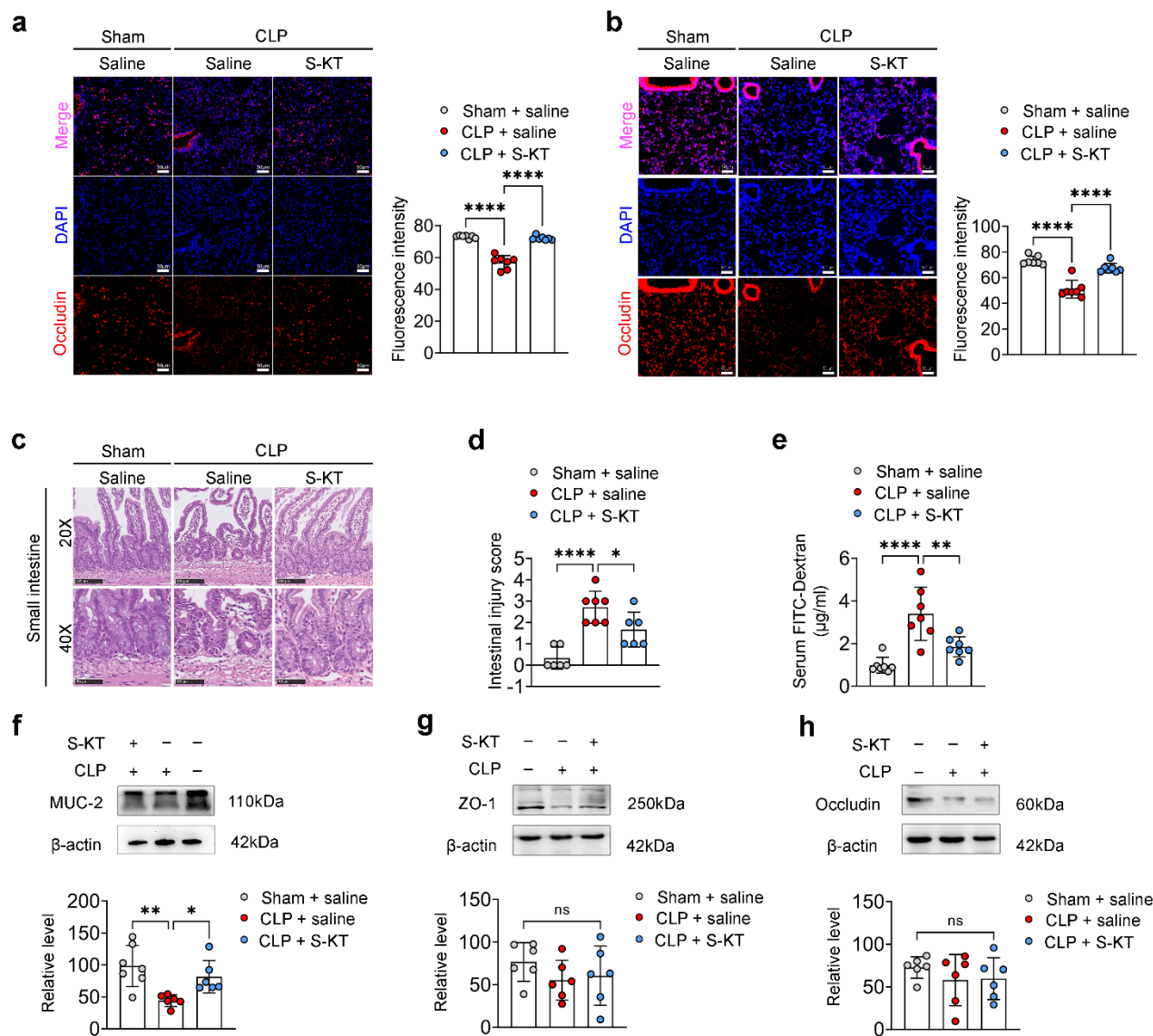


Supplementary Information

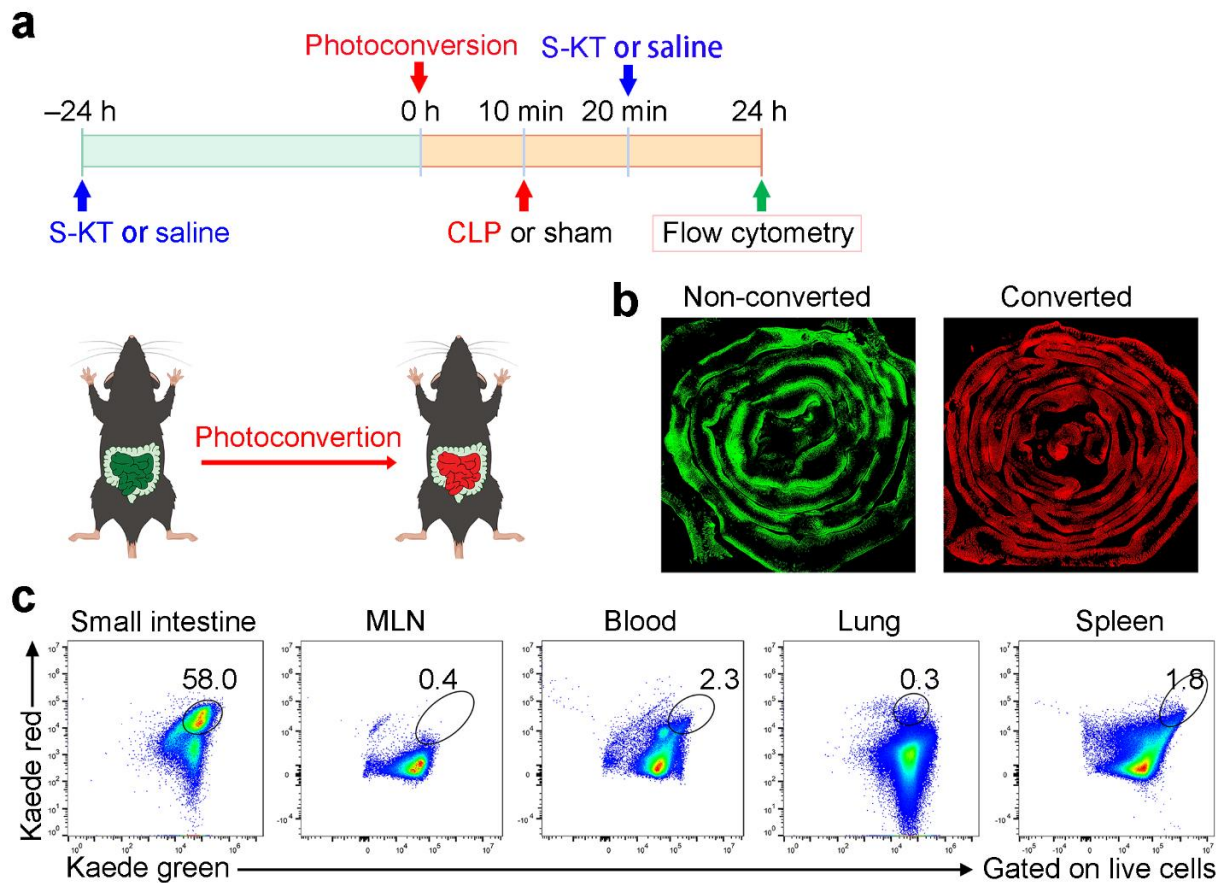
Gut-derived memory $\gamma\delta$ T17 cells exacerbate sepsis-induced acute lung injury in mice



Supplementary Fig. 1. Esketamine ameliorated sepsis-induced pulmonary and intestinal barrier disruption.

