## Supplementary materials

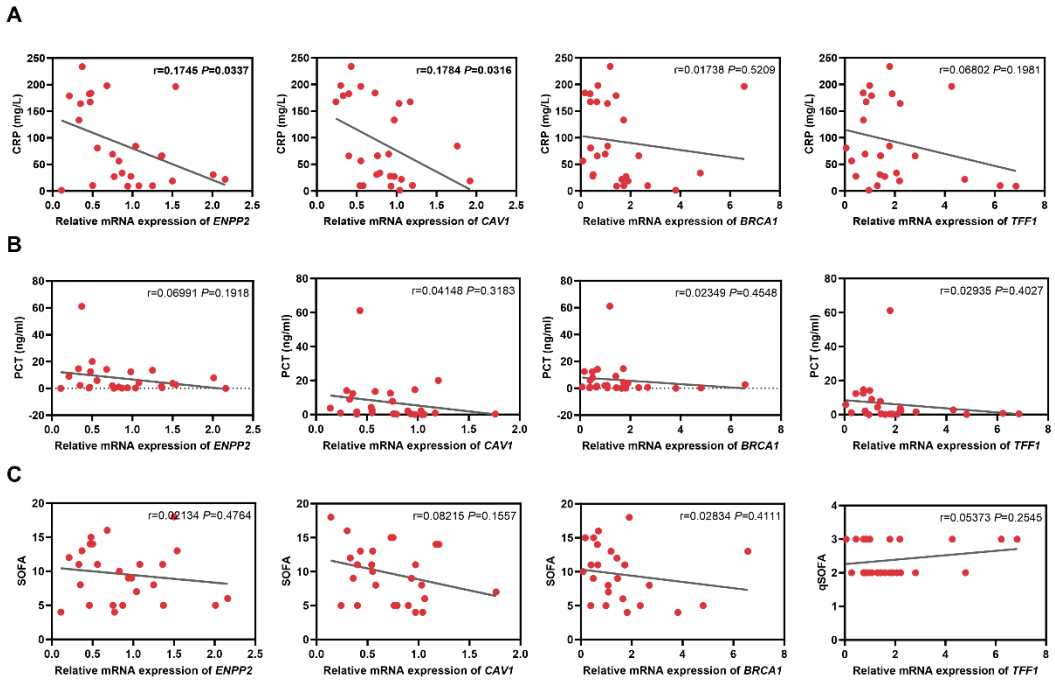



**Fig. S1 Correlation analysis of *ENPP2*, *CAV1*, *BRCA1*, *TFF1* expression and disease severity in patients with sepsis.** Whole blood samples from patients with sepsis were collected. n = 26. (A) Correlation between the mRNA expression of *ENPP2*, *CAV1*, *BRCA1*, *TFF1* and CRP levels in the blood samples of sepsis patients was analyzed by Pearson correlation analysis. (B) Correlation between the mRNA expression of *ENPP2*, *CAV1*, *BRCA1*, *TFF1* and PCT levels in the blood samples of sepsis patients was analyzed by Pearson correlation analysis. (C) Correlation between the mRNA expression of *ENPP2*, *CAV1*, *BRCA1*, *TFF1* and SOFA of sepsis patients was analyzed by Pearson correlation analysis. Pearson correlation analysis was used in A-C. Data are expressed as mean ± SEM. *r*, Pearson correlation coefficient.

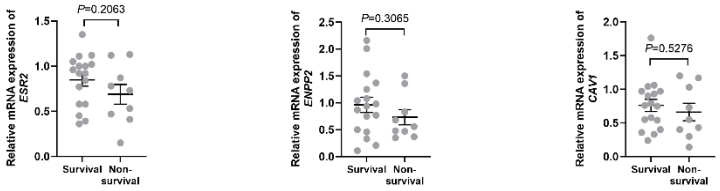



**Fig. S2 The mRNA expression of *ESR2*, *ENPP2* and *CAV1* in whole blood samples of patients with sepsis.** Sepsis patients were divided into 28 days survivors (n = 17) and non-survivors (n =9), and whole blood samples from patients with sepsis (n = 26) were collected. The mRNA expression of *ESR2*, *ENPP2* and *CAV1* in whole blood samples of survivors and non-survivors was analyzed using qPCR. Unpaired student *t*-test was performed in S2.

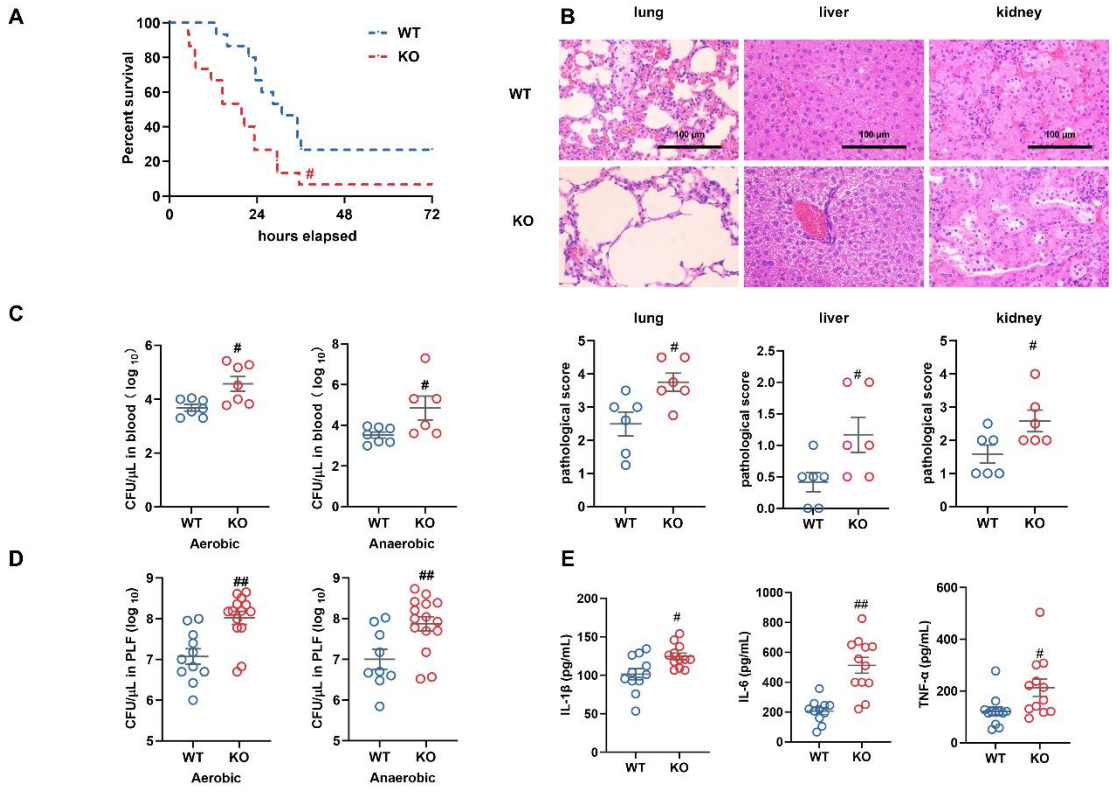



**Fig. S3 Effect of ERβ deficiency on disease severity in female septic mice.** The female WT and KO mice were subjected to CLP. (A) The survival curve of septic mice,

