Evidence of SARS-CoV-2 transcriptional activity in cardiomyocytes of COVID-19 patients without clinical signs of cardiac involvement

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Short title: SARS-CoV-2 in cardiomyocytes of COVID-19 patients

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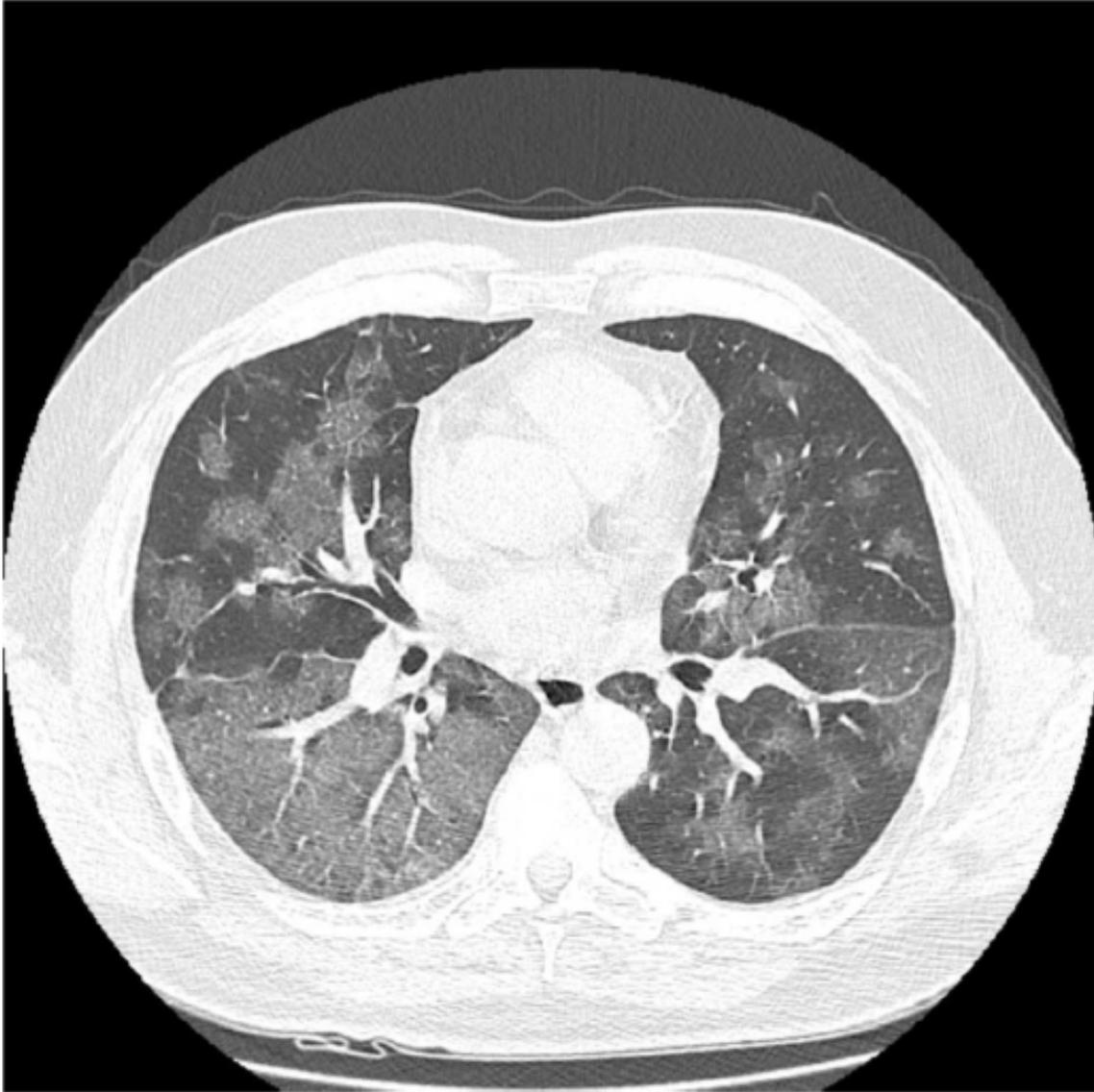
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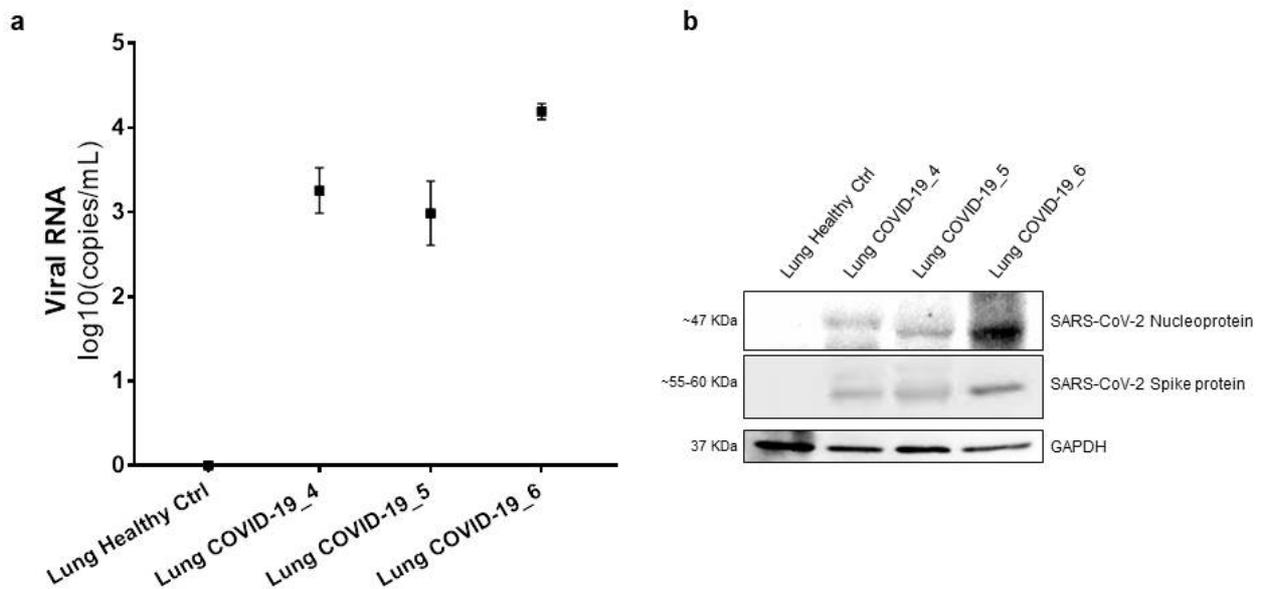
