Immunity, Volume *54*

Supplemental information

Leveraging the antiviral type I interferon system as a first line of defense against SARS-CoV-2 pathogenicity

Daisy A. Hoagland, Rasmus Møller, Skyler A. Uhl, Kohei Oishi, Justin Frere, Ilona Golynker, Shu Horiuchi, Maryline Panis, Daniel Blanco-Melo, David Sachs, Knarik Arkun, Jean K. Lim, and Benjamin R. tenOever

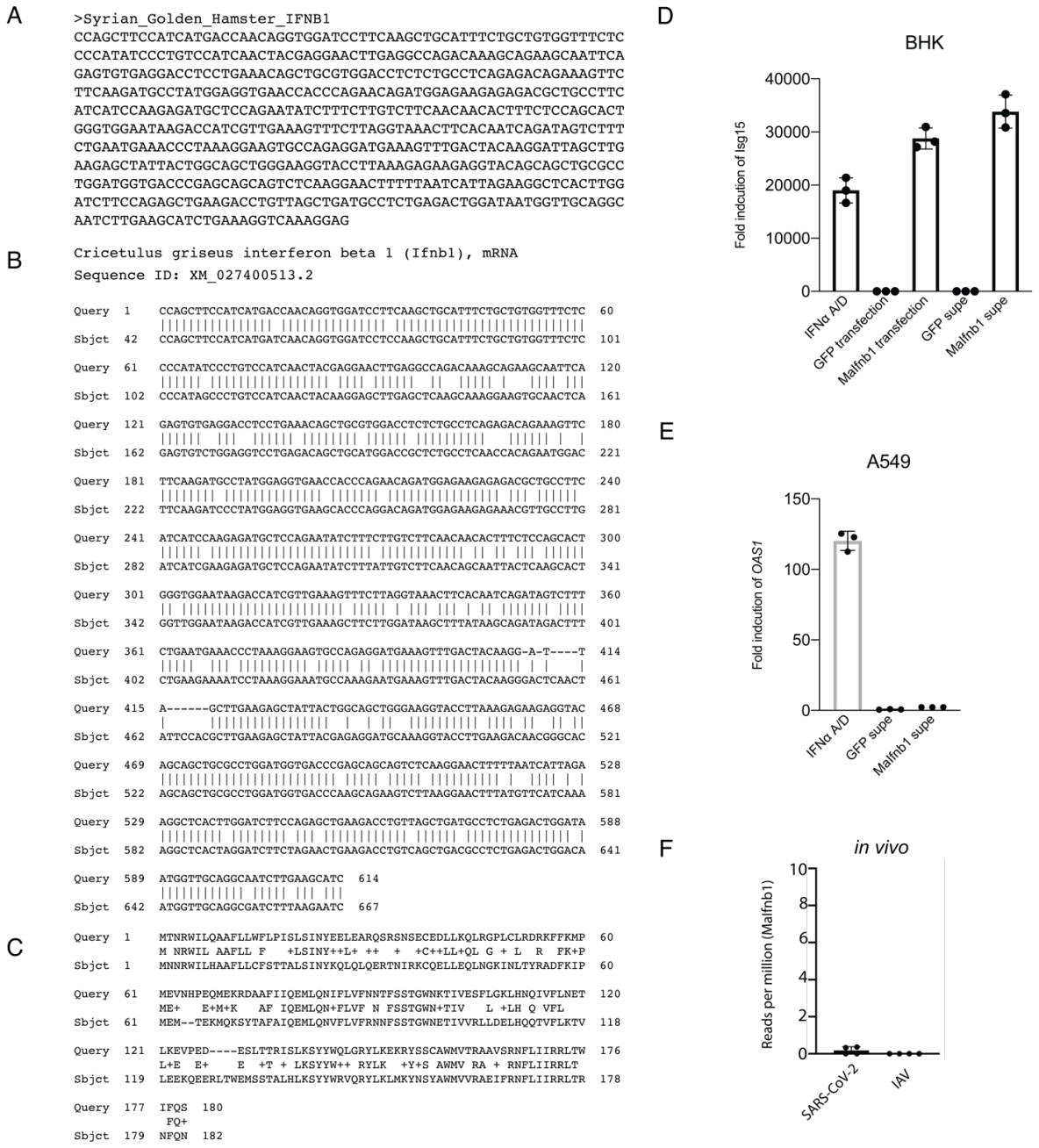

Figure S1. *De novo* assembly of *Mesocricetus auratus Ifnb1*. Related to Figure 1.

