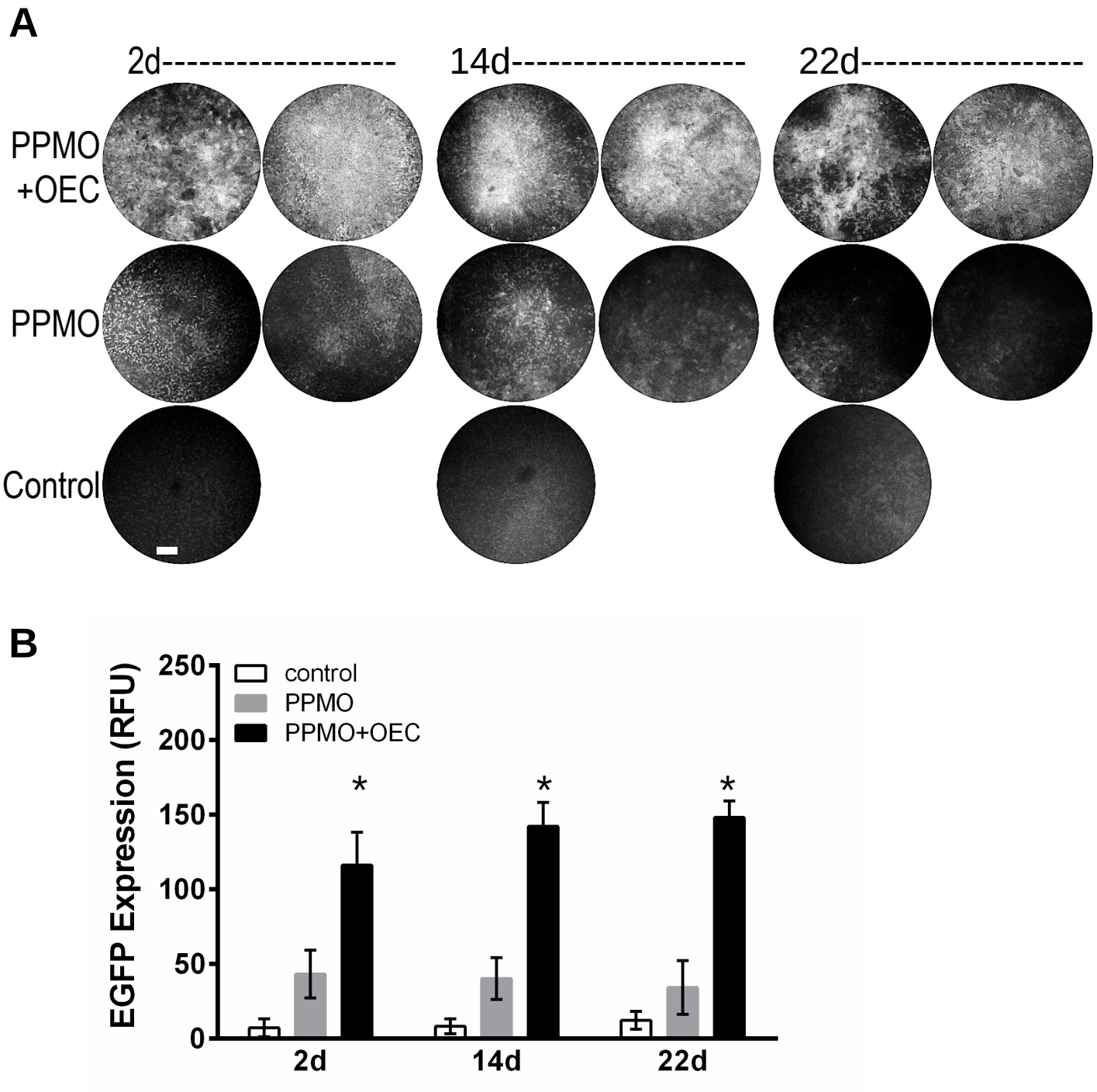
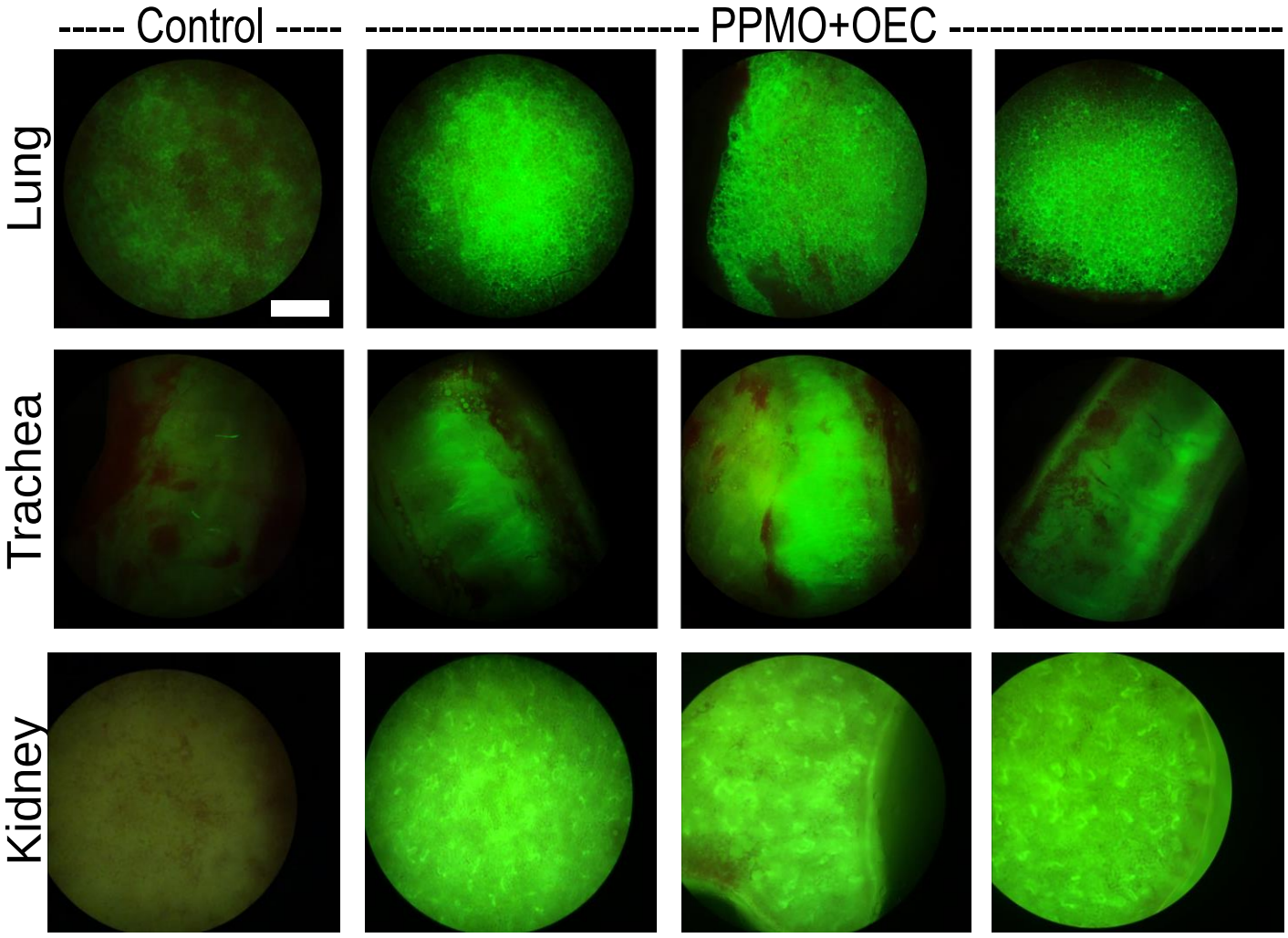
