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LETTER

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**Anti-COVID-19 efficacy of ivermectin in the golden hamster**

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1           **The devastating coronavirus disease 2019 (COVID-19) pandemic, due to SARS-CoV-2, has**  
2 **caused more than 47 million confirmed cases and more than 1.2 million human deaths around the**  
3 **globe<sup>1</sup>, and most of the severe cases of COVID-19 in humans are associated with neurological**  
4 **symptoms such as anosmia and ageusia, and uncontrolled inflammatory immune response<sup>2-5</sup>.**  
5 **Among therapeutic options<sup>6-8</sup>, the use of the anti-parasitic drug ivermectin (IVM), has been**  
6 **proposed, given its possible anti-SARS-CoV-2 activity<sup>9</sup>. Ivermectin is a positive allosteric modulator**  
7 **of the  $\alpha$ -7 nicotinic acetylcholine receptor<sup>10</sup>, which has been suggested to represent a target for**  
8 **the control of Covid-19 infection<sup>11</sup>, with a potential immunomodulatory activity<sup>12</sup>. We assessed**  
9 **the effects of IVM in SARS-CoV-2-intranasally-inoculated golden Syrian hamsters. Even though**  
10 **ivermectin had no effect on viral load, SARS-Cov-2-associated pathology was greatly attenuated.**  
11 **IVM had a sex-dependent and compartmentalized immunomodulatory effect, preventing clinical**  
12 **deterioration and reducing olfactory deficit in infected animals. Importantly, ivermectin**  
13 **dramatically reduced the *Il-6/Il-10* ratio in lung tissue, which likely accounts for the more favorable**  
14 **clinical presentation in treated animals. Our data support IVM as a promising anti-COVID-19 drug**  
15 **candidate.**

16           Coronaviruses cause respiratory disease in a wide variety of hosts. During the ongoing  
17 pandemic of COVID-19 caused by SARS-CoV-2, clinical signs other than respiratory symptoms have  
18 been linked to infection, frequently associated with an altered sense of smell and by high mortality in  
19 some COVID-19 patients. These features seem related to the over-responsiveness of patients'  
20 immune system to SARS-CoV-2, sometimes referred to as 'cytokine storm'<sup>4,5,13</sup>.

21           Ivermectin (IVM), a macrocyclic lactone, is a commercially-available anti-parasitic drug which  
22 induces effects in various endo- and ectoparasites, mycobacteria and even some viruses<sup>12,14</sup>. IVM is  
23 an efficient positive allosteric modulator of the  $\alpha$ -7 nicotinic acetylcholine receptor (nAChR) and of  
24 several ligand-gated ion channels, including the muscle receptor for glutamate (GluCl) in worms<sup>10</sup>.  
25 Furthermore, IVM has been shown to modulate the host's immune system<sup>12,14</sup> under conditions that  
26 are known to involve the  $\alpha$ -7 receptor<sup>15</sup>. An IVM anti-COVID-19 effect has been hypothesized due to  
27 similarities between SARS-CoV-2 and other nicotinic receptor ligand sequences<sup>11</sup>. *In vitro* IVM  
28 inhibition of SARS-CoV-2 replication in Vero/hSLAM cells<sup>9</sup> has also been reported, albeit at much  
29 higher concentrations (50- to 100-fold) than those clinically attainable in human patients (150-400  
30  $\mu\text{g}/\text{kg}$ )<sup>16-18</sup>.

31           Several clinical trials using IVM have been initiated either alone or in combination with other  
32 molecules<sup>19</sup>. IVM has already been administered to hospitalized COVID-19 patients, with contrasting  
33 outcomes: one study related no efficacy of late IVM administration (8-18 days after symptoms onset)  
34 in severe COVID-19 patients treated in combination with other drugs (hydroxychloroquine,  
35 azithromycin, tocilizumab, steroids)<sup>20</sup>, whereas another study observed lower mortality, especially in

1 severe COVID-19 patients treated with IVM in addition to usual clinical care (hydroxychloroquine,  
2 azithromycin, or both)<sup>21</sup>. Finally, in a pilot clinical trial, Gorial *et al.* reported a reduction in the  
3 hospital stay for the patients receiving IVM on admission, as add-on therapy with  
4 hydroxychloroquine/azithromycin<sup>22</sup>.

5 Consequently, the aim of this study was to investigate the effects of IVM alone on SARS-CoV-2  
6 infection using the golden Syrian hamster as a model for COVID-19<sup>23</sup>. Male and female adult golden  
7 Syrian hamsters were intranasally inoculated with  $6 \times 10^4$  PFU of SARS-CoV-2  
8 [BetaCoV/France/IDF00372/2020]. This inoculum size was selected as it invariably causes  
9 symptomatic infection in golden Syrian hamster, with a high incidence of anosmia and high viral  
10 loads in the upper and lower respiratory tracts within four days post-infection<sup>24</sup>. At the time of  
11 infection, animals received a single subcutaneous injection of IVM at the anti-parasitic dose of 400  
12  $\mu\text{g}/\text{kg}$  classically used in a clinical setting and were monitored over four days. Mock-infected animals  
13 received the physiological solution only.

14 IVM-treated and infected animals exhibited a significant reduction in the severity of clinical  
15 signs (Fig. 1a) and remarkably, IVM treatment reduced the olfactory deficit in infected animals:  
16 66.7% (12/18) of the saline-treated hamsters presented with hyposmia/anosmia, whereas only  
17 22.2% (4/18) of IVM-treated hamsters presented signs of olfactory dysfunction (Fisher's exact test  
18  $p=0.018$ ; Fig. 1a, Extended Data Fig. 1). This effect was sex-dependent: infected males presented a  
19 reduction in the clinical score (Fig. 1b) whereas a complete absence of signs was noticed in the  
20 infected females (Fig. 1c). Regarding the olfactory performance, 83.3% (10/12) of the saline-treated  
21 males presented with hyposmia/anosmia, in contrast to only 33.3% (4/12) of IVM-treated males  
22 (Fisher's exact test  $p=0.036$ ). Furthermore, no olfactory deficit was observed in IVM-treated females  
23 (0/6), while 33.3% (2/6) of saline-treated females presented with hyposmia/anosmia (Fisher's exact  
24 test  $p=0.455$ ). The IVM-treated and infected animals presented, however, a transient decrease of  
25 body weight similar to that observed in saline-treated and infected hamsters (Extended Data Fig. 1).

