

Nasal mucosa exploited by SARS-COV-2 for replicating and shedding during reinfection

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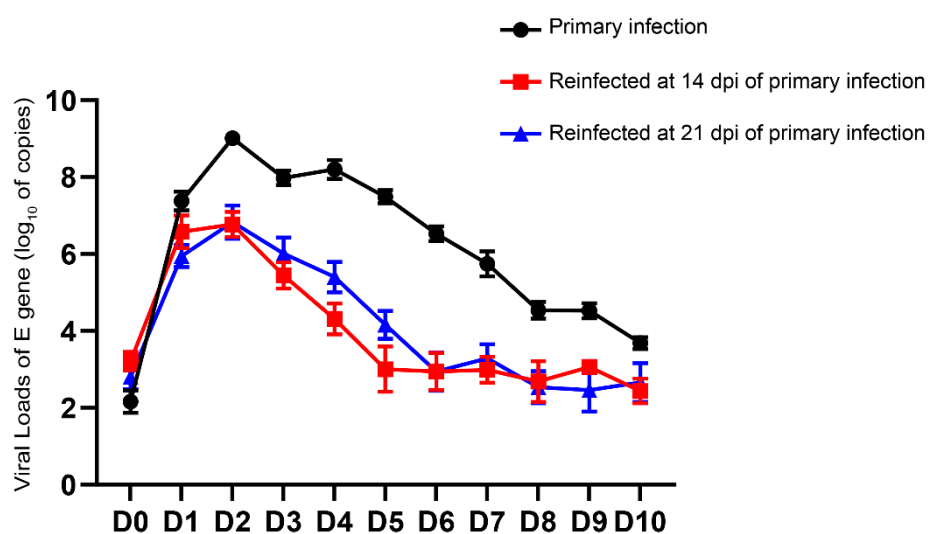


Figure S1. Viral shedding in 1 ml nasal washes from 0 dpi to 10 dpi in the primary infection and re-infection.