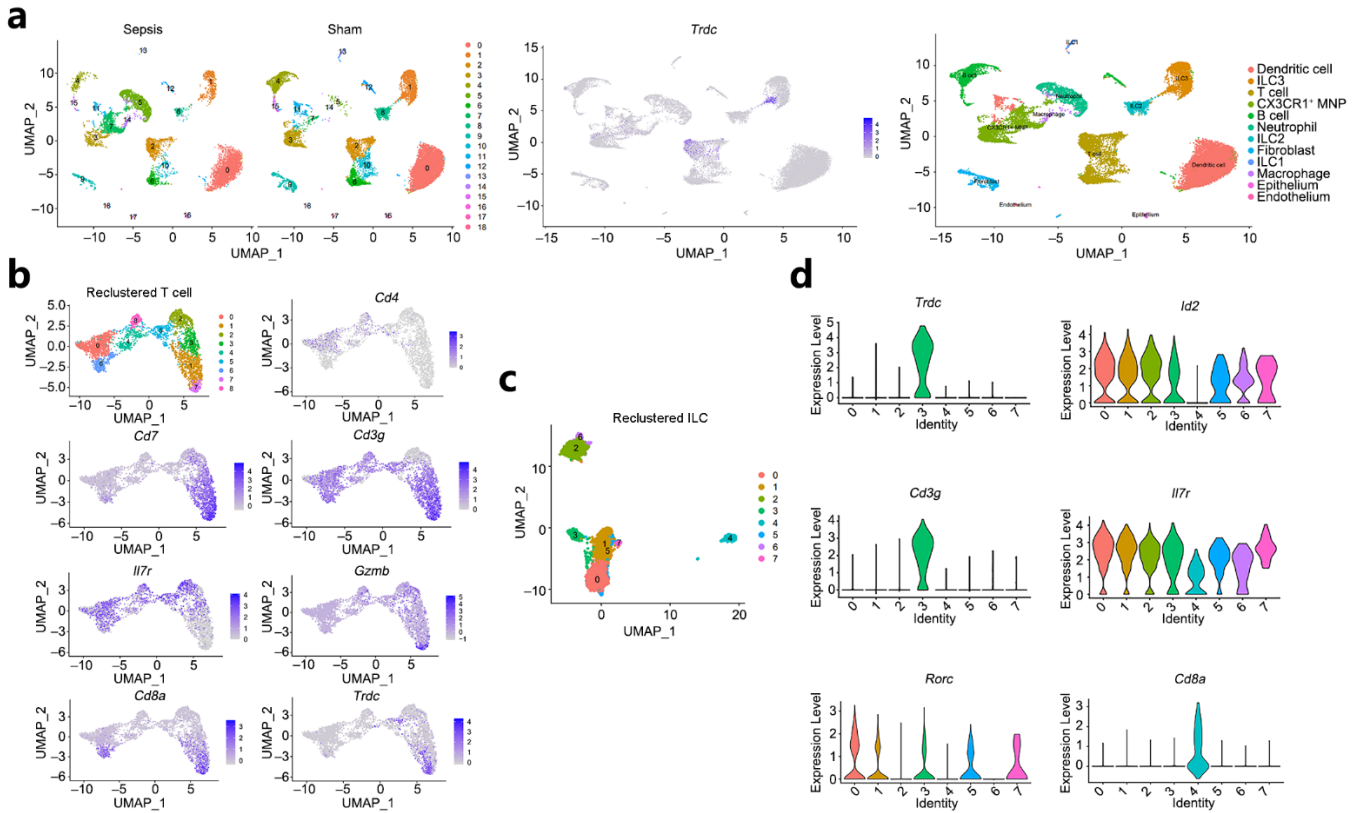
(a, b) Immunofluorescence staining and semi-quantitative analysis of occludin and ZO-1 in mice after combined prophylactic and therapeutic administration of S-KT or 0.9% saline (n = 7). 20X, Scale bar = 50 μ m. **(c, d)** Representative images of H&E staining of the small intestine and the quantitative score of H&E pathological injury in mice after combined prophylactic and therapeutic administration of S-KT or 0.9% saline (n = 6 for sham + saline and CLP + S-KT, 7 for CLP + saline). 20X, Scale bar = 100 μ m. 40X, Scale bar = 50 μ m. **(e)** Intestinal permeability measured by FITC-dextran in the serum of mice after combined prophylactic and therapeutic administration of S-KT or 0.9% saline (n = 7). Western blotting analysis of **(f)** MUC-2, **(g)** ZO-1 and **(h)** occludin in small intestine of mice after combined prophylactic and therapeutic administration

of S-KT or 0.9% saline (n = 6). Data are shown as mean $\pm$ SD. * P < 0.05, ** P < 0.01, **** P < 0.0001. ns, not significant. P values were calculated using one-way ANOVA followed by Tukey's multiple-comparison test (b, c, e–g). Source data and exact P values are provided as a Source Data file.



