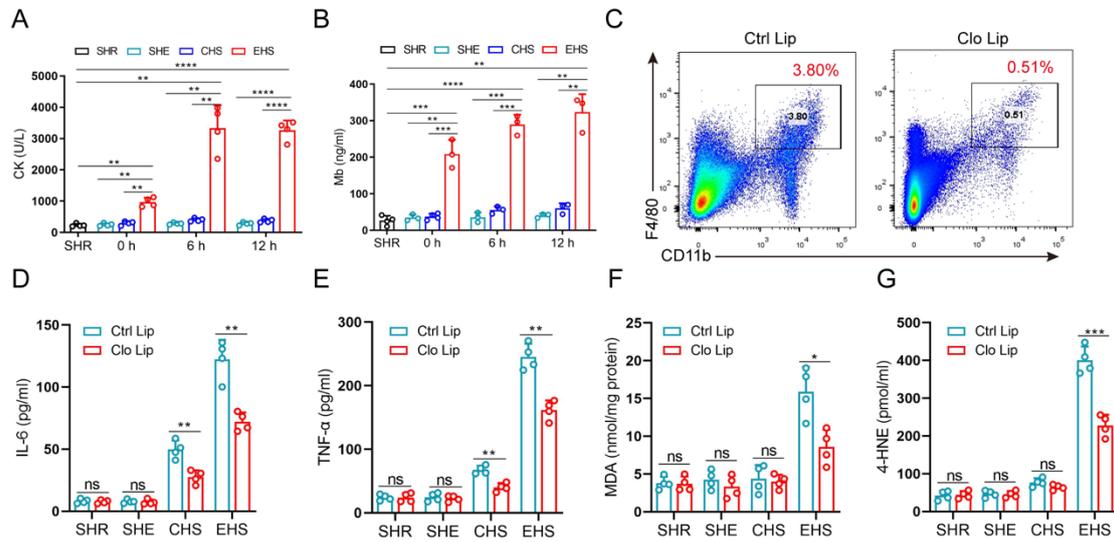


1 **Supplementary figures**

2



3

4 **Fig. S1 The role of smRTM ferroptosis in promoting skeletal muscle injury in**

5 **EHS mice (A-B)** Plasma CK and Mb levels were measured (n=3-5 mice/group). (C)

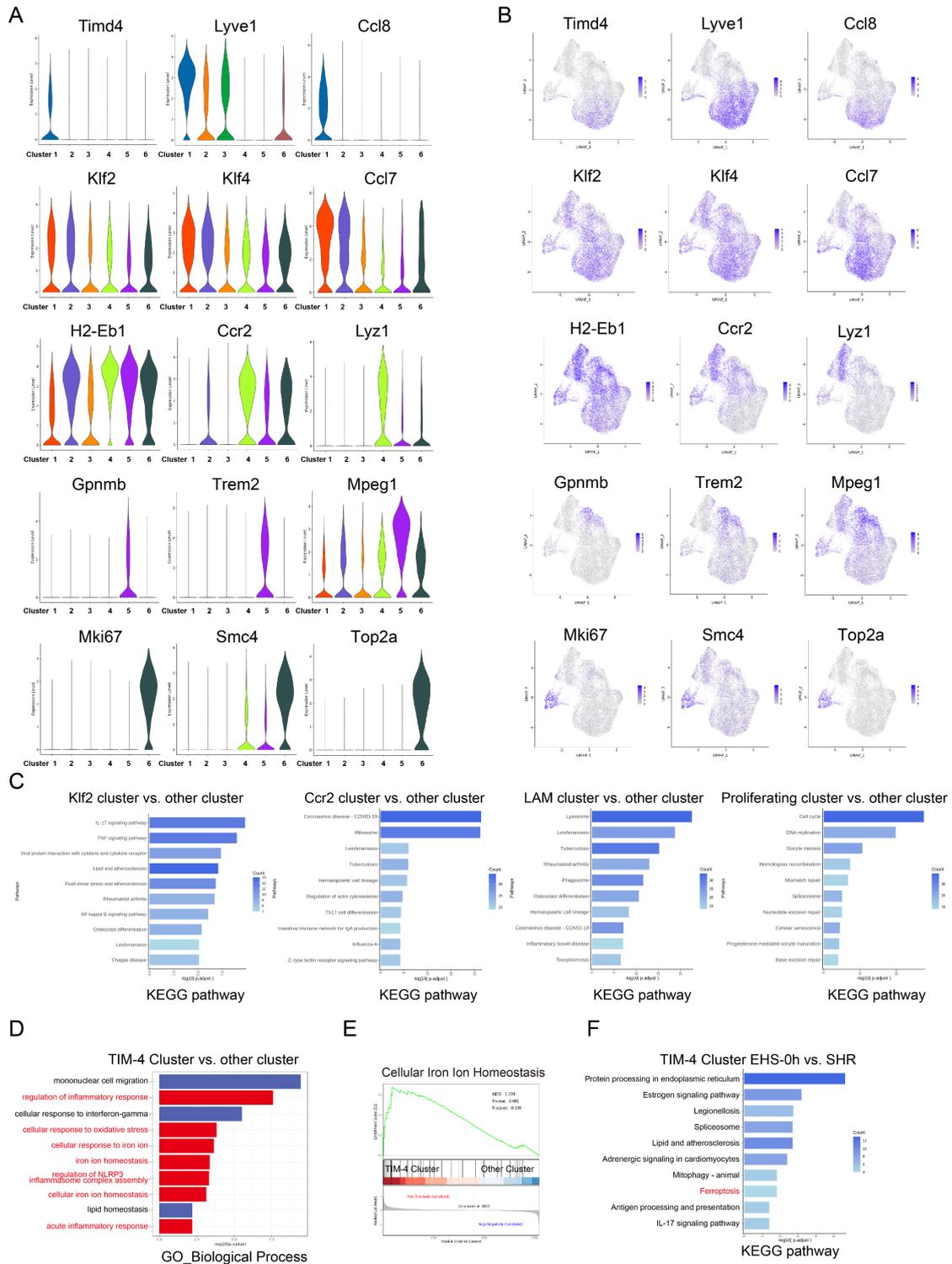
6 Flow cytometry was used to analyze the change in the number of smRTM after Clo