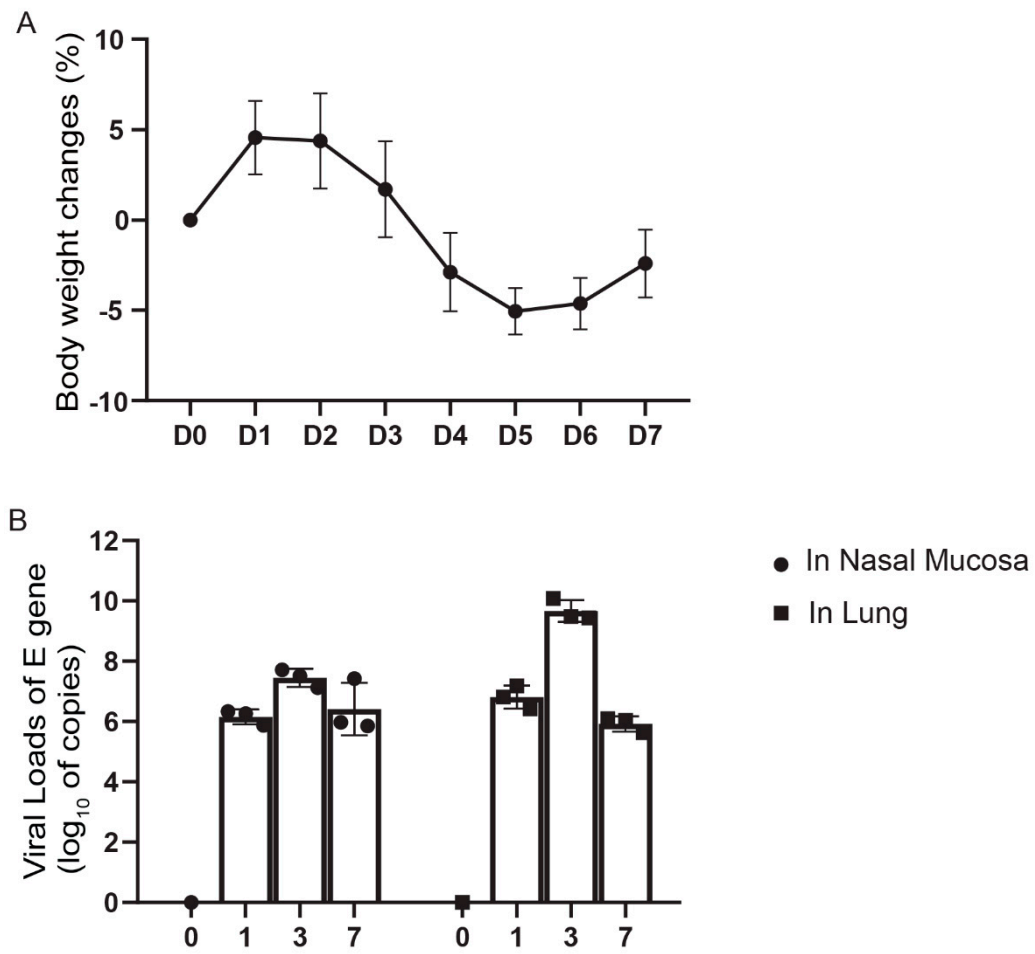


Figure S2. The infectivity in healthy Syrian hamsters of the shedding from upper respiratory tract in the primary infection and reinfection.

