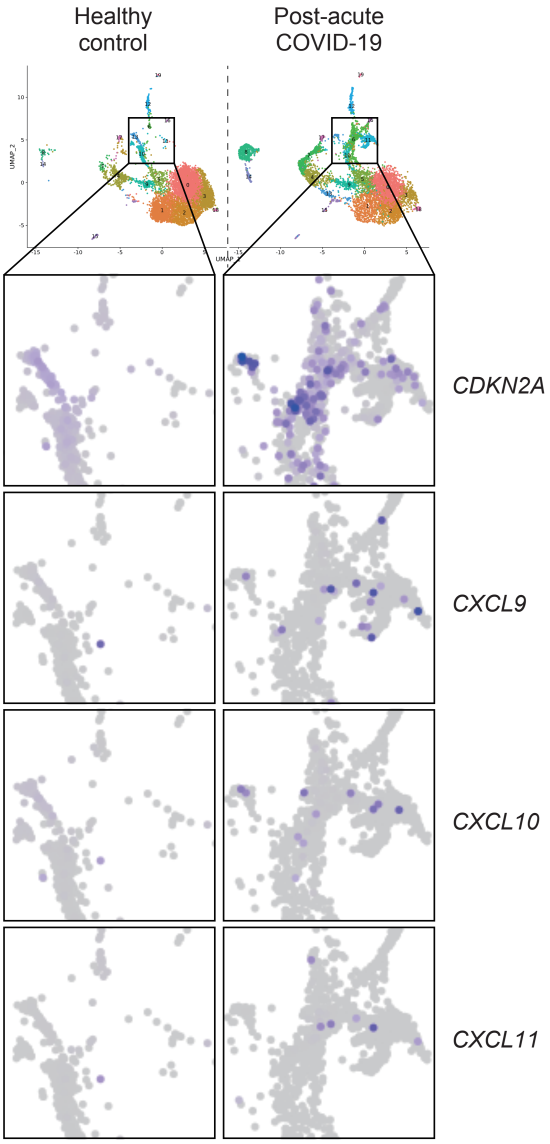
nature portfolio

https://doi.org/10.1038/s43587-022-00170-7

## Supplementary information

# SARS-CoV-2 infection triggers paracrine senescence and leads to a sustained senescence-associated inflammatory response

