Cell Reports Medicine, Volume *1*

Supplemental Information

Oral SARS-CoV-2 Inoculation Establishes Subclinical Respiratory Infection with Virus Shedding in Golden Syrian Hamsters

Andrew Chak-Yiu Lee, Anna Jinxia Zhang, Jasper Fuk-Woo Chan, Can Li, Zhimeng Fan, Feifei Liu, Yanxia Chen, Ronghui Liang, Siddharth Sridhar, Jian-Piao Cai, Vincent Kwok-Man Poon, Chris Chung-Sing Chan, Kelvin Kai-Wang To, Shuofeng Yuan, Jie Zhou, Hin Chu, and Kwok-Yung Yuen

SUPPLEMENTARY TABLES AND FIGURES

Table S1. Semi-quantitative histopathological scoring criteria, related to STAR methods

Categories	**Score criteria**	**Score (Total score=27 per lung)**[a]
General evaluation	Assessment percentage of lung damage area under 4x objective lens	0 none 1 focal lung inflammation within one lobe or <30% of total lung cutting area[b] 2 diffuse lung inflammation within one lobe or involving 30-70% total lung cutting area 3 diffuse lung inflammation involving more than one lobe or 70% total lung cutting area
Bronchioles	Assessing appearance and severity of peribronchiolar infiltration, intra-bronchiolar wall infiltration and bronchiolar epithelial cell death/desquamation	0 none 1 peribronchiolar infiltration 2 bronchiolar epithelial cell death 3 bronchiolar wall infiltration and or severe epithelium desquamation
Alveoli	Assessing the appearance, scope involved, and severity of alveolar septal infiltration, alveolar space infiltration, alveolar space exudation and hemorrhage	0 none 1 only alveolar wall thickening 2 focal area alveolar space infiltration, exudation or hemorrhage involving <30% of lung cutting area 3 diffuse alveolar space infiltration, exudation or hemorrhage involving >70% of lung cutting area
Vasculature[4]	Severity of vasculature inflammation	0 none, 1 only perivascular edema and or perivascular infiltration 2 mild infiltration within the vessel wall without endothelium infiltration 3 intensive infiltration into the smooth muscle, or infiltration beneath the endothelium

[a] Total score = the scores of general assessment × the scores accumulated from individual category assessment

[b] More than one lung lobes should be examined for histological assessment, if only one lobe were studied, percentage of the total lung cutting area would be used for scoring.

Table S2. Semi-quantitative lung histological scores of SARS-CoV-2 infected hamster, Related to Figure 5

		Histology score (average score)			
Inoculum PFU (route)	**Sample No.**	**2dpi**	**P value[a]**	**4dpi**	**P value[a]**
10^2 (i.n.)	3	4, 2, 4 (3.3)	N/A	18, 27, 27 (24)	N/A
10^3 (i.n.)	3	12, 6, 9 (9)	0.019	18, 27, 27 (24)	0.50
10^4 (i.n.)	3	14, 16, 21 (17)	0.0017	27, 27, 18 (24)	0.50
10^5 (i.n.)	3	27, 27, 27 (27)	<0.0001	27, 27, 27 (27)	0.19
10^5 (oral)	4	ND	N/A	9, 8, 8, 6 (7.8)	<0.0001[b]

[a] comparing with 10^2 PFU.
[b] comparing with 10^5 PFU i.n. at 4dpi.
ND not determined
N/A not applicable

Table S3. Numbers of hamsters included in this study, related to STAR methods

	Time post infection	No. sacrificed	No. for study[b]	No. for virus shedding[c]
Oral inoculation (Total No. hamsters[a] = 15)	12hpi	3	3	ND
	4dpi	6	6	12
	7dpi	3	3	6 (at 6dpi)
	14dpi	3	0	3
Intranasal inoculation (Total No. hamsters[a] =11)	12hpi	3	3	ND
	4dpi	3	3	8
	7dpi	0	0	5 (at 6dpi)
	14dpi	5	0	5

[a] The number of hamsters is the sum from three independent experiments.
[b] The number included in viral load, histology and cytokine studies.
[c] The number of hamsters from which oral swabs and faeces were collected
ND, not determined.

