

1 Oral and intranasal Ad5 SARS-CoV-2 vaccines decrease disease and viral transmission
2 in a golden hamster model

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18 **Abstract**

19 Transmission-blocking strategies that slow the spread of SARS-CoV-2 and protect
20 against COVID-19 are needed. We have developed a shelf-stable, orally-delivered Ad5-
21 vectored SARS-CoV-2 vaccine candidate that expresses the spike protein. Here we
22 demonstrated that oral and intranasal SARS-CoV-2 vaccination of this candidate
23 protected against disease in index hamsters, and decreased aerosol transmission to
24 unvaccinated, naïve hamsters. We confirmed that mucosally-vaccinated hamsters had
25 robust antibody responses. We then induced a post-vaccination infection by inoculating
26 vaccinated index hamsters with SARS-CoV-2. Oral and IN-vaccinated hamsters had
27 decreased viral RNA and infectious virus in the nose and lungs and experienced less
28 lung pathology compared to mock-vaccinated hamsters post challenge. Naive hamsters
29 exposed in a unidirectional air flow chamber to mucosally-vaccinated, SARS-CoV-2-
30 infected hamsters had lower nasal swab viral RNA and exhibited less clinical symptoms
31 of disease than control animals. Our data demonstrate that oral immunization is a viable
32 strategy to decrease SARS-CoV-2 disease and aerosol transmission.

33

34 **Main**

35 The injectable SARS-CoV-2 vaccines currently approved for use are capable of
36 protecting vaccinees from symptomatic infection, hospitalization and death from COVID-
37 19¹⁻³. However, they do not completely prevent infection, as evidenced by multiple
38 reports of infections in vaccinated individuals^{4,5}. Indeed, mRNA vaccinated individuals

39 infected with the B.1.617 (delta) variant can shed viral RNA and infectious titers and
40 potentially spread SARS-CoV-2 to others^{6,7}. Considering most of the world is
41 unimmunized, including children below 12, the possibility that a vaccinated individual
42 with a post-vaccination infection can spread SARS-CoV-2 to unimmunized family or
43 community members poses a public health risk. There would be a substantial benefit to
44 develop vaccines that protect against disease *and* reduce SARS-CoV-2 transmission
45 from vaccinated to unvaccinated individuals.

46 Since the mucosal surface of the upper respiratory tract (URT) is the initial site of
47 SARS-CoV-2 replication and primary site of infection⁸, interventions that induce robust
48 mucosal immune responses may have the greatest impact on reduction of SARS-CoV-2
49 transmission. We have created a replication-defective, shelf-stable oral adenoviral
50 vector vaccine candidate expressing the spike (S) protein from SARS-CoV-2 (r-Ad-S)
51 that is designed to induce both systemic and mucosal immunity. Importantly, immune
52 activation via the intestine may represent an important organ for oral immunization as
53 antibody secreting plasmablasts and plasma cells can traffic from the gut to the nose,
54 trachea, and lung^{9,10}. Prior work in a human influenza challenge study with the same
55 platform has shown an ability to limit viral RNA shedding of influenza virus¹¹,
56 highlighting the utility of this vaccination strategy for respiratory viruses.

57 To study the potential impact of oral vaccination on transmission to naïve individuals,
58 we used a hamster infection and aerosol transmission system. We vaccinated index
59 hamsters with oral r-Ad-S, using intranasal (IN) r-Ad-S as a control for mucosal
60 stimulation, intramuscular spike protein (IM S) as a control for systemic stimulation, and
61 oral PBS as a mock control. We then infected animals, via IN delivery, with a high titer

62 of SARS-CoV-2 in order to replicate a post-vaccination infection. One-day post viral
63 challenge, index hamsters were placed upstream of vaccine-naïve hamsters in a
64 chamber that allowed aerosol movement but not direct contact or fomite transmission.
65 We demonstrated that post-vaccination, the oral and IN r-Ad-S groups had higher
66 serum IgG and IgA, as well as higher bronchoalveolar lavage (BAL) IgA compared to
67 control animals. This corresponded to decreased SARS-CoV-2 RNA and infectious
68 virus in the URT and decreased weight loss and lung pathology. Importantly, oral and IN
69 r-Ad-S vaccination decreased aerosol transmission of SARS-CoV-2 and reduced
70 disease indicators such as lung inflammation and weight loss in unvaccinated naïve
71 animals, despite the presence of substantial viral RNA in nasal swabs of index
72 immunized animals. These data demonstrate that oral r-Ad-S immunization resulted in
73 reduced disease and decreased SARS-CoV-2 transmission in a hamster model.

74

75 **RESULTS**

76 **Oral and IN r-Ad-S vaccination induced robust systemic and mucosal antibody** 77 **responses**

78 Index hamsters were immunized at weeks 0 and 4 with oral r-Ad-S, IN r-Ad-S (mucosal
79 positive control), IM S protein or mock (oral PBS) prior to SARS-CoV-2 challenge at
80 week 7 (Figure 1A). To determine immunogenicity of these vaccines, serum was
81 collected at weeks 0, 3 and 6 post immunization. BAL was collected upon necropsy
82 (day 5 post-inoculation) (Figure 1A). Oral and IN r-Ad-S vaccinated groups had
83 significantly higher S-specific IgG antibody titers in serum at week 3 compared to mock-

84 dosed hamsters; this was not true in IM S vaccinated hamsters (Figure 1B). Using a
85 surrogate virus neutralizing test (sVNT), serum from IN r-Ad-S hamsters had greater
86 ability to block binding of SARS-CoV-2 S to ACE-2 after the booster vaccination (week
87 6) compared to serum from mock-vaccinated hamsters (Figure 1C).

88 Serum anti-S IgA antibodies increased post-oral and IN r-Ad-S vaccination but not in IM
89 S or mock groups (Figure 1D). As expected from our oral immunization platform, oral r-
90 Ad-S vaccinated hamsters demonstrated similar anti-S IgA levels in BAL compared to
91 the IN r-Ad-S positive control group, suggesting similar stimulation of mucosal immunity
92 (Figure 1E). There were no differences in serum or BAL IgA responses between IM S
93 and mock-vaccinated hamsters, suggesting that IgA was not induced by systemic
94 immunization with the IM S protein. These data demonstrate that oral r-Ad-S and IN r-
95 Ad-S vaccinated hamsters generated robust systemic and mucosal humoral immunity.

