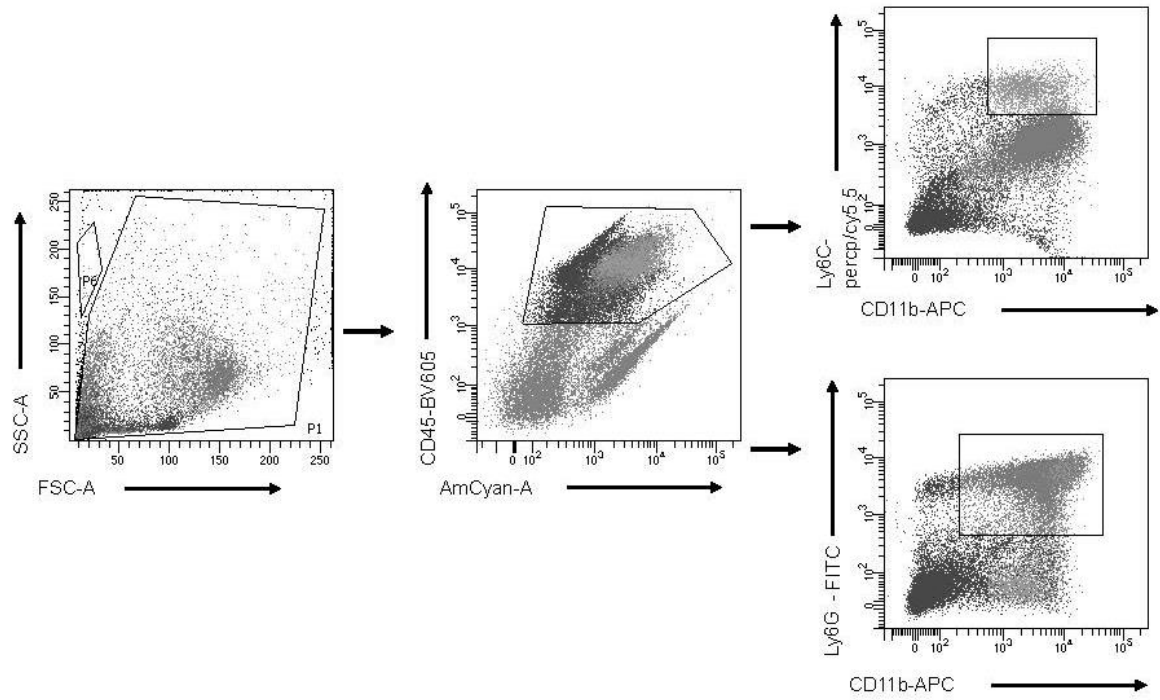


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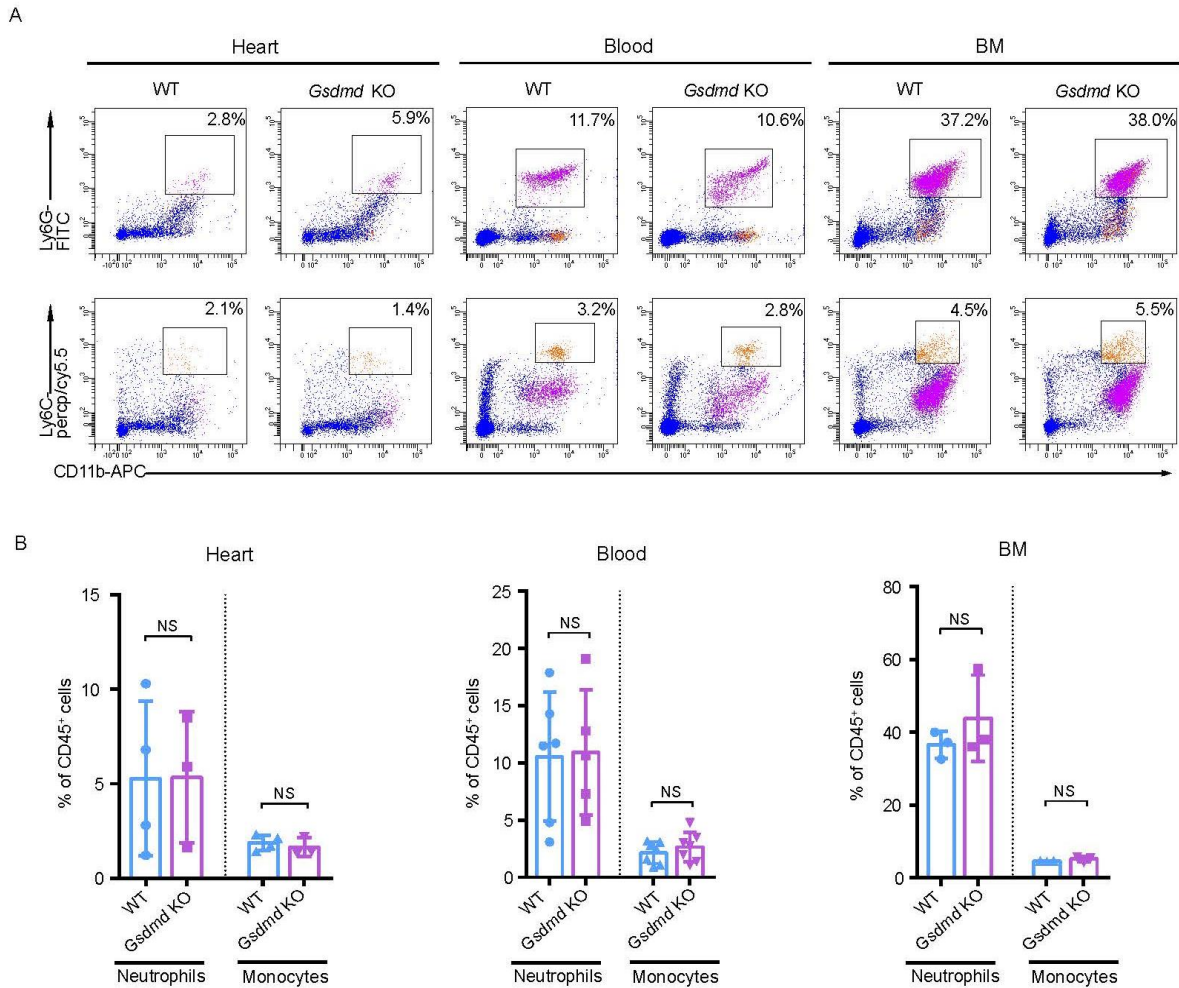
2 **Figure S1. Gene expression analysis by RNA-Seq.**

3 (A) The distribution of samples was determined by the 1st and 2nd principal component generated
 4 by principal component analysis (PCA). (B) Represented one of the dynamic expression patterns
 5 of genes across Sham, MI (Day 1) and MI (Day 7), with (C) showing the functional analysis of
 6 such genes by GO Biological Process based on hypergeometric distribution (the adjusted P-value
 7 < 0.05).

