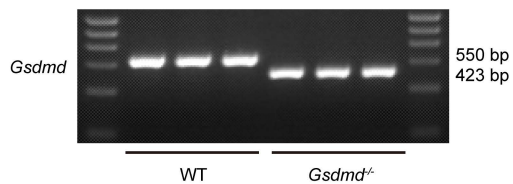
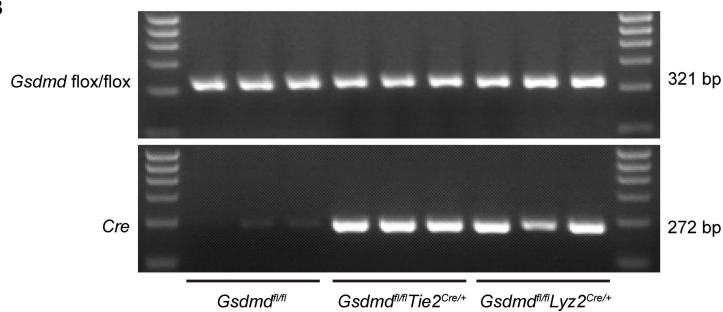


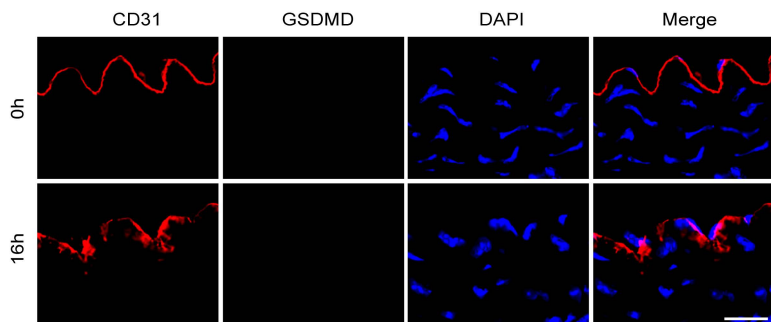
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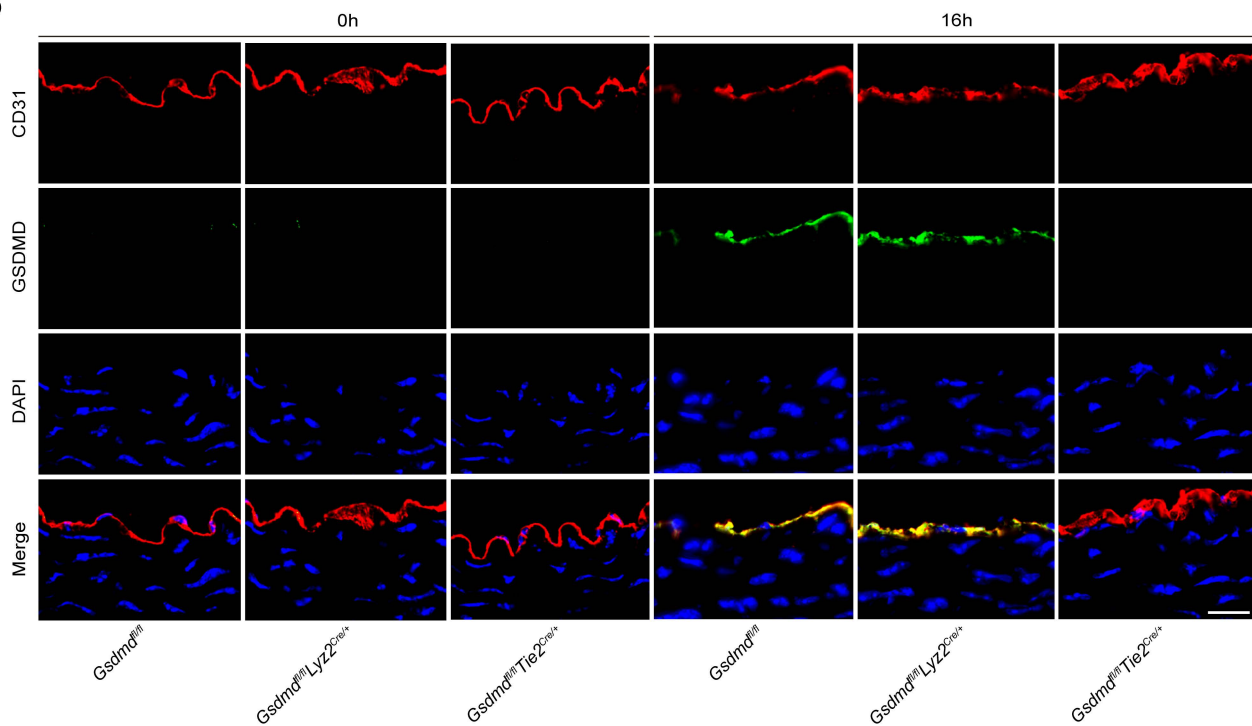
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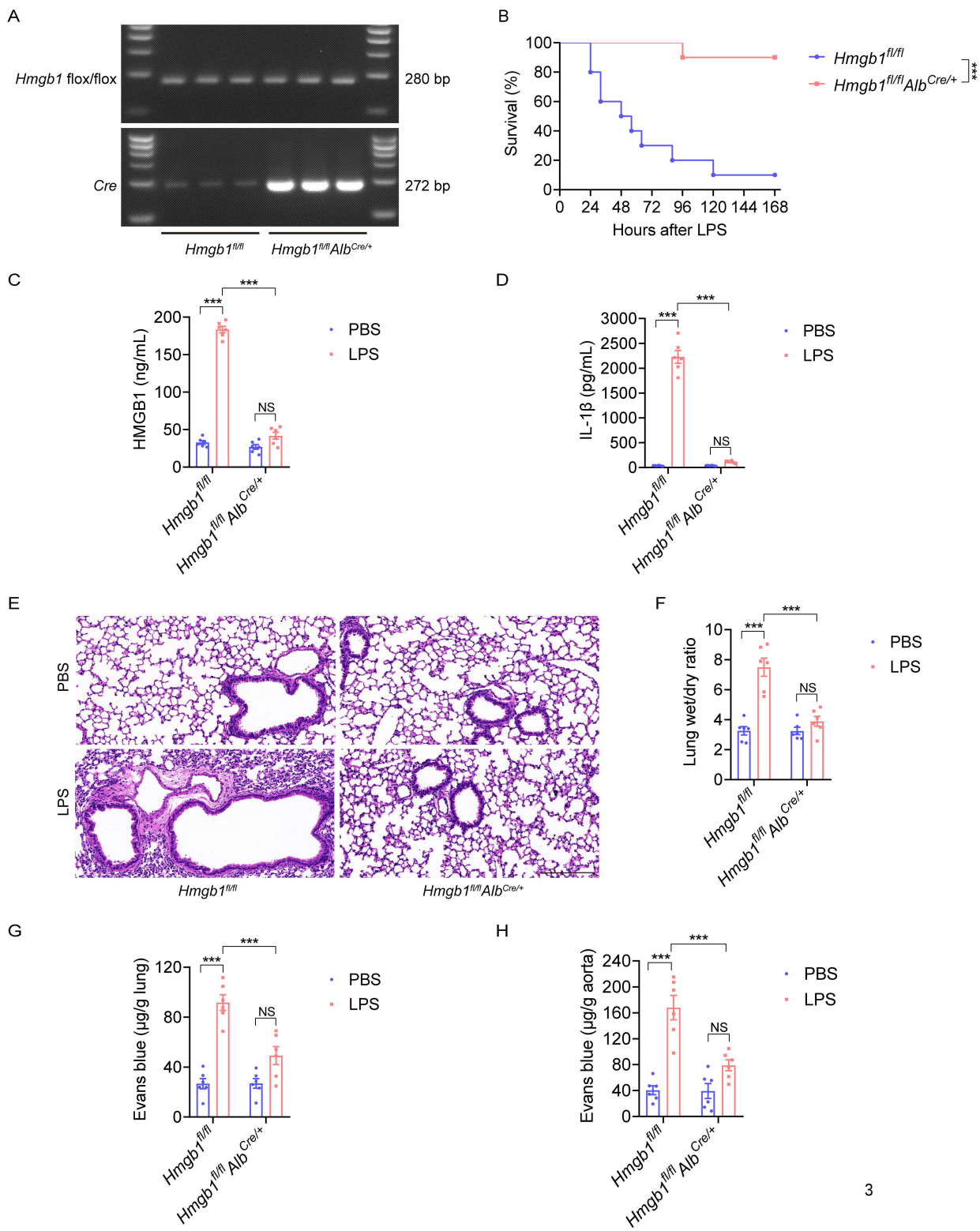
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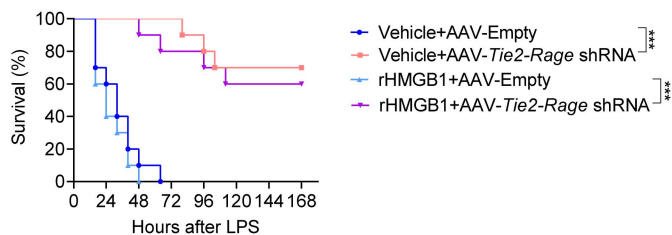