96

97 **Oral and IN r-Ad-S vaccination accelerated SARS-CoV-2 viral RNA clearance and**
98 **protected against disease in hamsters**

99 In index animals, SARS-CoV-2 RNA from nasal swabs at 3 and 5 days post-inoculation
100 was lower in oral and IN r-Ad-S-vaccinated animals compared to mock-vaccinated
101 animals; this was not true for IM-vaccinated animals (Figure 2A). Nasal swabs of index
102 animals immunized with IN r-Ad-S had significantly lower TCID₅₀ values compared to
103 mock animals on day 1 (Figure 2B), but all groups still had detectable infectious virus.
104 Oral and IN r-Ad-S-vaccinated index animals had lower viral RNA loads in the lungs at
105 terminal collection (day 5) when compared to mock-vaccinated animals, which was not

106 true for IM S-vaccinated animals (Figure 2C). All vaccinees had significantly reduced
107 lung infectious viral loads, with all appearing below the limit of detection at day 5 (Figure
108 2D). These data demonstrated that oral and IN r-Ad-S vaccination decreased SARS-
109 CoV-2 replication post infection, and accelerated viral clearance.

110 During vaccination but prior to SARS-CoV-2 inoculation, all animals were gaining weight
111 at a similar rate (Extended data Fig. 1). After SARS-CoV-2 inoculation, we observed
112 significantly greater weight loss in unvaccinated hamsters (AUC = 767 ± 2.5) compared
113 to uninfected hamsters (AUC = 809.6 ± 4.0) (Figure 3A), a characteristic of disease in
114 this model. Oral and IN r-Ad-S vaccinated animals lost less weight by the termination of
115 the study compared to mock-vaccinated animals (Figure 3B). To quantify pulmonary
116 inflammation, lung weights were measured, and lungs were scored for gross pathology.
117 In index animals, lung weights (normalized to total body weight) (Figure 3C) and
118 average gross pathology scores (Figure 3D) were decreased in both oral and IN r-Ad-S-
119 vaccinated groups when compared to mock-treated hamsters.

120

121 **Oral and IN r-Ad-S vaccination limited SARS-CoV-2 transmission to unvaccinated,**
122 **naïve hamsters leading to decreased clinical indicators of disease**

123 As a test of transmissibility, unvaccinated naïve hamsters were exposed to vaccinated,
124 SARS-CoV-2-infected index animals at a 1:4 ratio of index:naive, where each vaccine
125 exposure group has 4 index animals and 16 vaccine naïve animals. Exposure was
126 performed by putting an index animal in a chamber (index chamber) connected to a
127 second chamber containing the four naïve animals (naïve chamber), separated by a 5

128 inch connecting chamber (Extended data Fig. 2). Screens at either end of the
129 connecting chamber prevented direct contact. Air was circulated in the index chamber
130 by fan, and was pulled into the naïve chamber by vacuum. Exposure of naïve hamsters
131 to aerosols produced by index hamsters was performed for 8 hours prior to moving
132 each hamster to an individual cage. On day 1 post infection the amount of viral RNA
133 loads in all index animals was above 7.8×10^7 gene copies per swab (Figure 2A).

134 In naïve hamsters exposed to the oral and IN r-Ad-S-immunized groups, SARS-CoV-2
135 RNA was significantly lower on days 1 and 3 compared to mock-immunized animals
136 (Figure 4A). The number of vaccine naïve animals with nasal swab viral RNA levels
137 above a threshold of 1×10^5 gene copies was also determined. On day 1 post exposure
138 of the index and naïve animals, there were 3 naïve hamsters (3/16) exposed to the oral
139 r-Ad-S index group that were above the threshold compared to eleven mock exposed
140 hamsters (11/16) ($p=0.011$, Fisher's exact test) (Figure 4A, Extended data Table 1). The
141 naïve hamsters exposed to IN r-Ad-S index hamsters had 0 (0/16) animals above the
142 threshold which was not significantly different than oral, but significantly lower than
143 naïve animals exposed to aerosol from mock-vaccination animals ($p=0.22$, $p<0.0001$ by
144 Fisher's exact test respectively) (Figure 4A, Extended data Table 1). On day 3, in the
145 oral exposed group, 11 (11/16) had nasal swab viral RNA levels above the threshold
146 compared to 16 (16/16) for the naïve exposed group ($p=0.043$ by Fisher's exact test)
147 (Figure 4A, Extended data Table 1). The naïve animals exposed to IN index animals
148 had 6 (6/16) which was not significantly different than oral ($p=0.16$ by Fisher's exact
149 test) (Figure 4A, Extended data Table 1). These data demonstrate decreased SARS-
150 CoV-2 transmission from oral and IN r-Ad-S-vaccinated index to naïve animals. On day

151 5, nasal viral RNA did not differ between groups. However, lung viral RNA and
152 infectious virus were significantly lower in IN r-Ad-S vaccinated compared to
153 unvaccinated hamsters (Figure 4B-D).

154 While oral and IN r-Ad-S vaccination didn't completely prevent transmission, the
155 vaccination likely reduced the effective dose reaching the naïve animals. In a prior virus
156 titering experiment, we demonstrated animals lost more weight and had increased
157 pulmonary inflammation when they received higher titers of SARS-CoV-2 inoculum,
158 suggesting that the severity of disease acquired is dependent on the original infectious
159 dose (Extended data Fig. 3). Similar dose-dependent disease severity findings have
160 been shown by others in hamsters, mice, ferrets and nonhuman primates¹²⁻¹⁶.

