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Article

The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice

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Severe acute respiratory syndrome CoV-2 (SARS-CoV-2) caused the corona virus disease 2019 (COVID-19) cases in China and has become a public health emergency of international concern[1]. Because angiotensin-converting enzyme 2 (ACE2) is the cell entry receptor of SARS-CoV[5], we used transgenic mice bearing human ACE2 and infected with SARS-CoV-2 to study the pathogenicity of the virus. Weight loss and virus replication in lung were observed in hACE2 mice infected with SARS-CoV-2. The typical histopathology was interstitial pneumonia with infiltration of significant macrophages and lymphocytes into the alveolar interstitium, and accumulation of macrophages in alveolar cavities. Viral antigens were observed in the bronchial epithelial cells, macrophages and alveolar epithelia. The phenomenon was not found in wild-type mice with SARS-CoV-2 infection. Notably, we have confirmed the pathogenicity of SARS-CoV-2 in hACE2 mice. The mouse model with SARS-CoV-2 infection will be valuable for evaluating antiviral therapeutics and vaccines as well as understanding the pathogenesis of COVID-19.

In late December of 2019, the coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome CoV-2 (SARS-CoV-2), linked to a seafood market in which exotic animals were also sold and consumed, were identified and reported from Wuhan City, Hubei Province, China[1,2]. The number of confirmed cases has since soared, with almost 78,000 cases reported and over 2,700 deaths as of February 25, 2020 in China[3], and imported cases from travelers of mainland China in several other countries. It is critical to find the pathogenicity and biology of the virus for prevention and treatment of the disease.

Because SARS-CoV-2 was highly homologous with severe acute respiratory syndrome coronavirus (SARS-CoV), human angiotensin-converting enzyme 2 (hACE2), which was the entry receptor of SARS-CoV, was also considered to have a high binding ability with the SARS-CoV-2 by molecular biological analysis[4,5]. Therefore, we used the hACE2 transgenic and wild type (WT) mice infected with SARS-CoV-2 to study the pathogenicity of the virus.

Specific pathogen-free, 6-11-month-old, male and female WT mice (WT-HB-01, n=15) and hACE2 mice (ACE2-HB-01, n=19) were inoculated intranasally with SARS-CoV-2 stock virus (HB-01) at a dosage of 10^5 $TCID_{50}$/50 μL inoculum volume per mouse after the mice were intraperitoneally anesthetized by 2.5% avertin, and the mock-treated hACE2 mice (ACE2-Mock, n=15) were used as control. Clinical manifestations were recorded from thirteen mice (WT-HB-01, n=3; ACE2-Mock, n=3; ACE2-HB-01, n=7). Compared to WT-HB-01 mice and ACE2-Mock mice, slight bristled fur and weight loss were only observed in ACE2-HB-01 mice during the 14 days observation, and other clinical symptoms such as arched back and decreased response of external stimuli were not found. Notably, the weight loss of ACE2-HB-01 mice was up to 8% at 5 days post infection (dpi) (Figure 1a).

Next, viral replication and pathological changes were examined from three animals per group at each time point, and the primary organs were collected periodically, including heart, liver, spleen, lung, kidney, brain, intestine and testis (male mice). As shown in Figure 1b, viral loads were detectable by qRT-PCR at 1 dpi, 3 dpi, 5 dpi and 7 dpi in the lungs of ACE2-HB-01 mice but not in that of WT-HB-01mice (data not shown), and the viral RNA copies reached a peak at 3 dpi ($10^{6.77}$ copies/mL). Interestingly, the viral RNA was also detectable at 1 dpi in the intestine of ACE2-HB-01 mice, which was failed to be detected in other tissues along the timeline (Figure 1b). Although the viral loads were detectable in the intestine, no virus in intestine was isolated at 1 dpi, which was speculated the residual input inoculum from the nasal mucosa to intestines by swallowing. Consistent to the results of viral loads in lung, infectious virus was respectively isolated from the lungs of ACE2-HB-01 mice at 1 dpi, 3 dpi and 5 dpi, and the highest virus titers were detected at 3 dpi ($10^{2.44}$ $TCID_{50}$/100 μL) (Figure 1c). Meanwhile, the infectious virus was isolated by Vero E6 cells culture from lung, and the SARS-CoV-2 particles were observed by electron microscopy (Figure 1d). However, the virus was failed to be isolated from the lungs

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in WT-HB-01 mice and ACE2-Mock mice along the detecting timeline (Figure 1c), which suggested that hACE2 was essential for SARS-CoV-2 infection and replication in mice. Moreover, specific IgG antibodies against S protein of SARS-CoV-2 were positively measured in the sera of ACE2-HB-01 mice at 21 dpi (Figure 1e).

There were no obviously gross and histopathological changes at 1 dpi in all the animals of each group. Compared to WT-HB-01 mice or ACE2-mock mice with homogeneously pink and slightly deflated lung lobes, all the ACE2-HB-01 mice at 3 dpi displayed comparable gross lesions with focal to multifocal dark red discoloration in certain lung lobes. The lesions progressed into multifocal to coalescent scattered-dark reddish purple areas and focal palpable nodules throughout the lung lobes at 5 dpi (Figure 2a). The damaged lungs became swollen and enlarged in size. Microscopically, the lung tissues from ACE2-HB-01 mice at 3 dpi displayed moderate interstitial pneumonia characterized by thickened alveolar septa accompanied with infiltration of inflammatory cells in 70-80% areas of the lung tissues, and accumulation of inflammatory cells in partial alveolar cavities (20-30%). Inflammatory cells including lymphocytes, macrophages, and neutrophils accumulated in the alveolar interstitium and caused thickening of the alveolar walls. At 5 dpi, the lung progressed into coalescing interstitial pneumonia with diffuse lesions characterized by more severe thickened alveolar septa accompanied with infiltration of inflammatory cells, and accumulation of inflammatory cells in more alveolar cavities (40-50%). The thickened alveolar septa were filled with macrophages, lymphocytes, and neutrophils (Figure 2a). A small amount of collagen fiber could be observed in the thickened alveolar interstitium in the ACE2-HB-01 mice by Modified Masson's Trichrome stain at 5 dpi (Extended Data Figure 1a). The bronchiolar epithelial cells showed swelling, degeneration, some of which fragmentated (Figure 2a). A few periodic acid-Schiff (PAS) positive-exudation or denatured and detached bronchiolar epithelium could be occasionally observed in affected bronchioles (Extended Data Figure 1b) at 5 dpi. The alveolar cavities were distended mainly by swollen and degenerative macrophages, lymphocytes, and neutrophils (Figure 2a). To investigate the infiltration of specific inflammatory cells, immunohistochemical (IHC) was carried out to identify $MAC2^+$ macrophages (Extended Data Figure 2a), $CD3^+$ T lymphocytes, and $CD19^+$ B lymphocytes (Extended Data Figure 2b). Compared to the WT-HB-01 mice, more macrophages and T lymphocytes were found in the lungs of ACE2-HB-01 mice along with lasting prolonged infection time. The $MAC2^+$ macrophages were diffusely infiltrated in the alveolar cavities (at 3 dpi) or focally aggregated together in the thickened alveolar septa (at 5 dpi, Extended Data Figure 2a). $CD3^+$ T lymphocytes were scattered dispersed or occasionally aggregated in the alveolar interstitium in the ACE2-HB-01 mice at 3 dpi and 5 dpi, and some $CD19^+$ B lymphocytes were also observed at 5 dpi (Extended Data Figure 2b). Perivascular infiltrated inflammatory cells, including lymphocytes, macrophages, and neutrophils, were observed multifocally within and adjacent to affected areas of the lungs. In the lesional areas of the lungs, IHC staining of sequential section revealed that viral antigens were found in the macrophages, alveolar epithelia, and the degenerative and being desquamated bronchial epithelial cells (Figure 2b). Meanwhile, we also observed small amounts of viral antigens in respiratory epithelial cells within non-lesional areas of the lungs (data not shown). However, there were no significant histopathological changes (Extended Data Figure 3) or viral antigens of SARS-CoV-2 (Extended Data Figure 4) in the other organs, including myocardium, liver, spleen, kidney, cerebrum, intestine, and testis. At 7 dpi, the pneumonia became mild with focal lesion areas (data not shown).

