# Supplementary Materials

## Supplementary Materials and Methods

### Animal experiments

All animal experiments were carried out in accordance with the Animal Welfare Act, and all applicable international, national, and institutional guidelines for the care and use of animals were strictly followed. All animal sample collection protocols complied with the current laws of China. The animal experiments were conducted under the authority of a license issued by the government of Yunnan Province, with approval from the Animal Experimentation Ethics Committee and Care and Use of Laboratory Animal Committee of the Kunming Institute of Zoology (approval license number: SMKX-20200301-01). All SARS-CoV-2 research was performed in an animal biosafety level 3 (ABSL-3) facility at the Kunming Institute of Zoology, Chinese Academy of Sciences.

The humanized ACE2 (hACE2) mice, C57BL/6NAce2$^{em2(hACE2\text{-}WPRE,\ pgk\text{-}puro)/CCLA}$, were kindly provided by the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences. The mice were divided into five groups: i.e., Sham, Angiotensin II (Ang II), SARS-CoV-2, Ang II+SARS-CoV-2, and Ang II+SARS-CoV-2+Captopril (Cap) groups. Mice in the Sham group only received saline treatment. Mice in the Ang II group were treated with Ang II through Ang II pumps. Mice in the Ang II+SARS-CoV-2 and Ang II+SARS-CoV-2+Cap groups were first treated with Ang II, then intranasally challenged with SARS-CoV-2 at indicated time points. Mice in the Ang II+SARS-CoV-2+Cap group were also treated with Cap. The Ang II (#HY-13948) was purchased from MedChemExpress

(MCE, USA) and the ALZET mini-osmotic pumps (model 1002) were purchased from Alza Corp (Cupertino, CA, USA).

Mice were first anaesthetized with isoflurane. A midline incision was made on the dorsum and a subcutaneous pocket was created in the right flank area. Mini-osmotic pumps loaded with saline or Ang-II (100 μL) were inserted to deliver saline or Ang-II at 1.4 mg/kg/day for a period of 22 days. After mice were persistently treated with saline or Ang-II for 2 weeks, their heart rate and systolic and diastolic blood pressure were measured using tail cuff plethysmography (MRBP system, IITC Life Science, USA) to verify elevated blood pressure. After successful modeling of hypertension, the mice were treated intragastrically with Cap (10 mg/kg/day, i.g.) or vehicle at 14 days post-Ang II treatment (dpa) before intranasal inoculation with SARS-CoV-2 ($2\times10^6$ TCID50, i.n.) at 15 dpa (also termed 0 days post-infection (dpi)). Mice were orally treated with Cap or vehicle daily until sacrifice at the indicated time points. To collect samples, mice were sacrificed at 3 or 7 dpi, and their lungs were collected and frozen at −80 °C to extract RNA in the future or stored at room temperature in 4% paraformaldehyde for histopathological analysis. Feces were collected daily until sacrifice and frozen at −80 °C for viral loads measurements.

**Measurement of viral RNA loads**

To measure the viral load in lungs, total RNA was extracted from frozen lung samples using TRIzol Reagent (Invitrogen) according to the protocols of the manufacturer. Viral RNA quantification was performed by quantitative real-time polymerase chain reaction (qRT-PCR) targeting SARS-CoV-2 nucleoprotein genes using a THUNDERBIRD® Probe One-step

qRT-PCR Kit (Toyobo). The sequences of the primers and probes can be found in our previous studies (Liu et al., 2021; Song et al., 2020; Xu et al., 2020).

Fecal viral loads were measured as per our previous research (Liu et al., 2021). Briefly, frozen fecal samples were homogenized in 1 ml of Dulbecco's Modified Eagle Medium (DMEM), with the suspension then collected. Viral RNA was purified from 200 μl of fecal suspension using a High Pure Viral RNA Kit (Roche). Nucleoprotein gene copies of SARS-CoV-2 in the purified viral RNA were measured, as above.

