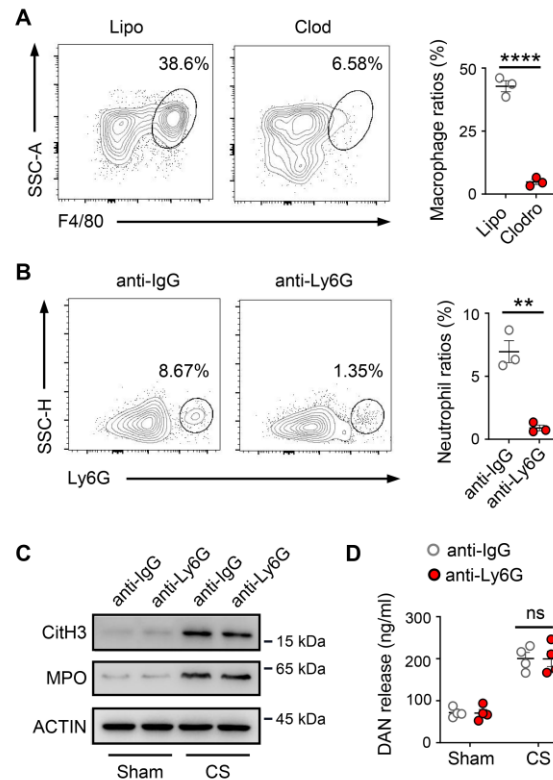
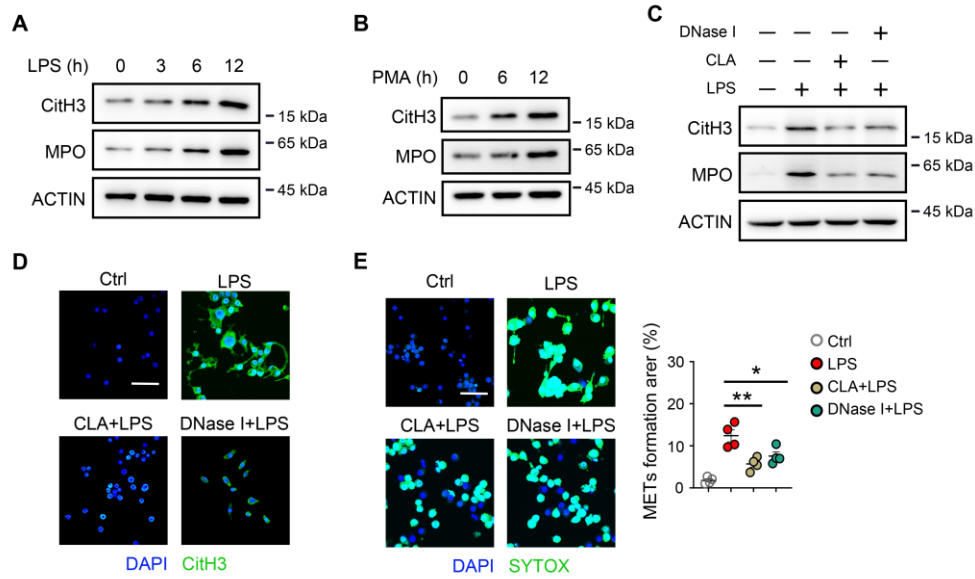


HIF-1 α Promotes Macrophage Extracellular Trap Formation and Exacerbates Acute Lung Injury in Neonatal Sepsis

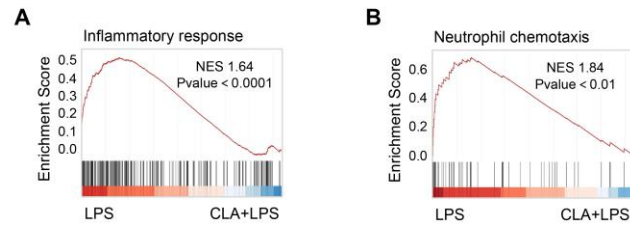
Huiling Zhang^{1, †}, Wei Huang^{2, †}, Xinlong Dai^{3, †}, Jundi Zheng⁴, Xinyao Jiang³, Yutao Yang³, Hanhui Zhong^{3, *}, Guang Yang^{1, *}



