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Supplementary information

Gasdermin D-dependent platelet pyroptosis exacerbates NET formation and inflammation in severe sepsis

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Gasdermin D-dependent platelet pyroptosis exacerbates NET formation and inflammation in severe sepsis

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Supplementary Tables

Supplementary Table 1: Demographic characteristics and laboratory findings of the cohort on admission.

Variable	Total (n = 93)	Sepsis (n = 37)	Severe sepsis		P value			
			Severe (n = 22)	Shock (n = 34)	Overall	Severe vs Sepsis	Shock vs Sepsis	Shock vs Severe
Demographics								
Age < 1 year	50 (54)	20 (54)	16 (73)	14 (41)	0.0689	-	-	-
Age 1~5 years	32 (34)	16 (43)	4 (18)	12 (35)	0.1453	-	-	-
Age 5~12 years	9 (10)	1 (3)	1 (5)	7 (21)	0.0349	>0.9999	0.0721	0.1948
Age 12~18 years	2 (2)	0 (0)	1 (5)	1 (3)	0.4691	-	-	-
Gender male, n (%)	53 (57)	25 (68)	12 (55)	16 (47)	0.2111	-	-	-
Medications								
Cephalosporin antibiotics, n (%)	32 (34)	17 (46)	7 (32)	8 (24)	0.1333	-	-	-
Vancomycin antibiotics, n (%)	16 (17)	1 (3)	4 (18)	11 (32)	0.0017	00593	0.0010	0.3562
Penicillin antibiotics, n (%)	15 (16)	6 (16)	5 (23)	4 (12)	0.5567	-	-	-
Typical Symptoms								
Fever, n (%)	80 (86)	36 (97)	17 (68)	27 (79)	0.0192	0.0360	0.0360	>0.9999
Dyspnea, n (%)	56 (60)	0 (0)	22 (100)	34 (100)	<0.0001	<0.0001	-<0.0001	>0.9999
Primary source of infection, n (%)								
Respiratory tract	61 (66)	12 (32)	22 (100)	27 (79)	<0.0001	<0.0001	0.0003	0.0627
Abdomen	14 (15)	9 (24)	2 (9)	3 (9)	0.1556	-	-	-
Skin	14 (15)	7 (19)	0 (0)	7 (21)	0.0528	-	-	-
Cardiovascular	10 (11)	3 (8)	0 (0)	7 (21)	0.0366	0.2860	0.2670	0.1050

