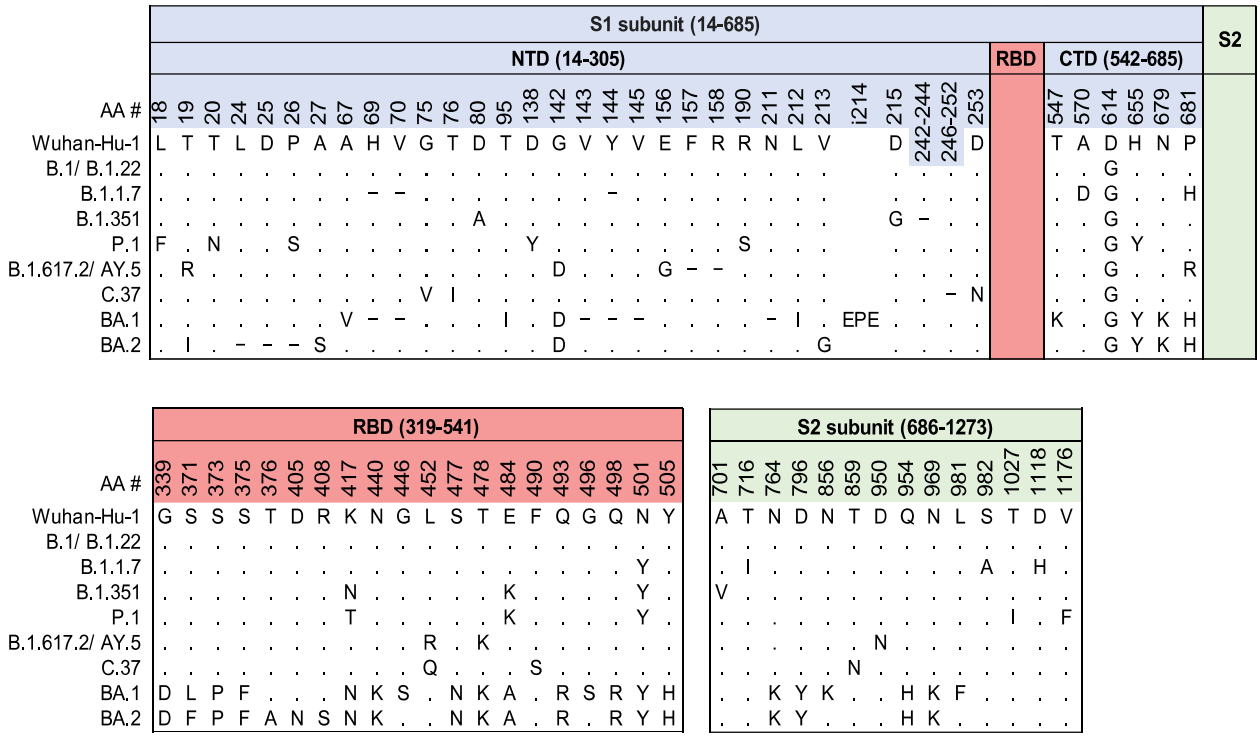
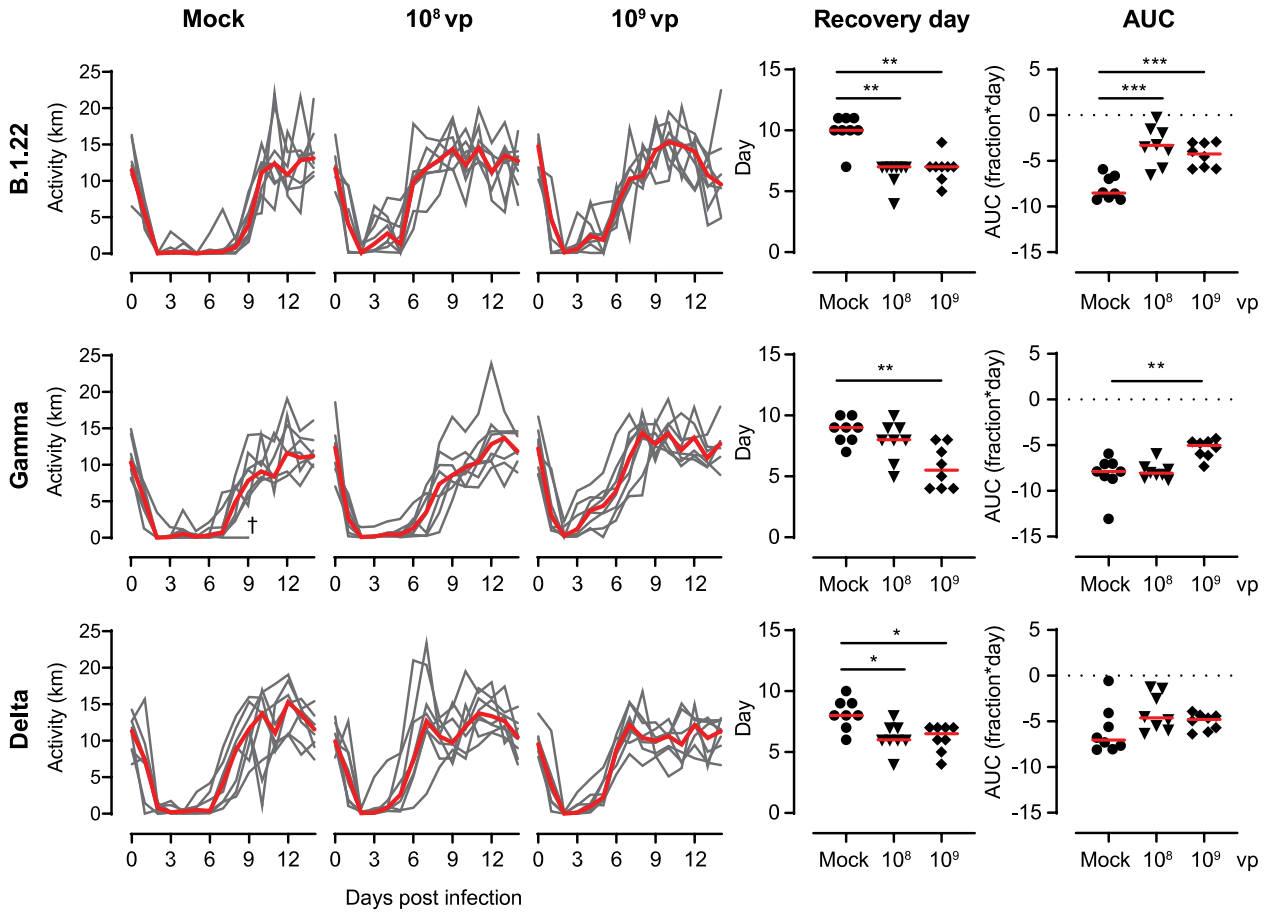
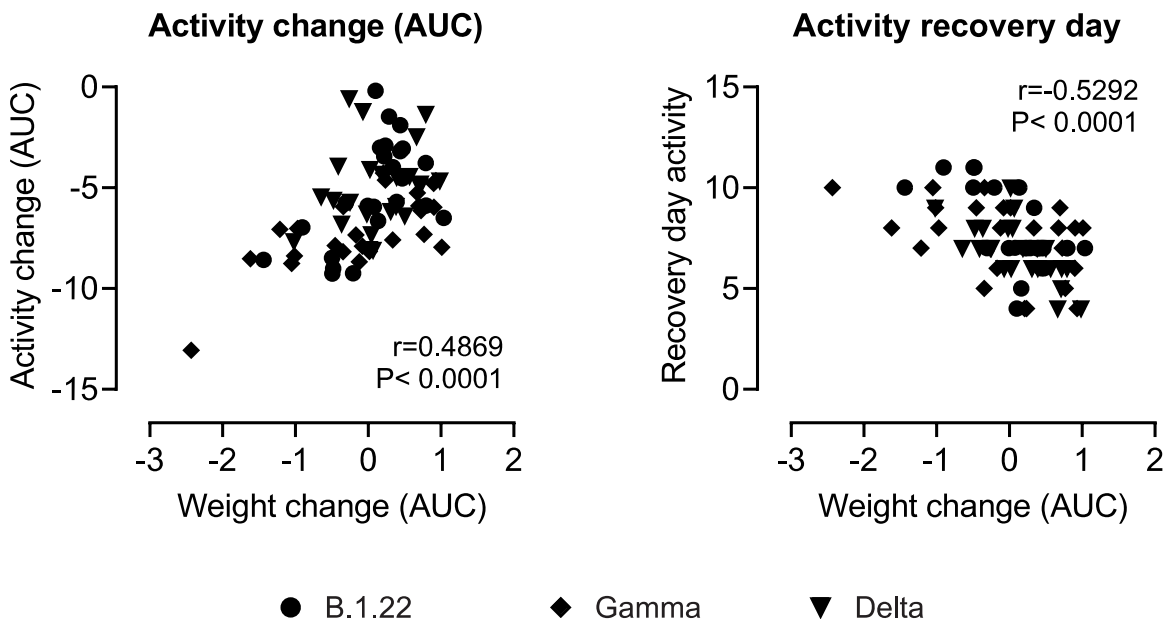


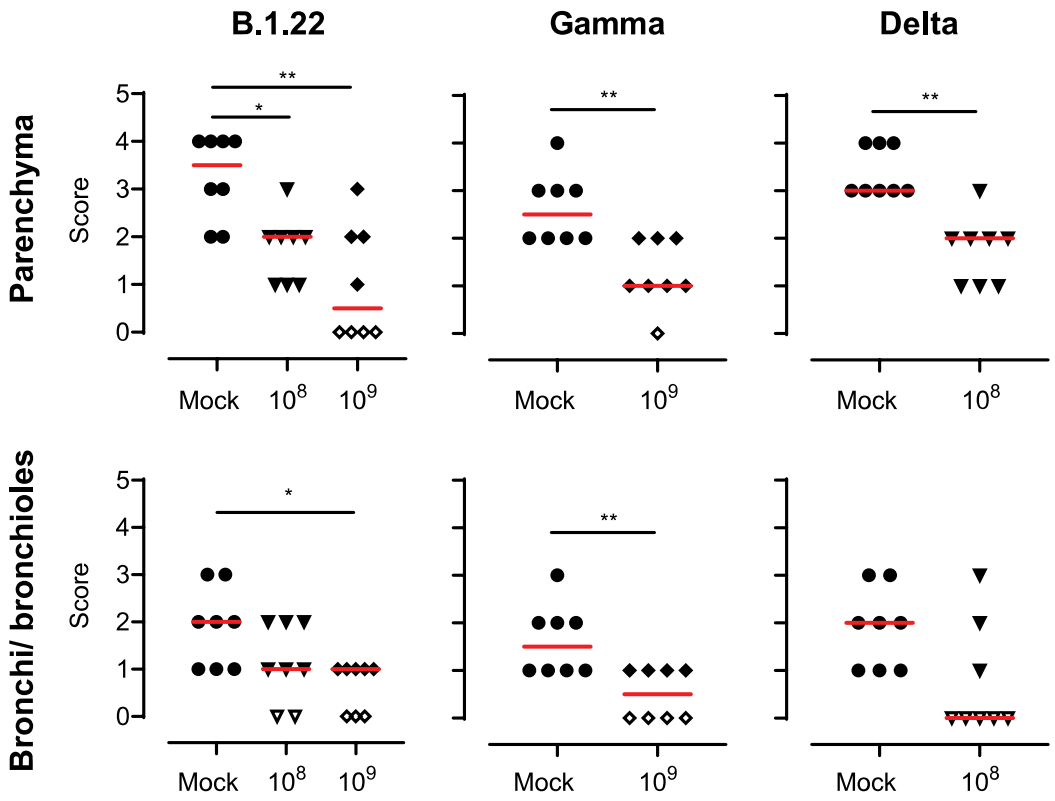
Supplemental table 1. Spike mutations in SARS-CoV-2 challenge stocks compared with Wuhan-Hu-1. The mutations/ deletions in spike are shown compared with the Wuhan-Hu-1 isolate (MN908947; lineage B). N-terminal domain (NTD), receptor-binding domain (RBD), C-terminal domain (CTD), dots indicate no differences to the Wuhan-Hu-1 sequence and deletions are indicated with a dash. B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2/ AY.5 (Delta), C.37 (Lambda), BA.1/ BA.2 (Omicron).



