Supplemental information

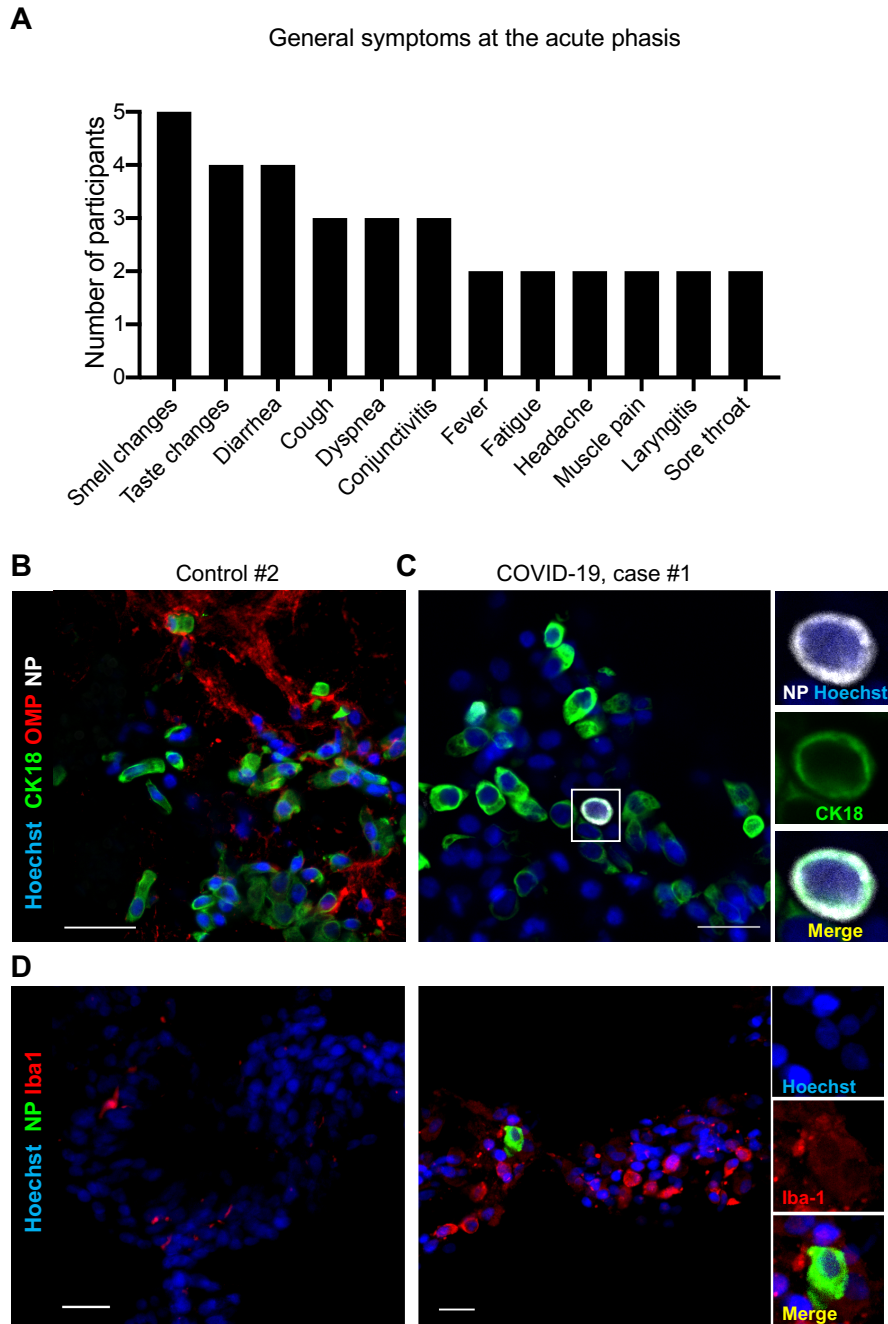SARS-CoV-2 neuroinvasion and persistence Study, De Melo et al.