161 Reduction in effective dose may have led to a greater proportion of naïve animals
162 exposed to oral r-Ad-S (14/16) and IN r-Ad-S (15/16) vaccinated hamsters gaining
163 weight by the end of the study (Figure 4E). This is compared to naïve animals exposed
164 to the IM (S) vaccinated (8/16) and unvaccinated (10/16) hamsters (Figure 4E) where
165 fewer naïve animals increased in size. Additionally, oral and IN r-Ad-S-vaccinated
166 hamsters had lower lung weights (Figure 4F) and pathology (Figure 4G) scores
167 compared to unvaccinated hamsters. These data demonstrate that oral and IN r-Ad-S-
168 vaccinated animals with a post-vaccination SARS-CoV-2 infection transmit less
169 infectious virus via aerosol to unvaccinated, naïve hamsters than infected but
170 unvaccinated (or IM vaccinated) animals, and that this difference in transmission
171 resulted in less severe clinical outcomes.

172

173 **DISCUSSION**

174 SARS-CoV-2 emerged in late 2019 and quickly spread around the globe, leading to
175 hundreds of millions of cases and over 4 million deaths. Despite reports of decreased
176 viral RNA shedding from mRNA-vaccinated individuals compared to unvaccinated
177 individuals^{7,17}, recent evidence demonstrates that vaccinated individuals can get
178 infected with SARS-CoV-2 and shed infectious virus, which can lead to onward
179 transmission. Improved mucosal responses that stimulate local immunity at the site of
180 SARS-CoV-2 replication may have a much greater impact on human transmission. In
181 this study, we show that oral and IN r-Ad-S vaccination decreases disease in vaccinated
182 hamsters and reduces SARS-CoV-2 transmission to unvaccinated naïve hamsters.

183

184 To induce post-vaccination infection, we infected index animals with a high dose of
185 SARS-CoV-2, and subsequently exposed naïve animals to these vaccinated, infected
186 index animals in an aerosol exposure chamber. We observed faster clearance of viral
187 RNA in oral and IN r-Ad-S-vaccinated compared to mock-vaccinated hamsters.
188 Additionally, aerosol transmission to naïve animals during the 8-hr exposure window
189 was substantially reduced from oral and IN r-Ad-S-vaccinated hamsters as evidenced
190 by reduced nasal swab viral RNA loads in naïve animals one and three days post
191 transmission exposure. We hypothesize that mucosal antibodies in the URT were able
192 to enhance SARS-CoV-2 clearance in vaccinated animals and limit the infectiousness of
193 transmitted aerosols. Consistent with this hypothesis, anti-S IgA in the BAL of the oral
194 and IN r-Ad-S immunized animals was higher than IM or mock vaccinated animals.

195

196 Other groups have also tested SARS-CoV-2 transmission in a hamster model. For
197 example, van Doremalen and colleagues determined that IN-vaccinated hamsters were
198 protected from SARS-CoV-2 when placed in the same cage as unvaccinated hamsters
199 inoculated with 1×10^4 TCID₅₀ of virus¹⁸. These experiments determined whether IN-
200 vaccinated individuals were protected against transmission from unvaccinated
201 individuals. In our study, we tested whether mucosal vaccination reduces the ability of
202 the virus replicating in vaccinated individuals from being transmitted specifically via
203 aerosol to naïve animals. We used a relatively high dose of virus to induce a post-
204 vaccine infection (1×10^5 TCID₅₀) and physically separated animals in the exposure
205 chamber to remove the possibility of contact/fomite exposure as a means of
206 transmission. We show for the first time that mucosal vaccination can reduce SARS-
207 CoV-2 transmission from vaccinated to unvaccinated animals. These data are of high
208 relevance because of its implication in global public health, especially in areas in which
209 a significant percentage of the people are unvaccinated. Additionally, if new variants
210 arise that result in an increase in post-vaccine infections, reducing transmission even in
211 vaccinated populations would contribute to limiting circulating virus. Mucosal vaccination
212 could be considered for implementation not only because they protect the vaccinee, but
213 because they likely have a greater effect on the community as a whole. Reducing
214 SARS-CoV-2 transmission to the unprotected is likely to lead to decreased
215 hospitalization and deaths.

216

217 Our study does have limitations. Firstly, we do not measure mucosal T cell responses
218 which are shown to play a role in limiting SARS-CoV-2 infection at mucosal sites¹⁹.

219 Additionally, the SAR-CoV-2 challenge dose we used was above physiological dose
220 likely to be picked up by an environmental exposure, as evidenced by the high viral
221 RNA load in the nose at 1-day post infection in all index hamsters. It is likely that
222 mucosal immunization would provide greater protection against SARS-CoV-2
223 transmission when lower doses of challenge virus are used. Our study was meant to be
224 a stringent challenge to clearly identify advantages between vaccine groups. For
225 practical reasons, such as the limitation of the number of aerosol transmission
226 chambers, lower dose levels or evaluation at later time points were not done.
227 Additionally, future work should include challenging mucosally-vaccinated hamsters with
228 the Delta variant and other variants of concern.

229 An orally-delivered, temperature-stable SARS-CoV-2 vaccine is ideal for global
230 vaccination, where adequate storage and qualified health care providers maybe in short
231 supply. IN delivery has some of the same advantages, but translating IN SARS-CoV-2
232 vaccination efficacy in humans has proven to be more difficult than in animals^{20,21}.
233 Implementing oral vaccine campaigns around the world has been done, as evidenced
234 by the rotavirus and poliovirus vaccination efforts^{22,23}. We have previously demonstrated
235 that our SARS-CoV-2 clinical candidate vaccine VXA-COV2-1 generated robust
236 humoral immune responses in mice²⁴. Additionally, it was tolerated and immunogenic in
237 a phase I clinical trial where the oral vaccine was delivered to people as tablets
238 (<https://clinicaltrials.gov/show/NCT04563702>) and protected golden hamsters from
239 SARS-CoV-2 challenge when given by oral gavage (Johnson, 2021, submitted). An
240 additional phase 2 study using the vaccine candidate tested in this study and given as a
241 tablet will begin clinical studies shortly. In summary, the data presented here

242 demonstrate that oral immunization is a viable strategy to decrease SARS-CoV-2
243 transmission and disease, and should be considered for vaccination efforts that
244 increase global immunity to SARS-CoV-2.

