Supplementary Materials and Methods

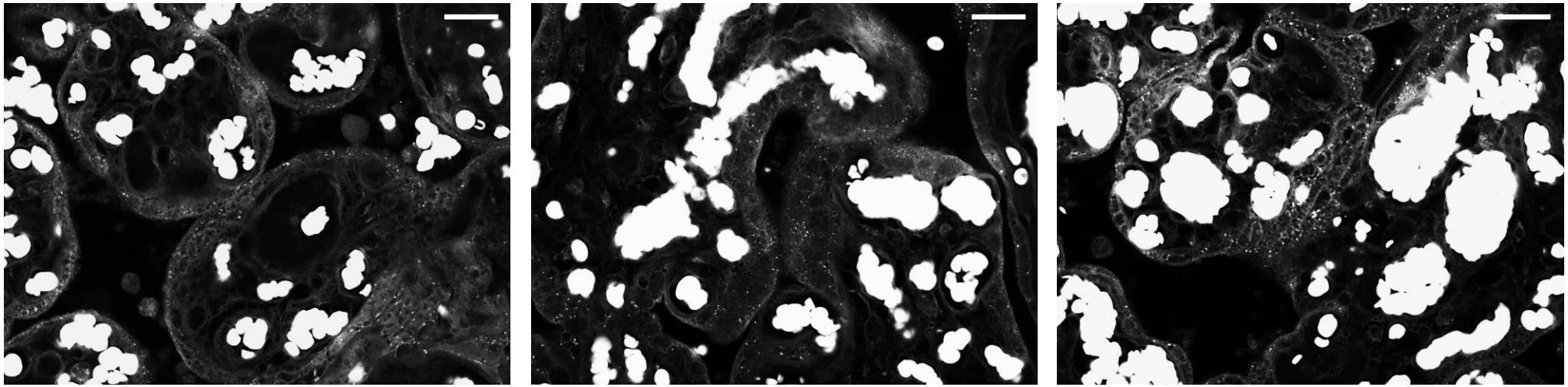
PadLock Assay

Paraffin-embedded sections were dewaxed by treat**ment** with xylene overnight. Sections were then hydrated in 100%, 95%, 90%, 80%, 70%, 50% and 30% ethanol for 2 minutes each and rinsed in distilled water. To preserve RNA during the following procedure, sections were fixed once again in 4% formaldehyde for 20 minutes. Samples were incubated for 30 minutes at 80 °C in the unmasking solution (10 mM Sodium Citrate, 0.05% Tween 20, pH 6.0) and then allowed to cool to room temperature for 10 minutes. After rinse in PBS, the slides were processed for the padlock assay. Sections were incubated overnight at 37°C with the specific padlock probe to the target mRNA. The reaction was carried out in 50 ml of a solution containing 7.5 ml of phosphorylated padlock probe (10 mM), 2.5 ml of DTT (100 mM), 1.25 ul of RiboLock RNase Inhibitor (40 U ml-1) and 38.75 ml of DEPC-treated H_2O. After a wash in PBS/0.01% Tween-20 the samples were incubated for 2 hours at 37°C in 50 ml with the ligation reaction mixture (1X SplintR ligase buffer, 2.5 U ml-1Splint R ligase and 1 U ml-1 RiboLock RNase Inhibitor). Subsequently, the sections were washed in PBS/0.01% Tween-20 and incubated for 1 hour at 37°C with the RCA primer mixture (0.2 mM RCA primer, 1X SSC, 10% formamide, 5 mM DTT and 0.5 U ml-1 RiboLock RNase Inhibitor). RCA reaction was then carried out for 2 hours at 37°C, the sample was incubated with 50 ml of a mixture containing 5 ml of 10X phi29 DNA polymerase reaction Buffer, 2.5 ml di phi29 DNA polymerase (10 U ml-1), 15 ml of dNTPs (10 mM of each dATP, dCTP, dGTP and dTTP), 1.25 ml of RiboLock RNase Inhibitor (40 U ml-1) and 26.25 ml of DEPC-treated H2O. The incubation was followed by a wash in PBS/0.01% Tween-20. Finally, slides were incubated with a mixture containing 100 nM AlexaFluor 595-labelled detection probe, 2X SSC and 15% formamide for 30 min at 37°C, and then washed in PBS/0.01% Tween-20, three times for 5 minutes each. Slides were mounted with ProLong with Dapi (Thermo Fisher Scientific) and examined by a conventional epifluorescence microscope (Olympus BX53; Milano, Italy). Images were captured by a SPOT RT3 camera and elaborated by IAS software.