In addition, the co-localization of SARS-CoV-2 S protein (Figure 3f) and hACE2 receptor (Figure 3g) was demonstrated in alveolar epithelial cells of ACE2-HB-01 mice by immunofluorescence at 3 dpi (Figure 3h). The phenomenon was not found in the ACE2-Mock mice (Figures 3a, 3b, 3c and 3d) or WT-HB-01 mice (data not shown), indicating that the SARS-CoV-2, as same as SARS-CoV, also utilizes the hACE2 as a receptor for entry[4].

The speed of geographical spread of COVID-19 caused by SARS-CoV-2 has been declared as public health emergency of international concern (PHEIC), with cases reported on multiple continents only weeks after the disease was first reported[6]. Although it has been determined by bioinformatics that the pathogen of this epidemic is a novel coronavirus, it is necessary to be confirmed by animal experiments following Koch's postulates[7]. The previous clinical studies have confirmed the isolation of the virus from diseased hosts and cultivation in host cells[1]. In present study, after the experimental infection of hACE2 transgenic mice with one of the earliest known isolates of SARS-CoV-2, the mouse model of SARS-CoV-2 infection has exhibited viral replication in the lungs characterized by moderate interstitial pneumonia, similar to initial clinical reports of pneumonia caused by SARS-CoV-2[8]. Moreover, we also observed specific antibodies against SARS-CoV-2 and re-isolated the virus from infected mice.

The case fatality rate of currently reported cases is about 2%, which implies that so far, this novel coronavirus does not seem to cause the high fatality rates as SARS-CoV (9-11%)[9], which suggested the difference in pathogenicity between the two viruses. The pathogenicity of SARS-CoV-2 seems mild compared to SARS-CoV in mice, the latter caused extrapulmonary organ damage, includes brain, kidney, intestine, heart and liver, furthermore, the neurons are susceptible for SARS-CoV infection, and cerebral vasculitis and hemorrhage were observed in hACE2 transgenic mice[10,11]. However, only interstitial pneumonia was observed in SARS-CoV-2-infected hACE2 mice, implying the disparity in pathogenicity of the coronavirus.

Taken together, our results demonstrated the pathogenicity of SARS-CoV-2 in mice, together with the previous clinical studies[1], completely fulfills the Koch's postulates[7] and confirmed SARS-CoV-2 was the pathogen of COVID-19. The mouse model with SARS-CoV-2 infection will be valuable for evaluating antiviral therapeutics and vaccines as well as understanding the disease pathogenesis.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2312-y.

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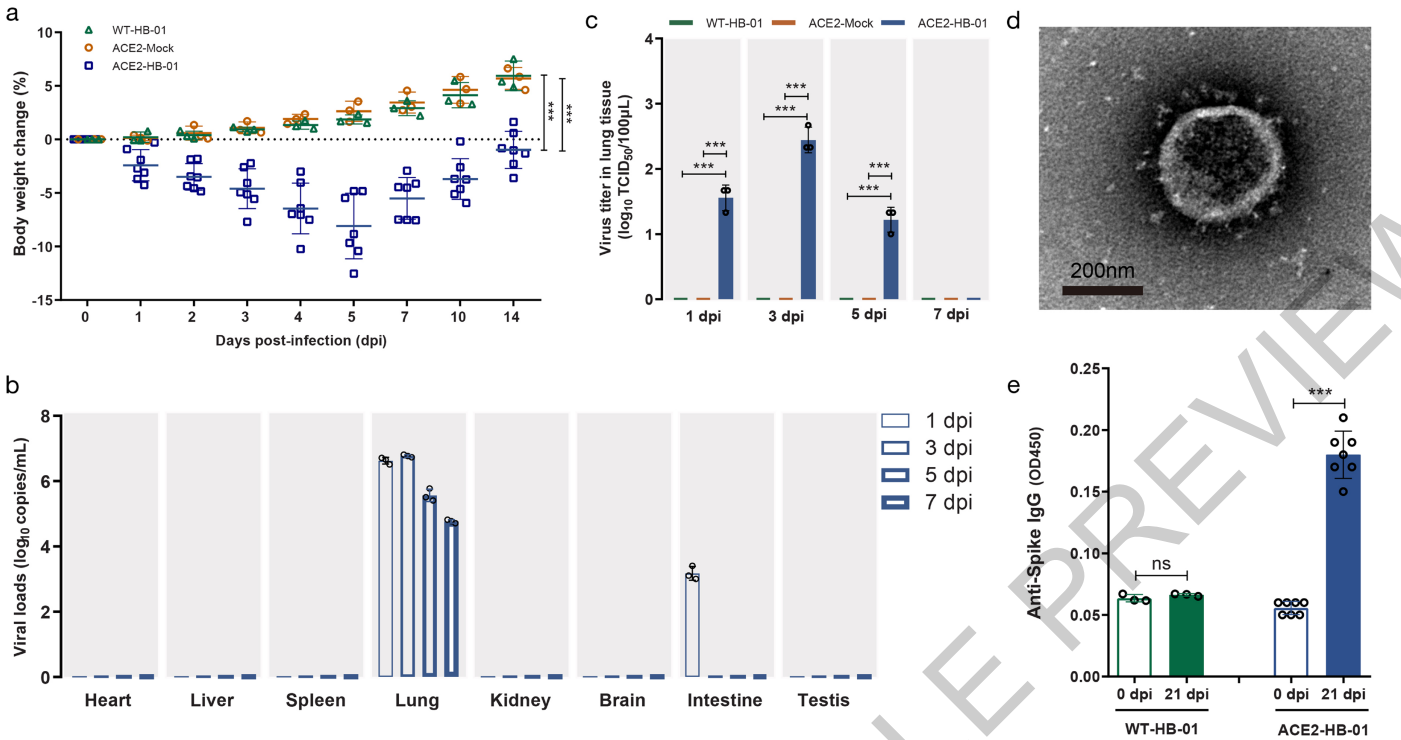


Fig. 1 | Weight loss, virus replication and specific IgG production in hACE2 mice after SARS-CoV-2 infection. a, weight loss was recorded for 14 days. hACE2 mice (n=7) and WT mice (n=3) were experimentally challenged intranasally with SARS-CoV-2, and the ACE2-Mock mice (n=3) were used as control. According to two-tailed Mann-Whitney U test, weight loss in ACE2-HB-01 mice displayed a significant decline compared with that in WT-HB-01 or ACE2-Mock mice (***$p=0.0005$). **b**, to measure viral RNA, 12 mice were infected in each group, and 3 mice per group were sacrificed and their major organs (testis from male mice) harvested for viral loads and virus titer at 1 dpi, 3 dpi, 5 dpi and 7 dpi respectively. The distribution of SARS-CoV-2 in the primary organs of ACE2-HB-01 mice was detected by qRT-PCR. **c**, virus titers of lungs were determined on Vero E6 cells. According to two-tailed unpaired Welch's *t*-test, viral titers in lung from ACE2-HB-01 mice (n=3) showed a significant increase compared with that in WT-HB-01 (n=3) or ACE2-Mock mice (n=3) at 1 dpi (**$p=0.0053$), 3 dpi (**$p=0.0022$) and 5 dpi (**$p=0.0081$). **d**, the virus isolated from lungs of ACE2-HB-01 mice at 3 dpi was observed by electron microscope. Black scale bar=200 nm. Data are representatives of three independent experiments. **e**, the specific IgG against SARS-CoV-2 was detected from the sera of mice (WT-HB-01 mice, n=3 or ACE2-HB-01 mice, n=7) at 0 dpi and 21 dpi by ELISA (two-tailed unpaired Student's *t*-test, ns, $p=0.2193$; two-tailed unpaired Welch's *t*-test, ***$p=3.11E-06$). Data (**a**-**c**, **e**) represent mean±SD.

