

Combined treatment of bronchial epithelial Calu-3 cells with Peptide Nucleic Acids Targeting miR-145-5p and miR-101-3p: Synergistic Enhancement of the Expression of the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) Gene

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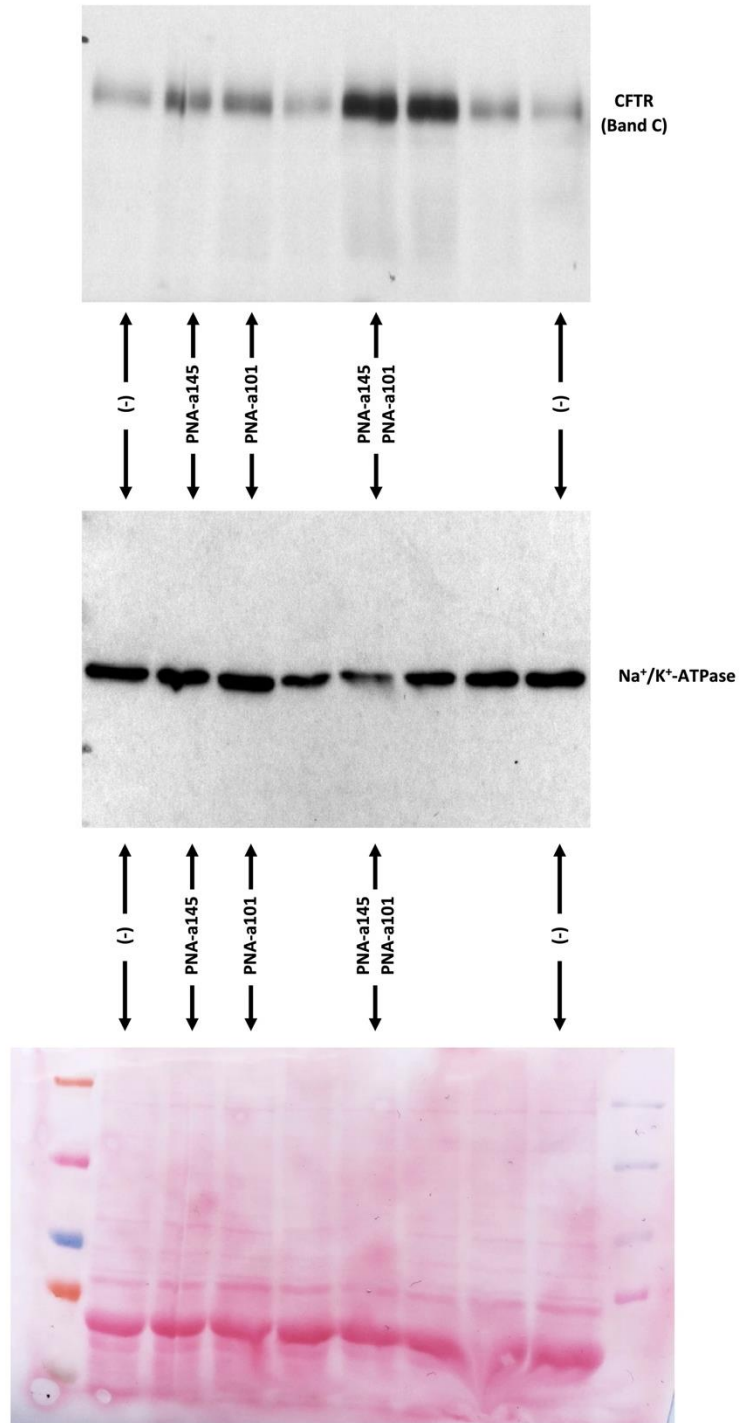
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SUPPLEMENTARY MATERIALS

FIGURE S1

Uncut version of the Western blotting data



These data have been used for Figure 3A, upper part of the panel (up) and Figure 3A, lower part of the panel (down).

