

Supplemental Information

Host liver-derived extracellular

vesicles deliver miR-142a-3p induces neutrophil

extracellular traps via targeting WASL to block the development of

Schistosoma japonicum

Lifu Wang, Zifeng Zhu, Yao Liao, Lichao Zhang, Zilong Yu, Ruibing Yang, Ji Wu, Zhongdao Wu, and Xi Sun

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Supplemental Information

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Neutrophil Extracellular Traps via Targeting WASL to Block the Development

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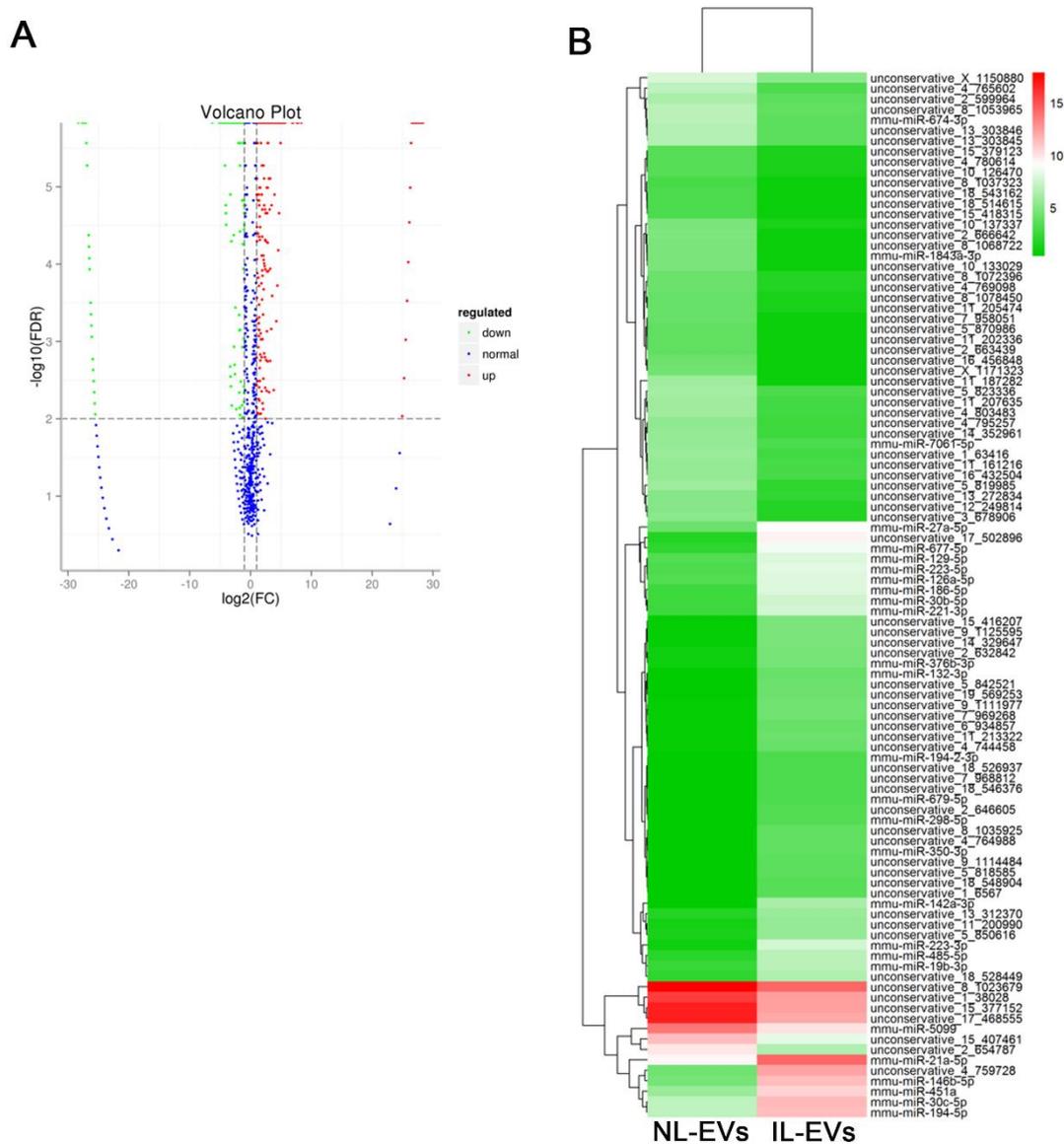
of *Schistosoma japonicum*