7 Lip treatment. (D-E) The level of IL-6 and TNF- $\alpha$ . (F-G) The levels of MDA and

8 4-HNE at 6 h were assayed (n = 3-5 mice/group). Data are mean  $\pm$  SEM. \* $P < 0.05$ ,

9 \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$  by Student's t-test.

10

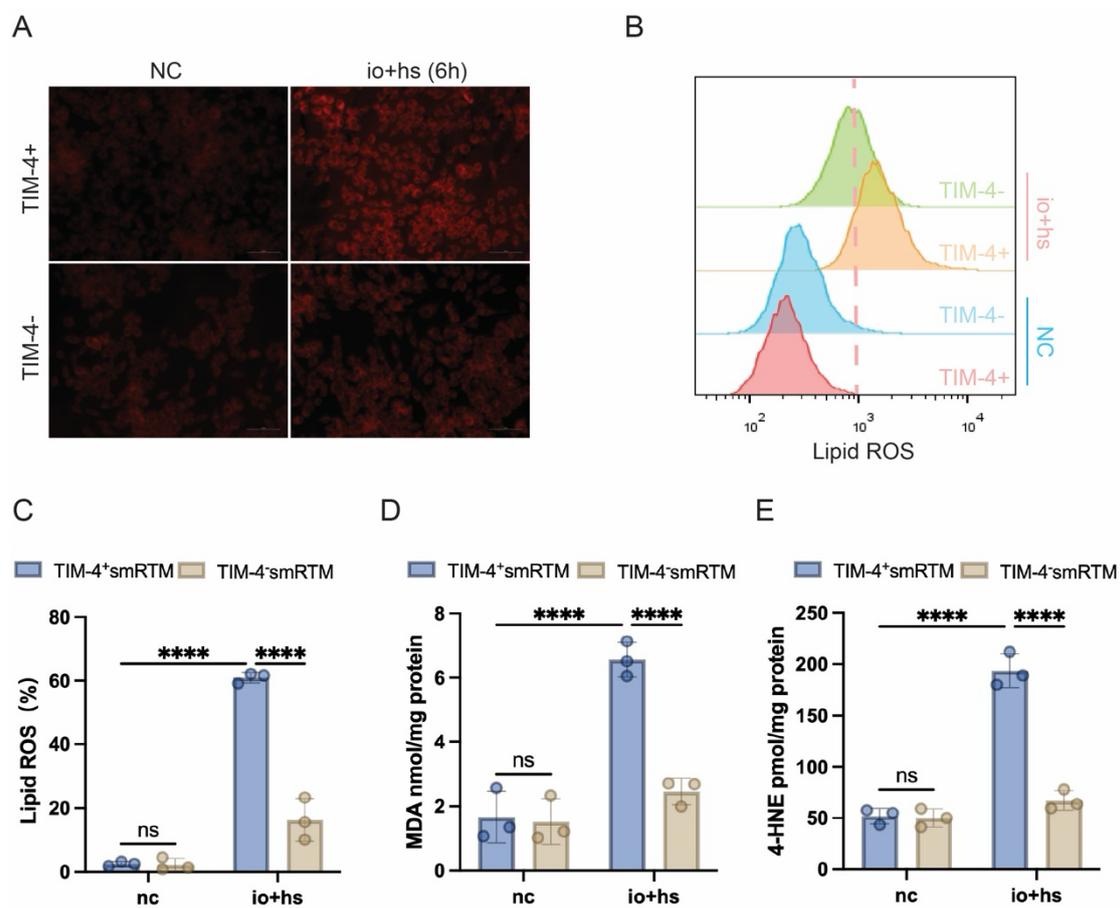


11

12 **Fig. S2. Single-cell RNA-seq reveals macrophage heterogeneity and dynamic**  
 13 **changes after EHS. (A–B) Violin plots (A) and feature plots (B) show marker gene**  
 14 **expression across clusters: *Timd4*, *Lyve1*, *Ccl8* (TIM-4<sup>+</sup> cluster); *Klf2*, *Klf4*, *Ccl7***  
 15 **(Klf2<sup>+</sup> cluster); *H2-Eb1*, *Ccr2*, *Lyz1* (Ccr2<sup>+</sup> cluster); *Gpmb*, *Trem2*, *Mpeg1* (LAM**