Primary infection	TTGTTTGTTCCTTCTGTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCGAAGCTCAATTACCCCTGCATACACTAATTCCTTTCACAC	100
Reinfection at 14 dpi	TTGTTTGTTCCTTCTGTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCGAAGCTCAATTACCCCTGCATACACTAATTCCTTTCACAC	100
Reinfection at 21 dpi	TTGTTTGTTCCTTCTGTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCGAAGCTCAATTACCCCTGCATACACTAATTCCTTTCACAC	99
Consensus	atggttggtttttctgtttttattgccaactagctctctagtcagtggttaattcttaaacacaggaactcaattaccocctgcatacactaattctcttccacac	
Primary infection	GTGGTGTTTATTACCTGACAAAGTTTTCAGATCTCAGTTTACATCTCAACTCAGGACTGTTCCTTACCTTCTCTTTCCAAATGTACTTGGTTCATGCG	200
Reinfection at 14 dpi	GTGGTGTTTATTACCTGACAAAGTTTTCAGATCTCAGTTTACATCTCAACTCAGGACTGTTCCTTACCTTCTCTTTCCAAATGTACTTGGTTCATGCG	200
Reinfection at 21 dpi	GTGGTGTTTATTACCTGACAAAGTTTTCAGATCTCAGTTTACATCTCAACTCAGGACTGTTCCTTACCTTCTCTTTCCAAATGTACTTGGTTCATGCG	199
Consensus	gtggtgtttattaccctgacaaagttttcagatcctcagttttacatccaactcaggactgttcttacccttctcttccaatgttacttgggtccatgctc	
Primary infection	TATACATGTCCTCGGACCAATGGTACTAAGAGGTTTGATAACCCCTGTCTACCATTTAATGATGGTGTTTATTTTGCTTCCACTGAGAAGCTCTAACATA	300
Reinfection at 14 dpi	TATACATGTCCTCGGACCAATGGTACTAAGAGGTTTGATAACCCCTGTCTACCATTTAATGATGGTGTTTATTTTGCTTCCACTGAGAAGCTCTAACATA	300
Reinfection at 21 dpi	TATACATGTCCTCGGACCAATGGTACTAAGAGGTTTGATAACCCCTGTCTACCATTTAATGATGGTGTTTATTTTGCTTCCACTGAGAAGCTCTAACATA	299
Consensus	tatacatgtctctgggaccaaagtggtactaagaggtttgataacccctgtcctaccatttaatgatggtgtttattttgtcttccactgagaaggtctaacata	
Primary infection	ATAAGAGGCTGGATTTTGTGTACTACTTTAGATTGGAAGACCCAGTCCCTACTTATTTGTTAATAACGCTACTAATGTGTATTAAAGCTGTGAATTC	400
Reinfection at 14 dpi	ATAAGAGGCTGGATTTTGTGTACTACTTTAGATTGGAAGACCCAGTCCCTACTTATTTGTTAATAACGCTACTAATGTGTATTAAAGCTGTGAATTC	400
Reinfection at 21 dpi	ATAAGAGGCTGGATTTTGTGTACTACTTTAGATTGGAAGACCCAGTCCCTACTTATTTGTTAATAACGCTACTAATGTGTATTAAAGCTGTGAATTC	399
Consensus	ataagaggtggatttttgggtactactttagattcgaagaccagctccctacttattgttaataacgctactaatgtgtttataaagctctgtgaatttc	
Primary infection	AAITTTGTAATGATCCATTTTGGGTGTTTATTACACAAAAACAACAAAGTTGGATGGAAGTGAGTTCAGAGTTTATCTAGTGCGAATAATTGCAC	500
Reinfection at 14 dpi	AAITTTGTAATGATCCATTTTGGGTGTTTATTACACAAAAACAACAAAGTTGGATGGAAGTGAGTTCAGAGTTTATCTAGTGCGAATAATTGCAC	500
Reinfection at 21 dpi	AAITTTGTAATGATCCATTTTGGGTGTTTATTACACAAAAACAACAAAGTTGGATGGAAGTGAGTTCAGAGTTTATCTAGTGCGAATAATTGCAC	499
Consensus	aattttgtaatgatccatttttgggtgtttattaccacaaaaacaaacaaagttggatggaaagtgaagtcagagtttattctagtgcgaataattgcac	
Primary infection	TTTTGAATATGTCCTCAGCCCTTTCTTATGGACCTTGAAGGAAAAACAGGGTAATTTCAAATACTTAGGGAATTTGTGTTTAAAGATATTGATGGTTAT	600
Reinfection at 14 dpi	TTTTGAATATGTCCTCAGCCCTTTCTTATGGACCTTGAAGGAAAAACAGGGTAATTTCAAATACTTAGGGAATTTGTGTTTAAAGATATTGATGGTTAT	600
Reinfection at 21 dpi	TTTTGAATATGTCCTCAGCCCTTTCTTATGGACCTTGAAGGAAAAACAGGGTAATTTCAAATACTTAGGGAATTTGTGTTTAAAGATATTGATGGTTAT	599
Consensus	ttttgaatattgtctctcagcccttttcttatggaccttgaaggaaaaacagggtaatttcaaaaactcttagggaatttgggtttaagaatattgatggttat	
Primary infection	TTTAAATATATCTAAGCACACGCCCTATTAAATTTAGTGGGTGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGGCCATAGGTATTA	700
Reinfection at 14 dpi	TTTAAATATATCTAAGCACACGCCCTATTAAATTTAGTGGGTGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGGCCATAGGTATTA	700
Reinfection at 21 dpi	TTTAAATATATCTAAGCACACGCCCTATTAAATTTAGTGGGTGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTGGCCATAGGTATTA	699
Consensus	tttcaaatatattctaagcacacgccctattaatttagtggtgtatctccctcagggtttttcgggtttagaaccattggtagatttgcgaataggtattta	