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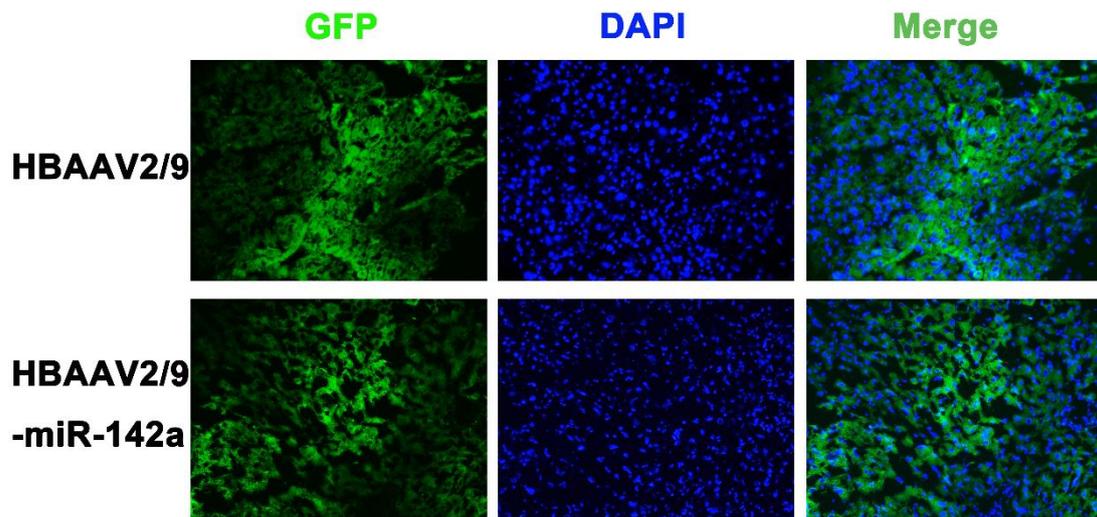
Zhongdao Wu, and Xi Sun



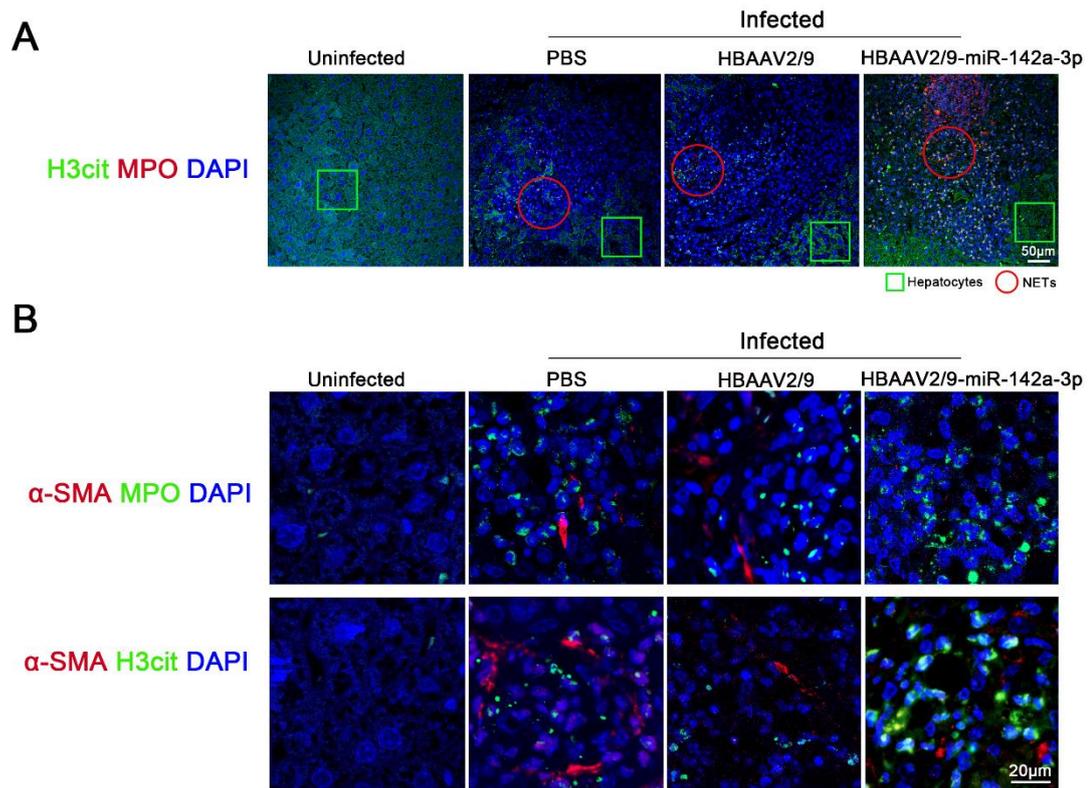
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8 **Figure S1. miRNAs of EVs derived from liver of *S. japonicum*-infected mice (IL-**