16 cluster); and *Mki67*, *Smc4*, *Top2a* (proliferating cluster). (C) KEGG enrichment of  
 17 upregulated genes in *Klf2*, *Ccr2*, LAM, and proliferating clusters. (D) GO analysis of  
 18 DEGs in the TIM-4<sup>+</sup> cluster highlights roles in inflammation and iron homeostasis. (E)  
 19 GSEA confirms enrichment of iron ion homeostasis pathways in TIM-4<sup>+</sup> macrophages.  
 20 (F) KEGG pathway analysis comparing TIM-4<sup>+</sup> cells from EHS-0 h and SHR reveals  
 21 enrichment in ferroptosis-related pathways.

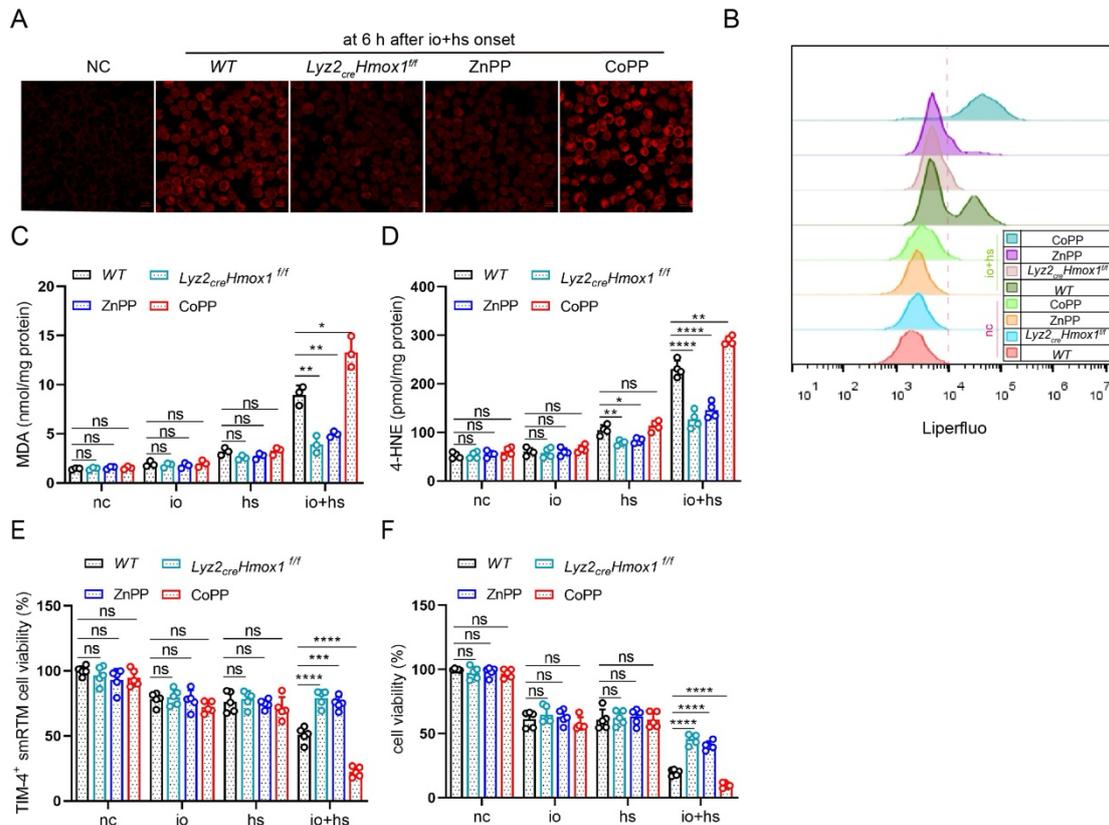
22



23  
 24 **Fig. S3. TIM-4<sup>+</sup> smRTMs exhibit elevated lipid peroxidation under EHS**  
 25 **conditions. (A–B)** Representative images (A) and flow cytometry (B) showing  
 26 increased lipid ROS in TIM-4<sup>+</sup> smRTMs compared to TIM-4<sup>-</sup> cells after io+hs. (C–E)  
 27 Quantification of lipid ROS (C), MDA (D), and 4-HNE (E) levels confirms enhanced

28 lipid peroxidation in TIM-4<sup>+</sup> smRTMs under EHS. Data are mean ± SEM. \**P*<0.05,  
 29 \*\**P*<0.01, \*\*\**P*<0.001, \*\*\*\**P*<0.0001 by Student's t-test.