**Figure S1 – General symptoms at the acute phasis and infected cell types in olfactory mucosa of COVID-19 patients at the acute phasis. (A)** Histogram depicting the general symptoms of the COVID-19 patients #1 to #5 at the acute phase. **(B, C)** Immunofluorescence of olfactory mucosa of control (B) and COVID-19 (C) patients, showing olfactory neurons (OMP+ cells) or sustentacular cells (cytokeratin-18 (CK18)+ cells). Inset in (C) shows an infected sustentacular cell. **(D)** Immunofluorescence of COVID-19 patients olfactory mucosa showing infected myeloid cells (Iba1+ cells). Scale bar = 20μm (B, C) or 10μm (D).
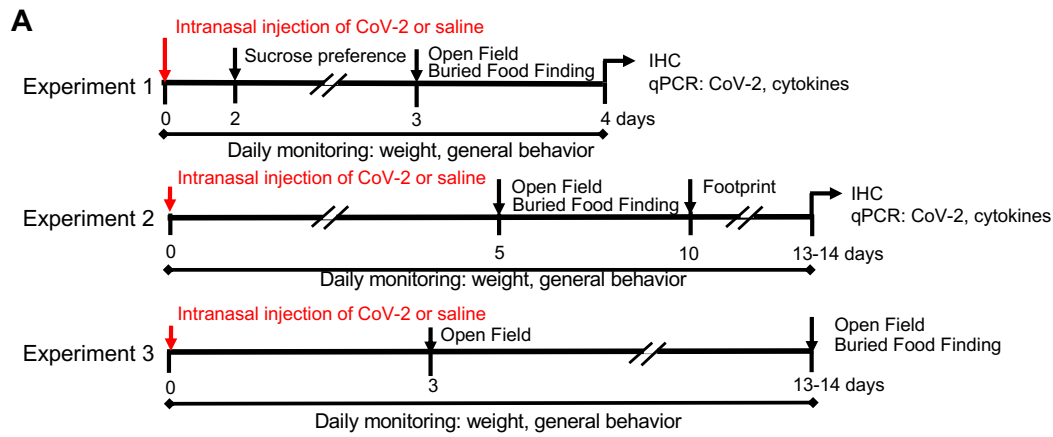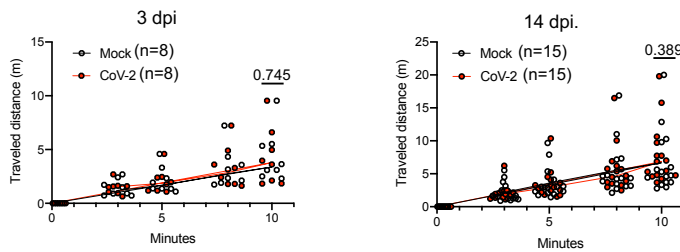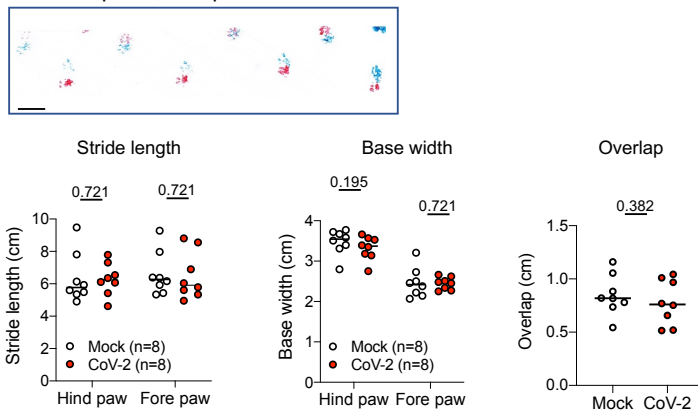
**Figure S2 – Experimental design of the experiments and complementary behavioral tests with golden hamsters.