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9 **Figure S2. Gating strategy for flow cytometric analysis.**
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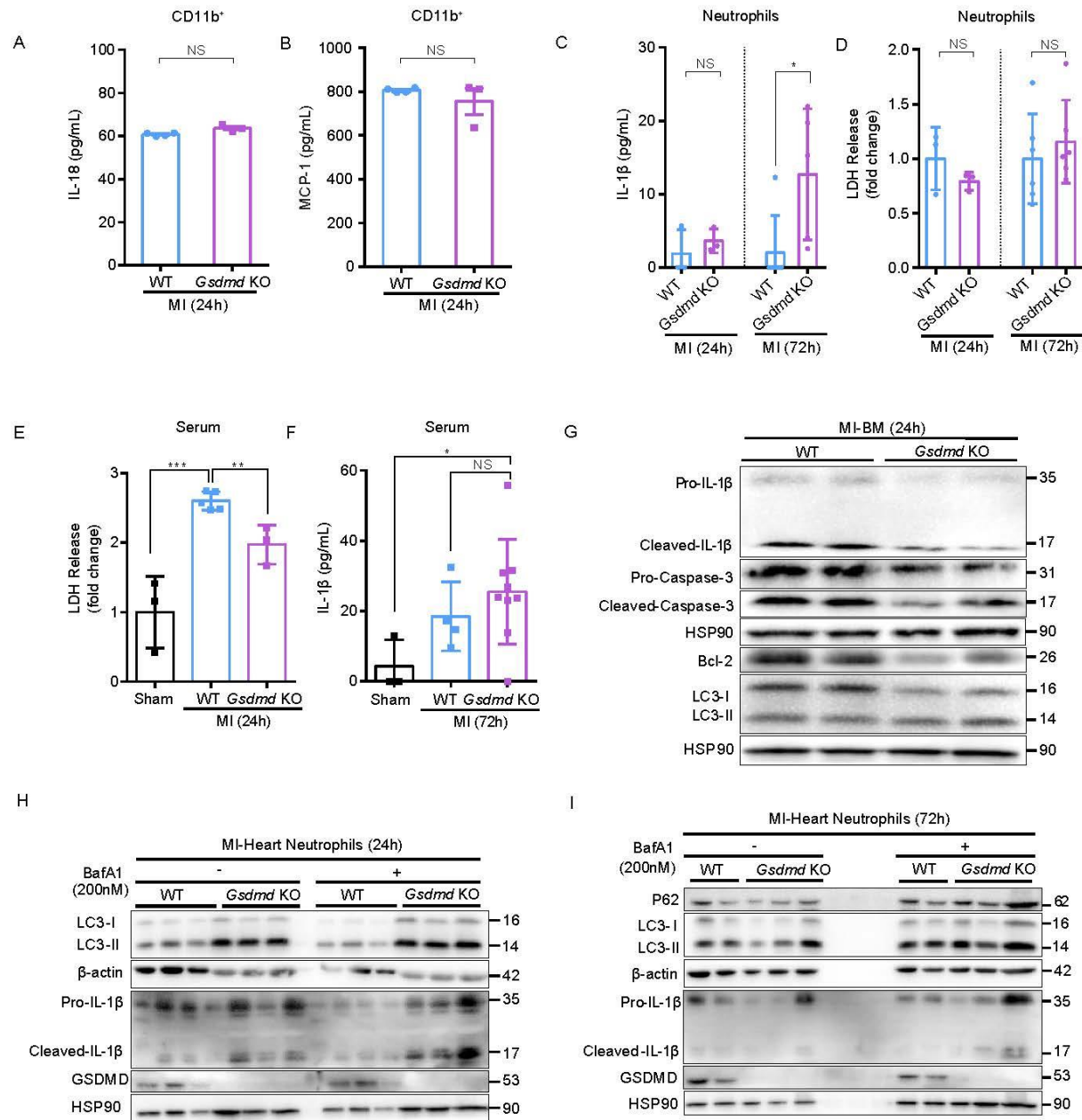
12 **Figure S3. The baseline of WT or *Gsdmd*^{-/-} mice leukocytes.**

13 Flow cytometric analysis (A) and quantification (B) of Cd11b⁺Ly6G⁺ neutrophils and
 14 Cd11b⁺Ly6C⁺ monocytes in blood from WT or *Gsdmd*^{-/-} mice at baseline without a MI surgery
 15 (n= 3-7). Data are mean ±SD and were analyzed by unpaired two-tailed Student's *t* test (B). NS,
 16 not significant.