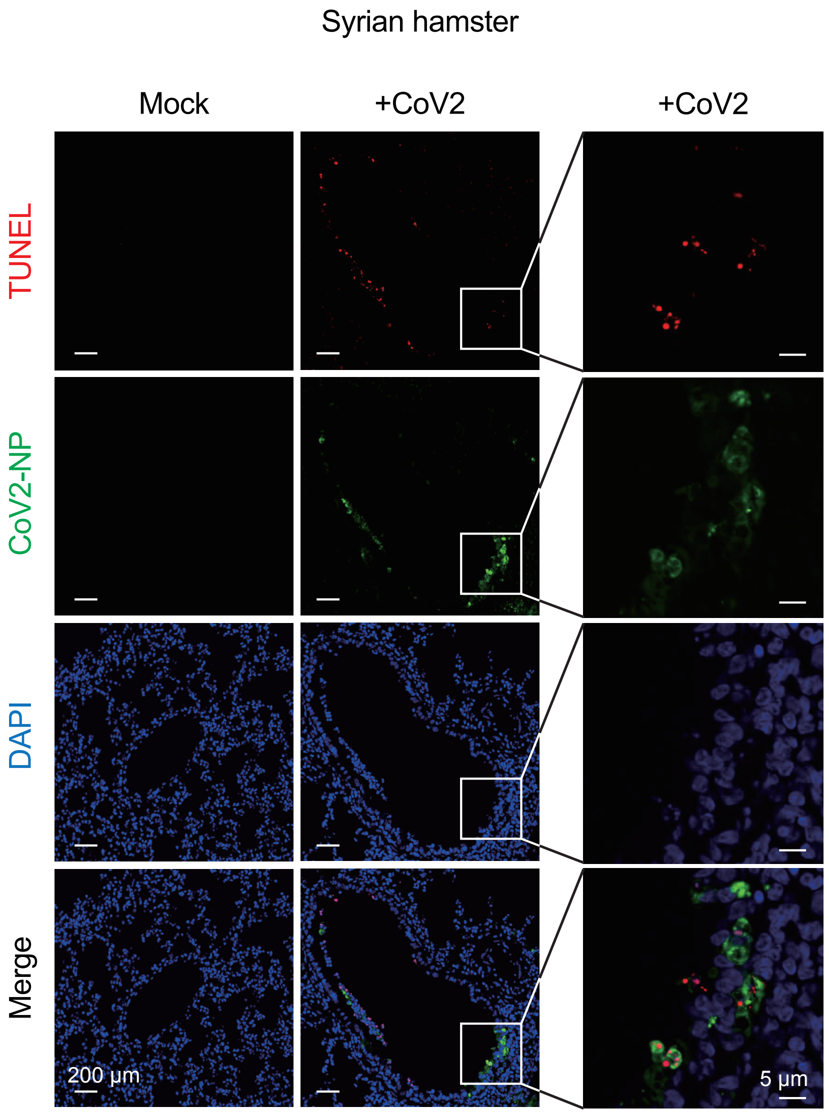
## Supplementary information



**Supplementary Fig. 1**

**Persistent inflammatory response in patients with COVID-19.**

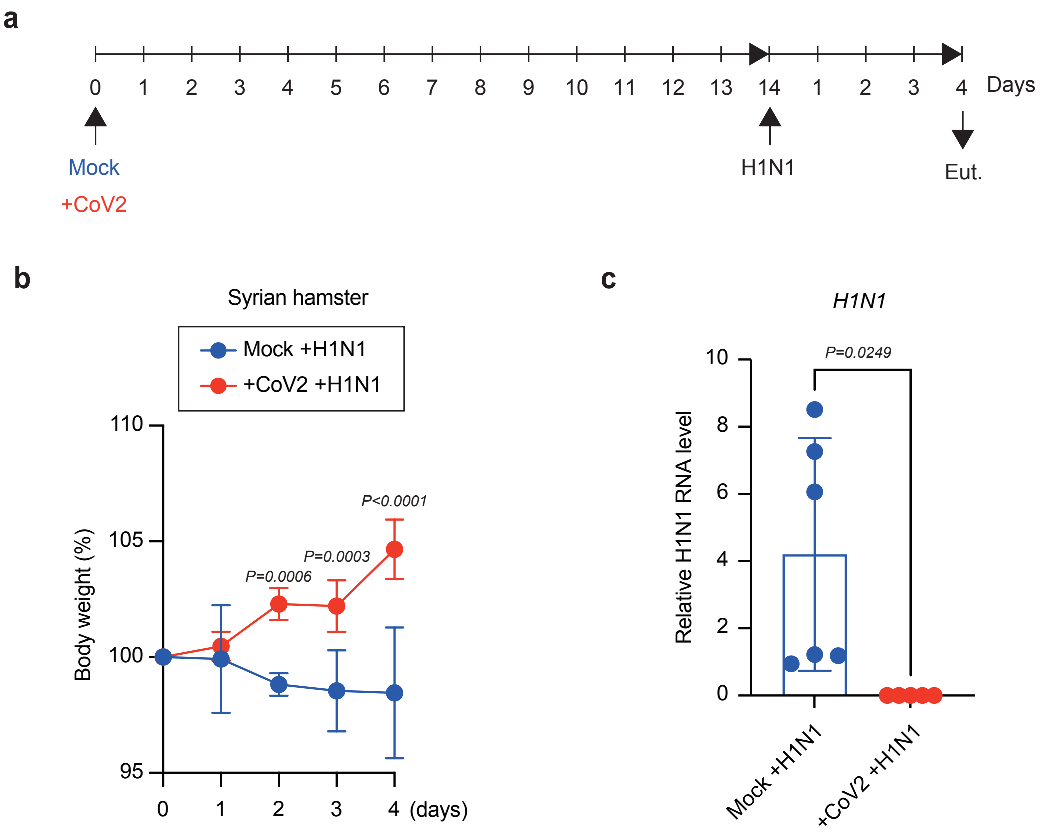
Single-cell RNA transcriptomic analysis of lung tissue from patients with severe COVID-19[28]. Epithelial cells population of Uniform Manifold Approximation and Projection (UMAP) plots was divided into healthy controls (n=2) and COVID-19 patients (n=3), and The distribution of cells expressing the cellular senescence marker (*CDKN2A* gene) and the SASP factor gene, which are also expressed in the Syrian hamster model of SARS-CoV-2 infection.



**Supplementary Fig. 2**

**SARS-CoV-2 infection induces cell death.**

Syrian hamsters infected Mock (n=3) or SARS-CoV-2 (n=3) were euthanized on days 5 post-infection and lung sections were subjected to TUNEL staining for SARS-CoV-2 N protein (CoV2-NP [green]), TUNEL [red], and DAPI [blue]. Representative images were shown.