245

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251 Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Wuhan-Hu-1 with C-
252 Terminal Histidine Tag, Recombinant from Baculovirus, NR-52308. Thanks to Dr. Anne
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254

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267

268 **Contributions**

269 S.N.L., S.J., E.G.B., A.W. and S.N.T. conceived, designed and analyzed the
270 experiments. P.J.K. and H.I. designed and constructed the aerosol transmission
271 chamber. H.I. and A.W. carried out the in vivo aerosol transmission experiments. N.P.
272 and E.G.D. created the vaccine material. C.I.M. and S.N.T. performed the
273 immunological experiments. S.N.L., S.J., A.W. and S.N.T wrote the manuscript with
274 input from all co-authors.

275

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278

279 **Competing Interests**

280 S.N.T, S.J., N.P., E.G.D., C.I.M., and S.N.T. are employees of Vaxart, Inc., and/or have
281 received stock options. H.I., P.J.K, E.G.B, and A.W. are employees of Lovelace

282 Biomedical. EGD and SNT are named as inventors covering a SARS- CoV-2 (nCoV-
283 19) vaccine. SNT is named as an inventor on patent covering the vaccine platform.
284 S.N.L. reports no conflicts.

285

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287

288 **METHODS**

289 *Vaccine constructs*

290 r-Ad-S is a rAd5 vector containing full-length SARS-CoV-2 S gene under control of the
291 CMV promoter. rAd5 vaccine constructs were created based on the published DNA
292 sequence of SARS-CoV-2 publicly available as Genbank Accession No. MN908947.3.
293 The published amino acid sequences of the SARS-CoV-2 S were used to create
294 recombinant plasmids containing transgenes cloned into the E1 region of Adenovirus
295 Type 5 (rAd5)²⁵, using the same vector backbone used in prior clinical trials for oral rAd
296 tablets^{11,26}. All vaccines were grown in the Expi293F suspension cell-line (Thermo
297 Fisher Scientific) and purified by CsCl density centrifugation. The S Protein vaccine
298 was provided by BEI Resources, NR-52308. Spike Glycoprotein (Stabilized) from
299 SARS-Related Coronavirus 2, Recombinant from Baculovirus, NR-52308.

300 *Animals and study design*

301 Male Syrian Hamsters (*Mesocricetus auratus*) approximately 12-14 weeks of age with a
302 weight range of 106-136g were sourced from Charles River Laboratory. Animal work
303 was performed at Lovelace Biomedical, with approval from the Institutional Animal Care

304 and Use Committee. Hamsters were singly housed in filter-topped cage systems and
305 were supplied with a certified diet, filtered municipal water, and dietary and
306 environmental enrichment. The study was powered to compare viral RNA loads in naïve
307 hamsters exposed to vaccinated, SARS-CoV-2 infected index animals between vaccine
308 groups, where beta was set to 0.2, alpha=0.05. Assuming an attack rate of 80%
309 infected in the placebo group and a vaccine efficacy of 70%, an N=15 was calculated
310 with continuity correction²⁷. The study was rounded to 16 naïve, 4 index to maintain the
311 1:4 ratio. The study was not powered to directly compare index groups, but many
312 statistical significances were achieved with N=4.

313 All r-Ad-S vaccinations were given at a dose of 1e9 IU (1:100 of a human dose¹¹). Oral
314 vaccine was delivered by gavage in 300µl of PBS subsequent to delivery of 300µl 7.5%
315 bicarbonate buffer. IN vaccination was delivered in PBS by pipette, 50µl/nostril. The
316 control group received PBS via oral gavage. One group received recombinant SARS-
317 CoV-1 S protein, made in insect cells, by the IM immunization group (BEI, #NR-722). All
318 index animals were challenged by IN inoculation of SARS-CoV-2 at approximately
319 1E+05 TCID₅₀/animal in 300µl volume seven weeks after the initial vaccination. Index
320 animals were then housed for 24 hrs individually before placing in aerosol chamber.
321 Each vaccine/index group had 4 animals, and matched with a corresponding N=16
322 naïve exposed animals (1:4 ratio of index to naïve, with 1 index animal exposed to 4
323 naïve in a chamber setup). All animals were sacrificed five-days post inoculation (index)
324 or aerosol chamber exposure (naïve) for terminal assays.

325 *Transmission chamber*

326 The aerosol transmission chamber was based on previous transmission work¹⁵ and
327 adapted for hamsters. The chamber consists of multiple subchambers that support
328 unidirectional flow. Starting from left to right in Extended data Fig. 2: 1) chamber for the
329 index hamster, 2) a connector chamber, and 3) a chamber for the naïve hamster(s). All
330 chambers were fitted with access doors with air tight seals and appropriate safety
331 features to ensure animals cannot interact with fans, sampling, flow, etc. Approximate
332 dimensions of the first chamber (1) is 4"x10"x9" (length, width, height). The
333 unidirectional flow (5 L/min) was controlled by regulated house exhaust flow from the 3rd
334 chamber. This drew room air into chamber 1 via a HEPA filter (F; HEPA Vacuum Filter
335 Compatible with Kenmore 86880, EF-2, Panasonic MC-V194H Vacuum Cleaner).
336 Chamber 1 was also fitted with a recirculating fan (D; ANVISION 40mm x 10mm DC 5V
337 USB Brushless Cooling Fan, Dual Ball Bearing, Model YDM4010B05) to ensure
338 homogeneity of the aerosol prior to transitioning into chambers 2 and 3. The connector
339 chamber (chamber 2) (approximate dimensions of 4"x5"x5"), connected chambers 1
340 and 3 in order to separate the hamsters, but allows for air passage. Chamber 3
341 (approximately 10"x10"x9") houses the naïve hamsters. A wire mesh screen with 0.25"x
342 0.25" holes was placed at each end of chambers 1 and 3 to prevent hamsters moving to
343 another chamber. Additionally, chamber 2 was raised off the ground to prevent feces
344 and/or urine from moving between chambers.