26 Since males presented a high index of anosmia/hyposmia, we subsequently performed a dose-  
27 response curve to test the effect of IVM on the clinical presentation of infected males: lower doses of  
28 IVM (100 or 200  $\mu\text{g}/\text{kg}$ ) elicited similar clinical outcomes as the anti-parasitic dose of 400  $\mu\text{g}/\text{kg}$   
29 (Extended Data Fig. 2). As expected, no signs of olfactory deficit were observed in the mock-infected  
30 hamsters (Extended Data Fig. 1). Despite sex differences, IVM treatment reduced clinical  
31 deterioration in SARS-CoV-2-infected hamsters, and even prevented the occurrence of anosmia, a  
32 typical symptom of COVID-19 in humans<sup>5,13</sup>.

33 A panel of selected cytokines (*Il-6*, *Il-10*, *Il-1 $\beta$* , *Tnf- $\alpha$*  and *Ifn- $\gamma$* ) and chemokines (*Cxcl10* and  
34 *Ccl5*), already known to be affected in COVID-19<sup>4</sup> disease progression in humans and animal

1 models<sup>23</sup>, were used to assess the impact of IVM treatment on the immune response of SARS-CoV-2  
2 infected hamsters. We assayed two airway compartments: nasal turbinates and lungs.

3 In the nasal turbinates, upon treatment with IVM, there were marked differences between sex  
4 groups: females presented an important down-regulation of several mediators (*Il-6*, *Il-10*, *Tnf- $\alpha$*  and  
5 *Cxcl10*) while males presented an increase in two pro-inflammatory mediators (*Ifn- $\gamma$*  and *Ccl5*) (Fig.  
6 2a, Extended Data Fig.3). Further, the expression of *Cxcl10*<sup>25</sup> –a key mediator known to be involved in  
7 respiratory disease and olfaction dysfunction in COVID-19 patients–, was remarkably lower in the  
8 nasal turbinates of IVM-treated females without significant changes in males. These findings are in  
9 line with the better performance of IVM-treated females observed in the food finding tests (Fig. 1).

10 In the lungs, however, the significant overexpression of *Il-10* was a common feature of IVM-  
11 treated males and females (Fig. 2b, Extended Data Fig.4). This effect may be related to a modulation  
12 of the inflammatory response in the lung (down-regulation of *Tnf- $\alpha$*  and *Cxcl10* in males, and of *IL-6*  
13 in females) associated with the reduced clinical signs. Additionally, the *Il-6/Il-10* ratio in the lung of  
14 IVM-treated hamsters was significantly lower than in non-treated animals (Fig. 2b).

15 The modulation of some immune mediators in the IVM-treated hamsters confirms what was  
16 observed in other infectious contexts and in other animal species<sup>12,14</sup>. In particular, the low *Il-6/Il-10*  
17 ratio observed in the lung of IVM-treated hamsters may predict their better clinical presentation, as  
18 observed in humans, as lower plasmatic *IL-6/IL-10* ratios are detected in hospitalized COVID-19  
19 patients who do not require intensive care<sup>26</sup>.

20 The viral RNA load in the respiratory tract remained unaffected by IVM treatment in both nasal  
21 turbinates and lung samples. These were tested using both classical RT-qPCR (Fig. 2c) and the highly  
22 sensitive technique of digital droplet PCR<sup>27</sup> (Fig. 2d, Extended Data Fig.5). Furthermore, IVM  
23 treatment did not influence the viral replication rate, as evaluated by the ratio between structural  
24 and non-structural gene transcription (Fig. 2d, Extended Data Fig.5). Finally, IVM treatment did not  
25 alter infectious viral titers in the lungs (Fig. 2e). These results differ from a previous report suggesting  
26 that IVM, albeit used at far higher concentrations, inhibits the replication of SARS-CoV-2 *in vitro*<sup>9</sup>.  
27 Therefore, the action of IVM on COVID-19 signs in the golden hamster model does not result from its  
28 antiviral activity.

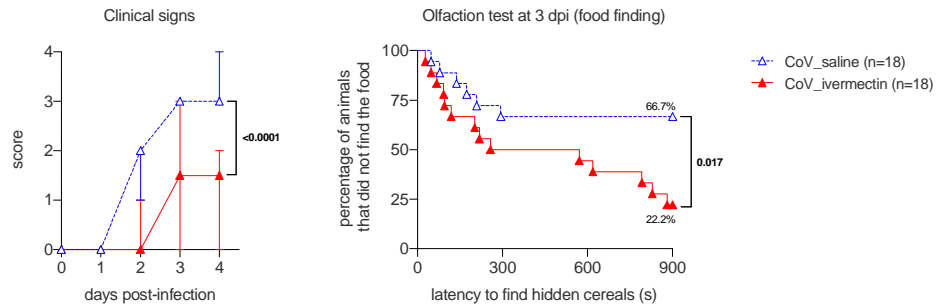
29 In humans, IVM is widely used as anti-helminthic and anti-scabies at therapeutic doses (150-  
30 400  $\mu\text{g}/\text{kg}$ )<sup>16</sup> that are in the range of those used in our golden hamster experiments. Moreover,  
31 considerable sex differences are observed in terms of clinical presentation in hamsters, as is seen in  
32 human COVID-19 patients, where men tend to develop more severe disease than women<sup>28,29</sup>.  
33 Further studies are needed to assess the effects of IVM when administered at later time-points  
34 during the course of the disease. Considering the results observed in our golden hamster model,  
35 together with their limitations, IVM may be considered as a new category of therapeutic agent

1 against COVID-19 which would not modify SARS-CoV-2 replication but affect the pathophysiological  
2 consequences of the virus *in vivo*. Indeed, a characteristic modulation of the cytokine gene  
3 expression in the airways was observed in IVM-treated hamsters that led to a cytokine profile similar  
4 with that observed in humans exhibiting milder symptoms and presenting improved prognosis<sup>26</sup>.

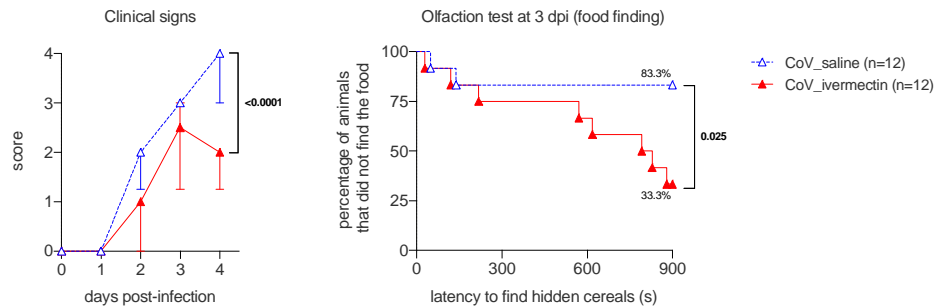
5 The molecular mechanism of IVM's anti-COVID-19 effect remains largely unknown. Even if the  
6 effects observed may, to some extent, share similarities with the anti-inflammatory action of  
7 dexamethasone<sup>7</sup> and tocilizumab (anti-IL-6)<sup>8</sup>, IVM effects are steady and strong in the golden  
8 hamsters and thought to occur upstream of inflammatory pathways. Among possible molecular  
9 targets, nAChRs have been suggested to directly or indirectly interact with SARS-CoV-2<sup>11</sup>. An  
10 interaction of IVM with the  $\alpha$ -7 receptor<sup>10</sup> may account for the modulation of the immune response  
11 in treated hamsters, an interpretation coherent with the mobilization of the cholinergic anti-  
12 inflammatory pathway (CAP) under vagus nerve control<sup>15</sup>, as observed in other models<sup>30</sup>. On the  
13 basis of these findings, and given its innocuity, we conclude that IVM should be considered as a  
14 promising anti-COVID-19 drug candidate, alone or as add-on therapy.