Supplemental Figure 1. The level of endothelial GSDMD in global *Gsdmd* deletion mice and tissue-conditioned *Gsdmd* knockout mice with endotoxemia. Genotyping of (A) wild-type (WT) mice and global *Gsdmd* knockout (*Gsdmd*^{-/-}) mice and (B) endothelial *Gsdmd*-deficient (*Gsdmd*^{fl/fl}*Tie2*^{Cre/+}) mice, myeloid cell *Gsdmd*-deficient (*Gsdmd*^{fl/fl}*Lyz2*^{Cre/+}) mice and their *Cre*-negative littermates (*Gsdmd*^{fl/fl} mice). *Gsdmd*^{-/-} mice, *Gsdmd*^{fl/fl}*Tie2*^{Cre/+} mice, *Gsdmd*^{fl/fl}*Lyz2*^{Cre/+} mice and *Gsdmd*^{fl/fl} mice were intraperitoneally injected with a lethal dose of LPS (17.5 mg/kg). Representative aortic immunofluorescence images of CD31 (red), GSDMD (green) and DAPI (blue) at 0 h and 16 h after exposure to LPS in (C) *Gsdmd*^{-/-} mice, (D) *Gsdmd*^{fl/fl}*Tie2*^{Cre/+} mice, *Gsdmd*^{fl/fl}*Lyz2*^{Cre/+} mice and *Gsdmd*^{fl/fl} mice. The scale bar represents 20 μm.