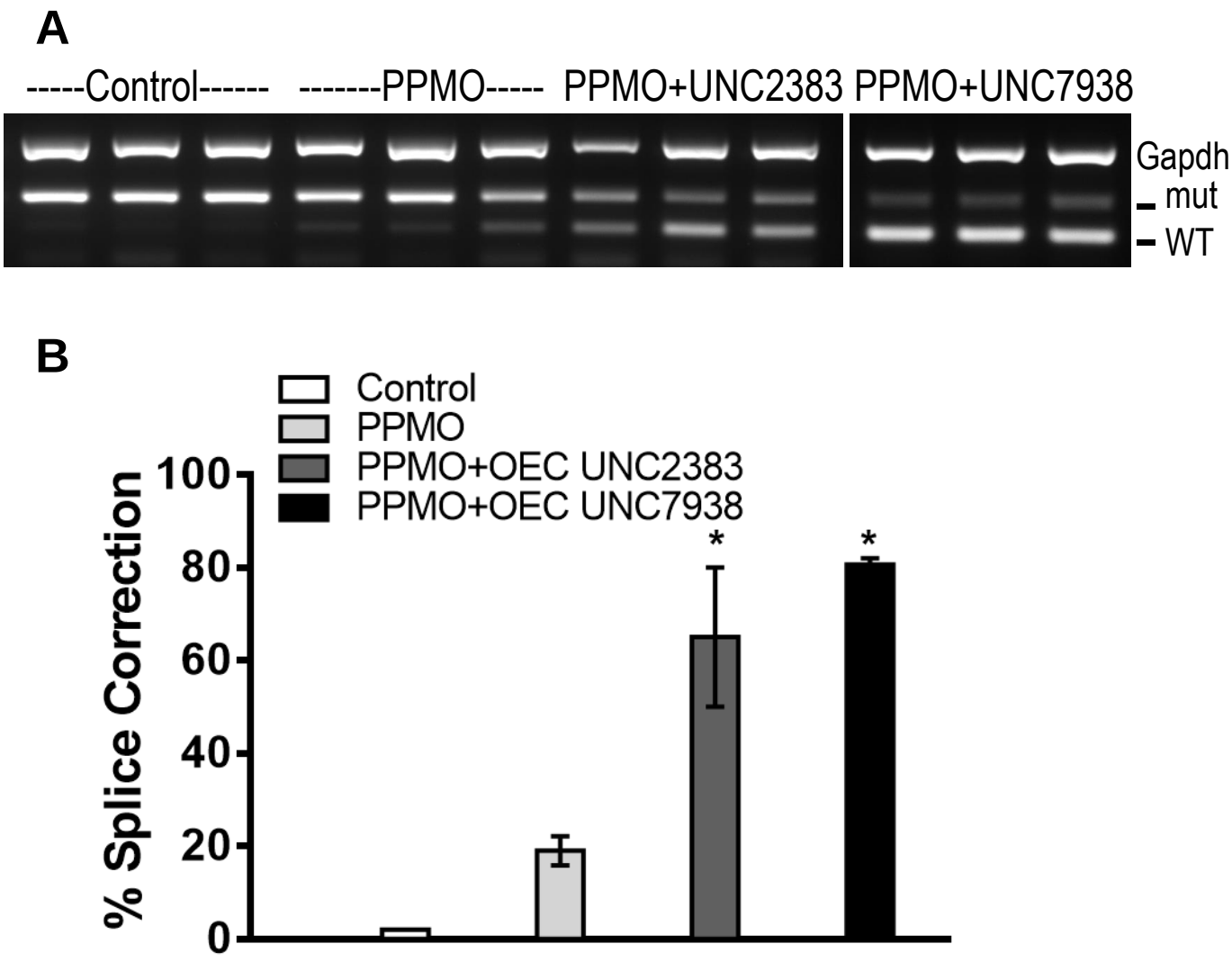
Supplemental Figures and Tables