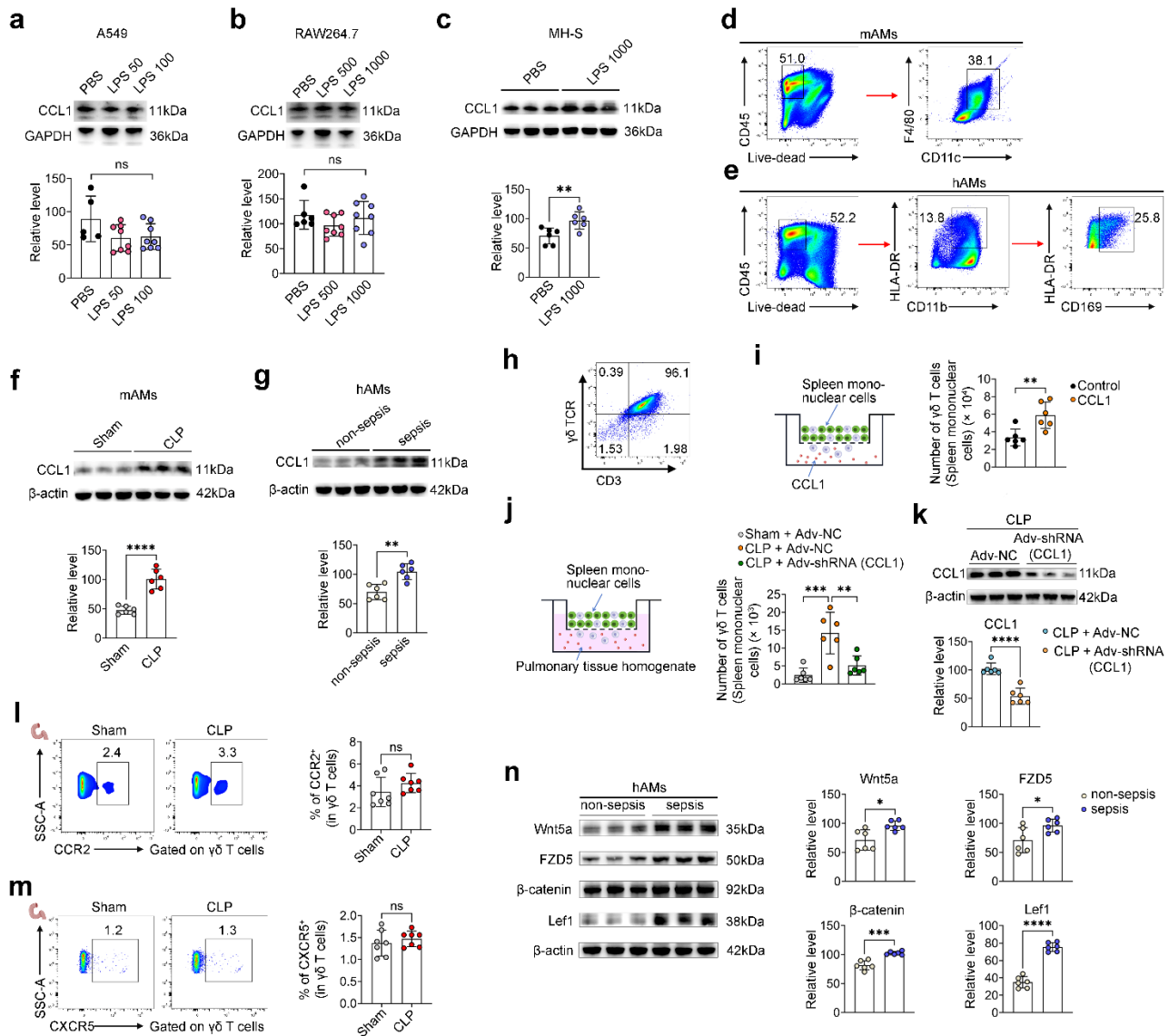
Supplementary Fig. 2. The ultraviolet exposure protocol and fluorescence conversion efficiency in the small intestine of Kaede-tg mice.

(a) Treatment schedule. The small intestine of Kaede transgenic mice was subjected to a 405-nm laser for a duration of 10 min prior to the CLP or sham operation. S-KT, 15 mg/kg) was administered 24 h before and immediately after CLP. 24 h after CLP or sham operation, flow cytometric analysis were carried out on the small intestine and the lung tissues. **(b)** The photoconverted Kaede signal in the small intestine was monitored by fluorescence detection 24 h after photoconversion. **(c)** The Kaede red expression on live cells were detected by fluorescence detection immediately after photoconversion. As we exposed the small intestine to violet light only, the expression of Kaede red on the cells of mesenteric lymph nodes (mLN), blood, lung, and spleen was notably scarce.



Supplementary Fig. 3. Expression pattern of marker genes of $\gamma\delta$ T cell in main cell types, reclustered T cells and innate lymphoid-like cells.

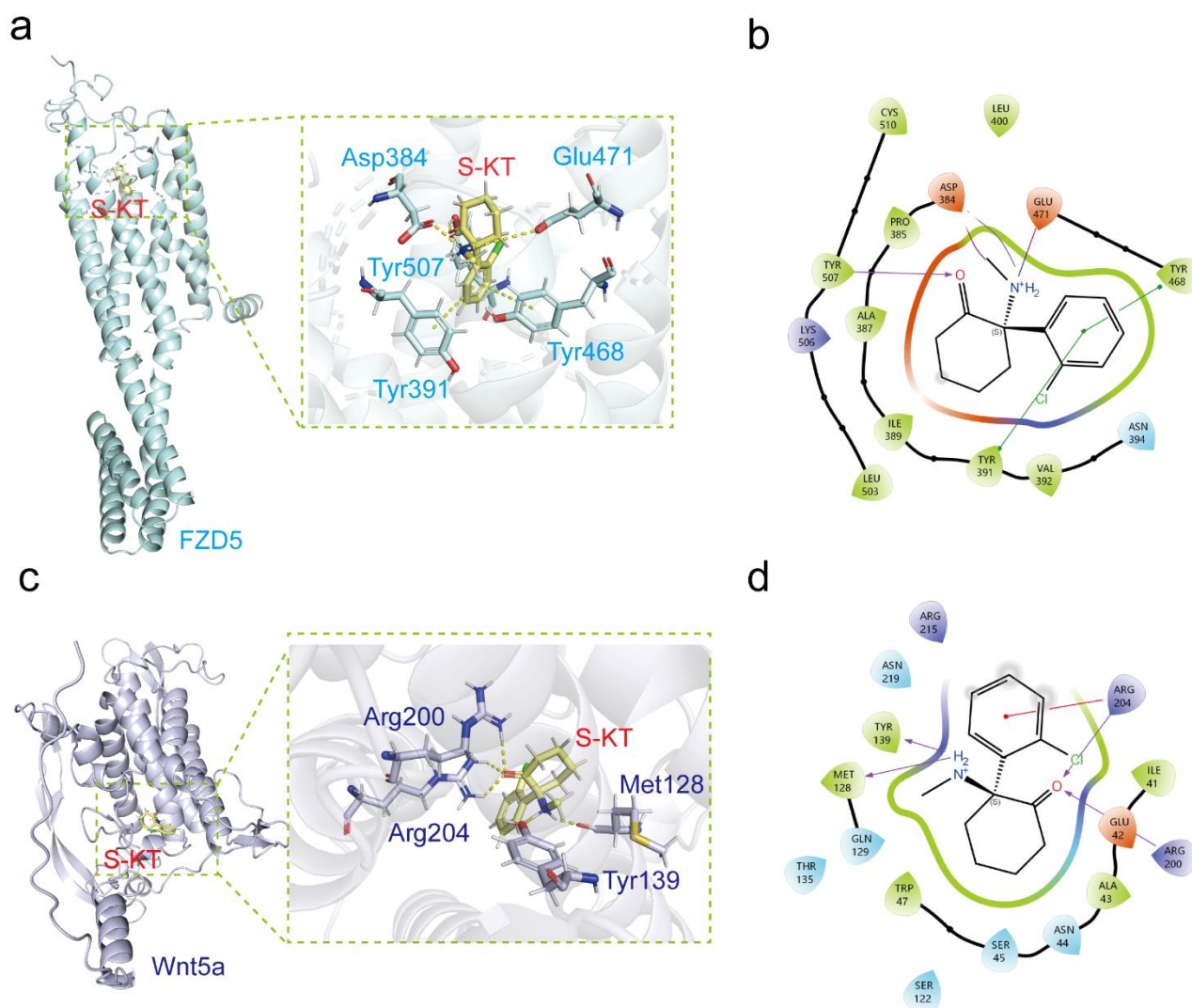
(a) Left, UMAP plot showing clusters of all the cells separately by sample groups. Right, identification of major cell types. Middle, expression levels of *Trdc* in all cells. **(b)** UMAP plots showing expression patterns of marker genes in reclustered T cells, defined for $\gamma\delta$ T cell subsets. **(c)** UMAP plot showing reclustered innate lymphoid-like cells coloured by subclusters. **(d)** Violin plots showing the expression level of marker genes in reclustered innate lymphoid-like cells, defined for $\gamma\delta$ T cell subsets.