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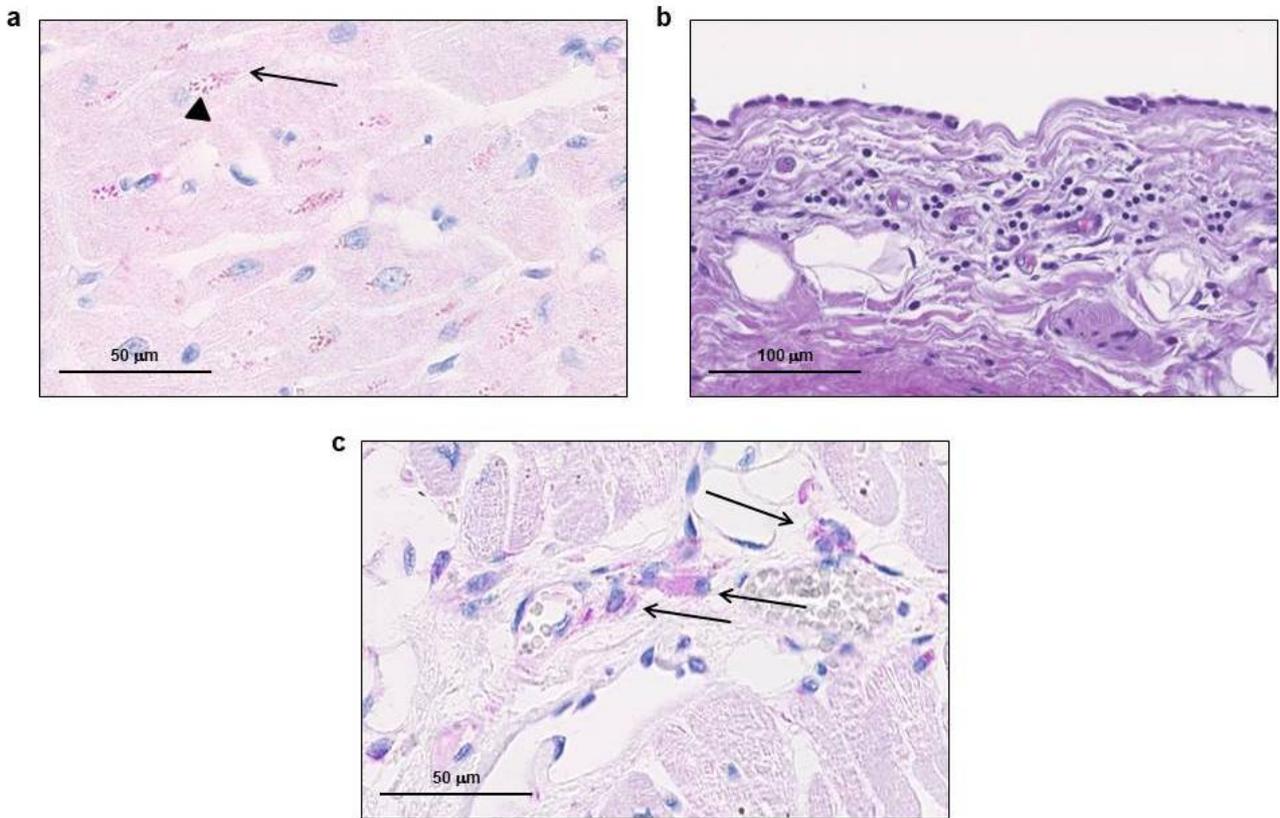
Supplementary Figures

Supplementary Figure 1 – Representative Computed Tomography image of a COVID-19 patient.
Bilateral pathognomonic lung lesion patterns typical of COVID-19 pneumonia are visible.

Supplementary Figure 2 - RNA and protein of SARS-CoV-2 are present in lung samples of COVID-19 patients. a) Chip-based digital PCR for SARS-CoV-2 N sequence in total RNA extracts from lung tissues of 3 out of 6 different patients. Raw expression values were expressed as log10(copies/mL). The results are expressed as median and confidence interval (CI 95%). **b)** Western blot analysis for SARS-CoV-2 nucleoprotein and spike protein on total protein extracts from lung tissues of 3 out of 6 different patients. GAPDH has been adopted as loading control.
