Supporting information

For

High mobility group box 1 enables bacterial lipids to trigger receptor-interacting protein kinase 3 (RIPK3)-mediated necroptosis and apoptosis in mice

Ran Meng[1,2], Lan Gu[1,3], Yanyan Lu[1,3], Kai Zhao[1,3], Jianfeng Wu[4], Haichao Wang[5], Jiahuai Han[4], Yiting Tang[1,5*] and Ben Lu[1,2,5,7*]

From the [1]Department of Hematology and Key Laboratory of non-resolving inflammation and tumor, The 3rd Xiangya Hospital, Central South University, Changsha, Hunan Province, 410000 P.R. China; [2]Postdoctoral Research Station of Clinical Medicine, the 3rd Xiangya Hospital, Central South University, Changsha, Hunan Province, 410000 P.R.China; [3]Key Laboratory of Medical Genetics, School of Biological Science and Technology, Central South University, Changsha, Hunan Province, 410000 P.R. China; [3]State Key Laboratory of Cellular Stress Biology, Innovation Center for Cell Signaling Network, School of Life Sciences, Xiamen University, Xiamen, Fujian 361005, China; [4]Department of Emergency Medicine, North Shore University Hospital, Northwell Health, 350 Community Drive, Manhasset, NY 11030, USA; [5]Department of Physiology, School of Basic Medical Science, Central South University, Changsha, Hunan Province, 410000, People's Republic of China. [6]Key Laboratory of Sepsis Translational Medicine of Hunan, Central South University, Changsha, Hunan Province, 410000 P.R. China; [7]Department of Pathophysiology, School of Basic Medical Science, Jinan University, Guangzhou, Guangdong Province, 510632 P.R. China

Running title: *HMGB1 orchestrates RIPK3-mediated cell death*

[*]To whom correspondence should be addressed: Yiting Tang, M.D.; 168, Tongzipo Road, Department of Physiology, School of Basic Medical Science, Central South University, Changsha 410013, P. R. China; Telephone: 86-0731-88618214, fax: 0731-88921910, E-mail: 109356513@qq.com. Ben Lu, Ph.D., M.D.; 138, Tongzipo Road, Department of Hematology, the Third Xiangya Hospital, Central South University, Changsha 410013, P. R. China; Telephone: 86-0731-88618214, fax:0731-88921910, E-mail: xybenlu@csu.edu.cn

Supplemental information including:

Figure S1: HMGB1, Lipid IVa or Lipid A alone Failed to Trigger Necrosis or Apoptosis.
Figure S2: Endogenous HMGB1 Enables Microbial Lipids to Trigger Cytokines Release.
Figure S3: HMGB1 Enables Lipid A to Trigger Cytokines Release in Human Cells.
Figure S4: HMGB1+Lipid A Increase the mRNA Levels of Cytokines
Figure S5: Inhibition of Necroptosis attenuates HMGB1/Microbial Lipids-Induced Cytokines' Release.
Figure S6: Neutralization of HMGB1 Attenuates necrosis/apoptosis and Cytokines' Release *in vivo*.