Primary infection	ACATCCTAGGTTTCAAACTTTACTTGCTTTACATAGAAGTTTATGACTCTGGTGATTTCTTCTCAGGTTGGACAGCTGGTGCAGCTTATTATGT	800
Reinfection at 14 dpi	ACATCCTAGGTTTCAAACTTTACTTGCTTTACATAGAAGTTTATGACTCTGGTGATTTCTTCTCAGGTTGGACAGCTGGTGCAGCTTATTATGT	800
Reinfection at 21 dpi	ACATCCTAGGTTTCAAACTTTACTTGCTTTACATAGAAGTTTATGACTCTGGTGATTTCTTCTCAGGTTGGACAGCTGGTGCAGCTTATTATGT	799
Consensus	acatacctagggttcaaaacttacttgctttacatagaagttatttgaacttctggtgattctcttccaggttggacagctgggtcgtcagcttattatgt	
Primary infection	GGGTTATCTTCAACCTAGGACTTTTCTATTAAATATATAAGAAATGGAACCATTAACAGATGCTGTAGACTGTGCATTTGACCTCTCTCAGAAACAAAG	900
Reinfection at 14 dpi	GGGTTATCTTCAACCTAGGACTTTTCTATTAAATATATAAGAAATGGAACCATTAACAGATGCTGTAGACTGTGCATTTGACCTCTCTCAGAAACAAAG	900
Reinfection at 21 dpi	GGGTTATCTTCAACCTAGGACTTTTCTATTAAATATATAAGAAATGGAACCATTAACAGATGCTGTAGACTGTGCATTTGACCTCTCTCAGAAACAAAG	899
Consensus	gggttatcttcaacctaggacttttctattaaatataatgaaatggaaccattacagatgctgtagactgtgacattgacctctcttcagaacaaag	
Primary infection	TGTACGTTGAAATCCTTCACTGTAGAAAAGGAATCTATCAAACTTCTAATCTTAGAGTCCAAACCAACAGAATCTATTGTTAGATTTCCTAATATTACAA	1000
Reinfection at 14 dpi	TGTACGTTGAAATCCTTCACTGTAGAAAAGGAATCTATCAAACTTCTAATCTTAGAGTCCAAACCAACAGAATCTATTGTTAGATTTCCTAATATTACAA	1000
Reinfection at 21 dpi	TGTACGTTGAAATCCTTCACTGTAGAAAAGGAATCTATCAAACTTCTAATCTTAGAGTCCAAACCAACAGAATCTATTGTTAGATTTCCTAATATTACAA	999
Consensus	tgtacgttgaatcccttcaactgtagaaaaggaatctatcaaaacttctcaacttttagagtccaaccaacagaaatctattgttagatttctctaattataac	
Primary infection	ACITGTGCCCTTTTGGTGAAGTTTTTAACGCCACAGATTGTGCATCTGTTTATGCTTGGAAACGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGT	1100
Reinfection at 14 dpi	ACITGTGCCCTTTTGGTGAAGTTTTTAACGCCACAGATTGTGCATCTGTTTATGCTTGGAAACGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGT	1100
Reinfection at 21 dpi	ACITGTGCCCTTTTGGTGAAGTTTTTAACGCCACAGATTGTGCATCTGTTTATGCTTGGAAACGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGT	1099
Consensus	acttgtgcccttttgggtgaagtttttaacgccacacagatttgcattctgtttatgcttggaaacaggaagagaatcagcaactggtgtgctgattattctgt	
Primary infection	CCATATAAATTCGCGATCATTTTCCACTTTTAAAGTGTTATGGAAGTGTCTCTACTAATTAATGATCTCTGCTTTACTAATGTCTATGAGATTTCATT	1200
Reinfection at 14 dpi	CCATATAAATTCGCGATCATTTTCCACTTTTAAAGTGTTATGGAAGTGTCTCTACTAATTAATGATCTCTGCTTTACTAATGTCTATGAGATTTCATT	1200
Reinfection at 21 dpi	CCATATAAATTCGCGATCATTTTCCACTTTTAAAGTGTTATGGAAGTGTCTCTACTAATTAATGATCTCTGCTTTACTAATGTCTATGAGATTTCATT	1199
Consensus	cctatataattccgcatcatctttccacttttaagtgatttgagtgctctcctactaaataaataatgatctcgtttacttaagtgtctatgcagatcatctt	
Primary infection	GTAATTAGAGTGATGAAGTCAGACAAATCGCTCCAGGCAAACTGGAAGAGTTGCTGATTATAATTATAAATACAGATGATTTCACAGCTGCGTTA	1300
Reinfection at 14 dpi	GTAATTAGAGTGATGAAGTCAGACAAATCGCTCCAGGCAAACTGGAAGAGTTGCTGATTATAATTATAAATACAGATGATTTCACAGCTGCGTTA	1300
Reinfection at 21 dpi	GTAATTAGAGTGATGAAGTCAGACAAATCGCTCCAGGCAAACTGGAAGAGTTGCTGATTATAATTATAAATACAGATGATTTCACAGCTGCGTTA	1299
Consensus	gtaattagagtgatgaagtcagacaaatcgctccagggcaaaactggaagagttgctgattataattataaataaccagatgattttacaggtcgtgta	
Primary infection	TAGCTTGGAAATCTCAACAACTTGTATTTAAGGTTTGGTGGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTAATCTCAAACTTTTGGAGAGA	1400
Reinfection at 14 dpi	TAGCTTGGAAATCTCAACAACTTGTATTTAAGGTTTGGTGGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTAATCTCAAACTTTTGGAGAGA	1400
Reinfection at 21 dpi	TAGCTTGGAAATCTCAACAACTTGTATTTAAGGTTTGGTGGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTAATCTCAAACTTTTGGAGAGA	1399