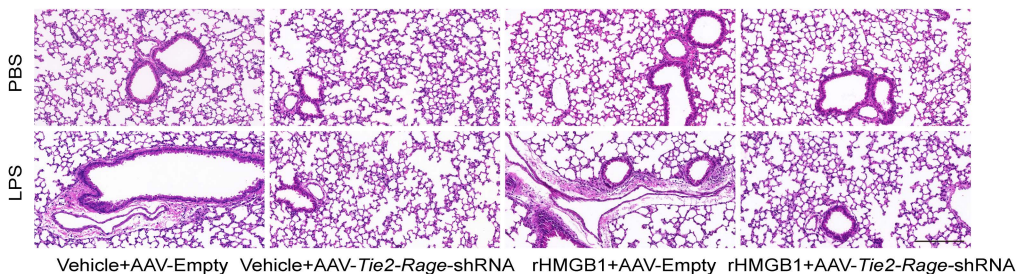


Supplemental Figure 2. Hepatocyte *Hmgb1* deletion reduces circulating HMGB1 levels and vascular injury in endotoxemia. (A) Genotyping of hepatocellular *Hmgb1*-deficient (*Hmgb1*^{fl/fl}*Alb*^{Cre/+}) mice and their *Cre*-negative littermates (*Hmgb1*^{fl/fl} mice). *Hmgb1*^{fl/fl}*Alb*^{Cre/+} mice and *Hmgb1*^{fl/fl} mice were intraperitoneally injected with LPS (17.5 mg/kg). (B) Mouse survival was assessed on the indicated days and is shown as a Kaplan–Meier plot. n=10 per group. Survival data were analyzed by a log-rank (Mantel–Cox) test. Blood, aortas and lungs were obtained from *Hmgb1*^{fl/fl}*Alb*^{Cre/+} mice and *Hmgb1*^{fl/fl} mice after treatment with LPS (17.5 mg/kg) or PBS for 16 h and were analyzed. The plasma (C) HMGB1 and (D) IL-1 β levels were determined in the indicated groups. n=6 per group. The data are shown as the means $\pm$ SEMs. Two-way ANOVA with Bonferroni post hoc correction was performed. (E) Representative HE-stained images of the lung sections are presented. The scale bar represents 200 μ m. (F) The lung wet/dry ratio was quantitatively analyzed. n=6 per group. Lung microvascular permeability and aortic permeability were detected via an Evans blue–albumin extravasation assay. (G) The amount of extracted pulmonary Evans blue dye in the formamide extracts was quantified by measuring the dye at 620 nm. n=6 per group. (H) The aortic Evans blue dye content was quantitatively analyzed. n=6 per group. The data are shown as the means $\pm$ SEMs. Two-way ANOVA with Bonferroni post hoc correction was used. All the data shown are representative of a minimum of 3 independent experiments. *** $P < 0.001$.

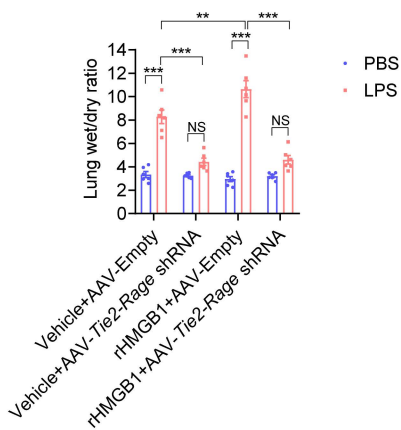
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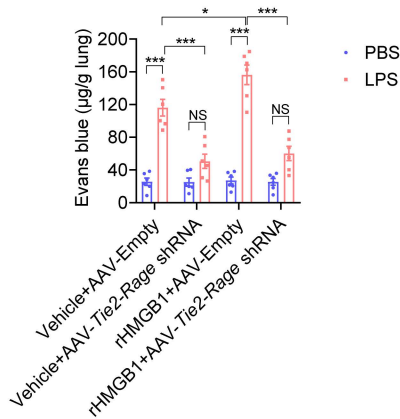
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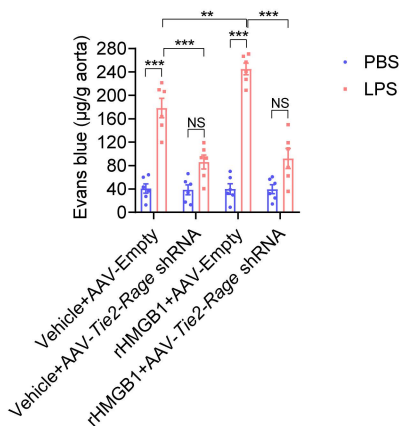
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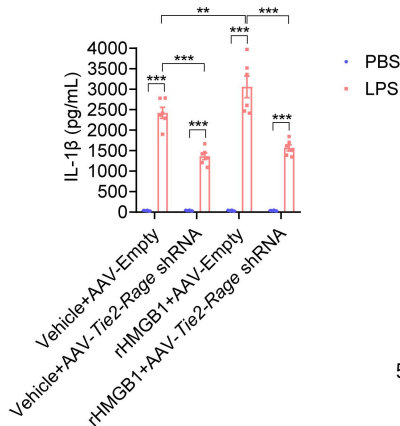
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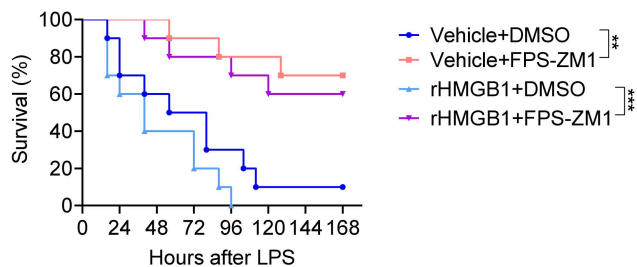