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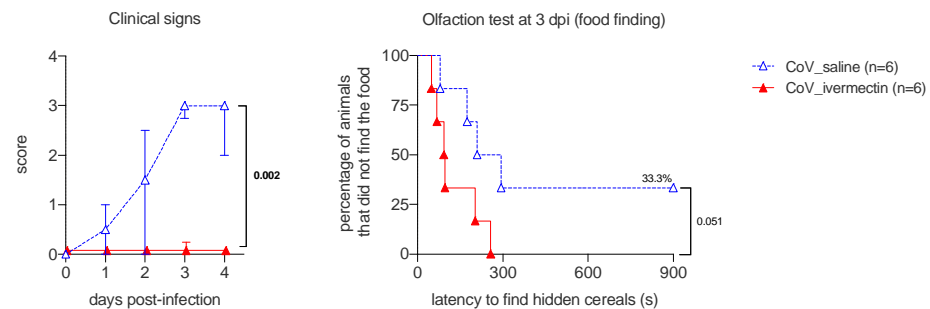
**a. All infected hamsters**



**b. Infected males**



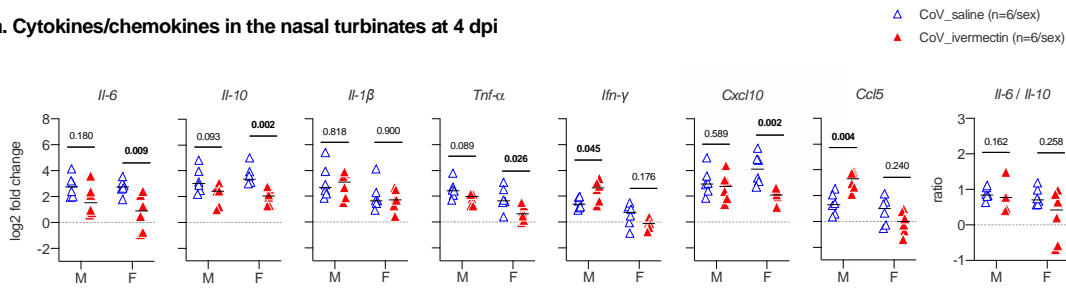
**c. Infected females**



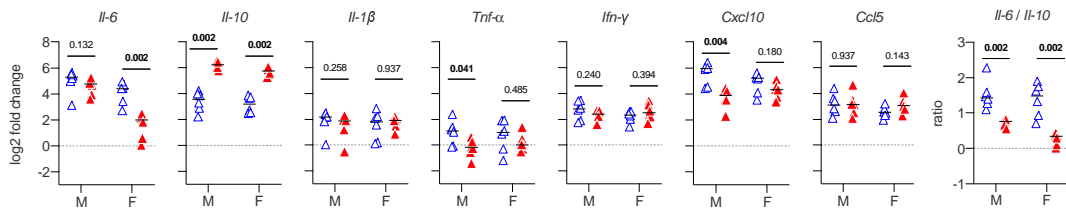
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**Fig. 1 | Clinical presentation and olfaction test of SARS-CoV-2-infected hamsters with and without ivermectin treatment. a.** clinical signs and olfactory deficit in all infected hamsters. **b.** clinical signs and olfactory deficit in infected male hamsters only. **c.** clinical signs and olfactory deficit in infected female hamsters only. The clinical score is based on a cumulative 0-4 scale: ruffled fur; slow movements; apathy; stress when manipulated. The olfaction test is based on the buried food finding test. Curves represent the percentage of animals that did not find the buried food. Food finding assays were performed at 3 days post-infection. Mann-Whitney test at 4 dpi (clinical signs) and Log-rank (Mantel-Cox) test (olfaction tests). The p value is indicated in bold when significant at a 0.05 threshold. Symbols indicate the median  $\pm$  interquartile range. Data were obtained from three independent experiments for males and two independent experiments for females. See extended Fig. 1.

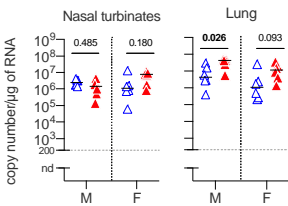
**a. Cytokines/chemokines in the nasal turbinates at 4 dpi**



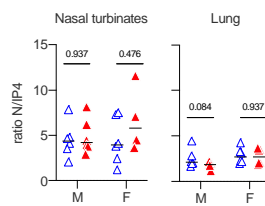
**b. Cytokines/chemokines in the lung at 4 dpi**



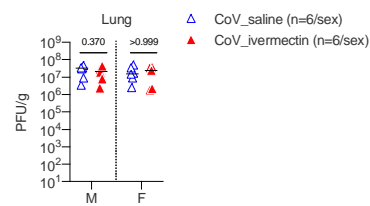
**c. Viral RNA load at 4 dpi**



**d. ddPCR at 4 dpi**



**e. Viral titer at 4 dpi**



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**Fig. 2 | Immunological and virologic aspects in the nasal turbinates and in the lungs at 4 days-post-infection of SARS-CoV-2-infected hamsters with and without ivermectin treatment.** **ab**, cytokines and chemokines transcripts in the nasal turbinates (a) and in the lungs (b) at 4dpi in male and female SARS-CoV-2 infected hamsters, treated with saline or with 400  $\mu\text{g}/\text{kg}$  ivermectin. **c**, viral load in the nasal turbinates and in the lungs at 4 dpi. **d**, ratio between the CPD (copy per droplets, normalized to  $\gamma$ -actin and *Hprt* reference genes relative expression) of structural [N, nucleocapsid] and non-structural [IP4: RdRp, RNA-dependent RNA polymerase] viral gene expression determined by digital droplet PCR (ddPCR) in the nasal turbinates and in the lungs at 4 dpi. **e**, infectious viral titer in the lung at 4 dpi expressed as Plaque Forming Units (PFU)/g of tissue. Mann-Whitney test. The p value is indicated in bold when significant at a 0.05 threshold. Horizontal lines indicate medians. M: male hamsters; F: female hamsters. Data were obtained from two independent experiments for each sex. See extended Fig. 3-5.