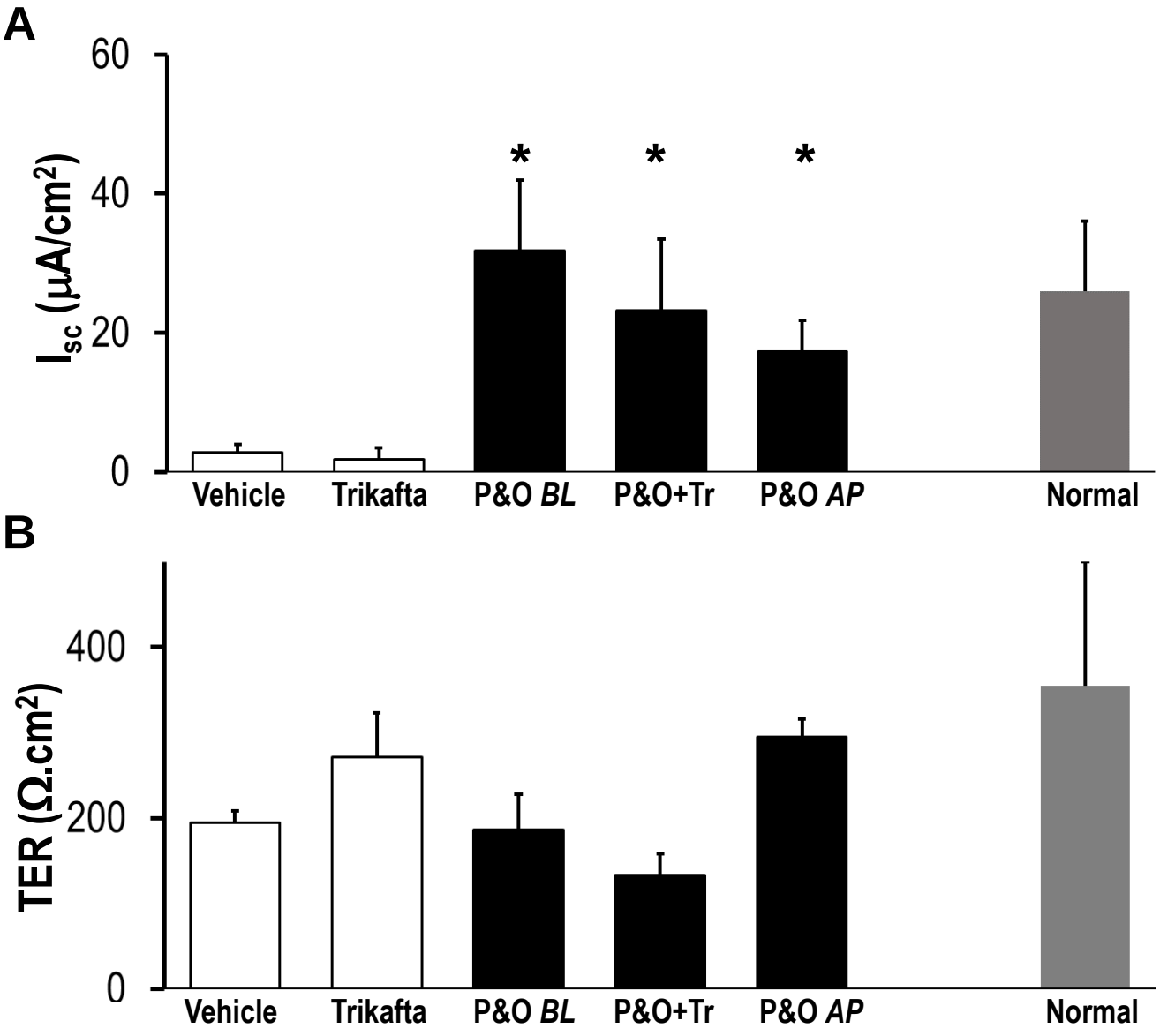
Supplemental Figure 1. Extended effect of one treatment of PPMO & OEC in airway epithelial cultures. Mouse tracheal cells derived from the EGFP654 splice mutant mouse were differentiated on permeable supports and treated with PPMO654 (1uM) followed by vehicle or OEC (UNC7938, 10uM). Both drugs were administrated one time, via the basolateral side. (**A**) Fluorescence in live cultures was imaged at low magnification (10x) in a Leica DMRB microscope at 2, 14, and 22 days post-treatment (n=2-3), bar=100μm. (**B**) Fluorescence intensity quantification, * =p<0.01 vs. PPMO alone.