n = 15. (B) H&E staining and histopathological scores of the lung, liver and kidney from septic mice. n=6. (C and D) Quantification of bacterial colonies in the blood (C) and PLF (D) from septic mice. n = 6-15. (E) Quantification of cytokines in the sera from septic mice. n = 11-12. Long rank (Mantel Cox) test was adopted to compare the significance in A. Unpaired student *t*-test was performed in B-E. Data are expressed as mean ± SEM. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs*. WT group. Scale bar, 100 μm.

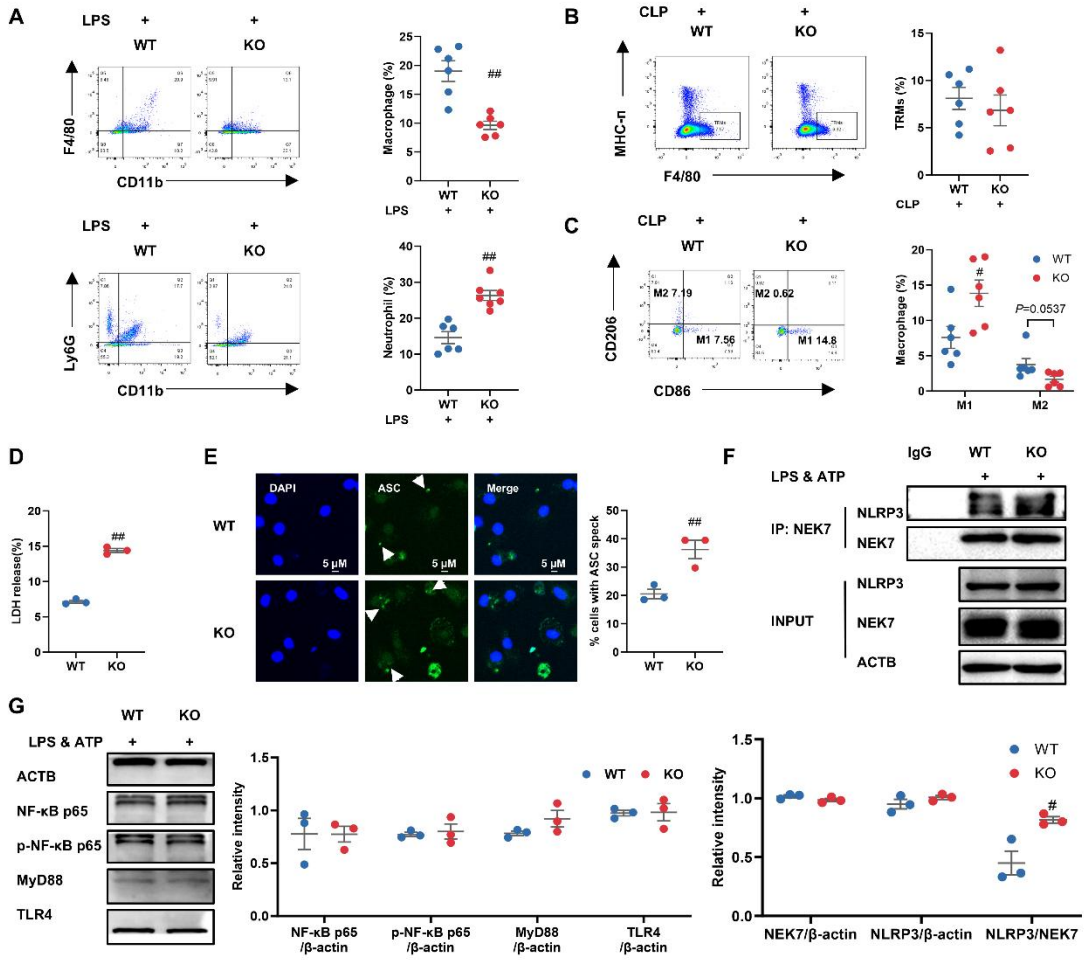



**Fig. S4 Effect of ERβ deficiency on the proportion of phagocytic cells in the PLF of septic mice induced by LPS or CLP and effect of ERβ deficiency on the pyroptosis of primary mouse PMs.** (A) Flow cytometric analysis of macrophages or

neutrophils in PLF from WT and KO septic mice subjected to intraperitoneal injection of LPS. n=6-7. (B) Flow cytometric analysis of TRMs in PLF from septic mice subjected to CLP. n=6. (C) Flow cytometric analysis of M1 ($CD86^+CD206^-$ cells) and M2 ($CD86^-CD206^+$ cells) macrophages in PLF from septic mice subjected to CLP. n=6. (D-G) Primary mouse PMs were treated with LPS (2 μg/mL) for 3 h, followed by ATP (5 mM) treatment for 1 h, n=3. (D) LDH release. (E) The ASC oligomerization in PMs was detected by immunofluorescence assay. (F) The interaction between NLRP3 and NEK7 in PMs was measured by using Co-IP. (G) The protein expression of NF-κB p65, p-NF-κB p65, MyD88 and TLR4 of PMs was analyzed by western blot. Unpaired student *t*-test was performed in A-G. Data are expressed as mean ± SEM. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs*. WT group. Scale bar, 5 μm.

