

Supplementary Methods

Mice. *Ace2* mutant mice were generated as described¹⁶ and backcrossed to C57Bl/6 more than 5 times. Only sex, age, and background matched mice were used as controls. Mice were genotyped by PCR and Southern blotting¹⁶ and maintained at the animal facilities of the Institute of Molecular Pathology, Vienna, and for SARS infections at the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, P.R. China, in accordance with each institutional guidelines.

SARS-coronavirus. The SARS-coronavirus (Beijing strain, PUMC01 isolate, Genbank access number AY350750) used in this study was kindly provided by Z. Wang and Y. Liu of PUMC hospital²⁹. This isolate was certified by National Institute for the Control of Pharmaceutical and Biological Products (No. SH200301298). The virus was isolated and passaged eight times to generate a virus stock with a titer of $10^{6.23}$ 50% tissue culture infective doses (TCID₅₀)/ml. All work with infectious virus was performed inside a biosafety cabinet in a biosafety containment level 3 facility. All work with SARS-Coronavirus PUMC01 isolate was approved by the Ministry of Health and performed according to "Laboratory Biosafety Management of Pathogen" guidelines of the state council of the People's Republic of China.

***In vivo* SARS infections.**

All mouse studies were approved by the Ministry of Health Science and Technology division and were carried out in an approved animal biosafety level 3 facility. Five wild type mice and five *Ace2* knockout mice were infected at each experiment with a total of 15 infected mice for each genotype. Eight female and seven male mice of each wild type and ACE2 deficient were used. Mice three to five weeks of age were anesthetized with isoflurane and intranasally inoculated with 100 μ l virus ($10^{5.23}$ TCID₅₀). At day 2 mice were euthanized with carbon dioxide, and the lungs were removed and frozed at -70°C. Frozen tissues were thawed and homogenized in a 10% suspension in DMEM medium (Invitrogen). Virus titers were determined using Vero cell monolayers in 24- and 96-well plates. Virus titers are expressed as TCID₅₀ per gram of lung tissue. Total RNA and protein was isolated from homogenized lung tissues using Trizol reagent (Invitrogen) and stored at -70°C. For detection of Spike RNA, samples were analyzed by real-time PCR (BioAsia Inc., Shanghai, China). ACE2 and ACE proteins were detected by Western blotting¹⁸. The mouse β -actin housekeeping gene was used for sample normalization. For histological analysis, mice were sacrificed on day 6 after the SARS infection and lungs isolated. 5- μ m thick lung sections were cut and stained with hematoxylin and eosin (H&E).

SARS-Spike protein binding experiments.

The coding sequence of SARS spike protein (amino acids 1-1190 from Urbani strain) or a Spike sequence that only contains the previously mapped²¹ ACE2 binding domain (aa318-510) were codon optimized, synthesized, and subcloned into the PEAK vector (kind gift from Dr. Brian Seed) to generate a fusion protein with the Fc portion of human IgG1. CHO cells were transfected with the Spike-Fc expression vector, supernatants harvested, and Spike-Fc protein purified by affinity chromatography using a Protein A Sepharose column. For *in vitro* binding assays, A549 human alveolar epithelial cells or IMCD murine kidney epithelial cells were homogenized in lysis buffer (50 mM Tris-HCl, pH 7.4, 20 mM EDTA, and 1% triton-X100) supplemented with "Complete"