345 *Assessment of infectious SARS-CoV-2 load in lung homogenate*

346 Lung tissue samples from euthanized hamsters infected with SARS-CoV-2 were
347 collected, weighed and homogenized with beads using a Tissue Lyser (Qiagen). Each
348 sample was serially diluted 10-fold in Dulbecco's Modified Eagle's Medium containing

349 2% fetal bovine serum (FBS) and 1% Pen-Strep solution. VeroE6 cell monolayers at \geq
350 90% confluency in 96-well plates were rinsed with PBS. The plates were inoculated with
351 100 μ l of each sample dilution in five technical replicates. Negative control wells
352 contained dilution medium only. The plates were incubated at 37°C and 5% CO₂ for 72
353 hours. Cytopathic effect was scored after fixing the cell monolayers with 10% formalin
354 and staining with 0.5% crystal violet. Viral load was determined in tissue culture
355 infectious dose 50%/ml of lung homogenate using the Reed and Muench method²⁸.
356 Infectious virus titers in infected lungs were expressed as TCID₅₀/gr of tissue.

357 *SARS-CoV-2 Surrogate Neutralization Assay*

358 Neutralizing antibodies were measured using a SARS-CoV-2 Surrogate Virus
359 Neutralization Test Kit (Genscript). Hamster sera was diluted from 1:20 to 1:500
360 incubated at a 1:1 ratio with HRP conjugated SARS-CoV-2 RBD protein for 30 min at
361 37°C. Following incubation, 100 μ l was added to hACE2 pre-coated plates and
362 incubated for 15 min at 37 °C. Plates were washed 4x with 260 μ l per well of supplied
363 wash solution, followed by the addition of 100 μ l per well of supplied TMB solution.
364 Plates were developed for 15 min at RT in the dark before development was stopped
365 with 50 μ l per well of supplied stop solution. OD was measured at 450 nm with a
366 Spectra Max M2 microplate reader.

367 *IgG ELISA*

368 Purified SARS-CoV-2 S1 protein (GenScript) in carbonate buffer, pH 9.4, (Thermo
369 Scientific) as coated onto microtiter plates (Maxisorp, Nunc) at 1 μ g/ml and incubated
370 overnight at 4°C before blocking with 100 μ l of PBS-0.05% Tween (PBST) + 1% BSA

371 for 1 hr. Serum samples were serially diluted in PBST. After a 2-hr incubation at room
372 temperature (RT) the plates were washed 3x with PBST, followed by the addition of
373 100µl per well of 1:3000 goat anti-hamster IgG-HRP (Thermo Fisher) in PBST + 1%
374 BSA. Plates were incubated at RT for 1 hr before washing 3x with PBST before the
375 addition of 50µl per well of TMB substrate (Rockland). The plates were developed for 10
376 minutes, then stopped with 50µl per well of 2M sulfuric acid. Optical densities (OD) were
377 measured at 450nm with a Spectra Max M2 microplate reader.

378 *IgA MSD*

379 S1 protein was biotinylated according to manufacturer's instructions (EZ-link,
380 Thermofisher), and was conjugated to a U-Plex MSD linker (Mesoscale Diagnostics).
381 The linked S1 protein was coated on 2-spot U-plex 96 well plates (Mesoscale
382 Diagnostics) for a final concentration of 66nM per well over night. The next day the
383 plates were blocked with PBS-T for 1 hour prior to the addition of hamster serum or
384 BAL. Samples from each individual hamster acquired on D0, D28 and D55 were
385 quantified on the same plate. The serum samples were diluted to 1:200 and the BAL
386 samples were added to the plate neat. Following 2-hr sample incubation, the plate was
387 washed and SULFO-TAG (MSD) anti-hamster IgA (Brookwood Biomedical) detection
388 antibody was added. The plate was washed and 1X MSD read buffer was added and
389 each plate was analyzed using MSD QuickPlex.

390 *SARS-CoV-2 Challenge*

391 All animals were challenged by IN inoculation of 1×10^5 TCID₅₀/animal SARS-CoV-2
392 (isolate USA-WA1/2020) at 100µl/nostril eight weeks post initial vaccination. SARS-

393 CoV-2, isolate USA-WA1/2020, was sourced from BEI Resources and propagated in
394 Vero E6 African Green Monkey kidney cells (BEI, catalog #N596) at the University of
395 Texas Medical Branch (UTMB). Virus was stored in a biosafety level 3 compliant facility.

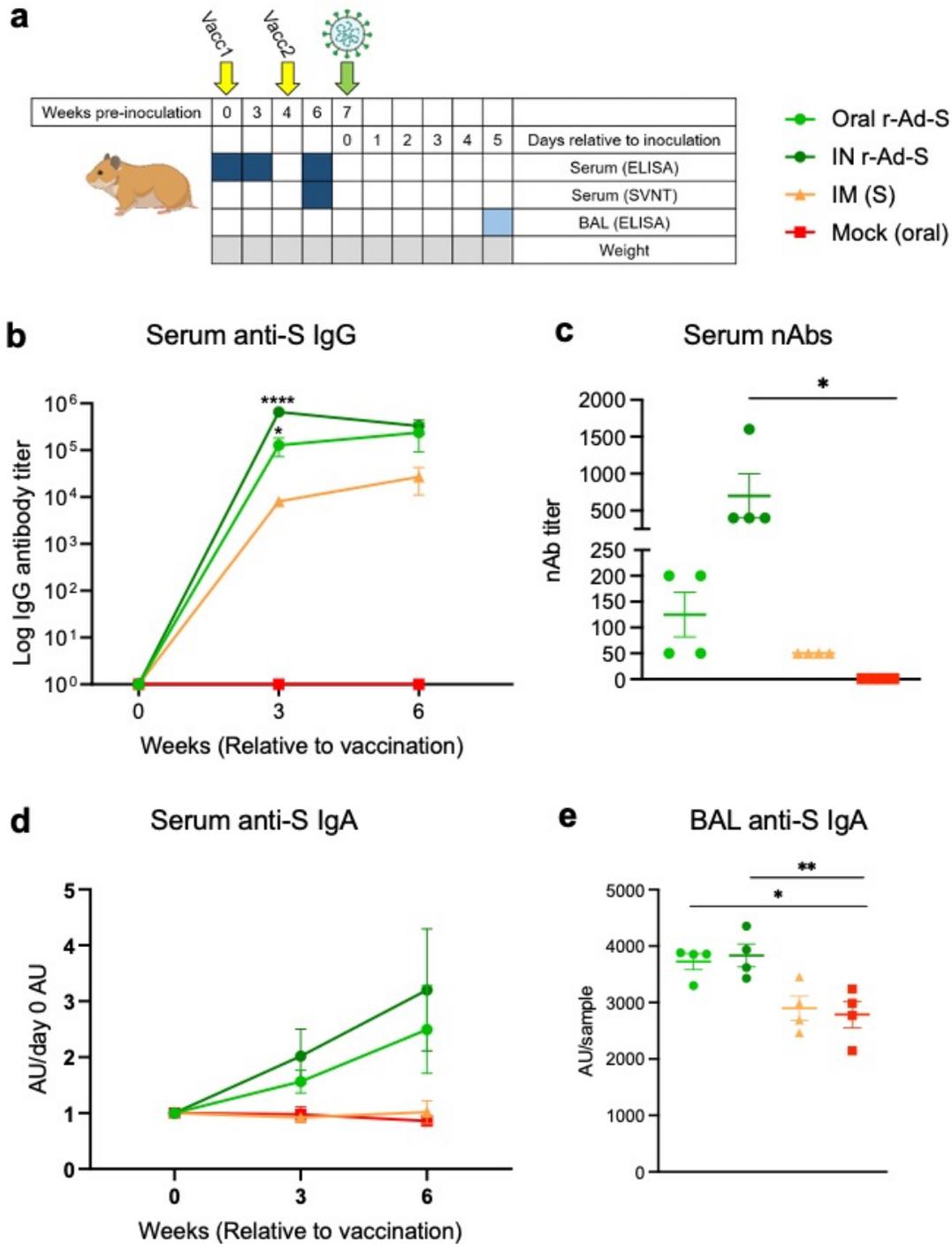
396 *Detection of SARS-CoV-2 in lung homogenates by qRT-PCR*