Table S4. Primers and probes sequences, Related to STAR methods

Gene name	**Forward primer (5' to 3')**	**Reverse Primer (5' to 3')**
RdRp	CGCATACAGTCTTRCAGGCT	GTGTGATGTTGAWATGACATGGTC
	Probe (5' to 3'): FAM- TTAAGATGTGGTGCTTGCATACGTAGAC -lABkFQ	
β-actin	ATGGCCAGGTCATCACCATTG	CAGGAAGGAAGGCTGGAAAAG
	Probe (5' to 3'): Cy5-AGCGGTTCCGTTGCCCTGAG-IABkFQ	
IL-6	TGTCTTCTTGGGACTGCTGC	CCAAACCTCCGACTTGTTGA
TNF-α	CACCCACCGTCAAGGATTCA	TTGGCTGGGCAATGAAGAGT
IFN-α	AGACTGGGAGTTGCCTGTGA	GAGGAATCCAGGGCTTTCCAG
IFN-γ	TGCATCTTGGCTTTGTTGCTC	TCCCCTCCATTCACGACATC
MIP-1α	GGTCCAAGAGTACGTCGCTG	GAGTTGTGGAGGTGGCAAGG
RANTES	TCAGCTTGGTTTGGGAGCAA	TGAAGTGCTGGTTTCTTGGGT
IP-10	TACGTCGGCCTATGGCTACT	TTGGGGACTCTTGTCACTGG

