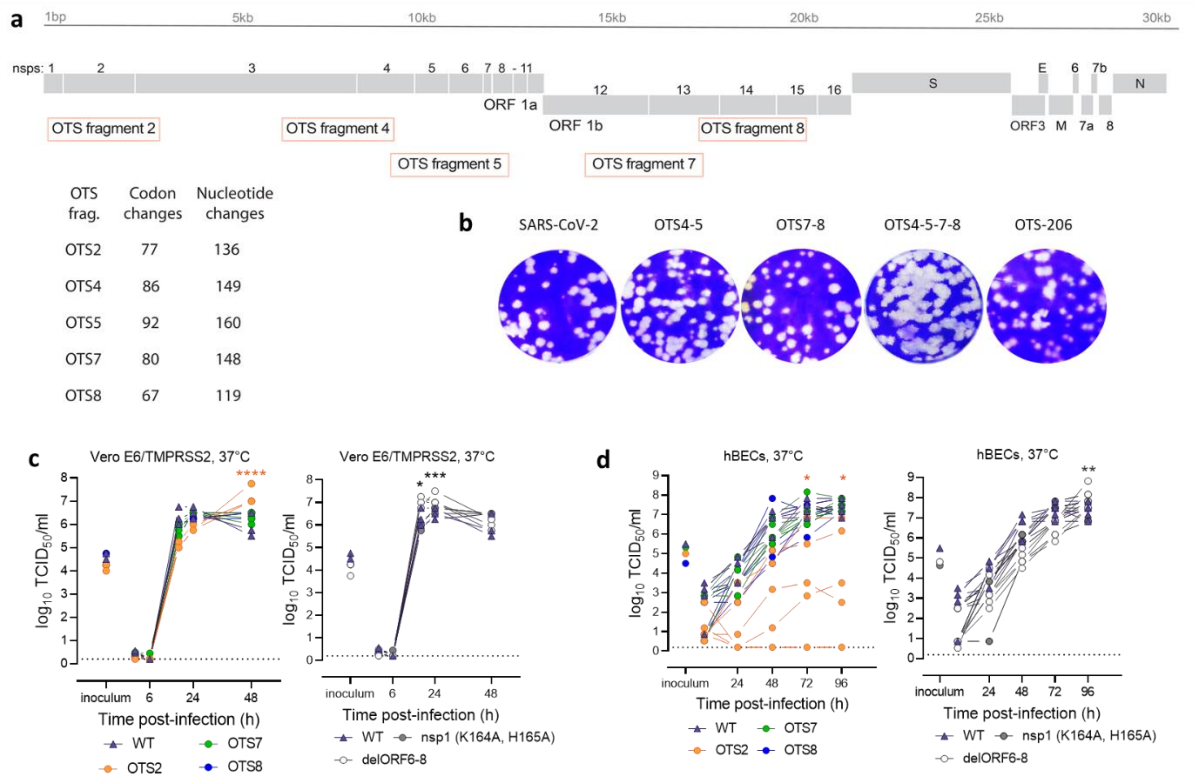
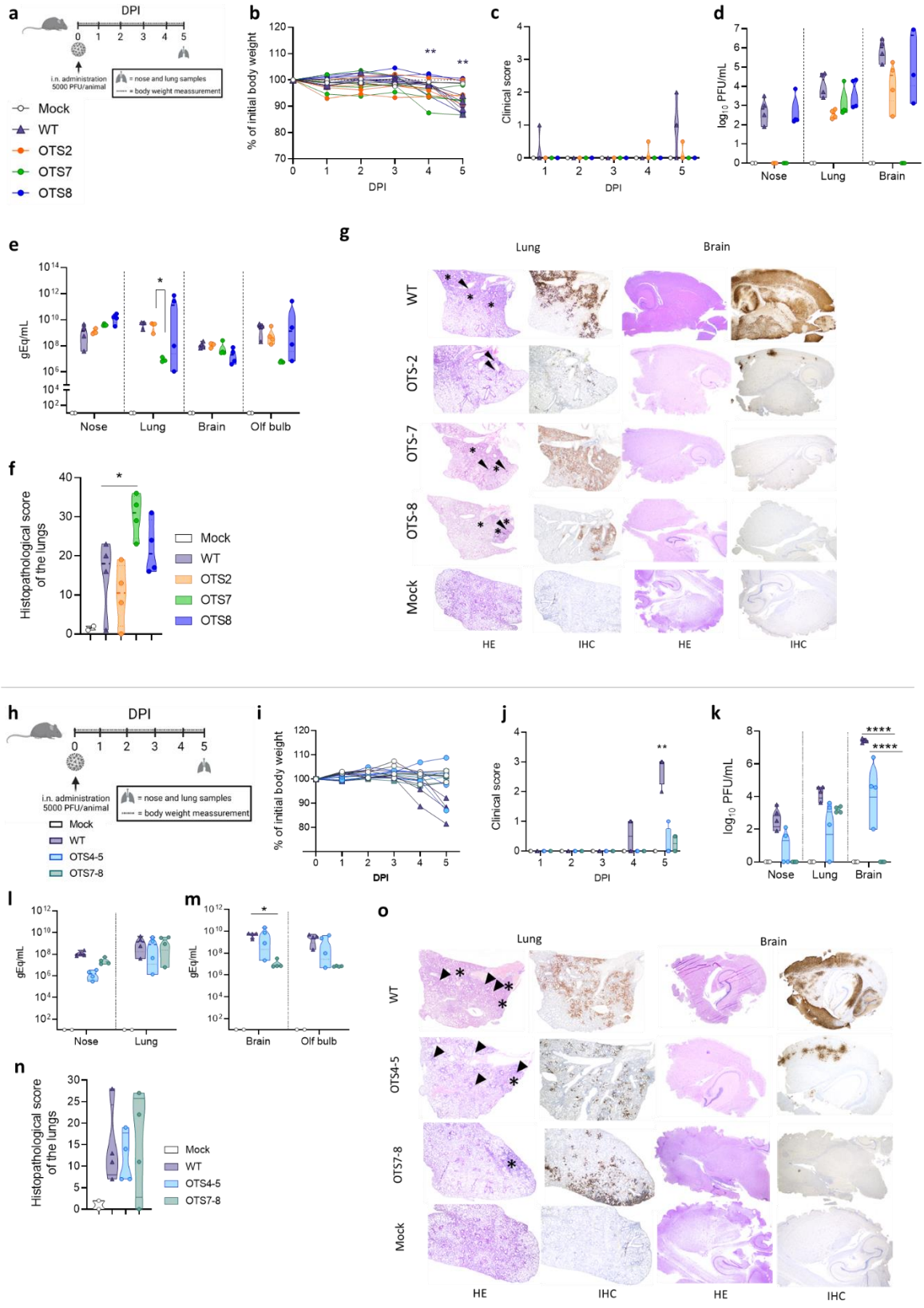
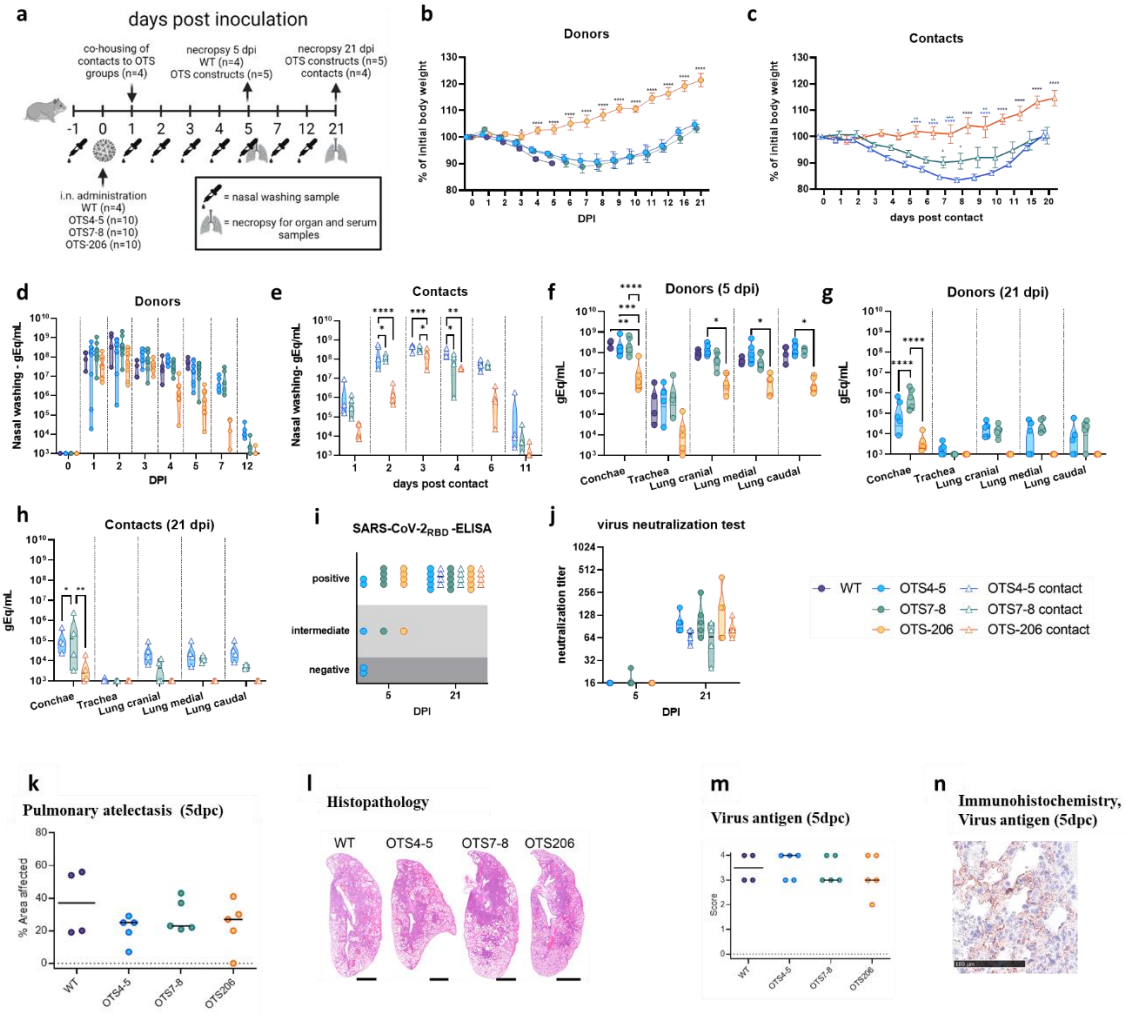