397 Lung samples were weighed and homogenized with beads using a Tissue Lyser
398 (Qiagen) in 1 ml of TRI reagent, before RNA was isolated and purified from tissue
399 samples using the Direct-Zol 96- RNA kit (Zymo Research). Copies of SARS-CoV-2 N
400 were measured by qRT-PCR TaqMan Fast Virus 1-step assay (Applied Biosystems).
401 SARS-CoV-2 specific primers and probes from the 2019-nCoV RUO Assay kit
402 (Integrated DNA Technologies) were used: (L Primer:TTACAAACATTGGCCGCAAA; R
403 primer: GCGCGACATTCCGAAGAA; probe:6FAM-
404 ACAATTTGCCCCCAGCGCTTCAG-BHQ-1). Reactions were carried out on a
405 Stratagene MX3005P or BioRad CFX384 Touch instrument according to the
406 manufacturer's specifications. A semi-logarithmic standard curve of synthesized SARS-
407 CoV-2 N gene RNA (LBRI) was obtained by plotting the Ct values against the logarithm
408 of cDNA concentration and used to calculate SARS-CoV-2 N gene in copies per gram
409 of tissue.

410 *Gross pathology scoring*

411 Gross necropsy observations of the lung will be recorded in Provantis using consistent
412 descriptive terminology to document location(s), size, shape, color, consistency, and
413 number. Gross observations will include a severity grade for red discoloration of the
414 lung (likely to be associated with pneumonia) based on a 0 to 4 scale indicating percent

415 of whole lung affected: none (no grade), minimal (1), mild (2), moderate (3), marked (4)
416 correlating to 0, 1-25, 26-50, 51-75, and 76-100% affected, respectively.



