A new treatment approach for cystic fibrosis

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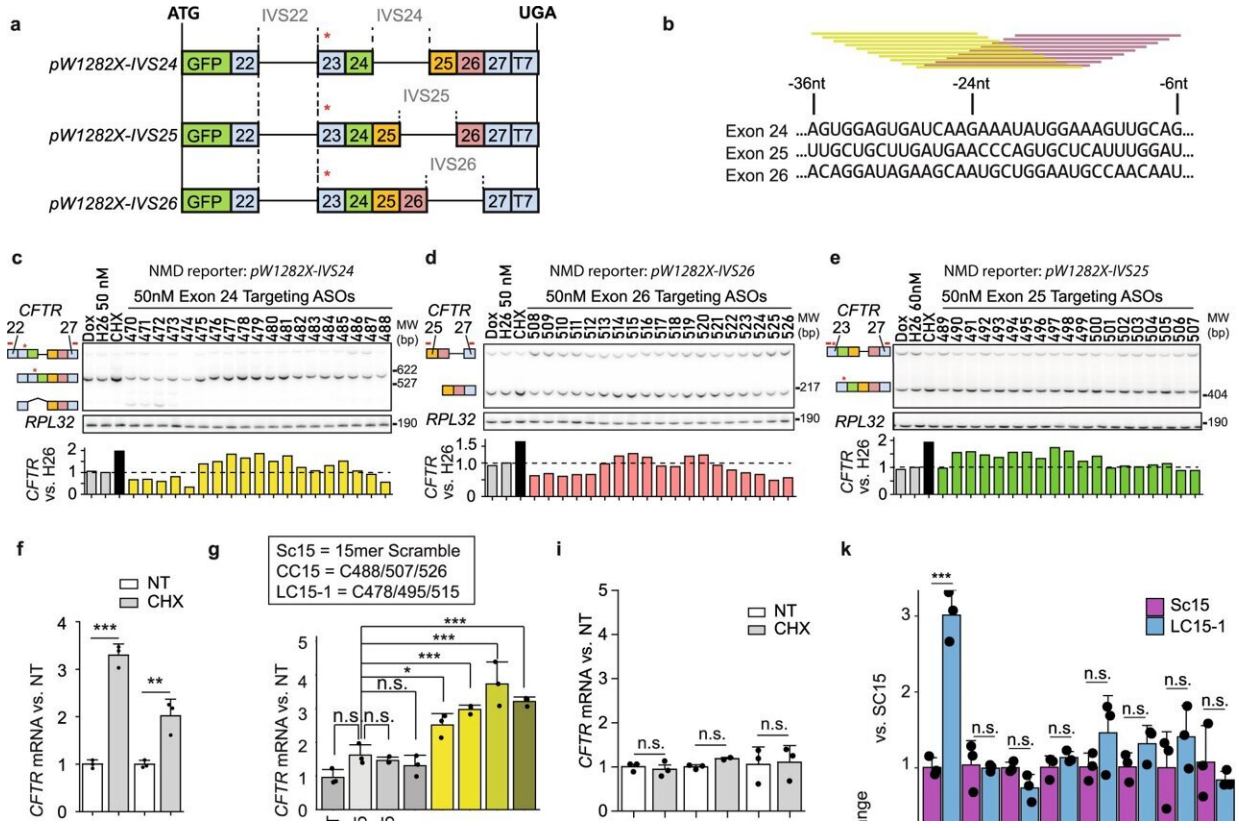

Identification of NMD-inhibiting ASOs and assessment of their specificity. **a** Schematic of NMD reporters. The numbers show the *CFTR* exons in the NMD reporters. The red asterisk (*) indicates the location of the W1282X mutation. **b** Schematic of ASO screening. 19 MOE-PS-modified 15-mer ASOs (yellow and magenta bars) were designed to cover the presumptive EJC binding sites on exons 24, 25, and 26 at 1-nt resolution. U2OS cells stably expressing each NMD reporter were transfected with individual ASOs targeting EJC binding regions on *CFTR* exon (**c**) 24, (**d**) 25, or (**e**) 26, respectively. Reporter mRNA levels were measured by radioactive RT-PCR, using primers (red bars above the target exon) listed in Supplementary Table 5. **f** Effect of cycloheximide on *CFTR* expression

in 16HBE-W1282X and DLD1-W1282X cells. **g** Effect of the 15-mer ASO cocktail LC15-1 on *CFTR* expression in 16HBE-W1282X cells. **h** Comparison between the effects of LC15-1 or LC18 on *CFTR* expression in 16HBE-W1282X cells. **i** Effects of cycloheximide on *CFTR* expression in DLD1-WT, 16HBE-G551D, and 16HBE-F508del cells. **j** *CFTR* mRNA levels in DLD1-WT, 16HBE-G551D, and 16HBE-F508del transfected with Sc15 or LC15-1 at a nominal total concentration of 120 nM. **k** Endogenous NMD-sensitive mRNA levels in 16HBE-W1282X cells treated with cycloheximide, 120 nM Sc15 or LC15-1. All mRNA levels in (**f**)–(**k**) were measured by RT-qPCR; *CFTR* mRNA levels were measured using forward and reverse primers targeting exon 22 and exon 23, respectively. *RPL32* served as internal reference for all panels except **h**, in which *HPRT* served as internal reference. NT = No treatment; Dox: doxycycline 1 μg/mL; Sc15/18 = 15/18-mer Scramble ASO; CC15 = ASO cocktail C488/C507/C526; LC15-1=ASO cocktail C478/C495/C515; LC18 = 18-mer ASO cocktail C24/25/26; CHX = 1-h incubation with 100 μg/mL cycloheximide. Data are represented as mean values ± SD. All data points represent independent biological replicates. **c**–**e** ($n = 1$). **f**–**k** ($n = 3$ for all treatments, except $n = 2$ in LC18-mer 120 nM in **h**). For all statistical tests, n.s. $P > 0.05$, *P