Supplementary Materials: Biofilm-Forming Methicillin-Resistant *Staphylococcus aureus* Survive in Kupffer Cells and Exhibit High Virulence in Mice

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Table S1. List of Antibodies for WB.

No.	Antibody	Manufacturer	applied dilution times	Catalog Number
1	LC3B	Cell Signaling Technology	1000	#3868
2	SQSTM1/p62	Cell Signaling Technology	1000	#5114
3	Atg5	Cell Signaling Technology	1000	#12994
4	ATG12	Cell Signaling Technology	1000	#4180
5	Beclin-1	Cell Signaling Technology	1000	#3495
6	GAPDH	Cell Signaling Technology	2000	#97166
7	XPC	Cell Signaling Technology	1000	#14768
8	ERCC1	Cell Signaling Technology	1000	#12345
9	β-actin	Cell Signaling Technology	2000	#3700
10	HO-1	Abcam	1000	#ab13248
11	(HRP)-linked anti-rabbit IgG	Cell Signaling Technology	10000	#7074
12	(HRP)-linked anti-mouse IgG	Cell Signaling Technology	10000	#7076

Supplementary methods

1. In vitro transfection and construction of stable HO-1 overexpression and silence cell lines

For overexpression of HO-1 in Hepa 1–6 cells (HO-1^{OE} Hepa 1–6 cells), the HMOX-1 sequence was inserted into the pHBLV-CMVIE-ZsGreen-Puro vector (Fig. S2a) (Hanbio Technology Co. Ltd, Shanghai, China). Primers include: m-HMOX1-Eco/Eco-F primer, and m-HMOX1-Eco/Eco-R primer. For silence of HO-1 in Hepa 1–6 cells (HO-1shRNA Hepa 1–6 cells), three different shRNAs were inserted into the pHBLV-U6-Scramble-ZsGreen-Puro vector (Fig. S2a) (Hanbio). Stable HO-1 overexpression and silence cell lines were harvested for quantitative polymerase chain reaction (qPCR) after 14 h transfection at 37 °C and were selected using 1 μg/mL puromycin before DON administration.

2. AAV8-mediated overexpression and silence of HO-1 in the liver

For construction of HO-1 overexpression recombinant adeno-associated virus serotype 8 (HO-1OE AAV8), mouse HMOX-1 gene sequence was packaged into

pHBAAV-CMV-MCS-ZsGreen vector (Fig. S2b). Primers include: m-HMOX1-Bam-kpn-F primer, and m-HMOX1-Bam-kpn-R primer. For construction of HO-1 silence recombinant AAV8 (HO-1shRNA AAV8) viral vectors, shRNA3 was packaged into pHBAAV-U6-ZsGreen vector (Fig. S2b). All recombinant AAV8 were purified by HanBio Technology Co. Ltd (Shanghai, China). Accordingly, based on a separate pre-experiment of determining an optimal virus injection dose, mice were injected HO-1OE or HO-1shRNA AAV8 (1 × 1012 viral particles, 100 μL/mouse) via tail vein after 1-week acclimation and then housed for 4 weeks (Fig.S1). Liver samples were collected after DON administration and western blot assays were used to examine the HO-1 expression in liver.

3. Verification of the efficiency of HO-1 overexpression and silencing

During low-dose DON exposure, the viral transfection efficiency of the HO-1 gene in mouse liver and Hepa 1-6 cells was verified by Western blot. Compared to the control group and the DON group, the expression level of HO-1 in HO-1OE group was signifi-cantly increased, and in HO-1shRNA group was significantly reduced, regardless of mouse liver or Hepa 1-6 cells (Fig. 3a-c). These results indicate that HO-1 overexpres-sion/silencing cells or animal models have been successfully constructed.

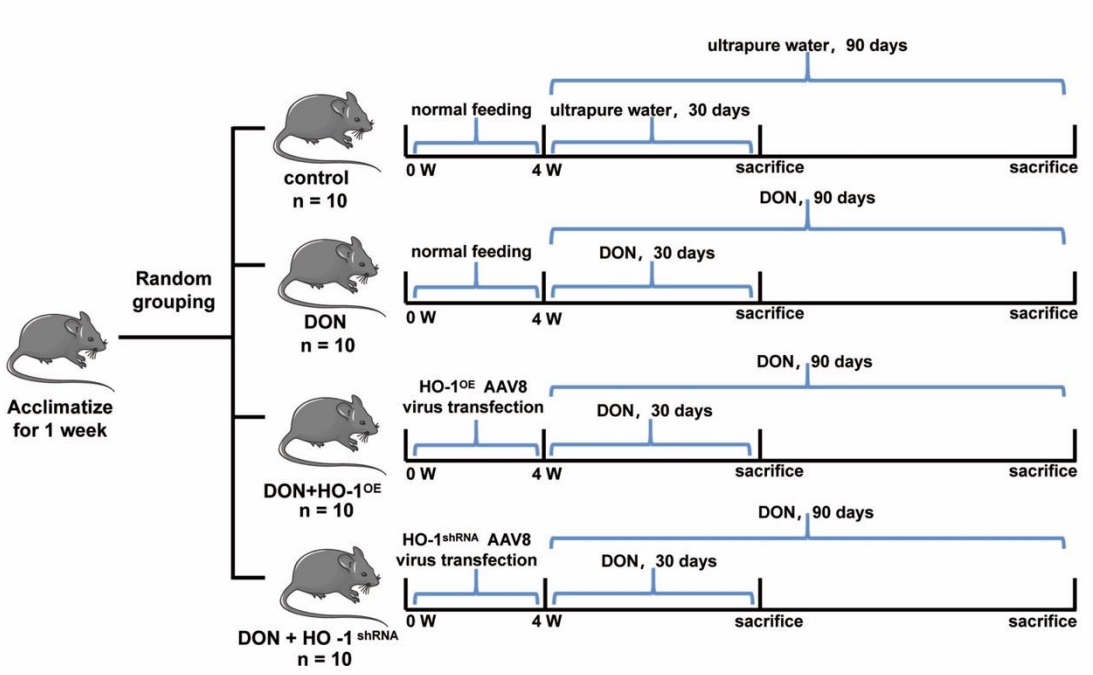

Figure S1. The grouping and treatment of experimental animals.