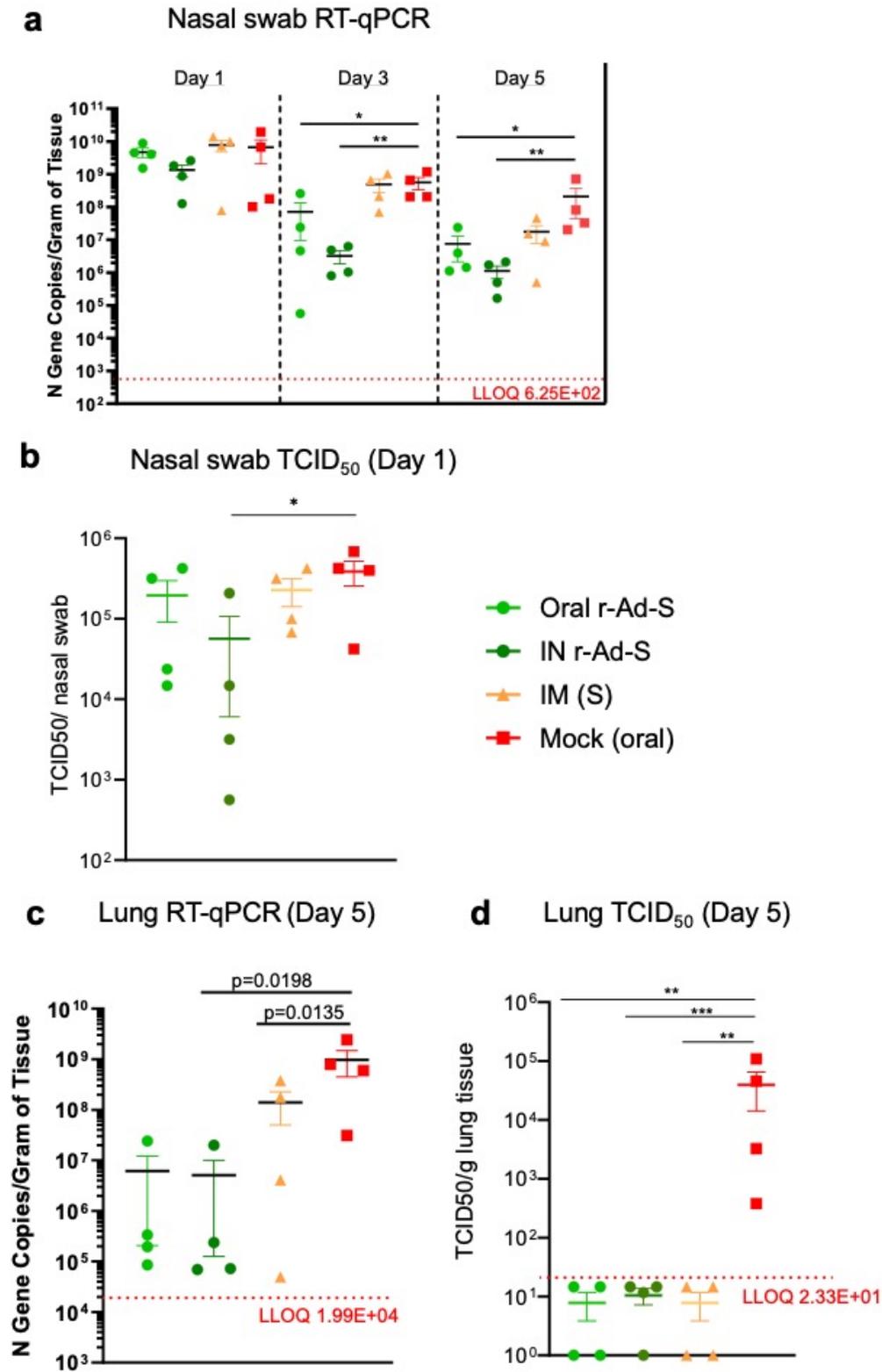
418

419 **Fig.1: Oral and intranasal r-Ad-S vaccination induced robust IgG and IgA**
420 **antibodies in golden hamsters**

421 **a-d**, Index hamsters were immunized with oral r-Ad-S, intranasal (IN) r-Ad-S,
422 intramuscular (IM) spike (S) or mock (oral) ($n = 4$ per group) and inoculated with SARS-
423 CoV-2 seven weeks later. **a**, Experimental design schematic. **b**, Serum anti-S IgG
424 antibody titers were measured at weeks 0, 3 and 6 post immunization via ELISA. **c**,
425 SARS-CoV-2 neutralizing antibody (nAb) titers were measured in serum at week 6. **d**,
426 Serum anti-S IgA antibody titers were measured (AU/sample) at weeks 0, 3 and 6 post
427 immunization via Meso scale discovery (MSD) and normalized to day 0 AU values. **e**,
428 anti-S IgA antibodies were measured in bronchoalveolar lavage (BAL) fluid via MSD.
429 Data were analyzed by a one-way ANOVA and Dunnett's multiple comparisons. Error
430 bars represent the standard error of the mean (SEM). * $P < 0.05$, ** $P < 0.01$, **** $P <$
431 0.0001.

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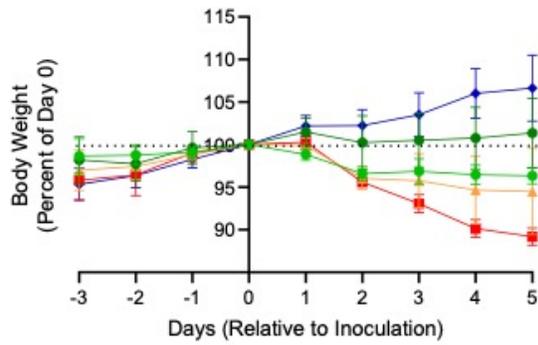
435 **Fig.2: Oral and intranasal r-Ad-S vaccination decreased SARS-CoV-2 RNA and**
436 **infectious virus in nasal swabs and lungs**

437 **a**, Nasal swabs were collected on days 1, 3 and 5 in index hamsters and viral RNA
438 loads in these samples were determined by quantitative reverse transcription PCR
439 (qRT-PCR) of the N gene. **b**, Nasal swabs were collected on day 1 and infectious virus
440 titers were determined by TCID₅₀. **c**, Lung tissue was collected at necropsy (day 5) and
441 RNA was isolated for SARS-CoV-2 detection by qRT-PCR of the N gene and **d**,
442 infectious viral titers by TCID₅₀. The dotted line represents Lower Limit of Quantification
443 (LLOQ), with data below the limit of detection plotted at ½ LLOQ. Data were analyzed
444 by a one-way ANOVA and Dunnett's multiple comparisons. Error bars represent the
445 SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

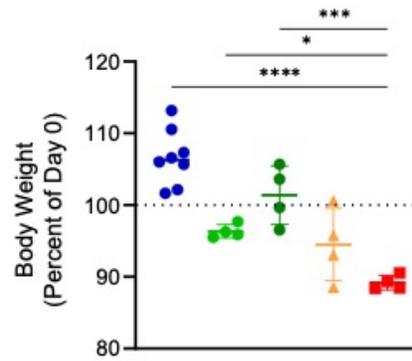
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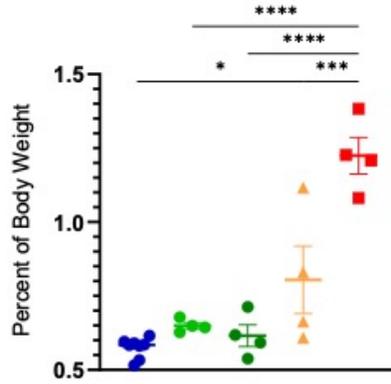
a Body weights