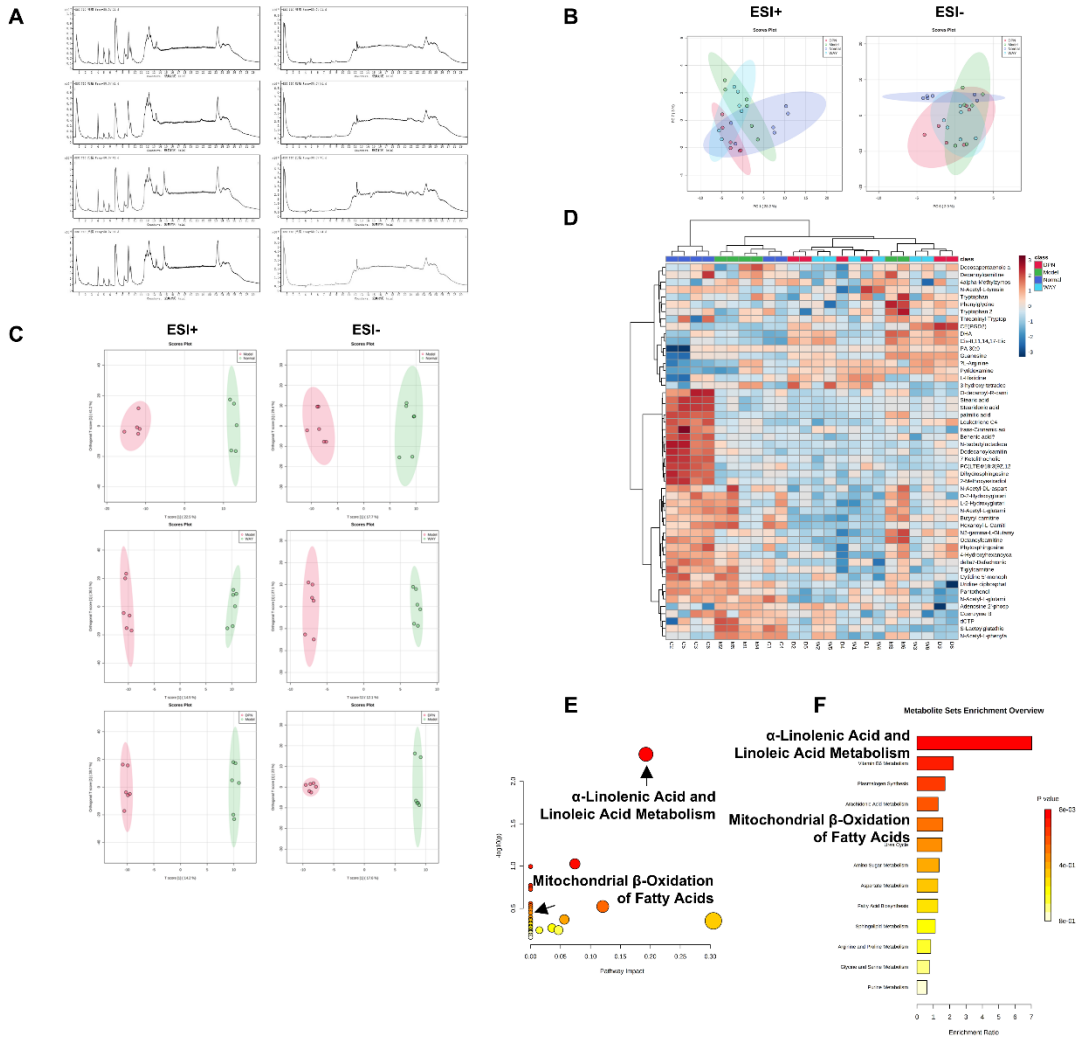



**Fig. S5 Effect of ERβ activation on macrophage metabolism under pyroptosis conditions.** Primary mouse PMs were stimulated with LPS (2 μg/mL), treated with or without ERβ specific agonists WAY200070 (WAY, 3 μM) or diarylpropionitrile (DPN, 3 μM) for 3 h, and then treated with ATP (5 mM). n=6. (A) The total positive and negative ion chromatograms of PMs. (B) The PCA plots of cell samples obtained from the normal group, model group, WAY (3 μM) and DPN (3 μM) groups. (C) S-plots generated from the OPLS-DA model between the normal group and model group in the ESI positive ion mode and negative ion mode. (D) Heatmap based on the relative abundance of metabolites. (E and F) Pathway analysis between Normal group and

model group.

