

A Peptide nucleic acid (PNA) masking the miR-145-5p binding site of the 3'-UTR of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mRNA enhances CFTR expression in Calu-3 cells

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Supplementary Material

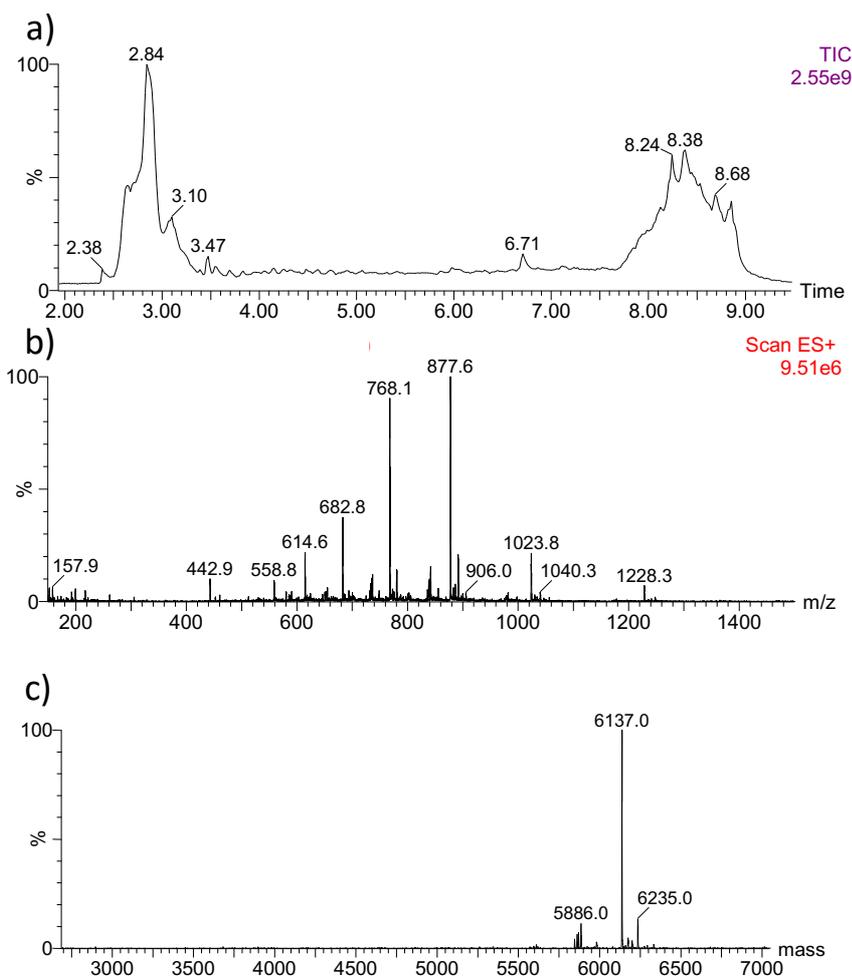


Figure S1. (a) UPLC/MS Chromatogram of the purified **PNA-1** ("**miR145-maskingPNA**"), used for the present work; peak at 2.84 min corresponds to the target PNA. (b) Mass spectrum of the peak at 2.84 min; reconstructed molecular mass obtained from deconvolution of the spectrum in (c).

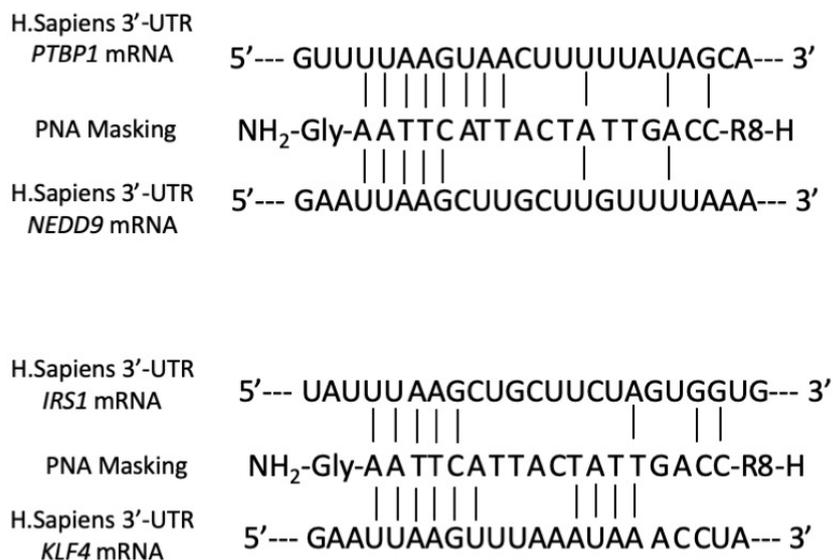


Figure S2. Interactions between the miR145-maskingPNA and the PTBP1 (Polypyrimidine Tract Binding Protein 1) [1], NEDD9 (Neural Precursor Cell Expressed, Developmentally Down-Regulated 9) [2], IRS1 (Insulin Receptor Substrate 1) [3] and KLF4 (Kruppel Like Factor 4) [4] mRNAs. No inhibition in all these examples is appreciable even at the highest concentrations used (Figure 3 of the main text). The miR145-maskingPNA is fully complementary with the 3'UTR region of the CFTR mRNA (Figure 2 of the main text).