9 EVs) and normal liver-derived EVs (IL-EVs) were sequenced and analyzed. (A)
10 Volcano diagrams of miRNAs derived from the comparative gene expression analyses.
11 Red dots represent up-regulated miRNAs, and green dots represent down-regulated
12 miRNAs. (B) Heat map analysis shows differentially expressed miRNAs. Bright
13 green=low expression; bright red=high expression.



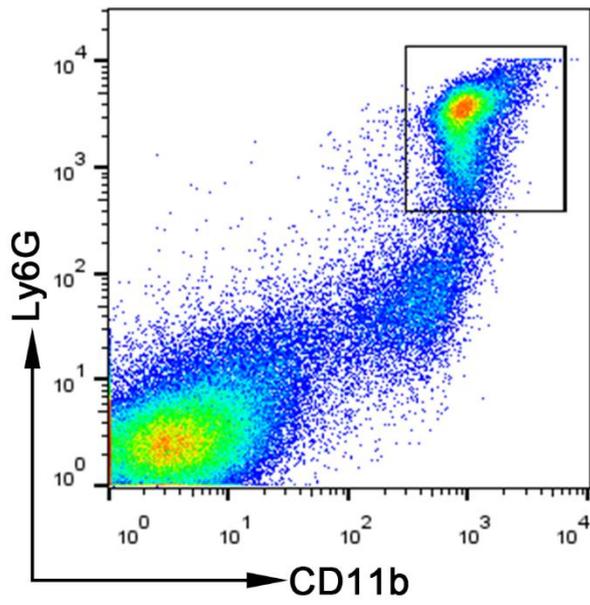
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15 **Figure S2. HBAAV2/9-miR-142a-3p was constructed and successfully transfected**
16 **into mice (HBAAV2/9 expresses GFP). HBAAV2/9-miR-142a-3p colonization in**
17 **the liver was analyzed by fluorescence microscopy.**



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19 **Figure S3. NETs were observed in the granulomas of *S. japonicum*.** (A) Location
 20 of NETs in liver sections was observed based on H3cit and MPO co-localization. (B)
 21 Co-localization of H3cit, MPO, and α -SMA was observed by immunofluorescence.

