Data S1

Treatment of IB3-1 cells with the BNT162b2 vaccine. The BNT162b2 vaccine (Comirnaty™; Lot. no. FP8191) was obtained from the Hospital Pharmacy of the University of Padova. For treatment with the BNT162b2 vaccine, IB3-1 cells were seeded at a density of 40,000 cells/ml and were then treated with 0.5, 1 and 2 μg/ml of the vaccine for 24 h (48,49). When needed, cells were transfected with pre-miR-93-5p and/or co-treated with AGE.

BNT162b2 Spike mRNA quantitative analyses. For the quantification of the relative content of the BNT162b2 Spike mRNA, total RNA from BNT162b2 treated cells was isolated after 24 hours treatment and reverse transcribed into cDNA, as described by Gasparello *et al* (1). Quantitative (q)PCR analysis for BNT162b2 Spike mRNA was performed according to the study by Fertig *et al* (2). In this study, primers were designed to be specific to the publicly available, codon-optimised vaccine mRNA sequence (2,3) but not to viral RNA encoding the S-protein. The sequences of the employed primers were 5'-GTGGATCTGCCCATCGGC ATC (forward) and 5'-GTCCATCCGCTGCTGCTATCG (reverse) and were used at a final concentration of 200 nM. For the PCR assay, after an initial step of 10 min at 95˚C, 40 cycles of amplification were performed (15 sec at 95˚C, 1 min at 65˚C) and a final step of heating from 60 to 95˚C. PCR amplification was carried out as previously described using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) (1). Endogenous b-actin was used as internal control.

Statistical analysis. The differences among different groups were compared using one-way ANOVA (analyses of variance between groups, http://vassarstats.net/anova1u.html). Prism (v. 9.02) by GraphPad software was also employed. Differences were considered statistically significant when $P<0.05$ and highly significant when $P<0.01$ (1,4).

References

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3. Jeong DE, Mccoy M, Artiles K, Ilbay O, Fire A, Nadeau K, Park H, Betts B, Boyd S, Hoh R and Shoura M: Assemblies of putative SARS-CoV2-spike-encoding mRNA sequences for vaccines BNT-162b2 and mRNA-1273. Available online: https://virological.org/t/assemblies-of-putative-sars-cov2-spike-encoding-mrna-sequences-for-vaccines-bnt-162b2-and-mrna-1273/663 (Accessed on 19 December, 2024).
4. Gasparello J, Marzaro G, Papi C, Gentili V, Rizzo R, Zurlo M, Scapoli C, Finotti A and Gambari R: Effects of Sulforaphane on SARS-CoV-2 infection and NF-κB dependent expression of genes involved in the COVID-19 'cytokine storm'. Int J Mol Med 52: 76, 2023.

Figure S1. (A) Representative examples showing the RT-qPCR analysis of SARS-CoV-2 S-protein mRNA. (B) Content of the SARS-CoV-2 mRNA (fold increase with respect to trace amount of hybridizable material found in control cells, taken as 1). The content of SARS-CoV-2 S-protein mRNA has been evaluated by RT-qPCR using as described by Fertig *et al* (70) and further detailed in Data S1. Endogenous β-actin was used as internal control. Results represent the average ± standard deviation of three independent experiments. RT-qPCR, reverse transcription-quantitative PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