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 (*MaIfnb1), or treated with supernatant (supe) from cells transfected with the constructs indicated for 16 hours. (E) Relative expression of *OAS1* measured by qRT-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 *MaIfnb1* in mRNA-seq datasets of lungs from hamsters infected with either SARS-CoV-2 or IAV.

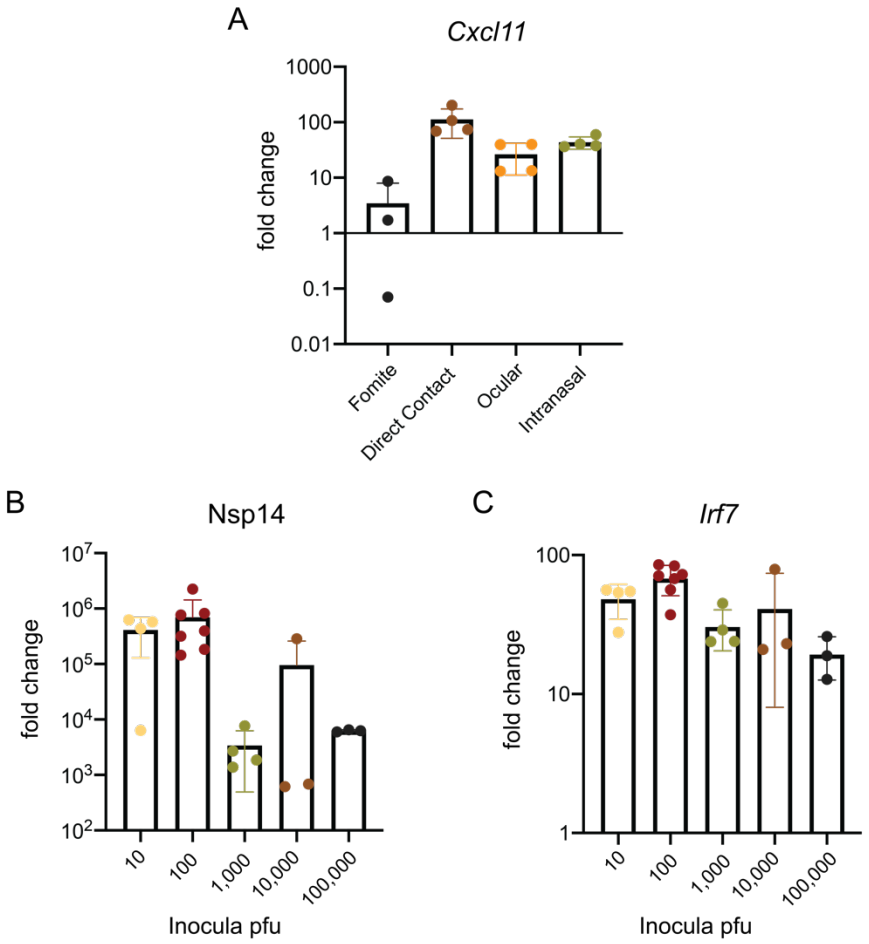

Figure S2. Corroborating immune response and vRNA-levels of different infection parameters. Related to Figure 2. (A) Relative *Cxcl11* expression at four days post infection from either fomite, direct contact with an infected animal, ocular or intranasal direct infection (n = 4; fomite transmission, 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).