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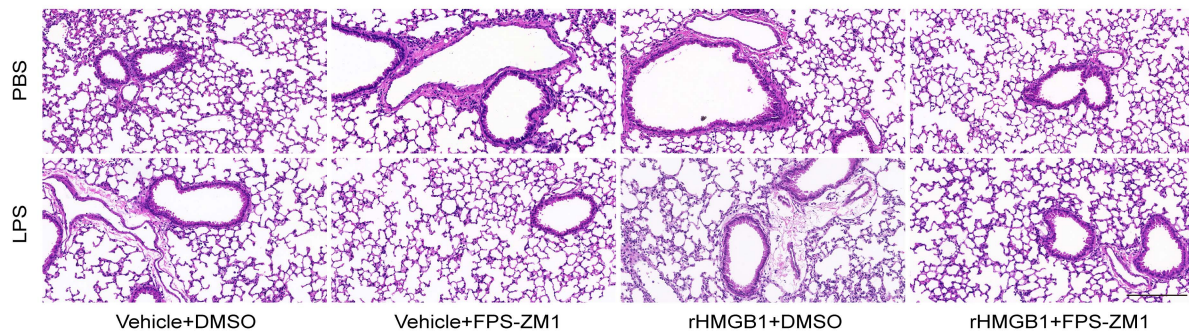


Supplemental Figure 3. The HMGB1/RAGE axis regulated vascular injury and death in mice intratracheally instilled with LPS. Five-week-old WT mice were injected with a null AAV9 vector or an endothelial conditional *Rage* shRNA-knockdown AAV9 vector via the tail vein. After 6 weeks, these mice were intratracheally instilled with LPS (17.5 mg/kg). Subsequently, vehicle or Recombinant HMGB1 (rHMGB1) protein was administered intravenously at a dose of 5 µg at 2, 16, 28, and 40 h after LPS injection. **(A)** Mouse survival was monitored on the indicated days and is presented as a Kaplan–Meier plot. n=10 per group. Survival data were compared via a log-rank (Mantel–Cox) test. Five-week-old WT mice were injected with a null AAV9 vector or an endothelial conditional *Rage* shRNA-knockdown AAV9 vector via the tail vein and were intratracheally instilled with LPS (17.5 mg/kg) or PBS after 6 weeks. Then, vehicle or rHMGB1 protein was administered intravenously at a dose of 5 µg at 2 and 16 h, and the blood, aortas and lungs were excised from the mice and analyzed. **(B)** HE staining of the lung sections is presented. The scale bar represents 200 µm. **(C)** The ratio of the wet lung weight to the dry lung weight was determined. n=6 per group. Lung microvascular permeability and aortic permeability were detected via an Evans blue–albumin extravasation assay. **(D)** The amount of extracted pulmonary Evans blue dye in the formamide extracts was quantified by measuring the dye at 620 nm. n=6 per group. **(E)** The aortic Evans blue dye content was quantitatively analyzed. n=6 per group. **(F)** The plasma IL-1β concentration was determined. n=6 per group. The data are expressed as the means ± SEMs. The data were analyzed by two-way ANOVA with Bonferroni post hoc correction. All the data shown are representative of a minimum of 3 independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

