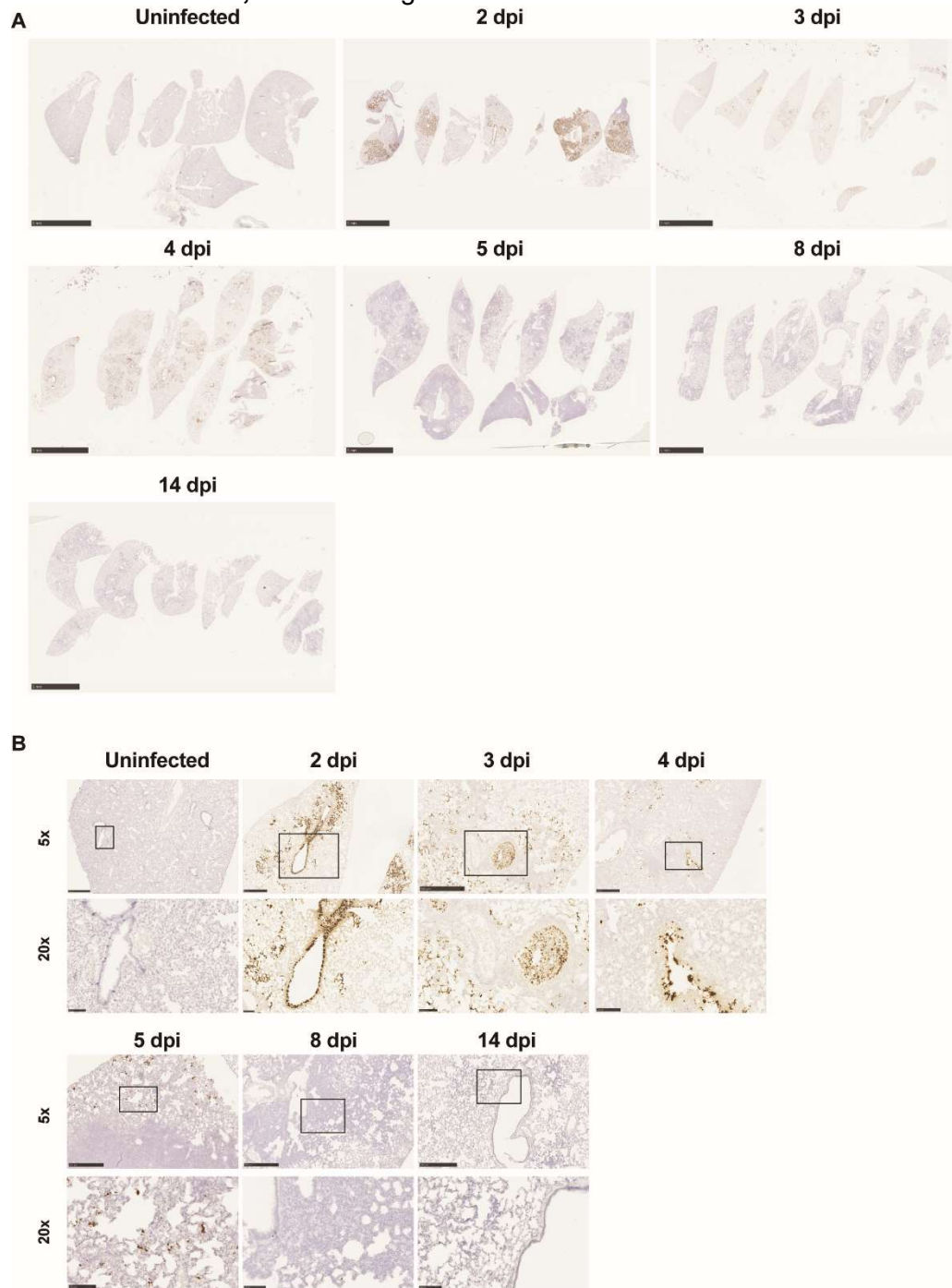


Supplemental information

**A single intranasal or intramuscular immunization
with chimpanzee adenovirus-vectored SARS-CoV-2
vaccine protects against pneumonia in hamsters**