** (**A**) Hamsters were infected intranasally with SARS-CoV-2 or received physiological water (Mock). They were assessed at different timepoints for sensorial, motor and cognitive functions, then terminated at different timepoints for tissue and fluid sampling. Schematic experimental pipeline. (**B**) Total distance traveled in open field at 3 dpi (left) and 14 (right) dpi. (**C**) Analysis of painted footprints leaved by hamsters at 10 days post inoculation, using blue paints on the forepaws and red paints on the hind paws. Up: Picture of footprint patterns. Bottom: Stride length for hind-paw and fore-paw strides (left) Base width hind-paw (middle) and fore-paw steps (right) Overlap between forepaw and hindpaw placement. P values indicated in (A, B, C) are calculated by Mann-Whitney test and are in bold when significant. Scale bar: 5 cm.
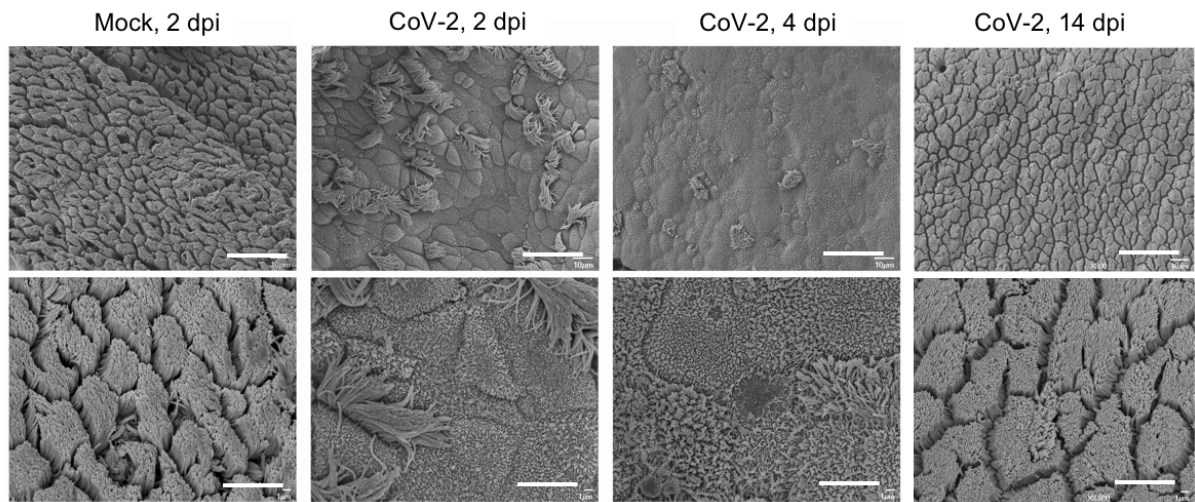