Table S1: oligos used in this study

Padlock Probe NSP7	5'ACATTGAGCCCACATTTTTTTCTCAATTCTGCTACTTTACTACCTCAATTCTGCTACTG TACTACTTTTTTCATTGTGTAACTGGA 3'
RCA Primer	AGTACAGTAGCAGAATTGAG
AlexaFluor 595-labelled probe	CTCAATTCTGCTACTTTACTAC

SARS-CoV-2 RNA-positive



SARS-CoV-2 RNA-negative

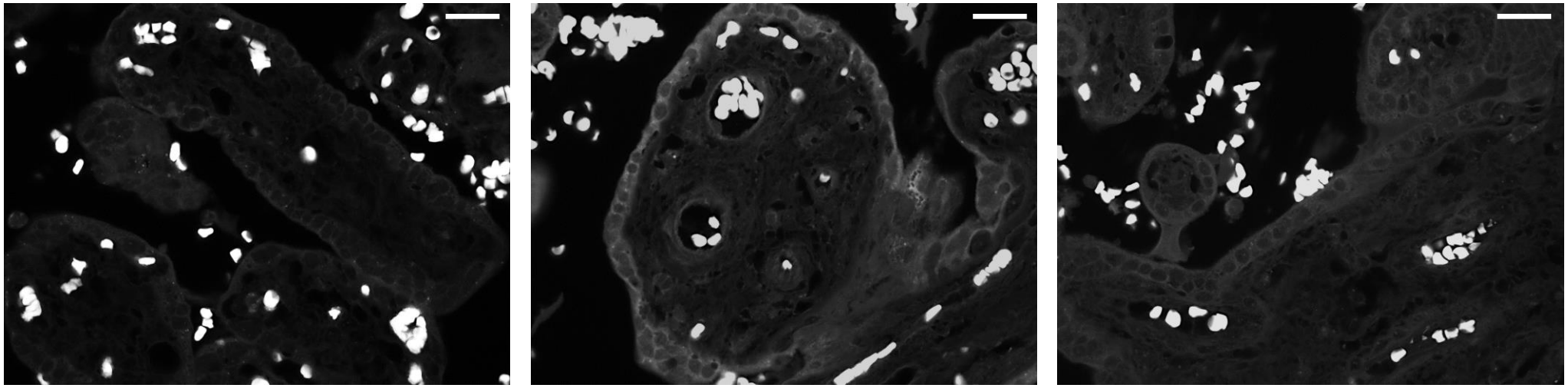


Figure S1. SARS-CoV-2 RNA detection by Padlock Assay. Microscope fields (*black and white*) of padlock assay targeting the SARS-CoV-2 RNA in a SARS-CoV-2-positive placenta. No specific signals are detectable in sections from a SARS-CoV-2-negative placenta. Scale bar, 20 μm