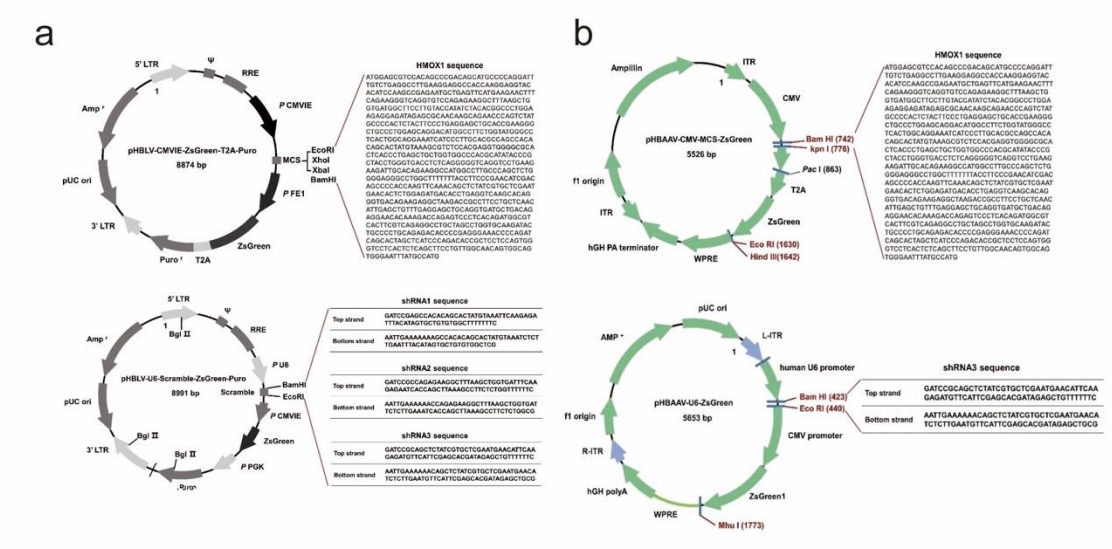

Figure S2. Virus transfection vector. (a) Lentiviral vector. (b) Adeno-associated virus vector.

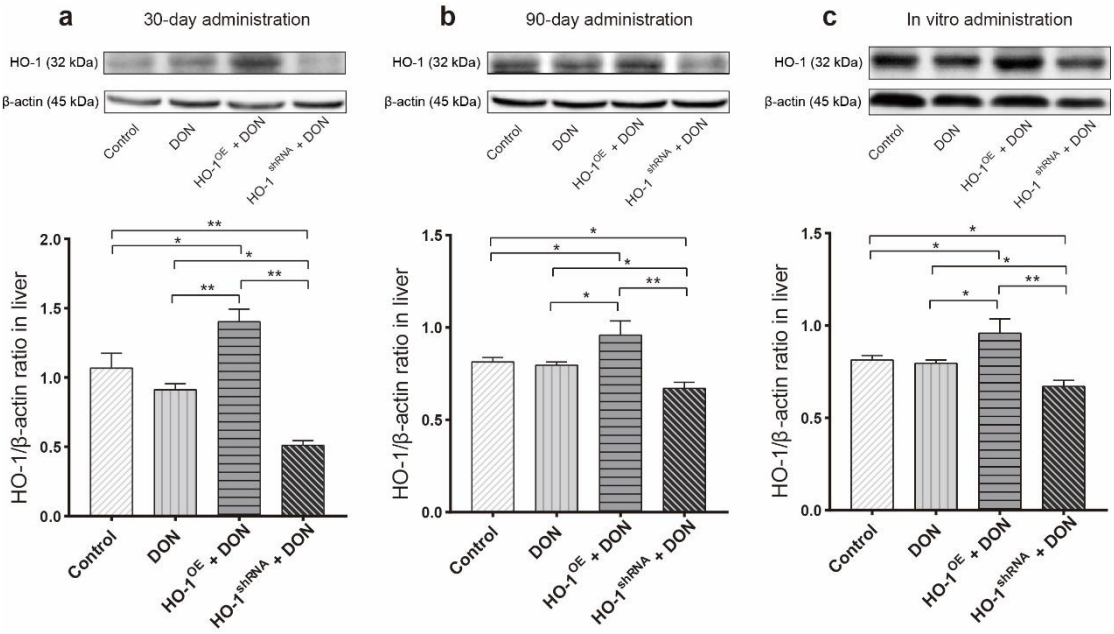

Figure S3. Validation of the efficiency of AAV8 transfecting the HO-1 gene. HO-1 overexpres-sion/silencing efficiency in mouse liver after 30 days (**a**) and 90 days (**b**) of DON administration. (**c**) HO-1 overexpression/silencing efficiency in Hepa 1-6. Data are expressed as mean ± SD; "*" means P <0.05; "**" means P <0.01.