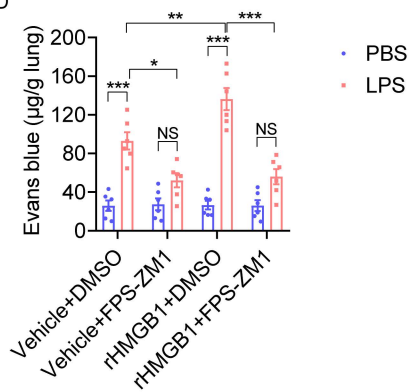
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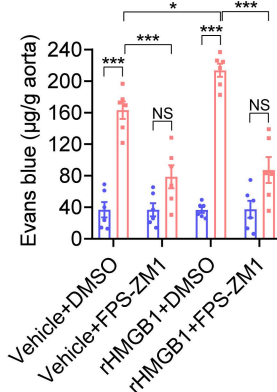
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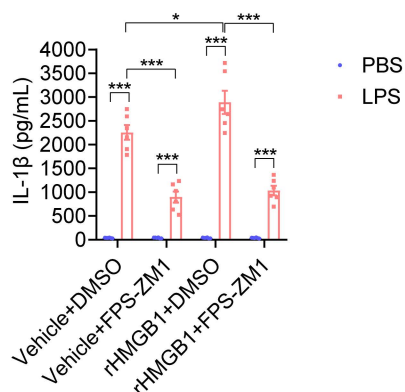
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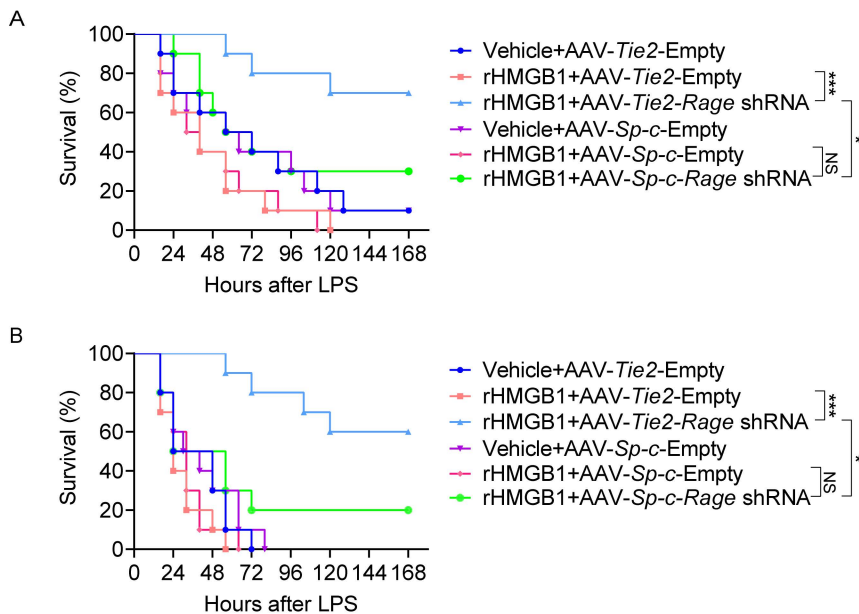
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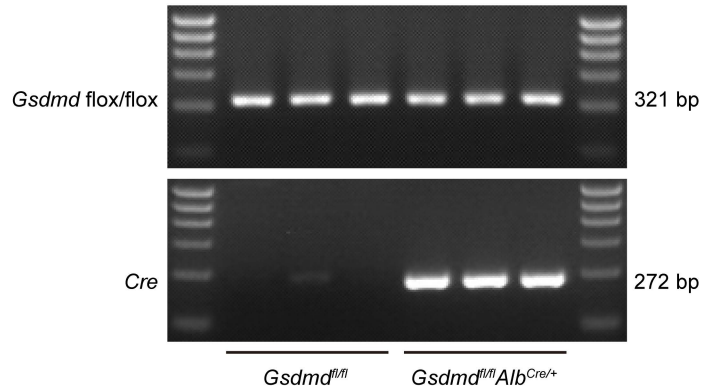
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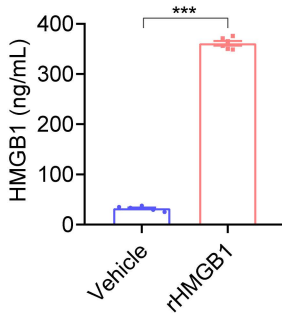
Supplemental Figure 4. HMGB1 binding to RAGE is responsible for systemic vascular injury and death in endotoxemia. WT mice were intraperitoneally injected with FPS-ZM1 (3 mg/kg) or dimethyl sulfoxide (DMSO) at 72, 48, 24, and 1 h before LPS (17.5 mg/kg) injection and at 24, 48, 72, 96, 120, 144 and 168 h after LPS injection. Subsequently, vehicle or rHMGB1 protein was administered intravenously at a dose of 5 µg at 2, 16, 28, and 40 h after LPS injection. **(A)** Mouse survival was monitored on the indicated days and is presented as a Kaplan–Meier plot. n=10 per group. Survival data were compared via a log-rank (Mantel–Cox) test. WT mice were intraperitoneally injected with FPS-ZM1 (3 mg/kg) or DMSO at 72, 48, 24, and 1 h before LPS (17.5 mg/kg) or PBS injection. Then, vehicle or rHMGB1 protein was administered intravenously at a dose of 5 µg at 2 and 16 h, and the blood, aortas and lungs were excised from the mice and analyzed. **(B)** HE staining of the lung sections is presented. The scale bar represents 200 µm. **(C)** The ratio of the wet lung weight to the dry lung weight was determined. n=6 per group. Lung microvascular permeability and aortic permeability were detected via an Evans blue–albumin extravasation assay. **(D)** The amount of extracted pulmonary Evans blue dye in the formamide extracts was quantified by measuring the dye at 620 nm. n=6 per group. **(E)** The aortic Evans blue dye content was quantitatively analyzed. n=6 per group. **(F)** The plasma IL-1β concentration was determined. n=6 per group. The data are expressed as the means ± SEMs. The data were analyzed by two-way ANOVA with Bonferroni post hoc correction. All the data shown are representative of a minimum of 3 independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



Supplemental Figure 5. The role of type II alveolar epithelial RAGE in endotoxemia. Five-week-old WT mice were injected with a null AAV9 vector, an endothelial conditional *Rage* shRNA-knockdown AAV9 vector or a type II alveolar epithelial conditional *Rage* shRNA-knockdown AAV9 vector via the tail vein. After 6 weeks, these mice were intraperitoneally injected with LPS (17.5 mg/kg) or intratracheally instilled with LPS (17.5 mg/kg). Subsequently, vehicle or rHMG1 protein was administered intravenously at a dose of 5 μ g at 2, 16, 28, and 40 h after LPS injection or instillation. The survival of the mice **(A)** that were intraperitoneally injected with LPS or **(B)** intratracheally instilled with LPS was monitored on the indicated days and is presented as a Kaplan–Meier plot. n=10 per group. Survival data were compared via a log-rank (Mantel–Cox) test. All the data shown are representative of a minimum of 3 independent experiments. * $P < 0.05$, *** $P < 0.001$.