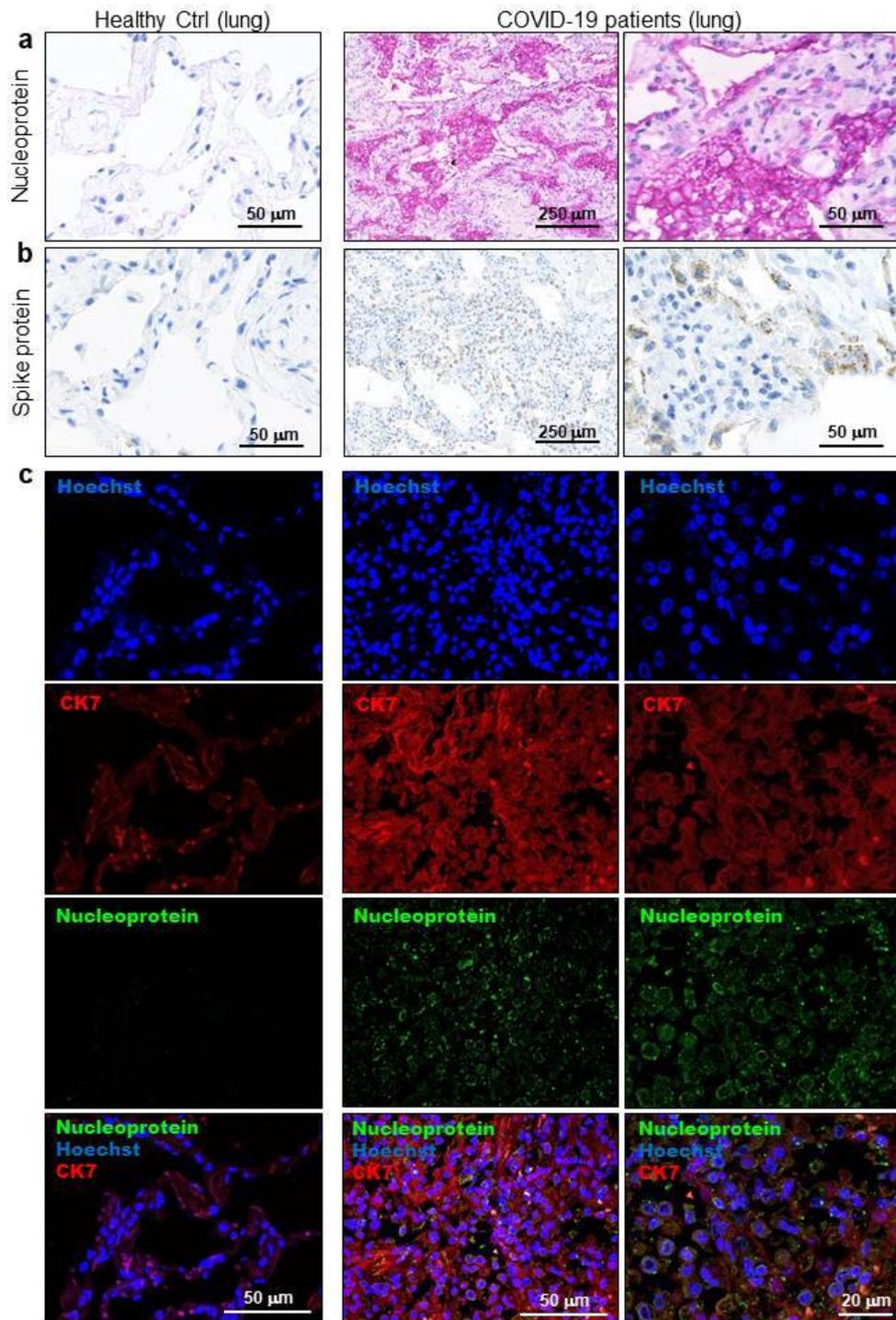
Supplementary Figure 3 - SARS-CoV-2 proteins are detectable in lung tissue of COVID-19 patients. a-b) Representative images of immunohistochemistry assays on 3µm-slides of formalin-fixed paraffin-embedded lung specimens from healthy control (Healthy Ctrl, left panels) and COVID-19 patients (COVID-19, central and right panels) for SARS-CoV-2 nucleoprotein (**a**, in red) and spike protein (**b**, in brown). **c)** Representative images of immunofluorescence assay on 3µm-slides of formalin-fixed paraffin-embedded lung specimens from healthy control (Healthy Ctrl, left panels) and COVID-19 patients (COVID-19, central and right panels) for SARS-CoV-2 nucleoprotein (green) and cytokeratin 7 (CK7, red). Nuclei have been stained by Hoechst (blue). Magnification as scale bars.

