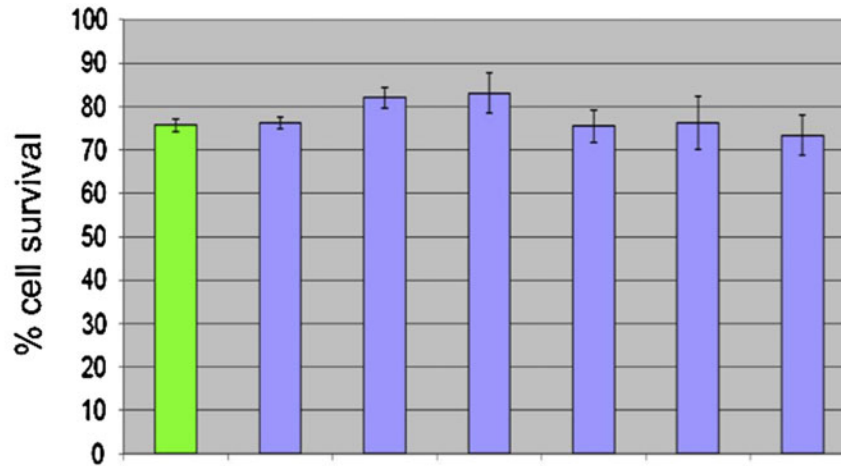


Supplementary Data



pEGFP	1 μg	1 μg	1 μg	1 μg	1 μg	1 μg	1 μg
CFi9-R ^{WT}		1 μg				1 μg	
CFi9-L ^{WT}			1 μg			1 μg	
CFi9-R ^{KK}				1 μg			1 μg
CFi9-L ^{EL}					1 μg		1 μg
pcDNA3.1+	3 μg	2 μg	2 μg	2 μg	2 μg	1 μg	1 μg

SUPPLEMENTARY FIG. S1. Percentage survival of cells transfected with pEGFP and pZFNs expressed as mean ± standard error of the mean. The amount of each plasmid used in transfections is shown in μg. ZFN1/2^{WT} and ZFN 1^{KK}/2^{EL} do not cause toxicity in the CFTE cell line. 300,000 CFTE cells were transfected with 4 μg DNA/10 μL lipofectamine²⁰⁰⁰. The number of EGFP-positive cells was recorded at 24 and 72 h post-transfection by FACS (Beckman Coulter), with 100% representing the level of EGFP-positive cells after 24 h in EGFP-only-transfected cells. The ratio of EGFP-positive cells in ZFN transfections compared to the ratio of EGFP-positive cells in pcDNA3.1+ only transfections was determined. None of the ZFN combinations tested displayed any significant decrease in cell survival, relative to cells transfected with pcDNA3.1+. It should be noted that transfection of CFTE cells with pEGFP results in a 25% reduction in cell survival over 72 h. However, this level of cell death has been reported by other groups¹⁶ after a lipid-based transfection. All transfections were carried out in triplicate. ZFN, zinc-finger nucleases; CFTE, cystic fibrosis tracheal epithelial; EGFP, enhanced green fluorescent protein.