Figure S3 – SARS-CoV-2 induces loss of ciliation in the olfactory epithelium. Scanning electron microscope imaging showing changes in olfactory epithelium following CoV-2 infection at 2, 4 and 14 day post intranasal inoculation. Scale bars: 10 µm (up), 1 µm (bottom).
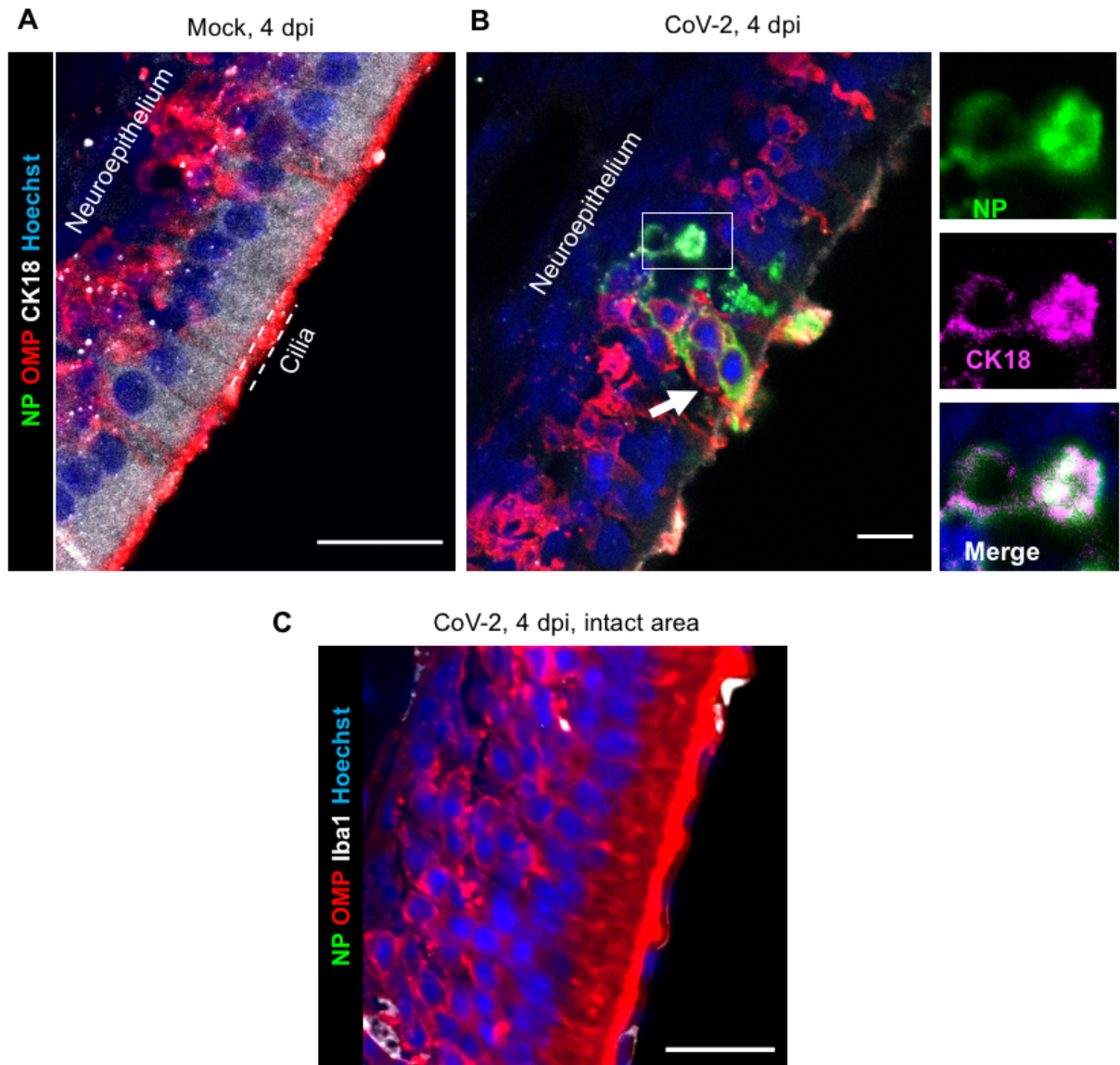
Figure S4– Cell types infected by SARS-CoV-2 in hamster olfactory mucosa and olfactory bulb. Olfactory epithelium of mock- (**A**) and SARS-CoV-2 (**B, C**) inoculated hamsters at 4 dpi. Infected sustentacular cells (CK18+) are depicted (inset). Neuro-epithelium containing infected cells are often disorganized (**B**) while adjacent areas without SARS-CoV-2 nucleoprotein staining retained an intact structure (**C**). Mature olfactory neurons express olfactory marker protein (OMP), immature neurons express Tuj1 and myeloid cells express Iba1. SARS-CoV-2 is detected by antibodies raised against the viral nucleoprotein (NP). Scale bars: 20μm.
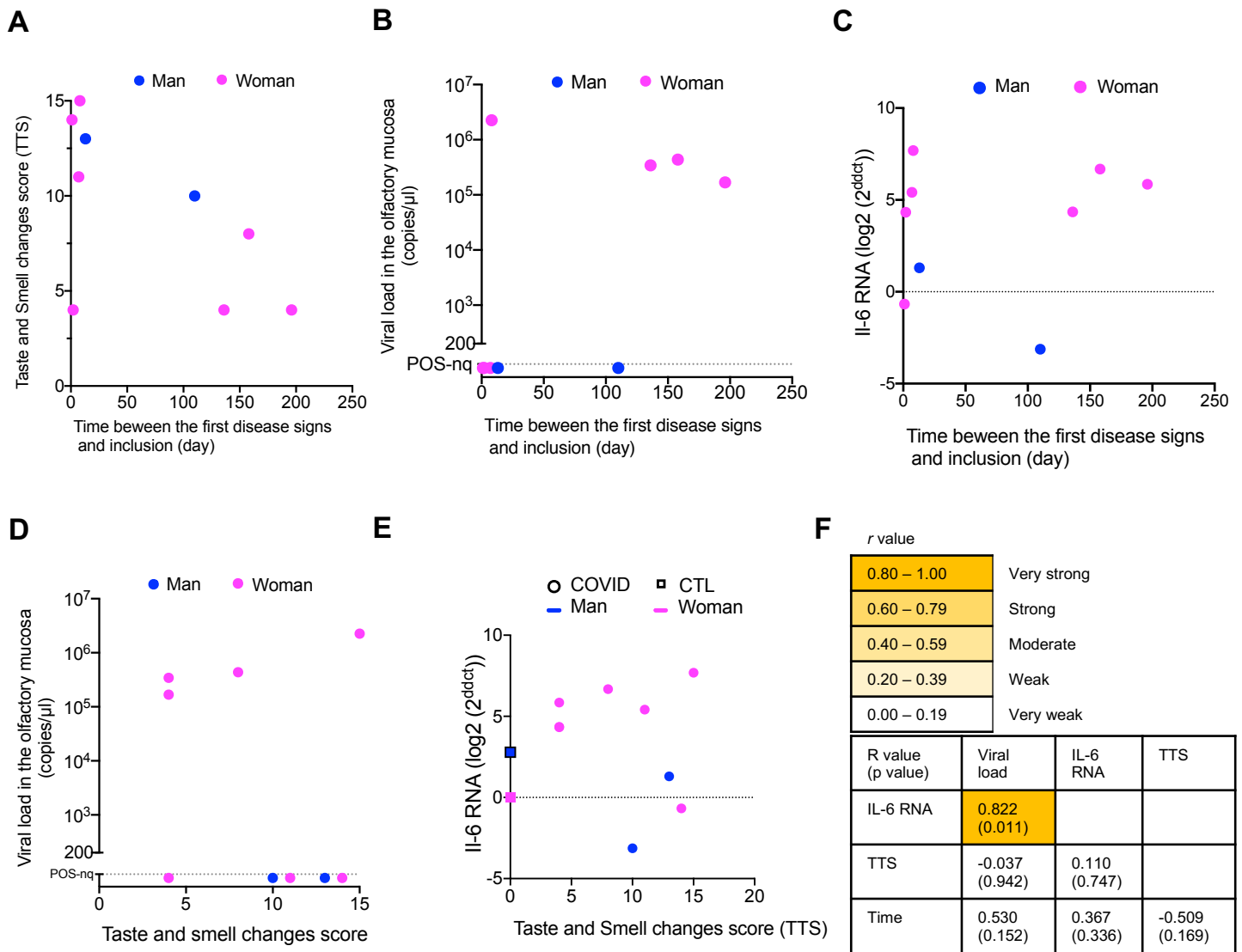
Figure S5 – Clinical and virologic profiles from patients with persistent olfactory dysfunction post- COVID-19 compared to COVID-19 patients with loss of smell at early onset and controls Graphs depicting taste and smell changes scores and time between the first disease signs and inclusion (**A**), the viral load in the olfactory mucosa and time between the first disease signs and inclusion (**B**), the level of IL-6 transcripts in the olfactory mucosa and time between the first disease signs and inclusion (**C**), the viral load in the olfactory mucosa and the taste and smell changes scores (**D**), and the taste and smell changes scores and the level of IL-6 transcripts (**E**). TTS: score range 0-15 from 0 no change to 15= anosmia and ageusia."); n=5 COVID-19 patients with loss of smell included in the early infection phase ("acute"); N=4 patients with persistent olfactory dysfunction post-COVID-19 ("persistent; n=2 control subjects. (**F**) Spearman test r and p are shown.