Extended Data Fig. 1: OTS constructs show comparable replication kinetics to WT in vitro, but higher sensitivity to treatment with antivirals. a, Schematic overview of the mutations introduced to SARS-CoV-2 genome to generate OTS codons. Fragments 2, 4, 5, 7, and 8, which are used for TAR cloning of recombinant SARS-CoV-2 clones have been modified to enrich the number of one-to-stop codons. The number of codons and nucleotides that have been changed are indicated for each fragment. For the OTS-206 construct, two additional point mutations were introduced in Nsp1 (K164A/H165A) and open reading frames ORF6 to ORF8 were deleted. The number of codons and nucleotides that have been changed in each fragment are listed in **Supplementary Table 1**. **b**, Representative pictures of the plaque sizes of viruses in 6-well plates 2 dpi. **c**, Vero E6/TMPRSS2 cells (n=3) and **d**, human bronchial epithelial cell (hBECs) (n=6 (3 replicates from 2 donors)) cultures were infected with 0.1 MOI or $5x10^4$ PFU, respectively, of the SARS-CoV-2 WT and OTS viruses from the apical side and incubated at 37 °C for 1 h. After 1 h, supernatant was discarded and the cells were washed 3 times with PBS or HBSS, respectively, and the third wash was kept for analysis. Following the addition of new sera on the cells, they were incubated 37 °C. Samples were collected on 6, 18, 24 and 48 h post-infection. Infectious particle titers were assessed by TCID50 assays on VeroE6/TMPRSS2 cells. Each line in the graphs shows the titers obtained from one individual sample. Statistical significance was determined using two-way ANOVA and p-values were adjusted using Tukey's multiple comparison test; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