Consensus	tagcttgggaattctaacaaactcttgattctaaaggttggtggttaattataattactctgtatagattgttttaggaagtctaatctcaaaccttttgagagaga	
Primary infection	TATTTCAACTGAATCTATCAGGCCGTTAGCACACCTTGTAATGGTGTGGAAGGTTTAAATGTTTACTTTTCTTACAATCATATGGTTTCCAAACCCACT	1500
Reinfection at 14 dpi	TATTTCAACTGAATCTATCAGGCCGTTAGCACACCTTGTAATGGTGTGGAAGGTTTAAATGTTTACTTTTCTTACAATCATATGGTTTCCAAACCCACT	1500
Reinfection at 21 dpi	TATTTCAACTGAATCTATCAGGCCGTTAGCACACCTTGTAATGGTGTGGAAGGTTTAAATGTTTACTTTTCTTACAATCATATGGTTTCCAAACCCACT	1499
Consensus	tatttcaactgaaactatcagggcggtagcacaccttgtaatgggttggaaggttttaattgtttacttttcttacaatcatatggtttccaaacccact	
Primary infection	AATGGTGTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTGAACCTTCTACATGCACACCACTGTTTGTGGACCTAAAAAGTCTACTAATTGG	1600
Reinfection at 14 dpi	AATGGTGTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTGAACCTTCTACATGCACACCACTGTTTGTGGACCTAAAAAGTCTACTAATTGG	1600
Reinfection at 21 dpi	AATGGTGTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTGAACCTTCTACATGCACACCACTGTTTGTGGACCTAAAAAGTCTACTAATTGG	1599
Consensus	aatggtgttggttaccacacacacagagtagtagtactttctttgaacttctacatgcaccagcaactggttggagacctaaaaagtcactaatttgg	
Primary infection	TTAAAAACAATGTGTCAATTTCAACTTCAATGGTTTAAACAGGCACAGGTGTTCTTACTGAGTCTAACAAAAAGTTTCTGCCCTTTCCAACAATTTGGCAG	1700
Reinfection at 14 dpi	TTAAAAACAATGTGTCAATTTCAACTTCAATGGTTTAAACAGGCACAGGTGTTCTTACTGAGTCTAACAAAAAGTTTCTGCCCTTTCCAACAATTTGGCAG	1700
Reinfection at 21 dpi	TTAAAAACAATGTGTCAATTTCAACTTCAATGGTTTAAACAGGCACAGGTGTTCTTACTGAGTCTAACAAAAAGTTTCTGCCCTTTCCAACAATTTGGCAG	1699
Consensus	ttaaaaacaatgtgtcaatttcaacttcaatggttttaaaccaggcacaggtgtcttactgagtgctaaacaaagtttctgctttccaacaaatttggcag	
Primary infection	AGACATTGCTGACACTACTGATGCTGCTGGTATGATCCACAGACACTTGAGATTCTTGACATTACACCATGTTCTTTTGGTGGTGCAGTGTATTAACACCA	1800
Reinfection at 14 dpi	AGACATTGCTGACACTACTGATGCTGCTGGTATGATCCACAGACACTTGAGATTCTTGACATTACACCATGTTCTTTTGGTGGTGCAGTGTATTAACACCA	1800
Reinfection at 21 dpi	AGACATTGCTGACACTACTGATGCTGCTGGTATGATCCACAGACACTTGAGATTCTTGACATTACACCATGTTCTTTTGGTGGTGCAGTGTATTAACACCA	1799
Consensus	agacattgctgacactactgatgctgctggtatccacagacacttgagattcttgacattacacacatgtctcttttgggtggtcagtgctgtataacacca	
Primary infection	GGAAACAATACTTCTAACCAAGTTGCTGTTCTTTACAGGATGTTAACTGCACAGAGTCCCTGTTGCTATTATGAGTGTGACATCACTTACTCTACTTGGC	1900
Reinfection at 14 dpi	GGAAACAATACTTCTAACCAAGTTGCTGTTCTTTACAGGATGTTAACTGCACAGAGTCCCTGTTGCTATTATGAGTGTGACATCACTTACTCTACTTGGC	1900
Reinfection at 21 dpi	GGAAACAATACTTCTAACCAAGTTGCTGTTCTTTACAGGATGTTAACTGCACAGAGTCCCTGTTGCTATTATGAGTGTGACATCACTTACTCTACTTGGC	1899
Consensus	ggaaacaatacttctaaccaggttgctgtctttatcaggtatgtaactgcacagaagtcctctgttgctattcatgcagatcaacttactcctacttggc	
Primary infection	GTGTTTATCTACAGGTTCTAATGTTTCTTCAACACAGTGCAGGCTGTTTAAATAGGGGCTGAACATGTCAACAACTCATATGAGTGTGACATACCCATTGG	2000
Reinfection at 14 dpi	GTGTTTATCTACAGGTTCTAATGTTTCTTCAACACAGTGCAGGCTGTTTAAATAGGGGCTGAACATGTCAACAACTCATATGAGTGTGACATACCCATTGG	2000
Reinfection at 21 dpi	GTGTTTATCTACAGGTTCTAATGTTTCTTCAACACAGTGCAGGCTGTTTAAATAGGGGCTGAACATGTCAACAACTCATATGAGTGTGACATACCCATTGG	1999
Consensus	gtgtttattctacaggtttctaagtgttttcaaacacagctgagcgtgtttaaagggcgtgaacatgtcaacaactcatatgagtgtagacataccattgg	
Primary infection	TGCAGGTATATGCGCTAGTTATCAGACTCAGACTAATTCCTCC	2045
Reinfection at 14 dpi	TGCAGGTATATGCGCTAGTTATCAGACTCAGACTAATTCCTCC	2043
Reinfection at 21 dpi	TGCAGGTATATGCGCTAGTTATCAGACTCAGACTAATTCCTCC	2043
Consensus	tcaggttatatgctgtagttatcagactcagactaattctcctc	