TABLE S1

List of miRNAs targeting CFTR and location of their binding sites within the CFTR 3'UTR sequence

| miRNA | Binding sites | Reference |
|-------------|--------------------------|--|
| miR-505-3p | 15 - 21 | Viart et al., 2015 |
| miR-943 | 56 - 62 | Viart et al., 2015 |
| miR-1827 | 97 - 104 | Gilllen et al., 2011 |
| miR-335-5p | 170 - 183 | Tavazoie et al., 2008 |
| miR-377-3p | 212 – 219 1549 - 1556 | Viart et al., 2015 |
| miR-145-5p | 427 - 434 | Gilllen et al., 2011 Oglesby et al., 2013 Viart et al., 2015 Fabbri et al., 2017 Lutful et al., 2017 |
| miR-939-5p | 477 - 483 | Gilllen et al., 2011 |
| miR-200b-3p | 545 - 551 | Bartoszewska et al., 2017 |
| miR-143-5p | 573 - 580 | De Santi et al., 2018 |
| miR-331-3p | 740 – 746 1304 - 1310 | Gilllen et al., 2011 |
| miR-433-3p | 1060 - 1067 | Amato et al., 2013 |
| miR-509-3p | 1056 - 1063 | Amato et al., 2013 Ramachandran et al., 2013 |
| miR-384 | 1118 - 1125 | Gilllen et al., 2011 |
| miR-494-3p | 1140 - 1147 | Gilllen et al., 2011 Viart et al., 2015 Megiorni et al., 2011 Oglesby et al., 2013 Ramachandran et al., 2013 |

| | | |
|-------------|-------------|--|
| miR-607 | 1216 - 1223 | Gilllen et al., 2011 |
| miR-376b-3p | 1250 - 1257 | Gilllen et al., 2011 |
| miR-223-3p | 1475 - 1481 | Oglesby et al., 2013 |
| miR-101-3p | 1508 - 1515 | Megiorni et al., 2011 Hassan et al., 2012 Viart et al., 2015 |
| miR-144-3p | 1508 - 1514 | Hassan et al., 2012 |
| miR-600 | 1510 - 1517 | Gilllen et al., 2011 Viart et al., 2015 |
| miR-1290 | 1535 - 1542 | Gilllen et al., 2011 |
| miR-1246 | 1537 - 1544 | Gilllen et al., 2011 Lukosevicius et al., 2022 |

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TABLE S2

List of the mRNAs targeted by miR-145-5p, miR-101-3p and miR-335-5p and list of the relative publications

| miR-145-5p | | | |
|----------------|---|-------------------|---------------------------------------|
| SOX9 | Zhuang, et al. 2022 | FLI1 | Chen et al., 2021 |
| YES1 | Sun et al., 2022 | NF- κ Bp65 | Tu et al., 2021 |
| Smad3 | Ye at al., 2022 Shen et al., 2020 | CDCA3 | Gu et al., 2021 Chen et al., 2021 |
| ABRACL | Fan et al., 2021 | SOCS7 | Zeng et al., 2021 |
| TMOD3 | Li et al., 2021 | KLF4 | Tuo et al., 2021 |
| IGF1R | Capik et al., 2021 | RBBP5 | Xie et al., 2021 |
| SERPINE1 | Li et al., 2021 | S1PR1 | Gao et al. 2021 |
| CDH22 | Zeng et al., 2021 | CD36 | Yuan et al., 2021 |
| MYO6 | Yang et al., 2021 | Smad2 | Shen et al., 2020 |
| WIP1 | Sun et al., 2021 | HDAC11 | Wang et al., 2020 |
| NRAS | Yu et al., 2021 Ding et al., 2020 Li et al., 2020 | CPEB4 | Liu et al., 2020 |
| MEST | Yu et al., 2021 | ABCC1 | Zheng et al., 2020 |
| MORC2 | Su et al., 2021 | BACH2 | Liu et al., 2020 Li et al., 2020 |
| BRD4 | Wang et al, 2021 | Smad4 | Liu et al., 2020 Zhou et al., 2020 |
| SPOP | Zhang et al., 2021 | ANGPT2 | Zhou et al., 2020 |
| TGF- β 2 | Qi et al., 2021 | TNFAIP2 | Li et al., 2020 |
| PAK7 | Chen et al., 2021 | SPATS2 | Dong et al., 2020 |
| PXN | Lin et al, 2021 | E2F3 | Li et al., 2020 |
| NOH-1 | Tan et al., 2021 | IGF1 | Chen et al., 2020 |
| AIFM1 | Zhou et al., 2021 | Notch1 | Wei et al., 2020 |
| NURR1 | Jiang et al., 2021 | ITGB8 | Wei et al., 2020 |
| CXCL1 | Zhuang et al., 2021 | CXCL3 | Pei et al., 2020 |
| ITGA2 | Zhuang et al., 2021 | CDK6 | Shi et al., 2020 Shao et al., 2013 |
| MUC1 | Jara et al., 2021 | SOX2 | Tang et al., 2019 |
| PLD5 | Liu et al., 2021 | FLT1 | Lv et al., 2019 Dang et al., 2019 |
| MAL2 | He et al., 2021 | KLF5 | Zhou et al., 2019 Cao et al., 2020 |

