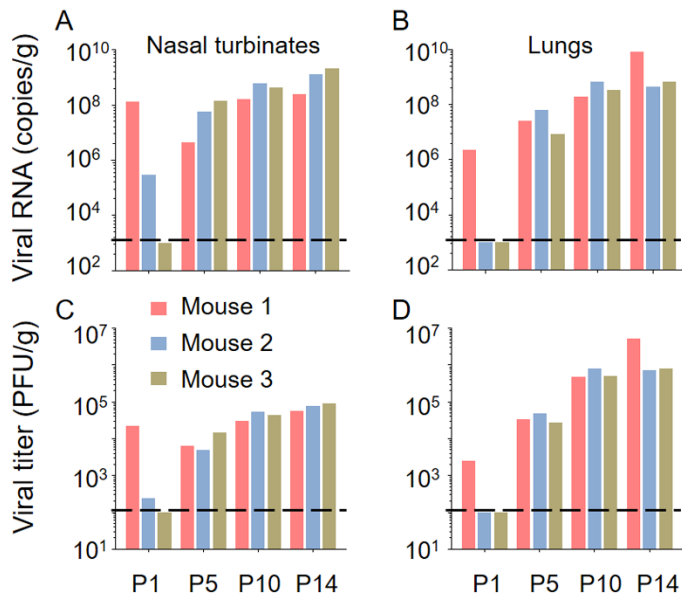
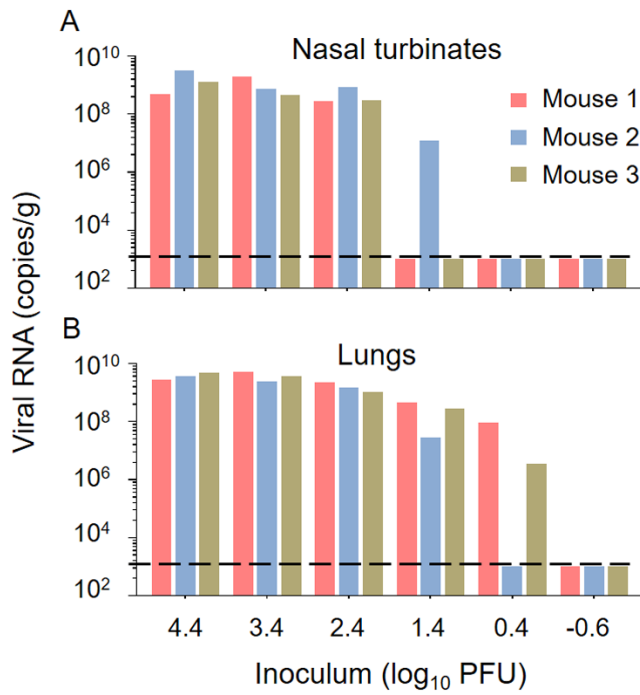