Urinary tract	7 (8)	5 (14)	0 (0)	2 (6)	0.1804	-	-	-
Hematologic and inflammatory data								
WBC (10^9/L)	15.04 ± 11.07	19.87 ± 11.72	12.59 ± 9.81	11.25 ± 9.20	0.0023	0.0383	0.0034	>0.9999
Lymphocyte (10^9/L)	3.82 ± 4.62	5.19 ± 5.99	3.53 ± 3.45	2.47± 2.91	0.0004	0.1228	0.0003	0.4718
PMNs (10^9/L)	8.91 ± 8.00	12.51 ± 8.54	7.06 ± 8.12	6.12 ± 5.59	0.0009	0.0113	0.0023	>0.9999
Monocyte (10^9/L)	1.41 ± 1.31	1.88 ± 1.41	1.15 ± 0.81	1.05 ± 1.34	0.0004	0.0904	0.0002	0.5715
Eosinophil (10^9/L)	0.24 ± 0.68	0.16 ± 0.25	0.58 ± 1.28	0.11 ± 0.24	0.0036	0.9984	0.0364	0.0053
Basophil (10^9/L)	0.04 ± 0.10	0.04 ± 0.04	0.04 ± 0.05	0.03 ± 0.06	0.0139	>0.9999	0.0571	0.0255
N (%)	52.99 ± 19.75	55.73 ± 17.76	50.18 ± 20.97	51.82 ± 21.17	0.3938	-	-	-
L (%)	28.23 ± 18.09	27.38 ± 14.47	31.36 ± 19.15	27.12 ± 21.02	0.4530	-	-	-
RBC (10^9/L)	3.59 ± 0.81	3.98 ± 0.64	3.51 ± 0.79	3.21 ± 0.81	0.0001	0.0546	<0.0001	0.2881
HGB (g/L)	102.59 ± 26.02	111.22 ± 19.98	107.14 ± 28.29	90.26 ± 26.25	0.0003	>0.9999	0.0003	0.0210
PCT (ng/L)	21.12 ± 32.74	2.71 ± 4.62	22.35 ± 35.86	35.85 ± 36.92	<0.0001	0.0281	<0.0001	0.2447
CRP (mg/L)	81.02 ± 78.02	89.37 ± 70.34	48.93 ± 65.90	92.68 ± 88.91	0.0396	0.0485	>0.9999	0.0994
hsCRP (mg/L)	88.95 ± 83.63	106.52 ± 76.45	57.91 ± 93.35	91.02 ± 80.73	0.0124	0.0098	>0.9999	0.1150
APTT (S)	50.41 ± 14.54	43.94 ± 8.94	53.60 ± 18.21	55.20 ± 14.54	0.0006	0.0432	0.0006	>0.9999
PT (S)	17.73 ± 5.12	14.64 ± 1.59	18.32 ± 5.45	20.61 ± 5.62	<0.0001	0.0011	<0.0001	0.2702
FIB (g/L)	3.79 ± 2.25	5.48 ± 2.03	2.63 ± 1.78	2.76 ± 1.59	<0.0001	<0.0001	<0.0001	>0.9999
ALT (U/L)	99.22 ± 329.72	22.05 ± 18.13	31.14 ± 27.79	227.24 ± 524.96	0.0004	>0.9999	0.0005	0.0158
AST (U/L)	103.06 ± 146.28	37.05 ± 23.21	87.09 ± 79.47	185.24 ± 207.45	0.0021	0.0515	0.0025	>0.9999
LDH (U/L)	566.17 ± 703.68	256.43 ± 166.42	644.41 ± 644.76	861.30 ± 948.26	<0.0001	0.0006	<0.0001	>0.9999
ALB (g/L)	33.76 ± 7.41	37.59 ± 6.06	31.04 ± 5.24	31.36 ± 8.27	0.0001	0.0016	0.0006	0.9838
LAC (mmol/L)	3.00 ± 2.88	2.45 ±1.42	2.11 ± 1.33	4.11 ± 4.11	0.221	-	-	-
D-dimer (mg/L)	6.94 ± 6.09	1.11 ± 0.84	7.14 ± 4.55	8.52 ± 6.33	0.0007	0.0218	0.0005	>0.9999
Severity of disese								
APACHE IV score	89.69 ± 34.62	59.29 ± 20.79	114.18 ± 33.40	105.15 ± 22.04	<0.0001	<0.0001	<0.0001	>0.9999
Hospital mortality	35.55 ± 28.07	10.74 ± 10.20	58.66 ± 28.31	46.13 ± 20.18	<0.0001	<0.0001	<0.0001	0.8134

assessment (%)								
Hospital mortality, n (%)	20 (22)	0 (0)	3 (14)	17 (50)	<0.0001	0.0431	<0.0001	0.0145
Mechanical ventilation (%)	57 (61)	1 (3)	22 (100)	34 (100)	<0.0001	<0.0001	<0.0001	>0.9999

Data are expressed as n/N (%) or mean ± SD unless otherwise stated. For variables with overall p values greater than 0.05, we did not perform group comparisons. Categorical variables were analyzed using Chi-square test or Fisher's exact test. One-way ANOVA and Tukey's multiple comparisons test for RBC and ALB. Kruskall-Wallis test and Dunn's multiple comparisons test for WBC, N, L, HGB, ALT, AST, LDH, LAC, D-dimer and APACHE IV score. WBC, white blood cell; PMNs, polymorphonuclear neutrophils; N%, PMN to white blood cell ratio; L%, lymphocyte to white blood cell ratio; RBC, red blood cell; HGB, hemoglobin; PCT, procalcitonin; CRP, C-reactive protein; hsCRP, high-sensitivity C-reactive protein; APTT, activated partial thromboplastin time; PT, prothrombin time; FIB, fibrinogen; ALT, alanine transaminase; AST; aspartate aminotransferase; LDH, lactic dehydrogenase; ALB, albumin; LAC, lactate; APACHE, acute physiology and chronic health evaluation.