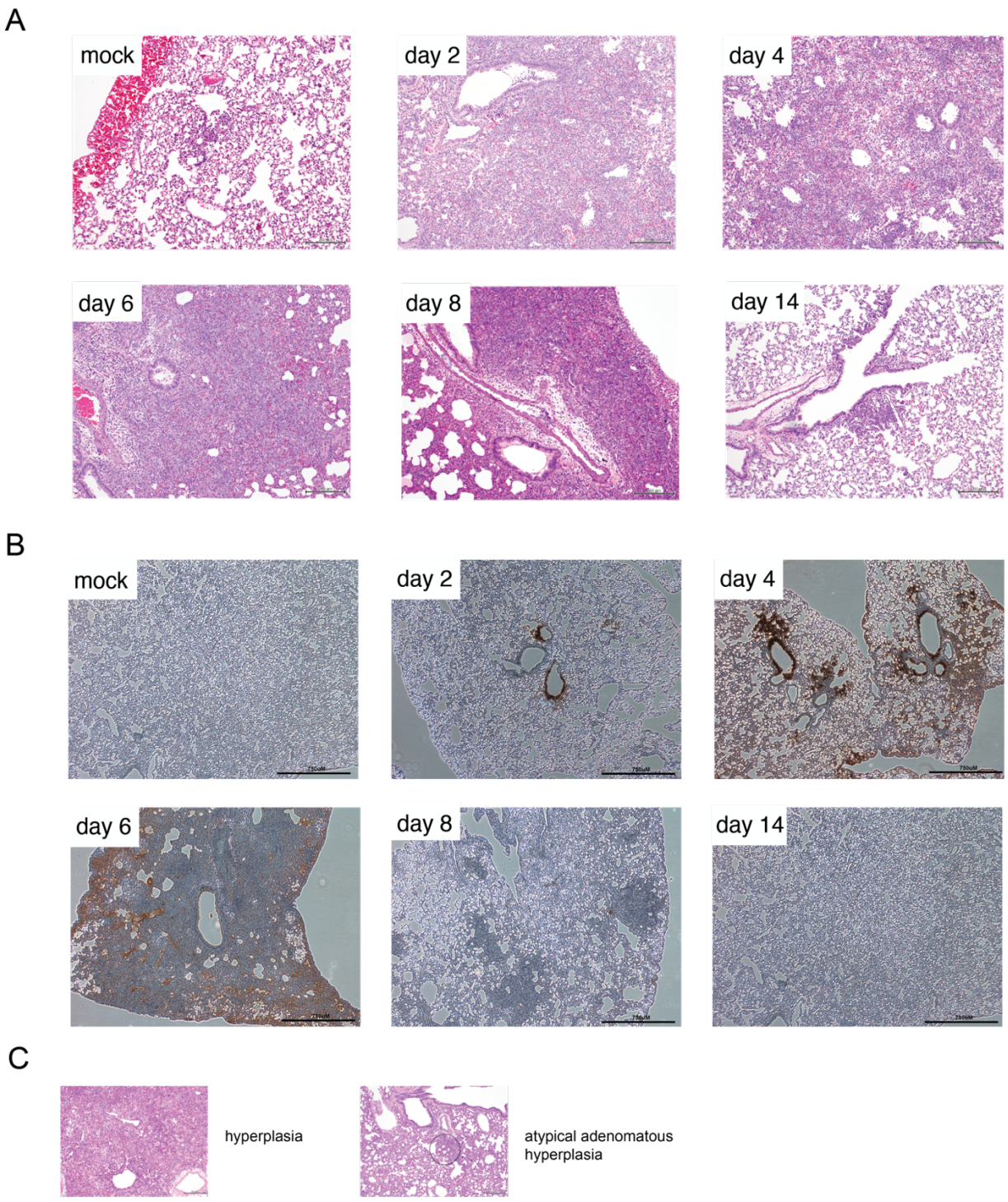

Figure S3. Representative images of lung pathology time course. Related to Figure 3.
Representative images of lungs assessed for pathology at a lower magnification from hamsters infected intranasally with 100 pfu of SARS-CoV-2 and harvested at day two, four, six, eight, or 14 post infection of (A) H&E staining (scale bar = 200 μm) and (B) immunohistochemistry with nucleocapsid-specific antibody (scale bar = 750 μm). (C) Representative images of hyperplasia and atypical adenomatous hyperplasia in hamster lungs (scale bar = 200 μm).