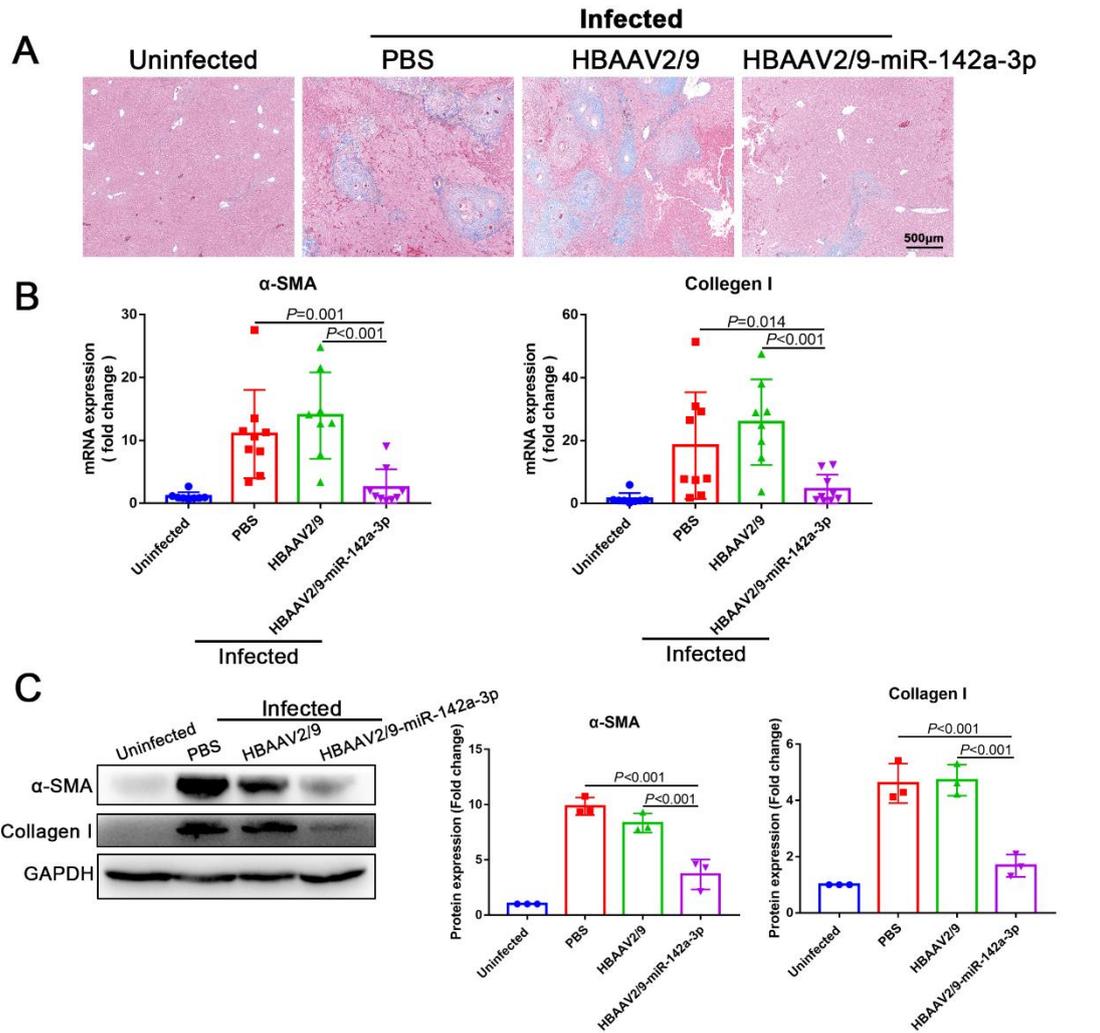
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24 **Figure S4. Neutrophils were isolated using Percoll density gradient**

25 **centrifugation and flow cytometry.**



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27 **Figure S5. miR-142a-3p attenuated liver fibrosis in *S. japonicum* infection. (A)**

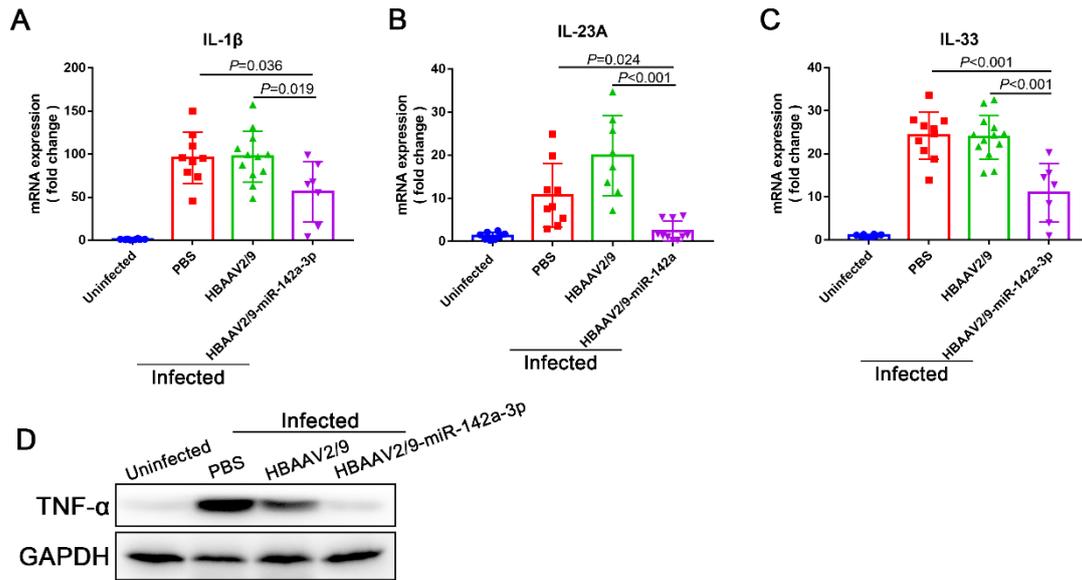
28 Fibrosis was observed in liver sections stained with Masson's trichrome. **(B)**

29 Expression levels of α -SMA and collagen I in mouse liver were analyzed by qRT-PCR

30 (n=8-9 mice per group). **(C)** Expression levels of α -SMA and collagen I in mouse