Supplementary Table 2: The platelet parameters of HS, sepsis, severe sepsis and septic shock patients

Variable	HS (n = 75)	Sepsis (n = 37)	Severe sepsis	Septic shock	P value						
					Overall	Sepsis vs. HS	Severe sepsis vs. HS	Septic shock vs. HS	Severe sepsis vs. Sepsis	Septic shock vs. Sepsis	Septic shock vs. Severe sepsis
PLT (10^9/L)	292.08 ± 53.78	358.68± 113.35	171.32 ± 105.35	72.26 ± 58.25	<0.0001	0.0477	0.001	<0.0001	<0.0001	<0.0001	0.0798
MPV (fL)	10.04 ± 0.78	9.97 ± 1.06	11.19 ± 1.24	11.15 ± 1.25	<0.0001	>0.9999	0.0014	0.0001	0.0012	0.0001	>0.9999
PDW (fL)	11.13 ± 1.66	10.79 ± 2.15	14.19 ± 3.79	13.26 ± 3.39	<0.0001	0.6901	0.0029	0.0111	<0.0001	0.0003	>0.9999
P-LCR (%)	24.55 ± 5.76	23.88 ± 8.43	33.10 ± 9.81	33.68 ± 9.65	<0.0001	>0.9999	0.0046	<0.0001	0.0017	<0.0001	>0.9999

Data are expressed as mean ± SD. For variables with overall p values greater than 0.05, we did not perform group comparisons. Kruskall-Wallis test and Dunn's multiple comparisons test for PLT, MPV, PDW, P-LCR. PLT, platelet; MPV, mean platelet volume, PDW, platelet distribution width; P-LCR, platelet large cell ratio.

Supplementary Table 3: The parameters of platelets from platelet-specific *Gsdmd* KO mice and *Gsdmd*$^{fl/fl}$ mice

	Platelet-specific *Gsdmd* KO mice	*Gsdmd*$^{fl/fl}$ mice	P value
Platelet count (10^9/L)	933.60 ± 83.74	973.20 ± 140.92	0.6038
Mean platelet volume (MPV, fL)	6.34 ± 0.05	6.40 ± 0.19	0.6587
Platelet distribution width (PDW, fL)	5.38 ± 0.04	5.48 ± 0.08	0.119
Platelet large cell ratio (P-LCR, %)	2.20 ± 0.64	2.72 ± 1.06	0.375

Platelet parameters were compared between platelet-specific *Gsdmd* KO mice and *Gsdmd*$^{fl/fl}$ mice. The morphological parameters: Platelet count, mean platelet volume, platelet distribution width, and platelet large cell ratio were compared in platelet-specific *Gsdmd* KO mice and *Gsdmd*$^{fl/fl}$ mice (n = 5) (mean ± SD). Unpaired t test with two-tailed for platelet count and P-LCR. Mann Whitney test with two-tailed for MPV and PDW. P value of 0.05 or less was considered statistically significant. Abbreviation is as follow: GSDMD, Gasdermin D; KO, knockout; MPV, high mean platelet volume, PDW, high platelet distribution width; P-LCR, high platelet large cell ratio.