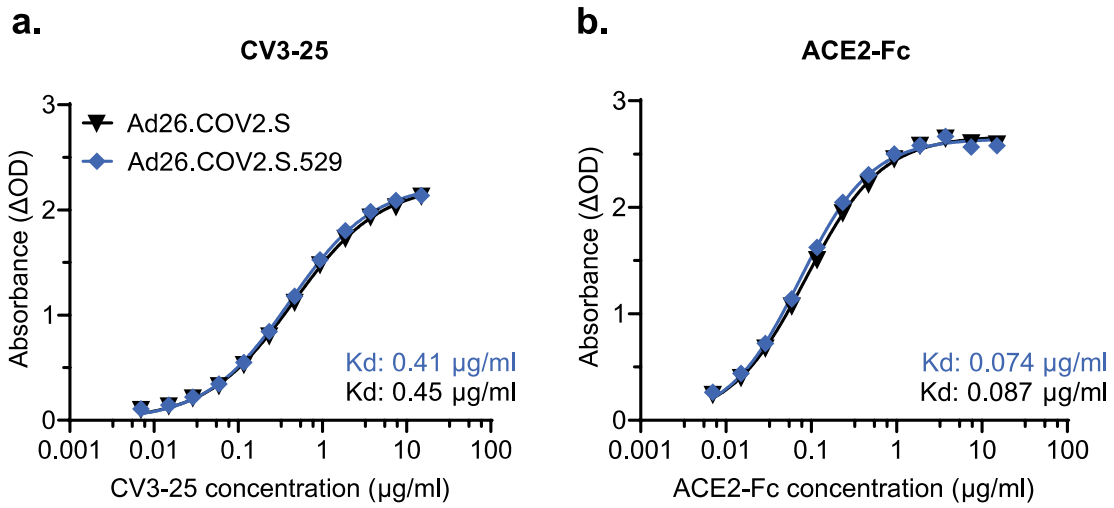
Supplemental figure 1. Ad26.COV2.S protects against activity loss upon SARS-CoV-2 B.1.22, Gamma and Delta challenge. Hamsters were vaccinated with formulation buffer (mock), 10^8 or 10^9 vp Ad26.COV2.S at day -28 (n=8 per group). The animals were intranasally challenged with 10^3 $TCID_{50}$ SARS-CoV-2 B.1.22, 10^4 $TCID_{50}$ SARS-CoV-2 Gamma or 10^4 $TCID_{50}$ SARS-CoV-2 Delta on day 0 and followed up till day 14. Individual activity traces, the day at which animals recovered from activity loss (defined by regaining at least 2.5 km activity a day for 2 consecutive days) and the area under the curve (AUC) as a fraction*day compared with the pre-challenge activity are shown. Red lines indicate group medians. † indicates a humane endpoint was reached. Comparisons to the mock group were performed by ANOVA with a post-hoc t-test or a Mann-Whitney U-test with a 2-fold Bonferroni correction. Statistical differences are indicated by asterisks: * P<0.05, ** P<0.01, *** P<0.001.