protease inhibitor cocktail (Roche) and 1 mM Na₃VO₄. Cell lysates were incubated with Spike-Fc or control human IgG-Fc protein with gentle agitation for 2 hours at 4°C. Spike-Fc or control human IgG-Fc protein were pulled down by Protein G Sepharose, proteins separated by SDS-polyacrylamide gel electrophoresis (PAGE), and transferred to a Nitrocellulose membrane. Pulled-down human and mouse ACE2 were detected by human ACE2-specific polyclonal antibodies (R&D systems) and a mouse ACE2-specific polyclonal antibody generated in our laboratory¹⁶, respectively. For flow cytometry, Vero E6 cells are detached by 2mM EDTA/PBS and incubated with Spike-Fc or control human IgG-Fc protein at 4°C or 37°C for 3hrs. Cells were then incubated with ACE2-specific antibodies, followed by FITC-conjugated mouse IgG-specific antibodies (Jackson ImmunoResearch Laboratories, Inc). In addition, a FITC-conjugated human IgG-specific polyclonal antibody was used for detection of Spike-Fc protein and control IgG-Fc bound to Vero E6 cells. In both experimental systems, similar results were obtained. Full length mouse and human ACE2 coding regions were cloned into the PEAK vector and transfected into 293 cells. Spike-Fc binding was detected as above. All samples were analyzed by flow cytometry using a FACScan (Becton Dickinson).

Recombinant Spike-Fc *in vivo* challenge in mice

We used the mouse model of acid aspiration-induced acute lung injury¹⁸ for all Spike-Fc *in vivo* experiments. Mice (2.5-3 months old) received Spike (S1190)-Fc, Spike-(S318-510)-Fc, or control-Fc (5.5 nmol/kg each) i.p. three time at 30 min before and at 1 and 2 hrs after acid treatment. After HCl instillation, animals were randomized into the indicated experimental cohorts. All animals were then ventilated for 3 hrs and lung elastance analyzed as described in Imai et al. Briefly, total PEEP (PEEP_T) and plateau pressure (P_{plat}) were measured at the end of expiratory and inspiratory occlusion, respectively, and elastance was calculated as (P_{plat} minus PEEP_T)/V_T every 30 minutes during the ventilation periods. At the end of the ventilation, left lungs were sampled for the measurement of lung wet/dry mass ratios or snap frozen in liquid nitrogen for subsequent biochemical analysis. Right lungs were fixed in 10 % buffered formalin for histological examination. To detect the i.p. injected Spike-Fc protein or ACE2 protein, lung homogenates were prepared from Spike-Fc and control-Fc treated mice with acid lung injury after 3 hrs of the ventilation. Spike-Fc and control-Fc were pulled down by Protein G Sepharose and detected by Western Blot using a human IgG-specific polyclonal antibody (Sigma). For AT1R inhibition of Spike-Fc-mediated acute lung injury, we treated the Spike(S1190)-Fc challenged mice with the AT1R inhibitor Losartan (15 mg/kg). For histological analysis, 5-µm thick sections were cut and stained with hematoxylin and eosin (H&E). For detection of AngII peptide levels, lungs were homogenized on ice in 80% ethanol / 0.1% HCl containing peptidase inhibitors as described¹⁸.

Immunohistochemistry

Lung tissues were fixed in 3.7% formalin and embedded in paraffin. 5 µm sections were pretreated with EDTA buffer at 72°C and stained with goat human Fc-specific polyclonal antibody (Jackson Immunological Research, Inc.). Vectastatin ABC kit was used for the detection of specific staining (Vectar Laboratories).

Lung injury scores

For semiquantitative assessment of SARS-CoV-associated inflammation in the lung, H&E-stained section was examined by light microscope as described previously³⁰. Three randomly chosen fields of each section (30 fields, each group) were scored for leukocyte infiltrates in a blinded fashion as follows: 0 = minimal (little) damage, 1 = mild damage, 2 = moderate damage, 3 = severe damage, and 4 = maximal damage. For semiquantitative assessment of the lung injury in Spike-Fc or control-Fc treated mice after acid-aspiration, 4 randomly chosen fields of each section (16 fields, each group) were scored in a blinded fashion using a previously defined scale consisting of alveolar congestion, haemorrhage, neutrophil infiltration, thickness of the alveolar wall, and hyaline membrane formation, as follows: 0 = minimal (little) damage, 1 = mild damage, 2 = moderate damage, 3 = severe damage and 4 = maximal damage³⁰.