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## Supplemental information

### Leveraging the antiviral type I interferon

#### system as a first line of defense

### against SARS-CoV-2 pathogenicity

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А >Syrian\_Golden\_Hamster\_IFNB1

С

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CCAGCTTCCATCATGACCAACAGGTGGATCCTTCAAGCTGCATTTCTGCTGTGGTTTCTC CCCATATCCCTGTCCATCAACTACGAGGAACTTGAGGCCAGACAAAGCAGAAGCAAATTCA GAGTGTGAGGACCTCCTGAAACAGCTGCGTGGACCTCTCTGCCTCAGAGACAGAAAGTTC ATCATCCAAGAGATGCTCCAGAATATCTTTCTTGTCTTCAACAACACTTTCTCCAGCACT GGGTGGAATAAGACCATCGTTGAAAGTTTCTTAGGTAAACTTCACAATCAGATAGTCTTT  ${\tt CTGAATGAAACCCTAAAGGAAGTGCCAGAGGATGAAAGTTTGACTACAAGGATTAGCTTG$ AAGAGCTATTACTGGCAGCTGGGAAGGTACCTTAAAGAGAAGAGGTACAGCAGCTGCGCC TGGATGGTGACCCGAGCAGCAGTCTCAAGGAACTTTTTAATCATTAGAAGGCTCACTTGG ATCTTCCAGAGCTGAAGACCTGTTAGCTGATGCCTCTGAGACTGGATAATGGTTGCAGGC AATCTTGAAGCATCTGAAAGGTCAAAGGAG