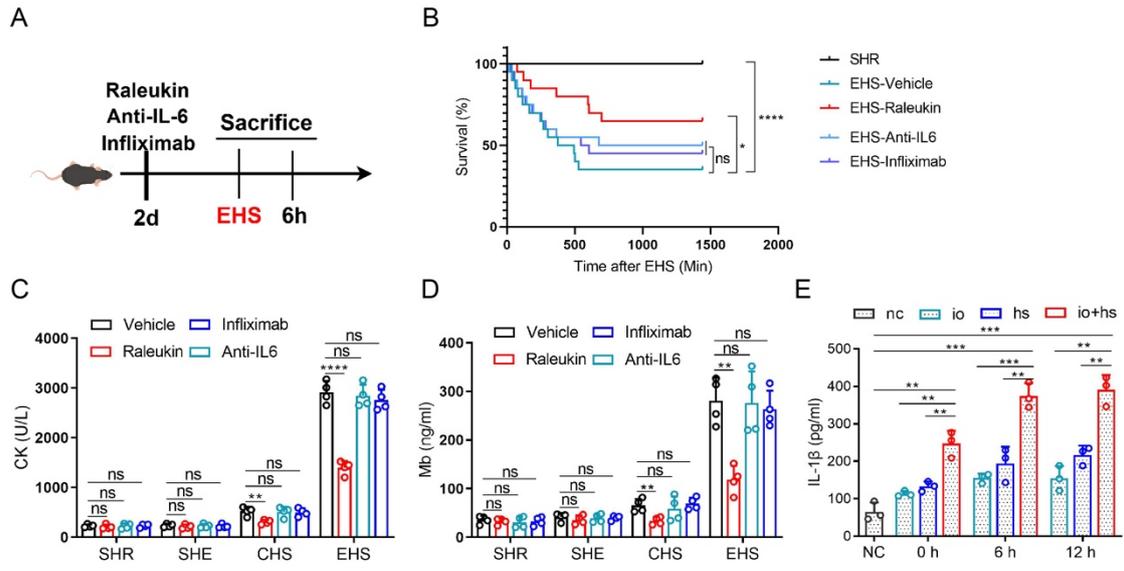
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31

32 **Fig. S4 HMOX1 induced TIM-4<sup>+</sup> smRTM ferroptosis *in vitro*** (A) Representative  
 33 images of FerroOrange staining in TIM-4<sup>+</sup> smRTM following pretreatment with ZnPP,  
 34 CoPP, or *Hmox1* knockout at 6 h post io+hs exposure. (B) Flow cytometry was  
 35 employed to analyze lipid peroxidation (n = 3). (C-D) The levels of MDA and 4-HNE  
 36 were assayed (n = 3). (E) Cell viability was assessed (F) Cell viability was assessed in  
 37 C2C12 cell co-cultured with pretreated TIM-4<sup>+</sup> smRTM. Data are mean ± SEM.  
 38 \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, \*\*\*\**P*<0.0001 by Student's t-test.

39



40

41 **Fig. S5 Inhibition of IL-1 $\beta$  secretion could reduce skeletal muscle injury (A)**

42 Schematic illustration of Raleukin, Anti-IL-6, and Infliximab in modulating

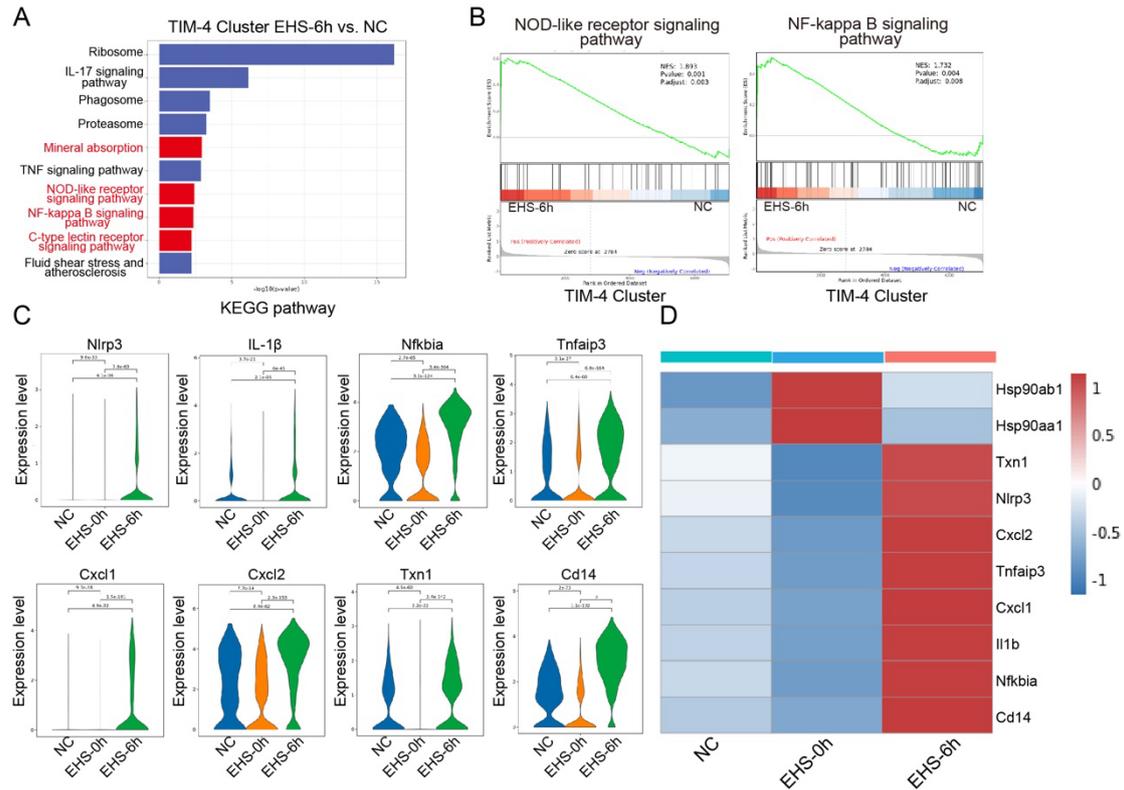
43 inflammatory cytokine activity. (B) Survival curves are shown for mice pretreated