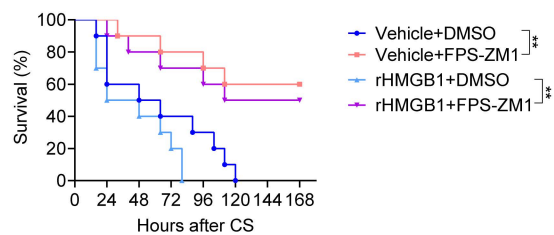


Supplemental Figure 6. Construction of hepatocyte-specific *Gsdmd*-deficient mice. Genotyping of hepatocellular *Gsdmd*-deficient (*Gsdmd*^{fl/fl} *Alb*^{Cre/+}) mice and their *Cre*-negative littermates (*Gsdmd*^{fl/fl} mice).

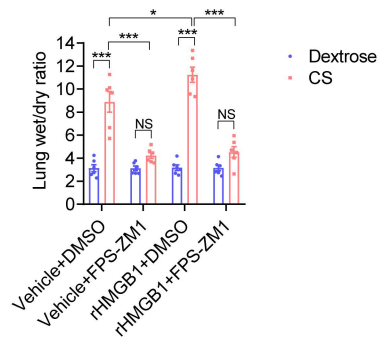


Supplemental Figure 7. The plasma HMGB1 level after rHMGB1 protein injection. *Gsdmd^{fl/fl}Alb^{Cre/+}* mice were intraperitoneally injected with LPS (17.5 mg/kg). Subsequently, vehicle or rHMGB1 protein was administered intravenously at a dose of 5 μ g at 2 and 16 h after LPS injection. The HMGB1 concentration in the plasma was subsequently measured. n=6 per group. The data are expressed as the means $\pm$ SEMs. An unpaired 2-tailed Student *t* test was performed. All the data shown are representative of a minimum of 3 independent experiments. ****P* < 0.001.