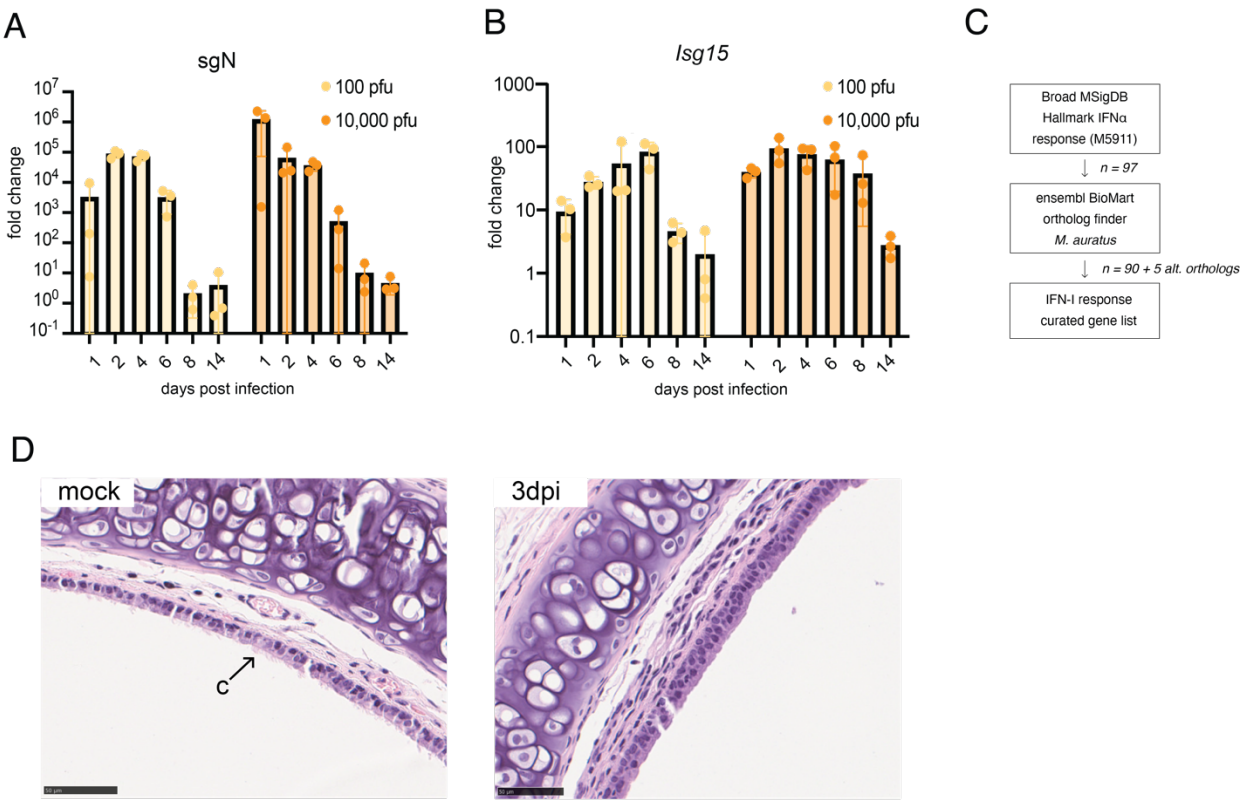

Figure S4. Defining temporal viral sgRNA levels and ISG signature in Golden Syrian hamsters across time at two different challenge doses. Related to Figure 4. (A) Nucleocapsid sgRNA and (B) *Isg15* qRT-PCR of RNA from total perfused lung harvested at indicated days post infection with 100 pfu or 10,000 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).