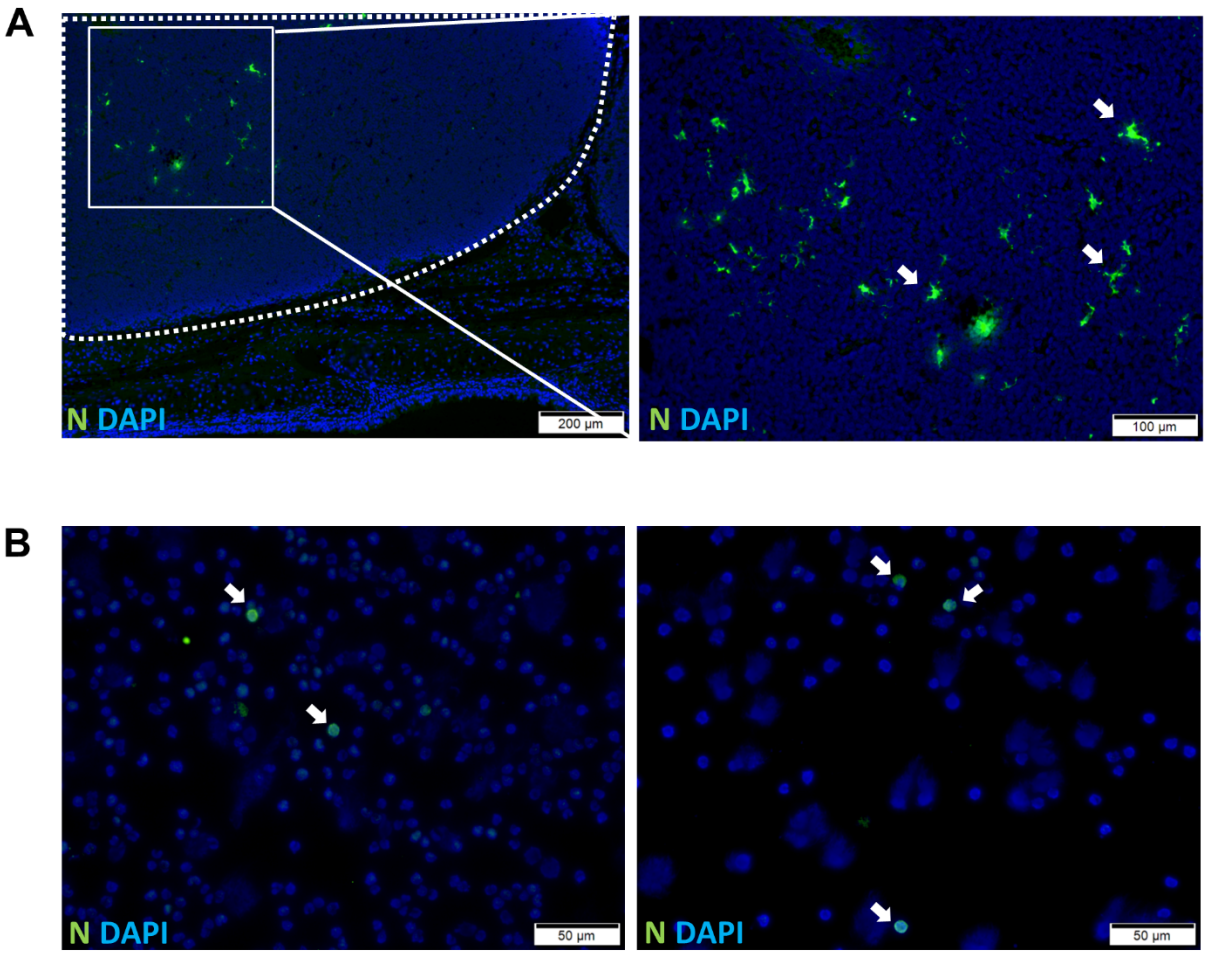

Figure S1. The expression Viral N protein in lymph nodes and peripheral white blood cells from orally inoculated hamsters, Related to Figure 3

Hamsters were inoculated with 10^5PFU of SARS-CoV-2 by oral inoculation. (A) One lymph node (area within dashed line) associated with oropharynx tissue taken at 4dpi was stained with viral N protein (arrows in magnified images). (B) peripherial mononuclear cells isolated from two hamsters at 4dpi after oral inoculation were stained viral N protein (arrows). N protein, Green; DAPI, blue.