| | | | |
|----------|---|-----------|---|
| ARF6 | Zhong et al., 2021 Wang et al., 2021 Hsu et al., 2020 | Sema3A | Liu et al., 2019 |
| TOP2A | Du et al., 2021 | TPT1 | Jian et al., 2019 Du et al., 2019 |
| Arhgap24 | Zhang et al., 2021 | RAB18 | Wang et al., 2019 |
| Srgap1 | Zhang et al., 2021 | RHBDD1 | Niu et al., 2019 |
| ROCK1 | Cheng et al., 2021 Zhuang et al., 2021 | FSCN1 | Gao et al., 2019 |
| SF-1 | Liang et al., 2021 | TGFbetaR2 | García-García et al., 2019 Dong et al., 2021 |
| FGF5 | Zhu et al., 2021 Zhang et al., 2019 | DUSP6 | Wu et al., 2019 Gu et al., 2019 |
| SOX4 | Zhu et al., 2021 | CDK6 | Sun et al., 2019 |
| TRPC6 | Whang et al., 2021 | MYD88 | Sun et al., 2019 |
| HMGA2 | Tan et al., 2021 | CTGF | Hang et al., 2019 |
| TLR4 | Jiang et al., 2021 Jin et al., 2019 Jara et al., 2021 | PCBP2 | Wang et al., 2019 |
| PAFAH1B2 | Xu et al., 2021 | AKT3 | Feng et al., 2019 |
| VRK2 | Mu et al., 2021 | NEDD9 | Yu et al., 2019 |
| TRIM2 | Xu et al., 2021 | MTDH | Wang et al., 2015 |
| TBX15 | Zheng et al., 2021 | SOX11 | Chang et al., 2017 |
| ATF3 | Pan et al., 2021 | ADAM17 | Yang et al., 2014 |
| EGFR | Cho et al., 2011 | CFTR | Fabbri et al., 2017 |
| ABRACL | Fan et al., 2021 | JAM-A | Yang et al., 2020 |
| CD40 | Yuan et al., 2017 | HBXIP | Jiang et al., 2019 |
| BNIP3 | Du et al., 2017 | WNT2B | Li et al., 2019 |
| UHRF1 | Matsushita et al., 2016 | AMAD19 | Wang et al., 2019 |
| FOXO1 | Hao et al., 2018 | REV3L | Chen et al., 2019 |
| CCND2 | Jin et al., 2020 | EPHA4 | Cai et al., 2019 |
| TP53 | Sheykhhasan et al., 2021 | PAI-1 | Liu et al., 2020 |
| NUAK1 | Xiong et al., 2018 | TAGLN2 | Zhang et al., 2018 |
| STAT1 | Gregersen et al., 2010 | MRP1 | Zhan et al., 2016 |
| ADD3 | Ye et al., 2017 | PAK1 | Kou et al., 2014 |
| PDE8A | Booiman et al., 2014 | ABCG2 | Shi et al., 2014 |
| DNMT3A | Li et al., 2020 | TWIST | Shi et al., 2014 |
| VEGFA | Zou et al., 2012 | TNFRSF11B | Wang et al., 2017 |
| HDAC2 | Noh et al., 2013 | LMNB2 | Su et al., 2020 |