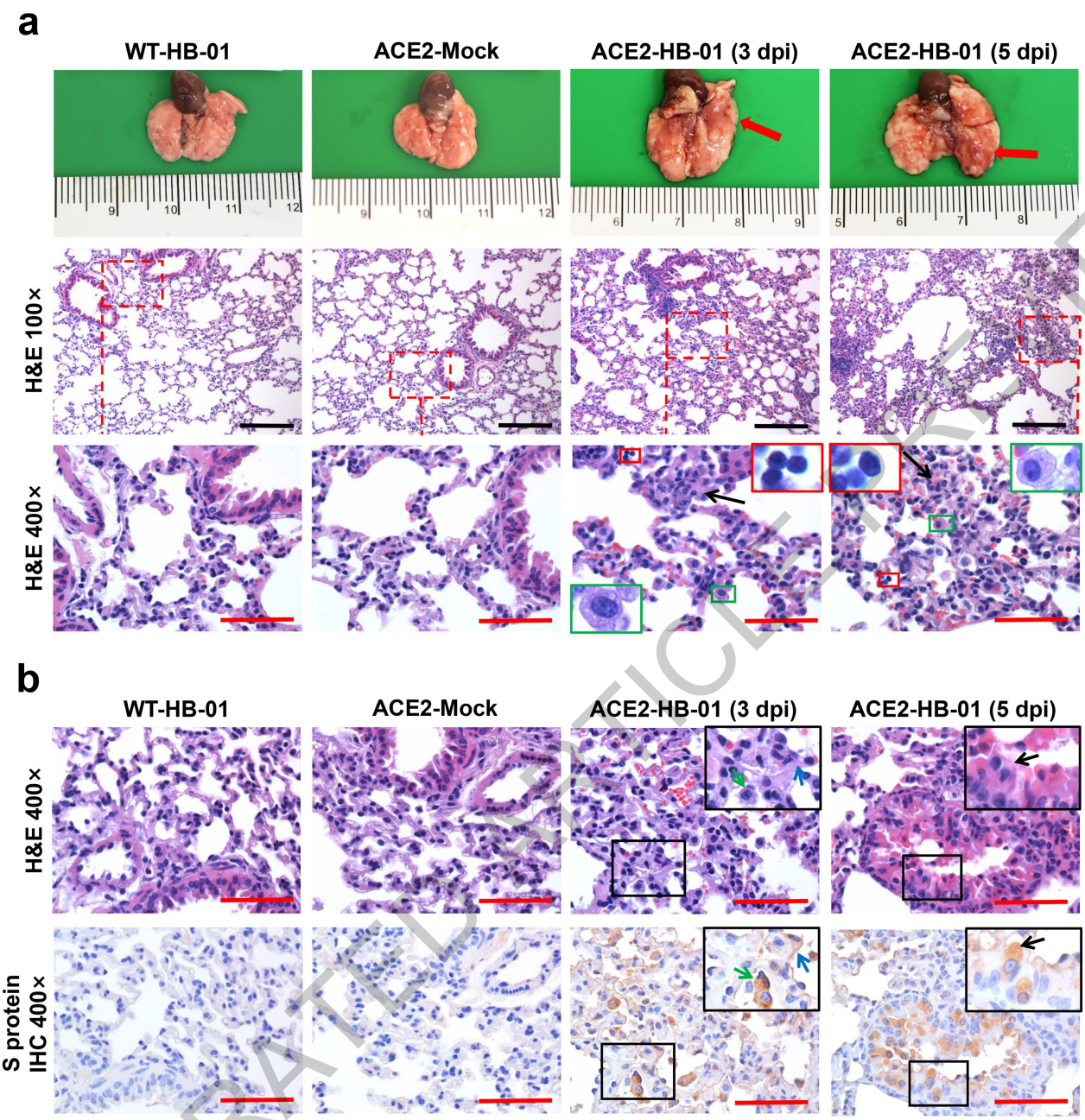


Fig. 2 | Gross pathology, histopathology, and immunohistochemistry of the lungs in SARS-CoV-2-infected hACE2 mice. a, gross pathology and histopathology of lungs from WT-HB-01 mice (3 dpi), ACE2-Mock mice (3 dpi) and ACE2-HB-01 mice (3 dpi and 5 dpi). Postmortem examinations showed focal dark red lesions (red arrow) throughout the dorsal of the right middle lobe of the lung at 3 dpi. The lesions progressed into multifocally scattered-dark reddish purple areas and palpable nodules (red arrow) throughout the right lobe of the lung at 5 dpi. Histopathological observation indicated that moderate interstitial pneumonia with thickened alveolar septa (black arrows) and infiltration of lymphocytes (red frames, 1000 magnification). The swollen and degenerative mononuclear cells (green frames, 1000 magnification) are scattered within the alveolar cavities at 3 dpi and 5 dpi. **b**, immunohistochemical examination of lungs of each group. The sequential sections were stained by HE and IHC, respectively. The viral antigens were observed in the mononuclear cells (green arrow), the alveolar epithelia (blue arrows), and the degenerative and being desquamated bronchial epithelial cells (black arrows). The black frames on the upper-right corner is the magnification. Black scale bar = 100 μm, red scale bar = 50 μm. Data (**a**, **b**) are representatives of three independent experiments.