Supplementary Figure 4 – SARS-CoV-2 nucleoprotein localization in lipofuscin granule area of cardiomyocytes and in macrophages, and inflammatory infiltrates of heart samples of COVID-19 patients. a) Representative image of immunohistochemistry on 3μm-slides of formalin-fixed paraffin-embedded left ventricle specimens from COVID-19-positive patient for SARS-CoV-2 nucleoprotein. The lipofuscin granules (in light brown) are indicated with arrowhead, while viral nucleoprotein (in red), localized also in same sub-cellular area, is indicated with black arrow. **b)** Haematoxylin-eosin staining on left ventricle specimens from COVID-19-positive patient. The inflammatory infiltrate is clearly localized in sub-epicardial region. **c)** Representative image of immunohistochemistry on left ventricle specimens from COVID-19-positive patient for SARS-CoV-2 nucleoprotein (in red). The high immunoreactive interstitial macrophages are indicated with black arrows. Magnification as scale bars.
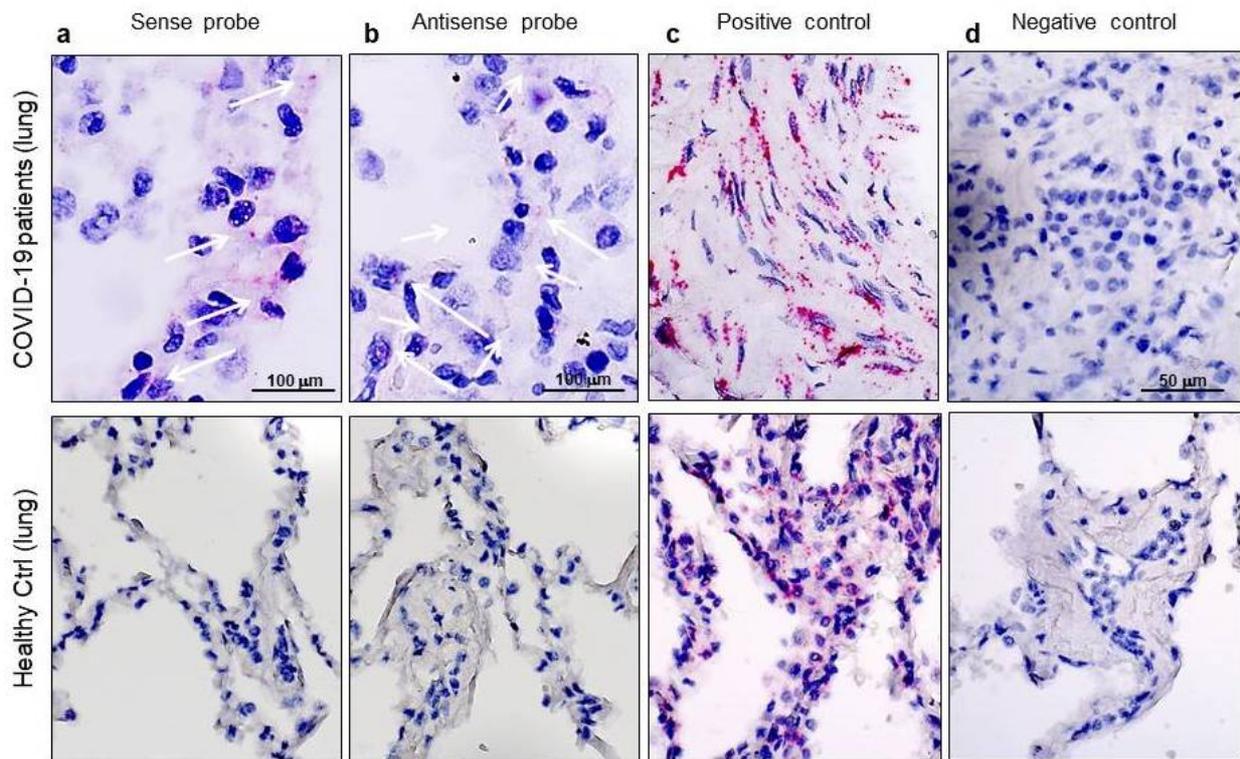
Supplementary Figure 5 - SARS-CoV-2 sense and antisense RNA are localized in the lung specimens of COVID-19 patients. a-b) Representative images of spatially resolved viral RNA detection by RNAScope assay on 3μm-slides of formalin-fixed paraffin-embedded lung specimens from COVID-19 patients (upper panels) and healthy control (Healthy Ctrl, lower panels) for SARS-CoV-2 sense (**a**) and antisense (**b**) probes for spike protein RNA sequences, as well as probes for human PPIB gene (**c**, positive control) and for bacterial DapB gene (**d**, negative control). Fast Red dots indicate viral RNA presence (white arrows). Nuclei have been counterstained with haematoxylin. Magnification as scale bars.