Supplementary Fig. 4. LPS induces an increased secretion of CCL1 by MH-S cells.

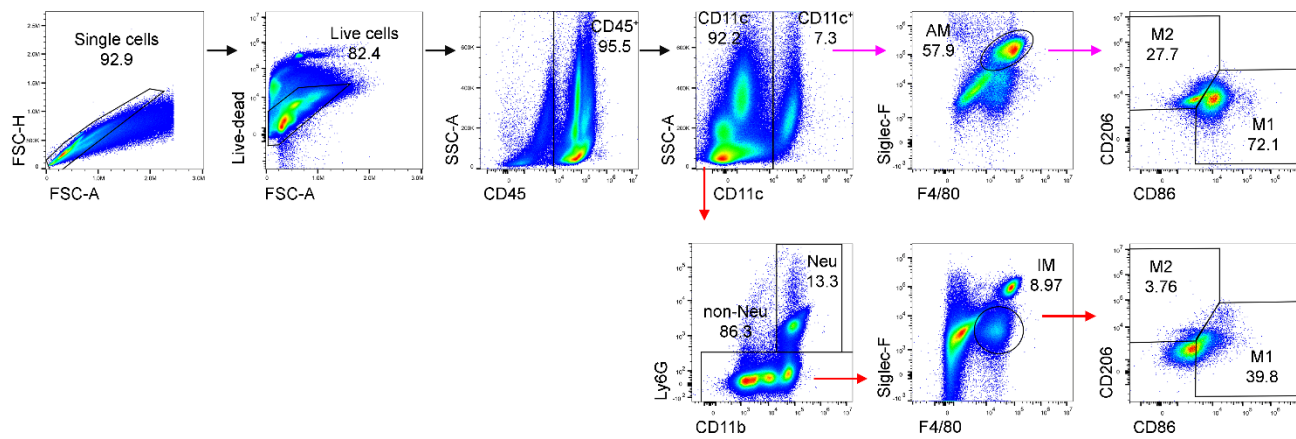
(a) The protein level of CCL1 in the A549 cells at 24 h after LPS (50 or 100 $\mu\text{g ml}^{-1}$) stimulation ($n = 5$ for PBS, 8 for LPS (50 or 100 $\mu\text{g ml}^{-1}$)). **(b)** The protein level of CCL1 in the RAW264.7 cell line at 24 h after LPS (500 or 1000 ng ml^{-1}) stimulation ($n = 6$ for PBS, 8 for LPS (50 or 100 $\mu\text{g ml}^{-1}$)). **(c)** The protein level of CCL1 in the MH-S cell line at 24 h after LPS (1000 ng ml^{-1}) stimulation ($n = 6$). **(d)** FACS strategy to obtain primary mouse AMs (mAMs). **(e)** FACS strategy to obtain primary human AMs (hAMs). **(f)** The protein level of CCL1 in the mAMs at 24 h after CLP ($n = 6$). **(g)** The protein level of CCL1 in the hAMs ($n = 6$). **(h)** Representative FCM plots showing the purity of $\gamma\delta$ T cells after sorting from spleen mononuclear cells. **(i)** Transwell assay was used to assess the ability of CCL1 to chemotaxis $\gamma\delta$ T cells contained in the spleen mononuclear cells ($n = 6$). **(j)** Transwell assay was used to detect the ability of pulmonary tissue

homogenate to chemotaxis $\gamma\delta$ T cells (n = 6). **(k)** The protein level of CCL1 in the lung of septic mice subjected to tracheal administration of CCL1-shRNA Adv or control Adv (n = 6). **(l, m)** CCR2 and CXCR5 expression by intestinal $\gamma\delta$ T cells was measured by FCM analysis (n = 7). **(n)** The protein levels of the components of Wnt signaling pathway (Wnt5a, FZD5, β -catenin, and Lef1) in the hAMs (n = 6). Data are shown as mean $\pm$ SD. ** $P < 0.01$. ns, not significant. P values were calculated using one-way ANOVA followed by Tukey's multiple-comparison test (a, b, k), two-sided Student's unpaired t-test (c, f, g, i, j, h, l–n). Source data and exact P values are provided as a Source Data file.