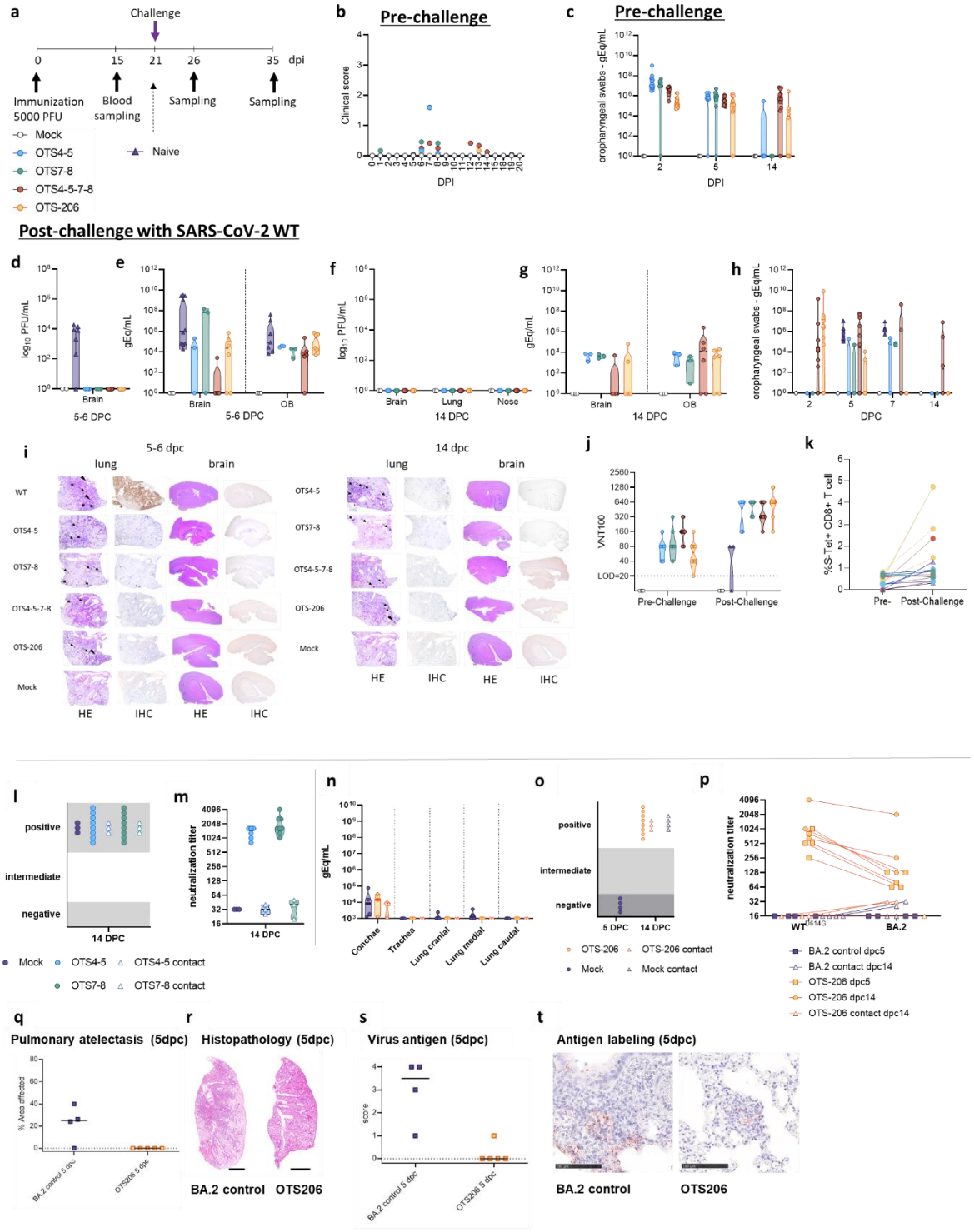


Extended Data Fig. 2: Attenuation of OTS2, OTS7, OTS8, OTS4-5 and OTS7-8 in K18-hACE2 mice. a, Experimental setup of comparison of OTS2, OTS7, OTS8 to WT infection in short-term. K18-hACE2 mice (7-16 week-old, n=4 mice/group) were infected with 5'000 PFU of either OTS2, OTS7, OTS8 and SARS-CoV-2 WT virus, or only with medium for 5 days. **b, c**, Mice were monitored for body weight change and clinical symptoms over the 5-day course of infection. On 5 dpi, mice were euthanized and samples from the nose, lungs, brain and olfactory bulbs are collected for evaluation of infectious virus titers, viral genome copy numbers, and pathology.