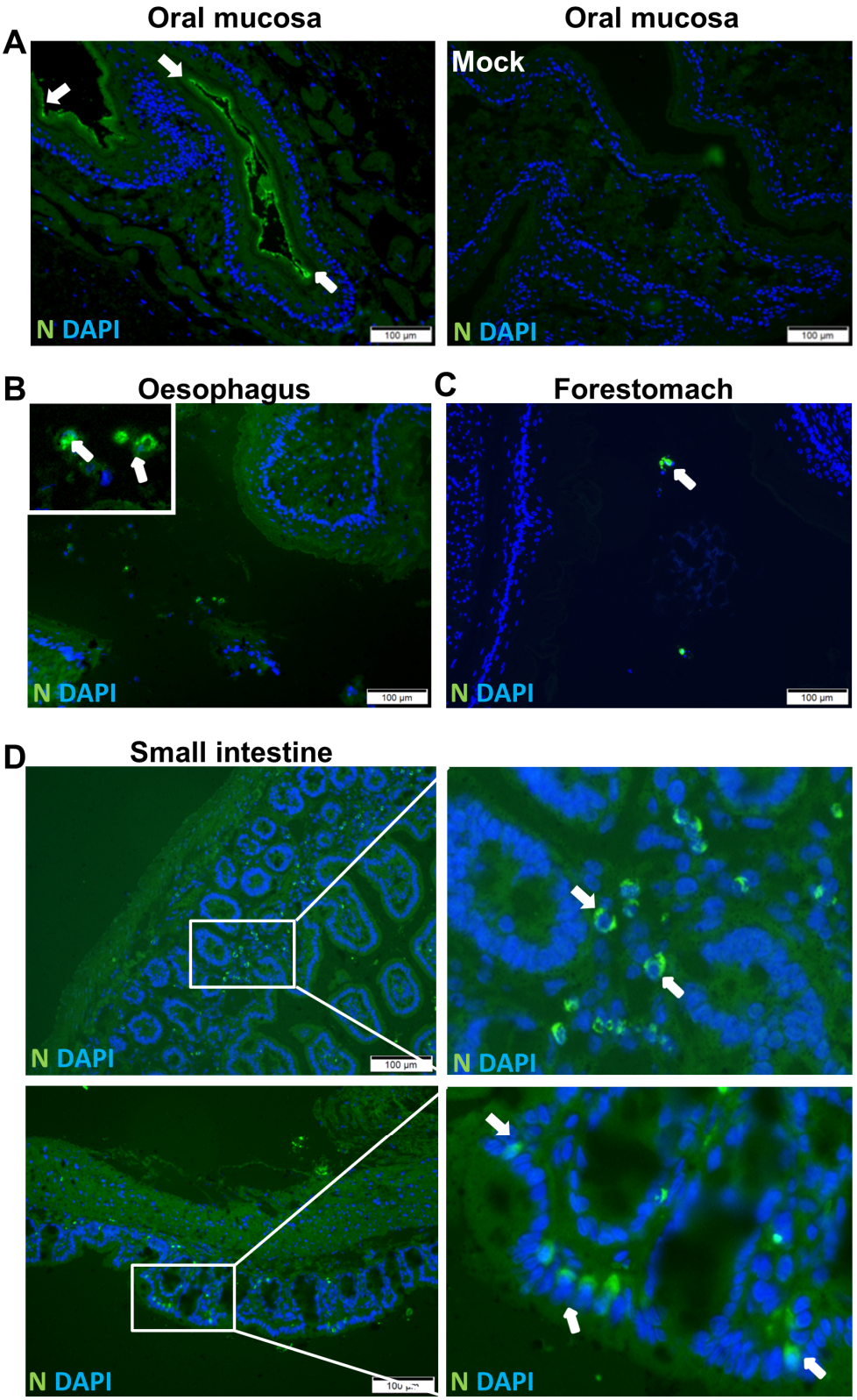

Figure S2. Viral N protein in gastrointestinal tissue of orally inoculated hamsters, Related to Figure 3.
Hamsters were inoculated with 10^5PFU of SARS-CoV-2 by oral inoculation, tissues taken at 4dpi. (A) Tissue sections of oral mucosa showed N protein positive on the surface (arrows) but not in the epithelial cells; the image on the right was the oral mucosa of mock infected hamster stained with anti-SARS-CoV N protein. (B) oesophagus section (C) forestomach section showed a few N protein positive cells associated with luminal debris. No N protein expression cells were detected in the epithelium. (D) Small intestinal sections showed a few histiocytes expressing N protein in the larmina propria (upper, arrows); and a few enterocytes expressing viral N protein (lower, arrows). N protein, Green; DAPI, blue.