44 with Raleukin, Anti-IL-6, and Infliximab, followed by EHS exposure (n =20

45 mice/group). (C-D) Detection of CK and Mb in plasma of pretreated mice (n=3-5

46 mice/group). (E) Detection of IL-1 $\beta$  content in cell supernatant. Data are mean  $\pm$

47 SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$  by Student's t-test.



48

49 **Fig. S6 Single-cell RNA sequencing reveals NLRP3 inflammasome activation**

50 **under EHS conditions (A)** KEGG enrichment analysis was performed to display the

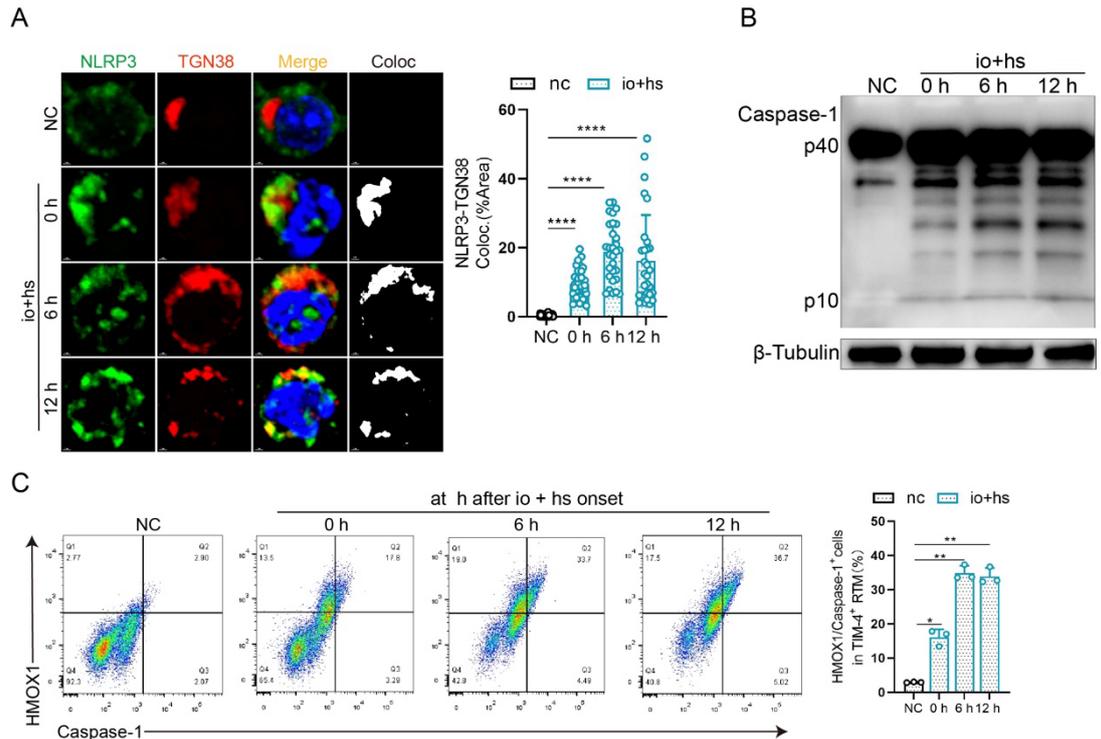
51 top 10 enriched pathways in EHS-recovered mice at 6 h compared to SHR mice. **(B)**

52 Gene set enrichment analysis (GSEA) was conducted to analyze EHS-recovered mice

53 at 6 h and SHR mice. **(C-D)** Violin plots **(C)** and Heat maps **(D)** were used to

54 visualize the expression of genes associated with the NOD-receptor signaling pathway