d, Infectious virus titers from the nose, lung and brain samples were determined using plaque assays in VeroE6 cells. **e**, genome copy numbers (genome equivalence per ml, gEq/mL) in the nose, lung, brain and olfactory bulb samples of mice infected with different viruses were quantified using probe-specific RT–qPCR. **f**, Histopathological lung score was given for characterization and comparison of the severity of lung lesions. **g**, Hematoxylin and eosin stain (left panel) and immunohistochemical analysis specific for SARS-CoV-2 nucleocapsid protein (right panel) of lung and brain sections (n=4 per group). **h**, Experimental setup of comparison of OTS4-5, OTS7-8 to WT infection in short-term. K18-hACE2 mice (7–16 week-old, n=4 mice/group) were infected with 5'000 PFU of either OTS4-5, OTS7-8 and SARS-CoV-2 WT virus, or only with medium for 5 days. **i, j**, Mice were monitored for body weight change and clinical symptoms over the 5-day course of infection. On 5 dpi, mice were euthanized and samples from the nose, lungs, brain and olfactory bulbs are collected for evaluation of infectious virus titers, viral genome copy numbers, and pathology. **k**, Infectious virus titers from the nose, lung and brain samples were determined using plaque assays in VeroE6 cells. **l, m**, genome copy numbers (genome equivalence per ml, gEq/mL) in the nose, lung, brain and olfactory bulb samples of mice infected with different viruses were quantified using probe-specific RT–qPCR. **n**, Histopathological lung score was given for characterization and comparison of the severity of lung lesions. **o**, Hematoxylin and eosin stain (left panel) and immunohistochemical analysis specific for SARS-CoV-2 nucleocapsid protein (right panel) of lung and brain sections (n = 4 per group) (magnification 50x). Consolidated lung areas are highlighted with an asterisk, and perivascular and peribronchiolar lymphohistiocytic inflammation highlighted with an arrowhead. Viruses were visualized in the lungs of the infected animals by immunhistochemistry by anti-N SARS-CoV Antibody (Rockland). Statistical significance was determined using one-way or two-way ANOVA (a–d) and P values were adjusted using Tukey's multiple-comparison test; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data were obtained from one experiment. Each data point represents one biological replicate. Body weight changes, clinical scores and histopathological score of the lungs of all K18-hACE2 mice experiments are shown in **Supplementary Table 6**.

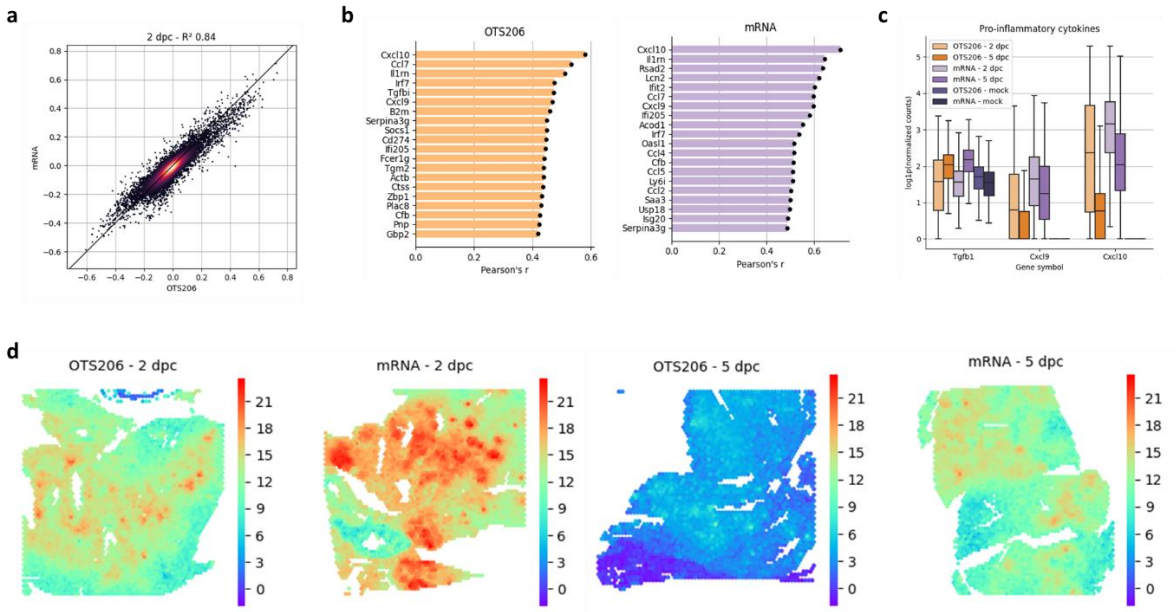


Extended Data Fig. 3: Safety study of OTS4-5, OTS7-8 and OTS-206 in Syrian hamster model. a, Experimental setup of intranasal inoculation of Syrian hamsters (Donors) with OTS4-5, OTS7-8, or OTS-206 SARS-CoV-2, including co-housing of naïve contact animals (Contacts) 1 day post inoculation (dpi). **b, c,** Body weight changes of inoculated and contact hamsters in percent. **d, e,** Virus genome copy numbers in nasal washings of donor and contact hamsters. **f, g,** Virus genome copy numbers in the organ samples of donors at 5 and 21 dpi. **h,** Virus genome copy numbers in the organ samples of contact hamsters at 21 dpi. **i,** Serum samples of 5 and 21 dpi analyzed by SARS-CoV-2RBD-ELISA. **j,** Serum samples that reacted positive in the ELISA, were analyzed in addition by live virus neutralization assay (capacity to neutralize 100 TCID50) against ancestral WT SARS-CoV-2. Statistical significance was determined using two-way ANOVA and p-values were adjusted using uncorrected Fisher´s LSD, with individual variances computed for each comparison. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