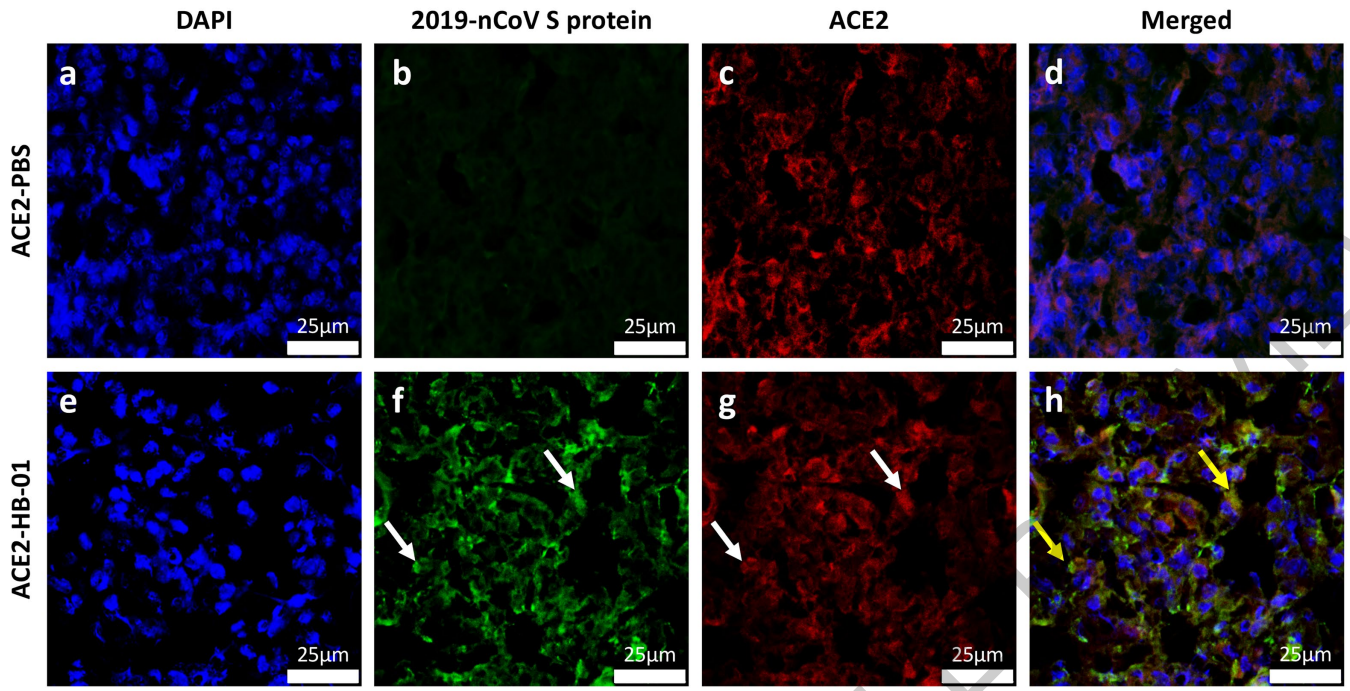


Fig. 3 | Immunofluorescence analysis of viral antigens in lungs of SARS-CoV-2-infected hACE2 mice at 3 dpi. Co-localization of SARS-CoV-2 S protein and hACE2 receptor in hACE2 mouse lungs, the sections were incubated with anti-SARS-CoV-2 S protein antibody, anti-human ACE2 antibody, and DAPI. **a-d**, the lung sections of ACE2-Mock mice. **e-h**, the lung sections of ACE2-HB-01 mice. The white arrows showed the viral S protein (**f**) and hACE2 (**g**), respectively, the yellow arrow showed the merge of viral S protein and hACE2 (**h**). White scale bar=25 μm. Data (**a** - **h**) are representatives of three independent experiments.

Methods

Ethics statement

Murine studies were performed in an animal biosafety level 3 (ABSL3) facility using HEPA-filtered isolators. All procedures in this study involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Institute of Laboratory Animal Science, Peking Union Medical College (BLL20001). All the experiments were complied with all relevant ethical regulations.

Viruses and cells

The SARS-CoV-2 (strain HB-01) was kindly provided by Professor Wenjie Tan[1], from the China Centers for Disease Control and Prevention (China CDC). The complete genome for this SARS-CoV-2 was submitted to GISAID (BetaCoV/Wuhan/IVDC-HB-01/2020|EPI_ISL_402119), and deposited in the China National Microbiological Data Center (accession number NMDC10013001 and genome accession numbers MDC60013002-01). Seed SARS-CoV-2 stocks and virus isolation studies were performed in Vero cells, which are maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 μg/ml streptomycin, and incubated at 37 °C, 5% CO_2. For infected mice, the lung homogenates were used for virus titration tests using endpoint titration in Vero E6 cells. Virus titer of the supernatant were determined using a standard 50% tissue culture infection dose (TCID50) assay.

Animal experiments

For the animal experiments, specific pathogen-free, 6-11-month-old, male and female transgenic hACE2 mice were obtained from the Institute of Laboratory Animal Science, Peking Union Medical College, China. Transgenic mice were generated by microinjection of the mice ACE2 promoter driving the human ACE2 coding sequence into the pronuclei of fertilized ova from ICR mice, and then human ACE2 integrated was identified by PCR as previous described[10], the hACE2 mainly expressed in lung, heart, kidney, and intestine of transgenic mice. After intraperitoneally anesthetized by 2.5% avertin with 0.02 mL/g body weight, the hACE2 mice or WT (ICR) mice were respectively inoculated intranasally with SARS-CoV-2 stock virus at a dosage of 10^5 $TCID_{50}$ and hACE2 mice intranasally inoculated with equal volume of PBS was used as mock-infection control. The infected animals were continuously observed daily to record body weights, clinical symptoms, response to external stimuli and death. And the mice were dissected at 1 dpi 3 dpi, 5 dpi and 7 dpi respectively to collect different tissues to screen virus replication and histopathological changes.

Preparation of homogenate supernatant

Tissues homogenates (1 g/mL) were prepared by homogenizing perfused tissues using an electric homogenizer for 2 min 30 s in DMEM. The homogenates were centrifuged at 3,000 rpm for 10 min at 4 °C. The supernatant was collected and stored at −80 °C for viral titer and viral loads detection.

RNA extraction and qRT-PCR

Total RNA was extracted from tissues homogenates of organs using the RNeasy Mini Kit (Qiagen, Hilden, Germany), and reverse transcription was performed using the PrimerScript RT Reagent Kit (TaKaRa, Japan) following manufacturer instructions. A quantitative real-time reverse transcription-PCR (qRT-PCR) reactions were performed using the PowerUp SYBG Green Master Mix Kit (Applied Biosystems, USA), in which samples were processed in duplicate using the following cycling protocol: 50 °C for 2 min, 95 °C for 2 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 30 s, and then 95 °C for 15 s, 60 °C for 1 min, 95 °C for 45 s. The primer sequences used for RT-PCR are targeted against the envelope (E) gene of SARS-CoV-2 and are as follows: Forward: 5′-TCGTTTCGGAAGAGACAGGT-3′, Reverse: 5′-GCGCAGTAAGGATGGCTAGT-3′. The PCR products were verified by sequencing using the dideoxy method on an ABI 3730 DNA sequencer (Applied Biosystems, CA, USA). During the sequencing process, amplification was performed using specific primers. The sequences for this process are available upon request. The sequencing reads obtained were linked using DNAMAN, and the results were compared using the Megalign module in the DNAStar software package. The SYBR Green real-time PCR standard curve was generated by serial 10-fold dilutions of recombinant plasmid with a known copy number (from 1.47×10^9 to 1.47×10^1 copies/μL). These dilutions were tested and used as quantification standards to construct the standard curve by plotting the plasmid copy number against the corresponding threshold cycle values (CT). Results were expressed as log10 numbers of genome equivalent copies (GEC) per ml of sample.

ELISA method

The specific IgG against SARS-CoV from ACE2-HB-01 mice and WT-HB-01 mice were determined by enzyme-linked immunosorbent assay (ELISA). 96-well plates were coated with Spike 1 protein of SARS-CoV-2 (0.1 μg/100 μL, Sino Biological, 40591-V08H), the tested sera were diluted at 1:100 and added to each well, and 3 multiple wells were set for each sample, and then incubated at 37°C for 30 minutes, followed by the goat anti-mouse secondary antibodies conjugated with HRP (ZB-2305, zhongshan,1:10,000 dilution), and incubated at room temperature for 30 minutes. The reaction was developed by TMB substrate and the optical densities at 450 nm were determined (Metertech960 enzyme marker with 450 nm wavelength).

