

Supplementary Figure 1

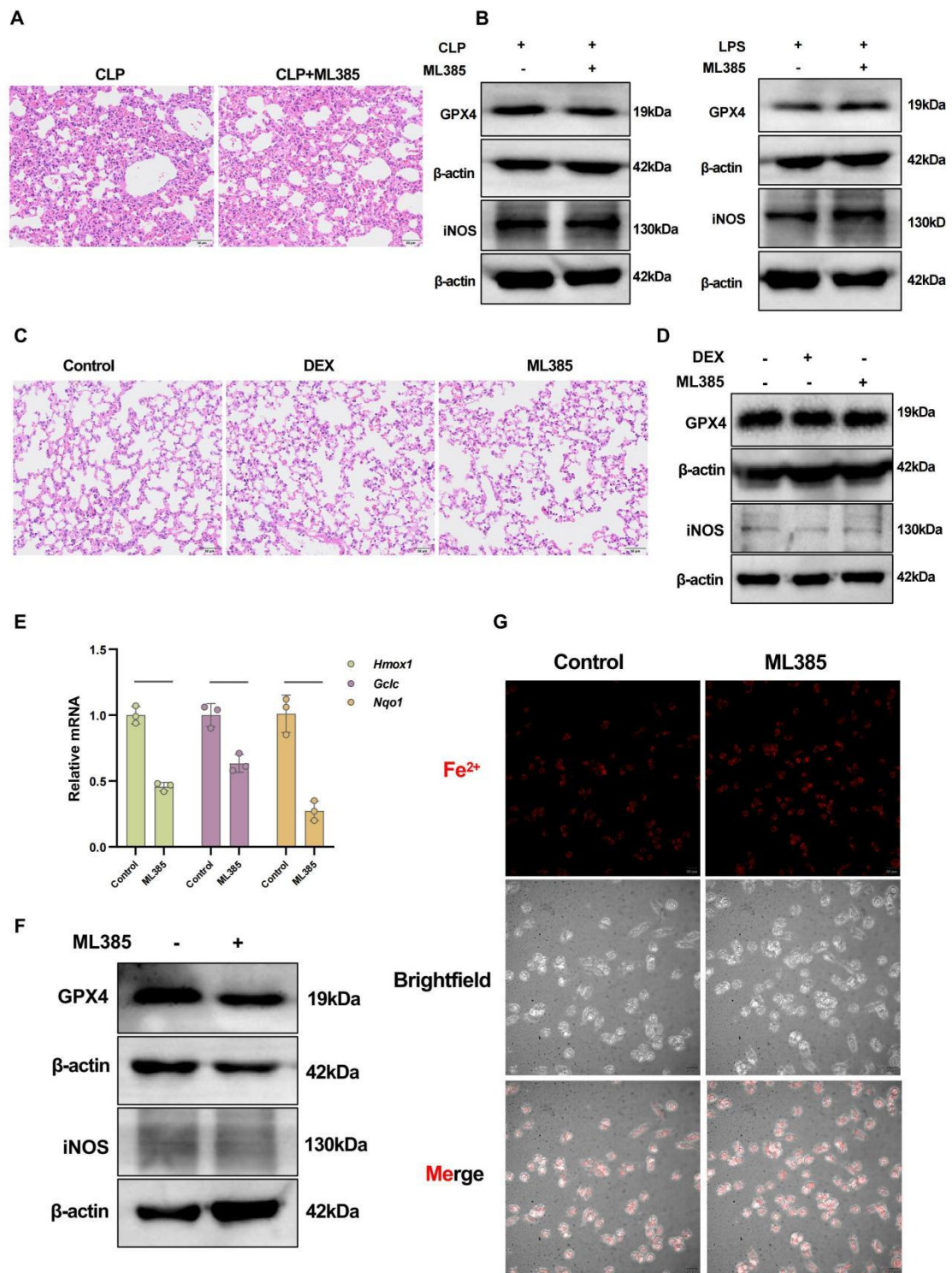


Figure 1. Validation of DEX and ML385 specificity and drug safety in vivo and in vitro.
(A) Representative H&E staining images of lung tissues from CLP-induced septic mice treated with or without ML385. Scale bar = 50µm.