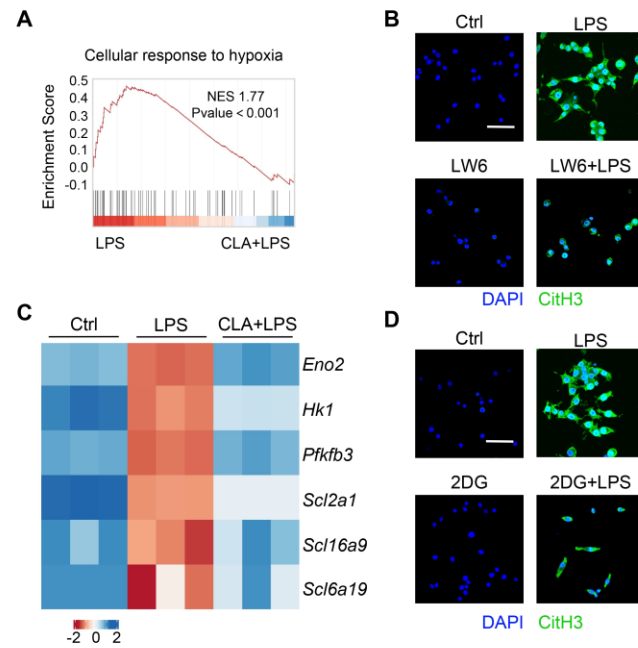
Supplementary Figure S1. Neutrophil depletion did not affect the formation of ETs. (A) Neonatal mice (postnatal day 5-7) were treated with PBS liposomal (Lipo) or clodronate liposomal (Clod, intraperitoneal injection). Flow cytometry analysis of lung macrophage ratio in live, CD45⁺ cells (n=3). (B-D) Neonatal mice (postnatal day 5-7) were injected with 50 µg mouse IgG Isotype control (IgG) or anti-Ly6G antibody (i. p.), followed sham and sepsis for 24 h. (B) Flow cytometry analysis of lung neutrophil ratio in live, CD45⁺ cells (n=3). (C) The protein levels of CitH3 and MPO in lung tissue were measured by western blot (n=4). (D) Extracellular DNA levels in BALF were quantified (n=4). **P<0.01, ****P<0.0001, ns: not significant.



Supplementary Figure S2. LPS or PMA induced METs formation in vitro. (A, B) RAW264.7 cells were exposed to LPS (100 ng/mL) or PMA (100 nM) at different time. The protein levels of CitH3 and MPO were measured by western blot (n=4). **(C-E)** RAW264.7 cells were pretreated with CLA (200 μ M) or DNase I (10 U/mL) for 1 h, followed by LPS (100 ng/mL) for 12 h. **(C)** The protein levels of CitH3 and MPO were measured by western blot (n=4). **(D)** Immunofluorescence staining of CitH3 in RAW264.7 cells. Scale bar, 50 μ m. **(E)** SYTOX Green staining was performed to observe the rate of METs and analysis of METs rate (n=4). *P<0.05, **P<0.01.



Supplementary Figure S3. METs formation was associated with macrophage activation. RNA-seq analysis of cultured RAW264.7 with control, LPS-treated, or CLA+LPS-treated. GSEA of inflammation response gene **(A)** and neutrophil chemotaxis gene **(B)** set.



Supplementary Figure S4. HIF-1 α -driven glycolysis contributed to METs formation. **(A)** GSEA of Cellular response to hypoxia gene set. **(B)** RAW264.7 cells were pretreated with 20 μ M LW6 for 1 h, followed by 100 ng/mL LPS for 12 h. Immunofluorescence staining of CitH3 in RAW264.7 cells. Scale bar, 50 μ m. **(C)** Heatmap showing differentially expression of glycolysis-associated genes (*Eno2*, *Hk1*, *Pfkfb3*, *Slc2a1*, *Slc16a9*, *Slc6a19*). **(D)** RAW264.7 cells were pretreated with 50 mM 2DG for 1 h, followed by 100 ng/mL LPS for 12 h. Immunofluorescence staining of CitH3 in RAW264.7 cells. Scale bar, 50 μ m. Data shown represents four independent experiments.

Supplementary Table S1 | List of primers used qRT-PCR.

Primers		Species
<i>18S</i> -Forward	GTAACCCGTTGAACCCCAT	Mouse
<i>18S</i> -Reverse	CCATCCAATCGGTAGTAGCG	Mouse
<i>Nos2</i> -Forward	GAGACAGGGAAGTCTGAAGCAC	
<i>Nos2</i> -Reverse	CCAGCAGTAG TTGCTCCTCTTC	Mouse
<i>Tnf</i> -Forward	AAGCCTGTAGCC CACGTCGTA	
<i>Tnf</i> -Reverse	GGCACCACTAGTTGGTTGTCTTTG	Mouse
<i>Il1b</i> -Forward	GAAATGCCACCTTTTGACAGTG	
<i>Il1b</i> -Reverse	TGGATGCTCTCATCAGGACAG	Mouse
<i>Eno2</i> -Forward	CGTTACTTAGGCAAAGGTGTCC	
<i>Eno2</i> -Reverse	CTCCAGCATCAGGTTGTCCAGT	Mouse
<i>Cxcl2</i> -Forward	CCTGGTTCAGAAAATCATCCA	
<i>Cxcl2</i> -Reverse	CTTCCGTTGAGGGACAGC	