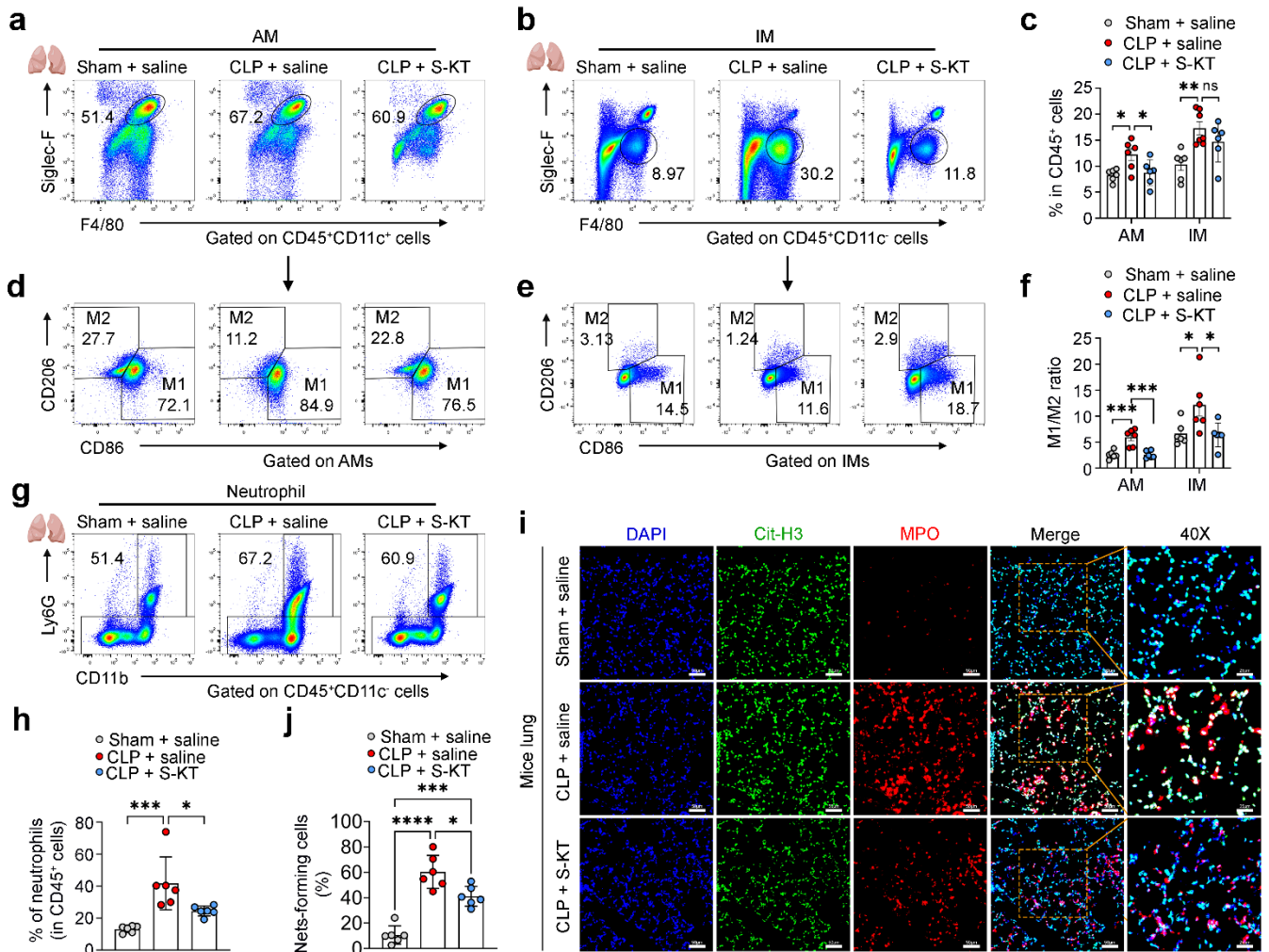
Supplementary Fig. 5. Interaction and binding pocket of FZD5/Wnt5a with esketamine.

(a) The three-dimensional structure diagram and the substrate binding pocket diagram of FZD5. The structure of FZD5 is represented as cartoon in cyan color, esketamine (S-KT) is shown as yellow colored stick diagram, and atoms are colored by element (carbon: orange, green or blue; nitrogen: dark blue; oxygen: red; phosphorus: dark orange). **(b)** The two-dimensional interactions between S-KT and the residues of FZD5. Hydrogen bonds are represented by yellow dashed lines. Atoms are shown in stick models and are colored by element. **(c)** The three-dimensional structure diagram and the substrate binding pocket diagram of Wnt5a. The structure of Wnt5a is represented as cartoon in purple color. **(d)** The two-dimensional interactions between S-KT and the residues of Wnt5a. Hydrogen bonds are represented by yellow dashed lines.



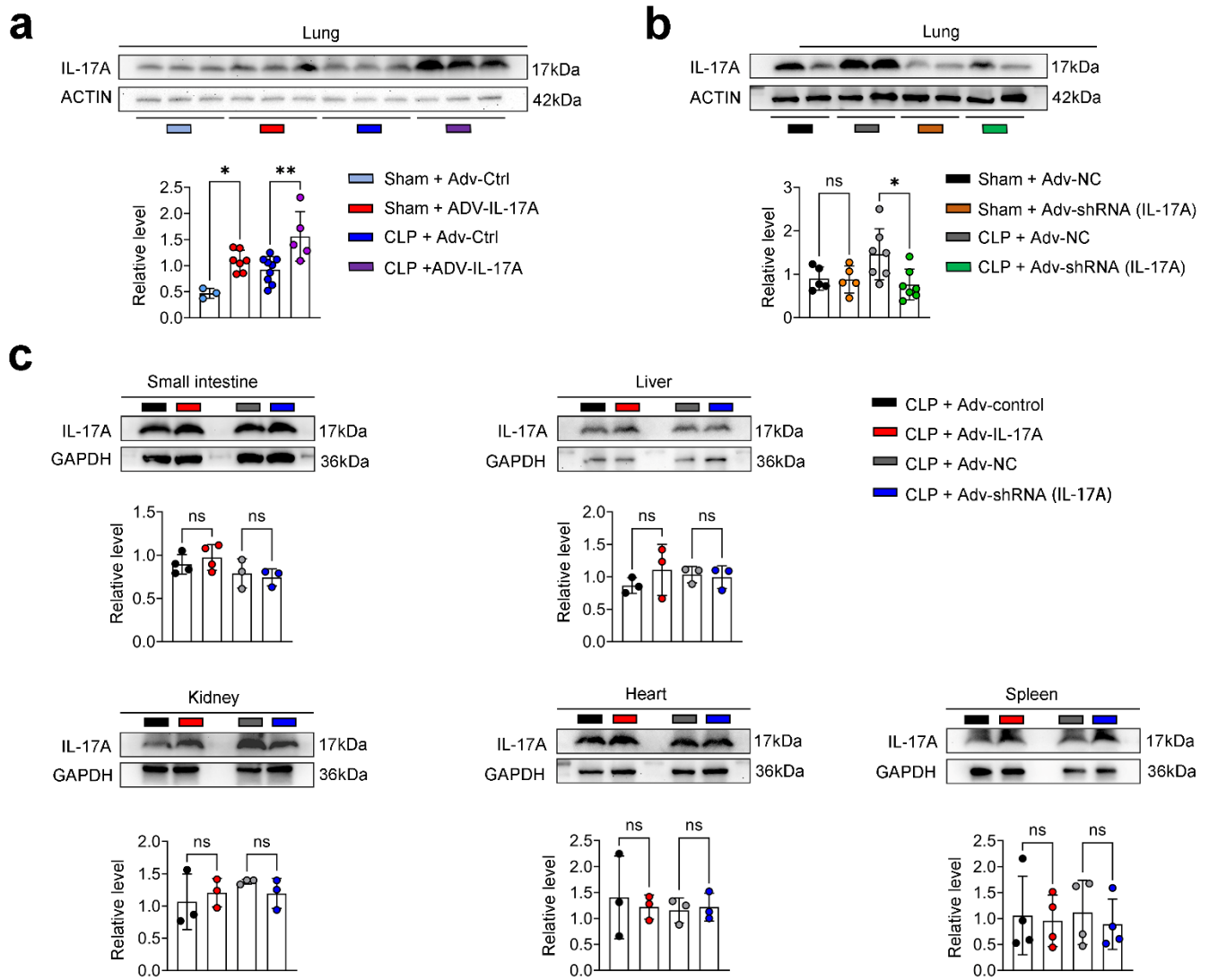
Supplementary Fig. 6. Flow cytometric gating strategy used to identify pulmonary AMs, IMs and neutrophils.