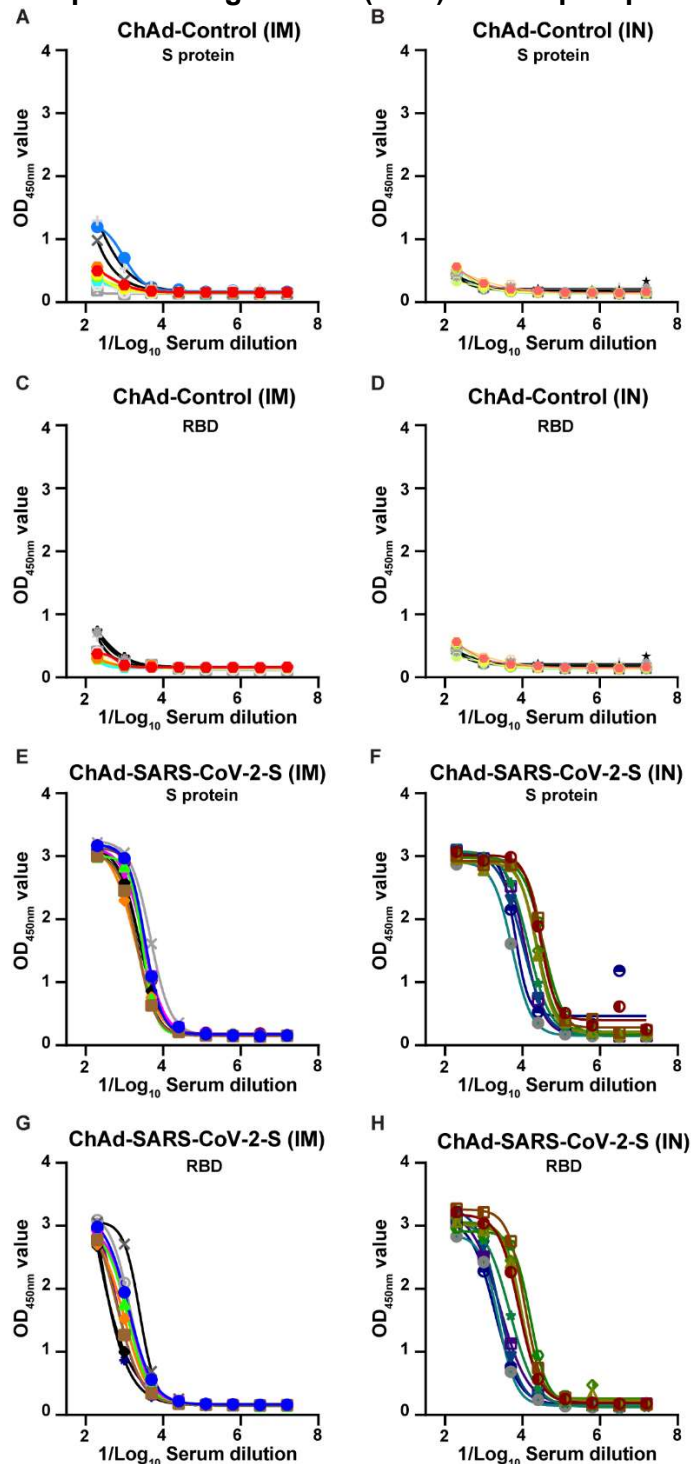
Traci L. Bricker, Tamarand L. Darling, Ahmed O. Hassan, Houda H. Harastani, Allison Soung, Xiaoping Jiang, Ya-Nan Dai, Haiyan Zhao, Lucas J. Adams, Michael J. Holtzman, Adam L. Bailey, James Brett Case, Daved H. Fremont, Robyn Klein, Michael S. Diamond, and Adrianus C.M. Boon

Figure S1: RNA *in situ* (ISH) hybridization on lung tissue sections from SARS-CoV-2 infected hamsters, related to Figure 1