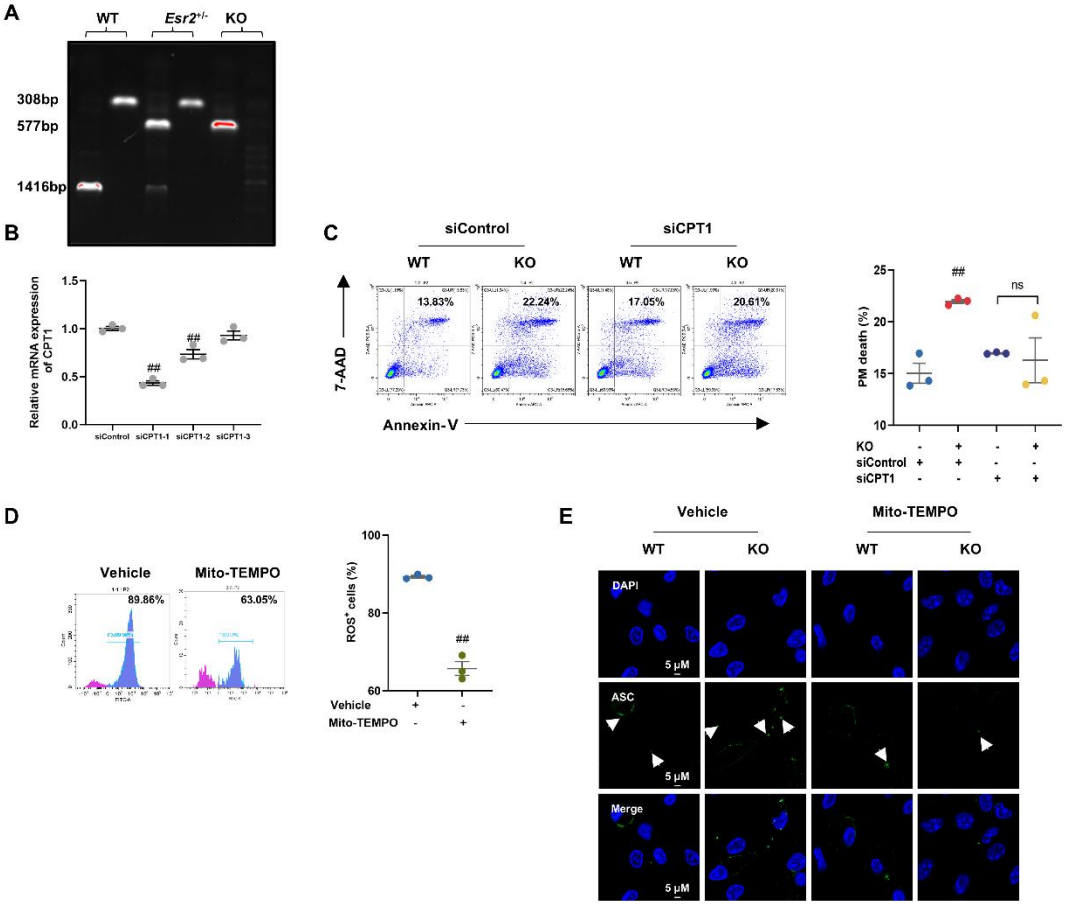



**Fig. S6 Verification of gene knockout efficiency in mice and siRNA knockdown efficiency in PMs.** (A) The identification of wild-type mice (WT), *Esr2*$^{+/-}$ mice, and *Esr2*$^{-/-}$ mice (KO) were analyzed by DNA gel electrophoresis. (B and C) The primary mouse PMs were transfected with siControl or siCPT1, n=3. (B) The mRNA expression was detected by using qPCR. (C) Cell death analysis of PMs. (D and E) The primary mouse PMs, pre-treated with mitochondrial-specific ROS inhibitor Mito-TEMPO (50 nM, HY-112879, MCE) or vehicle for 24 h, were treated with LPS (2 μg/mL) for 3 h, followed by ATP (5 mM) treatment for 1 h, n=3. The proportion of ROS-positive cells was quantified by flow cytometry. (E) The display diagram of ASC oligomerization in

PMs. Unpaired student *t*-test was performed in B and D. Two-way ANOVA was employed in C. Data are expressed as the means ± S.E.M. n=3 $^{\#\#}P < 0.01$ *vs.* siControl group or vehicle group.

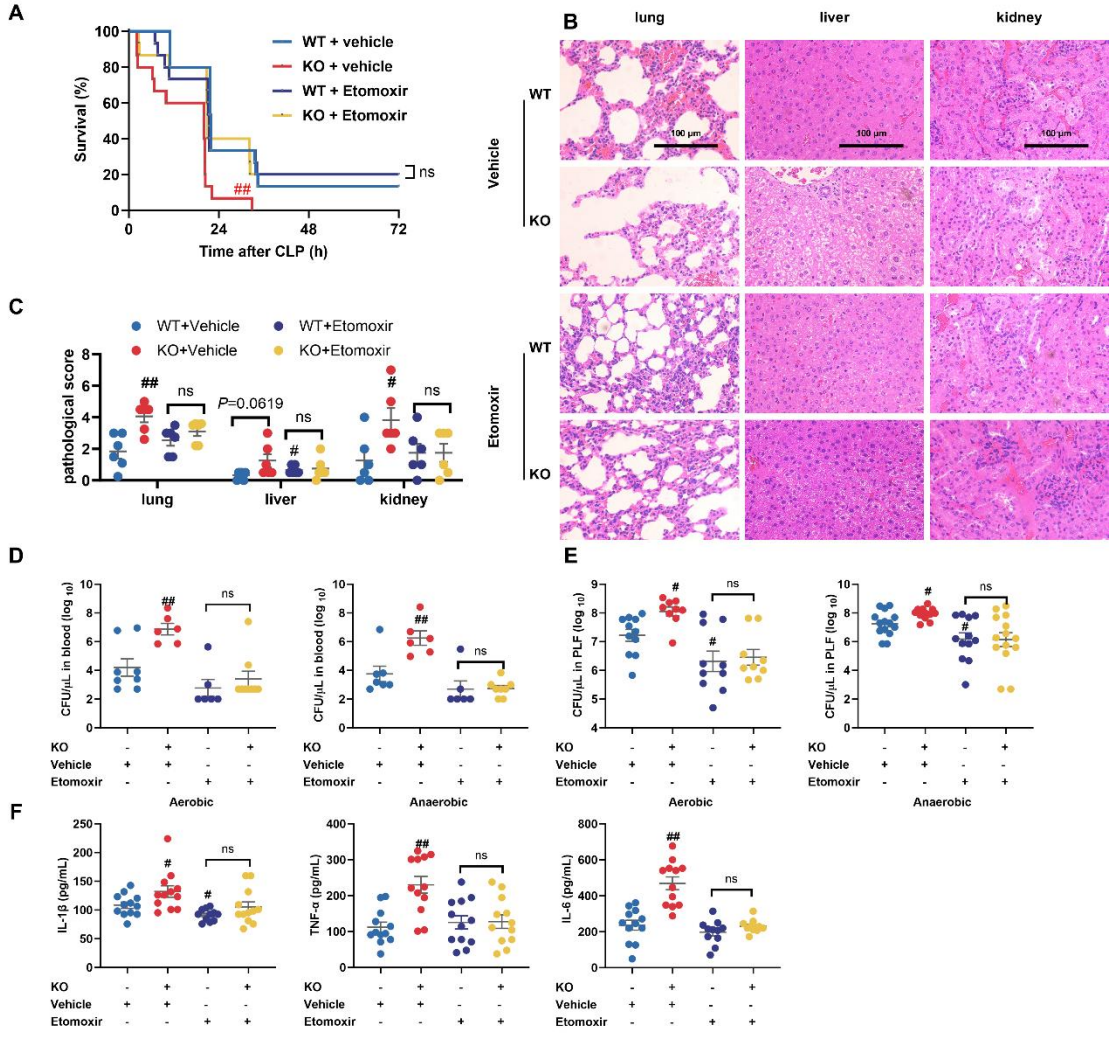



**Fig. S7 Effect of etomoxir on the exacerbation of sepsis outcomes induced by CLP in the context of ERβ deficiency.** Mice receiving etomoxir treatment were administered an intraperitoneal injection of 100 μL of etomoxir (3 mg/mL, Cayman Chemical) dissolved in sterile PBS containing 10% DMSO 1 h prior to the establishment of the CLP model. (A) The survival curve of septic mice, n = 15. (B and

C) H&E staining and histopathological scores of the lung, liver and kidney from septic mice. n=6. (D and E) Quantification of bacterial colonies in the blood and PLF from septic mice. n = 6-14. (F) Quantification of cytokines in the sera from septic mice. n = 11-12. Long rank (Mantel Cox) test was adopted to compare the significance in A. Two-way ANOVA was employed in C-F. Data are presented as mean ± SEM. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs*. WT + control group. ns, no significance. Scale bar, 100 μm.