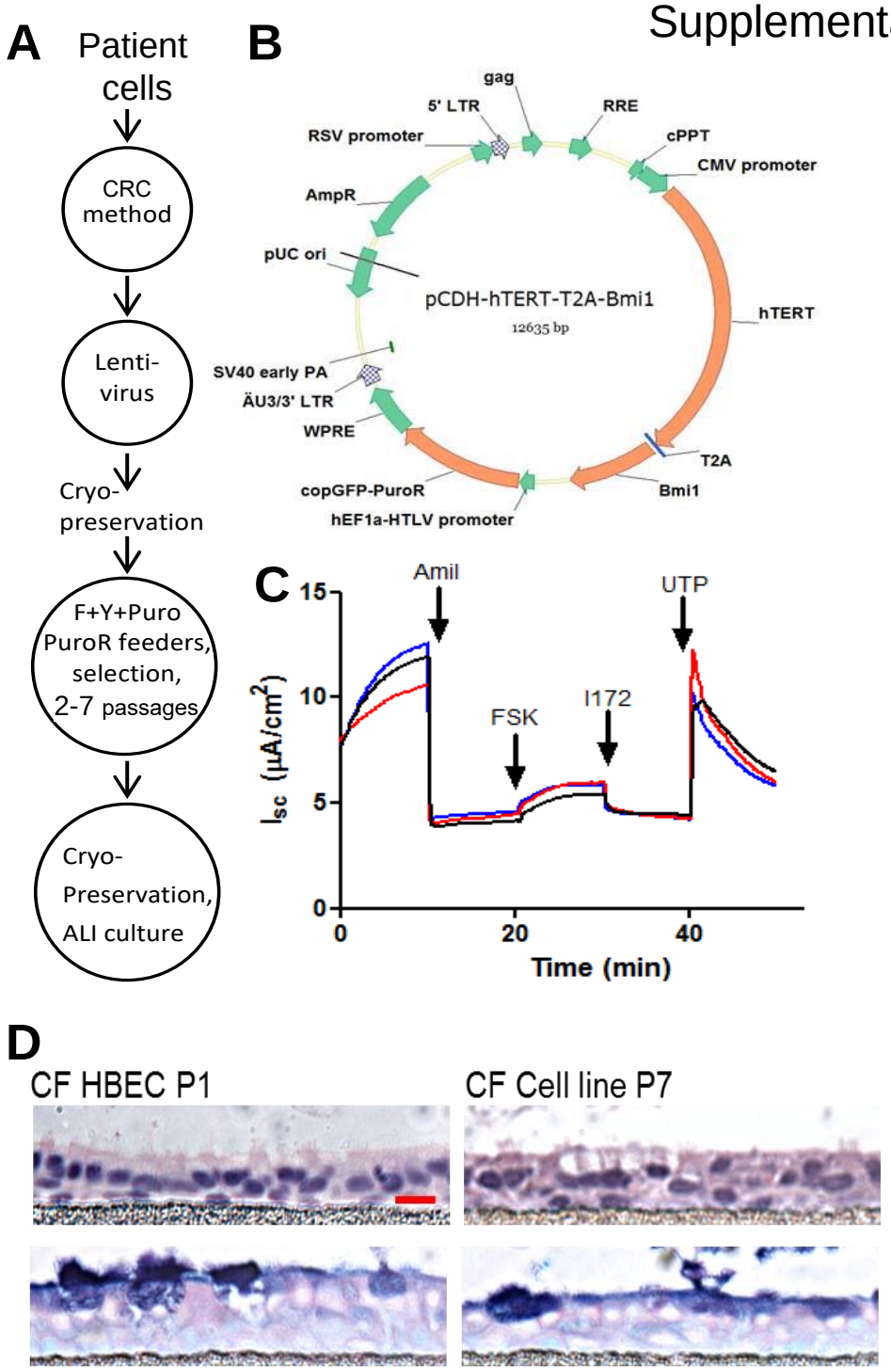
Supplemental Figure 2. PPMO & OEC splicing correction in vivo in the EGFP654 splice mutant mouse. Whole organs (one control and three PPMO+OEC-treated mice) from experiment described in fig 3 were quickly dissected 48hs post-treatment and imaged at low magnification (4x) in a Leica DMRB microscope. Increased EGFP fluorescence was observed in fresh lung, trachea, and kidney by effective in vivo delivery of PPMO654 followed by OEC compared to vehicle controls, n=3, bar=1mm.