Laboratory preparation of the antibody of SARS-CoV-2 spike-1 (S1) protein

Mice were immunized with purified SARS-CoV-2 S1 protein (Sino biological) and splenocytes of hyper immunized mice were fused with myeloma cells. Positive clones were selected by ELISA using SARS-CoV-2 S1 protein (Extended Data Figure 5). The cell supernatant of 7D2 clone, binding to SARS-CoV-2 S1 protein, was collected for immunofluorescence analysis.

Pathological examination

Autopsies were performed in the animal biosafety level 3 (ABSL3) laboratory. Major organs were grossly examined and then fixed in 10% buffered formalin solution, and paraffin sections (3-4 μm in thickness) were prepared routinely. Hematoxylin and Eosin (H&E) stain, periodic acid-Schiff (PAS) stain and modified Masson's Trichrome stain were used to identify histopathological changes in all the organs. The histopathology of the lung tissue was observed by light microscopy.

Immunohistochemistry (IHC)

The organs were fixed in 10% buffered formalin solution, and paraffin sections (3-4 μm in thickness) were prepared routinely. Sections were treated with an antigen retrieval kit (Boster, AR0022) for 1 min at 37°C and quenched for endogenous peroxidases in 3% H_2O_2 in methanol for 10 min. After blocking in 1% normal goat serum, the sections were incubated with 7D2 monoclonal antibody (laboratory preparation) at 4°C overnight, followed by HRP-labeled goat anti-mouse IgG secondary antibody (HRP) (Beijing ZSGB Biotechnology, ZDR-5307). Alternatively, the sections were stained with rat IgG2a antibody (Abcam, ab18450, RTK2758), MAC2 antibody (Cedarlane Laboratories, CL8942AP), CD3 antibody (Dako, A0452) or CD19 antibody (Cell Signaling Technology, 3574) at 4°C overnight. Subsequently, the sections were goat anti-rat IgG secondary antibody (HRP) (Beijing ZSGB Biotechnology, PV9004), goat anti-rabbit IgG secondary antibody (HRP) (Beijing ZSGB Biotechnology, PV9001) for 60 min, and visualized by incubation with 3,30-diaminobenzidine tetrahydrochloride (DAB). The slices were counterstained with hematoxylin, dehydrated and mounted on a slide and viewed under an Olympus microscope. The sections from

WT-HB-01, ACE2-Mock or ACE2-HB-01 mice were directly incubated with HRP-labeled goat anti-rat/mouse/rabbit IgG were used as the negative control of MAC2, CD19, CD3 or viral antigen staining. Rat IgG2a antibody was used as isotype control of MAC2 staining. For the expression of viral antigen, the sections from WT-HB-01 or ACE2-Mock mice incubated with anti-S protein antibody was also used as the negative control.

Confocal microscopy

For viruses and hACE2 receptor co-localization analysis, the lung tissue sections were washed twice with PBS, fixed by Immunol Staining Fix Solution (P0098, Beyotime Biotechnology), blocked 1 hour at room temperature by Immunol Staining Blocking Buffer (P0102, Beyotime Biotechnology) and then incubated overnight at 4 °C with the appropriate primary and secondary antibodies. The nuclei were stained with DAPI. Anti-S protein antibody (mouse monoclonal 7D2, laboratory preparation, 1:200) and anti-hACE2 antibody (rabbit polyclonal, ab15348, Abcam, 1:200) were used as the primary antibody. The sections were washed with PBS and incubated with secondary antibodies conjugated with FITC (goat anti-mouse, ZF-0312, Beijing ZSGB Biotechnology, 1:200) and TRITC (goat anti rabbit, ZF-0317, Beijing ZSGB Biotechnology, 1:200), dried at room temperature and observed via fluorescence microscopy. The sections from ACE2-Mock or ACE2-HB-01 mice were directly incubated with FITC-conjugated goat anti-mouse IgG or TRITC-conjugated goat anti-rabbit IgG were used as the negative control. For the expression of hACE2, the sections from WT mice stained with anti-ACE2 antibody were used as the negative control, and the stable cell line expressing hACE2 was used as the positive control. For the viral antigen, the sections from ACE2-Mock mice incubated with anti-S protein antibody were used as the negative control.

Transmission electron microscopy

Supernatant from Vero E6 cell cultures that showed cytopathic effects was collected, inactivated with 2% paraformaldehyde for at least 2 hours, and ultracentrifuged to sediment virus particles. The enriched supernatant was negatively stained on film-coated grids for examination. The negative stained grids were observed under transmission electron microscopy.

Statistical analysis

All data were analyzed with GraphPad Prism 8.0 software. Statistically significant differences were determined using unpaired *t*-tests. Student's *t*-tests, Welch's *t*-tests or Mann-Whitney U tests were applicable according to test requirements. A two-sided p value <0.05 was considered statistically significant. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

All raw data are available from the corresponding author on reasonable request.

Acknowledgements We thank Dr George F Gao for his advice and coordination on this work. We thank Hongkui Deng, Xiuhong Yang and Lianfeng Zhang for providing the hACE2 mice as a gift. We also thank Gary Wong for helping us proofread the language. This work was supported by National Research and Development Project of China (Grant No. 2020YFC0841100), Fundamental Research Funds for CAMS of China (2020HY320001), National Key Research and Development Project of China (Grant No. 2016YFD0500304), CAMS initiative for Innovative Medicine of China (Grant No. 2016-I2M-2-006), and National Mega projects of China for Major Infectious Diseases (2017ZX10304402, 2018ZX10301403).

Author contributions Conceptualization: C.Q. and G.W.; Methodology: L.B., W.D., B.H., H.G. and J.L.; Investigation: L.B., W.D., B.H., H.G., J.L., L.R., Q.W., P.Y., Y.X., F.Q., Y.Q., F.L., Q.L., W.W., J.X., S.G., M.L., G.W., S.W., Z.S., L.Z., P.L., L.Z., F.Y., H.W., W.Z., N.Z., W.Z., H.Y., X.Z., L.G., L.C., C.W., Y.W., X.W., Y.X., Q.S., H.L., F.Z., C.M., L.Y., M.Y., J.H., W.X., W.T., X.P., Q.J.; Writing – Original Draft: L.B., J.L., J.X. and Z.S.; Writing –Review and Editing: C.Q. and G.W.; Funding Acquisition: C.Q. and L.B.; Resources: C.Q.; Supervision: C.Q. and G.W.