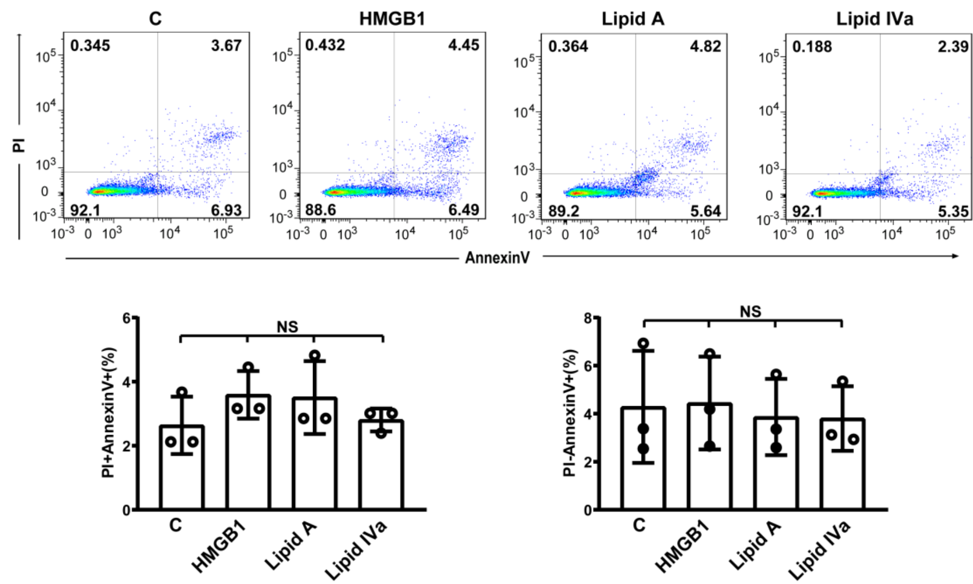

Figure S1. HMGB1, Lipid IVa or Lipid A alone Failed to Trigger Necrosis or Apoptosis. Flow cytometry analysis of WT peritoneal macrophages undergoing necrosis (PI+) or apoptosis (PI-) after stimulated with HMGB1 (0.4 μg/ml), lipid IVa or lipid A (1 μg/ml). NS, not significant. Results are presented as mean ± SD.

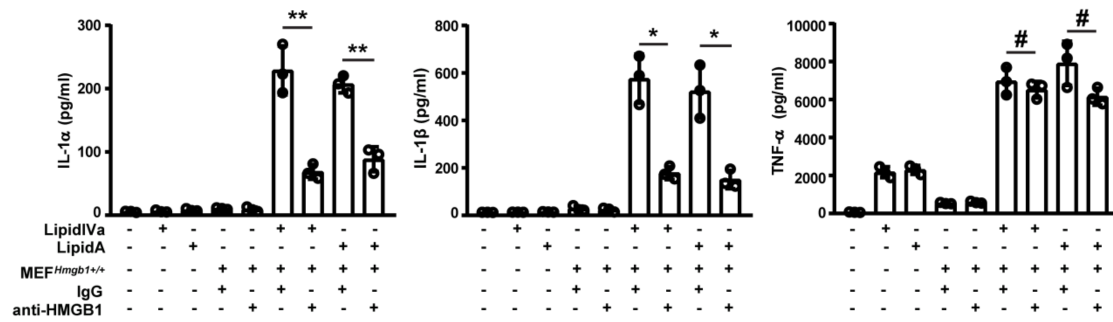

Figure S2. Endogenous HMGB1 Enables Microbial Lipids to Trigger Cytokines Release. Necrotic lysate of *Hmgb1*$^{+/+}$ MEFs were pretreated with HMGB1 neutralizing antibody or isotype IgG controls. IL-1α, IL-1β and TNF-α measured from culture supernatants of peritoneal macrophages from WT upon exposure to the anti-HMGB1 or isotype IgG pretreated necrotic *Hmgb1*$^{+/+}$ MEF in the presence or the absence of lipid IVa or lipid A (1 μg/ml). $^*P < 0.05$; $^{**}P < 0.01$; #, not significant. Results are presented as mean ± SD.

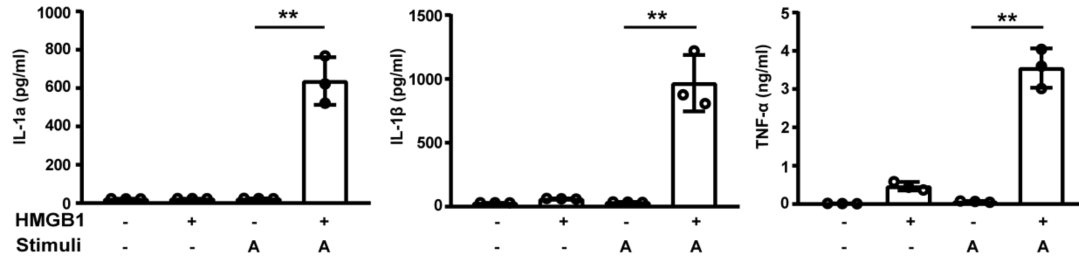

Figure S3. HMGB1 Enables Lipid A to Trigger Cytokines Release in Human Cells. Total PBMCs were isolated from healthy peripheral blood by Ficoll. IL-1α, IL-1β and TNF-α measured from culture supernatants of PBMC following stimulated with lipid A (1 μg/ml) in the absence or the presence of HMGB1 (0.4 μg/ml). **P＜0.01. Results are presented as mean ±SD.