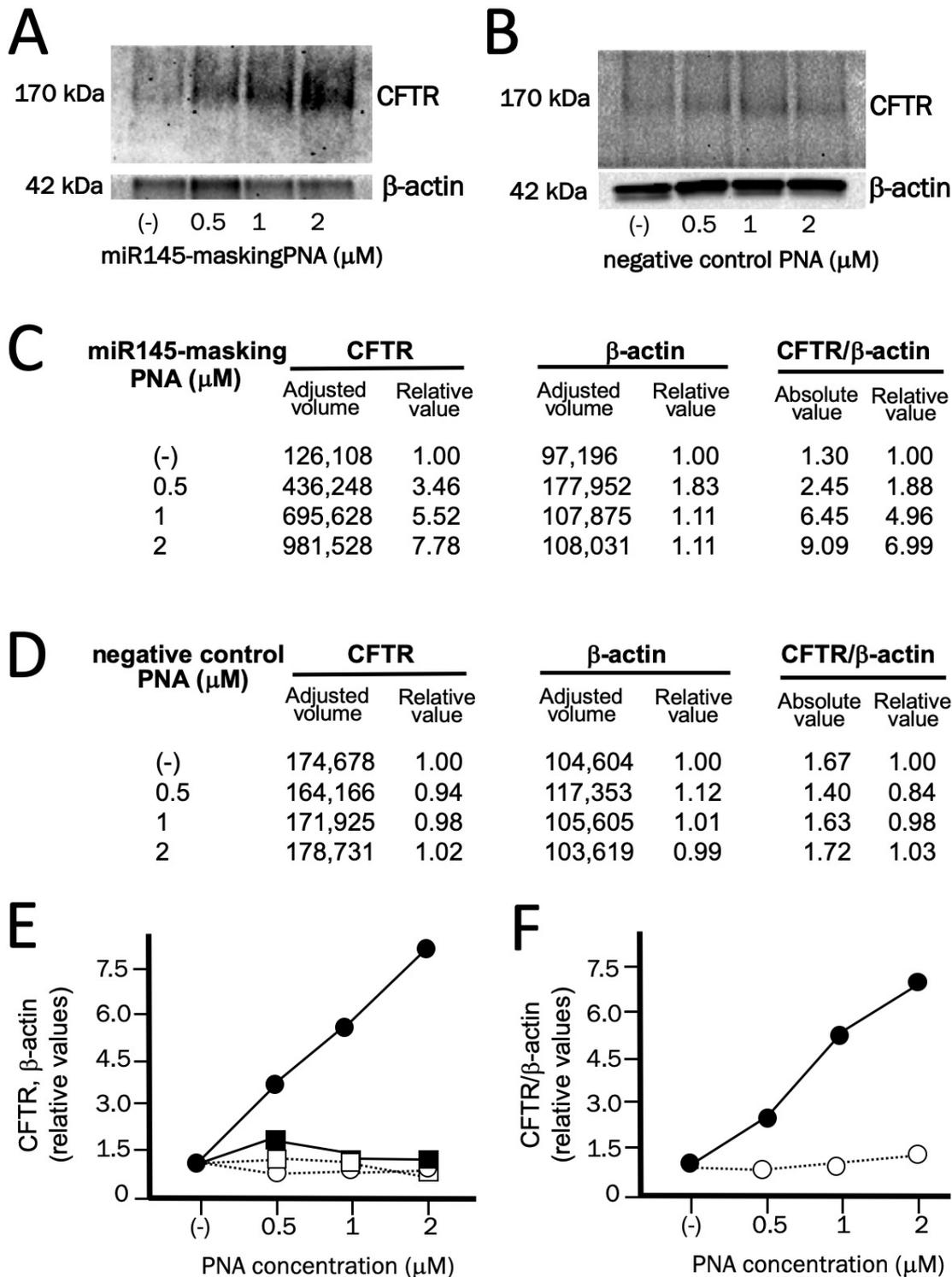


Figure S3. Increase of CFTR expression in Calu-3 cells treated with the miR145-maskingPNA. Raw data of the Western blotting performed using the CFTR- and β -actin directed monoclonal antibodies are presented in panels A-D. Calu-3 cells have been treated for 3 days with the indicated concentrations of miR145-maskingPNA and negative control PNA, as indicated. Panels A and B show the original autoradiography film obtained after exposure of the filter, treated with the CFTR- and β -actin specific antibody, to horseradish peroxidase (HRP) substrate; panel C and D show raw data and representative calculations relative to the densitometric analysis of the autoradiography films shown in A and B. E, F. Relative values of Adjusted volumes (E) and of the calculated CFTR/ β -actin ratios (F). Treatment with the miR145-maskingPNA: black symbols; treatment with the negative control PNA: open symbols; CFTR, circles; β -actin: squares.

Results

Densitometric analysis of Western blotting. For densitometry analysis, the ChemiDoc XRS+ System (Bio-Rad) was used. The Image Lab Software for ChemiDoc and Gel Doc Imaging Systems (Version 6.0.1) was employed in order to acquire, display, edit and analyze the Western blot images, and to display and export data [5,6]. In our case, we performed all the analyses without ambiguity relative to the obtained results. In order to calculate the correct volumes, tools were used to highlight the band section and subtract the background signal. Further, the whole volume analysis Table was saved in excel file to perform further statistical analysis. The raw data of the Western blot experiment shown in Figure 4 (B and C) are reported in the upper part of Figure S3. An increase of the Adjusted volume values was found only when samples from Calu-3 cells treated with miR145-maskingPNA were bound to the CFTR-directed monoclonal antibody 596. No increase in the Adjusted volume values was found in samples from cells cultured in the presence of the negative control PNA. On the other hand, the Adjusted volume values relative to β -actin did not exhibit major changes in samples isolated from cells treated with the two PNAs. In the last two columns the CFTR/ β -actin ratios (including both absolute and relative values) are shown. These calculations generated the summary panel depicted in Figure 4C.

References

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