**Supplementary Fig. 3**

**SARS-CoV-2 infection prevents superinfection of influenza virus.**

**a-c.** Syrian hamsters were intranasally inoculated with SARS-CoV-2 (B.1.1.7) 5.6 x $10^5$ PFU (in 80 μL), medium (mock). After 14 days post-infection of SARS-CoV-2, mock or SARS-CoV-2 infected hamsters were intranasally inoculated influenza A (H1N1) pdm09 5.6 x $10^5$ PFU (in 80 μL). Hamsters infected with mock followed by H1N1 (mock+H1N1; n=6) and SARS-CoV-2 followed by H1N1 (CoV2+H1N1; n=6). The timeline of the experiment was shown (**a**). The body weight was monitored until day 4 post H1N1 infection (**b**). These hamsters were euthanized on day 4 after H1N1 infection, and the expression of H1N1 genomic RNA was analyzed using RT-qPCR. (**c**). For all graphs, error bars indicate mean ± standard deviation (s.d.). Statistical significance was determined two-way ANOVA followed by sidak's multiple comparison test in (b), and with two-tailed unpaired Student's t-test in (c).

# Supplementary Table. 1

| RT-qPCR Primer Information | |
|---|---|
| Primer ID | Sequence |
| *human ACE2 Forward* | 5'- CGAAGCCGAAGACCTGTTCTA -3' |
| *human ACE2 Reverse* | 5'- GGGCAAGTGTGGACTGTTC -3' |
| *human p16 Forward* | 5'- ACCAGAGGCAGTAACCATGC -3' |
| *human p16 Reverse* | 5'- AAGTTTCCCGAGGTTTCTCA -3' |
| *human p21 Forward* | 5'- AGCGATGGAACTTCGACTTTG -3' |
| *human p21 Reverse* | 5'- CGAAGTCACCCTCCAGTGGT -3' |
| *human LMNB1 Forward* | 5'- GATTGCCCAGTTGGAAGCCT -3' |
| *human LMNB1 Reverse* | 5'- TGGTCTCGTTAATCTCCTCTTCATACA -3' |
| *human TNFa Forward* | 5'- GCCCCCAGAGGGAAGAGTTCCCCA -3' |
| *human TNFa Reverse* | 5'- GCT TGAGGGTTTGCTACAACATGGGC -3' |
| *human IL6 Forward* | 5'- CCAGGAGCCCAGCTATGAAC -3' |
| *human IL6 Reverse* | 5'- CCCAGGGAGAAGGCAACTG -3' |
| *human IFNb Forward* | 5'- AAACTCATGAGCAGTCTGCA -3' |
| *human IFNb Reverse* | 5'- AGGAGATCTTCAGTTTCGGAGG -3' |
| *human IL1B Forward* | 5'- CTGTCCTGCGTGTTGAAAGA -3' |
| *human IL1B Reverse* | 5'- TTGGGTAATTTTTGGGATCTACA -3' |
| *human IL8 Forward* | 5'- AAGGAAAACTGGGTGCAGAG -3' |
| *human IL8 Reverse* | 5'- ATTGCATCTGGCAACCCTAC -3' |
| *human β-actin Forward* | 5'- TGGATCAGCAAGCAGGAGTATG -3' |
| *human β-actin Reverse* | 5'- GCATTTGCGGTGGACGAT -3' |
| *human GAPDH Forward* | 5'- CAACTACATGGTTTACATGTTC -3' |
| *human GAPDH Reverse* | 5'- GCCAGTGGACTCCACGAC -3' |
| *syrian hamster p16 Forward* | 5'- AGAGGTTCGGGCTTTGCT -3' |
| *syrian hamster p16 Reverse* | 5'- CTACTTGGGTGTTGCCCATC -3' |
| *syrian hamster p21 Forward* | 5'- TTGTTCGGAGGCACCACCAT -3' |
| *syrian hamster p21 Reverse* | 5'- TCGAAGTTCCACCGTTCTCG -3' |
| *syrian hamster Ccl17 Forward* | 5'- GTGCTGCCTGGAGATCTTCA -3' |
| *syrian hamster Ccl17 Reverse* | 5'- TGGCATCCCTGGGACACT -3' |
| *syrian hamster Ifng Forward* | 5'- TGTTGCTCTGCCTCACTCAGG -3' |
| *syrian hamster Ifng Reverse* | 5'- AAGACGAGGTCCCCTCCATTC -3' |
| *syrian hamster Cxcl9 Forward* | 5'- ACTCTGCTCTGCCATGAAGT -3' |
| *syrian hamster Cxcl9 Reverse* | 5'- TTCCTTATGACTAGGGTTCCTTGAA -3' |
| *syrian hamster Cxcl10 Forward* | 5'- GCCATTCATCCACAGTTGACA -3' |
| *syrian hamster Cxcl10 Reverse* | 5'- CATGGTGCTGACAGTGGAGTCT -3' |
| *syrian hamster Cxcl11 Forward* | 5'- CCGCCTCATACGGGAAATGT -3' |
| *syrian hamster Cxcl11 Reverse* | 5'- AAGACAGAAGGTTGGGCTCG -3' |
| *syrian hamster Gapdh Forward* | 5'- TTGTTGCCATCAATGACCCCTT -3' |
| *syrian hamster Gapdh Reverse* | 5'- CGTTCTCAGCCTTGACTGTGCCTT -3' |
| *mouse β-actin Forward* | 5'- GATGACCCAGATCATGTTTGA -3' |
| *mouse β-actin Reverse* | 5'- GGAGAGCATAGCCCTCGTAG -3' |
| *mouse Gapdh Forward* | 5'- CAACTACATGGTCTACATGTTC -3' |
| *mouse Gapdh Reverse* | 5'- CGCCAGTAGACTCCACGAC -3' |
| *mouse p16Ink4a Forward* | 5'- GAACTCTTTCGGTCGTACCC -3' |
| *mouse p16Ink4a Reverse* | 5'- CGAATCTGCACCGTAGTTGA -3' |
| *mouse p19Arf Forward* | 5'- GCCGCACCGGAATCCT -3' |
| *mouse p19Arf Reverse* | 5'- TTGAGCAGAAGAGCTGCTACGT -3' |
| *mouse Ifnb Forward* | 5'- CAGCTCCAAGAAAGGACGAAC -3' |
| *mouse Ifnb Reverse* | 5'- GGCAGTGTAACTCTTCTGCAT -3' |
| *mouse Ifng Forward* | 5'- ATGAACGCTACACACTGCATC -3' |
| *mouse Ifng Reverse* | 5'- CCATCCTTTTGCCAGTTCCTC -3' |
| *mouse Cxcl9 Forward* | 5'- CCTAGTGATAAGGAATGCACGATG -3' |
| *mouse Cxcl9 Reverse* | 5'- CTAGGCAGGTTTGATCTCCGTTC -3' |
| *mouse Cxcl10 Forward* | 5'- ATCATCCCTGCGAGCCTATCCT -3' |
| *mouse Cxcl10 Reverse* | 5'- GACCTTTTTTGGCTAAACGCTTTC -3' |
| *mouse Mmp10 Forward* | 5'- TGCTGCCTATGAGGCTCACAAC -3' |
| *mouse Mmp10 Reverse* | 5'- GGAGGAAAACCGAGAGTGTGGA -3' |
| *SARS-CoV-2 N Forward* | 5'- TTACAAACATTGGCCGCAAA -3' |
| *SARS-CoV-2 N Reverse* | 5'- GCGCGACATTCCGAAGAA -3' |
| *SARS-CoV subgenomic N Forward* | 5'- CCAGGTAACAAACCAACCAACTTTCG -3' |
| *SARS-CoV subgenomic N Reverse* | 5'- GGTTACTGCCAGTTGAATCTGAGG -3' |
| *H1N1 pdm09 Forward* | 5'- GGAGCAAAAAGCTTCTACAA -3' |
| *H1N1 pdm09 Reverse* | 5'- ACTTTGTTGGTCAGCACTA -3' |