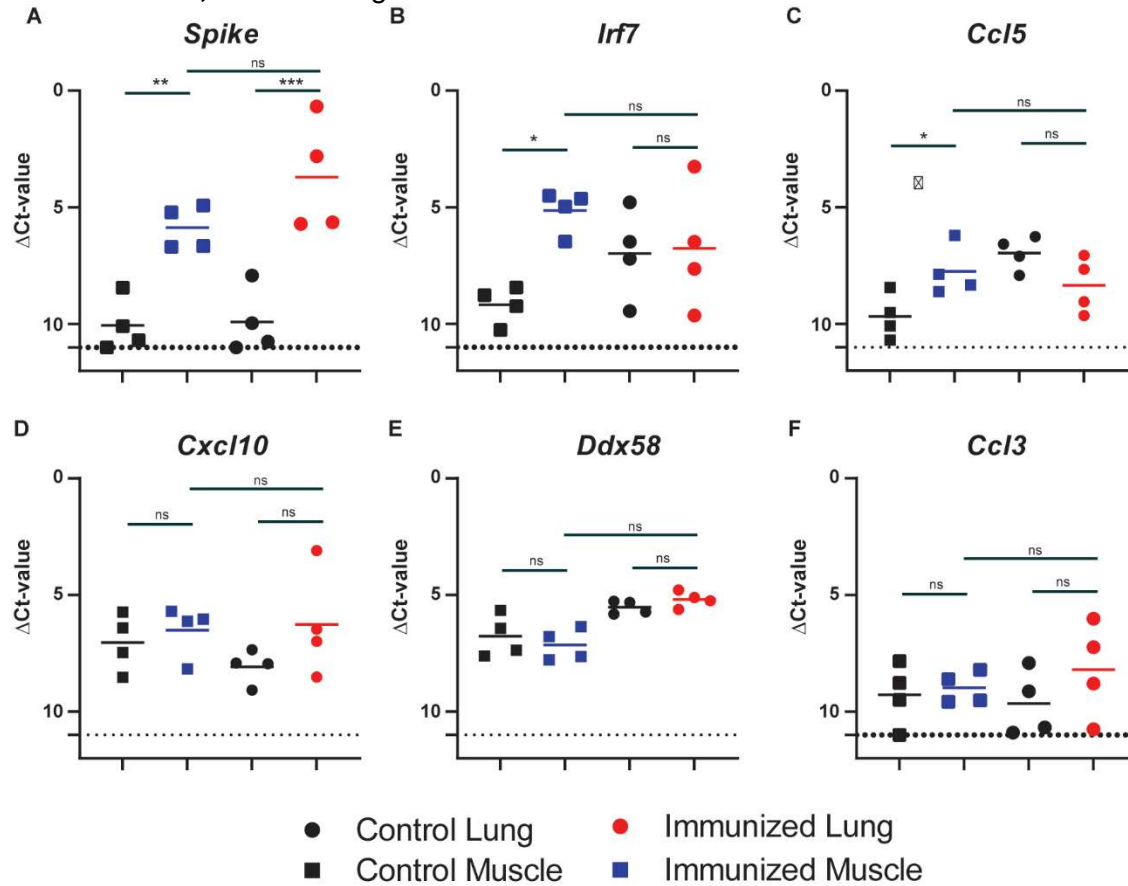
Representative images at 0.5x (**A**), 5x (**B**), and 20x (**B**) magnification of RNA-ISH of SARS-CoV-2 infected hamsters sacrificed at different time points after inoculation (n = 5 for 2 dpi, n = 3 for 3 dpi, n = 3 for 4 dpi, n = 5 for 5 dpi, n = 2 for 8 dpi, n = 3 for 14 dpi, n = 3 for uninfected). The scale bar is 1 mm in size.

Figure S2: IgG2/IgG3 serum antibody titers against recombinant spike protein and the receptor binding domain (RBD) of the spike protein, related to Figure 2