31 liver were analyzed by Western blotting. Results are shown as mean \pm SD (one-way

32 ANOVA with Dunnett's multiple comparison test).

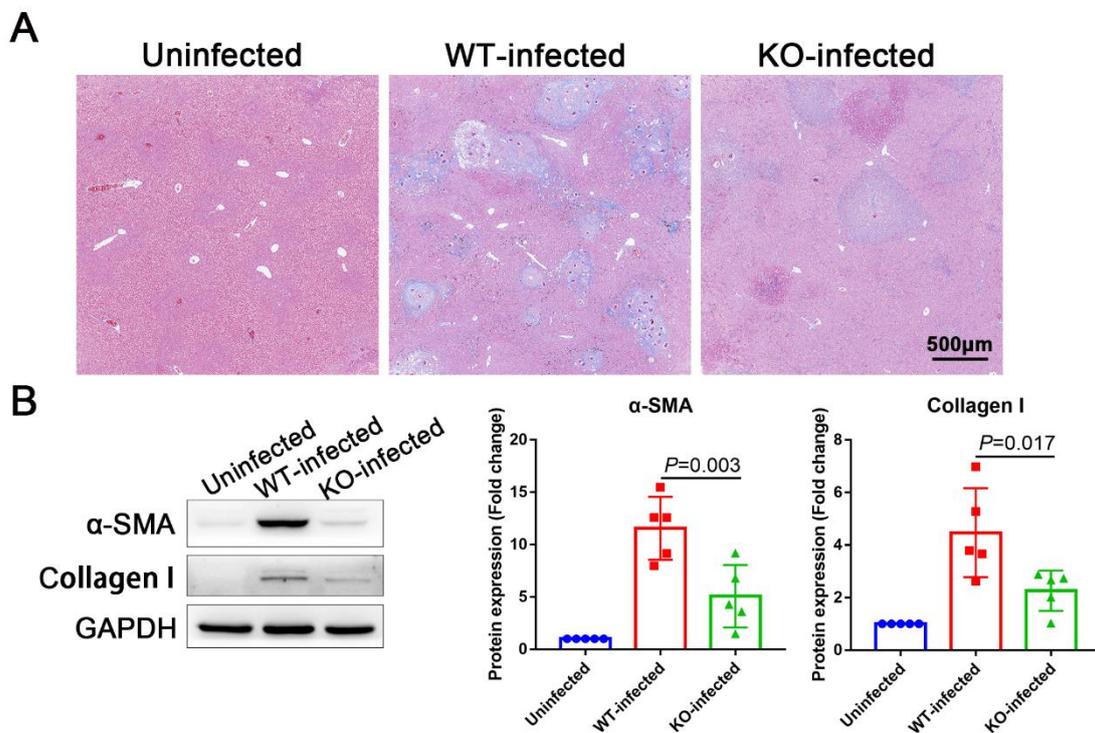


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34 **Figure S6.** High expression of the inflammatory cytokines IL-1 β (A), IL-23A (B), IL-

35 33 (C), and TNF- α (D) in liver tissues during *S. japonicum* infection decreased

36 significantly after HBAAV2/9-miR-142a-3p treatment (n=7-12 mice per group).



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38 **Figure S7.** WASL-deletion attenuated liver fibrosis in *S. japonicum* infection. (A)

39 Fibrosis was observed in liver sections stained with Masson's trichrome. (B)

40 Expression levels of α -SMA and collagen I in mouse liver were analyzed by Western
 41 blotting. Results are shown as mean \pm SD (one-way ANOVA with Dunnett's multiple
 42 comparison test).

43 **Table S1. Particle characteristics and yield of NL-EVs and IL-EVs**

	NL-EVs	IL-EVs
Mice	n=10	n=10
Cell culture time of liver tissues	24 h	24 h
Cell density (cells/mL)	1×10^6	1×10^6
Particle concentration (particles/mL)	9.72×10^{10}	6.69×10^{10}
Protein concentration (mg/mL)	7.34	5.21
Total volume of EVs (in PBS)	300 μ L	400 μ L

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45 **Table S2. The top ten differentially-expressed miRNA (IL-EVs/NL-EVs).**

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NO.	miRNA	log2FC	regulated
1	mmu-miR-142a-3p	7.753542806	Up
2	mmu-miR-223-3p	7.615122837	Up
3	mmu-miR-677-5p	7.006138383	Up
4	mmu-miR-146b-5p	6.866236731	Up
5	mmu-miR-186-5p	5.572362953	Up
6	mmu-miR-132-3p	5.49367909	Up
7	mmu-miR-350-3p	5.333210545	Up