After excluding doublets and exclusion of the dead cells, AMs were identified as CD45⁺ CD11c⁺ F4/80⁺ Siglec-F⁺ cells, IMs were identified as CD45⁺ CD11c⁻ ly6G⁻ F4/80⁺ Siglec-F⁻ cells, and neutrophils were identified as CD45⁺ CD11c⁻ ly6G⁺ CD11b⁺ cells. Finally, using CD206 and CD86, AMs and IMs were classified as M1 (CD86⁺ CD206⁻) and M2 (CD86⁻ CD206⁺) subpopulations.



Supplementary Fig. 7. The impact of esketamine on the primary immune cells in septic lung.

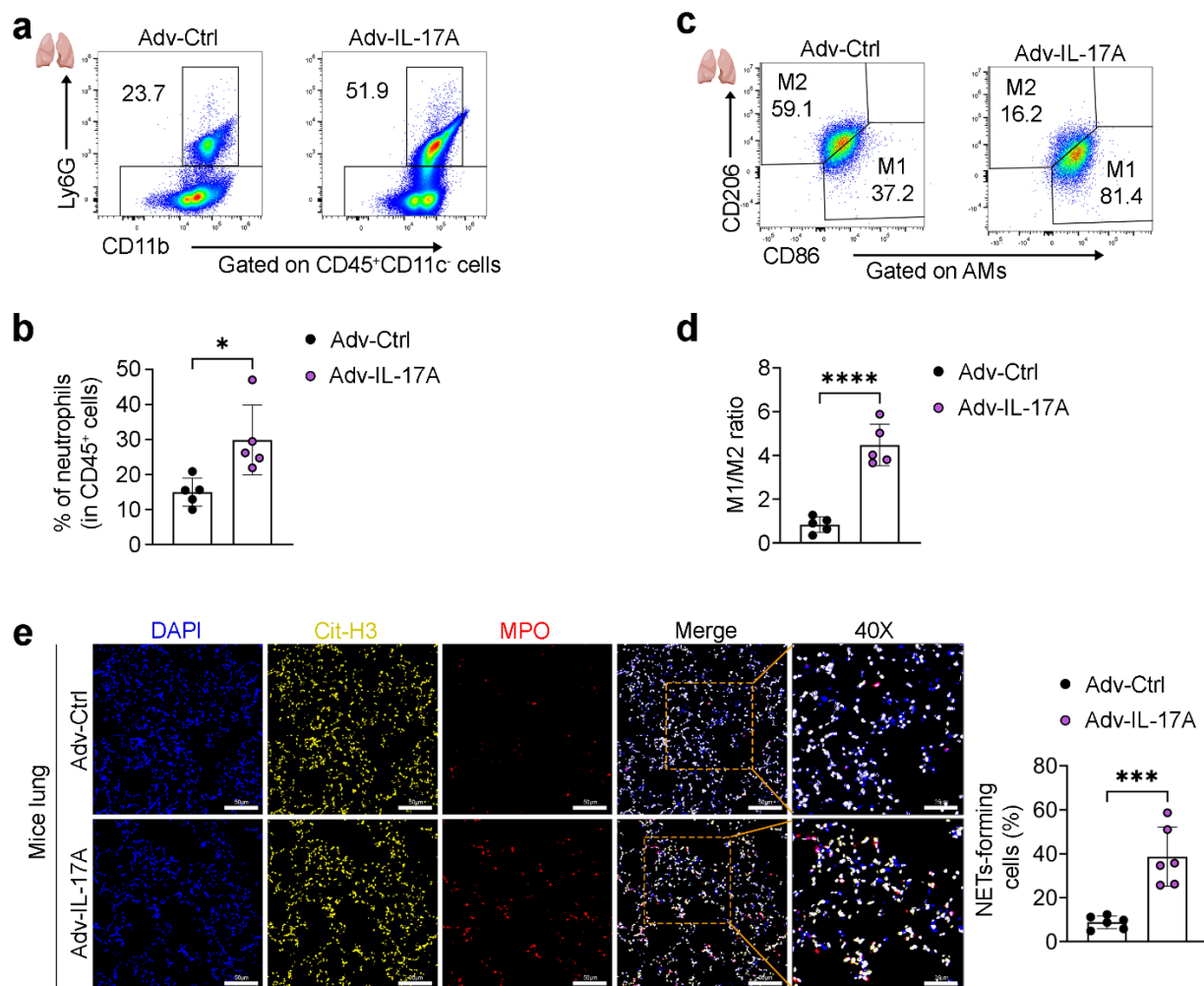
(a–c) Representative FCM plots and percentage of AMs and IMs in CD45⁺ cells in the lung of mice after prophylactic and therapeutic administration of S-KT or 0.9% saline (n = 6 for sham + saline, CLP + S-KT and AM in CLP + saline, 7 for IM in CLP + saline). **(d–f)** Representative FCM plots and the ratio of M1 to M2 subpopulations of AMs and IMs in the lung of mice after prophylactic and therapeutic administration of S-KT or 0.9% saline (n = 6). **(g, h)** Representative FCM plots and percentage of neutrophils in the lung of mice after prophylactic and therapeutic administration of S-KT or 0.9% saline (n = 6). **(i, j)** Representative images and semi-quantitative analysis of NETs formation in the lung detected by immunofluorescence staining with Cit-H3 and MPO (n = 6), 20X, Scale bar = 50 μ m. 40X, Scale bar = 25 μ m. Data are shown as mean $\pm$ SD. **P* < 0.05, ****P* < 0.001, *****P* < 0.0001, ns, not significant. *P* values were calculated using one-way ANOVA followed by Tukey's multiple-comparison test (c, f, h, j). Source data and exact *P* values are provided as a Source Data file.



Supplementary Fig. 8. The influence of tracheal administration of IL-17A overexpression adenovirus and IL-17A-shRNA adenovirus on IL-17A expression in various organs.

