In vitro induction of interleukin-8 by SARS-CoV-2 Spike protein is inhibited in bronchial epithelial IB3-1 cells by a miR-93-5p agomiR

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Abbreviations: RT-qPCR, reverse transcription quantitative polymerase chain reaction; miR/miRNA, microRNA; S-protein, SARS-CoV-2 Spike protein; IL, interleukin.

SUPPLEMENTARY MATERIALS

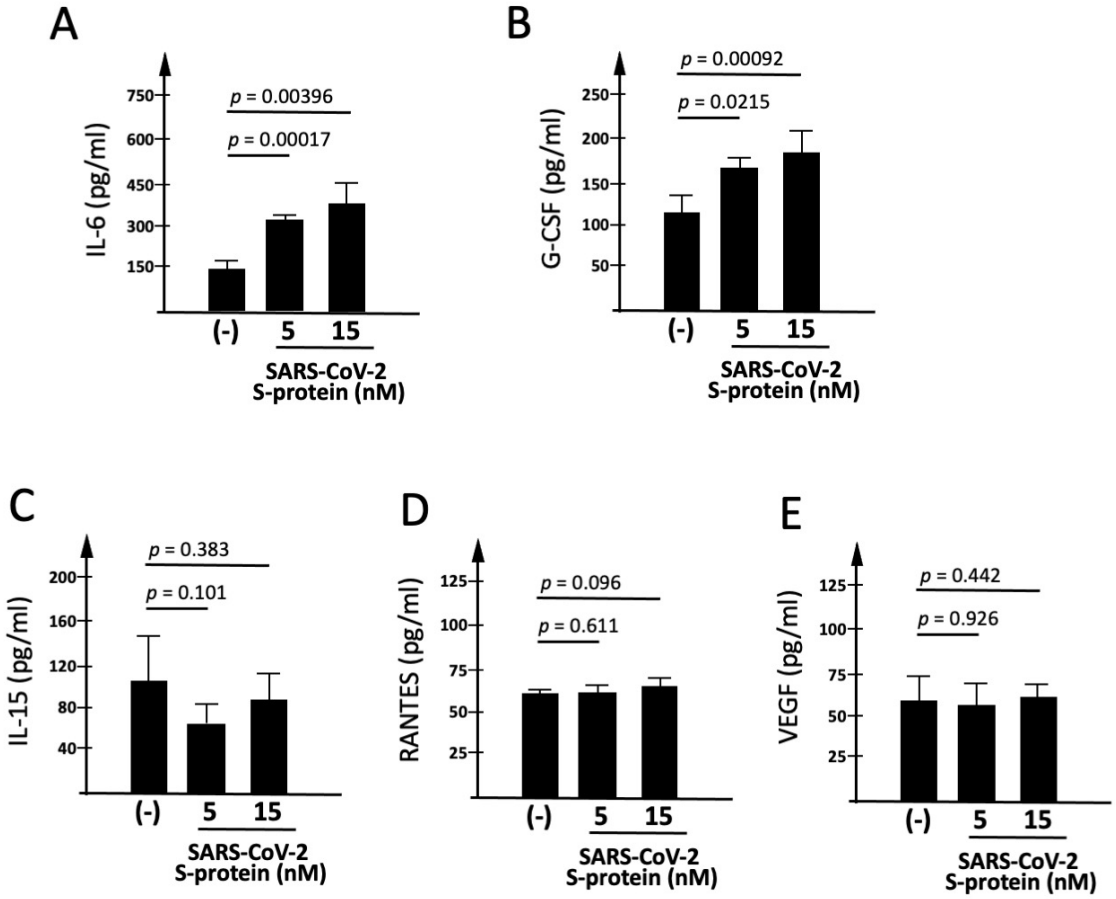

Fig. S1. Differential effects of SARS-CoV-2 Spike protein on IL-6, G-CSF, IL-15, RANTES, VEGF. IB3-1 cells were either untreated (-) or exposed to 5 nM and 15 nM S-protein. Medium was harvested after 24 hours culture, starting from 30% confluence seeding cells (results are the average ± S.D. of three independent experiments). The protein release was quantified by Bio-plex analysis and expressed as pg/ml. **A** = IL-6; **B** = G-CSF; **C** = IL-15; **D** = RANTES and **E** = VEGF. The significance of the difference between S-protein treated vs untreated cells is shown ($p > 0.05$, not significant; $p < 0.05$, significant; $p < 0.01$, highly significant). The effects of SARS-CoV-2 Spike protein on IL-8 is reported in Fig.1B of the main text.