Supplementary Table 4: The parameters of platelets from *Tlr4*$^{-/-}$ mice and WT mice

	***Tlr4*$^{-/-}$ mice**	**WT mice**	**P value**
Platelet count (10^9/L)	978.80 ± 145.55	938.80 ± 35.54	0.567
Mean platelet volume (MPV, fL)	6.44 ± 0.17	6.48 ± 0.08	0.6454
Platelet distribution width (PDW, fL)	5.60 ± 0.22	5.78 ± 0.18	0.1975
Platelet large cell ratio (P-LCR, %)	2.34 ± 0.96	2.04 ± 0.95	0.6334

Platelet parameters were compared between *Tlr4*$^{-/-}$ mice and WT mice. The morphological parameters: Platelet count, mean platelet volume, platelet distribution width, and platelet large cell ratio were compared in *Tlr4*$^{-/-}$ mice and WT mice (n = 5) (mean ± SD). Unpaired t test with two-tailed for platelet count, MPV, PDW and P-LCR. P value of 0.05 or less was considered statistically significant. Abbreviation is as follow: TLR4, toll-like receptor 4; WT, wild type; MPV, high mean platelet volume, PDW, high platelet distribution width; P-LCR, high platelet large cell ratio.

Supplementary Table 5: Age specific vital signs and laboratory variables in pediatric sepsis

Age group	Temperature (°C)	WBC ($\times 10^9$/L)	Heart rate (beats/minute)	Respiratory rate (breaths/minute)	Systolic blood pressure (mmHg)
0 days~1 week	< 36 or > 38.5	> 34	> 180 or < 100	> 50	< 65
1 week~1 month	< 36 or > 38.5	> 19.5 or < 6	> 180 or < 100	> 40	< 75
1 month~1 year	< 36 or > 38.5	> 17.5 or < 6	> 180 or < 90	> 34	< 100
1~5 years	< 36 or > 38.5	> 15.5 or < 6	> 140	> 22	< 4
5~12 years	< 36 or > 38.5	> 13.5 or < 4.5	> 130	> 18	< 104
12~18 years	< 36 or > 38.5	> 11 or < 4.5	> 110	> 14	< 117

Extended Data Figure Legends

Extended Data Fig.1: Identified purity of platelet, representative proteins related to different cell death signal pathways and platelet pyroptosis in sepsis.

a, Purified platelets were obtained from human. Purity of platelet preparation was determined by FACS analysis using FITC anti-human CD41a and PE anti-human CD45 (n = 3). **b**, Heatmap of representative proteins expression related to different cell deaths signal pathways in purified platelet samples from HS (n = 3) and severe sepsis (with or without septic shock) (n = 3) using high-throughput proteomics analysis. **c**, Bar graphs displaying the percentage of activations of caspase 1 in platelets from sepsis and severe sepsis (with or without septic shock) and HS using FACS **(**HS: n = 13, Sepsis: n = 10, Severe sepsis with or without septic shock: n = 13). Data was presented as mean ± SD. Kruskall-Wallis test and Dunn's multiple comparisons test for **c**. HS, healthy subjects; Severe sepsis, severe sepsis/septic shock; PLTs, platelets.

Extended Data Fig.2: TEM images of platelets induced by apoptosis, autophagy or pyroptosis agonists and apoptosis in severe sepsis patients.

a, Platelets were induced to apoptosis (10 μM ABT-737 induces apoptosis), autophagy (10 μM FCCP induces autophagy) and pyroptosis (10 μg/ml LPS and 5 μM Nigericin induces pyroptosis). TEM imaging of different states in platelets. **b**, Representative lower and higher power TEM field demonstrating loss of platelet ultrastructure in severe sepsis (with or without septic shock) patients (n = 5), with reduced granules/organelles and increased vacuolation. Apoptosis, red arrowheads indicate shrinkage of cell membrane and apoptotic bodies in apoptosis of platelet. Scale bars: 1 μm and 500 nm. Severe sepsis, severe sepsis/septic shock; PLTs, platelets.

