



Article scientifique

Article

2020

Supplemental data

Open Access

This file is a(n) Supplemental data of:

---

Interferon-dependent and respiratory virus-specific interference in dual  
infections of airway epithelia

---

Essaidi, Manel; Geiser, Johan; Huang, Song; Constant, Samuel; Kaiser, Laurent; Tapparel, Caroline

This publication URL:

<https://archive-ouverte.unige.ch//unige:139844>

Publication DOI:

[10.1038/s41598-020-66748-6](https://doi.org/10.1038/s41598-020-66748-6)

1 INTERFERON-DEPENDENT AND RESPIRATORY VIRUS-SPECIFIC  
2 INTERFERENCE IN DUAL INFECTIONS OF AIRWAY EPITHELIA

3

4 SHORT TITLE: Viral interference in human respiratory epithelia

5

6 Manel Essaidi-Laziosi<sup>1</sup>; Johan Geiser<sup>1</sup>; Song Huang<sup>3</sup>; Samuel Constant<sup>3</sup>; Laurent Kaiser<sup>1,2</sup> and Caroline Tapparel

7 <sup>1,2\*</sup>

8 1. Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva,  
9 Switzerland

10 2. Division of Infectious diseases, Geneva University Hospital, Switzerland

11 3. Epithelix Sàrl, Plan les Ouates, Geneva, Switzerland

12

13 \*Correspondence to: [caroline.tapparel@unige.ch](mailto:caroline.tapparel@unige.ch)

14

15 SUPPLEMENTARY METHODS

16 LACTATE DEHYDROGENASE ASSAY.

17 LDH release was measured with the Cytotoxicity Detection Kit (Roche 04744926001) according  
18 to the manufacturer's instruction. Fifty  $\mu$ l of basal sample was incubated with 50 $\mu$ l of catalyst/dye  
19 solution (1/45) for 15' at RT in the dark. The reaction was stopped with 25 $\mu$ l of stop solution. Sample  
20 absorbance was measured at 490nm using the photometer reader (Thermo Fisher Scientific Multiskan  
21 Go). Percentage of cytotoxicity was calculated with the equation: % of cytotoxicity (sample) =  
22  $100 * [OD(\text{sample}) - OD(\text{non-infected control})] / [OD(\text{triton treated tissue}) - OD(\text{non-infected control})]$ ,  
23 where triton treated and non-infected tissues constitute the positive and negative controls  
24 respectively.

25 CYTOKINE SECRETION

26 Interleukin-8 (IL-8 or CXCL-8), CXC motif chemokine 10 (CXCL10 or IP-10) C-C motif chemokine 5  
27 (CCL5 or RANTES), interferon lambda (IFN- $\lambda$ 1/  $\lambda$ 3, IL-29 /IL-28B), and interleukin-6 (IL-6) were  
28 measured in the basal medium by ELISA according to the manufacturer's instruction (R&D DY208-05,  
29 DY266-05, DY278-05, DY1598B-05, and DY206-05). Sample absorbance was measured at 450nm using  
30 the photometer reader (BioTek, FilterMax F5 Multi-Mode Microplate Reader).