**qRT-PCR**

To examine the impact of SARS-CoV-2 on Ang II-induced hypertension, lung tissue expression of two inflammatory markers, i.e., *Il1b* and *Cxcl2*, was analyzed by qRT-PCR using a Real-Time PCR Kit (Takara) containing TB green dye II. Each reaction was performed in triplicate. The relative expression levels of *Il1b* and *Cxcl2* were determined using primer pairs *Il1b*-F and *Il1b*-R and *Cxcl2*-F and *Cxcl2*-R. *Gapdh* was used as the control and the $2^{-\Delta Ct}$ method was applied for data analysis. Primer sequences are as follows. Mouse *Il1b*-F: CTACAGGCTCCGAGATGAACAAC, *Il1b*-R: TCCATTGAGGTGGAGAGCTTTC; Mouse *Cxcl2*-F: CTCCTTTCCAGGTCAGTTAGC, *Cxcl2*-R: CAGAAGTCATAGCCACTCTCAA; Mouse *Gapdh*-F: AGAACATCATCCCTGCATCC, *Gapdh*-R: CACATTGGGGGTAGGAACAC.

**Western blotting**

Mouse tissues were homogenized in cell lysis buffer (Beyotime) supplemented with cOmplete$^{TM}$ Protease Inhibitor (Roche). The denatured protein lysates were separated using

5%–12% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After polyvinylidene fluoride (PVDF) membrane transfer, CXCL2 antibody (Affinity, 1:1 000), IL-1β antibody (Abcam, 1:1 000), and β-actin antibody (Abcam, 1:1 000) were added to probe target proteins, followed by incubation with horseradish peroxidase (HRP)-conjugated anti-mouse and rabbit IgG (H+L) secondary antibody (Invitrogen, 1:5 000) for 1 hour at room temperature. The Pierce ECL Western Blotting Substrate (CST) was used for development. The intensity of immunoblotted bands was quantified using ImageJ software (National Institutes of Health, Bethesda, Md, USA).

**Histopathological analysis**

Histopathological analysis was performed using hematoxylin and eosin (H&E) staining, as described previously (Sun et al., 2020). In brief, mouse tissues were excised, fixed with 10% neutral buffered formalin, dehydrated, and embedded in paraffin. Each embedded tissue was sectioned into 4 mm thick longitudinal sections. Five tissue sections derived from different parts of each tissue were stained with H&E according to standard procedures for examination by light microscopy. The degree of lung damage under light microscopy was assessed by the degeneration of alveolar epithelial cells, expansion of parenchymal wall, edema, hemorrhage, and inflammatory cell infiltration.

**Multiplex immunofluorescent assay**

Paraffin sections were deparaffinized in xylene and rehydrated in a series of graded alcohol. Antigen retrievals were performed in Tris-EDTA buffer (pH 9) in a pressure cooker at sub-boiling. After blocking, samples were incubated with rabbit monoclonal antibodies

against IL-6 (Servicebio, 1:500), IL-1β (Servicebio, 1:800), Ly6G (Servicebio, 1:500), CCR2 (Servicebio, 1:1 000), and F4/80 (Servicebio, 1:500) overnight at 4 °C to mark inflammatory cells. Next, paraffin sections were incubated for 1h at room temperature with HRP-conjugated goat anti-rabbit secondary antibody to catalyze iFluor™ 488/Cy3/Cy5-tyramide (AAT Bioquest) and amplify the staining signal according to tyramide signal amplification. Afterward, the primary and secondary antibodies were thoroughly eliminated by heating the slides in citrate buffer (pH 6) for 10 min at 95 ℃ using a microwave. In a serial fashion, each antigen was labeled by distinct fluorophores. Finally, the sections were stained with 4',6-diamidino-2-phenylindole (DAPI) and mounted. All antibodies were detected sequentially, and slices were imaged using a confocal laser scanning microscopy platform (Zeiss LSM880).

**Statistical analysis**

All values are expressed as mean±SEM (n), where n denotes the number of independent experiments. Significant differences between two groups were determined using the Mann-Whitney test. All analyses were performed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). Differences were considered statistically significant at $P<0.05$.

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