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Supplemental Figure 4



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20 **Figure S4. Biochemical analysis of myeloid-originated cells from the heart.**

21 **(A-B)** Secretion levels of IL-18 and MCP-1 from myeloid-originated cells from the heart of WT (n
 22 = 4) or *Gsdmd*^{-/-} (n = 3) mice at 24 hours after MI assessed by ELISA. **(C)** Secretion levels of LDH
 23 from neutrophils (2 \times 10⁶) from the heart of WT or *Gsdmd*^{-/-} mice at 24 hours and 72 hours after MI.
 24 **(D)** Production of IL-1 β from neutrophils from the heart of WT or *Gsdmd*^{-/-} mice at 24 hours and

25 72 hours after AMI assessed by ELISA. The corresponding n values were indicated in the plot. **(E)**
26 Serum levels of LDH from WT or *Gsdmd*^{-/-} mice at 24 hours after MI or a sham surgery. **(F)**
27 Serum levels of IL-1 β from WT or *Gsdmd*^{-/-} mice at 72 hours after MI or a sham surgery. **(G)**
28 Representative immunoblotting images of protein levels of BM cells from WT or *Gsdmd*^{-/-} mice
29 at 24 hours after MI or a sham surgery (n = 2 per group). **(H-I)** Representative immunoblotting
30 images of protein levels of neutrophils cells with or without BafA1 (200 nM) from the heart of WT
31 or *Gsdmd*^{-/-} mice at 24 hours or 72 hours after MI (n = 2 - 3 per group). Data represent mean \pm SD
32 and were analyzed by unpaired two-tailed Student's *t* test **(A, C and D)** or One-way analysis of
33 variance followed by Tukey's multiple comparison test **(E)**. NS, not significant; *, $P < 0.05$; **,
34 $P < 0.01$; ***, $P < 0.001$.

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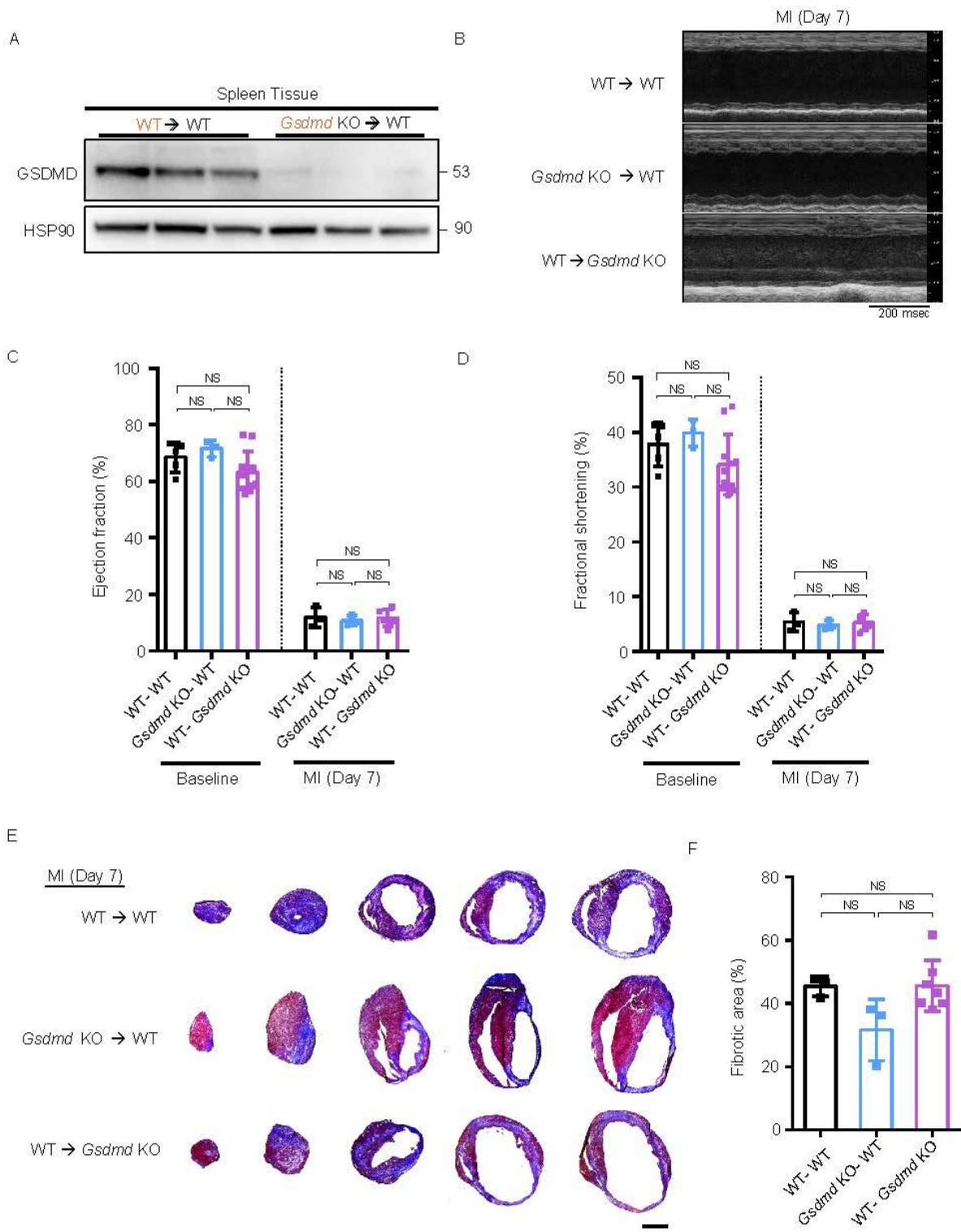
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Supplemental Figure 5