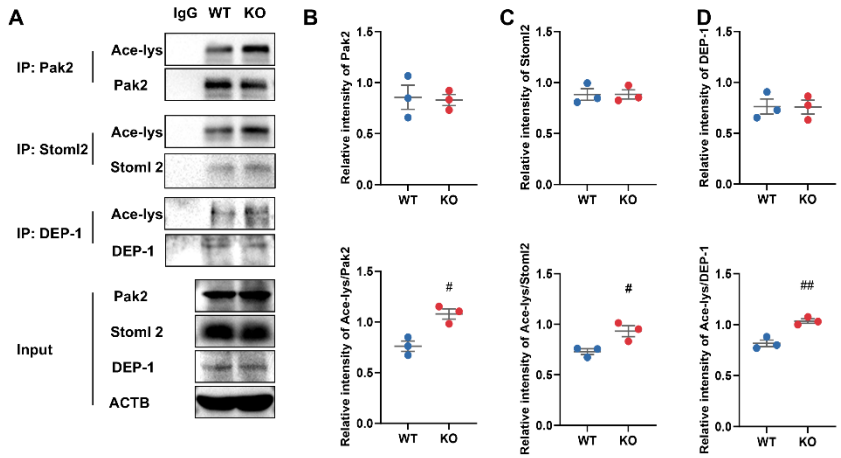



**Fig. S8 Effect of ERβ deficiency on the acetylation of lysine of pak2, stoml2 and DEP-1.** (A-D) The primary mouse PMs were treated with LPS (2 μg/mL) for 3 h, followed by ATP (5 mM) treatment for 1 h, n=3. The acetylation of lysine of Pak2, Stoml2 and DEP-1 in PMs was measured by using CO-IP. Unpaired student *t*-test was performed in B-D. Data are expressed as the means ± S.E.M. n = 3. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs*. WT group.

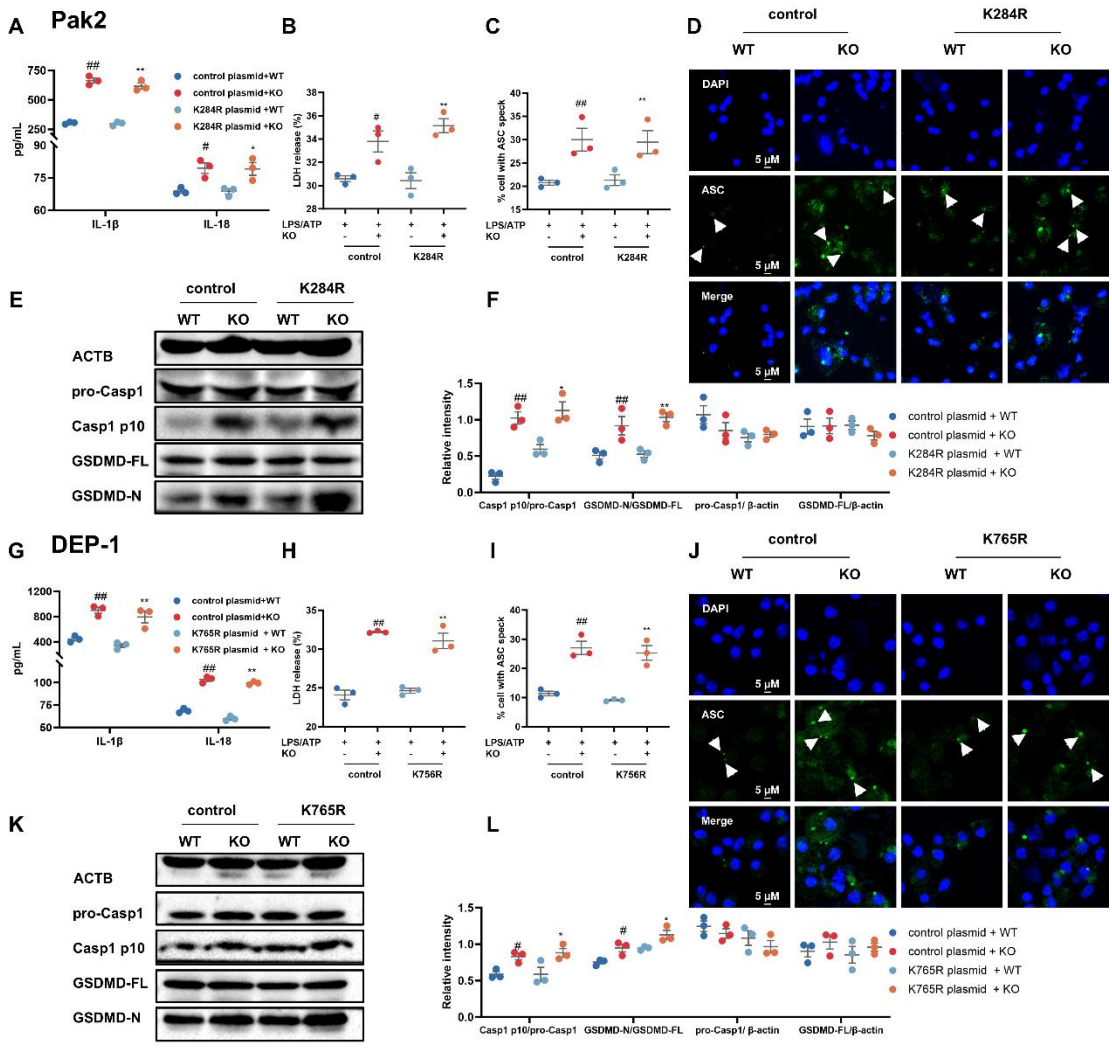



**Fig. S9 Effect of mutations in the acetylated lysine site of Pak2 or DEP-1 on ERβ deficiency-drove macrophage pyroptosis.** (A-F) The primary mouse PMs, transfected with either control plasmid or K284R plasmid, were treated with LPS (2 μg/mL) for 3 h, followed by ATP (5 mM) treatment for 1 h, n=3. (A) IL-1β and IL-18 levels, (B) LDH release were measured in the supernatants of PMs. (C and D) The ASC oligomerization in PMs was measured by using immunofluorescence assay. (E and F) The protein expression of Casp1 p10, Pro-Casp1, GSDMD-FL and GSDMD-N of PMs was analyzed by western blot. (G-L) The primary mouse PMs, transfected with either control plasmid or K765R plasmid, were treated with LPS (2 μg/mL) for 3 h, followed

by ATP (5 mM) treatment for 1 h, n=3. (G) IL-1β and IL-18 levels, (H) LDH release were measured in the supernatants of PMs. (I and J) The ASC oligomerization in PMs was measured by using immunofluorescence assay. (K and L) The protein expression of Casp1 p10, Pro-Casp1, GSDMD-FL and GSDMD-N of PMs was analyzed by western blot. Two-way ANOVA was employed in A-C, F, G-I and L. Data are presented as mean ± S.E.M. n = 3. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs.* control plasmid + WT group; $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs.* K284R plasmid + WT group or K765R plasmid + WT group. Scale bars: 5 μm.