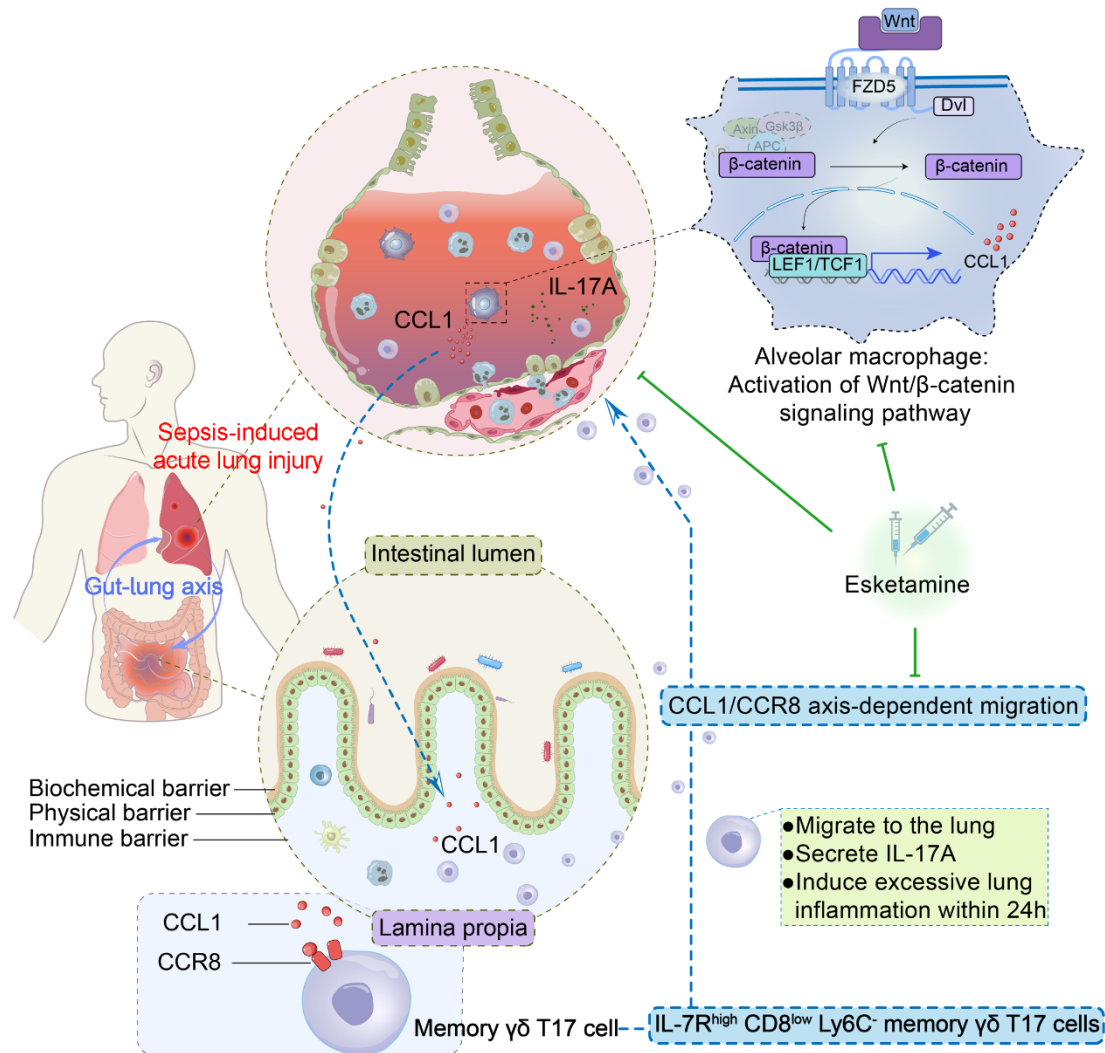
(a) The protein level of IL-17A in the lung of sham-operated and septic mice subjected to tracheal administration of IL-17A overexpression Adv or control Adv ($n = 3$ for sham + Adv-Ctrl, 7 for sham + Adv-IL-17A, 9 for CLP + Adv-Ctrl, 5 for CLP + Adv-IL-17A). **(b)** The protein level of IL-17A in the lung of sham-operated and septic mice subjected to tracheal administration of IL-17A-shRNA Adv or control Adv ($n = 5$ for sham + Adv-Ctrl and sham + Adv-IL-17A, 7 for CLP + Adv-Ctrl and CLP + Adv-IL-17A). **(c)** Alterations in the protein levels of IL-17A in the small intestine, liver, kidney, heart, and spleen in mice treated with Adv-IL-17A (or Adv-Ctrl) or Adv-shRNA (IL-17A) (or Adv-NC) (For the small intestine: $n = 4$ for CLP + Adv-Ctrl and CLP + Adv-IL-17A, 3 for CLP + Adv-NC and CLP + Adv-shRNA (IL-17A); For the liver, kidney and heart: $n = 3$; For the spleen: $n = 4$). Data are shown as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$. ns, not significant.

P values were calculated using two-sided Student's unpaired t-test (a–c). Source data and exact *P* values are provided as a Source Data file.



Supplementary Fig. 9. The influence of IL-17A overexpression on non-septic mice lung.

Mice which received tracheal administration of IL-17A overexpression adenovirus (Adv-IL-17A) or control Adv (Adv-Ctrl) were subjected to relevant experiments 4 days later. **(a, b)** Representative FCM plots and percentage of neutrophils in CD45⁺ cells in the lung of mice treated with Adv-IL-17A or Adv-Ctrl (n = 5). **(c, d)** Representative FCM plots and ratio of M1 to M2 subpopulations of AMs in the lung of mice treated with Adv-IL-17A or Adv-Ctrl (n = 5). **(e)** Representative images and semi-quantitative analysis of NETs formation in the lung detected by immunofluorescence staining with Cit-H3 and MPO (n = 6), 20X, Scale bar = 50 μ m. 40X, Scale bar = 25 μ m. Data are shown as mean $\pm$ SD. * P < 0.05, *** P < 0.001, **** P < 0.0001. P values were calculated using two-sided Student's unpaired t-test (b, d, e). Source data and exact P values are provided as a Source Data file.



Supplementary Fig. 10. Esketamine mitigates pulmonary injury in early sepsis by targeting Wnt/ β -catenin signaling and $\gamma\delta$ T17 cell migration.

In the initial 24 hours of sepsis, pulmonary alveolar macrophages activate the Wnt/ β -catenin signaling pathway, inducing the upregulation of CCL1 via the transcription factor Lef1. This prompts Ly6C⁻ IL-7R^{high} CD8^{low} memory $\gamma\delta$ T17 cells from the small intestine to migrate to the lungs, exacerbating inflammation and damage. S-KT inhibits the pulmonary Wnt/ β -catenin pathway, curbing $\gamma\delta$ T17 cell migration to the lungs and mitigating septic ALI.