42 **Figure S5. Bone marrow transplantation and functional analysis of mice.**

43 **(A)** Immunoblotting for GSDMD validating the success of BMT experiment. **(B-D)**
44 Echocardiography images **(B)** and M-mode quantification of ejection fraction **(C)** and fractional
45 shortening **(D)** for WT or *Gsdmd*^{-/-} mice before or at 1 week after MI (baseline: WT → WT, n = 5;
46 *Gsdmd* KO → WT, n = 3; WT → *Gsdmd* KO, n=11; 1 week: WT → WT, n = 3; *Gsdmd* KO→WT,
47 n = 3; WT→ *Gsdmd* KO, n=5). **(E-F)** Masson's Trichrome staining **(E)** and quantification **(F)** of
48 fibrotic area of short-axis heart sections from WT → WT (n = 3), *Gsdmd* KO → WT (n = 3) or
49 WT→ *Gsdmd* KO (n=7) mice at 1 week after MI (scale bar, 1 mm). Data are mean ±SD and were
50 analyzed by One-way analysis of variance followed by Tukey's multiple comparison test **(C, D**
51 **and F)**. NS, not significant.

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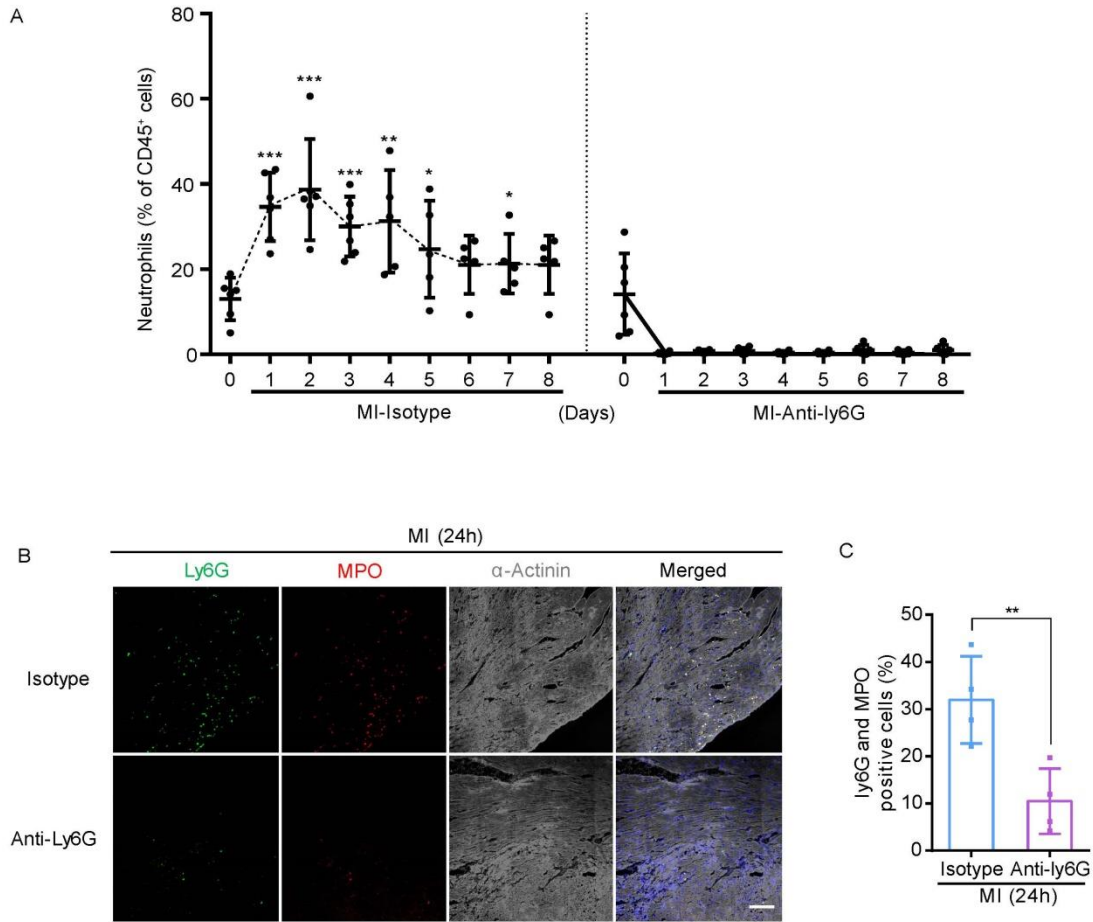
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Supplementary Figure 6



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63 **Figure S6. Validation of Cd11b⁺Ly6G neutrophils depletion**

64 (A) Flow cytometric quantification of Cd11b⁺Ly6G⁺ neutrophils in blood from WT or anti-ly6G
65 treated mice at different time points. The corresponding n values were indicated in the plot. (B)

66 Immunofluorescence imaging on heart sections from Isotype or Anti-ly6G mice at 24h after MI
67 showing α -Actinin (gray), Ly6G (green), MPO (red) and DAPI (blue). Representative fields are