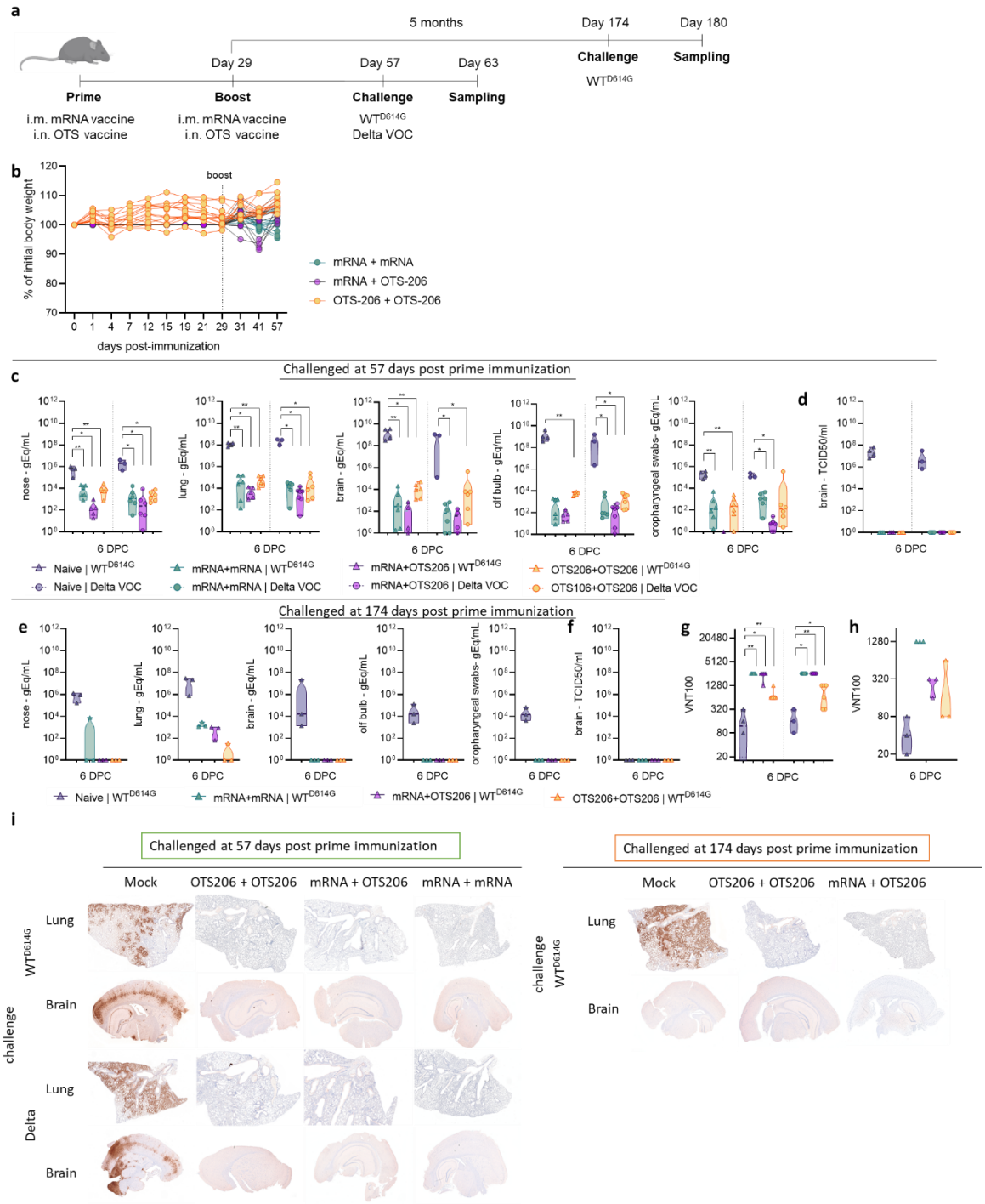


Extended Data Fig. 4: Immunization with OTS4-5, OTS7-8, OTS4-5-7-8, and OTS-206 protects K18-hACE2 mice and Syrian hamsters from infection with SARS-CoV-2 Wuhan WT. a, K18-hACE2 transgenic mice (7-16-weeks-old, n=8 mice/group) were immunized with 5'000 PFU of either OTS viruses and SARS-CoV-2 WT virus, or only with medium (mock). **b**, Mice were monitored for clinical symptoms over the course of infection and **c**, oropharyngeal swabs were taken on the indicated days. On day 15 post-immunization blood samples were taken to have pre-challenge serum samples. 21-dpi, mice were challenged with 5'000 PFU of SARS-CoV-2 WT, and mice were euthanized on 5-days and 14-days post-challenge (dpc) (26 and 35 dpi, respectively). On 5 and 14 dpc, mice were euthanized and samples from the nose, lungs, brain, and olfactory bulbs are collected for evaluation of infectious virus titers, viral genome copy numbers, and pathology. **d, f,** Infectious virus titers from the brain, lung and nose samples were determined using plaque assays in VeroE6 cells. **e, g, h,** genome copy numbers (genome equivalence per ml, gEq/mL) in the post-challenge samples of mice infected with different viruses were