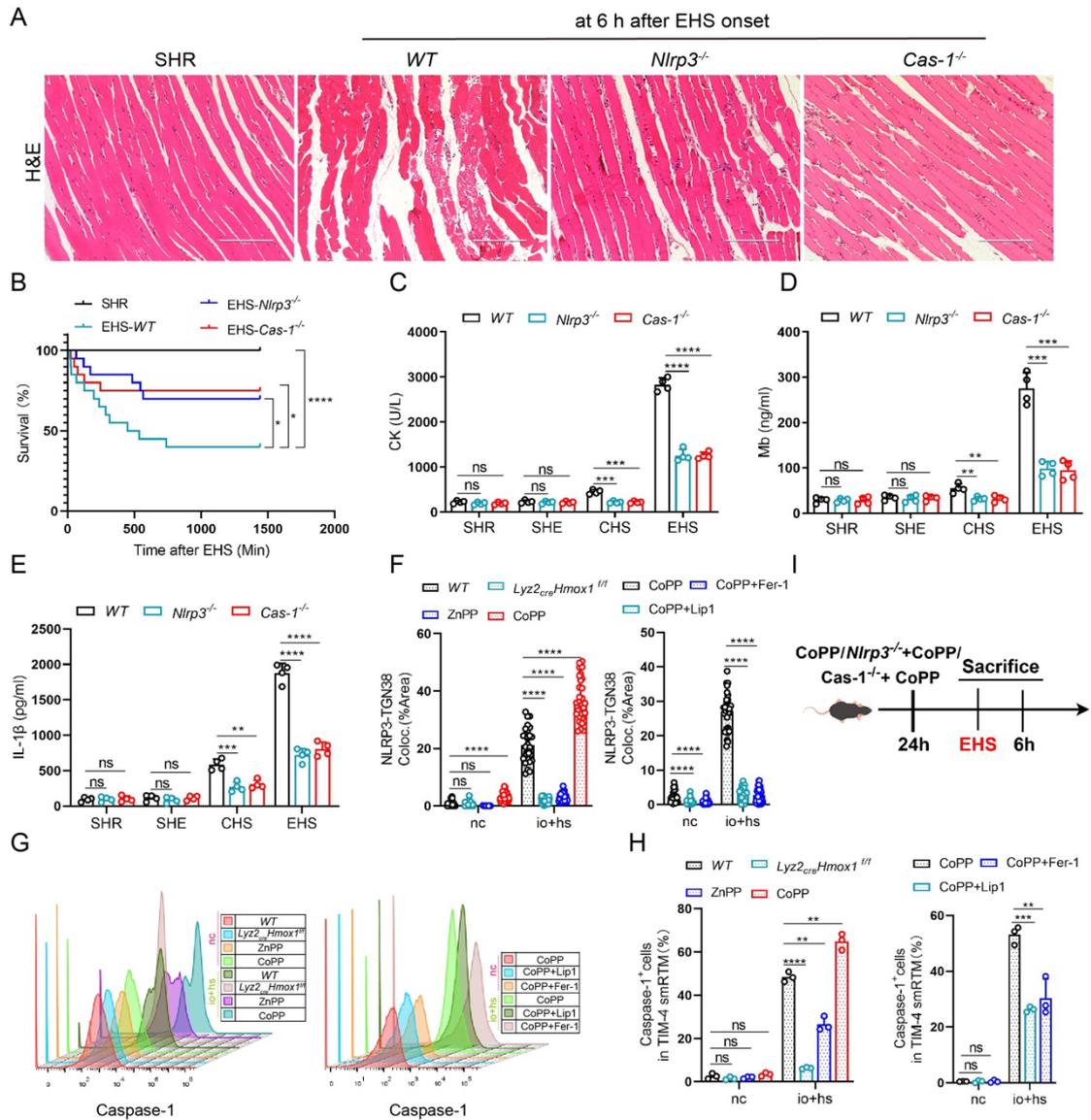
55 under EHS conditions.



56

57 **Fig. S7 The relationship between EHS and NLRP3 activation (A)** Representative  
 58 images of immunofluorescence staining in TIM-4<sup>+</sup> smRTM and statistical analysis of  
 59 co-localization of NLRP3 and TGN38. **(B)** Western Blotting was employed to analyze  
 60 Caspase-1 expression in TIM-4<sup>+</sup> smRTM. **(C)** Flow cytometry was used to analyze  
 61 the proportion of Hmox1<sup>+</sup>/Caspase-1<sup>+</sup> cells in TIM-4<sup>+</sup> smRTM (n = 3). Data are mean  
 62 ± SEM. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, \*\*\*\**P*<0.0001 by Student's t-test.