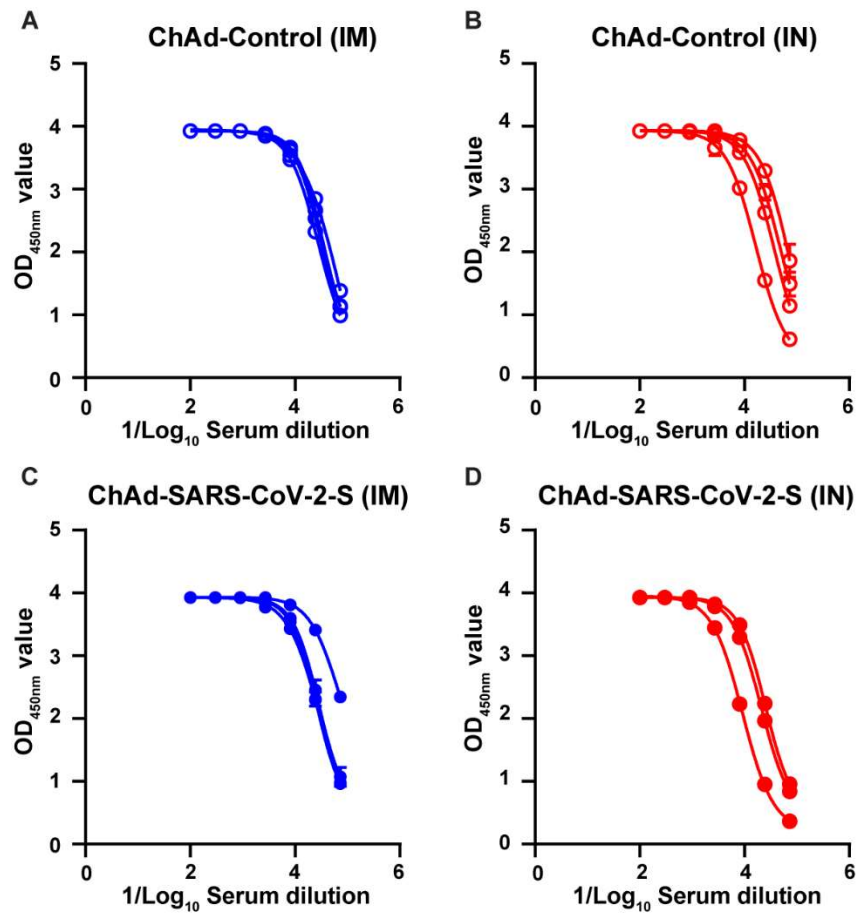
(A-B) S protein-specific serum IgG2/IgG3 titers in hamsters immunized IM **(A)** or IN **(B)** with ChAd-Control. **(C-D)** RBD-specific serum IgG2/IgG3 titers in hamsters immunized IM **(C)** or IN **(D)** with ChAd-Control. **(E-F)** S protein-specific serum IgG2/IgG3 titers in hamsters immunized IM **(E)** or IN **(F)** with ChAd-SARS-CoV-2-S. **(G-H)** RBD-specific serum IgG2/IgG3 titers in hamsters immunized IM **(G)** or IN **(D)** with ChAd-SARS-CoV-2-S. Each line is an individual animal.

Figure S3: Spike gene and hamster inflammatory host gene expression after IN and IM immunization, related to Figure 2