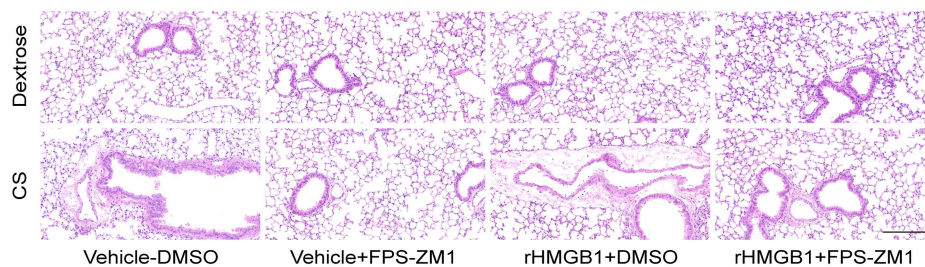
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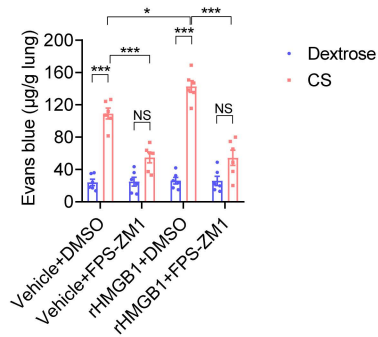
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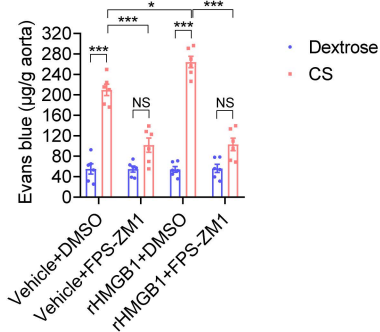
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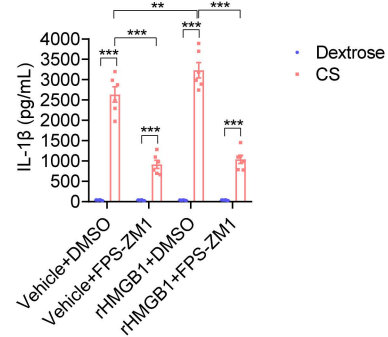
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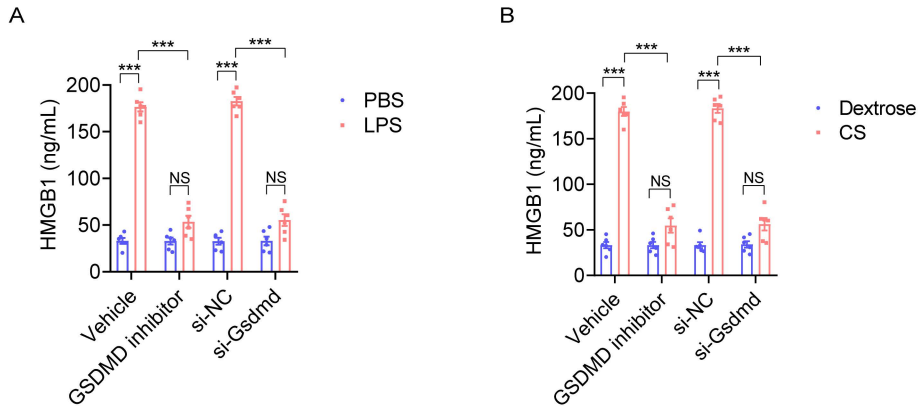
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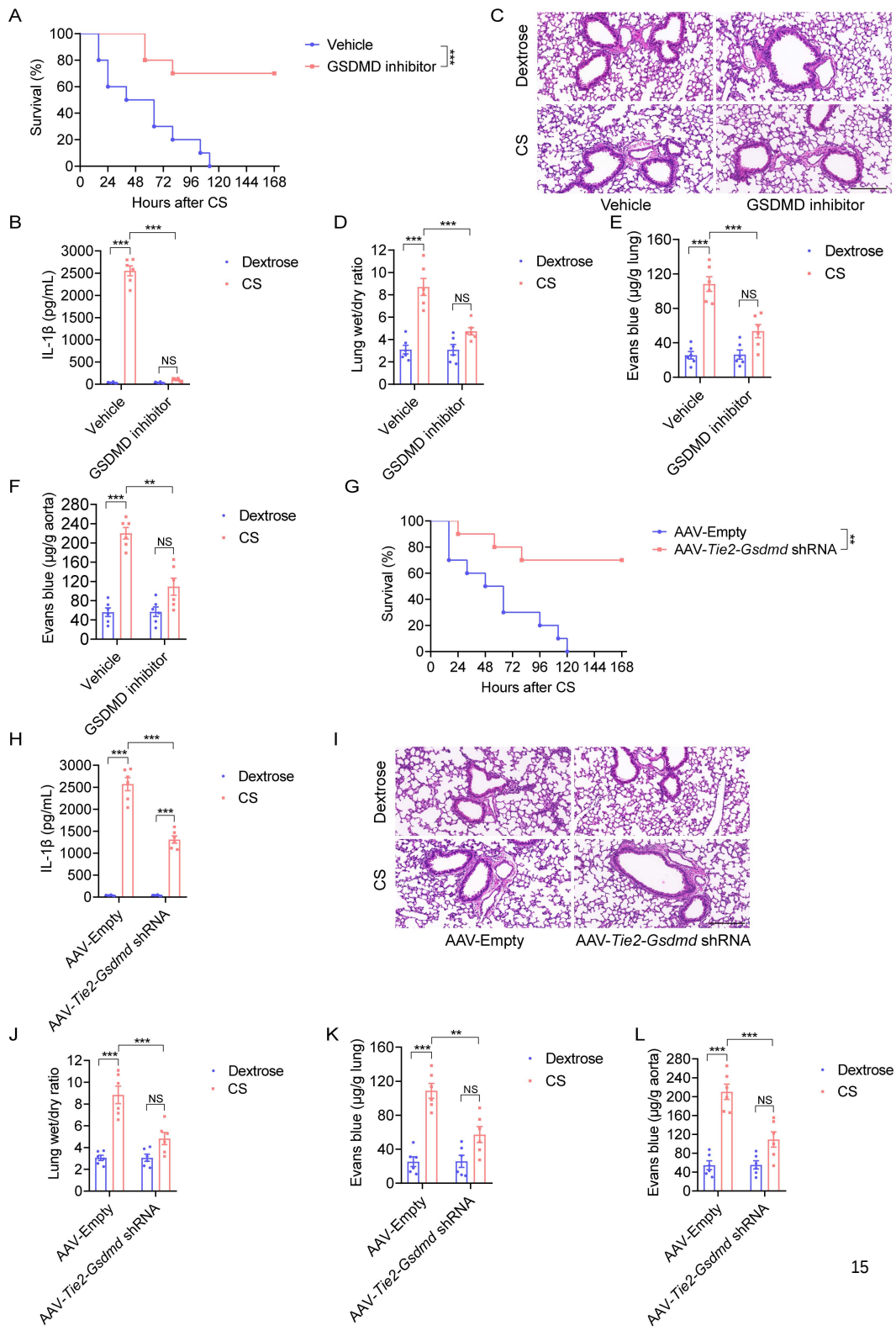
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Supplemental Figure 8. HMGB1 binding to RAGE causes systemic vascular injury and death in sepsis. WT mice were intraperitoneally injected with FPS-ZM1 (3 mg/kg) or DMSO at 72, 48, 24, and 1 h before cecal slurry (CS) (2.5 mg/g) injection and at 24, 48, 72, 96, 120, 144 and 168 h after CS injection. Subsequently, vehicle or rHMGB1 protein was administered intravenously at a dose of 5 μ g at 2, 16, 28, and 40 h after CS injection. **(A)** Mouse survival was monitored on the indicated days and is presented as a Kaplan–Meier plot. n=10 per group. Survival data were compared via a log-rank (Mantel–Cox) test. WT mice were intraperitoneally injected with FPS-ZM1 (3 mg/kg) or DMSO at 72, 48, 24, and 1 h before CS (2.5 mg/g) or 5% dextrose injection. Then, vehicle or rHMGB1 protein was administered intravenously at a dose of 5 μ g at 2 and 16 h, and the blood, aortas and lungs were excised from the mice and analyzed. **(B)** HE staining of the lung sections is presented. The scale bar represents 200 μ m. **(C)** The ratio of the wet lung weight to the dry lung weight was determined. n=6 per group. Lung microvascular permeability and aortic permeability were detected via an Evans blue–albumin extravasation assay. **(D)** The amount of extracted pulmonary Evans blue dye in the formamide extracts was quantified by measuring the dye at 620 nm. n=6 per group. **(E)** The aortic Evans blue dye content was quantitatively analyzed. n=6 per group. **(F)** The plasma IL-1 β concentration was determined. n=6 per group. The data are expressed as the means $\pm$ SEMs. The data were analyzed by two-way ANOVA with Bonferroni post hoc correction. All the data shown are representative of a minimum of 3 independent experiments. * P < 0.05, ** P < 0.01, *** P < 0.001.