Extended Data Fig.3: The expression and localization of NLRP3 and ASC in severe sepsis platelets or rhS100A8/A9-induced platelets.

a, **b**, Immunofluorescence analysis showing the co-localization of CD41 (green), ASC (red) and NLRP3 (blue) in platelets from severe sepsis (with or without septic shock) patients (**a**) (n = 8); and platelets treated with 1 μg/ml rhS100A8/A9 or 10 μM Paquinimod (**b**) (n = 6); purple indicates overlap. Scale bars: 5 μm and 1 μm. HS,

healthy subjects; Severe sepsis, severe sepsis/septic shock; NLRP3, NOD-like receptors containing domain pyrin 3 inflammasome; ASC, adaptor-apoptosis-associated speck-like protein; Paq, Paquinimod.

Extended Data Fig.4: The identification and classic functions of platelets from platelet-specific *Gsdmd* KO mice.

a, b, The *Gsdmd*$^{fl/fl}$ PF4-Cre mice were identified by PCR **(a)** and confirmed by western blot **(b)**, respectively (n = 6). **c**, The platelet (isolated from mice) suspensions were incubated with 0.1 U/ml thrombin for 30 minutes. P selectin translocation to membrane was assessed by FACS after stimulation with thrombin. The representative plots were presented as the number of counts over the log of associated fluorescence (baseline refers to the group without thrombin). Quantification of data presented as percentage of platelet activation. Data are expressed as mean ± SD (*Gsdmd*$^{fl/fl}$+HBSS, n = 5; *Gsdmd*$^{fl/fl}$+Thrombin, n = 6; *Gsdmd*$^{fl/fl}$ PF4-Cre+Thrombin, n = 4). **d**, Tail bleeding times of mouse was measured with the tail dipped into warmed saline to assess haemostasis using a tail-guillotine**.** Data are expressed as mean ± SD (*Gsdmd*$^{fl/fl}$ mice, n = 7; *Gsdmd*$^{fl/fl}$ PF4-Cre mice, n = 6). One-way ANOVA and Tukey's multiple comparisons test for **c**. Unpaired t test with two-tailed for **d**. MT, mutation; WT, wild type; Tg, transgene; Ctrl, control; GSDMD, Gasdermin D; PLTs, platelets; HBSS, hank's balanced salt solution.

Extended Data Fig.5: The levels of S100A8/A9 in sepsis patients/mice and the caspase 1 activity in platelets from the CLP or rmS100A8/A9-injected mice.

a-c, Boxplots displaying the level of heterodimer S100A8/A9 in plasma from **(a)** severe sepsis (with or without septic shock) patients (HS: n = 53, Severe sepsis: n = 51), **(b)** CLP-induced sepsis mice (Sham, n = 10, CLP: n = 20) and **(c)** LPS-induced sepsis mice (PBS, n = 8, LPS: n = 8) by ELISA. The boxes indicate the 25% quantile, median, and 75% quantile. **d**, In a mouse model, mice that were injected intravenously with rmS100A8/A9 (30 μg/kg) or normal saline (n = 4 mouse/group) for 6 hours. Another mouse model, mice were induced CLP for 6 hours. FACS analysis displaying the caspase 1 activity in platelets. Mann Whitney test with two-tailed for **a-b**. Unpaired t test with two-tailed for **c**. One-way ANOVA and Tukey's multiple comparisons test for **d**. Data was presented as mean ± SD. HS, healthy subjects; Severe sepsis, severe sepsis/septic shock; Sham, sham-operated mice; CLP, CLP-induced sepsis mice; PBS, PBS-injected mice; LPS, LPS-injected mice.

Extended Data Fig.6: Putative receptors for S100A8/A9-induced platelet pyroptosis.

The platelet (isolated from HS) suspensions were incubated in the presence of neutralizing monoclonal antibodies (20 μg/ml) against control IgG, CD36, RAGE, or

TLR4, and then treated with 1 μg/ml rmS100A8/A9 for 4 hours. **a**, FACS analysis displaying the caspase 1 activity in human platelets after stimulation. The quantified results are shown on the below. **b**, P selectin translocation to membrane (CD62P) was assessed by flow cytometry after stimulation. The quantified results are shown on the below. Data was presented as mean ± SD, n = 4. One-way ANOVA and Tukey's multiple comparisons test for **a, b**. HS, healthy subjects; TLR4, toll-like receptor 4; RAGE, advanced glycation end products.