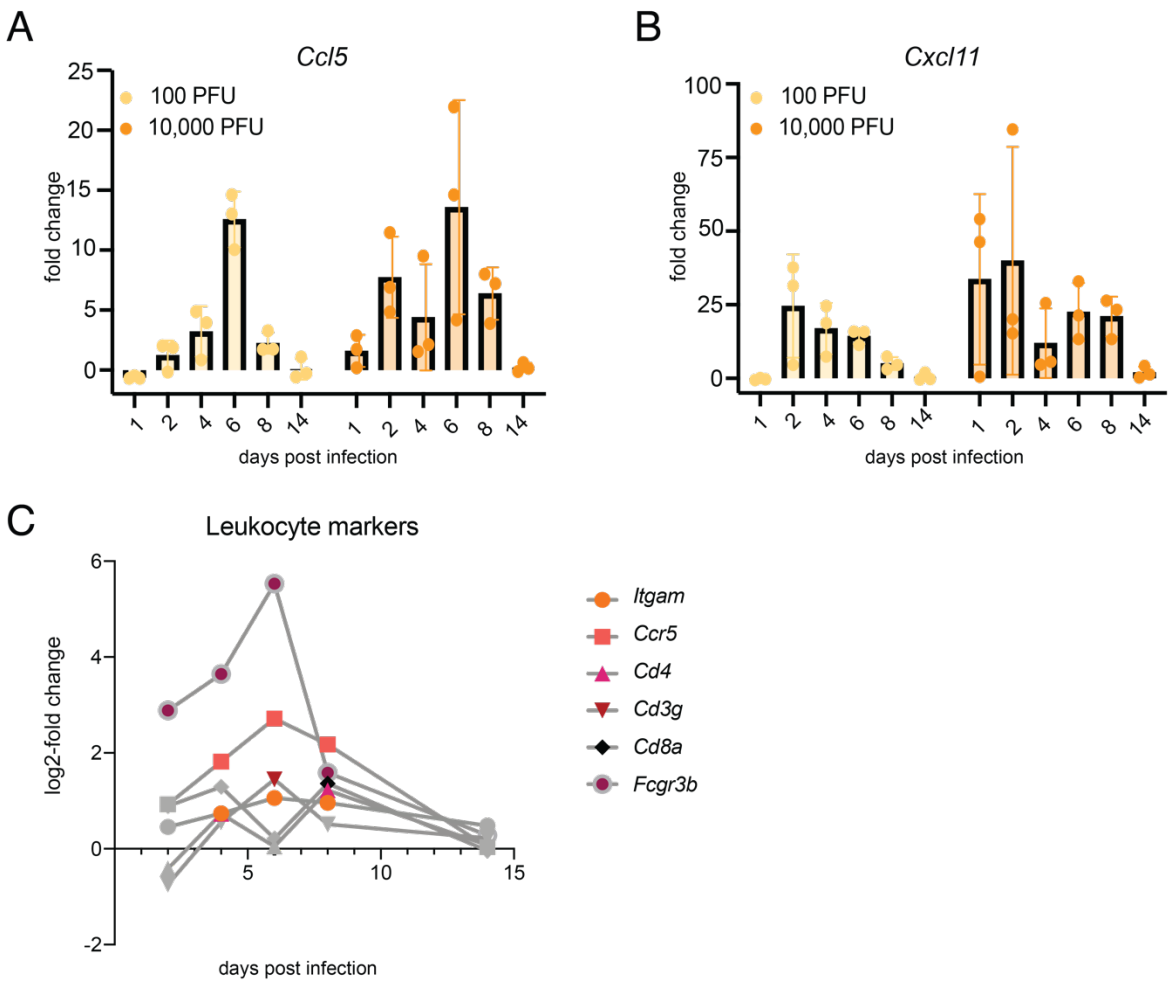

Figure S5. Defining the temporal cytokine response in Golden Syrian hamsters across time at two different challenge doses. Related to Figure 5. (A) *Cxcl11* and (B) *Ccl5* qRT-PCR of RNA from total perfused lung harvested at indicated days post infection with 100pfu or 10,000pfu of SARS-CoV-2. RNA levels 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.