1 **SUPPLEMENTAL MATERIALS**



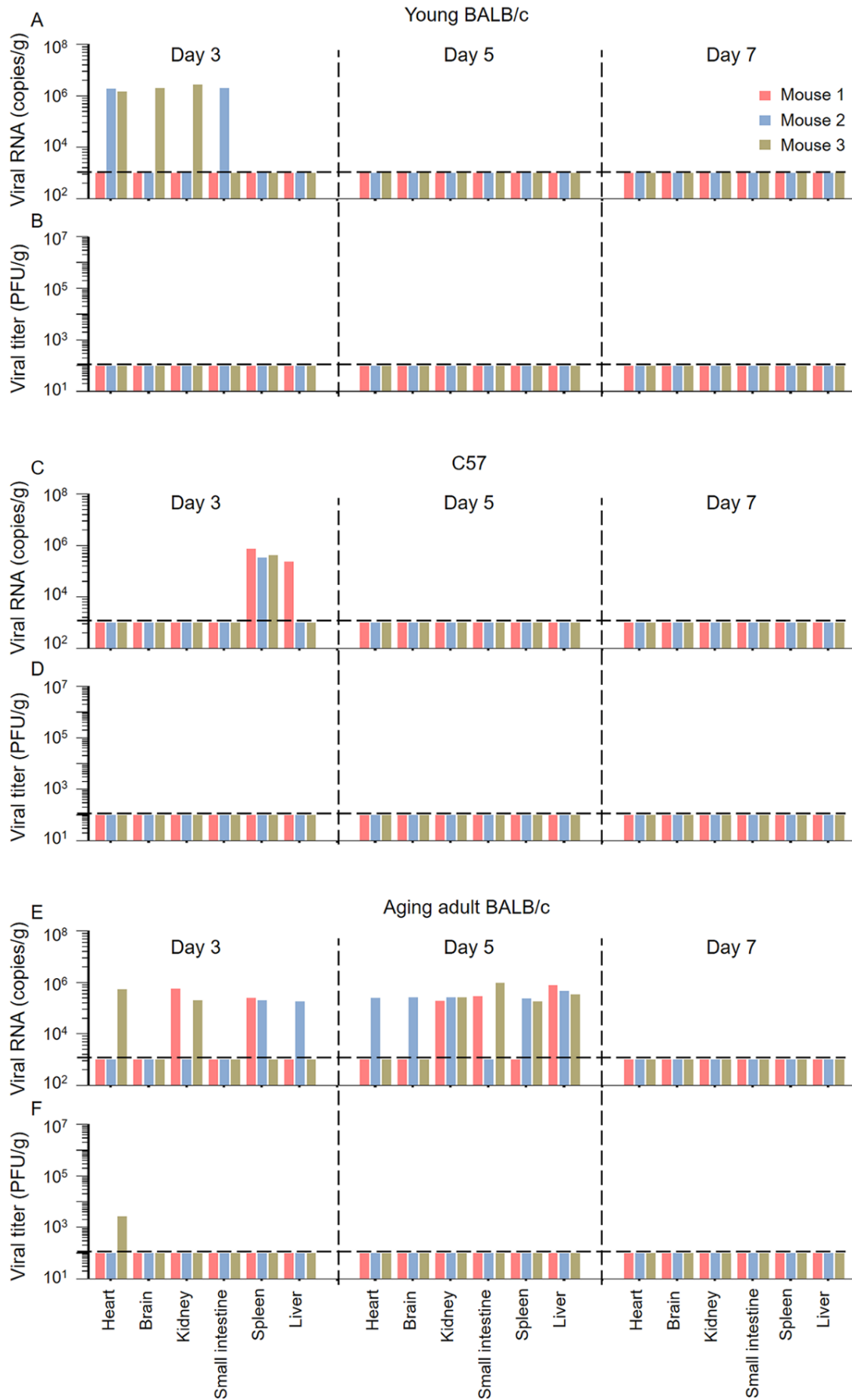
2

3 **Figure S1 Adaptation of SARS-CoV-2 in BALB/c mice.** 4–6-week-old female mice were  
4 inoculated i.n. with 10<sup>6.2</sup> PFU of SARS-CoV-2 virus HRB26. On day 3 p.i., nasal turbinates  
5 and lungs were collected and a mixture of nasal turbinate and lung homogenates with the  
6 highest viral RNA copies was defined as P1 and serially passaged to 3 mice by intranasal  
7 inoculation until P14. Viral RNA copies (A, B) and infectious titres (C, D) in the nasal  
8 turbinates (A, C) and lungs (B, D) from the indicated passages were detected by qPCR and  
9 virus titration. The horizontal dashed lines indicate the limit of detection.

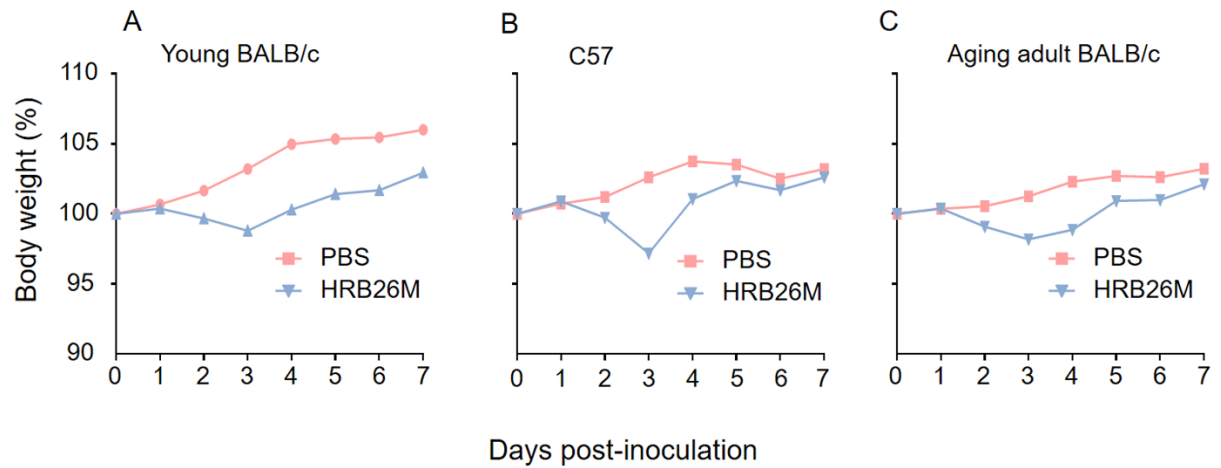


10

11 **Figure S2 50% mouse infectious dose (MID<sub>50</sub>) of mouse-adapted virus in young female**  
 12 **BALB/c mice.** Groups of three 4–6-week-old female BALB/c mice were inoculated i.n. with  
 13 10-fold serially diluted HRB26M. On day 3 p.i., three mice in each group were euthanized  
 14 and the viral RNA copies in the nasal turbinates (A) and lungs (B) were detected by qPCR.  
 15 The horizontal dashed lines indicate the limit of detection.