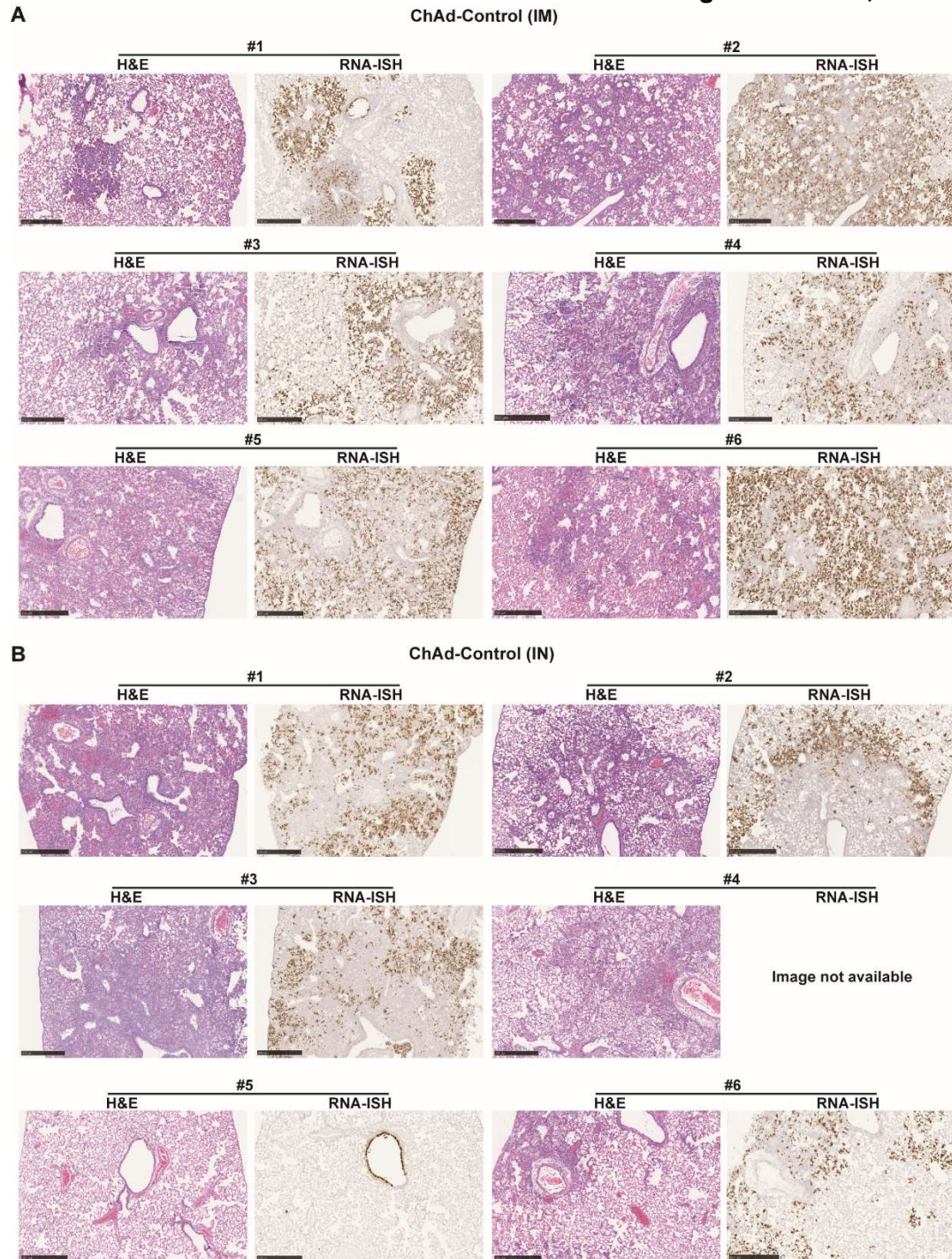
Spike gene and inflammatory gene-expression was quantified by RT-PCR in RNA extracted from lung or muscle homogenates 3 days after intranasal (IN) or intramuscular (IM) immunization with ChAd-SARS-CoV-2-S vaccine. (A) ΔCt -values between spike gene and *Rpl18* housekeeping gene in lung of IN immunized animals (red circles) and muscle of IM immunized (blue squares) animals. The lung and muscle of the IM (black circles) and IN (black squares) immunized animals respectively served as tissue control for this analysis. (ns = not significant, **** $P < 0.0001$, *** $P < 0.001$ by one-way ANOVA with a Šidák correction for multiple comparisons). (B-G) ΔCt -values for *Irf7*, *Ccl5*, *Cxcl10*, *Ddx58* and *Ccl3* in the lung and muscle of IN (red circles) and IM (blue squares) immunized animals. The lung and muscle of the IM (black circles) and IN (black squares) immunized animals respectively served as tissue control for this analysis. (ns = not significant, *** $P < 0.001$, ** $P < 0.01$ by one-way ANOVA with a Šidák correction for multiple comparisons). Each dot is an individual animal from two experiments. Bars indicate average values.

Figure S4: IgG(H+L) serum antibody titers against SARS-CoV-2 nucleoprotein 10 days after infection in vaccinated and control hamsters, related to Figure 3