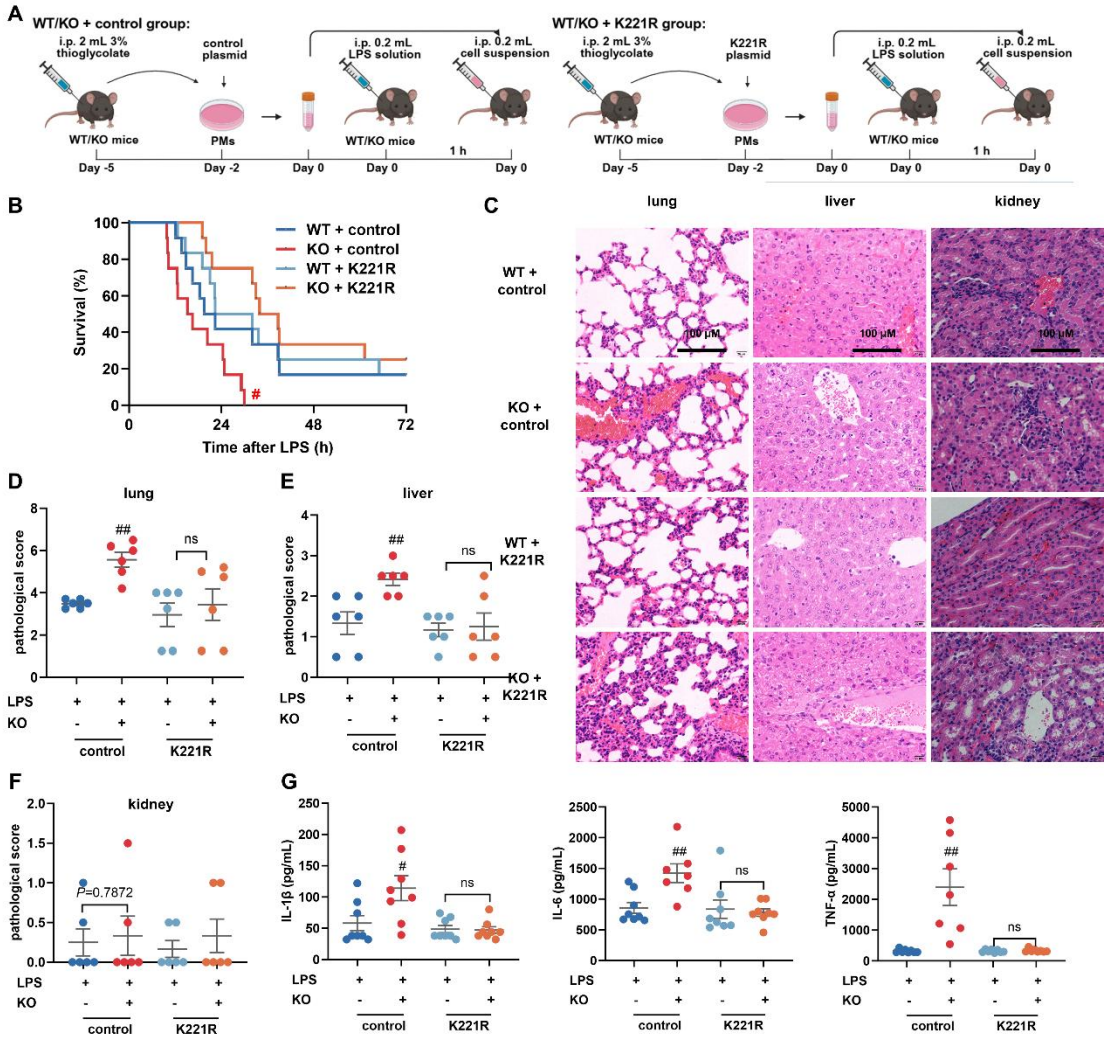


**Fig. S10 Effect of mutations in the acetylated lysine site of Stoml2 on the exacerbation of sepsis outcomes induced by LPS in the context of ERβ deficiency.** (A) The study protocol is shown. Adoptive transfer of PMs with the Stoml2 K221 point mutation into corresponding LPS-induced sepsis mice. (B) The survival curve of septic mice, n = 12. (C-F) H&E staining and histopathological score of the lung, liver and kidney from septic mice. n=6. (G) Quantification of cytokine levels in the sera from septic mice. n=7-8. Long rank (Mantel Cox) test was adopted to compare the significance in B. Two-way ANOVA was employed in D-G. Data are expressed as mean ± SEM. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs*. WT + control group. ns, no significance. Scale bar, 100 μm.

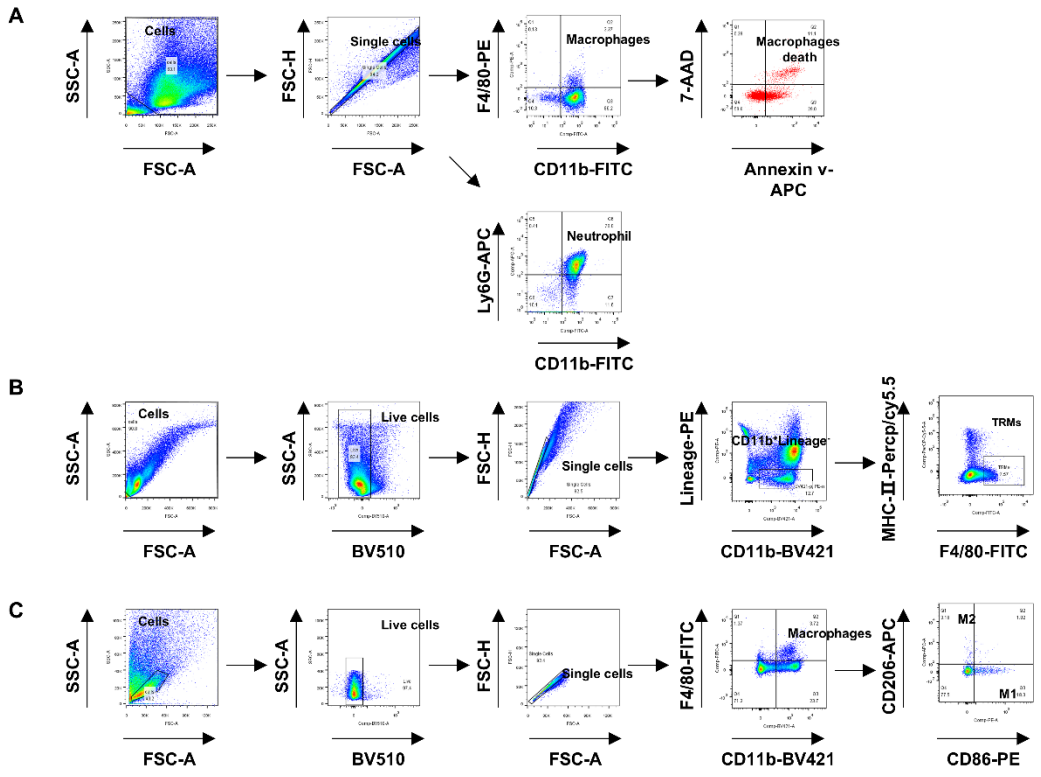



**Fig. S11 Gating strategies used for flow cytometry.** (A) Gating strategy to analyze PMs, peritoneal neutrophils, and the proportion of macrophage death in experimental