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1 **Methods**

2 **Ethics.** All animal experiments were performed according to the French legislation and in compliance  
3 with the European Communities Council Directives (2010/63/UE, French Law 2013–118, February 6,  
4 2013) and according to the regulations of Pasteur Institute Animal Care Committees. The Animal  
5 Experimentation Ethics Committee (CETEA 89) of the Institut Pasteur approved this study (200023;  
6 APAFIS#25326-2020050617114340 v2) before experiments were initiated. Hamsters were housed by  
7 groups of 4 animals in isolators and manipulated in class III safety cabinets in the Pasteur Institute  
8 animal facilities accredited by the French Ministry of Agriculture for performing experiments on live  
9 rodents. All animals were handled in strict accordance with good animal practice.

10

11 **Production and titration of SARS-CoV-2 virus.** The isolate BetaCoV/France/IDF00372/2020 (EVAg  
12 collection, Ref-SKU: 014V-03890) was kindly provided by Sylvie Van der Werf. Viral stocks were  
13 produced on Vero-E6 cells infected at a multiplicity of infection of  $1 \times 10^{-4}$  PFU (plaque-forming units).  
14 The virus was harvested 3 days post-infection, clarified and then aliquoted before storage at  $-80^{\circ}\text{C}$ .  
15 Viral stocks were titrated on Vero-E6 cells by classical plaque assays using semisolid overlays (Avicel,  
16 RC581-NFDR080I, DuPont)<sup>31</sup>.

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18 **SARS-CoV-2 model and ivermectin treatment of hamsters.** Male and female Syrian hamsters  
19 (*Mesocricetus auratus*) of 5-6 weeks of age (average weight 60-80 grams) were purchased from  
20 Janvier Laboratories and handled under specific pathogen-free conditions. The animals were housed  
21 and manipulated in isolators in a Biosafety level-3 facility, with *ad libitum* access to water and food.  
22 Before manipulation, animals underwent an acclimation period of one week.

23 Animals were anesthetized with an intraperitoneal injection of 200 mg/kg ketamine (Imalgène 1000,  
24 Merial) and 10 mg/kg xylazine (Rompun, Bayer), and received one single subcutaneous injection of  
25 200  $\mu\text{L}$  of freshly-diluted ivermectin (I8898, Sigma-Aldrich) at the classical anti-parasitic dose of 400  
26  $\mu\text{g}/\text{kg}$ <sup>32</sup> (or at 100-200  $\mu\text{g}/\text{kg}$  for the dose-response experiment). Non-treated animals received one  
27 single subcutaneous injection of 200  $\mu\text{L}$  of physiological solution. 100  $\mu\text{L}$  of physiological solution  
28 containing  $6 \times 10^4$  PFU of SARS-CoV-2 was then administered intranasally to each animal (50  
29  $\mu\text{L}/\text{nostril}$ ). Mock-infected animals received the physiological solution only.

30 Infected and mock-infected animals were housed in separate isolators and all hamsters were  
31 followed-up daily during four days at which the body weight and the clinical score were noted. The  
32 clinical score was based on a cumulative 0-4 scale: ruffled fur, slow movements, apathy, stress when  
33 manipulated.

34 At day 3 post-infection (dpi), animals underwent a food finding test to assess olfaction as previously  
35 described<sup>24,33</sup>. Briefly, 24 hours before testing, hamsters were fasted and then individually placed

1 into a fresh cage (37 x 29 x 18 cm) with clean standard bedding for 10 minutes. Subsequently,  
2 hamsters were placed in another similar cage for 2 minutes when about 5 pieces of cereals were  
3 hidden in 1.5 cm bedding in a corner of the test cage. The tested hamsters were then placed in the  
4 opposite corner and the latency to find the food (defined as the time to locate cereals and start  
5 digging) was recorded using a chronometer. The test was carried out during a 15 min period. As soon  
6 as food was uncovered, hamsters were removed from the cage. One minute later, hamsters  
7 performed the same test but with visible chocolate cereals, positioned upon the bedding. The tests  
8 were realized in isolators in a Biosafety level-3 facility that were specially equipped for that.

9 At 4 dpi, animals were euthanized with an excess of anesthetics (ketamine and xylazine) and  
10 exsanguination<sup>34</sup>, and samples of nasal turbinates and lungs were collected and immediately frozen  
11 at -80°C.

12

### 13 **RNA isolation and transcriptional analyses by quantitative PCR from golden hamsters' tissues.**

14 Frozen tissues were homogenized with Trizol (15596026, Invitrogen) in Lysing Matrix D 2 mL tubes  
15 (116913100, MP Biomedicals) using the FastPrep-24™ system (MP Biomedicals) at the speed of 6.5  
16 m/s during 1 min. Total RNA was extracted using the Direct-zol RNA MicroPrep Kit (R2062, Zymo  
17 Research: nasal turbinates) or MiniPrep Kit (R2052, Zymo Research: lung) and reverse transcribed to  
18 first strand cDNA using the SuperScript™ IV VILO™ Master Mix (11766050, Invitrogen). qPCR was  
19 performed in a final volume of 10 μL per reaction in 384-well PCR plates using a thermocycler  
20 (QuantStudio 6 Flex, Applied Biosystems). Briefly, 2.5 μL of cDNA (12.5 ng) were added to 7.5 μL of  
21 a master mix containing 5 μL of Power SYBR green mix (4367659, Applied Biosystems) and 2.5 μL of  
22 nuclease-free water with nCoV\_IP2 primers (nCoV\_IP2-12669Fw: 5'-ATGAGCTTAGTCCTGTG-3';  
23 nCoV\_IP2-12759Rv: 5'-CTCCCTTGTGTGTGTGT-3') at a final concentration of 1 μM<sup>35</sup>. The  
24 amplification conditions were as follows: 95°C for 10 min, 45 cycles of 95°C for 15 s and 60°C for 1  
25 min; followed by a melt curve, from 60°C to 95°C. Viral load quantification of hamster tissues was  
26 assessed by linear regression using a standard curve of eight known quantities of plasmids containing  
27 the *RdRp* sequence (ranging from 10<sup>7</sup> to 10<sup>0</sup> copies). The threshold of detection was established as  
28 200 viral copies/μg of RNA. The Golden hamster gene targets were selected for quantifying host  
29 inflammatory mediator transcripts in the tissues using the *Hprt* (hypoxanthine  
30 phosphoribosyltransferase) and the *γ-actin* genes as reference (Table 1). Variations in gene  
31 expression were calculated as the n-fold change in expression in the tissues from the infected  
32 hamsters compared with the tissues of the uninfected ones using the 2<sup>-ΔΔCt</sup> method<sup>36</sup>.