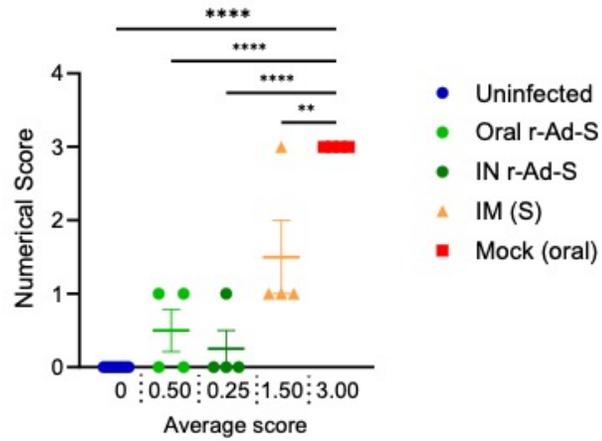
b Terminal body weights



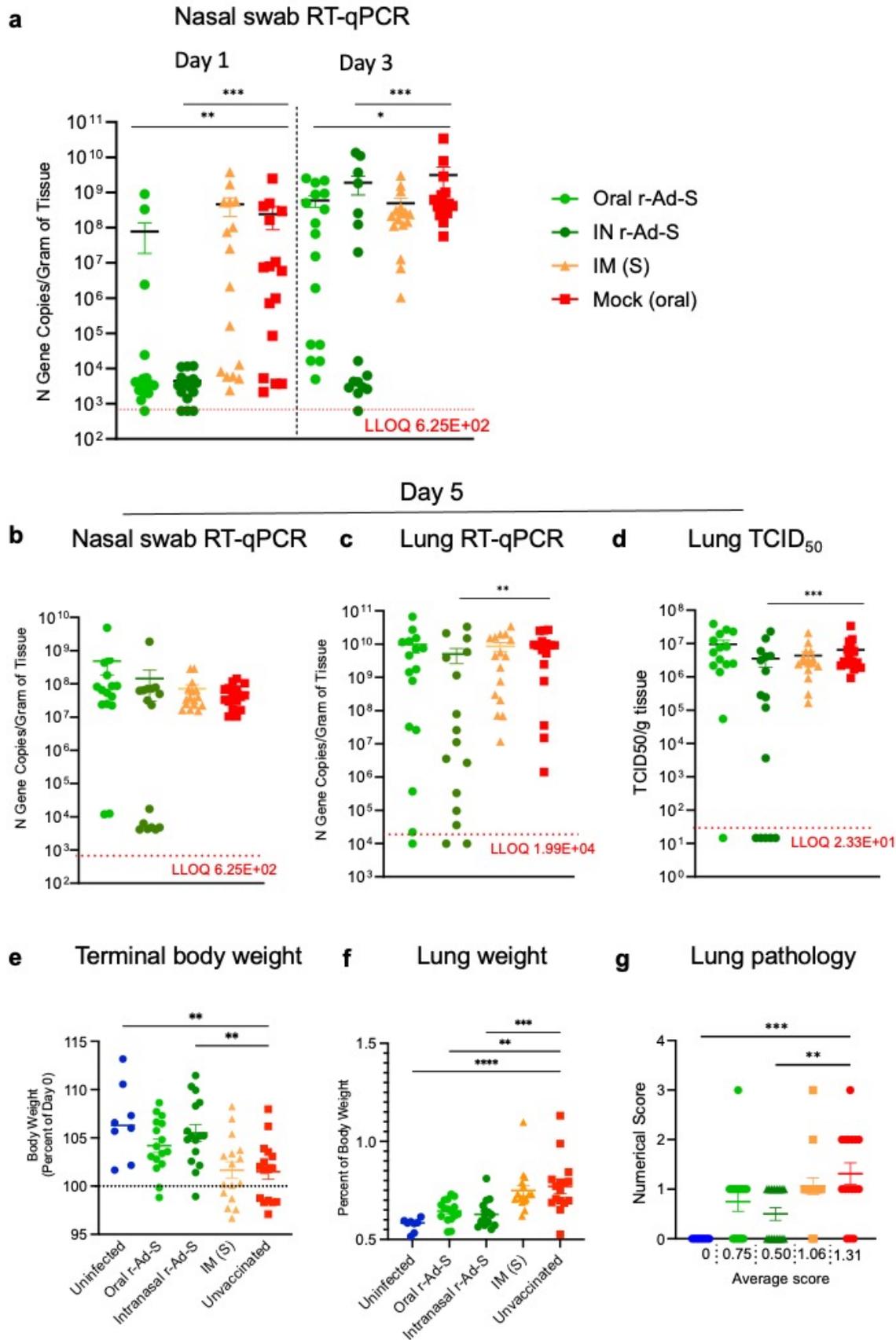
c Lung weights



d Lung pathology



449 **Fig.3: Oral and intranasal r-Ad-S vaccination reduced disease indicators**
450 **including body weight, lung weight and lung pathology scores in index hamsters.**
451 **a**, Daily weight change and **b**, terminal body weights were determined by the percent of
452 day 0 (relative to SARS-CoV-2 inoculation). **c**, terminal lung weights and **d**, lung
453 pathology scores were determined. Severity grade for red discoloration of the lung was
454 based on a 0 to 4 scale indicating percent of whole lung affected: none (no grade),
455 minimal (1), mild (2), moderate (3), marked (4) correlating to 0, 1-25, 26-50, 51-75, and
456 76-100% affected, respectively. Data were analyzed by a one-way ANOVA and Tukey's
457 multiple comparisons. Error bars represent the SEM. * $P < 0.05$, ** $P < 0.01$, *** $P <$
458 0.001 , **** $P < 0.0001$.



460 **Fig.4: Oral and intranasal SARS-CoV-2 vaccination decreased SARS-CoV-2**
461 **transmission and clinical indicators of disease.**

462 **a**, Nasal swabs were collected in naïve animals on days 1, 3 and **b**, 5 after exposure to
463 index, infected hamsters in aerosol chamber. Viral RNA loads in these samples were
464 determined by quantitative reverse transcription PCR (qRT-PCR) of the N gene. **c**, Lung
465 tissue was collected at necropsy (day 5) and RNA was isolated for SARS-CoV-2
466 detection by qRT-PCR of the N gene and **d**, infectious viral titers were determined by
467 TCID₅₀. **a-d**, The dotted line represents LOD, with data below the limit of detection
468 plotted at ½ LOD. Data were analyzed by a one-way ANOVA and Dunnett's multiple
469 comparisons. **e**, terminal body weights were determined by the percent of day 0
470 (relative to SARS-CoV-2 inoculation). **f**, Terminal lung weights and **g**, lung pathology
471 scores were determined. Severity grade for red discoloration of the lung was based on a
472 0 to 4 scale indicating percent of whole lung affected: none (no grade), minimal (1), mild
473 (2), moderate (3), marked (4) correlating to 0, 1-25, 26-50, 51-75, and 76-100%
474 affected, respectively. **e-g**, Data were analyzed by a one-way ANOVA and Tukey's
475 multiple comparisons. **a-g**, Error bars represent the SEM. **P* <0.05, ** *P* <0.01, *** *P* <
476 0.001, **** *P* <0.0001.

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