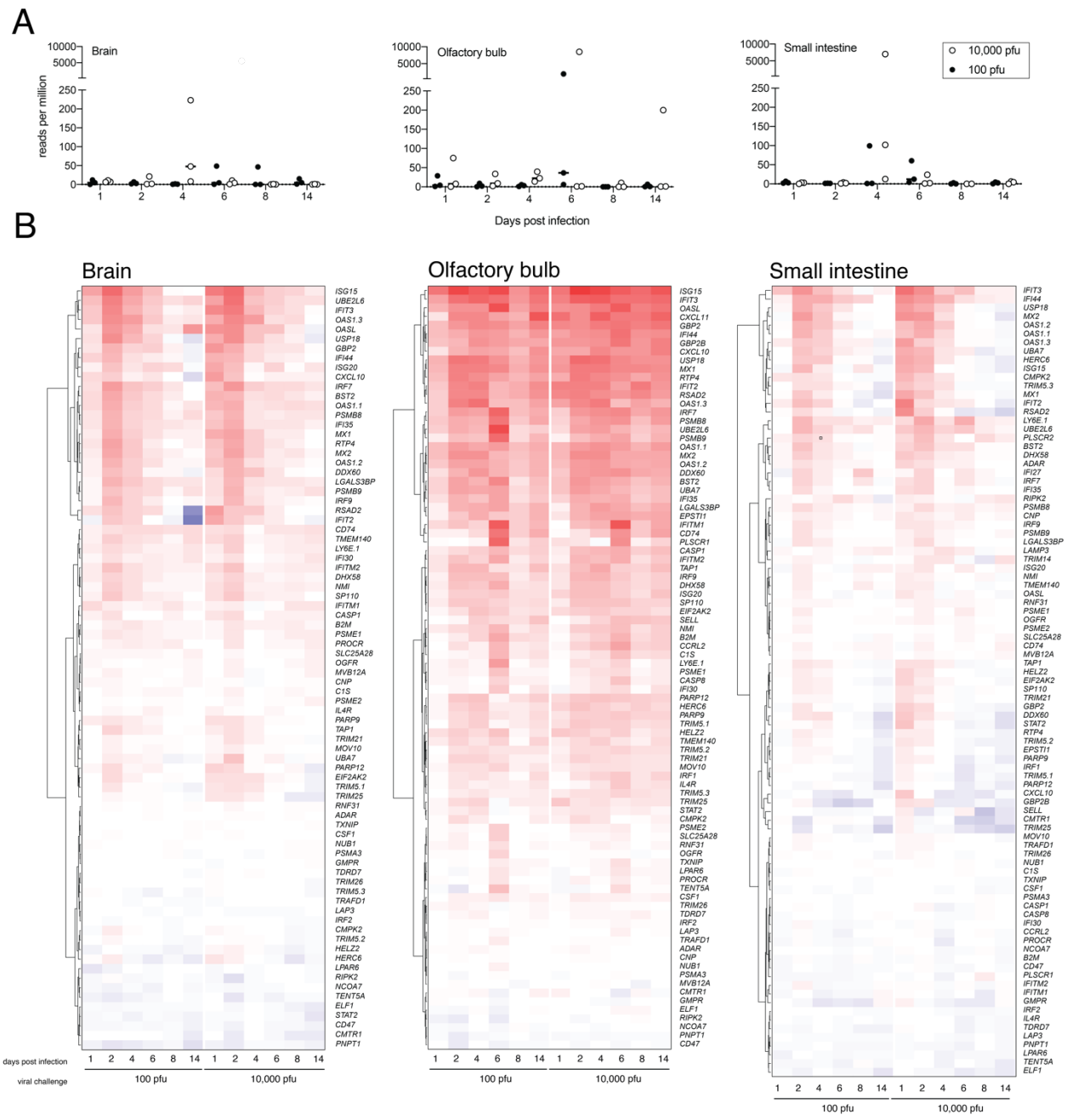

Figure S6. IFN-I signature of distal tissues. Related to Figure 6.

(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).