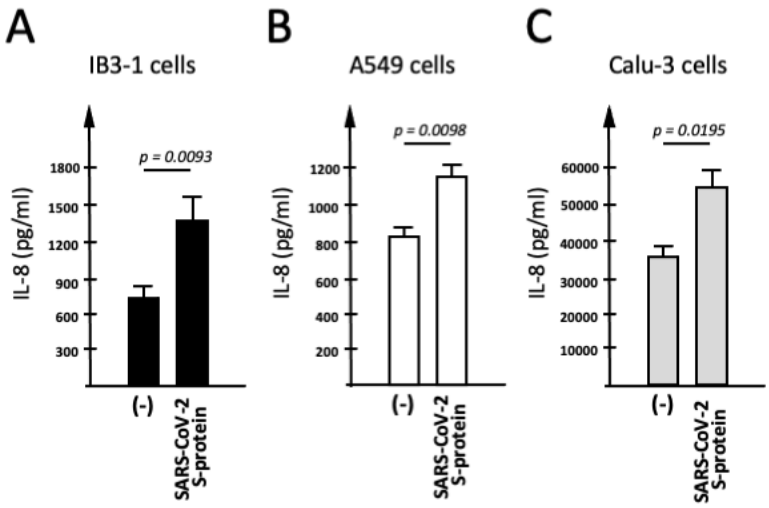

Fig. S2. Effects of SARS-CoV-2 Spike protein on IL-8 release in IB3-1, A549 and Calu-3 cells. Cells were exposed to 5 nM S-protein, medium was harvested after 48 hours culture, and IL-8 was quantified by Bio-plex analysis. The results obtained are reported for IB3-1 cells (**A**; N = 5), A549 cells (**B**; N = 3) and Calu-3 cells (**C**; N = 3). IL-8 quantification was performed using HCYTA-60K-02 Human Cyto Panel A (Merck Life Science S.r.l. cat. HCYTA-60K-02).