8	mmu-miR-485-5p	5.322722633	Up
9	mmu-miR-30b-5p	5.315091289	Up
10	mmu-miR-221-3p	5.176670648	Up

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66 **WASL gene knockout mice**

67 Gene: ENSMUSG00000031165

68 Description: Wiskott-Aldrich syndrome [Source:MGI Symbol;Acc:MGI:105059]

69 Location: Chromosome X: 7,947,692-7,956,737 reverse strand.

70 Primers' sequences: "GGCCCTGGAGGACTTATTTC" and

71 "AGCTCAGGGGGTCACTGATA"

72 PCR products: WT (811 bp) and KO (~200 bp)

PCR Reaction

2×Taq Master Mix	7.5 µL
Forward primers (10 µM)	0.3 µL
Reverse primers (10 µM)	0.3 µL
Genomic DNA (20 ng/µL)	1.0 µL
Add H2O up to	15 µL

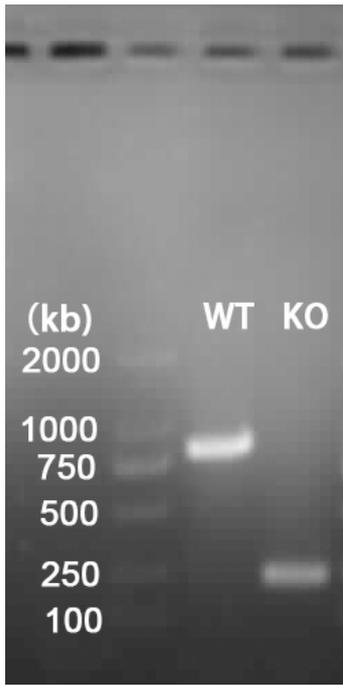
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PCR Program

	94 °C	5 min	
35 cycles	} 94 °C	30 sec	
		60 °C	30 sec
		72 °C	70 sec
	72 °C	10 min	

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75 **PCR products: WT (811 bp) and KO (~200 bp)**



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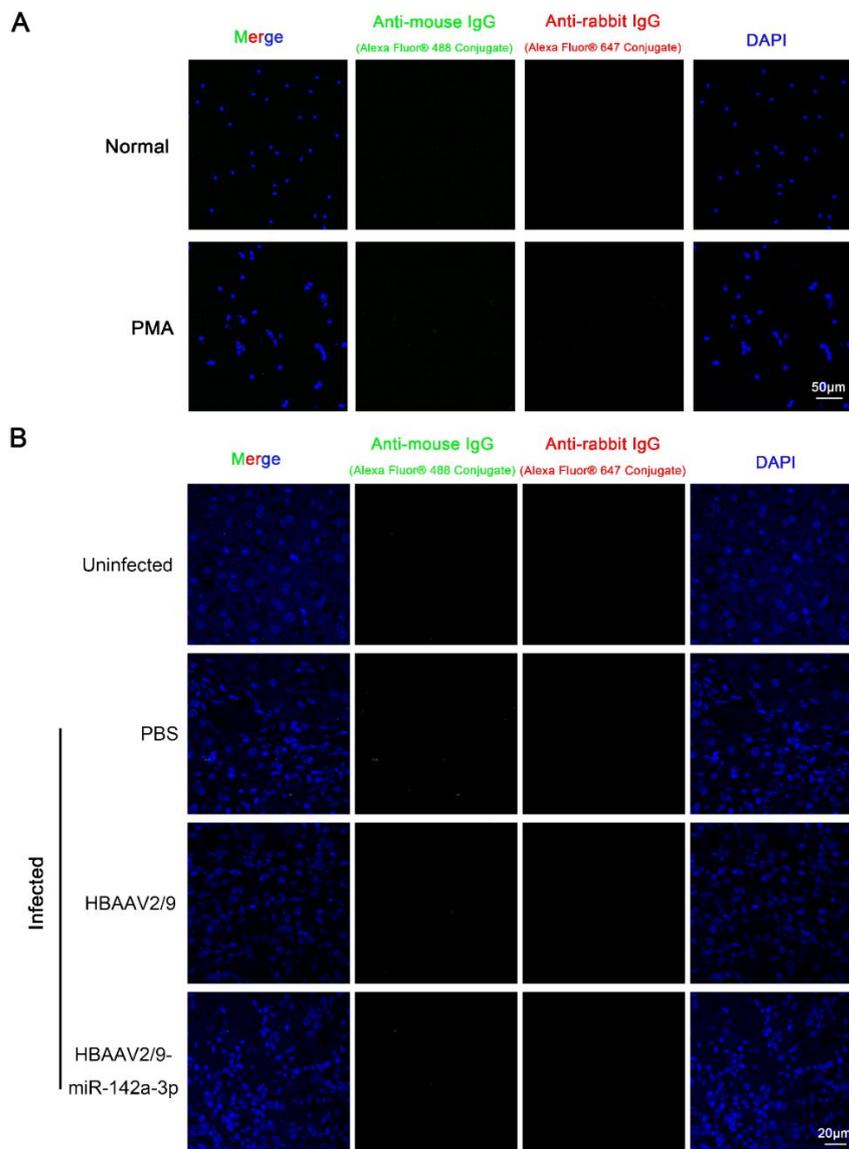
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90 **Negative control for immunofluorescence**