Figure S3: No difference in the S1 gene sequence from the nasal washes between the

primary infection and reinfection.

Table S1. *p*-value among viral loads in various tissues in the primary and secondary infection by SPSS PASW statistical software version 18.0

		Viral load in nose	Viral load in nasal mucosa	Viral load in lung	Viral load in trachea	Viral load in bulbous olfactorius	Viral load in cerebrum	Viral load in jaw	Viral load in NALF	Viral load in BALF	sgRNA in nose	sgRNA in nasal mucosa
D0	B A	0.000	0.000	0.000							0.386	
	C A	0.000	0.000	0.000							0.001	
	B C	0.360	0.000	0.014							0.001	
D3	B A	0.000	0.000	0.000	0.000	0.000	0.000	0.081	0.000	0.000	0.019	0.155
	C A	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.010	0.007
	B C	0.863	0.891	0.001	0.003	0.362	0.380	0.061	0.066	0.473	0.592	0.054
D5	B A	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
	C A	0.000	0.000	0.000	0.004	0.000	0.000	0.001	0.000	0.000	0.000	0.000
	B C	0.007	0.006	0.080	0.038	0.236	0.000	0.037	0.000	1.000	0.219	0.000
D7	B A	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.003	0.001	0.000	0.000
	C A	0.000	0.000	0.000	0.014	0.000	0.001	0.003	0.002	0.000	0.000	0.000
	B C	0.006	0.698	0.050	0.002	0.210	0.279	0.747	0.703	0.783	0.297	1.000
D10	B A	0.000	0.009	0.000	0.001	0.037	0.038	0.043		0.037	0.558	0.000
	C A	0.000	0.001	0.000	0.061	0.007	0.035	0.023		0.004	0.063	0.000
	B C	0.071	0.089	0.015	0.010	0.237	0.952	0.646		0.113	0.027	1.000