33

34

35

1

2 **Table 1. Primer sequences used for qPCR in the golden hamster tissues.**

Gene	Primer sequence (5' – 3')	Reference
ha- $\gamma$ -actin	For ACAGAGAGAA GATGACGCAGATAAT	37
	Rev GCCTGAATGGCCACGTACA	
ha-Hprt	For TGC GGATGATATCTCAACTTAACTG	38
	Rev AAAGGAAAGCAAAGTTTGTATTGTCA	
ha-Il-6	For GGACAATGACTATGTGTTGTTAGAA	37
	Rev AGG CAA ATT TCC CAA TTG TAT CCA GGTTGCCAAACCTTATCAGAAATG	
ha-Il-10	For TTCACCTGTTCCACAGCCTTG	37
	Rev	
ha-Il-1 $\beta$	For GGCTGATGCTCCCATTCG	38
	Rev CACGAGGCATTTCTGTTGTTC A	
ha-Tnf- $\alpha$	For TGAGCCATCGTGCCAATG	37
	Rev AGCCCGTCTGCTGGTATCAC	
ha-Ifn- $\gamma$	For TGTTGCTCTGCCTCACTCAGG	37
	Rev AAGACGAGGTCCCCTCCATTC	
ha-Cxcl10	For GCCATTCATCCACAGTTGACA	38
	Rev CATGGTGCTGACAGTGGAGTCT	
ha-Ccl5	For ACTGCCTCGTGTTACATCA	39
	Rev CCCACTTCTTCTTTGGGTTG	

3

4

5 **Droplet digital PCR (ddPCR).** Reverse transcription: 200 ng of RNA was reverse transcribed using  
6 iScript Advanced cDNA Synthesis kit for RT-qPCR (1702537, Bio-Rad) according to the manufacturer's  
7 specifications. Quantitative PCR for  $\gamma$ -actin and Hprt reference genes: Real-time PCR was performed  
8 in a CFX96 qPCR machine (Bio-Rad). All samples were measured in duplicate. The 10  $\mu$ L PCR reaction  
9 included 0.8 ng of cDNA, 1 $\times$  PowerUp PCR master mix (A25742, Applied Biosystems) and 0.5  $\mu$ M of  
10 each primer (Table 1). The reactions were incubated in a 96-well optical plate at 95°C for 2 min,  
11 followed by 40 cycles of 95°C for 15s and 60°C for 1 min. Droplet digital PCR: ddPCR reactions were  
12 performed on the QX200 Droplet Digital PCR system according to manufacturer's instructions (Bio-  
13 Rad). Briefly, reaction mixture consisted in 10  $\mu$ L ddPCR Supermix for probe no dUTP (1863023, Bio-  
14 Rad), 0.25 to 1 ng of cDNA, primers and probes for E/IP4 and N/nsp13 duplex reactions used at  
15 concentration listed in Table 2 in a final volume of 20  $\mu$ L. PCR amplification was conducted in a  
16 iCycler PCR instrument (Bio-Rad) with the following condition: 95 °C for 10 min, 40 cycles of 94 °C for

1 30 s with a ramping of 2°/s, 59 °C for 1 min with a ramping of 2°/s, followed by 98 °C for 5 min with a  
 2 ramping of 2°/s and a hold at 4 °C. After amplification, the 96-well plate was loaded onto the QX200  
 3 droplet reader (Bio-Rad) that measures automatically the fluorescence intensity in individual  
 4 droplets. Generated data were subsequently analyzed with QuantaSoft™ software (Bio-Rad) based  
 5 on positive and negative droplet populations. Data are expressed as CPD (copy per droplets)  
 6 normalized to *γ-actin* and *Hprt* reference genes relative expression.

7  
 8 **Table 2. Primer and probes sequences used for ddPCR in the golden hamster lung.**

Gene	Primer sequence (5' – 3')	Concentration (nM)	Reference
N	For GGGGAAGTCTCCTGCTA	900	27
	Rev CAGACATTTTGCTCTCAA	900	
	Probe FAM-TTGCTGCTGCTTGACAGATT-IBFQ	250	
IP4	For GGTAAGTGGTATGATTTTCG	900	35
	Rev CTGGTCAAGGTTAATATAGG	900	
	Probe HEX-TCATACAAACCACGCCAGG-IBFQ	300	
E	For ACAGGTACGTTAATAGTTAATAGCGT	200	40
	Rev ATATTGCAGCAGTACGCACACA	200	
	Probe FAM-ACACTAGCCATCCTTACTGCGCTTCG-IFBQ	150	
nsp13	For TAAGGGCACTAGAACCAG	900	this paper
	Rev ACAATTTAGCAGGACAACG	900	
	Probe HEX-AGGTCCAGACATGTTCTCGGAA-IFBQ	250	

9

10

11 **Viral titration in golden hamsters' lung.** Frozen lungs fragments were weighted and homogenized  
 12 with 1 mL of ice-cold DMEM supplemented with 1% penicillin/streptomycin (15140148, Thermo  
 13 Fisher) in Lysing Matrix M 2 mL tubes (116923050-CF, MP Biomedicals) using the FastPrep-24™  
 14 system (MP Biomedicals), and the following scheme: homogenization at 4.0 m/s during 20 sec,  
 15 incubation at 4°C during 2 min, and new homogenization at 4.0 m/s during 20 sec. The tubes were  
 16 centrifuged at 10.000 x g during 1 min at 4°C, and the supernatants were titrated on Vero-E6 cells by  
 17 classical plaque assays using semisolid overlays (Avicel, RC581-NFDR080I, DuPont)<sup>31</sup>.

18

19 **Statistics.** Statistical analysis was performed using Prism software (GraphPad, version 9.0.0, San  
 20 Diego, USA), with *p* < 0.05 considered significant. Quantitative data was compared across groups  
 21 using Log-rank test or two-tailed Mann-Whitney test.

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17 [of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6\\_2](https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2)> (2020).
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32

33

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44 manuscript.

45

#### 46 **Author Contributions**

- 1 JPC and HB conceived the experimental hypothesis.
- 2 GDM, FLaz, FLar and HB designed the experiments.
- 3 GDM, FLaz, FLar, LF, LK and AM performed the experiments.
- 4 GDM, FLaz, FLar, LF, AM, PP, ML and PML analyzed the data.
- 5 GDM, JPC and HB wrote the manuscript.

6

#### 7 **Competing interests**

- 8 The authors declare no competing interests.