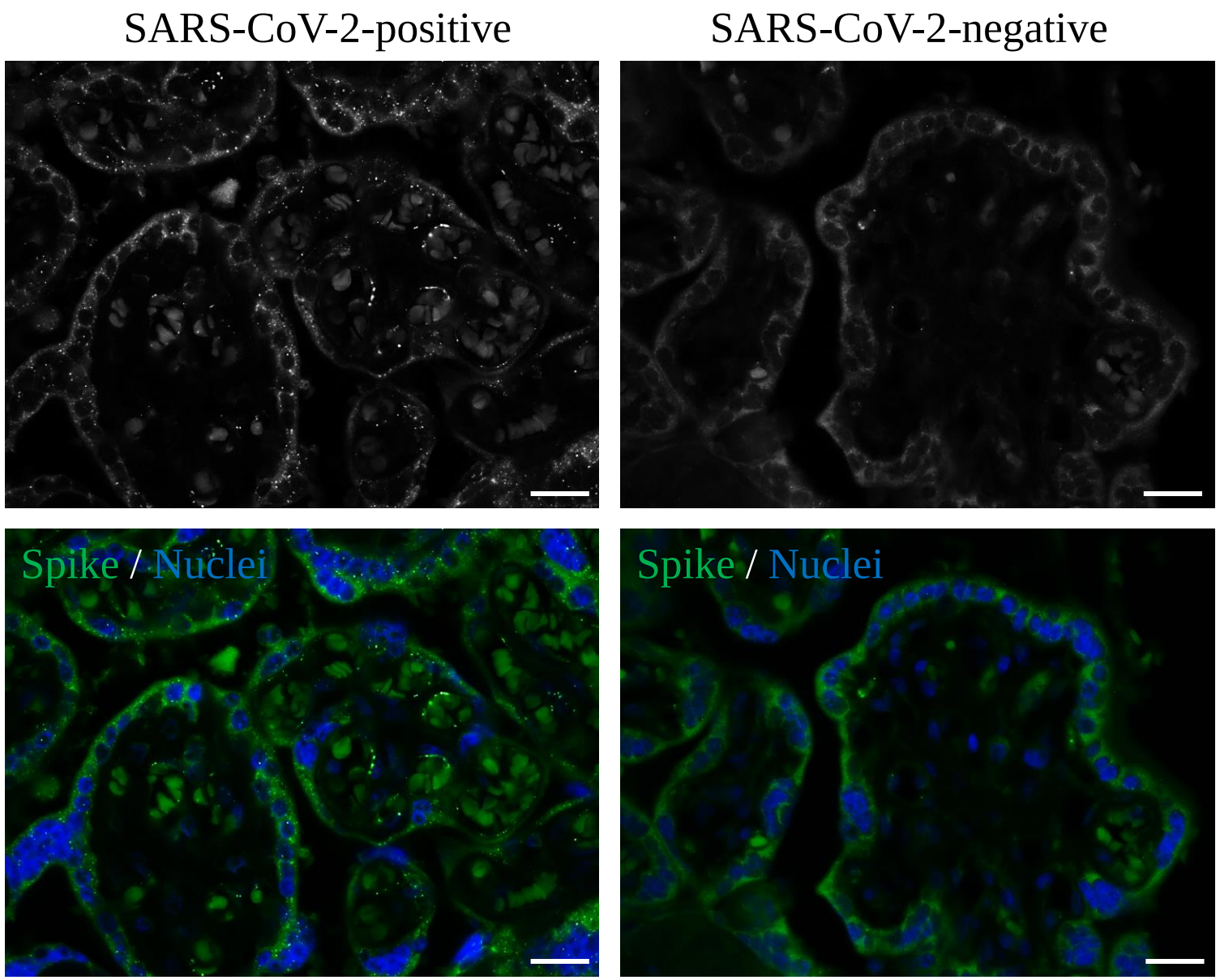


Figure S2. Immunostaining of spike protein. Representative images of the immunofluorescence analysis showing the distribution of spike protein in deparaffinized sections of the SARS-CoV-2-positive placenta. No specific signals are detectable in sections from the SARS-CoV-2-negative placenta. Nuclei were labelled with DAPI (blue). (both *black and white* and *coloured* microscope fields). Scale bar, 20 μm