Table S1. Characteristics of smell and taste abnormalities at inclusion of the participants with recent loss of smell associated to COVID-19.

	Total	COVID #1	COVID #2	COVID #3	COVID #4	COVID #5
Years/Sex		53/W	31/W	61/W	40/W	46/M
Smell abnormalities	**5/5**	Yes	Yes	Yes	Yes	Yes
Severity of smell loss						
Partial	2/5	Yes	-	Yes	-	-
Complete	3/5	-	Yes	-	Yes	Yes
Reduced acuity	5/5	Yes	Yes	Yes	Yes	Yes
Increased acuity	0/5	No	No	No	No	No
« Food smell different »	2/5	Yes	Yes	Yes	Yes	Yes
Deemed severe	4/5	Severe	Severe	Moderate	Severe	Severe
First symptom of COVID-19	1/5	No	No	No	No	Yes
Preceded of classical symptoms of COVID-19 or minor symptoms	3/5	Yes	Yes	Yes	No	No
Concomitant with other symptoms of COVID-19	1/5	No	No	No	No	No
Sudden onset smell loss	4/5	No	Yes	Yes	Yes	Yes
Progressive onset smell loss	1/5	Yes	No	No	No	No
Taste abnormalities	**4/5**	Yes	Yes	No	Yes	Yes
Severity of Taste loss						
Partial	4/4	Yes	Yes	-	Yes	Yes
Complete	0/4	-	-	-	-	-
Reduced acuity for bitter	2/4	No	Partial	-	No*	Partial
Reduced acuity for salt	3/4	No	Partial	-	Complete	Partial
Reduced acuity for sour	3/4	No	Complete	-	Complete	Partial
Reduced acuity for sweet	4/4	Partial	Partial	-	Complete	Complete
"Food tastes different "	4/4	Yes	Yes	-	Yes	No
Deemed severe	4/4	Severe	Severe	-	Severe	Severe
Bad taste in the mouth	4/4	Yes	Yes (bitter)	-	Yes (bitter)	Yes (bitter)
Able to discriminate between						
Two meats	1/3		Yes	-	Yes	No
Two vegetables	1/3		Yes	-	Yes	No
Two fruits	1/3		Yes	-	Yes	No
Meat and fish	1/2	*LD*	Yes	-	Yes	No