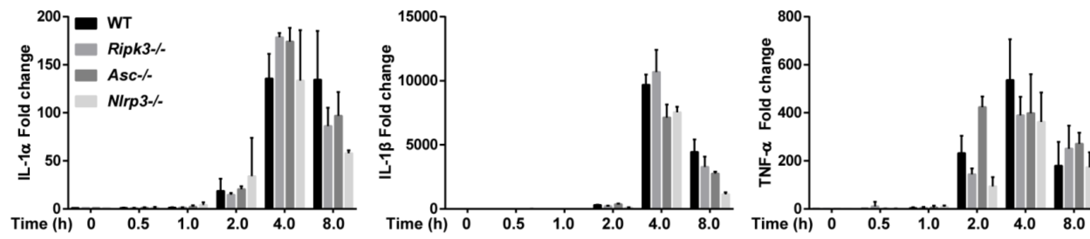

Figure S4. HMGB1+Lipid A increase the mRNA levels of cytokines

The mRNA levels of IL-1α, IL-1β and TNF-α in WT peritoneal macrophages measured by quantitative real-time PCR analysis after stimulated with HMGB1 + Lipid A. Quantitative real-time PCR fold change values for target genes were normalized against the level of β-actin. Results are presented as mean ± SD.

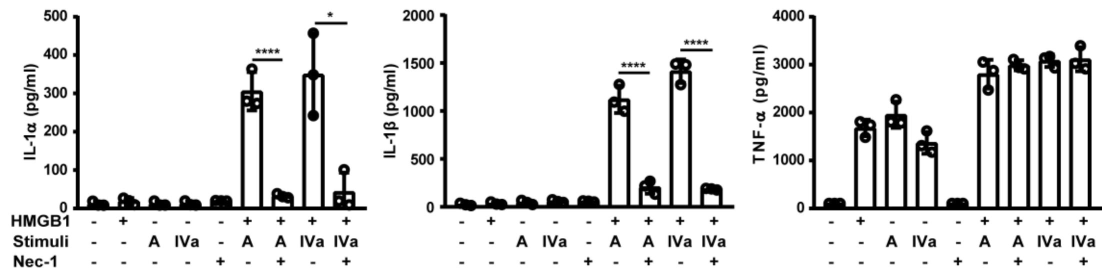

Figure S5. Inhibition of Necroptosis attenuates HMGB1/Microbial Lipids-Induced Cytokines' release.

IL-1α, IL-1β and TNF-α measured from culture supernatants of peritoneal macrophages from WT mice stimulated with HMGB1+lipid IVa/A in the absence or the presence of necrostatin-1. *P＜0.05; ****P<0.0001. Results are presented as mean ±SD.

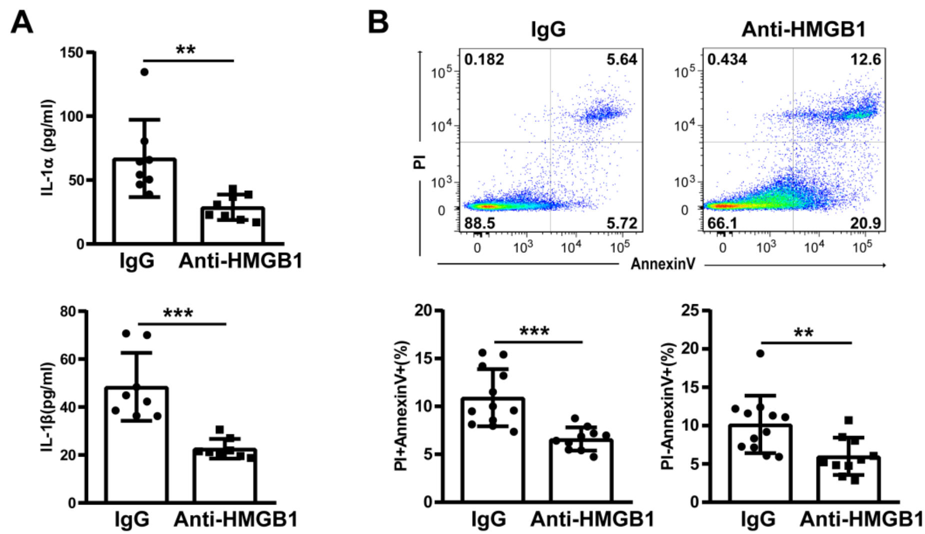

Figure S6. Neutralization of HMGB1 Attenuates necrosis/apoptosis and Cytokines' Release *in vivo*.

Air-pouch inflammatory infiltration was induced in the absence or presence of HMGB1-neutralizing or normal control IgGs. Then air-pouch lavage fluid was collected. Levels of IL-1α and IL-1β were measured by ELISA analysis (A), necroptosis and apoptosis were measured by flow cytometry (B). **P＜0.01; ***P＜0.001. Results are presented as mean ±SD.