Supplemental Figure 9. Effects of a GSDMD activation inhibitor on plasma HMGB1 levels in endotoxemia and sepsis. WT mice were intraperitoneally injected with LPS (17.5 mg/kg) or PBS. Then, the vehicle control or GSDMD inhibitor was administered intraperitoneally at a dose of 5 μ g at 2 and 12 h. In addition, WT mice were injected with *Gsdmd*-siRNA (si-*Gsdmd*) (10 nmol) or negative control (si-NC) (10 nmol) on days 2, 4, and 6 via the tail vein before LPS (17.5 mg/kg) or PBS injection. Blood was excised from the mice and analyzed after treatment with LPS or PBS for 16 h. **(A)** The plasma HMGB1 concentration was determined. n=6 per group. WT mice were intraperitoneally injected with CS (2.5 mg/g) or 5% dextrose. Then, the vehicle control or GSDMD inhibitor was administered intraperitoneally at a dose of 5 μ g at 2 and 12 h. In addition, WT mice were injected with *Gsdmd*-siRNA (si-*Gsdmd*) (10 nmol) or negative control (si-NC) (10 nmol) on days 2, 4, and 6 via the tail vein before CS (2.5 mg/g) or 5% dextrose injection. The blood was excised from the mice and analyzed after treatment with CS or 5% dextrose for 16 h. **(B)** The plasma HMGB1 concentration was determined. n=6 per group. The data are shown as the means $\pm$ SEMs. Two-way ANOVA with Bonferroni post hoc correction was used. All the data shown are representative of a minimum of 3 independent experiments. *** $P < 0.001$.



Supplemental Figure 10. The GSDMD activation inhibitor alleviated systemic vascular injury and death in sepsis. WT mice were intraperitoneally injected with CS (2.5 mg/g). The vehicle control or GSDMD inhibitor was subsequently administered intraperitoneally at a dose of 200 μ g at 2, 12, 24, and 36 h after CS. **(A)** Mouse survival was monitored on the indicated days and is presented as a Kaplan–Meier plot. n=10 per group. A log-rank (Mantel–Cox) test was used to compare survival curves. WT mice were intraperitoneally injected with CS (2.5 mg/g) or 5% dextrose. Then, the vehicle control or GSDMD inhibitor was administered intraperitoneally at a dose of 5 μ g at 2 and 12 h. The blood, aortas and lungs were excised from the mice and analyzed after treatment with CS or 5% dextrose for 16 h. **(B)** The plasma IL-1 β concentration was determined. n=6 per group. **(C)** HE staining of the lung sections. The scale bar represents 200 μ m. **(D)** The lung wet/dry ratio was quantitatively analyzed. n=6 per group. Lung microvascular permeability and aortic permeability were detected via an Evans blue–albumin extravasation assay. **(E)** The amount of extracted pulmonary Evans blue dye in the formamide extracts was quantified by measuring the dye at 620 nm. n=6 per group. **(F)** The aortic Evans blue dye content was quantitatively analyzed. n=6 per group. The data are shown as the means $\pm$ SEMs. Two-way ANOVA with Bonferroni post hoc correction was used. Five-week-old WT mice were injected with a null AAV9 vector or an endothelial-conditioned *Gsdmd* shRNA-knockdown AAV9 vector via the tail vein. After 6 weeks, these mice were intraperitoneally injected with CS (2.5 mg/g). **(G)** A Kaplan–Meier survival plot of mice on the indicated days is presented. n=10 per group. Survival data were analyzed by a log-rank (Mantel–Cox) test. Five-week-old WT mice were injected with a null AAV9 vector or an endothelial conditional *Gsdmd* shRNA-knockdown AAV9 vector via the tail vein and were intraperitoneally injected with CS (2.5 mg/g) or 5% dextrose after 6 weeks. Blood, aortas and lungs were obtained from the mice after treatment

with CS or 5% dextrose for 16 h and were subsequently assessed. **(H)** The plasma IL-1 β concentration was determined. n=6 per group. **(I)** Representative HE-stained images of the lung sections are presented. The scale bar represents 200 μ m. **(J)** The ratio of wet lung weight to dry lung weight was determined. n=6 per group. Lung microvascular permeability and aortic permeability were detected via an Evans blue–albumin extravasation assay. **(K)** The amount of extracted pulmonary Evans blue dye in the formamide extracts was quantified by measuring the dye at 620 nm. n=6 per group. **(L)** The aortic Evans blue dye content was quantitatively analyzed. n=6 per group. The data are expressed as the means $\pm$ SEMs. The data were analyzed by two-way ANOVA with Bonferroni post hoc correction. All the data shown are representative of a minimum of 3 independent experiments. ** $P < 0.01$, *** $P < 0.001$.