*Stronger perception; LD: Lacking data

Table S2. Characteristics of smell and taste abnormalities at inclusion of the participants with persistent olfactory dysfunction.

	Total	COVID #6	COVID #8	COVID #9	COVID #10
Years/Sex		24/M	43/W	71/W	56/W
Smell abnormalities	**4/4**	Yes	Yes	Yes	Yes
Severity of smell loss					
Partial	0/4	-	-	-	-
Complete	4/4	Yes	Yes	Yes	Yes
Reduced acuity	3/4	Yes	No	Yes	Yes
Increased acuity	0/4	No	Nos	No	No
« Food smell different »	4/4	Yes	Yes	Yes	Yes
Deemed severe	1/4	Severe	Moderate	Moderate	Unimportant
First symptom of COVID-19	0/4	No	No	No	No
Preceded of classical symptoms of COVID-19 or minor symptoms	2/4	LD	Yes	Yes	No
Concomitant with other symptoms of COVID-19	1/4	LD	No	No	Yes
Sudden onset smell loss	4/4	Yes	Yes	Yes	Yes
Progressive onset smell loss	0/4	No	No	No	No
Taste abnormalities	**3/4**	Yes	No	Yes	Yes
Severity of Taste loss					
Partial	3/3	Yes	-	Yes	Yes
Complete	0/3	-	-	-	-
Reduced acuity for bitter	0/3	No	-*	No	LD
Reduced acuity for salt	0/3	No	-	No	No
Reduced acuity for sour	0/3	No	-	No	No
Reduced acuity for sweet	0/3	No	-	No	No
"Food tastes different "	3/3	Yes	-	Yes	Yes
Deemed severe	1/3	Severe	-	Moderate	Unimportant
Bad taste in the mouth	2/4	Yes (bitter, sour)	No	Yes	No
Able to discriminate between					
Two meats	0/3	No	-	No	No
Two vegetables	0/3	No	-	No	No
Two fruits	0/3	No	-	No	No
Meat and fish	0/2	DNK	-	No	No

DNK: Does not know, LD: Lacking data

Table S3. Primer sequences used for qPCR in the golden hamster tissues.

Gene		Primer sequence (5' – 3')	Reference
ha-*γactin*	For	ACAGAGAGAAGATGACGCAGATAAT	(1)
	Rev	GCCTGAATGGCCACGTACA	
ha-*Hprt*	For	TGCGGATGATATCTCAACTTTAACTG	(2)
	Rev	AAAGGAAAGCAAAGTTTGTATTGTCA	
ha-*Il-6*	For	GGACAATGACTATGTGTTGTTAGAA	(1)
	Rev	AGG CAA ATT TCC CAA TTG TAT CCA	
ha-*Cxcl10*	For	GCCATTCATCCACAGTTGACA	(2)
	Rev	CATGGTGCTGACAGTGGAGTCT	
ha-*Ccl5*	For	ACTGCCTCGTGTTCACATCA	(3)
	Rev	CCCACTTCTTCTTTGGGTTG	
ha-*Irf7*	For	CACTATCCGTGGCTACACTCTG	(3)
	Rev	GGTCCTACTCTGTGATGTGCTG	
ha-*Mx2*	For	CCAGTAATGTGGACATTGCC	(2)
	Rev	CATCAACGACCTTGTCTTCAGTA	
ha-*Stat1*	For	CAATATAGCCGCTTTTCTTTGG	(3)
	Rev	TGTACAGGATCCTCCTGGAAGT	
ha-*Ddx58*	For	CGCGGAACTTTGAAGAGAAG	(3)
	Rev	TTGGTCTCCGGCTTTAAGTG	
ha-*Il-1β*	For	GGCTGATGCTCCCATTCG	(2)
	Rev	CACGAGGCATTTCTGTTGTTCA	
ha-*Ifnβ*	For	ACCCTAAAGGAAGTGCCAG	(4)
	Rev	CCAGCTGCCAGTAATAGCTC	

Supplemental methods

Behavioral tests

Open field: Open Field was employed to check spontaneous locomotor activity. The test was assessed by individually videotracking animals in a 37 x 29 x 18 cm cage with a camera (C920 HD Pro, Logitech) and a single-mouse-tracker (http://icy.bioimageanalysis.org/plugin/single-mouse-tracker/) (5). Total distance performed by the animals during the 10 min of exploration of the arena was recorded.

Painted footprints: In the footprint test, which is used to analyze abnormal gait, the fore- and hind-paws of hamsters were painted in blue and red, respectively, and made to walk straight on a laboratory-made 60 cm length runway (9 x 20 cm) covered with white paper toward a dark goal-box at the end. The footprint patterns obtained on the white paper were then manually analyzed for stride length and base width for both fore- and hind-paws (6).