17 **Figure S3 Replication of mouse-adapted SARS-CoV-2 virus in the organs of young and**  
18 **aging adult male BALB/c and C57 mice.** Groups of nine 4–6-week-old female BALB/c (A,  
19 B), 4–6-week-old female C57 (C, D) or 8–9-month-old male BALB/c mice (E, F) were  
20 inoculated i.n. with  $10^{4.4}$  PFU of HRB26M in a volume of 50  $\mu$ L. On days 3, 5, and 7 p.i.,  
21 three mice in each group were euthanized. The viral RNA copies (A, C, E) and infectious  
22 titres (B, D, F) in the hearts, brains, kidneys, small intestines, spleens, and livers were  
23 detected by qPCR and virus titration. The horizontal dashed lines indicate the limit of  
24 detection.  
25



26

27 **Figure S4 Body weight changes.** Groups of three 4–6-week-old (young) female BALB/c

28 (A), 4–6-week-old female C57 (B), or 8–9-month-old (aging adult) male BALB/c (C) mice

29 were lightly anesthetized with CO<sub>2</sub> and i.n. inoculated with 10<sup>4.4</sup> PFU of HRB26M or PBS.

30 Body weights were monitored daily for 7 days and are presented as a percentage of the

31 weight on the day of inoculation (day 0).

32

## 33 MATERIALS AND METHODS

### 34 Biosafety and Facility

35 All experiments with infectious SARS-CoV-2 were performed in the biosafety level 4 and  
36 animal biosafety level 4 facilities in the Harbin Veterinary Research Institute (HVRI) of the  
37 Chinese Academy of Agricultural Sciences (CAAS), which is approved for such use by the  
38 Ministry of Agriculture and Rural Affairs of China.

### 39 Cells and Viruses

40 Vero E6 cells were maintained in Dulbecco's modified Eagle's medium (DMEM)  
41 containing 10% fetal bovine serum (FBS) and antibiotics and incubated at 37°C with 5%  
42 CO<sub>2</sub>. SARS-CoV-2/HRB26/human/2020/CHN (HRB26, GISAID access no.  
43 EPI\_ISL\_459909) was isolated from a patient in Vero E6 cells. Viral stocks were prepared in  
44 Vero E6 cells with DMEM containing 5% FBS. Mouse-adapted SARS-CoV-  
45 2/HRB26/human/2020/CHN (HRB26M, GISAID access no. EPI\_ISL\_459910) was obtained  
46 by serially passaging the HRB26 virus in 4–6-week-old female mice until passage 14 and  
47 was propagated in Vero E6 cells. Infectious virus titers were determined by using a plaque  
48 forming unit (PFU) assay in Vero E6 cells.

### 49 qPCR and sequencing of viral genomes

50 Viral genomic RNA of SARS-CoV-2 was extracted by using a QIAamp vRNA Minikit  
51 (Qiagen, Hilden, Germany). Reverse transcription was performed by using the HiScript II Q  
52 RT SuperMix (Vazyme, Nanjing, China) for qPCR. qPCR was performed to quantitate the  
53 viral N gene RNA copies by using the Applied Biosystems QuantStudio 5 Real-Time PCR  
54 System (Thermo Fisher Scientific, Waltham, MA, USA) with Premix Ex Taq (probe qPCR)

55 (Takara, Dalian, China). The *N* gene-specific primers (forward, 5'-  
56 GGGGAACTTCTCCTGCTAGAAAT-3'; reverse, 5'-CAGACATTTTGCTCTCAAGCTG-3')  
57 and probe (5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3') were utilized according to  
58 the information provided by the National Institute for Viral Disease Control and Prevention,  
59 China (<http://nmdc.cn/nCoV>). The amount of vRNA for the target SARS-CoV-2 *N* gene was  
60 normalized to the standard curve obtained by using a plasmid (pBluescriptIISK-N, 4,221 bp)  
61 containing the full-length cDNA of the SARS-CoV-2 *N* gene. Viral genome sequencing was  
62 performed on an Applied Biosystems DNA analyzer. Sequences were assembled and  
63 analyzed with the Seqman module of the DNASTar package.