| | | | |
|--------|---|---------|------------------------|
| SP1 | Zhu et al., 2014 | p70S6K1 | Xu et al., 2012 |
| FKBP3 | Zhu et al., 2017 | OTC4 | Hu et al., 2012 |
| ERBB2 | Sheykhhasan et al., 2021 | HMGB3 | Xie et al., 2020 |
| PDGFRB | Xu et al., 2017 | LRP5 | Cai et al., 2020 |
| MAPK1 | Yang et al., 2018 | LASP1 | Wang et al., 2016 |
| CBFB | Fukuda et al., 2015 Ostenfeld et al., 2010 | SLC7A1 | Wang et al., 2018 |
| PPP3CA | Ostenfeld et al., 2010 | C-MYC | Shao et al., 2013 |
| CLINT1 | Ostenfeld et al., 2010 | SWAP70 | Chiyomaru et al., 2011 |
| IRS1 | Guo et al., 2012 | ESR1 | Hu et al., 2018 |
| NUDT1 | Cho et al., 2011 | | |

miR-335-5p

| | | | |
|---------|-------------------------------------|--------------|--------------------|
| ARGLU1 | Zhao et al., 2022 | TPX2 | Gu et al., 2020 |
| TTK | Suyal et al., 2022 | SNIP1 | Xie et al., 2019 |
| HBP1 | Lu et al., 2021 | IGF1R | Qi et al., 2019 |
| MAPK10 | Gao et al., 2021 | LDHB | Zhang et al., 2019 |
| SLC2A4 | Li et al., 2021 | ROCK1 | Du et al., 2019 |
| Oct4 | Ji et al., 2021 | OTC4 | Yang et al., 2019 |
| OCT4A | Taheri Bajgan et al., 2021 | RB1 | Shi et al., 2012 |
| COL11A1 | Kang et al., 2021 | ZEB2 | Sun et al., 2014 |
| CCNB2 | Wang et al., 2020 | SOX17 | Yang et al., 2014 |
| ADCY3 | Zou et al., 2020 | RASA1 | Wang et al., 2010 |
| JNK3 | Wang et al., 2020 | BCL2L2 | Liu et al., 2018 |
| ROCK1 | Li et al., 2020 She et al., 2020 | ICAM1 | Luo et al., 2018 |
| SIX2 | Jia et al., 2020 | PYGO2 | Qian et al., 2021 |
| CUL4B | Yu et al., 2020 | ZEB1 | Zhang et al., 2018 |
| NUCB2 | Huo et al., 2020 | CFTR | Song et al., 2021 |
| CPNE1 | Tang et al., 2018 | CRIM1 | Jiang et al., 2021 |
| POU5F1 | Gou et al., 2017 | SP1 | Wang et al., 2020 |
| LRP1 | Legaki et al., 2020 | VEGF-C | Ping et al., 2021 |
| SGK3 | Yao et al., 2018 | Tra2 β | Liu et al., 2018 |
| BIRC5 | Zu et al., 2012 | c-MET | Gao et al., 2015 |
| TRIM29 | Zhou et al., 2016 | HIF1AN | Wu et al., 2018 |
| c-MET | Gao et al., 2015 | SGMS2 | Pan et al., 2021 |

| | | | |
|--------|--------------------|-------|-------------------|
| MAP3K2 | Wang et al., 2021 | NFIB | Lin et al., 2022 |
| MEF2D | Wang et al., 2019 | EphA4 | Dong et al., 2018 |
| EGR3 | Zhang et al., 2019 | E2F3 | Song et al., 2021 |