A Primary infection; B Reinfected at 14 dpi of primary infection; C Reinfected at 21 dpi of primary infection

NALF were nasal pharyngeal larvage fluid, BALF were bronchoalveolar larvage fluid.

Table S2. Histology score standards of the lung damage

Categories	Score
Pulmonary parenchymal	0 none
	1 The lung structure was clear, and the alveolar septum was slightly thickened, very few inflammatory cells infiltrate
	2 Local edema, local alveolar septum thickening, alveolar cells had slightly exfoliated necrosis, a small amount of inflammatory cell infiltration
	3 Some alveolar septum was thickened, accompanied by mild parenchymal lesions, a small amount of alveolar cell degeneration necrosis, moderate amount of inflammatory cell infiltration
	4 Large area of the alveolar septum was thickened, some substantial lesions, large area of alveolar structure has disappeared, alveolar

	<p>cells have degeneration and necrosis, a large number of inflammatory cell infiltration</p> <p>5 Lung structure disappeared with extensive consolidation and diffuse infiltration of inflammatory cells</p>
Bronchus and Bronchiole	<p>0 none</p> <p>1 Local tracheal epithelium was slightly diseased with slight cell abscission</p> <p>2 Part of trachea epithelial cells were shed, and mild amount of exudate was observed in the lumen</p> <p>3 The cells were denaturated and necrotic, part of the mucosa is detached, and a large number of inflammatory exudates and tissue fragments could be seen in the lumen</p> <p>4 The cells were denaturated and necrotic, and a large number of mucosa detached, part of the lumen was blocked</p> <p>5 The cells were denaturated and necrotic, and a large number of the mucosa was detached, A large area or the total lumen was blocked</p>
Pulmonary vasculitis	<p>0 none</p> <p>1 Mild edema and hyperemia, minimal inflammatory cell infiltrated around the lumen</p> <p>2 Vascular edema, local endothelial cells were necrotic, a small number of inflammatory cell infiltration</p> <p>3 Endothelial cells were denaturated and necrotic with vascular wall hyperplasia, inflammatory cell infiltration</p> <p>4 Vascular wall cells were denaturated and necrotic, with lumen stenosis and inflammatory cell infiltration</p> <p>5 Vascular structures were ruptured or many cells were denaturated and necrotic, diffuse infiltration of inflammatory cells</p>

Table S3. Sequences of primers for real time RT-qPCR detection of mRNA gene expression of host cytokines

Gene name	Forward primer (5' to 3')	Reverse Primer (5' to 3')
<i>β-actin</i>	ATGGCCAGGTCATCACCATT G	CAGGAAGGAAGGCTGGAAAA G
<i>IL-1β</i>	GTGGACAACAAAGCTCGTG G	AGCCCGTCAACCTCAAAGAA
<i>IL-6</i>	TGTCTTCTTGGGACTGCTGC	CCAAACCTCCGACTTGTTGA
<i>TNF-α</i>	CACCCACCGTCAAGGATTCA	TTGGCTGGGCAATGAAGAGT
<i>IFN-α</i>	AGACTGGGAGTTGCCTGTGA	GAGGAATCCAGGGCTTTCCAG
<i>IFN-γ</i>	TGCATCTTGGCTTTGTTGCTC	TCCCCTCCATTACGACATC
<i>MIP-1α</i>	GGTCCAAGAGTACGTCGCTG	GAGTTGTGGAGGTGGCAAGG
<i>RANTES</i>	TCAGCTTGGTTTGGGAGCAA	TGAAGTGCTGGTTTCTTGGGT

<i>IP-10</i>	TACGTCGGCCTATGGCTACT	TTGGGGACTCTTGTCACTGG
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