9

#### 10 **Additional information**

- 11 Extended data is available for this paper.

12

- 13 **Reporting Summary.** Further information on research design is available in the Nature Research

- 14 Reporting Summary linked to this article.

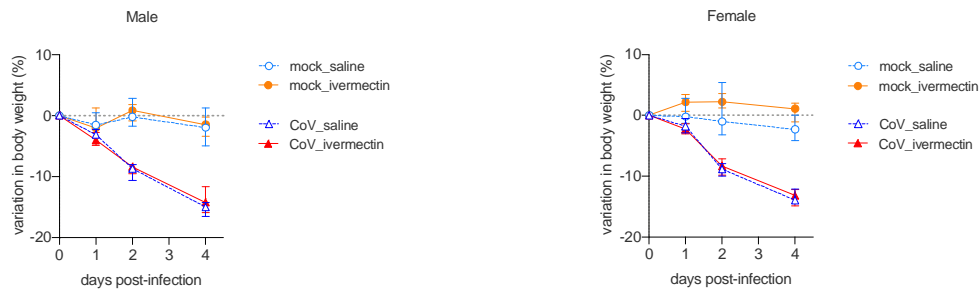
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#### 16 **Data availability**

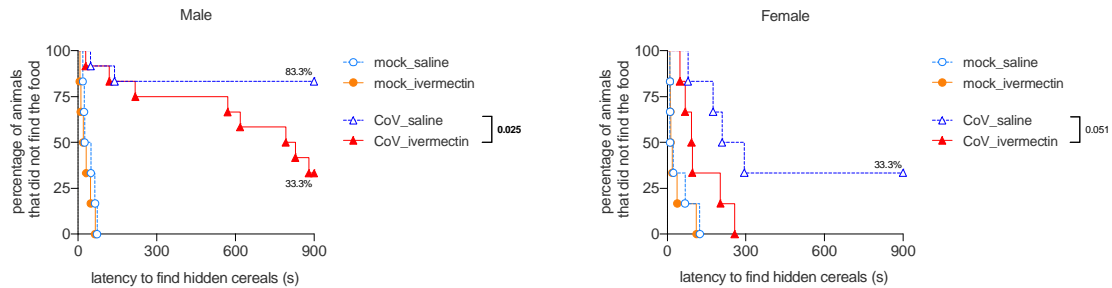
- 17 All the data generated or analyzed during this study are included in this article along with its
- 18 extended data.

19

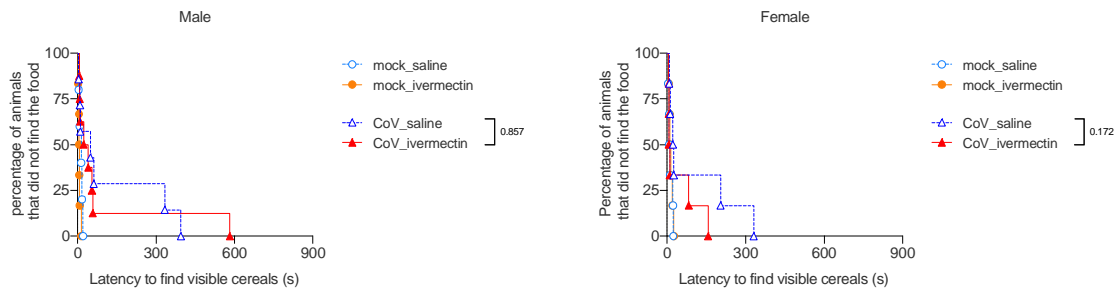
### a. Body weight



### b. Food finding test at 3 dpi (hidden food)



### c. Food finding test at 3 dpi (visible food)



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2

**Extended data Fig. 1 | Body weight variation and Food finding test. a,** progression of body weight in

3

male and female hamsters, mock-infected or SARS-CoV-2 infected, treated with saline or with 400

4

$\mu\text{g}/\text{kg}$  ivermectin. **b.** curves represent the percentage of animals that did not find the hidden (buried)

5

food. **c.** curves represent the percentage of animals that did not find the visible (unburied) food.

6

Food finding assays were performed at 3 days post-infection.  $n=12/\text{group}$  (males CoV\_saline and

7

males CoV\_ivermectin),  $n=6/\text{group}$  (females CoV\_saline and females CoV\_ivermectin),  $n=4/\text{group}$

8

(males and females mock\_ivermectin),  $n=3/\text{group}$  (males and females mock\_saline). Log-rank

9

(Mantel-Cox) test. The p value is indicated in bold when significant at a 0.05 threshold. Data were

10

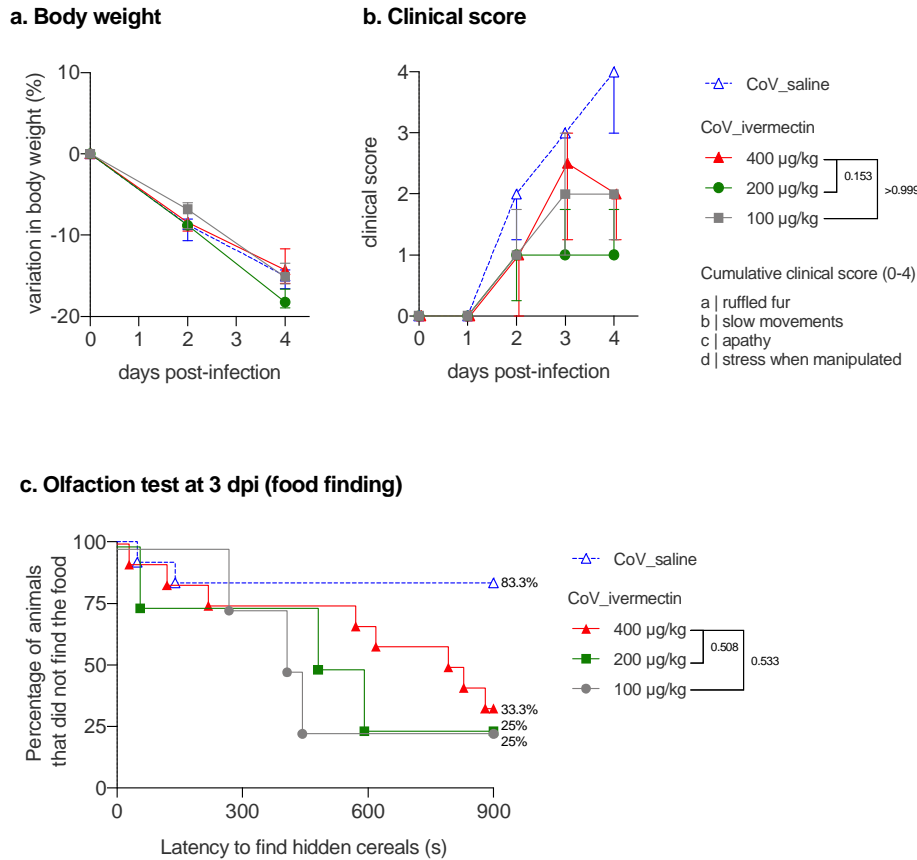
obtained from three independent experiments for males and two independent experiments for