(B) Western blot analysis of GPX4 and iNOS protein expression in macrophages isolated from CLP mice or stimulated with LPS in vitro.

(C) H&E staining of lung tissues from healthy Sham mice treated with DEX or ML385 alone. Scale bar = 50 μ m.

(D) Western blot analysis of GPX4 and iNOS in lung homogenates from Sham + DEX and Sham + ML385 groups. Basal protein expression remained unaltered, confirming drug safety in healthy tissue.

(E) qPCR analysis of Nrf2 downstream target genes (Hmox1, Gclc, Nqo1) in normal macrophages treated with ML385 alone.

(F) Western blot analysis of GPX4 and iNOS in normal macrophages treated with ML385 alone.

(G) Representative FerroOrange live-cell imaging of intracellular Fe^{2+} in RAW264.7 cells.

Supplementary Figure 2

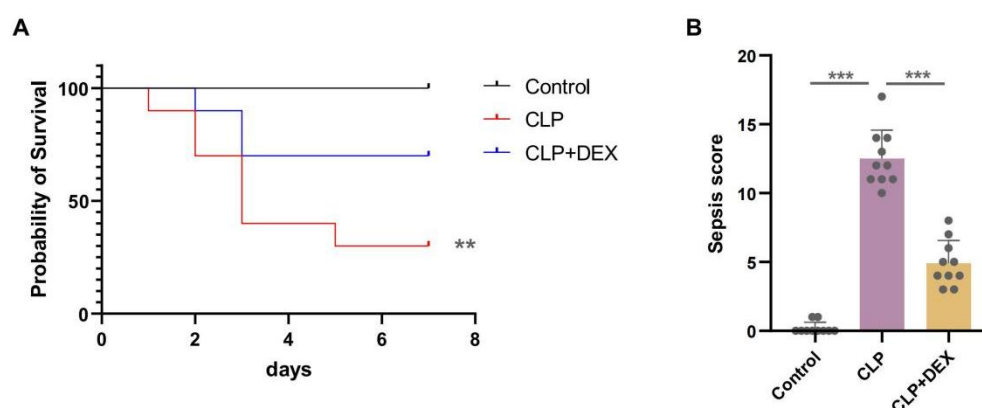


Figure 2. DEX alleviates sepsis-induced mortality and sepsis score in CLP mice.

(A) Survival curves of CLP-induced septic mice treated with vehicle or DEX for 7 days. Data are presented as Kaplan-Meier survival plots (n = 10 per group). ** p < 0.01).

(B) Sepsis scores assessed at 24 h post-CLP based on clinical parameters (n = 8–10 per group). Data are mean $\pm$ SEM. *** p < 0.001.

Survival analysis

10 mice per group, with a total of three groups. The mouse model of sepsis was established using cecal ligation and puncture (CLP). In the Sham group, only laparotomy was performed, followed by closure. In the CLP + DEX group, DEX was injected intraperitoneally at a dose of 50 μ g/kg 30 min after CLP operation, while the other groups received an equal volume of normal saline. Survival was monitored continuously for 7 days postoperatively. The Kaplan-Meier method was used to plot survival curves, and differences between groups were compared using the Log-rank test.

Sepsis severity

Sepsis severity was assessed using a scoring system comprising seven clinical indicators: (1) general appearance, (2) level of consciousness, (3) spontaneous activity, (4) responsiveness to external stimuli, (5) eye condition, (6) respiratory rate, and (7) respiratory pattern. Each indicator was rated on a scale from 0 to 4. The findings demonstrated that the Mouse Sepsis Score (MSS) was significantly higher in the CLP group, indicating pronounced sepsis-associated behavioral abnormalities. Notably, treatment with DEX effectively ameliorated these clinical manifestations.