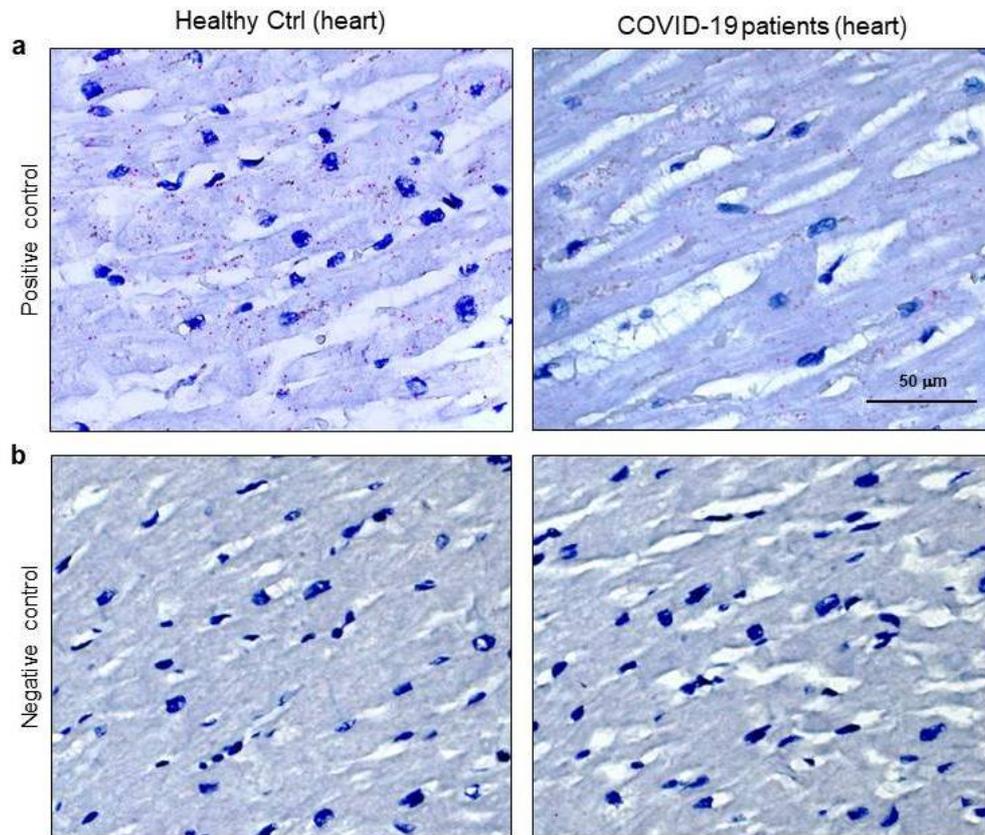
Supplementary Figure 6 – RNAScope positive and negative controls on heart samples from healthy subject and COVID-19 patients. a-b) Representative images of spatially resolved RNA detection by RNAScope assay on 3μm-slides of formalin-fixed paraffin-embedded left ventricle specimens from healthy control (Healthy Ctrl, left panels) and COVID-19-positive patients (right panels). The positive control probe targets human PPIB gene (**a**), while the negative control the bacterial DapB gene (**b**). Fast Red dots indicate RNA. Nuclei have been counterstained with haematoxylin. Magnification as scale bars.