Extended Data Fig.7: NLRP3 inflammasome and caspase 1 activity of platelets in mice transfused with *Tlr4*$^{-/-}$ or WT platelets.

a, *In vitro*, FACS analysis displaying the caspase 1 activity in platelets (*Tlr4*$^{-/-}$ or WT) treated with 1 μg/ml rmS100A8/A9 for 4 hours (n = 5). The quantified results are shown on the right. **b-f,** *In vivo*, a total of 1.2 x 10^7 purified platelets (volume: 200 μl, concentration: 6 x 10^{10} platelets/L) from *Tlr4*$^{-/-}$ or WT mice were intravenously transfused to mT/mG: PF4-Cre mouse. (**b**) After platelet depletion, platelet counts in mice were assessed at 0, 2, 4, 6, 8, 24 and 26 hours using a hematology analyzer (n = 3). (**c**) Platelet counts in mice before and after transfused with WT or *Tlr4*$^{-/-}$ platelets were detected at 0, 0.5, 2, 4 and 6 hours using a hematology analyzer (n = 3). (**d**) The percentages of transfused *Tlr4*$^{-/-}$ platelets in total platelets of mice were detected at 0, 0.5, 2, 4 and 6 hours using FACS analysis (n = 3). (**e**) Caspase 1 activity was measured using FACS analysis (n = 6). (**f**) The association of ASC and NLRP3 inflammasome in murine platelets was measured by immunofluorescence analysis. Platelets were stained for CD41 (green), ASC (red) and NLRP3 (blue); scale bars: 5 μm and 1 μm; n = 6. Data was presented as mean ± SD. One-way ANOVA and Tukey's multiple comparisons test for **a**. Two-way ANOVA test for **c**, **d**. Abbreviation is as follow: HBSS, hank's balanced salt solution; Saline, mice transfused with normal saline; WT PLTs, LPS-injected mice transfused with WT platelets; *Tlr4*$^{-/-}$ PLTs, LPS-injected mice transfused with *Tlr4*$^{-/-}$ platelets. PBS, PBS-injected mice; LPS+saline, LPS-injected mice transfused with normal saline; LPS+WT PLTs, LPS-injected mice transfused with WT platelets; LPS+*Tlr4*$^{-/-}$ PLTs, LPS-injected mice transfused with *Tlr4*$^{-/-}$ platelets; LPS, lipopolysaccharide; NLRP3, NOD-like receptors containing domain pyrin 3 inflammasome; ASC, adaptor-apoptosis-associated speck-like protein.

Extended Data Fig.8: The function of mitochondria in septic platelets and S100A8/A9-induced platelets.

a, In platelets from severe sepsis (with or without septic shock) patients, bar graphs displaying change of mitochondrial membrane potential ($\Delta\Psi_m$) by staining with 40 nM TMRM using FACS analysis (HS: n = 20, Severe sepsis: n = 25). **b**, **c**, *In vitro*, bar graphs displaying change of mitochondrial $\Delta\Psi_m$ (**b**) and ROS production (**c**) in platelets (*Tlr4*$^{-/-}$ or WT) treated with 1 μg/ml rmS100A8/A9 for 4 hours using FACS

analysis (n = 5). **d**, **e**, In the LPS induced murine model, mice with platelets depletion were transfused with a total of 1.2 x 10^7 purified platelets (volume: 200 μl, concentration: 6 x 10^{10} platelets/L) from *Tlr4*$^{-/-}$ or WT mice (n = 6/group). After 6 hours, bar graphs displaying change of mitochondrial $\Delta\Psi_m$ (**d**) and ROS production (**e**) in platelets (*Tlr4*$^{-/--}$ or WT) using FACS analysis (n = 6). Data was presented as mean fluorescence ± SD. Unpaired t test with two-tailed for **a**. One-way ANOVA and Tukey's multiple comparisons test for **b-e**. Abbreviation is as follow: HS, healthy subjects; Severe sepsis, severe sepsis/septic shock; HBSS, hank's balanced salt Solution. PBS, PBS-injected mice; LPS+saline, LPS-injected mice transfused with normal saline; LPS+WT PLTs, LPS-injected mice transfused with WT platelets; LPS+*Tlr4*$^{-/-}$ PLTs, LPS-injected mice transfused with *Tlr4*$^{-/-}$ platelets; TMRM, tetramethylrhodamine methyl ester; ROS, reactive oxygen species.