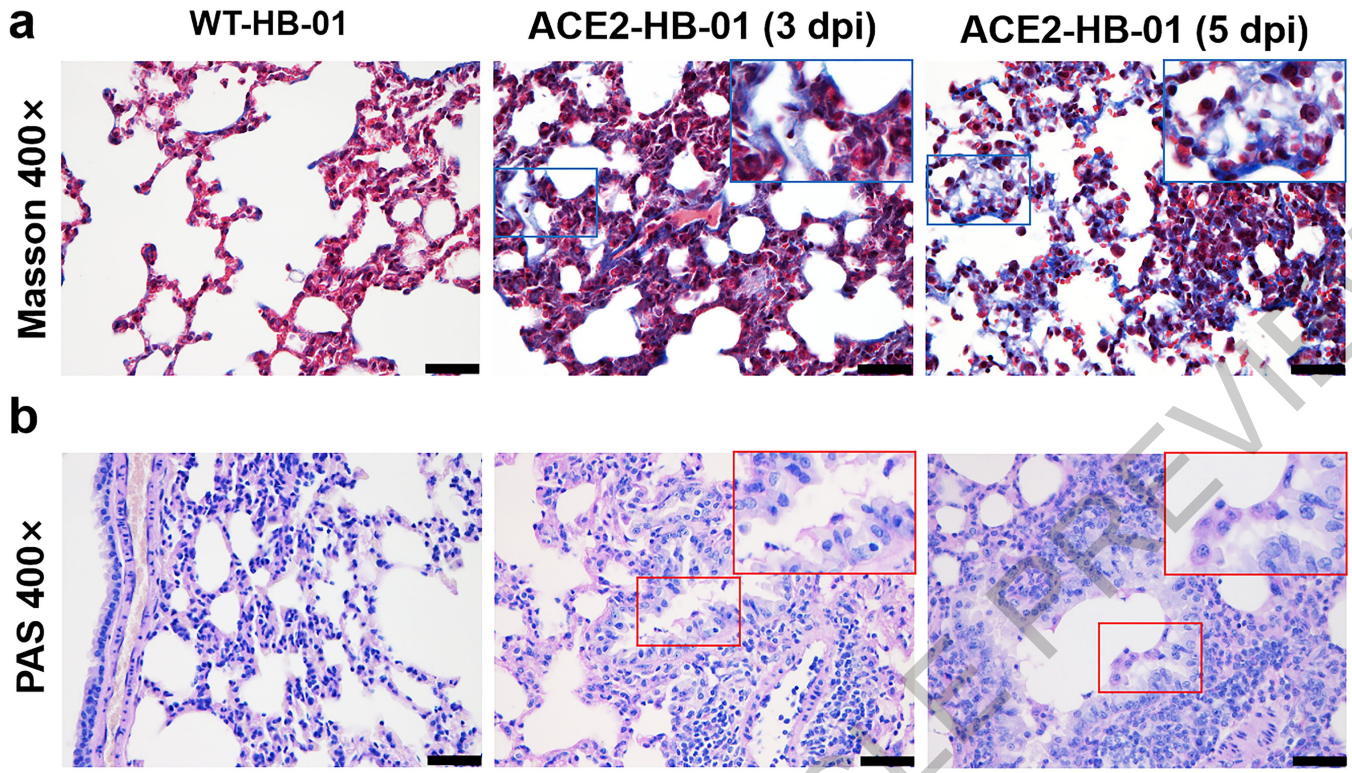
Competing interests The authors declare no competing interests.

Additional information

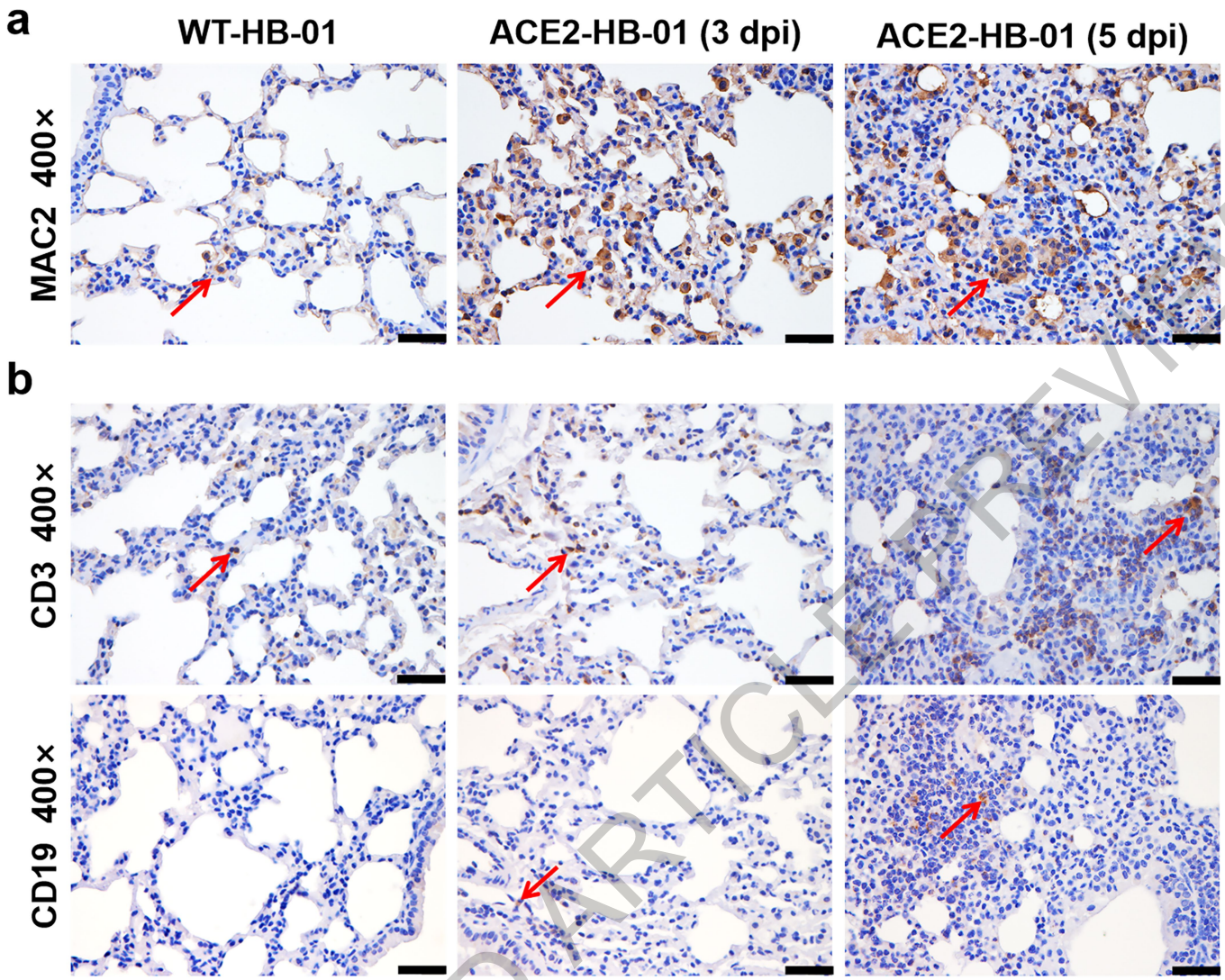
Supplementary information is available for this paper at https://doi.org/10.1038/s41586-020-2312-y.

Correspondence and requests for materials should be addressed to G.W. and C.Q.