#### 64 **Mouse study**

65 The mice for this study, 4–6-week-old female and 8–9-month-old male BALB/c, and 4–  
66 6-week-old female C57BL/6J (C57) were obtained from Beijing Charles River Labs  
67 (Beijing, China). Mice were lightly anesthetized with CO<sub>2</sub> and intranasally (i.n.) inoculated  
68 with 50 µL dilutions of SARS-CoV-2. Body weights and clinical symptoms were monitored  
69 daily. On days 3, 5, or 7 post-inoculation (p.i.), animals were euthanized and their organs,  
70 including nasal turbinates, lungs, hearts, brains, kidneys, small intestines, spleens, and  
71 livers, were collected for viral RNA detection by qPCR, virus titration by use of a PFU  
72 assay, and histopathological study.

73 To determine the 50% mouse infectious dose (MID<sub>50</sub>) of HRB26M virus, groups of three  
74 young female mice were inoculated i.n. with 10-fold serially diluted HRB26M virus. On  
75 day 3 p.i., three mice in each group were euthanized and their nasal turbinates and lungs  
76 were collected for virus detection; 50% mouse infectious dose (MID<sub>50</sub>) at day 3 p.i. were

77 calculated by the method of Reed and Muench (Reed and Muench, 1938).

## 78 **Histopathologic and immunohistochemical studies**

79 Animal tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, and cut  
80 into 4- $\mu$ m sections. The sections were stained with hematoxylin-eosin for histopathologic  
81 observation. The sections used for immunohistochemistry were dewaxed in xylene and  
82 hydrated through a series of descending concentrations of alcohol to water. For viral antigen  
83 retrieval, sections were immersed in citric acid/sodium citrate solution, treated with 3%  
84 hydrogen peroxide, and blocked with 8% skim milk. Primary rabbit anti-SARS-CoV-2  
85 nucleoprotein monoclonal antibody (1:500; Frdbio, Wuhan, China) and HRP-conjugated anti-  
86 rabbit IgG (whole molecule) secondary antibody (1:600, Sigma-Aldrich) were used.  
87 Immunostaining was visualized with DAB and counterstained with hematoxylin.

## 88 **Evaluation of antiviral activity in cells**

89 Remdesivir (GS-5734) was obtained from Hanxiang BioTec (Shanghai, China). Vero E6  
90 cells were pretreated with the indicated concentrations of remdesivir in DMSO or with  
91 DMSO alone for 1 hour. The cells were then infected with HRB26 or HRB26M at an MOI  
92 of 0.005 and incubated for 1 hour at 37°C. Cells were washed with PBS and virus growth  
93 medium containing the indicated amounts of remdesivir in DMSO or DMSO alone was  
94 added. Supernatants were collected at 24 hours post-infection for virus titration by use of a  
95 PFU assay in Vero E6 cells. Relative viral titers were calculated on the basis of the ratios to  
96 the viral titers in the mock-treated counterparts. Data shown are the mean values with  
97 standard deviations for the results of three independent experiments.

98 Cell viability was determined by using the Cell Titer-Glo kit (Promega, Madison, WI,

99 USA) following the manufacturer's instructions. Briefly, Vero E6 cells were seeded in 96-  
100 well plates with opaque walls. After 12 to 16 hours, the indicated concentrations of  
101 remdesivir in DMSO were added. Twenty-four hours later, Cell Titer-Glo reagent was  
102 added to each well. Luminescence was measured with a GloMax 96 Microplate  
103 Luminometer (Promega, Madison, WI, USA).

#### 104 **Evaluation of antiviral activity in mice**

105 Groups of six 4–6-week-old female mice were treated intramuscularly (i.m.) with a loading  
106 dose of 50 mg/kg (high dose) or 10 (low dose) mg/kg remdesivir, followed by a daily  
107 maintenance dose of 25 mg/kg (high dose) or 5 mg/kg (low dose). Alternatively, mice were  
108 treated i.n. alone or a combination of i.n. and i.m. with a loading dose of 50 mg/kg  
109 remdesivir, followed by a daily maintenance dose of 25 mg/kg. As a control, mice were  
110 administered vehicle solution (12% sulfobutylether- $\beta$ -cyclodextrin, pH 3.5) daily. One hour  
111 after administration of the loading dose of remdesivir or vehicle solution, each mouse was  
112 inoculated i.n. with  $10^{3.6}$  PFU of HRB26M in 50  $\mu$ L. Three mice from each group were  
113 euthanized on days 3 and 5 p.i.. The nasal turbinates and lungs were collected for virus  
114 detection by qPCR and PFU assay.