63



64

65 **Fig. S8 NLRP3 inflammasome activation promotes skeletal muscle injury**

66 **induced by EHS (A)** Representative HE-staining shows skeletal muscle sections

67 from *Nlrp3*<sup>-/-</sup> mice or *Cas-1*<sup>-/-</sup> mice, followed by EHS. **(B)** Survival curves of *Nlrp3*<sup>-/-</sup>

68 mice or *Cas-1*<sup>-/-</sup> mice (n = 20 mice/group). **(C-D)** Measurement of CK and Mb levels

69 (n = 3-5 mice/group). Samples were collected at 6 h post EHS onset. **(E)** Plasma IL-1 $\beta$

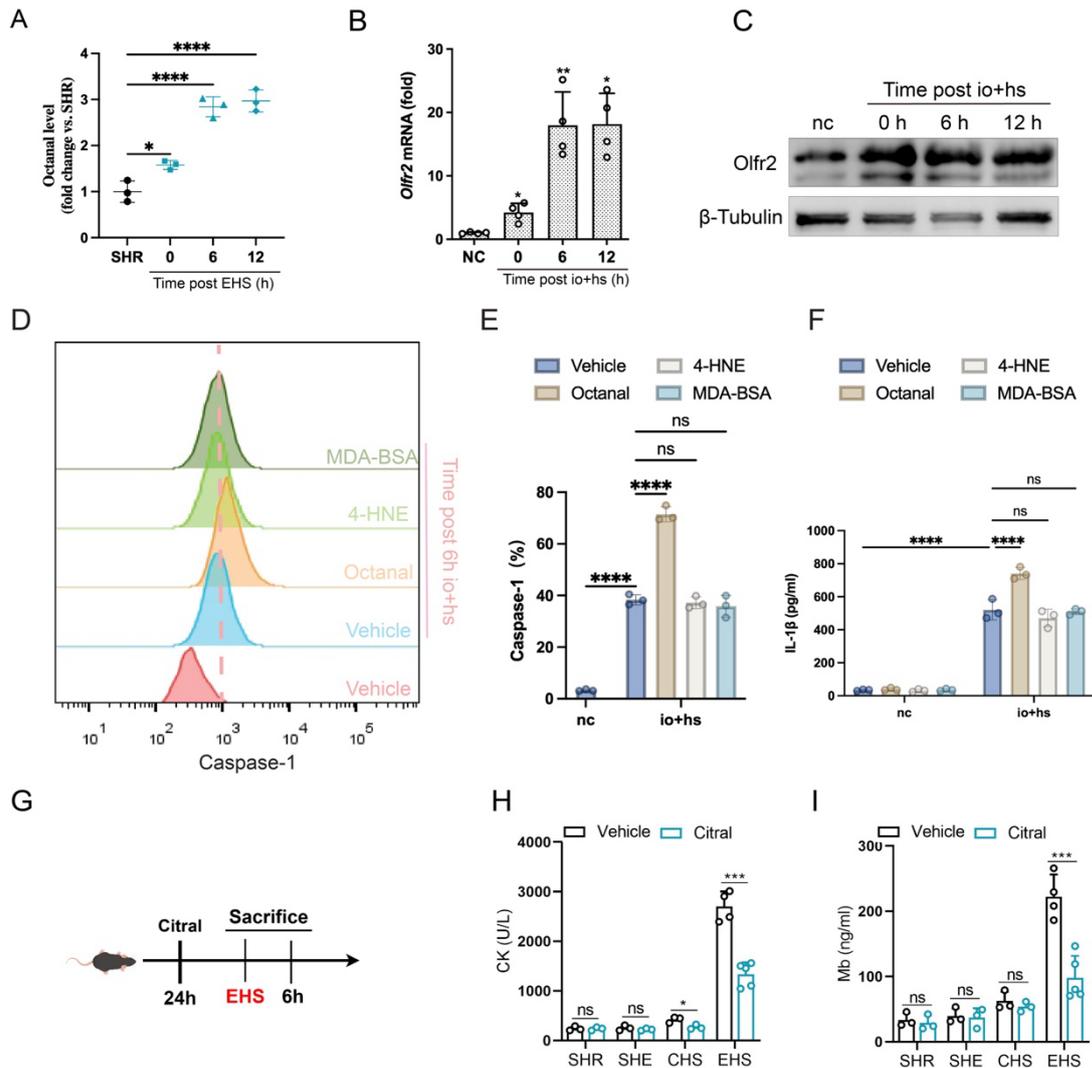
70 content were detected (n = 3-5 mice/group). **(F)** Statistical analysis of NLRP3 and

71 TGN38 co-localization in Fig. 5E is presented. **(G-H)** Analysis of Caspase-1

72 expression in pretreated TIM-4<sup>+</sup>smRTM and statistical analysis (n = 3). **(I)** Schematic

73 diagram illustrating NLRP3 inflammasome regulation through *Nlrp3* or *Cas-1*  
 74 knockout and CoPP. Data are mean  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ,  
 75 \*\*\*\* $P < 0.0001$  by Student's t-test.