11

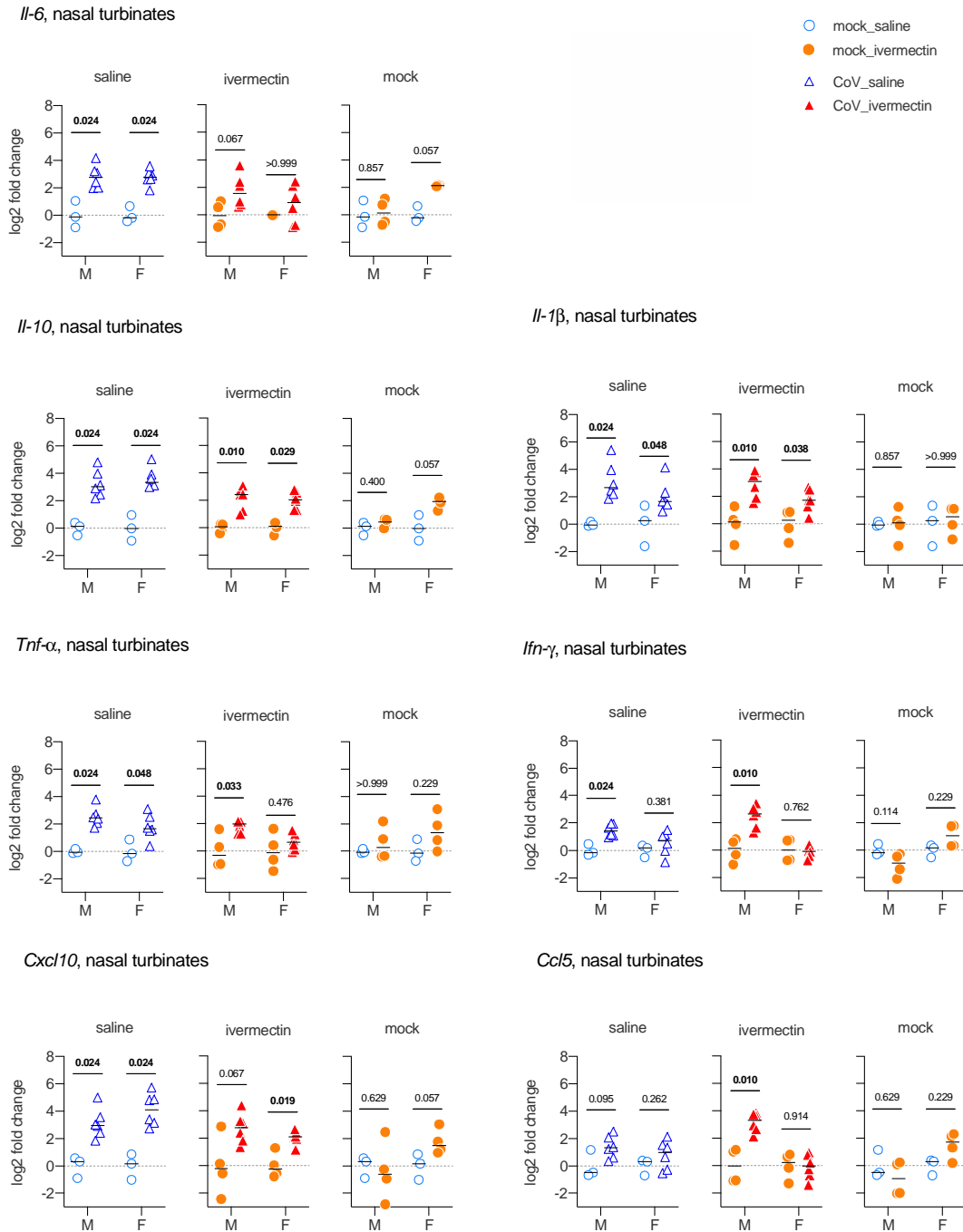
females.