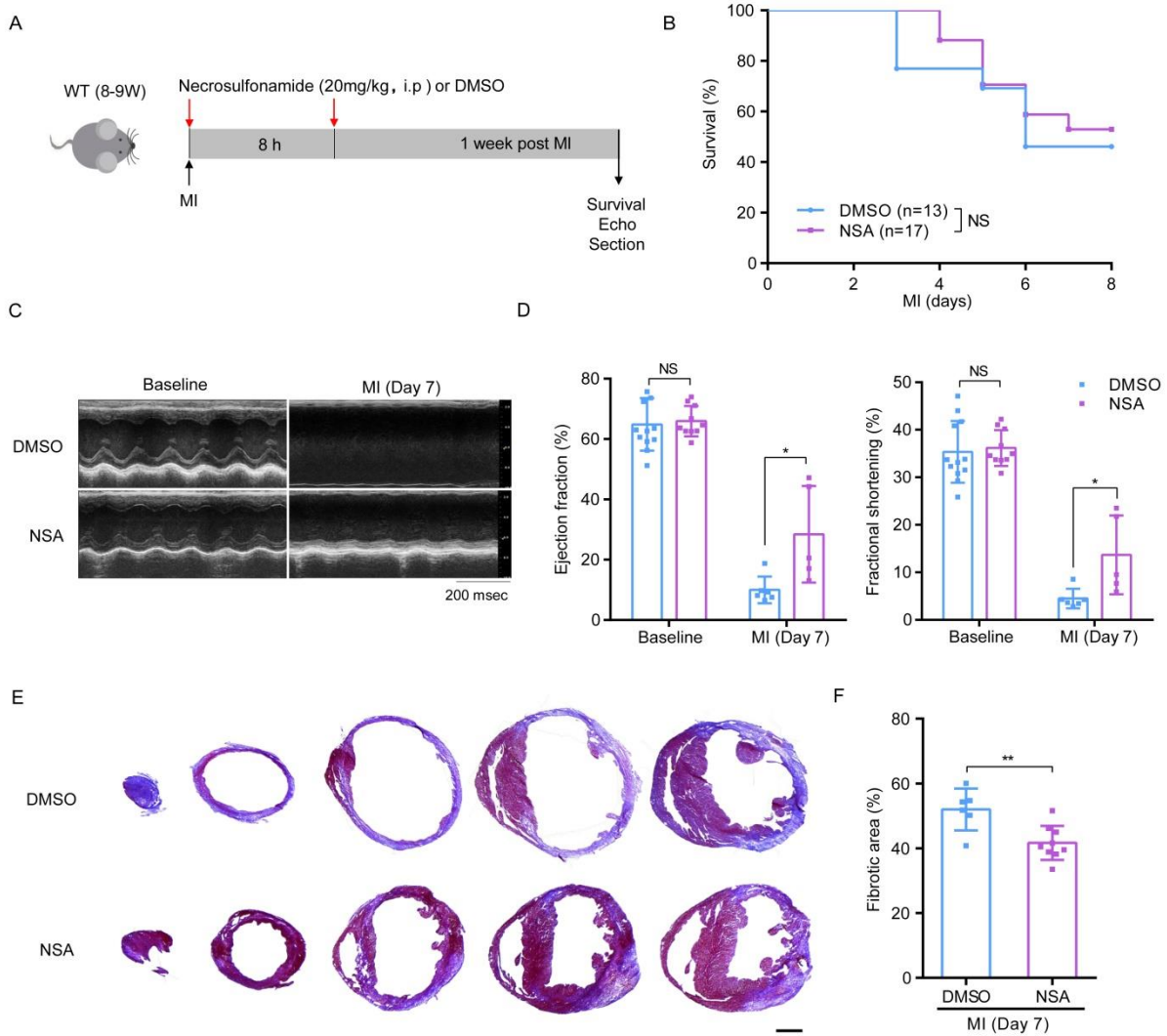
68 presented (scale bar, 100 μ m). (C) Quantification of Ly6G and MPO positive area proportion in
69 the field of heart sections from Isotype or Anti-ly6G mice, each value was averaged from the

70 values of 5 fields of view from the same mouse (n = 4 per group). Data represent mean \pm SD and

71 were analyzed by One-way analysis of variance followed by Tukey's multiple comparison test (A)
72 or unpaired two-tailed Student's *t* test (C). NS, not significant; *, P<0.05; **, P<0.01; ***,
73 P<0.001; ****, P<0.0001.