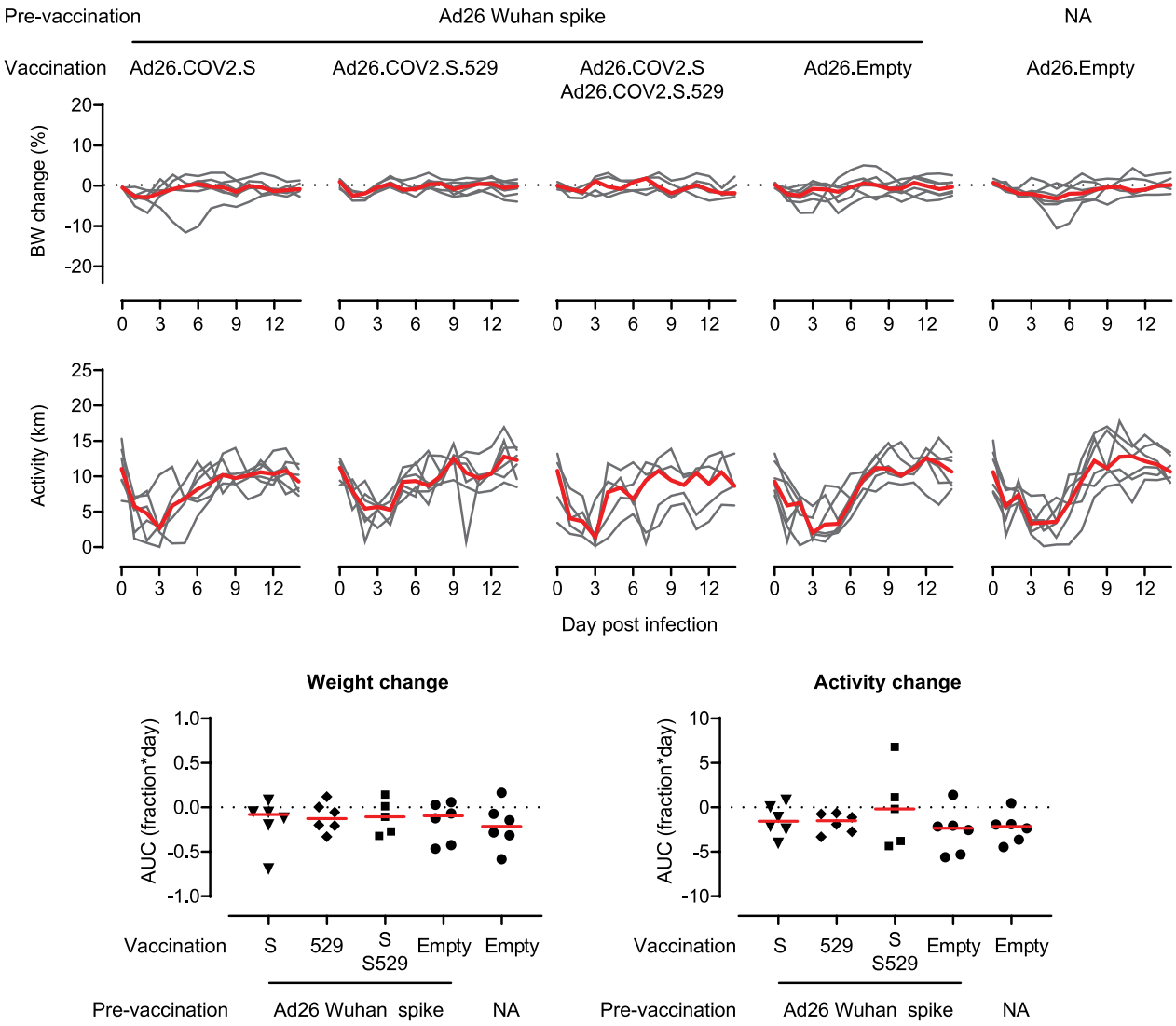
Supplemental figure 2. Correlation activity change with weight change post SARS-CoV-2 challenge. Hamsters were vaccinated with formulation buffer (mock), 10^8 or 10^9 vp Ad26.COV2.S at day -28. Hamsters were intranasally challenged with 10^3 $TCID_{50}$ SARS-CoV-2 B.1.22, 10^4 $TCID_{50}$ SARS-CoV-2 Gamma or 10^4 $TCID_{50}$ SARS-CoV-2 Delta on day 0. The correlation between the area under the curve (AUC as fraction*day compared with pre-challenge weight/ activity) for weight change with the AUC for weight change or the recovery day post-activity change is shown. The day at which animals recovered from activity loss was defined as regaining at least 2.5 km activity a day for 2 consecutive days. Correlation coefficients were calculated across all 3 VOC using two-sided Spearman rank correlation.