12



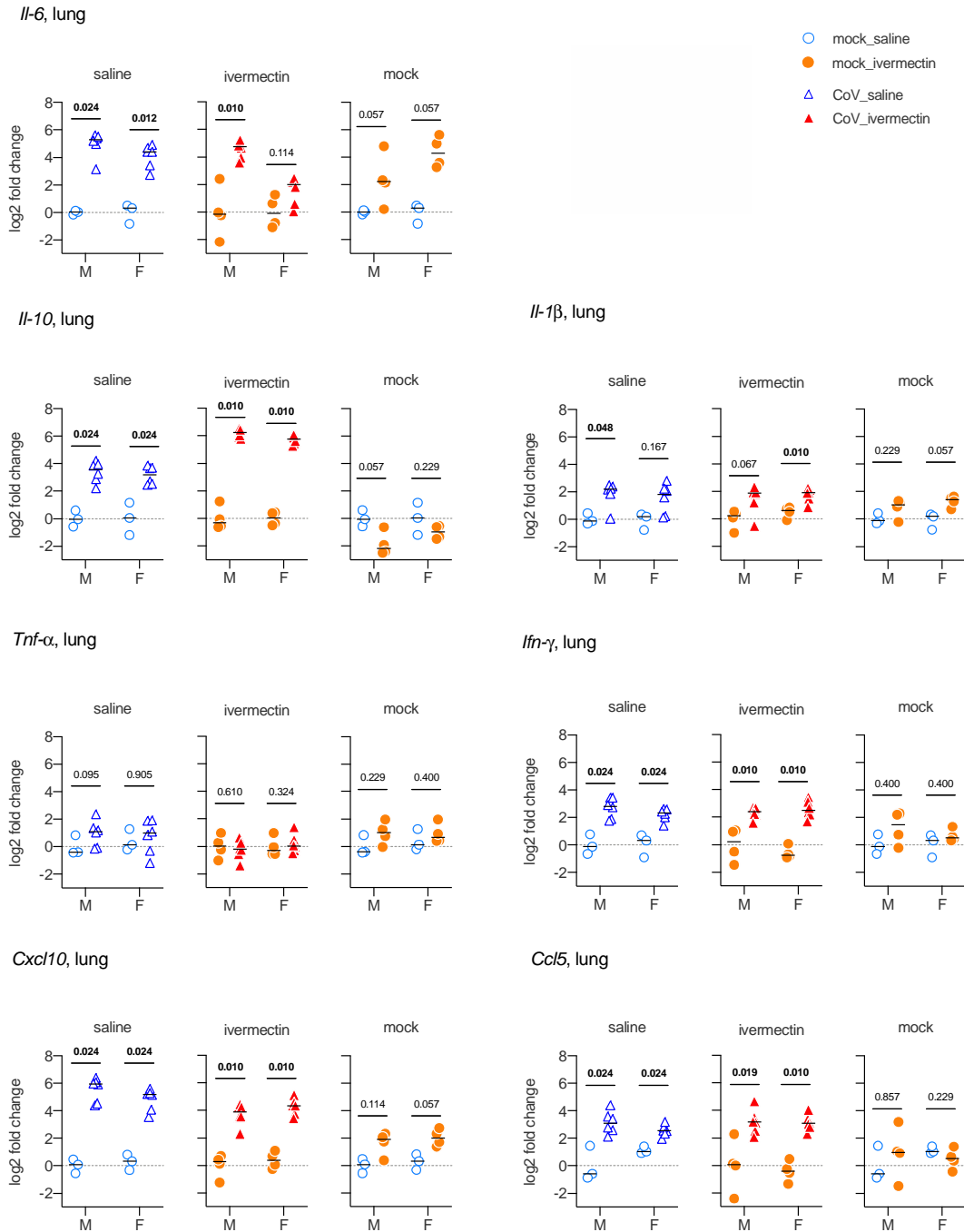


1  
2 **Extended data Fig. 2 | Clinical aspects of SARS-CoV-2-infected male hamsters and treated with**  
3 **different doses of ivermectin. a**, progression of body weight in male hamsters, treated with saline or  
4 with 400 µg/kg, 200 µg/kg or 100 µg/kg ivermectin. **b**, clinical score based on a cumulative 0-4 scale:  
5 ruffled fur; slow movements; apathy; stress when manipulated. **c**, olfaction deficit based on the  
6 buried food finding test. Curves represent the percentage of animals that did not find the buried  
7 food. Food finding assays were performed at 3 days post-infection. n=12/group (CoV\_saline and  
8 CoV\_ivermectin 400 µg/kg, as shown in Fig. 1) or n=4 (CoV\_ivermectin 200 µg/kg and 100 µg/kg).  
9 Mann-Whitney test at 4 dpi (b) and Log-rank (Matel-Cox) test (c). The p value is indicated in bold  
10 when significant at a 0.05 threshold. Symbols indicate the median ± interquartile range.



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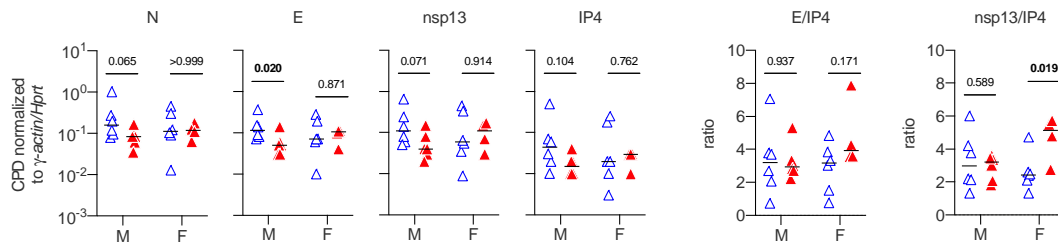
2 **Extended data Fig. 3 | Immunological aspects in the nasal turbinates at 4 days post-infection of**  
 3 **SARS-CoV-2-infected hamsters with and without ivermectin treatment.** Cytokines and chemokines  
 4 transcripts in the nasal turbinates at 4 dpi. n=6/group (CoV\_saline and CoV-ivermectin), n=4/group  
 5 (mock\_ivermectin), n=3/group (mock\_saline). Mann-Whitney test. The p value is indicated in bold  
 6 when significant at a 0.05 threshold. Horizontal lines indicate medians. M: male hamsters; F: female  
 7 hamsters. Data were obtained from two independent experiments for each sex.



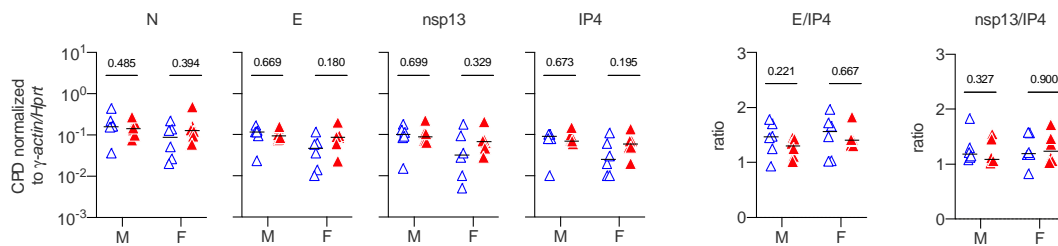
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2 **Extended data Fig. 4 | Immunological aspects in the lungs at 4 days post-infection of SARS-CoV-2-**  
 3 **infected hamsters with and without ivermectin treatment.** Cytokines and chemokines transcripts in  
 4 the lungs at 4 dpi. n=6/group (CoV\_saline and CoV-ivermectin), n=4/group (mock\_ivermectin),  
 5 n=3/group (mock\_saline). Mann-Whitney test. The p value is indicated in bold when significant at a  
 6 0.05 threshold. Horizontal lines indicate medians. M: male hamsters; F: female hamsters. Data were  
 7 obtained from two independent experiments for each sex.

**a. ddPCR in the nasal turbinates at 4 dpi**



**b. ddPCR in the lung at 4 dpi**



1

2 **Extended data Fig. 5 | Virologic aspects in the nasal turbinates and in the lungs at 4 days post-**  
 3 **infection of SARS-CoV-2-infected hamsters with and without ivermectin treatment. ab, viral gene**  
 4 **expression of N (nucleocapsid), E (envelope), nsp13 (non-structural protein 13), IP4 (RdRp, RNA-**  
 5 **dependent RNA polymerase), and E/IP4 and nsp13/IP4 ratios determined by digital droplet PCR**  
 6 **(ddPCR) in the nasal turbinates (a) and in the lungs (b) at 4 dpi. Data are expressed as CPD (copy per**  
 7 **droplets) normalized to  $\gamma$ -actin and Hprt reference genes relative expression. n=6/group (except**  
 8 **nasal turbinates from female CoV\_ivermectin, where n=4). Mann-Whitney test. The p value is**  
 9 **indicated in bold when significant at a 0.05 threshold. Horizontal lines indicate the medians. M: male**  
 10 **hamsters; F: female hamsters. Data were obtained from two independent experiments for each sex.**

11