quantified using probe-specific RT–qPCR. **i**, Hematoxylin and eosin stain of lung sections (n=4 per group and time point). Consolidated lung areas corresponding to interstitial pneumonia are highlighted with an asterisk, perivascular and peribronchiolar cuffings are highlighted with an arrowhead and tertiary lymphoid follicle formations with an arrow. No viral antigen was detected by immunohistochemistry by anti-N SARS-CoV Antibody (Rockland). **j**, Sera collected on 15 dpi (pre-challenge) and 5 and 14 dpc (post-challenge) were tested against SARS-CoV-2 Wuhan WT virus in a serum neutralization test. **k**, Whole blood cells collected on 15 dpi (pre-challenge) and 5 and 14 dpc (post-challenge) were labeled with Alexa fluor 647 labeled tetramer against SARS-CoV-2 Spike 539-546 (VNFNFNGL). Gating strategy and the antibodies used for the flow cytometry analysis is given in **Supplementary Figure 1** and the staining protocol is given in the **Supplementary Document 1**. Statistical significance was determined using one-way or two-way ANOVA and P values were adjusted using Tukey's multiple-comparison test; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data were obtained from one experiment. Each data point represents one biological replicate. Body weight changes, clinical and histopathological scores of the lungs of all K18-hACE2 mice experiments are shown in **Supplementary Table 6**. **l**, Sera samples of OTS4-5 or OTS7-8 vaccinated and subsequently SARS-CoV-2 WT challenged Syrian hamsters (**Fig. 2l**), as well as sera of co-housed contact animals, were analyzed by SARS-CoV-2-RBD specific ELISA, confirming all samples, including the naïve contact animals positive, 14 days post-challenge. **m**, For those samples it was confirmed that they have virus neutralizing capacity too, while mock, OTS4-5 contact, and OTS7-8 contact animals had only low titers of around 32, OTS4-5 and OTS7-8 vaccinated and subsequently challenged animals had titers in average of 1406 (OTS4-5) and 2055 (OTS7-8). **n,** Organ samples of OTS-206 vaccinated and subsequently SARS-CoV-2 Omicron BA.2 challenged Syrian hamsters (Fig. 2r) were analyzed by RT-qPCR. Only residual amount of challenge virus genome was detectable in individual lung samples of mock group animals and in the conchae samples at 14 dpc. **o**, Serological evaluation by SARS-CoV-2-RBD specific ELISA confirmed transmission of BA.2 challenge virus to the naïve contact animals for the mock vaccinated as well as for the OTS-206 vaccinated animals. **p**, Comparing the live virus neutralizing capacity, revealed substantial neutralizing titers of the OTS-206 vaccinated animals against ancestral SARS-CoV-2 and Omicron BA.2 VOC, while the post BA-2 challenge seroconverted mock and contact animals only exhibit minimal neutralizing capacity against the BA.2 variant. **q,** Pneumonia-induced pulmonary atelectasis 5 dpi given in % affected area. **r,** Histopathology, lung whole slide images showing atelectasis, hematoxylin-eosin stain, bar 2.5 mm. **s,** Virus antigen score, 0 = no antigen, 1 = focal, 2 = multifocal, 3 = coalescing, 4 = diffuse. **t,** Virus antigen, immunohistochemistry for SARS-CoV nucleocapsid protein detection, mainly in type-1 pneumocytes, bar 100 µm.

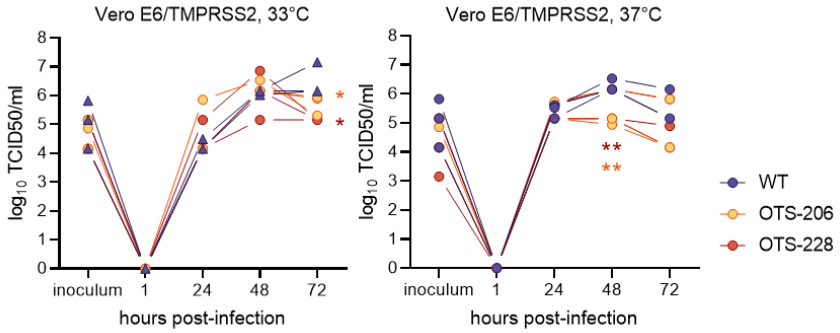


Extended Data Figure 5: Spatial transcriptomics shows that OTS-206 vaccination induces similar activation of genes related to the immune response to viral infection and reduced inflammatory response. a, Pearson's correlation coefficients were calculated between total SARS-CoV-2 gene counts and all host genes to determine spatial correlations. These values are plotted against each other on the x and y axis for the OTS and mRNA 2 dpc samples to show that the spatial gene expression signatures are very similar, as their correlation coefficients are nearly identical. **b,** Top 20 spatially most correlated genes in the lungs of infected mice vaccinated with OTS-206 or mRNA vaccine. **c,** Changes in proinflammatory cytokine expression between conditions. **d**, Spatial JAK-STAT pathway activity in the lung. We can see the co-occurrence between SARS-CoV-2 transcripts from **d**, and the increased JAK-STAT activity. Spatial transcriptomics samples (n=11): OTS 2dpc - 2, OTS 5dpc - 2, mRNA 2dpc - 2, mRNA 5dpc - 3, mRNA mock - 1, OTS mock - 1.