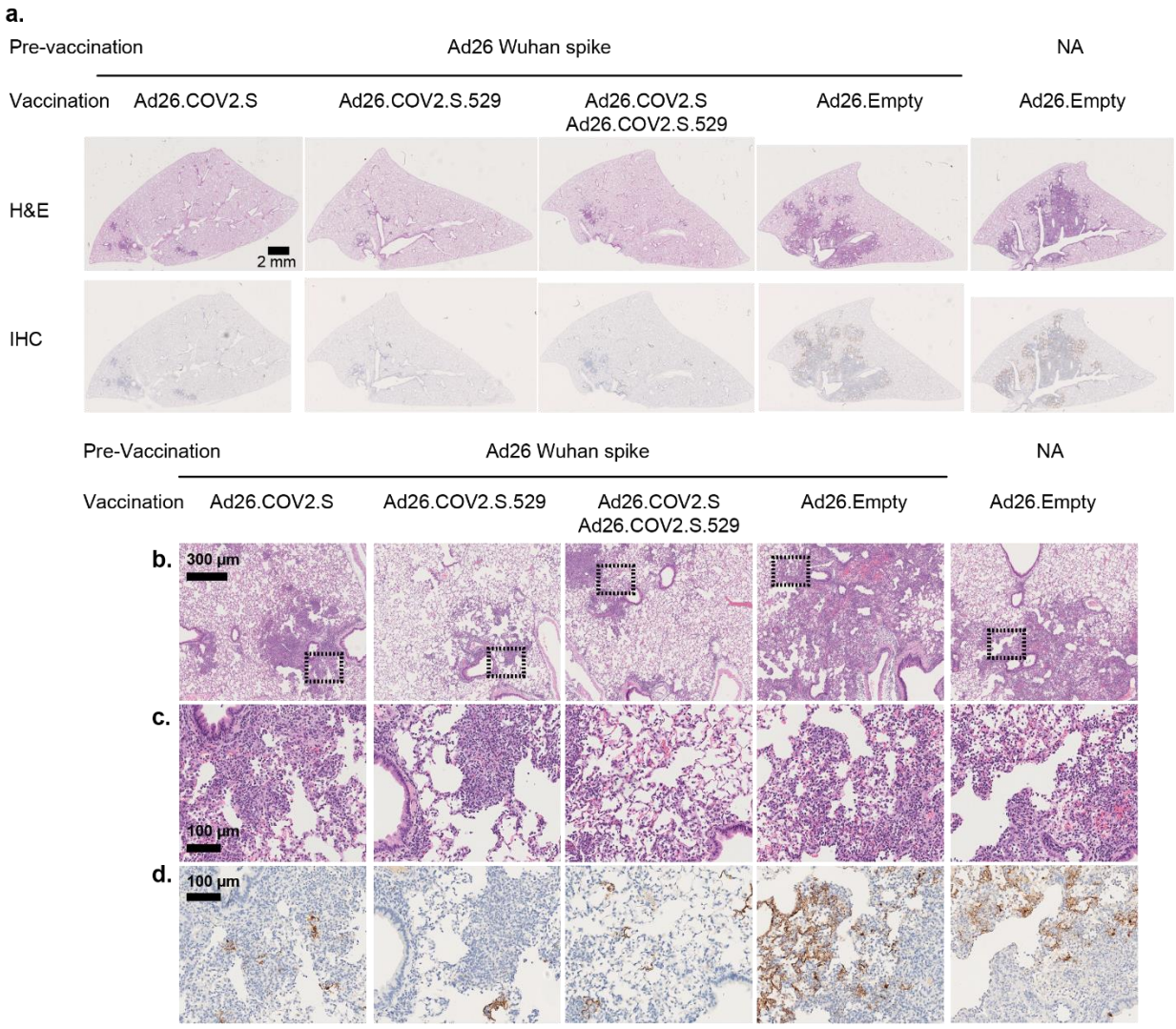
Supplemental figure 3. Nucleocapsid positivity in hamsters after SARS-CoV-2 B.1.22, Gamma and Delta challenge. Hamsters were vaccinated with formulation buffer (mock), 10^8 or 10^9 vp Ad26.COV2.S at day -28 (n=8 per group). The animals were intranasally challenged with 10^3 $TCID_{50}$ SARS-CoV-2 B.1.22, 10^4 $TCID_{50}$ SARS-CoV-2 Gamma or 10^4 $TCID_{50}$ SARS-CoV-2 Delta on day 0. While animals vaccinated with 10^8 and 10^9 vp were evaluated at day 4 after challenge with SARS-CoV-2 B.1.22, only animals vaccinated with 10^9 vp were examined at day 4 after challenge with SARS-CoV-2 Gamma and only 10^8 vp-vaccinated animals were evaluated at day 4 after challenge with the Delta variant. Lungs were stained and scored for SARS-CoV-2 nucleocapsid-protein positivity (score 0-5) in lung parenchyma and in bronchi/bronchioles. Red horizontal bars indicate the median response per group. Open symbols indicate a score of 0. Comparisons were performed by a Mann-Whitney U-test with a 2-fold Bonferroni correction. Statistical differences are indicated by asterisks: * $P<0.05$; ** $P<0.01$.