31

32

33

34

35

36

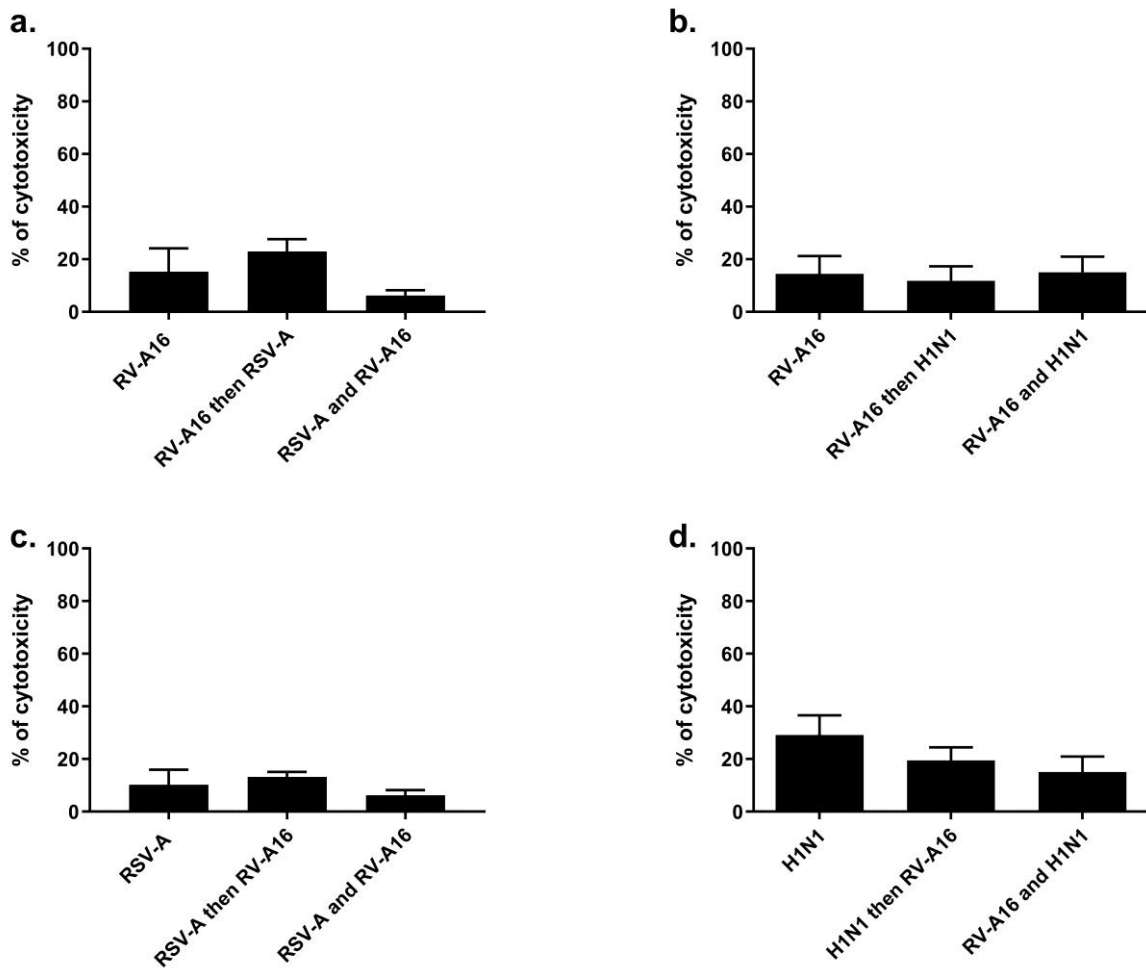
37

38

39

40 SUPPLEMENTARY FIGURES

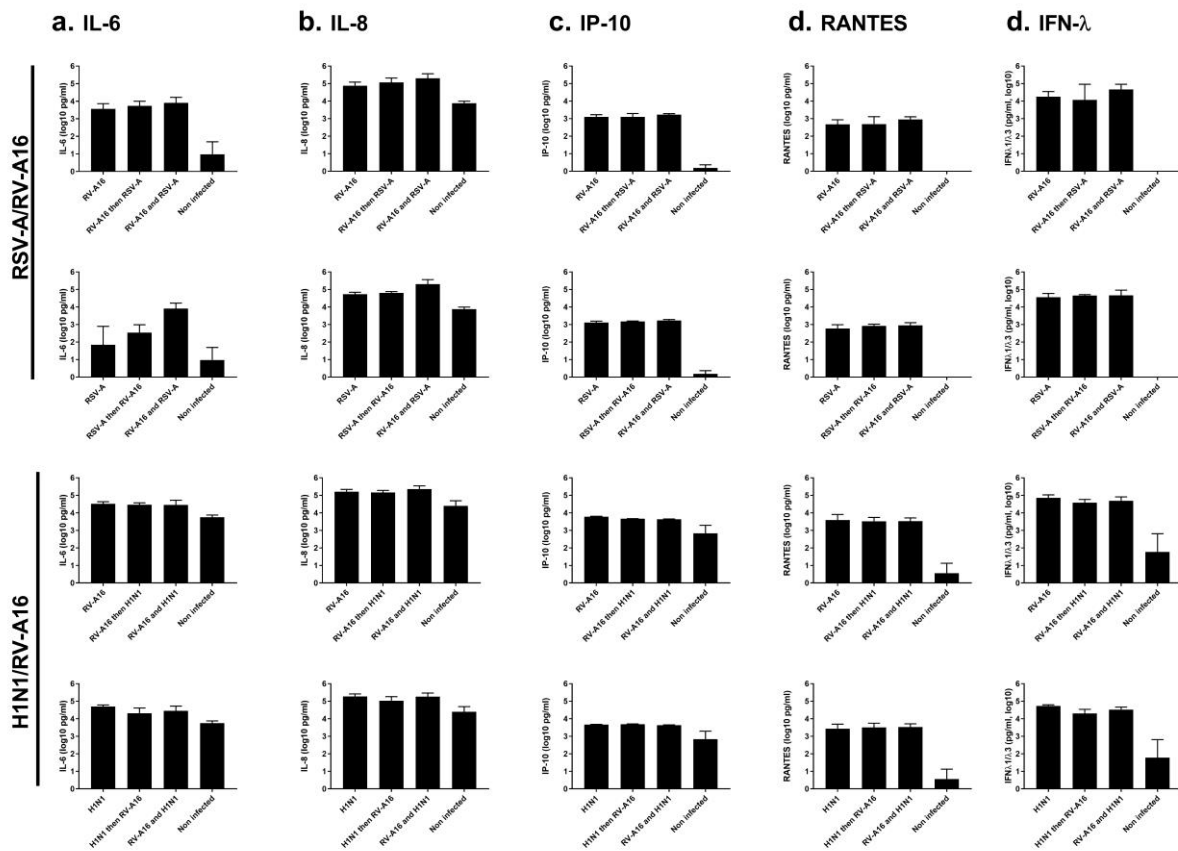
41



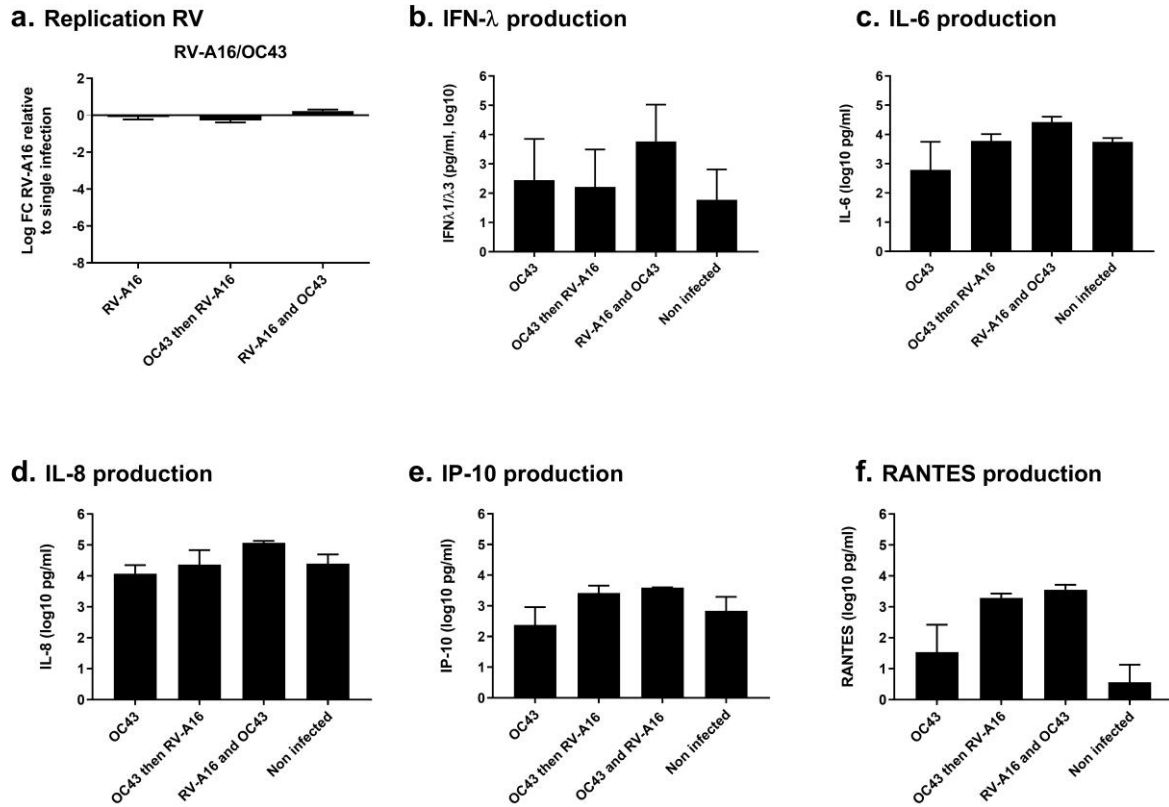
42

43 **Figure S1:** Viral toxicity in dual versus single infections of reconstituted human airway epithelia. Each  
 44 virus was inoculated alone or in combination with the other virus at the same time or two days after,  
 45 respectively. Lactase dehydrogenase release was measured as a marker of cell death as previously  
 46 described (20) at five days after inoculation with the first virus. The percentage of cytotoxic effect was  
 47 then calculated in comparison with non-infected tissue (0%) and Triton X-100–treated tissue (100%).  
 48 The analyzed viral couple is specified on the top of each panel, while the chronology of infection is  
 49 shown on the X-axis (‘then’, after two days; ‘and’, at the same time). Statistical significance relative to  
 50 single infection was calculated using one-way ANOVA. Data are expressed as mean and SEM of at least  
 51 three replicates.

52 The analyzed viral couple is indicated on the top of each panel while the chronology of infection is is  
 53 shown on the X-axis ('then', after two days; 'and', at the same time). Data represent the means and  
 54 SEMs of two independent experiments.