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92 **Negative control for immunofluorescence**

93 **Figure (A)** Neutrophils were treated with PMA (500 nM, 4 h), and the non-specific

94 staining effect of the secondary antibody was excluded by direct use of secondary

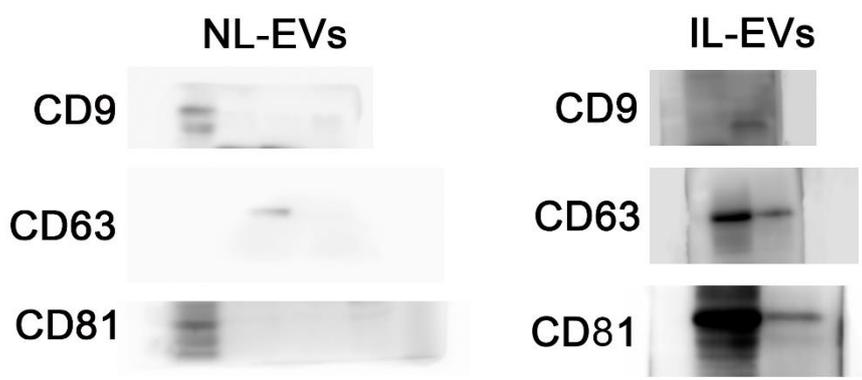
95 antibody without adding primary antibody. **(B)** The non-specific staining effect of the

96 secondary antibody on liver slices was excluded by direct use of secondary antibody

97 without adding primary antibody.