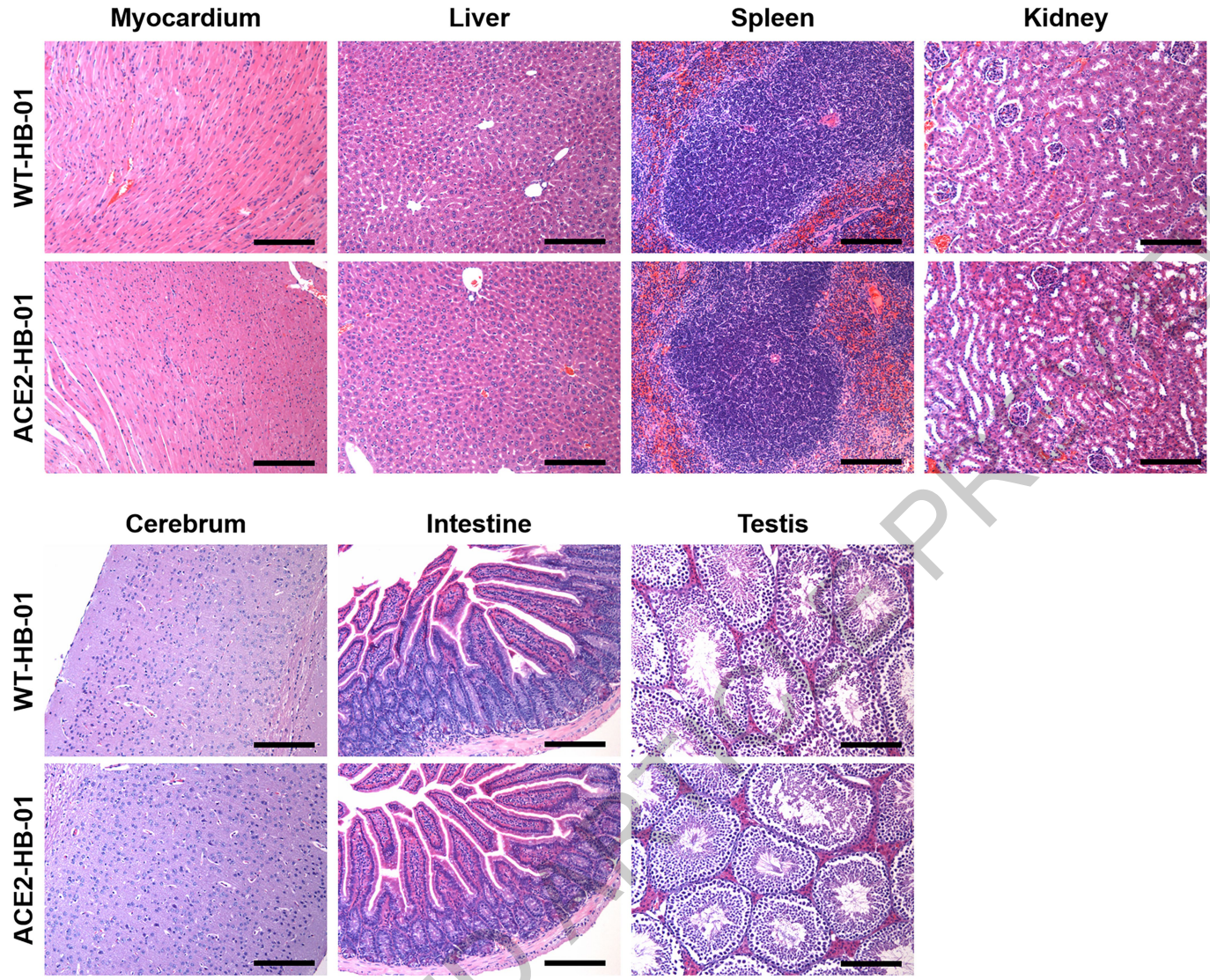
Peer reviewer reports are available.



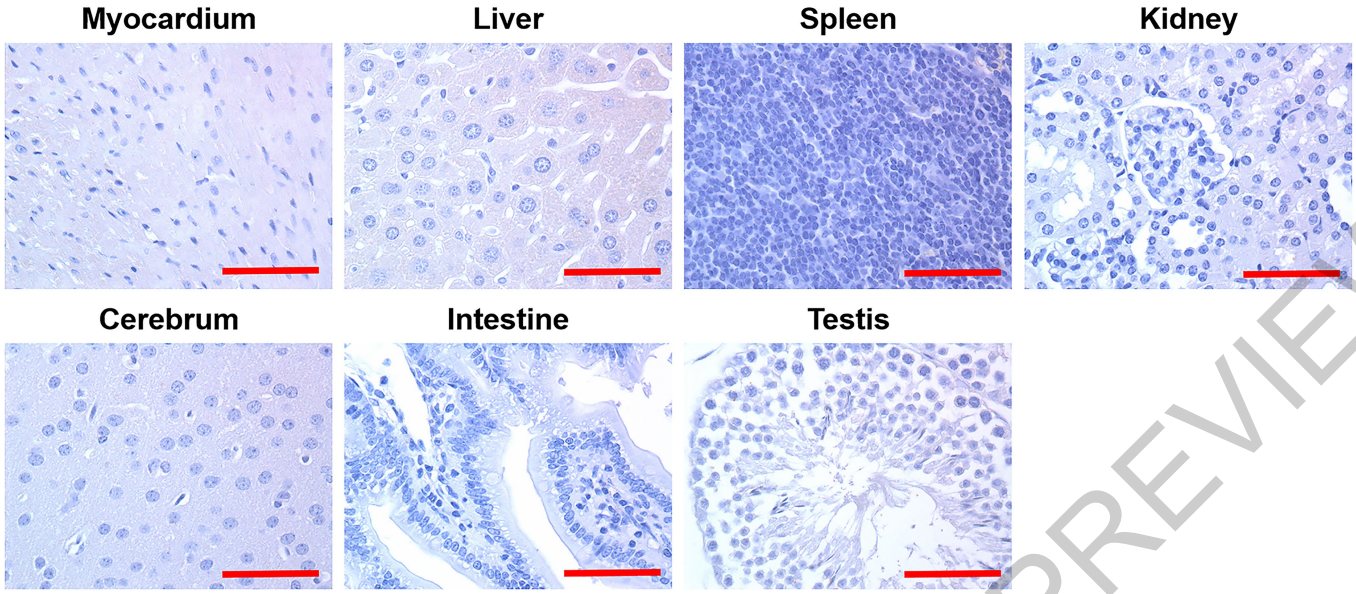
Extended Data Fig. 1 | Special stains of the lungs in the WT-HB-01 and ACE2-HB-01 mice at 3dpi and 5 dpi. a, modified Masson's Trichrome stain of the lung. Compared to the WT-HB-01 group, the increased collagen fiber (blue-stained fibers) (blue frames on the upper-right corner is the magnification) in the thickened alveolar interstitium were observed in both the ACE2-HB-01 mice at 3dpi and 5 dpi. **b**, periodic acid-Schiff (PAS) stain of the respiratory epithelium in bronchioles. A small amount of mucus was accumulated on the surface of bronchial epithelial cells (red frames on the upper-right corner is the magnification). Black scale bar=40 μm. Data (**a**, **b**) are representatives of three independent experiments.



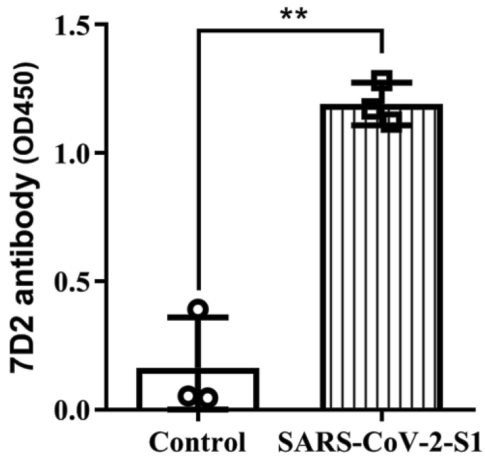
Extended Data Fig. 2 | IHC was carried out to identify macrophages with MAC2, T lymphocytes with CD3, and B lymphocytes with CD19. a, Diffuse infiltration of macrophages (red arrow) in the expanded alveolar septa in the ACE2-HB-01 mice at 3 dpi and 5 dpi. **b**, Many T lymphocytes (red arrow) infiltrated the thickened alveolar septa in the first row of b at 5 dpi in the ACE2-HB-01 mice. A few B lymphocytes (red arrow) were observed in the ACE2-HB-01 mice. Black scale bar=40 μm. Data (**a**, **b**) are representatives of three independent experiments.