Extended Data Fig.9: The formation of NET with different treatments and the release of ox-mtDNA from S100A8/A9-induced platelets after MitoTempo treatment.

a, Representative immunofluorescence of platelets treated with rhS100A8/A9 alone or supernatants of rhS100A8/A9-induced platelets for 4 hours. Cells were stained with Hoechst for DNA (blue), anti-citrullinated H3 for PMNs or NETs (cyan); scale bars: 5 μm; n = 4. **b-c**, PMNs isolated from HS were incubated with PBS, 50 nM PMA, and resting platelets, 0.1 U/ml thrombin activated platelets or 1 μg/ml S100A8/A9-induced platelets for 4 hours. Representative immunofluorescence of NET formation treated with PMA, resting platelets, thrombin or S100A8/A9-induced platelets (**b**). Cells were stained with Hoechst for DNA (blue), anti-citrullinated H3 for PMNs or NETs (green), CD41 for platelet (red). (**c**) Quantification of MPO-DNA and dsDNA in the supernatant of NET formation using PicoGreen fluorescent dye and MPO-DNA-ELISA, respectively (n = 6). **d-e**, Purified platelets suspensions were treated with rhS100A8/A9 (1 μg/ml) and MitoTempo (5 mM) for 4 hours, and then 50 nM PMA, S100A8/A9-induced platelets or MitoTempo-S100A8/A9-induced platelets induced NET formation. (**d**) The levels of ox-mtDNA in supernatant of S100A8/A9-induced platelets were determined by General 8-OHdG ELISA Kit (n = 4). **(e**) Quantification of MPO-DNA and dsDNA (NETosis) in the supernatant of cells using PicoGreen fluorescent dye and MPO-DNA-ELISA, respectively (n = 3). Data was presented as mean ± SD. One-way ANOVA and Tukey's multiple comparisons test for **c-e**. Abbreviation is as follow: HS, healthy subjects; PLTs, platelets; PMNs, polymorphonuclear neutrophils; PMA, phorbol myristate acetate; MPO, myeloperoxidase; dsDNA, double-stranded DNA; NET, neutrophil extracellular trap; ox-mtDNA, oxidized mitochondrial DNA.

Extended Data Fig.10: Immunofluorescence of pyroptotic platelets in NETs and the change of platelet counts in *Gsdmd*$^{fl/fl}$ PF4-Cre mice by CLP.

a, PMNs (*S100a9*$^{-/-}$ or WT) were incubated with 50 nM PMA to induced NET

formation for 4 hours, and then incubated with platelets for another 4 hours. Representative immunofluorescence of PMNs incubated with platelets. Cells were stained with Hoechst for DNA (blue), anti-MPO for PMNs or NETs (cyan), CD41 for platelet (red) and activated caspase 1 for pyroptosis (green); scale bars: 25 μm and 5 μm. **b**, In the CLP-induced sepsis model, platelet counts in *Gsdmd*$^{fl/fl}$ PF4-Cre mice and littermate control *Gsdmd*$^{fl/fl}$ mice were assessed at 0, 2, 4, and 6 hours using a hematology analyzer (n = 5). Data was presented as mean ± SD. Two-way ANOVA and Tukey's multiple comparisons test for b. Abbreviation is as follow: PLT, platelet; PMNs, polymorphonuclear neutrophils; PMA, phorbol myristate acetate; MPO, myeloperoxidase; Sham, sham-operated mice; CLP, CLP-induced sepsis mice; GSDMD, Gasdermin D.