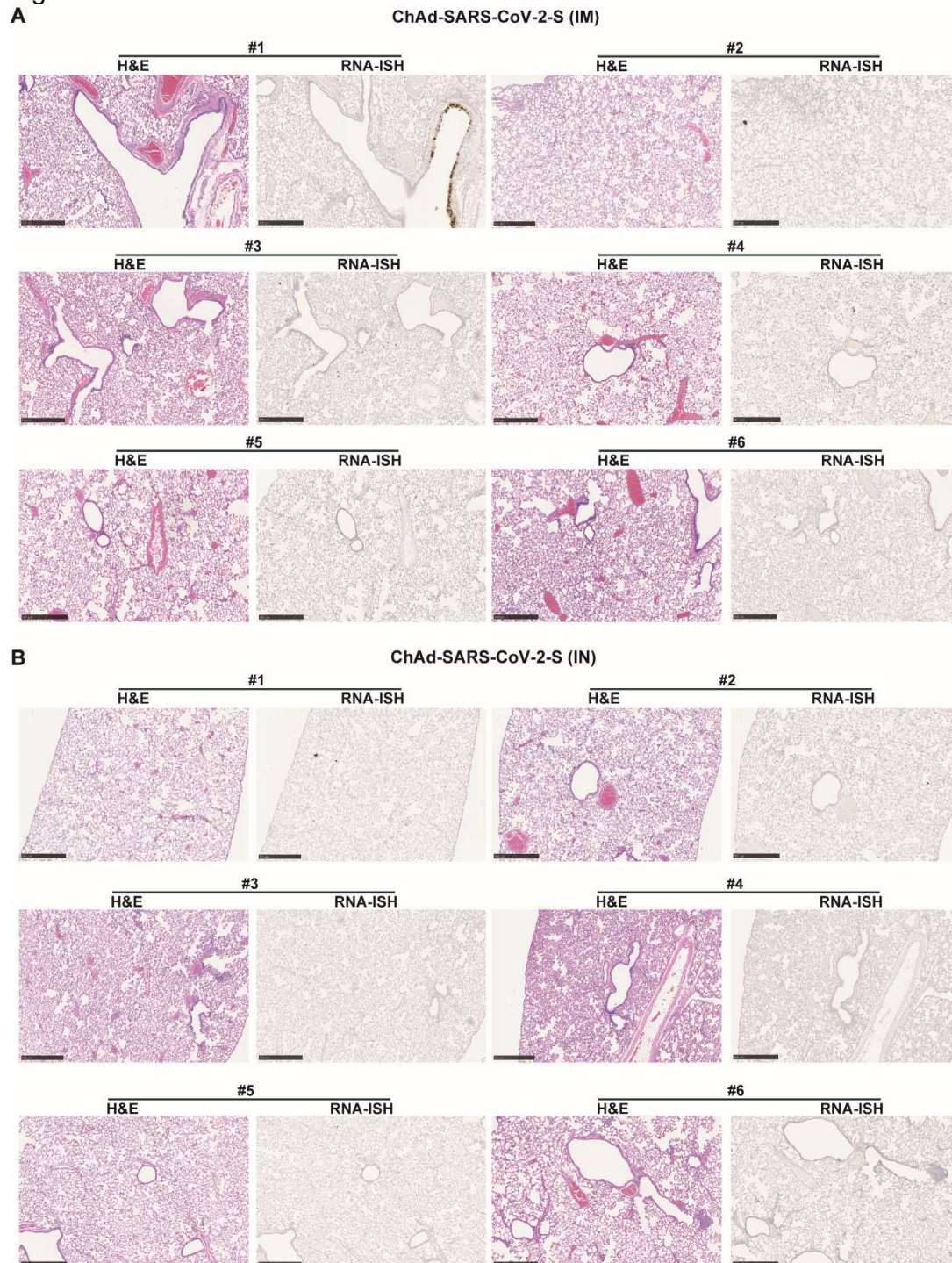
Nucleoprotein-specific serum antibody titers in ChAd-Control (**A-B**) or ChAd-SARS-CoV-2-S (**C-D**) immunized and SARS-CoV-2 challenged Golden Syrian hamsters 10 days post challenge. Each line is an individual animal.

Figure S5: Histological and RNA *in situ* (ISH) hybridization analysis of lung tissue sections from ChAd-Control vaccinated and SARS-CoV-2 challenged hamsters, related to Figure 4