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Supplementary Figure 7



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76 **Figure S7. Pharmacological inhibition of GSDMD reduces infarct size post MI.**

77 **(A)** Schematic diagram showing the strategy of postoperative NSA administration to the mice. **(B)**

78 Kaplan-Meier survival curves comparing post-MI survival of control (DMSO administration)

79 mice (n = 13) to that of mice administrated with NSA (n = 17). Significance was determined by

80 Mantel-Cox test. **(C-D)** Echocardiography images **(C)** and M-mode quantification **(D)** of ejection

81 fraction (left) and fractional shortening (right) for control mice or mice with NSA administration

82 before or at 1 week after MI. (baseline: DMSO, n = 12; NSA, = 10; 1 week: DMSO, n = 6; NSA, n

83 = 5). **(E-F)** Masson's Trichrome staining **(E)** and quantification of fibrotic area **(F)** of short-axis
84 heart sections from control mice or mice with NSA administration at 1 week after MI (DMSO, n =
85 6; NSA, n = 9) (scale bar, 1 mm). Data are mean \pm SD and were analyzed by One-way analysis of
86 variance followed by Tukey's multiple comparison test **(D)** or unpaired two-tailed Student's *t* test
87 **(F)**. NS, not significant; *, $P < 0.05$; **, $P < 0.01$.

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89 **Supplemental Table 1: Patients' characteristics.**

Demographics	n=234
Age (years)	63.97±11.991
Sex (Male/Female)	41/193
Clinical history	
Previous PCI, n(%)	5(2.1)
Previous CABG, n(%)	0(0)
Previous Stroke, n(%)	13(5.6)
Hypertension, n(%)	111(47.4)
Diabetes, n(%)	41(17.5)
Dyslipidemia, n(%)	2(0.9)
Medications	
β-blockers, n(%)	176(75.2)
Aspirin, n(%)	216(92.3)
Statin, n(%)	215(91.9)
ACEI/ARB, n(%)	149(63.7)
ADP inhibitors, n(%)	217(92.7)

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98 **Supplemental Table 2: Key Resources and Reagents**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Anti-GSDMD	Abcam	Ab209845
Anti-CD68	Abcam	Ab53444
Anti-Sarcomeric Alpha Actinin	Abcam	Ab9465
Caspase-1+p10+p20	Abcam	Ab179515
Anti-mouse Caspase-1	Adipogen	AG-20B-0042-C100
Anti-mouse IL-1 β	R&D Systems	AF-401-NA
MPO	R&D Systems	AF3667
Bcl-2	SantaCruz	Sc-7382
Anti-mouse NLRP3	Cell Signaling	1510S
SQSTM1/P62 Antibody	Cell Signaling	5114S
LC3 A/B Antibody	Cell Signaling	4108S
Caspase-3	Cell Signaling	9662S
HSP90	Cell Signaling	4877S
β -actin	Cell Signaling	4970S
β -tubulin	Cell Signaling	2146S
GAPDH	Cell Signaling	3683S
anti-Ly6G/Ly6C antibody	BioLegend	108436
CD45-BV605	BioLegend	103140
CD11b-AlexaFluor647	BioLegend	101218
Ly6G-FITC	BioLegend	127606
Ly6C-Perp-cy5.5	BioLegend	128012
Anti-Ly6G antibody	BioLegend	127632
Goat anti-mouse IgG, HRP	Invitrogen	31430
Goat anti-rabbit IgG, HRP	Invitrogen	31460
TEXAS-Red goat anti-rat IgG	Invitrogen	T2767
Alexa Fluor 488 goat anti-rat IgG	Invitrogen	A11034
Alexa Fluor 594 goat anti-mouse IgG	Invitrogen	A11032
Alexa Fluor 488 goat anti-rat IgG	Invitrogen	A11006
Alexa Fluor 555 donkey anti-goat IgG	Invitrogen	A21432
Alexa Fluor 488 goat anti-mouse IgG	Invitrogen	A11001
Alexa Fluor 647 goat anti-rat IgG	Invitrogen	A21247
CD11b microbeads UltraPure mouse	Miltenyi	130-126-725
Neutrophil separation kit	Miltenyi	130-097-658
Mouse chow	Changzhou SYSE Bio-Tec.Co.Ltd	PD450J
Bafilomycin A1	MCE	HY-100558

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