models. (B) Gating strategy to analyze TRMs in the PLF of septic mice. Lineage (Ly6G CD19 CD90.2)(26). (C) Gating strategy to analyze M1 and M2 in the PLF of septic mice.

1 **Table. S1 Patients Characteristics**

| Demographics | Sepsis | | | Non sepsis |
|---|---|---|---|---|
| | All patients | Patients with high ERβ expression | Patients with low ERβ expression | |
| Age (years, mean ± SEM) | 76.30 ± 13.79 | 89.33 ± 7.17 | 75.42 ± 13.16 | 45.36 ± 24.29 |
| Male (n, %) | 18, 69.23 % | 6, 100% | 12, 100% | 12, 40% |
| **Source of infection** | | | | |
| ≥2 (n, %) | 10, 38.46 % | 2, 33.33% | 3, 25.00% | |
| Abdominal (n, %) | 2, 7.69 % | 1, 16.67% | 0, 0.00% | |
| Pulmonary (n, %) | 25, 92.31 % | 4, 66.67% | 12, 100% | |
| Urinary (n, %) | 7, 26.92 % | 1, 16.67% | 2, 16.67% | |
| Soft tissue (n, %) | 2, 7.69 % | 1, 16.67% | 1, 8.33% | |
| Others (n, %) | 1, 3.84 % | 1, 16.67% | 0, 0.00% | |
| **Comorbidities** | | | | |
| ≥2 (n, %) | 16, 61.54 % | 5, 83.33% | 7, 58.33% | |
| None (n, %) | 2, 7.69 % | 0, 0.00% | 1, 8.33% | |
| Diabetes (n, %) | 7, 26.92 % | 2, 33.33% | 1, 8.33% | |
| Trauma/Surgery (n, %) | 3, 11.54 % | 1, 16.67% | 2, 16.67% | |
| Cancer (n, %) | 9, 34.62 % | 2, 33.33% | 4, 33.33% | |

| | | | |
|---|---|---|---|
| Heart failure (n, %) | 16, 61.54 % | 4, 66.67% | 8, 66.67% |
| Kidney disease (n, %) | 13, 50.00 % | 5, 83.33% | 4, 33.33% |
| **Disease scores** | | | |
| PCT (ng/ml) | 6.14 ± 11.94 | 1.57 ± 2.24 | 10.09 ± 16.91 |
| CRP (mg/L) | 93.00 ± 74.03 | 87.40 ± 72.01 | 116.98 ± 81.95 |
| SOFA | 9.62 ± 4.02 | 7.33 ± 2.42 | 11.5 ± 3.48 |
| **28-day mortality rate** | | | |
| | 9, 34.62% | 2, 33.33% | 6, 50% |
| **Complete blood count (CBC) data** | | | |
| Neutrophil percentage (%, mean ± SEM) | 83.00 ± 11.64 | 86.05 ± 4.30 | 86.58 ± 11.39 |
| Lymphocyte percentage (%, mean ± SEM) | 10.40 ± 10.23 | 6.2 ± 2.73 | 8.33 ±10.17 |
| Mononuclear macrophage percentage (%, mean ± SEM) | 5.67 ± 2.56 | 6.83 ± 1.73 | 4.19 ± 2.67 |
| Eosinophil percentage (%, mean ± SEM) | 0.73 ± 1.78 | 0.63 ± 1.06 | 0.75 ± 2.47 |

| | | | |
|---|---|---|---|
| Basophil percentage (%, mean ± SEM) | 0.20 ± 0.22 | 0.28 ± 0.24 | 0.15 ± 0.09 |

1

2

**Table. S2 Human primer pairs used in real-time PCR**

| Gene | Sequence (5'-3') | Length (bp) |
|---|---|---|
| *ESR2* | Forward | CCTTAATTCTCCTTCCTCCTACAACTG |
| | Reverse | CTGGATATTCATGGTGGCTGTCTAC |
| *ENPP2* | Forward | GTGGACCAATCTTCGACTATGACTATG |
| | Reverse | GGTGATGATGCTGTAGTAGTGAGTTG |
| *CAV1* | Forward | GCATTTACTTCGCCATTCTCTCTTTC |
| | Reverse | GTAGATGGAATAGACACGGCTGATG |
| *ESR1* | Forward | CTCCTCATCCTCTCCCACATCAG |
| | Reverse | AGCATCTCCAGCAGCAGGTC |
| *BRCAa1* | Forward | ACCGTTGCTACCGAGTGTCTG |
| | Reverse | TGATGTTCCTGAGATGCCTTTGC |
| *TFF1* | Forward | GCCGAGGCCCAGACAGAG |
| | Reverse | GTCGAAACAGCAGCCCTTATTTG |
| *GPER1r* | Forward | CTCTCGGAGCACCAGCAGTAC |
| | Reverse | TCACCACCAGGATCAGGATGTTG |
| *ACTB* | Forward | CATGTACGTTGCTATCCAGGC |
| | Reverse | CTCCTTAATGTCACGCACGAT |

**Table. S3 Mouse primer pairs used in real-time PCR**

| Gene | Sequence (5'-3') | Length (bp) |
|---|---|---|
| *Cpt1* | Forward | CTACATCACCCCAACCCATATT |
| | Reverse | GATCCCAGAAGACGAATAGGTT |
| *Actb* | Forward | AGCAAGCAGGAGTACGATGAG |
| | Reverse | GGTGTAAAACGCAGCTCAGTAA |