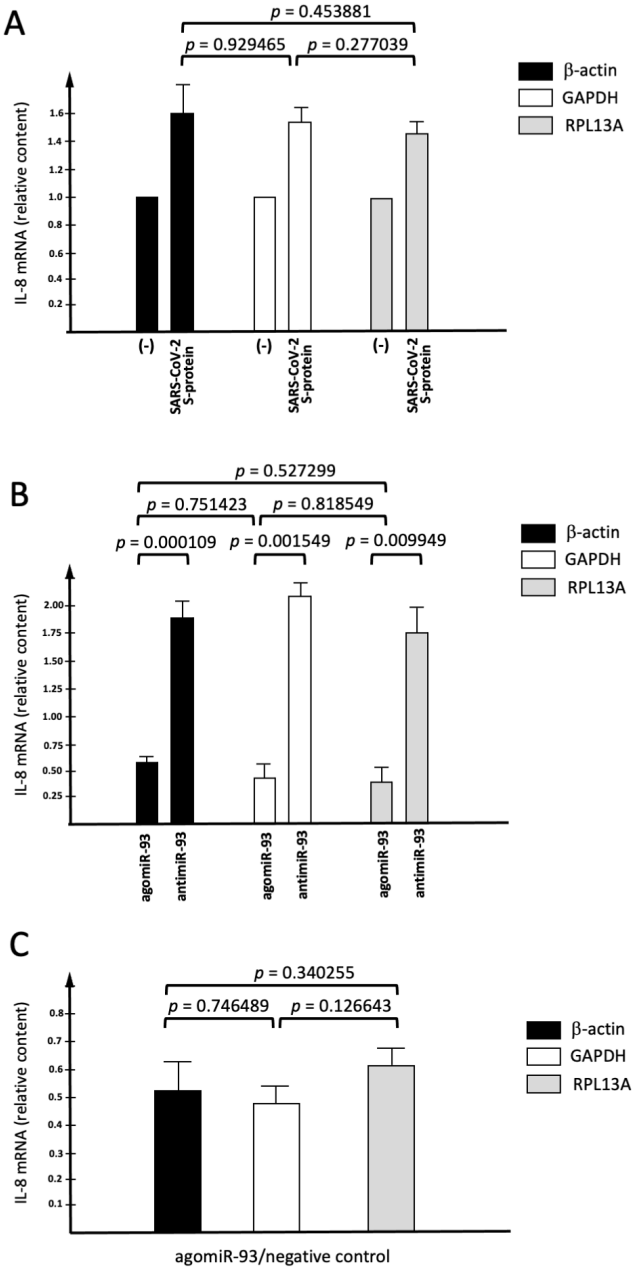

Fig. S3. Reproducibility of the RT-qPCR results using β-actin, GAPDH and RPL13 as reference sequences. Results obtained with the housekeeping β-actin (black boxes), GAPDH (white boxes) and RPL13A (grey boxes) are shown. IB3-1 cells were employed in these experiments. **(A)** Reproducibility of the increase of IL-8 mRNA content following exposed to SARS-CoV-2 S-protein (N = 3). **(B)** Reproducibility of the differential effects on IL-8 mRNA following treatment with the agomiR-93-5p and an antagomiR-93-5p. Please note that when the results concerning the treatments with AgomiR-93-5p and the antagomiR-93-5p are compared highly significance ($p < 0.01$) was obtained using the three reference sequences; on the contrary, when the effects of the agomiR-93 obtained using β-actin, GAPDH and RPL13A as housekeeping sequences are compared, the results were, as expected, similar (N = 4). **(C)** Reproducibility of the results obtained when the effects on IL-8 mRNA of the agomiR-93 are compared with those of a negative control sequence. Also in this case, no significant difference was obtained using β-actin, GAPDH and RPL13A as housekeeping sequences (N = 3).

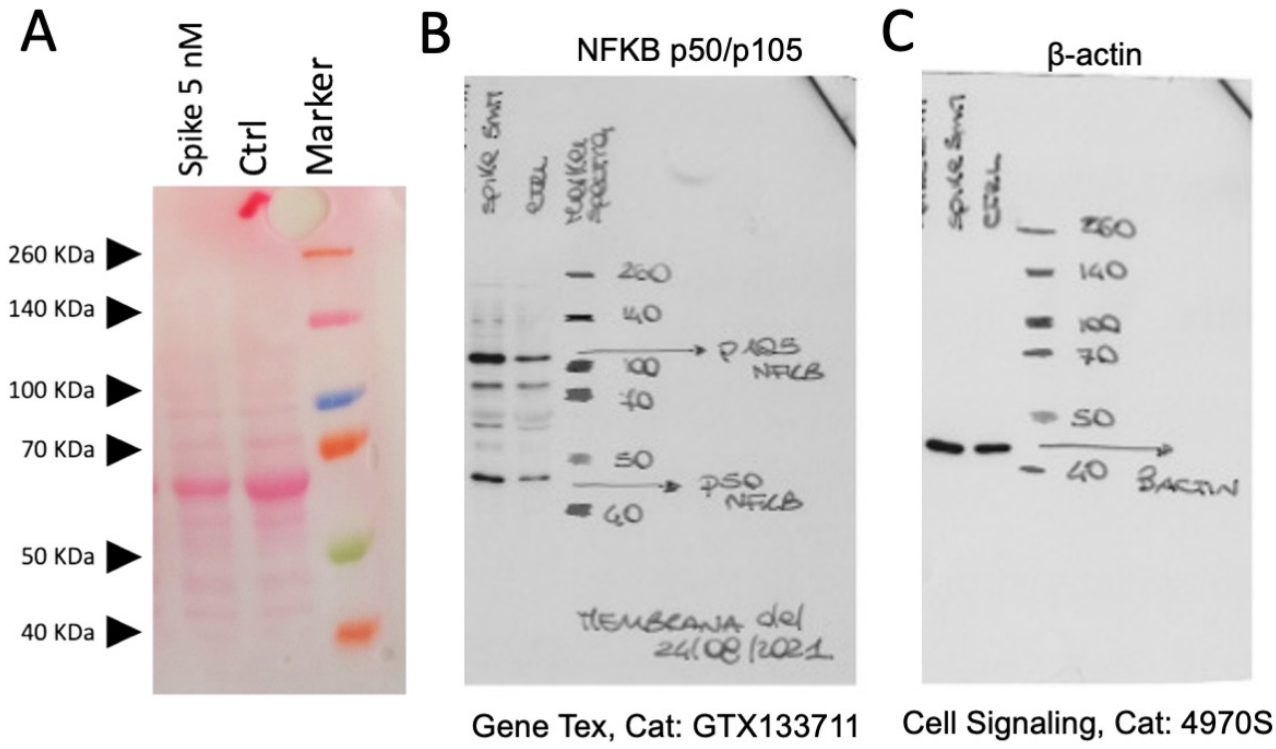

Fig. S4. Western blotting. Original version of the Western blotting depicted in Figure 2, panels B and C. (**A**) Ponceau staining. (**B,C)** Binding of the filters with the indicated antibodies recognizing NF-kB p105/p50 (**B**) and β-actin (**C**).