Transcriptomics analysis in Golden hamsters' olfactory bulb

The RNA-seq analysis was performed with the Sequana framework (7). We used the RNA-seq pipeline (v0.9.16), which is available online (https://github.com/sequana/sequana_rnaseq)). It is built on top of Snakemake 5.8.1 (8). Reads were trimmed from adapters using Cutadapt 2.10 (9) then mapped to the golden hamster MesAur1.0 genome assembly from Ensembl using STAR 2.7.3a (10). FeatureCounts 2.0.0 (11) was used to produce the count matrix, assigning reads to features using annotation MesAur1.0.100 with strand-specificity information. Quality control statistics were summarized using MultiQC 1.8 (12). Statistical analysis on the count matrix was performed to identify differentially regulated genes, comparing infected versus non-infected samples considering all samples and separating by sex. Clustering of transcriptomic profiles were assessed using a Principal Component Analysis (PCA). Differential expression testing was conducted using DESeq2 library 1.24.0 (13) scripts based on SARTools 1.7.0 (14) indicating the significance (Benjamini-Hochberg adjusted p-values, false discovery rate FDR < 0.05) and the effect size (fold-change) for each comparison. Finally, enrichment analysis was performed using modules from Sequana, first by converting golden hamster ensembl ids to gene names and then using human annotations for GO terms and KEGG pathways. The GO enrichment module uses PantherDB (15) and QuickGO (16) services; the KEGG pathways enrichment uses gseapy (https://github.com/zqfang/GSEApy/), EnrichR (17), KEGG (18) and BioMart services. All programmatic access to the online web services were performed via BioServices (19).

Supplemental references

1. Ribeiro-Romão RP, et al. Development of real-time PCR assays for evaluation of immune response and parasite load in golden hamster (Mesocricetus auratus) infected by Leishmania (Viannia) braziliensis. *Parasit Vectors.* 2016;9(1):361-.
2. Zivcec M, et al. Validation of assays to monitor immune responses in the Syrian golden hamster (Mesocricetus auratus). *J Immunol Methods.* 2011;368(1-2):24-35.
3. Schountz T, et al. Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters. *Viruses.* 2019;11(3):291.
4. Gowen BB, et al. MP-12 virus containing the clone 13 deletion in the NSs gene prevents lethal disease when administered after Rift Valley fever virus infection in hamsters. *Front Microbiol.* 2015;6(651).
5. de Chaumont F, et al. Icy: an open bioimage informatics platform for extended reproducible research. *Nat Methods.* 2012;9(7):690-6.
6. Brooks SP, and Dunnett SB. Tests to assess motor phenotype in mice: a user's guide. *Nat Rev Neurosci.* 2009;10(7):519-29.
7. Cokelaer T, et al. Sequana': a Set of Snakemake NGS pipelines. *J Open Source Softw.* 2017;2(16):352.
8. Köster J, and Rahmann S. Snakemake—a scalable bioinformatics workflow engine. *Bioinformatics.* 2012;28(19):2520-2.
9. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *2011.* 2011;17(1):3.
10. Dobin A, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics.* 2012;29(1):15-21.
11. Liao Y, et al. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics.* 2014;30(7):923-30.
12. Ewels P, et al. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics.* 2016;32(19):3047-8.
13. Love MI, et al. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15(12):550.
14. Varet H, et al. SARTools: A DESeq2- and EdgeR-Based R Pipeline for Comprehensive Differential Analysis of RNA-Seq Data. *PLoS One.* 2016;11(6):e0157022.
15. Mi H, et al. PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. *Nucleic Acids Res.* 2019;47(D1):D419-D26.
16. Huntley RP, et al. The GOA database: Gene Ontology annotation updates for 2015. *Nucleic Acids Res.* 2014;43(D1):D1057-D63.
17. Chen EY, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC bioinformatics.* 2013;14:128.
18. Kanehisa M, and Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* 2000;28(1):27-30.
19. Cokelaer T, et al. BioServices: a common Python package to access biological Web Services programmatically. *Bioinformatics.* 2013;29(24):3241-2.