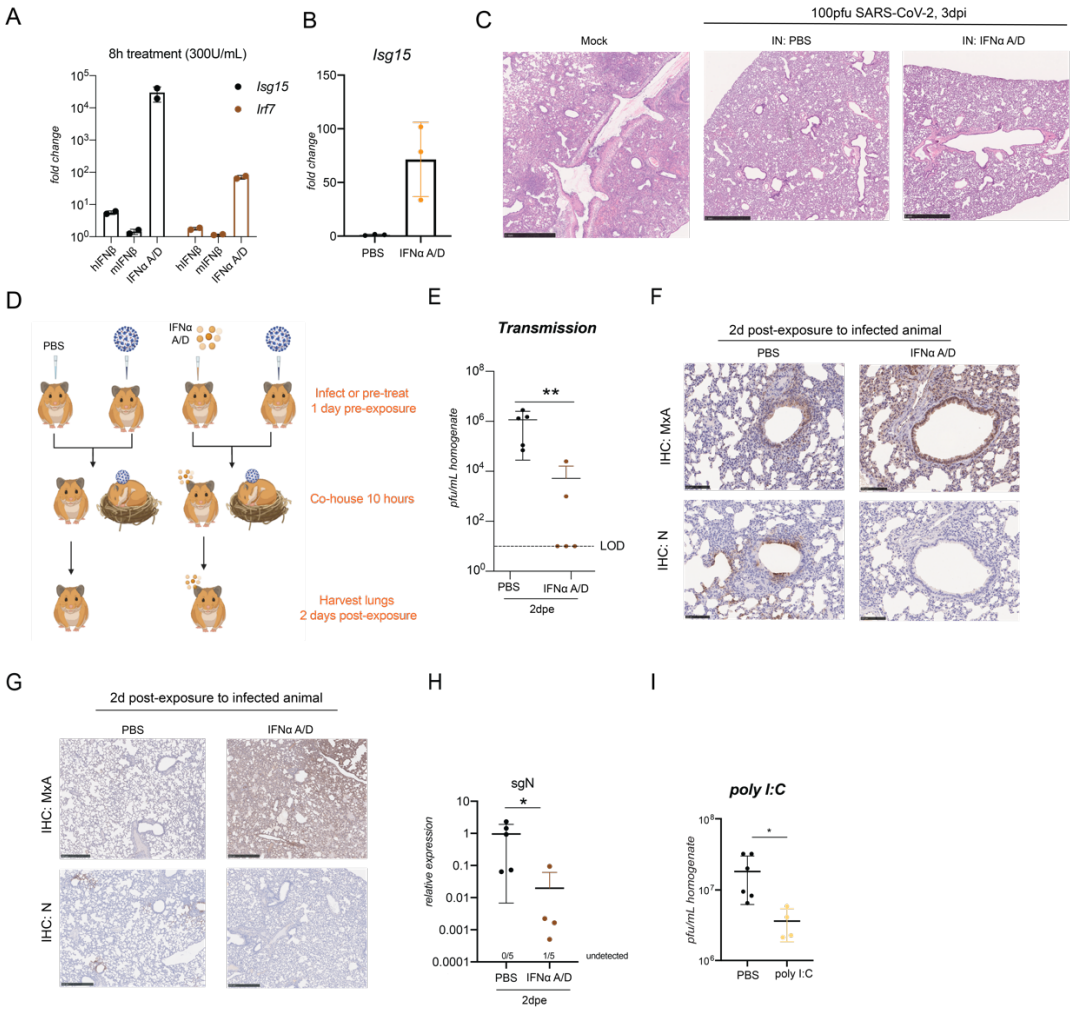

Figure S7. IFNα A/D induces ISGs in hamster cell lines and reduces viral load after 10pfu SARS-CoV-2 challenge. Related to Figure 7. (A) qRT-PCR for *Isg15* or *Irf7* after treatment of BHK-21 cells after 8h treatment with 300 U/mL of either hIFNβ, mIFNβ, or IFNα A/D displayed as log fold change compared to untreated cells. (B) qRT-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, IFNα A/D, or infected with 100 pfu SARS-CoV-2. The next day, hamsters treated either with PBS or IFNα 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 IFNα 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 5'-CCAAGGCCAACCGTGAAAAG-3'	This paper	N/A
M. auratus actin reverse primer 5'-ATGGCTACGTACATGGCTGG-3'	This paper	N/A
M. auratus Isg15 forward primer 5'-TCTATGAGGTCCGGCTGACA-3'	This paper	N/A
M. auratus Isg15 reverse primer 5'-GCACTGGGGCTTTAGGTCAT-3'	This paper	N/A
M. auratus Cxcl11 forward primer 5'-CCGCCTCATACGGGAAATGT-3'	This paper	N/A
M. auratus Cxcl11 reverse primer 5'-AAGACAGAAGGTTGGGCTCG-3'	This paper	N/A
M. auratus Irf7 forward primer 5'-ATTTCGGTCGCAGGGATCTG-3'	This paper	N/A
M. auratus Irf7 reverse primer 5'-TGCAAGATAAAGCGTCCCGT-3'	This paper	N/A
M. auratus Ccl5 forward primer 5'-ACTGCCTCGTGTTCACATCA-3'	This paper	N/A
M. auratus Ccl5 reverse primer 5'-TTCGGGTGACAAAAACGACT-3'	This paper	N/A
SARS-CoV-2 Nsp14 (genomic) forward primer 5'-TGGGGYTTTACRGGTAACCT-3'	Chu et al., 2020	N/A
SARS-CoV-2 Nsp14 (genomic) reverse primer 5'-AACRCGCTTAACAAAGCACTC-3'	Chu et al., 2020	N/A
SARS-CoV-2 sgRNA (TRS-L) forward primer 5'-CTCTTGTAGATCTGTTCTCTAAACGAAC-3'	Yang et al., 2020	N/A
SARS-CoV-2 N sgRNA reverse primer 5'-GGTCCACCAAACGTAATGCG-3'	Yang et al., 2020	N/A
SARS-CoV-2 M sgRNA reverse primer 5'-TTACTGTACAAGCAAAGCAATATTGTCA-3'	This paper	N/A
M. auratus Il6 forward primer 5'-GGTATGCTAAGGCACAGCACACT-3'	Sanchez-Felipe et al., 2020)	N/A
M. auratus Il6 reverse primer 5'- CCTGAAAGCACTTGAAGAATTCC-3'	Sanchez-Felipe et al., 2020)	N/A
M. auratus Il10 forward primer 5'-GAAGGACCAGCTGGACAACA-3'	This paper	N/A
M. auratus Il10 reverse primer 5'-TGGCAACCCAAGTAACCCTTA-3'	This paper	N/A