Supplemental Figure 3. Kidney splicing correction in vivo. EGFP654 splice mutant mice were treated by co-administration of PPMO654 and UNC7938 or UNC2383 as described in Fig. 6. Freshly dissected tissues were subjected to RNA isolation and analysis as before; (A) PCR gels for the EGFP and Gapdh reactions and (B) EGFP correction quantification, $p<0.05$ PPMO+OEC vs. PPMO, n=3.



Supplemental Figure 4. CFTR functional correction by Ussing chamber analysis. Post-Amiloride forskolin short circuit peak values (**A**) and transepithelial resistances (TER) (**B**) from the experiment described in Fig 7; sem ± sd; n=4, *=p<0.01 vs. vehicle or Trikafta®-like combination (Tr). The forskolin peak values for basolateral (BL) and apical (AP) treatments of PPMO+OEC (P&O) were not significantly different than values obtained in cell cultures derived from non-CF (Normal) patients. CFTR activity was restored using PPMO+OEC but not Trikafta®-like combination in HBEC from a 3849+10kbC->T homozygous CF patient. TERs were not significantly affected by PPMO/OEC treatments (basolateral or apical). The grey bar "Normal" indicate historical data from non-CF HBEC cultured and analyzed in comparable conditions (non-CF HBEC derived from 10 different donors; sem ± sd).