Extended Data Fig. 3 | The histopathological observation of the organs in the WT-HB-01 and ACE2-HB-01 mice. There were no significant histopathological changes in the organs, including myocardium, liver, spleen, kidney, cerebrum, intestine and testis in the ACE2-HB-01 mice compared with the ACE2-Mock mice. Black scale bar = 100 μm. Data are representatives of three independent experiments.



Extended Data Fig. 4 | The immunohistochemical (IHC) observation of the organs in the ACE2-HB-01 mice. There were no SARS-CoV-2 antigens in the organs, including myocardium, liver, spleen, kidney, cerebrum, intestine and testis. Red scale bar = 50 μm. Data are representatives of three independent experiments.



Extended Data Fig. 5 | Identification of 7D2 antibody against SARS-CoV-2 S1 protein. The plate coated by 0.2 ug SARS-CoV-2 S1 protein was incubated with 7D2 antibody as primary antibody (1:200) and detected using HRP-conjugated goat anti-mouse secondary antibody. The titer of antibody was determined using enzyme-linked immunosorbent assay (ELISA) assay. Data represent mean±SD. Significant differences are indicated with asterisks (n=3, two-tailed unpaired Student's *t*-test, ***p=0.0011*).

nature research

Corresponding author(s): Chuan Qin

Last updated by author(s): Mar 20, 2020

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	All data were collected with GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA)
Data analysis	All data analysis were performed with GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The complete genome for this SARS-CoV-2 was submitted to GISAID (BetaCoV/Wuhan/IVDC-HB-01/2020|EPI_ISL_402119), and deposited in the Novel Coronavirus National Science and Technology Resource Service System (http://nmdc.cn/nCoV, accession number NMDC10013001 and genome accession numbers MDC60013002-01). All data are available without any restrictions.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size. For the animal study, there are three groups investigated, including hACE2 transgenic mice group (ACE2-HB-01, n=19) and wild type ICR mice group (WT-HB-01, n=15) with SARS-CoV-2 infection, and hACE2 transgenic mice group without infection (ACE2-Mock, n=15). The numbers of mice in each group meet the requirement for statistical analysis (at least 3 for each group per time-point), which is sufficient given the excellent technical reproducibility.
Data exclusions	No data were excluded from the analyses.
Replication	Nineteen or fifteen mice were included for each group, and the results were compared with the same trend. As for the viral loads, viral titers, IHC, IF, HE stain, Masson stain and PAS stain experiments, 3 mice for each group (at each detecting time-point) were tested, and some of them were repeated twice separately by two technicians. All attempts at replication were successful.
Randomization	For the animal study, we have designed a time-dependent pathological experiments in 12 WT-HB-01 mice, 12 ACE2-mock and 12 ACE2-HB-01 for euthanasia. These 12 mice per group were randomly divided in to the four groups to undergo four time-point measurements (1 dpi, 3 dpi, 5 dpi and 7 dpi). The tests were also randomly selected from all samples. The pictures were representatively shown.
Blinding	During the study, all mice and all samples were coded. The technicians did not know which animal or sample is hACE2 transgenic or wild type, with or without virus infection.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access and import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

7D2 antibody for anti-SARS-CoV-2 S protein (mouse antibody, laboratory preparation, Supplementary Figure 5,1:200), rat lgG2a antibody (rat antibody, ab18450, Abcam, RTK2758, Low endotoxin, Azide Free,1:1000) , anti-ACE2 antibody (rabbit antibody, Abcam, ab15348, 1:500) , anti-MAC2 antibody (rat antibody, Cedarlane Laboratories, CL8942AP, 1:1000) , anti-CD3 antibody (rabbit antibody, Dako, A0452,1:100), anti-CD19 antibody (rabbit antibody, Cell Signaling Technology, 3574,1:50) , goat anti rabbit lgG conjugated TRITC (goat anti rabbit, ZF-0317, Beijing ZSGB Biotechnology,1:200), goat anti-mouse lgG conjugated FITC, (goat anti-mouse, ZF-0312, Beijing ZSGB Biotechnology,1:200), goat-anti rat lgG conjugated HRP (goat anti-mouse, ZF-0312, Beijing ZSGB Biotechnology,1:200), goat-anti mouse lgG conjugated HRP (ZDR-5307, Beijing ZSGB Biotechnology,1:200), goat-anti rabbitt lgG conjugated HRP (PV9001, Beijing ZSGB Biotechnology,1:200).

Validation

7D2 antibody for anti-SARS-CoV-2 S protein (mouse antibody, laboratory preparation, Supplementary Figure 5,1:200)

Anti-ACE2 antibody (rabbit antibody, Abcam, ab15348, 1:500)
https://www.abcam.cn/ace2-antibody-ab15348.html

Anti-MAC2 antibody (rat antibody, Cedarlane Laboratories, CL8942AP, 1:1000)
https://www.cedarlanelabs.com/products/detail/cl8942ap?lob=AllProducts

Rat lgG2a antibody (rat antibody, ab18450, Abcam, RTK2758, Low endotoxin, Azide Free,1:1000)
https://www.abcam.cn/rat-igg2a-kappa-monoclonal-rtk2758-isotype-control-low-endotoxin-azide-free-ab18450.html

Anti-CD3 antibody (rabbit antibody, Dako, A0452,1:100)
https://www.agilent.com/cs/library/packageinsert/public/P05193CN_02%20A0452.pdf

Anti-CD19 antibody (rabbit antibody, Cell Signaling Technology, 3574,1:50)
https://www.cst-c.com.cn/products/primary-antibodies/cd19-antibody/3574

Secondary antibodies conjugated TRITC (goat anti rabbit, ZF-0317, Beijing ZSGB Biotechnology,1:200)
http://www.zsbio.com/product/ZF-0317

Secondary antibodies conjugated FITC (goat anti-mouse, ZF-0312, Beijing ZSGB Biotechnology,1:200)
http://www.zsbio.com/product/ZF-0312

Goat anti-rat lgG secondary antibody conjugated HRP (PV9004, Beijing ZSGB Biotechnology,1:200)
http://www.zsbio.com/product/PV-9004

Goat anti-mouse lgG secondary antibody conjugated HRP (ZDR-5307, Beijing ZSGB Biotechnology,1:200)
http://www.zsbio.com/product/ZDR-5307

Goat anti-rabbit lgG secondary antibody conjugated HRP (PV9001, Beijing ZSGB Biotechnology,1:200)
http://www.zsbio.com/product/PV-9001

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero E6 cells were obtained from ATCC (CRL-1586).
Authentication	To follow the protocol provided by ATCC. None of the cell lines have been authenticated.
Mycoplasma contamination	The cell line tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	There is no commonly misidentified cell lines used in this study.

Palaeontology

Specimen provenance	*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).*
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Male and female transgenic hACE2 mice (specific pathogen-free, 6-11-month-old) were obtained from the Institute of Laboratory Animal Science, Peking Union Medical College, China. Transgenic mice were generated by microinjection of the cytomegalovirus promoter driving the human ACE2 coding sequence into Institute of Cancer Research (ICR) mice, the presence of human ACE2 was confirmed in the mice used by PCR.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All procedures in this study involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Institute of Laboratory Animal Science, Peking Union Medical College (BLL20001).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone*

Antibodies	*name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference
(See Eklund et al. 2016)

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.