## Sauce Data of Supplementary figure. 3

| S. Fig. 3b | | | | | |
|---|---|---|---|---|---|
| | Body weght (%) | | | | |
| days | 0 | 1 | 2 | 3 | 4 |
| Mock+H1N1 | 100 | 98.3298539 | 99.0605428 | 100 | 99.3736952 |
| | 100 | 99.7918835 | 98.855359 | 96.2539022 | 96.0457856 |
| | 100 | 99.5014955 | 97.9062812 | 97.4077767 | 97.108674 |
| | 100 | 104.314995 | 98.8133765 | 97.3031284 | 95.0377562 |
| | 100 | 99.9016716 | 98.9183874 | 100 | 101.966568 |
| | 100 | 97.6626016 | 99.3902439 | 100.304878 | 101.219512 |
| CoV2+H1N1 | 100 | 101.117886 | 101.727642 | 101.626016 | 103.556911 |
| | 100 | 100.209424 | 102.513089 | 104.293194 | 106.910995 |
| | 100 | 100.432432 | 102.378378 | 101.513514 | 103.459459 |
| | 100 | 99.7402597 | 102.467532 | 101.688312 | 105.324675 |
| | 100 | 100 | 101.354402 | 102.708804 | 104.288939 |
| | 100 | 101.317365 | 103.353293 | 101.437126 | 104.431138 |
| Adjusted P Value | >0.9999 | 0.9711 | 0.0006 | 0.0003 | <0.0001 |
| Two-way ANOVA Šídák's multiple comparisons test | | | | | |

| S. Fig. 3c | |
|---|---|
| Relative (to GAPDH) H1N1 genomic RNA level | |
| Mock+H1N1 | CoV2+H1N1 |
| 7.260153243 | 2.0215E-08 |
| 8.51496146 | 9.10938E-09 |
| 0.946057647 | 7.60713E-09 |
| 1.189207115 | 5.41647E-09 |
| 6.062866266 | 1.43936E-08 |
| 1.222640278 | ND |
| Unpaired t test | Two-tailed |
| P value | 0.0249 |