Supplemental Figure 5. Creation and testing of human CF bronchial epithelial UNCCF8T cells from patient-derived cells. (**A)** Overall approach for cell line generation. (**B)** Lentiviral vector for creation of growth extended cell line. (**C).** Ussing chamber traces of UNCCF8T cells (homozygous 3849+10kbC->T) at passage seven (P7). CRC = conditionally reprogrammed cell, F+Y = CRC medium, Puro = puromycin / R=resistance, ALI = air-liquid interface, Amil = Amiloride, FSK = forskolin, I172=CFTR inhibitor 172, UTP=uridine triphosphate. **D.** Microscopy images of UNCCF8T cells and the parental patient-derived HBEC stained by H&E (top) and Alcian Blue-Periodic acid–Schiff (bottom) protocols; bar=15µm.

Supplemental Table 1
Peptide-Morpholino Oligonucleotides and Primers

NAME	PEPTIDE SEQUENCE	OLIGONUCLEOTIDE SEQUENCE
PPMO SSO 654	RXRRXRRXRRXRXB	*ATATTGCTATTACCTTAACCCAGAA*
PMO SSO 654		*ATATTGCTATTACCTTAACCCAGAA*
PPMO mismatch	RXRRXRRXRRXRXB	*ATTTTACTATTATCTTAACACAGTA*
PPMO SSO 3849	RXRRXRRXRRXRXB	*CCTTTCAGGGTGTCTTACTCACCAT*
PCR primer 654 forward		GTGGTGCCCATCCTGGTCGAG
PCR primer 654 reverse		CCGTAGGTCAGGGTGGTCACG
PCR primer 3849 forward		CCATACAAGAATGGCCAACTC
PCR primer 3849 reverse		GTTCTTCCCAAGAGGCCCA
PCR primer Gapdh forward		GTCTCCTCTGACTTCAACAGCG
PCR primer Gapdh reverse		ACCACCCTGTTGCTGTAGCCAA

Oligonucleotide symbols: PMO=italic
Peptide symbols: R=arginine, B= β–alanine, X=6-aminohexanoic acid

Supplemental Table 2
Clinical Chemistry Analysis for OEC 7938 Sequential Administration (IV)
Experiment in Fig. 3

Clinical Chemistry Data (n=3)	**ALP U/L**	**ALT U/L**	**AST U/L**	**BUN mg/dl**	**CREATININE mg/dl**
Average for Controls	34+/-10	85+/-46	229+/-118	22+/-2	0.35+/-0.07
Average for PPMO only	41+/-14	86+/-21	174+/-96	24+/-6	0.33+/-0.08
Average for PPMO+OEC	35+/-3	120+/-173	221+/-134	19+/-0.5	0.35+/-0.1

Plasma samples taken 48 h after OEC administration
ALP= alkaline phosphatase, ALT=alanine aminotransferase,
AST=aspartate aminotransferase, BUN=blood urea nitrogen

Supplemental Table 3A
Clinical Chemistry Analysis for OEC 7938 Co-Administration (IV) Experiment (48h)

Clinical Chemistry Data (n=3)	**ALP U/L**	**ALT U/L**	**AST U/L**	**BUN mg/ml**	**CREATININE mg/ml**
Average for Controls	65+/-7	60+/-1	77+/-1	26+/-1	0.28+/-0.06
Average for PPMO only	83+/-1	45+/-10	57+/-16	19+/-6	0.45+/-0.10
Average for PPMO+OEC	72 +/-20	36+/-14	60+/-9	19+/-2	0.37+/-0.02

Supplemental Table 3B
Clinical Chemistry Analysis for OEC 2383 Co-Administration (IV) Experiment (48h)

Clinical Chemistry Data (n=3)	**ALP U/L**	**ALT U/L**	**AST U/L**	**BUN mg/ml**	**CREATININE mg/ml**
Average for Controls	61+/-1	75+/-29	68+/-23	21+/-6	0.45+/-0.14
Average for PPMO only	89+/-11	56+/-38	92+/-20	19+/-6	0.45+/-0.08
Average for PPMO+OEC	90+/-16	38+/-20	59+/-8	11+/-12	0.34+/-0.08

Supplemental Table 4
Clinical Chemistry Analysis of OEC 7938 Co-administration Experiment (6 days)

Clinical Chemistry Data (n=4)	**ALP U/L**	**ALT U/L**	**AST U/L**	**BUN mg/ml**	**CREATININE mg/ml**
Average for Controls	54+/-3	27+/-1	51+/-10	17+/-1	0.36+/-0.02
Average for PPMO only	48+/-4	27+/-4	64+/-26	17+/-2	0.27+/-0.03
Average for PPMO+OEC	58+/-5	24+/-2	66+/-21	16+/-5	0.27+/-0/04