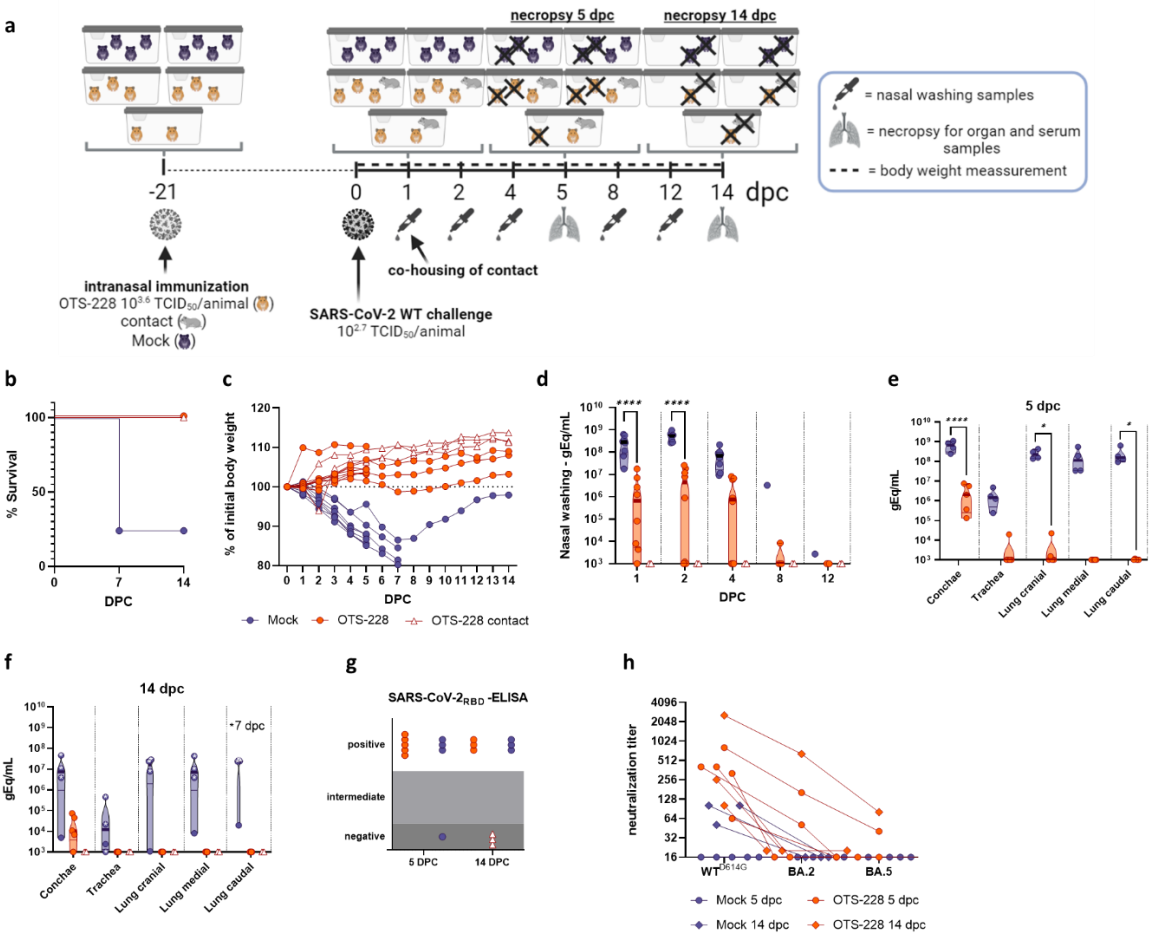


Extended Data Figure 6: OTS-206 show comparable efficacy to mRNA-vaccines and inducing long-term immunity in K18-hACE2 mice. a, K18-hACE2 transgenic mice (7-15 weeks old, n=8 mice/group) were immunized (prime & boost) either intramuscularly with a single dose of 1 µg of mRNA-Vaccine Spikevax (Moderna), or intranasally with 5'000 PFU of OTS-206. At 57 dpi a group of mice was intranasally inoculated with 10^4 $TCID_{50}$ of SARS-CoV-2D614G, or SARS-CoV-2 Delta VOC (**c-d**). The rest of the immunized mice were kept for approximately 5 months and then intranasally inoculated with 104 TCID50 of SARS-CoV-2D614G (**e-h**). **b**, During immunization, mice were regularly monitored for body weight changes. Each line in the body weight loss graphs represents a mouse. Six days post-challenge, mice were euthanized and organ samples were collected for evaluation of infectious virus titers, viral genome copy numbers, and pathology. **c, e**, Genome copy numbers (genome equivalence per ml, gEq/mL) in nose, lung, brain, olfactory bulb and oropharyngeal swab samples of mice infected with different viruses were quantified using probe-specific RT-qPCR. **d, f**, Infectious virus titers from the brain samples were determined using plaque assays in VeroE6 cells. **g, h,** Sera collected on 6 dpc (post-challenge) were tested against SARS-CoV-2 Wuhan WT virus in a serum neutralization test. **i**,

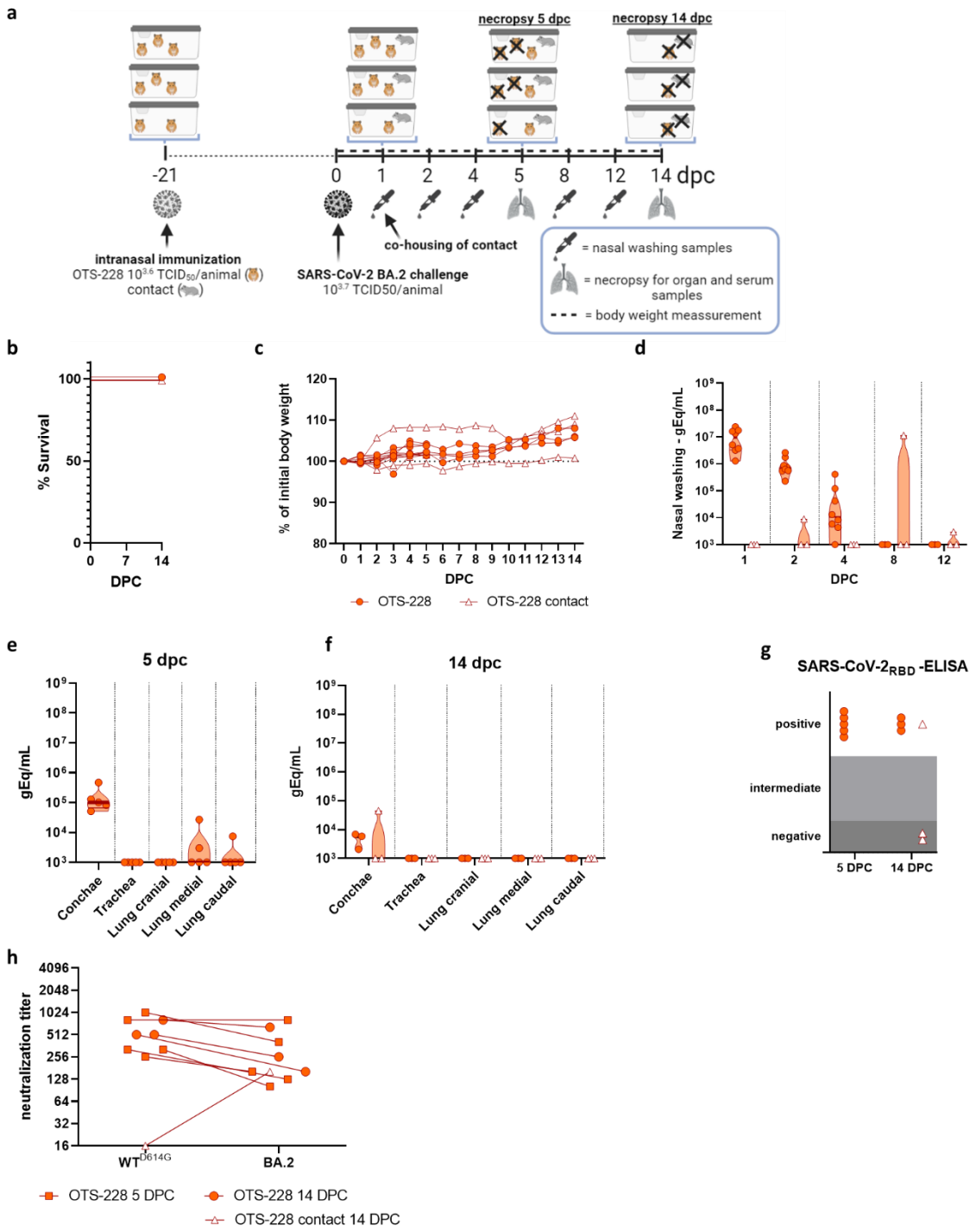
Immunohistochemical analysis specific for SARS-CoV-2 nucleocapsid protein (magnification 50x). Statistical significance between non-immunized and immunized mice was determined using unpaired nonparametric t-test (Mann Whitney test) (panels c-h); *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Data were obtained from one experiment. Each data point represents one biological replicate. Body weight changes, clinical scores and histopathological score of the lungs of all K18-hACE2 mice experiments are shown in **Supplementary Table 6**.