Supplementary table 1.**Key resources table**

Reagent or resource	Company	Identifier
Antibodies		
CCL1	R&D Systems	Cat# MAB845; RRID: AB_2070618
CCL1	ABclonal	Cat# A10670; RRID: AB_2768750
IL-17A	Santa Cruz	Cat# sc-374218, RRID: AB_10988239
β -actin	Proteintech	Cat# 66009-1-Ig, RRID: AB_2687938
GAPDH	Proteintech	Cat# 60004-1-Ig, RRID: AB_2107436
IL-6	Abcam	Cat# ab229381, RRID: AB_2861234
IL-1 β	ABclonal	Cat# A1112, RRID: AB_2758416
MUC-2	Proteintech	Cat# 27675-1-AP, RRID: AB_2880943
ZO-1	Proteintech	Cat# 21773-1-AP, RRID: AB_10733242
Occludin	Proteintech	Cat# 27260-1-AP, RRID: AB_2880820
Wnt5a	Zen Bio	Cat# 619919, RRID: AB_2927750
FZD5	Zen Bio	Cat# 222996, RRID: AB_3075327
β -Catenin	Proteintech	Cat# 51067-2-AP, RRID: AB_2086128
Lef1	Proteintech	Cat# 14972-1-AP, RRID: AB_2265677
	Cell Signaling	
Lef1	Technology	Cat# 76010, RRID: AB_2799877
TCF1/7	Abclonal	Cat# A20835, RRID: AB_3066057
HRP-goat anti-mouse IgG (HL)	AntGene	Cat# ANT019, RRID: AB_3101880
PerCP-Cy TM 5.5 anti-mouse CD45	BD Biosciences	Cat# 550994, RRID: AB_394003
FITC anti-mouse CD11b	BD Biosciences	Cat# 561688, RRID: AB_10898180

PE-Cy TM 7 anti-mouse Ly-6G	BD Biosciences	Cat# 560601, RRID: AB_1727562
APC anti-mouse CD86	BD Biosciences	Cat# 558703, RRID: AB_2075114
BV421 anti-mouse F4/80	BD Biosciences	Cat# 565411, RRID: AB_2734779
BV650 anti-mouse CD11c	BD Biosciences	Cat# 564079, RRID: AB_2725779
PE-CF594 anti-mouse Siglec-F	BD Biosciences	Cat# 552125, RRID: AB_394340
BV605 anti-mouse CD206	Biolegend	Cat# 141721, RRID: AB_2562340
Fixable Viability Stain 510	BD Biosciences	Cat# 564406, RRID: AB_2869572
FITC anti-mouse CD45	BD Biosciences	Cat# 553079, RRID: AB_394609
PE-Cy TM 7 anti-mouse CD3e	BD Biosciences	Cat# 552774, RRID: AB_394460
APC anti-mouse TCR γ/δ	Biolegend	Cat# 118115, RRID: AB_1731824
Brilliant Violet 421 TM anti-mouse IL-17A	Biolegend	Cat# 506925, RRID: AB_10900442
APC/Fire750 anti-mouse CD8a	Biolegend	Cat# 100766, RRID: AB_2572113
Purified rat anti-mouse CD16/CD32	BD Biosciences	Cat# 553141, RRID: AB_39465
BV786 anti-mouse CD44	BD Biosciences	Cat# 563736, RRID: AB_2738395
Alexa Fluor 700 anti-Mouse Ly-6C (AL-21)	BD Biosciences	Cat# 561237, RRID: AB_10612017
FITC anti-mouse IFN- γ (XMG1.2)	BD Biosciences	Cat# 554411, RRID: AB_395375
PE anti-mouse CD198 (CCR8)	Biolegend	Cat# 150312, RRID: AB_2617032
PE anti-mouse CD185 (CXCR5)	Biolegend	Cat# 145504, RRID: AB_2561968
BV421 anti-mouse CD192 (CCR2)	Biolegend	Cat# 150605, RRID: AB_2571913
BV421 anti-mouse Ki67	Biolegend	Cat# 652411, RRID: AB_2562663
Purified hamster anti-mouse TCR γ/δ (GL4)	BD Biosciences	Cat# 553180, RRID: AB_394690

	Thermo Fisher	
BV605 anti-mouse IL-7R	Scientific	Cat# 416-1271-82, RRID: AB_2929104
FITC anti-mouse/human CD11b	Biolegend	Cat# 101205, RRID: AB_312788
PerCP-Cy™5.5 anti-human CD45	Biolegend	Cat# 304027, RRID: AB_1236444
PE-Cy™7 anti-human HLA-DR	Biolegend	Cat# 361611, RRID: AB_2783278
APC anti-human CD169 (Siglec-1)	Biolegend	Cat# 307609, RRID: AB_314688
Goat anti-rabbit IgG H&L (Alexa Fluor® 488)	Abcam	Cat# ab150077, RRID: AB_2630356
Goat anti-mouse IgG H&L (Alexa Fluor® 594)	Abcam	Cat# ab150116, RRID: AB_2650601
Goat anti-rabbit IgG H&L (HRP)	Abcam	Cat# ab205718, RRID: AB_2819160
Cith3	Abcam	Cat# ab5103, RRID: AB_304752
MPO	Abcam	Cat# ab208670, RRID: AB_2864724
Chemicals, adenoviruses and recombinant proteins		
Esketamine	Hengrui	
LPS (Escherichia coli; 055:B5)	Sigma-Aldrich	Cat# L2880
Recombinant mouse CCL1 protein	R&D Systems	Cat# 845-TC
iCRT14	Selleck	Cat# S8704
pADV-U6-shRNA (Ccl1)-CMV- MCS	Obio	Cat# Y25327
pADV-U6-shRNA (NC2)-CMV- MCS	Obio	Cat# Y5068

pADV-U6-shRNA (Il17a)-CMV-EGFP	Obio	Cat# Y22590
pADV-U6-shRNA (NC2)-CMV-EGFP	Obio	Cat# Y201NC2
pAdeno-EF1A(S)-mNeonGreen-CMV-MCS-3FLAG	Obio	Cat# H12588
pcADV-EF1-mNeonGreen-CMV-Il17a-3xFLAG	Obio	Cat# H27226
Critical commercial assays		
TCR g/d cell isolation kit	Miltenyi	Cat# 130-092-125
Cytometric bead array (CBA) mouse Th1/Th2/Th17 cytokine kit	BD Biosciences	Cat# 560485
MPO assay kit	Nanjing Jiancheng	Cat# A044-1-1
QuantiCyto® Mouse IL-6 ELISA kit	Neobioscience	Cat# EMC004.96
QuantiCyto® Mouse IL-17/IL-17A ELISA kit	Neobioscience	Cat# EMC008.96
QuantiCyto® Mouse TNF- α ELISA kit	Neobioscience	Cat# EMC102a.96
Bio-plex pro mouse chemokine Panel 31-Plex	Bio-rad	Cat# 12009159