Supplemental Table 4

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| **Antibodies** | | |
| Recombinant anti-pro caspase-1 (pro-Casp1) + p10 (Casp1 p10) + p12 antibody | Abcam | Cat# ab179515 |
| APC anti-mouse Ly6G antibody | Biolegend | Cat# 127613 |
| Alexa Fluor® 488 anti-mouse/human CD11b antibody | Biolegend | Cat# 101217 |
| Bv421 anti-mouse/human CD11b antibody | Biolegend | Cat# 101235 |
| PE anti-mouse F4/80 antibody | Biolegend | Cat# 123110 |
| FITC anti-mouse F4/80 antibody | Biolegend | Cat# 123107 |
| PerCP/Cyanine5.5 anti-mouse I-A/I-E antibody | Biolegend | Cat# 107626 |
| PE anti-mouse Ly6G antibody | Biolegend | Cat# 127608 |
| PE anti-mouse CD19 antibody | Biolegend | Cat# 115507 |
| PE anti-mouse CD90.2 antibody | Biolegend | Cat# 105307 |
| PE-conjugated anti-mouse CD86 | Biolegend | Cat# 105007 |

| | | |
|---|---|---|
| APC-conjugated anti-mouse CD206 antibody | Biolegend | Cat# 141707 |
| Acetyl-histone H3 monoclonal antibody | Bioworld | Cat# MB65867 |
| Gasdermin D (GSDMD-FL) rabbit mAb antibody | CST | Cat# 39754 |
| Cleaved gasdermin D (GSDMD-N) rabbit mAb antibody | CST | Cat# 36425 |
| β-actin rabbit mAb antibody | CST | Cat# 4970 |
| NLRP3 rabbit mAb antibody | CST | Cat# D4D8T |
| NF-κB p65 mouse antibody | Proteintech | Cat# 66535-1-Ig |
| p-NF-κB p65 mouse antibody | Proteintech | Cat# 82335-1-RR |
| TLR4 mouse antibody | Proteintech | Cat# 66350-1-Ig |
| ASC mouse antibody | Santa | Cat# sc-22514-R |
| NIMA Related Kinase 7 (NEK7) mouse antibody | Santa | Cat# sc-393539 |
| CPT1 mouse antibody | Santa | Cat# sc-514555 |
| PAK2 mouse antibody | Santa | Cat# sc-373740 |
| Stoml2 mouse antibody | Santa | Cat# sc-376869 |
| DEP-1 mouse antibody | Santa | Cat# sc-390404 |
| MyD88 mouse antibody | Wanleibio | Cat# WL02494 |
| **Biological Samples** | | |

| | | |
|---|---|---|
| Septic patient samples | This study | See Table. S1 |
| **Chemicals** | | |
| NP-40 buffer | Beyotime | Cat# P0013F |
| Phenylmethanesulfonyl fluoride | Beyotime | Cat# ST505 |
| Lipo6000 | Beyotime | Cat# C0526 |
| Blood agar plate medium | Bikeman | Cat# 110701019 |
| VX765 | MCE | Cat# HY-13205 |
| Necrostatin-1 | MCE | Cat# HY-15760 |
| Z-DEVD-FMK | MCE | Cat# HY-12466 |
| Ferrostatin-1 | MCE | Cat# HY-100579 |
| ATP | MCE | Cat# HY-B2176 |
| Protein A/G magnetic beads | MCE | Cat# HY-K0202 |
| Mito-TEMPO | MCE | Cat# HY-112879 |
| 2-DG | MCE | Cat# HY-13966 |
| Mouse GM-CSF recombinant protein | PeproTech | Cat# 315-03 |
| Isoflurane | RWD | Cat# R510-22-10 |
| LPS | Sigma | Cat# L2880 |
| Thioglycolate | Sigma | Cat# 106691 |
| Diamidino-2-phenylindole (DAPI) | Sigma | Cat# 10236276001 |
| **Critical Commercial Assays** | | |
| Seahorse XF palmitate oxidation stress test kit | Agilent | Cat# 103693-100 |

| | | |
|---|---|---|
| Seahorse XF Glycolytic Rate Assay kit | Agilent | Cat# 103344-100 |
| BCA protein colorimetric assay kit | Beyotime | Cat# P0010S |
| LDH cytotoxicity assay kit | Beyotime | Cat# C0016 |
| MMP assay kit | Beyotime | Cat# C2006 |
| Reactive oxygen species assay kit | Beyotime | Cat# S0033S |
| Acetyl-CoA ELISA kit | mlbio | Cat# YJ258897 |
| Mouse IL-18 ELISA kit | MultiSciences Biotech | Cat# EK218 |
| Annexin V-APC/7-AAD apoptosis kit | MultiSciences Biotech | Cat# AP105 |
| Pyruvate assay kit | njjcbio | Cat# A081-1-1 |
| PDH activity assay kit | Solarbio | Cat# BC0385 |
| Mouse IL-1β uncoated ELISA kit | ThermoFisher | Cat# 88-7013A-88 |
| Mouse IL-6 uncoated ELISA kit | ThermoFisher | Cat# 88-7064-88 |
| Mouse TNF alpha uncoated ELISA kit | ThermoFisher | Cat# 88-7324-76 |
| Mouse IL-10 uncoated ELISA kit | ThermoFisher | Cat# 88-7105-88 |
| **Experimental Models:** | | |
| *Esr2* (the gene encoding ERβ) KO mice | Shanghai Model Organisms Center, Inc. | N/A |
| **Oligonucleotides** | | |

| | | |
|---|---|---|
| Primers for qPCR | This study | See Table. S2 and Table. S3 |
| si-CPT1-1 CCAAAUUGCAGUGGUAUUUTT | This study | N/A |
| si-CPT1-2 CCUGCAUUCCUUCCCAUUUTT | This study | N/A |
| si-CPT1-3 CCACCUCUUCUGCCUCUAUTT | This study | N/A |
| **Recombinant DNA** | | |
| Pak2 plasmid | This study | N/A |
| Pak2 K284R plasmid | This study | N/A |
| Stoml2 plasmid | This study | N/A |
| Stoml2 K221R plasmid | This study | N/A |
| DEP-1 plasmid | This study | N/A |
| DEP-1 K765R plasmid | This study | N/A |
| **Deposited data** | | |
| Non-target metabolomics data | This study | OMIX014575 |
| Quantitative acetylome analysis data | This study | PXD073393 |
| **Software** | | |
| ImageJ software | NIH | https://imagej.nih.gov/ij/ |

| | | |
|---|---|---|
| Graphpad Prism 8 software | GraphPad | https://www.graphpad.com/scientific-software/prism/ |
| FlowJo V10 | BD | https://www.flowjo.com/ |
| ZEN lite 2.6 software | Zeiss | https://www.zeiss.com/ |