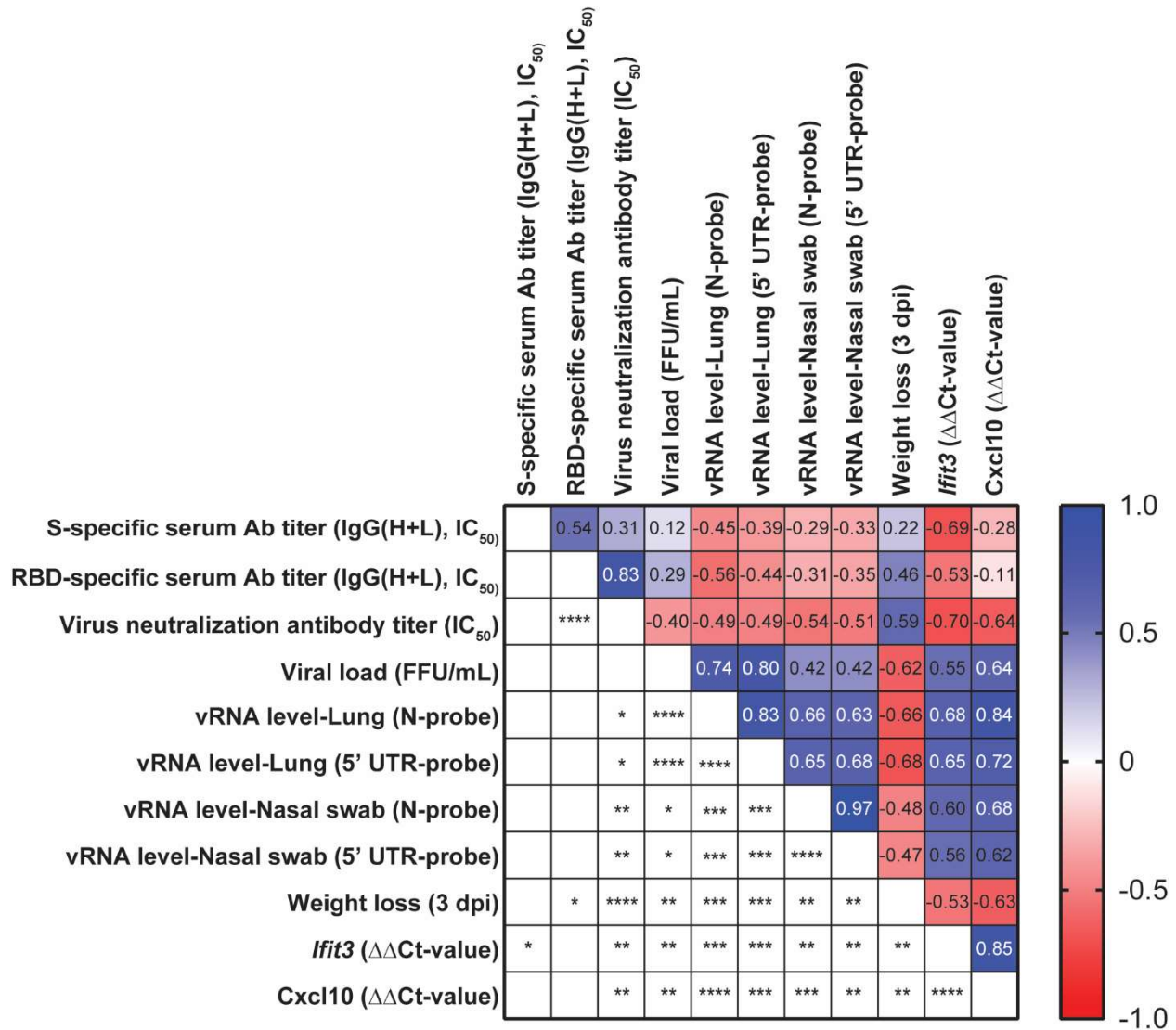
Representative images at 5x magnification of H & E staining and RNA-ISH of hamsters immunized IM (n = 6) and IN (n = 6) with ChAd-Control and challenged 28 days later with SARS-CoV-2. Lungs were collected 3 days post challenge, fixed in 10% formalin and paraffin embedded prior to sectioning and staining. The scale bar is 1 mm in size.

Figure S6: Histological and RNA *in situ* (ISH) hybridization analysis of lung tissue sections from ChAd-SARS-CoV-2-S vaccinated and SARS-CoV-2 challenged hamsters, related to Figure 4



Representative images at 5x magnification of H & E staining and RNA-ISH of hamsters immunized IM (n = 6) and IN (n = 6) with ChAd-SARS-CoV-2S and challenged 28 days later with SARS-CoV-2. Lungs were collected 3 days post challenge, fixed in 10% formalin and paraffin embedded prior to sectioning and staining. The scale bar is 1 mm in size.

Figure S7: Immune correlates of vaccine-mediated protection SARS-CoV-2.
 Related to Figures 2, 3, and 5



Correlations between % weight-loss/gain (3 dpi), RNA levels in the lungs and nasal swabs, infectious virus titers, serum antibody responses, serum virus neutralization titer, and inflammatory hamster gene expression were analyzed for all animals in the vaccine study using a Pearson correlation matrix. The top right side is the correlation coefficient and the bottom left side has the *P*-value for every combination (**** *P* < 0.0001, *** *P* < 0.001, ** *P* < 0.01, * *P* < 0.05 by Pearson's correlation analysis).