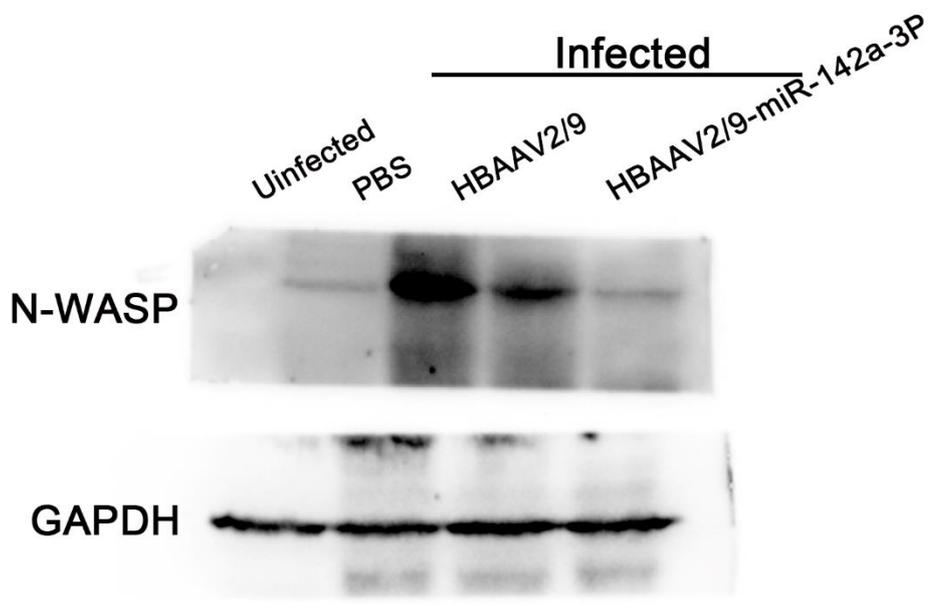
98 **Uncropped western blotting**

99 **Figure 1B**



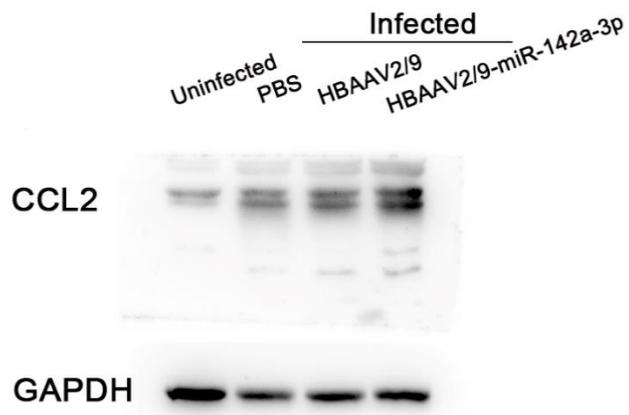
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101 **Figure 5C**

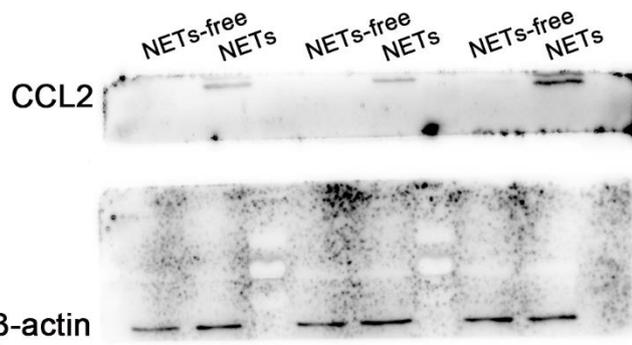


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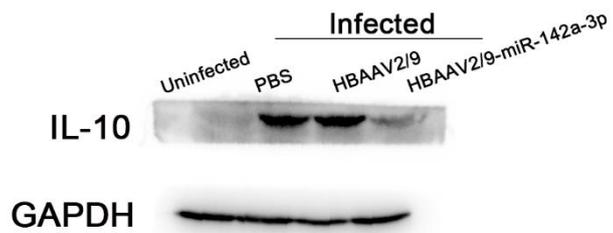
103 **Figure 7B**



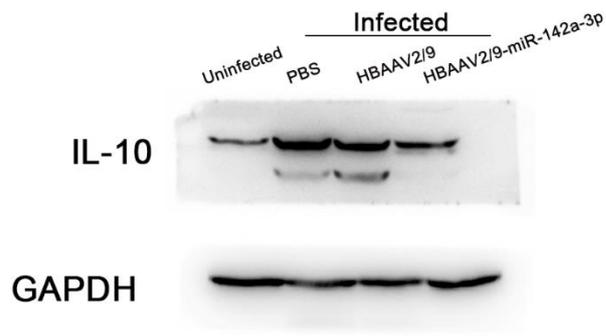
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105 **Figure 7D**



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107 **Figure 8E**

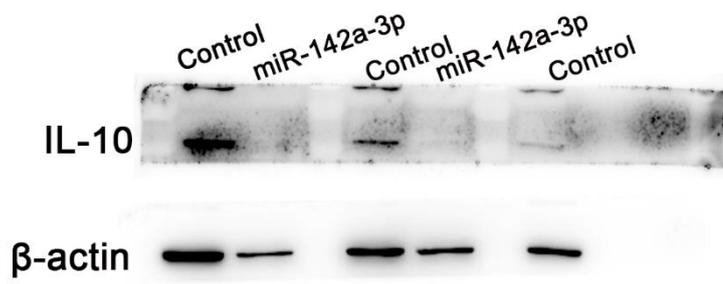


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109 **Figure 8K**



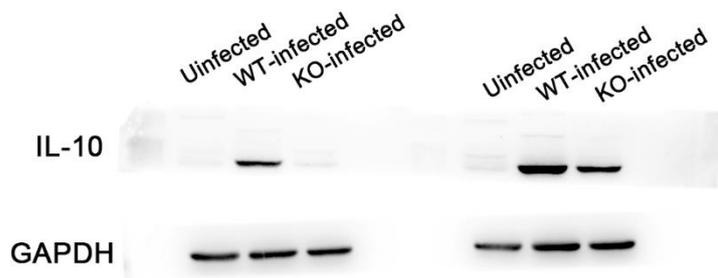
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111 **Figure 8L**



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113 **Figure 8N**



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