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Cricetulus griseus interferon beta 1 (Ifnb1), mRNA
В
       Sequence ID: XM 027400513.2
```

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	Sbjct	42	CCAGCTTCCATCATCAACAGGTGGATCCTCCAAGCTGCATTTCTGCTGTGGTTTCTC	101
	Query	61	CCCATATCCCTGTCCATCAACTACGAGGAACTTGAGGCCAGACAAAGCAAAGCAATTCA	120
	Sbjct	102	CCCATAGCCCTGTCCATCAACTACAAGGAGCTTGAGCTCAAGCAAAGGAAGTGCAACTCA	161
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	Sbjct	162	GAGTGTCTGGAGGTCCTGAGACAGCTGCATGGACCGCTCTGCCTCAACCACAGAATGGAC	221
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	Sbjct	222	TTCAAGATCCCTATGGAGGTGAAGCACCCAGGACAGATGGAGAAGAAAACGTTGCCTTG	281
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	Sbjct	282	ATCATCGAAGAGATGCTCCAGAATATCTTTATTGTCTTCAACAGCAATTACTCAAGCACT	341
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	Sbjct	342	GGTTGGAATAAGACCATCGTTGAAAGCTTCTTGGATAAGCTTTATAAGCAGATAGACTTT	401
	Query	361	CTGAATGAAACCCTAAAGGAAGTGCCAGAGGATGAAAGTTTGACTACAAGG-A-TT	414
	Sbjct	402	CTGAAGAAAATCCTAAAGGAAATGCCAAAGAATGAAAGTTTGACTACAAGGGACTCAACT	461
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	Sbjct	462	ATTCCACGCTTGAAGAGCTATTACGAGAGGAGGATGCAAAGGTACCTTGAAGACAACGGGCAC	521
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	Sbjct	522	AGCAGCTGCGCCTGGATGGTGACCCAAGCAGAAGTCTTAAGGAACTTTATGTTCATCAAA	581
	Query	529	AGGCTCACTTGGATCTTCCAGAGCTGAAGACCTGTTAGCTGATGCCTCTGAGACTGGATA	588
	Sbjct	582	AGGCTCACTAGGATCTTCTAGAACTGAAGACCTGTCAGCTGACGCCTCTGAGACTGGACA	641
ç	Query	589	ATGGTTGCAGGCAATCTTGAAGCATC 614	
	Sbjct	642	ATGGTTGCAGGCGATCTTTAAGAATC 667	
	Query	1	MTNRWILQAAFLLWFLPISLSINYEELEARQSRSNSECEDLLKQLRGPLCLRDRKFFKMP M NRWIL AAFLL F +LSINY++L+ ++ + +C++LL+OL G + L R FK+P	60
	Sbjct	1	MNNRWILHAAFLLCFSTTALSINYKQLQLQERTNIRKCQELLEQLNGKINLTYRADFKIP	
	Query	61	MEVNHPEQMEKRDAAFIIQEMLQNIFLVFNNTFSSTGWNKTIVESFLGKLHNQIVFLNET ME+ E+M+K AF IOEMLON+FLVF N FSSTGWN+TIV L +LH O VFL	120
	Sbjct	61	MEMTEKMQKSYTAFAIQEMLQNVFLVFRNNFSSTGWNETIVVRLLDELHQQTVFLKTV	118
	Query	121	LKEVPEDESLTTRISLKSYYWQLGRYLKEKRYSSCAWMVTRAAVSRNFLIIRRLTW L+E E+ E +T + LKSYYW++ RYLK +Y+S AWMV RA + RNFLIIRRLT	176
	Sbjct	119	LEEKQEERLTWEMSSTALHLKSYYWRVQRYLKLMKYNSYAWMVVRAEIFRNFLIIRRLTR	178
	Query	177	IFQS 180 FQ+	
	Sbjct	179	NFON 182	





De novo assembly was performed using mRNA-seq reads from hamster trachea on day one after SARS-CoV-2 infection, (A) displaying the mRNA sequence deposited to NCBI. (B) Alignment to Cricetulus griseus Ifnb1 mRNA. (C) Alignment of Mesocricetus auratus IFNB1 to Peromyscus leucopus IFNB1 at the protein level. (D) Relative expression of *Isg15* measured by qRT-PCR in BHK-21 cells treated with either IFNα A/D, transfected with constructs encoding green fluorescent protein (GFP) or Mesocricetus autatus Ifnb1 (Malfnb1), or treated with supernatant (supe) from cells transfected with the constructs indicated for 16 hours. (E) Relative expression of OAS1 measured by gRT-PCR in human A549 cells treated with either IFNα A/D or supernatant from BHK-21 cells transfected with the constructs indicated. (F) Reads per million of Mallfnb1 in mRNA-seq datasets of lungs from hamsters infected with either SARS-CoV-2 or IAV.



Figure S2. Corroborating immune response and vRNA-levels of different infection parameters. Related to Figure 2. (A) Relative *Cxcl11* expression at four days post infection from either fomite, direct contact with an infected animal, ocular or intranasal direct infection (n = 4; fomite transmission, n = 3). (B) SARS-CoV-2 Nsp14 vRNA-levels and (C) *Irf7* expression measured by qRT-PCR and graphed as fold change following infection with 10, 100, 1,000, 10,000, or 100,000 pfu of SARS-CoV-2, shown as fold change of transcript abundance compared to uninfected controls. (n = 4 for 10 pfu and 1,000 pfu, n = 6 for 100 pfu, and n = 3 for 10,000 pfu and 100,000 pfu).



#### Figure S3. Representative images of lung pathology time course. Related to Figure 3.

Representative images of lungs assessed for pathology at a lower magnification from hamsters infected intranasally with 100 pfu of SARS-CoV-2 and harvested at day two, four, six, eight, or 14 post infection of (A) H&E staining (scale bar = 200  $\mu$ m) and (B) immunohistochemistry with nucleocapsid-specific antibody (scale bar = 750  $\mu$ m). (C) Representative images of hyperplasia and atypical adenomatous hyperplasia in hamster lungs (scale bar = 200  $\mu$ m).



Figure S4. Defining temporal viral sgRNA levels and ISG signature in Golden Syrian hamsters across time at two different challenge doses. Related to Figure 4. (A) Nucleocapsid sgRNA and (B) *Isg15* qRT-PCR of RNA from total perfused lung harvested at indicated days post infection with 100 pfu or 10,000 pfu of SARS-CoV-2. RNA levels were normalized to those of RNA from mock-infected hamsters and represented as fold change, (n = 3 for all time points and treatment groups). (C) Curated list of IFN-I response genes used to generate the heat maps in Figure 4A-B. (D) Representative H&E images of trachea from a mock-infected hamster and hamsters infected with 100 pfu SARS-CoV-2 and collected three days post infection. "C" indicates cilia on the cells of the tracheal epithelium (scale bar = 50 µm).