Supplemental figure 4. Comparison of CV3-25 and ACE2-Fc antibody binding to spike after expression by Ad26.COV2.S and Ad26.COV2.S.529. CV3-25 is an antibody that binds to the stem region of the SARS-CoV-2 spike S2[1], which is conserved between the Wuhan-Hu-1 and BA.1 spike protein and ACE2-binding affinity is reported to be similar between Wuhan-Hu-1 Spike and Omicron spike[2,3] **a.** CV3-25 or **b.** ACE2-Fc fusion protein binding to the spike protein in A549 cells after transduction with 2000 infectious units/cell Ad26.COV2.S.529 (blue line) or Ad26.COV2.S (black line). Binding affinity (Kd) values are shown, each point represents the mean value of a duplicate measurement.



Supplemental figure 5. Weight and activity change after Omicron BA.2 challenge in hamsters. Hamsters with pre-existing immunity were generated by pre-vaccination with 10^7 viral particles (vp) of Ad26NCOV006 (Ad26 vector encoding Wuhan-Hu-1 spike, pre-immune animals) at week -27. Naïve control hamsters were not vaccinated (NA). At week -4, Ad26 Wuhan-Hu-1 spike-vaccinated hamsters were vaccinated with 10^9 vp Ad26.COV2.S (S), Ad26.COV2.S.529 (529), a 1:1 combination of S and 529 or Ad26.Empty (all n=5-6). In addition, naïve hamsters were vaccinated with Ad26.Empty (n=6). The animals were intranasally challenged with $10^{4.7}$ $TCID_{50}$ SARS-CoV-2 BA.2 in week 0. Individual weight and activity traces and the area under the curve (AUC) as a fraction compared with the pre-challenge weight/ activity are shown. Red lines indicate group medians. Comparisons to the pre-vaccinated-only and pairwise comparisons were performed by a t-test with a 3-fold Bonferroni correction. No significant differences were found.



Supplemental figure 6. Representative histopathology images after Omicron BA.2 challenge. Hamsters with pre-existing immunity were generated by pre-vaccination with 10^7 viral particles (vp) of Ad26NCOV006 (Ad26 vector encoding Wuhan-Hu-1 spike, pre-immune animals) at week -27. Naïve control hamsters were not vaccinated (NA). At week -4, Ad26 Wuhan-Hu-1 spike-vaccinated hamsters were vaccinated with 10^9 vp Ad26.COV2.S (S), Ad26.COV2.S.529 (529), a 1:1 combination of S and 529 or Ad26.Empty (all n=8). In addition, naïve hamsters were vaccinated with Ad26.Empty (n=8). The animals were intranasally challenged with $10^{4.7}$ $TCID_{50}$ SARS-CoV-2 BA.2 on week 0. **a.** Representative overview of H&E-stained left lung lobes, and SARS-CoV-2 nucleocapsid positivity visualized using immunohistochemistry (IHC, brown staining). **b.** Representative H&E-stained lung images are shown per group at a 4x magnification. **c.** A 20x magnification of the H&E staining in the rectangular box is shown. **d.** SARS-CoV-2 nucleocapsid positivity shown for the rectangular box in **b** at a 20x magnification.

Supplemental references

1. Jennewein, M. F. *et al.* Isolation and characterization of cross-neutralizing coronavirus antibodies from COVID-19+ subjects. *Cell Rep.* **36**, 109353 (2021).
2. Han, P. *et al.* Receptor binding and complex structures of human ACE2 to spike RBD from omicron and delta SARS-CoV-2. *Cell* **185**, 630-640.e10 (2022).
3. Mannar, D. *et al.* SARS-CoV-2 Omicron variant: Antibody evasion and cryo-EM structure of spike protein–ACE2 complex. *Science* **375**, 760–764 (2022).