76



77

78 **Fig. S9 Octanal promotes NLRP3 inflammasome activation and exacerbates**

79 **muscle injury under EHS conditions. (A)** Octanal levels in plasma were quantified

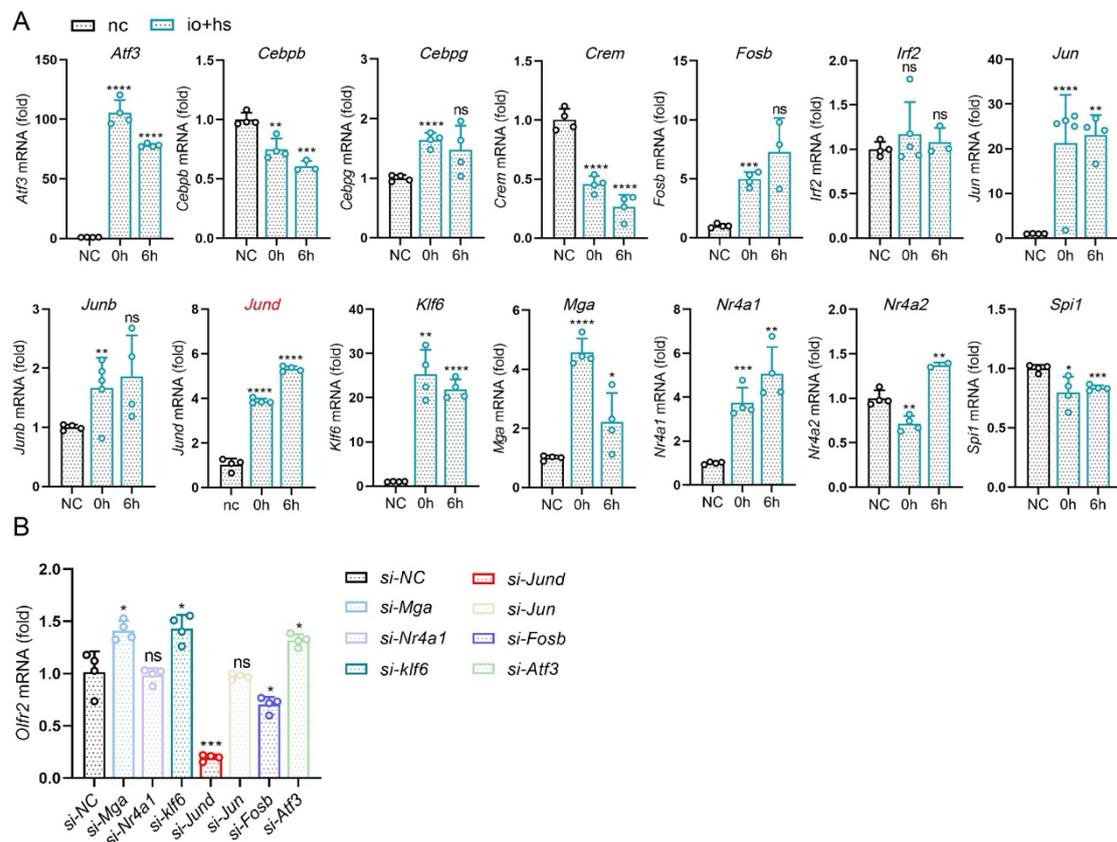
80 **by LC-MS at indicated time points post-EHS. (B, C)** qPCR **(B)** and Western blot **(C)**

81 **showing time-dependent upregulation of the octanal receptor Olfr2 in TIM-4+**

82 **smRTMs after io+hs stimulation. (D)** Flow cytometry plots of Caspase-1 activation in

83 macrophages treated with Octanal, MDA-BSA, or 4-HNE. **(E, F)** Quantification of  
 84 Caspase-1<sup>+</sup> cells **(E)** and IL-1 $\beta$  secretion **(F)** following lipid aldehyde stimulation  
 85 under io+hs. **(G)** Experimental scheme for Citral (an Olf2 antagonist) administration  
 86 and EHS induction. **(H, I)** Plasma CK **(H)** and Mb **(I)** levels showing that Citral  
 87 administration attenuated muscle damage during EHS. Data are presented as mean  $\pm$   
 88 SEM; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$  by one-way ANOVA or  
 89 Student's t-test, as appropriate.

90



91

92 **Fig. S10 JunD regulates the transcription of *Olf2*.** **(A)** qPCR showing the  
 93 expression of *Atf3*, *Cebpb*, *Cebpg*, *Crem*, *Fosb*, *Irf2*, *Jun*, *Junb*, *Jund*, *Klf6*, *Mga*,  
 94 *Nr4a1*, *Nr4a2*, and *Spi1* in TIM4<sup>+</sup> smRTM under io+hs conditions compared to the  
 95 NC group. **(B)** qPCR analysis of *Olf2* expression after knocking down *Mga*, *Nr4a1*,

96 *Klf6*, *Jund*, *Jun*, *Fosb*, and *Atf3* in TIM-4<sup>+</sup> smRTM. Summary data are presented as  
97 the mean ± SEM. Significance was calculated using the Student's t-test. (\* $P < 0.05$ ,  
98 \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ ).

99