Figure S5. Defining the temporal cytokine response in Golden Syrian hamsters across time at two different challenge doses. Related to Figure 5. (A) *Cxcl11* and (B) *Ccl5* qRT-PCR of RNA from total perfused lung harvested at indicated days post infection with 100pfu or 10,000pfu of SARS-CoV-2. RNA levels were normalized to those of RNA from mock-infected hamsters and represented as fold change, (n = 3 per condition for all time points and treatment groups). (C) log2 fold change of indicated cell surface markers from RNA-seq dataset of hamsters infected with 100 pfu SARS-CoV-2 (n = 3 per condition). Timepoints of markers with differential expression with p < 0.05 have colored symbols; data points in grey do not reach significance.





(A) Reads per million mapping to the SARS-CoV-2 genome in brain, olfactory bulb, and small intestine at the time points and inocula indicated (n = 3, except for small intestine, day 8, 10,000 pfu n = 2). (B) Differential gene expression of a curated list of interferon-stimulated genes calculated from bulk RNA sequencing of mRNA from brain, olfactory bulb and small intestine of hamsters infected with SARS-CoV-2 for the times and dosage indicated compared to uninfected controls. The heatmaps represent the log2 fold change of each gene indicated on the right (human ortholog) at the time points below (control group n = 5, experimental groups n = 3 apart from small intestine 10,000 pfu dose on day eight n = 2).



Figure S7. IFNα A/D induces ISGs in hamster cell lines and reduces viral load after 10pfu SARS-CoV-2 challenge. Related to Figure 7. (A) gRT-PCR for Isg15 or Irf7 after treatment of BHK-21 cells after 8h treatment with 300 U/mL of either hIFN $\beta$ , mIFN $\beta$ , or IFN $\alpha$  A/D displayed as log fold change compared to untreated cells. (B) gRT-PCR of lungs from hamsters treated either with PBS or IFNα A/D for eight hours, (n = 3 per condition). (C) Representative images of hematoxylin and eosin stains from perfused hamster lungs harvested three days after infection with 100 pfu SARS-CoV-2 and daily IN treatment with PBS or 200,000 units of IFNα A/D, scale bar = 1mm. (D) Transmission experimental design from Figure 7F-G. Hamsters were either treated with PBS, IFNa A/D, or infected with 100 pfu SARS-CoV-2. The next day, hamsters treated either with PBS or IFNa A/D were intranasally treated again and cohoused with a SARS-CoV-2-infected hamster for ten hours. They were then removed and caged separately, treated 24 hours later with PBS or IFNα A/D respectively, before lung collection two days after exposure. (E) Plaque assay of lung homogenates from hamsters treated intranasally with PBS or IFNa A/D and exposed to SARS-CoV-2 by transmission (see Figure S7E and Methods), (n = 5, p = 0.004, Mann-Whitney test). (F) sgN levels by qRT-PCR of animals described in (F), (n = 5, p = 0.0293). (G-H) Representative images from IHC staining for MxA or N protein of perfused lungs from transmission experiment, scale bar = 100 µm, and at lower magnification, scale bar = 500 µm. (I) Plaque assay of lung homogenate from hamster lungs infected with 100 pfu SARS-CoV-2 harvested two days after infection after daily IN treatment with PBS or 20µg of poly (I:C) starting 24 hours prior to infection, (PBS n = 6, poly (I:C) n = 4, p-value = 0.007). See Methods for statistical analyses.

# Supplemental Table S5. Related to Key Resources Table.

#### OLIGONUCLEOTIDE SEQUENCES

REAGENT or RESOURCE	SOURCE	IDENTIFIER			
Oligonucleotides					
M. auratus actin forward primer	This paper	N/A			
5'-CCAAGGCCAACCGTGAAAAG-3'					
M. auratus actin reverse primer	This paper	N/A			
5'-ATGGCTACGTACATGGCTGG-3'					
<i>M. auratus</i> lsg15 forward primer	This paper	N/A			
5'-TCTATGAGGTCCGGCTGACA-3'					
<i>M. auratus</i> lsg15 reverse primer	This paper	N/A			
5'-GCACTGGGGCTTTAGGTCAT-3'					
<i>M. auratus</i> Cxcl11 forward primer	This paper	N/A			
5'-CCGCCTCATACGGGAAATGT-3'					
<i>M. auratus</i> Cxcl11 reverse primer	This paper	N/A			
5'-AAGACAGAAGGTTGGGCTCG-3'					
<i>M. auratus</i> Irf7 forward primer	This paper	N/A			
5'-ATTICGGTCGCAGGGATCTG-3'					
<i>M. auratus</i> Irf7 reverse primer	This paper	N/A			
5'-IGCAAGATAAAGCGTCCCGT-3'					
<i>M. auratus</i> Ccl5 forward primer	This paper	N/A			
5'-ACTGCCTCGTGTTCACATCA-3'					
<i>M. auratus</i> Ccl5 reverse primer	This paper	N/A			
	01	N1/A			
SARS-Cov-2 Nsp14 (genomic) forward primer	Chu et al., 2020	N/A			
5-IGGGGYIIIACRGGIAACCI-3	Church al. 2020				
	Chu et al., 2020	N/A			
SARCOV 2 agRNA (TRS L) forward primer	Vana at al. 2020	NI/A			
5' CTCTTCTACATCTCTCTCTAAACCAAC 2'	rang et al., 2020	N/A			
SARS CoV 2 N og RNA rovorgo primor	Vana at al. 2020	NI/A			
	1 ally et al., 2020	N/A			
SARS-CoV/-2 M sqRNA reverse primer	This paper	Ν/Δ			
		17/7			
M auratus II6 forward primer	Sanchez-Feline et	Ν/Δ			
	al 2020)				
M auratus II6 reverse primer	Sanchez-Feline et	N/A			
5'- CCTGAAAGCACTTGAAGAATTCC-3'	al 2020)	1.07			
<i>M. auratus</i> II10 forward primer	This paper	N/A			
5'-GAAGGACCAGCTGGACAACA-3'					
<i>M. auratus</i> II10 reverse primer	This paper	N/A			
5'-TGGCAACCCAAGTAACCCTTA-3'					