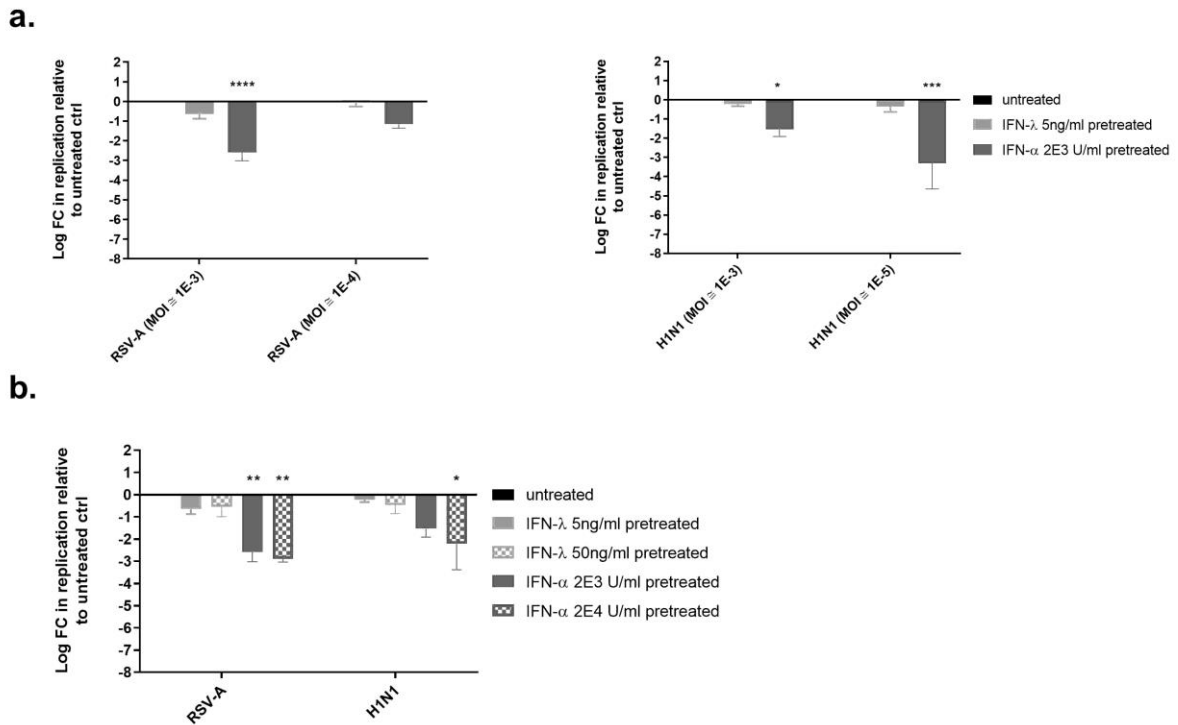
55  
 56 **Figure S2: Cytokine induction in dual versus single infections of reconstituted human airway**  
 57 **epithelia.** Each virus was inoculated alone or in combination with the other virus at the same time or  
 58 two days after, respectively. IL-6 (a), IL-8 (b), IP-10 (c), RANTES (d) and IFN-λ (e) released in the basal  
 59 medium were measured by ELISA four DPI with the first virus, as previously described (20). The  
 60 analyzed viral couple is indicated on the top of each panel while the chronology of infection is shown  
 61 on the X-axis ('then', after two days; 'and', at the same time). Data represent the means and SEMs of  
 62 three replicates.



63

64 **Figure S3: Change in viral replication and cytokine response in dual versus single infections of**  
 65 **reconstituted human airway epithelia with OC43 and RV-A16. A.** RV-A16 was inoculated alone, at the  
 66 same time ('and') or two days after (then') OC43. The log fold change (FC) in apically released RV-A16  
 67 (measured by RT-qPCR five days post infection) in dual versus single infection is indicated on the Y-  
 68 axis. The chronology of infection is shown on the X-axis ('then', after two days; 'and', at the same time).  
 69 **b to f:** IFN-λ (b), IL-6 (c), IL-8 (d), IP-10 (e), and RANTES (f) released in the basal medium four DPI with  
 70 OC43 were measured by ELISA as previously described [20]. Data are expressed as mean and SEM of  
 71 at least three replicates.

72



73

74 **Figure S4: Susceptibility of RSV-A and H1N1 to IFNs after variation of the viral inoculum (a) or the**  
 75 **IFN dose (b).** Tissues were treated with IFN before and during infection as in figure 4 but with different  
 76 viral inoculum (MOI around 1E-3 and 1E-4 virus/accessible cell for RSV and 1E-3 or 1E-5 for H1N1) in  
 77 panel a or different doses of IFN (5ng/ml or 50ng/ml of IFN-λ and 2E3 U/ml or 2E4 U/ml of IFN-α) in  
 78 panel b. For each condition, the log fold change (FC) in apically released virus (measured by RT-qPCR  
 79 three days post infection) relative to untreated Ctrl is indicated on the Y-axis. Statistical significance  
 80 relative to the untreated control was calculated using two-way ANOVA (\*\*\*\*P < 0.0001, \*\*\*P < 0.001,  
 81 \*\*P < 0.01, \*P < 0.01). Data are expressed as mean and SEM of at least two replicates.