Table S1: Primer and probe sequences for gene-expression analysis in the Golden Syrian hamster (*Mesocricetus auratus*)
Related to STAR Methods section

Gene	Accession number	Forward Primer	Probe	Reverse Primer	Exons spanned
RPL18	XM_005084699.3	GTTTATGAGTCGCACTAACCG	TCTGTCCCTGTCCCGGATGATC	TGTTCTCTCGGCCAGGAA	2
β 2M	XM_005068531.3	GGCTCACAGGGAGTTGTAC	CTGCGACTGATAAATACGCCTGCA	TGGGCTCCTTCAGAGTTATG	1
C3	XM_021233717.1	TCTCCATGATGACTGGCTTTG	ACACAAACGACCTGGAAGTCTGA	GGCTTTGGTCATCTCGTACTT	2
CCL2	XM_005076967.3	CTCACCTGCTGCTACTCATT	CAGCAGCAAGTGTCCCAAAGAAGC	CTCTCTTTGAGCTTGGTGATG	2
CCL3	NM_001281338.1	CCTCCTGCTGCTTCTTCTATG	TCCCACAAATTCATCGCCGACTAT	TGCCGGTTTCTTTGGTTAG	2
CCL5	XM_005076936.3	TGCTTTGACTACCTCTCCTTTAC	TGCCTCGTGTTACATCAAGGAGT	GGTTCCTTCGGGTGACAAA	2
IFIT3	XM_021224964.1	CTGATACCAACTGAGACTCCTG	ACCGTACAGTCCACACCCAACTTT	CTTCTGCTTCTCCTCGGATTAG	2
IFN γ	NM_001281631.1	TTGTTGCTCTGCCCTCACTC	TACTGCCAGGGCACACTCATTGAA	CCCTCCATTCACGACATCTAAG	2
IL-10	XM_005079860.2	AGCGCTGTCATCGATTTCTC	AAGGCTGTGGAACAGGTGAAGGAT	CGCCTTTCTCTTGAGCTTAT	3
IL-12p40	NM_001281689.1	GAGGCCAGCACAAGTATAA	ATCATCAAACCGGACCCACCCAAA	AGTCAGGATACTCCAGGATAA	2
IL-15	XM_005077725.3	AGGCTGAGTTCTCCGTCTAA	TCAGAGAGGTCAGGAAAGGAGGTGT	AGTGTTGAAGAGCTGGCTATG	2
IL-1 β	XM_005068610.3	TTCCTGAACTCGACAGTGAAT	TCTTTGAGGTTGACGGGCTCCAAA	GCTTTGGAAACAGCTCTTCATC	2
IL-2	NM_001281629.1	TGCCTGGAAGAAGAAGTGG	CGTGCTGGATTTGGCTCAAAGCAA	ATGTGCTTTCAGAGCCCTTA	4
IL-4	AF046213	CCACGGAGAAAGACCTCATCTG	CAGGGCTTCCAGGTGCTTCGCAAGT	GGGTCACCTCATGTTGGAATAAA	2
IL-6	XM_005087110.2	CCAGATCTACCTGGAGTTTGTG	AAGCCAGAGTCATTCAGAGCACCA	CTGGACCCTTTACCTCTTGTTT	2
IL-7	XM_021225270.1	GTGTGGCTTCTGTGGACATATTA	TTCCAGTCTCCAGAGTTGCCAAA	GAGATTCGGCTAAGAGGCTTTC	1
IP10	NM_001281344.1	AGAGCCTCTTAACCAGAGAGAA	AAAGCCCGTCTCTCCATCACTTCT	TAGCCATAGGCCGACGTATAA	1
IRF7	XM_005063345.3	AGCACGGGACGCTTTATC	AGTTTGGATGTAAGGCCCCGG	GACGGTCACTTCTTCCCTATTC	2
Rig-I	NM_001310553.1	GTGCAACCTGGTCATTCTTTATG	AAACCAGAGGCAGAGGAAGAGCAA	GTCAGGAGGAAGCACTTACTATC	2
STAT1	NM_001281685.1	AGGTCGTCAGCAGCTTAA	TCTGAATGAGCTGCTGGAAGAGGACA	GCCGTTCCACCACAAAT	2
STAT6	XM_005079747.3	AGCACCTCATTACCTTCAG	ACCAAGACAACAATGCCAAAGCCA	AAGCATTGTCCACAGGATAG	2
TNF α	XM_005086799.3	GGAGTGGCTGAGCCATCGT	CCAATGCCCTCCTGGCCAACG	AGCTGGTTGTCTTTGAGAGACATG	1

Primer-probes for RPL18, β 2M, IL4, STAT1 and TNF α were found in PMID: 21334343. All other primer-probes were designed using IDT's PrimerQuest Design Tool.