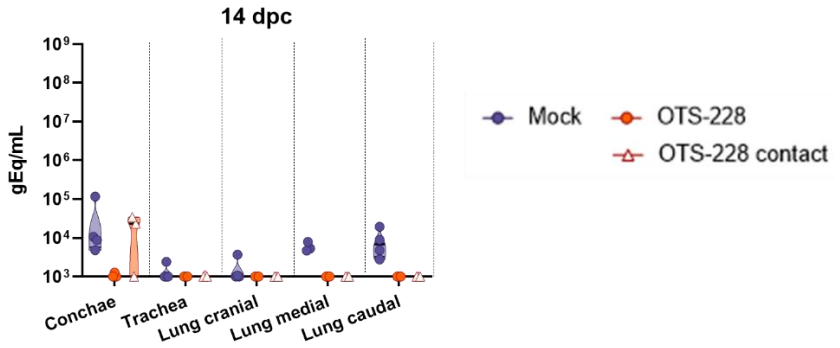
Extended Data Figure 7: OTS-228 shows replication kinetics comparable to WT and OTS-206 in VeroE6/TMPRSS2 cells. Vero E6/TMPRSS2 cells were infected with 0.1 MOI of the indicated viruses and incubated at 37°C for 1 h. After 1 h, supernatant was discarded and the cells were washed 3 times with PBS, and the third wash was kept for analysis. Following the addition of new sera on the cells, they were incubated 37°C. Samples were collected on designated time points post-infection. Infectious particle titers were assessed by TCID50 assays on VeroE6/TMPRSS2 cells. Each line in the graphs shows one replicate of samples. Statistical significances in the titer differences of OTS viruses vs WT on given times were determined using two-way ANOVA and p-values were adjusted using Tukey's multiple-comparison test; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.



Extended Data Figure 8: SARS-CoV-2 WT challenge infection of OTS-228 immunized Syrian hamsters. a, Experimental setup. **b**, Survival post-challenge infection. **c**, Relative body weight in percent. **d**, **e,** Virus genome copy numbers in nasal washing and organ samples of 5 dpc. **f**, Serum samples of 5 and 14 dpc were analyzed by SARS-CoV-2RBD-ELISA. **g**, Serum samples that reacted positively in the ELISA, were analyzed in addition by live virus neutralization assay (capacity to neutralize 100 $TCID_{50}$) against ancestral (B.1) SARS-CoV-2 as well as against Omicron BA.2 and BA.5 variants. Whenever calculated the statistical significance was determined using ordinary one-way ANOVA with p-values adjusted by Fisher´s LSD test, calculated p-values are as indicated. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.



Extended Figure 9: Omicron BA.2 challenge of OTS-228 vaccinated Syrian hamsters. a, Experimental setup. **b**, Survival post challenge infection. **c**, Relative body weight in percent. Shown are the mean with standard error. **d-g**, Virus genome copy numbers in nasal washing and organ samples of 5 dpc and 14 dpc. Serum samples of 5 and 14 dpc were analyzed by SARS-CoV-2RBD-ELISA. **h**, Serum samples which reacted positive in the ELISA, were analyzed in addition by live virus neutralization assay (capacity to neutralize 100 $TCID_{50}$) against ancestral (B.1) as well as against Omicron BA.2 variant. Whenever calculated the statistical significance was determined using ordinary one-way ANOVA with p-values adjusted by Fisher´s LSD test, calculated p-values are as indicated. *P<0.05, **P < 0.01, ***P<0.001, ****P<0.0001.



Extended Figure 10: Organ samples following Omicron BA.5 challenge of OTS-228 vaccinated Syrian hamsters. Virus genome copy numbers in organ samples 14 dpc.