miR-101-3p

| | | | |
|----------------|---|---------|--|
| FOXP4 | Xue et al., 2022 | RAB5A | Sheng et al., 2014 |
| HIPK3 | Tao et al, 2022 | KIF2A | Zhao et al., 2020 |
| WEE1 | Sun et al., 2022 Chen et al., 2019 | SRF | Wu et al., 2017 |
| USP47 | Park et al., 2022 | BICC1 | Wang et al., 2020 |
| BIRC5 | Meng et al., 2021 | SOX9 | Liu et al., 2017 |
| EZH2 | Dong et al., 2021 Wang et al., 2018 Li et al., 2019 | FOXP1 | Sun et al., 2018 |
| MAPK1 | Zhao et al., 2021 | PDCD4 | Zhao et al., 2019 |
| KPNA2 | Wang et al., 2021 | TLR2 | Dong et al., 2015 |
| DUSP1 | Xin et al., 2021 | APP | Barbato et al., 2019 |
| SKP1 | Zhang et al., 2021 | RAC1 | Lin et al., 2014 |
| CUL4B | Gu et al., 2021 Zhang et al., 2019 Xie et al., 2021 | IRF2BP2 | Yao et al., 2019 |
| MTOR | Gu et al., 2021 | TRIB1 | Niespolo et al., 2020 |
| Notch1 | Jiang et al., 2021 | CMIP | Zhang et al., 2017 |
| EIF4G2 | Wang et al., 2021 | CCN4 | Pommier et al., 2021 |
| VEGFA | Guo et al., 2021 | MCL-1 | Cui et al., 2018 Zhu et al., 2017 |
| CLDN1 | Du et al., 2021 | COX2 | Ma et al., 2016 |
| TET2 | Chen et al., 2020 | ATP5B | Zheng et al., 2014 |
| BICC1 | Wang et al., 2020 | CDH5 | Cao et al., 2019 |
| C/EBP α | Zhao et al., 2020 | CPEB1 | Xiaoping et al., 2013 |
| KLF6 | Zhao et al., 2020 | ZEB2 | Lin et al., 2019 |
| STC1 | An et al., 2020 Yuan et al., 2021 | PIM1 | Liu et al., 2015 |
| KRAS | Ding et al., 2020 | ATG4D | Frankel et al., 2011 |
| PTGS2 | Wei et al., 2020 | RAB5A | Frankel et al., 2011 |
| HDAC9 | Sun et al., 2020 | STMN1 | Frankel et al., 2011 Zhu et al., 2018 |
| RAP2B | Zhou et al., 2020 | C-MET | Zhu et al., 2017 |
| TRIM44 | Li et al., 2019 | HGF | Liu et al., 2020 |

| | | | |
|----------|-------------------------------------|----------------|------------------------------|
| ATX | Wang et al., 2019 | NLK | Shen et al., 2014 |
| MED19 | Zhang et al., 2019 | CFTR | Hassan et al., 2012 |
| ZEB1 | Fan et al., 2019 | BICC1 | Wang et al., 2020 |
| Beclin-1 | Sun et al., 2019 | CXCR7 | Yang et al., 2019 |
| AMPK | Cao et al., 2019 | MKP-1 | Qiu et al., 2020 |
| VEGF-C | Liu et al., 2019 Li et al., 2019 | KDM1A | Huang et al., 2019 |
| RAP1B | Zhou et al., 2020 | KPNB1 | Liu et al., 2019 |
| | | SOCS5 | Zhang et al., 2020 |
| CREB1 | Yang et al., 2019 | MEK1 | Huang et al., 2019 |
| HMGA2 | Jiang et al., 2016 | ANXA2 | Bao et al., 2017 |
| SOX4 | Tavazoie et al., 2008 | Nrf2 | Dong et al., 2019 |
| PTPRN2 | Tavazoie et al., 2008 | USP22 | Zhao et al., 2016 |
| MERTK | Tavazoie et al., 2008 | FOXO1 | Wang et al., 2017 |
| TNC | Tavazoie et al., 2008 | ABCC1 | Shao et al., 2021 |
| TIGAR | Xu et al., 2017 | Jak2 | Wang et al., 2014 |
| CXCL12 | Zhang et al., 2015 | RAP1A | Chen et al., 2020 |
| FOS | Liang et al 2014 | TGF- β 1 | Wang et al., 2021 |
| FZD4 | Chen et al., 2019 | RUNX1 | Wang et al., 2015 |
| ROCK2 | Ye et al., 2016 | PTGER4 | Chandramouli et al., 2012 |

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TABLE S3

Levels of CFTR protein content in Calu-3 cells treated with PNA-a145, PNA-a101, and PNA-a335 administrated singularly or in combination

| Treatment | CFTR (Fold average) | Standard Deviation (SD) |
|---------------------|---------------------|-------------------------|
| PNA-a145 | 1.94 | 0.19 |
| PNA-a101 | 1.96 | 0.07 |
| PNA-a335 | 1.57 | 0.14 |
| PNA-a145 + PNA-a101 | 9.33 | 0.99 |
| PNA-a145 + PNA-a335 | 4.95 | 0.26 |
| PNA-a101 + PNA-a335 | 1.93 | 0.20 |