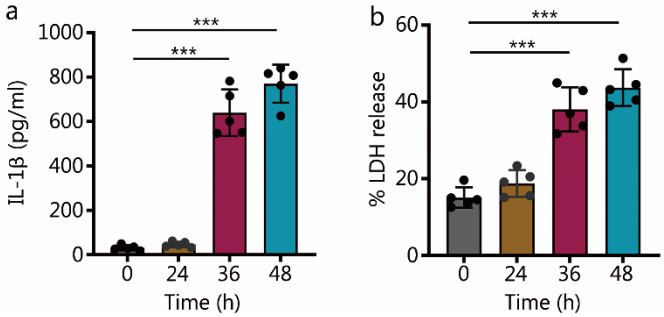

Fig. S4 LPS and rmCIRP induce PANoptosis in MPVECs. **a** Secreted IL-1β measured by ELISA. **b** Cell death quantified by LDH release. The data are presented as the mean ± SD. $^{***}P < 0.001$. MPVECs mouse pulmonary vascular endothelial cells, IL-1β interleukin-1β, ELISA enzyme-linked immunosorbent assay, LDH lactate dehydrogenase, LPS lipopolysaccharide, rmCIRP recombinant mouse cold-inducible RNA-binding protein