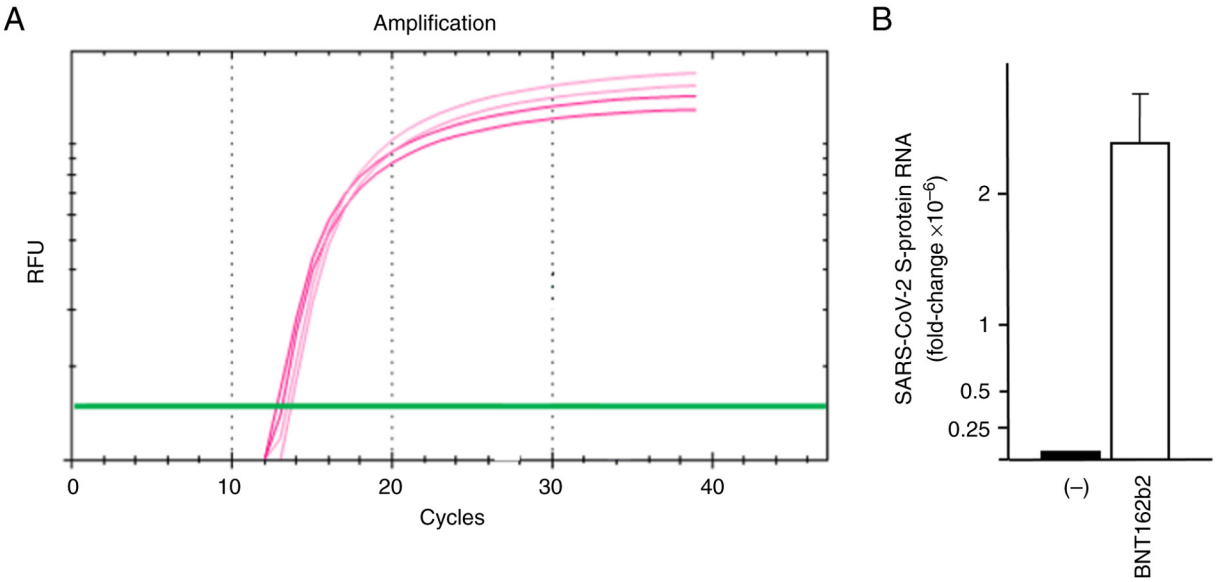

Figure S2. Representative examples showing the increase of the content of miR-93-5p (fold increase with respect to endogenous miR-93-5p) in IB3-1 cells transfected with the pre-miR-93-5p. The content of miR-93-5p has been evaluated by RT-qPCR using the assays described in Table SI. The relative expression of miR-93-5p was calculated as elsewhere described (39) using human Let-7c-5p (white) and U6 (grey) RNAs as normalizers. Results represent the average ± standard deviation of five independent experiments. RT-qPCR, reverse transcription-quantitative PCR; miR, microRNA; n.s., not significant.

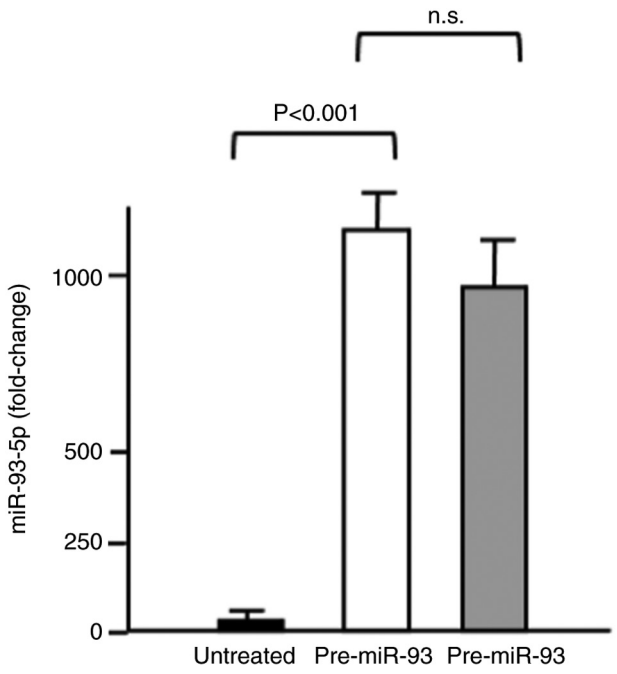

Figure S3. Representative examples showing the increase of the expression of pro-inflammatory genes upon treatment of IB3-1 cells with 5 nm SARS-CoV2 S-protein and the effect of transfection with 200 nM pre-miR-93-5p (agomiR-93) and miR-93 negative control using (A) Bio-plex analysis and (B) RT-qPCR. The relative expression of IL-8 mRNA was calculated as elsewhere described (1,4) using human β-actin as normalizer. Results represent the average ± standard deviation of four independent experiments. Modified from the study reported by Gasparello *et al* (1). Copyright can be found at: https://pmc.ncbi.nlm.nih.gov/articles/PMC8492649/. IL, interleukin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. Further information is included in Table SII.