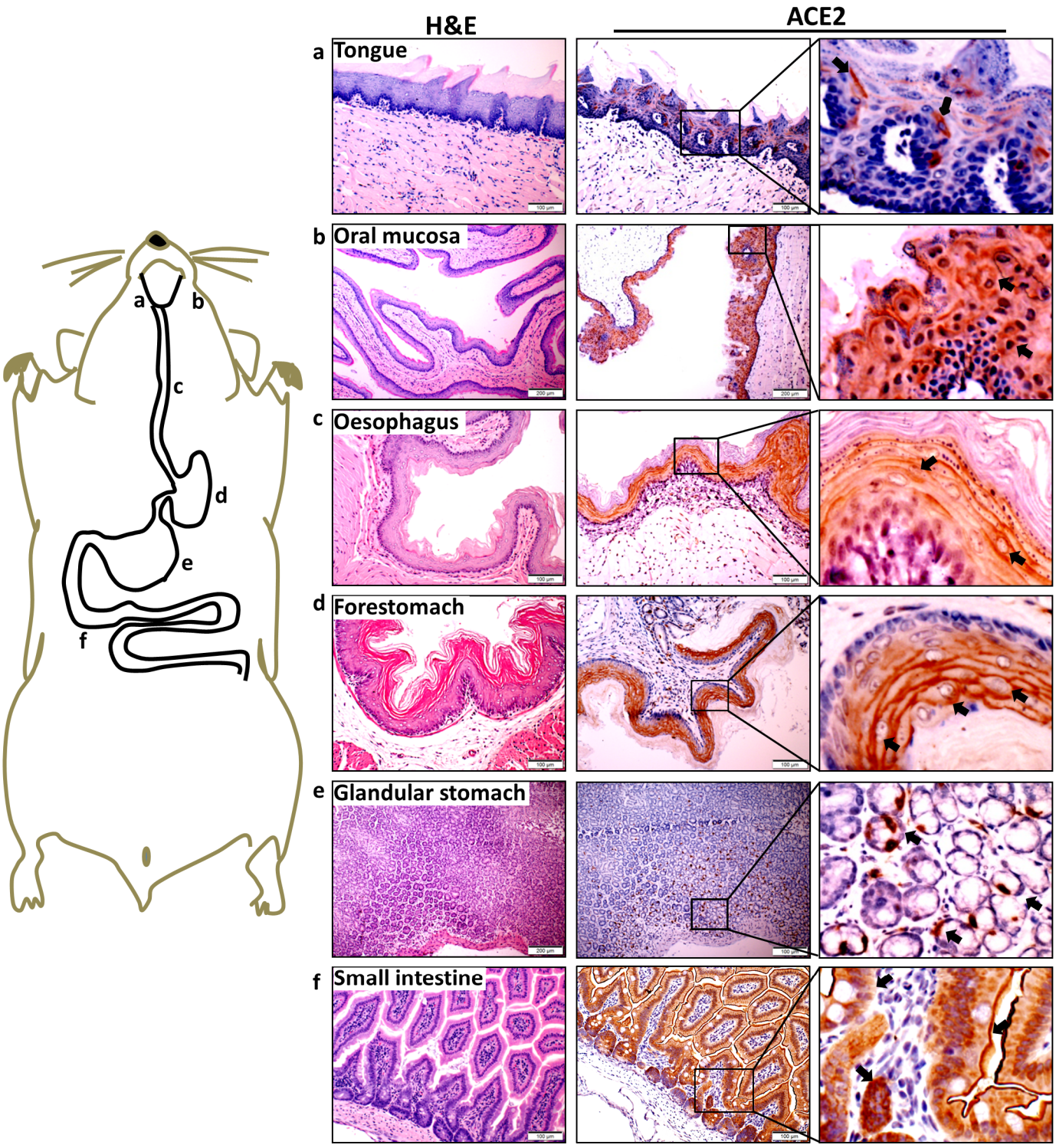

Figure S3. Images of H&E stained hamster gastrointestinal tissues and immunohistochemistry stained ACE2 expression, Related to Figure 3.

Illustration of the studied hamster tissues (left). a-c, representative H&E images and ACE2 expression in tongue, oral mucosa and oesophagus, respectively. The mucosa of these tissues is lined with keratinizing stratified squamous epithelium. ACE2 stained in brown localized in the cytoplasmic in the middle layer (solid arrows in magnified image). d, forestomach which is also lined with keratinizing stratified squamous epithelium, ACE2 expressed in the cells of middle layer (solid arrows in magnified). e, H&E images and ACE2 expression in the gland cells of glandular stomach (solid arrows in magnified image). f, small intestine showed abundant ACE2 expression in enterocytes.